Complications of type 2 diabetes

Prevalence and association with mannose-binding lectin

PhD dissertation

Anne Gedebjerg

Aarhus University - Health
Department of Clinical Medicine

&

Aarhus University Hospital
Department of Clinical Epidemiology

2020

Supervisors

Reimar Wernich Thomsen
Associate professor, MD, PhD (main supervisor)
Department of Clinical Epidemiology
Aarhus University Hospital, Aarhus, Denmark

Mette Bjerre
Associate professor, MSc, PhD (project supervisor)
Medical/Steno Diabetes Center Aarhus Research Laboratory
Aarhus Hospital, Aarhus, Denmark

Troels Krarup Hansen
Professor, MD, PhD, DMSc (project supervisor)
Steno Diabetes Center Aarhus
Aarhus University Hospital, Aarhus, Denmark

The assessment committee

Anne-Mette Hvas (chairman)
Professor, MD, PhD
Department of Clinical Biochemistry
Aarhus University Hospital, Aarhus, Denmark

Lars Christian Mørch Stene
Senior Researcher, PhD
Department of Chronic Diseases and Ageing
Norwegian Institute of Public Health, Oslo, Norway

Morten Dahl
Professor, MD, PhD, DMSc
Department of Clinical Biochemistry
Zealand University Hospital, Køge, Denmark

To my family

Acknowledgements

I would like to start by expressing my sincere gratitude to a number of people who made this work possible.

First, I would like to express my sincere gratitude to my main-supervisor, Reimar Wernich Thomsen. You believed in me from the start and opened the world of research to me. Thank you for your support, guidance, and for always answering my e-mails quickly (day and night) during this PhD. In particular, I would like to thank you for teaching me to take leadership of the project.

My sincere thanks to my supervisors, Mette Bjerre and Troels Krarup Hansen, for generously sharing your extensive research experience and clinical knowledge. Thank you for introducing me to the wonders of mannose-binding lectin (MBL) and your priceless feedback and guidance during this PhD. A special thanks to you Mette, for the enormous work involving storage and measurements of MBL on more than 7000 individuals.

Henrik Toft Sørensen, thank you for your advice, your insightful comments, and skillful linguistic revisions on manuscripts. It has been an honor to work with you at such an inspiring research department.

A special thanks to Alisa Devedzic Kjærgaard for sharing your enormous knowledge on genetics and Mendelian Randomization, and for your excellent skills in scientific writing. I could not have done it without your guidance and mentorship.

A thank you to the Danish Centre for Strategic Research in Type 2 Diabetes (DD2) project, all the participants in the DD2 study, and Jens Steen Nielsen for making all the ends of the DD2 come together.

I sincerely would like to thank all my wonderful colleagues at the Department of Clinical Epidemiology throughout the years, for great company and support. Sia Kromann Nicolaisen, thank you for your work with the DD2 data. Mette Kielsholm Thomsen, Mette Lauge Kristensen, and Sigrid Bjerge Gribsholt, thank you for all the laughs and conversations at the office. A special thanks to Diana Hedevang Christensen, who really helped me a lot.

Finally, my warmest thanks to friends and family. My parents, Lise and Dan Gedebjerg, a heartfelt thank you for teaching me that anything is possible if you set your mind to it and for giving me every opportunity in life. Troels, thank you for always being there and believing in me – you are my dearest friend, the love of my life, and the best father. Asta and Ella, thank you for putting perspective into my life.

Anne Gedebjerg, March 2020

Grants

The thesis was made possible with financial support from:

- The Danish Diabetes Academy
- The Danish Heart Foundation
- The Graduate School of Health, Aarhus University
- Danielsen Foundation
- Augustinus Foundation
- The A.P. Møller Foundation
- Direktør Emil C. Hertz og hustru Inger Hertz' Foundation
- Direktør Kurt Bønnelycke og hustru Grethe Bønnelyckes Foundation
- The MEA/KEA Foundation

List of papers

The thesis is based on the following three original studies, which will be referred in the following text by their roman numerals:

I. **Prevalence of micro- and macrovascular diabetes complications at time of type 2 diabetes diagnosis and associated clinical characteristics: A cross-sectional baseline study of 6958 patients in the Danish DD2 cohort.**
Gedebjerg A, Almdal TP, Berencsi K, Rungby J, Nielsen JS, Witte DR, Friborg S, Brandslund I, Vaag A, Beck-Nielsen H, Sørensen HT, Thomsen RW. *J Diabetes Complications.* 2018 Jan;32(1):34-40. doi: 10.1016/j.jdiacomp.2017.09.010. Epub 2017 Sep 19

II. **Mannose-binding lectin and Risk of Cardiovascular Events and Mortality in Type 2 Diabetes: A Danish Cohort Study.**
Gedebjerg A, Bjerre M, Kjaergaard AD, Steffensen R, Nielsen JS, Rungby J, Friborg S, Brandslund I, Thiel S, Beck-Nielsen H, Sørensen HT, Hansen TK, Thomsen RW. Submitted to *Diabetes Care*

III. **Mannose-binding Lectin and Risk of Infections in Type 2 Diabetes: A Danish Cohort Study.**
Gedebjerg A, Thomsen RW, Kjaergaard AD, Steffensen R, Nielsen JS, Rungby J, Friborg S, Brandslund I, Thiel S, Beck-Nielsen H, Sørensen HT, Hansen TK, Bjerre M.
In draft

Abbreviations

ATC	Anatomical Therapeutic Chemical Classification
BMI	Body Mass Index
CCI	Charlson comorbidity index
CI	Confidence interval
CPR	Central Personal Registration
CRS	Civil Registration System
CRP	C-reactive protein
DDDA	Danish Diabetes Database for Adults
DD2	The Danish Centre for Strategic Research in Type 2 Diabetes
DNA	Deoxyribonucleic acid
DNHSP	The Danish National Health Service Prescription Database
DNPR	The Danish National Patient Registry
DRCD	The Danish Register of Causes of Death
GP	General Practitioner
HbA1c	Hemoglobin A1c
HDL	High-density lipoprotein
HR	Hazard ratio
hs-CRP	High-sensitivity C-reactive protein
ICD	International Classification of Diseases
LDL	Low-density lipoprotein
MBL	Mannose-binding lectin (or mannan-binding lectin)
MBP	Mannan-binding protein
MASP	MBL-associated serine proteases
MeSH	Medical Subject Heading
MVNI	Multivariate normal imputation
PCR	Polymerase chain reaction
PR	Prevalence ratio
RCT	Randomized controlled trial
SNP	Single nucleotide polymorphism
TRIFMA	Time resolved immune-fluorometric assay
WHO	World health organization

Table of contents

1. Background

1.1 Diabetes mellitus

Diabetes mellitus, also known as diabetes, is a chronic, metabolic disorder characterized by elevated blood glucose levels (i.e., hyperglycemia). Diabetes can be classified into four overall categories: type 1 diabetes, type 2 diabetes, gestational diabetes mellitus, and other types of diabetes.[1] However, type 1 and type 2 diabetes, the two main types of diabetes, are probably more heterogeneous diseases than previously thought.[1] Type 1 diabetes is an autoimmune disorder characterized by autoimmune-mediated pancreatic β-cell destruction leading to absolute insulin deficiency. Type 2 diabetes is considered a metabolic disorder characterized by peripheral insulin resistance (i.e., liver, muscles, and adipose tissue) and deficient β-cell insulin secretion leading to relative insulin deficiency.[1,2] Type 2 diabetes accounts for around 90% of all diabetes[3] and are the focus of this thesis.

The world is currently experiencing a pandemic of diabetes.[4] According to the International Diabetes Federation Diabetes Atlas 2019, 463 million adults (i.e., 20–79 years) are living with diagnosed diabetes worldwide and this number is expected to increase to 700 million adults by 2045, with the largest increase in developing contries.[3] The number of individuals with diabetes is likely to be even higher as an estimated 232 million adults are living with undiagnosed diabetes.[3,4] The emerging diabetes pandemic is mainly driven by population ageing, improved diabetes survival, increasing obesity and inactivity, and stronger exposure to diabetes risk factors.[3,4] The onset of diabetes frequently occurs years before the actual diabetes diagnosis and individuals with undiagnosed and untreated diabetes are at increased risk of diabetes complications.[5] Individuals may therefore have diabetes complications already at time of diabetes diagnosis, and complications in newly diagnosed type 2 diabetes will be the focus for study I of this thesis.

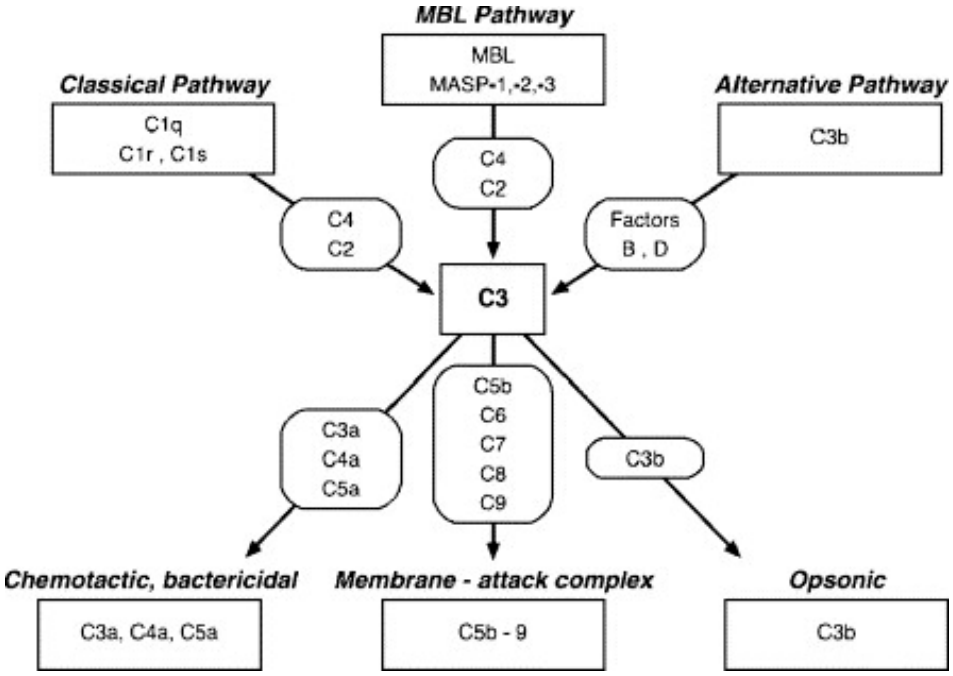
1.2 Diabetes complications

Diabetes is associated with increased morbidity and mortality, and much of the global burden of type 2 diabetes is due to the development of diabetes complications.[4,5] Diabetes complications have traditionally been divided into microvascular complications (i.e., diabetic retinopathy, diabetic neuropathy, and diabetic nephropathy) due to damage to the small blood vessels and macrovascular complications (i.e., ischemic heart disease, cerebrovascular disease, and peripheral vascular disease) due to damage to the arteries.[5,6] Globally, it is estimated that 8.4% of deaths are attributable to diabetes.[7] Macrovascular complications remains the predominant cause of accelerated mortality in type 2 diabetes.[8] Overall, mortality and rates of diabetes complications have decreased in individuals with diabetes in recent years, likely related to improved management of risk factors (e.g., hyperglycemia, hypertension, and dyslipidemia) and improved diabetes care.[6,9] However, due to the increasing absolute numbers of individuals with diabetes, the actual numbers of individuals with diabetes complications is still increasing on the population level.[5]

A number of non-vascular diabetes complications exist including infections.[10,11] Individuals with diabetes are more susceptible to infection and often have a more severe disease course once the infection is established, reflected by increased rates of hospital admission, length of stay, and complications.[11] The elderly population with diabetes is at particularly high risk of infections.[12] Type 2 diabetes is associated with 1.5–3-fold increased risk of a number of infections.[13] Thus, infectious disease risk in individuals with diabetes is an important area of research and has been a rather neglected research topic compared to traditional microvascular and macrovascular complications.[11] Infections will be the focus for study III of this thesis.

In general, our understanding of why only some individuals develop diabetes complications and others do not is limited. In order to prevent complications, we must improve our understanding of the pathological pathways and risk factors of these complications. It is well known that the immune system and inflammation play an important role in development of diabetes and its complications.[14] However, the exact mechanisms are still unknown. The complement system, especially the mannose-binding lectin (MBL) pathway, may play an important role in affecting both the susceptibility to infections[15] and the pathogenesis of diabetes vascular complications[16] and will be the focus for study II and study III of this thesis.

Figure 1. Simplified illustration of the complement system. The complement system is activated by three different pathways: the classical pathway, the lectin pathway, and the alternative pathway.
Reprinted from Ostergaard *et al.*[16], with permission from Elsevier.



1.3 The complement system

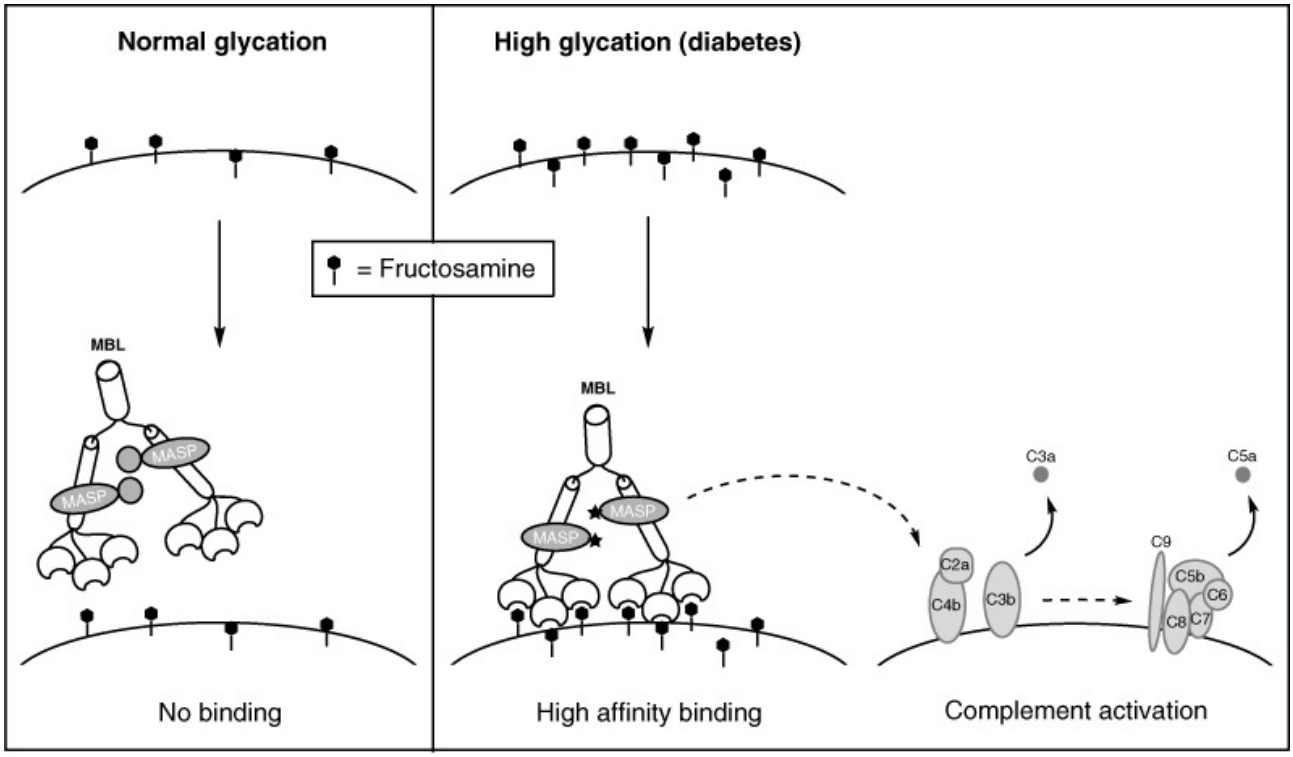
The innate immune system is the first-line of the host defense system.[17] The complement system is part of the innate immune system and is activated through three different pathways, i.e. the classical pathway (by antibody recognition), the lectin pathway (e.g., by MBL binding), and the alternative pathway (e.g., directly binding of microbes).[16] The three pathways leading to the activation of C3 (common for all three pathways) are a system of triggered enzyme cascades (Figure 1).[18,19] Activation of the complement system may promote different physiological activities concerning host defense against infection[19]: chemotaxis (e.g., recruitment of inflammatory cells), opsonization (e.g., opsonization of pathogens which may increase phagocytosis), and lysis of bacteria and cells (e.g., generation of the membrane-attack complex penetrating the cell membrane leading to osmotic instability and cell death). MBL plays an important role in the innate immune system[20] and MBL deficiency is associated with an increased susceptibility to infections[15,21-27], particularly if other immunological abnormalities are present as well.[28,29]

1.4 Mannose-binding lectin

The MBL protein was discovered in 1978 and named mannan-binding protein (MBP) because of its affinity for mannan (i.e., a glycoprotein).[30] The abbreviation MBP was used for other protein (e.g., maltose-binding protein and myelin basic protein) and to avoid confusion with the MBP abbreviation, the names mannose-binding lectin and mannan-binding lectin were introduced.[31,32] Mannose-binding lectin is the most used search term in the literature (i.e., 2922 hits on PubMed with the search term “Mannose-binding lectin” compared to 699 hits with “Mannan-binding lectin”) and will be used throughout this thesis.

MBL is a protein primarily (>95%[17]) synthesized in the liver by hepatocytes, and it belongs to the C-type lectin family of blood proteins. MBL has been suggested to act as an acute-phase reactant[33] but much slower and weaker reacting than C-reactive protein (CRP)[20], and MBL increases only 2-3-fold during infection/inflammation.[34] In contrast to the classical complement pathway, the lectin pathway is antibody-independent, and initiated by MBL binding in a calcium-dependent manner to carbohydrate patterns (e.g., patterns of mannose and fucose) present on the surfaces of microorganisms.[20] Upon binding to the target, MBL initiates the lectin pathway through association with MBL-associated serine proteases (MASP-1, -2, and -3) and activates the complement system.[35,36] Under normal circumstances, MBL does not recognize the body’s own tissues (Figure 2).[37-39] However, glycosylation changes (e.g., in individuals with diabetes) might cause increased autoreactivity[38-40] and MBL binding to fructosamine (i.e., glycation reaction between glucose and a protein) points to a potential link between hyperglycemia (e.g., in individuals with diabetes) and complement activation (Figure 2).[39,41] In addition, changes in cell surface glycosylation after cellular hypoxia may lead to increased MBL binding and complement activation.[42,43]

Figure 2. A plausible mechanism for complement activation in diabetes. The increase in fructosamines on the cell surface, as observed in diabetes, induces MBL binding and facilitates complement activation. Reprinted from Fortpied *et al*.[41], with permission from Wiley & Sons.



1.4.1 Genetics

Functional serum MBL levels are largely genetically determined by six common single nucleotide polymorphisms (SNPs) in the gene encoding MBL, termed *MBL2* (*MBL1* is a pseudogene), which is located on chromosome 10.[44,45] While the three SNPs (termed H/L, Y/X, and P/Q [in the untranslated sequence]) in the promoter region regulate MBL expression, the three missense SNPs (termed B, C, and D) in exon 1 decrease the serum MBL levels.[44] The wild-type allele on exon 1 is termed A.[45] The presence of one of the three polymorphisms on exon 1 is termed O.[20] Due to linkage disequilibrium the six SNPs give rise to only seven common haplotypes: HYPA, LYQA, LYPA, LXPA, LYPB, LYQC, and HYPD.[45] Two rare haplotypes have been reported, including the haplotype: LYPD.[46] MBL deficiency is a common immunodeficiency.[22,27] One third of the general population have MBL concentrations less than 500 µg/L, and 12% of the population have MBL concentrations less than 50 µg/L.[40,47] Around 5% of Caucasians have an inherited MBL deficiency (i.e., O/O homozygotes)[48], which are similar to reports in individuals with type 1 diabetes.[20] No clear definition of MBL deficiency exists and various cutoffs of serum MBL levels have been used previously to define MBL deficiency[49], ranging from 100ng/ml to 1 µg/ml.[17]

1.4.2 Serum levels

Functional serum MBL levels are stable over time and there are no circadian or day-to-day variation in healthy individuals, which is an advantage for the potential role as a biomarker.[50] Serum MBL levels are constant after the second decade of life.[50] The median serum MBL concentration in healthy individuals is 800–1000 µg/L.[20,47] Large inter-individual variation in serum MBL levels exist, from undetectable to above 10000 µg/L in some individuals with diabetes, mainly due to the above-mentioned MBL polymorphisms.[20,50] However, also for individuals with identical genotypes, the serum MBL levels differ considerable.[47]

1.5 Mendelian randomization

Observational study designs are prone to confounding and reverse causation that limits their ability to identify causal associations.[51] The observed association between serum MBL levels and cardiovascular disease is potentially biased by confounders associated with both serum MBL levels and cardiovascular disease. In addition, since serum MBL may act as a weak acute-phase reactant, it is possible that cardiovascular disease causes increased serum MBL levels (i.e., reverse causation), and not the other way around. Randomized trials are often considered the "gold standard" to proving causality; however, observational studies may also suggest causality, e.g., with the Mendelian randomization study design.[51] Mendelian randomization studies utilizes Mendel's second law of inheritance stating that alleles of SNPs are passed independently of one another and randomly at the time of conception.[51,52] Central in a Mendelian randomization study design is the use of an instrumental variable, that is a measurable variable (i.e., a genotype in Mendelian randomization) which is associated with the exposure, but not with any other factors or confounders.[51] Figure 3 illustrates the principle of a complete Mendelian randomization study design. In study II and III, we conducted the first three steps in a Mendelian randomization study design, and the third step aids in substantiating a causal association.[51]

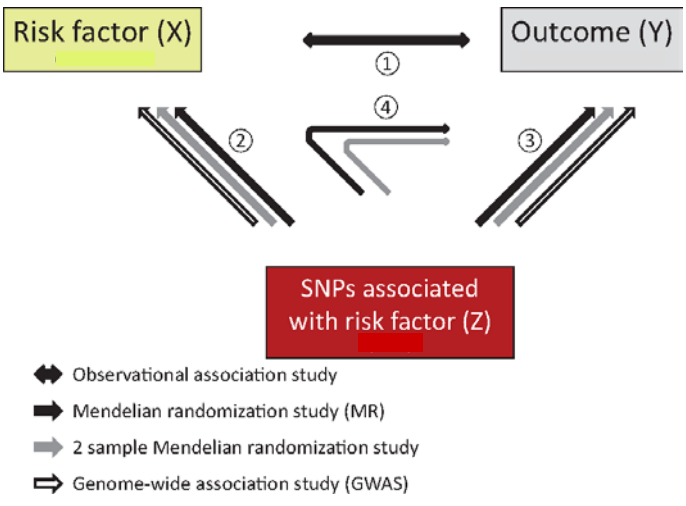


Figure 3. Mendelian randomization study design shown with a risk factor, an outcome, and a genotype associated with the risk factor. 1. Testing whether the risk factor (serum MBL) is associated with the outcome. 2. Testing whether the genotype (MBL expression genotype) is associated with the risk factor (serum MBL). 3. Testing whether the genotype (MBL expression genotype) is associated with the outcome, as an indication of a causal association of the risk factor on disease. 4. Causal estimate using instrumental variable analysis. Modified and reprinted from Benn *et al.*[51], with permission from Oxford University Press.

1.6 Literature review

A review of selected relevant background literature for this thesis was performed. I identified studies on the prevalence of diabetes complications and associated clinical characteristics in newly diagnosed type 2 diabetes (study I), and the association between MBL and risk of cardiovascular disease, early death, and infections in individuals with type 2 diabetes (study II and III). I searched MEDLINE (PubMed) on October 24, 2019, using the major and non-major Medical Subject Heading (MeSH) search terms: “prevalence”, “risk factors”, “diabetes complications”, “diabetic complications”, “newly diagnosed type 2 diabetes”, “mannose-binding lectin”, “mannan-binding lectin”, “MBL”, “diabetes mellitus”, “cardiovascular disease”, “mortality”, and “infections”. In addition, I did a search of PubMed and EMBASE for studies using the same or similar non-MeSH terms. Searches were supplemented by review of reference lists of retrieved relevant full-text articles. I restricted the searches to individuals with newly diagnosed type 2 diabetes in study I and to individuals with diabetes in study II and III. I excluded non-human studies. The last search date was January 24, 2020.

The titles and abstracts for papers listed in the search results were assessed as an initial screening for relevance. Retrieval of full text papers was performed where abstracts suggested that inclusion criteria were met. Table 1 summarizes the result of the literature review.

The search for studies on MBL and risk of cardiovascular disease in individuals with diabetes yielded 19, 30, and 79 papers in the initial search from PubMed (MeSH and non-MeSH) and EMBASE, respectively. I identified 8 studies on the association between MBL and cardiovascular disease in individuals with diabetes. The search for studies on MBL and mortality in individuals with diabetes yielded 0, 16, and 38 papers in the initial search from PubMed (MeSH and non-MeSH) and EMBASE, respectively. I selected 2 studies on the association between MBL and mortality in individuals with diabetes. The search for studies on MBL and infections in individuals with diabetes yielded 5, 16, and 54 papers in the initial search from PubMed (MeSH and non-MeSH) and EMBASE, respectively. I found no relevant studies on the association between MBL and infections in individuals with diabetes.

Table 1. Summary of the literature

Study I – Prevalence of diabetes complications in individuals with newly diagnosed type 2 diabetes			
Author, journal, year	**Design, setting, registries, period**	**Population, exposure, controls (if applicable), outcome**	**Results, limitations**
Gylfadottir *et al.*[53] - 2019	- Cross-sectional questionnaire study - Denmark - the DD2 cohort - Enrolled 2010-2016	- **R**ecently diagnosed type 2 diabetes patients (n=5514) - Prevalence of symptom-based diabetic polyneuropathy and patient characteristics	- Prevalence of symptom-based diabetic polyneuropathy was 18%. - Female sex, age, diabetes duration, body mass index, and smoking associated with symptom-based polyneuropathy - A median of 2.8 yr since DD2 enrollment
Uddin *et al.*[54] - 2018	- Cross-sectional study - Pakistan - Enrolled 2015-2016	- Newly diagnosed type 2 diabetes patients (n=891) - Prevalence of micro- and macrovascular complications	- Overall prevalence of micro- and macrovascular complications was 68.8% and 9.0%, respectively. Neuropathy, nephropathy, and retinopathy were reported in 59.6%, 24.4%, and 15.9% of the patients, respectively. Angina, myocardial infarction, peripheral arterial disease, stroke were reported in 5.2%, 3.3%, 2.2%, and 2.0% of the patients, respectively
Martinell *et al.*[55] - 2015	- Cross-sectional study - Sweden - Enrolled 2008-2013	- Recently/newly diagnosed type 2 diabetes (n=2174, 93% type 2 diabetes, 7% LADA) - Prevalence of diabetic retinopathy and contributing risk by socio and clinical characteristics	- Prevalence of 12% of diabetic retinopathy (DR) - Low educational level increased risk of DR (OR: 1.44, 1.07-1.93) - <50% beta-cell function increased risk of DR (aOR: 1.77, 1.28-2.44)
Bansal *et al.*[56] - 2014	- Cross-sectional study - India - Enrolled 2011-2013	- Newly diagnosed type 2 diabetes patients (n=449) - Prevalence of microvascular complications and associated risk factors	- Prevalence of any microvascular complications was 18%. Prevalence of neuropathy, retinopathy, and nephropathy was 8.2%, 9.5%, and 2.8%, respectively.
Kostev *et al.*[57] - 2014	- Cross-sectional study - UK/Germany - 2008-2012	- Newly diagnosed type 2 diabetes patients from primary care practices (n=45633 Germany and n=14205 UK) - Prevalence and risk factors of diabetic neuropathy	- Prevalence of 5.7% (Germany) and 2.4% (UK) of diabetic neuropathy - At baseline: 3.6% (Germany) and 17.7% (UK) retinopathy; 9.4% (Germany) and 13.4% (UK) nephropathy; 9.5% (Germany) and 39.5% (UK) peripheral artery disease; 23.2% (Germany) and 7.9% (UK) coronary heart disease. - Diabetic neuropathy was associated with male sex, age, antidiabetic treatment, and antihypertensive treatment - registry data
Sandbæk *et al.*[58] - 2014	- Randomized controlled trial - Denmark - ADDITION-Europe study - 2001-2006	- Screen-detected type 2 diabetes (n=3057) - Prevalence of diabetes complications	- At baseline: 20% albuminuria. - At 5 years follow-up: 23% albuminuria, 11% retinopathy, 5.5% neuropathy
Ruta *et al.*[59] - 2013	- Review article - Australia	- Newly diagnosed type 2 diabetes patients (n number not stated) - prevalence of diabetic retinopathy	- Prevalence estimates of diabetic retinopathy varied from 1.5%- 31%
Kostev *et al.*[60] - 2013	- Cross-sectional study - UK - 2005-2009	- Newly diagnosed type 2 diabetes patients in general practices (n=12524) - Prevalence and risk factors of diabetic retinopathy	- Prevalence of 19.0% of diagnosed diabetic retinopathy - Diabetic retinopathy was associated with age, male sex, HbA1c, systolic blood pressure, and antihypertensive drugs
Looker *et al.* [61] - 2012	- Cross-sectional study - Scotland - 2005-2008	- Newly diagnosed type 2 diabetes patients (n=51526) - Prevalence and risk factors of diabetic retinopathy	- Prevalence of any retinopathy 19.3% and for referable retinopathy 1.9%. - Diabetic retinopathy was associated with male sex, HbA1c, systolic blood pressure, and obesity
Sinclair *et al.*[62] - 2012	- Cohort study - UK - 2003-2005	- Newly diagnosed type 2 diabetes patients (n=9158) - Prevalence of diabetes complications	- 1.7% presented with microvascular complications - 19.2% presented with cardiovascular complications
UKPDS 33[63] -1998	- Randomized clinical trial - UK - enrolled from general practitioners(GPs)	- Newly diagnosed type 2 diabetes patients (n=3867) - Prevalence of diabetes complications	- 36% retinopathy, 1.9% proteinuria, 11.5% neuropathy

	- Included between 1977-1997		
UKPDS 17[64] -1996	- Randomized clinical trial - UK - enrolled from GPs - Included between 1977-1997	- Newly diagnosed type 2 diabetes patients (n=4209) - Prevalence of diabetes complications	- Approximately 50% had an diabetes-related tissue damage at recruitment: 8% had cardiovascular disease, 37% had retinopathy, 18% had microalbuminuria

Study II – MBL and cardiovascular disease in individuals with diabetes

Author, journal, year	Design, setting, registries, period	Population, exposure, controls (if applicable), outcome	Results, limitations
Hertle *et al.*[65] - 2016	- Prospective cohort study - the population based CODAM cohort - the Netherlands	- Normal/impaired glucose metabolism/type 2 diabetes (52/22/26 %) (n=495) - plasma MBL concentrations - MBL genotype (3 SNPs: B,C,D) - median 7 yr follow-up - Outcomes: Carotid.intima-media thickness, low-grade inflammation, cardiovascular disease, cardiovascular events	- Non-linear association between plasma MBL (tertiles) and low-grade inflammation - Association of MBL genotype and incident CVE: Low/intermediate MBL genotype compared to high MBL genotype: aOR 1.60 (0.73-3.51) - Association of plasma MBL (tertiles) and incident CVE: aOR of 2.57 (0.89-7.44) for the low compared to the middle plasma MBL and 2.12 (0.65-6.90) for the high compared to the middle plasma MBL - Only genotyped for 3 of the 6 SNPs. Not all diabetic patients. - Low and intermediate MBL genotype grouped together
Káplár *et al.*[66] - 2015	- Cross-sectional study - Hungary	- Patients with type 2 diabetes (n=103) - Age matched controls (n=98) - serum MBL levels (divided into 4 groups: <100, 100-500, 500-1000, >1000 ng/mL) - Outcome: Carotid.intima-media thickness	- U shaped association between serum MBL levels (categories) and carotid.intima-media thickness in type 2 diabetes. No association in controls. - study size and no MBL genotype information
Zhao *et al.*[67] - 2015	- Meta-analysis (12 published studies on MBL and vascular complications in diabetes) - China - Published between 2004-2014	- Patients with diabetes (n=2714) - data on MBL expression levels - vascular complications - 400 µg/L as the cutoff – 2256 cases with high expression levels of MBL and 458 cases with low expression MBL	- The combined ORs for the high MBL expression levels with vascular complications were 1.60 (1.24-2.08) and 1.94 (1.00-3.76) - No MBL genotype information - Cutoff at 400 µg/L - also included microvascular complications
Siezenga *et al.*[68] - 2011	- Prospective cohort study - the Netherlands	- type 2 diabetes patients (n =168) - 122 with known type 2 diabetes and 46 newly diagnosed - MBL genotype (3 SNPs: B,C,D) - serum MBL levels - Outcomes: cardiovascular events - median follow-up 7.7 yr	- Compared to the high MBL genotype, the low MBL genotype was associated with cardiovascular events (HR 3.43, 1.24-9.49) - Compared to the high MBL genotype the HR for the intermediate genotype was 0.65 (0.20-2.07) - Log-transformed serum MBL levels were not associated with CVE (HR 0.93, 0.50-1.73; aHR 1.19, 0.61-2.30) - log-transformation of serum MBL – will not show the true association between serum MBL and cardiovascular events because both low and high serum MBL levels are potentially associated with cardiovascular events
Mellbin *et al.*[69] - 2010	- Prospective cohort study - Substudy of the DIGAMI 2 trial - Sweden/Denmark	- Patients with type 2 diabetes and myocardial infarction (n=387) - serum MBL (dichotomized below or above 1000µg/L) - MBL genotype (n= 287) categorized as high or low - Outcome: A composite of cardiovascular events (cardiovascular disease death, reinfarction, stroke) - mean follow-up 2.5 yr	- No association between cardiovascular events and serum MBL (continuous variable) (HR 0.93, 0.85-1.01) or genotype (0.92, 0.82-1.02) - Patients with high MBL levels (sMBL >1000) had lower event rates (unadjusted HR 0.68, 0.48-0.95) compared to low MBL levels - Patients with high genotype/high MBL levels had a lower event rate (unadjusted HR 0.49, 0.26-0.92) compared to low genotype/low MBL levels - log-transformation of serum MBL – will not show the true association between serum MBL and cardiovascular events because both low and high serum MBL levels are potentially associated with cardiovascular events - Grouped low and intermediate MBL genotype into one category

Saevarsdottir *et al.*[70] - 2005	- case control studies (cross-sectional and follow-up groups – nested case-control study) - Iceland - subsample from the prospective Reykjavik study	- Population based cohort - Cross-sectional group – myocardial infarction (MI) cases (n=457), gender matched controls (n=530) - Follow-up group – MI cases (n=867), event-free controls (n=442). - Follow-up time 27 yr - outcome: nonfatal MI and cardiovascular disease death - stratification to diabetes - Serum MBL (dichotomized – high >1000 and low <1000µg/L)	Overall: high serum MBL (>1000) associated with decreased risk of MI compared to low serum MBL (<1000) - Cross-sectional group (aOR=0.64;0.50-0.82) - Follow-up group (aOR=0.88;0.69-1.1) Stratification to diabetes ptt: High MBL associated with decreased risk of MI in patients with diabetes - Cross-sectional group (aOR=0.38;0.17-0.88) - Follow-up group (aOR=0.15;0.03-0.78) - small groups when stratified for diabetes
Hansen *et al.*[20] - 2004	- case-control study - Steno Diabetes Center, Denmark -	- Cases: 199 type 1 diabetes patients with diabetic nephropathy and 192 type 1 diabetes patients with normoalbuminuria matched for sex, age, diabetes duration - Controls: 100 age matched healthy subjects - serum MBL and MBL genotype (categorized as high or low) - Outcomes: cardiovascular disease (ischemic heart disease, stroke, claudication)	- Serum MBL concentrations higher among ptt with previous CVD compared to ptt without CVD - This difference persisted in patients with high genotype but not with low genotype - Grouped low and intermediate MBL genotype into one category
Best *et al.*[71] - 2004	- Case-control study - Subsample from The strong Heart Study - USA - Enrolled between 1989 and 1992	- 3 American Indian communities (n=434) - 69% of cases had diabetes and 37% of controls had diabetes - MBL genotype categorized in low and high - Cases – experiencing an outcomes (n=217) - Controls matched on SHS center, sex, and age – without an outcome (n=217) - Outcome: coronary artery disease	- Low MBL genotype was associated with increased risk of Coronary artery disease - aOR 3.2 (1.5-7.0) - Categorized MBL genotype into low and high

Study II – MBL and all-cause mortality in individuals with diabetes

Author, journal, year	Design, setting, registres, period	Population, exposure, controls (if applicable), outcome	Results, limitations
Østergaard *et al.*[72] - 2015	- Prospective cohort study - Steno Diabetes Center Denmark - Included in 1993	- Patients with type 1 diabetes (n=372) - Serum MBL levels (dichotomized in high and low MBL levels – above/low the median) - MBL genotype (dichotomized in high and low MBL expression genotypes) - Outcome: all-cause mortality	- The adjusted HR for all-cause mortality was 1.47 (0.97-2.21) for high genotype compared to low genotype - the same tendency was found for MBL genotype and CVD mortality (data not shown) - The adjusted HR for all-cause mortality was aHR 1.79 (1.19-2.71) for high MBL levels compared to low MBL levels. Increased CVD mortality rate for those with high MBL levels compared to low MBL levels (data not shown)
Hansen *et al.*[73] - 2006	- Prospective cohort study - Steno Diabetes Center Denmark - Included between Jan and Dec 1987	- Patients with type 2 diabetes (n=326) - Serum MBL levels (dichotomized <1000 and ≥1000µg/L) - Outcome: all-cause mortality - median follow-up 15.6 yr.	- High MBL levels was associated with increased risk of dying compared to low MBL levels (HR 1.2, 0.7-2.1) after 5 years follow-up. - Small study size, no MBL genotype

Study III – MBL and infection in individuals with diabetes

Author, journal, year	Design, setting, registres, period	Population, exposure, controls (if applicable), outcome	Results, limitations
No relevant studies were found			

1.7 Prevalence of diabetes complications (study I)

As seen from Table 1, a number of studies have reported the prevalence of microvascular and/or macrovascular complications in individuals with newly diagnosed type 2 diabetes. Reported estimates vary widely between 1.7%-68.8% for early microvascular complications[54,56,62] and 9% for early macrovascular complications.[54] The reported estimates also vary widely between 2.4%-59.6% for diabetic neuropathy[53,54,56-58,63], 1.5%-37% for diabetic retinopathy[54-61,63,64], 2.8%-24.4% for diabetic nephropathy[54,56-58,64], 7.9%-23.2% for cardiovascular disease[54,57,62,64], 2% for cerebrovascular disease[54], and 2%-39.5% for peripheral vascular disease.[54,57] These large variations may be partly explained by some of the following. Increased diabetes case-finding in populations-at-risk[74] in recent years, lead to earlier type 2 diabetes diagnosis and lower prevalence of complications at time of diagnosis[75,76], compared to older studies.[63,64] A recent study reported a large decline in hemoglobin A1c (HbA1c) at first diabetes treatment from 2000-2017, suggesting earlier diabetes diagnosis.[77] Various ways of assessing diabetes complications may also play a role, i.e., complication data derived from randomized clinical trials[58,63] (i.e., structured interview and clinical examination), exclusively through hospital contact diagnoses[57] or in combination with questionnaire data.[53] Other explanations include differences in: 1) study populations, e.g., ethnic differences[78], sampled from outpatients clinics or primary care, non-similar health care access, and obesity prevalence; and 2) diabetes duration, may lead to the reported difference in prevalence of early diabetes complications. In some studies of "newly diagnosed" diabetes, individuals had been known with diabetes for a varying period before complications assessment.[53,55,58,60] Increasing diabetes duration is a well-known risk factor for development of diabetes complications.[79] Thus, the timing of diabetes complication assessment in relation to diabetes diagnosis may have an impact on the prevalence of diabetes complications.

1.8 MBL and cardiovascular disease in individuals with diabetes (study II)

The association between MBL and cardiovascular disease is ambiguous. Some studies have associated low MBL levels and other studies high MBL levels with increased risk of cardiovascular disease. In 2004, Hansen *et al.*[20] were among the first to show that serum MBL levels were higher in individuals with type 1 diabetes with previous cardiovascular disease compared to individuals with type 1 diabetes without cardiovascular disease. This difference persisted in individuals with high MBL genotype, but not with low genotype. In 2004, Best *et al*.[71] were the first to show an association between low MBL genotype and incident coronary artery disease in the Strong Heart Study (69% of cases with diabetes). In 2005, Saevarsdottir *et al.*[70] showed that high serum MBL levels were associated with decreased risk of myocardial infarction in individuals with diabetes. Mellbin *et al.*[69] found no association between cardiovascular events and serum MBL or MBL genotype in individuals with type 2 diabetes and myocardial infarction. After dichotomizing serum MBL, the risk of cardiovascular events was lower in individuals with high MBL genotype and high serum levels compared to individuals with low MBL genotype and low serum levels.

Siezenga *et al*.[68] reported that low MBL genotype was associated with increased risk of cardiovascular events compared to high MBL genotype in individuals with type 2 diabetes. In addition, intermediate MBL genotype seemed associated with decreased risk of cardiovascular events compared to high MBL genotype. Serum MBL levels were not associated with cardiovascular events. In a metaanalysis, Zhao *et al.*[67] reported an association between high serum MBL levels and increased risk of vascular complications (also including microvascular complications) in diabetes.

It is biologically plausible that MBL may play a double-edged role in the development of cardiovascular disease. Low MBL levels (such as in MBL deficiency) may impair pathogen clearance and reduce removal of atherogenic lipoproteins.[68,80] High MBL levels may amplify a low-grade immune response through complement activation, in particular if hyperglycemia is present.[42,81] In support of this, Káplár *et al.*[66] showed for the first time in individuals with type 2 diabetes, that both high serum MBL levels and low serum MBL levels were associated with increases in carotid intima-media thickness as a marker of subclinical atherosclerosis.[82] Hertle *et al.*[65] also found a non-linear association between plasma MBL levels (tertiles) and cardiovascular events and low-grade inflammation. In addition, low MBL genotype was associated with increased risk of cardiovascular events compared to high MBL genotype.

Overall, the literature review shows that examinations of the association between MBL and cardiovascular disease in individuals with diabetes have yielded mixed and contradictory findings regarding the direction of the association. Selected studies on the association between MBL and cardiovascular disease in the general population[48,83-86] and in other selected populations[87-89] (e.g., individuals with rheumatoid arthritis) will be discussed in the Discussion section of this thesis.

1.9 MBL and mortality in individuals with diabetes (study II)

The association between MBL and mortality is less well studied compared to the association with cardiovascular disease. In 2006, Hansen *et al.*[73] showed that mortality was higher in individuals with type 2 diabetes with high MBL levels compared to low MBL levels. Østergaard *et al.*[72] also showed an association in individuals with type 1 diabetes between high serum MBL levels and increased mortality compared to low serum levels. In addition, compared to low MBL genotype, they reported an association between high MBL genotype and increased mortality. These two studies does not report on cause-specific mortality. A large Danish general population study showed no clear association with death from any cause, whereas MBL deficiency showed a trend towards an association with death from cardiovascular disorders.[48]

1.10 MBL and infections in individuals with diabetes (study III)

To my knowledge, no previous studies have investigated the association between MBL and infections in individuals with diabetes. It is well known that there is an association between MBL deficiency and susceptibility to infections.[15,17] Especially in individuals with additional risk factors (e.g., immature adaptive

immune response in infants, chemotherapy, or cancer), MBL deficiency may play a pivotal role in predisposition to infections.[22,27-29] MBL binds to a wide variety of pathogens, e.g., bacteria, fungi, viruses, and parasites.[15] A number of studies have reported an association between MBL deficiency and lower respiratory tract infections.[49,90-92] Since there is no local production of MBL in the lungs, this can be explained biologically by "leakage" of MBL protein from bloodstream into infected airways, thereby activating the complement system.[22] In addition, there are multiple studies reporting strong associations between MBL deficiency and severe bacterial infections in patients undergoing chemotherapy.[15] This may be explained by a compromised adaptive immune response. Mannan is a major component of fungal cell walls and MBL has been shown to bind to a variety of fungi, thus, causing activation of the complement system.[15] Previous studies have reported increased susceptibility to HIV infection in individuals with MBL deficiency.[15] Studies on the association between MBL and viral infection are however limited.

2. Hypotheses and aims

The exact prevalence of micro- and macrovascular complications and associated clinical characteristics in newly diagnosed type 2 diabetes is uncertain. Moreover, after two decades of research, the exact role of MBL in the development of cardiovascular disease and early death remains ambiguous. Several studies have found an association between decreased MBL levels and increased risk of infections, but none in individuals with type 2 diabetes who are at high risk of infections. Thus, the overall aim of this thesis was to examine the prevalence of early complications among individuals newly diagnosed with type 2 diabetes, and the association between MBL and subsequent cardiovascular events, early death, and infections in individuals newly diagnosed with type 2 diabetes. The hypotheses and specific aims of the three studies included in this thesis are listed below:

Study I

Hypothesis: Baseline clinical characteristics may differ between recently diagnosed individuals with type 2 diabetes presenting with either microvascular or macrovascular complications.

Aim: To examine the prevalence of micro- and macrovascular complications and associated clinical characteristics among individuals with recently diagnosed type 2 diabetes in the Danish DD2 cohort.

Study II

Hypothesis: Compared to intermediate MBL levels, both low and high serum MBL levels are directly associated with increased risk of cardiovascular events and all-cause mortality in individuals with type 2 diabetes.

Aim: To investigate the association between MBL and risk of cardiovascular events and all-cause mortality in individuals with recently diagnosed type 2 diabetes from the DD2 cohort.

Study III

Hypothesis: Low baseline MBL levels are associated with increased risk of future infections compared with intermediate or high levels in individuals with type 2 diabetes.

Aim: To examine the association between MBL and risk of hospital-treated infections and community-based antimicrobial prescriptions in individuals with recently diagnosed type 2 diabetes from the DD2 cohort.

Cross-sectional study I describes the baseline DD2 cohort, on which longitudinal studies II and III were based.

3. Methods

This section describes the methods used for studies I-III. An overview is provided in Table 2. Methodological considerations will be discussed in section 5.2 Methodological considerations.

3.1 Setting

Denmark has a universal tax-financed health care system, guaranteeing free access to health care for all residents and partial reimbursement of most costs for prescription medications.[93] General practitioners (GPs) are essential for a well-functioning primary health care sector and serve as gatekeepers to the secondary health care sector as they are responsible for the majority of referrals to the secondary sector.[94] In Denmark, the majority of individuals with type 2 diabetes are treated by GPs, while the remaining ~20% receive diabetes care at Danish hospital outpatient clinics.[95]

3.2 Data sources

All three studies used prospectively collected data recorded in medical and administrative databases. The unique and permanent central personal registration (CPR) number, assigned to each Danish resident at birth or immigration by the Civil Registration System (CRS), allowed for accurate and unambiguous individual-level linkage of all the databases with complete follow-up.[93]

3.2.1 Administrative and health care databases

The Civil Registration System[93](studies I, II, and III) was established in 1968 and provides a complete account of all changes in vital statistics on a daily basis. The CRS registry records exact date of birth, death, immigration, emigration, civil status, address, and kinship (children, partner, and parents) for all Danish residents.

The Danish National Patient Registry (DNPR)[96](studies I, II, and III) records data on all Danish non-psychiatric hospital admissions since 1977, on all Danish non-psychiatric emergency visits and hospital outpatient clinics contacts since 1995, and on all Danish psychiatric hospital contacts since 1995. Reporting to the DNPR is mandatory. The DNPR records exact dates of admission and discharge, a primary diagnosis (the primary reason for contact), an optional number of secondary diagnoses (additional conditions), and any surgical procedures performed. The primary and secondary diagnoses were coded according to the International Classification of Diseases (ICD), 8th revision, until the end of 1993, and by the ICD-10 thereafter. Since 1996, Nordic Medico-Statistical Committee classification was used for surgical procedures.

The Danish Registry of Causes of Death (DRCD)[97](study II) records data on all Danish death certificates since 1943 and the classification of cause of death is based on ICD-10 codes since 1994. The physician in

Table 2: Summary of materials and methods.

	Study I	Study II	Study III
Objectives	To examine the prevalence of micro- and macrovascular complications and associated clinical characteristics in individuals with recently diagnosed type 2 diabetes.	To examine the association between MBL and risk of cardiovascular events (CVE) and early death in individuals with recently diagnosed type 2 diabetes.	To examine the association between MBL and risk of future infection in individuals with recently diagnosed type 2 diabetes.
Setting	Denmark, 2010-2016.	Denmark, 2010-2018.	Denmark, 2010-2018.
Design	Cross-sectional.	Prospective cohort study.	Prospective cohort study.
Data sources	DD2 core data, DD2 biobank data, and linked Danish health register data (CRS, DNPR, DNHSP, DDDA)	DD2 core data, DD2 biobank data, and linked Danish health register data (CRS, DNPR, DNHSP, DDDA, DRCD)	DD2 core data, DD2 biobank data, and linked Danish health register data (CRS, DNPR, DNHSP, DDDA)
Study population	All DD2 participants enrolled by February 2016, n=6,958. Subcohort linkable to the DDDA, n=5,115.	All DD2 participants enrolled by December 2016 and with a stored blood sample in the DD2 biobank, n=7,588.	All DD2 participants enrolled by December 2016 and with a stored blood sample in the DD2 biobank, n=7,588.
Exposures	Age, sex, central obesity, waist-hip ratio, physical activity, blood glucose, c-peptide, CRP. Subcohort linkable to the DDDA: blood pressure, smoking, BMI, lipid levels, HbA1c.	Serum MBL level (n=7,305) and MBL expression genotype (n=3,043).	Serum MBL level (n=7,305) and MBL expression genotype (n=3,043).
Outcomes	Micro- and macrovascular complications diagnosed prior to or up to 6 months after the DD2 enrolment date.	A composite of CVE (first occurrence of myocardial infarction, ischemic stroke, unstable angina, coronary revascularization, or cardiovascular death), individual subtypes of CVE, and all-cause mortality.	First hospital-treated infections (classified into bacterial, viral, and fungal). First community-based antimicrobial prescriptions and individual subtypes
Covariates	-	Age, sex, diabetes duration, waist circumference, waist-hip ratio, BMI, physical activity, smoking, blood pressure, CCI, anti-diabetes, lipid-lowering, anti-hypertensive, and anti-thrombotic drug use, blood glucose, HbA1c, C-peptide, albumin:creatinine ratio, lipid levels, and hs-CRP. Missing covariates were treated with multiple imputation.	Age, sex, diabetes duration, waist circumference, waist-hip ratio, BMI, physical activity, smoking, alcohol consumption, CCI, anti-diabetes, and lipid-lowering drug use, blood glucose, HbA1c, lipid levels, and hs-CRP. Missing covariates were treated with multiple imputation.
Statistical analysis	Calculation of prevalence. Calculation of prevalence ratios using Log-binomial and Poisson regression.	Restricted cubic spline models. Cumulative incidence of CVE, considering non-cardiovascular death as a competing risk, using STATA's stcompet command. Cumulative mortality using the Kaplan–Meier method. Hazard ratios using the Cox regression analysis. Multiple imputation.	Restricted cubic spline models. Cumulative incidence of outcomes, considering death as a competing risk, using STATA's stcompet command. Hazard ratios using the Cox regression analysis. Multiple imputation.
Sensitivity analyses	Restriction to individuals with type 2 diabetes with no glucose-lowering treatment, registration in the DDDA, or hospital diagnoses of type 2 diabetes >1 year prior to DD2 enrolment.	1. Exclusion of individuals with CRP levels >10 mg/L (n=641). 2. Exclusion of individuals with a registered diabetes duration >1 year (n=4,042). 3. Exclusion of individuals with any prior record of cardiovascular disease (n=1,451).	1. Exclusion of individuals with CRP levels >10 mg/L (n=641).

charge assigns one diagnosis (the underlying cause of death) and additional diagnoses (contributory or direct causes of death).

The Danish Diabetes Database for Adults (DDDA)[98](studies I, II, and III) is a nationwide clinical quality database, monitoring the quality-of-care for Danish diabetes patients since 2005. Danish hospital outpatient clinics and GP offices provide data on a number of variables (e.g., smoking habits, blood pressure, lipid levels, HbA1c, anthropometric measures) annually with up to one year of latency. In 2013, it became mandatory for the GP offices to report to the DDDA database.

The Danish National Health Service Prescription Database (DNHSP)[99](studies I, II, and III) records data on all filled prescriptions of reimbursable drugs at Danish community pharmacies since 2004. Data includes the following variables: name and type of the drug dispensed according to the Anatomical Therapeutic Chemical (ATC) classification system, strength and amount of the drug dispensed, and data and place of the dispensing.

3.2.2 The Danish Centre for Strategic Research in Type 2 Diabetes Cohort

The Danish Centre for Strategic Research in Type 2 Diabetes (DD2) cohort[100] (studies I, II, and III) was established in 2009 and the first participant was enrolled November 10, 2010. The DD2 project is still ongoing with continuous enrolment, and the cohort, by February 2020, consists of 8218 participants. The DD2 project's overall goal is to provide a large, data-rich, and extensive resource for type 2 diabetes research, including studies of type 2 diabetes complications, and improved genotypic, and clinical characterization of type 2 diabetes. The DD2 participants are individuals newly or recently diagnosed with type 2 diabetes (the WHO criteria for diabetes) in the routine clinical practice throughout Denmark, and the DD2 enrolment takes place from GPs or hospital outpatient clinics. The DD2 project aims to enroll individuals with type 2 diabetes around the time of their diagnosis and encourages clinicians to do so, but some individuals may have been diagnosed years before enrolment.[101] The DD2 project enrolls an estimated 5% of individuals with newly diagnosed type 2 diabetes nationwide in Denmark.[100] By 2016, 53% of the DD2 participants were enrolled from GPs and 47% from hospital outpatient clinics.[100] An estimated 80% of individuals with type 2 diabetes in Denmark receive their diabetes care by GPs, and the remainder at Danish hospital outpatient clinics.[100] In brief, the physician/nurse completes an online questionnaire eliciting lifestyle information (e.g. physical activity, alcohol consumption) and clinical examination data (e.g. waist- and hip circumference) for each entered DD2 participant at the time of enrollment (DD2 core data). In addition, urine- and fasting blood samples are obtained and stored in the DD2 biobank[102] and have currently been analyzed for a number of biomarkers, e.g., fasting blood glucose and C-peptide. High-sensitivity C-reactive protein (hs-CRP) and MBL (both serum and genotype) have been measured as a part of this PhD.

3.3 Study designs and study populations

Study I[103] is a cross-sectional study and the study population was all DD2 participants enrolled by February 2016. Study II and III are cohort studies and the study populations were all DD2 participants enrolled by December 2016 and with a stored blood sample in the DD2 biobank (for overview, see the flow diagram in Figure 5 of the Result section).

3.4 Exposures

3.4.1 Patient characteristics (study I)

In study I[103], we extracted data on patient characteristics present at time of DD2 enrolment from the DD2 core data and the linked health databases: DDDA (we used the variable closest to the DD2 enrolment date) and DNHSP (we used yes/no to a redeemed prescription the previous year). Our focus was to examine micro- and macrovascular complications and their associated clinical characteristics among individuals with recently diagnosed type 2 diabetes. As mentioned previously, DD2 aims to enroll individuals with newly and recently diagnosed type 2 diabetes, thus, we used time of DD2 enrolment as a proxy for time of type 2 diabetes diagnosis. Patient characteristics of interest were age, sex, central obesity (defined as waist circumference ≥94 cm for men and ≥80 cm for women), waist-hip ratio, regular physical activity (yes/no), fasting blood glucose (mmol/L), C-peptide (pmol/L), CRP (mg/L), and lipid-lowering, and anti-hypertensive drug use. Patient characteristics of interest available for a subcohort currently linkable to the DDDA database (n=5115, 75%), were HbA1c (%), blood pressure (mmHg), body mass index (BMI, kg/m^2), tobacco smoking (defined as smoking or never/previous smoking), plasma lipids (mmol/L).

3.4.2 Serum MBL levels (study II and III)

For study II and III, functional serum MBL was measured in the DD2 baseline blood samples using an in-house time resolved immune-fluorometric assay (TRIFMA).[104] Serum levels of MBL were determined in duplicates. In brief, microtiter wells were coated with mannan, followed by incubation with diluted serum samples. After washing, biotin-labelled monoclonal anti-MBL antibody was added followed by europium-labelled streptavidin, and after incubation and washing, the amount of bound MBL was assessed by time-resolved fluorometry. Serum samples with known high, intermediate, and low MBL concentration were included as internal controls on every plate. The limit of quantification was 10 μg/L at the dilution used, thus, MBL deficiency was set to 10 μg/L. The intra- and interassay coefficients of variation were below 10%. We analyzed serum MBL levels as a continuous variable to examine a potential nonlinear association with outcomes, and categorized as low (≤100 μg/L), intermediate (101–1000 μg/L), or high (>1000 μg/L), cut points often used in MBL research.[37] This TRIFMA method requires MBL binding to mannan, thus, this method assays biologically functional MBL.[17]

3.4.3 MBL expression genotypes (study II and III)

In study II and III, we used purified DNA, from the DD2 baseline blood samples, genotyping the human *MBL2* gene. *MBL2* genotyping was performed on the first consecutive individuals with type 2 diabetes enrolled in the DD2 cohort (n=3042). The six SNPs located within the promoter region (rs11003125, rs7096206, rs7095891) and exon 1 (rs5030737, rs1800451, rs1800450) of the *MBL2* gene were genotyped by real-time polymerase chain reaction (PCR) technique using TaqMan genotyping assays as previously described.[45] In brief, for PCR reactions, a GeneAmp 9700 thermal cycler (ABI, Foster City, CA, USA) were used for amplification. The PCR reaction mixes contained 20 ng DNA, 900 nM primers, and 200 nM probes, as well as TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA) in a final volume of 5 µL in 384-well plates. The PCR profile included 2 minutes at 50 °C, 10 minutes at 95 °C, followed by 40 cycles of 15 seconds at 95 °C and 1 minute at an annealing temperature of 60 °C. PCR primers are designed to amplify a small piece of DNA around a SNP target and allele specific probes are labeled with reporter dye.[105] Samples only emitting the dye of the common allele were homozygous for the common allele (A/A), while samples only emitting the dye of the rare allele were homozygous for the rare allele (O/O), and finally, samples emitting both dyes were heterozygous (A/O). The endpoint reading of the fluorescence generated during PCR amplification were carried out on the real-time PCR instrument Quant Studio 12K Flex using the Quant Studio 12K Flex software. Due to linkage disequilibrium, the six SNPs give rise to seven major haplotypes: HYPA, LYQA, LYPA, LXPA, LYPB, LYQC and HYPD.[47] We categorized the MBL haplotypes into three MBL expression genotypes, low (YO/YO, XA/YO), intermediate (XA/XA, YA/YO), or high (YA/YA, XA/YA)[49,106], that previously have been correlated with serum MBL levels (Figure 4).[37] See the Appendix II, supplemental for additional information.

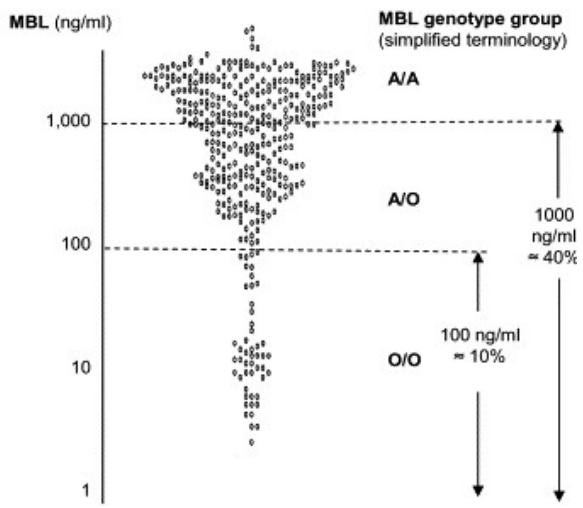


Figure 4. Correlation between plasma MBL levels and genotypes. A/A indicates the wild type, A/O indicates the heterozygotes; and O/O indicates the homozygotes. Adapted and reprinted from Gadjeva *et al*.[37], with permission from Elsevier.

3.5 Outcomes

3.5.1 Micro- and macrovascular complications (study I)

For each DD2 cohort participant included in study I[103], we retrieved information on presence or absence of micro- and macrovascular complications (both inpatient hospital admission or hospital outpatient clinic contact with a primary or secondary diagnosis) recorded in the DNPR[96] between 10 years before and until 6 months after the DD2 enrollment date. Our focus was to examine the prevalence of micro- and macrovascular complications at time of type 2 diabetes diagnosis. Again, time of DD2 enrolment was used as a proxy for time at type 2 diabetes diagnosis. We categorized micro- and macrovascular complications as: no complications, microvascular complications, macrovascular complications, or both micro- and macrovascular complications at DD2 enrolment. Microvascular complications included: retinopathy (including any diabetes related eye disease, atherosclerotic eye disease, blindness/severely impaired vision, or use of retinal photocoagulation therapy); neuropathy (including any diabetes related neurological complication); and nephropathy (including any diabetes related kidney disease, albuminuria, chronic dialysis, or renal failure). Macrovascular complications included: ischemic heart disease (including angina pectoris and coronary surgery); atherosclerotic cerebrovascular disease (including thrombolysis and thrombectomy); and atherosclerotic peripheral vascular disease (including vascular surgery, amputation, and any operation for macroangiopathy). See Appendix I, Appendix Supplementary table A.1 for diagnosis and procedure codes.

3.5.2 Cardiovascular events (study II)

A composite endpoint of cardiovascular events and the individual subtypes of cardiovascular events were outcomes in study II. The composite endpoint of cardiovascular events were defined as first-time inpatient hospital admission of myocardial infarction, ischemic stroke, unstable angina pectoris, coronary revascularization, or cardiovascular death after the DD2 enrolment date. Individuals with an event prior to DD2 enrollment (n=434 for myocardial infarction; n=248 for ischemic stroke; n=602 for coronary revascularization; n=163 for unstable angina pectoris; and n=924 for any cardiovascular events) were excluded from the analyses for the relevant outcome. We retrieved information on diagnosis of cardiovascular events (both as the primary or secondary discharge diagnosis) from the DNPR[96] and on cardiovascular death (both as the immediate or underlying cause of death) from the DRCD.[97] See Appendix II, Supplemental material, Supplementary Table 1 for diagnosis and procedure codes.

3.5.3 All-cause mortality (study II)

All-cause mortality was another outcome in study II. Information on vital status, including exact date of death was assessed from the CRS.[93]

3.5.4 Hospital-treated infections (study III)

Any hospital-treated infections and individual subtypes were outcomes in study III. Any hospital-treated infections were defined as first-time inpatient hospital admissions or hospital outpatient clinic contacts with a primary or secondary diagnosis of any infections after the DD2 enrolment date. We retrieved information on hospital-treated infections based on diagnosis from the DNPR.[96] We categorized infections into subtypes consistent with previous studies[107,108]: bacterial, viral, and fungal. Hospital-treated bacterial infections were: pneumonia, skin infections, urinary tract infections, sepsis, intra-abdominal infections, abscesses, diarrheal diseases, and other bacterial infections. Hospital-treated viral infections were: influenza and other viral infection. See Appendix III, Supplemental material, Supplementary Table 1 for diagnostic codes.

3.5.5 Community-based antimicrobial prescriptions (study III)

Community-based antimicrobial prescriptions and individual subtypes were outcomes in study III and we used these as a proxy for community-treated infections. Any community-based antimicrobial prescriptions were defined as first-time redeemed antimicrobial prescriptions recorded in the DNHSP after the DD2 enrolment date, consistent with previous studies.[107,109] We categorized any community-based antimicrobial prescriptions into subtypes as previously used[107]: all antimicrobial drugs prescribed for oral treatment of bacterial, viral, and fungal infection. As a proxy for respiratory tract infections, we identified the number of redeemed prescriptions for oral treatment with phenoxymethylpenicillin and specific macrolides (erythromycin, roxithromycin, and clarithromycin).[107] As a proxy for skin infections, we identified redeemed prescriptions of dicloxacillin and flucloxacillin.[107] As a proxy for urinary tract infections, we used redeemed prescriptions of pivmecillinam, sulfamethizole, nitrofurantoin, and trimethoprim.[107] In an additional analysis, we included pivampicillin as an expanded proxy for urinary tract infections. Furthermore, we evaluated redeemed prescriptions with commonly prescribed broad-spectrum penicillins (amoxicillin and amoxicillin with enzyme inhibitor). See Appendix III, Supplemental material, Supplementary Table 2 for ATC codes.

3.6 Covariates

For study II and III, we extracted information on various covariates to describe the study population[110] (of which some could be associated with subsequent outcome risk) and to perform subgroup analyses. From the DD2 online questionnaire[111] and linked administrative and health care registries, we obtained baseline information on other covariates present at the time of DD2 enrollment.

3.6.1 Demographic information

From the CRS, we extracted information on sex and age at time of DD2 enrolment.

3.6.2 Diabetes duration

As previously mentioned, some of the individuals with type 2 diabetes in the DD2 cohort have been diagnosed with diabetes years before enrolment. In study II and III we defined a diabetes duration variable as time from first of the following events until the DD2 enrollment date: prescription of glucose-lowering drugs, first diabetes-related diagnosis in the Danish National Patient Registry, or DDDA registration. In the absence of information from a prior drug prescription, diabetes diagnosis from the DNPR, or DDDA registration, diabetes duration was set to 0 at DD2 enrollment date.

3.6.3 High-sensitive C-reactive protein

In study II and III serum hs-CRP was measured in the DD2 baseline blood samples by in-house TRIFMA[112] based on commercially available monoclonal antibodies (MAB17071 and BAM17072; R&D Systems, Minneapolis, MN, USA) and calibrated against recombinant human CRP WHO 85/506 (National Institute for Biological Standards and Control). Samples were diluted 1000-fold and measured in duplicate. Intra- and interassay coefficients of variations were <5% and <6%, respectively.

3.6.4 Other covariates

In study II and III, we extracted information on baseline patient characteristics from the DD2 core data, the DNPR, the DDDA, and the DNHSP that could be associated with subsequent outcome risk. In brief, these included physical activity, the Charlson Comorbidities Index (CCI), HbA1c, BMI, central obesity, waist-hip ratio, waist circumference, smoking, blood pressure, fasting blood-glucose level, C-peptide level, plasma lipid level, use of anti-hypertensive and lipid-lowering drugs, and albumin/creatinine ratio. From the DNPR, we obtained information on inpatient and outpatient discharge diagnoses recorded within a period of 10 years (older diagnosis would less likely affect the outcome occurrence) before the DD2 enrolment date. We computed the CCI scores[113] using this information, and categorized the overall comorbidity level as: low (CCI score = 0), medium (CCI score = 1–2), and high (CCI score ≥ 3). The Diabetes category was not included in the CCI scoring system, as it constituted the index disease of our cohort. An overview, that lists covariates, definitions, and codes, is provided in appendix II and III, supplemental material, Supplementary Table 1. In study II and III, DD2 participants with missing covariates were not excluded from the analyses but treated with multiple imputation.[114] For further information on multiple imputation, see statistical analyses in the Method section (section 3.7.3 page 24-25).

3.7 Statistical analyses

In all three studies, we provided descriptive tables of the study populations included in each study. In study I according to micro- and macrovascular complication groups and in study II and III according to serum MBL and MBL expression genotype categories.

3.7.1 Prevalence of micro-/macrovascular complications (study I)

The prevalence of microvascular, macrovascular, and both types of complications at time of type 2 diabetes diagnosis was calculated as the number of individuals diagnosed with a micro- and/or macrovascular complications at time of DD2 enrolment divided by the total number of all DD2 cohort members.[103] We further calculated the prevalence of individual complications, i.e. retinopathy, neuropathy, nephropathy, ischemic heart disease, atherosclerotic cerebrovascular disease, and atherosclerotic peripheral vascular disease.

3.7.2 Characteristics associated with micro-/macrovascular complications (study I)

We calculated prevalence ratios (PRs) with 95% confidence intervals (CIs) of microvascular, macrovascular, and both micro- and macrovascular complications associated with presence of each of the patient characteristics under study[103] using log-binomial and Poisson regression.[115-118] The exact pathophysiological pathways between the metabolic syndrome and other cardiovascular-related and dysglycemia-related factors are incompletely understood and various factors may act as clusters in the same causal pathway.[119] Thus, in our main analysis we only adjusted all our PRs for age and sex to assess whether the associations were independent of age and sex. We did a supplementary analysis where we also adjusted all associations for waist-hip ratio (as a marker of central obesity) because obesity and in particular abdominal obesity might be a fundamental factor preceding several of the other metabolic risk factors.

In order to maximize the probability of type 2 diabetes being a newly detected diagnosis, we performed a sensitivity analysis where we excluded all individuals with type 2 diabetes from the DD2 cohort with an earlier glucose-lowering treatment, hospital diagnosis of type 2 diabetes, or registration in the DDDA database recorded >1 year prior to the DD2 enrolment date.

3.7.3 MBL and outcomes (study II and III)

In study II and III, we used time-to-event data to examine the association between MBL and outcomes (study II: cardiovascular events, individual subtypes, and all-cause mortality; study III: hospital-treated infections, community-based antimicrobial prescriptions, and individual subtypes). Individuals with type 2 diabetes were followed from DD2 enrolment date until an event, death, migration, or end of follow-up, whichever came first. We used study time as the time scale in the analyses. Individuals with an event prior to DD2 enrolment were excluded from the analyses for the relevant outcome. We did not consider recurrent events.

To circumvent confounding and reverse causation, and to assess a potential causal association between serum MBL levels and outcomes, we investigated the first three steps of a Mendelian randomization study design[51]: 1) the association between serum MBL levels and risk of outcomes; 2) the association between MBL expression genotype and serum MBL levels; and 3) whether MBL expression genotype was associated

with outcomes. According to the Mendelian randomization study design, this third step aids in substantiating a causal association between serum MBL levels and outcomes.[51]

We examined a potential nonlinear association between serum MBL levels, as a continuous variable, and risk of outcomes using restricted cubic spline models.[120,121] We used STATA's stcompet command to plot the cumulative incidence of cardiovascular events, hospital-treated infections, and community-based antimicrobial prescriptions for the three MBL categories, with risk for non-cardiovascular death and death as a competing risks, respectively.[122] We plotted cumulative mortality for the three MBL categories using the Kaplan–Meier method. Incidence and mortality rates of cardiovascular events, hospital-treated infections, community-based antimicrobial prescriptions, and all-cause mortality for the three MBL categories, were calculated by dividing the total number of new events/deaths by the total person-time of the at risk population expressed per 1000 person-years using the STATA's stptime command. We used Cox proportional hazard regression analysis to compute hazard ratios (HRs) of cardiovascular events, all-cause mortality, hospital-treated infections, and community-based antimicrobial prescriptions with 95% CIs according to the three MBL (both serum levels and MBL expression genotypes) exposure categories. The intermediate MBL (both serum levels and MBL expression genotypes) categories were used as references based on previous studies indicating a nonlinear association between serum MBL and cardiovascular outcomes.[65,66,88] Multivariable Cox regression was used to adjust for potential confounders. We performed extensive adjustments for a priori decided potential confounders to ensure robustness of the potential associations. We graphically verified the proportional hazards assumption by plotting –ln(survival probability) against ln(analysis time) and detected no violations.

In study II, we created three models to adjust for potential confounders. In Model 1, HRs were adjusted for sex and age. In Model 2, HRs were adjusted for sex, age, diabetes duration, and levels of hs-CRP. In Model 3, HRs were adjusted for sex, age, diabetes duration, hs-CRP, waist circumference, waist–hip ratio, BMI, physical activity, smoking, systolic and diastolic blood pressure, CCI, fasting blood glucose, HbA1c, C-peptide, albumin/creatinine ratio, total cholesterol, low-density lipoprotein, high-density cholesterol, triglycerides, and use of anti-diabetic, lipid-lowering, anti-hypertensive, or anti-thrombotic drugs.

In study III, we created three models to adjust for potential confounders. In Model 1, HRs were adjusted for sex and age. In Model 2, HRs were adjusted for sex, age, diabetes duration, and levels of hs-CRP. In Model 3, HRs were adjusted for sex, age, diabetes duration, hs-CRP, waist circumference, waist–hip ratio, BMI, physical activity, smoking, alcohol consumption, CCI, fasting blood glucose, HbA1c, total cholesterol, low-density lipoprotein, high-density cholesterol, triglycerides, and use of anti-diabetic, or lipid-lowering treatment.

We used multiple imputation to handle missing data.[114] Missing data on covariates (n=5–3966; 0.1%–54%; Appendix II, Supplemental material, Supplementary Table 2) used for adjustment in the Cox

regression models were imputed to maximize power and avoid selection bias. We used multivariate normal imputation (MVNI)[123] to impute 20 complete data sets. MVNI assumes that all variables in the imputation model follow a multivariate normal distribution and that missing data are missing at random. Continuous variables with clearly non-normal (skewed) distributions were zero-skewness log-transformed, i.e., transformed to approximate normality before imputation. Then the imputed values were transformed back to the original scale before analysis.[114] Smoking (categorical variable) was also imputed using MVNI, which has been shown to perform well even in the presence of binary and ordinal variables.[123] All covariates used in the analysis model, as well as the outcomes, were included in the imputation model to ensure maximum recovery of information about the association of interest. See Appendix II, Supplemental material for the full text on multiple imputation. We did not impute the exposure MBL expression genotype where this information was missing (n=4262, 58%).

In study II and III, to assess risk of genotype misclassification, we used the Hardy–Weinberg equilibrium and the χ^2 test, comparing the observed allele frequency distribution in the study population with the expected distribution.[124] We performed the Cuzick non-parametric test for trend across ordered groups[125] and calculated R^2 (coefficient of determination) by simple linear regression to evaluate the association between MBL expression genotype and serum MBL levels.

We performed several sensitivity analyses. In study II, we first excluded DD2 participants with a serum CRP level >10 mg/L (n=641; 9%) to decrease the risk of misclassification of serum MBL levels related to acute inflammation and/or infection.[34,108,126] Second, we excluded anyone with a registered diabetes duration >1 year (n=4042; 55%) to focus solely on individuals with newly diagnosed type 2 diabetes. Third, we excluded individuals with any previous record of cardiovascular disease (See Appendix II, Supplemental material, Supplementary Table 1 for diagnosis and procedure codes), including any atherosclerotic disease or heart failure (n=1451; 20%). In study III, we performed a sensitivity analysis excluding individuals with serum CRP levels >10 mg/L (n=641; 9%) to prevent ongoing infection at the time of testing.[108]

In study I, we used SAS version 9.4 (SAS Institute, Inc., Cary, North Carolina, USA) for data management and analyses. In study II and III, we used SAS version 9.4 (SAS Institute) for data management and STATA statistical software package version 14.2 (Stata Corp, College Station, TX, USA) for analyses.

3.8 Ethical considerations

All three studies included in this thesis were approved by the Danish Data Protection Agency and by the Regional Committees on Health Research Ethics for Southern Denmark. All DD2 participants volunteered and gave their written informed consent to participate in the DD2 project.

4. Results

The main findings from study I-III are summarized below. See appendices I-III for the paper and manuscripts.

4.1 Prevalence of micro-/macrovascular complications (study I)

Overall, 35% (n=2,456) of the 6,958 DD2 participants had a micro- and/or macrovascular complication at time of DD2 enrollment.[103] The prevalence of microvascular complications, macrovascular complications, and both micro- and macrovascular complications was 11.9% (n=828), 17.1% (n=1,186), and 6.4% (n=442), respectively.[103] Of the individuals with recently diagnosed type 2 diabetes with any microvascular complications, 3.4% (n=234) had nephropathy, 3.8% (n=264) had neuropathy, and 12.8% (n=887) had retinopathy. Of the individuals with recently diagnosed type 2 diabetes with any macrovascular complications, 2.2% (n=151) had peripheral vascular disease, 5.3% (n=365) had cerebrovascular disease, and 15.2% (n=1,059) had ischemic heart disease.[103]

4.2 Characteristics associated with micro-/macrovascular complications (study I)

Tables 3 and 4 shows the patient characteristics according to micro- and macrovascular complications among individuals recently diagnosed with type 2 diabetes (i.e. time of DD2 enrollment). Patient characteristics according to retinopathy, neuropathy, nephropathy, ischemic heart disease, cerebrovascular disease, and peripheral vascular disease are shown in Appendix I, Supplementary Tables A8–A11.

We found that higher baseline HbA1c levels were associated with presence of microvascular and both micro- and macrovascular complications at type 2 diabetes diagnosis but not with presence of macrovascular complications. In contrast, we found that higher C-peptide levels were associated with presence of macrovascular complications at diagnosis but not with microvascular complications (Tables 3 and 4).[103]

Overall, we found that the remaining metabolic syndrome related factors were all associated with presence of macrovascular complications at type 2 diabetes diagnosis. We found that absence of lipid-lowering drug use, higher blood pressure, and higher triglyceride levels were associated with presence of microvascular complications at diagnosis (Tables 3 and 4).[103] Male sex, higher age, and low-grade inflammation (i.e., CRP >3.0 mg/L[107]) were associated with presence of macrovascular and both micro- and macrovascular complications at type 2 diabetes diagnosis but not with microvascular complications, except for age over 70 years (Table 3).[103] Smoking was associated with presence of macrovascular complications at diagnosis, but not with microvascular and both micro- and macrovascular complications (Table 4).[103]

The associations were robust to further adjustment for waist-hip ratio (Appendix I, Supplementary Table A2 and A3).[103] All sensitivity analyses restricted to DD2 participants with a newly detected type 2 diabetes diagnosis, showed robustness of results in the main analyses (Appendix I, Supplementary Table A6 and A7).[103]

Table 3. Patient characteristics according to micro-/macrovascular complications among 6958 individuals with type 2 diabetes at DD2 enrollment, with corresponding age- and gender-adjusted prevalence ratios.

Characteristic	All T2D patients	No complication (%)	Microvascular complication (%)	aPR (95% CI)	Macrovascular complication (%)	aPR (95% CI)	Micro- and macrovascular complications (%)	aPR (95% CI)
Main cohort	6958							
Sex								
Female	2927	2047 (69.9)	365 (12.5)	Ref (1.00)	374 (12.8)	Ref (1.00)	141 (4.8)	Ref (1.00)
Male	4031	2455 (60.9)	463 (11.5)	0.92 (0.81-1.05)	812 (20.1)	1.60 (1.43-1.79)	301 (7.5)	1.59 (1.32-1.93)
Age (years)								
<50	1220	973 (79.8)	142 (11.6)	Ref (1.00)	82 (6.7)	Ref (1.00)	23 (1.9)	Ref (1.00)
50-59	1790	1262 (70.5)	180 (10.1)	0.87 (0.70-1.07)	276 (15.4)	2.31 (1.82-2.92)	72 (4.0)	2.17 (1.36-3.44)
60-69	2517	1550 (61.6)	291 (11.6)	0.99 (0.82-1.20)	505 (20.1)	2.99 (2.40-3.74)	171 (6.8)	3.61 (2.35-5.55)
≥70	1431	717 (50.1)	215 (15.0)	1.28 (1.05-1.56)	323 (22.6)	3.44 (2.73-4.32)	176 (12.3)	6.60 (4.31-10.12)
Central obesity[a]								
No	570	393 (69.0)	63 (11.1)	Ref (1.00)	83 (14.6)	Ref (1.00)	31 (5.4)	Ref (1.00)
Yes	6378	4099 (64.3)	765 (12.0)	1.08 (0.85-1.38)	1103 (17.3)	1.33 (1.09-1.63)	411 (6.4)	1.38 (0.97-1.97)
Waist-hip ratio[b]								
≤0.95m/≤0.80f	772	506 (65.5)	95 (12.3)	Ref (1.00)	131 (17.0)	Ref (1.00)	40 (5.2)	Ref (1.00)
0.96-1.0m/0.81-0.85f	1437	911 (63.4)	180 (12.5)	0.99 (0.79-1.26)	261 (18.2)	1.12 (0.93-1.36)	85 (5.9)	1.22 (0.85-1.75)
>1.0m/>0.85f	4737	3074 (64.9)	553 (11.7)	0.92 (0.74-1.14)	793 (16.7)	1.21 (1.02-1.43)	317 (6.7)	1.75 (1.27-2.40)
Regular physical exercise								
Yes	2725	1847 (67.8)	304 (11.2)	Ref (1.00)	430 (15.8)	Ref (1.00)	144 (5.3)	Ref (1.00)
No	4232	2655 (62.7)	524 (12.4)	1.12 (0.98-1.28)	755 (17.8)	1.10 (0.99-1.23)	298 (7.0)	1.31 (1.08-1.58)
Use of lipid-lowering drugs								
No	2072	1537 (74.2)	304 (14.7)	Ref (1.00)	171 (8.3)	Ref (1.00)	60 (2.9)	Ref (1.00)
Yes	4886	2965 (60.7)	524 (10.7)	0.70 (0.62-0.80)	1015 (20.8)	2.31 (1.98-2.69)	382 (7.8)	2.35 (1.81-3.07)
Use of anti-hypertensive drugs								
No	1967	1548 (78.7)	232 (11.8)	Ref (1.00)	151 (7.7)	Ref (1.00)	36 (1.8)	Ref (1.00)
Yes	4991	2954 (59.2)	596 (11.9)	0.94 (0.81-1.10)	1035 (20.7)	2.28 (1.93-2.70)	406 (8.1)	3.19 (2.27-4.49)
Fasting blood glucose (mmol/L)								
<6.5	1548	996 (64.3)	159 (10.3)	Ref (1.00)	278 (18.0)	Ref (1.00)	115 (7.4)	Ref (1.00)
6.5-7.0	917	611 (66.6)	86 (9.4)	0.92 (0.71-1.17)	167 (18.2)	1.00 (0.85-1.19)	53 (5.7)	0.80 (0.59-1.09)
7.0-7.5	751	482 (64.2)	80 (10.7)	1.05 (0.82-1.36)	147 (19.6)	1.07 (0.90-1.28)	42 (5.6)	0.75 (0.54-1.06)
≥7.5	2146	1424 (66.4)	245 (11.4)	1.16 (0.96-1.41)	350 (16.3)	0.95 (0.83-1.10)	127 (5.9)	0.92 (0.72-1.16)
C-peptide (pmol/L)								
<550	295	202 (68.5)	36 (12.2)	Ref (1.00)	39 (13.2)	Ref (1.00)	18 (6.1)	Ref (1.00)
550 - 800	853	606 (71.0)	90 (10.6)	0.84 (0.58-1.21)	120 (14.1)	1.00 (0.72-1.40)	37 (4.3)	0.65 (0.38-1.12)
≥800	4652	2932 (63.0)	552 (11.9)	0.97 (0.71-1.33)	847 (18.2)	1.34 (1.00-1.80)	321 (6.9)	1.07 (0.68-1.69)
CRP (mg/L)								
≤3.0	627	429 (68.4)	62 (9.9)	Ref (1.00)	114 (18.2)	Ref (1.00)	22 (3.5)	Ref (1.00)
>3.0	403	254 (63.0)	35 (8.7)	0.86 (0.58-1.29)	87 (21.6)	1.42 (1.11-1.81)	27 (6.7)	2.34 (1.34-4.07)

Table modified from Gedebjerg A. *et al.*[103] J Diabetes Complications. 2018. Appendix I.

Abbreviations: DD2=the Danish Centre for Strategic Research in Type 2 Diabetes; T2D=type 2 diabetes; aPR=adjusted prevalence ratio; CI=confidence interval.

[a]Central obesity=waist circumference >94 (men) and >80 (women). [b]Waist-hip ratio: m=males; f=females.

Table 4. Patient characteristics according to micro-/macrovascular complications in the subcohort of 5115 inidividuals at DD2 enrollment, with corresponding age- and gender-adjusted prevalence ratios.

