Online-Only Supplemental Material

Natriuretic peptides and risk of type 2 diabetes: Results from the Biomarkers for Cardiovascular Risk Assessment in Europe (BiomarCaRE) Consortium

Short running title: Natriuretic peptides and type 2 diabetes

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Table of Contents

Supplementary Table S1. Overview of contributing BiomarCaRE cohorts

Cohort	Country	Short description
Kooperative Gesundheits-forschung in der Region Augsburg (KORA)	Germany	The WHO Multinational Monitoring of Trends and Determinants in Cardiovascular Diseases (MONICA) / Cooperative Health Research in the Region of Augsburg (KORA) Survey 3 (S3) and Survey 4 (S4) are population-based health surveys that comprise randomly selected respondents aged 25-74 years from the city of Augsburg in Bavaria, Southern Germany and its two surrounding counties. List of municipalities and population registers were used as sampling frames. The MONICA/KORA S3 baseline examination was carried out in 1994-1995 as a part of the WHO MONICA project and consists of 4,856 men and women with a response rate of 75%. The S4 baseline examination was carried out in 1999-2001 and includes 4,261 participants (response rate: 66%); out of these 3,080 participated were re-examined in 2006-2008 (F4). The BiomarCaRE project includes 4,692 participants from the S3 and 4,221 participants from the S4 (including 3,060 participants with additional data from F4). Follow-up questionnaires were send to all MONICA/KORA S3 study participants in 1997/1998, 2002/2003, 2008/2009 and 2016 and to all S4 study participants in 2008/2009 and 2016 to obtain information on the occurrence of chronic diseases and risk factors. In addition, follow-up examinations were conducted for S3 participants in 2004/2005 (F3) and for S4 participants in 2006-2008 (F4) and 2013/2014 (FF4). The core BiomarCaRE database includes follow-up data until 2009. In the current analysis, we included incident cases of type 2 diabetes, which had been diagnosed up to 2016. Prevalent or incident type 2 diabetes was defined as self-reported clinically diagnosed diabetes that could be validated by the responsible physician or hospital discharge letters, or by self-reported use of glucose-lowering medication. Furthermore, participants with clinical diagnoses or comorbidity ICD-code (ICD-9: 250) on the death certificate were coded as prevalent or incident diabetes. For incident diabetes cases, the self-reported date of diagnosis was assessed and generally verified by contacting the treating physician or medical chart review. When information on the type of diabetes was not available, it was considered to be type 2 diabetes if the age of the person was above 35 years at the time of diagnosis. https://www.thl.fi/publications/morgam/cohorts/full/germany/ger-auga.htm

FINRISK	Finland	The FINRISK study is a series of population-based cardiovascular risk factor surveys carried out every five years in five (or six in 2002) districts of Finland, including North Karelia, Northern Savo (former Kuopio), South-western Finland, Oulu Province, Lapland province (in 2002 only) and the region of Helsinki and Vantaa. A stratified random sample was drawn for each survey from the national population register; the age-range was 25-74 years. All individuals enrolled in the study received a physical examination, a self-administered questionnaire, and a blood sample examination. In 1997, altogether 11,500 individuals were invited and 8,444 (73%) participated in the clinical examination. During the follow-up time the National Hospital Discharge Register, the National Causes of Death Register and the National Drug Reimbursement Register were used to identify endpoints. Participants were followed up until December 31st, 2010. The cohorts were linked to the Hospital Discharge Register and Causes of Death Register and drug reimbursement registers. A hospitalization or death with the ICD-8/9 code 250 or with any of the ICD-10 codes E10, E11 and E14 was considered to indicate diabetes. Likewise, the appearance of "special reimbursements" for diabetes mellitus, i.e., KELA code 103, or purchases of drugs with the ATC code A10* were taken as diabetes. The type of diabetes was then determined as follows: If the age of the patient was < 35 at the time of diagnosis and the treatment was insulin only, the person was considered to have type 1 diabetes. All others were considered to have type 2 diabetes. http://www.thl.fi/publications/morgam/cohorts/full/finland/fin-fina.htm
Prospective Epidemiological Study of Myocardial Infarction (PRIME) Belfast	United Kingdom	The PRIME study examined the classic and putative cardiovascular risk factors to explain the large difference in heart disease incidence between Ireland and France. The study includes four cohorts of men aged 50-59; from Belfast, Northern Ireland (N= 2,745) and Lille (N= 2,633), Toulouse (N= 2,610) and Strasbourg (N= 2,612) in France. The current study only includes the Belfast cohort, since data on natriuretic peptides was not available for the French cohorts. Baseline examinations took place in 1991-1994 and targeted cohorts that had broadly similar social class structures to the background population. Study participants were followed up until 2012 through annual follow up questionnaires with verification against national death registers, medical records, hospital discharge diagnoses. Endpoints were validated by expert medical committee. Type 2 diabetes cases identified by contacting the practitioner of each subject who self-reported type 2 diabetes to validate diabetes type, treatment and diagnosis date.