Characteristic	All T2D patients	No complication (%)	Microvascular complication (%)	aPR (95% CI)	Macrovascular complication (%)	aPR (95% CI)	Micro- and macrovascular complications (%)	aPR (95% CI)
Subcohort	5115							
High blood pressure (mmhg)[a]								
No	1575	1012 (64.3)	173 (11.0)	Ref (1.00)	278 (17.7)	Ref (1.00)	112 (7.1)	Ref (1.00)
Yes	3261	2013 (61.7)	450 (13.8)	1.27 (1.08-1.50)	572 (17.5)	0.94 (0.83-1.07)	226 (6.9)	0.92 (0.74-1.14)
Smoking								
No	3903	2464 (63.1)	490 (12.6)	Ref (1.00)	676 (17.3)	Ref (1.00)	273 (7.0)	Ref (1.00)
Yes	941	579 (61.5)	127 (13.5)	1.08 (0.90-1.30)	177 (18.8)	1.20 (1.04-1.40)	58 (6.2)	1.07 (0.82-1.41)
BMI (kg/m2)								
<25	500	309 (61.8)	73 (14.6)	Ref (1.00)	78 (15.6)	Ref (1.00)	40 (8.0)	Ref (1.00)
25-29	1291	800 (62.0)	156 (12.1)	0.84 (0.65-1.08)	222 (17.2)	1.10 (0.87-1.40)	113 (8.8)	1.05 (0.74-1.48)
30-34	1147	692 (60.3)	141 (12.3)	0.84 (0.65-1.09)	231 (20.1)	1.37 (1.09-1.73)	83 (7.2)	1.01 (0.70-1.47)
≥35	897	554 (61.8)	145 (16.2)	1.11 (0.85-1.45)	150 (16.7)	1.39 (1.08-1.80)	48 (5.4)	0.85 (0.56-1.31)
HDL cholesterol (mmol/L)[b]								
≥1.3m/≥1.0f	2061	1246 (60.5)	297 (14.4)	Ref (1.00)	356 (17.3)	Ref (1.00)	162 (7.9)	Ref (1.00)
<1.3m/<1.0f	969	558 (57.6)	152 (15.7)	1.01 (0.84-1.22)	181 (18.7)	1.37 (1.16-1.61)	78 (8.1)	1.37 (1.06-1.78)
Triglycerides (mmol/L)								
<1.7	2410	1573 (65.3)	261 (10.8)	Ref (1.00)	415 (17.2)	Ref (1.00)	161 (6.7)	Ref (1.00)
≥1.7	2348	1430 (60.9)	330 (14.1)	1.31 (1.12-1.52)	424 (18.1)	1.16 (1.03-1.31)	164 (7.0)	1.23 (1.00-1.52)
HbA1C (%)								
<7.0	3592	2298 (64.0)	413 (11.5)	Ref (1.00)	651 (18.1)	Ref (1.00)	230 (6.4)	Ref (1.00)
7.0 - 8.0	824	500 (60.7)	126 (15.3)	1.35 (1.12-1.62)	129 (15.7)	0.91 (0.76-1.08)	69 (8.4)	1.48 (1.14-1.91)
8.0 - 9.0	303	182 (60.1)	43 (14.2)	1.30 (0.96-1.74)	57 (18.8)	1.19 (0.93-1.52)	21 (6.9)	1.50 (0.97-2.30)
≥9.0	313	187 (59.7)	52 (16.6)	1.53 (1.17-2.01)	47 (15.0)	1.00 (0.76-1.32)	27 (8.6)	2.14 (1.46-3.13)

Table modified from Gedebjerg A. *et al.*[103] J Diabetes Complications. 2018. Appendix I.

DD2: the Danish Centre for Strategic Research in Type 2 Diabetes; DDDA: the Danish Diabetes Database for Adults; aPR: adjusted prevalence ratio; CI: confidence interval.

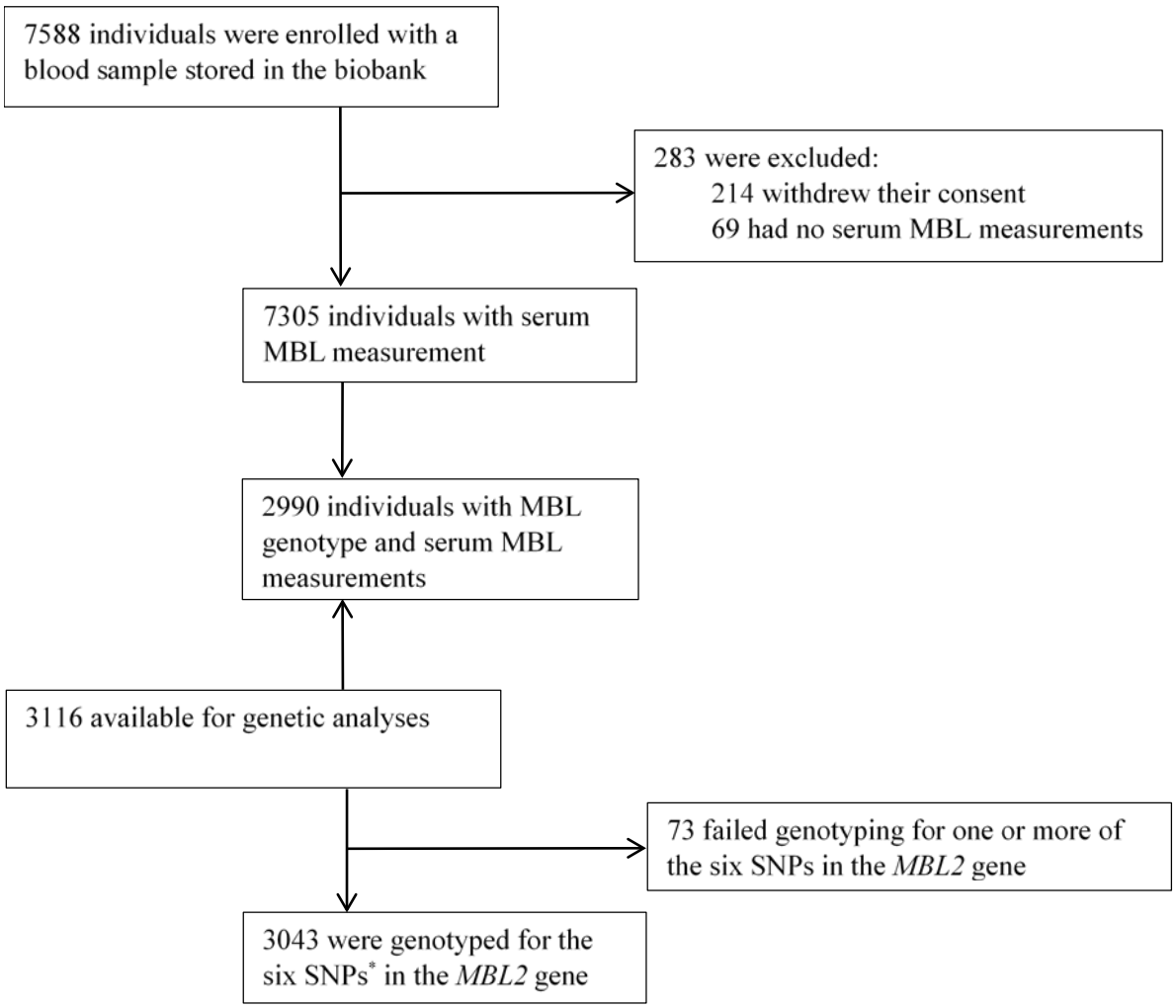
[a]High blood pressure = defined as no (systolic blood pressure <130 or diastolic bloodpressure <85) and as yes (systolic blood pressure ≥130 or diastolic bloodpressure ≥85)

[b]HDL cholesterol: m = males; f = females.

4.3 MBL, cardiovascular events, and all-cause mortality (study II)

Of the 7,588 DD2 participants with type 2 diabetes, 7,305 (96%) had a serum MBL measurement and 3,043 (42%) were genotyped for the six SNPs in the *MBL2* gene (Figure 5). During 2010 to 2018, we identified 324 individuals with type 2 diabetes from the DD2 cohort whom developed the composite endpoint of cardiovascular events, including 157 with coronary revascularization, 124 with ischemic stroke, 106 with myocardial infarction, 45 with unstable angina pectoris, and 73 with cardiovascular death (more than one outcome was possible). We identified 439 all-cause deaths. Table 1 and Supplementary Table 3 in the Appendix II shows baseline characteristics according to serum MBL and MBL expression genotype categories. We found no clear associations between serum MBL and MBL expression genotype categories and baseline characteristics.

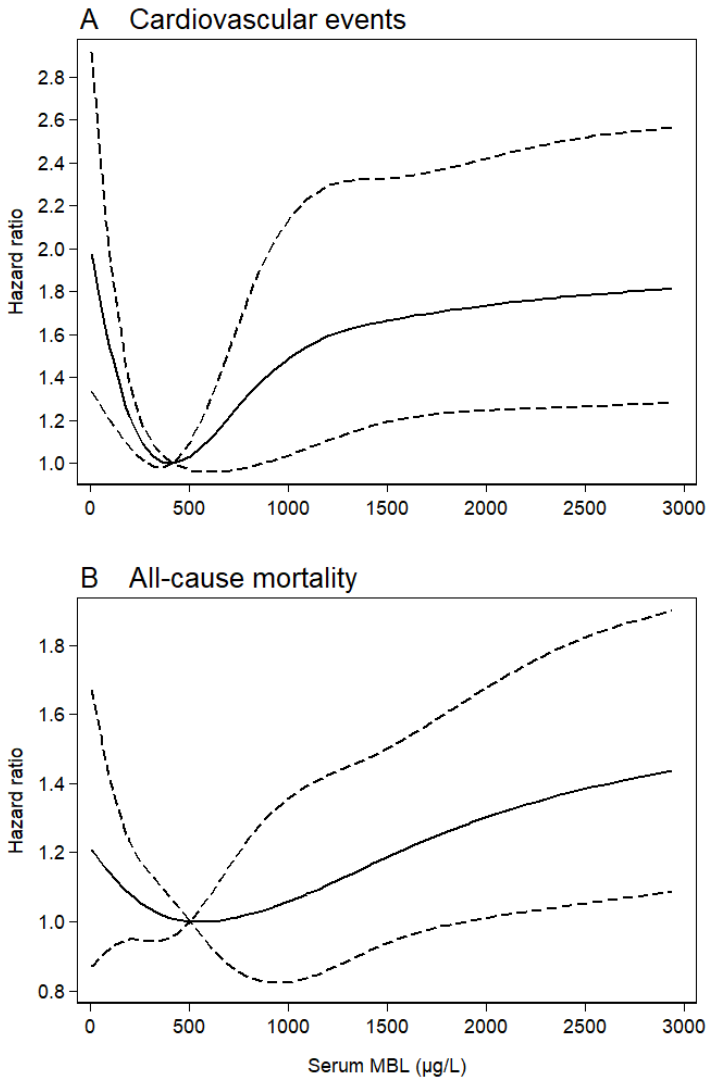
Figure 5. Flowchart of study populations (study II-III). *The six SNPs in the *MBL2* gene were: rs11003125, rs7096206, rs7095891, rs5030737, rs1800451, and rs1800450. Figure from Gedebjerg *et al.* Appendix II.



4.3.1 Serum MBL levels and risk of cardiovascular events/all-cause mortality

We found a U-shaped association between serum MBL levels and cardiovascular events in individuals with type 2 diabetes (Figure 6A). Both low and high serum MBL levels were associated with an increased risk of cardiovascular events. The association did not change when adjusted for potential confounders (Figure 7). The individual subtypes of cardiovascular events showed results consistent with the results of the composite endpoint of cardiovascular events (Appendix II, Supplemental material, Supplementary Figures 2–16). We found a similar but attenuated U-shaped association for all-cause mortality (Figures 6B and 7). All sensitivity analyses showed robustness of the main results (Appendix II, Supplemental material, Supplementary Figures 18–32).

Figure 6. Risk of cardiovascular events and all-cause mortality by serum MBL levels in individuals with type 2 diabetes. Cardiovascular events (A) and all-cause mortality (B). The solid lines indicate the hazard ratios and the dotted lines indicate the 95% CI. The continuous variable serum MBL was modeled with five restricted cubic splines. Figure from Gedebjerg *et al.* Appendix II.



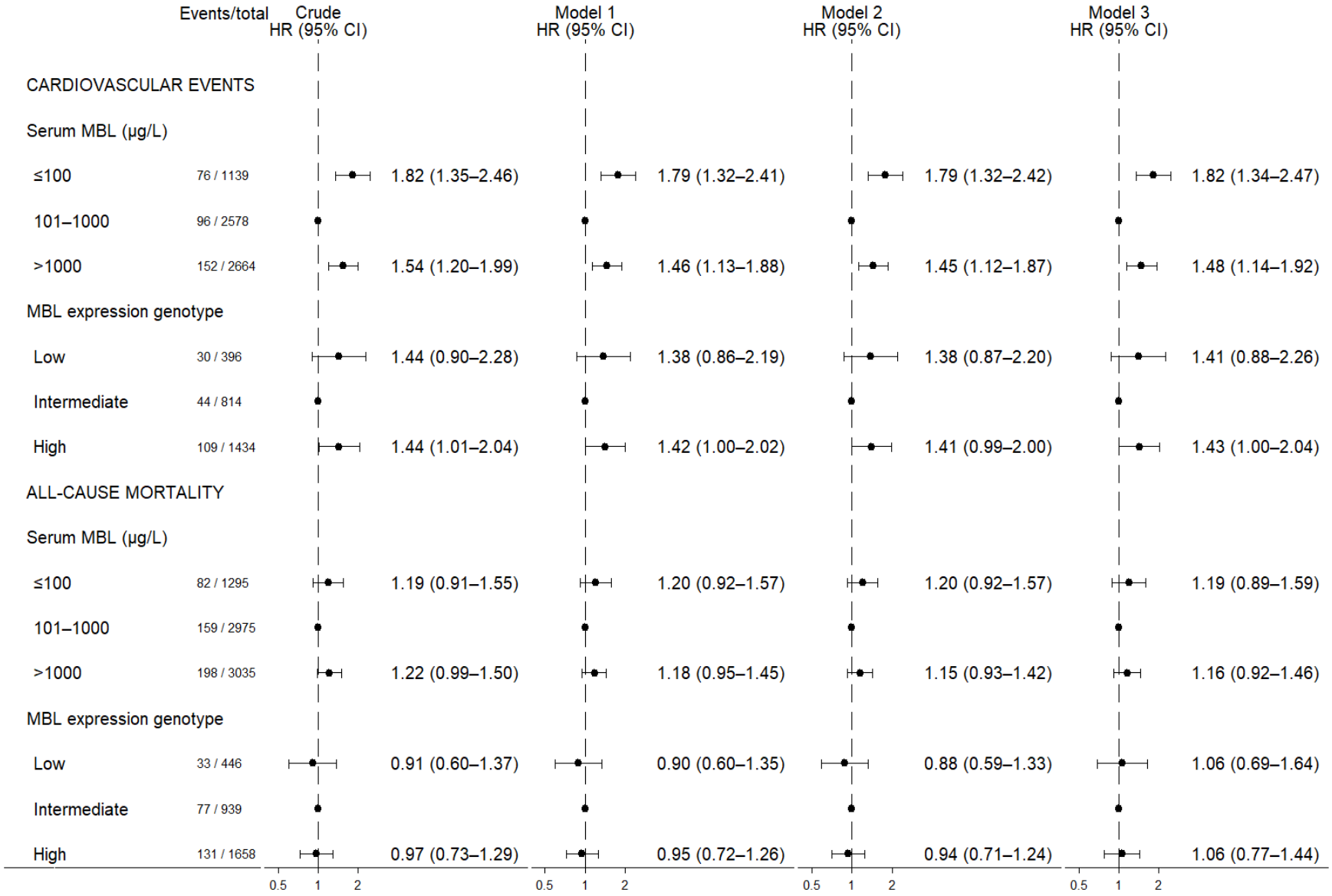


Figure 7. Hazard ratios of cardiovascular events and all-cause mortality by serum MBL and MBL expression genotype categories.

Model 1 adjusted for sex and age. Model 2 adjusted for sex, age, diabetes duration, and hs-CRP. Model 3 adjusted for sex, age, diabetes duration, waist circumference, waist–hip ratio, body mass index, physical activity, smoking, systolic and diastolic blood pressure, comorbidities, fasting blood glucose, HbA1c, C-peptide, albumin:creatinine ratio, total cholesterol, low-density lipoprotein, high-density cholesterol, triglycerides, hs-CRP, and use of anti-diabetic, lipid-lowering, anti-hypertensive, and anti-thrombotic drugs.

Missing covariates were treated with multiple imputation. Figure adapted from Gedebjerg *et al.* Appendix II.

4.3.2 MBL expression genotype and serum MBL levels

The distributions of the seven common MBL haplotypes and the uncommon LYPD haplotype (i.e., combining to form 30 haplotypes in this population) with corresponding median serum MBL levels are shown in Appendix II, Supplementary Table 5. The overall frequencies of A/A, A/O, and O/O MBL genotypes among individuals with type 2 diabetes were 59%, 36%, and 5%, respectively, which was similar to the general population[48] and individuals with type 1 diabetes.[20] Figure 8 shows that the continuous variable serum MBL was strongly associated with MBL expression genotypes.

Figure 8. Genotype–phenotype association. Serum MBL levels according to MBL expression genotypes. R^2 is the coefficient of determination. Cuzick's non-parametric test for trend ($P<1\times10^{-300}$).

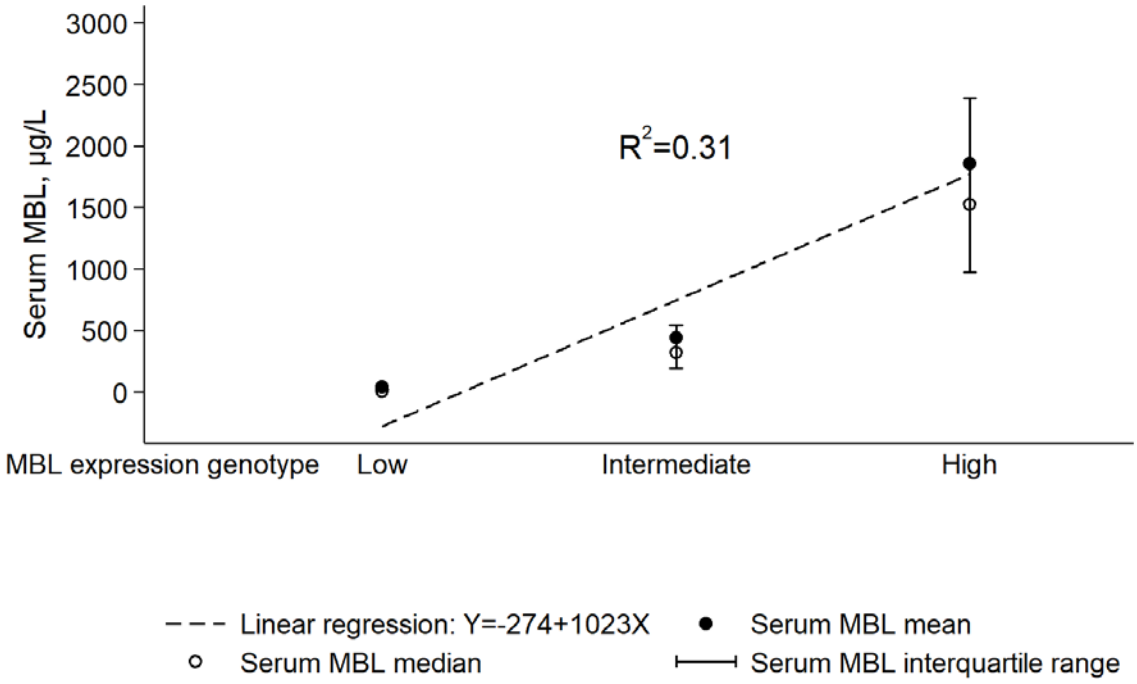


Figure from Gedebjerg *et al.* Appendix II.

4.3.2 MBL expression genotype and risk of cardiovascular events/all-cause mortality

In the crude analysis, we found that both low and high MBL expression genotypes were associated with a 44% increased risk of cardiovascular events, consistent with a U-shaped association (Figure 7). Again, the association did not change when adjusted for potential confounders (Figure 7). The individual subtypes of cardiovascular events showed results consistent with the results of the composite endpoint of cardiovascular events (Appendix II, Supplemental material, Supplementary Figures 2–16). We found no association between MBL expression genotype and all-cause mortality (Figure 7).

4.4 MBL and infections (study III)

Of the 7,588 DD2 participants, 7,305 (96%) had a serum MBL measurement and 3,043 (42%) were genotyped for the six SNPs in the *MBL2* gene (Figure 5). From 2010 to 2018, we identified 1140 individuals with type 2 diabetes (15.7% of all) who were hospitalized for an infections and we identified 5077 individuals (69.5% of all) who redeemed a community-based antimicrobial prescription. Supplementary Table 5 in the Appendix III shows the individual subtype events. More than one outcome was possible. Table 1 and Supplementary Table 6 in the Appendix III shows baseline characteristics according to serum MBL and MBL expression genotype categories. We found no clear associations between serum MBL and MBL expression genotype categories and baseline characteristics.

4.4.1 MBL and risk of hospital-treated infections

Serum MBL and the risk of any hospital-treated infections, bacterial infections, as well as major subtypes pneumonia, urinary tract infections, diarrheal diseases, and other bacterial infections showed a tendency towards an "L-shaped" association (Appendix III, Figure 2 and Supplementary Figure 3). In the fully adjusted Model 3 of serum MBL categories and infection outcomes, there was a modest association between low serum MBL level (≤100 µg/L) and increase in any hospital-treated infections (1.13 [0.96-1.33]), driven by bacterial infections (1.19 [1.01-1.41]) (Figure 9). The association of low MBL with bacterial infections was modest for urinary tract infections (1.14 [0.81-1.62]), and strongest for pneumonia (1.30 [0.98-1.70]), diarrheal diseases (1.77 [0.97-3.23]), and other bacterial infections (1.50 [1.00-2.24]) (Figure 9). Low MBL expression genotype was less strongly associated with increased risk of any hospital-treated infections (1.08 [0.84–1.38]) and bacterial infection (1.13 [0.88–1.46]) including an adjusted HR of 2.23 (1.04–4.80) for diarrheal diseases (Figure 9). Both high serum MBL levels and MBL expression genotype were associated with increased risk of fungal infections (Appendix III, Supplementary Figure 4). The sensitivity analysis excluding individuals with CRP above 10 mg/L, showed robustness of the main results (Appendix III, Supplemental material, Supplementary Figures 7–18).

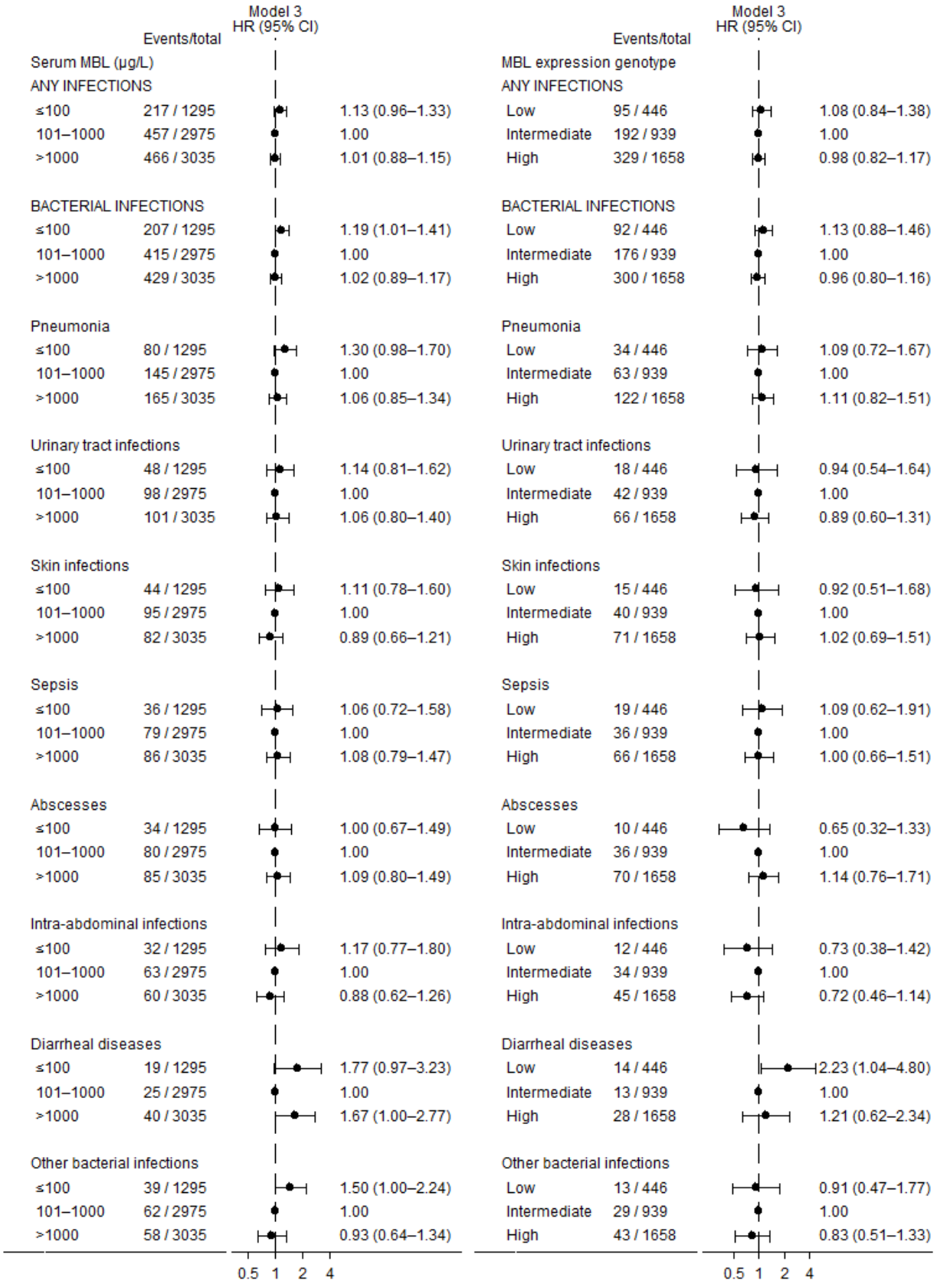


Figure 9. Risk of hospital-treated infections and bacterial subtypes by serum MBL and MBL expression genotype categories. Figure from Gedebjerg *et al.* Appendix III.

4.4.2 MBL and risk of community-based antimicrobial prescriptions

Overall, low serum MBL levels tended to show positive associations with increased antimicrobial prescriptions as with increased hospital-treated infections, but with lower risk estimates, e.g., adjusted HRs of 1.06 (0.98-1.15) for any prescriptions, 1.07 (0.99-1.16) for antibacterial prescriptions, 1.10 (1.00-1.21) for prescriptions for respiratory tract infections, and 1.09 (0.95-1.21) for prescriptions for urinary tract infections (Figure 10). Interestingly, the genetic association with low MBL expression genotype tended to be stronger than the observational association with serum MBL levels (Figure 10). Thus, modestly increased risk estimates were seen for most prescriptions with low MBL expression genotype, including for any prescriptions (1.18 [1.04-1.34]) and for antibacterial prescriptions (1.20 [1.05-1.36]), and prescriptions for respiratory tract infections (1.08 [0.93-1.25]) and skin infections (1.22 [0.97-1.53]) (Figure 10). Like for hospital-treated fungal infection, we found that high serum MBL levels were associated with antifungal prescriptions (Appendix III, Supplemental material, Supplementary Figure 6). In addition, high MBL expression genotype was associated with slightly increased risk estimates for any prescriptions (1.10 [1.00-1.20]) and also with slight increases for a number of individual prescription subtypes (Figure 10). The sensitivity analysis excluding individuals with CRP above 10 mg/L, showed similar results (Appendix III, Supplemental material, Supplementary Figures 7–18).

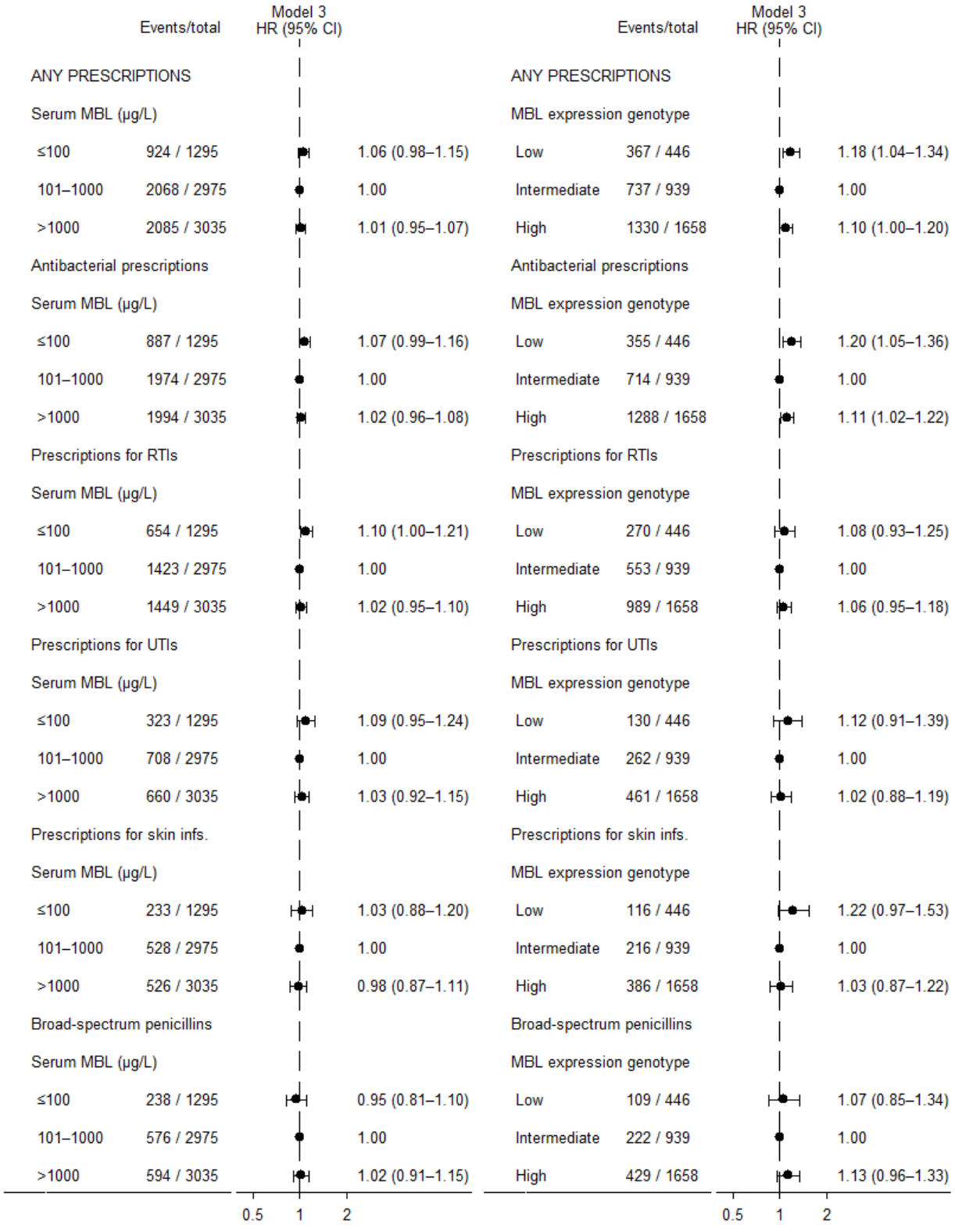


Figure 10. Risk of community-based antimicrobial prescriptions and subtypes of antibacterial prescriptions by serum MBL and MBL expression genotype categories.
Abbreviations: RTIs, respiratory tract infections; UTIs, urinary tract infections; infs, infections.
Figure from Gedebjerg *et al.* Appendix III.

5. Discussion

Among DD2 participants with recently diagnosed type 2 diabetes, we found that approximately 1 in 3 had a diabetes complication already at time of diagnosis. We showed that microvascular complication associates with hyperglycemia, absence of lipid-lowering drug use, higher blood pressure, and higher triglyceride levels, and whereas macrovascular complications associates with metabolic and lifestyle factors including general and central obesity, tobacco smoking, high C-peptide level, dyslipidemia, low-grade inflammation, and use of lipid-lowering and antihypertensive drug use.

We found a U-shaped association for both serum MBL and MBL expression genotype with cardiovascular event risk among individuals with type 2 diabetes in the DD2 cohort. We found a similar U-shaped association between serum MBL and all-cause mortality, but with lower risk estimates. MBL expression genotype was not associated with all-cause mortality.

Finally, we found an association between low serum MBL levels and increased risk of future hospital-treated bacterial infections among individuals with type 2 diabetes in the DD2 cohort, primarily driven by pneumonia, urinary tract infections, diarrheal diseases, and other bacterial infections. Low MBL expression genotype was only clearly associated with risk of diarrheal diseases. We found a similar association with community-based antimicrobial prescriptions, but with lower risk estimates and more clearly seen for low MBL genotype than for low serum MBL.

5.1 Comparison with existing literature

In the following sections, the study results will be compared to existing literature and possible explanations of our findings will be discussed. Relevant literature is summarized in Table 1. The discussion of studies II and III will go beyond studies of individuals with diabetes and will include selected studies of the general population and other selected populations.

5.1.1 Prevalence

The UKPDS studies[63,64], which enrolled individuals with newly diagnosed type 2 diabetes referred from GPs, reported that 36% had retinopathy, 11.5% had neuropathy, 18% had microalbuminuria, and 8% had cardiovascular disease at recruitment, whereas the ADDITION study[58] among screen-detected individuals with type 2 diabetes 5 years after diagnosis, reported that 11% had retinopathy, 23% had albuminuria, and 5.5% had neuropathy. Compared to our findings (13% had retinopathy, 4% had neuropathy, 3% had nephropathy, 15% had ischemic heart disease, 5% had cerebrovascular disease, and 2% had peripheral vascular disease[103]), the UKPDS studies[63,64] reported a much higher prevalence of retinopathy, neuropathy, and microalbuminuria but a lower prevalence of cardiovascular disease. This lower prevalence of individual subtypes of diabetes complications in our study compared to the older UKPDS studies[63,64] may be explained by earlier type 2 diabetes detection and improved clinical management over time.[127] Our retinopathy and

neuropathy prevalence's were more comparable with that reported in the ADDITION study[58] but they reported a much higher prevalence of nephropathy compared to ours, likely because we only assessed nephropathy through hospital diagnosis and not by albuminuria like in the ADDITION and UKPDS studies.[58,63,64] These discrepancies may be explained by the differences in study design (randomized controlled trials (RCTs) vs. registry-based studies) in assessment of diabetes complication data from a structured evaluation following diagnosis vs. hospital contact diagnosis, likely leading to an underestimation in our prevalence data.[103]

Several observational studies have reported the prevalence of diabetes complications and/or subtypes.[53-57,60-62,103] In another Danish cross-sectional study[53], also from the DD2 cohort, of 5514 individuals with recently diagnosed type 2 diabetes, the authors reported an 18% prevalence of symptom-based diabetic polyneuropathy (a median of 2.8 years of follow-up since DD2 enrollment) compared to a 4% prevalence of neuropathy in our study.[103] Again, this discrepancy may be explained by questionnaire-based evaluation of neuropathy[53] vs. hospital contact diagnosis.[103] In a UK cohort study of 9158 individuals newly diagnosed with type 2 diabetes, 19.2% presented with a cardiovascular complication (comparable with our findings of a 17% prevalence of macrovascular complications) but they only found that 1.7% presented with a microvascular complication compared to our findings of a 12% prevalence of microvascular complications. A cross-sectional study[55] from Sweden of 2174 individuals with recently diagnosed type 2 diabetes found a 12% prevalence of diabetic retinopathy, compatible with our findings. Three cross-sectional studies[57,60,61] from the UK/Germany/Scotland reported slightly higher prevalence's of retinopathy, nephropathy, and peripheral artery disease, but a lower prevalence of coronary heart disease compared to our findings. A cross-sectional study[54] from Pakistan of 891 individuals with newly diagnosed type 2 diabetes reported much higher presence of microvascular complications (68.8% vs 12% in our study) and lower presence of macrovascular complications (9% vs 17% in our study), possibly explained by ethnic differences.[78] However, an cross-sectional study[56] from India of 449 individuals with newly diagnosed type 2 diabetes found compatible results with ours, with only a slightly higher prevalence of microvascular complications (18%).

When comparing our prevalence data in individuals with recently diagnosed type 2 diabetes to other studies, it must be kept in mind that although the DD2 cohort aims to enroll individuals with newly diagnosed type 2 diabetes, some may have had diabetes some years before enrollment[101] (median diabetes duration of 1.4 years in study II and III) which may lead to an overestimation of our complication prevalence's in "newly diagnosed" diabetes.

5.1.2 Associated clinical characteristics

The pathophysiology leading to micro- and macrovascular complications in individuals with type 2 diabetes remains incompletely understood. In the following sections, different clinical characteristics will be

discussed briefly in relation to micro- and macrovascular complications, and individual subtypes, while keeping in mind the limitations of our cross-sectional design (see section Methodological considerations for further discussion).

5.1.2.1 Hyperglycemia

It is well documented that glycemic control reduces the risk of microvascular complications in individuals with type 2 diabetes.[128] In accordance, we found that higher baseline HbA1c levels (>7.0%) were associated with higher prevalence of overall microvascular complications and individual subtypes (retinopathy, neuropathy, and nephropathy) in our study.[103] Corroborating our findings, two cross-sectional studies[60,61], reported an association between HbA1c levels and diabetic retinopathy among individuals with newly diagnosed type 2 diabetes. In contrast, the exact role of tight and early glucose control on subsequent cardiovascular disease risk is still debated.[128-130] Although large clinical RCTs have not been able to show a clear beneficial effect of very intensive glucose control on cardiovascular events in type 2 diabetes[128], there may still be a beneficial effect of good early glycemic control.[130] We found no overall association between HbA1c and early macrovascular complications, but higher HbA1c levels (>8%) tended to be associated with ischemic heart disease.[103]

5.1.2.2 Metabolic syndrome and other cardiovascular risk factors

Recent clinical RCTs[131-134] suggest that newer glucose-lowering drugs reduce cardiovascular risk beyond their glucose-lowering effect, by affecting other cardiovascular risk factors.[135] In accordance, we found that baseline central obesity, high triglyceride levels (≥1.7), low HDL cholesterol (<1.3), and use of anti-hypertensive drugs were associated with higher prevalence of overall macrovascular complications and individual subtypes (ischemic heart disease, cerebrovascular disease, and peripheral vascular disease), except for cerebrovascular disease and high triglycerides. In addition, we found that other cardiovascular risk factors, e.g., baseline high waist-hip ratio, high BMI (>30), smoking, physical activity, and use of lipid-lowering drugs, were associated with higher prevalence of macrovascular but not microvascular complications, except for physical activity and microvascular complications that tended to be associated as well.[103] Thus, metabolic syndrome-related and other cardiovascular risk factors may be of greater importance than hyperglycemia for development of cardiovascular disease in individuals with type 2 diabetes.[103] C-peptide may reflect the insulin resistance underlying the metabolic syndrome.[136] Corroborating this, we found that high baseline C-peptide levels (≥ 800) were associated with macrovascular but not microvascular complications.[103] In a case-control study[137] of 2535 with type 2 diabetes (median duration of 14 years) they found an increased risk of microvascular disease with higher triglyceride levels and lower HDL levels, with the strongest association for diabetic kidney disease and a less strong association for retinopathy. Compatible with this, we found that baseline high triglyceride levels and low HDL cholesterol levels were strongly

associated with higher prevalence of nephropathy, and that baseline high triglyceride levels, but not low HDL cholesterol levels, were associated with retinopathy.[103] The longitudinal ADDITION study[138] of 1533 screen-detected individuals with type 2 diabetes (followed for 13 years) reported that low HDL cholesterol levels were associated with increased risk of diabetic polyneuropathy. Another study of 218 individuals with type 2 diabetes[139] reported an association between hypertriglyceridemia and increased risk of diabetic polyneuropathy. In contrast, we found no association between baseline high triglyceride levels and low HDL cholesterol levels and higher prevalence of neuropathy.[103] Two cross-sectional studies[60,61], reported an association between systolic blood pressure and diabetic retinopathy among individuals with newly diagnosed type 2 diabetes. Likewise, we found an association between high blood pressure and retinopathy.

5.1.2.3 Sex and age

Male sex is a well-known risk factor for development of cardiovascular disease. In accordance, we observed a higher prevalence of macrovascular complications and the individual subtypes among males. Male sex was not associated with overall microvascular complications in our study. However, for the individual subtypes, male sex was associated with a higher prevalence of both neuropathy and nephropathy, but not of retinopathy. Our findings are in disagreement with two previous studies[60,61] reporting an association between male sex and diabetic retinopathy but in agreement with another study[57] reporting an association between male sex and diabetic neuropathy. In contrast, the previously mentioned cross-sectional study[53] from the DD2 cohort, found a higher prevalence of diabetic polyneuropathy (questionnaire-based) among females with recently diagnosed type 2 diabetes, which may be explained by difference between males and females in reporting of symptoms.[57] Older age is also a well-recognized risk factor for cardiovascular disease. In accordance, we found strong associations between older age and macrovascular complications and the individual subtypes in individuals with recently diagnosed type 2 diabetes in our study. In contrast, age above 70 years was only associated with higher prevalence of microvascular complications. However, when examining the individual subtypes of microvascular complications, all age groups tended to be associated with the individual subtypes, corroborating previous findings.[57,60]

5.1.2.4 Low-grade inflammation

Low-grade inflammation (e.g., hs-CRP > 3mg/L) is associated with development of cardiovascular disease.[140] In accordance, we found that baseline low-grade inflammation (CRP > 3mg/L) was associated with higher prevalence of macrovascular and both micro- and macrovascular complications in our subgroup analysis of 1030 individuals recently diagnosed with type 2 diabetes with available CRP measurements.[103] We found no clear association between low-grade inflammation (CRP > 3mg/L) and microvascular complications in individuals recently diagnosed with type 2 diabetes.[103] This may represent a chance finding. A recent prospective study[141] of 1301 participant with type 2 diabetes, reported that baseline hs-CRP

predicted both coronary heart disease and microvascular complications in this study population. In accordance with this, reanalyzing our data on 6223 individuals with recently diagnosed type 2 diabetes from the DD2 cohort, we found a clear association between baseline low-grade inflammation (hs-CRP > 3mg/L) and microvascular complications (age and gender adjusted HR of 1.30, 95% CI 1.13–1.50, data not shown).

5.1.3 Mannose-binding lectin and cardiovascular disease (study II)

Previous studies of the association between MBL and cardiovascular disease have shown contradictory findings regarding the direction of the association. Some studies have linked only low MBL expression genotype[48,68,71,84,86,142] or low serum MBL levels[69,70,87] with increased risk of cardiovascular disease. For instance, the prospective cohort study by Siezenga *et al.*[68] found that in individuals with type 2 diabetes, the O/O MBL genotype (i.e., low MBL expression genotype) was associated with a 3-fold increased risk of cardiovascular events compared to the wild-type A/A (i.e., high MBL expression genotype). However, they found no association between serum MBL levels (log-transformed) and cardiovascular events. The Strong Heart Study[71] (high burden of diabetes) also found that the low MBL genotype was associated with a 3-fold increased risk of coronary heart disease. In the prospective Reykjavik study[70], they found that in individuals with type 2 diabetes, low serum MBL levels were associated with increased incidence of myocardial infarction. Opposite, some studies have linked only high MBL expression genotype or high serum MBL levels with increased risk.[20,67,83,85,89] For instance, a case-control study[20] found higher serum MBL levels in individuals with type 1 diabetes with previous cardiovascular disease compared to individuals without cardiovascular disease. This difference persisted in individuals with high MBL genotype but not with low genotype. The seeming discrepancies can possibly be explained by the fact that most previous studies: 1) analyzed serum MBL as a categorical variable not considering a potential non-linear association; 2) used different combinations of both serum MBL and *MBL2* genotype categories, e.g., low combined with intermediate versus high, or low versus intermediate combined with high ; 3) used different reference groups in the analyses of genotype and serum MBL levels; 4) log-transformation of serum MBL – see below; 5) were substantially smaller than our study; and 6) had heterogeneous study populations and designs not directly comparable with our results. MBL is not normally distributed and in our study 12% had no detectable MBL protein (MBL deficiency set to 10 µg/L as the limit of quantification). The distribution of MBL was skewed to the right and because of MBL-deficient individuals it is not possible to achieve normal distribution by standard log-transformation.[65] In addition, log-transformation of serum MBL will not show the true association between serum MBL and cardiovascular events because both low and high serum MBL levels are associated with cardiovascular events and thus, log-transformation of the continuous variable serum MBL will not detect this. Thus, we analyzed serum MBL as a continuous variable and in relevant clinical categories without log-transformation.

We found that both serum MBL and MBL expression genotype showed a U-shaped association with risk of cardiovascular events in individuals with type 2 diabetes. A biologically plausible explanation for these findings is that both low and high serum MBL may promote chronic inflammation and atherosclerosis in type 2 diabetes. Low MBL levels (e.g., MBL deficiency) by impairment of pathogen clearance[80,84,143] and reduced removal of atherogenic lipoproteins.[68] High MBL levels by amplifying a low-grade immune response through complement activation in connection with hyperglycemia.[42,81] In support of our findings and these explanations, three studies[65,66,88] (two in diabetes[65,66]) have reported a U-shaped association between serum MBL and low-grade inflammation and carotid intima-media thickness (a surrogate marker of atherosclerosis[82]), supporting the hypothesis of a potential dual role of MBL in the development of cardiovascular disease. A cohort study of 9245 participants from the Danish general population[48] reported a relative risk of 1.2 (95% CI: 1.0-1.4) with homozygote (i.e., low MBL expression genotype) versus noncarriers (i.e., high MBL expression genotype) MBL deficiency genotype, and 0.95 (95% CI: 0.87-1.0) with heterozygote (i.e., intermediate MBL expression genotype) versus noncarriers MBL deficiency genotype for hospitalization from cardiovascular disorders, compatible with a U-shaped association. Furthermore, compared to the noncarriers, the heterozygotes were associated with a reduced risk (relative risk of 0.73, 95% CI 0.55-0.95) of death from cardiovascular disorders. Had they used the heterozygote MBL deficiency genotype (i.e., intermediate MBL expression genotype A/O) as reference category, their results might have been comparable with our findings of a U-shaped association. These results suggest that our findings may be applicable to the general population and not only to individuals with type 2 diabetes.

5.1.4 Mannose-binding lectin and all-cause mortality (study II)

We found no association between MBL expression genotype and all-cause mortality in individuals with type 2 diabetes. This result was in agreement with the large Danish general population study previously mentioned[48] but in disagreement with another Danish cohort study[72] of 372 individuals with type 1 diabetes reporting an adjusted HR of 1.47 (95% CI: 0.97-2.21) for high expression genotype compared to low (combined intermediate and low) MBL expression genotype. This possible discrepancy may be explained by differences in individuals with type 1 and type 2 diabetes and the association with mortality, and differences in diabetes duration (in the type 1 diabetes study individuals had longstanding diabetes at baseline). Furthermore, a Danish cohort study[142] of 91 individuals with systemic lupus erythematosus reported a greater risk of death (HR 2.9, 95% CI: 0.6-13.8) among individuals with the low MBL expression genotype (i.e., O/O MBL genotype) compared to the high MBL expression genotype (i.e., combined A/A and A/O genotype). However, in the latter study, the study cohort was substantially smaller than our study, with only 2 events in the low genotype category and very wide confidence intervals, thus also compatible with a null association in line with our findings.

We found that both low and high serum MBL levels were associated with a 19% (1.19 [0.89–1.59]) and 16% (1.16 [0.92–1.46]) increased risk of all-cause mortality in individuals with type 2 diabetes, respectively, although we could not rule out neither a null association or a much larger effect (59% and 46%, respectively). Our results of a weaker association between MBL and all-cause mortality compared to the composite outcome of cardiovascular events, including cardiovascular mortality, suggest that the modest association between MBL and all-cause mortality may be driven by cardiovascular mortality. Dichotomizing the serum MBL levels (i.e., high [≥ 1000 μg/L] versus low and intermediate [< 1000 μg/L] serum MBL categories) yielded a HR of 1.15 (95% CI: 0.95-1.39) among the high serum MBL category compared to the combined low serum MBL category. This result is in agreement with a Danish cohort study[73] in individuals with type 2 diabetes reporting a HR of 1.2 (95% CI: 0.7-2.1) among individuals with high serum MBL levels (≥ 1000 μg/L) compared to low serum MBL levels (< 1000 μg/L) after 5 years follow-up. Dichotomizing the serum MBL levels (i.e., high [≥ 1895 μg/L] versus low and intermediate [< 1895 μg/L] serum MBL categories) yielded a HR of 1.35 (95% CI: 1.08-1.67) among the high serum MBL category compared to the combined low serum MBL category. Thus, our estimate increased a bit but was still at the low end of the previously mentioned Danish cohort study[72] in individuals with type 1 diabetes reporting an adjusted HR of 1.79 (95% CI: 1.19-2.71) among individuals with high serum MBL levels (≥ 1895 μg/L, i.e., above the median) compared to low serum MBL levels (< 1895 μg/L, i.e., below the median). In a cohort study of 107 hemodialysis patients, mortality was not associated with plasma MBL levels (HR not reported).[87]

5.1.5 Mannose-binding lectin and infections (study III)

The association between MBL and infections in general has been examined before[15,22,49,90-92], but the association has to my knowledge not been investigated specifically in individuals with diabetes. MBL is an important factor in the innate immune system, by initiation of the complete system hereby promoting pathogen clearance.[20,104] Thus, it is biologically plausible that MBL deficiency increases the risk of future infection. However, in the Danish general population study[48] mentioned above, low MBL expression genotype was not associated with increased risk of hospitalization due to infection. This may indicate that in individuals from the general population, other parts of the immune system (e.g., ficolins[144] – an additional group of soluble pattern recognition molecules – that can activate the lectin pathway[145]) are able to take over and compensate for MBL deficiency. In accordance with this, MBL deficiency may only increase susceptibility for infection when other parts of the immune system are compromised.[22,146] Several studies have thus examined the association between MBL deficiency and chemotherapy, cancer, and autoimmune and inflammatory diseases.[22,146] Chemotherapy induces neutropenia and thus increases risk of subsequent infection.[146] Peterslund *et al.*[29] showed that among 54 adults treated with chemotherapy for hematological malignancies, serum MBL levels were lower in patients developing a subsequent infection compared to patients not developing an infection. In a cohort study[147] of 255 adults with hematological malignancy

undergoing chemotherapy, they found an increased risk of severe infection in MBL-deficient patients (MBL concentration <500 ng/mL) compared to non-MBL-deficient patients (HR 1.50, 95% CI: 1.04-2.16). Inflammation and autoimmune disease are connected and thus, a number of studies on the association with MBL exist. In individuals with systemic lupus erythematosus, MBL deficiency increases the risk of respiratory tract infection.[148,149]

Several studies have found an association between low levels of serum MBL and *MBL2* genotype and increased risk of infection, in particular bacterial infection.[15,17,22,49,90-92] We found that in individuals with type 2 diabetes, low serum MBL levels were associated with an increased risk of future bacterial infections – mainly for hospital-treated infections and to a lesser extent community-based antimicrobial prescriptions – thus, supporting previous findings. This suggest that type 2 diabetes (like systemic lupus erythematosus) is an inflammatory disease[150] in which parts of the immune system are suppressed, increasing the susceptibility for infection in MBL-deficient patients. The association between MBL and increased susceptibility to future bacterial infections may be explained by reduced activation of the complement system by the lectin pathway and decreased opsonophagocytic killing.[17]

We found a weaker association between MBL expression genotype and risk of future infections, both when defining an infection event by hospital contact due to infections and as redeemed prescriptions of antimicrobial agents. A case-control study[151] of 120 MBL deficient adults showed that individuals with the O/O genotype (i.e., low MBL expression genotype) were associated with more gastrointestinal disease than individuals with the A/O (i.e., intermediate MBL expression genotype) and A/A genotype (i.e., high MBL expression genotype) corroborating our findings of an association between MBL deficiency and diarrheal disease. The association that we observed between both high serum MBL levels and high MBL expression genotype and increased risk of hospital-treated fungal infection needs to be confirmed in future studies.

5.2 Methodological considerations

The overall goal of an epidemiological study is to achieve valid and precise estimates of disease occurrence or the effect of an exposure on an outcome,[152] the latter with the ultimate goal of estimating causal inference. However, errors in estimation are inevitable. Errors in estimation are classified as either random error (precision) or systematic error (internal validity) of which the latter are selection bias, information bias, and confounding.[152] Traditionally, observational study designs have been ranked according to their potential validity, ranking the cohort study (study II and III) above the cross-sectional study (study I) due to the potential inherent limitations of this study design. Potential limitations to the cross-sectional study design include intrinsic uncertainty as to whether the exposure is leading to the outcome (premise for estimating causal association) or vice versa and whether a covariate is an intermediate factor on the causal pathway or a potential confounder. Study I was a cross-sectional study ascertaining exposure (patient characteristics) and outcome (microvascular and macrovascular complications) status simultaneously[153], making it hard to draw

firm conclusions concerning the direction of the exposure-outcome associations.[103] Study II and III were cohort studies defined by two or more groups of individuals that are free of disease (i.e. outcome(s) of interest) and that differ according to the extent of their exposure (serum MBL levels and MBL expression genotypes) to a potential cause of disease.[153] This provides the possibility of estimating a potential causal exposure-outcome association, with consideration of the remaining potential threats to the internal validity. In the following, a critical appraisal of the potential limitations related to internal validity of our findings are discussed.

5.2.1 Random error (chance)

Random error (or chance) is related to statistical precision. We used the width of the confidence intervals to quantify the precision, which together with the effect estimate enables an evaluation of inference.[154,155] We did not use P-values or locations of CI boundaries in which inferences are based on statistical hypothesis testing (a dichotomous declaration of a test result as either statistically significant or not) rather than considering both the strength and precision of the esimates.[154-156] In study II, compared to the intermediate MBL expression genotype, the adjusted HR for cardiovascular events was 1.41 (95% CI, 0.88 to 2.26) for the low expression genotype. Had we done a significance test or used the locations of the CI boundaries, we would have concluded that there was no association between low MBL expression genotype and cardiovascular events, i.e. 1 included in the confidence interval. Instead, we used the magnitude of the effect estimate and the width of the CI when concluding that low MBL expression genotype was associated with a potentially clinically important 41% increased risk of cardiovascular events, although statistical precision was limited and we could not rule out neither a null association or a much larger effect (126%).

The rather large number of individuals included in all of our studies reduces the random error, caused by sample variation, and increases the precision of the effect estimates.[156] However, although all main analyses yielded convincingly precise estimates (judged by the width of the CI), the precision in subgroup- (i.e., individual subtypes of cardiovascular events, infections, and micro- and macrovascular complications) and sensitivity analyses (e.g., maximize the probability of type 2 diabetes being a newly detected diagnosis) was compromised by wide CI and thus these results must be interpreted with caution.

5.2.2 Selection bias

Selection bias is a systematic error that occur when the association between the exposure and outcome is different for those participating in the study and all those theoretically eligible for the study, including those who do not participate.[152]

A common source of selection bias is self-selection, which refers to a bias that occurs if participation in the study is associated with the measured outcome.[152] This may have caused bias of our complication prevalence estimates in study I. Selection bias (self-selection) may have been introduced by invited

practitioners/outpatient clinics if factors related to inclusion are also related to the outcome, e.g., difference in type 2 diabetes phenotype of individuals recently diagnosed and difference in clinical management of type 2 diabetes across regions/practices/outpatient clinics in Denmark. Another potential source of self-selection bias into the DD2 cohort may be introduced if individuals with complications were more likely to participate. This would lead to an overestimation of the true diabetes complication prevalence. Opposite, individuals with a healthier lifestyle may also be more likely to participate, possibly leading to underestimation of complications. The initial enrollment into the DD2 cohort often took place in hospital outpatient clinics, potentially including individuals with a more severe type 2 diabetes phenotype than average in Denmark.[100] This may represent a Berksonian-like bias (Berksonian bias is a type of selection bias which occurs when both the exposure and the outcome affects participation into the study leading to bias of the relative estimates[152]) if an undiagnosed diabetes complication leads individuals to seek medical attention, thus causing type 2 diabetes to be diagnosed and the individual to be enrolled in the DD2 cohort possibly leading to an overestimation of diabetes complication prevalence in early type 2 diabetes.[103] Such bias may also have led to inflated associations between risk factors and complications in study 1, if both the risk factor exposure and the diabetes complication increased the likelihood of hospital clinic contact and DD2 cohort participation. Major selection bias seems unlikely, because the characteristics of the DD2 cohort appear very comparable and representative of population-based individuals with type 2 diabetes from the Northern Region of Denmark initiating their first glucose-lowering therapy.[76]

Loss to follow-up is potentially a major source of selection bias in cohort studies if the non-participation over time is associated with both the exposure and the outcomes. However, as the follow-up in the DD2 cohort was close to 100% due to linkage with the Danish health registries[103] and death as a competing risk has been handled in the analyses, this would unlikely bias our results. A potential selection bias may also exist in studies II and III affecting the relative estimates if non-participation is associated with genotype (MBL expression) and/or phenotype (serum MBL), and the outcome of interest. The participants were not aware of their exposure status and the studies encompassed in this thesis at the DD2 enrollment, thus this bias seems less likely. In addition, participants included in the DD2 cohort survived until DD2 enrollment where blood was drawn for serum MBL and *MBL2* genotype analyses. This may introduce survival bias (thought of as a special case of selection bias[152]). However, both situations (selection and survival bias) would lead to decreased participation of individuals with a severe type 2 diabetes phenotype or high outcome risk (e.g., cardiovascular) and would likely bias the results towards the null hypothesis, and therefore most likely cannot explain our findings for cardiovascular events, all-cause mortality, and infections. Overall, selection bias and survival bias are less likely because the patient characteristics and comorbidities of individuals enrolled in the DD2 cohort are similar to individuals with early treated type 2 diabetes in routine practice in Denmark.[76,130]

Although we were able to include more than 3,000 individuals with type 2 diabetes with a MBL expression genotype measurement, a considerable number of individuals in our genotype analyses were excluded because the MBL expression genotype was unavailable. However, DNA purification and *MBL2* genotyping were non-selective and analyzed on the first 3043 consecutive individuals with type 2 diabetes enrolled in the DD2 cohort; therefore, missing genotype data are unlikely to affect the validity of our findings.

5.2.3 Information bias

Information bias is a systematic error that arises because of mismeasurement of exposure or outcome variables.[152] Misclassification can be either differential (when the classification errors differ between groups, i.e., exposure or outcome groups) or non-differential (the classification is incorrect but is the same across groups, i.e., exposure or outcome groups).[152] All three studies in this thesis were based on prospectively recorded/collected data which avoids recall bias that can occur when outcome affects the exposure, thus reducing the risk of differential misclassification. In addition, the DD2 participants and treating physicians are unaware of the exposure status (at baseline) and a potential misclassification would likely be non-differential. The direction of bias by non-differential misclassification of a dichotomous variable is toward the null value.[152] With an exposure that is divided into more than two categories, however, non-differential misclassification can bias results in either direction.[152]

Misclassification of exposure

In studies II and III, serum MBL (levels and categories) and MBL expression genotype categories served as the exposures. By restriction to those individuals with available serum MBL and genotype measurements, misclassification of individuals with unavailable MBL measurements was eliminated. We used non-dichotomous exposure variables, and thus non-differential misclassification of high-exposure individuals (high MBL serum and expression genotype) as low-exposure individuals (low MBL serum and expression genotype) would lead to upward bias of the risk estimate for the low-exposure category.[152] This effect could be an explanation for the non-null finding observed between the low serum MBL category and cardiovascular events in study II. However, we find this explanation unlikely as serum MBL was determined in duplicates, had low intra- and interassay coefficients of variation, large study size (the probability decreases that a particular result will deviate from its expectation[152]), and good correlation between serum MBL concentrations and MBL expression genotypes. Furthermore, we find it biologically unlikely that high-exposure would be misclassified as low-exposure as serum MBL act as a weak acute phase reactant that would increase 2-3-fold during infection/inflammation causing it to be more likely that the low-exposure would be misclassified as the high-exposure. Non-differential misclassification of low-exposure individuals as high-exposure individuals would lead to downward bias of the risk estimate for the high-exposure

individuals[152], thus this cannot explain our findings. This was confirmed in sensitivity analyses excluding those individuals with CRP above 10 mg/L. In addition, misclassification of genotype is unlikely given the lack of major deviations from Hardy-Weinberg equilibrium and given that *MBL2* genotype frequencies and serum MBL levels were similar to previous studies.[20,37]

Misclassification of outcome

Correct classification of outcomes depends on several factors, e.g., patients seeking healthcare, quality of clinical diagnoses, prescribed treatment, and reporting to the registries.

In study I, micro- and macrovascular complications were the primary outcomes and identified by ICD-10 codes in the DNPR.[103] We included a 6 months after enrollment period to take account of prevalent micro- and macrovascular complications diagnosed shortly after diabetes diagnosis. Assessment of diabetes complications exclusively through hospital contact diagnoses leads to likely underestimation of the complication prevalence, especially for microvascular, but probably less so for macrovascular complications.[103] Macrovascular complications are often severe and most individuals with these diseases would have come into contact with a hospital, and thus would have been recorded in the registries. Opposite, individuals with diabetic retinopathy may largely be followed at private eye specialists, instead of at hospitals, and thus would not be recorded in the registries (misclassified). Likewise, diabetic nephropathy and diabetes neuropathy may also be misclassified when only based on hospital contact diagnoses, not including clinical examination (e.g., albuminuria). Thus, this should be kept in mind when interpreting our prevalence data.

In study II, a composite endpoint of cardiovascular events (first occurrence of myocardial infarction, ischemic stroke, coronary revascularization, unstable angina pectoris, and cardiovascular death) and all-cause mortality were the primary outcomes. The positive predictive value of cardiovascular diagnoses and procedure codes in the Danish national registries is high.[157-159] The positive predictive values of cardiovascular diagnoses and procedure codes in the DNPR has been estimated to be 97% for first-time myocardial infarction, 97% for ischemic stroke, 98% for coronary revascularization, and 88% for unstable angina pectoris, respectively.[157-159] The Danish Register of Causes of Death may have fairly low validity.[97,160] However, any misclassification of cardiovascular events would likely be non-differential and bias the association with MBL toward the null value, and therefore most likely cannot explain our findings for cardiovascular events. Information on all-cause mortality was based on vital status from the CRS, which is updated daily and holds complete and accurate data with virtually no losses to follow-up and thus misclassification of all-cause mortality is unlikely.[93]

In study III, outcomes were hospital-treated infections identified by ICD-10 codes in the DNPR and community-based antimicrobial prescriptions identified by ATC-codes in the DNHSP. ICD-10 discharge diagnoses have been shown to identify infections with a high degree of validity.[108,161,162] However, hospital-

treated infections identified by diagnosis codes may still be misclassified (misdiagnosis), but most likely be non-differential and bias the association with MBL toward the null value. Therefore, this may explain some of our findings for hospital-treated infections. A limited supply of topically low-dose antimicrobials (e.g., fusidin) are available over-the-counter, while systemic antimicrobials are available only on prescription, and thus, we may have missed some of the milder infections. In addition, individuals may have used previously prescribed antimicrobials for actual infections or individuals may receive a prescription for prophylactic usage (e.g., traveling). However, a potential misclassification would likely be non-differential and bias the results toward null. Again, this may explain some of our findings for community-based antimicrobial prescriptions. Furthermore, use of filled prescriptions may not always be an accurate surrogate for actual drug use.

5.2.4 Confounding

Confounding may be considered the confusion of effects that occurs when the exposure of interest is distorted because the effect of other factors is mixed with the actual effect of the exposure on the outcome.[152] To fulfill the criteria for a confounder, a factor has to have an effect on the outcome, must be unevenly distributed across exposure categories, and it cannot be an intermediate step on the causal pathway from exposure to outcome.[152] Confounders can be controlled for by design, e.g., restriction and matching, and in the statistical analysis, e.g. stratification, standardization, and adjustment.[152] Still, observational studies are vulnerable to residual and unmeasured confounding.

In study I, we handled potential confounding from a few basic patient factors by adjustment. As previously mentioned, we are fully aware that firm causal inference cannot be drawn from a cross-sectional study while conducting our association study between clinical characteristics and complications. In addition, the precise pathophysiological pathways between the metabolic syndrome-related and dysglycemia-related factors are incompletely understood and more factors may act as intermediates on the same causal pathway.[103,119] Thus, in our main analysis we only adjusted all our PRs for age and sex to assess whether the associations were independent of age and sex, similar to other studies.[138] Because abdominal obesity is thought to be an important factor preceding other risk factors of the metabolic syndrome, we conducted a supplementary analysis in which we also adjusted our risk estimates for waist-to-hip ratio as a marker of abdominal obesity.[103]

In studies II and III, we also handled potential confounding by adjustments. The exact association between MBL and other covariates that may have an independent effect on outcome risk (e.g., risk of cardiovascular disease) is unknown. Thus, it is unclear if factors such as obesity, hyperlipidemia, hypertension etc. may influence MBL level and independently influence cardiovascular risk, or if some of these factors may be effects of a longstanding high MBL level, and thus not be confounders but rather intermediate factors on the pathway from MBL to cardiovascular disease. We chose to perform several

regression models, first only adjusting for age and sex, and then for successively more factors, to examine if the association persisted even with detailed adjustment for a range of outcome risk factors. In the full model, we may have risked adjustment for some potential intermediate factors which may have falsely attenuated the association between MBL and outcomes.[163] However, crude and adjusted estimates (in the different models) were very similar. Still, incomplete measurement of some variables (e.g., ethnicity) may have led to residual confounding.

In studies II and III, a potential limitation is confounding by variation in nearby genes, i.e., if variants of another gene related to the risk of outcomes are in linkage disequilibrium with the MBL polymorphisms we have studied, this will confound our results.[164] However, this is unlikely because no SNPs in the *MBL2* gene were in linkage disequilibrium with any other genetic variants.

5.2.5 Statistical considerations

Overall, the methods used in this thesis are well-established statistical methods within epidemiology. However, some words are required on less conventional statistical methods, e.g., restricted cubic spline models to assess the association between serum MBL (as a continuous variable) and outcomes in study II and III, and multiple imputation to address problems with missing data in study II and III.

Categorization (two or more groups) of a continuous variable assumes that all values of the category (e.g., serum MBL) have the exact same association with the outcome.[121] This entails inefficient use of information stored in each category and may result in loss of power and precision.[165] Restricted cubic spline models does not assume constant risks within each interval and can take on practically any form, which allows for a precise description of the actual exposure-outcome association without categorization (jumps from one interval to the other) or imposing the assumption of a linear association on a continuous variable.[121] Thus, this method is ideal for exploration of potential non-linear continuous associations[121], like in our case with serum MBL and cardiovascular disease. As in categorical analyses, intervals needs to be defined, usually five or fewer knots are sufficient.[121] A restricted cubic spline has the additional property that the curve is linear before the first knot and after the last knot to overcome problems of instability at the extreme ends of the spline where data are sparse.[166] We used five knots based on Harrell *et al*.[166] Interpretation of a restricted cubic spline is not always straightforward and splines may be more valuable in determining the shape of the risk function, opposed to the actual risk estimates.[120]

Furthermore, in study II and study III, we did not estimate the magnitude of the potential causal effect on e.g., cardiovascular risk (i.e., the fourth step of a complete Mendelian randomization design[51] which assumes linear association between exposure and outcome[52]) because of our non-linear association findings. Several methods exist to assess non-linear causal effects[167-169], which we could have applied to our results in study II and study III. However, according to the Mendelian randomization design, the third step provides information on whether there is a causal association and the fourth step merely quantifies this relationship.[51]

Missing data are frequent in epidemiologic studies. We had missing data on some variables (e.g., C-peptide, BMI, and HDL cholesterol) both due to missingness in the original cohort data and because only around two-thirds of the DD2 participants were linkable to the DDDA. Improper handling of missing data can undermine the validity of study results.[114,170] In study I[103], we analyzed our data (prevalence ratios) using complete case analysis that reduces precision and may lead to bias[114], and thus this should be kept in mind when interpreting our results for the variables affected by missing data.[103] In studies II and III we used multiple imputation to handle the missing data. Missing data on covariates used for adjustment in the Cox regression models were imputed to maximize power and avoid selection bias. We used MVNI[123] to impute 20 complete data sets. MVNI assumes that all variables in the imputation model follow a multivariate normal distribution and that missing data are missing at random, meaning that the probability of a variable being missing depends only on the observed data but not on the unobserved data. Continuous variables (e.g., BMI) with clearly non-normal (skewed) distributions were zero-skewness log-transformed (i.e., transformed to approximate normality) before imputation. Then the imputed values were transformed back to the original scale before analysis.[114] Smoking (categorical variable) was also imputed using MVNI, which has been shown to perform well even in the presence of binary and ordinal variables.[123] The binary variable smoking (1: smoking – former/current vs. 0: never smoking) was imputed on a continuous scale and rounded to 0 or 1 by simple rounding.[171] Each variable in the data set was characterized as being 'imputed' or 'regular'. All covariates used in the analysis model, as well as the outcomes, were included in the imputation model to ensure maximum recovery of information about the association of interest. The imputed models were validated by comparing the mean, median, and inter-quartile range of the first and last imputed dataset with the complete dataset.

6. Main conclusions and perspectives

In this thesis, we have shown that one-third of individuals with recently diagnosed type 2 diabetes enrolled in the Danish DD2 cohort presented with a likely diabetes complication around the time of diabetes diagnosis. With the previous discussion of type 2 diabetes being true newly diagnosed in mind, our results show that in spite of earlier diagnosis and improved treatment, preventable diabetes complications are still very prevalent in individuals with recently diagnosed type 2 diabetes in the 2010s. This suggest that some individuals may be diagnosed on the basis of already having developed diabetes complications.

Again, taking into account the discussion concerning methodological considerations of the cross-sectional design in study I, the observed associations of hyperglycemia with microvascular complications, and of metabolic syndrome- and other cardiovascular disease-related factors with macrovascular complications, point to different underlying pathophysiological mechanisms. Therefore, individualized preventative treatment strategies may hold great potentials for reducing the risk of both micro- and macrovascular complications. Large longitudinal cohort studies on the exact association between a range of potential risk factors and biomarkers in early type 2 diabetes and the development of diabetes complications are needed.

Studies II and III combine the use of primary collected biomarkers and register data, and add to the scientific knowledge regarding the association between MBL and risk of cardiovascular events (study II), all-cause mortality (study II), and infectious disease (study III).

Previous studies have associated low levels or high levels of MBL expression genotype and serum MBL with increased risk of cardiovascular disease. The potential dual role of MBL in cardiovascular disease, and whether or not this association is causal, has not been formally investigated before. To our knowledge, we show for the first time a U-shaped association between serum MBL and cardiovascular events. According to the Mendelian randomization study design, the consistency of the U-shaped association for both serum MBL and MBL expression genotype suggests that serum MBL is directly involved in the development of cardiovascular disease in type 2 diabetes. As discussed in section 5.1.3 of this thesis, the U-shaped association might also be applicable to the general population. More research on the potential dual role of serum MBL in the development of cardiovascular disease in both diabetes populations and general populations are needed. As discussed under statistical considerations (Section 5.2.5) our aim was not to quantify this association, merely to clarify the nature of the association. Future studies could try to quantify this potential causal association by non-linear Mendelian randomization.

We found a similar but weaker U-shaped association between serum MBL and all-cause mortality. We suggest that this modest association may be driven by cardiovascular mortality, which is supported by our finding of no association between MBL expression genotype and all-cause mortality.

Taken together, our findings support previous studies showing that intermediate MBL levels are the most advantageous for healthy aging.[172,173] Individuals with low and high serum MBL levels may benefit

from more aggressive preventative treatment strategies. However, MBL replacement therapy is likely not the way to go due to the potential dual role of MBL in development of cardiovascular disease. MBL may be used as a biomarker for cardiovascular risk stratification, but this needs to be confirmed in future studies.

Study III adds new evidence suggesting that MBL deficiency may be a risk factor for future bacterial infections in individuals with type 2 diabetes. Even though we only found a modestly increased relative risk, the absolute risk of both hospital-treated infections and community-based antimicrobial prescriptions is high in diabetes. Thus, our findings merit attention to MBL deficient individuals with type 2 diabetes, as this group may be particularly vulnerable to bacterial infection.

We found an association, although weak, between MBL expression genotype and risk of infections in individuals with type 2 diabetes, which suggests that MBL is directly involved in development of infections in type 2 diabetes according to the principle of Mendelian randomization. MBL deficiency in combination with type 2 diabetes may act as a dual hit to the immune system and thus increase the susceptibility of bacterial infections.

7. Summary

The excess mortality and morbidity in diabetes is related to development of diabetes complications, divided into microvascular complications, macrovascular complications, and non-vascular complications e.g. infections. Understanding of why only some individuals develop e.g. macrovascular complications and others do not is limited. After two decades of research, the exact role of mannose-binding lectin (MBL, a multifunctional protein involved in innate immunity) in the development of cardiovascular disease and early death remains uncertain. Previous findings have been ambiguous, with some studies linking low levels and other studies linking high levels of MBL with increased cardiovascular risk. Several studies have found an association between MBL deficiency and increased risk of infections, but none in individuals with diabetes. In this thesis, based on the DD2 cohort, we aimed to study the prevalence of micro- and macrovascular complications and associated clinical characteristics among individuals with recently diagnosed type 2 diabetes (study I). Moreover, we wanted to investigate the association between MBL and risk of cardiovascular events and all-cause mortality in individuals with type 2 diabetes (study II). Finally, we wanted to examine the association between MBL and risk of hospital-treated infections and community-based antimicrobial prescriptions in individuals with type 2 diabetes (study III).

In study I, we reported a prevalence of diabetes complications of 35%, among individuals with recently diagnosed type 2 diabetes. Of these individuals, 12% had microvascular complications only, 17% had macrovascular complications only, and 6% had both micro- and macrovascular complications. Moreover, we showed that older age, higher HbA1c, absence of lipid-lowering drug use, higher blood pressure, and higher triglyceride levels were associated with microvascular complication, whereas male sex, older age, higher C-peptide level, central obesity, higher BMI, higher waist-hip ratio, dyslipidemia, low-grade inflammation, and use of lipid-lowering and antihypertensive drugs were associated with macrovascular complications.

In study II, we found that both serum MBL and MBL expression genotype showed a U-shaped association with cardiovascular event risk in individuals with type 2 diabetes. According to the Mendelian randomization study design, the consistency of the association for both serum MBL and MBL expression genotype suggests that serum MBL is directly involved in the development of cardiovascular disease in type 2 diabetes. Serum MBL levels showed a similar but attenuated association with all-cause mortality. MBL expression genotype was not associated with all-cause mortality.

In study III, we found an association between baseline low serum MBL levels (≤100 µg/L vs. 101–1000 µg/L) and increased risk of future hospital-treated bacterial infections in individuals with type 2 diabetes. Low MBL expression genotype showed similar, but weaker, associations with increased risk of hospital-treated bacterial infections, indicating direct involvement of serum MBL in bacterial infections. We found similar, although weaker, associations between MBL and community-based antimicrobial prescriptions in individuals with type 2 diabetes.

8. Dansk resumé (Danish summary)

Øget morbiditet og mortalitet blandt diabetikere er relateret til udviklingen af sendiabetiske komplikationer, opdelt i mikrovaskulære komplikationer (dvs. i små blodkar), makrovaskulære komplikationer (dvs. i store blodkar) og ikke-vaskulære komplikationer som f.eks. infektioner. Vores viden om hvorfor det kun er nogle personer, der udvikler f.eks. makrovaskulære komplikationer, mens andre ikke gør, er begrænset. På trods af mange års forskning, kender vi fortsat ikke den præcise rolle af mannose-bindende lektin (MBL, et multifunktionelt protein som spiller en rolle i det første immunforsvar) i udviklingen af hjertekarsygdom og tidlig død. Tidligere fund har været tvetydige, hvor nogle studier har koblet lave niveauer af MBL og andre studier har koblet høje niveauer af MBL sammen med øget risiko for hjertekarsygdom. Adskillige undersøgelser har fundet en sammenhæng mellem MBL mangel og øget risiko for infektioner, men ingen har undersøgt sammenhængen blandt personer med diabetes. Formålet med denne afhandling, som er baseret på DD2 kohorten, var at undersøge forekomsten af mikro- og makrovaskulære komplikationer og associerede kliniske karakteristika blandt relativ nydiagnosticerede type 2 diabetes personer (studie I). Desuden ønskede vi at undersøge sammenhængen mellem MBL og risikoen for udvikling af kardiovaskulære sygdomme og tidlig død hos personer med type 2 diabetes (studie II). Sluttelig ønskede vi at undersøge sammenhængen mellem MBL og risikoen for udvikling af hospitals-behandlede infektioner samt indløsning af recept på antimikrobiel medicin blandt personer med type 2 diabetes (studie III).

I studie I fandt vi en forekomst af diabetes komplikationer på 35% blandt personer med relativ nydiagnosticeret type 2 diabetes. Blandt disse personer havde 12% kun mikrovaskulære komplikationer, 17% havde kun makrovaskulære komplikationer og 6% havde både mikro- og makrovaskulære komplikationer. Mikrovaskulære komplikationer var relateret til højere alder, højere HbA1c, fravær af lipid-sænkende medicin brug, højere blodtryk, og højere triglycerid niveauer. Makrovaskulære komplikationer var relateret til mandligt køn, højere alder, højere C-peptid niveauer, mavefedme, højere BMI, højere talje-hofte mål, højere triglycerid- og lavere HDL kolesterol niveauer, højere CRP niveauer, og brug af lipid-sænkende og blodtrykssænkende medicin.

I studie II fandt vi en U-formet sammenhæng mellem både serum MBL niveauer og MBL ekspressions genotype og risikoen for udvikling af kardiovaskulære sygdomme hos personer med type 2 diabetes. Ifølge principperne bag Mendelsk randomisering indikerer overensstemmelsen mellem sammenhængen for både serum MBL niveauer og MBL ekspressions genotype, at serum MBL er direkte involveret i udviklingen af hjertekarsygdom i type 2 diabetes. Vi fandt en tilsvarende omend svagere sammenhæng mellem serum MBL niveauer og død. Vi fandt ingen sammenhæng mellem MBL ekspressions genotype og død.

I studie III fandt vi en sammenhæng mellem lave serum MBL niveauer (≤100 µg/L vs. 101–1000 µg/L) og øget risiko for udvikling af hospitals-behandlede bakterielle infektioner hos personer med type 2 diabetes. Vi fandt en tilsvarende, men svagere sammenhæng mellem lav MBL ekspressions genotype og øget risiko for bakterielle infektioner, hvilket kan indikere at serum MBL er direkte involveret i udviklingen af

bakterielle infektioner. Vi fandt en tilsvarende men svagere sammenhæng mellem serum MBL niveauer og indløsning af recept på antimikrobiel medicin blandt personer med type 2 diabetes.

9. References

1. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2019. Diabetes Care 2019;42:S13-s28.
2. Skyler JS, Bakris GL, Bonifacio E, et al. Differentiation of Diabetes by Pathophysiology, Natural History, and Prognosis. Diabetes 2017;66:241-55.
3. *IDF Diabetes Atlas, 9th edn.* . 2019. (Accessed 1/7, 2020, at http:///www.diabetesatlas.org.)
4. van Dieren S, Beulens JW, van der Schouw YT, Grobbee DE, Neal B. The global burden of diabetes and its complications: an emerging pandemic. European journal of cardiovascular prevention and rehabilitation : official journal of the European Society of Cardiology, Working Groups on Epidemiology & Prevention and Cardiac Rehabilitation and Exercise Physiology 2010;17 Suppl 1:S3-8.
5. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nature reviews Endocrinology 2018;14:88-98.
6. Forbes JM, Fotheringham AK. Vascular complications in diabetes: old messages, new thoughts. Diabetologia 2017;60:2129-38.
7. Group IDFDA. Update of mortality attributable to diabetes for the IDF Diabetes Atlas: Estimates for the year 2013. Diabetes Res Clin Pract 2015;109:461-5.
8. Nwaneri C, Cooper H, Bowen-Jones D. Mortality in type 2 diabetes mellitus: Magnitude of the evidence from a systematic review and meta-analysis. British Journal of Diabetes and Vascular Disease 2013;13:192-207.
9. Faerch K, Carstensen B, Almdal TP, Jorgensen ME. Improved survival among patients with complicated type 2 diabetes in Denmark: a prospective study (2002-2010). J Clin Endocrinol Metab 2014;99:E642-6.
10. Johnson JA, Carstensen B, Witte D, Bowker SL, Lipscombe L, Renehan AG. Diabetes and cancer (1): evaluating the temporal relationship between type 2 diabetes and cancer incidence. Diabetologia 2012;55:1607-18.
11. Pearson-Stuttard J, Blundell S, Harris T, Cook DG, Critchley J. Diabetes and infection: assessing the association with glycaemic control in population-based studies. The lancet Diabetes & endocrinology 2016;4:148-58.
12. McDonald HI, Nitsch D, Millett ER, Sinclair A, Thomas SL. New estimates of the burden of acute community-acquired infections among older people with diabetes mellitus: a retrospective cohort study using linked electronic health records. Diabet Med 2014;31:606-14.
13. Thomsen RW, Mor A. Diabetes and risk of community-acquired respiratory tract infections, urinary tract infections, and bacteremia. Open Infectious Diseases Journal 2012;6:27-39.
14. Goldberg RB. Cytokine and cytokine-like inflammation markers, endothelial dysfunction, and imbalanced coagulation in development of diabetes and its complications. J Clin Endocrinol Metab 2009;94:3171-82.
15. Eisen DP, Minchinton RM. Impact of mannose-binding lectin on susceptibility to infectious diseases. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2003;37:1496-505.
16. Ostergaard J, Hansen TK, Thiel S, Flyvbjerg A. Complement activation and diabetic vascular complications. Clinica chimica acta; international journal of clinical chemistry 2005;361:10-9.
17. Takahashi K. Mannose-binding lectin and the balance between immune protection and complication. Expert review of anti-infective therapy 2011;9:1179-90.
18. Walport MJ. Complement. Second of two parts. N Engl J Med 2001;344:1140-4.
19. Walport MJ. Complement. First of two parts. N Engl J Med 2001;344:1058-66.
20. Hansen TK, Tarnow L, Thiel S, et al. Association between mannose-binding lectin and vascular complications in type 1 diabetes. Diabetes 2004;53:1570-6.
21. Dean MM, Minchinton RM, Heatley S, Eisen DP. Mannose binding lectin acute phase activity in patients with severe infection. Journal of clinical immunology 2005;25:346-52.

22. Eisen DP. Mannose-binding lectin deficiency and respiratory tract infection. Journal of innate immunity 2010;2:114-22.
23. Garred P, Madsen HO, Hofmann B, Svejgaard A. Increased frequency of homozygosity of abnormal mannan-binding-protein alleles in patients with suspected immunodeficiency. Lancet (London, England) 1995;346:941-3.
24. Hibberd ML, Sumiya M, Summerfield JA, Booy R, Levin M. Association of variants of the gene for mannose-binding lectin with susceptibility to meningococcal disease. Meningococcal Research Group. Lancet 1999;353:1049-53.
25. Koch A, Melbye M, Sorensen P, et al. Acute respiratory tract infections and mannose-binding lectin insufficiency during early childhood. Jama 2001;285:1316-21.
26. Summerfield JA, Ryder S, Sumiya M, et al. Mannose binding protein gene mutations associated with unusual and severe infections in adults. Lancet 1995;345:886-9.
27. Super M, Thiel S, Lu J, Levinsky RJ, Turner MW. Association of low levels of mannan-binding protein with a common defect of opsonisation. Lancet 1989;2:1236-9.
28. Neth O, Hann I, Turner MW, Klein NJ. Deficiency of mannose-binding lectin and burden of infection in children with malignancy: a prospective study. Lancet 2001;358:614-8.
29. Peterslund NA, Koch C, Jensenius JC, Thiel S. Association between deficiency of mannose-binding lectin and severe infections after chemotherapy. Lancet 2001;358:637-8.
30. Kawasaki T, Etoh R, Yamashina I. Isolation and characterization of a mannan-binding protein from rabbit liver. Biochemical and biophysical research communications 1978;81:1018-24.
31. Reading PC, Hartley CA, Ezekowitz RA, Anders EM. A serum mannose-binding lectin mediates complement-dependent lysis of influenza virus-infected cells. Biochemical and biophysical research communications 1995;217:1128-36.
32. Tan SM, Chung MC, Kon OL, Thiel S, Lee SH, Lu J. Improvements on the purification of mannan-binding lectin and demonstration of its Ca(2+)-independent association with a C1s-like serine protease. The Biochemical journal 1996;319 (Pt 2):329-32.
33. Perez-Castellano M, Penaranda M, Payeras A, et al. Mannose-binding lectin does not act as an acute-phase reactant in adults with community-acquired pneumococcal pneumonia. Clin Exp Immunol 2006;145:228-34.
34. Thiel S, Holmskov U, Hviid L, Laursen SB, Jensenius JC. The concentration of the C-type lectin, mannan-binding protein, in human plasma increases during an acute phase response. Clin Exp Immunol 1992;90:31-5.
35. Degn SE, Thiel S, Jensenius JC. New perspectives on mannan-binding lectin-mediated complement activation. Immunobiology 2007;212:301-11.
36. Larsen JB, Hvas CL, Hvas AM. The Lectin Pathway in Thrombotic Conditions-A Systematic Review. Thrombosis and haemostasis 2018;118:1141-66.
37. Gadjeva M, Takahashi K, Thiel S. Mannan-binding lectin--a soluble pattern recognition molecule. Mol Immunol 2004;41:113-21.
38. Malhotra R, Wormald MR, Rudd PM, Fischer PB, Dwek RA, Sim RB. Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement via the mannose-binding protein. Nature medicine 1995;1:237-43.
39. Axelgaard E, Ostergaard JA, Thiel S, Hansen TK. Diabetes Is Associated with Increased Autoreactivity of Mannan-Binding Lectin. Journal of diabetes research 2017;2017:6368780.
40. Hansen TK, Thiel S, Knudsen ST, et al. Elevated levels of mannan-binding lectin in patients with type 1 diabetes. The Journal of clinical endocrinology and metabolism 2003;88:4857-61.
41. Fortpied J, Vertommen D, Van Schaftingen E. Binding of mannose-binding lectin to fructosamines: a potential link between hyperglycaemia and complement activation in diabetes. Diabetes/metabolism research and reviews 2010;26:254-60.
42. Collard CD, Lekowski R, Jordan JE, Agah A, Stahl GL. Complement activation following oxidative stress. Molecular immunology 1999;36:941-8.

43. Collard CD, Vakeva A, Morrissey MA, et al. Complement activation after oxidative stress: role of the lectin complement pathway. Am J Pathol 2000;156:1549-56.
44. Ip WK, Takahashi K, Ezekowitz RA, Stuart LM. Mannose-binding lectin and innate immunity. Immunological reviews 2009;230:9-21.
45. Molle I, Steffensen R, Thiel S, Peterslund NA. Chemotherapy-related infections in patients with multiple myeloma: associations with mannan-binding lectin genotypes. European journal of haematology 2006;77:19-26.
46. Swierzko AS, Szala A, Cedzynski M, et al. Mannan-binding lectin genotypes and genotype-phenotype relationships in a large cohort of Polish neonates. Human immunology 2009;70:68-72.
47. Steffensen R, Thiel S, Varming K, Jersild C, Jensenius JC. Detection of structural gene mutations and promoter polymorphisms in the mannan-binding lectin (MBL) gene by polymerase chain reaction with sequence-specific primers. Journal of immunological methods 2000;241:33-42.
48. Dahl M, Tybjaerg-Hansen A, Schnohr P, Nordestgaard BG. A population-based study of morbidity and mortality in mannose-binding lectin deficiency. J Exp Med 2004;199:1391-9.
49. Eisen DP, Dean MM, Boermeester MA, et al. Low serum mannose-binding lectin level increases the risk of death due to pneumococcal infection. Clin Infect Dis 2008;47:510-6.
50. Ytting H, Christensen IJ, Thiel S, et al. Biological variation in circulating levels of mannan-binding lectin (MBL) and MBL-associated serine protease-2 and the influence of age, gender and physical exercise. Scand J Immunol 2007;66:458-64.
51. Benn M, Nordestgaard BG. From genome-wide association studies to Mendelian randomization: novel opportunities for understanding cardiovascular disease causality, pathogenesis, prevention, and treatment. Cardiovascular research 2018;114:1192-208.
52. Teumer A. Common Methods for Performing Mendelian Randomization. Frontiers in cardiovascular medicine 2018;5:51.
53. Gylfadottir SS, Christensen DH, Nicolaisen SK, et al. Diabetic polyneuropathy and pain, prevalence, and patient characteristics: a cross-sectional questionnaire study of 5,514 patients with recently diagnosed type 2 diabetes. Pain 2019.
54. Uddin F, Ali B, Junaid N. Prevalence Of Diabetic Complications In Newly Diagnosed Type 2 Diabetes Patients In Pakistan: Findings From National Registry. Journal of Ayub Medical College, Abbottabad : JAMC 2018;30(Suppl 1):S652-s8.
55. Martinell M, Dorkhan M, Stalhammar J, Storm P, Groop L, Gustavsson C. Prevalence and risk factors for diabetic retinopathy at diagnosis (DRAD) in patients recently diagnosed with type 2 diabetes (T2D) or latent autoimmune diabetes in the adult (LADA). Journal of diabetes and its complications 2016;30:1456-61.
56. Bansal D, Gudala K, Esam HP, Nayakallu R, Vyamusani RV, Bhansali A. Microvascular Complications and Their Associated Risk Factors in Newly Diagnosed Type 2 Diabetes Mellitus Patients. International journal of chronic diseases 2014;2014:201423.
57. Kostev K, Jockwig A, Hallwachs A, Rathmann W. Prevalence and risk factors of neuropathy in newly diagnosed type 2 diabetes in primary care practices: a retrospective database analysis in Germany and U.K. Primary care diabetes 2014;8:250-5.
58. Sandbaek A, Griffin SJ, Sharp SJ, et al. Effect of early multifactorial therapy compared with routine care on microvascular outcomes at 5 years in people with screen-detected diabetes: a randomized controlled trial: the ADDITION-Europe Study. Diabetes care 2014;37:2015-23.
59. Ruta LM, Magliano DJ, Lemesurier R, Taylor HR, Zimmet PZ, Shaw JE. Prevalence of diabetic retinopathy in Type 2 diabetes in developing and developed countries. Diabet Med 2013;30:387-98.
60. Kostev K, Rathmann W. Diabetic retinopathy at diagnosis of type 2 diabetes in the UK: a database analysis. Diabetologia 2013;56:109-11.
61. Looker HC, Nyangoma SO, Cromie D, et al. Diabetic retinopathy at diagnosis of type 2 diabetes in Scotland. Diabetologia 2012;55:2335-42.

62. Sinclair AJ, Alexander CM, Davies MJ, Zhao C, Mavros P. Factors associated with initiation of antihyperglycaemic medication in UK patients with newly diagnosed type 2 diabetes. BMC endocrine disorders 2012;12:1-6823-12-1.
63. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet 1998;352:837-53.
64. Turner R, Cull C, Holman R. United Kingdom Prospective Diabetes Study 17: a 9-year update of a randomized, controlled trial on the effect of improved metabolic control on complications in non-insulin-dependent diabetes mellitus. Ann Intern Med 1996;124:136-45.
65. Hertle E, Arts IC, van der Kallen CJ, et al. Distinct Longitudinal Associations of MBL, MASP-1, MASP-2, MASP-3, and MAp44 With Endothelial Dysfunction and Intima-Media Thickness: The Cohort on Diabetes and Atherosclerosis Maastricht (CODAM) Study. Arteriosclerosis, thrombosis, and vascular biology 2016;36:1278-85.
66. Kaplar M, Sweni S, Kulcsar J, et al. Mannose-Binding Lectin Levels and Carotid Intima-Media Thickness in Type 2 Diabetic Patients. Journal of diabetes research 2016;2016:8132925.
67. Zhao Y, Lin W, Li Z, et al. High Expression of Mannose-Binding Lectin and the Risk of Vascular Complications of Diabetes: Evidence from a Meta-Analysis. Diabetes technology & therapeutics 2015;17:490-7.
68. Siezenga MA, Shaw PK, Daha MR, Rabelink TJ, Berger SP. Low Mannose-Binding Lectin (MBL) genotype is associated with future cardiovascular events in type 2 diabetic South Asians. A prospective cohort study. Cardiovasc Diabetol 2011;10:60.
69. Mellbin LG, Hamsten A, Malmberg K, et al. Mannose-binding lectin genotype and phenotype in patients with type 2 diabetes and myocardial infarction: a report from the DIGAMI 2 trial. Diabetes care 2010;33:2451-6.
70. Saevarsdottir S, Oskarsson OO, Aspelund T, et al. Mannan binding lectin as an adjunct to risk assessment for myocardial infarction in individuals with enhanced risk. The Journal of experimental medicine 2005;201:117-25.
71. Best LG, Davidson M, North KE, et al. Prospective analysis of mannose-binding lectin genotypes and coronary artery disease in American Indians: the Strong Heart Study. Circulation 2004;109:471-5.
72. Ostergaard JA, Thiel S, Lajer M, et al. Increased all-cause mortality in patients with type 1 diabetes and high-expression mannan-binding lectin genotypes: a 12-year follow-up study. Diabetes Care 2015;38:1898-903.
73. Hansen TK, Gall MA, Tarnow L, et al. Mannose-binding lectin and mortality in type 2 diabetes. Archives of Internal Medicine 2006;166:2007-13.
74. American Diabetes A. 2. Classification and Diagnosis of Diabetes. Diabetes care 2017;40:S11-S24.
75. Gregg EW, Sattar N, Ali MK. The changing face of diabetes complications. The lancetDiabetes & endocrinology 2016;4:537-47.
76. Thomsen RW, Baggesen LM, Svensson E, et al. Early Glycemic Control Among Patients With Type 2 Diabetes and InitialGlucose-lowering Treatment: A 13-year Population-based Cohort Study. Diabetes, obesity & metabolism 2015.
77. Knudsen JS, Hulman A, Ronn PF, et al. Trends in HbA1c and LDL Cholesterol in Patients With Type 2 Diabetes Receiving First-Time Treatment in Northern Denmark, 2000-2017: Population-Based Sequential Cross-Sectional Analysis. Diabetes Care 2020;43:e17-e9.
78. Spanakis EK, Golden SH. Race/ethnic difference in diabetes and diabetic complications. Current diabetes reports 2013;13:814-23.
79. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-Year Follow-Up of Intensive Glucose Control in Type 2 Diabetes. The New England journal of medicine 2008;359:1577-89.

80. Rugonfalvi-Kiss S, Endresz V, Madsen HO, et al. Association of Chlamydia pneumoniae with coronary artery disease and its progression is dependent on the modifying effect of mannose-binding lectin. Circulation 2002;106:1071-6.
81. Axelgaard E, Ostergaard JA, Haxha S, Thiel S, Hansen TK. Global Autorecognition and Activation of Complement by Mannan-Binding Lectin in a Mouse Model of Type 1 Diabetes. Mediators Inflamm 2017;2017:9403754.
82. Polak JF, Pencina MJ, Pencina KM, O'Donnell CJ, Wolf PA, D'Agostino RB, Sr. Carotid-wall intima-media thickness and cardiovascular events. N Engl J Med 2011;365:213-21.
83. Keller TT, van Leuven SI, Meuwese MC, et al. Serum levels of mannose-binding lectin and the risk of future coronary artery disease in apparently healthy men and women. Arteriosclerosis, thrombosis, and vascular biology 2006;26:2345-50.
84. Madsen HO, Videm V, Svejgaard A, Svennevig JL, Garred P. Association of mannose-binding-lectin deficiency with severe atherosclerosis. Lancet (London, England) 1998;352:959-60.
85. Pesonen E, Hallman M, Sarna S, et al. Mannose-binding lectin as a risk factor for acute coronary syndromes. Annals of Medicine 2009;41:591-8.
86. Vengen IT, Madsen HO, Garred P, Platou C, Vatten L, Videm V. Mannose-binding lectin deficiency is associated with myocardial infarction: the HUNT2 study in Norway. PloS one 2012;7:e42113.
87. Poppelaars F, Gaya da Costa M, Berger SP, et al. Strong predictive value of mannose-binding lectin levels for cardiovascular risk of hemodialysis patients. Journal of translational medicine 2016;14:236.
88. Troelsen LN, Garred P, Christiansen B, et al. Double role of mannose-binding lectin in relation to carotid intima-media thickness in patients with rheumatoid arthritis. Mol Immunol 2010;47:713-8.
89. Troelsen LN, Garred P, Madsen HO, Jacobsen S. Genetically determined high serum levels of mannose-binding lectin and agalactosyl IgG are associated with ischemic heart disease in rheumatoid arthritis. Arthritis and rheumatism 2007;56:21-9.
90. Ip WK, Chan KH, Law HK, et al. Mannose-binding lectin in severe acute respiratory syndrome coronavirus infection. The Journal of infectious diseases 2005;191:1697-704.
91. Roy S, Knox K, Segal S, et al. MBL genotype and risk of invasive pneumococcal disease: a case-control study. Lancet 2002;359:1569-73.
92. Garcia-Laorden MI, Sole-Violan J, Rodriguez de Castro F, et al. Mannose-binding lectin and mannose-binding lectin-associated serine protease 2 in susceptibility, severity, and outcome of pneumonia in adults. The Journal of allergy and clinical immunology 2008;122:368-74, 74.e1-2.
93. Schmidt M, Pedersen L, Sorensen HT. The Danish Civil Registration System as a tool in epidemiology. Eur J Epidemiol 2014;29:541-9.
94. Schmidt M, Schmidt SAJ, Adelborg K, et al. The Danish health care system and epidemiological research: from health care contacts to database records. Clin Epidemiol 2019;11:563-91.
95. Thomsen RW, Friborg S, Nielsen JS, Schroll H, Johnsen SP. The Danish Centre for Strategic Research in Type 2 Diabetes (DD2): organization of diabetes care in Denmark and supplementary data sources for data collection among DD2 study participants. Clinical epidemiology 2012;4:15-9.
96. Schmidt M, Schmidt SA, Sandegaard JL, Ehrenstein V, Pedersen L, Sorensen HT. The Danish National Patient Registry: a review of content, data quality, and research potential. Clin Epidemiol 2015;7:449-90.
97. Helweg-Larsen K. The Danish Register of Causes of Death. Scand J Public Health 2011;39:26-9.
98. The Danish Diabetes Datatbase for Adults. (Accessed 10/12, 2019, at https://www.rkkp.dk/om-rkkp/de-kliniske-kvalitetsdatabaser/voksendiabetes/.)
99. Johannesdottir SA, Horvath-Puho E, Ehrenstein V, Schmidt M, Pedersen L, Sorensen HT. Existing data sources for clinical epidemiology: The Danish National Database of Reimbursed Prescriptions. Clinical epidemiology 2012;4:303-13.

100. Christensen DH, Nicolaisen SK, Berencsi K, et al. Danish Centre for Strategic Research in Type 2 Diabetes (DD2) project cohort of newly diagnosed patients with type 2 diabetes: a cohort profile. BMJ open 2018;8:e017273.
101. Mor A, Berencsi K, Svensson E, et al. Prescribing practices and clinical predictors of glucose-lowering therapy within the first year in people with newly diagnosed Type 2 diabetes. Diabetic medicine : a journal of the British Diabetic Association 2015;32:1546-54.
102. Christensen H, Nielsen JS, Sorensen KM, Melbye M, Brandslund I. New national Biobank of The Danish Center for Strategic Research on Type 2 Diabetes (DD2). Clinical epidemiology 2012;4:37-42.
103. Gedebjerg A, Almdal TP, Berencsi K, et al. Prevalence of micro- and macrovascular diabetes complications at time of type 2 diabetes diagnosis and associated clinical characteristics: A cross-sectional baseline study of 6958 patients in the Danish DD2 cohort. Journal of diabetes and its complications 2017.
104. Thiel S, Moller-Kristensen M, Jensen L, Jensenius JC. Assays for the functional activity of the mannan-binding lectin pathway of complement activation. Immunobiology 2002;205:446-54.
105. Chen X, Sullivan PF. Single nucleotide polymorphism genotyping: biochemistry, protocol, cost and throughput. The pharmacogenomics journal 2003;3:77-96.
106. Swale A, Miyajima F, Kolamunnage-Dona R, et al. Serum mannose-binding lectin concentration, but not genotype, is associated with Clostridium difficile infection recurrence: a prospective cohort study. Clin Infect Dis 2014;59:1429-36.
107. Kaspersen KA, Dinh KM, Erikstrup LT, et al. Low-Grade Inflammation Is Associated with Susceptibility to Infection in Healthy Men: Results from the Danish Blood Donor Study (DBDS). PLoS One 2016;11:e0164220.
108. Zacho J, Benfield T, Tybjaerg-Hansen A, Nordestgaard BG. Increased Baseline C-Reactive Protein Concentrations Are Associated with Increased Risk of Infections: Results from 2 Large Danish Population Cohorts. Clinical chemistry 2016;62:335-42.
109. Mor A, Petersen I, Sorensen HT, Thomsen RW. Metformin and other glucose-lowering drug initiation and rates of community-based antibiotic use and hospital-treated infections in patients with type 2 diabetes: a Danish nationwide population-based cohort study. BMJ open 2016;6:e011523.
110. Rothman K. Epidemiology: An Introduction. 2nd edition ed: Oxford; 2012.
111. Nielsen JS, Thomsen RW, Steffensen C, Christiansen JS. The Danish Centre for Strategic Research in Type 2 Diabetes (DD2) study: implementation of a nationwide patient enrollment system. Clinical epidemiology 2012;4:27-36.
112. Ozbay LA, Moller N, Juhl C, et al. Calcineurin inhibitors acutely improve insulin sensitivity without affecting insulin secretion in healthy human volunteers. British journal of clinical pharmacology 2012;73:536-45.
113. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. Journal of chronic diseases 1987;40:373-83.
114. Sterne JA, White IR, Carlin JB, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. Bmj 2009;338:b2393.
115. Greenland S. Model-based estimation of relative risks and other epidemiologic measures in studies of common outcomes and in case-control studies. Am J Epidemiol 2004;160:301-5.
116. Petersen MR, Deddens JA. A comparison of two methods for estimating prevalence ratios. BMC Med Res Methodol 2008;8:9.
117. Zou G. A modified poisson regression approach to prospective studies with binary data. Am J Epidemiol 2004;159:702-6.
118. McNutt LA, Wu C, Xue X, Hafner JP. Estimating the relative risk in cohort studies and clinical trials of common outcomes. American Journal of Epidemiology 2003;157:940-3.
119. Cefalu WT, Leiter LA, de Bruin TW, Gause-Nilsson I, Sugg J, Parikh SJ. Dapagliflozin's Effects on Glycemia and Cardiovascular Risk Factors in High-Risk Patients With Type 2 Diabetes: A 24-Week,

Multicenter, Randomized, Double-Blind, Placebo-Controlled Study With a 28-Week Extension. Diabetes care 2015;38:1218-27.
120. Greenland S. Dose-response and trend analysis in epidemiology: alternatives to categorical analysis. Epidemiology 1995;6:356-65.
121. Gauthier J, Wu QV, Gooley TA. Cubic splines to model relationships between continuous variables and outcomes: a guide for clinicians. Bone marrow transplantation 2019.
122. Andersen PK, Geskus RB, de Witte T, Putter H. Competing risks in epidemiology: possibilities and pitfalls. International journal of epidemiology 2012;41:861-70.
123. Lee KJ, Carlin JB. Multiple imputation for missing data: fully conditional specification versus multivariate normal imputation. Am J Epidemiol 2010;171:624-32.
124. Wang J, Shete S. Testing Departure from Hardy-Weinberg Proportions. Methods in molecular biology (Clifton, NJ) 2017;1666:83-115.
125. Cuzick J. A Wilcoxon-type test for trend. Stat Med 1985;4:87-90.
126. Hansen TK, Forsblom C, Saraheimo M, et al. Association between mannose-binding lectin, high-sensitivity C-reactive protein and the progression of diabetic nephropathy in type 1 diabetes. Diabetologia 2010;53:1517-24.
127. Stark Casagrande S, Fradkin JE, Saydah SH, Rust KF, Cowie CC. The prevalence of meeting A1C, blood pressure, and LDL goals among people with diabetes, 1988-2010. Diabetes care 2013;36:2271-9.
128. Hemmingsen B, Lund SS, Gluud C, et al. Targeting intensive glycaemic control versus targeting conventional glycaemic control for type 2 diabetes mellitus. The Cochrane database of systematic reviews 2013;11:CD008143.
129. Garber AJ, Newcomer JW, Hennekens CH. Lower A1c targets in type 2 diabetes and increased mortality: causal or casual? Am J Med 2013;126:1033-4.
130. Svensson E, Baggesen LM, Johnsen SP, et al. Early Glycemic Control and Magnitude of HbA1c Reduction Predict Cardiovascular Events and Mortality: Population-Based Cohort Study of 24,752 Metformin Initiators. Diabetes care 2017;40:800-7.
131. Marso SP, Bain SC, Consoli A, et al. Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes. The New England journal of medicine 2016;375:1834-44.
132. Marso SP, Daniels GH, Brown-Frandsen K, et al. Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. The New England journal of medicine 2016;375:311-22.
133. Neal B, Perkovic V, Mahaffey KW, et al. Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. The New England journal of medicine 2017.
134. Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. The New England journal of medicine 2015;373:2117-28.
135. Abdul-Ghani M, DeFronzo RA, Del Prato S, Chilton R, Singh R, Ryder REJ. Cardiovascular Disease and Type 2 Diabetes: Has the Dawn of a New Era Arrived? Diabetes care 2017;40:813-20.
136. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet 2005;365:1415-28.
137. Sacks FM, Hermans MP, Fioretto P, et al. Association between plasma triglycerides and high-density lipoprotein cholesterol and microvascular kidney disease and retinopathy in type 2 diabetes mellitus: a global case-control study in 13 countries. Circulation 2014;129:999-1008.
138. Andersen ST, Witte DR, Dalsgaard EM, et al. Risk Factors for Incident Diabetic Polyneuropathy in a Cohort With Screen-Detected Type 2 Diabetes Followed for 13 Years: ADDITION-Denmark. Diabetes Care 2018;41:1068-75.
139. Smith AG, Singleton JR. Obesity and hyperlipidemia are risk factors for early diabetic neuropathy. J Diabetes Complications 2013;27:436-42.
140. Zacho J, Tybjaerg-Hansen A, Nordestgaard BG. C-reactive protein, genetically elevated levels and risk of ischemic heart and cerebrovascular disease. Scandinavian journal of clinical and laboratory investigation 2009;69:442-6.

141. Aryan Z, Ghajar A, Faghihi-Kashani S, Afarideh M, Nakhjavani M, Esteghamati A. Baseline High-Sensitivity C-Reactive Protein Predicts Macrovascular and Microvascular Complications of Type 2 Diabetes: A Population-Based Study. Annals of nutrition & metabolism 2018;72:287-95.
142. Ohlenschlaeger T, Garred P, Madsen HO, Jacobsen S. Mannose-binding lectin variant alleles and the risk of arterial thrombosis in systemic lupus erythematosus. N Engl J Med 2004;351:260-7.
143. Monsey L, Best LG, Zhu J, DeCroo S, Anderson MZ. The association of mannose binding lectin genotype and immune response to Chlamydia pneumoniae: The Strong Heart Study. PLoS One 2019;14:e0210640.
144. Holmskov U, Thiel S, Jensenius JC. Collections and ficolins: humoral lectins of the innate immune defense. Annual review of immunology 2003;21:547-78.
145. Endo Y, Matsushita M, Fujita T. The role of ficolins in the lectin pathway of innate immunity. The international journal of biochemistry & cell biology 2011;43:705-12.
146. Thiel S, Frederiksen PD, Jensenius JC. Clinical manifestations of mannan-binding lectin deficiency. Mol Immunol 2006;43:86-96.
147. Vekemans M, Robinson J, Georgala A, et al. Low mannose-binding lectin concentration is associated with severe infection in patients with hematological cancer who are undergoing chemotherapy. Clin Infect Dis 2007;44:1593-601.
148. Garred P, Voss A, Madsen HO, Junker P. Association of mannose-binding lectin gene variation with disease severity and infections in a population-based cohort of systemic lupus erythematosus patients. Genes and immunity 2001;2:442-50.
149. Takahashi R, Tsutsumi A, Ohtani K, et al. Association of mannose binding lectin (MBL) gene polymorphism and serum MBL concentration with characteristics and progression of systemic lupus erythematosus. Annals of the rheumatic diseases 2005;64:311-4.
150. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. Nature reviews Immunology 2011;11:98-107.
151. Bjarnadottir H TV, Jorgensen GH, Arnardottir M, Ludviksson BR. Mannan-Binding Lectin (MBL) Deficient Individuals with the O/O Genotype are Highly Susceptible to Gastrointestinal Diseases. J Clin Cell Immunol 2014;5:182.
152. Rothman KJ, Greenland S, Lash TL. Validity in epidemiological studies. Modern Epidemiology. 3 ed. ed. Philadelphia: Lippincott Williams & Wilkins 2008.
153. Kenneth R, Greenland S, Lash TL. Types of epidemiologic studies. Modern Epidemiology 3. ed2008.
154. Goodman S. A dirty dozen: twelve p-value misconceptions. Seminars in hematology 2008;45:135-40.
155. Rothman KJ. Six persistent research misconceptions. Journal of general internal medicine 2014;29:1060-4.
156. Rothman KJ, Greenland S, Lash TL. Precision and statistics in epidemiologic studies. Modern Epidemiology. 3. ed2008.
157. Adelborg K, Sundboll J, Munch T, et al. Positive predictive value of cardiac examination, procedure and surgery codes in the Danish National Patient Registry: a population-based validation study. BMJ open 2016;6:e012817.
158. Krarup LH, Boysen G, Janjua H, Prescott E, Truelsen T. Validity of stroke diagnoses in a National Register of Patients. Neuroepidemiology 2007;28:150-4.
159. Sundboll J, Adelborg K, Munch T, et al. Positive predictive value of cardiovascular diagnoses in the Danish National Patient Registry: a validation study. BMJ open 2016;6:e012832.
160. Madsen M, Davidsen M, Rasmussen S, Abildstrom SZ, Osler M. The validity of the diagnosis of acute myocardial infarction in routine statistics: a comparison of mortality and hospital discharge data with the Danish MONICA registry. Journal of clinical epidemiology 2003;56:124-30.
161. Benfield T, Jensen JS, Nordestgaard BG. Influence of diabetes and hyperglycaemia on infectious disease hospitalisation and outcome. Diabetologia 2007;50:549-54.

162. Henriksen DP, Nielsen SL, Laursen CB, Hallas J, Pedersen C, Lassen AT. How well do discharge diagnoses identify hospitalised patients with community-acquired infections?--a validation study. PLoS One 2014;9:e92891.
163. Schisterman EF, Cole SR, Platt RW. Overadjustment bias and unnecessary adjustment in epidemiologic studies. Epidemiology 2009;20:488-95.
164. Zacho J, Tybjaerg-Hansen A, Jensen JS, Grande P, Sillesen H, Nordestgaard BG. Genetically elevated C-reactive protein and ischemic vascular disease. The New England journal of medicine 2008;359:1897-908.
165. Bennette C, Vickers A. Against quantiles: categorization of continuous variables in epidemiologic research, and its discontents. BMC Med Res Methodol 2012;12:21.
166. Harrell FE, Jr., Lee KL, Pollock BG. Regression models in clinical studies: determining relationships between predictors and response. Journal of the National Cancer Institute 1988;80:1198-202.
167. Silverwood RJ, Holmes MV, Dale CE, et al. Testing for non-linear causal effects using a binary genotype in a Mendelian randomization study: application to alcohol and cardiovascular traits. International journal of epidemiology 2014;43:1781-90.
168. Staley JR, Burgess S. Semiparametric methods for estimation of a nonlinear exposure-outcome relationship using instrumental variables with application to Mendelian randomization. Genetic epidemiology 2017;41:341-52.
169. Sun Y-Q, Burgess S, Staley JR, et al. Body mass index and all cause mortality in HUNT and UK Biobank studies: linear and non-linear mendelian randomisation analyses. 2019;364:l1042.
170. White IR, Royston P, Wood AM. Multiple imputation using chained equations: Issues and guidance for practice. Stat Med 2011;30:377-99.
171. Bernaards CA, Belin TR, Schafer JL. Robustness of a multivariate normal approximation for imputation of incomplete binary data. Stat Med 2007;26:1368-82.
172. Eisen DP, Osthoff M. If there is an evolutionary selection pressure for the high frequency of MBL2 polymorphisms, what is it? Clin Exp Immunol 2014;176:165-71.
173. Tomaiuolo R, Ruocco A, Salapete C, et al. Activity of mannose-binding lectin in centenarians. Aging cell 2012;11:394-400.

10. Appendices

Paper I

● **Appendix I**

Paper II

● **Appendix II**

Paper III

● **Appendix III**

Paper I

● **Appendix I**

Journal of Diabetes and Its Complications 32 (2018) 34–40

Contents lists available at ScienceDirect

Journal of Diabetes and Its Complications

journal homepage: WWW.JDCJOURNAL.COM

Prevalence of micro- and macrovascular diabetes complications at time of type 2 diabetes diagnosis and associated clinical characteristics: A cross-sectional baseline study of 6958 patients in the Danish DD2 cohort

Anne Gedebjerg [a,b,*], Thomas Peter Almdal [c], Klara Berencsi [a], Jørgen Rungby [d], Jens Steen Nielsen [e], Daniel R. Witte [b,f], Søren Friborg [e], Ivan Brandslund [g], Allan Vaag [h], Henning Beck-Nielsen [e], Henrik Toft Sørensen [a], Reimar Wernich Thomsen [a]

[a] Department of Clinical Epidemiology, Institute of Clinical Medicine, Aarhus University Hospital, Aarhus, Denmark
[b] Danish Diabetes Academy, Odense University Hospital, Odense, Denmark
[c] Department of Endocrinology PE, Rigshospitalet, University of Copenhagen, Denmark
[d] Department of Endocrinology IC, Bispebjerg University Hospital, Copenhagen, Denmark
[e] Diabetes Research Centre, Department of Endocrinology, Odense University Hospital, Odense, Denmark
[f] Department of Public Health, Aarhus University, Aarhus, Denmark
[g] Department of Biochemistry, Lillebaelt Hospital, Vejle, Denmark
[h] AstraZenaca, Mölndal, Sweden

ARTICLE INFO

Article history:
Received 25 June 2017
Received in revised form 28 August 2017
Accepted 16 September 2017
Available online 19 September 2017

Keywords:
Type 2 diabetes
Microvascular complications
Macrovascular complications
Risk factors
Epidemiology

ABSTRACT

Aims: To examine the prevalence of micro- and macrovascular complications and their associated clinical characteristics at time of type 2 diabetes (T2D) diagnosis.
Methods: We examined the prevalence of complications and associated clinical characteristics among 6958 newly diagnosed T2D patients enrolled in the prospective Danish Center for Strategic Research in T2D cohort during 2010–2016. We calculated age- and gender-adjusted prevalence ratios (aPRs) of complications using log-binomial and Poisson regression.
Results: In total, 35% (n = 2456) T2D patients had diabetic complications around diagnosis; 12% (n = 828) had microvascular complications, 17% (n = 1186) macrovascular complications, and 6% (n = 442) had both. HbA1c levels of ≥7% were associated with microvascular complications [HbA1c 7%–8%; aPR: 1.35, 95% confidence interval (CI): 1.12–1.62] but not macrovascular complications [aPR: 0.91, 95% CI: 0.76–1.08]. High C-peptide ≥ 800 pmol/L was associated with macrovascular [aPR 1.34, 95% CI: 1.00–1.80] but not microvascular [aPR 0.97, 95% CI: 0.71–1.33] complications. Macrovascular complications were associated with male sex, age > 50 years, obesity, hypertriglyceridemia, low HDL cholesterol, smoking, elevated CRP levels, and antihypertensive therapy. Microvascular complications were associated with high blood pressure, hypertriglyceridemia, and absence of lipid-lowering therapy.
Conclusions: One-third of patients with T2D had diabetes complications around time of diagnosis. Our findings suggest different pathophysiological mechanisms behind micro- and macrovascular complications.

1. Introduction

It is a major clinical and public health problem that a variable proportion of individuals with T2D remains undiagnosed and untreated before developing diabetes complications.[1] Many patients with T2D thus present with complications already at time of diagnosis,[1] as the various pathophysiological abnormalities associated with T2D, such as hyperglycemia, dyslipidemia, and hypertension may have existed for several years.[2,3]

Recent data on the prevalence of diabetes-related complications at time of diagnosis are scarce. Many studies that examined this issue are 10–20 years old, and generally showed high prevalences of complications,[4] e.g., a ~36% prevalence of retinopathy in the UKPDS study.[4] Diabetes case-finding has been increasing in populations-at-

Conflicts of interest: none.
* Corresponding author at: Department of Clinical Epidemiology, Aarhus University Hospital, Olof Palmes Alle 43-45, DK-8200 Aarhus N, Denmark.
E-mail address: aged@clin.au.dk (A. Gedebjerg).

https://doi.org/10.1016/j.jdiacomp.2017.09.010
1056-8727/

risk,[5] likely leading to earlier diabetes diagnoses and possibly a lower prevalence of complications at onset.[6,7] For example, Thomsen et al.[6] found that the median baseline hemoglobin A1c (HbA1c) measurement before initial glucose-lowering treatment in Denmark declined from 8.9% in 2000–2003 to 7.0% in 2010–2012, suggesting earlier diagnosis and therapy.

It is not well understood at present whether pathogenic processes leading to micro- and macrovascular T2D complications differ.[8] Hyperglycemia per se seems to be an important risk factor for microvascular outcomes but less so for macrovascular outcomes, for which traditional cardiovascular risk factors may play a greater role.[4,9] Moreover, several recent randomized clinical trials (RCTs)[10–13] have found that newer diabetes drugs exert a CVD protective effect beyond their glucose-lowering effect.[9] In this context, we hypothesized that clinical characteristics at baseline may differ between T2D patients presenting with micro- and macrovascular complications, with dysglycemia-related factors being more important for microvascular complications and metabolic syndrome-related factors more important for macrovascular complications. In the present study, we examined the prevalence of micro- and macrovascular complications and associated characteristics among newly diagnosed T2D patients in a large prospective Danish cohort.

2. Materials and methods

2.1. Study population

We conducted this cross-sectional study using information from the Danish Centre for Strategic Research in Type 2 Diabetes (DD2) project, which includes a nationwide cohort of newly or recently diagnosed type 2 diabetes mellitus (T2D) patients enrolled from general practitioners' (GPs) offices and hospital specialist outpatient clinics in Denmark since November 2010.[14] The implementation and logistics of the DD2 project, patient enrollment, and the DD2 biobank[15] have been described in detail previously.[16] In brief, GPs or hospital physicians provide detailed interview and clinical examination data for each DD2 patient at time of enrollment. This information is recorded in the DD2 database together with each patient's civil registration number (CPR number). Blood samples (fasting) and urine samples are obtained from each patient, either on the day of the interview or later.[17]

Our main study population consisted of all 6958 incident T2D patients currently enrolled in the DD2 cohort. The unique CPR number provided to each Danish resident, at birth or upon immigration, allowed data linkage of this cohort with other Danish registries. We could thus obtain a complete hospital contact history for each DD2 participant through linkage with the Danish National Patient Registry (DNPR), which covers all Danish hospitals and contains discharge records from all inpatient hospitalizations since 1977 and all hospital outpatient clinic and emergency department visits since 1995.[18] Additionally, we obtained complete data on filled medication prescriptions for each DD2 participant through linkage with the Danish National Health Service Prescription Database (DNHSPD).[19] Through linkage with a nationwide quality-of-care database, the Danish Diabetes Database for Adults (DDDA), we were furthermore able to extract additional clinical data for a subcohort of 5115 (75%) DD2 patients.[14]

2.2. Micro- and macrovascular complications

For each cohort member, we assessed presence or absence of diabetes complications as recorded in the DNPR between 10 years before and up till 6 months after the DD2 enrolment date. The 6 months after period was included to allow for investigation and diagnosis of prevalent diabetes complications shortly after diabetes diagnosis. We categorized diabetes complications as: (1) no microvascular or macrovascular complications at enrolment; (2) microvascular complications; (3) macrovascular complications; and (4) both microvascular and macrovascular complications. Microvascular complications included a medical database history of the following conditions: retinopathy, including any diabetes-related eye disease, atherosclerotic eye disease, blindness or severely impaired vision, or use of retinal photocoagulation therapy; neuropathy, including any diabetes-related neurological complication; and nephropathy, including any diabetes-related kidney disease, albuminuria, chronic dialysis, or renal failure. Macrovascular complications included a medical registry history of any of the following conditions: history of ischemic heart disease including angina pectoris or coronary surgery; atherosclerotic cerebrovascular disease including thrombolysis and thrombectomy; atherosclerotic peripheral vascular disease including vascular surgery or amputation; or any operation for macroangiopathy (see Supplemental Table A1 for diagnosis and procedure codes).

2.3. Associated patient characteristics

From the DD2 cohort questionnaire and the linked medical databases, we extracted data on patient characteristics present at the time of DD2 enrollment. Patient characteristics of particular interest included age, sex, body mass index (BMI) ≥30 kg/m^2, central obesity (defined as waist circumference ≥ 94 cm for men and ≥80 cm for women), high waist-hip ratio (WHR) (defined as >1.0 in men and >0.85 in women), tobacco smoking, blood pressure (mm Hg), fasting blood-glucose level (mmol/L), C-peptide level (pmol/L), plasma lipid level (mmol/L), C-reactive protein (CRP) level (mg/L), and use of anti-hypertensive and lipid-lowering drugs. Data on age, sex, central obesity, WHR, physical activity, and use of lipid-lowering and anti-hypertensive drugs were available for the entire DD2 cohort, and data on HbA1c, blood pressure, BMI, tobacco smoking, and plasma lipids were available for the subcohort of 5115 patients (75%) currently linkable to the DDDA.[14] Concerning specific biomarkers, fasting blood glucose, C-peptide and CRP have currently been analyzed for the first 5563 (80%), 5800 (83%) and 1030 (15%) DD2 cohort patients in the DD2 biobank.

The DD2 project, including patient registration and sample collection, has been approved by The Regional Committees on Health Research Ethics for Southern Denmark (record number S-20100082) and the Danish Data Protection Agency (record number 2008-58-0035). After receiving detailed oral and written information approved by The Regional Committees on Health Research Ethics for Southern Denmark, patients volunteer to participate in the DD2 project. Their willingness to participate is documented by a signed written informed consent document.

2.4. Statistical analysis

We characterized patients according to factors as described above. Prevalence of microvascular, macrovascular, and both micro- and macrovascular complications at baseline was calculated as proportions (percentages) of all DD2 cohort members. We calculated prevalence ratios (PRs) with 95% confidence intervals (CIs) of the different complications associated with presence of each factor using log-binomial and Poisson regression.[20] The exact pathophysiological pathways between the different dysglycemia-related and metabolic syndrome-related factors are incompletely understood and several factors may act as clusters in the same causal pathway.[21] We therefore only adjusted our estimates for age and gender in the main analysis (aPRs) to assess whether associations were independent of these two factors.[20] Because obesity and in particular abdominal obesity is thought to be a fundamental factor preceding a number of other metabolic risk factors in many individuals; we did a supplementary analysis in which

we also adjusted all associations for WHR category as a marker of abdominal obesity.

Although the DD2 project aims to enroll newly or recently diagnosed T2D patients, we conducted a sensitivity analysis to maximize the probability of T2D being a newly detected diagnosis: in this analysis, we included only T2D patients who had no earlier glucose-lowering treatment, hospital diagnosis of T2D, or registration in the DDDA quality-of-care database recorded >1 year prior to DD2 enrollment.

In addition, we calculated aPRs separately for individual complications, i.e. retinopathy, neuropathy, nephropathy, ischemic heart disease, atherosclerotic cerebrovascular disease, and atherosclerotic peripheral vascular disease.

All analyses were performed using SAS version 9.4 (SAS Institute, Inc., Cary, North Carolina).

3. Results

3.1. Prevalence data

Among the 6958 patients with T2D, 35% (n = 2456) had diabetes complications around enrolment in the DD2 cohort. Of these patients, 12% (n = 828) had microvascular complications only, 17% (n = 1186) had macrovascular complications only, and 6% (n = 442) had both micro- and macrovascular complications (Table 1). Among all newly diagnosed T2D patients with any microvascular complications, 13% (n = 887) had retinopathy, 4% (n = 264) had neuropathy, and 3% (n = 234) had nephropathy (Supplementary Table A.8). Among all newly diagnosed T2D patients with any macrovascular complications, 15% (n = 1059) had ischemic heart disease, 5% (n = 365) had atherosclerotic cerebrovascular disease, and 2% (n = 151) had atherosclerotic peripheral vascular disease (Supplementary Table A.10).

3.2. Characteristics associated with micro- and macrovascular complications

Tables 1 and 2 present clinical and lifestyle characteristics according to diabetes complications around time of diagnosis, with corresponding aPRs and 95% CIs.

Compared with HbA1c levels < 7%, higher HbA1c levels at diagnosis were associated with a higher prevalence of microvascular complications [e.g., HbA1c 7–8%, aPR: 1.35, 95% confidence interval (CI): 1.12–1.62] and of both micro- and macrovascular complications [aPR: 1.48, 95% CI: 1.14–1.91], but not of macrovascular complications [aPR: 0.91, 95% CI: 0.76–1.08] (Table 2). Similarly, we found a relationship between high baseline fasting blood glucose levels (>7.5 mmol/L) and the presence of microvascular complications, but no association with either macro- or combined micro-/macrovascular complications (Table 1). In contrast, high C-peptide ≥ 800 pmol/L was associated with macrovascular complications [aPR 1.34, 95% CI: 1.00–1.80], but not microvascular complications [aPR 0.97, 95% CI: 0.71–1.33] or both micro- and macrovascular complications [aPR: 1.07, 95% CI: 0.68–1.69].

Higher age and male sex were both associated with presence of macrovascular complications and both micro- and macrovascular complications (Table 1). In contrast, the presence of microvascular complications was only increased in persons aged ≥70 years and did not differ by sex.

For the remaining metabolic syndrome-related factors, we found that central obesity, high baseline triglyceride (≥1.7), low baseline HDL cholesterol (<1.3), and use of anti-hypertensive drugs were all associated with presence of macrovascular and both micro- and macrovascular complications (Tables 1 and 2). Similar associations were found for high waist-hip ratio (>1.0) and BMI. Regarding microvascular complications, we found an association with high blood pressure [aPR: 1.27, 95% CI: 1.08–1.50] and high triglyceride level [aPR: 1.31, 95% CI: 1.12–1.52]. The prevalence of microvascular complications was lower in patients using lipid-lowering drugs (99.5% used statins), while the prevalence of macrovascular and both micro- and macrovascular complications was increased.

Smoking was associated with macrovascular complications, but not with microvascular or both micro- and macrovascular complications (Table 2). In the subsample with biobank information, high baseline CRP (>3.0 mg/L) was associated with presence of macrovascular and both micro- and macrovascular complications, but not with microvascular complications.

Of interest, most associations were very robust to further adjustment for central obesity assessed by WHR categories, as seen in Supplementary Tables A.2 and A.3.

Overall, the sensitivity analysis restricted to patients with maximized probability of having newly detected diabetes showed consistent results with the main analysis (Supplementary Tables A.4 and A.5). This included similar associations between HbA1c levels and complications as observed in the main analysis. Further adjustment for WHR categories in these subcohorts did not change the estimates materially (Supplementary Tables A.6 and A.7).

3.3. Characteristics associated with individual complications

Characteristics associated with individual micro- and macrovascular complications are presented in Supplementary Tables A.8–A.11. Statistical precision was limited, but some interesting tendencies were noted. For macrovascular complications, central obesity, high waist-hip ratio (>1.0), physical inactivity, high blood pressure, and smoking were more closely associated with peripheral vascular disease than with the other complications. High C-peptide was most strongly associated with cerebrovascular disease. In contrast, very high HbA1c values of 9% or more tended to be associated with ischemic heart disease, but not with cerebrovascular disease. For microvascular complications, an increased HbA1c level of 7% or more was associated with both retino-, neuro-, and nephropathy. Male sex was associated with neuropathy and nephropathy, but not with retinopathy. Obesity, high C-peptide, and dyslipidemia tended to be associated with nephropathy, but not with the other microvascular complications. Smoking was related to neuropathy.

4. Discussion

Our findings from the nationwide DD2 study cohort show that one-third of newly or recently diagnosed T2D patients have hospital-diagnosed micro- and macrovascular complications already around time of diagnosis. Of concern, this suggests that many patients have their T2D diagnosed on the basis of already having developed diabetes complications. While keeping in mind the limitations of a cross-sectional analysis, the observed associations of dysglycemia-related factors with microvascular complications, and metabolic syndrome related factors with macrovascular complications corroborate hypotheses about different underlying pathophysiological mechanisms.

4.1. Strengths and limitations

The main strength of this large cross-sectional study is its comprehensive and detailed assessment of lifestyle and clinical factors based on the DD2 database, DD2 biobank, and linkage with population-based health registries. These resources provide close to 100% completeness for demographic, clinical characteristics and prescription data for the newly diagnosed T2D patients in our study.[17]

Table 1
Prevalence of diabetes complications among 6958 patients with newly diagnosed type 2 diabetes in the DD2 cohort. Age- and gender-adjusted prevalence ratios are shown according to each patient characteristic.

Characteristic	All T2D patients	No complication (%)	Microvascular complication (%)	aPR (95% CI)	Macrovascular complication (%)	aPR (95% CI)	Micro- and macrovascular complications (%)	aPR (95% CI)
Main cohort	6958	4502 (64.7)	828 (11.9)		1186 (17.1)		442 (6.4)	
Sex								
Female	2927	2047 (69.9)	365 (12.5)	Ref (1.00)	374 (12.8)	Ref (1.00)	141 (4.8)	Ref (1.00)
Male	4031	2455 (60.9)	463 (11.5)	0.92 (0.81–1.05)	812 (20.1)	1.60 (1.43–1.79)	301 (7.5)	1.59 (1.32–1.93)
Age (years)								
<50	1220	973 (79.8)	142 (11.6)	Ref (1.00)	82 (6.7)	Ref (1.00)	23 (1.9)	Ref (1.00)
50–59	1790	1262 (70.5)	180 (10.1)	0.87 (0.70–1.07)	276 (15.4)	2.31 (1.82–2.92)	72 (4.0)	2.17 (1.36–3.44)
60–69	2517	1550 (61.6)	291 (11.6)	0.99 (0.82–1.20)	505 (20.1)	2.99 (2.40–3.74)	171 (6.8)	3.61 (2.35–5.55)
≥70	1431	717 (50.1)	215 (15.0)	1.28 (1.05–1.56)	323 (22.6)	3.44 (2.73–4.32)	176 (12.3)	6.60 (4.31–10.12)
Central obesity[a]								
No	570	393 (69.0)	63 (11.1)	Ref (1.00)	83 (14.6)	Ref (1.00)	31 (5.4)	Ref (1.00)
Yes	6378	4099 (64.3)	765 (12.0)	1.08 (0.85–1.38)	1103 (17.3)	1.33 (1.09–1.63)	411 (6.4)	1.38 (0.97–1.97)
Waist-hip ratio[b]								
≤0.95m/≤0.80f	772	506 (65.5)	95 (12.3)	Ref (1.00)	131 (17.0)	Ref (1.00)	40 (5.2)	Ref (1.00)
0.96–1.0m/0.81–0.85f	1437	911 (63.4)	180 (12.5)	0.99 (0.79–1.26)	261 (18.2)	1.12 (0.93–1.36)	85 (5.9)	1.22 (0.85–1.75)
>1.0m/>0.85f	4737	3074 (64.9)	553 (11.7)	0.92 (0.74–1.14)	793 (16.7)	1.21 (1.02–1.43)	317 (6.7)	1.75 (1.27–2.40)
Regular physical exercise								
Yes	2725	1847 (67.8)	304 (11.2)	Ref (1.00)	430 (15.8)	Ref (1.00)	144 (5.3)	Ref (1.00)
No	4232	2655 (62.7)	524 (12.4)	1.12 (0.98–1.28)	755 (17.8)	1.10 (0.99–1.23)	298 (7.0)	1.31 (1.08–1.58)
Use of lipid-lowering drugs								
No	2072	1537 (74.2)	304 (14.7)	Ref (1.00)	171 (8.3)	Ref (1.00)	60 (2.9)	Ref (1.00)
Yes	4886	2965 (60.7)	524 (10.7)	0.70 (0.62–0.80)	1015 (20.8)	2.31 (1.98–2.69)	382 (7.8)	2.35 (1.81–3.07)
Use of anti-hypertensive drugs								
No	1967	1548 (78.7)	232 (11.8)	Ref (1.00)	151 (7.7)	Ref (1.00)	36 (1.8)	Ref (1.00)
Yes	4991	2954 (59.2)	596 (11.9)	0.94 (0.81–1.10)	1035 (20.7)	2.28 (1.93–2.70)	406 (8.1)	3.19 (2.27–4.49)
Fasting blood glucose (mmol/L)								
<6.5	1548	996 (64.3)	159 (10.3)	Ref (1.00)	278 (18.0)	Ref (1.00)	115 (7.4)	Ref (1.00)
6.5–7.0	917	611 (66.6)	86 (9.4)	0.92 (0.71–1.17)	167 (18.2)	1.00 (0.85–1.19)	53 (5.7)	0.80 (0.59–1.09)
7.0–7.5	751	482 (64.2)	80 (10.7)	1.05 (0.82–1.36)	147 (19.6)	1.07 (0.90–1.28)	42 (5.6)	0.75 (0.54–1.06)
≥7.5	2146	1424 (66.4)	245 (11.4)	1.16 (0.96–1.41)	350 (16.3)	0.95 (0.83–1.10)	127 (5.9)	0.92 (0.72–1.16)
C-peptide (pmol/L)								
<550	295	202 (68.5)	36 (12.2)	Ref (1.00)	39 (13.2)	Ref (1.00)	18 (6.1)	Ref (1.00)
550–800	853	606 (71.0)	90 (10.6)	0.84 (0.58–1.21)	120 (14.1)	1.00 (0.72–1.40)	37 (4.3)	0.65 (0.38–1.12)
≥800	4652	2932 (63.0)	552 (11.9)	0.97 (0.71–1.33)	847 (18.2)	1.34 (1.00–1.80)	321 (6.9)	1.07 (0.68–1.69)
CRP (mg/L)								
≤3.0	627	429 (68.4)	62 (9.9)	Ref (1.00)	114 (18.2)	Ref (1.00)	22 (3.5)	Ref (1.00)
>3.0	403	254 (63.0)	35 (8.7)	0.86 (0.58–1.29)	87 (21.6)	1.42 (1.11–1.81)	27 (6.7)	2.34 (1.34–4.07)

DD2: the Danish Centre for Strategic Research in Type 2 Diabetes; aPR: adjusted prevalence ratio; CI: confidence interval.
Data available in total DD2 cohort (n = 6958) for central obesity (n = 10 missing); for waist-hip ratio (n = 12 missing); for regular physical activity (n = 1 missing). Data currently analyzed in the DD2 biobank for fasting blood glucose (n = 5362); for C-peptide (n = 5800); and for CRP (n = 1030).
[a] Central obesity = waist circumference > 94 (men) and >80 (women).
[b] Waist-hip ratio: m = males; f = females.

Our study also has important limitations. Firstly, the cross-sectional design of our study leads to intrinsic uncertainty as to whether given diabetes complications preceded or followed the diagnosis of T2D and some of the patient characteristics, making it difficult to draw firm conclusions about the direction of exposure-outcome associations. Secondly, although the DD2 project aims to enroll newly diagnosed T2D patients, some may have been diagnosed with diabetes several years before enrolment,[22] which may have led to an overestimation of the true complication prevalence at diabetes debut. Thirdly, the DD2 cohort may represent patients whose newly diagnosed T2D is more severe than average in Denmark, as initial enrolment took place in hospital specialist outpatient clinics in about half of the cases.[17] This may represent an example of Berksonian-like bias if an undiagnosed diabetic complication leads patients to seek medical attention in secondary health care, thus causing T2D to be diagnosed and the patient to be enrolled in the DD2, with a possible overestimation of the average complication prevalence in early T2D. However, since 2013, the number of patients recruited by GPs has increased rapidly and by 2016 characteristics of the cohort appear to be representative of all newly diagnosed T2D patients in Denmark.[6] Fourth, assessment of complications exclusively through hospital contact diagnoses leads to likely underestimation, especially for microvascular complications, but probably less so for macrovascular complications. Thus, when comparing with results from RCTs, it must be kept in mind that our complication data are not derived from a structured evaluation following briefly after diagnosis, leading to a likely underestimation.

4.2. Comparison with previous literature

The lower prevalence of individual diabetes complications in our cohort compared with older studies may originate in earlier and more complete detection of T2D cases in recent years, or be due to improvements over time in clinical management[23] which seem to have

Table 2
Prevalence of diabetes complications in the subcohort of 5115 DD2 patients who currently can be linked to the DDDA. Age- and gender-adjusted prevalence ratios are shown according to patient characteristics.

Characteristic	All T2D patients	No complication (%)	Microvascular complication (%)	aPR (95% CI)	Macrovascular complication (%)	aPR (95% CI)	Micro- and macrovascular complications (%)	aPR (95% CI)
Subcohort	5115							
High blood pressure (mm Hg)[a]								
No	1575	1012 (64.3)	173 (11.0)	Ref (1.00)	278 (17.7)	Ref (1.00)	112 (7.1)	Ref (1.00)
Yes	3261	2013 (61.7)	450 (13.8)	1.27 (1.08–1.50)	572 (17.5)	0.94 (0.83–1.07)	226 (6.9)	0.92 (0.74–1.14)
Smoking								
No	3903	2464 (63.1)	490 (12.6)	Ref (1.00)	676 (17.3)	Ref (1.00)	273 (7.0)	Ref (1.00)
Yes	941	579 (61.5)	127 (13.5)	1.08 (0.90–1.30)	177 (18.8)	1.20 (1.04–1.40)	58 (6.2)	1.07 (0.82–1.41)
BMI (kg/m^2)								
<25	500	309 (61.8)	73 (14.6)	Ref (1.00)	78 (15.6)	Ref (1.00)	40 (8.0)	Ref (1.00)
25–29	1291	800 (62.0)	156 (12.1)	0.84 (0.65–1.08)	222 (17.2)	1.10 (0.87–1.40)	113 (8.8)	1.05 (0.74–1.48)
30–34	1147	692 (60.3)	141 (12.3)	0.84 (0.65–1.09)	231 (20.1)	1.37 (1.09–1.73)	83 (7.2)	1.01 (0.70–1.47)
≥35	897	554 (61.8)	145 (16.2)	1.11 (0.85–1.45)	150 (16.7)	1.39 (1.08–1.80)	48 (5.4)	0.85 (0.56–1.31)
HDL cholesterol (mmol/L)[b]								
≥1.3m/≥1.0f	2061	1246 (60.5)	297 (14.4)	Ref (1.00)	356 (17.3)	Ref (1.00)	162 (7.9)	Ref (1.00)
<1.3m/<1.0f	969	558 (57.6)	152 (15.7)	1.01 (0.84–1.22)	181 (18.7)	1.37 (1.16–1.61)	78 (8.1)	1.37 (1.06–1.78)
Triglycerides (mmol/L)								
<1.7	2410	1573 (65.3)	261 (10.8)	Ref (1.00)	415 (17.2)	Ref (1.00)	161 (6.7)	Ref (1.00)
≥1.7	2348	1430 (60.9)	330 (14.1)	1.31 (1.12–1.52)	424 (18.1)	1.16 (1.03–1.31)	164 (7.0)	1.23 (1.00–1.52)
HbA1C (%)								
<7.0	3592	2298 (64.0)	413 (11.5)	Ref (1.00)	651 (18.1)	Ref (1.00)	230 (6.4)	Ref (1.00)
7.0–8.0	824	500 (60.7)	126 (15.3)	1.35 (1.12–1.62)	129 (15.7)	0.91 (0.76–1.08)	69 (8.4)	1.48 (1.14–1.91)
8.0–9.0	303	182 (60.1)	43 (14.2)	1.30 (0.96–1.74)	57 (18.8)	1.19 (0.93–1.52)	21 (6.9)	1.50 (0.97–2.30)
≥9.0	313	187 (59.7)	52 (16.6)	1.53 (1.17–2.01)	47 (15.0)	1.00 (0.76–1.32)	27 (8.6)	2.14 (1.46–3.13)

DD2: the Danish Centre for Strategic Research in Type 2 Diabetes; DDDA: the Danish Diabetes Database for Adults; aPR: adjusted prevalence ratio; CI: confidence interval.
Data available in DDDA cohort (n = 5115); for blood pressure (n = 279 missing); for smoking (n = 271 missing); for BMI (n = 1280 missing); for HDL (n = 2085 missing); for triglycerides (n = 357 missing); and for HbA1c (n = 83 missing).
[a] High blood pressure = defined as no (systolic blood pressure < 130 or diastolic blood pressure < 85) and as yes (systolic blood pressure ≥ 130 or diastolic blood pressure ≥ 85).
[b] HDL cholesterol: m = males; f = females.

decreased the gap in life expectancy for persons with diabetes versus the general population.[24] The UKPDS study,[4] which enrolled patients with newly diagnosed diabetes referred by GPs, reported a 36% prevalence of retinopathy and an 11.5% prevalence of neuropathy. Our observed prevalence of 12.8% with retinopathy and 3.8% with neuropathy was more comparable with that reported among screen-detected T2D patients 5 years after diagnosis in the ADDITION study[25] (11% and 5.5%, respectively). Compared to our findings, the ADDITION study reported a much higher prevalence of nephropathy (23% vs. 3.4% in our study), likely because we were only able to assess nephropathy through manifest hospital diagnoses and not by albuminuria[25] leading to an underestimation. Among 9158 people newly diagnosed with T2D during 2003–2005 in a UK cohort study,[26] a lower proportion (1.7%) presented with microvascular conditions and cardiovascular complications (19.2%), compared with our study. A large population-based cohort study conducted in Sweden among patients with T2D and mean diabetes duration of 7.4 years[27] reported microalbuminuria in 16.6% and cardiovascular disease in 18.2% during the follow-up time of maximum 6 years. Another large Swedish cross-sectional population-based study[28] reported a prevalence of 12% of diabetic retinopathy among newly diagnosed T2D patients which corresponds well with our findings.

The pathophysiology leading to micro- versus macrovascular complications in T2D patients remains incompletely understood. Overall, RCTs have not demonstrated a substantial clinical effect of intensive glucose-lowering therapy on macrovascular outcomes in T2D patients, although there may be beneficial legacy effects of good early glucose control.[29] Also, recent RCTs[10–13] suggest that newer glucose lowering drugs reduce CVD risk beyond their glucose-lowering effect, by affecting other CVD risk factors. Our cross-sectional observations confirms that risk factors related to the metabolic syndrome, e.g., central obesity, dyslipidemia, and hypertension, in addition to low physical activity, tobacco smoking, and older age, may be of greater importance than hyperglycemia per se for development of macrovascular complications. High C-peptide ≥ 800 pmol/L was associated with early macrovascular complications in our study in accordance with others' findings[30] and may reflect the insulin resistance underlying most of the metabolic syndrome.[31] In contrast, the fact that poor glycemic control per se reflected by a high HbA1c level was associated with microvascular but not macrovascular complications corroborates main findings from several randomized clinical trials[3,32–34] that tight glycemic control in T2D patients reduces the risk of microvascular complications by 10% - 28%. Of note, high triglyceride levels, but not HDL cholesterol levels, were also associated with microvascular complications, and absence of lipid-lowering drug use was associated with microvascular complications at diagnosis. In analyses of individual complications, high triglyceride and low HDL cholesterol levels were clearly associated with nephropathy, consistent with a large case-control study[35] that reported strong associations between diabetic kidney disease, high triglyceride levels, and low HDL cholesterol levels. We did not find an association between retinopathy or neuropathy and high triglyceride or low HDL cholesterol levels, as other studies have reported.[36,37]

4.3. Conclusion

In conclusion, almost one-third of newly or recently diagnosed T2D patients in the DD2 cohort presented with a likely diabetic

complication around disease onset. Our findings suggest that different phenotypical risk profiles exist for microvascular versus macrovascular complications, pointing to different pathophysiological mechanisms and a possible need to individualize preventive treatment strategies.

Acknowledgements

We are very grateful to all DD2 participants and thank you to Johnny A. Kahlert for assistance with the statistical analysis.

Funding

The Danish Centre for Strategic Research in Type 2 Diabetes Project (DD2) is supported by the Danish Agency for Science (grant nos. 09-067009 and 09-075724), the Danish Health and Medicines Authority, the Danish Diabetes Association, and an unrestricted donation from Novo Nordisk A/S. Project partners are listed on the website www.DD2.nu. The Department of Clinical Epidemiology, Aarhus University Hospital participates in the International Diabetic Neuropathy Consortium (IDNC) research programme, which is supported by a Novo Nordisk Foundation Challenge programme grant (Grant number NNF14SA000 6). AG and DW are supported by the Danish Diabetes Academy, which is funded by an unrestricted grant from the Novo Nordisk Foundation. Besides, AG has received funding from the Danish Heart Foundation (grant no.15-R99-A5866-22891), and from Aarhus University.

Author contributions

HBN, HTS and JR participated in designing the DD2 cohort. TPA, JR, JSN, DW, SF, AV, IB, JSC, HBN, HTS and RWT conceived of the study. IB was responsible for the biobank and the biochemical analyses. AG, RWT and HTS participated in the design of the study and KB performed the statistical analysis. AG initially drafted the article, with help by RWT and HTS. All other authors have critically reviewed the manuscript. All authors contributed substantially, revised the manuscript for intellectual content, and approved the final version to be submitted. AG and RWT are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jdiacomp.2017.09.010.

References

1. American Diabetes Association. 2. Classification and diagnosis of diabetes. *Diabetes Care.* 2017;40:S11-24.
2. Tabak AG, Jokela M, Akbaraly TN, Brunner EJ, Kivimaki M, Witte DR. Trajectories of glycaemia, insulin sensitivity, and insulin secretion before diagnosis of type 2 diabetes: an analysis from the Whitehall II study. *Lancet.* 2009;373:2215-21.
3. Faerch K, Witte DR, Tabak AG, Perreault L, Herder C, Brunner EJ, et al. Trajectories of cardiometabolic risk factors before diagnosis of three subtypes of type 2 diabetes: a post-hoc analysis of the longitudinal Whitehall II cohort study. *Lancet Diabetes Endocrinol.* 2013;1:43-51.
4. *Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK prospective diabetes study (UKPDS) group.Lancet.* 1998;352:837-53.
5. American Diabetes Association. 2. Classification and diagnosis of diabetes. *Diabetes Care.* 2016;39:S13-22.
6. Thomsen RW, Baggesen LM, Svensson E, Pedersen L, Noerrelund H, Buhl ES, et al. Early glycemic control among patients with type 2 diabetes and initial glucose-lowering treatment: a 13-year population-based cohort study. *Diabetes Obes Metab.* 2015;17:771-80.
7. Gregg EW, Sattar N, Ali MK. The changing face of diabetes complications. *Lancet Diabetes Endocrinol.* 2016;4:537-47.
8. Skyler JS, Bakris GL, Bonifacio E, Darsow T, Eckel RH, Groop L, et al. Differentiation of diabetes by pathophysiology, natural history, and prognosis. *Diabetes.* 2017;66:241-55.
9. Abdul-Ghani M, DeFronzo RA, Del Prato S, Chilton R, Singh R, Ryder REJ. Cardiovascular disease and type 2 diabetes: has the dawn of a new era arrived? *Diabetes Care.* 2017;40:813-20.
10. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med.* 2015;373:2117-28.
11. Marso SP, Daniels GH, Brown-Frandsen K, Kristensen P, Mann JF, Nauck MA, et al. Liraglutide and cardiovascular outcomes in type 2 diabetes. *N Engl J Med.* 2016;375:311-22.
12. Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N Engl J Med.* 2017;377:644-57.
13. Marso SP, Bain SC, Consoli A, Eliaschewitz FG, Jódar E, Leiter LA, et al. Semaglutide and cardiovascular outcomes in patients with type 2 diabetes. *N Engl J Med.* 2016;375:1834-44.
14. Thomsen RW, Friborg S, Nielsen JS, Schroll H, Johnsen SP. The Danish centre for strategic research in type 2 diabetes (DD2): organization of diabetes care in Denmark and supplementary data sources for data collection among DD2 study participants. *Clin Epidemiol.* 2012;4:15-9.
15. Christensen H, Nielsen JS, Sorensen KM, Melbye M, Brandslund I. New national biobank of the Danish center for strategic research on type 2 diabetes (DD2). *Clin Epidemiol.* 2012;4:37-42.
16. Nielsen JS, Thomsen RW, Steffensen C, Christiansen JS. The Danish centre for strategic research in type 2 diabetes (DD2) study: implementation of a nationwide patient enrollment system. *Clin Epidemiol.* 2012;4:27-36.
17. Thomsen RW, Nielsen JS, Ulrichsen SP, Pedersen L, Hansen AM, Nilsson T. The Danish centre for strategic research in type 2 diabetes (DD2) study: collection of baseline data from the first 580 patients. *Clin Epidemiol.* 2012;4:43-8.
18. Lynge E, Sandegaard JL, Rebolj M. The Danish national patient register. *Scand J Public Health.* 2011;39:30-3.
19. Johannesdottir SA, Horvath-Puho E, Ehrenstein V, Schmidt M, Pedersen L, Sorensen HT. Existing data sources for clinical epidemiology: the Danish national database of reimbursed prescriptions. *Clin Epidemiol.* 2012;4:303-13.
20. McNutt LA, Wu C, Xue X, Hafner JP. Estimating the relative risk in cohort studies and clinical trials of common outcomes. *Am J Epidemiol.* 2003;157:940-3.
21. Cefalu WT, Leiter LA, de Bruin TW, Gause-Nilsson I, Sugg J, Parikh SJ. Dapagliflozin's effects on glycemia and cardiovascular risk factors in high-risk patients with type 2 diabetes: a 24-week, multicenter, randomized, double-blind, placebo-controlled study with a 28-week extension. *Diabetes Care.* 2015;38:1218-27.
22. Mor A, Berencsi K, Svensson E, Rungby J, Nielsen JS, Friborg S, et al. Prescribing practices and clinical predictors of glucose-lowering therapy within the first year in people with newly diagnosed type 2 diabetes. *Diabet Med.* 2015;32:1546-54.
23. Stark Casagrande S, Fradkin JE, Saydah SH, Rust KF, Cowie CC. The prevalence of meeting A1C, blood pressure, and LDL goals among people with diabetes, 1988–2010. *Diabetes Care.* 2013;36:2271-9.
24. Gregg EW, Zhuo X, Cheng YJ, Albright AL, Narayan KM, Thompson TJ. Trends in lifetime risk and years of life lost due to diabetes in the USA, 1985–2011: a modelling study. *Lancet Diabetes Endocrinol.* 2014;2:867-74.
25. Sandbaek A, Griffin SJ, Sharp SJ, Simmons RK, Borch-Johnsen K, Rutten GE, et al. Effect of early multifactorial therapy compared with routine care on microvascular outcomes at 5 years in people with screen-detected diabetes: a randomized controlled trial: the ADDITION-Europe study. *Diabetes Care.* 2014;37:2015-23.
26. Sinclair AJ, Alexander CM, Davies MJ, Zhao C, Mavros P. Factors associated with initiation of antihyperglycaemic medication in UK patients with newly diagnosed type 2 diabetes. *BMC Endocr Disord.* 2012;12:1.
27. Svensson MK, Cederholm J, Eliasson B, Zethelius B, Gudbjornsdottir S, Swedish National Diabetes Register. Albuminuria and renal function as predictors of cardiovascular events and mortality in a general population of patients with type 2 diabetes: a nationwide observational study from the Swedish national diabetes register. *Diab Vasc Dis Res.* 2013;10:520-9.
28. Martinell M, Dorkhan M, Stalhammar J, Storm P, Groop L, Gustavsson C. Prevalence and risk factors for diabetic retinopathy at diagnosis (DRAD) in patients recently diagnosed with type 2 diabetes (T2D) or latent autoimmune diabetes in the adult (LADA). *J Diabetes Complications.* 2016;30:1456-61.
29. Svensson E, Baggesen LM, Johnsen SP, Pedersen L, Nørrelund H, Buhl ES, et al. Early glycemic control and magnitude of HbA1c reduction predict cardiovascular events and mortality: population-based cohort study of 24,752 metformin initiators. *Diabetes Care.* 2017;40:800-7.
30. Pikkemaat M, Melander O, Molstad S, Garberg G, Bostrom KB. C-peptide concentration, mortality and vascular complications in people with type 2 diabetes. The Skaraborg diabetes register. *Diabet Med.* 2015;32:85-9.
31. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet.* 2005;365:1415-28.
32. Gerstein HC, Miller ME, Ismail-Beigi F, Largay J, McDonald C, Lochnan HA, et al. Effects of intensive glycaemic control on ischaemic heart disease: analysis of data from the randomised, controlled ACCORD trial. *Lancet.* 2014;384:1936-41.
33. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med.* 2008;359:1577-89.
34. Griffin SJ, Borch-Johnsen K, Davies MJ, Khunti K, Rutten GE, Sandbaek A, et al. Effect of early intensive multifactorial therapy on 5-year cardiovascular outcomes in

individuals with type 2 diabetes detected by screening (ADDITION-Europe): a cluster-randomised trial. *Lancet.* 2011;378:156-67.
35. Sacks FM, Hermans MP, Fioretto P, Valensi P, Davis T, Horton E, et al. Association between plasma triglycerides and high-density lipoprotein cholesterol and microvascular kidney disease and retinopathy in type 2 diabetes mellitus: a global case-control study in 13 countries. *Circulation.* 2014;129:999-1008.
36. Nielsen SF, Nordestgaard BG. Statin use before diabetes diagnosis and risk of microvascular disease: a nationwide nested matched study. *Lancet Diabetes Endocrinol.* 2014;2:894-900.
37. Ostergaard L, Finnerup NB, Terkelsen AJ, Olesen RA, Drasbek KR, Knudsen L, et al. The effects of capillary dysfunction on oxygen and glucose extraction in diabetic neuropathy. *Diabetologia.* 2015;58:666-77.

APPENDIX

Prevalence of micro- and macrovascular diabetes complications at time of type 2 diabetes diagnosis and associated clinical characteristics: A cross-sectional baseline study of 6958 patients in the Danish DD2 cohort

APPENDIX

Table of contents:

Supplementary table A.1: ICD-10 and procedure codes used to identify micro- and macrovascular complications.

	ICD-10 codes
Diabetes with any microvascular complication	E104, E114, E144, G590, G632, E103, E113, E143, H340-H342, H280, H334, H450, H360, H540, H541, H544, H25, H268, H269, H430, H431, H438C, H439, H334A, H330, H335, KCKC10, KCKC15, KCKD65, E102, E112, E142, I120, N083, N06, N17-N19, R809, BJFD2
Diabetes with any macrovascular complication	KFNA, KFNB, KFNC, KFND, KFNE, KFNF, KFNG, KFNH, KFNW, KFLF, KPAE+F+H+N+P+Q, KPAW99, KPAU74, KPBE+F+H+N+P+Q, KPBW, KPGH10, KPCE+F+H+N+P+Q, KPCW99, KPCW20, KPCU74+82+83+84, KPGE+F+H+N+P+Q, KPGW99, KPGW20, KPEE+F+H+N+P+Q+W, KPFE+H+N+P+Q+W, KPGH20+21+22+23+30+31+40+99, KPDU74+82+83+84, KPEU74+82+83+84, KPFU74+82+83+84, KPGU74+83+84+99, KPGW, KPWG, KAAL10, KAAL11 I20-I25, T822A, T823 I61, I63-I66, I672, I678-I679, I691, I693-I698 I702, I742-I745, I739A, I739B, I739C, E105, E115, KNBQ, KNCQ, KNDQ, KNEQ, KNFQ, KNGQ, KNHQ I700, I708, I709, I740-I741, I748-I749, N280, I701, K550-K551, K558-559 H340-H342

Supplementary table A.2. Prevalence of diabetes complications among 6958 patients in the DD2 cohort. Prevalence ratios adjusted for age and gender, and additionally adjusted for waist-hip ratio.

Characteristic	All T2D patients	No complication (%)	Microvascular complication (%)	aPR (95% CI)	Macrovascular complication (%)	aPR (95% CI)	Micro- and macrovascular complications (%)	aPR (95% CI)
Main cohort	6958	4502 (64.7)	828 (11.9)		1186 (17.1)		442 (6.4)	
Sex								
Female	2927	2047 (69.9)	365 (12.5)	Ref (1.00)	374 (12.8)	Ref (1.00)	141 (4.8)	Ref (1.00)
Male	4031	2455 (60.9)	463 (11.5)	0.90 (0.78-1.03)	812 (20.1)	1.66 (1.47-1.86)	301 (7.5)	1.79 (1.47-2.17)
Age (years)								
<50	1220	973 (79.8)	142 (11.6)	Ref (1.00)	82 (6.7)	Ref (1.00)	23 (1.9)	Ref (1.00)
50-59	1790	1262 (70.5)	180 (10.1)	0.87 (0.70-1.07)	276 (15.4)	2.28 (1.81-2.89)	72 (4.0)	2.14 (1.34-3.40)
60-69	2517	1550 (61.6)	291 (11.6)	0.99 (0.82-1.20)	505 (20.1)	2.99 (2.39-3.73)	171 (6.8)	3.58 (2.33-5.50)
≥70	1431	717 (50.1)	215 (15.0)	1.27 (1.04-1.54)	323 (22.6)	3.43 (2.73-4.31)	176 (12.3)	6.76 (4.41-10.37)
Central obesity[a]								
No	570	393 (69.0)	63 (11.1)	Ref (1.00)	83 (14.6)	Ref (1.00)	31 (5.4)	Ref (1.00)
Yes	6378	4099 (64.3)	765 (12.0)	1.18 (0.89-1.56)	1103 (17.3)	1.26 (1.01-1.57)	411 (6.4)	1.05 (0.71-1.53)
Waist-hip ratio[b]								
≤0.95m/≤0.80f	772	506 (65.5)	95 (12.3)	Ref (1.00)	131 (17.0)	Ref (1.00)	40 (5.2)	Ref (1.00)
0.96-1.0m/0.81-0.85f	1437	911 (63.4)	180 (12.5)	0.99 (0.79-1.26)	261 (18.2)	1.12 (0.93-1.36)	85 (5.9)	1.22 (0.85-1.75)
>1.0m/>0.85f	4737	3074 (64.9)	553 (11.7)	0.92 (0.74-1.14)	793 (16.7)	1.21 (1.02-1.43)	317 (6.7)	1.75 (1.27-2.40)
Regular physical exercise								
Yes	2725	1847 (67.8)	304 (11.2)	Ref (1.00)	430 (15.8)	Ref (1.00)	144 (5.3)	Ref (1.00)
No	4232	2655 (62.7)	524 (12.4)	1.13 (0.99-1.29)	755 (17.8)	1.09 (0.98-1.22)	298 (7.0)	1.27 (1.05-1.54)
Use of lipid-lowering drugs								
No	2072	1537 (74.2)	304 (14.7)	Ref (1.00)	171 (8.3)	Ref (1.00)	60 (2.9)	Ref (1.00)
Yes	4886	2965 (60.7)	524 (10.7)	0.71 (0.62-0.81)	1015 (20.8)	2.29 (1.96-2.67)	382 (7.8)	2.29 (1.76-2.99)
Use of anti-hypertensive drugs								
No	1967	1548 (78.7)	232 (11.8)	Ref (1.00)	151 (7.7)	Ref (1.00)	36 (1.8)	Ref (1.00)
Yes	4991	2954 (59.2)	596 (11.9)	0.95 (0.82-1.11)	1035 (20.7)	2.26 (1.91-2.67)	406 (8.1)	3.06 (2.18-4.29)
Fasting blood glucose (mmol/L)								

Supplementary table A.2. Prevalence of diabetes complications among 6958 patients in the DD2 cohort. Prevalence ratios adjusted for age and gender, and additionally adjusted for waist-hip ratio.

Characteristic	All T2D patients	No complication (%)	Microvascular complication (%)	aPR (95% CI)	Macrovascular complication (%)	aPR (95% CI)	Micro- and macrovascular complications (%)	aPR (95% CI)
<6.5	1548	996 (64.3)	159 (10.3)	Ref (1.00)	278 (18.0)	Ref (1.00)	115 (7.4)	Ref (1.00)
6.5-7.0	917	611 (66.6)	86 (9.4)	0.92 (0.72-1.18)	167 (18.2)	1.00 (0.85-1.19)	53 (5.7)	0.79 (0.58-1.08)
7.0-7.5	751	482 (64.2)	80 (10.7)	1.06 (0.82-1.36)	147 (19.6)	1.07 (0.90-1.28)	42 (5.6)	0.75 (0.54-1.05)
≥7.5	2146	1424 (66.4)	245 (11.4)	1.18 (0.97-1.43)	350 (16.3)	0.95 (0.82-1.09)	127 (5.9)	0.91 (0.72-1.15)
C-peptide (pmol/L)								
<550	295	202 (68.5)	36 (12.2)	Ref (1.00)	39 (13.2)	Ref (1.00)	18 (6.1)	Ref (1.00)
550 - 800	853	606 (71.0)	90 (10.6)	0.80 (0.55-1.14)	120 (14.1)	1.02 (0.73-1.44)	37 (4.3)	0.59 (0.34-1.03)
≥800	4652	2932 (63.0)	552 (11.9)	1.03 (0.74-1.42)	847 (18.2)	1.26 (0.94-1.70)	321 (6.9)	0.97 (0.60-1.54)
CRP (mg/L)								
≤3.0	627	429 (68.4)	62 (9.9)	Ref (1.00)	114 (18.2)	Ref (1.00)	22 (3.5)	Ref (1.00)
>3.0	403	254 (63.0)	35 (8.7)	0.88 (0.59-1.30)	87 (21.6)	1.38 (1.08-1.76)	27 (6.7)	2.31 (1.32-4.04)

DD2: the Danish Centre for Strategic Research in Type 2 Diabetes; aPR: adjusted prevalence ratio; CI: confidence interval.

[a]Central obesity = waist circumference >94 (men) and >80 (women). [b]Waist-hip ratio: m = males; f = females.

Data available in total DD2 cohort (n=6958) for central obesity (n=10 missing); for waist-hip ratio (n=12 missing); for regular physical activity (n=1 missing). Data currently analysed in the DD2 biobank for fasting blood glucose (n=5362); for C-peptide (n=5800); and for CRP (n=1030).

Supplementary table A.3. Prevalence of diabetes complications in the subcohort of 5115 DD2 patients who currently can be linked to the DDDA. Prevalence ratios adjusted for age and gender, and additionally adjusted for waist-hip ratio.

Charac-teristic	All T2D patients	No complication (%)	Microvascular complication (%)	aPR (95% CI)	Macrovascular complication (%)	aPR (95% CI)	Micro- and macrovascular complications (%)	aPR (95% CI)
Subcohort	5115							
High blood pressure (mmhg)[a]								
No	1575	1012 (64.3)	173 (11.0)	Ref (1.00)	278 (17.7)	Ref (1.00)	112 (7.1)	Ref (1.00)
Yes	3261	2013 (61.7)	450 (13.8)	1.28 (1.09-1.51)	572 (17.5)	0.94 (0.82-1.06)	226 (6.9)	0.90 (0.73-1.12)
Smoking								
No	3903	2464 (63.1)	490 (12.6)	Ref (1.00)	676 (17.3)	Ref (1.00)	273 (7.0)	Ref (1.00)
Yes	941	579 (61.5)	127 (13.5)	1.09 (0.90-1.30)	177 (18.8)	1.20 (1.03-1.39)	58 (6.2)	1.06 (0.81-1.40)
BMI (kg/m2)								
<25	500	309 (61.8)	73 (14.6)	Ref (1.00)	78 (15.6)	Ref (1.00)	40 (8.0)	Ref (1.00)
25-29	1291	800 (62.0)	156 (12.1)	0.85 (0.65-1.11)	222 (17.2)	1.08 (0.85-1.37)	113 (8.8)	0.97 (0.68-1.38)
30-34	1147	692 (60.3)	141 (12.3)	0.88 (0.66-1.18)	231 (20.1)	1.37 (1.06-1.77)	83 (7.2)	0.83 (0.56-1.23)
≥35	897	554 (61.8)	145 (16.2)	1.15 (0.85-1.56)	150 (16.7)	1.28 (0.95-1.71)	48 (5.4)	0.81 (0.49-1.34)
HDL cholesterol (mmol/L)[b]								
≥1.3m/≥1.0f	2061	1246 (60.5)	297 (14.4)	Ref (1.00)	356 (17.3)	Ref (1.00)	162 (7.9)	Ref (1.00)
<1.3m/<1.0f	969	558 (57.6)	152 (15.7)	1.02 (0.85-1.23)	181 (18.7)	1.35 (1.14-1.59)	78 (8.1)	1.36 (1.04-1.76)
Triglycerides (mmol/L)								
<1.7	2410	1573 (65.3)	261 (10.8)	Ref (1.00)	415 (17.2)	Ref (1.00)	161 (6.7)	Ref (1.00)
≥1.7	2348	1430 (60.9)	330 (14.1)	1.33 (1.14-1.55)	424 (18.1)	1.15 (1.01-1.30)	164 (7.0)	1.20 (0.97-1.48)
HbA1C (%)								
<7.0	3592	2298 (64.0)	413 (11.5)	Ref (1.00)	651 (18.1)	Ref (1.00)	230 (6.4)	Ref (1.00)
7.0 - 8.0	824	500 (60.7)	126 (15.3)	1.36 (1.13-1.63)	129 (15.7)	0.91 (0.76-1.08)	69 (8.4)	1.46 (1.13-1.89)
8.0 - 9.0	303	182 (60.1)	43 (14.2)	1.31 (0.97-1.76)	57 (18.8)	1.18 (0.93-1.51)	21 (6.9)	1.47 (0.95-2.26)
≥9.0	313	187 (59.7)	52 (16.6)	1.57 (1.19-2.06)	47 (15.0)	1.00 (0.75-1.30)	27 (8.6)	2.08 (1.41-3.05)

DD2: the Danish Centre for Strategic Research in Type 2 Diabetes; DDDA: the Danish Diabetes Database for Adults; aPR: adjusted prevalence ratio; CI: confidence interval.
[a]High blood pressure = defined as no (systolic blood pressure <130 or diastolic bloodpressure <85) and as yes (systolic blood pressure ≥130 or diastolic bloodpressure ≥85)
[b]HDL cholesterol: m = males; f = females.
Data available in DDDA cohort (n=5115); for blood pressure (n=279 missing); for smoking (n=271 missing); for BMI (n=1280 missing); for HDL(n = 2085 missing); for triglycerides (n= 357 missing); and for HbA1c (n= 83 missing).

Supplementary table A.4: Prevalence of diabetes complications in the subgroup of 3077 patients DD2 with highest probability of having newly detected T2D: no earlier glucose-lowering treatment, hospital diagnosis of T2D, or registration in the DDDA quality-of-care database recorded more than 1 year prior to DD2 enrollment. Prevalence ratios adjusted for age and gender.

Characteristic	All T2D patients	No complication (%)	Microvascular complication (%)	aPR (95% CI)	Macrovascular complication (%)	aPR (95% CI)	Micro- and macrovascular complications (%)	aPR (95% CI)
Subcohort	3077	2041 (66.3)	374 (12.2)		502 (16.3)		160 (5.2)	
Sex								
Female	1302	920 (70.7)	163 (12.5)	Ref (1.00)	161 (12.4)	Ref (1.00)	58 (4.5)	Ref (1.00)
Male	1775	1121 (63.2)	211 (11.9)	0.96 (0.79-1.16)	341 (19.2)	1.63 (1.37-1.94)	102 (5.8)	1.41 (1.03-1.92)
Age (years)								
<50	618	485 (78.5)	72 (11.7)	Ref (1.00)	52 (8.4)	Ref (1.00)	9 (1.5)	Ref (1.00)
50-59	891	648 (72.7)	94 (10.6)	0.92 (0.68-1.22)	119 (13.4)	1.62 (1.19-2.21)	30 (3.4)	2.43 (1.15-5.12)
60-69	1053	643 (61.1)	142 (13.5)	1.16 (0.89-1.52)	210 (19.9)	2.48 (1.86-3.31)	58 (5.5)	3.86 (1.92-7.75)
≥70	515	265 (51.5)	66 (12.8)	1.06 (0.78-1.44)	121 (23.5)	2.95 (2.18-4.00)	63 (12.2)	8.57 (4.30-17.11)
Central obesity[a]								
No	246	181 (73.6)	26 (10.6)	Ref (1.00)	30 (12.2)	Ref (1.00)	9 (3.7)	Ref (1.00)
Yes	2823	1852 (65.6)	348 (12.3)	1.16 (0.80-1.70)	472 (16.7)	1.52 (1.09-2.13)	151 (5.4)	1.66 (0.85-3.25)
Waist-hip ratio[b]								
≤0.95m/≤0.80f	342	228 (66.7)	46 (13.5)	Ref (1.00)	54 (15.8)	Ref (1.00)	14 (4.1)	Ref (1.00)
0.96-1.0m/0.81-0.85f	655	426 (65.0)	83 (12.7)	0.92 (0.66-1.30)	110 (16.8)	1.09 (0.81-1.45)	36 (5.5)	1.39 (0.76-2.54)
>1.0m/>0.85f	2070	1378 (66.6)	245 (11.8)	0.83 (0.61-1.14)	337 (16.3)	1.26 (0.97-1.64)	110 (5.3)	1.64 (0.93-2.90)
Regular physical exercise								
Yes	1215	841 (69.2)	141 (11.6)	Ref (1.00)	179 (14.7)	Ref (1.00)	54 (4.4)	Ref (1.00)
No	1862	1200 (64.5)	233 (12.5)	1.08 (0.89-1.32)	323 (17.4)	1.17 (0.99-1.38)	106 (5.7)	1.29 (0.94-1.76)
Use of lipid-lowering drugs								
No	1170	885 (75.6)	170 (14.5)	Ref (1.00)	91 (7.8)	Ref (1.00)	24 (2.1)	Ref (1.00)
Yes	1907	1156 (60.6)	204 (10.7)	0.71 (0.59-0.87)	411 (21.6)	2.48 (2.00-3.08)	136 (7.1)	2.85 (1.86-4.37)
Use of anti-hypertensive drugs								
No	1065	831 (78.0)	132 (12.4)	Ref (1.00)	85 (8.0)	Ref (1.00)	17 (1.6)	Ref (1.00)

Supplementary table A.4: Prevalence of diabetes complications in the subgroup of 3077 patients DD2 with highest probability of having newly detected T2D: no earlier glucose-lowering treatment, hospital diagnosis of T2D, or registration in the DDDA quality-of-care database recorded more than 1 year prior to DD2 enrollment. Prevalence ratios adjusted for age and gender.