		http://www.thl.fi/publications/morgam/cohorts/full/uk/unk-bela.htm
Moli-sani Study	Italy	The cohort of the Moli-sani Study was recruited in the Molise region from city hall registries by a multistage sampling. First, townships were sampled in major areas by cluster sampling; then, within each township, participants aged 35 years or older were selected by simple random sampling. Exclusion criteria were pregnancy at the time of recruitment, lack in understanding, current multiple trauma or coma, or refusal to sign the informed consent. A total of 24,325 men (47%) and women (53%) over the age of 35 were examined at baseline from 2005 to 2010. Participation rate was 70%. The cohort was followed-up for a median of 4.2 years (maximum 6.5 years) in December 2011 and will be followed-up every 5 years. Follow up is achieved through record linkage to national mortality registries and hospital discharge registers, validation of events was achieved through hospital record linkage and doctors medical records using updated MORGAM criteria. Type 2 diabetes cases were defined as documented, i.e. through Hospital Discharge Records that revealed a hospitalization with ICD-9 code 250; or self-reported diagnosis of type 2 diabetes via a phone interview (2010). https://www.thl.fi/publications/morgam/cohorts/full/italy/ita-mola.htm
Northern Sweden MONICA	Sweden	The Northern Sweden MONICA project was initiated as part of the WHO MONICA study in 1985. The six population-based surveys consist of individuals from the counties of Västerbotten and Norrbotten, selected about every five years from 1986 to 2009. Individuals were randomly selected from population registers, stratified for 10-years age group (with age range from 25 to 64 years in 1986 and 1990, and from 25 to 74 years from 1994 to 2009) and sex. In total, 10,450 individuals participated, equalling an overall participation rate of 74.6%. Follow-up was achieved through linkage with the national death register and the National registers at the National Board of Health and Welfare (Cause of Death Register, Inpatient Diagnosis Register, Cancer Register, and Medication Register) with endpoint diagnosis based on MORGAM criteria. Follow-up was completed in December 31st, 2011. All cases of incident type 2 diabetes were identified through linkage to the Diabetes register. http://www.thl.fi/publications/morgam/cohorts/full/sweden/swe-nswa.htm.

Supplementary Table S2. Intra-assay and inter-assay coefficients of variation for each natriuretic peptide by participating BiomarCaRE cohort

Cohort	NT-proBNP		MR-proANP	
	Intra-assay (%)	Inter-assay (%)	Intra-assay (%)	Inter-assay (%)
KORA S3-S4	1.17	5.52 – 9.18	NA	NA
KORA F4	NA	NA	3.50	2.63 – 3.36
FINRISK	2.58	1.38	3.65	2.33
PRIME Belfast	2.58	1.38	3.65	2.33
Moli-sani	2.30	5.44 – 6.50	NA	NA
Northern Sweden	1.48	5.88 – 8.70	NA	NA

Abbreviations: MR-proANP, mid-regional pro-atrial natriuretic peptide; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NA, data not available

Supplementary Table S3. Characteristics of the total study population in the quarters of NT-proBNP baseline concentrations