Characteristic	All T2D patients	No complication (%)	Microvascular complication (%)	aPR (95% CI)	Macrovascular complication (%)	aPR (95% CI)	Micro- and macrovascular complications (%)	aPR (95% CI)
Yes	2012	1210 (60.1)	242 (12.0)	0.92 (0.75-1.14)	417 (20.7)	2.18 (1.73-2.74)	143 (7.1)	2.98 (1.79-4.94)
Fasting blood glucose (mmol/L)								
<6.5	718	473 (65.9)	77 (10.7)	Ref (1.00)	120 (16.7)	Ref (1.00)	48 (6.7)	Ref (1.00)
6.5-7.0	400	275 (68.8)	33 (8.3)	0.77 (0.52-1.14)	67 (16.8)	1.01 (0.77-1.32)	25 (6.3)	0.97 (0.62-1.53)
7.0-7.5	316	204 (64.6)	28 (8.9)	0.83 (0.55-1.25)	69 (21.8)	1.28 (0.99-1.66)	15 (4.8)	0.73 (0.41-1.29)
≥7.5	835	578 (69.2)	91 (10.9)	1.05 (0.78-1.40)	133 (15.9)	1.02 (0.82-1.27)	33 (4.0)	0.70 (0.46-1.06)
C-peptide (pmol/L)								
<550	108	71 (65.7)	17 (15.7)	Ref (1.00)	14 (13.0)	Ref (1.00)	6 (5.6)	Ref (1.00)
550 - 800	353	251 (71.1)	38 (10.8)	0.69 (0.40-1.17)	50 (14.2)	1.09 (0.64-1.85)	14 (4.0)	0.73 (0.29-1.85)
≥800	2110	1364 (64.6)	260 (12.3)	0.78 (0.50-1.22)	362 (17.2)	1.41 (0.87-2.26)	124 (5.9)	1.17 (0.52-2.62)
CRP (mg/L)								
≤3.0	393	273 (69.5)	34 (8.7)	Ref (1.00)	76 (19.3)	Ref (1.00)	10 (2.5)	Ref (1.00)
>3.0	251	164 (65.3)	20 (8.0)	0.95 (0.56-1.61)	53 (21.1)	1.41 (1.04-1.92)	14 (5.6)	2.86 (1.28-6.42)

DD2: the Danish Centre for Strategic Research in Type 2 Diabetes; DDDA: the Danish Diabetes Database for Adults; aPR: adjusted prevalence ratio; CI: confidence interval.
[a]Central obesity = waist circumference >94 (men) and >80 (women). [b]Waist-hip ratio: m = males; f = females.
Data available in the subgroup of the DD2 cohort (n=3077) for central obesity (n=8 missing); for waist-hip ratio (n=10 missing). Data currently analysed in the DD2 biobank for fasting blood glucose (n=2269); for C-peptide (n=2571); and for CRP (n=644).

Supplementary table A.5: Prevalence of diabetes complications in the subgroup of 2054 patients who currently can be linked to the DDDA and have highest probability of having newly detected T2D: no earlier glucose-lowering treatment, hospital diagnosis of T2D, or registration in the DDDA quality-of-care database recorded more than 1 year prior to DD2 enrollment. Prevalence ratios adjusted for age and gender.

Characteristic	All T2D patients	No complication (%)	Microvascular complication (%)	aPR (95% CI)	Macrovascular complication (%)	aPR (95% CI)	Micro- and macrovascular complications (%)	aPR (95% CI)
Subcohort	2054							
High blood pressure (mmhg)[a]								
No	623	395 (63.4)	75 (12.0)	Ref (1.00)	112 (18.0)	Ref (1.00)	41 (6.6)	Ref (1.00)
Yes	1325	811 (61.2)	202 (15.3)	1.28 (1.00-1.64)	228 (17.2)	0.94 (0.77-1.15)	84 (6.3)	0.97 (0.68-1.39)
Smoking								
No	1573	993 (63.1)	217 (13.8)	Ref (1.00)	262 (16.7)	Ref (1.00)	101 (6.4)	Ref (1.00)
Yes	363	212 (58.4)	62 (17.1)	1.21 (0.93-1.57)	71 (19.6)	1.35 (1.07-1.70)	18 (5.0)	0.99 (0.61-1.62)
BMI (kg/m2)								
<25	206	124 (60.2)	34 (16.5)	Ref (1.00)	35 (17.0)	Ref (1.00)	13 (6.3)	Ref (1.00)
25-29	541	328 (60.6)	76 (14.1)	0.85 (0.58-1.24)	94 (17.4)	1.05 (0.74-1.48)	43 (8.0)	1.22 (0.66-2.25)
30-34	472	298 (63.1)	56 (11.9)	0.71 (0.47-1.06)	88 (18.6)	1.17 (0.82-1.65)	30 (6.4)	1.17 (0.59-2.31)
≥35	378	226 (59.8)	74 (19.6)	1.11 (0.76-1.63)	60 (15.9)	1.32 (0.89-1.95)	18 (4.8)	0.95 (0.44-2.04)
HDL cholesterol (mmol/L)[b]								
≥1.3m/≥1.0f	704	397 (56.4)	125 (17.8)	Ref (1.00)	123 (17.5)	Ref (1.00)	59 (8.4)	Ref (1.00)
<1.3m/<1.0f	372	211 (56.7)	76 (20.4)	1.03 (0.79-1.35)	62 (16.7)	1.36 (1.02-1.80)	23 (6.2)	1.05 (0.64-1.72)
Triglycerides (mmol/L)								
<1.7	931	606 (65.1)	107 (11.5)	Ref (1.00)	155 (16.7)	Ref (1.00)	63 (6.8)	Ref (1.00)
≥1.7	960	577 (60.1)	158 (16.5)	1.41 (1.12-1.77)	173 (18.0)	1.21 (1.00-1.47)	52 (5.4)	0.95 (0.67-1.36)
HbA1C (%)								
<7.0	1428	899 (63.0)	181 (12.7)	Ref (1.00)	264 (18.5)	Ref (1.00)	84 (5.9)	Ref (1.00)
7.0 - 8.0	320	194 (60.6)	53 (16.6)	1.29 (0.98-1.70)	46 (14.4)	0.81 (0.61-1.08)	27 (8.4)	1.60 (1.06-2.41)
8.0 - 9.0	121	81 (66.9)	17 (14.1)	1.14 (0.71-1.84)	18 (14.9)	0.96 (0.62-1.48)	5 (4.1)	1.06 (0.44-2.53)
≥9.0	153	87 (56.9)	33 (21.6)	1.72 (1.23-2.40)	24 (15.7)	1.01 (0.69-1.48)	9 (5.9)	1.46 (0.76-2.82)

DD2: the Danish Centre for Strategic Research in Type 2 Diabetes; DDDA: the Danish Diabetes Database for Adults; aPR: adjusted prevalence ratio; CI: confidence interval.

Supplementary table A.5: Prevalence of diabetes complications in the subgroup of 2054 patients who currently can be linked to the DDDA and have highest probability of having newly detected T2D: no earlier glucose-lowering treatment, hospital diagnosis of T2D, or registration in the DDDA quality-of-care database recorded more than 1 year prior to DD2 enrollment. Prevalence ratios adjusted for age and gender.

Characteristic	All T2D patients	No complication (%)	Microvascular complication (%)	aPR (95% CI)	Macrovascular complication (%)	aPR (95% CI)	Micro- and macrovascular complications (%)	aPR (95% CI)

[a]High blood pressure = defined as no (systolic blood pressure <130 or diastolic bloodpressure <85) and as yes (systolic blood pressure ≥130 or diastolic bloodpressure ≥85)
[b]HDL cholesterol: m = males; f = females.Data available in the subgroup of the DDDA cohort (n=2054); for blood pressure (n=106 missing); for smoking (n=118 missing); for BMI (n=457 missing); for HDL(n = 978 missing); for triglycerides (n= 163 missing); and for HbA1c (n= 32 missing).

Supplementary table A.6: Prevalence of diabetes complications in the subgroup of 3077 patients DD2 with highest probability of having newly detected T2D: no earlier glucose-lowering treatment, hospital diagnosis of T2D, or registration in the DDDA quality-of-care database recorded more than 1 year prior to DD2 enrollment. Prevalence ratios adjusted for age and gender, and additionally adjusted for waist-hip ratio.

Characteristic	All T2D patients	No complication (%)	Microvascular complication (%)	aPR (95% CI)	Macrovascular complication (%)	aPR (95% CI)	Micro- and macrovascular complications (%)	aPR (95% CI)
Subcohort	3077	2041 (66.3)	374 (12.2)		502 (16.3)		160 (5.2)	
Sex								
Female	1302	920 (70.7)	163 (12.5)	Ref (1.00)	161 (12.4)	Ref (1.00)	58 (4.5)	Ref (1.00)
Male	1775	1121 (63.2)	211 (11.9)	0.93 (0.76-1.14)	341 (19.2)	1.72 (1.43-2.05)	102 (5.8)	1.53 (1.12-2.11)
Age (years)								
<50	618	485 (78.5)	72 (11.7)	Ref (1.00)	52 (8.4)	Ref (1.00)	9 (1.5)	Ref (1.00)
50-59	891	648 (72.7)	94 (10.6)	0.92 (0.69-1.22)	119 (13.4)	1.60 (1.18-2.18)	30 (3.4)	2.38 (1.13-5.00)
60-69	1053	643 (61.1)	142 (13.5)	1.17 (0.90-1.53)	210 (19.9)	2.47 (1.85-3.29)	58 (5.5)	3.82 (1.90-7.66)
≥70	515	265 (51.5)	66 (12.8)	1.05 (0.77-1.43)	121 (23.5)	2.97 (2.19-4.02)	63 (12.2)	8.78 (4.36-17.67)
Central obesity[a]								
No	246	181 (73.6)	26 (10.6)	Ref (1.00)	30 (12.2)	Ref (1.00)	9 (3.7)	Ref (1.00)
Yes	2823	1852 (65.6)	348 (12.3)	1.35 (0.89-2.04)	472 (16.7)	1.45 (1.01-2.07)	151 (5.4)	1.39 (0.69-2.78)
Waist-hip ratio[b]								
≤0.95m/≤0.80f	342	228 (66.7)	46 (13.5)	Ref (1.00)	54 (15.8)	Ref (1.00)	14 (4.1)	Ref (1.00)
0.96-1.0m/0.81-0.85f	655	426 (65.0)	83 (12.7)	0.92 (0.66-1.30)	110 (16.8)	1.09 (0.81-1.45)	36 (5.5)	1.39 (0.76-2.54)
>1.0m/>0.85f	2070	1378 (66.6)	245 (11.8)	0.83 (0.61-1.14)	337 (16.3)	1.26 (0.97-1.64)	110 (5.3)	1.64 (0.93-2.90)
Regular physical exercise								
Yes	1215	841 (69.2)	141 (11.6)	Ref (1.00)	179 (14.7)	Ref (1.00)	54 (4.4)	Ref (1.00)
No	1862	1200 (64.5)	233 (12.5)	1.10 (0.90-1.33)	323 (17.4)	1.15 (0.98-1.36)	106 (5.7)	1.27 (0.92-1.73)
Use of lipid-lowering drugs								
No	1170	885 (75.6)	170 (14.5)	Ref (1.00)	91 (7.8)	Ref (1.00)	24 (2.1)	Ref (1.00)
Yes	1907	1156 (60.6)	204 (10.7)	0.72 (0.59-0.87)	411 (21.6)	2.45 (1.98-3.05)	136 (7.1)	2.81 (1.83-4.30)
Use of anti-hypertensive drugs								
No	1065	831 (78.0)	132 (12.4)	Ref (1.00)	85 (8.0)	Ref (1.00)	17 (1.6)	Ref (1.00)
Yes	2012	1210 (60.1)	242 (12.0)	0.93 (0.75-1.15)	417 (20.7)	2.15 (1.70-2.70)	143 (7.1)	2.92 (1.77-4.83)
Fasting blood glucose (mmol/L)								

Supplementary table A.6: Prevalence of diabetes complications in the subgroup of 3077 patients DD2 with highest probability of having newly detected T2D: no earlier glucose-lowering treatment, hospital diagnosis of T2D, or registration in the DDDA quality-of-care database recorded more than 1 year prior to DD2 enrollment. Prevalence ratios adjusted for age and gender, and additionally adjusted for waist-hip ratio.

Characteristic	All T2D patients	No complication (%)	Microvascular complication (%)	aPR (95% CI)	Macrovascular complication (%)	aPR (95% CI)	Micro- and macrovascular complications (%)	aPR (95% CI)
<6.5	718	473 (65.9)	77 (10.7)	Ref (1.00)	120 (16.7)	Ref (1.00)	48 (6.7)	Ref (1.00)
6.5-7.0	400	275 (68.8)	33 (8.3)	0.78 (0.53-1.15)	67 (16.8)	1.02 (0.78-1.34)	25 (6.3)	0.97 (0.62-1.53)
7.0-7.5	316	204 (64.6)	28 (8.9)	0.84 (0.55-1.26)	69 (21.8)	1.29 (1.00-1.67)	15 (4.8)	0.74 (0.42-1.31)
≥7.5	835	578 (69.2)	91 (10.9)	1.06 (0.79-1.42)	133 (15.9)	1.03 (0.82-1.28)	33 (4.0)	0.70 (0.46-1.06)
C-peptide (pmol/L)								
<550	108	71 (65.7)	17 (15.7)	Ref (1.00)	14 (13.0)	Ref (1.00)	6 (5.6)	Ref (1.00)
550 - 800	353	251 (71.1)	38 (10.8)	0.67 (0.38-1.15)	50 (14.2)	1.18 (0.68-2.04)	14 (4.0)	0.66 (0.27-1.66)
≥800	2110	1364 (64.6)	260 (12.3)	0.83 (0.52-1.33)	362 (17.2)	1.28 (0.79-2.06)	124 (5.9)	1.11 (0.49-2.52)
CRP (mg/L)								
≤3.0	393	273 (69.5)	34 (8.7)	Ref (1.00)	76 (19.3)	Ref (1.00)	10 (2.5)	Ref (1.00)
>3.0	251	164 (65.3)	20 (8.0)	1.00 (0.59-1.71)	53 (21.1)	1.38 (1.01-1.87)	14 (5.6)	3.01 (1.30-6.97)

DD2: the Danish Centre for Strategic Research in Type 2 Diabetes; DDDA: the Danish Diabetes Database for Adults; aPR: adjusted prevalence ratio; CI: confidence interval.
[a]Central obesity = waist circumference >94 (men) and >80 (women). [b]Waist-hip ratio: m = males; f = females.
Data available in the subgroup of the DD2 cohort (n=3077) for central obesity (n=8 missing); for waist-hip ratio (n=10 missing). Data currently analysed in the DD2 biobank for fasting blood glucose (n=2269); for C-peptide (n=2571); and for CRP (n=644).

Supplementary table A.7: Prevalence of diabetes complications in the subgroup of 2054 patients who currently can be linked to the DDDA and have highest probability of having newly detected T2D: no earlier glucose-lowering treatment, hospital diagnosis of T2D, or registration in the DDDA quality-of-care database recorded more than 1 year prior to DD2 enrollment. Prevalence ratios adjusted for age and gender, and additionally adjusted for waist-hip ratio.

Characteristic	All T2D patients	No complication (%)	Microvascular complication (%)	aPR (95% CI)	Macrovascular complication (%)	aPR (95% CI)	Micro- and macrovascular complications (%)	aPR (95% CI)
Subcohort	2054							
High blood pressure (mmhg)[a]								
No	623	395 (63.4)	75 (12.0)	Ref (1.00)	112 (18.0)	Ref (1.00)	41 (6.6)	Ref (1.00)
Yes	1325	811 (61.2)	202 (15.3)	1.31 (1.02-1.68)	228 (17.2)	0.92 (0.75-1.13)	84 (6.3)	0.95 (0.66-1.35)
Smoking								
No	1573	993 (63.1)	217 (13.8)	Ref (1.00)	262 (16.7)	Ref (1.00)	101 (6.4)	Ref (1.00)
Yes	363	212 (58.4)	62 (17.1)	1.23 (0.95-1.59)	71 (19.6)	1.33 (1.06-1.68)	18 (5.0)	0.98 (0.60-1.59)
BMI (kg/m2)								
<25	206	124 (60.2)	34 (16.5)	Ref (1.00)	35 (17.0)	Ref (1.00)	13 (6.3)	Ref (1.00)
25-29	541	328 (60.6)	76 (14.1)	0.90 (0.61-1.34)	94 (17.4)	1.02 (0.71-1.44)	43 (8.0)	1.12 (0.59-2.12)
30-34	472	298 (63.1)	56 (11.9)	0.69 (0.44-1.08)	88 (18.6)	1.16 (0.78-1.72)	30 (6.4)	1.09 (0.50-2.36)
≥35	378	226 (59.8)	74 (19.6)	1.14 (0.73-1.78)	60 (15.9)	1.20 (0.78-1.85)	18 (4.8)	0.96 (0.39-2.39)
HDL cholesterol (mmol/L)[b]								
≥1.3m/≥1.0f	704	397 (56.4)	125 (17.8)	Ref (1.00)	123 (17.5)	Ref (1.00)	59 (8.4)	Ref (1.00)
<1.3m/<1.0f	372	211 (56.7)	76 (20.4)	1.04 (0.79-1.36)	62 (16.7)	1.33 (1.00-1.77)	23 (6.2)	1.06 (0.64-1.76)
Triglycerides (mmol/L)								
<1.7	931	606 (65.1)	107 (11.5)	Ref (1.00)	155 (16.7)	Ref (1.00)	63 (6.8)	Ref (1.00)
≥1.7	960	577 (60.1)	158 (16.5)	1.45 (1.15-1.83)	173 (18.0)	1.20 (0.99-1.45)	52 (5.4)	0.94 (0.66-1.34)
HbA1C (%)								
<7.0	1428	899 (63.0)	181 (12.7)	Ref (1.00)	264 (18.5)	Ref (1.00)	84 (5.9)	Ref (1.00)
7.0 - 8.0	320	194 (60.6)	53 (16.6)	1.30 (0.99-1.72)	46 (14.4)	0.81 (0.61-1.08)	27 (8.4)	1.61 (1.07-2.43)
8.0 - 9.0	121	81 (66.9)	17 (14.1)	1.16 (0.72-1.86)	18 (14.9)	0.95 (0.62-1.47)	5 (4.1)	1.05 (0.44-2.50)
≥9.0	153	87 (56.9)	33 (21.6)	1.77 (1.26-2.47)	24 (15.7)	1.00 (0.68-1.47)	9 (5.9)	1.45 (0.75-2.81)

DD2: the Danish Centre for Strategic Research in Type 2 Diabetes; DDDA: the Danish Diabetes Database for Adults; aPR: adjusted prevalence ratio; CI: confidence interval.

Supplementary table A.7: Prevalence of diabetes complications in the subgroup of 2054 patients who currently can be linked to the DDDA and have highest probability of having newly detected T2D: no earlier glucose-lowering treatment, hospital diagnosis of T2D, or registration in the DDDA quality-of-care database recorded more than 1 year prior to DD2 enrollment. Prevalence ratios adjusted for age and gender, and additionally adjusted for waist-hip ratio.

Characteristic	All T2D patients	No complication (%)	Microvascular complication (%)	aPR (95% CI)	Macrovascular complication (%)	aPR (95% CI)	Micro- and macrovascular complications (%)	aPR (95% CI)

[a]High blood pressure = defined as no (systolic blood pressure <130 or diastolic bloodpressure <85) and as yes (systolic blood pressure ≥130 or diastolic bloodpressure ≥85)
[b]HDL cholesterol: m = males; f = females.
Data available in the subgroup of the DDDA cohort (n=2054); for blood pressure (n=106 missing); for smoking (n=118 missing); for BMI (n=457 missing); for HDL(n = 978 missing); for triglycerides (n= 163 missing); and for HbA1c (n= 32 missing).

Supplementary table A.8: Prevalence of individual microvascular complications (retinopathy, neuropathy, and nephropathy) among 6958 patients with newly diagnosed type 2 diabetes from the DD2 cohort. Age- and gender-adjusted prevalence ratios are shown according to patient characteristics.

Characteristic	All T2D patients	Retinopathy (%)	aPR (95% CI)	Neuropathy (%)	aPR (95% CI)	Nephropathy (%)	aPR (95% CI)
Main cohort	6958	887 (12.8)		264 (3.8)		234 (3.4)	
Sex							
Female	2927	384 (13.1)	Ref (1.00)	80 (2.7)	Ref (1.00)	70 (2.4)	Ref (1.00)
Male	4031	503 (12.5)	0.96 (0.85-1.09)	184 (4.6)	1.67 (1.29-2.17)	164 (4.1)	1.71 (1.30-2.26)
Age (years)							
<50	1220	116 (9.5)	Ref (1.00)	32 (2.6)	Ref (1.00)	30 (2.5)	Ref (1.00)
50-59	1790	145 (8.1)	0.86 (0.68-1.08)	79 (4.4)	1.70 (1.13-2.54)	53 (3.0)	1.22 (0.78-1.89)
60-69	2517	317 (12.6)	1.32 (1.08-1.62)	102 (4.1)	1.55 (1.05-2.29)	83 (3.3)	1.34 (0.89-2.03)
≥70	1431	309 (21.6)	2.26 (1.85-2.76)	51 (3.6)	1.38 (0.89-2.13)	68 (4.8)	1.95 (1.28-2.99)
Central obesity[a]							
No	570	72 (12.6)	Ref (1.00)	18 (3.2)	Ref (1.00)	9 (1.6)	Ref (1.00)
Yes	6378	815 (12.8)	1.05 (0.84-1.32)	246 (3.9)	1.34 (0.83-2.15)	225 (3.5)	2.51 (1.29-4.88)
Waist-hip ratio[b]							
≤0.95m/≤0.80f	772	103 (13.3)	Ref (1.00)	25 (3.2)	Ref (1.00)	18 (2.3)	Ref (1.00)
0.96-1.0m/0.81-0.85f	1437	183 (12.7)	0.95 (0.76-1.19)	62 (4.3)	1.35 (0.86-2.14)	46 (3.2)	1.43 (0.83-2.48)
>1.0m/>0.85f	4737	601 (12.7)	0.97 (0.79-1.19)	177 (3.7)	1.42 (0.93-2.16)	170 (3.6)	1.92 (1.17-3.15)
Regular physical exercise							
Yes	2725	325 (11.9)	Ref (1.00)	86 (3.2)	Ref (1.00)	71 (2.6)	Ref (1.00)
No	4232	562 (13.3)	1.13 (0.99-1.28)	178 (4.2)	1.29 (1.00-1.66)	163 (3.9)	1.43 (1.09-1.89)
Use of lipid-lowering drugs							
No	2072	270 (13.0)	Ref (1.00)	75 (3.6)	Ref (1.00)	54 (2.6)	Ref (1.00)
Yes	4886	617 (12.6)	0.88 (0.77-1.00)	189 (3.9)	1.04 (0.80-1.37)	180 (3.7)	1.32 (0.98-1.78)
Use of anti-hypertensive drugs							
No	1967	200 (10.2)	Ref (1.00)	60 (3.1)	Ref (1.00)	24 (1.2)	Ref (1.00)
Yes	4991	687 (13.8)	1.09 (0.94-1.26)	204 (4.1)	1.31 (0.97-1.76)	210 (4.2)	3.15 (2.06-4.82)
Fasting blood glucose (mmol/L)							
<6.5	1548	192 (12.4)	Ref (1.00)	56 (3.6)	Ref (1.00)	46 (3.0)	Ref (1.00)
6.5-7.0	917	102 (11.1)	0.91 (0.73-1.13)	26 (2.8)	0.77 (0.49-1.22)	24 (2.6)	0.87 (0.54-1.42)

Supplementary table A.8: Prevalence of individual microvascular complications (retinopathy, neuropathy, and nephropathy) among 6958 patients with newly diagnosed type 2 diabetes from the DD2 cohort. Age- and gender-adjusted prevalence ratios are shown according to patient characteristics.

Characteristic	All T2D patients	Retinopathy (%)	aPR (95% CI)	Neuropathy (%)	aPR (95% CI)	Nephropathy (%)	aPR (95% CI)
7.0-7.5	751	87 (11.6)	0.96 (0.76-1.21)	29 (3.9)	1.04 (0.67-1.62)	21 (2.8)	0.91 (0.55-1.52)
≥7.5	2146	235 (11.0)	0.98 (0.82-1.17)	91 (4.2)	1.18 (0.85-1.64)	83 (3.9)	1.37 (0.95-1.95)
C-peptide (pmol/L)							
<550	295	39 (13.2)	Ref (1.00)	18 (6.1)	Ref (1.00)	4 (1.4)	Ref (1.00)
550 - 800	853	101 (11.8)	0.85 (0.60-1.20)	27 (3.2)	0.52 (0.29-0.93)	13 (1.5)	1.07 (0.34-3.43)
≥800	4652	599 (12.9)	0.95 (0.70-1.29)	177 (3.8)	0.62 (0.39-0.99)	172 (3.7)	2.67 (1.00-7.13)
CRP (mg/L)							
≤3.0	627	59 (9.4)	Ref (1.00)	14 (2.2)	Ref (1.00)	14 (2.2)	Ref (1.00)
>3.0	403	37 (9.2)	1.04 (0.70-1.55)	16 (4.0)	1.75 (0.83-3.68)	12 (3.0)	1.57 (0.71-3.45)

DD2: the Danish Centre for Strategic Research in Type 2 Diabetes; aPR: adjusted prevalence ratio; CI: confidence interval.
[a]Central obesity = waist circumference >94 (men) and >80 (women). [b]Waist-hip ratio: m = males; f = females.
Data available in total DD2 cohort (n=6958) for central obesity (n=10 missing); for waist-hip ratio (n=12 missing); for regular physical activity (n=1 missing).
Data currently analysed in the DD2 biobank for fasting blood glucose (n=5362); for C-peptide (n=5800); and for CRP (n=1030).

Supplementary table A.9: Prevalence of individual microvascular complications (retinopathy, neuropathy, and nephropathy) among 5115 patients with newly diagnosed type 2 diabetes from the DD2 cohort who currently can be linked to the DDDA. Age- and gender-adjusted prevalence ratios are shown according to patient characteristics.

Characteristic	All T2D patients	Retinopathy (%)	aPR (95% CI)	Neuropathy (%)	aPR (95% CI)	Nephropathy (%)	aPR (95% CI)
Subcohort	5115						
High blood pressure (mmhg)[a]							
No	1575	195 (12.4)	Ref (1.00)	63 (4.0)	Ref (1.00)	54 (3.4)	Ref (1.00)
Yes	3261	480 (14.7)	1.17 (1.00-1.36)	139 (4.3)	1.03 (0.77-1.38)	118 (3.6)	1.00 (0.73-1.37)
Smoking							
No	3903	546 (14.0)	Ref (1.00)	152 (3.9)	Ref (1.00)	134 (3.4)	Ref (1.00)
Yes	941	122 (13.0)	1.02 (0.84-1.22)	47 (5.0)	1.29 (0.94-1.78)	30 (3.2)	0.96 (0.65-1.41)
BMI (kg/m2)							
<25	500	87 (17.4)	Ref (1.00)	23 (4.6)	Ref (1.00)	10 (2.0)	Ref (1.00)
25-29	1291	190 (14.7)	0.87 (0.69-1.09)	62 (4.8)	0.97 (0.61-1.55)	43 (3.3)	1.50 (0.76-2.94)
30-34	1147	155 (13.5)	0.84 (0.66-1.07)	57 (5.0)	1.04 (0.64-1.68)	44 (3.8)	1.92 (0.95-3.86)
≥35	897	123 (13.7)	0.87 (0.67-1.12)	39 (4.4)	0.97 (0.56-1.68)	47 (5.2)	2.76 (1.35-5.63)
HDL cholesterol (mmol/L)[b]							
≥1.3m/≥1.0f	2061	340 (16.5)	Ref (1.00)	95 (4.6)	Ref (1.00)	66 (3.2)	Ref (1.00)
<1.3m/<1.0f	969	160 (16.5)	1.07 (0.89-1.27)	47 (4.9)	1.10 (0.77-1.55)	44 (4.5)	1.65 (1.11-2.46)
Triglycerides (mmol/L)							
<1.7	2410	308 (12.8)	Ref (1.00)	89 (3.7)	Ref (1.00)	58 (2.4)	Ref (1.00)
≥1.7	2348	348 (14.8)	1.25 (1.09-1.44)	94 (4.0)	1.09 (0.81-1.47)	100 (4.3)	1.90 (1.37-2.63)
HbA1C (%)							
<7.0	3592	454 (12.6)	Ref (1.00)	130 (3.6)	Ref (1.00)	110 (3.1)	Ref (1.00)
7.0 - 8.0	824	132 (16.0)	1.37 (1.15-1.64)	46 (5.6)	1.52 (1.09-2.10)	40 (4.9)	1.59 (1.12-2.28)
8.0 - 9.0	303	45 (14.9)	1.43 (1.08-1.90)	19 (6.3)	1.77 (1.10-2.87)	13 (4.3)	1.51 (0.85-2.68)
≥9.0	313	60 (19.2)	1.94 (1.52-2.49)	11 (3.5)	0.99 (0.53-1.86)	11 (3.5)	1.33 (0.70-2.52)

DD2: the Danish Centre for Strategic Research in Type 2 Diabetes; DDDA: the Danish Diabetes Database for Adults; aPR: adjusted prevalence ratio; CI: confidence interval.

[a]High blood pressure = defined as no (systolic blood pressure <130 or diastolic bloodpressure <85) and as yes (systolic blood pressure ≥130 or diastolic bloodpressure ≥85). [b]HDL cholesterol: m = males; f = females.

Data available in DDDA cohort (n=5115); for blood pressure (n=279 missing); for smoking (n=271 missing); for BMI (n=1280 missing); for HDL(n = 2085 missing); for triglycerides (n= 357 missing); and for HbA1c (n= 83 missing).

Supplementary table A.10: Prevalence of individual macrovascular complications (ischemic heart disease, cerebrovascular disease, and peripheral vascular disease) among 6958 patients with newly diagnosed type 2 diabetes from the DD2 cohort. Age- and gender-adjusted prevalence ratios are shown according to patient characteristics.

Characteristic	All T2D patients	Ischemic heart disease (%)	aPR (95% CI)	Cerebrovascular disease (%)	aPR (95% CI)	Peripheral vascular disease (%)	aPR (95% CI)
Main cohort	6958	1059 (15.2)		365 (5.3)		151 (2.2)	
Sex							
Female	2927	320 (10.9)	Ref (1.00)	127 (4.3)	Ref (1.00)	44 (1.5)	Ref (1.00)
Male	4031	739 (18.3)	1.70 (1.51-1.92)	238 (5.9)	1.39 (1.13-1.71)	107 (2.7)	1.82 (1.29-2.58)
Age (years)							
<50	1220	67 (5.5)	Ref (1.00)	20 (1.6)	Ref (1.00)	5 (0.4)	Ref (1.00)
50-59	1790	222 (12.4)	2.28 (1.75-2.96)	72 (4.0)	2.46 (1.51-4.02)	21 (1.2)	2.88 (1.10-7.60)
60-69	2517	448 (17.8)	3.25 (2.54-4.16)	148 (5.9)	3.60 (2.26-5.71)	66 (2.6)	6.43 (2.60-15.90)
≥70	1431	322 (22.5)	4.20 (3.27-5.39)	125 (8.7)	5.38 (3.38-8.58)	59 (4.1)	10.27 (4.15-25.41)
Central obesity[a]							
No	570	75 (13.2)	Ref (1.00)	23 (4.0)	Ref (1.00)	8 (1.4)	Ref (1.00)
Yes	6378	984 (15.4)	1.34 (1.08-1.66)	342 (5.4)	1.50 (0.99-2.25)	143 (2.2)	1.91 (0.95-3.83)
Waist-hip ratio[b]							
≤0.95m/≤0.80f	772	118 (15.3)	Ref (1.00)	44 (5.7)	Ref (1.00)	10 (1.3)	Ref (1.00)
0.96-1.0m/0.81-0.85f	1437	226 (15.7)	1.09 (0.89-1.33)	84 (5.9)	1.08 (0.76-1.54)	27 (1.9)	1.71 (0.87-3.36)
>1.0m/>0.85f	4737	714 (15.1)	1.27 (1.06-1.51)	237 (5.0)	1.04 (0.76-1.43)	114 (2.4)	2.67 (1.43-4.97)
Regular physical exercise							
Yes	2725	379 (13.9)	Ref (1.00)	138 (5.1)	Ref (1.00)	40 (1.5)	Ref (1.00)
No	4232	679 (16.0)	1.12 (1.00-1.26)	227 (5.4)	1.05 (0.85-1.28)	111 (2.6)	1.74 (1.22-2.49)
Use of lipid-lowering drugs							
No	2072	127 (6.1)	Ref (1.00)	45 (2.2)	Ref (1.00)	22 (1.1)	Ref (1.00)
Yes	4886	932 (19.1)	2.80 (2.35-3.35)	320 (6.6)	2.68 (1.97-3.64)	129 (2.6)	2.16 (1.38-3.37)
Use of anti-hypertensive drugs							
No	1967	93 (4.7)	Ref (1.00)	37 (1.9)	Ref (1.00)	23 (1.2)	Ref (1.00)

Supplementary table A.10: Prevalence of individual macrovascular complications (ischemic heart disease, cerebrovascular disease, and peripheral vascular disease) among 6958 patients with newly diagnosed type 2 diabetes from the DD2 cohort. Age- and gender-adjusted prevalence ratios are shown according to patient characteristics.

Characteristic	All T2D patients	Ischemic heart disease (%)	aPR (95% CI)	Cerebrovascular disease (%)	aPR (95% CI)	Peripheral vascular disease (%)	aPR (95% CI)
Yes	4991	966 (19.4)	3.34 (2.71-4.12)	328 (6.6)	2.69 (1.91-3.80)	128 (2.6)	1.49 (0.95-2.36)
Fasting blood glucose (mmol/L)							
<6.5	1548	263 (17.0)	Ref (1.00)	86 (5.6)	Ref (1.00)	30 (1.9)	Ref (1.00)
6.5-7.0	917	149 (16.3)	0.95 (0.79-1.14)	49 (5.3)	0.96 (0.69-1.35)	22 (2.4)	1.27 (0.74-2.19)
7.0-7.5	751	118 (15.7)	0.91 (0.75-1.10)	51 (6.8)	1.23 (0.88-1.72)	23 (3.1)	1.60 (0.95-2.71)
≥7.5	2146	312 (14.5)	0.92 (0.79-1.07)	100 (4.7)	0.94 (0.71-1.25)	50 (2.3)	1.40 (0.90-2.17)
C-peptide (pmol/L)							
<550	295	38 (12.9)	Ref (1.00)	10 (3.4)	Ref (1.00)	6 (2.0)	Ref (1.00)
550 - 800	853	90 (10.6)	0.77 (0.54-1.10)	35 (4.1)	1.12 (0.56-2.22)	10 (1.2)	0.52 (0.19-1.43)
≥800	4652	779 (16.8)	1.26 (0.94-1.69)	266 (5.7)	1.62 (0.87-3.02)	116 (2.5)	1.15 (0.51-2.58)
CRP (mg/L)							
≤3.0	627	89 (14.2)	Ref (1.00)	25 (4.0)	Ref (1.00)	14 (2.2)	Ref (1.00)
>3.0	403	65 (16.1)	1.37 (1.02-1.83)	24 (6.0)	1.77 (1.02-3.07)	19 (4.7)	2.71 (1.42-5.18)

DD2: the Danish Centre for Strategic Research in Type 2 Diabetes; aPR: adjusted prevalence ratio; CI: confidence interval.

[a]Central obesity = waist circumference >94 (men) and >80 (women). [b]Waist-hip ratio: m = males; f = females.

Data available in total DD2 cohort (n=6958) for central obesity (n=10 missing); for waist-hip ratio (n=12 missing); for regular physical activity (n=1 missing). Data currently analysed in the DD2 biobank for fasting blood glucose (n=5363); for C-peptide (n=5800); and for CRP (n=1030).

Supplementary table A.11: Prevalence of individual macrovascular complications (ischemic heart disease, cerebrovascular disease, and peripheral vascular disease) among 5115 patients with newly diagnosed type 2 diabetes from the DD2 cohort who currently can be linked to the DDDA. Age- and gender-adjusted prevalence ratios are shown according to patient characteristics.

Characteristic	All T2D patients	Ischemic heart disease (%)	aPR (95% CI)	Cerebrovascular disease (%)	aPR (95% CI)	Peripheral vascular disease (%)	aPR (95% CI)
Subcohort	5115						
High blood pressure (mmhg)[a]							
No	1575	279 (17.7)	Ref (1.00)	94 (6.0)	Ref (1.00)	26 (1.7)	Ref (1.00)
Yes	3261	521 (16.0)	0.85 (0.75-0.97)	166 (5.1)	0.81 (0.64-1.04)	83 (2.6)	1.45 (0.94-2.23)
Smoking							
No	3903	648 (16.6)	Ref (1.00)	209 (5.4)	Ref (1.00)	81 (2.1)	Ref (1.00)
Yes	941	144 (15.3)	1.05 (0.89-1.24)	51 (5.4)	1.19 (0.88-1.61)	29 (3.1)	1.82 (1.20-2.77)
BMI (kg/m2)							
<25	500	78 (15.6)	Ref (1.00)	25 (5.0)	Ref (1.00)	9 (1.8)	Ref (1.00)
25-29	1291	210 (16.3)	1.00 (0.79-1.27)	86 (6.7)	1.37 (0.89-2.10)	39 (3.0)	1.66 (0.80-3.46)
30-34	1147	222 (19.4)	1.33 (1.06-1.68)	64 (5.6)	1.28 (0.82-1.99)	22 (1.9)	1.14 (0.53-2.46)
≥35	897	131 (14.6)	1.23 (0.95-1.60)	39 (4.4)	1.26 (0.76-2.08)	15 (1.7)	1.34 (0.59-3.05)
HDL cholesterol (mmol/L)[b]							
≥1.3m/≥1.0f	2061	340 (16.5)	Ref (1.00)	116 (5.6)	Ref (1.00)	46 (2.2)	Ref (1.00)
<1.3m/<1.0f	969	185 (19.1)	1.52 (1.29-1.78)	57 (5.9)	1.34 (0.98-1.82)	26 (2.7)	1.77 (1.09-2.86)
Triglycerides (mmol/L)							
<1.7	2410	379 (15.7)	Ref (1.00)	136 (5.6)	Ref (1.00)	46 (1.9)	Ref (1.00)
≥1.7	2348	393 (16.7)	1.20 (1.06-1.37)	125 (5.3)	1.09 (0.85-1.38)	57 (2.4)	1.52 (1.03-2.24)
HbA1C (%)							
<7.0	3592	585 (16.3)	Ref (1.00)	201 (5.6)	Ref (1.00)	80 (2.2)	Ref (1.00)
7.0 - 8.0	824	135 (16.4)	1.08 (0.91-1.27)	42 (5.1)	1.00 (0.72-1.38)	19 (2.3)	1.15 (0.70-1.89)
8.0 - 9.0	303	50 (16.5)	1.21 (0.93-1.58)	18 (5.9)	1.35 (0.84-2.16)	4 (1.3)	0.80 (0.29-2.18)
≥9.0	313	52 (16.6)	1.32 (1.02-1.71)	12 (3.8)	0.94 (0.52-1.67)	7 (2.2)	1.51 (0.70-3.25)

Supplementary table A.11: Prevalence of individual macrovascular complications (ischemic heart disease, cerebrovascular disease, and peripheral vascular disease) among 5115 patients with newly diagnosed type 2 diabetes from the DD2 cohort who currently can be linked to the DDDA. Age- and gender-adjusted prevalence ratios are shown according to patient characteristics.

Characteristic	All T2D patients	Ischemic heart disease (%)	aPR (95% CI)	Cerebrovascular disease (%)	aPR (95% CI)	Peripheral vascular disease (%)	aPR (95% CI)

DD2: the Danish Centre for Strategic Research in Type 2 Diabetes; DDDA: the Danish Diabetes Database for Adults; aPR: adjusted prevalence ratio; CI: confidence interval.

[a]High blood pressure = defined as no (systolic blood pressure <130 or diastolic bloodpressure <85) and as yes (systolic blood pressure ≥130 or diastolic bloodpressure ≥85). [b]HDL cholesterol: m = males; f = females.

Data available in DDDA cohort (n=5115); for blood pressure (n=279 missing); for smoking (n=271 missing); for BMI (n=1280 missing); for HDL(n = 2085 missing); for triglycerides (n= 357 missing); and for HbA1c (n= 83 missing).

Paper II

● **Appendix II**

Title page

Running head: Mannose-binding Lectin and Outcomes in Type 2 Diabetes

Gedebjerg A et al.

Mannose-binding Lectin and Risk of Cardiovascular Events and Mortality in Type 2 Diabetes: A Danish Cohort Study

A. Gedebjerg, MD[1,2], M. Bjerre, PhD[3], A.D. Kjaergaard, PhD[1], R. Steffensen, PhD[4], J.S. Nielsen, PhD[5], J. Rungby, DMSc[6,7], S. Friborg, MD[8], I. Brandslund, DMSc[9], S. Thiel, PhD[10], H. Beck-Nielsen, DMSc[5,8], H.T. Sørensen, DMSc[1,11,12], T.K. Hansen, DMSc[13], R.W. Thomsen, PhD[1]

[1]Department of Clinical Epidemiology, Aarhus University Hospital, Aarhus, Denmark
[2]Danish Diabetes Academy, Odense University Hospital, Odense, Denmark
[3]Medical Research Laboratory, Department of Clinical Medicine, Aarhus University, Aarhus, Denmark
[4]Department of Immunology, Aalborg University Hospital, Aalborg, Denmark
[5]DD2, Steno Diabetes Center Odense, Odense University Hospital, Odense, Denmark
[6]Department of Endocrinology IC, Bispebjerg University Hospital, Copenhagen, Denmark
[7]Copenhagen Center for Translational Research, Bispebjerg University Hospital, Copenhagen
[8]Diabetes Research Centre, Department of Endocrinology, Odense University Hospital, Odense, Denmark
[9]Department of Biochemistry, Lillebaelt Hospital, Vejle, Denmark
[10]Department of Biomedicine, Aarhus University, Aarhus, Denmark
[11]Department of Epidemiology, Boston University, MA, USA
[12]Center for Population Health Sciences, Stanford University, CA, USA
[13]Steno Diabetes Center Aarhus, Aarhus University Hospital, Aarhus, Denmark

Corresponding author: Anne Gedebjerg, Department of Clinical Epidemiology, Aarhus University Hospital, Olof Palmes Alle 43-45, DK-8200 Aarhus N, Denmark; Tel: +45 87168253; Fax: +45 87167215
Email: aged@clin.au.dk

Word count:

Title (words): 17; summary (words): 252; main text (words): 3040 (excluding summary, tables, figure legends, reference list, and supplementary tables); number of references: 40; number of tables: 1; number of figures: 3; number of supplementary tables: 5; number of supplementary figures: 32.

Abstract

Objective: Mannose-binding lectin (MBL) is linked to risk of cardiovascular disease in diabetes, but the nature of the association is unclear. We investigated the association between MBL and risk of cardiovascular events (CVE) and all-cause mortality in type 2 diabetes.

Research Design and Methods: In a cohort study of 7588 patients with type 2 diabetes, we measured serum MBL in 7305 and performed MBL expression genotyping in 3043. We grouped serum MBL and MBL expression genotypes into three categories: low, intermediate, and high. Outcomes were CVE (myocardial infarction, stroke, coronary revascularization, unstable angina, and cardiovascular death) and all-cause mortality. The association with outcomes was examined by spline and Cox regression analyses.

Results: Serum MBL and CVE showed a U-shaped association. Compared to the intermediate serum MBL category, the adjusted hazard ratio (HR) for CVE was 1.82 (95% confidence interval [CI], 1.34 to 2.47) for the low-MBL category and 1.48 (95% CI, 1.14 to 1.92) for the high-MBL category. We found a similar U-shaped association for all-cause mortality, but with lower risk estimates. Compared to the intermediate MBL expression genotype, the adjusted HR for CVE was 1.41 (95% CI, 0.88 to 2.26) for the low-expression genotype and 1.43 (95% CI, 1.00 to 2.04) for the high-expression genotype. MBL expression genotype was not associated with all-cause mortality.

Conclusions: Both serum MBL and MBL expression genotype showed a U-shaped association with CVE risk in individuals with type 2 diabetes. Our findings suggest that serum MBL is a risk factor for cardiovascular disease in this population.

Keywords: Type 2 diabetes; complement system; cohort study, mannose-binding lectin; cardiovascular event; mortality; association

Mannose-binding lectin (MBL, also known as mannan-binding lectin) is a multifunctional serum protein primarily produced in the liver.(1) It belongs to the lectin family of blood proteins and is instrumental in innate immunity(1), initiating the complement cascade via the lectin pathway and promoting pathogen clearance. In contrast to the classical complement pathway, which recognizes an antibody bound to its target, the lectin pathway starts with MBL binding to carbohydrate structures (e.g., patterns of mannose) on the surface of pathogens.(1; 2) Functional serum MBL levels are relatively stable over time(3) and largely determined genetically by six common single nucleotide polymorphisms (SNPs).(4) These SNPs can be categorized into three MBL expression genotypes – low, intermediate, and high(1) – with corresponding serum MBL levels.(4)

The association of MBL with cardiovascular disease (CVD) is mixed. Some studies have linked low levels and other studies high levels of serum MBL and MBL expression genotypes with increased risk of CVD(1; 5-11) and all-cause mortality(6; 12; 13) in individuals with diabetes and in the general population. MBL thus may play two roles in the development of CVD. Low levels (as in MBL deficiency) could impair pathogen clearance and reduce removal of atherogenic lipoproteins.(10; 14) High levels may amplify a low-grade immune response through complement activation, in particular if hyperglycemia is present.(15; 16) In support of this potential dual role, three studies(17-19) (two in patients with diabetes(17; 18)) have reported a U-shaped association of serum MBL levels with carotid intima-media thickness and low-grade inflammation. If both low and high serum MBL levels are directly involved in development of CVD and all-cause mortality, then both low and high MBL expression genotypes also would be expected to show a similar association.(20) The potential dual role of both serum MBL and MBL expression genotypes in CVD and all-cause mortality in type 2 diabetes has not been formally investigated.

We hypothesized that compared to intermediate levels, both low and high serum MBL levels are directly associated with increased risk of cardiovascular events (CVE) and all-cause mortality in

patients with early type 2 diabetes. To test this hypothesis, we conducted a Danish cohort study of 7588 patients with type 2 diabetes followed for up to 8 years. We first investigated the link between serum MBL levels and risk of CVE and all-cause mortality. Second, we examined the association between MBL expression genotype and serum MBL levels. Third, we investigated whether MBL expression genotype was associated with risk of CVE and all-cause mortality. According to the Mendelian randomization study design, this third step aids in substantiating a causal association between serum MBL and CVE and all-cause mortality.(20)

RESEARCH DESIGN AND METHODS

Study Cohort

We accessed information from the Danish Centre for Strategic Research in Type 2 Diabetes (DD2) cohort, an ongoing cohort of patients recently diagnosed with type 2 diabetes.(21) Enrollment in the cohort has been continuous from general practitioners' offices and hospital specialist outpatient clinics since November 1, 2010.(21) In brief, general practitioners or hospital physicians identify newly/recently diagnosed individuals with type 2 diabetes and complete an online questionnaire(22) eliciting lifestyle (e.g., physical activity) and clinical examination data for each entered participant at the time of enrollment. Urine and fasting blood samples are collected from each patient and stored in the DD2 biobank.(23) The study cohort was all DD2 participants enrolled by December 2016 and with a stored blood sample in the DD2 biobank.

Ethics

This study was approved by the Danish Data Protection Agency (record number 2008-58-0035) and by the Regional Committees on Health Research Ethics for Southern Denmark (record number S-20100082). All cohort participants gave written informed consent.

Outcomes

Outcomes were a composite of CVE (first occurrence of myocardial infarction, ischemic stroke, unstable angina pectoris, coronary revascularization, or cardiovascular death), individual subtypes of CVE, and all-cause mortality. We obtained all diagnoses (primary and secondary discharge diagnoses) from the Danish National Patient Registry, which covers all hospitals in Denmark and contains records of discharge diagnoses from all inpatient hospitalizations since 1977 and all emergency department visits and hospital outpatient clinic since 1995.(24) Exact dates of death were obtained from the Danish Civil Registration System(25), and information on cardiovascular death (both as the immediate or underlying cause of death) was accessed from the Danish Registry of Causes of Death.(26; 27) Diagnoses and procedure codes are shown in Supplementary Table 1.

Serum MBL Levels

Functional serum MBL levels were measured using an in-house time resolved immuno-fluorometric assay, as described elsewhere.(2) Mannan-coated microtiter wells were incubated with serum samples, and bound MBL was detected with biotin-labeled monoclonal anti-MBL antibody followed by europium-labeled streptavidin and detection by time-resolved fluorometry. The limit of quantification was 10 μg/L at the dilution used, and MBL deficiency (n=849; 12%) was set to 10 μg/L. The intra- and interassay coefficients of variation were <10%. Serum MBL levels were categorized as low (≤100 μg/L), intermediate (101–1000 μg/L), or high (>1000 μg/L), with the cut points often used in MBL research.(28) Consistent with previous studies indicating a nonlinear association between serum MBL and cardiovascular outcomes(17-19), the intermediate serum MBL category (101–1000 μg/L) was used as reference.

MBL Expression Genotypes

Six SNPs located within the promoter region (rs11003125, rs7096206, rs7095891) and exon 1 (rs5030737, rs1800451, rs1800450) of the *MBL2* gene were genotyped on the first 3043 consecutive individuals in the DD2 cohort, using TaqMan genotyping assays as previously described.[4] Because of linkage disequilibrium, the six SNPs give rise to seven major haplotypes: HYPA, LYQA, LYPA, LXPA, LYPB, LYQC, and HYPD. These MBL haplotypes were categorized into three MBL expression genotypes – low, intermediate, and high(1; 4) – that have previously been correlated with serum MBL levels.(28) Complete methods are described in the supplemental material.

Covariates

From the DD2 cohort questionnaire(22) and linked medical and administrative registries, we extracted baseline information on covariates present at the time of DD2 enrollment that could be associated with subsequent cardiovascular risk. Covariates, definitions, and codes are listed in Supplementary Table 1.

Statistical Analysis

We used restricted cubic spline models with five degrees of freedom to examine the association between serum MBL levels, as a continuous variable, and risks of CVE and all-cause mortality. The cumulative incidence of CVE, with risk of non-cardiovascular death as a competing risk, was plotted using STATA's stcompet command, and we plotted cumulative incidence of all-cause mortality using the Kaplan–Meier method. Incidence and mortality rates were calculated using STATA's stptime command, and to calculate hazard ratios (HRs) and 95% confidence intervals (CIs), we used Cox regression analysis. We performed extensive adjustments to ensure robustness

of the potential associations. In Model 1, HRs were adjusted for sex and age. In Model 2, HRs were adjusted for sex, age, diabetes duration, and levels of high-sensitivity C-reactive protein (hs-CRP). In Model 3, HRs were adjusted for sex, age, diabetes duration, hs-CRP, waist circumference, waist–hip ratio, body mass index, physical activity, smoking, systolic and diastolic blood pressure, comorbidities, fasting blood glucose, HbA1c (hemoglobin), C-peptide, albumin:creatinine ratio, total cholesterol, low-density lipoprotein, high-density cholesterol, triglycerides, and use of anti-diabetes, lipid-lowering, anti-hypertensive, or anti-thrombotic drugs. Missing covariates (n=5–3966; 0.1%–54%; Supplementary Table 2) were treated with multiple imputation (the full text on multiple imputation is shown in the supplemental material). We did not impute MBL expression genotype where this information was missing (n=4262; 58%).

For Cox regression analyses, we graphically verified the proportional hazards assumption by plotting –ln(survival probability) against ln(analysis time) and detected no violations. Individuals with type 2 diabetes were followed from the enrollment date until an event, death, or August 10, 2018, whichever came first. All-cause and cardiovascular death data were available up to August 22, 2018, and December 28, 2016, respectively. Individuals with an event prior to enrollment (myocardial infarction, n=434; ischemic stroke, n=248; coronary revascularization, n=602; unstable angina pectoris: n=163; any cardiovascular events, n=924) were excluded from the analyses for the relevant outcome. We did not consider recurrent events.

To assess risk of genotype misclassification, we used the Hardy–Weinberg equilibrium and the χ^2 test. We performed a Cuzick non-parametric test for trend and calculated R^2 by simple linear regression to evaluate the association between MBL expression genotype and serum MBL levels.

We also performed a number of sensitivity analyses. First, to decrease risk of misclassification of serum MBL related to acute infection and/or inflammation, we excluded individuals with serum hs-CRP levels >10 mg/L (n=641; 9%).(29) Second, to focus exclusively on

individuals newly diagnosed with type 2 diabetes, we excluded anyone with a registered diabetes duration >1 year (n=4042; 55%). Third, we excluded individuals with any previous record of CVD, including any atherosclerotic disease or heart failure (n=1451; 20%). For statistical analyses, we used STATA version 14.2.

RESULTS

Baseline Characteristics

The study included 7588 participants with type 2 diabetes, of whom 7305 (96%) had a serum MBL measurement available and 3043 (42%) had been genotyped for the six SNPs in the *MBL2* gene (Supplementary Fig. 1). The cohort was followed for a median period of 4.7 years (inter-quartile range [IQR]: 3.3–5.7) for CVE and 4.8 years (IQR: 3.6–5.8) for all-cause mortality. Between 2010 and 2018, 324 patients developed the composite outcome of CVE, including 106 with myocardial infarction, 124 with ischemic stroke, 73 with cardiovascular death, 157 with coronary revascularization, and 45 with unstable angina pectoris. More than one outcome was possible. There were 439 all-cause deaths.

Table 1 shows baseline characteristics according to serum MBL categories (≤100, 101–1000, or >1000 µg/L), and Supplementary Table 3 shows them according to MBL expression genotypes (low, intermediate, and high). Serum MBL and MBL expression genotype categories showed no clear associations with any baseline characteristics. No MBL categories were associated with hs-CRP levels.

Serum MBL Levels and Risk for Cardiovascular Events/All-Cause Mortality

Serum MBL levels and CVE showed a U-shaped association (Fig. 1A). Compared to intermediate serum MBL levels, both low and high serum MBL levels were associated with increased CVE risk

(Fig. 1A and Fig. 2A). All-cause mortality had a similar but attenuated association (Fig. 1B and Fig. 2C). Supplementary Table 4 lists incidence rates.

Fig. 3 shows the HRs for CVE and all-cause mortality by serum MBL categories and MBL expression genotypes. Compared to the intermediate serum MBL category, the adjusted HR (Model 3) for CVE was 1.82 (95% CI, 1.34–2.47) for the low serum MBL category and 1.48 (95% CI, 1.14–1.92) for the high serum MBL category. Results for the CVE subtypes were consistent with the analysis of the composite outcome of CVE (Supplementary Fig. 2–16): there was a U-shaped association between serum MBL levels and all individual subtypes of CVE, but with limited statistical precision because of fewer events. The spline for all-cause mortality was similar to the spline for cardiovascular mortality, but attenuated. Compared to the intermediate serum MBL category, the adjusted HR (Model 3) for all-cause mortality was 1.19 (95% CI, 0.89–1.59) for the low serum MBL category and 1.16 (95% CI, 0.92–1.46) for the high category.

MBL Expression Genotypes and Serum MBL Levels

The distributions of all MBL haplotypes with corresponding median serum MBL levels are shown in Supplementary Table 5. We identified no major deviations from Hardy–Weinberg equilibrium (χ^2 test for rs11003125, P=0.97; for rs7095891, P=0.10; for rs7096206, P=0.66; for rs1800451, P=0.91; for rs1800450, P=0.78; and for rs5030737, P=0.01; Supplementary Table 6). Median serum MBL levels for individuals with low, intermediate, and high MBL expression genotypes were 10 µg/L (IQR: 10–26 µg/L), 321 µg/L (IQR: 199–545 µg/L), and 1527 µg/L (IQR: 974–2394 µg/L), respectively (Supplementary Table 5). Serum MBL levels were strongly associated with MBL expression genotypes (R^2=0.31, P for trend $<1\times10^{-300}$) (Supplementary Fig. 17).

MBL Expression Genotypes and Risk for Cardiovascular Events/All-Cause Mortality

Both low and high MBL expression genotypes were associated with increased risk of CVE (Fig. 2B). There was no clear association for all-cause mortality (Fig. 2D). Supplementary Table 4 shows incidence rates.

Compared to the intermediate MBL expression genotype, the adjusted HR (Model 3) for CVE was 1.41 (95% CI, 0.88–2.26) for the low MBL expression genotype and 1.43 (95% CI, 1.00–2.04) for the high MBL expression genotype (Fig. 3). Individual subtypes of CVE showed results consistent with those from the analysis of the composite outcome of CVE (Supplementary Fig. 2–16), with limited precision because of fewer events.

Compared to the intermediate MBL expression genotype, the adjusted HR (Model 3) for all-cause mortality was 1.06 (95% CI, 0.69–1.64) for the low MBL expression genotype and 1.06 (95% CI, 0.77–1.44) for the high MBL expression genotype (Fig. 3).

Sensitivity Analyses

Overall, the sensitivity analyses restricted to individuals with hs-CRP below 10 mg/L, newly diagnosed type 2 diabetes, and no previous CVD yielded results similar to analyses of the composite CVE and all-cause mortality (Supplementary Fig. 18–32).

CONCLUSIONS

In this prospective study of 7305 patients with type 2 diabetes, compared to the intermediate expression genotype, both low and high MBL expression genotypes were associated with a 41% and 43% increased risk of CVE. The consistency of this association for serum levels and MBL expression genotypes suggests a causal role for serum MBL in development of CVD in type 2 diabetes.

Previous studies of the association between MBL and CVD have yielded contradictory findings regarding the direction of the associations. Some studies have shown links only for low serum MBL levels(30; 31) or low MBL expression genotype(5; 6; 10; 11) and increased risk of CVD. Other groups have reported that only high serum MBL levels(1; 7; 32; 33) or high MBL expression genotype were associated with increased risk. A possible explanation for these apparent discrepancies is that the studies used different combinations of MBL categories (low and intermediate versus high, or low versus high and intermediate). They also had heterogeneous study populations and designs that preclude direct comparison with the present results. As an example, a previous cohort study of 9245 individuals from the Danish general population(6) yielded a relative risk of 1.2 with low versus high MBL expression genotype, and 0.95 with intermediate versus high MBL expression genotype, respectively, compatible with a U-shaped association. If the authors had used the intermediate MBL expression genotype as the reference category, the identified risks might have been comparable with the current values. This suggests that our findings might be applicable to the general population, not only to patients with type 2 diabetes. Taken together, these previous observations support our findings that intermediate MBL levels are the most advantageous for healthy aging(34; 35) and that both low and high MBL levels may raise risk of CVD.

We found no association between MBL expression genotype and risk of all-cause mortality, in agreement with the Danish general population study mentioned above(6) but in contrast with another Danish study of 372 patients with type 1 diabetes.(13) These latter authors reported a 47% increased risk of all-cause mortality with a high versus low MBL expression genotype. We also found that low and high serum MBL levels were compatible with a 16% to 19% increased risk of all-cause mortality, although we could not rule out a null association. These findings are at the low end of previously reported values, including the results of the earlier study of patients with type 1 diabetes(13) and another Danish study of 326 individuals with type 2 diabetes(12) suggesting that

high serum MBL levels were associated with a 20% to 79% increased risk of all-cause mortality. Our findings of a weaker association of MBL with all-cause mortality than with CVE (a composite outcome also including cardiovascular mortality) suggests that the modest association between MBL and all-cause mortality is driven by cardiovascular mortality.

It is biologically plausible that both low and high serum MBL could promote chronic inflammation and atherosclerosis in individuals with type 2 diabetes. Low MBL levels (such as in MBL deficiency) could do so by impairment of pathogen clearance(8; 14; 36) and reduced removal of atherogenic lipoproteins.(10) High MBL levels could have this effect by amplifying a low-grade immune response through complement activation in an interplay with hyperglycemia.(15; 16) In support of these explanations, two cross-sectional studies(18; 19) and one prospective study(17) have shown a U-shaped association of serum MBL with carotid intima-media thickness and low-grade inflammation, supporting the hypothesis of a dual role for MBL in the development of CVD.

Limitations of this study include the possibility of survival bias and selection bias in the DD2 cohort. Both situations would likely lead to decreased participation of individuals with a severe type 2 diabetes phenotype or high cardiovascular risk and likely bias results towards the null hypothesis. Moreover, survival and selection bias are unlikely because the characteristics and comorbidities of individuals participating in DD2 are similar to those of individuals with early treated type 2 diabetes in routine practice in Denmark.(21; 37) Another potential concern include misclassification of diagnoses, which we do not believe happened to a large degree because the validity of cardiovascular diagnoses and procedures in the national Danish registries is high.(38; 39) In addition, misclassification of genotype is unlikely given the *MBL2* genotype frequencies (and serum levels) that were similar to those of previous studies and the lack of major deviations from Hardy–Weinberg equilibrium.(1; 28) Another limitation is potential pleiotropy. According to Ldlink (https://ldlink.nci.nih.gov/), no SNPs in the *MBL2* gene were in linkage disequilibrium with any

genetic variants outside the *MBL2* gene. Confounding by variation in nearby genes can therefore not explain our findings. Pleiotropy is often impossible to refute completely, but is unlikely as the six SNPs we used were located within the promoter region and exon1 of the *MBL2* gene, were highly associated with serum MBL levels, and were not associated with any potential confounders. More than 90% of Denmark's population is Caucasian, and our results may not necessarily apply to other ethnic groups. Finally, we did not estimate the magnitude of the potential causal effect (i.e., the fourth step of a complete Mendelian randomization design(20)) because of the non-linear association between serum MBL levels and outcomes.(40)

In conclusion, in this prospective study of individuals with type 2 diabetes, we found a U-shaped association for both serum MBL and MBL expression genotype with risk of CVE. This result suggests that serum MBL is directly involved in the development of CVD. Individuals with low and high serum MBL might benefit from a more aggressive preventative treatment.

Acknowledgments

We are grateful to all participants in the DD2 study. We thank Hanne Petersen and Karen Mathiassen for their assistance with the large-volume measurements of serum MBL and hs-CRP. We gratefully acknowledge Sia Kromann Nicolaisen for assistance with data management.

Funding

The Danish Centre for Strategic Research in Type 2 Diabetes Project (DD2) is supported by the Danish Agency for Science (grant nos. 09-067009 and 09-075724), the Danish Health and Medicines Authority, the Danish Diabetes Association, and an unrestricted donation from Novo Nordisk A/S. Project partners are listed on the www.DD2.nu website. The work of A.G. was supported by the Danish Diabetes Academy, which is funded by an unrestricted grant from the Novo Nordisk Foundation, and by grants from the Danish Heart Foundation (grant nos. 15-R99-A5866-22891) and Aarhus University. In addition, A.G. has received funding from the Danielsen Foundation, Augustinus Foundation, A.P. Møller Foundation, Hertz Foundation, and Bønnelycke Foundation. The Department of Clinical Epidemiology, Aarhus University Hospital, participates in the International Diabetic Neuropathy Consortium research program, which is supported by a Novo Nordisk Foundation Challenge program grant (grant number NNF14SA000 6). The funding source had no role in study design, data collection, data analysis, data interpretation, or writing of the report. AG and RWT had full access to all study data and had final responsibility for the decision to submit for publication.

Duality of Interest

AG, MB, ADK, RS, JSN, JR, SF, IB, ST, HBN, HTS, TKH, RWT have reported no personal conflicts of interest relevant to this article. The Danish Centre for Strategic Research in Type 2 Diabetes Project (DD2) is supported by the Danish Agency for Science [grant number 09-067009, 09-075724], the Danish Health and Medicines Authority, the Danish Diabetes Association, and an

unrestricted donation from Novo Nordisk A/S. Project partners are listed on the website www.DD2.nu. The Department of Clinical Epidemiology, Aarhus University Hospital, receives funding for other studies from companies in the form of research grants to (and administered by) Aarhus University. None of these studies has any relation to the present study.

Author Contributions

H.B.N., H.T.S., and J.R. participated in conceiving and designing the parent DD2 project cohort study. M.B., J.S.N., J.R., S.F., I.B., H.B.N., H.T.S., T.K.H., and R.W.T. conceived of the current study. M.B. was responsible for serum MBL and hs-CRP measurements, and R.S. was responsible for MBL genotyping. I.B. was responsible for the biobank and the other biochemical analyses. A.G., A.D.K., R.W.T., and H.T.S. participated in the design of the current study, and A.G. performed the statistical analyses. A.G. drafted the article, with help from A.D.K., R.W.T., and H.T.S. All other authors have critically reviewed the manuscript. All authors contributed substantially to the study, revised the manuscript for intellectual content, and approved the final version to be submitted. A.G. and R.W.T. are the guarantors of this work and, as such, had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Prior presentation

This study was presented at an oral presentation at the annual meeting of the European Association for the Study of Diabetes (EASD), Barcelona, Spain, 16-20 September, 2019.

REFERENCES

1. Hansen TK, Tarnow L, Thiel S, Steffensen R, Stehouwer CD, Schalkwijk CG, Parving HH, Flyvbjerg A. Association between mannose-binding lectin and vascular complications in type 1 diabetes. Diabetes 2004;53:1570-1576
2. Thiel S, Moller-Kristensen M, Jensen L, Jensenius JC. Assays for the functional activity of the mannan-binding lectin pathway of complement activation. Immunobiology 2002;205:446-454
3. Ytting H, Christensen IJ, Thiel S, Jensenius JC, Svendsen MN, Nielsen L, Lottenburger T, Nielsen HJ. Biological variation in circulating levels of mannan-binding lectin (MBL) and MBL-associated serine protease-2 and the influence of age, gender and physical exercise. Scand J Immunol 2007;66:458-464
4. Molle I, Steffensen R, Thiel S, Peterslund NA. Chemotherapy-related infections in patients with multiple myeloma: associations with mannan-binding lectin genotypes. European journal of haematology 2006;77:19-26
5. Best LG, Davidson M, North KE, MacCluer JW, Zhang Y, Lee ET, Howard BV, DeCroo S, Ferrell RE. Prospective analysis of mannose-binding lectin genotypes and coronary artery disease in American Indians: the Strong Heart Study. Circulation 2004;109:471-475
6. Dahl M, Tybjaerg-Hansen A, Schnohr P, Nordestgaard BG. A population-based study of morbidity and mortality in mannose-binding lectin deficiency. J Exp Med 2004;199:1391-1399
7. Keller TT, van Leuven SI, Meuwese MC, Wareham NJ, Luben R, Stroes ES, Hack CE, Levi M, Khaw KT, Boekholdt SM. Serum levels of mannose-binding lectin and the risk of future coronary artery disease in apparently healthy men and women. Arteriosclerosis, thrombosis, and vascular biology 2006;26:2345-2350
8. Madsen HO, Videm V, Svejgaard A, Svennevig JL, Garred P. Association of mannose-binding-lectin deficiency with severe atherosclerosis. Lancet (London, England) 1998;352:959-960
9. Mellbin LG, Hamsten A, Malmberg K, Steffensen R, Ryden L, Ohrvik J, Hansen TK. Mannose-binding lectin genotype and phenotype in patients with type 2 diabetes and myocardial infarction: a report from the DIGAMI 2 trial. Diabetes care 2010;33:2451-2456
10. Siezenga MA, Shaw PK, Daha MR, Rabelink TJ, Berger SP. Low Mannose-Binding Lectin (MBL) genotype is associated with future cardiovascular events in type 2 diabetic South Asians. A prospective cohort study. Cardiovasc Diabetol 2011;10:60
11. Vengen IT, Madsen HO, Garred P, Platou C, Vatten L, Videm V. Mannose-binding lectin deficiency is associated with myocardial infarction: the HUNT2 study in Norway. PloS one 2012;7:e42113
12. Hansen TK, Gall MA, Tarnow L, Thiel S, Stehouwer CD, Schalkwijk CG, Parving HH, Flyvbjerg A. Mannose-binding lectin and mortality in type 2 diabetes. Archives of Internal Medicine 2006;166:2007-2013
13. Ostergaard JA, Thiel S, Lajer M, Steffensen R, Parving HH, Flyvbjerg A, Rossing P, Tarnow L, Hansen TK. Increased all-cause mortality in patients with type 1 diabetes and high-expression mannan-binding lectin genotypes: a 12-year follow-up study. Diabetes Care 2015;38:1898-1903
14. Rugonfalvi-Kiss S, Endresz V, Madsen HO, Burian K, Duba J, Prohaszka Z, Karadi I, Romics L, Gonczol E, Fust G, Garred P. Association of Chlamydia pneumoniae with coronary artery disease and its progression is dependent on the modifying effect of mannose-binding lectin. Circulation 2002;106:1071-1076
15. Collard CD, Lekowski R, Jordan JE, Agah A, Stahl GL. Complement activation following oxidative stress. Molecular immunology 1999;36:941-948
16. Axelgaard E, Ostergaard JA, Haxha S, Thiel S, Hansen TK. Global Autorecognition and Activation of Complement by Mannan-Binding Lectin in a Mouse Model of Type 1 Diabetes. Mediators Inflamm 2017;2017:9403754
17. Hertle E, Arts IC, van der Kallen CJ, Feskens EJ, Schalkwijk CG, Hoffmann-Petersen IT, Thiel S, Stehouwer CD, van Greevenbroek MM. Distinct Longitudinal Associations of MBL, MASP-1, MASP-2, MASP-3, and MAp44 With Endothelial Dysfunction and Intima-Media Thickness: The Cohort on Diabetes and Atherosclerosis Maastricht (CODAM) Study. Arteriosclerosis, thrombosis, and vascular biology 2016;36:1278-1285

18. Kaplar M, Sweni S, Kulcsar J, Cogoi B, Esze R, Somodi S, Papp M, Olah L, Magyar MT, Szabo K, Czuriga-Kovacs KR, Harsfalvi J, Paragh G. Mannose-Binding Lectin Levels and Carotid Intima-Media Thickness in Type 2 Diabetic Patients. Journal of diabetes research 2016;2016:8132925
19. Troelsen LN, Garred P, Christiansen B, Torp-Pedersen C, Christensen IJ, Narvestad E, Jacobsen S. Double role of mannose-binding lectin in relation to carotid intima-media thickness in patients with rheumatoid arthritis. Mol Immunol 2010;47:713-718
20. Benn M, Nordestgaard BG. From genome-wide association studies to Mendelian randomization: novel opportunities for understanding cardiovascular disease causality, pathogenesis, prevention, and treatment. Cardiovascular research 2018;114:1192-1208
21. Christensen DH, Nicolaisen SK, Berencsi K, Beck-Nielsen H, Rungby J, Friborg S, Brandslund I, Christiansen JS, Vaag A, Sorensen HT, Nielsen JS, Thomsen RW. Danish Centre for Strategic Research in Type 2 Diabetes (DD2) project cohort of newly diagnosed patients with type 2 diabetes: a cohort profile. BMJ open 2018;8:e017273
22. Nielsen JS, Thomsen RW, Steffensen C, Christiansen JS. The Danish Centre for Strategic Research in Type 2 Diabetes (DD2) study: implementation of a nationwide patient enrollment system. Clinical epidemiology 2012;4:27-36
23. Christensen H, Nielsen JS, Sorensen KM, Melbye M, Brandslund I. New national Biobank of The Danish Center for Strategic Research on Type 2 Diabetes (DD2). Clinical epidemiology 2012;4:37-42
24. Schmidt M, Schmidt SA, Sandegaard JL, Ehrenstein V, Pedersen L, Sorensen HT. The Danish National Patient Registry: a review of content, data quality, and research potential. Clin Epidemiol 2015;7:449-490
25. Schmidt M, Pedersen L, Sorensen HT. The Danish Civil Registration System as a tool in epidemiology. Eur J Epidemiol 2014;29:541-549
26. Helweg-Larsen K. The Danish Register of Causes of Death. Scand J Public Health 2011;39:26-29
27. Schmidt M, Schmidt SAJ, Adelborg K, Sundboll J, Laugesen K, Ehrenstein V, Sorensen HT. The Danish health care system and epidemiological research: from health care contacts to database records. Clin Epidemiol 2019;11:563-591
28. Gadjeva M, Takahashi K, Thiel S. Mannan-binding lectin--a soluble pattern recognition molecule. Mol Immunol 2004;41:113-121
29. Hansen TK, Forsblom C, Saraheimo M, Thorn L, Waden J, Hoyem P, Ostergaard J, Flyvbjerg A, Groop PH, FinnDiane Study G. Association between mannose-binding lectin, high-sensitivity C-reactive protein and the progression of diabetic nephropathy in type 1 diabetes. Diabetologia 2010;53:1517-1524
30. Poppelaars F, Gaya da Costa M, Berger SP, Assa S, Meter-Arkema AH, Daha MR, van Son WJ, Franssen CF, Seelen MA. Strong predictive value of mannose-binding lectin levels for cardiovascular risk of hemodialysis patients. Journal of translational medicine 2016;14:236
31. Saevarsdottir S, Oskarsson OO, Aspelund T, Eiriksdottir G, Vikingsdottir T, Gudnason V, Valdimarsson H. Mannan binding lectin as an adjunct to risk assessment for myocardial infarction in individuals with enhanced risk. The Journal of experimental medicine 2005;201:117-125
32. Pesonen E, Hallman M, Sarna S, Andsberg E, Haataja R, Meri S, Persson K, Puolakkainen M, Ohlin H, Truedsson L. Mannose-binding lectin as a risk factor for acute coronary syndromes. Annals of Medicine 2009;41:591-598
33. Troelsen LN, Garred P, Madsen HO, Jacobsen S. Genetically determined high serum levels of mannose-binding lectin and agalactosyl IgG are associated with ischemic heart disease in rheumatoid arthritis. Arthritis and rheumatism 2007;56:21-29
34. Eisen DP, Osthoff M. If there is an evolutionary selection pressure for the high frequency of MBL2 polymorphisms, what is it? Clin Exp Immunol 2014;176:165-171
35. Tomaiuolo R, Ruocco A, Salapete C, Carru C, Baggio G, Franceschi C, Zinellu A, Vaupel J, Bellia C, Lo Sasso B, Ciaccio M, Castaldo G, Deiana L. Activity of mannose-binding lectin in centenarians. Aging cell 2012;11:394-400
36. Monsey L, Best LG, Zhu J, DeCroo S, Anderson MZ. The association of mannose binding lectin genotype and immune response to Chlamydia pneumoniae: The Strong Heart Study. PLoS One 2019;14:e0210640

37. Gedebjerg A, Almdal TP, Berencsi K, Rungby J, Nielsen JS, Witte DR, Friborg S, Brandslund I, Vaag A, Beck-Nielsen H, Sorensen HT, Thomsen RW. Prevalence of micro- and macrovascular diabetes complications at time of type 2 diabetes diagnosis and associated clinical characteristics: A cross-sectional baseline study of 6958 patients in the Danish DD2 cohort. Journal of diabetes and its complications 2017;
38. Sundboll J, Adelborg K, Munch T, Froslev T, Sorensen HT, Botker HE, Schmidt M. Positive predictive value of cardiovascular diagnoses in the Danish National Patient Registry: a validation study. BMJ open 2016;6:e012832
39. Adelborg K, Sundboll J, Munch T, Froslev T, Sorensen HT, Botker HE, Schmidt M. Positive predictive value of cardiac examination, procedure and surgery codes in the Danish National Patient Registry: a population-based validation study. BMJ open 2016;6:e012817
40. Teumer A. Common Methods for Performing Mendelian Randomization. Frontiers in cardiovascular medicine 2018;5:51

Legends

Figure 1. Risk of cardiovascular events and all-cause mortality by serum MBL levels.

Cardiovascular events (A) and all-cause mortality (B). The solid lines indicate the hazard ratios, and the dotted lines indicate 95% confidence intervals. The continuous variable serum MBL was modeled with five restricted cubic splines.

Figure 2. Time-to-event curves of cardiovascular events and all-cause mortality by serum MBL and MBL expression genotype categories.

Cumulative incidence plots of cardiovascular events (A and B) and all-cause mortality (C and D) by serum MBL (A and C) and MBL expression genotype (B and D) categories. Cumulative incidence estimates are based on time from DD2 enrollment date to first event, with risk of non-cardiovascular death as a competing risk (A and B).

Figure 3. Hazard ratios of cardiovascular events and all-cause mortality by serum MBL and MBL expression genotype categories.

Model 1 is adjusted for sex and age. Model 2 is adjusted for sex, age, diabetes duration, and hs-CRP. Model 3 is adjusted for sex, age, diabetes duration, waist circumference, waist–hip ratio, body mass index, physical activity, smoking, systolic and diastolic blood pressure, comorbidities, fasting blood glucose, HbA1c, C-peptide, albumin:creatinine ratio, total cholesterol, low-density lipoprotein, high-density cholesterol, triglycerides, hs-CRP, and use of anti-diabetes, lipid-lowering, anti-hypertensive, and anti-thrombotic drugs. Missing covariates were treated with multiple imputation.

Table 1. Characteristics of DD2 Cohort Members at Baseline by Serum MBL Category.

	Low serum MBL (≤100 µg/L)	Intermediate serum MBL (101-1000 µg/L)	High serum MBL (>1000 µg/L)
Total, N (%)	1295 (17.7)	2975 (40.7)	3035 (41.6)
Male sex, n (%)	727 (56.1)	1612 (54.2)	1939 (63.9)
Median age (IQR), years	61.6 (52.7–69.0)	61.9 (53.1–68.7)	62.3 (53.0–68.8)
Median diabetes duration (IQR), years	1.3 (0.3–2.9)	1.4 (0.4–2.9)	1.2 (0.3–2.9)
Median waist circumference (IQR), cm	106 (97–117)	107 (97–117)	105 (96–115)
Median waist–hip ratio (IQR)	0.98 (0.92–1.04)	0.98 (0.92–1.04)	0.98 (0.93–1.04)
Median body mass index (IQR), kg/m^2	30.5 (27.1–34.5)	30.7 (27.4–34.7)	29.7 (26.4–33.7)
Physical activity* (IQR), days/week	3 (2–7)	3 (2–7)	4 (2–7)
Smoking, n (%)			
Never	434 (45.5)	1039 (47.7)	1052 (46.3)
Former	351 (36.8)	749 (34.4)	750 (33.0)
Current	170 (17.8)	389 (17.9)	471 (20.7)
Median systolic blood pressure (IQR), mmHg	130 (124–140)	130 (123–140)	130 (124–140)
Median diastolic blood pressure (IQR), mmHg	80 (74–85)	80 (74–85)	80 (75–86)
CCI score†, n (%)			
0	882 (68.1)	2034 (68.4)	2109 (69.5)
1-2	339 (26.2)	783 (26.3)	763 (25.1)
3	74 (5.7)	158 (5.3)	163 (5.4)
Anti-diabetes drug use, n (%)	1080 (83.4)	2547 (85.6)	2582 (85.1)
Lipid-lowering drug use, n (%)	932 (72.0)	2176 (73.1)	2037 (67.1)
Anti-hypertensive drug use, n (%)	926 (71.5)	2175 (73.1)	2143 (70.6)
Anti-thrombotic drug use, n (%)	372 (28.7)	873 (29.3)	855 (28.2)
Median fasting blood glucose (IQR), mmol/L	7.1 (6.3–8.1)	7.1 (6.3–8.2)	7.2 (6.4–8.3)
Median HbA1c (IQR), %	6.6 (6.2–7.2)	6.6 (6.1–7.2)	6.6 (6.2–7.3)
Median HbA1c (IQR), mmol/mol	49 (44–55)	49 (43–55)	49 (44–56)
Median C-peptide (IQR), pmol/L	1151 (865–1579)	1197 (890–1605)	1131 (838–1528)
Median albumin:creatinine ratio (IQR), mg/g	9 (4–22)	9 (4–22)	9 (4–22)
Median total cholesterol (IQR), mmol/L	4.4 (3.8–5.2)	4.3 (3.7–5.1)	4.3 (3.7–5.1)
Median LDL cholesterol (IQR), mmol/L	2.1 (1.7–2.7)	2.2 (1.7–2.8)	2.2 (1.7–2.9)
Median HDL cholesterol (IQR), mmol/L	1.2 (1–1.4)	1.2 (1–1.4)	1.2 (1–1.5)
Median triglycerides (IQR), mmol/L	1.7 (1.2–2.5)	1.7 (1.2–2.4)	1.6 (1.1–2.3)
Median hs-CRP (IQR), mg/L	2.0 (0.8–4.7)	2.0 (0.9–4.5)	1.9 (0.8–4.3)

Abbreviations: MBL, mannose-binding lectin; IQR, interquartile range; CCI, Charlson Comorbidity Index; HbA1c, hemoglobin A1c; LDL, low-density lipoprotein; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein

*Days per week with a minimum of 30 minutes of physical activity

†CCI (Charlson Comorbidity Index) score excluding diabetes.

Number of participants varied because availability of data (missing covariates are listed in Supplementary Table 2).

FIGURE 1

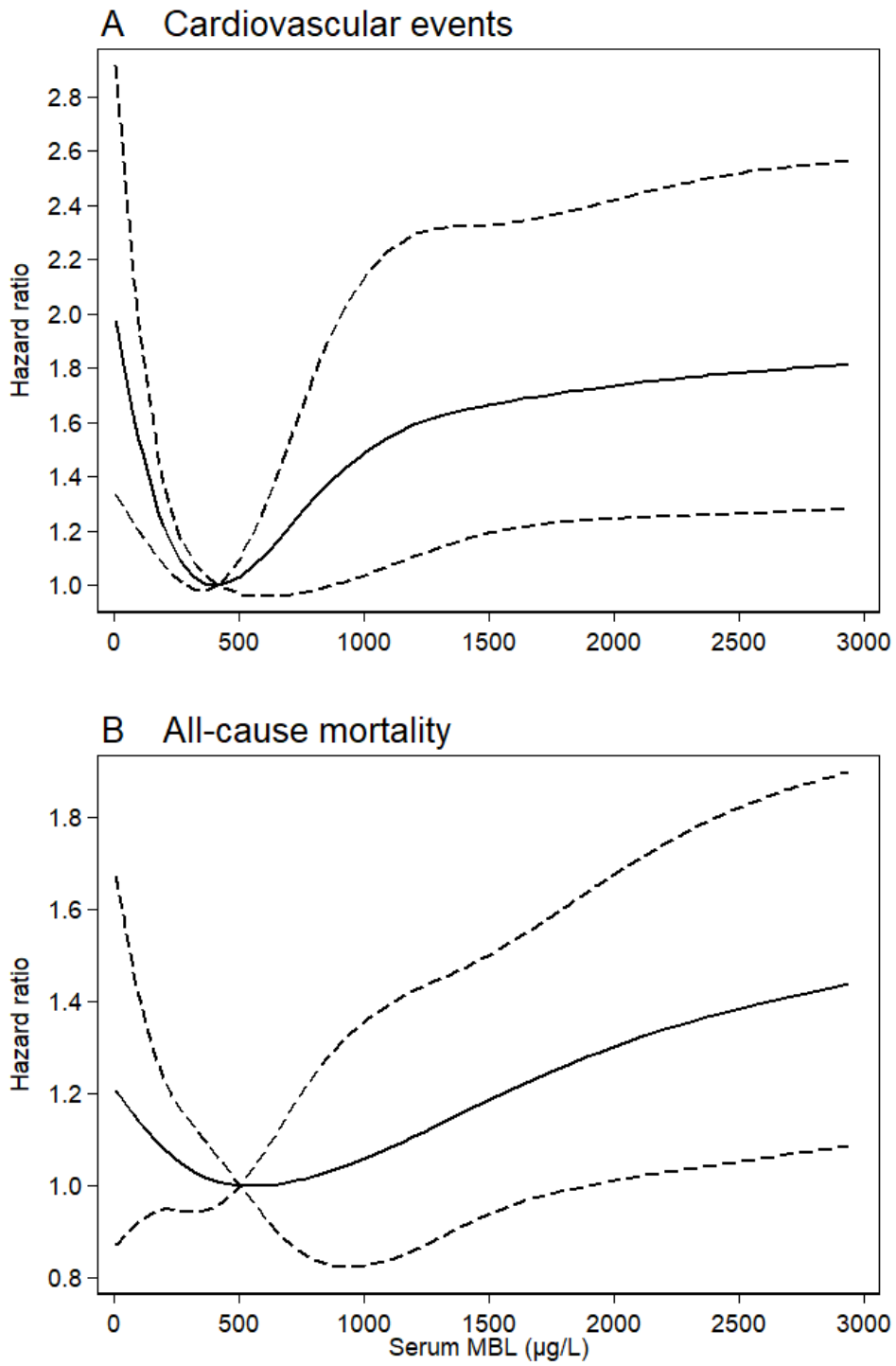


Figure 1. Risk of cardiovascular events and all-cause mortality by serum MBL levels.
Cardiovascular events (A) and all-cause mortality (B). The solid lines indicate the hazard ratios, and the dotted lines indicate 95% confidence intervals. The continuous variable serum MBL was modeled with five restricted cubic splines.

FIGURE 3

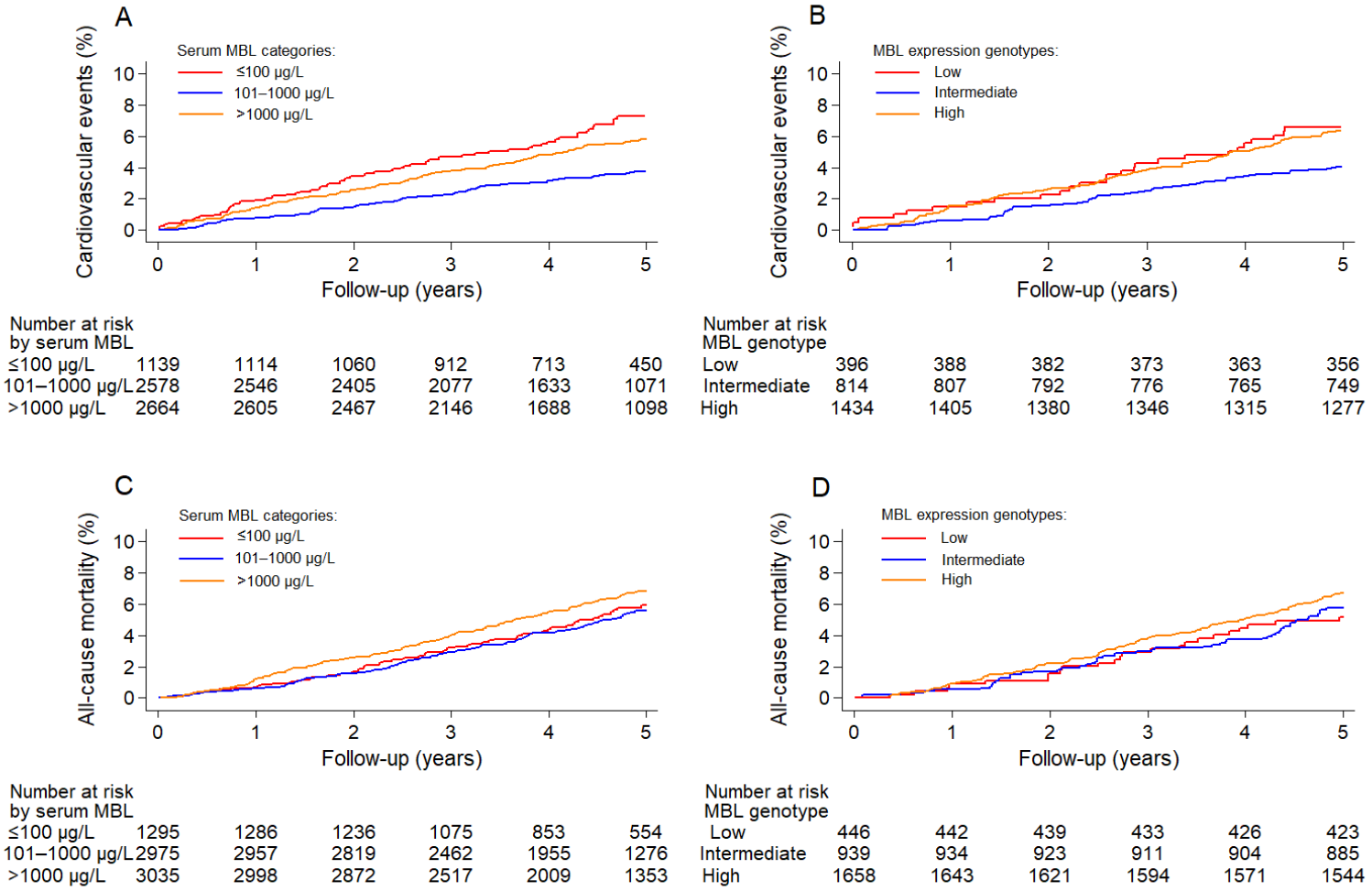


Figure 3. Time-to-event curves of cardiovascular events and all-cause mortality by serum MBL and MBL expression genotype categories. Cumulative incidence plots of cardiovascular events (A and B) and all-cause mortality (C and D) by serum MBL (A and C) and MBL expression genotype (B and D) categories. Cumulative incidence estimates are based on time from DD2 enrollment date to first event, with risk of non-cardiovascular death as a competing risk (A and B).

FIGURE 4

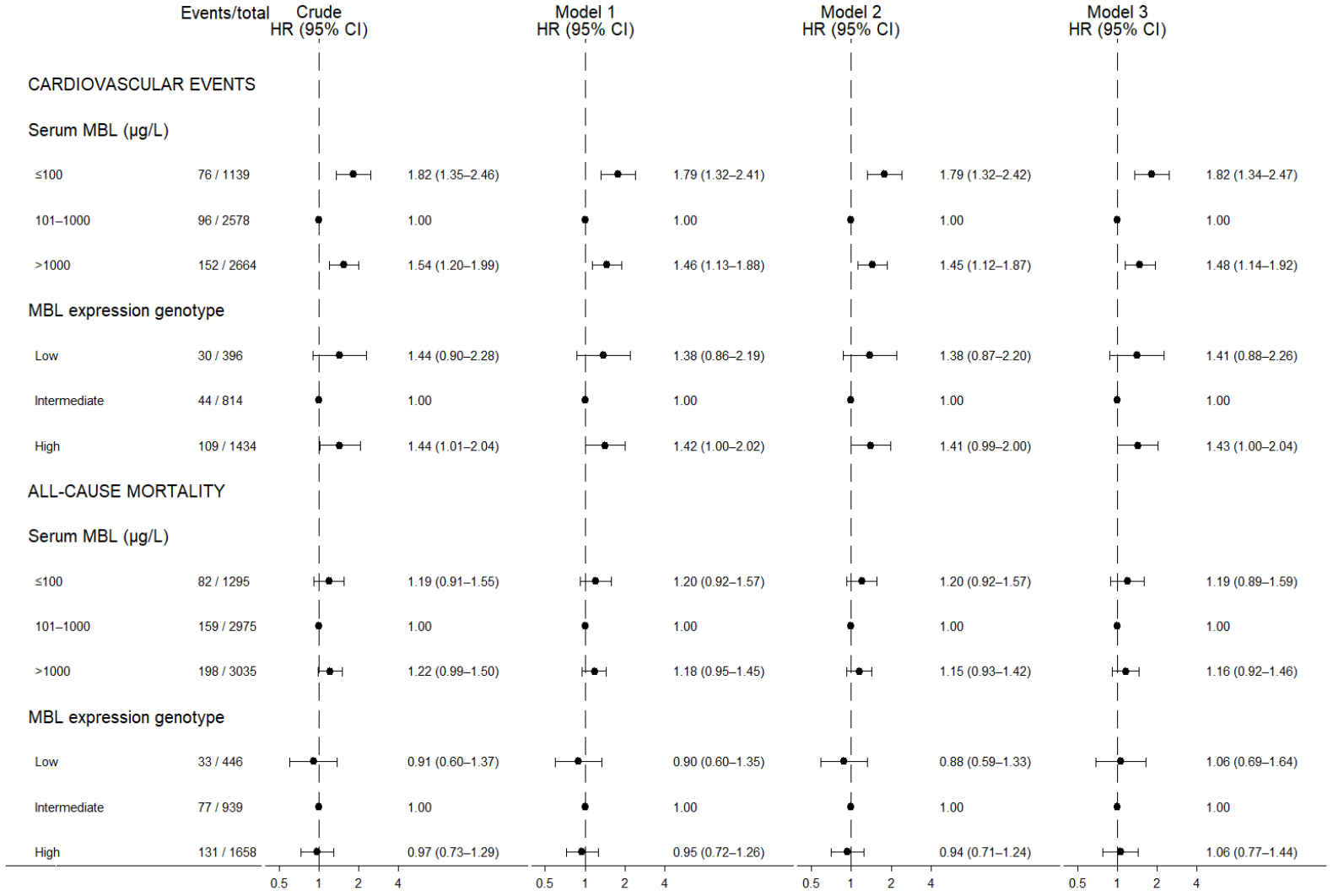


Figure 4. Hazard ratios of cardiovascular events and all-cause mortality by serum MBL and MBL expression genotype categories.
Model 1 is adjusted for sex and age. Model 2 is adjusted for sex, age, diabetes duration, and hs-CRP. Model 3 is adjusted for sex, age, diabetes duration, waist circumference, waist–hip ratio, body mass index, physical activity, smoking, systolic and diastolic blood pressure, comorbidities, fasting blood glucose, HbA1c, C-peptide, albumin:creatinine ratio, total cholesterol, low-density lipoprotein, high-density cholesterol, triglycerides, hs-CRP, and use of anti-diabetic, lipid-lowering, anti-hypertensive, and anti-thrombotic drugs. Missing covariates were treated with multiple imputation.

Mannose-binding Lectin and Risk of Cardiovascular Events and Mortality in Type 2 Diabetes: A Danish Cohort Study

SUPPLEMENTAL MATERIAL

Table of Contents

EXPANDED METHODS SECTION

MBL Expression Genotypes

Six SNPs located within the promoter region (rs11003125, rs7096206, rs7095891) and exon 1 (rs5030737, rs1800451, rs1800450) of the *MBL2* gene were genotyped using TaqMan genotyping assays, as previously described. From the six MBL polymorphisms, we generated seven common haplotypes (HYPA, LYPA, LYQA, LXPA, LYPB, LYQC, and HYPD) and ranked the haplotype combinations according to increasing serum MBL concentrations. The MBL haplotypes were divided into three MBL expression genotypes (low, intermediate, and high) based on the resulting serum MBL concentration. To compare genotype frequencies (for the six SNPs in the *MBL2* gene) between the DD2 cohort and other European cohorts, we performed a search in the Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org/) and the Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org/). ExAC spans 60,706 exome sequences and gnomAD spans 125,748 exome sequences and 15,708 whole-genome sequences from unrelated individuals sequenced as part of various disease-specific and population genetic studies (URL: http://exac.broadinstitute.org/ and https://gnomad.broadinstitute.org/). Because of differences in allele frequencies across different populations, we present information only for the European non-Finnish population from the ExAC and gnomAD databases, comprising approximately half of the total sequenced population (Supplemental Table 6). To examine possible confounding by variation in nearby genes, we searched for SNPs in linkage disequilibrium with the six SNPs used in this study. The search was performed on LDlink (https://ldlink.nci.nih.gov/), a suite of web-based applications designed to interrogate linkage disequilibrium in population groups.

Multiple Imputation

Missing data on covariates used for adjustment in the Cox regression models (Supplementary Table 3) were imputed to maximize power and avoid selection bias. We used multivariate normal imputation (MVNI)[1] to impute 20 complete data sets using a Bayesian approach with a Markov chain Monte Carlo algorithm. Missing values were sampled from the predictive distribution based on the observed data. MVNI assumes that all variables in the imputation model follow a multivariate normal distribution and that missing data are missing at random (MAR), meaning that the probability of a variable being missing depends only on the observed values. Continuous variables (fasting blood glucose, C-peptide, systolic and diastolic blood pressure, BMI, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, HbA1c, and albumin/creatinine ratio) with clearly non-normal (skewed) distributions were zero-skewness log-transformed, i.e., transformed to approximate normality before imputation. Then the imputed values were transformed back to the original scale before analysis.[2] Smoking (categorical variable) was also imputed using MVNI, which has been shown to perform well even in the presence of binary and ordinal variables.[1] The binary variable smoking (1: smoking – former/current vs. 0: never smoking) was imputed on a continuous scale and rounded to 0 or 1 by simple rounding.[3] Each variable in the data set was characterized as being 'imputed' or 'regular'. Imputed variables contain missing values, and those values are imputed. Regular variables usually do not contain missing values, or if they do, the missing values are not imputed. All covariates used in the analysis model, as well as the outcomes, were included in the imputation model to ensure maximum recovery of information about the association of interest. The following variables were characterized as 'imputed': fasting blood glucose, C-peptide, HbA1c, systolic blood pressure, diastolic blood pressure, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, albumin/creatinine ratio, height, weight, hs-CRP, waist circumference, waist-to-hip ratio, and smoking. The

following variables were characterized as ‘regular’: all-cause mortality, cardiovascular events, age, sex, diabetes duration, central obesity, anti-diabetic treatment, antihypertensive treatment, use of lipid-lowering drugs, anti-thrombotic treatment, and Charlson Comorbidity Index. The imputed models were validated by comparing the mean, median, and inter-quartile range of the first and last imputed dataset with the complete dataset.

Supplementary Table 1. Definitions and codes used in this study.

Registries	Variables	Definitions and Codes
The Danish Centre for Strategic Research in Type 2 Diabetes (DD2) cohort		A nationwide Danish cohort of individuals recently diagnosed with T2D. Cohort members have been enrolled continuously from general practitioners' offices and hospital specialist outpatient clinics since November 1, 2010. Concerning specific biomarkers in the DD2 biobank, fasting blood glucose, C-peptide, and hs-CRP (mg/L) were available for the first 5277 (72%), 5703 (78%), and 7300 (100%) DD2 cohort patients, respectively.
	-Serum high-sensitivity C-reactive protein (hs-CRP, mg/L)	-Continuous variable. hs-CRP was determined by in-house Time Resolved Immuno-fluorometric Assay, as previously described[4]. Samples were diluted 1000-fold and measured in duplicate. Intra- and interassay coefficients of variation were <5% and <6%, respectively.
	-C-peptide, pmol/L	-Continuous variable
	-Physical activity, days/week	-Categorical variable (0, 1–2, ≥3 days/week). Physical activity was defined as "number of days per week with a minimum of 30 minutes of physical activity."
	-Fasting blood glucose, mmol/L	-Continuous variable
	-Waist circumference, cm	-Continuous variable
	-Waist–hip ratio	-Continuous variable, defined as >1.0 in men and >0.85 in women
	-BMI (see below)	
The Danish Diabetes Database of Adults (DDDA)		A nationwide quality-of-care database were available for a subcohort of 5847 patients (~80%). For all DDDA variables **except height**: We used the measure closest to the DD2 enrollment date. All measures before or after DD2 enrollment were eligible for use. If a variable was measured exactly the same number of days before and after the DD2 enrollment date, we used the measure prior to DD2 enrollment.
	-Blood pressure, mmHg	-Continuous variables: systolic and diastolic blood pressure
	-Lipids, mmol/L	-Continuous variables: LDL, HDL, triglycerides, total cholesterol
	-HbA1c, %	-Continuous variable
	-Smoking	-Categorical variable: never, former, current (daily + occasionally)
	-Albumin:creatinine ratio	-Continuous variable
	-BMI (see below)	
BMI, kg/m^2		
	Height	Data on height were available from 3 sources: DD2 enrollment (2010 onwards), DDDA data (repeated measures), questionnaire data 2016 (self-reported).

	BMI DD2 enrollment	Continuous variable Weight: If weight was recorded during the DD2 enrollment process (few), we used this weight; otherwise, we used the DDDA weight. Height: We did not expect height to change over time among the adults in our study. Thus, we used available heights in the following hierarchical order: height obtained at DD2 enrollment, height obtained at DDDA enrollment, and questionnaire data obtained in 2016.
Diabetes duration		Time from first of the following events until the DD2 enrollment date: prescription of glucose-lowering drugs, first diabetes-related diagnosis in the Danish National Patient Registry, or DDDA registration. In the absence of information from a prior drug prescription, diabetes diagnosis from the DNPR, or DDDA registration, diabetes duration was set to DD2 enrollment date = 0.
The Civil Registration System	-Age -Sex	-Continuous variable -Male/female
The Danish Health Service Prescription database		For all prescription data, the relevant time period was around baseline (DD2 enrollment). The look-back period was 1 year prior to the DD2 enrollment date. Yes/no redemption of a drug prescription during the year prior to the index date.
	-Anti-diabetic drugs -Lipid-lowering drugs -Statins -Anti-hypertensive drugs -Anti-thrombotic drugs	ATC: A10A, A10B ATC: C10 ATC: C10AA, C10BA, C10BX ATC: C02, C03A, C03B, C03D, C03E, C07, C08, C09A, C09B, C09C, C09D, C09X B01AC04, B01AC06, B01AC07, B01AC22, B01AC24, B01AC30, N02BA01
ICD-10 and procedure codes used to identify CVE from the Danish National Patient Registry or the Danish Registry of Causes of Death		First-time inpatient hospital admission (with a date after the index date) with one of the following ICD-10 or procedure codes as primary or secondary discharge diagnosis:

	Acute myocardial infarction	DI21
	Combined ischemic stroke	DI63, DI64
	Cardiovascular death	DI00-DI99
	Coronary revascularization	KFNA, KFNB, KFNC, KFND, KFNE, KFNF, KFNG, KFNH20,
	Unstable angina pectoris	DI200
The Danish National Patient Registry		For all variables, the relevant time period was before DD2 enrollment (as a proxy for medical history prior to the diabetes diagnosis). Thus, the look-back period extended from the DD2 enrollment date back to 1994 (based on *International Classification of Diseases, Tenth Revision*, diagnosis codes).
	Any macrovascular complications	DI21, DI23, DI24, DT822A (ischemic heart disease); DT823 (acute ischemic heart disease with/without complications); DI20 (angina pectoris); DI25 (chronic ischemic heart disease); KFNA, KFNB, KFNC, KFND, KFNE, KFNF, KFNG, KFNH, KFNW, KFLF (coronary bypass or percutaneous coronary intervention); DI500, DI501, DI502, DI503, DI508, DI509, DI110, DI130, DI132, DI420, DI426, DI427, DI428, DI429 (heart failure); DI61 (cerebral bleeding); DI63, DI64, DI65, DI66 (cerebrovascular infarct); DG45 (transient cerebrovascular disease); DI672, DI678, DI679 (unspecified cerebrovascular disease); DI691, DI693, DI694, DI698 (previous cerebrovascular disease); KAAL10, KAAL11 (cerebral thrombolysis or thromboendarterectomy) (Abdominal- and peripheral vascular disease); DE105, DE115, DE125, DE135, DE145 (diabetes with peripheral vascular complications); DI700, DI701, DI702, DI708, DI709, DI739, DI74, DN280, DK550, DK551, DH340, DH341, DH342 (peripheral/abdominal vascular disease); KNBQ, KNCQ, KNDQ, KNEQ, KNFQ, KNGQ, KNHQ, KPAE, KPAF, KPAH, KPAN, KPAP, KPAQ, KPAW99, KPAU74, KPBE, KPBF, KPBH, KPBN, KPBP, KPBQ, KPBW, KPGH10, KPCE, KPCF, KPCH, KPCN, KPCP, KPCQ, KPCW99, KPCW20, KPCU74, KPCU82, KPCU83, KPCU84, KPGE, KPGF, KPGH, KPGN, KPGP, KPGQ, KPGW99, KPGW20, KPEE, KPEF, KPEH, KPEN, KPEP, KPEQ, KPEW, KPFE, KPFH, KPFN, KPFP, KPFQ, KPFW, KPGH20, KPGH21, KPGH22, KPGH23, KPGH30, KPGH31, KPGH40, KPGH99, KPDU74, KPDU82, KPDU83, KPDU84, KPEU74, KPEU82, KPEU83, KPEU84, KPFU74, KPFU82, KPFU83, KPFU84, KPGU74, KPGU83, KPGU84, KPGU99 (vascular surgery)
Modified Charlson Comorbidity Index		We categorized comorbidities according to the Charlson Comorbidity Index (CCI) within the 10-year period before the DD2 enrollment date. Diabetes was not included in the CCI scoring system because it constituted the index disease for our cohort.
	Score 1	
	Myocardial infarction	DI21, DI22, DI23

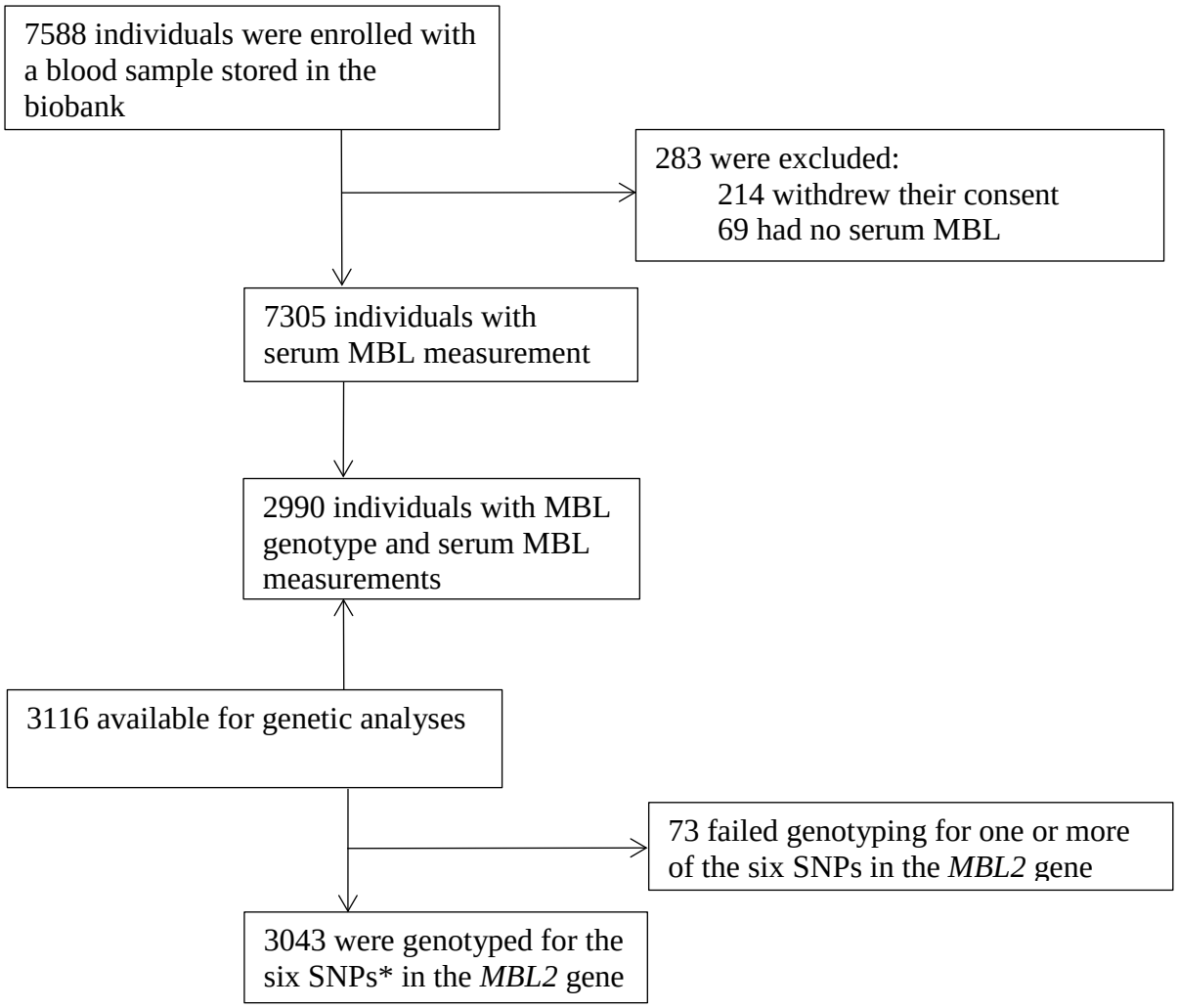
Congestive heart failure	DI50, DI110, DI130, DI132
Peripheral vascular disease	DI70, DI71, DI72, DI73, DI74, DI77
Cerebrovascular disease	DI60, DI61, DI62, DI63, DI64, DI65, DI66, DI67, DI68, DI69, DG45, DG46
Dementia	DF00, DF02, DF03, DF051, DG30
Chronic pulmonary disease	DJ40, DJ41, DJ42, DJ43, DJ44, DJ45, DJ46, DJ47, DJ60, DJ61, , DJ62, , DJ63, , DJ64, , DJ65, , DJ66, , DJ67, DJ684, DJ701, DJ703, DJ841, DJ920, DJ961, DJ982, DJ983
Connective tissue disease	DM05, DM06, DM08, DM09, DM30, DM31, DM32, DM33, DM34, DM35, DM36, DD86
Ulcer disease	DK221, DK25, DK26, DK27, DK28
Mild liver disease	DB18, DK700, D701, DK702, DK703, DK709, DK71, DK73, DK74, DK760
Score 2	
Hemiplegia	DG81, DG82
Moderate to severe renal disease	D12, DI13, DN00, DN01, DN02, DN03, DN04, DN05, DN07, DN11, DN14, DN17, DN18, DN19, DQ61
Any tumor (except basocellular carcinoma)	C00–C75, (excluding C44)
Leukemia	DC91, DC92, DC93, DC94, DC95
Lymphoma	DC81, DC82, DC83, DC84, DC85, DC90, DC96
Score 3	
Moderate to severe liver disease	DB150, DB160, DB162, DB190, DK704, DK72, DK766, DI85
Score 6	
Metastatic solid tumor	DC76, DC77, DC78, DC79, DC80
AIDS	DB21, DB22, DB23, DB24

Supplementary Table 2. Missing covariates for the serum MBL and MBL expression genotype cohorts.

	Serum MBL cohort		**MBL expression genotype cohort**	
	Missing, n (%)	Total	Missing, n (%)	Total
Sex	0 (0.0)	7305	0 (0.0)	3043
Age	0 (0.0)	7305	0 (0.0)	3043
Diabetes duration	0 (0.0)	7305	0 (0.0)	3043
Waist circumference	13 (0.18)	7305	<5** (0.2)	3043
Waist–hip ratio	11 (0.15)	7305	<5** (0.2)	3043
BMI*	569 (7.79)	7305	245 (8.05)	3043
Physical activity	<5† (0.0)	7305	0 (0.0)	3043
Smoking*	1900 (26.01)	7305	612 (20.11)	3043
Systolic blood pressure*	1774 (24.28)	7305	547 (17.98)	3043
Diastolic blood pressure*	1774 (24.28)	7305	547 (17.98)	3043
CCI score	0 (0.0)	7305	0 (0.0)	3043
Anti-diabetes drug use	0 (0.0)	7305	0 (0.0)	3043
Lipid-lowering drug use	0 (0.0)	7305	0 (0.0)	3043
Anti-hypertensive drug use	0 (0.0)	7305	0 (0.0)	3043
Anti-thrombotic drug use	0 (0.0)	7305	0 (0.0)	3043
Fasting blood glucose	2028 (27.76)	7305	368 (12.09)	3043
HbA1C*	1548 (21.19)	7305	486 (15.97)	3043
C-peptide	1602 (21.93)	7305	24 (0.79)	3043
Albumin:creatinine ratio*	2074 (28.39)	7305	653 (21.46)	3043
Total cholesterol*	3966 (54.29)	7305	1244 (40.88)	3043
LDL cholesterol*	1763 (24.13)	7305	550 (18.07)	3043
HDL cholesterol*	3951 (54.09)	7305	1245 (40.91)	3043
Triglycerides*	1849 (25.31)	7305	572 (18.80)	3043
hs-CRP	5 (0.07)	7305	56 (1.84)	3043

*By August 2018, a total of 5847 DD2 patients (80%) in the serum MBL cohort and 2597 DD2 patients (85%) in the MBL genotype cohort had been linked to the Danish Diabetes Database for Adults.
†Exact number of missing too low to be displayed according to Danish data protection regulations.

EXPANDED RESULTS SECTION



Supplementary Figure 1. Flow diagram of the study population.

*The six SNPs in the *MBL2* gene were: rs11003125, rs7096206, rs7095891, rs5030737, rs1800451, and rs1800450.

Supplementary Table 3. Characteristics of DD2 cohort members at baseline by MBL expression genotype category.

	Low MBL expression genotype	Intermediate MBL expression genotype	High MBL expression genotype
Total, N (%)	446 (14.7)	939 (30.8)	1658 (54.5)
Male sex, n (%)	277 (62.1)	533 (56.8)	944 (56.9)
Median age (IQR), years	61.3 (52.5–68.3)	61.3 (52.9–67.7)	62.0 (53.3–68.1)
Median diabetes duration (IQR), years	0.8 (0.2–2.2)	0.8 (0.2–2.0)	0.8 (0.3–2.1)
Median waist circumference (IQR), cm	104 (96–114)	105 (96–116)	106 (97–116)
Median waist–hip ratio (IQR)	0.97 (0.92–1.03)	0.97 (0.91–1.04)	0.97 (0.92–1.03)
Median body mass index (IQR), kg/m^2	29.7 (26.6–33.8)	30.4 (27.0–34.1)	30.3 (26.8–34.3)
Physical activity* (IQR), days/week	3 (2–7)	3 (1–7)	3 (2–7)
Smoking, n (%)			
Never	158 (45.0)	382 (51.2)	667 (50.0)
Former	131 (37.3)	220 (29.5)	416 (31.2)
Current	62 (17.7)	144 (19.3)	251 (18.8)
Median systolic blood pressure (IQR), mmHg	130 (124–140)	130 (124–140)	130 (124–140)
Median diastolic blood pressure (IQR), mmHg	80 (74–85)	80 (74–85)	80 (75–86)
CCI score†, n (%)			
0	296 (66.4)	636 (67.7)	1129 (68.1)
1–2	125 (28.0)	253 (26.9)	430 (25.9)
3	25 (5.6)	50 (5.3)	99 (6.0)
Anti-diabetes drug use, n (%)	356 (79.8)	784 (83.5)	1388 (83.7)
Lipid-lowering drug use, n (%)	295 (66.1)	652 (69.4)	1176 (70.9)
Anti-hypertensive drug use, n (%)	306 (68.6)	663 (70.6)	1201 (72.4)
Anti-thrombotic drug use, n (%)	122 (27.4)	309 (32.9)	551 (33.2)
Median fasting blood glucose (IQR), mmol/L	7.2 (6.4–8.3)	7.0 (6.3–8.2)	7.1 (6.4–8.3)
Median HbA1c (IQR), %	6.5 (6.2–7.1)	6.5 (6.1–7.2)	6.5 (6.1–7.1)
Median HbA1c (IQR), mmol/mol	48 (44–54)	48 (43–55)	48 (43–54)
Median C-peptide (IQR), pmol/L	1114 (837–1539)	1143 (846–1558)	1191 (908–1595)
Median albumin/creatinine ratio (IQR), mg/g	9 (4–22)	9 (4–22)	9 (4–25)
Median total cholesterol (IQR), mmol/L	4.3 (3.6–5.1)	4.5 (3.8–5.2)	4.3 (3.7–5.1)
Median LDL cholesterol (IQR), mmol/L	2.2 (1.8–2.9)	2.3 (1.8–2.9)	2.2 (1.8–2.8)
Median HDL cholesterol (IQR), mmol/L	1.2 (1–1.4)	1.2 (1–1.5)	1.2 (1–1.5)
Median triglycerides (IQR), mmol/L	1.5 (1.1–2.3)	1.6 (1.1–2.3)	1.6 (1.2–2.4)
Median hs-CRP (IQR), mg/L	1.8 (0.7–4.3)	2.0 (0.8–4.1)	1.9 (0.8–4.6)

MBL: mannose-binding lectin; IQR: interquartile range; CCI: Charlson Comorbidity Index; HbA1c: hemoglobin A1c; LDL: low-density lipoprotein; HDL: high-density lipoprotein; hs-CRP: high-sensitivity C-reactive protein
*Days per week with minimum 30 minutes of physical activity
†CCI score excluding diabetes
Number of participants varies because of availability of data (see Supplementary Table 2 for missing covariates).

Supplementary Table 4. Incidence/mortality rates per 1,000 person-years among 7305 individuals with T2D according to MBL categories.

	Cardiovascular Events	All-cause mortality
	Incidence rates (95% CI)	**Mortality rates (95% CI)**
Serum MBL (µg/L)		
≤100	14.9 (11.9–18.7)	13.7 (11.0–17.0)
101–1000	8.2 (6.7–10.0)	11.5 (9.8–13.4)
>1000	12.6 (10.8–14.8)	14.0 (12.2–16.1)
MBL expression genotype		
Low	13.3 (9.3–19.0)	12.5 (8.9–17.6)
Intermediate	9.3 (6.9–12.5)	13.8 (11.0–17.2)
High	13.3 (11.0–16.1)	13.4 (11.3–15.9)

Supplementary Table 5. Haplotypes and serum MBL levels in 3043 individuals with T2D according to low, intermediate, and high MBL expression genotypes.

Haplotype	Number (%)	Median serum MBL concentration (µg/L)
All low MBL expression genotypes	446 (14.7)	10 (10–26)
LXPA/LYQC	19 (0.6)	10 (10–42)
LXPA/LYPB	180 (5.9)	10 (10–20)
LXPA/HYPD	99 (3.3)	28 (10–80)
LYPB/LYPB	63 (2.1)	10 (10–10)
LYPB/LYQC	14 (0.5)	10 (10–10)
LYPB/HYPD	40 (1.3)	10 (10–10)
LYPB/LYPD*	<10 (0.3)	116
LYQC/LYQC*	<10 (0.3)	10
LYQC/HYPD*	<10 (0.3)	10 (10–12)
HYPD/HYPD	23 (0.8)	10 (10–10)
All intermediate MBL expression genotypes	939 (30.9)	321 (199–545)
LXPA/LXPA	151 (5.0)	253 (102–594)
HYPA/LYPB	285 (9.4)	313 (225–407)
LYPA/LYPB	56 (1.8)	195 (115–297)
LYQA/LYPB	172 (5.7)	259 (179–338)
HYPA/LYQC	27 (0.9)	293 (210–402)
LYPA/LYQC*	<10 (0.3)	292 (244–488)
LYQA/LYQC	22 (0.7)	247 (197–358)
HYPA/HYPD	109 (3.6)	740 (501–1050)
LYPA/HYPD	35 (1.2)	441 (276–746)
LYQA/HYPD	75 (2.5)	631 (465–832)
LYQA/LYPD*	<10 (0.3)	873
All high MBL expression genotypes	1658 (54.5)	1527 (974–2394)
HYPA/HYPA	276 (9.1)	1911 (1185–2974)
HYPA/LYPA	98 (3.2)	2010 (1381– 2611)
HYPA/LYQA	347 (11.4)	2022 (1349–2888)
HYPA/LXPA	400 (13.1)	1162 (784–1714)
LYPA/LYPA*	<10 (0.3)	2571 (1141–2738)
LYPA/LYQA	67 (2.2)	1785 (1151–2706)
LYPA/LXPA	86 (2.8)	1157 (613–1812)
LYQA/LYQA	129 (4.2)	2025 (1317–2769)
LYQA/LXPA	250 (8.2)	1129 (716–1643)

*Exact number of haplotypes too low to be displayed according to Danish data protection regulations.

Supplementary Table 6. Allele frequencies of the six SNPs in the *MBL2* gene.

SNP	Genotype*	Serum MBL† µg/L	DD2 cohort N (%)	HWE	ExAC database‡ N (%)	HWE	gnomeAD database‡ N (%)	HWE
Promoter region								
rs11003125	HH	1333 (761–2375)	408 (13.4)		NA		1081 (14.1)	
(-550 G>C)	LH	889 (321–1816)	1411 (46.4)	0.97	NA	NA	3647 (47.4)	0.45
	LL	404 (68–1254)	1224 (40.2)		NA		2964 (38.5)	
rs7096206	YY	828 (281–1997)	1857 (61.0)		NA		4594 (59.7)	
(-221 G>C)	XY	835 (73–1432)	1035 (34.0)	0.66	NA	NA	2700 (35.0)	0.69
	XX	253 (102–594)	151 (5.0)		NA		407 (5.3)	
rs7095891§	QQ	1807 (1046–2579)	152 (5.0)		NA		304 (3.9)	
(c.+4 C>T)	PQ	1087 (416–2036)	986 (32.4)	0.10	NA	NA	2563 (33.3)	0.12
	PP	546 (118–1381)	1905 (62.6)		NA		4841 (62.8)	
Exon 1								
rs5030737	AA	909 (274–1851)	2653 (87.1)		28,503 (85.4)		55,308 (85.8)	
(p. Arg52Cys)	AD (or AO)	344 (26–748)	367 (12.1)	0.01	4632 (13.9)	0.03	8743 (13.6)	0.39
	DD (or OO)	10 (10–10)	23 (0.8)		221 (0.7)		363 (0.6)	
rs1800451	AA	1188 (597–2071)	2224 (73.1)		32,115 (96.4)		62,059 (96.4)	
(p. Gly54Asp)	AB (or AO)	203 (18–331)	754 (24.8)	0.91	1198 (3.6)	0.41	2308 (3.6)	0.45
	BB (or OO)	10 (10–10)	65 (2.1)		14 (0.0)		25 (0.0)	
rs1800450	AA	826 (250–1716)	2948 (96.9)		24,337 (73.0)		47,173 (73.3)	
(p. Gly57Glu)	AC (or AO)	181 (10–313)	94 (3.1)	0.78	8285 (24.8)	0.54	15836 (24.6)	0.18
	CC (or OO)	10 (10–10)	1 (0.0)		724 (2.2)		1387 (2.1)	

Abbreviations: DD2, the Danish Centre for Strategic Research in Type 2 Diabetes; HWE, Hardy–Weinberg equilibrium

NA=not available or not applicable because the ExAC database spans only exome sequences and thus does not cover the promoter region.

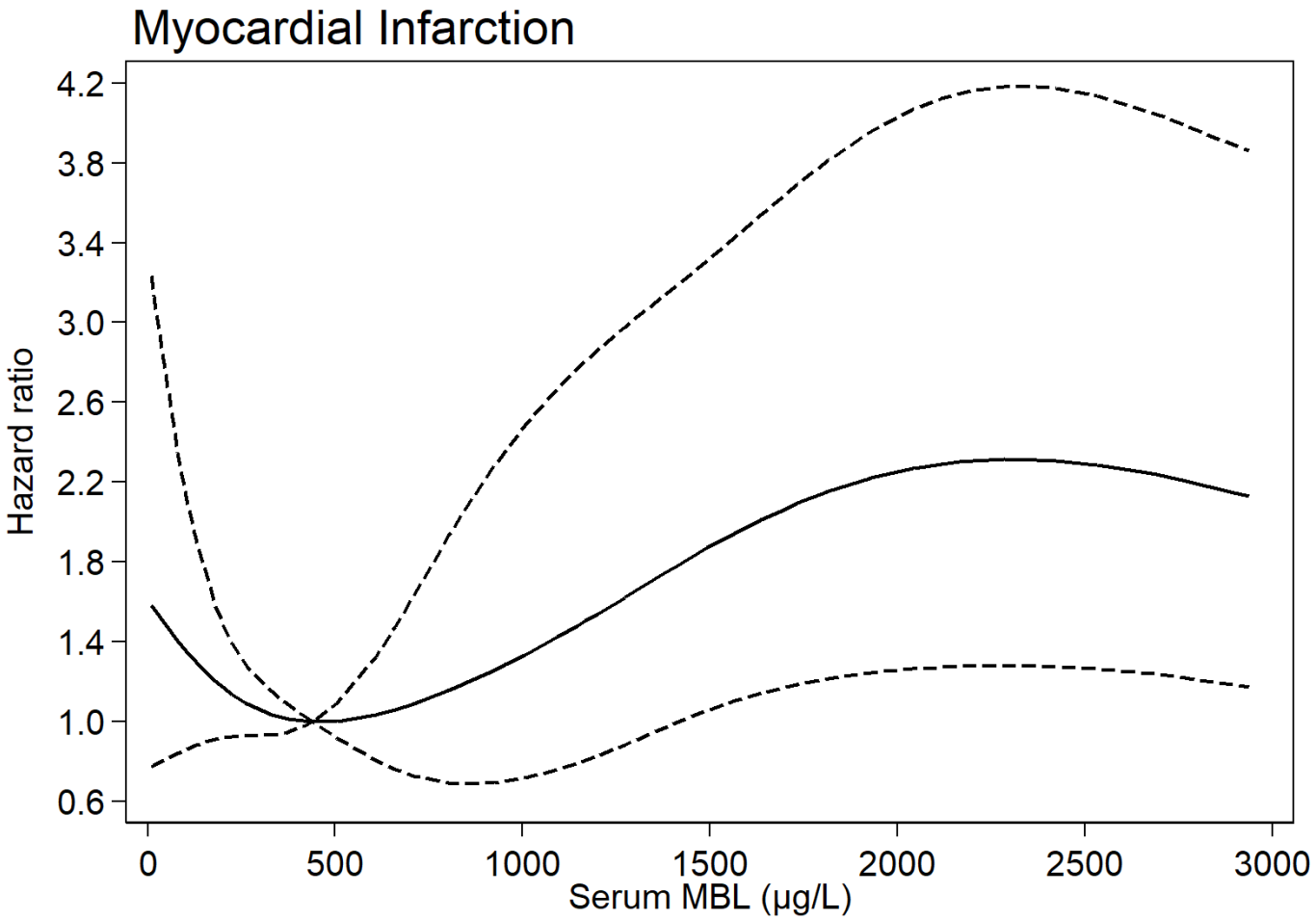
* The major alleles of the three SNPs in exon 1 are all referred to as the A allele, while the minor alleles (B, C, and D) are collectively referred to as the O allele.

† Serum MBL is expressed as median (interquartile range).

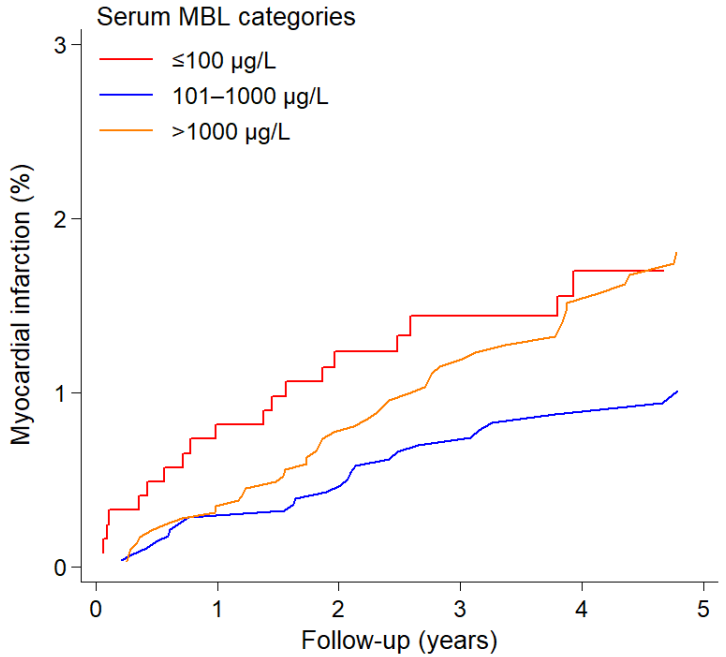
‡The Exome Aggregation Consortium (ExAC) spans 60,706 exome sequences and the Genome Aggregation Database (gnomAD) spans 125,748 exome sequences and 15,708 whole-genome sequences from unrelated individuals sequenced as part of various disease-specific and population genetic studies (URL: http://exac.broadinstitute.org/ and https://gnomad.broadinstitute.org/) [August 2019].

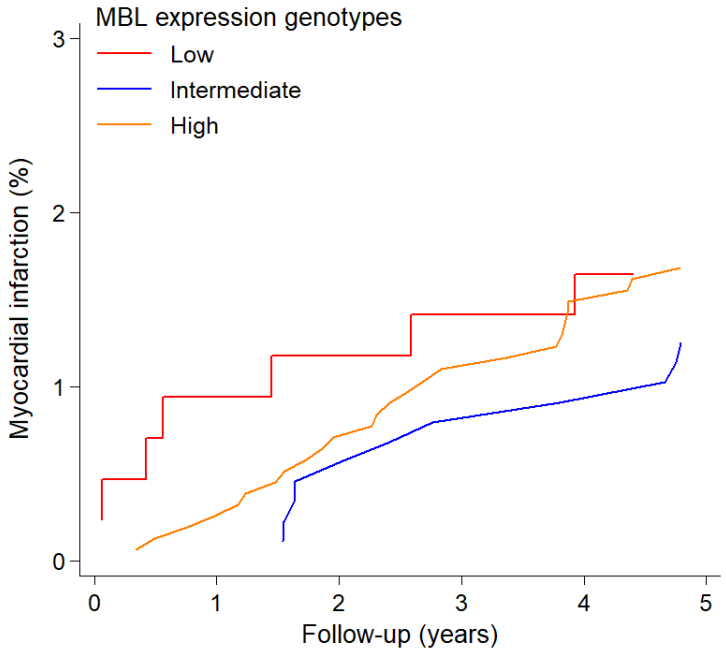
Because of differences in allele frequencies across different populations, we present only information for the European non-Finnish population from the ExAC and gnomAD databases, comprising around half of the total population sequenced.
§Previously known as rs12780112.
A minor deviation was observed for rs5030737 because of a slightly higher number of rare homozygotes than expected. We believe that this results from chance. However, even if this resulted from a minor genotyping error, potential misclassification of the MBL expression genotype (based on the seven haplotypes) is unlikely to be extensive and would possibly bias the results toward the null hypothesis.

SUBTYPES OF CARDIOVASCULAR EVENTS



Supplementary Figure 2. Risk of Myocardial Infarction by Serum MBL Levels.



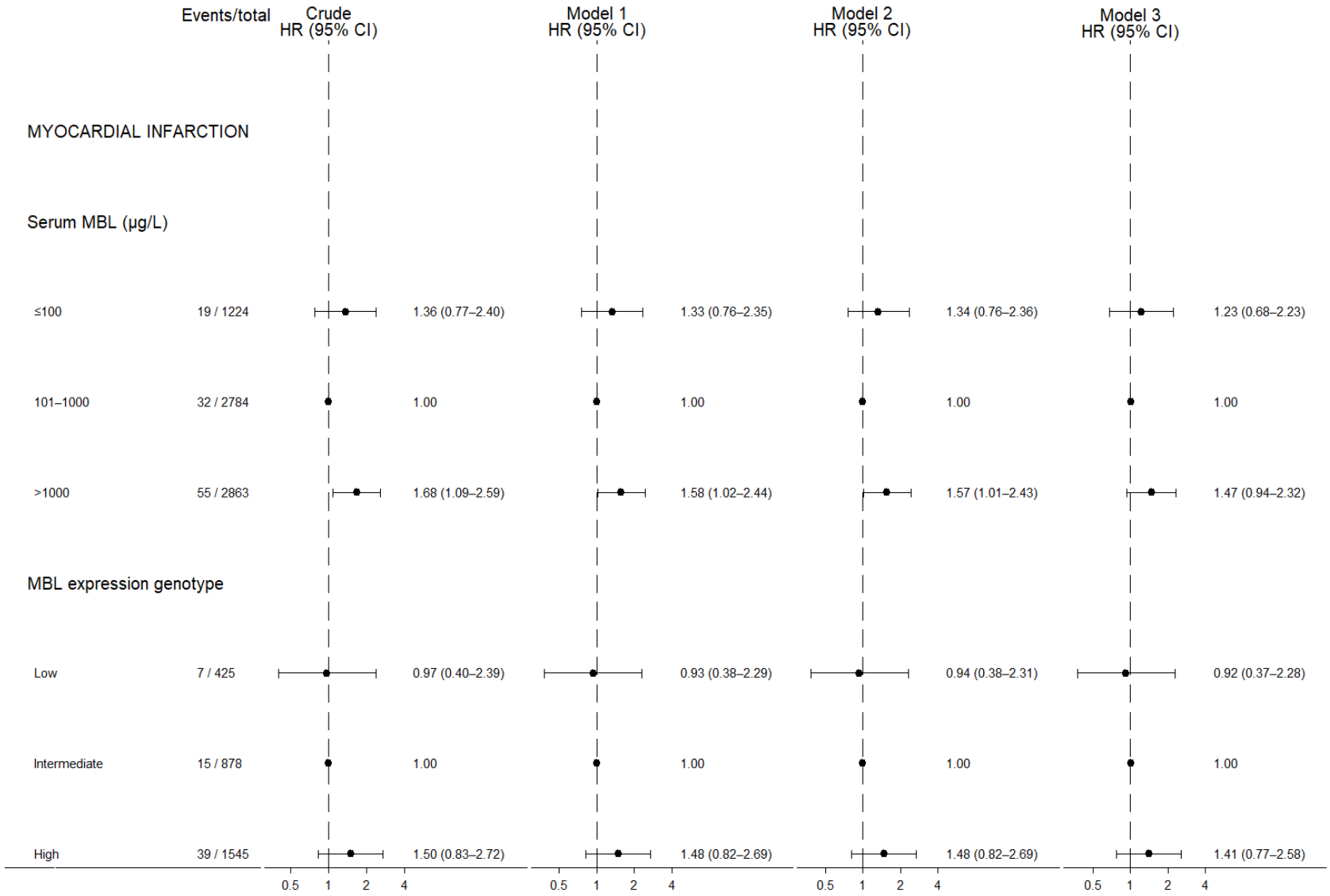


Number at risk by serum MBL						
≤100 µg/L	1224	1208	1156	1001	789	511
101–1000 µg/L	2784	2760	2627	2286	1801	1180
>1000 µg/L	2863	2821	2683	2334	1849	1215

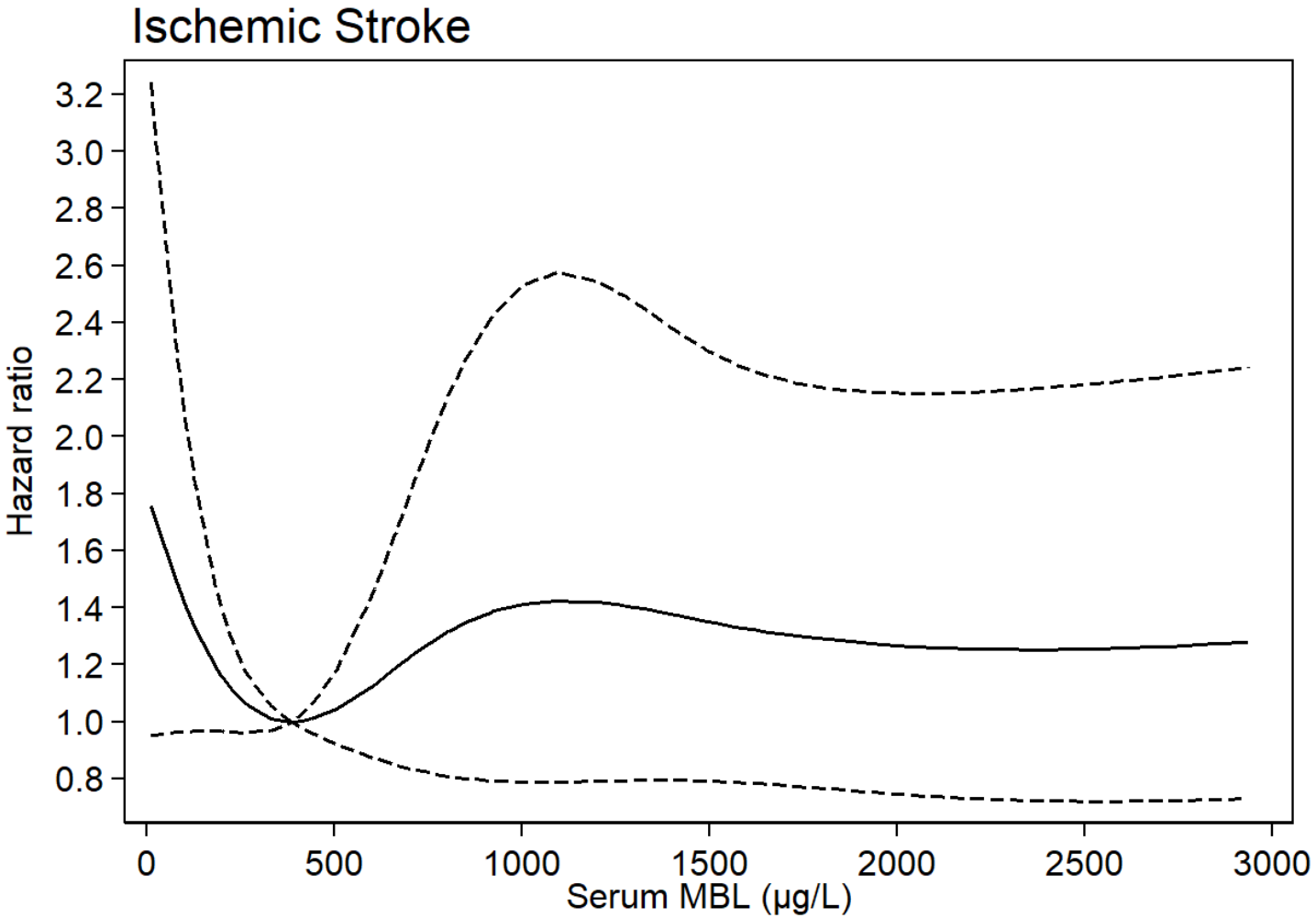
Number at risk MBL genotype						
Low	425	419	415	411	403	400
Intermediate	878	875	862	849	841	822
High	1545	1527	1503	1473	1448	1419

Supplementary Figure 3 Time-to-Event Curves of Myocardial Infarction by Serum MBL and MBL Expression Genotype Categories.

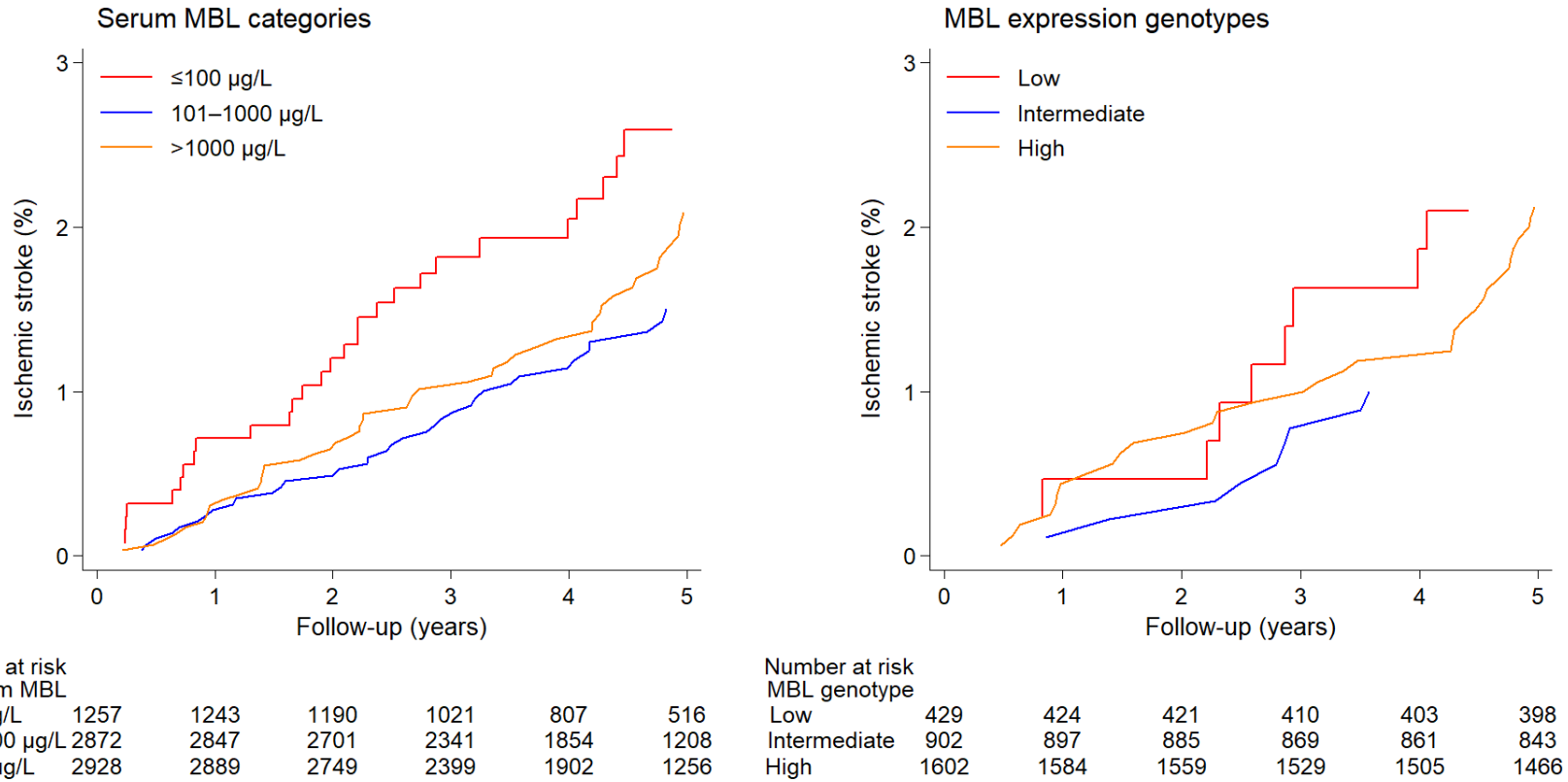
Time-to-event curves of myocardial infarction (considering death as a competing risk) divided into 3 groups of serum MBL (left) in 6871 individuals with type 2 diabetes. Right panel shows the association between low, intermediate, and high MBL expression genotypes in 2848 individuals with type 2 diabetes.



Supplementary Figure 4. Hazard Ratios of Myocardial Infarction by Serum MBL and MBL Expression Genotype Categories.

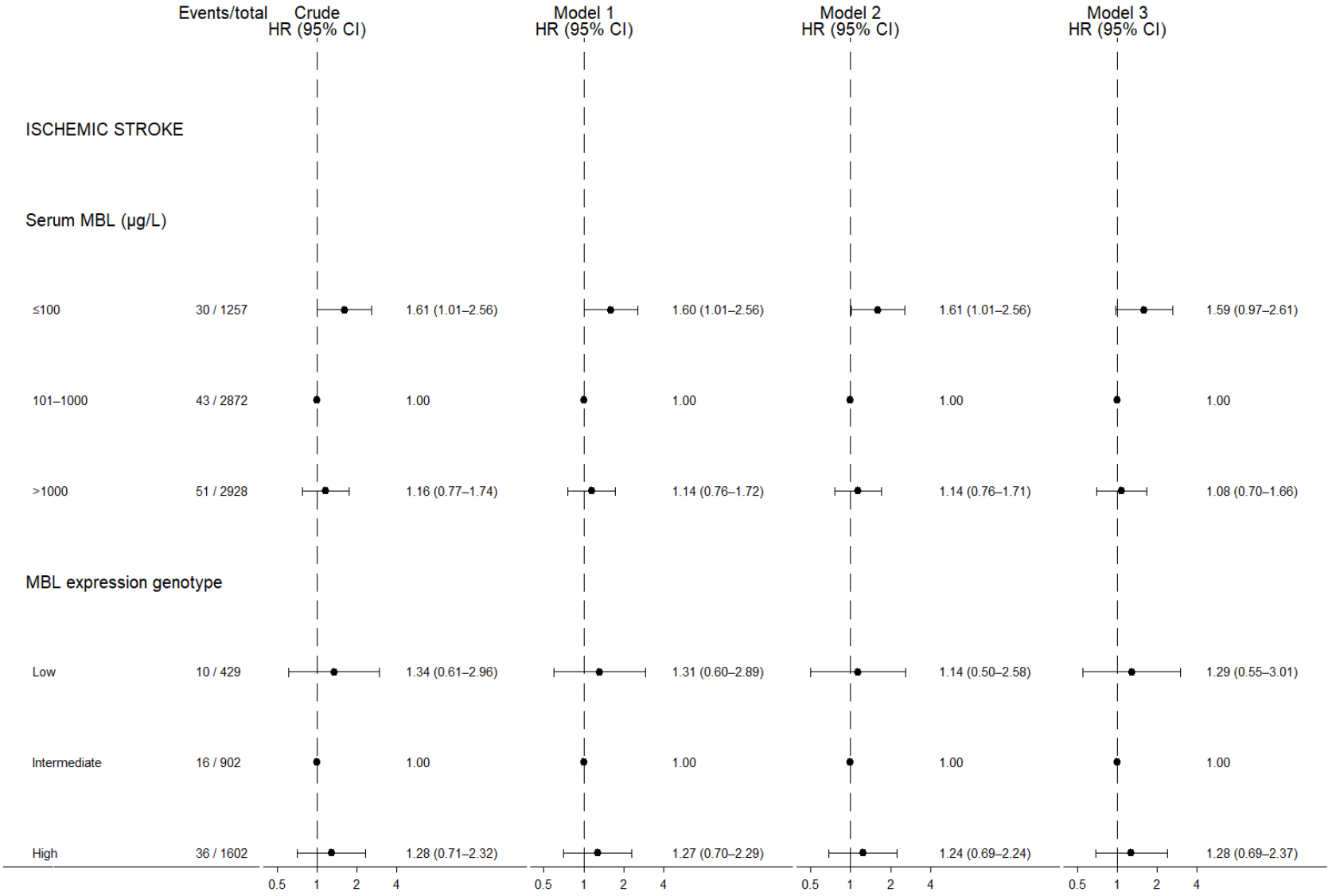


Supplementary Figure 5. Risk of Ischemic Stroke by Serum MBL Levels.

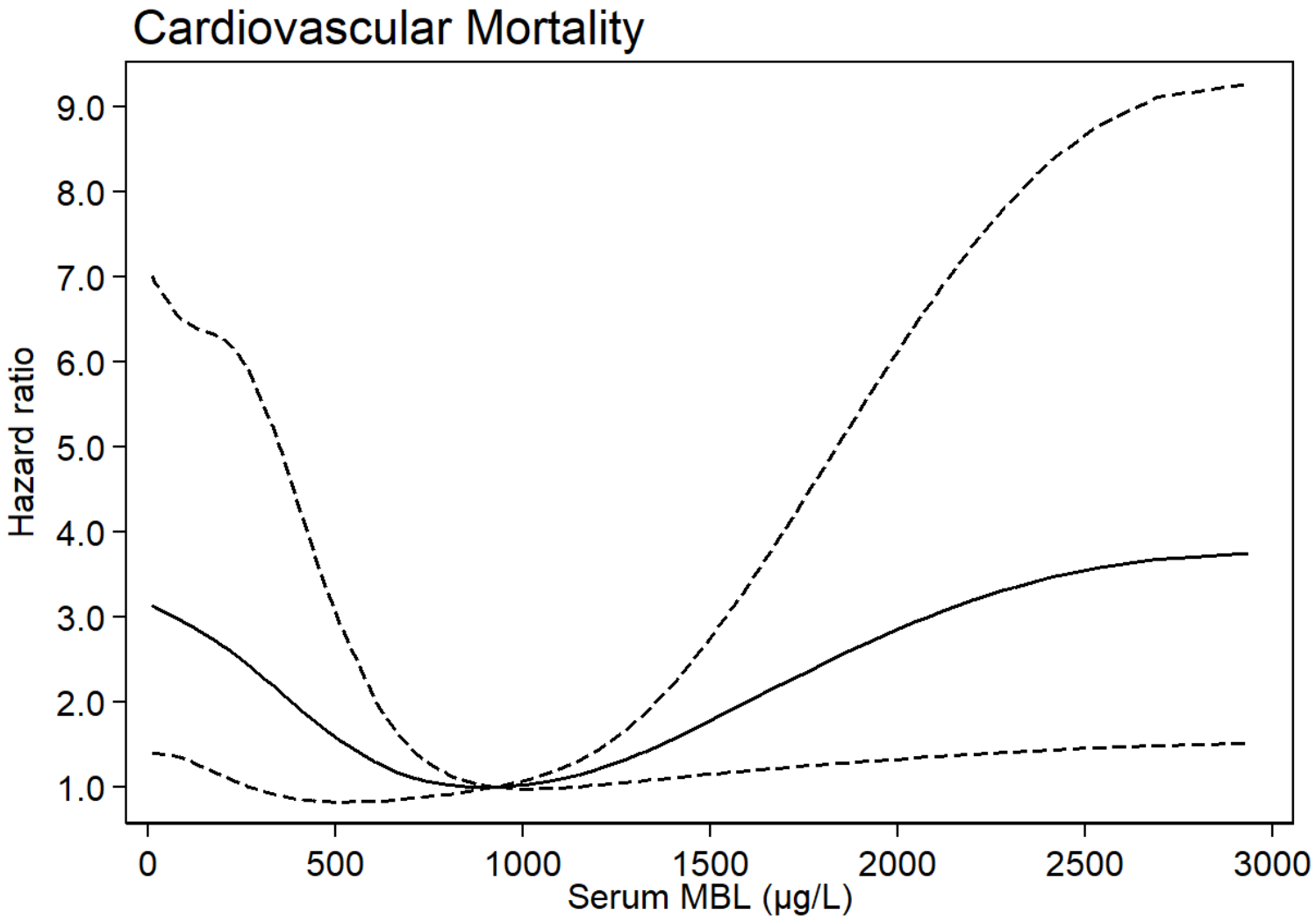


Supplementary Figure 6. Time-to-Event Curves of Ischemic Stroke by Serum MBL and MBL Expression Genotype Categories.

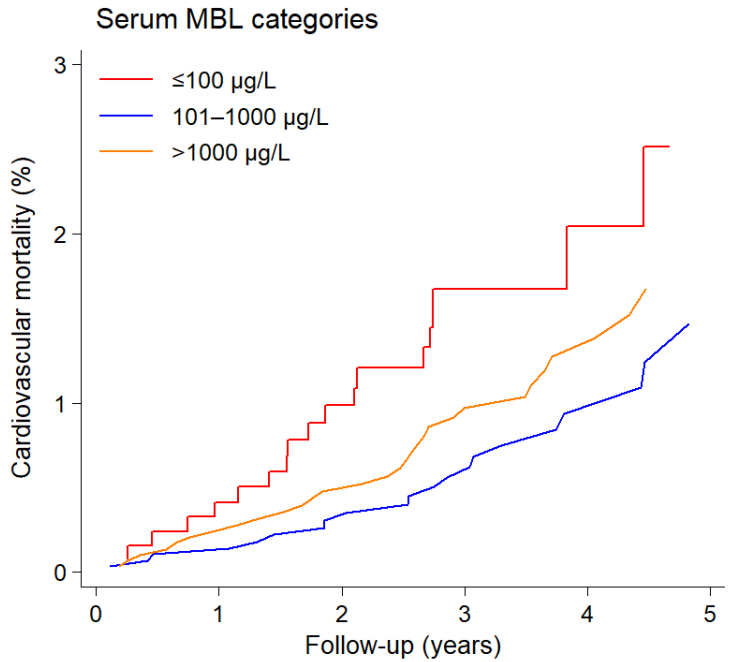
Time-to-event curves of ischemic stroke (considering death as a competing risk) divided into 3 groups of serum MBL (left) in 7057 individuals with type 2 diabetes. Right panel shows the association between low, intermediate, and high MBL expression genotypes in 2933 individuals with type 2 diabetes.



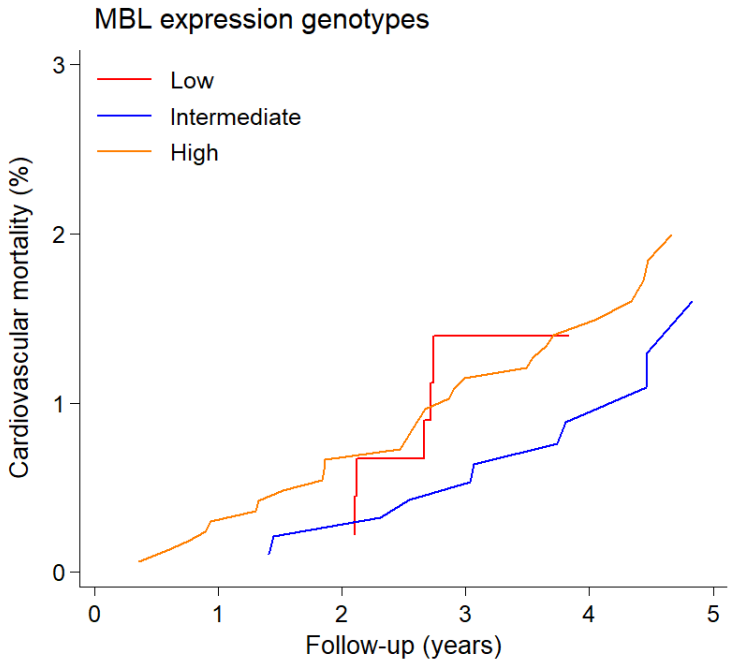
Supplementary Figure 7. Hazard Ratios of Ischemic Stroke by Serum MBL and MBL Expression Genotype Categories.



Supplementary Figure 8. Risk of Cardiovascular Mortality by Serum MBL Levels.



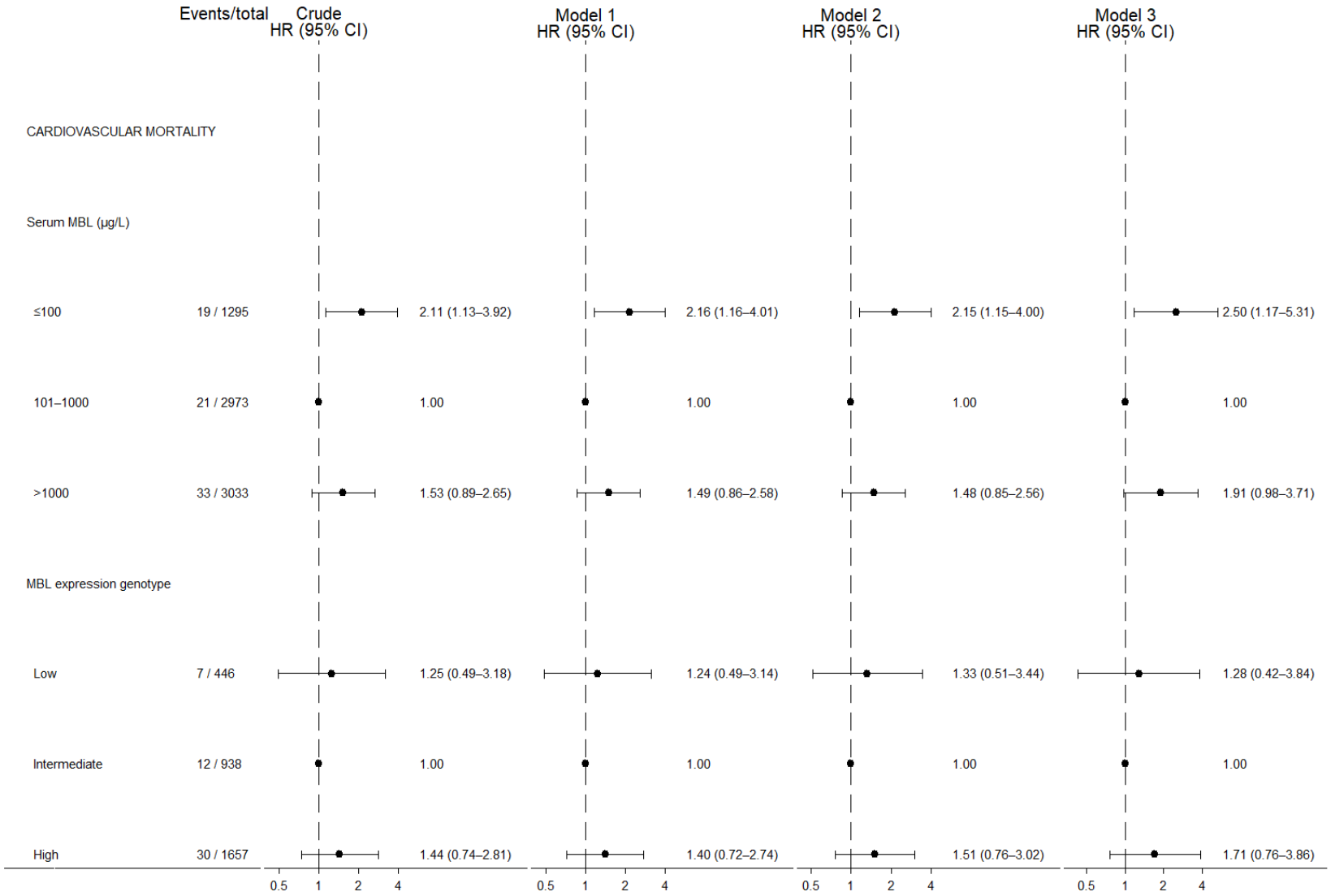
Number at risk by serum MBL						
≤100 µg/L	1295	1169	961	734	359	113
101–1000 µg/L	2973	2678	2245	1613	837	293
>1000 µg/L	3033	2740	2284	1733	883	286



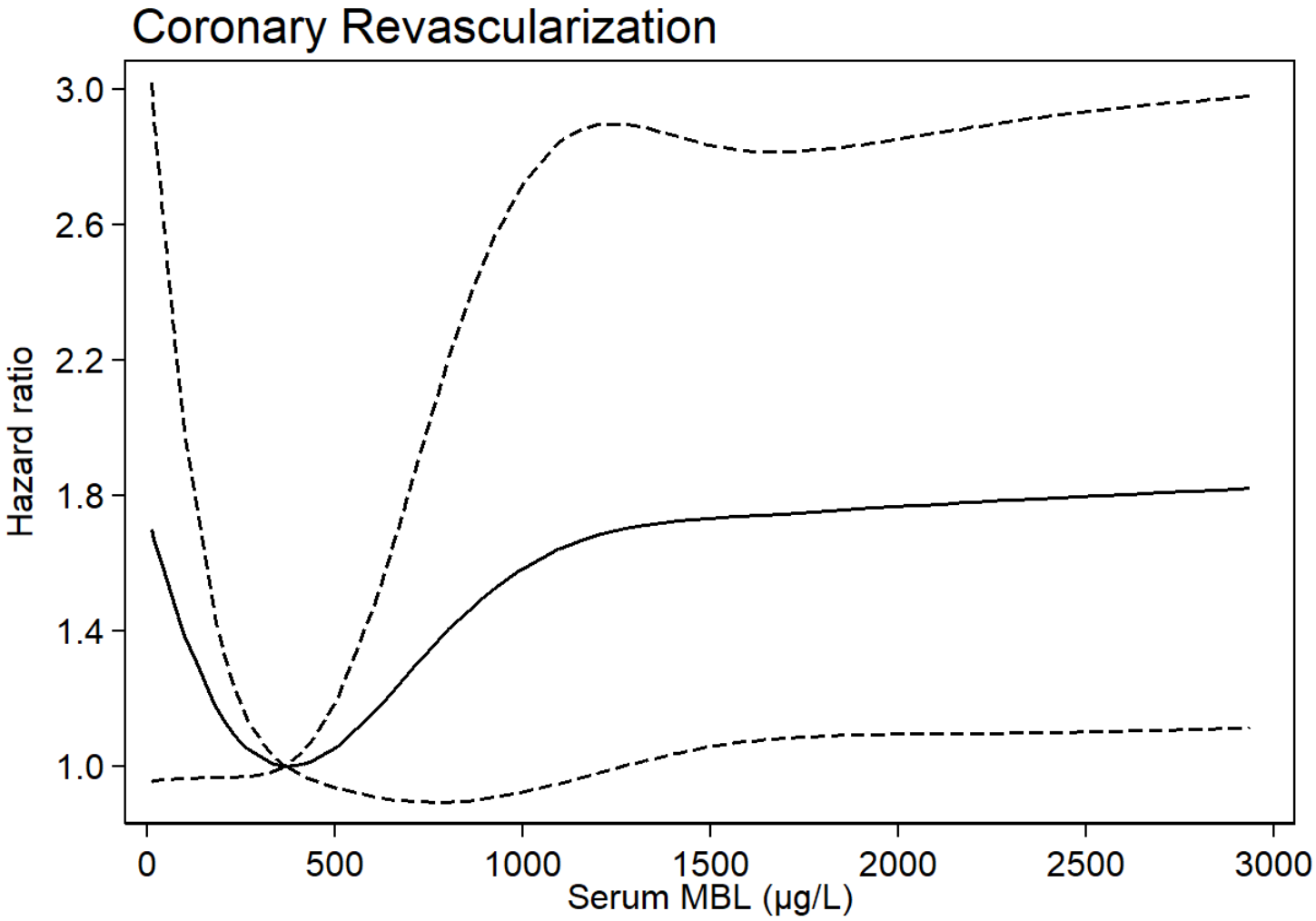
Number at risk MBL genotype						
Low	446	442	439	433	297	95
Intermediate	938	933	923	911	640	213
High	1657	1642	1619	1592	1113	359

Supplementary Figure 9. Time-to-Event Curves of Cardiovascular Mortality by Serum MBL and MBL Expression Genotype Categories.

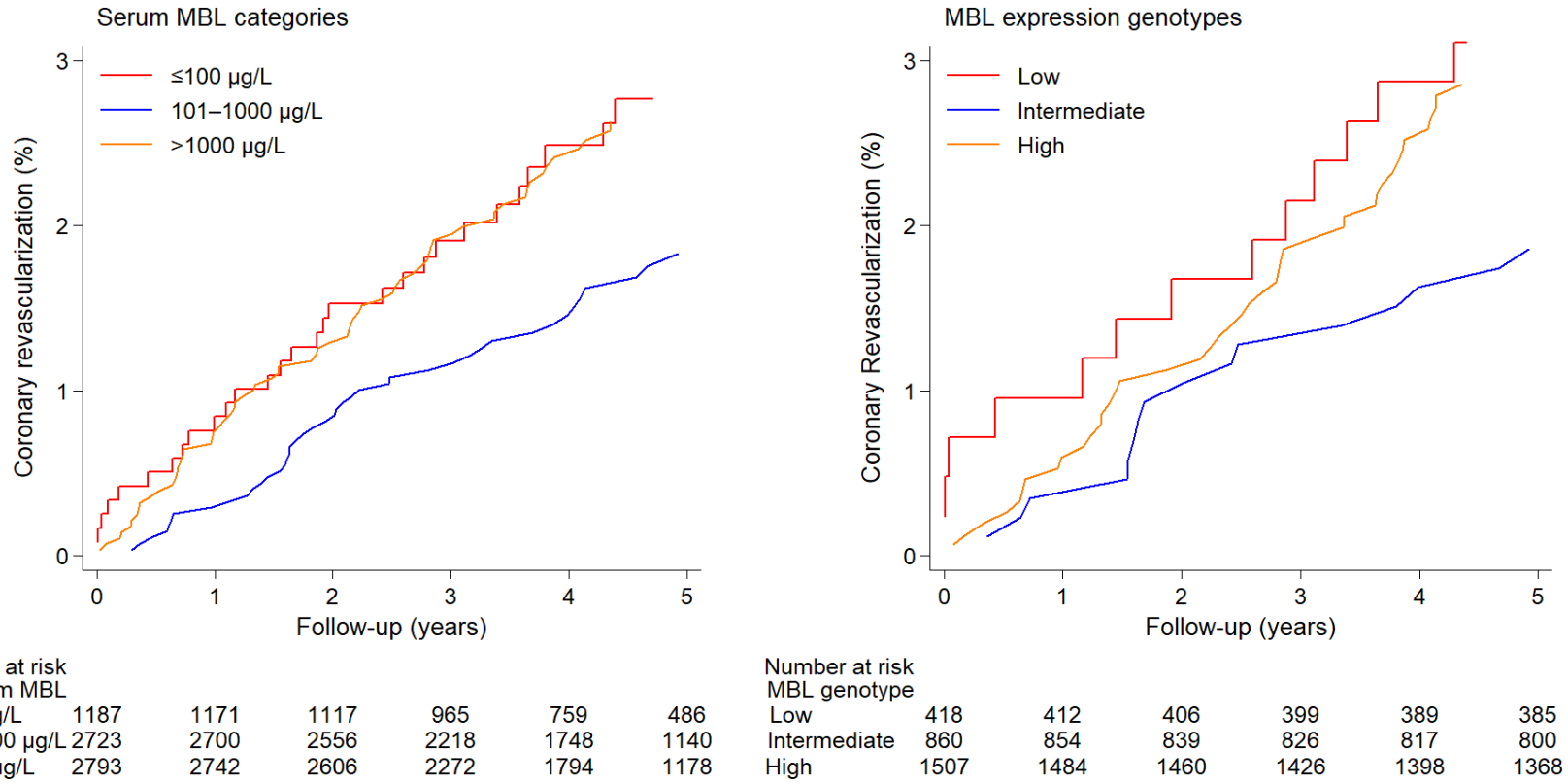
Time-to-event curves of cardiovascular mortality (considering death from other courses as a competing risk) divided into 3 groups of serum MBL (left) in 7301 individuals with type 2 diabetes. Right panel shows the association between low, intermediate, and high MBL expression genotypes in 3041 individuals with type 2 diabetes.



Supplementary Figure 10. Hazard Ratios of Cardiovascular Mortality by Serum MBL and MBL Expression Genotype Categories.

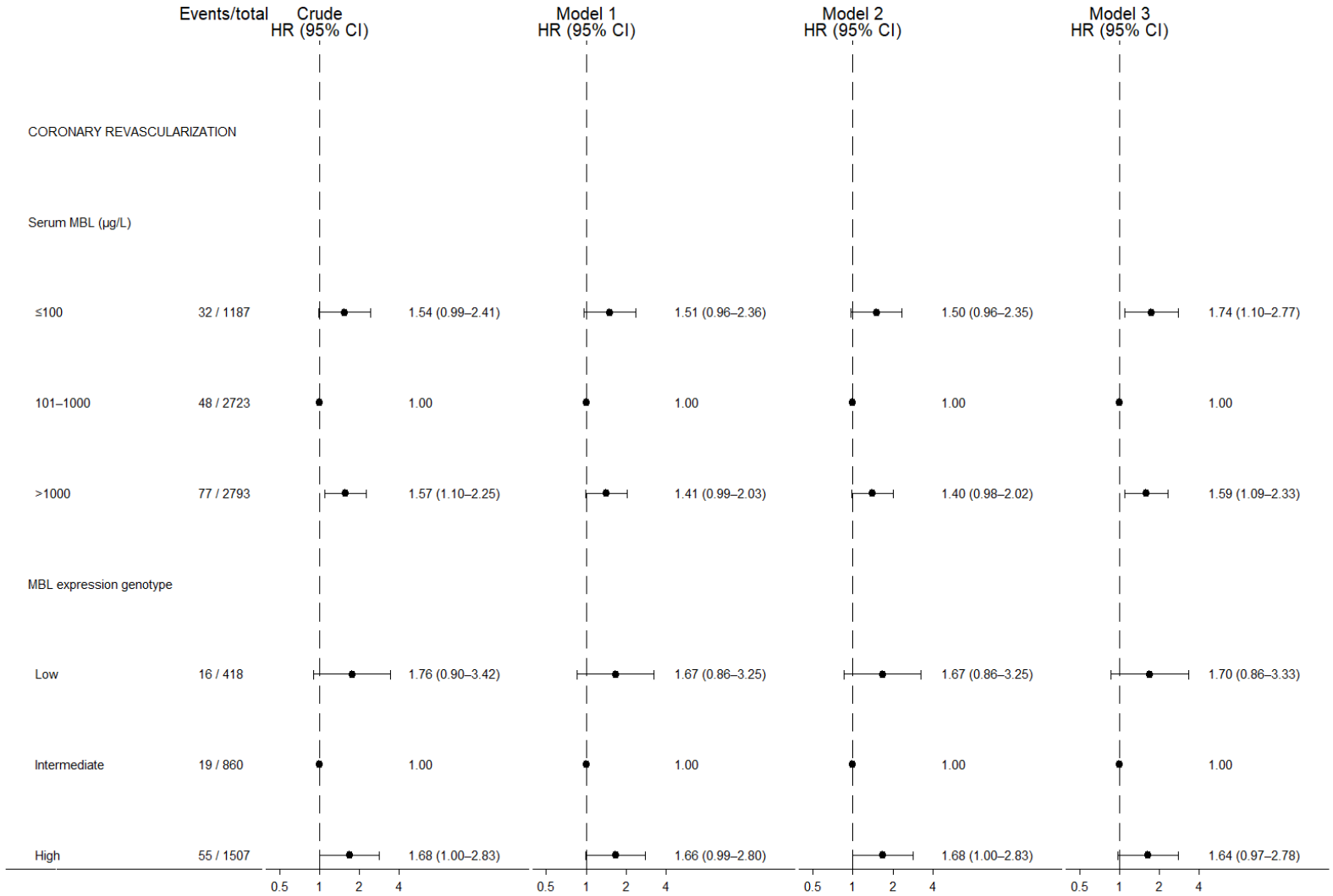


Supplementary Figure 11. Risk of Coronary Revascularization by Serum MBL Levels.

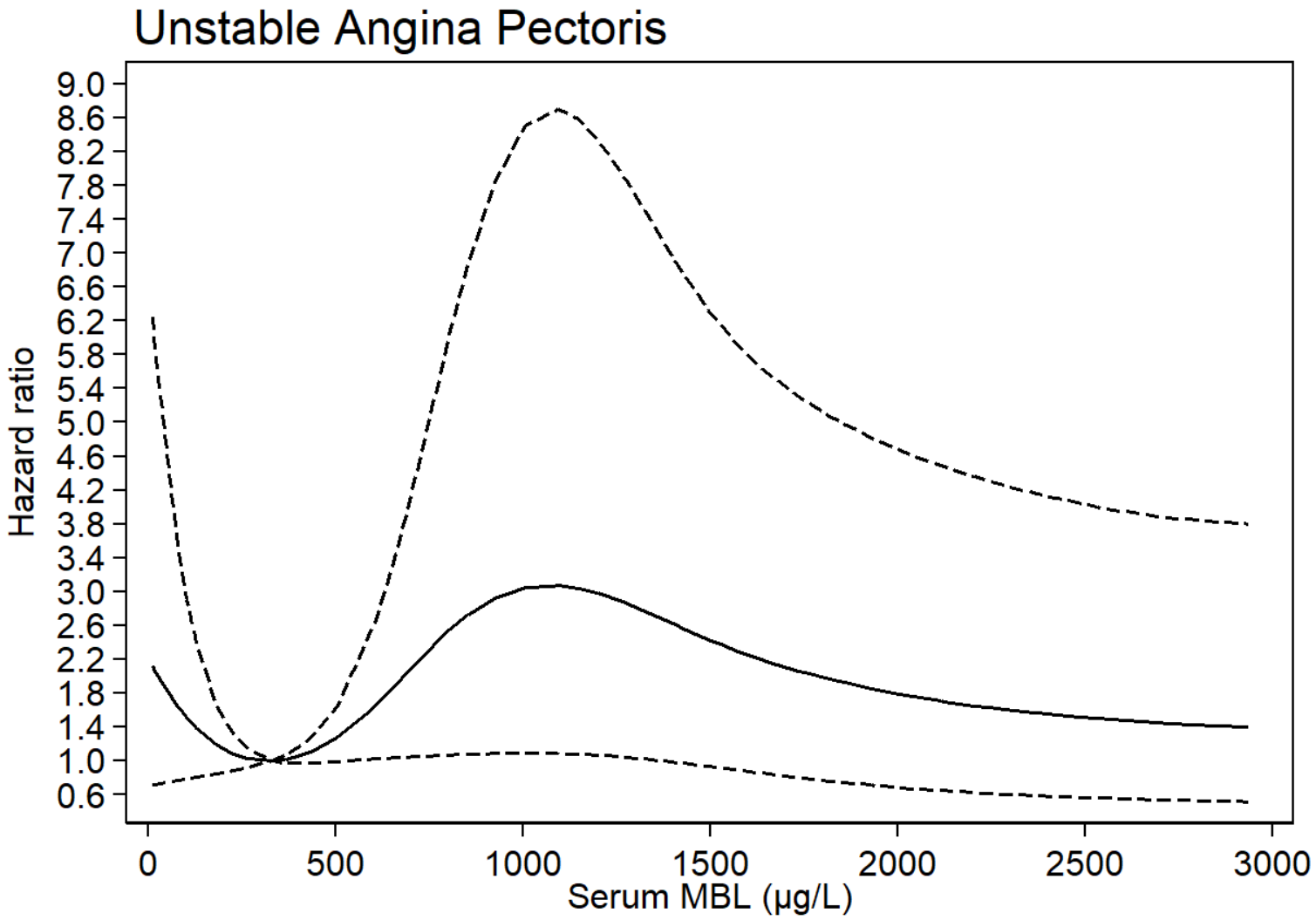


Supplementary Figure 12. Time-to-Event Curves of Coronary Revascularization by Serum MBL and MBL Expression Genotype Categories.

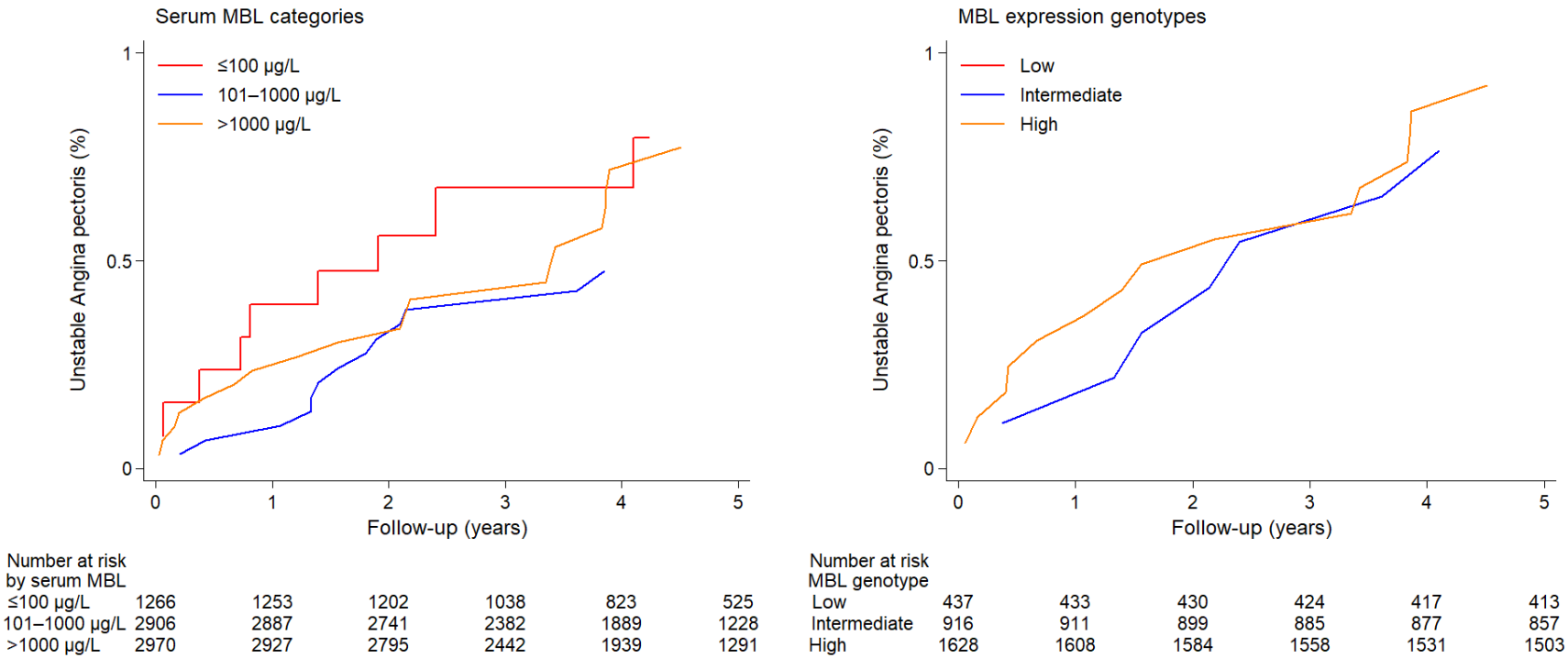
Time-to-event curves of coronary revascularization (considering death as a competing risk) divided into 3 groups of serum MBL (left) in 6703 individuals with type 2 diabetes. Right panel shows the association between low, intermediate, and high MBL expression genotypes in 2785 individuals with type 2 diabetes.



Supplementary Figure 13. Hazard Ratios of Coronary Revascularization by Serum MBL and MBL Expression Genotype Categories.

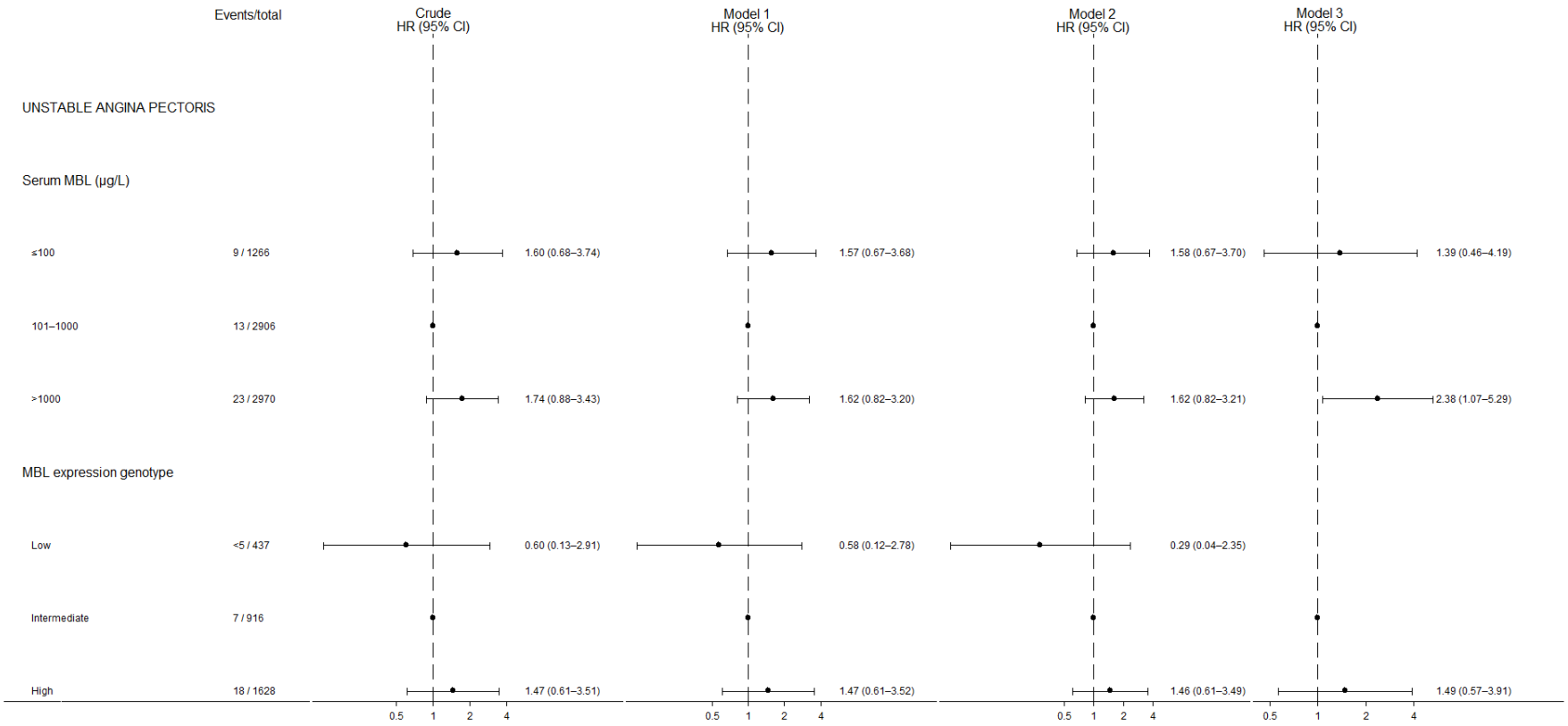


Supplementary Figure 14. Risk of Unstable Angina Pectoris by Serum MBL Levels.



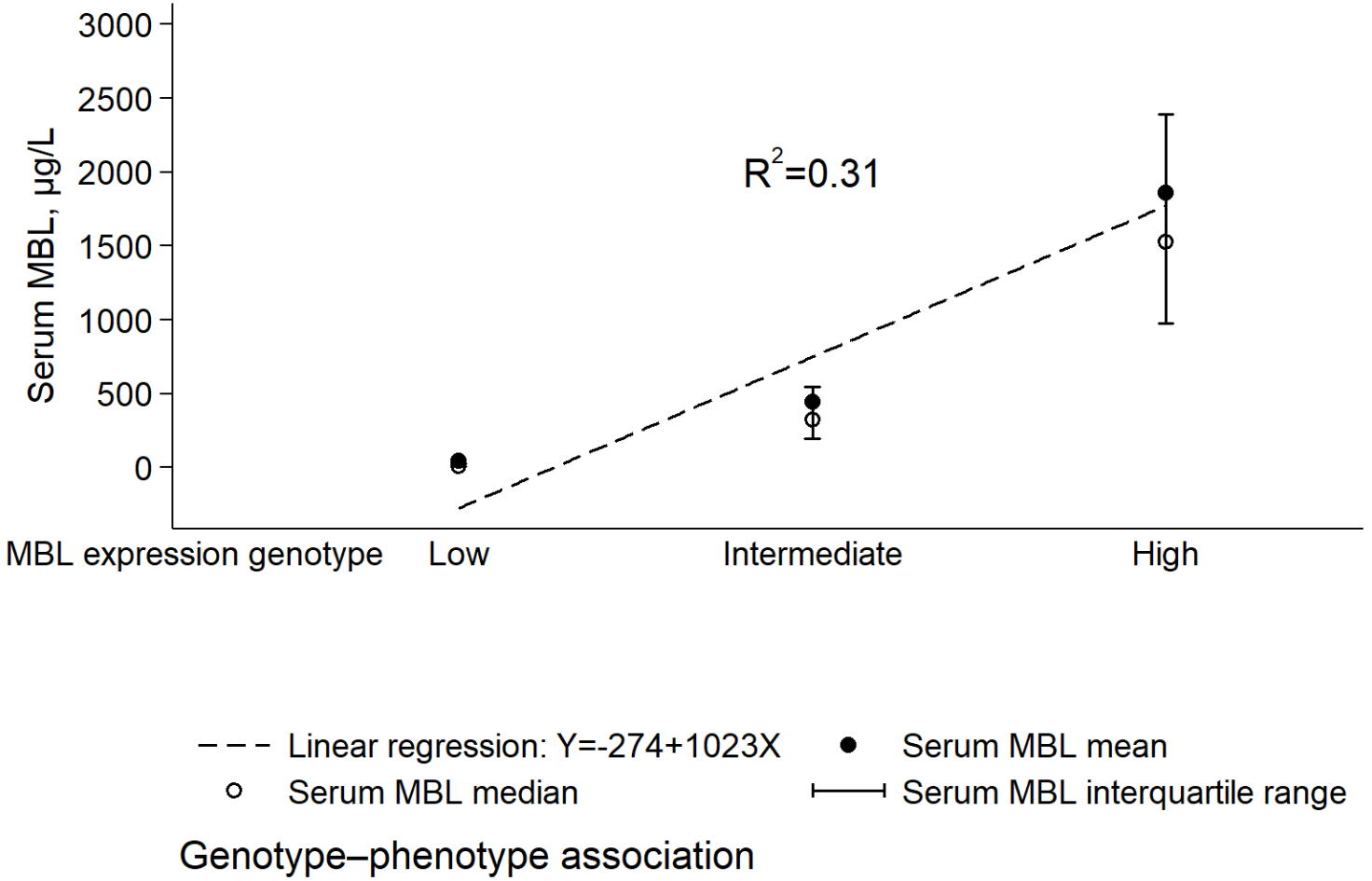
Supplementary Figure 15. Time-to-Event Curves of Unstable Angina Pectoris by Serum MBL and MBL Expression Genotype Categories.

Time-to-Event curves of unstable angina pectoris (considering death as a competing risk) divided into 3 groups of serum MBL (left) in 7142 individuals with type 2 diabetes. Right panel shows the association between low, intermediate, and high MBL expression genotypes in 2981 individuals with type 2 diabetes.



Supplementary Figure 16. Hazard Ratios of Unstable Angina Pectoris by Serum MBL and MBL Expression Genotype Categories.

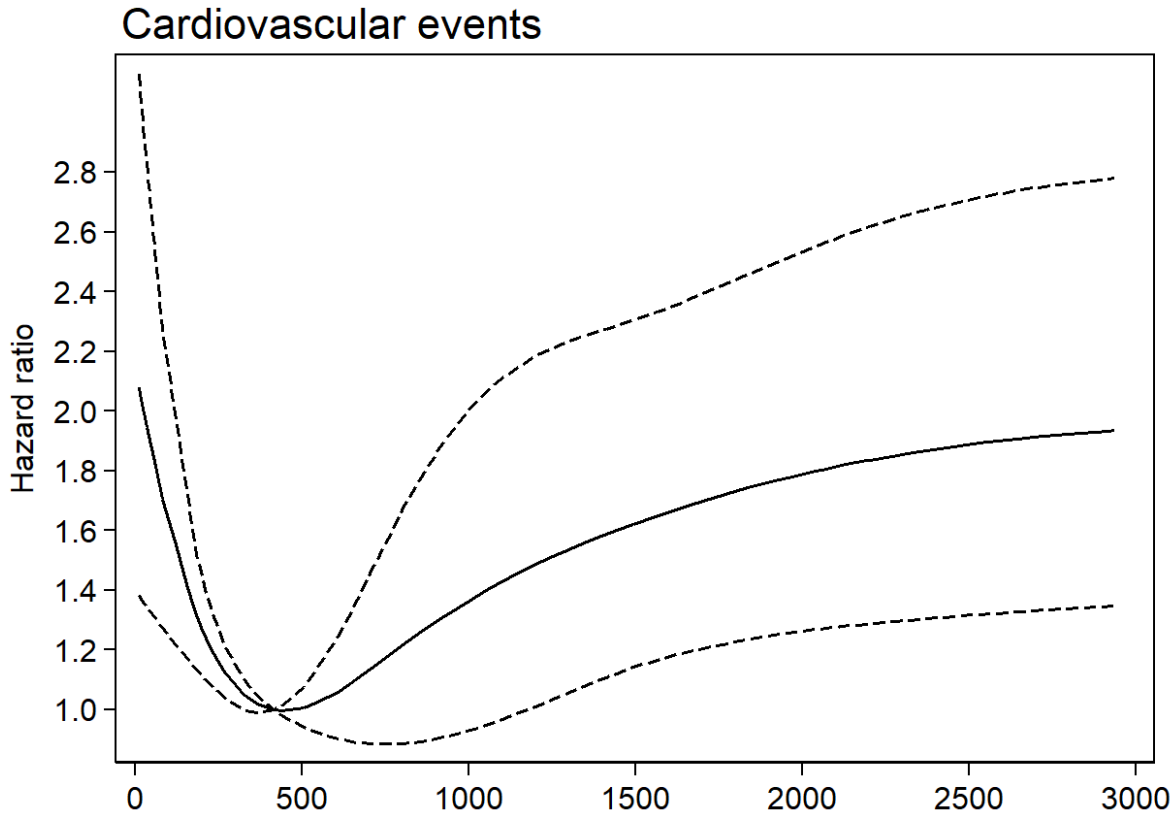
<5 = Exact number of events too low to be displayed according to Danish data protection regulations.



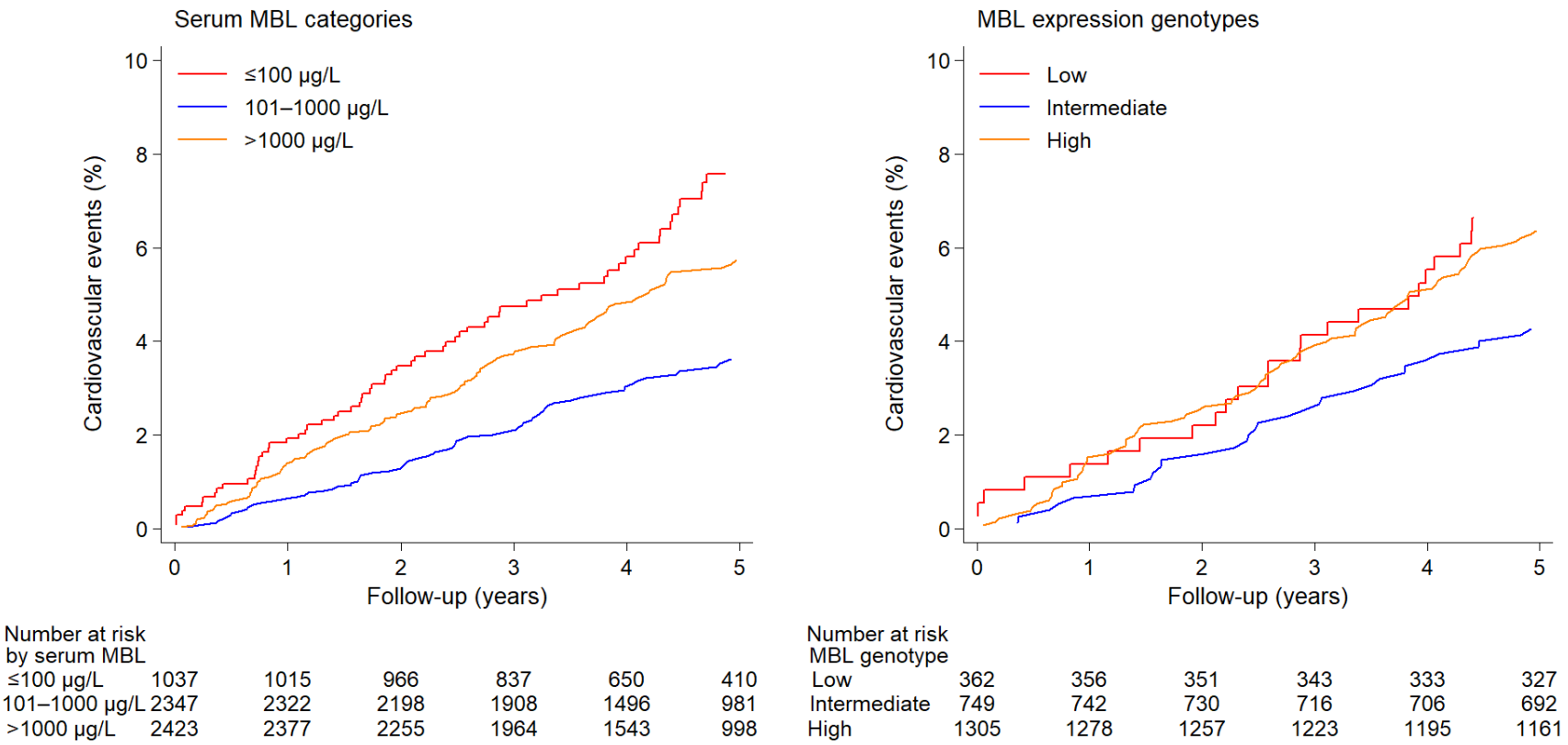
Supplementary Figure 17. Genotype–phenotype association.

Serum MBL levels according to MBL expression genotypes. R^2 is the coefficient of determination. Cuzick's non-parametric test for trend ($P<1\times10^{-300}$).

SENSITIVITY ANALYSES

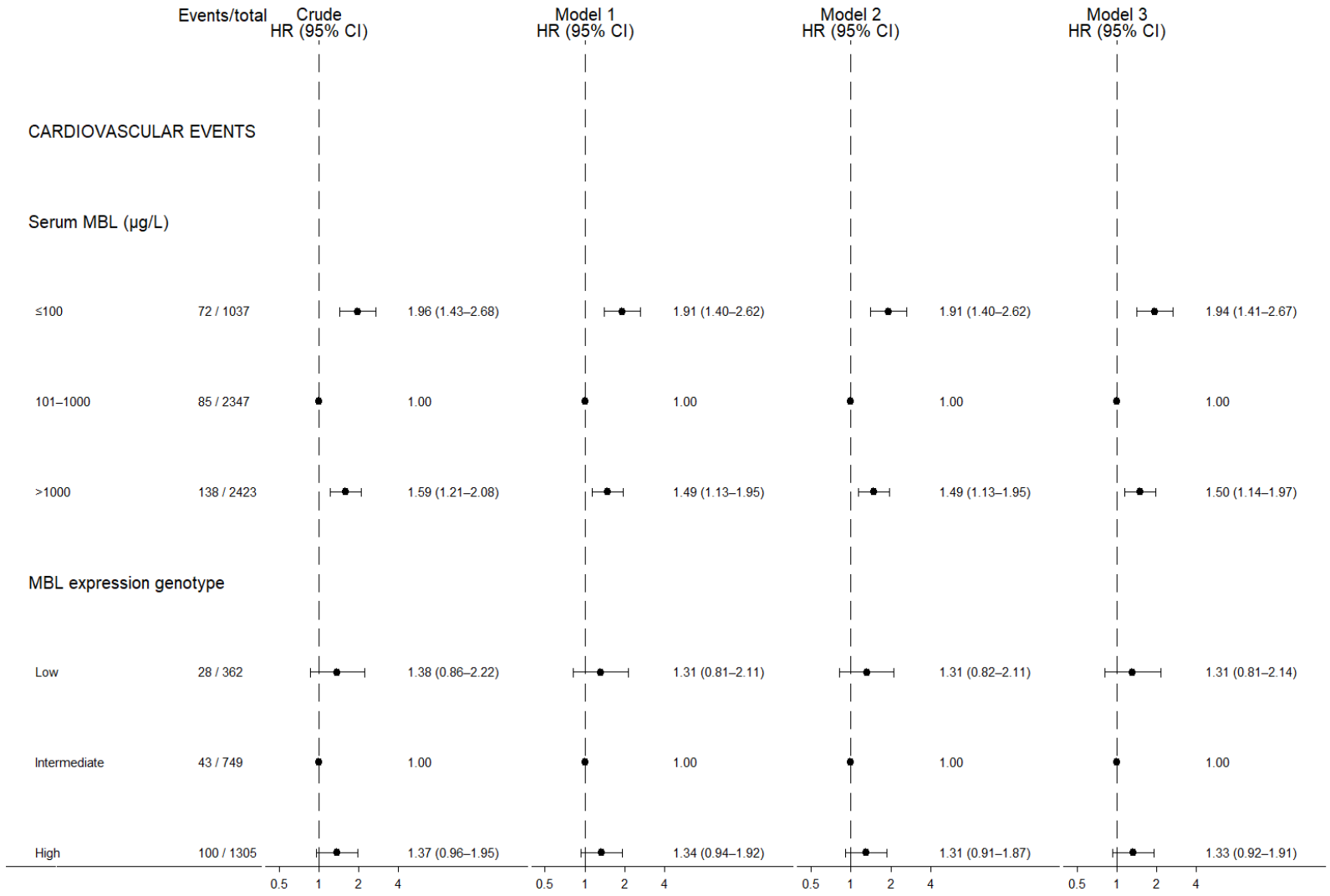


Supplementary Figure 18. Risk of Cardiovascular Events by Serum MBL Levels Excluding Individuals with CRP>10 mg/L.

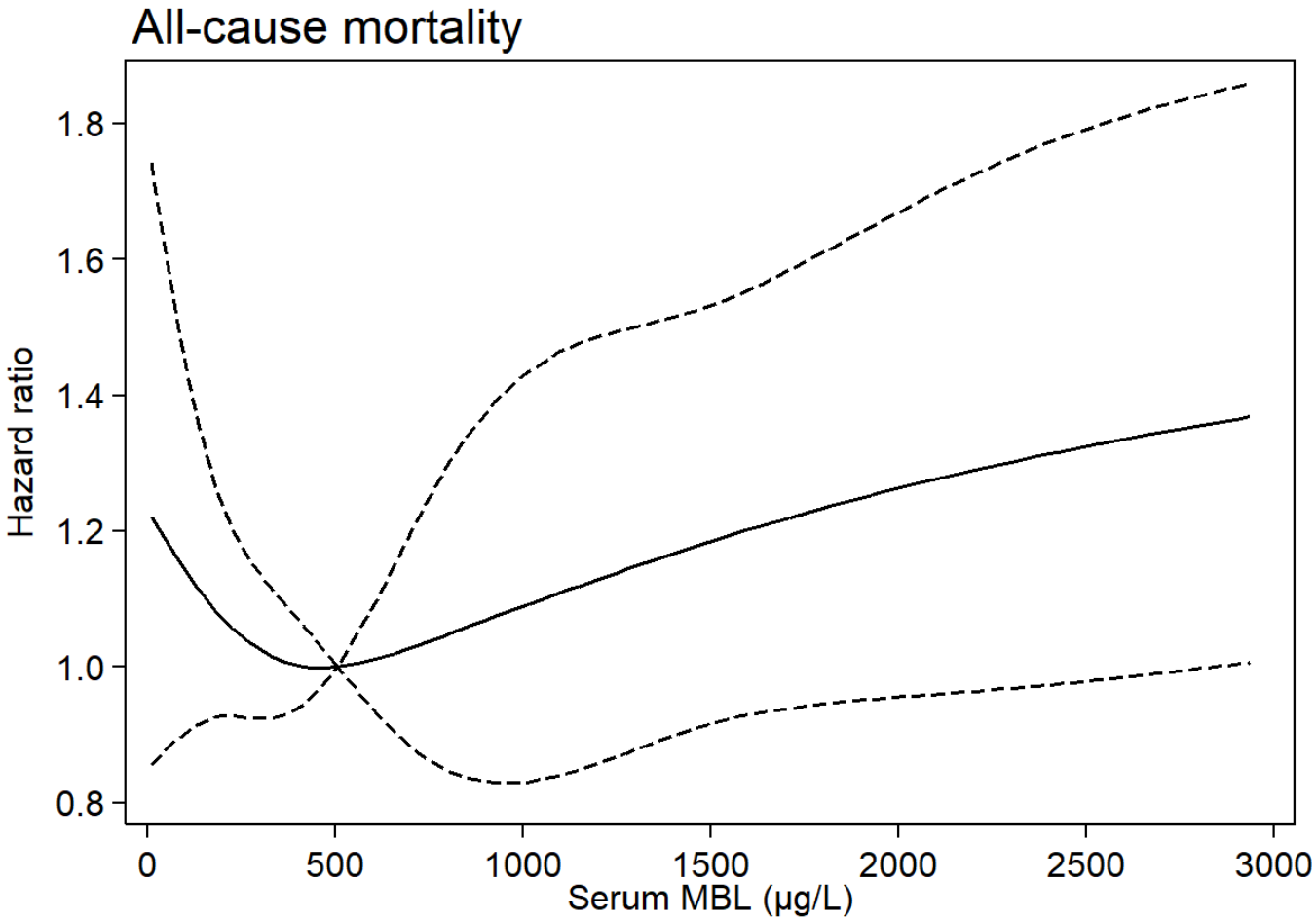


Supplementary Figure 19. Time-to-Event Curves of Cardiovascular Events by Serum MBL and MBL Expression Genotype Categories Excluding Individuals with CRP>10 mg/L.

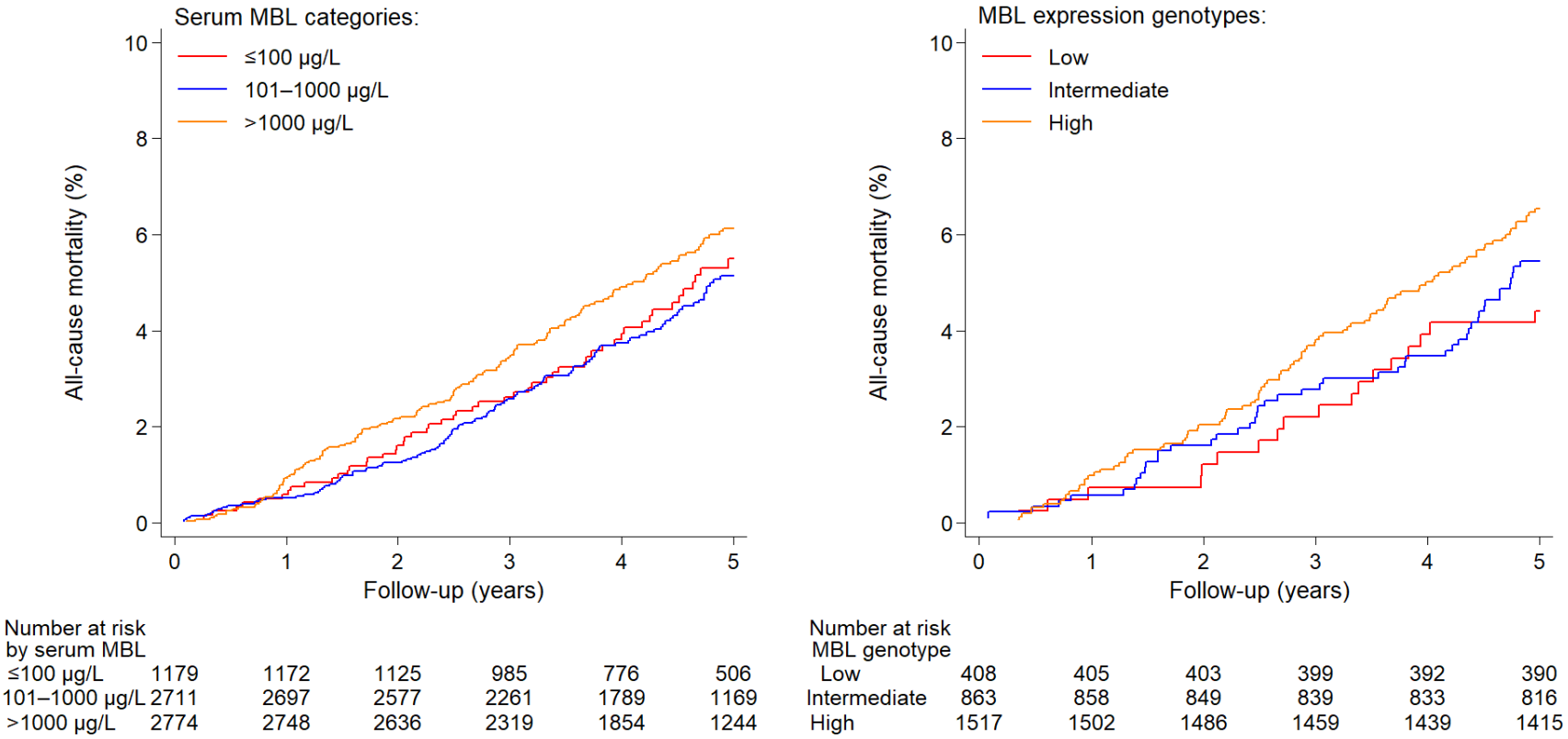
Time-to-event curves of cardiovascular events (considering death as a competing risk) excluding individuals with a CRP >10 mg/L divided into 3 groups of serum MBL (left) in 5807 individuals with type 2 diabetes. Right panel shows the association between low, intermediate, and high MBL expression genotypes in 2416 individuals with type 2 diabetes.



Supplementary Figure 20. Hazard Ratios of Cardiovascular Events by Serum MBL and MBL Expression Genotype Categories Excluding Individuals with CRP>10 mg/L.

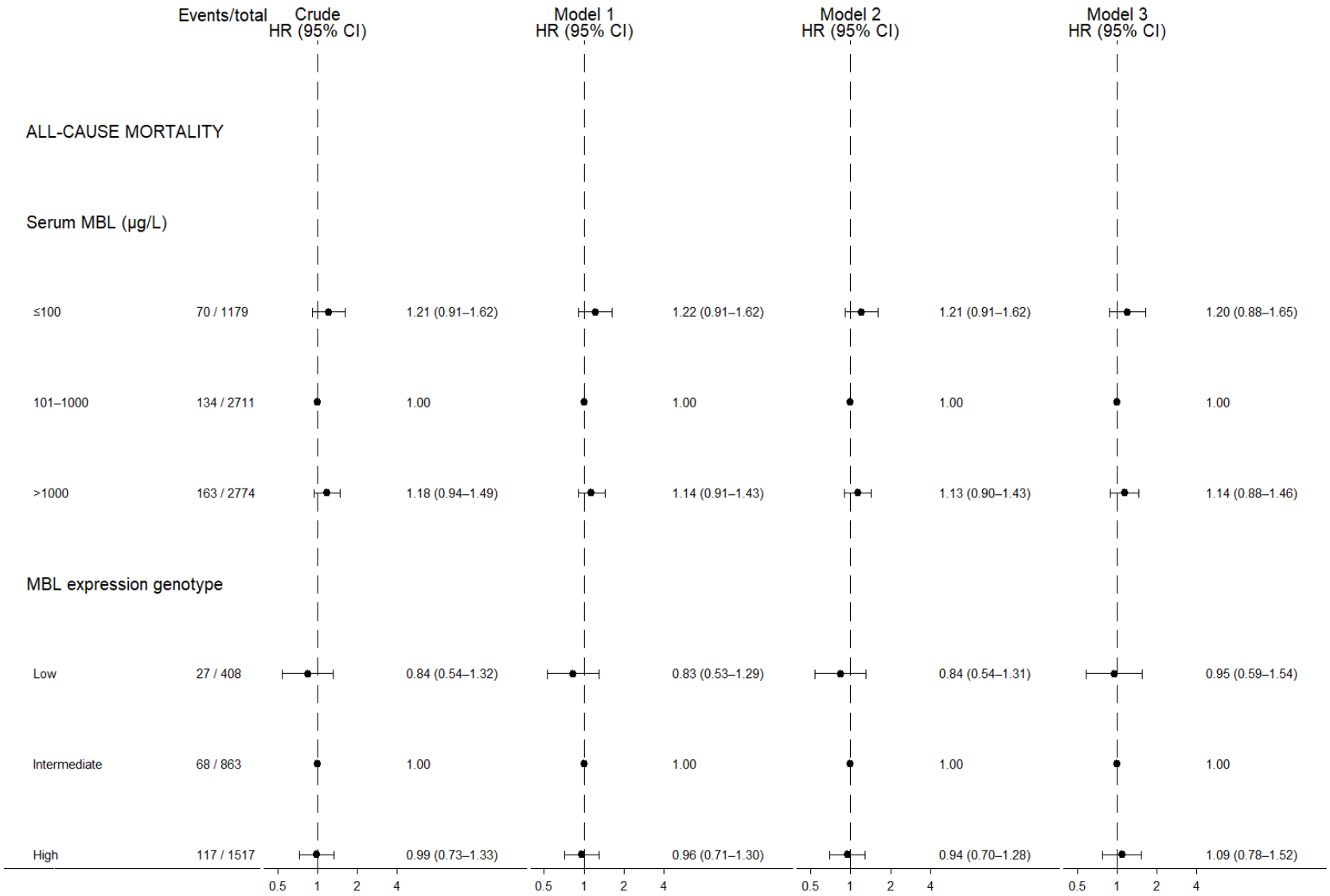


Supplementary Figure 21. Risk of All-cause Mortality by Serum MBL Levels Excluding Individuals with CRP>10 mg/L.

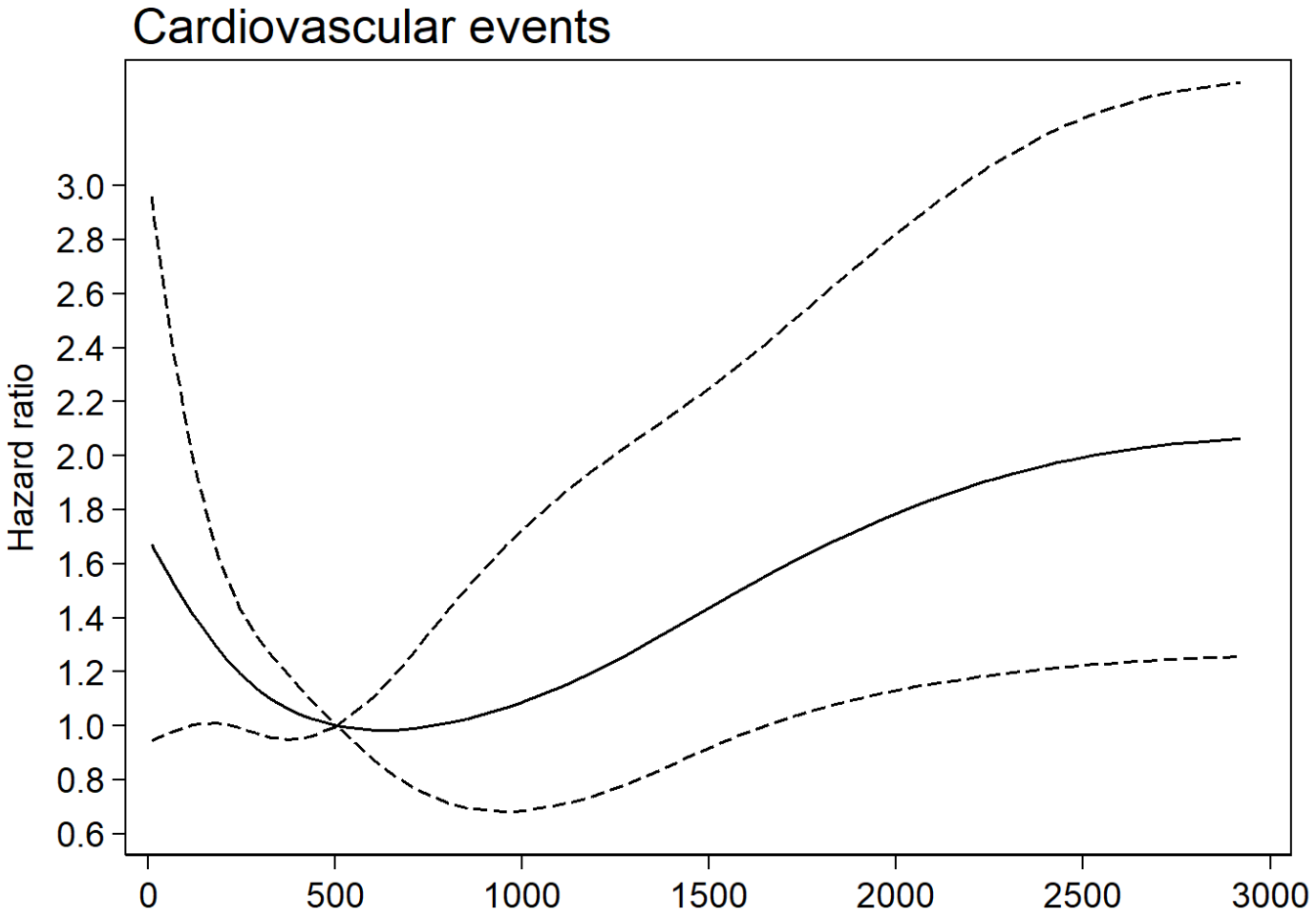


Supplementary Figure 22. Time-to-Event Curves of All-cause Mortality by Serum MBL and MBL Expression Genotype Categories Excluding Individuals with CRP>10 mg/L.

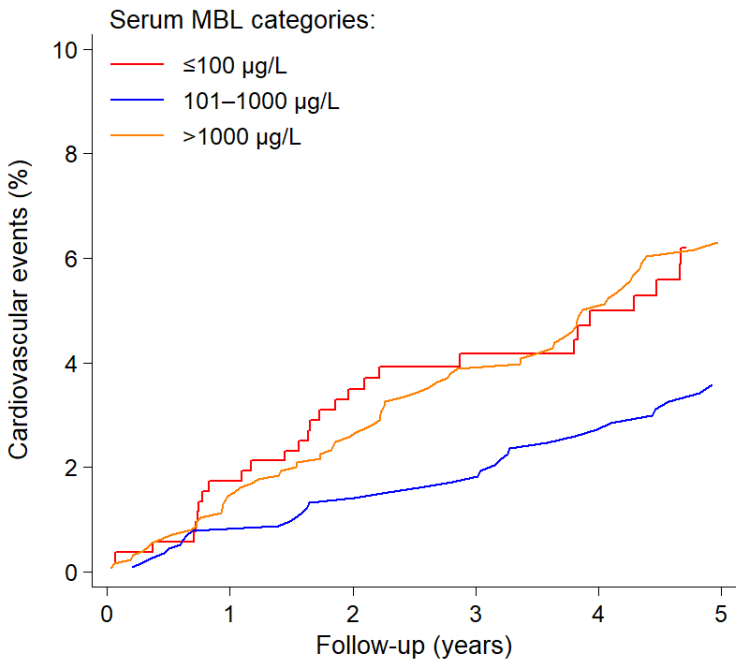
Time-to-event curves of all-cause mortality excluding individuals with a CRP >10 mg/L divided into 3 groups of serum MBL (left) in 6664 individuals with type 2 diabetes. Right panel shows the association between low, intermediate, and high MBL expression genotypes in 2788 individuals with type 2 diabetes.



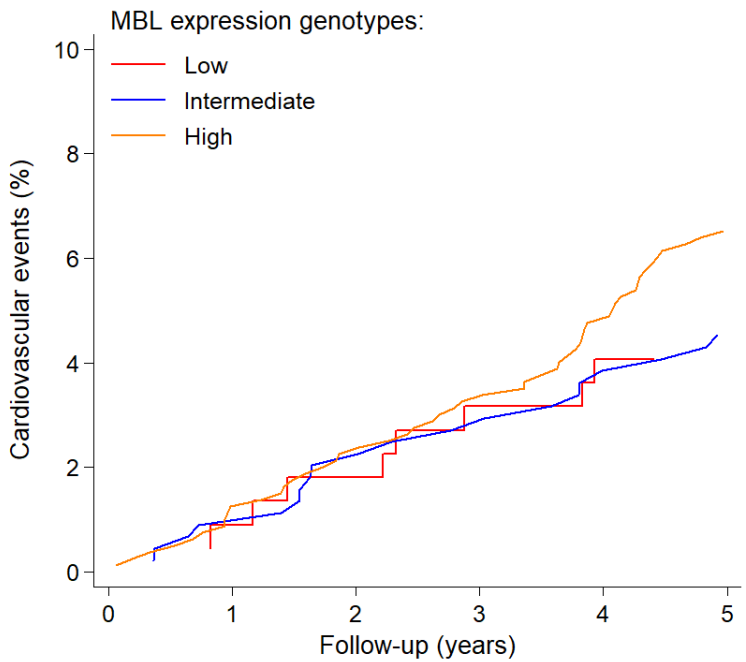
Supplementary Figure 23. Hazard Ratios of All-cause Mortality by Serum MBL and MBL Expression Genotype Categories Excluding Individuals with CRP>10 mg/L.



Supplementary Figure 24. Risk of Cardiovascular Events by Serum MBL Levels Excluding Individuals with Diabetes Duration >1 year.



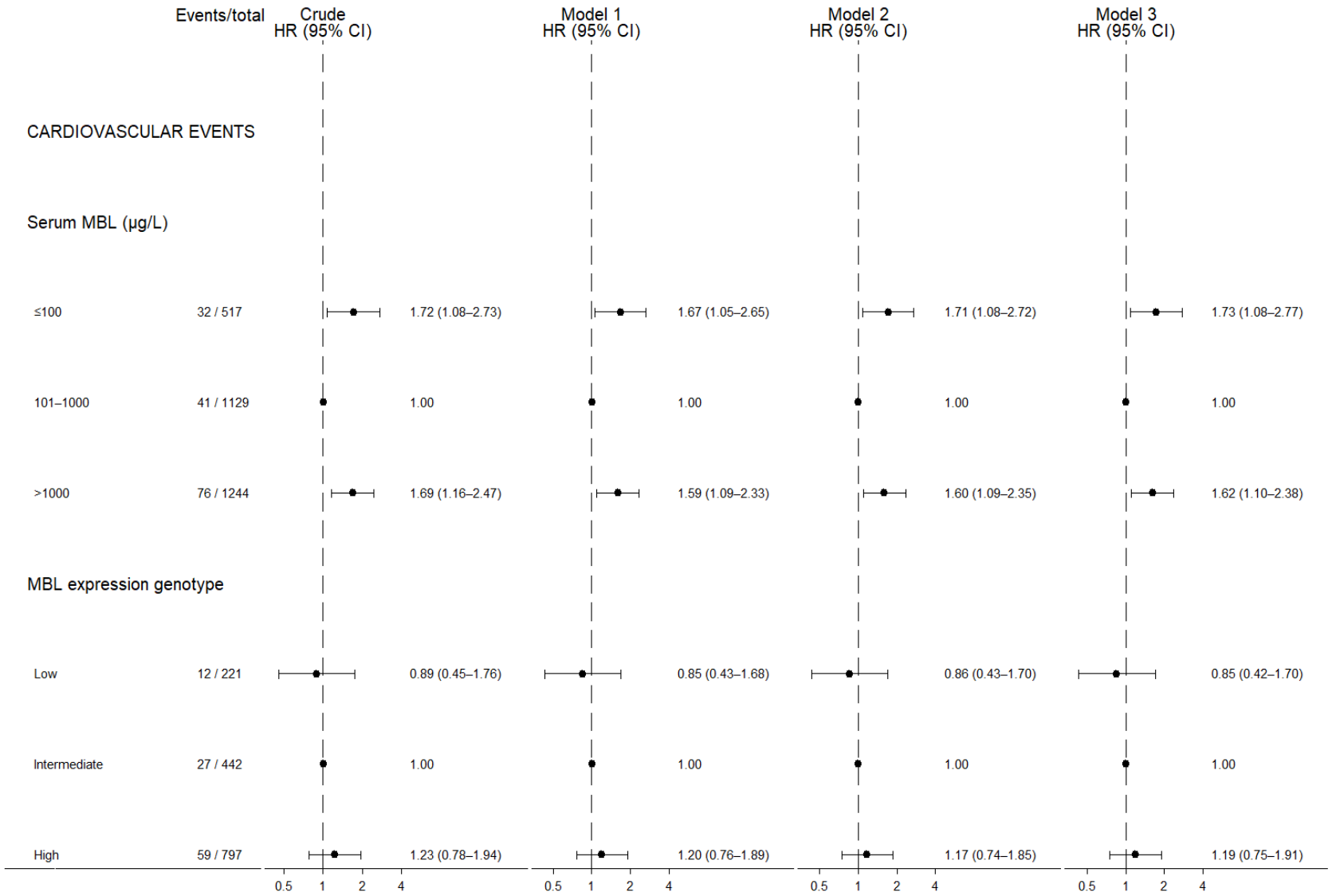
Number at risk by serum MBL						
≤100 µg/L	517	507	482	422	336	250
101–1000 µg/L	1129	1112	1043	901	749	558
>1000 µg/L	1244	1217	1144	1009	829	612



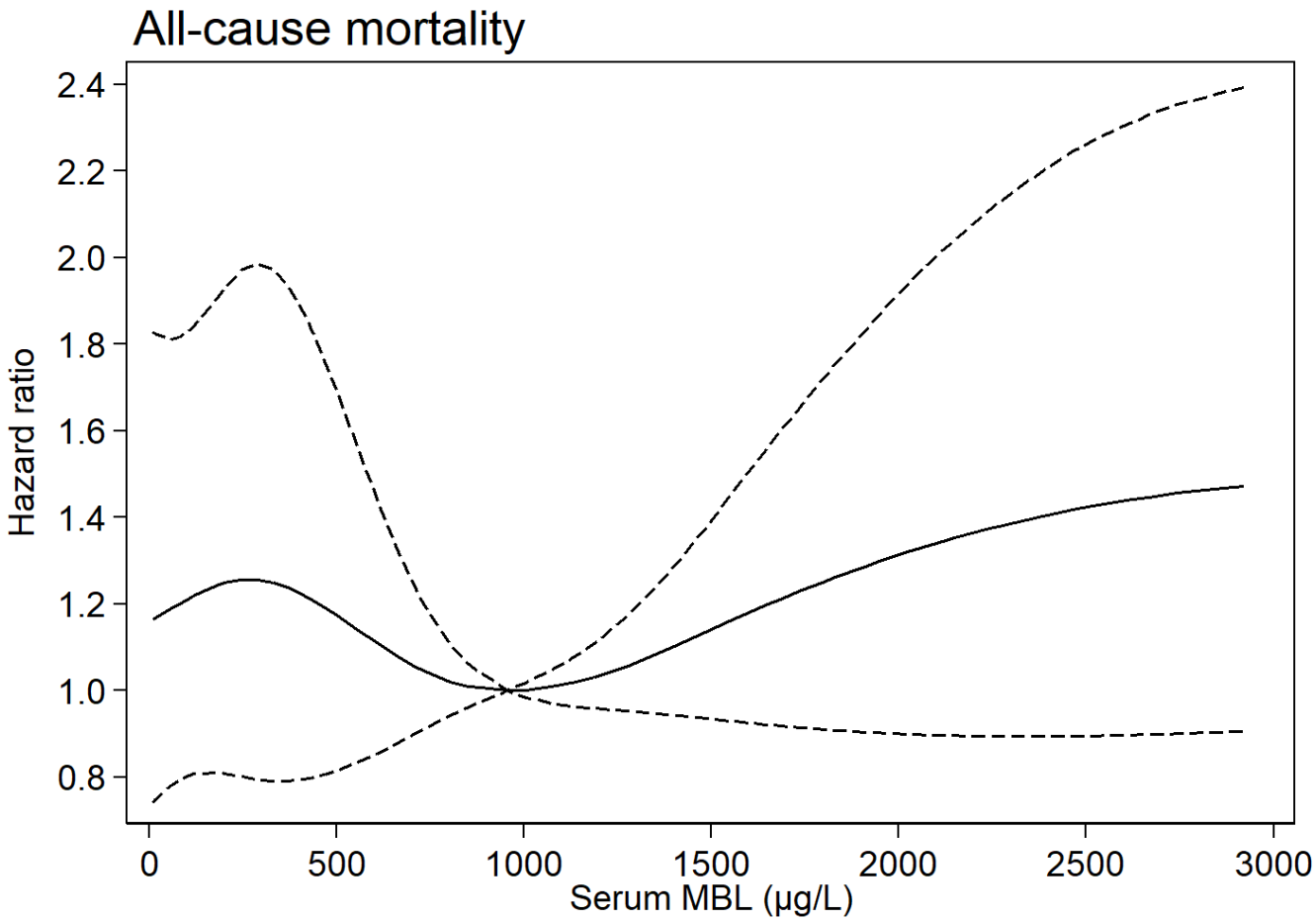
Number at risk MBL genotype						
Low	221	218	215	211	206	205
Intermediate	442	437	428	419	414	410
High	797	785	769	753	733	711

Supplementary Figure 25. Time-to-Event Curves of Cardiovascular Events by Serum MBL and MBL Expression Genotype Excluding Individuals with diabetes duration >1year.

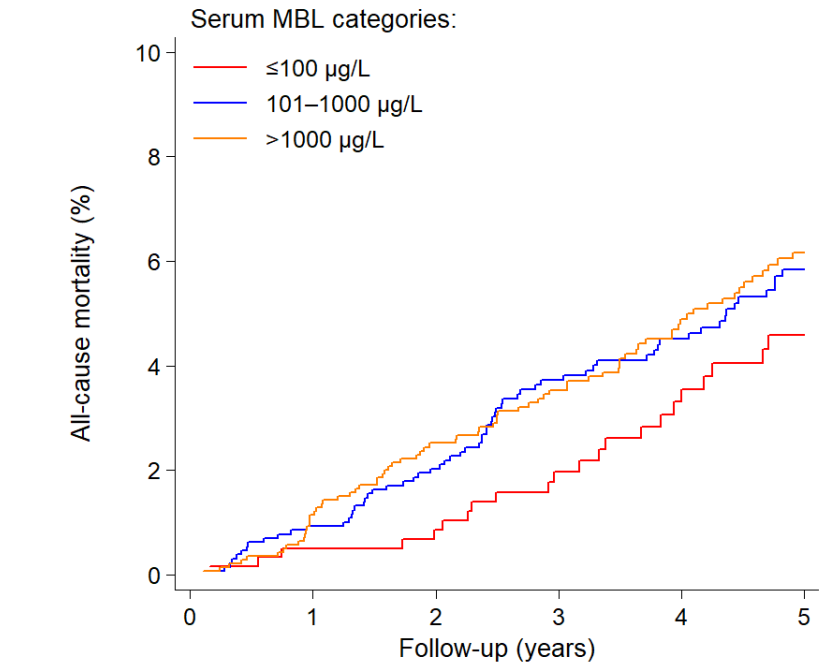
Time-to-event curves of cardiovascular events (considering death as a competing risk) excluding individuals with a diabetes duration >1 year divided into 3 groups of serum MBL (left) in 2890 individuals with type 2 diabetes. Right panel shows the association between low, intermediate, and high MBL expression genotypes in 1460 individuals with type 2 diabetes.



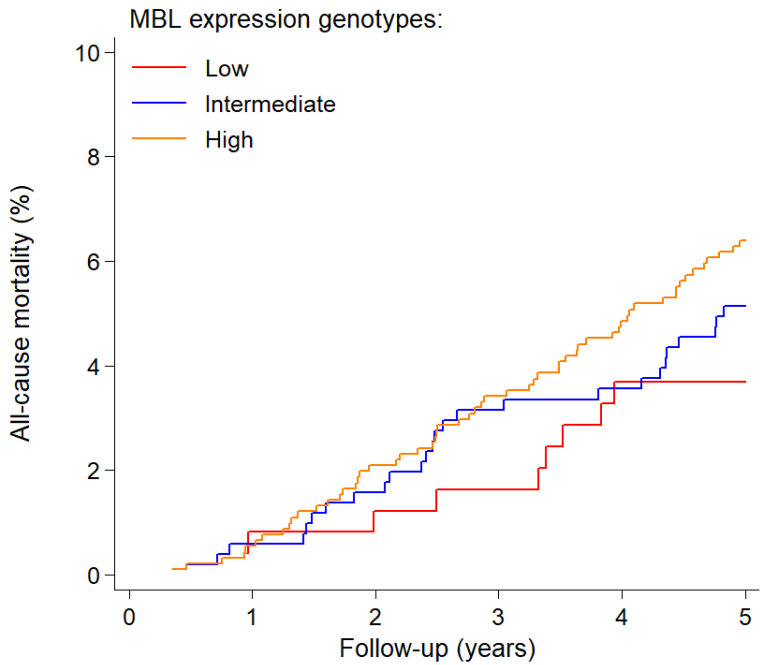
Supplementary Figure 26. Hazard Ratios of Cardiovascular Events by Serum MBL and MBL Expression Genotype Excluding Individuals with diabetes duration >1 year.



Supplementary Figure 27. Risk of All-cause Mortality by Serum MBL Levels Excluding Individuals with Diabetes Duration >1 year.



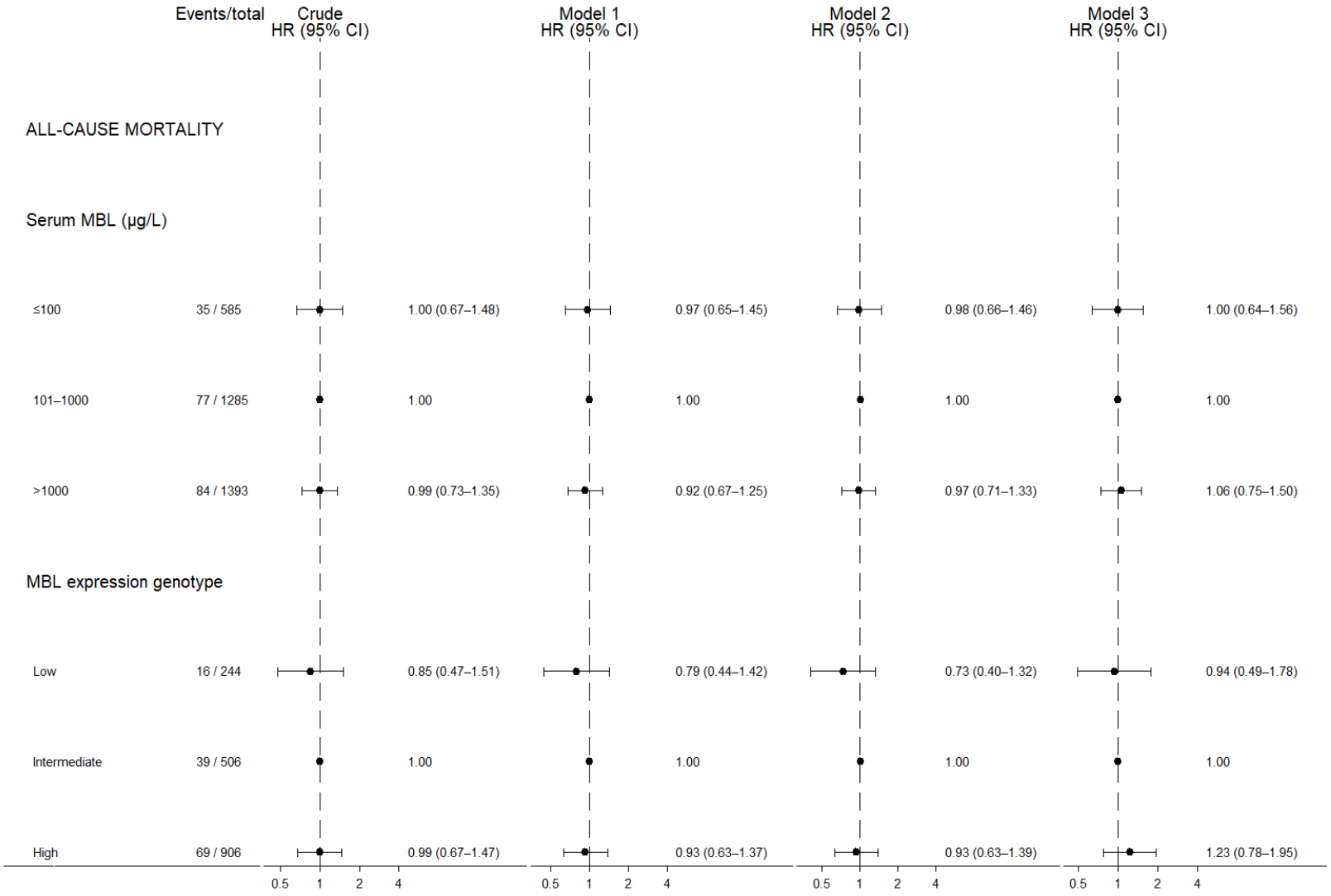
Number at risk by serum MBL						
≤100 µg/L	585	582	563	490	395	300
101–1000 µg/L	1285	1273	1205	1047	876	654
>1000 µg/L	1393	1377	1314	1167	978	739



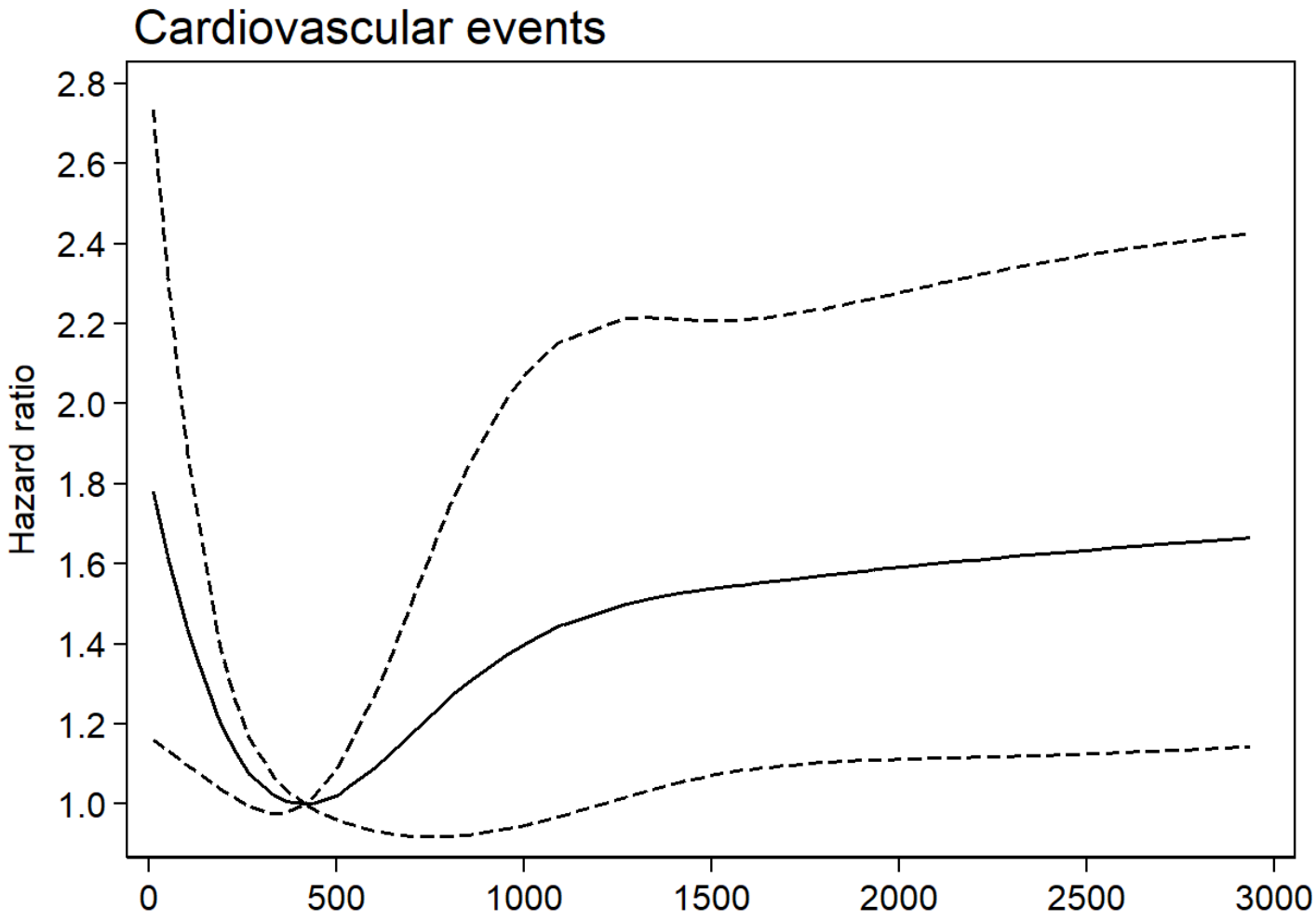
Number at risk MBL genotype						
Low	244	242	241	240	235	235
Intermediate	506	503	498	490	488	480
High	906	901	887	875	862	848

Supplementary Figure 28. Time-to-Event Curves of All-cause Mortality by Serum MBL and MBL Expression Genotype Excluding Individuals with Diabetes Duration >1year.

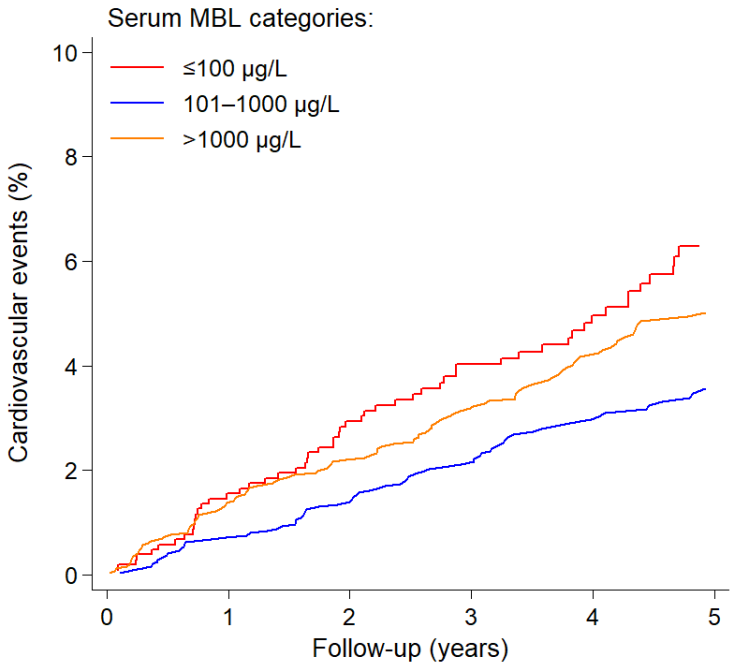
Time-to-event curves of all-cause mortality excluding individuals with a diabetes duration >1 year divided into 3 groups of serum MBL (left) in 3263 individuals with type 2 diabetes. Right panel shows the association between low, intermediate, and high MBL expression genotypes in 1656 individuals with type 2 diabetes.



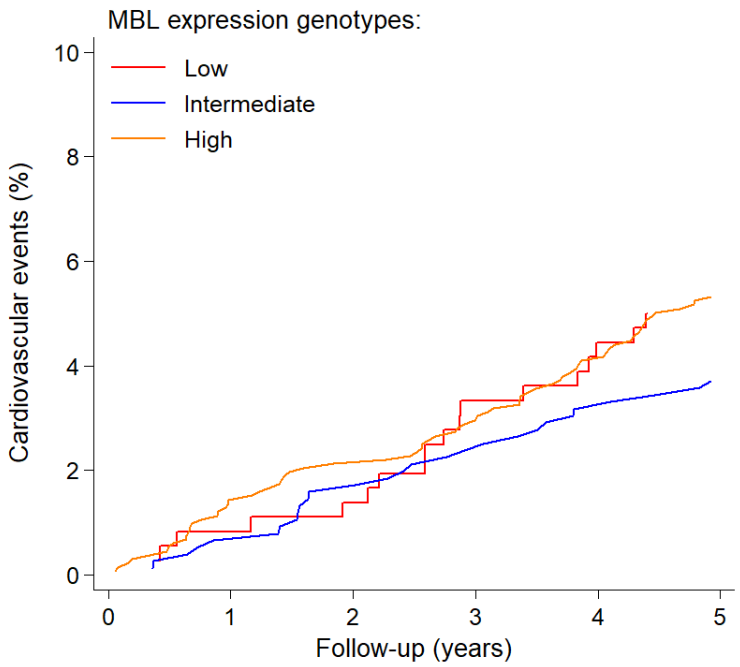
Supplementary Figure 29. Hazard Ratios of All-cause Mortality by Serum MBL and MBL Expression Genotype Excluding Individuals with Diabetes Duration >1 year.



Supplementary Figure 30. Risk of Cardiovascular Events by Serum MBL Levels Excluding Individuals with Any Previous Cardiovascular Disease.



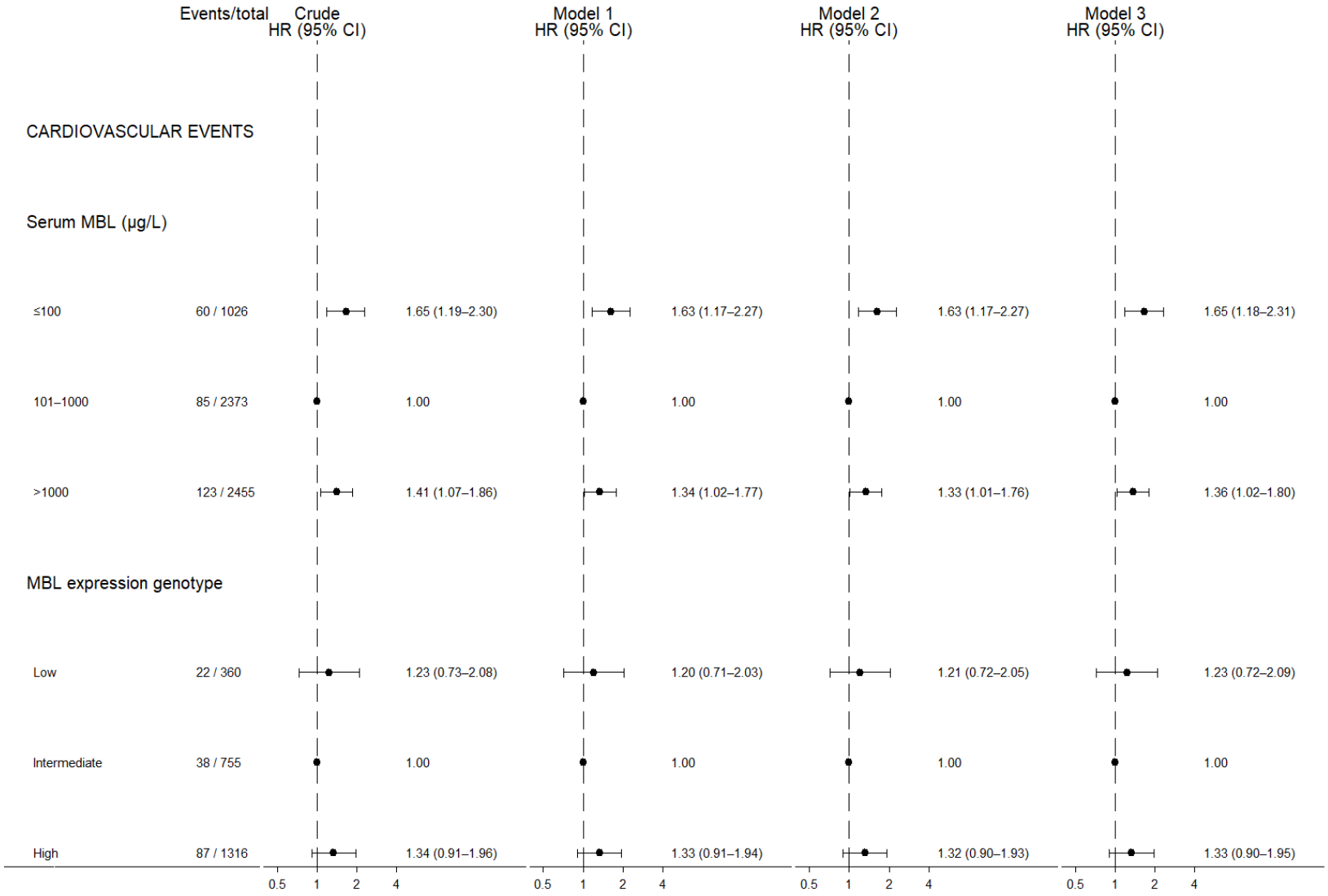
Number at risk by serum MBL						
≤100 µg/L	1026	1007	957	826	654	416
101–1000 µg/L	2373	2347	2222	1924	1510	999
>1000 µg/L	2455	2404	2284	1993	1563	1017



Number at risk MBL genotype						
Low	360	355	350	343	335	330
Intermediate	755	749	736	723	715	703
High	1316	1291	1272	1248	1221	1190

Supplementary Figure 31. Time-to-Event Curves of Cardiovascular Events by Serum MBL and MBL Expression Genotype Excluding Individuals with Any previous Cardiovascular Disease.

Time-to-event curves of cardiovascular events (considering death as a competing risk) excluding individuals with any previous cardiovascular disease divided into 3 groups of serum MBL (left) in 5854 individuals with type 2 diabetes. Right panel shows the association between low, intermediate, and high MBL expression genotypes in 2431 individuals with type 2 diabetes.



Supplementary Figure 32. Hazard Ratios of Cardiovascular Events by Serum MBL and MBL Expression Genotype Excluding Individuals with Any Previous Cardiovascular Disease.

References

1. Lee KJ, Carlin JB. Multiple imputation for missing data: fully conditional specification versus multivariate normal imputation. Am J Epidemiol 2010;171:624-32.
2. Sterne JA, White IR, Carlin JB, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. Bmj 2009;338:b2393.
3. Bernaards CA, Belin TR, Schafer JL. Robustness of a multivariate normal approximation for imputation of incomplete binary data. Stat Med 2007;26:1368-82.

Paper III

● **Appendix III**

Title page

Mannose-binding Lectin and Risk of Infections in Type 2 Diabetes: A Danish Cohort Study

A. Gedebjerg, MD[1,2], R.W. Thomsen, PhD[1], A.D. Kjaergaard, PhD[1], R. Steffensen, PhD[3], J.S. Nielsen, PhD[4], J. Rungby, DMSc[5,6], S. Friborg, MD[7], I. Brandslund, DMSc[8], S. Thiel, PhD[9], H. Beck-Nielsen, DMSc[4,7], H.T. Sørensen, DMSc[1], T.K. Hansen, DMSc[10], M. Bjerre, PhD[11]

Target: 1. Clinical Infectious Diseases. 2. The Journal of Infectious Diseases. 3. Clinical Microbiology and Infection

Short title: Mannose-binding Lectin, Diabetes and Infections

[1]Department of Clinical Epidemiology, Aarhus University Hospital, Aarhus, Denmark

[2]Danish Diabetes Academy, Odense University Hospital, Odense, Denmark

[3]Department of Immunology, Aalborg University Hospital, Aalborg, Denmark

[4]DD2, Steno Diabetes Center Odense, Odense University Hospital, Odense, Denmark

[5]Department of Endocrinology IC, Bispebjerg University Hospital, Copenhagen, Denmark

[6]Copenhagen Center for Translational Research, Bispebjerg University Hospital, Copenhagen

[7]Diabetes Research Centre, Department of Endocrinology, Odense University Hospital, Odense, Denmark

[8]Department of Biochemistry, Lillebaelt Hospital, Vejle, Denmark

[9]Department of Biomedicine, Aarhus University, Aarhus, Denmark

[10]Steno Diabetes Center Aarhus, Aarhus University Hospital, Aarhus, Denmark

[11]Medical Research Laboratory, Department of Clinical Medicine, Aarhus University, Aarhus, Denmark

Corresponding author:

Anne Gedebjerg, Department of Clinical Epidemiology, Aarhus University Hospital, Olof Palmes Alle 43-45, DK-8200 Aarhus N, Denmark; Tel: +45 87168253; Fax: +45 87167215

Email: aged@clin.au.dk

Word count:

Title (words): 14; abstract (words): 279; main text (words): 3042 (excluding abstract, tables, figure legends, reference list, and supplementary tables); number of references: 38; number of tables: 1; number of figures: 4; number of supplementary tables: 9; number of supplementary figures: 18.

ABSTRACT

Objective: Mannose-binding lectin (MBL) has been associated with risk of infection, but the nature and causality of this association in type 2 diabetes (T2D) is unclear.

Methods: We measured serum MBL in 7305 patients with early T2D in the nationwide Danish Center for Strategic Research in Type 2 Diabetes cohort. We then performed MBL expression genotyping in 3043 patients. Outcomes were hospital-treated infections and community-based antimicrobial prescriptions. The associations were examined by spline and Cox regression analyses.

Results: Risks of hospital-treated infections (n=1140 events) and antimicrobial prescriptions (n=5077 events) were increased in T2D patients with low serum MBL (≤100 μg/L) followed for up to 8 years, yielding an L-shaped risk profile. Compared to the intermediate serum MBL category (100–1000 μg/L), the adjusted hazard ratio (aHR) for the low MBL category were 1.13 (95% confidence interval, 0.96–1.33) for any hospital-treated infections and 1.19 (1.01–1.41) for bacterial infections, including 1.14 (0.81–1.62) for urinary tract infections, 1.30 (0.98–1.70) for pneumonia, 1.77 (0.97–3.23) for diarrheal diseases, and 1.50 (1.00–2.24) for other bacterial infections. A similar but attenuated association with low serum MBL was present for antimicrobial prescriptions (aHR 1.06 [0.98–1.15]). For the low MBL expression genotype, the aHR was 1.08 (0.84–1.38) for any infections, including an aHR of 2.23 (1.04–4.80) for diarrheal diseases, and the aHR was 1.18 (1.04–1.34) for antimicrobial prescriptions.

Conclusions: In patients with early T2D, low serum MBL levels are associated with increased risk of future bacterial infections. A low MBL expression genotype was associated with increased risk of community antimicrobial prescriptions and hospital-treated diarrheal disease, indicating a causal involvement of serum MBL in development of infections in T2D.

Keywords: Type 2 diabetes; complement system; cohort study, mannose-binding lectin; infection; association

INTRODUCTION

Mannose-binding lectin (MBL) belongs to the C-type lectin family of blood proteins and plays an important role in innate immunity.[1] MBL recognizes and binds to carbohydrate structures (e.g., patterns of mannose) on pathogens surfaces. This initiates the complement system, independent of antibodies via the lectin pathway, and promotes clearance of pathogens.[1,2] Serum MBL levels are rather stable over lifetime[3], but vary widely between individuals mainly because of six common single nucleotide polymorphisms (SNPs).[4] Due to linkage disequilibrium the six SNPs give rise to seven major haplotypes: HYPA, LYQA, LYPA, LXPA, LYPB, LYQC and HYPD, which can be further combined into three MBL expression genotypes – low, intermediate, and high[5,6] – corresponding to approximate serum MBL levels of ≤100, 101-1000, and >1000 µg/L.[4]

Low MBL concentrations (i.e., below 100 µg/L, as seen in ~12% of the general population[7]) may have impaired normal innate immune function and thus increased susceptibility of infections.[5,8-13] However, a large general population study found no clear association between MBL deficiency and increased risk of infections.[14] Due to the redundancy of the immune system, the increased risk of infections may only be apparent in individuals with additional risk factors, such as cancer, chemotherapy, autoimmune diseases[15-18], and possibly diabetes.

463 million adults now live with diabetes worldwide[19], and there is clear evidence that diabetes is associated with increased risk and severity of infections[20], in particular bacterial infections.[21] The causal role of serum MBL in infection risk in T2D has to our knowledge never been investigated before.

We tested the hypothesis that low MBL is associated with increased risk of infections in patients with T2D. To test this, we conducted a Danish cohort study of 7588 patients with T2D followed for up to 8 years. We first investigated the link between baseline serum MBL levels and subsequent risk of any hospital-treated infections, any community-based antimicrobial

prescriptions, and subtypes hereof. Second, we investigated whether MBL expression genotype was associated with risk of infection outcomes. According to the Mendelian randomization study design, this step aids in substantiating a causal association between serum MBL and infection risk.[22]

MATERIAL AND METHODS

Study Population

The Danish Centre for Strategic Research in Type 2 Diabetes (DD2) cohort has been described in detail previously.[23] Briefly, the DD2 cohort was initiated in November 2010 and is an ongoing cohort of patients recently diagnosed with T2D.[23] Enrollment has been continuous from hospital specialist outpatient clinics and general practitioners' offices. Hospital physicians and general practitioners identify newly/recently diagnosed patients with T2D and complete an online questionnaire[24] on health-related items (e.g., physical activity, alcohol consumption, and anthropometric measurements) and clinical examination data (e.g., waist–hip ratio) for each participants at the time of DD2 enrollment. Fasting blood and urine samples have been collected from each participant at enrolment and stored at -80C in the DD2 biobank.[25]

Identification of hospital-treated infections

We identified first hospital-treated infections after the DD2 enrollment date based on all diagnoses (primary and secondary discharge diagnoses) from the Danish National Patient Registry, which contains all Danish hospital records of discharge diagnoses from all inpatient hospitalizations since 1977 and all emergency department visits and hospital outpatient clinic since 1995.[26] Discharge diagnoses were based on the International Classification of Diseases, 10th revision. Supplementary Table 1 gives diagnosis codes. Hospital-treated infections were classified into subtypes consistent with previous studies[27,28]: the major category of bacterial infections, as well as viral and fungal

infections. Bacterial diseases were pneumonia, urinary tract infections, skin infections, sepsis, abscesses, intra-abdominal infections, diarrheal diseases, and other bacterial infections. Viral diseases included influenza and other viral infections.

Identification of community-based antimicrobial prescriptions

We identified first community-based antimicrobial prescriptions after the DD2 enrollment date from the Danish Health Service Prescription Database, which contains information on all prescribed drugs dispensed from all Danish pharmacies.[29] Antimicrobial prescriptions were based on the Anatomical Therapeutic Chemical (ATC) classification system. Supplementary Table 2 gives ATC codes. Community-based antimicrobial prescriptions were classified into subtypes consistent with previous studies[27]: dispensed prescriptions of all antimicrobial agents prescribed for oral treatment of bacterial, viral, and fungal infections were identified. As a proxy for respiratory tract infections, we identified the number of dispensed prescriptions for oral treatment with phenoxymethylpenicillin (which is recommended first-line treatment for respiratory tract infections in Denmark) and specific macrolides (erythromycin, roxithromycin, and clarithromycin).[27] As a proxy for skin infections, we identified dispensed prescriptions of dicloxacillin and flucloxacillin.[27] As a proxy for urinary tract infections, we used dispensed prescriptions of pivmecillinam, sulfamethizole, nitrofurantoin, and trimethoprim. In an additional analysis, we also included pivampicillin in an expanded proxy for urinary tract infections.[27] Furthermore, we evaluated dispensed prescriptions with commonly prescribed broad-spectrum penicillin in Denmark (amoxicillin and amoxicillin with enzyme inhibitor), which may be used for several infection types.[27]

Serum MBL Levels

Functional serum MBL levels at time of cohort enrolment were measured in the DD2 biobank using an in-house time-resolved immuno-fluorometric assay, as described in detail elsewhere.[2] In brief, mannan-coated microtiter wells were incubated with serum samples, and bound MBL was detected with biotin-labeled monoclonal anti-MBL antibody followed by europium-labeled streptavidin and detection by time-resolved fluorometrics. The limit of quantification was 10 μg/L and the intra- and interassay coefficients of variation were <10%. Serum MBL levels were categorized as previously defined (ref): low (≤100 μg/L), intermediate (101–1000 μg/L), or high (>1000 μg/L). Consistent with our previous finding indicating that intermediate MBL levels are the most advantageous for lowest mortality and cardiovascular outcomes, the intermediate serum MBL category (101–1000 μg/L) was used as reference (ref).

MBL Expression Genotypes

TaqMan genotyping assays were used to genotype the six single nucleotide polymorphisms (SNPs) in the *MBL2* gene (rs11003125, rs7096206, rs7095891, rs5030737, rs1800451, and rs1800450) on the first 3043 consecutive patients in the DD2 cohort[4], as described in detail in the Supplemental Material. Due to linkage disequilibrium, the six SNPs give rise to seven major haplotypes: HYPA, LYQA, LYPA, LXPA, LYPB, LYQC, and HYPD. These MBL haplotypes were categorized as previously defined (ref): low, intermediate, and high, previously shown to correlate with serum MBL levels (ref).[30]

Covariates

From the online DD2 questionnaire[24] and linked administrative and medical registries, we extracted information on covariates present at the time of DD2 enrollment. Selection of covariates was based

on their known association with serum MBL levels and/or risk of infections. Covariates, definitions, and codes are listed in Supplementary Table 3.

Biochemical analysis

High-sensitivity C-reactive protein (hs-CRP, mg/L) was determined by in-house Time Resolved Immuno-fluorometric Assay, as previously described.[31] Samples were diluted 1000-fold and measured in duplicate. Intra- and interassay coefficients of variation were <5% and <6%, respectively. HbA1c (hemoglobin), fasting blood glucose, and lipids were measured by hospital routine analysis.

Statistical Analysis

Analyses were performed using STATA version 14.2. Hardy–Weinberg equilibrium was assessed by a χ^2 test for assess risk of genotype misclassification. To evaluate the association between MBL expression genotype and serum MBL levels, we performed a Cuzick non-parametric test for trend and calculated R^2 by a simple linear regression.

The cumulative incidence of hospital-treated infections and community-based antimicrobial prescriptions, with death as a competing risk, was plotted using STATA's stcompet command and incidence rates were calculated using STATA's stptime command. We used restricted cubic spline models with five degrees of freedom to examine the association between serum MBL levels, as a continuous variable, and risks of hospital-treated infections and community-based antimicrobial prescriptions. Cox regression analysis, with time as the time scale, were used to compute hazard ratios (HRs) and 95% confidence intervals (CIs) for hospital-treated infections and community-based antimicrobial prescriptions. We detected no major violations of the proportional hazard assumption. We performed extensive adjustments to ensure robustness of the potential associations

between MBL and infections. In Model 1, HRs were adjusted for sex and age. In Model 2, HRs were adjusted for sex, age, diabetes duration, and levels of hs-CRP to examine whether this inflammatory biomarker could attenuate the association between MBL and risk of infections. In the fully adjusted Model 3, HRs were adjusted for sex, age, diabetes duration, hs-CRP, waist circumference, waist–hip ratio, body mass index, physical activity, smoking, alcohol consumption, comorbidities, fasting blood glucose, HbA1c, total cholesterol, low-density lipoprotein, high-density cholesterol, triglycerides, and use of anti-diabetic, and lipid-lowering treatment. Missing values for each covariate (n=5–3966; 0.1%–54%; Supplementary Table 4) were imputed, in order to use a complete dataset. The multiple imputation is described in detail in the Supplemental Material. We did not impute MBL expression genotype where this information was missing (n=4262; 58%).

For all analyses, we ignored any infectious disease and antimicrobial prescriptions prior to the DD2 enrollment date, and thus did not exclude patients with these events. We assumed that most patients with previous infectious disease would have fully recovered from the disease if they were able to attend the DD2 enrollment examination. We followed the patients from the DD2 enrollment date until a first infection event (separately for hospital-diagnosed infections and antimicrobial prescriptions), emigration, death, or end of follow-up, whichever came first. We did not consider recurrent infectious disease. For hospital-treated infections, end of follow-up was August 10, 2018. For community-based antimicrobial prescriptions, end of follow-up was December 31, 2017. Vital and emigration status, as well as exact dates of death were obtained from the Danish Civil Registration System.[32]

We performed a sensitivity analysis excluding patients with serum CRP levels above 10 mg/L (n=641; 9%) at the time of enrolment, to exclude possible ongoing infections at the time of MBL testing.[28]

Ethics

This study was approved by the Regional Committees on Health Research Ethics for Southern Denmark (record number S-20100082) and by the Danish Data Protection Agency (record number 2008-58-0035). All DD2 participants gave written informed consent.

RESULTS

The study included 7588 patients with T2D, of whom 7305 (96%) had a serum MBL measurement available and 3043 (42%) had been genotyped for the six SNPs in the *MBL2* gene (Supplementary Figure 1). The cohort was followed for up to 8 years, with a median follow-up of 4.5 years [inter-quartile range (IQR): 3.0–5.5] for any hospital-treated infections and 1.5 years [IQR: 0.5–3.1] for any community-based antimicrobial prescriptions. Between 2010 and 2018, 1140 patients (16%) were hospitalized with an infection and a total of 5077 patients (70%) had redeemed a community-based antimicrobial prescription. See supplementary Table 5 for the patient subtype events. More than one event was possible.

Table 1 shows baseline characteristics of T2D patients according to serum MBL categories (≤100, 101–1000, or >1000 µg/L) and Supplementary Table 6 shows them according to MBL expression genotype categories (low, intermediate, and high). We found no clear differences in baseline characteristics between the different serum MBL and MBL expression genotype categories.

The MBL genotype frequencies in T2D were 59% for A/A (wildtype), 36% for A/O (heterozygotes), and 5% for O/O (deficiency homozygotes), similar to the general population[14] and patients with type 1 diabetes.[1] The median serum MBL levels for patients with low, intermediate, and high MBL expression genotypes were 10 µg/L (IQR: 10–26 µg/L), 321 µg/L (IQR: 199–545 µg/L), and 1527 µg/L (IQR: 974–2394 µg/L), consistent with previous studies.[1] The distributions of

the MBL haplotypes with corresponding median serum MBL levels are shown in Supplementary Table 7. We detected no major deviations in Hardy-Weinberg equilibrium (Supplementary Table 8). Serum MBL levels were strongly associated with MBL expression genotypes (Supplementary Figure 2; R^2=0.31, *P* for trend $<1\times10^{-300}$).

MBL and risk of hospital-treated infections

Low serum MBL and low MBL expression genotypes were associated with the highest cumulative incidence of infections (Figure 1). Incidence rates are shown in Supplementary Table 9. When assessing serum MBL on a continuous scale (Figure 2 and Supplementary Figure 3), the risk of any hospital-treated infections, bacterial infections, as well as the main subtypes pneumonia, urinary tract infections, diarrheal diseases, and other bacterial infections showed a tendency towards an "L-shaped" association with serum MBL levels. Figure 3 shows aHRs for any hospital-treated infections and for the main categories of bacterial infections, by serum MBL and MBL expression genotype categories. In the fully adjusted Model 3, there was a modest association between low serum MBL level (≤100 μg/L) and increased risk of any hospital-treated infections (aHR 1.13, 95% CI 0.96–1.33), driven by bacterial infections (aHR 1.19, 95% CI 1.01–1.41) (Figure 3). The association between low serum MBL levels and bacterial infections was modest for urinary tract infections (aHR 1.14, 95% CI 0.81–1.62), and strongest for pneumonia (aHR 1.30, 95% CI 0.98–1.70), diarrheal diseases (aHR 1.77, 95% CI 0.97–3.23), and other bacterial infections (aHR 1.50, 95% CI 1.00–2.24) (Figure 3). Low MBL expression genotype was less strongly associated with increased risk of hospital-treated infections, with an aHR of 1.08 (0.84–1.38) for any hospitalized infections and 1.13 (0.88–1.46) for bacterial infections, including an aHR of 2.23 (1.04–4.80) for diarrheal diseases. As an additional finding, both high serum MBL levels and MBL expression genotype were associated with increased risk of fungal infection (Supplementary Figure 4).

MBL and risk of community-based antimicrobial prescriptions

Overall, low serum MBL levels tended to show the same positive associations with increased antimicrobial prescriptions as with hospital-treated infection (Figure 1). Incidence rates are shown in Supplementary Table 9. However, spline associations were less clear (Figure 2 and Supplementary Figure 5), and adjusted risk estimates were lower, e.g., aHR of 1.06 (95% CI, 0.98–1.15) for any antimicrobial prescriptions, 1.07 (95% CI, 0.99–1.16) for antibacterial prescriptions, 1.10 (95% CI, 1.00–1.21) for prescriptions for respiratory tract infections, and 1.09 (95% CI, 0.95–1.21) for prescriptions for urinary tract infections (Figure 4). Interestingly, the genetic associations with MBL expression genotypes tended to be stronger than the observational associations with serum MBL, e.g. for any antimicrobial prescriptions (aHR 1.18, 95% CI 1.04–1.34) and antibacterial prescriptions (aHR 1.20, 95% CI 1.05–1.36) (Figure 4). Like for hospital-treated fungal infection, high serum MBL levels (but not high MBL expression genotype) were associated with increased risk of antifungal prescriptions (Supplementary Figure 6). In addition, high MBL expression genotype was associated with a slightly increased risk for any antimicrobial prescriptions (aHR 1.10, 95% CI 1.00–1.20) and antibacterial prescriptions (aHR 1.11, 95% CI 1.02–1.22) (Figure 4).

Sensitivity Analyses

Overall, the sensitivity analyses restricted to patients with CRP below 10 mg/L (Supplementary Figures 7–18) yielded results similar to the main analyses of risk of future infections.

DISCUSSION

In this prospective study of 7305 patients with recently diagnosed T2D, low serum MBL levels were associated with increased risk of future bacterial infections. The association was evident both when defining an infectious disease events as a hospital contact due to infection and as a redeemed prescription of an antimicrobial agent. Importantly, the increased risk was shown mainly for bacterial infections, and was supported by similar, although weaker, associations with low MBL expression genotype, suggesting a causal role of MBL in infection risk according to the principle of Mendelian randomization.

MBL is a pivotal factor in the innate immune system, initiating the complement cascade and promoting pathogen clearance.[1,2] However, MBL deficiency did not increase risk of infection in a Danish cohort study of 9245 individuals from the general population.[14] This indicates that other immune systems, e.g., ficolins[33], are able to compensate for the MBL function in adults with MBL deficiency.[14] In accordance, MBL deficiency may only increase risk of infections when other parts of the immune system are compromised[8], e.g., by chemotherapy[17], autoimmune and inflammatory diseases[34], or cancer.[16] We demonstrate for the first time that low serum MBL levels increase risk of bacterial infections in patients with T2D. The biological mechanisms associated with increased susceptibility to infections may include reduced opsonophagocytic killing and reduced activation of the complement system by the lectin pathway.[35]

The consistency that both low serum MBL levels and MBL expression genotypes were strongly associated with diarrheal diseases suggests an important direct involvement of serum MBL in gastrointestinal infections in T2D. In accordance with this, a case-control study of 120 MBL deficient adults showed that individuals with the low MBL expression genotype (O/O) were more likely to suffer from gastrointestinal disease than individuals with the intermediate genotype (A/O) and high MBL expression genotype (A/A).[36]

Mannan is a major component of fungal cell walls, and MBL binds to different fungal pathogens.[9] We observed that high MBL serum levels and MBL expression genotypes were associated with increased risk of hospital-treated fungal infections. On the basis of transcriptomic data from The Cancer Genome Atlas it was very recently revealed that the level of MBL is influencing the oncogenic potential of pancreatic ductal adenocarcinoma patients, i.e. low levels of MBL were associated with longer survival.[37] This may be based on fungal dysbiosis driven by the lectin pathway of the complement system. It was confirmed by studies in mice that MBL induced a more pathogenic phenotype in this setting (ref).

Limitations of this study include the possibility of survival bias and selection bias in the DD2 cohort. However, these situations would likely result in underrepresentation of patients with a severe T2D phenotype or patients predisposed to infectious diseases, and would likely bias the results towards the null hypothesis. Another potential limitation includes misclassification of diagnoses, genotypes, and prescriptions. However, the validity of major diagnoses including infectious disease diagnoses in the Danish registries is high.[38] In addition, genotype misclassification is unlikely due to the lack of major deviations from Hardy-Weinberg equilibrium.[30] Prescription antimicrobials serve only as a proxy for the occurrence of an infectious disease in the community, as antimicrobial agents might be prescribed outside their proper indication and general practitioners' approach to treating infectious diseases may vary.[27] This may result in some outcome misclassification, but we expect such misclassification to be non-differential according to MBL level, likely biasing the results towards the null hypothesis. We were unfortunately unable to investigate the specific type of infection based on microbiological assessments. Finally, another limitation is pleiotropy. According to Ldlink (https://ldlink.nci.nih.gov/), no SNPs in the *MBL2* gene were in linkage disequilibrium with any

genetic variants outside the *MBL2* gene and confounding by variation in nearby genes can therefore not explain our findings.

In conclusion, in this prospective study, we found evidence that patients with early T2D and low MBL levels have an increased risk of future bacterial infections. T2D in combination with MBL deficiency may act as a dual hit to the immune system and increase susceptibility to bacterial infections.

ACKNOWLEDGMENTS

We are grateful to all participants in the DD2 study. We thank Hanne Petersen and Karen Mathiassen for their assistance with the large-volume measurements of serum MBL and hs-CRP. We gratefully acknowledge Sia Kromann Nicolaisen for assistance with data management.

Funding

The Danish Centre for Strategic Research in Type 2 Diabetes Project (DD2) is supported by the Danish Agency for Science (grant nos. 09-067009 and 09-075724), the Danish Health and Medicines Authority, the Danish Diabetes Association, and an unrestricted donation from Novo Nordisk A/S. Project partners are listed on the www.DD2.nu website. The work of A.G. was supported by the Danish Diabetes Academy, which is funded by an unrestricted grant from the Novo Nordisk Foundation, and by grants from the Danish Heart Foundation (grant nos. 15-R99-A5866-22891) and Aarhus University. In addition, A.G. has received funding from the Danielsen Foundation, Augustinus Foundation, A.P. Møller Foundation, Hertz Foundation, and Bønnelycke Foundation. The Department of Clinical Epidemiology, Aarhus University Hospital, participates in the International Diabetic Neuropathy Consortium research program, which is supported by a Novo Nordisk Foundation Challenge program grant (grant number NNF14SA000 6). The funding source had no role in study design, data collection, data analysis, data interpretation, or writing of the report. AG and RWT had full access to all study data and had final responsibility for the decision to submit for publication.

Declaration of Conflicts of Interest

None.

Author Contributions

H.B.N., H.T.S., and J.R. participated in conceiving and designing the parent DD2 project cohort study. R.W.T, A.D.K., J.S.N., J.R., S.F., I.B., H.B.N., H.T.S., T.K.H., and M.B. conceived of the

current study. M.B. was responsible for serum MBL and hs-CRP measurements, and R.S. was responsible for MBL genotyping. I.B. was responsible for the biobank and the other biochemical analyses. A.G., A.D.K., and R.W.T. participated in the design of the current study, and A.G. performed the statistical analyses. A.G. drafted the article, with help from A.D.K., R.W.T., and M.B. All other authors have critically reviewed the manuscript. All authors contributed substantially to the study, revised the manuscript for intellectual content, and approved the final version to be submitted. A.G., R.W.T., and M.B. are the guarantors of this work and, as such, had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

REFERENCES

1. Hansen TK, Tarnow L, Thiel S, et al. Association between mannose-binding lectin and vascular complications in type 1 diabetes. Diabetes 2004;53:1570-6.
2. Thiel S, Moller-Kristensen M, Jensen L, Jensenius JC. Assays for the functional activity of the mannan-binding lectin pathway of complement activation. Immunobiology 2002;205:446-54.
3. Ytting H, Christensen IJ, Thiel S, et al. Biological variation in circulating levels of mannan-binding lectin (MBL) and MBL-associated serine protease-2 and the influence of age, gender and physical exercise. Scand J Immunol 2007;66:458-64.
4. Molle I, Steffensen R, Thiel S, Peterslund NA. Chemotherapy-related infections in patients with multiple myeloma: associations with mannan-binding lectin genotypes. European journal of haematology 2006;77:19-26.
5. Eisen DP, Dean MM, Boermeester MA, et al. Low serum mannose-binding lectin level increases the risk of death due to pneumococcal infection. Clin Infect Dis 2008;47:510-6.
6. Swale A, Miyajima F, Kolamunnage-Dona R, et al. Serum mannose-binding lectin concentration, but not genotype, is associated with Clostridium difficile infection recurrence: a prospective cohort study. Clin Infect Dis 2014;59:1429-36.
7. Steffensen R, Thiel S, Varming K, Jersild C, Jensenius JC. Detection of structural gene mutations and promoter polymorphisms in the mannan-binding lectin (MBL) gene by polymerase chain reaction with sequence-specific primers. Journal of immunological methods 2000;241:33-42.
8. Eisen DP. Mannose-binding lectin deficiency and respiratory tract infection. Journal of innate immunity 2010;2:114-22.
9. Eisen DP, Minchinton RM. Impact of mannose-binding lectin on susceptibility to infectious diseases. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2003;37:1496-505.
10. Garcia-Laorden MI, Sole-Violan J, Rodriguez de Castro F, et al. Mannose-binding lectin and mannose-binding lectin-associated serine protease 2 in susceptibility, severity, and outcome of pneumonia in adults. The Journal of allergy and clinical immunology 2008;122:368-74, 74.e1-2.
11. Ip WK, Chan KH, Law HK, et al. Mannose-binding lectin in severe acute respiratory syndrome coronavirus infection. The Journal of infectious diseases 2005;191:1697-704.
12. Roy S, Knox K, Segal S, et al. MBL genotype and risk of invasive pneumococcal disease: a case-control study. Lancet 2002;359:1569-73.
13. Rantala A, Lajunen T, Juvonen R, et al. Mannose-binding lectin concentrations, MBL2 polymorphisms, and susceptibility to respiratory tract infections in young men. The Journal of infectious diseases 2008;198:1247-53.
14. Dahl M, Tybjaerg-Hansen A, Schnohr P, Nordestgaard BG. A population-based study of morbidity and mortality in mannose-binding lectin deficiency. J Exp Med 2004;199:1391-9.
15. Garred P, Voss A, Madsen HO, Junker P. Association of mannose-binding lectin gene variation with disease severity and infections in a population-based cohort of systemic lupus erythematosus patients. Genes and immunity 2001;2:442-50.
16. Neth O, Hann I, Turner MW, Klein NJ. Deficiency of mannose-binding lectin and burden of infection in children with malignancy: a prospective study. Lancet 2001;358:614-8.
17. Peterslund NA, Koch C, Jensenius JC, Thiel S. Association between deficiency of mannose-binding lectin and severe infections after chemotherapy. Lancet 2001;358:637-8.
18. Super M, Thiel S, Lu J, Levinsky RJ, Turner MW. Association of low levels of mannan-binding protein with a common defect of opsonisation. Lancet 1989;2:1236-9.
19. *IDF Diabetes Atlas, 9th edn.* . 2019. (Accessed 1/7, 2020, at http:///www.diabetesatlas.org.)

20. Pearson-Stuttard J, Blundell S, Harris T, Cook DG, Critchley J. Diabetes and infection: assessing the association with glycaemic control in population-based studies. The lancet Diabetes & endocrinology 2016;4:148-58.
21. Thomsen RW, Mor A. Diabetes and risk of community-acquired respiratory tract infections, urinary tract infections, and bacteremia. Open Infectious Diseases Journal 2012;6:27-39.
22. Benn M, Nordestgaard BG. From genome-wide association studies to Mendelian randomization: novel opportunities for understanding cardiovascular disease causality, pathogenesis, prevention, and treatment. Cardiovascular research 2018;114:1192-208.
23. Christensen DH, Nicolaisen SK, Berencsi K, et al. Danish Centre for Strategic Research in Type 2 Diabetes (DD2) project cohort of newly diagnosed patients with type 2 diabetes: a cohort profile. BMJ open 2018;8:e017273.
24. Nielsen JS, Thomsen RW, Steffensen C, Christiansen JS. The Danish Centre for Strategic Research in Type 2 Diabetes (DD2) study: implementation of a nationwide patient enrollment system. Clinical epidemiology 2012;4:27-36.
25. Christensen H, Nielsen JS, Sorensen KM, Melbye M, Brandslund I. New national Biobank of The Danish Center for Strategic Research on Type 2 Diabetes (DD2). Clinical epidemiology 2012;4:37-42.
26. Schmidt M, Schmidt SA, Sandegaard JL, Ehrenstein V, Pedersen L, Sorensen HT. The Danish National Patient Registry: a review of content, data quality, and research potential. Clin Epidemiol 2015;7:449-90.
27. Kaspersen KA, Dinh KM, Erikstrup LT, et al. Low-Grade Inflammation Is Associated with Susceptibility to Infection in Healthy Men: Results from the Danish Blood Donor Study (DBDS). PLoS One 2016;11:e0164220.
28. Zacho J, Benfield T, Tybjaerg-Hansen A, Nordestgaard BG. Increased Baseline C-Reactive Protein Concentrations Are Associated with Increased Risk of Infections: Results from 2 Large Danish Population Cohorts. Clinical chemistry 2016;62:335-42.
29. Johannesdottir SA, Horvath-Puho E, Ehrenstein V, Schmidt M, Pedersen L, Sorensen HT. Existing data sources for clinical epidemiology: The Danish National Database of Reimbursed Prescriptions. Clinical epidemiology 2012;4:303-13.
30. Gadjeva M, Takahashi K, Thiel S. Mannan-binding lectin--a soluble pattern recognition molecule. Mol Immunol 2004;41:113-21.
31. Holt CB, Ostergaard JA, Thiel S, et al. Circulating lectin pathway proteins do not predict short-term cardiac outcomes after myocardial infarction. Clin Exp Immunol 2019.
32. Schmidt M, Pedersen L, Sorensen HT. The Danish Civil Registration System as a tool in epidemiology. Eur J Epidemiol 2014;29:541-9.
33. Holmskov U, Thiel S, Jensenius JC. Collections and ficolins: humoral lectins of the innate immune defense. Annual review of immunology 2003;21:547-78.
34. Thiel S, Frederiksen PD, Jensenius JC. Clinical manifestations of mannan-binding lectin deficiency. Mol Immunol 2006;43:86-96.
35. Takahashi K. Mannose-binding lectin and the balance between immune protection and complication. Expert review of anti-infective therapy 2011;9:1179-90.
36. Bjarnadottir H TV, Jorgensen GH, Arnardottir M, Ludviksson BR. Mannan-Binding Lectin (MBL) Deficient Individuals with the O/O Genotype are Highly Susceptible to Gastrointestinal Diseases. J Clin Cell Immunol 2014;5:182.
37. Aykut B, Pushalkar S, Chen R, et al. The fungal mycobiome promotes pancreatic oncogenesis via activation of MBL. Nature 2019;574:264-7.
38. Henriksen DP, Nielsen SL, Laursen CB, Hallas J, Pedersen C, Lassen AT. How well do discharge diagnoses identify hospitalised patients with community-acquired infections?--a validation study. PLoS One 2014;9:e92891.

Legends

Figure 1. Time-to-event curves of any hospital-treated infections and any community-based antimicrobial prescriptions by serum MBL and MBL expression genotype categories. Cumulative incidence plots of any hospital-treated infections (A and B) and any community-based antimicrobial prescriptions (C and D) by serum MBL (A and C) and MBL expression genotype (B and D) categories. Cumulative incidence estimates are based on time from DD2 enrollment date to first event, with risk of death as a competing risk.

Figure 2. Risk of hospital-treated infections and community-based antimicrobial prescriptions by serum MBL levels. Any infections (A), bacterial infections (B), any prescriptions (C), and antibacterial prescriptions (D). The solid lines indicate the hazard ratios, and the dotted lines indicate 95% confidence intervals. The continuous variable serum MBL was modeled with five restricted cubic splines.

Figure 3. Hazard ratios of hospital-treated infections and bacterial subtypes by serum MBL and MBL expression genotype categories.
Model 3: sex, age, diabetes duration, hs-CRP, waist circumference, waist–hip ratio, body mass index, physical activity, smoking, alcohol consumption, comorbidities, fasting blood glucose, HbA1c, total cholesterol, low-density lipoprotein, high-density cholesterol, triglycerides, and use of anti-diabetic, and lipid-lowering treatment. Missing covariates were treated with multiple imputation.

Figure 4. Hazard ratios of community-based antimicrobial prescriptions and subtypes of antibacterial prescriptions by serum MBL and MBL expression genotype categories.
Model 3: sex, age, diabetes duration, hs-CRP, waist circumference, waist–hip ratio, body mass index, physical activity, smoking, alcohol consumption, comorbidities, fasting blood glucose, HbA1c, total cholesterol, low-density lipoprotein, high-density cholesterol, triglycerides, and use of anti-diabetic, and lipid-lowering treatment. Missing covariates were treated with multiple imputation.

Table 1. Characteristics of DD2 Cohort Participants at Baseline by Serum MBL Category.

	Low serum MBL (≤100 µg/L)	Intermediate serum MBL (101-1000 µg/L)	High serum MBL (>1000 µg/L)
Total, N (%)	1295 (17.7)	2975 (40.7)	3035 (41.6)
Male sex, n (%)	727 (56.1)	1612 (54.2)	1939 (63.9)
Median age (IQR), years	61.6 (52.7–69.0)	61.9 (53.1–68.7)	62.3 (53.0–68.8)
Median diabetes duration (IQR), years	1.3 (0.3–2.9)	1.4 (0.4–2.9)	1.2 (0.3–2.9)
Median waist circumference (IQR), cm	106 (97–117)	107 (97–117)	105 (96–115)
Median waist–hip ratio (IQR)	0.98 (0.92–1.04)	0.98 (0.92–1.04)	0.98 (0.93–1.04)
Median body mass index (IQR), kg/m^2	30.5 (27.1–34.5)	30.7 (27.4–34.7)	29.7 (26.4–33.7)
High alcohol intake[a], n (%)	74 (5.7)	211 (7.1)	190 (6.3)
Physical activity[b] (IQR), days/week	3 (2–7)	3 (2–7)	4 (2–7)
Smoking, n (%)			
Never	434 (45.5)	1039 (47.7)	1052 (46.3)
Former	351 (36.8)	749 (34.4)	750 (33.0)
Current	170 (17.8)	389 (17.9)	471 (20.7)
CCI score[c], n (%)			
0	882 (68.1)	2034 (68.4)	2109 (69.5)
1-2	339 (26.2)	783 (26.3)	763 (25.1)
3	74 (5.7)	158 (5.3)	163 (5.4)
Anti-diabetes drug use, n (%)	1080 (83.4)	2547 (85.6)	2582 (85.1)
Lipid-lowering drug use, n (%)	932 (72.0)	2176 (73.1)	2037 (67.1)
Median fasting blood glucose (IQR), mmol/L	7.1 (6.3–8.1)	7.1 (6.3–8.2)	7.2 (6.4–8.3)
Median HbA1c (IQR), %	6.6 (6.2–7.2)	6.6 (6.1–7.2)	6.6 (6.2–7.3)
Median total cholesterol (IQR), mmol/L	4.4 (3.8–5.2)	4.3 (3.7–5.1)	4.3 (3.7–5.1)
Median LDL cholesterol (IQR), mmol/L	2.1 (1.7–2.7)	2.2 (1.7–2.8)	2.2 (1.7–2.9)
Median HDL cholesterol (IQR), mmol/L	1.2 (1–1.4)	1.2 (1–1.4)	1.2 (1–1.5)
Median triglycerides (IQR), mmol/L	1.7 (1.2–2.5)	1.7 (1.2–2.4)	1.6 (1.1–2.3)
Median hs-CRP (IQR), mg/L	2.0 (0.8–4.7)	2.0 (0.9–4.5)	1.9 (0.8–4.3)

Abbreviations: MBL, mannose-binding lectin; IQR, interquartile range; CCI, Charlson Comorbidity Index; HbA1c, hemoglobin A1c; LDL, low-density lipoprotein; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein

[a]High alcohol intake defines as ≥14/21 alcoholic drinks/week for women/men.

[b]Days per week with a minimum of 30 minutes of physical activity

[c]CCI (Charlson Comorbidity Index) score excluding diabetes.

Number of participants varied because availability of data (see Supplementary Table 4 for missing covariates).

FIGURE 1

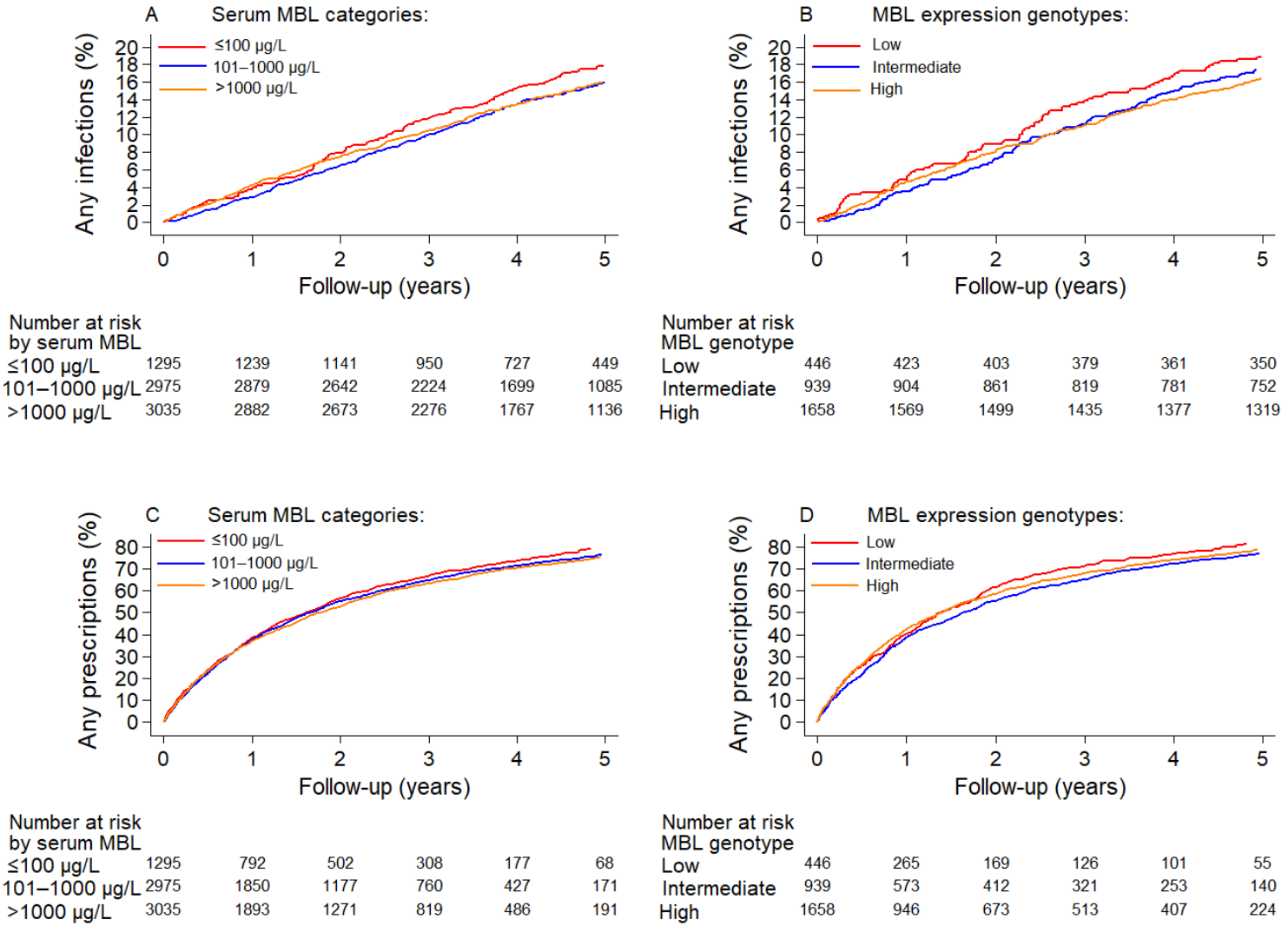

A
Serum MBL categories:
≤100 µg/L
101–1000 µg/L
>1000 µg/L
Any infections (%)
Follow-up (years)
Number at risk by serum MBL
≤100 µg/L 1295 1239 1141 950 727 449
101–1000 µg/L 2975 2879 2642 2224 1699 1085
>1000 µg/L 3035 2882 2673 2276 1767 1136
B
MBL expression genotypes:
Low
Intermediate
High
Any infections (%)
Follow-up (years)
Number at risk MBL genotype
Low 446 423 403 379 361 350
Intermediate 939 904 861 819 781 752
High 1658 1569 1499 1435 1377 1319
C
Serum MBL categories:
≤100 µg/L
101–1000 µg/L
>1000 µg/L
Any prescriptions (%)
Follow-up (years)
Number at risk by serum MBL
≤100 µg/L 1295 792 502 308 177 68
101–1000 µg/L 2975 1850 1177 760 427 171
>1000 µg/L 3035 1893 1271 819 486 191
D
MBL expression genotypes:
Low
Intermediate
High
Any prescriptions (%)
Follow-up (years)
Number at risk MBL genotype
Low 446 265 169 126 101 55
Intermediate 939 573 412 321 253 140
High 1658 946 673 513 407 224

FIGURE 2

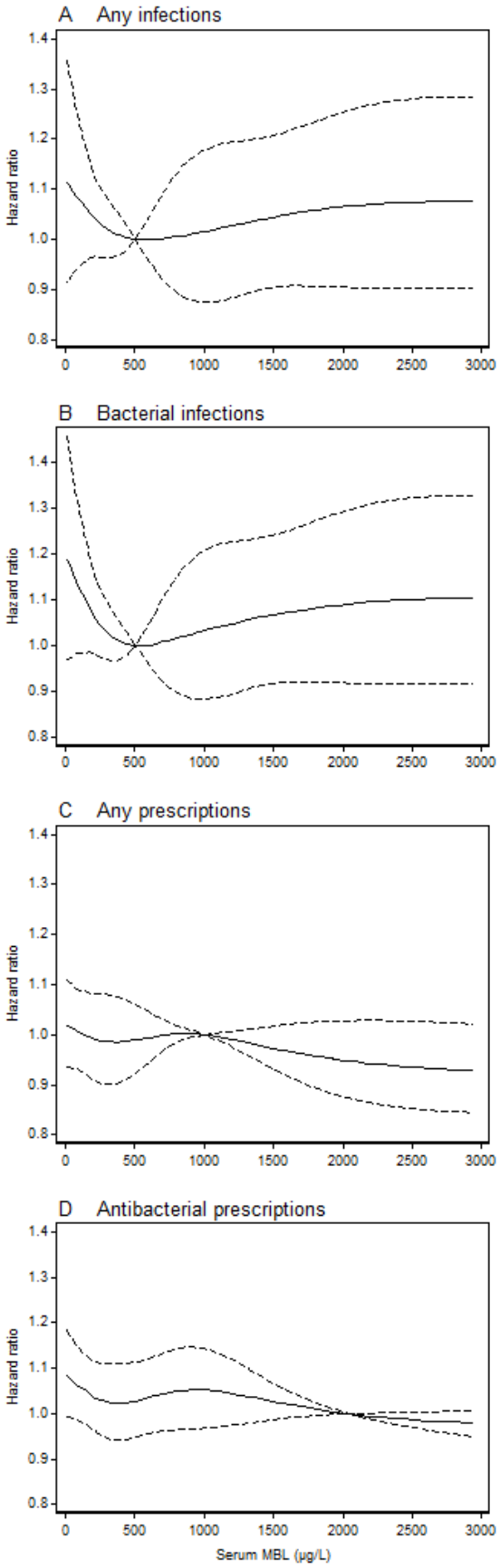

A Any infections
B Bacterial infections
C Any prescriptions
D Antibacterial prescriptions
Hazard ratio
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0
500
1000
1500
2000
2500
3000
Serum MBL (µg/L)

FIGURE 3

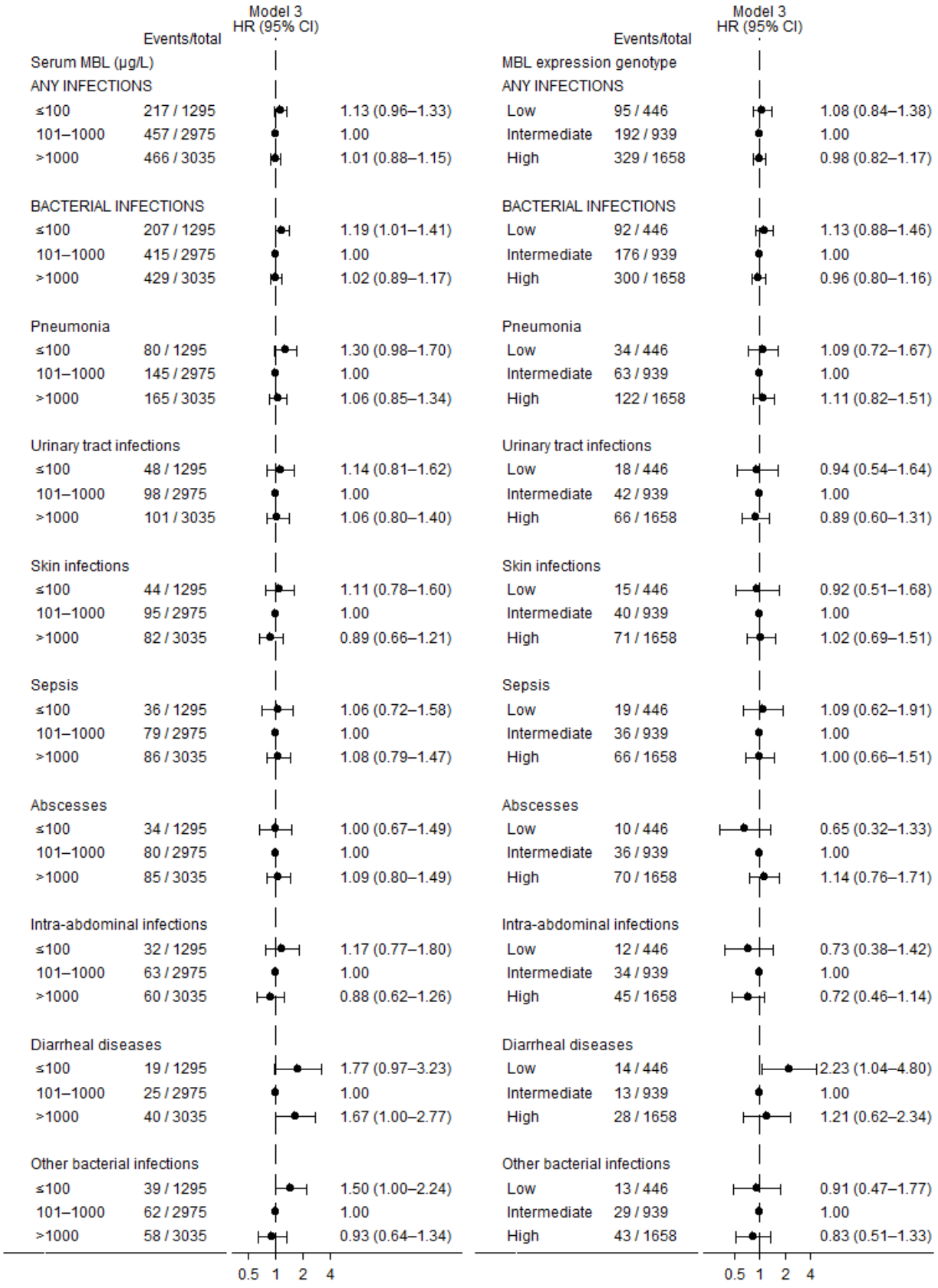
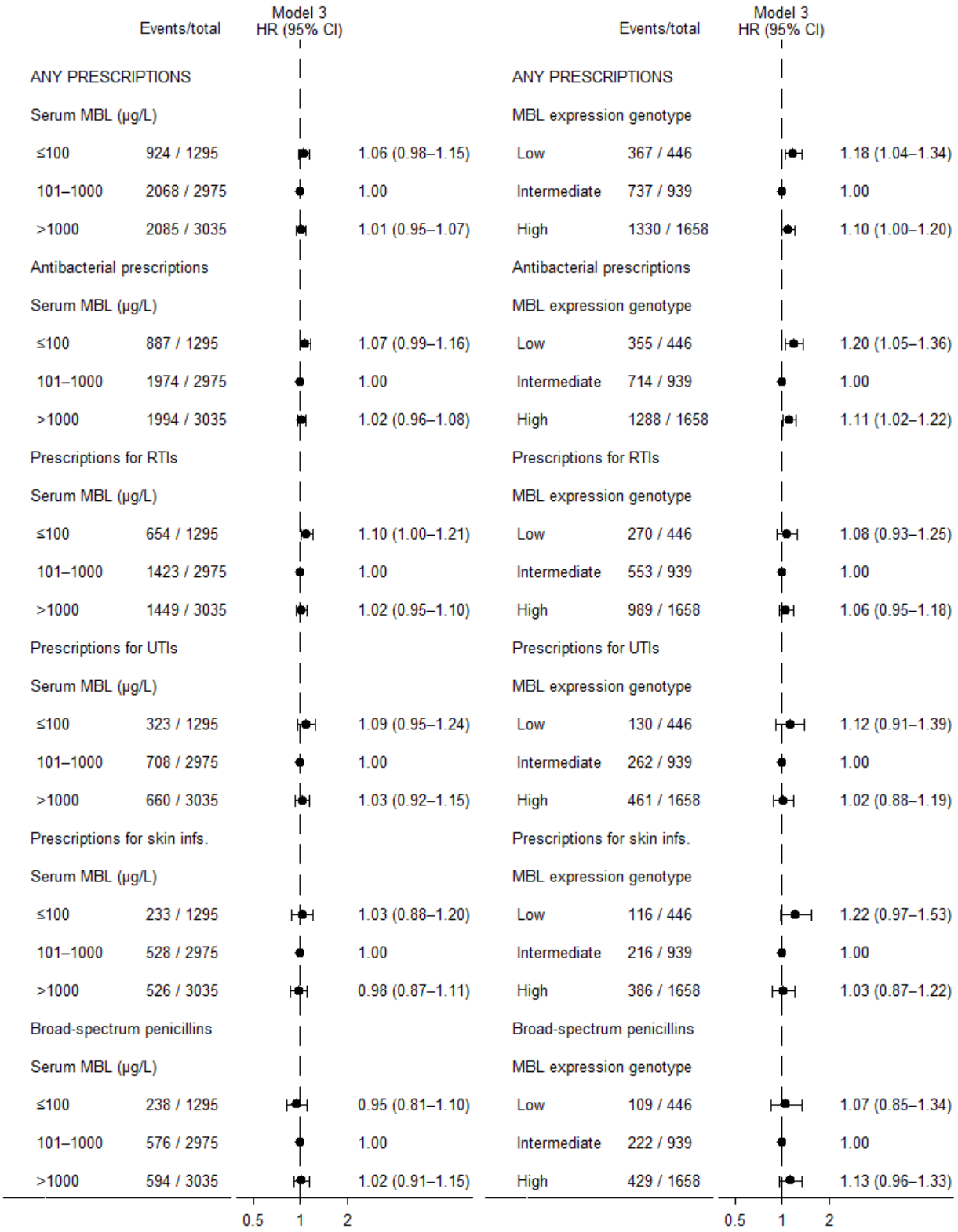

Model 3
HR (95% CI)
Events/total
Serum MBL (µg/L)
ANY INFECTIONS
≤100 217 / 1295 1.13 (0.96–1.33)
101–1000 457 / 2975 1.00
>1000 466 / 3035 1.01 (0.88–1.15)
BACTERIAL INFECTIONS
≤100 207 / 1295 1.19 (1.01–1.41)
101–1000 415 / 2975 1.00
>1000 429 / 3035 1.02 (0.89–1.17)
Pneumonia
≤100 80 / 1295 1.30 (0.98–1.70)
101–1000 145 / 2975 1.00
>1000 165 / 3035 1.06 (0.85–1.34)
Urinary tract infections
≤100 48 / 1295 1.14 (0.81–1.62)
101–1000 98 / 2975 1.00
>1000 101 / 3035 1.06 (0.80–1.40)
Skin infections
≤100 44 / 1295 1.11 (0.78–1.60)
101–1000 95 / 2975 1.00
>1000 82 / 3035 0.89 (0.66–1.21)
Sepsis
≤100 36 / 1295 1.06 (0.72–1.58)
101–1000 79 / 2975 1.00
>1000 86 / 3035 1.08 (0.79–1.47)
Abscesses
≤100 34 / 1295 1.00 (0.67–1.49)
101–1000 80 / 2975 1.00
>1000 85 / 3035 1.09 (0.80–1.49)
Intra-abdominal infections
≤100 32 / 1295 1.17 (0.77–1.80)
101–1000 63 / 2975 1.00
>1000 60 / 3035 0.88 (0.62–1.26)
Diarrheal diseases
≤100 19 / 1295 1.77 (0.97–3.23)
101–1000 25 / 2975 1.00
>1000 40 / 3035 1.67 (1.00–2.77)
Other bacterial infections
≤100 39 / 1295 1.50 (1.00–2.24)
101–1000 62 / 2975 1.00
>1000 58 / 3035 0.93 (0.64–1.34)
0.5 1 2 4
Model 3
HR (95% CI)
Events/total
MBL expression genotype
ANY INFECTIONS
Low 95 / 446 1.08 (0.84–1.38)
Intermediate 192 / 939 1.00
High 329 / 1658 0.98 (0.82–1.17)
BACTERIAL INFECTIONS
Low 92 / 446 1.13 (0.88–1.46)
Intermediate 176 / 939 1.00
High 300 / 1658 0.96 (0.80–1.16)
Pneumonia
Low 34 / 446 1.09 (0.72–1.67)
Intermediate 63 / 939 1.00
High 122 / 1658 1.11 (0.82–1.51)
Urinary tract infections
Low 18 / 446 0.94 (0.54–1.64)
Intermediate 42 / 939 1.00
High 66 / 1658 0.89 (0.60–1.31)
Skin infections
Low 15 / 446 0.92 (0.51–1.68)
Intermediate 40 / 939 1.00
High 71 / 1658 1.02 (0.69–1.51)
Sepsis
Low 19 / 446 1.09 (0.62–1.91)
Intermediate 36 / 939 1.00
High 66 / 1658 1.00 (0.66–1.51)
Abscesses
Low 10 / 446 0.65 (0.32–1.33)
Intermediate 36 / 939 1.00
High 70 / 1658 1.14 (0.76–1.71)
Intra-abdominal infections
Low 12 / 446 0.73 (0.38–1.42)
Intermediate 34 / 939 1.00
High 45 / 1658 0.72 (0.46–1.14)
Diarrheal diseases
Low 14 / 446 2.23 (1.04–4.80)
Intermediate 13 / 939 1.00
High 28 / 1658 1.21 (0.62–2.34)
Other bacterial infections
Low 13 / 446 0.91 (0.47–1.77)
Intermediate 29 / 939 1.00
High 43 / 1658 0.83 (0.51–1.33)
0.5 1 2 4

FIGURE 4


Events/total
Model 3 HR (95% CI)
ANY PRESCRIPTIONS
Serum MBL (µg/L)
≤100 924 / 1295 1.06 (0.98–1.15)
101–1000 2068 / 2975 1.00
>1000 2085 / 3035 1.01 (0.95–1.07)
Antibacterial prescriptions
Serum MBL (µg/L)
≤100 887 / 1295 1.07 (0.99–1.16)
101–1000 1974 / 2975 1.00
>1000 1994 / 3035 1.02 (0.96–1.08)
Prescriptions for RTIs
Serum MBL (µg/L)
≤100 654 / 1295 1.10 (1.00–1.21)
101–1000 1423 / 2975 1.00
>1000 1449 / 3035 1.02 (0.95–1.10)
Prescriptions for UTIs
Serum MBL (µg/L)
≤100 323 / 1295 1.09 (0.95–1.24)
101–1000 708 / 2975 1.00
>1000 660 / 3035 1.03 (0.92–1.15)
Prescriptions for skin infs.
Serum MBL (µg/L)
≤100 233 / 1295 1.03 (0.88–1.20)
101–1000 528 / 2975 1.00
>1000 526 / 3035 0.98 (0.87–1.11)
Broad-spectrum penicillins
Serum MBL (µg/L)
≤100 238 / 1295 0.95 (0.81–1.10)
101–1000 576 / 2975 1.00
>1000 594 / 3035 1.02 (0.91–1.15)
0.5
1
2
Events/total
Model 3 HR (95% CI)
ANY PRESCRIPTIONS
MBL expression genotype
Low 367 / 446 1.18 (1.04–1.34)
Intermediate 737 / 939 1.00
High 1330 / 1658 1.10 (1.00–1.20)
Antibacterial prescriptions
MBL expression genotype
Low 355 / 446 1.20 (1.05–1.36)
Intermediate 714 / 939 1.00
High 1288 / 1658 1.11 (1.02–1.22)
Prescriptions for RTIs
MBL expression genotype
Low 270 / 446 1.08 (0.93–1.25)
Intermediate 553 / 939 1.00
High 989 / 1658 1.06 (0.95–1.18)
Prescriptions for UTIs
MBL expression genotype
Low 130 / 446 1.12 (0.91–1.39)
Intermediate 262 / 939 1.00
High 461 / 1658 1.02 (0.88–1.19)
Prescriptions for skin infs.
MBL expression genotype
Low 116 / 446 1.22 (0.97–1.53)
Intermediate 216 / 939 1.00
High 386 / 1658 1.03 (0.87–1.22)
Broad-spectrum penicillins
MBL expression genotype
Low 109 / 446 1.07 (0.85–1.34)
Intermediate 222 / 939 1.00
High 429 / 1658 1.13 (0.96–1.33)
0.5
1
2

Mannose-binding Lectin and Risk of Infections in Type 2 Diabetes: A Danish Cohort Study

SUPPLEMENTAL MATERIAL

Table of Contents

EXPANDED METHODS SECTION

MBL Expression Genotypes

Six SNPs located within the promoter region (rs11003125, rs7096206, rs7095891) and exon 1 (rs5030737, rs1800451, rs1800450) of the *MBL2* gene were genotyped using TaqMan genotyping assays, as previously described. From the six MBL polymorphisms, we generated seven common haplotypes (HYPA, LYPA, LYQA, LXPA, LYPB, LYQC, and HYPD) and ranked the haplotype combinations according to increasing serum MBL concentrations. The MBL haplotypes were divided into three MBL expression genotypes (low, intermediate, and high) based on the resulting serum MBL concentration. To compare genotype frequencies (for the six SNPs in the *MBL2* gene) between the DD2 cohort and other European cohorts, we performed a search in the Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org/) and the Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org/). ExAC spans 60,706 exome sequences and gnomAD spans 125,748 exome sequences and 15,708 whole-genome sequences from unrelated individuals sequenced as part of various disease-specific and population genetic studies (URL: http://exac.broadinstitute.org/ and https://gnomad.broadinstitute.org/). Because of differences in allele frequencies across different populations, we present information only for the European non-Finnish population from the ExAC and gnomAD databases, comprising approximately half of the total sequenced population (Supplemental Table 6). To examine possible confounding by variation in nearby genes, we searched for SNPs in linkage disequilibrium with the six SNPs used in this study. The search was performed on LDlink (https://ldlink.nci.nih.gov/), a suite of web-based applications designed to interrogate linkage disequilibrium in population groups.

Multiple Imputation

Missing data on covariates used for adjustment in the Cox regression models (Supplementary Table 3) were imputed to maximize power and avoid selection bias. We used multivariate normal imputation (MVNI)[1] to impute 20 complete data sets using a Bayesian approach with a Markov chain Monte Carlo algorithm. Missing values were sampled from the predictive distribution based on the observed data. MVNI assumes that all variables in the imputation model follow a multivariate normal distribution and that missing data are missing at random (MAR), meaning that the probability of a variable being missing depends only on the observed values. Continuous variables (fasting blood glucose, C-peptide, systolic and diastolic blood pressure, BMI, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, HbA1c, and albumin/creatinine ratio) with clearly non-normal (skewed) distributions were zero-skewness log-transformed, i.e., transformed to approximate normality before imputation. Then the imputed values were transformed back to the original scale before analysis.[2] Smoking (categorical variable) was also imputed using MVNI, which has been shown to perform well even in the presence of binary and ordinal variables.[1] The binary variable smoking (1: smoking – former/current vs. 0: never smoking) was imputed on a continuous scale and rounded to 0 or 1 by simple rounding.[3] Each variable in the data set was characterized as being 'imputed' or 'regular'. Imputed variables contain missing values, and those values are imputed. Regular variables usually do not contain missing values, or if they do, the missing values are not imputed. All covariates used in the analysis model, as well as the outcomes, were included in the imputation model to ensure maximum recovery of information about the association of interest. The following variables were characterized as 'imputed': fasting blood glucose, C-peptide, HbA1c, systolic blood pressure, diastolic blood pressure, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, albumin/creatinine ratio, height, weight, hs-CRP, waist circumference, waist-to-hip ratio, and smoking. The following variables were characterized as 'regular': all-cause mortality, cardiovascular events, age, sex, diabetes duration, central obesity, anti-diabetic treatment, antihypertensive treatment, use of lipid-lowering drugs, anti-thrombotic treatment, and Charlson Comorbidity Index. The imputed models were validated by comparing the mean, median, and inter-quartile range of the first and last imputed dataset with the complete dataset.

Supplementary Table 1: Diagnostic ICD-10 codes used to identify hospital-treated infections		
	ICD-10 codes (first post-index-date occurrence of a hospital in- or outpatient clinic contact associated with a primary or secondary discharge diagnosis of infection – ICD10 codes)	**Exceptions**
Any hospital-treated infections	The below mentioned ICD-10 codes	
Bacterial infections	The below mentioned ICD-10 codes for pneumonia, urinary tract infection, skin infection, diarrheal infection, sepsis, intra-abdominal infection, abscess, and other bacterial infection, meningitis, tuberculosis, joint and connective tissue disease, endocarditis, myocarditis, pericarditis	
Pneumonia	J12-J18, J851, A70, A481	
Urinary tract infections	N10-N12, N151, N300, N308A, N309, N340, N341, N390	
Skin infections	A46, L00-L08, L303, L308F, L738H	
Sepsis	A282B, A267, A327, A392, A394, A40, A41, A427, A499A	
Abscesses	A541, B43, D733, E060A, E236A, E321, G06-G07, H000A, H050A, H440A, H600, J340A, J36, J383D, J387G, J390-J391, J398A, J851-J853, K113, K122, K130A, K140A, K209A, K353A, K353B, K570, K572, K574, K578, K61, K630, K650, K750, K810A, K858A, L02, L050, L059, M608A, M868A, M869A, N151, N340, N412, N450, N482, N492A, N619A, N619B, N700A, N700B, N710A, N730A, N730B, N732A, N732B, N733A, N735A, N738A, N738C, N751, N764, N768A	A541B, B430, B438, B439, K570B, K570C, K572B, K572C, K574A, K650M, K650N, K650O, K650P
Intra-abdominal infections	K209A, K35, K37, K570, K572, K574, K578, K61, K630, K650, K658I, K678, K750, K751, K770, K800, K803, K804, K810, K830, K858A, K858E, K819, K659	
Diarrheal infections	A00-A09	
Other bacterial infections	A20-A28, A30, A31, A320, A328, A329, A35-A37, A38, A42-A44, A48, A49, A65-A69, A71, A74, A75, A77-A79, B96, B96, B98, B99	
Meningitis	A170, A87, A203, A390, B003, B010, B021, B051, B261, G00, G01, G020, G03	
Tuberculosis	A15-A19, B90, N330, K230, K673, K930, M011, M490, M900	
Endocarditis, myocarditis, pericarditis	I33, I38, I398, I400, I41, I301, I302, I321	
Joint and connective tissue infection	M00, M01, M463, M465, M491-M493, M600, M608A, M630-M632, M650, M651, M680, M710, M711, M726, M868, M869A, M901, M902	
Upper respiratory tract infection	J00-J06, J340A, J340D, J340I, J36, J382A, J383D, J387G, J39	
Other lower respiratory tract infection	J20-J22, J440, J86	
Other infections of heart and blood vessels	I430, I520, I521, I681, I981	
Viral infection	B00-B09, B25-B27, B30, B33, B34, B97, A92-A99, J09-J11, B15-B17, B19, B20-B24	

Fungal infection	B35-B49	
Parasitic infection	B50-B58, B60, B64	

Supplementary Table 2: Diagnostic ATC codes used to identify community-based antimicrobial prescriptions	
	ATC codes (first post-index-date redemption of a prescription from a primary care physician for an antiinfective agent for systemic use – ATC codes)
Any community-based antimicrobial prescriptions	J01, J02, J04A, J05, A07AA, G01AA10, D01B"
Antibacterial prescriptions	J01CE02, J01FA01, J01FA09, J01FA06, J01CA08, J01EB02, J01XE01, J01EA01, J01CA08, J01EB02, J01XE01, J01EA01, J01CA02, J01CF01, J01CF05, J01CA04, J01CR02, J01FA10, J01M, J01A, J01D, J04A
Prescriptions for respiratory tract infections	J01CE02, J01FA01, J01FA09, J01FA06
Prescriptions for urinary tract infections	J01CA08, J01EB02, J01XE01, J01EA01
Prescriptions for expanded urinary tract infections	J01CA08, J01EB02, J01XE01, J01EA01, J01CA02
Prescriptions for skin infections	J01CF01, J01CF05
Broad-spectrum penicillins	J01CA04, J01CR02
Antiviral prescriptions	J05
Antifungal prescriptions	J02, D01B

Supplementary Table 3. Definitions and codes used in this study.

Registries	Variables	Definitions and Codes
The Danish Centre for Strategic Research in Type 2 Diabetes (DD2) cohort		A nationwide Danish cohort of patients recently diagnosed with T2D. Cohort members have been enrolled continuously from general practitioners' offices and hospital specialist outpatient clinics since November 1, 2010. Concerning specific biomarkers in the DD2 biobank, fasting blood glucose, C-peptide, and hs-CRP (mg/L) were available for the first 5277 (72%), 5703 (78%), and 7300 (100%) DD2 cohort patients, respectively.
	-Serum high-sensitivity C-reactive protein (hs-CRP, mg/L)	-Continuous variable.
	-C-peptide, pmol/L	-Continuous variable
	-Physical activity, days/week	-Categorical variable (0, 1–2, ≥3 days/week). Physical activity was defined as "number of days per week with a minimum of 30 minutes of physical activity."
	-Fasting blood glucose, mmol/L	-Continuous variable
	-Waist circumference, cm	-Continuous variable
	-Waist–hip ratio	-Continuous variable, defined as >1.0 in men and >0.85 in women
	-BMI (see below)	
The Danish Diabetes Database of Adults (DDDA)		A nationwide quality-of-care database were available for a subcohort of 5847 patients (~80%). For all DDDA variables **except height**: We used the measure closest to the DD2 enrollment date. All measures before or after DD2 enrollment were eligible for use. If a variable was measured exactly the same number of days before and after the DD2 enrollment date, we used the measure prior to DD2 enrollment.
	-Blood pressure, mmHg	-Continuous variables: systolic and diastolic blood pressure
	-Lipids, mmol/L	-Continuous variables: LDL, HDL, triglycerides, total cholesterol
	-HbA1c, %	-Continuous variable
	-Smoking	-Categorical variable: never, former, current (daily + occasionally)
	-Albumin:creatinine ratio	-Continuous variable
	-BMI (see below)	
BMI, kg/m^2		
	Height	Data on height were available from 3 sources: DD2 enrollment (2010 onwards), DDDA data (repeated measures), questionnaire data 2016 (self-reported).
	BMI DD2 enrollment	Continuous variable

		Weight: If weight was recorded during the DD2 enrollment process (few), we used this weight; otherwise, we used the DDDA weight. Height: We did not expect height to change over time among the adults in our study. Thus, we used available heights in the following hierarchical order: height obtained at DD2 enrollment, height obtained at DDDA enrollment, and questionnaire data obtained in 2016.
Diabetes duration		Time from first of the following events until the DD2 enrollment date: prescription of glucose-lowering drugs, first diabetes-related diagnosis in the Danish National Patient Registry, or DDDA registration. In the absence of information from a prior drug prescription, diabetes diagnosis from the DNPR, or DDDA registration, diabetes duration was set to DD2 enrollment date = 0.
The Civil Registration System	-Age -Sex	-Continuous variable -Male/female
The Danish Health Service Prescription database		For all prescription data, the relevant time period was around baseline (DD2 enrollment). The look-back period was 1 year prior to the DD2 enrollment date. Yes/no redemption of a drug prescription during the year prior to the index date.
	-Anti-diabetic drugs -Lipid-lowering drugs -Statins	ATC: A10A, A10B ATC: C10 ATC: C10AA, C10BA, C10BX
Modified Charlson Comorbidity Index		We categorized comorbidities according to the Charlson Comorbidity Index (CCI) within the 10-year period before the DD2 enrollment date. Diabetes was not included in the CCI scoring system because it constituted the index disease for our cohort.
	Score 1	
	Myocardial infarction	DI21, DI22, DI23
	Congestive heart failure	DI50, DI110, DI130, DI132
	Peripheral vascular disease	DI70, DI71, DI72, DI73, DI74, DI77
	Cerebrovascular disease	DI60, DI61, DI62, DI63, DI64, DI65, DI66, DI67, DI68, DI69, DG45, DG46
	Dementia	DF00, DF02, DF03, DF051, DG30
	Chronic pulmonary disease	DJ40, DJ41, DJ42, DJ43, DJ44, DJ45, DJ46, DJ47, DJ60, DJ61, , DJ62, , DJ63, , DJ64, , DJ65, , DJ66, , DJ67, DJ684, DJ701, DJ703, DJ841, DJ920, DJ961, DJ982, DJ983

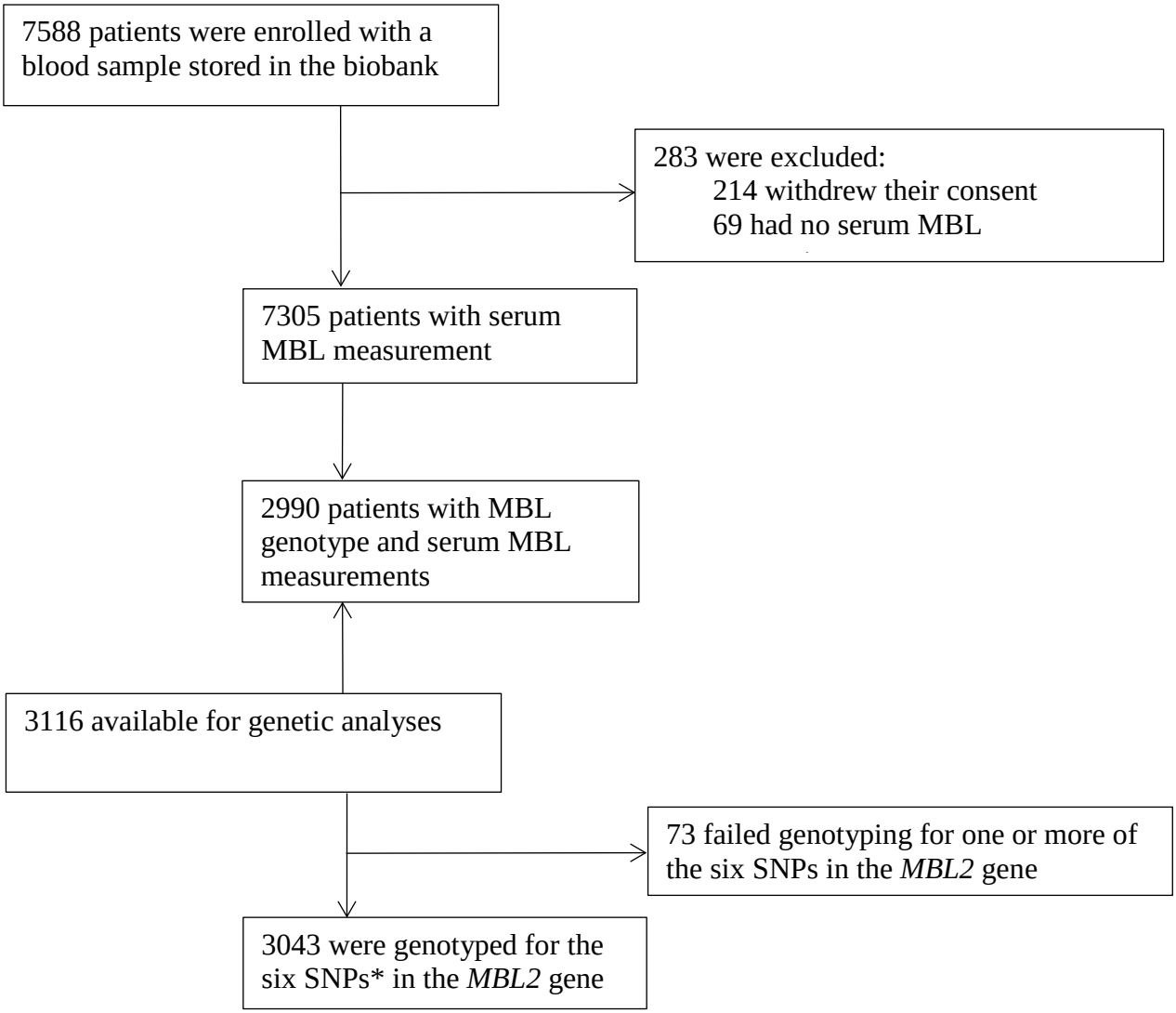
Connective tissue disease	DM05, DM06, DM08, DM09, DM30, DM31, DM32, DM33, DM34, DM35, DM36, DD86
Ulcer disease	DK221, DK25, DK26, DK27, DK28
Mild liver disease	DB18, DK700, D701, DK702, DK703, DK709, DK71, DK73, DK74, DK760
Score 2	
Hemiplegia	DG81, DG82
Moderate to severe renal disease	D12, DI13, DN00, DN01, DN02, DN03, DN04, DN05, DN07, DN11, DN14, DN17, DN18, DN19, DQ61
Any tumor (except basocellular carcinoma)	C00–C75, (excluding C44)
Leukemia	DC91, DC92, DC93, DC94, DC95
Lymphoma	DC81, DC82, DC83, DC84, DC85, DC90, DC96
Score 3	
Moderate to severe liver disease	DB150, DB160, DB162, DB190, DK704, DK72, DK766, DI85
Score 6	
Metastatic solid tumor	DC76, DC77, DC78, DC79, DC80
AIDS	DB21, DB22, DB23, DB24

Supplementary Table 4. Missing covariates for the serum MBL and MBL expression genotype cohorts.

	Serum MBL cohort		**MBL expression genotype cohort**	
	Missing, n (%)	Total	Missing, n (%)	Total
Sex	0 (0.0)	7305	0 (0.0)	3043
Age	0 (0.0)	7305	0 (0.0)	3043
Diabetes duration	0 (0.0)	7305	0 (0.0)	3043
Waist circumference	13 (0.18)	7305	<5** (0.2)	3043
Waist–hip ratio	11 (0.15)	7305	<5** (0.2)	3043
BMI*	569 (7.79)	7305	245 (8.05)	3043
Alcohol intake	0 (0.0)	7305	0 (0.0)	3043
Physical activity	<5† (0.0)	7305	0 (0.0)	3043
Smoking*	1900 (26.01)	7305	612 (20.11)	3043
CCI score	0 (0.0)	7305	0 (0.0)	3043
Anti-diabetes drug use	0 (0.0)	7305	0 (0.0)	3043
Lipid-lowering drug use	0 (0.0)	7305	0 (0.0)	3043
Fasting blood glucose	2028 (27.76)	7305	368 (12.09)	3043
HbA1C*	1548 (21.19)	7305	486 (15.97)	3043
Total cholesterol*	3966 (54.29)	7305	1244 (40.88)	3043
LDL cholesterol*	1763 (24.13)	7305	550 (18.07)	3043
HDL cholesterol*	3951 (54.09)	7305	1245 (40.91)	3043
Triglycerides*	1849 (25.31)	7305	572 (18.80)	3043
hs-CRP	5 (0.07)	7305	56 (1.84)	3043

*By August 2018, a total of 5847 DD2 patients (80%) in the serum MBL cohort and 2597 DD2 patients (85%) in the MBL genotype cohort had been linked to the Danish Diabetes Database for Adults.
†Exact number of missing too low to be displayed according to Danish data protection regulations.

EXPANDED RESULTS SECTION



Supplementary Figure 1. Flow diagram of the study population.

*The six SNPs in the *MBL2* gene were: rs11003125, rs7096206, rs7095891, rs5030737, rs1800451, and rs1800450.

Supplemental Table 5. Outcomes among 7305 patients with type 2 diabetes

Hospital-treated infections	Outcomes	Community-based antimicrobial prescriptions	Outcomes
Any infections	1140	Any prescriptions	5077
Bacterial infections	1051	Antibacterial prescriptions	4855
Pneumonia	390	Prescriptions for respiratory tract infections	3526
Urinary tract infections	247	Prescriptions for urinary tract infections	1691
Skin infections	221	Presciptions for skin infections	1287
Sepsis	201	Broad-spectrum penicillins	1408
Abscesses	199	Antiviral prescriptions	340
Diarrheal diseases	84	Antifungal prescriptions	845
Intra abdominal infections	155		
Other bacterial infections	159		
Viral infections	74		
Fungal infections	33		

Supplemental Table 6. Characteristics of DD2 cohort members at baseline by MBL expression genotype category.

	Low MBL expression genotype	Intermediate MBL expression genotype	High MBL expression genotype
Total, N (%)	446 (14.7)	939 (30.8)	1658 (54.5)
Male sex, n (%)	277 (62.1)	533 (56.8)	944 (56.9)
Median age (IQR), years	61.3 (52.5–68.3)	61.3 (52.9–67.7)	62.0 (53.3–68.1)
Median diabetes duration (IQR), years	0.8 (0.2–2.2)	0.8 (0.2–2.0)	0.8 (0.3–2.1)
Median waist circumference (IQR), cm	104 (96–114)	105 (96–116)	106 (97–116)
Median waist–hip ratio (IQR)	0.97 (0.92–1.03)	0.97 (0.91–1.04)	0.97 (0.92–1.03)
Median body mass index (IQR), kg/m^2	29.7 (26.6–33.8)	30.4 (27.0–34.1)	30.3 (26.8–34.3)
High alcohol intake[a], n (%)	27 (6.1)	65 (6.9)	112 (6.8)
Physical activity[b] (IQR), days/week	3 (2–7)	3 (1–7)	3 (2–7)
Smoking, n (%)			
Never	158 (45.0)	382 (51.2)	667 (50.0)
Former	131 (37.3)	220 (29.5)	416 (31.2)
Current	62 (17.7)	144 (19.3)	251 (18.8)
CCI score[c], n (%)			
0	296 (66.4)	636 (67.7)	1129 (68.1)
1–2	125 (28.0)	253 (26.9)	430 (25.9)
3	25 (5.6)	50 (5.3)	99 (6.0)
Anti-diabetes drug use, n (%)	356 (79.8)	784 (83.5)	1388 (83.7)
Lipid-lowering drug use, n (%)	295 (66.1)	652 (69.4)	1176 (70.9)
Median fasting blood glucose (IQR), mmol/L	7.2 (6.4–8.3)	7.0 (6.3–8.2)	7.1 (6.4–8.3)
Median HbA1c (IQR), %	6.5 (6.2–7.1)	6.5 (6.1–7.2)	6.5 (6.1–7.1)
Median total cholesterol (IQR), mmol/L	4.3 (3.6–5.1)	4.5 (3.8–5.2)	4.3 (3.7–5.1)
Median LDL cholesterol (IQR), mmol/L	2.2 (1.8–2.9)	2.3 (1.8–2.9)	2.2 (1.8–2.8)
Median HDL cholesterol (IQR), mmol/L	1.2 (1–1.4)	1.2 (1–1.5)	1.2 (1–1.5)
Median triglycerides (IQR), mmol/L	1.5 (1.1–2.3)	1.6 (1.1–2.3)	1.6 (1.2–2.4)
Median hs-CRP (IQR), mg/L	1.8 (0.7–4.3)	2.0 (0.8–4.1)	1.9 (0.8–4.6)

MBL: mannose-binding lectin; IQR: interquartile range; CCI: Charlson Comorbidity Index; HbA1c: hemoglobin A1c; LDL: low-density lipoprotein; HDL: high-density lipoprotein; hs-CRP: high-sensitivity C-reactive protein

[a]High alcohol intake defines as ≥14/21 alcoholic drinks/week for women/men.

[b]Days per week with minimum 30 minutes of physical activity

[c]CCI score excluding diabetes

Number of participants varies because of availability of data (see Supplementary Table 4 for missing covariates).

Supplementary Table 7. Haplotypes and serum MBL levels in 3043 patients with T2D according to low, intermediate, and high MBL expression genotypes.

Haplotype	Number (%)	Median serum MBL concentration (µg/L)
Low MBL expression genotypes (XA/YO, YO/YO)	446 (14.7)	10 (10–26)
LXPA/LYQC	19 (0.6)	10 (10–42)
LXPA/LYPB	180 (5.9)	10 (10–20)
LXPA/HYPD	99 (3.3)	28 (10–80)
LYPB/LYPB	63 (2.1)	10 (10–10)
LYPB/LYQC	14 (0.5)	10 (10–10)
LYPB/HYPD	40 (1.3)	10 (10–10)
LYPB/LYPD*	<10 (0.3)	116
LYQC/LYQC*	<10 (0.3)	10
LYQC/HYPD*	<10 (0.3)	10 (10–12)
HYPD/HYPD	23 (0.8)	10 (10–10)
Intermediate MBL expression genotypes (XA/XA, YA/YO)	939 (30.9)	321 (199–545)
LXPA/LXPA	151 (5.0)	253 (102–594)
HYPA/LYPB	285 (9.4)	313 (225–407)
LYPA/LYPB	56 (1.8)	195 (115–297)
LYQA/LYPB	172 (5.7)	259 (179–338)
HYPA/LYQC	27 (0.9)	293 (210–402)
LYPA/LYQC*	<10 (0.3)	292 (244–488)
LYQA/LYQC	22 (0.7)	247 (197–358)
HYPA/HYPD	109 (3.6)	740 (501–1050)
LYPA/HYPD	35 (1.2)	441 (276–746)
LYQA/HYPD	75 (2.5)	631 (465–832)
LYQA/LYPD*	<10 (0.3)	873
High MBL expression genotypes (YA/YA, XA/YA)	1658 (54.5)	1527 (974–2394)
HYPA/HYPA	276 (9.1)	1911 (1185–2974)
HYPA/LYPA	98 (3.2)	2010 (1381– 2611)
HYPA/LYQA	347 (11.4)	2022 (1349–2888)
HYPA/LXPA	400 (13.1)	1162 (784–1714)
LYPA/LYPA*	<10 (0.3)	2571 (1141–2738)
LYPA/LYQA	67 (2.2)	1785 (1151–2706)
LYPA/LXPA	86 (2.8)	1157 (613–1812)
LYQA/LYQA	129 (4.2)	2025 (1317–2769)
LYQA/LXPA	250 (8.2)	1129 (716–1643)

*Exact number of haplotypes too low to be displayed according to Danish data protection regulations.

Supplementary Table 8. Allele frequencies of the six SNPs in the *MBL2* gene.

SNP	Genotype*	Serum MBL† µg/L	DD2 cohort N (%)	HWE	ExAC database‡ N (%)	HWE	gnomeAD database‡ N (%)	HWE
Promoter region								
rs11003125	HH	1333 (761–2375)	408 (13.4)		NA		1081 (14.1)	
(-550 G>C)	LH	889 (321–1816)	1411 (46.4)	0.97	NA	NA	3647 (47.4)	0.45
	LL	404 (68–1254)	1224 (40.2)		NA		2964 (38.5)	
rs7096206	YY	828 (281–1997)	1857 (61.0)		NA		4594 (59.7)	
(-221 G>C)	XY	835 (73–1432)	1035 (34.0)	0.66	NA	NA	2700 (35.0)	0.69
	XX	253 (102–594)	151 (5.0)		NA		407 (5.3)	
rs7095891§	QQ	1807 (1046–2579)	152 (5.0)		NA		304 (3.9)	
(c.+4 C>T)	PQ	1087 (416–2036)	986 (32.4)	0.10	NA	NA	2563 (33.3)	0.12
	PP	546 (118–1381)	1905 (62.6)		NA		4841 (62.8)	
Exon 1								
rs5030737	AA	909 (274–1851)	2653 (87.1)		28,503 (85.4)		55,308 (85.8)	
(p. Arg52Cys)	AD (or AO)	344 (26–748)	367 (12.1)	0.01	4632 (13.9)	0.03	8743 (13.6)	0.39
	DD (or OO)	10 (10–10)	23 (0.8)		221 (0.7)		363 (0.6)	
rs1800451	AA	1188 (597–2071)	2224 (73.1)		32,115 (96.4)		62,059 (96.4)	
(p. Gly54Asp)	AB (or AO)	203 (18–331)	754 (24.8)	0.91	1198 (3.6)	0.41	2308 (3.6)	0.45
	BB (or OO)	10 (10–10)	65 (2.1)		14 (0.0)		25 (0.0)	
rs1800450	AA	826 (250–1716)	2948 (96.9)		24,337 (73.0)		47,173 (73.3)	
(p. Gly57Glu)	AC (or AO)	181 (10–313)	94 (3.1)	0.78	8285 (24.8)	0.54	15836 (24.6)	0.18
	CC (or OO)	10 (10–10)	1 (0.0)		724 (2.2)		1387 (2.1)	

Abbreviations: DD2, the Danish Centre for Strategic Research in Type 2 Diabetes; HWE, Hardy–Weinberg equilibrium

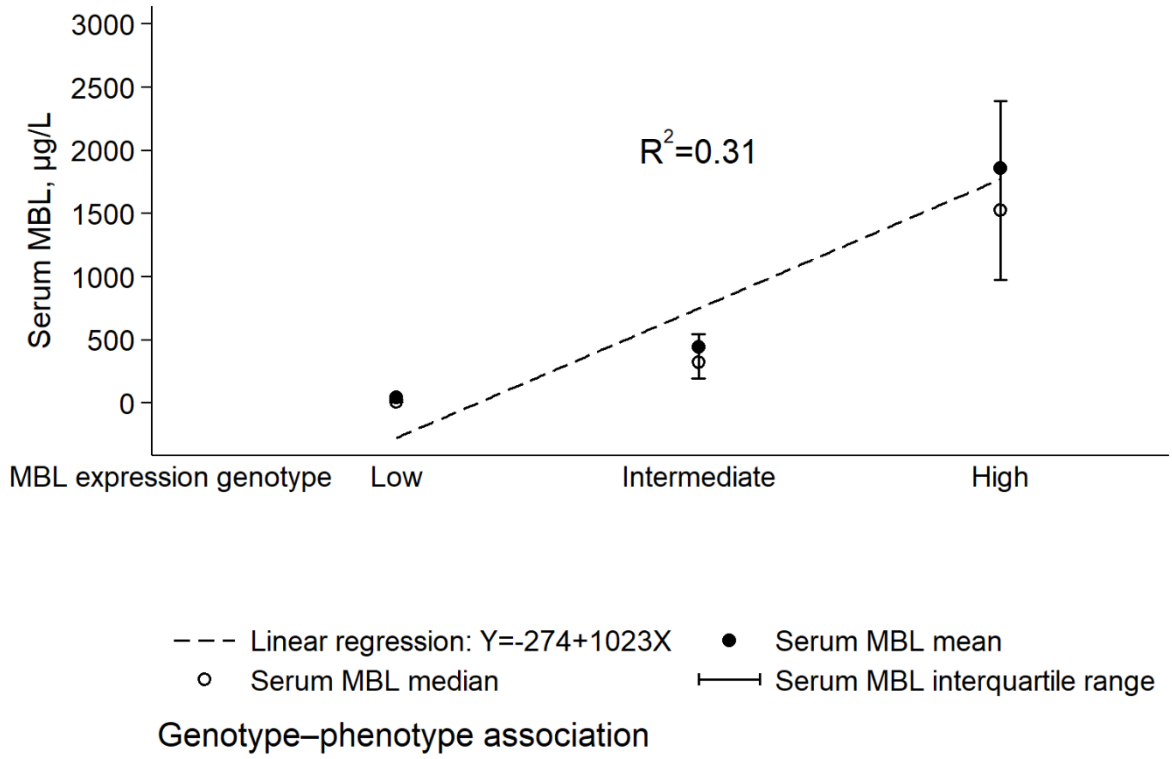
NA=not available or not applicable because the ExAC database spans only exome sequences and thus does not cover the promoter region.

* The major alleles of the three SNPs in exon 1 are all referred to as the A allele, while the minor alleles (B, C, and D) are collectively referred to as the O allele.

† Serum MBL is expressed as median (interquartile range).

‡The Exome Aggregation Consortium (ExAC) spans 60,706 exome sequences and the Genome Aggregation Database (gnomAD) spans 125,748 exome sequences and 15,708 whole-genome sequences from unrelated individuals sequenced as part of various disease-specific and population genetic studies (URL: http://exac.broadinstitute.org/ and https://gnomad.broadinstitute.org/) [August 2019].

Because of differences in allele frequencies across different populations, we present only information for the European non-Finnish population from the ExAC and gnomAD databases, comprising around half of the total population sequenced.
§Previously known as rs12780112.
A minor deviation was observed for rs5030737 because of a slightly higher number of rare homozygotes than expected. We believe that this results from chance. However, even if this resulted from a minor genotyping error, potential misclassification of the MBL expression genotype (based on the seven haplotypes) is unlikely to be extensive and would possibly bias the results toward the null hypothesis.

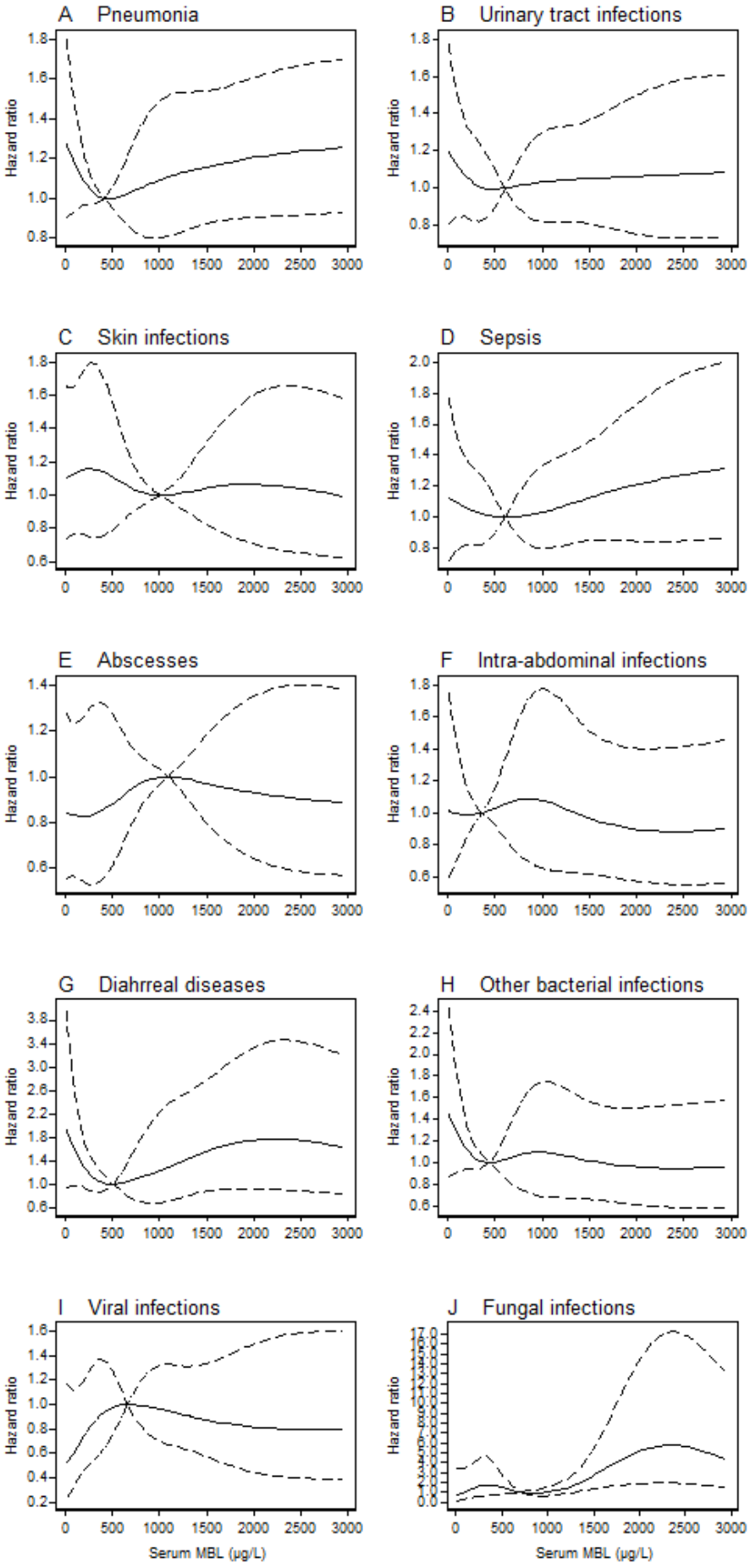


Supplementary Figure 2. Genotype–phenotype association.
Serum MBL levels according to MBL expression genotypes. R^2 is the coefficient of determination. Cuzick's non-parametric test for trend ($P<1\times10^{-300}$).

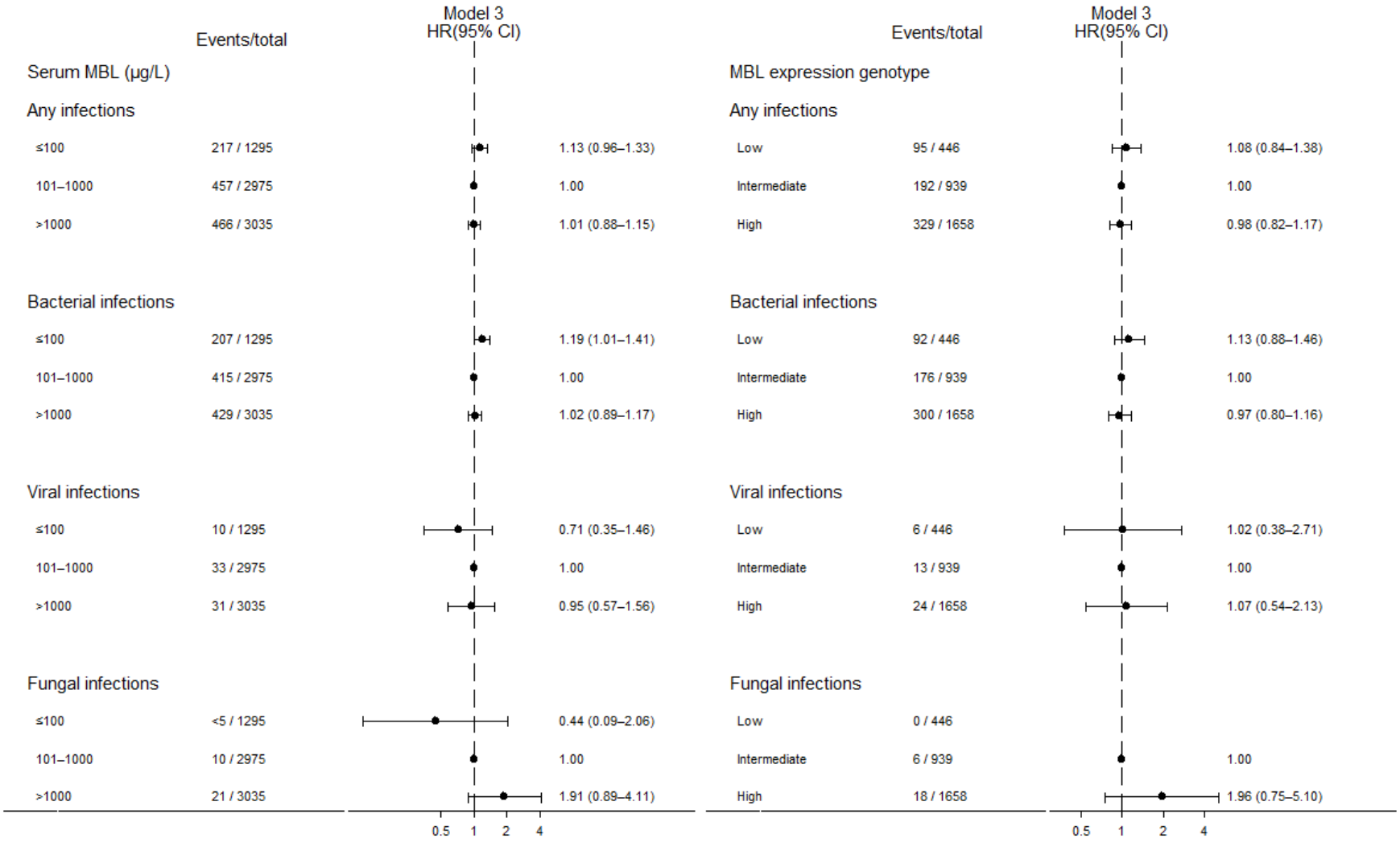
Supplementary Table 9. Incidence rates per 1,000 person-years among 7305 patients with T2D according to MBL categories.

	Any hospital-treated infection	Any community-based antimicrobial prescriptions
	Incidence rates (95% CI)	**Incidence rates (95% CI)**
Serum MBL (µg/L)		
≤100	40.0 (35.0-45.7)	37.8 (35.4-40.3)
101–1000	36.0 (32.8-39.4)	35.8 (34.3-37.4)
>1000	36.0 (32.9-39.4)	34.4 (32.9-35.9)
MBL expression genotype		
Low	40.8 (33.4-49.9)	39.4 (35.6-43.6)
Intermediate	38.2 (33.1-44.0)	33.6 (31.3-36.2)
High	37.5 (33.7-41.8)	37.2 (35.3-39.3)

Splines: Hospital-treated infection

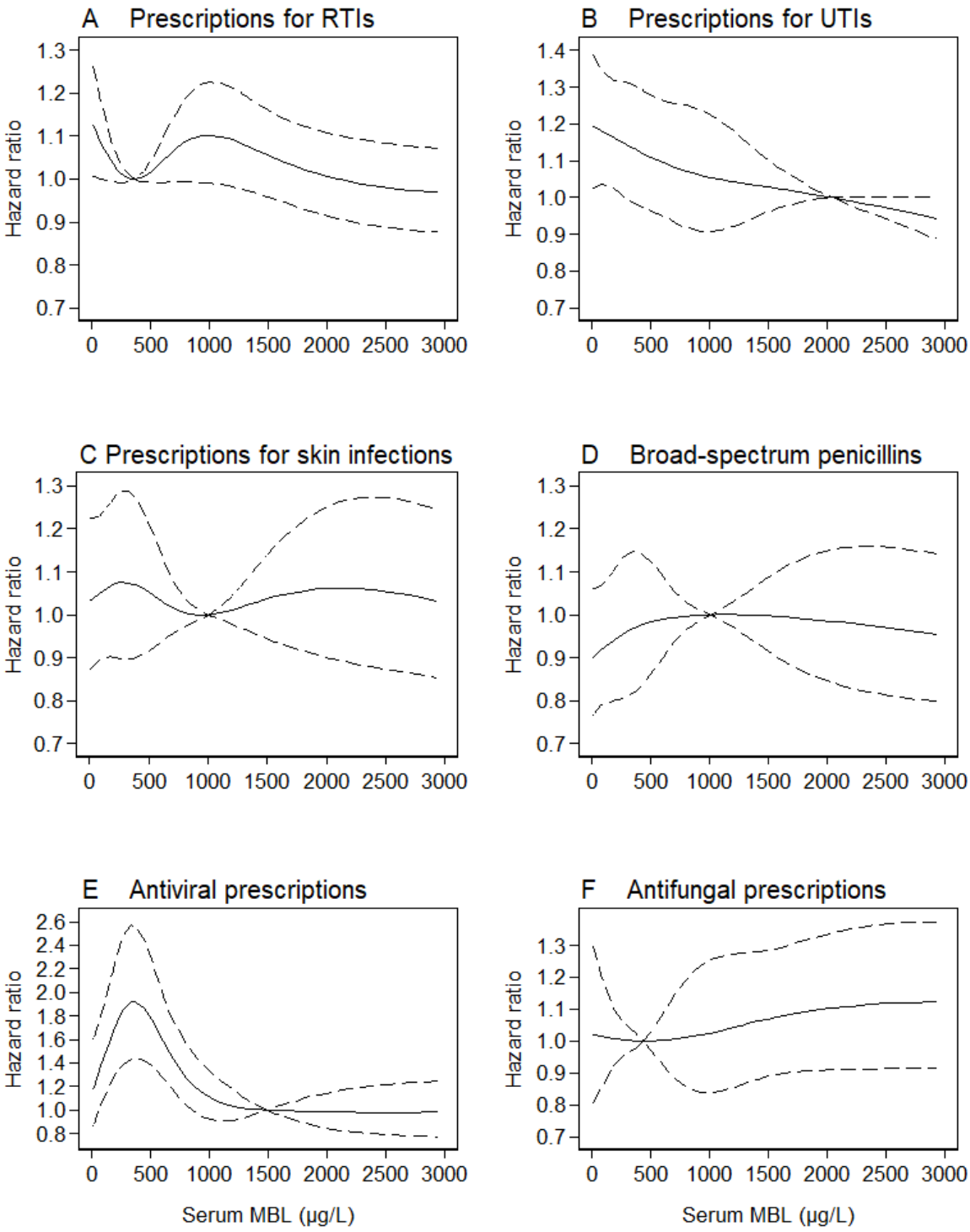


Supplementary Figure 3. Risk of hospital-treated infections by serum MBL

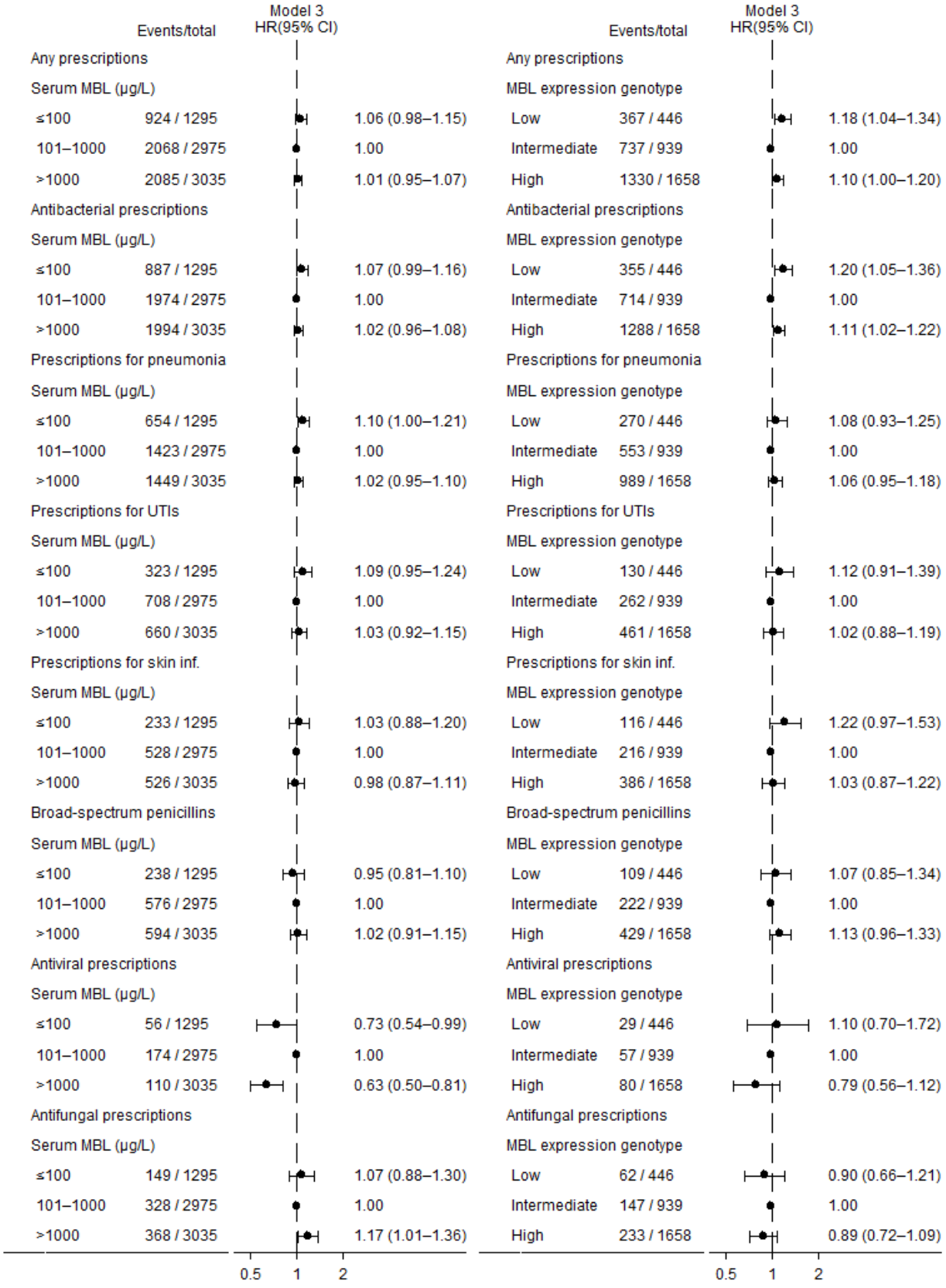


Supplementary Figure 4. Risk of Hospital-treated infections by serum MBL and MBL expression genotype categories.

Splines: Community-based antimicrobial prescriptions



Supplementary Figure 5. Risk of community-based antimicrobial prescriptions bysSerum MBL Levels.

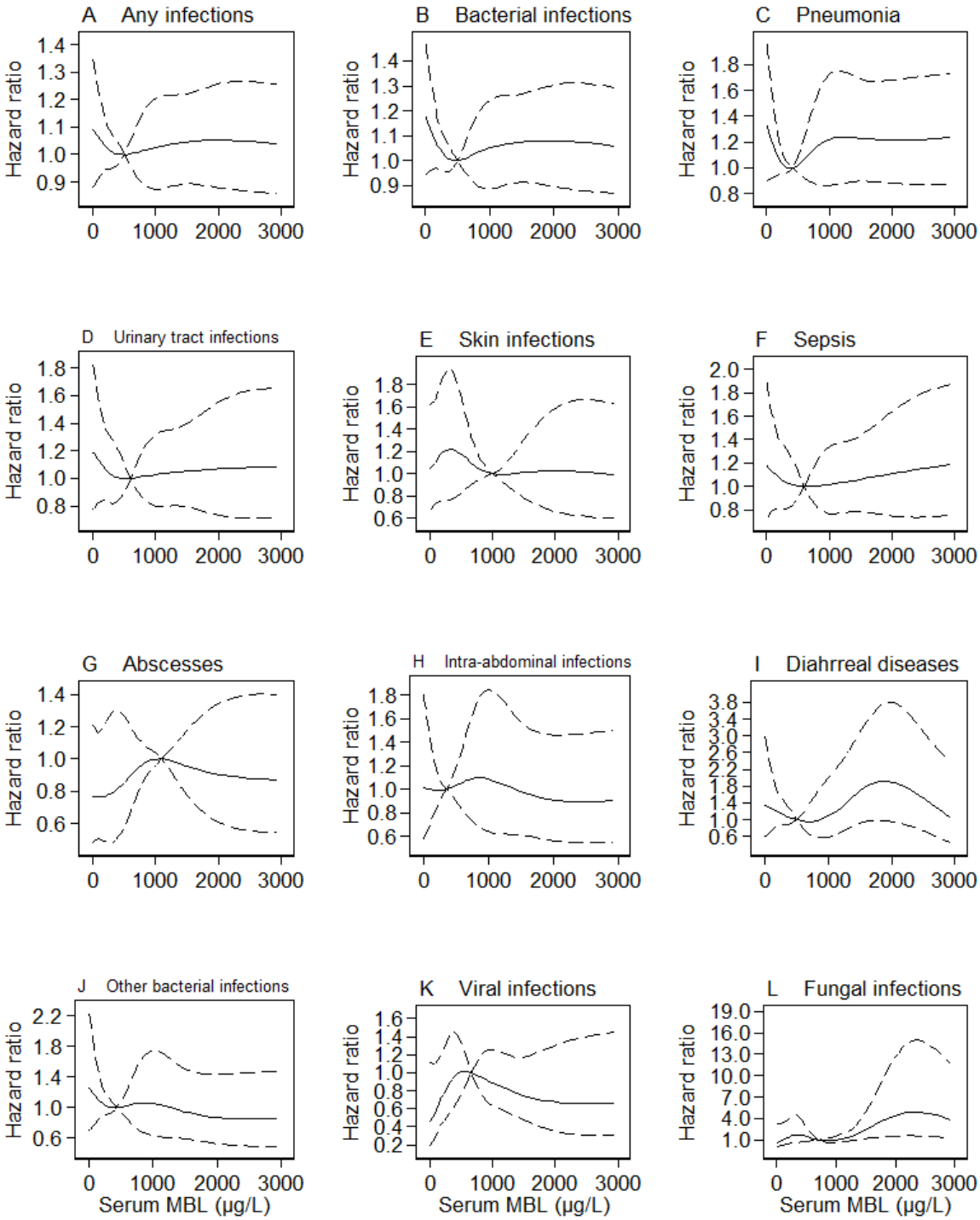


Supplementary Figure 6. Risk of Community-based antimicrobial prescriptions by serum MBL and MBL expression genotype categories.

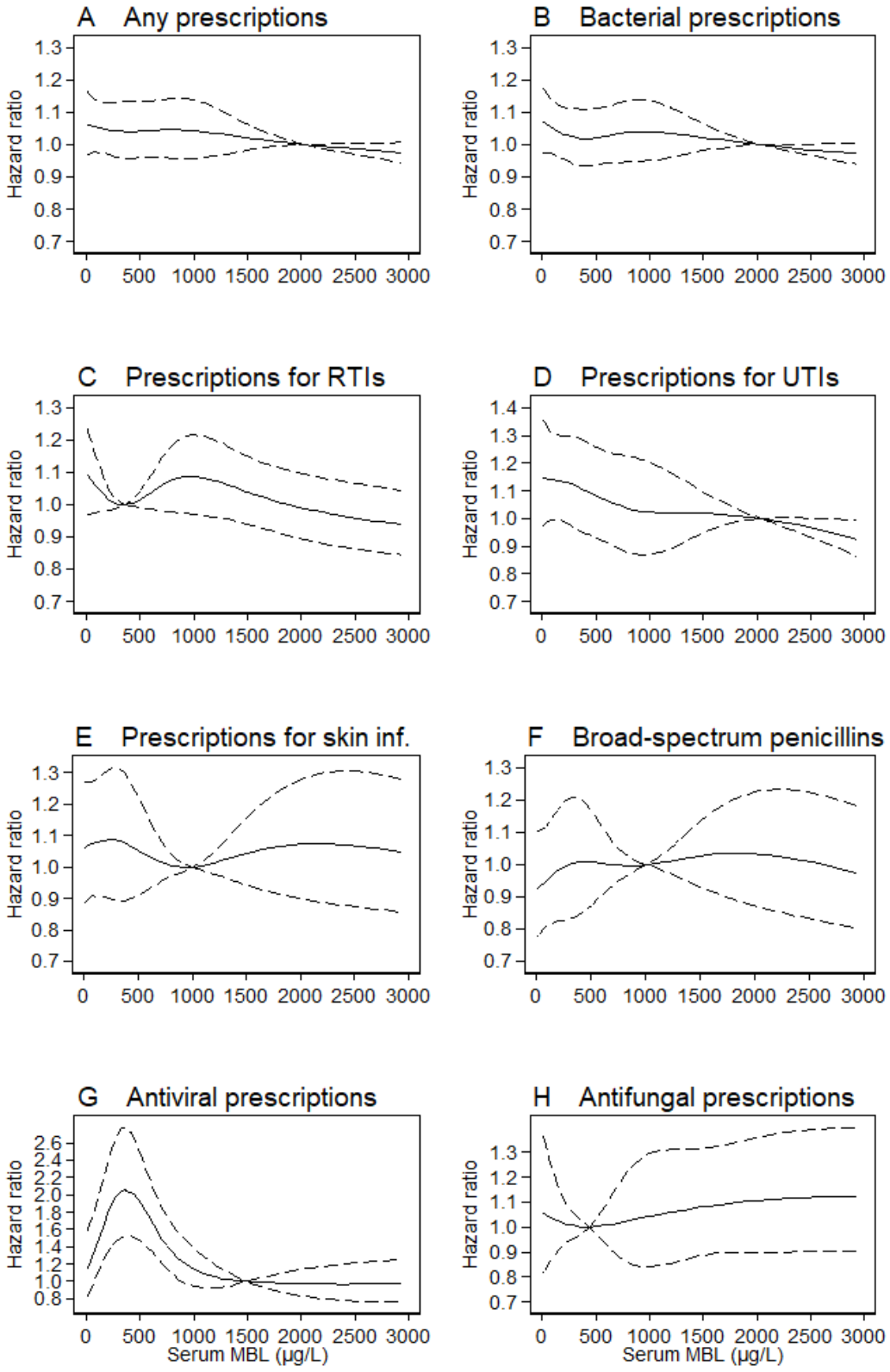
SENSITIVITY ANALYSIS

Exclusion of patients with CRP above 10 mg/L

Splines: Exclusion of patients with CRP above 10 mg/L

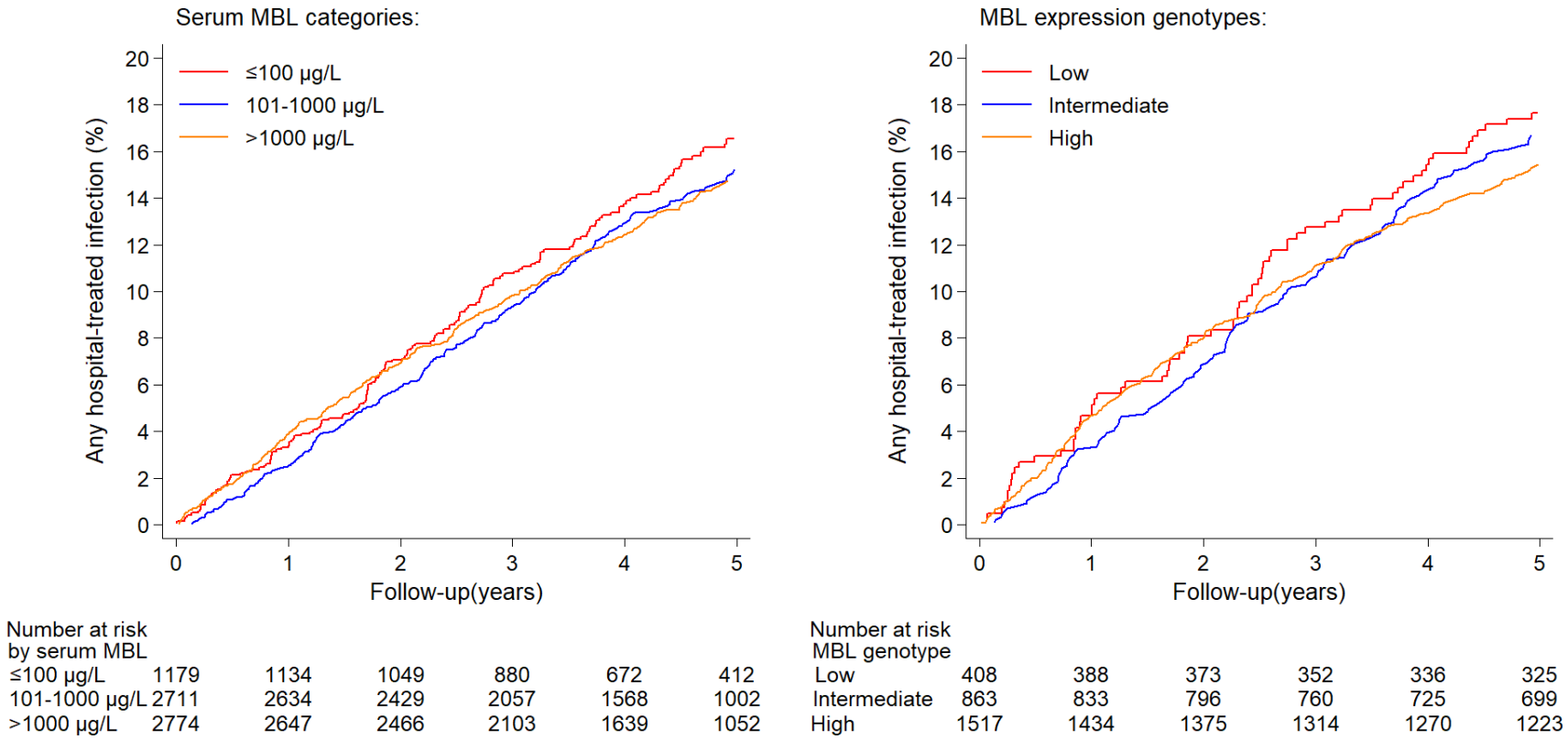


Supplementary Figure 7. Risk of hospital-treated infection by serum MBL levels, excluding patients with CRP >10mg/L.

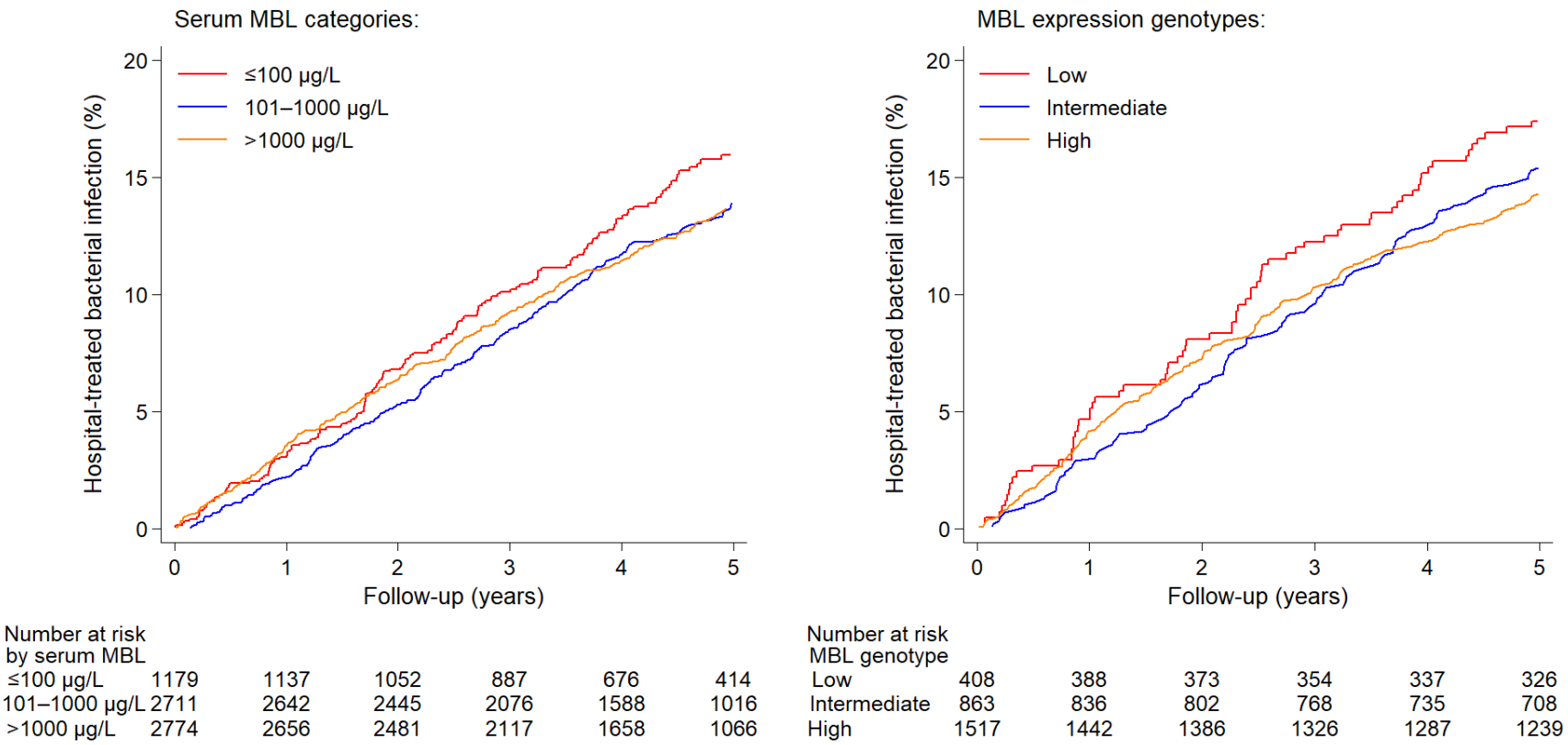


Supplementary Figure 8. Risk of Any community-based antimicrobial use by serum MBL Levels, excluding individuals with CRP >10mg/L**.**

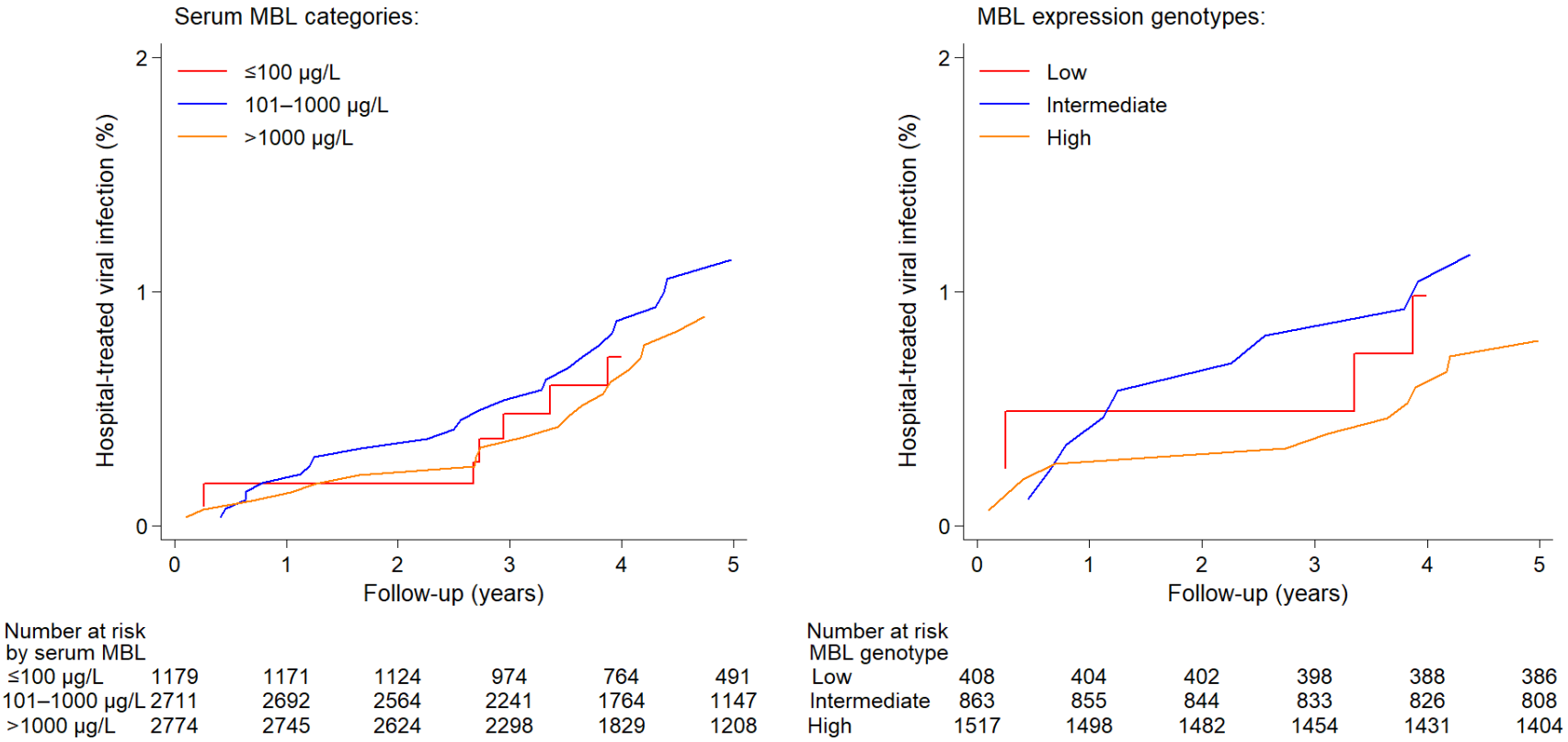
Cumulative incidence curves: Exclusion of patients with CRP above 10 mg/L



Supplementary Figure 9. Cumulative incidence curves of Any hospital-treated infection by serum MBL and MBL expression genotype categories excluding patients with CRP >10mg/L.



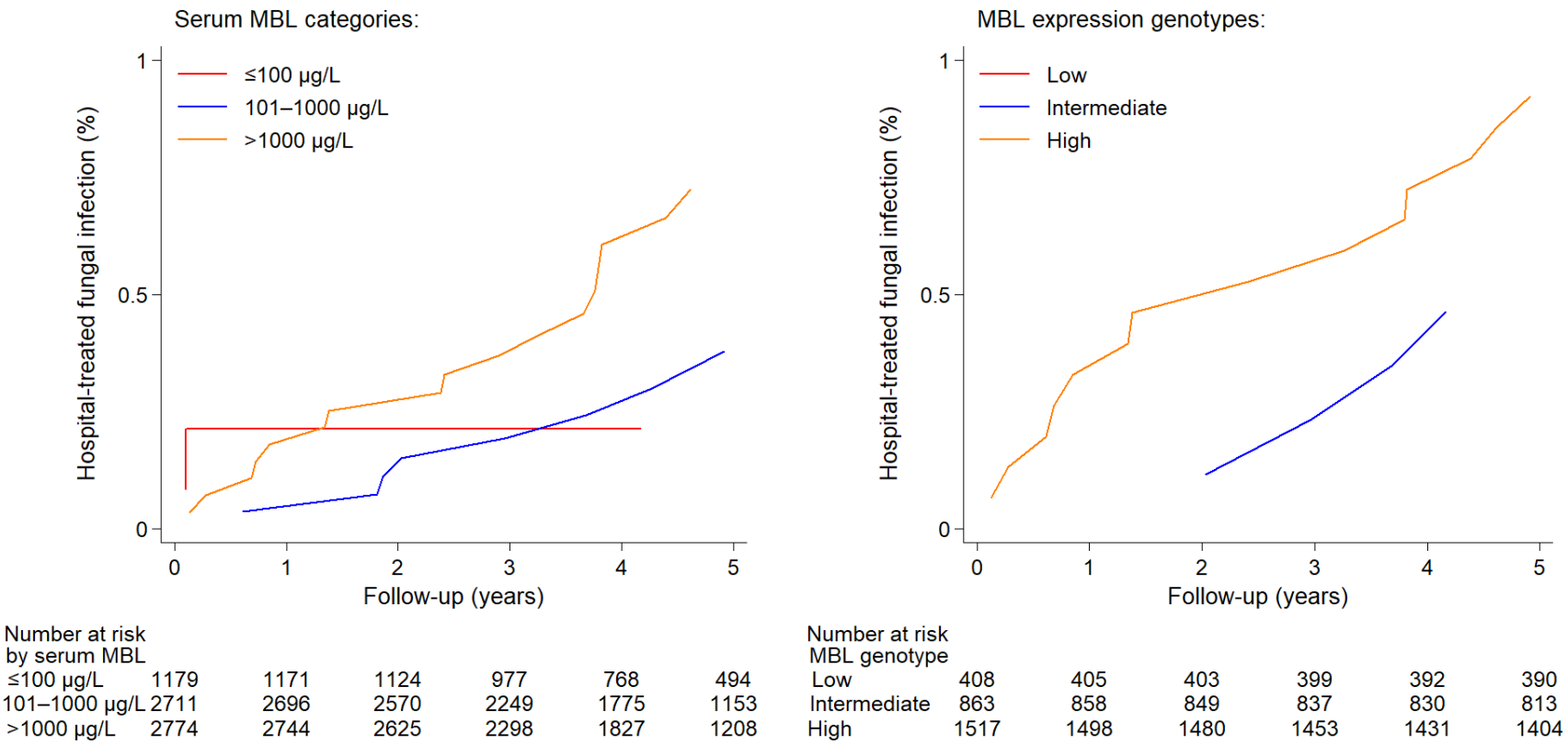
Supplementary Figure 10. Cumulative incidence curves of Hospital-treated bacterial infection by serum MBL and MBL expression genotype categories excluding patients with CRP >10mg/L**.**



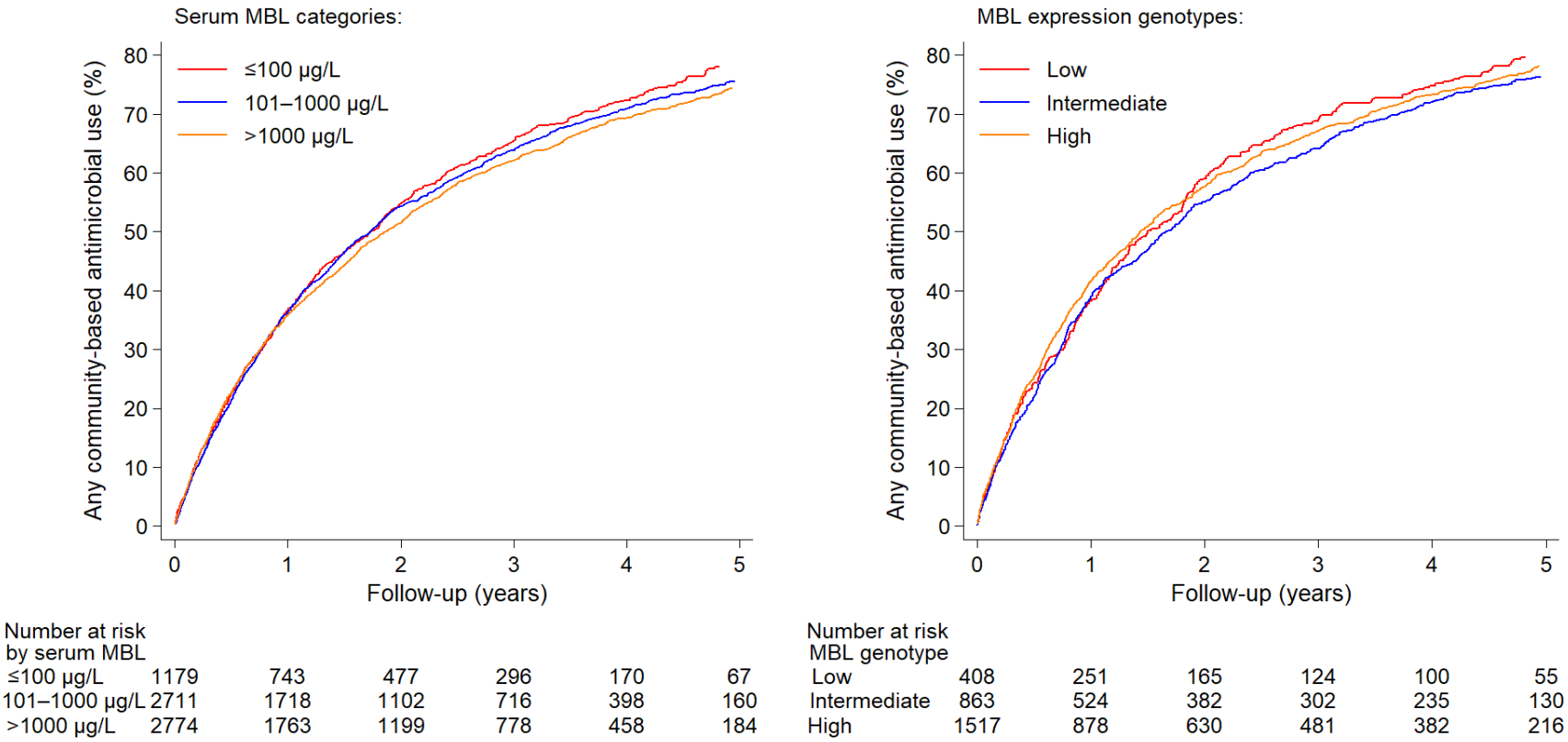
Number at risk by serum MBL						
≤100 µg/L	1179	1171	1124	974	764	491
101–1000 µg/L	2711	2692	2564	2241	1764	1147
>1000 µg/L	2774	2745	2624	2298	1829	1208

Number at risk MBL genotype						
Low	408	404	402	398	388	386
Intermediate	863	855	844	833	826	808
High	1517	1498	1482	1454	1431	1404

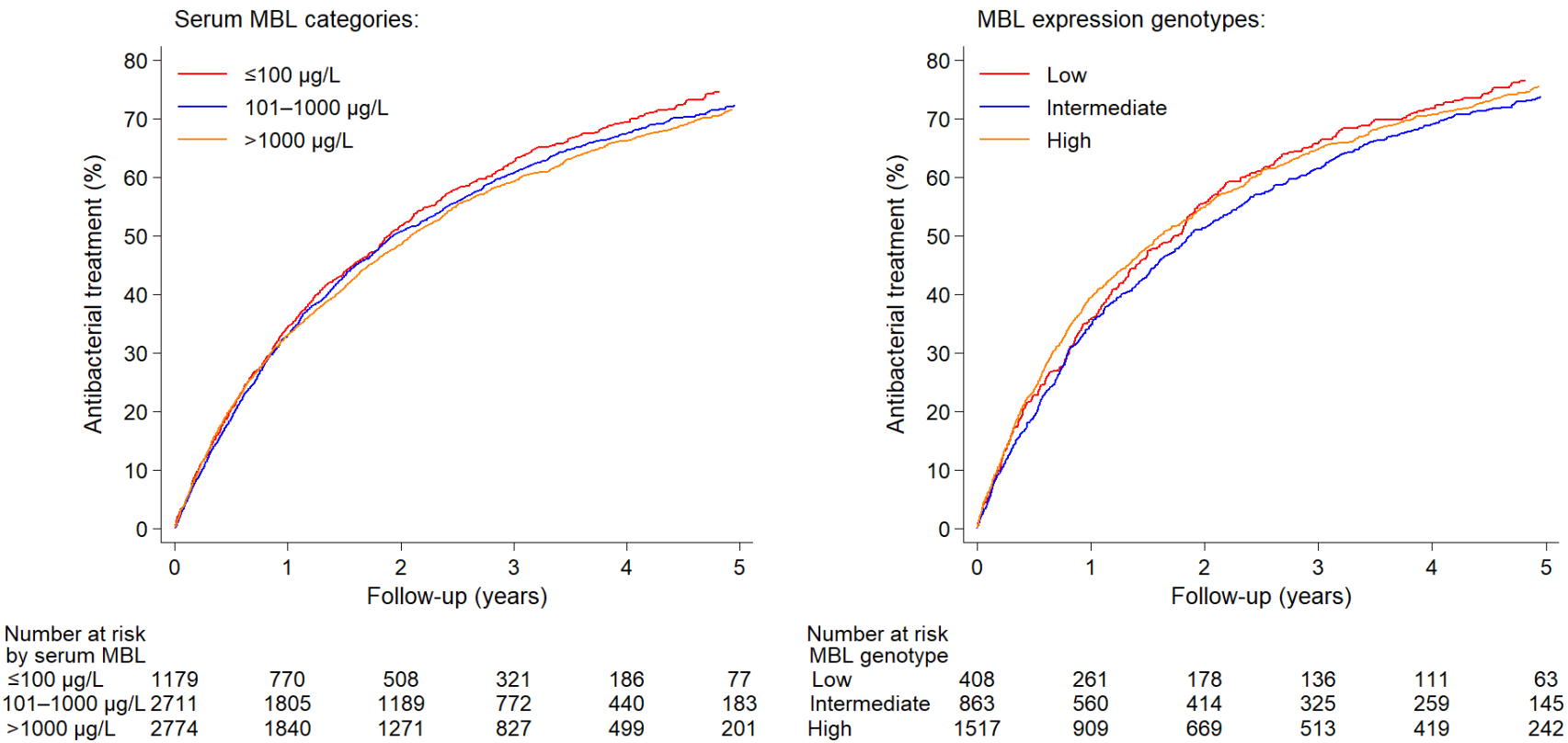
Supplementary Figure 11. Cumulative incidence curves of Hospital-treated viral infection by serum MBL and MBL expression genotype categories excluding patients with CRP >10mg/L**.**



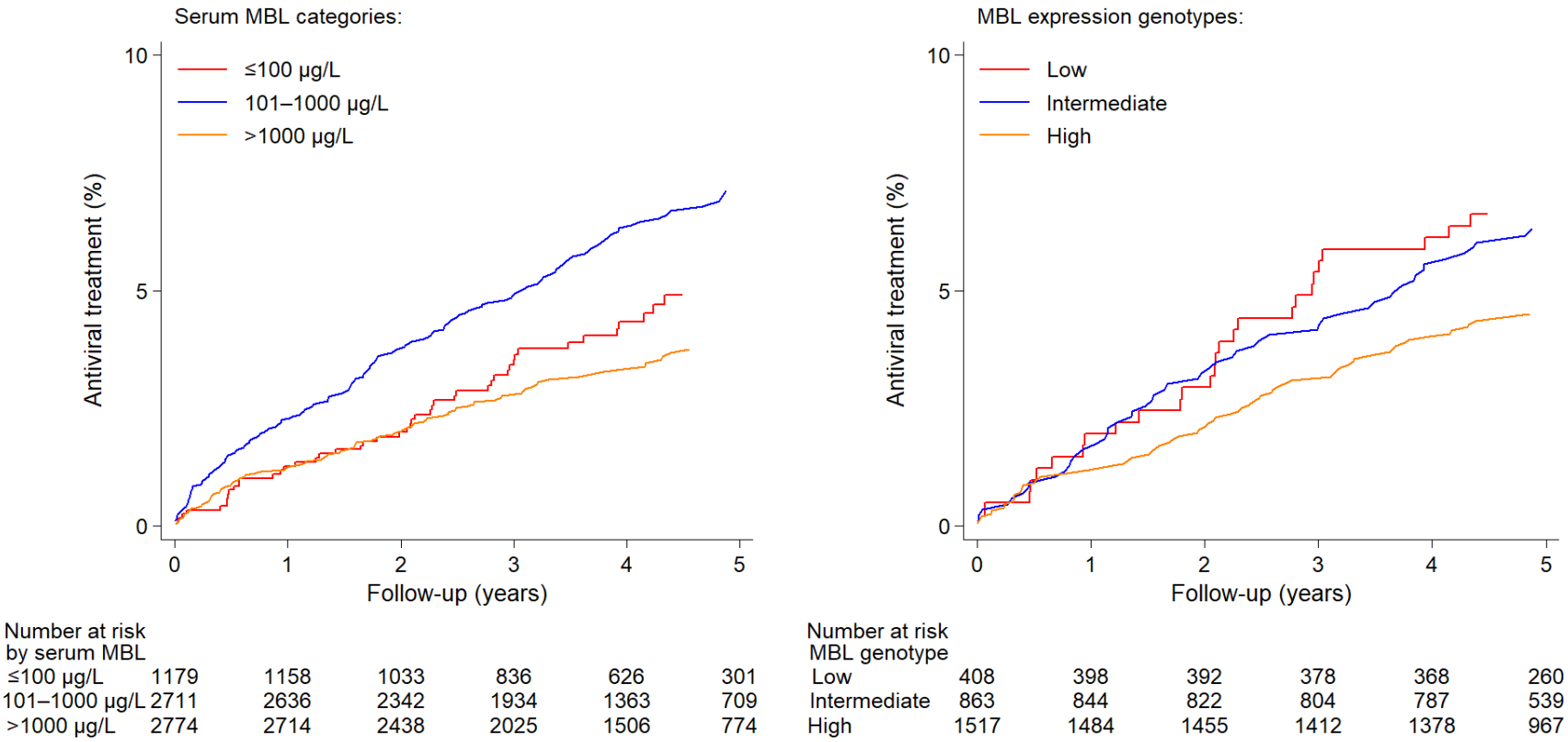
Supplementary Figure 12. Cumulative incidence curves of Hospital-treated fungal infection by serum MBL and MBL expression genotype categories excluding patients with CRP >10mg/L**.**



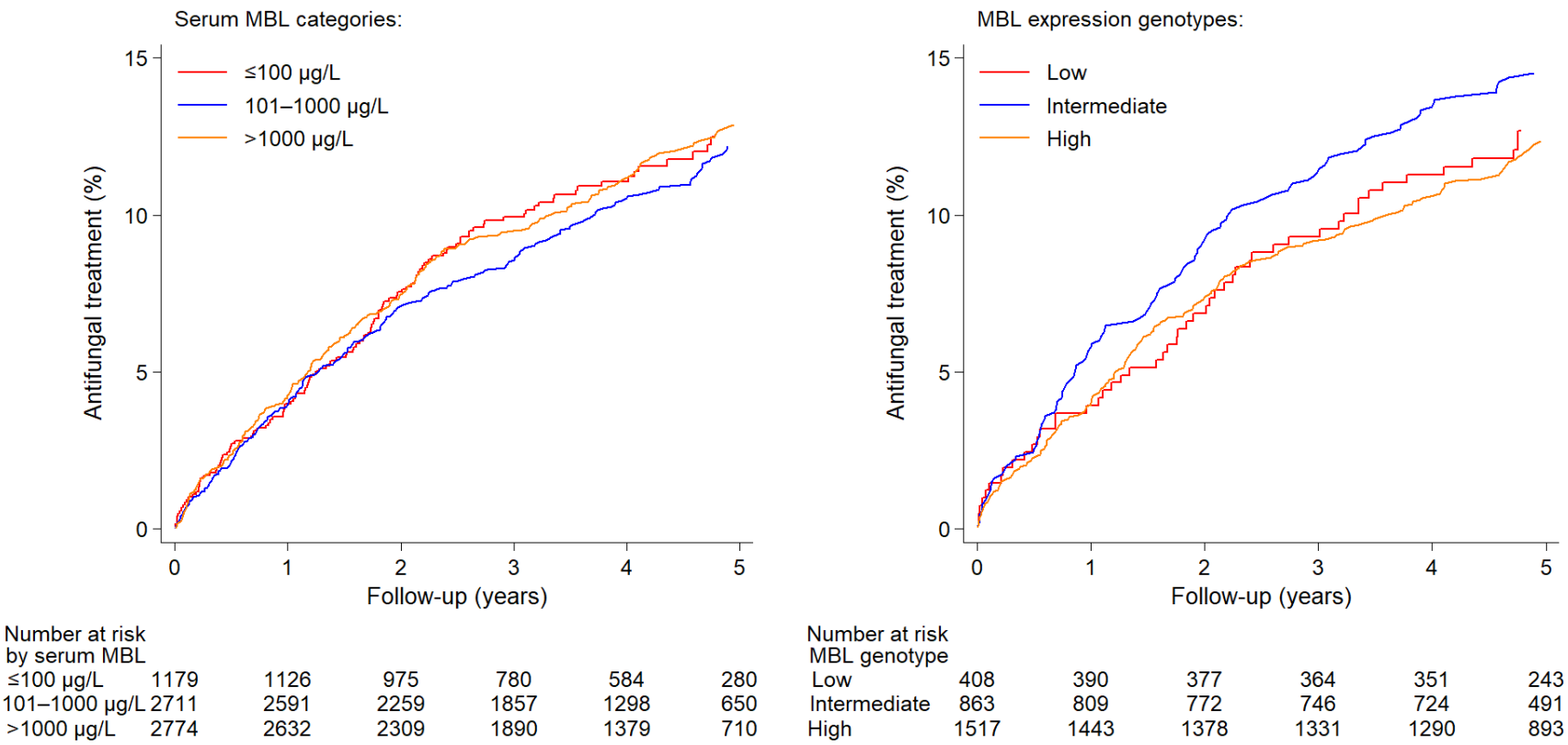
Supplementary Figure 13. Cumulative incidence curves of Any community-based antimicrobial use by serum MBL and MBL expression genotype categories excluding patients with CRP >10mg/L**.**



Supplementary Figure 14. Cumulative incidence curves of Antibacterial treatment by serum MBL and MBL expression genotype categories excluding patients with CRP >10mg/L**.**

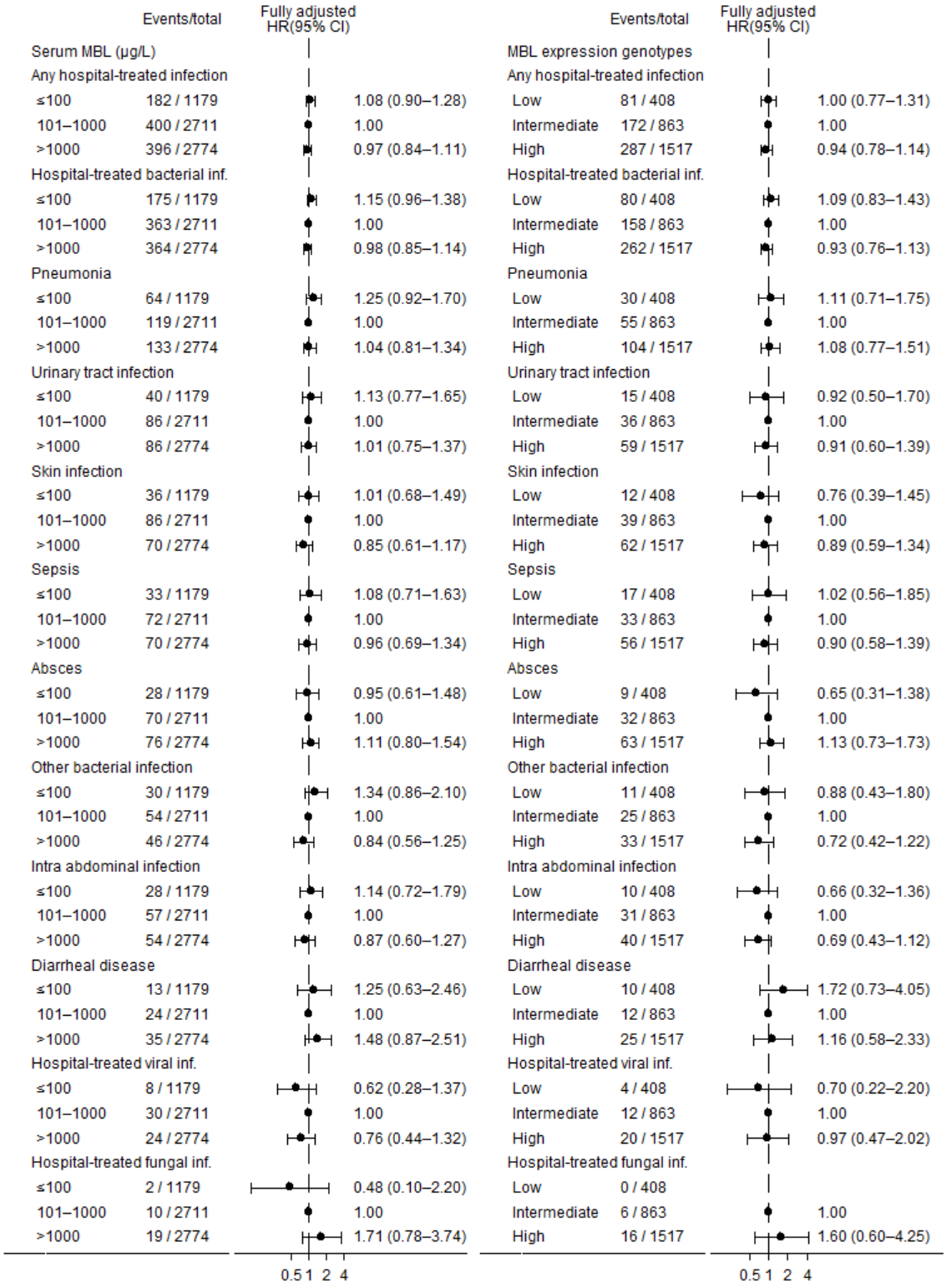


Supplementary Figure 15. Cumulative incidence curves of Antiviral treatment by serum MBL and MBL expression genotype categories excluding patients with CRP >10mg/L**.**

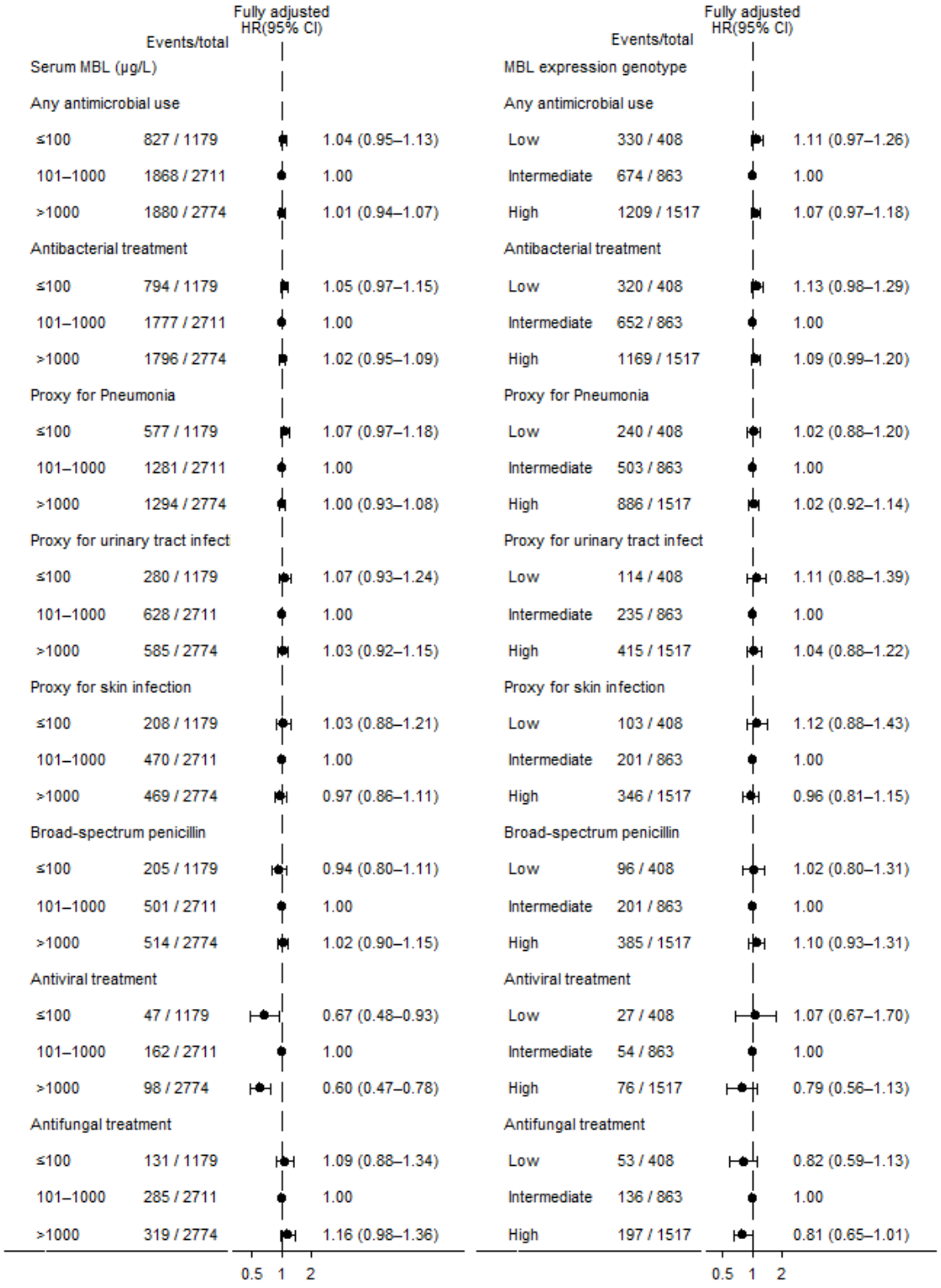


Supplementary Figure 16. Cumulative incidence curves of Antiviral treatment by serum MBL and MBL expression genotype categories excluding patients with CRP >10mg/L**.**

Forestplots: Exclusion of patients with CRP above 10 mg/L



Supplementary Figure 17. Risk of Hospital-treated infections by serum MBL and MBL expression genotype categories excluding patients with CRP >10mg/L**.** Fully adjusted includes all variables included in Model 3.



Supplementary Figure 18. Risk of Community-based antimicrobial use by serum MBL and MBL expression genotype categories excluding patients with CRP >10mg/L**.** Fully adjusted includes all variables included in Model 3.

References

1. Lee KJ, Carlin JB. Multiple imputation for missing data: fully conditional specification versus multivariate normal imputation. Am J Epidemiol 2010;171:624-32.
2. Sterne JA, White IR, Carlin JB, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. Bmj 2009;338:b2393.
3. Bernaards CA, Belin TR, Schafer JL. Robustness of a multivariate normal approximation for imputation of incomplete binary data. Stat Med 2007;26:1368-82.

Reports/PhD theses from Department of Clinical Epidemiology

1. Ane Marie Thulstrup: Mortality, infections and operative risk in patients with liver cirrhosis in Denmark. Clinical epidemiological studies. PhD thesis. *2000*.

2. Nana Thrane: Prescription of systemic antibiotics for Danish children. PhD thesis. *2000*.

3. Charlotte Søndergaard. Follow-up studies of prenatal, perinatal and postnatal risk factors in infantile colic. PhD thesis. *2001*.

4. Charlotte Olesen: Use of the North Jutland Prescription Database in epidemiological studies of drug use and drug safety during pregnancy. PhD thesis. *2001*.

5. Yuan Wei: The impact of fetal growth on the subsequent risk of infectious disease and asthma in childhood. PhD thesis. *2001*.

6. Gitte Pedersen. Bacteremia: treatment and prognosis. PhD thesis. *2001*.

7. Henrik Gregersen: The prognosis of Danish patients with monoclonal gammopathy of undertermined significance: register-based studies. PhD thesis. *2002*.

8. Bente Nørgård: Colitis ulcerosa, coeliaki og graviditet; en oversigt med speciel reference til forløb og sikkerhed af medicinsk behandling. PhD thesis. *2002*.

9. Søren Paaske Johnsen: Risk factors for stroke with special reference to diet, Chlamydia pneumoniae, infection, and use of non-steroidal anti-inflammatory drugs. PhD thesis. *2002*.

10. Elise Snitker Jensen: Seasonal variation of meningococcal disease and factors associated with its outcome. PhD thesis. *2003*.

11. Andrea Floyd: Drug-associated acute pancreatitis. Clinical epidemiological studies of selected drugs. PhD thesis. *2004*.

12. Pia Wogelius: Aspects of dental health in children with asthma. Epidemiological studies of dental anxiety and caries among children in North Jutland County, Denmark. PhD thesis. *2004*.

13. Kort-og langtidsoverlevelse efter indlæggelse for udvalgte kræftsygdomme i Nordjyllands, Viborg og Århus amter 1985-2003. *2004*.

14. Reimar W. Thomsen: Diabetes mellitus and community-acquired bacteremia: risk and prognosis. PhD thesis. *2004*.

15. Kronisk obstruktiv lungesygdom i Nordjyllands, Viborg og Århus amter 1994-2004. Forekomst og prognose. Et pilotprojekt. *2005*.

16. Lungebetændelse i Nordjyllands, Viborg og Århus amter 1994-2004. Forekomst og prognose. Et pilotprojekt. *2005*.

17. Kort- og langtidsoverlevelse efter indlæggelse for nyre-, bugspytkirtel- og leverkræft i Nordjyllands, Viborg, Ringkøbing og Århus amter 1985-2004. *2005*.

18. Kort- og langtidsoverlevelse efter indlæggelse for udvalgte kræftsygdomme i Nordjyllands, Viborg, Ringkøbing og Århus amter 1995-2005. *2005*.

19. Mette Nørgaard: Haematological malignancies: Risk and prognosis. PhD thesis. *2006*.

20. Alma Becic Pedersen: Studies based on the Danish Hip Arthroplastry Registry. PhD thesis. *2006*.

    Særtryk: Klinisk Epidemiologisk Afdeling - De første 5 år. *2006*.

21. Blindtarmsbetændelse i Vejle, Ringkjøbing, Viborg, Nordjyllands og Århus Amter. *2006*.

22. Andre sygdommes betydning for overlevelse efter indlæggelse for seks kræftsygdomme i Nordjyllands, Viborg, Ringkjøbing og Århus amter 1995-2005. *2006*.

23. Ambulante besøg og indlæggelser for udvalgte kroniske sygdomme på somatiske hospitaler i Århus, Ringkjøbing, Viborg, og Nordjyllands amter. *2006*.

24. Ellen M Mikkelsen: Impact of genetic counseling for hereditary breast and ovarian cancer disposition on psychosocial outcomes and risk perception: A population-based follow-up study. PhD thesis. *2006*.

25. Forbruget af lægemidler mod kroniske sygdomme i Århus, Viborg og Nordjyllands amter 2004-2005. *2006*.

26. Tilbagelægning af kolostomi og ileostomi i Vejle, Ringkjøbing, Viborg, Nordjyllands og Århus Amter. *2006*.

27. Rune Erichsen: Time trend in incidence and prognosis of primary liver cancer and liver cancer of unknown origin in a Danish region, 1985-2004. Research year report. *2007*.

28. Vivian Langagergaard: Birth outcome in Danish women with breast cancer, cutaneous malignant melanoma, and Hodgkin's disease. PhD thesis. *2007*.

29. Cynthia de Luise: The relationship between chronic obstructive pulmonary disease, comorbidity and mortality following hip fracture. PhD thesis. *2007*.

30. Kirstine Kobberøe Søgaard: Risk of venous thromboembolism in patients with liver disease: A nationwide population-based case-control study. Research year report. *2007*.

31. Kort- og langtidsoverlevelse efter indlæggelse for udvalgte kræftsygdomme i Region Midtjylland og Region Nordjylland 1995-2006. *2007*.

32. Mette Skytte Tetsche: Prognosis for ovarian cancer in Denmark 1980-2005: Studies of use of hospital discharge data to monitor and study prognosis and impact of comorbidity and venous thromboembolism on survival. PhD thesis. *2007*.

33. Estrid Muff Munk: Clinical epidemiological studies in patients with unexplained chest and/or epigastric pain. PhD thesis. *2007*.

34. Sygehuskontakter og lægemiddelforbrug for udvalgte kroniske sygdomme i Region Nordjylland. *2007*.

35. Vera Ehrenstein: Association of Apgar score and postterm delivery with neurologic morbidity: Cohort studies using data from Danish population registries. PhD thesis. *2007*.

36. Annette Østergaard Jensen: Chronic diseases and non-melanoma skin cancer. The impact on risk and prognosis. PhD thesis. *2008*.

37. Use of medical databases in clinical epidemiology. *2008*.

38. Majken Karoline Jensen: Genetic variation related to high-density lipoprotein metabolism and risk of coronary heart disease. PhD thesis. *2008*.

39. Blodprop i hjertet - forekomst og prognose. En undersøgelse af førstegangsindlæggelser i Region Nordjylland og Region Midtjylland. *2008*.

40. Asbestose og kræft i lungehinderne. Danmark 1977-2005. *2008*.

41. Kort- og langtidsoverlevelse efter indlæggelse for udvalgte kræftsygdomme i Region Midtjylland og Region Nordjylland 1996-2007. *2008*.

    Sandra Kruchov Thygesen. Atrial fibrillation in patients with ischemic stroke: A population-based study. Research year report. *2008*.

42. Akutte indlæggelsesforløb og skadestuebesøg på hospiter i Region Midtjylland og Region Nordjylland 2003-2007. Et pilotprojekt. *Not published*.

43. Peter Jepsen: Prognosis for Danish patients with liver cirrhosis. PhD thesis. *2009*.

44. Lars Pedersen: Use of Danish health registries to study drug-induced birth defects – A review with special reference to methodological issues and maternal use of non-steroidal anti-inflammatory drugs and Loratadine. PhD thesis. *2009*.

45. Steffen Christensen: Prognosis of Danish patients in intensive care. Clinical epidemiological studies on the impact of preadmission cardiovascular drug use on mortality. PhD thesis. *2009*.

46. Morten Schmidt: Use of selective cyclooxygenase-2 inhibitors and nonselective nonsteroidal antiinflammatory drugs and risk of cardiovascular events and death after intracoronary stenting. Research year report. *2009*.

47. Jette Bromman Kornum: Obesity, diabetes and hospitalization with pneumonia. PhD thesis. *2009*.

48. Theis Thilemann: Medication use and risk of revision after primary total hip arthroplasty. PhD thesis. *2009*.

49. Operativ fjernelse af galdeblæren. Region Midtjylland & Region Nordjylland. 1998-2008. *2009*.

50. Mette Søgaard: Diagnosis and prognosis of patients with community-acquired bacteremia. PhD thesis. *2009*.

51. Marianne Tang Severinsen. Risk factors for venous thromboembolism: Smoking, anthropometry and genetic susceptibility. PhD thesis. *2010*.

52. Henriette Thisted: Antidiabetic Treatments and ischemic cardiovascular disease in Denmark: Risk and outcome. PhD thesis. *2010*.

53. Kort- og langtidsoverlevelse efter indlæggelse for udvalgte kræftsygdomme. Region Midtjylland og Region Nordjylland 1997-2008. *2010*.

54. Prognosen efter akut indlæggelse på Medicinsk Visitationsafsnit på Nørrebrogade, Århus Sygehus. *2010*.

55. Kaare Haurvig Palnum: Implementation of clinical guidelines regarding acute treatment and secondary medical prophylaxis among patients with acute stroke in Denmark. PhD thesis. *2010*.

56. Thomas Patrick Ahern: Estimating the impact of molecular profiles and prescription drugs on breast cancer outcomes. PhD thesis. *2010*.

57. Annette Ingeman: Medical complications in patients with stroke: Data validity, processes of care, and clinical outcome. PhD thesis. *2010*.

58. Knoglemetastaser og skeletrelaterede hændelser blandt patienter med prostatakræft i Danmark. Forekomst og prognose 1999-2007. *2010*.

59. Morten Olsen: Prognosis for Danish patients with congenital heart defects - Mortality, psychiatric morbidity, and educational achievement. PhD thesis. *2010*.

60. Knoglemetastaser og skeletrelaterede hændelser blandt kvinder med brystkræft i Danmark. Forekomst og prognose 1999-2007. *2010*.

61. Kort- og langtidsoverlevelse efter hospitalsbehandlet kræft. Region Midtjylland og Region Nordjylland 1998-2009. *2010*.

62. Anna Lei Lamberg: The use of new and existing data sources in non-melanoma skin cancer research. PhD thesis. *2011*.

63. Sigrún Alba Jóhannesdóttir: Mortality in cancer patients following a history of squamous cell skin cancer – A nationwide population-based cohort study. Research year report. *2011*.

64. Martin Majlund Mikkelsen: Risk prediction and prognosis following cardiac surgery: the EuroSCORE and new potential prognostic factors. PhD thesis. *2011*.

65. Gitte Vrelits Sørensen: Use of glucocorticoids and risk of breast cancer: a Danish population-based case-control study. Research year report. *2011*.

66. Anne-Mette Bay Bjørn: Use of corticosteroids in pregnancy. With special focus on the relation to congenital malformations in offspring and miscarriage. PhD thesis. *2012*.

67. Marie Louise Overgaard Svendsen: Early stroke care: studies on structure, process, and outcome. PhD thesis. *2012*.

68. Christian Fynbo Christiansen: Diabetes, preadmission morbidity, and intensive care: population-based Danish studies of prognosis. PhD thesis. *2012*.

69. Jennie Maria Christin Strid: Hospitalization rate and 30-day mortality of patients with status asthmaticus in Denmark – A 16-year nationwide population-based cohort study. Research year report. *2012*.

70. Alkoholisk leversygdom i Region Midtjylland og Region Nordjylland. 2007-2011. *2012*.

71. Lars Jakobsen: Treatment and prognosis after the implementation of primary percutaneous coronary intervention as the standard treatment for ST-elevation myocardial infarction. PhD thesis. *2012*.

72. Anna Maria Platon: The impact of chronic obstructive pulmonary disease on intensive care unit admission and 30-day mortality in patients undergoing colorectal cancer surgery: a Danish population-based cohort study. Research year report. *2012*.

73. Rune Erichsen: Prognosis after Colorectal Cancer - A review of the specific impact of comorbidity, interval cancer, and colonic stent treatment. PhD thesis. *2013*.

74. Anna Byrjalsen: Use of Corticosteroids during Pregnancy and in the Postnatal Period and Risk of Asthma in Offspring - A Nationwide Danish Cohort Study. Research year report. *2013*.

75. Kristina Laugesen: In utero exposure to antidepressant drugs and risk of attention deficit hyperactivity disorder (ADHD). Research year report. *2013*.

76. Malene Kærslund Hansen: Post-operative acute kidney injury and five-year risk of death, myocardial infarction, and stroke among elective cardiac surgical patients: A cohort study. Research year report. *2013*.

77. Astrid Blicher Schelde: Impact of comorbidity on the prediction of first-time myocardial infarction, stroke, or death from single-photon emission computed tomography myocardial perfusion imaging: A Danish cohort study. Research year report. *2013*.

78. Risiko for kræft blandt patienter med kronisk obstruktiv lungesygdom (KOL) i Danmark. (Online publication only). *2013*.

79. Kirurgisk fjernelse af milten og risikoen for efterfølgende infektioner, blodpropper og død. Danmark 1996-2005. (Online publication only). *2013*.

    Jens Georg Hansen: Akut rhinosinuitis (ARS) – diagnostik og behandling af voksne i almen praksis. *2013*.

80. Henrik Gammelager: Prognosis after acute kidney injury among intensive care patients. PhD thesis. *2014*.

81. Dennis Fristrup Simonsen: Patient-Related Risk Factors for Postoperative Pneumonia following Lung Cancer Surgery and Impact of Pneumonia on Survival. Research year report. *2014*.

82. Anne Ording: Breast cancer and comorbidity: Risk and prognosis. PhD thesis. *2014*.

83. Kristoffer Koch: Socioeconomic Status and Bacteremia: Risk, Prognosis, and Treatment. PhD thesis. *2014*.

84. Anne Fia Grann: Melanoma: the impact of comorbidities and postdiagnostic treatments on prognosis. PhD thesis. *2014*.

85. Michael Dalager-Pedersen: Prognosis of adults admitted to medical departments with community-acquired bacteremia. PhD thesis. *2014*.

86. Henrik Solli: Venous thromboembolism: risk factors and risk of subsequent arterial thromboembolic events. Research year report. *2014*.

87. Eva Bjerre Ostenfeld: Glucocorticoid use and colorectal cancer: risk and postoperative outcomes. PhD thesis. *2014*.

88. Tobias Pilgaard Ottosen: Trends in intracerebral haemorrhage epidemiology in Denmark between 2004 and 2012: Incidence, risk-profile and case-fatality. Research year report. *2014*.

89. Lene Rahr-Wagner: Validation and outcome studies from the Danish Knee Ligament Reconstruction Registry. A study in operatively treated anterior cruciate ligament injuries. PhD thesis. *2014*.

90. Marie Dam Lauridsen: Impact of dialysis-requiring acute kidney injury on 5-year mortality after myocardial infarction-related cardiogenic shock - A population-based nationwide cohort study. Research year report. *2014*.

91. Ane Birgitte Telén Andersen: Parental gastrointestinal diseases and risk of asthma in the offspring. A review of the specific impact of acid-suppressive drugs, inflammatory bowel disease, and celiac disease. PhD thesis. *2014*.

    Mikkel S. Andersen: Danish Criteria-based Emergency Medical Dispatch – Ensuring 112 callers the right help in due time? PhD thesis. *2014*.

92. Jonathan Montomoli: Short-term prognosis after colorectal surgery: The impact of liver disease and serum albumin. PhD thesis. *2014*.

93. Morten Schmidt: Cardiovascular risks associated with non-aspirin non-steroidal anti-inflammatory drug use: Pharmacoepidemiological studies. PhD thesis. *2014*.

94. Betina Vest Hansen: Acute admission to internal medicine departments in Denmark - studies on admission rate, diagnosis, and prognosis. PhD thesis. *2015*.

95. Jacob Gamst: Atrial Fibrillation: Risk and Prognosis in Critical Illness. PhD thesis. *2015*.

96. Søren Viborg: Lower gastrointestinal bleeding and risk of gastrointestinal cancer. Research year report. *2015*.

97. Heidi Theresa Ørum Cueto: Folic acid supplement use in Danish pregnancy planners: The impact on the menstrual cycle and fecundability. PhD thesis. *2015*.

98. Niwar Faisal Mohamad: Improving logistics for acute ischaemic stroke treatment: Reducing system delay before revascularisation therapy by reorganisation of the prehospital visitation and centralization of stroke care. Research year report. *2015*.

99. Malene Schou Nielsson: Elderly patients, bacteremia, and intensive care: Risk and prognosis. PhD thesis. *2015*.

100. Jens Tilma: Treatment Injuries in Danish Public Hospitals 2006-2012. Research year report. *2015*.

101. Thomas Lyngaa: Intensive care at the end-of-life in patients dying of cancer and non-cancer chronic diseases: A nationwide study. Research year report. *2015*.

102. Lone Winther Lietzen: Markers of immune competence and the clinical course of breast cancer. PhD thesis. *2015*.

103. Anne Høy Seemann Vestergaard: Geographical Variation in Use of Intensive Care in Denmark: A Nationwide Study. Research year report. *2015*.

104. Cathrine Wildenschild Nielsen: Fecundability among Danish pregnancy planners. Studies on birth weight, gestational age and history of miscarriage. PhD thesis. *2015*.

105. Kathrine Dyhr Lycke: Preadmission use of antidepressants and quality of care, intensive care admission and mortality of colorectal cancer surgery – a nationwide population-based cohort study. Research year report. *2015*.

106. Louise Bill: Hyponatremia in acute internal medicine patients: prevalence and prognosis. PhD thesis. *2015*.

107. Kirstine Kobberøe Søgaard: Risk and prognosis of venous thromboembolism in patients with liver disease. PhD thesis. *2015*.

108. Rikke Nørgaard Pedersen: Reoperation due to surgical bleeding in breast cancer patients and breast cancer recurrence: A Danish population-based cohort study. Research year report. *2015*.

109. Thomas Deleuran: Cirrhosis of the liver and diseases of the large joints. PhD Thesis. *2016*.

110. Anne Mette Falstie-Jensen: Hospital accreditation – what's in it for the patients? PhD thesis. *2016*.

111. Sandra Kruchov Thygesen: Respiratory distress syndrome in moderately late and late preterm infants and selected long-term neurodevelopmental outcomes. PhD thesis. *2016*.

112. Alma Bečić Pedersen: Total hip replacement surgery - occurrence and prognosis. Doctoral dissertation. *2016*.

113. Anil Mor: Type 2 Diabetes and Risk of Infections. PhD thesis. *2016*.

114. Aske Hess Rosenquist: Behavioral Development Following Early Life Organochlorine Exposure. Research year report. *2016*.

115. Simon Ramsdal Sørensen: Anti-platelet and Anti-coagulant Prescriptions and Breast Cancer Recurrence: a Danish Nationwide Prospective Cohort Study. Research year report. *2016*.

116. Regional Differences in Treatment of Patients with Inflammatory Bowel Disease in Denmark

117. Clara Reece Medici: Impact of preadmission anti-inflammatory drug use on risk of depression and anxiety after intensive care requiring mechanical ventilation. Research year report. *2016*.

118. Johan Frederik Håkonsen Arendt. Clinical implications and biochemical understanding of high plasma vitamin B12 levels. PhD thesis. *2016*.

119. Manual for using the LABKA database for research projects. *2016*.

120. Søren Christiansen: Timing of renal replacement therapy and long-term risk of chronic kidney disease and death in intensive care patients with acute kidney injury. Research year report. *2017*.

121. Ellen Hollands Steffensen: Preadmission antidepressant use and bladder cancer: a population-based cohort study of stage at diagnosis, time to surgery, and surgical outcomes. Research year report. *2017*.

122. Sara Søndergaard Schwartz: Incidence and mortality of pulmonary hypertension in adults with congenital heart disease. Research year report. *2017*.

123. Jesper Smit: Community-acquired Staphylococcus aureus bacteremia: Studies of risk and prognosis with special attention to diabetes mellitus and chronic heart failure. PhD thesis. *2017*.

124. Carina Nørskov Bagge: Risk of Dementia in Adults with Congenital Heart Disease: A Nationwide Population-Based Cohort Study. Research year report. *2017*.

125. Pia Kjær Kristensen: Hip fracture in Denmark: Quality of in-hospital care and clinical outcomes. PhD thesis. *2017*.

126. Anne Nakano Jensen: Incident heart failure in Denmark: Studies on a nationwide quality improvement initiative. PhD thesis. *2017*.

127. Kasper Adelborg: Neurological and psychiatric comorbidity in patients with heart failure − Risk and prognosis. PhD thesis. *2017*.

128. Jens Sundbøll: Depression, stroke, and dementia in patients with myocardial infarction — Studies of risk and prognosis. PhD thesis. *2017*.

129. Sigrún Alba Jóhannesdóttir Schmidt: Herpes zoster: Occurrence and risk factors. PhD thesis. *2017*.

130. Mette Glavind Bülow Pedersen: Ischemic Stroke in Adults with Congenital Heart Disease: a population-based cohort study. Research year report. *2017*.

131. Sidse Høyer: Male alcohol intake and couples' time to pregnancy - A prospective cohort study. Research year report. *2017*.

132. Cecilie Daugaard: Socioeconomic inequality in drug reimbursement during end-of-life care: A nationwide study. Research year report. *2018*.

133. Stine Bakkensen Bruun: Selective Serotonin Reuptake Inhibitor Use and Mortality, Postoperative Complications, and Quality of Care in Hip Fracture Patients - A population-based cohort study. Research year report. *2018*.

134. Maria Møller Pedersen: Prognostic role of neutrophil-lymphocyte ratio in localized and metastatic renal cell carcinoma: A population-based cohort study. Research year report. *2018*.

135. Hanne Vangsgaard Nielsen: Proton pump inhibitor prescriptions and breast cancer recurrence: A Danish nationwide prospective cohort study. Research year report. *2018*.

136. Jaimie Gradus: The Long-Term Consequences of Trauma and Stress Disorders: Evidence from the Danish Registries. Doctoral dissertation. *2018*.

137. Mette Holland-Fischer: Rheumatoid arthritis, ankylosing spondylitis and hospitalization with pneumonia. PhD thesis. *2018*.

138. Kaja Kristiane Eriksrud Kjørholt: Increasing risk of postoperative infections among hip fracture patients: A nationwide study 2005-2016. Research year report. *2018*.

139. Troels Munch: Use of opioids prior to hospital admission among the nonsurgical critically ill: Studies on risk and prognosis. PhD thesis. *2018*.

140. Charlotte Slagelse Jensen-Haarup: Colorectal Cancer Surgery and Acute Kidney Injury: A Review with Special Reference to the Risk and Prognosis of Renin-Angiotensin System Blocker Use. PhD thesis. *2018*.

141. Lisbeth Wellejus Albertsen: Development and validation of the DANish Comorbidity index for Acute Myocardial Infarction (DANCAMI): One-year mortality prediction. Research year report. *2019*.

142. Frederikke Schønfeldt Troelsen: Risk and prognosis of primary liver, gallbladder, bile duct, and pancreatic cancer a negative CT scan of the abdomen: A Danish population-based cohort study. Research year report. *2019*.

143. Emil Zâl Bjerregaard Riahi: Thromboembolic and bleeding complications in patients with liver cirrhosis and atrial fibrillation – a population-based cohort study. Research year report. *2019*.

144. Kristine Fogh Andersen: Hematuria and subsequent long-term risk of chronic kidney disease. A Danish population-based cohort study. Research year report. *2019*.

145. Diana Hedevang Christensen: Diabetic polyneuropathy in type 2 diabetes. Prevalence, risk factors, mental health, and diagnostic validity. PhD thesis. *2019*.

146. Kristina Laugesen: Glucocorticoid treatment: Population-based studies on utilization and selected adverse effects. PhD thesis. *2020*.

147. Frederik Pagh Kristensen: Statin therapy and risk of diabetic polyneuropathy: A population-based cohort study. Research year report. *2020*.

148. Jeppe Damgren Vesterager: Hospital variation in the risk of infection after hip fracture surgery: A population-based cohort study. Research year report. *2020*.

149. Katrine Hjuler Lund: Perceived stress and in-home assessed semen quality - A cross-sectional study. Research year report. *2020*.

150. Jakob Schöllhammer Knudsen: Type 2 diabetes in real-life: Population-based Danish studies of incidence, prognosis, and treatment effectiveness. PhD thesis. *2020*.