	1st Quarter ≤ 21.04 pg/ml	2nd Quarter 21.05 – 42.87 pg/ml	3rd Quarter 42.88 – 81.54 pg/ml	4th Quarter ≥ 81.55 pg/ml
N	11,374	11,368	11,367	11,368
Incident type 2 diabetes (%)	463 (4.1)	384 (3.4)	385 (3.4)	475 (4.2)
Person-years	114,397	111,360	106,703	97,159
Study cohort (%)				
KORA S3-S4	966 (8.5)	1,375 (12.1)	1,405 (12.4)	1,384 (12.2)
FINRISK	1,837 (16.2)	1,779 (15.6)	1,818 (16.0)	1,806 (15.9)
PRIME Belfast	729 (6.4)	716 (6.3)	519 (4.6)	368 (3.2)
Moli-sani	4,854 (42.7)	5,249 (46.2)	5,520 (48.6)	5,734 (50.4)
Northern Sweden	2,988 (26.3)	2,249 (19.8)	2,105 (18.5)	2,076 (18.3)
Age in years (SD)	45.6 (10.3)	48.7 (11.1)	52.1 (11.9)	59.8 (13.0)
Male (%)	8,555 (75.2)	6,067 (53.4)	4,284 (37.7)	4,139 (36.4)
Body mass index in kg/m^2 (SD)	27.4 (4.3)	27.0 (4.5)	27.0 (4.7)	27.6 (4.94)
Current smoking (%)	3,331 (29.3)	3,072 (27.0)	2,703 (23.8)	2,227 (19.6)
Systolic blood pressure in mmHg (SD)	131.8 (16.5)	132.2 (18.4)	134.6 (20.6)	143.3 (23.9)
Use of antihypertensive medication (%)	1,093 (9.6)	1,447 (12.7)	2,024 (17.8)	3,860 (34.0)
Total cholesterol in mmol/l (SD)	5.74 (1.13)	5.65 (1.11)	5.64 (1.12)	5.64 (1.16)
HDL cholesterol in mmol/l (SD)	1.34 (0.36)	1.43 (0.38)	1.50 (0.40)	1.51 (0.42)
History of CVD (%)	150 (1.3)	221 (1.9)	373 (3.3)	1,251 (11.0)

Data are presented as frequency (percentage) for categorical variables and as mean (SD) for continuous variables. Abbreviations: CVD, cardiovascular disease; HDL, high-density lipoprotein; KORA, the Cooperative Health Research in the Augsburg Region Study; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PRIME, the Prospective Epidemiological Study of Myocardial Infarction; SD, standard deviation.

Supplementary Table S4. Characteristics of the total study population in the quarters of MR-proANP baseline concentrations

	1st Quarter ≤ 35.20 pmol/l	2nd Quarter 35.21 – 48.60 pmol/l	3rd Quarter 48.61 – 68.04 pmol/l	4th Quarter ≥ 68.05 pmol/l
N	2,885	2,892	2,876	2,884
Incident type 2 diabetes (%)	204 (7.1)	205 (7.1)	209 (7.3)	239 (8.3)
Person-years	36,626	35,681	35,194	33,705
Study cohort (%)				
KORA S3-S4	297 (10.3)	558 (19.3)	657 (22.8)	753 (26.1)
FINRISK	2,492 (86.4)	2,016 (69.7)	1,535 (53.4)	1,258 (43.6)
PRIME Belfast	96 (3.3)	318 (11.0)	684 (23.8)	873 (30.3)
Age in years (SD)	41.1 (10.3)	47.0 (11.1)	52.7 (9.9)	58.7 (9.0)
Male (%)	1,756 (60.9)	1,562 (54.0)	1,578 (54.9)	1,781 (61.8)
Body mass index in kg/m^2 (SD)	26.3 (4.4)	26.4 (4.4)	26.7 (4.2)	27.0 (4.3)
Current smoking (%)	1,024 (35.5)	798 (27.6)	653 (22.7)	523 (18.1)
Systolic blood pressure in mmHg (SD)	130.1 (16.5)	129.9 (19.0)	132.8 (20.4)	137.4 (23.7)
Use of antihypertensive medication (%)	171 (5.9)	242 (8.4)	335 (11.6)	785 (27.2)
Total cholesterol in mmol/l (SD)	5.39 (1.07)	5.54 (1.04)	5.68 (1.02)	5.70 (1.03)
HDL cholesterol in mmol/l (SD)	1.34 (0.34)	1.39 (0.36)	1.39 (0.38)	1.38 (0.40)
History of CVD (%)	61 (2.1)	91 (3.1)	148 (5.1)	483 (16.7)

Data are presented as frequency (percentage) for categorical variables and as mean (SD) for continuous variables. Abbreviations: CVD, cardiovascular disease; HDL, high-density lipoprotein; KORA, the Cooperative Health Research in the Augsburg Region Study; MR-proANP, mid-regional pro-atrial natriuretic peptide; PRIME, the Prospective Epidemiological Study of Myocardial Infarction; SD, standard deviation.

Supplementary Table S5. Participant characteristics by cohort

	KORA S3-S4	KORA F4	FINRISK	PRIME Belfast	Moli-sani	Northern Sweden
N	5,130	2,265	7,553	2,596	21,357	9,418
Examination years	1994-1995 & 1999-2001	2006-2008	1997	1991-1994	2005-2010	1986-2009
Median of follow-up time in years (IQR)	15.8 (14.1; 20.8)	8.1 (7.5; 8.5)	13.8 (13.8; 13.9)	18.0 (13.4; 18.0)	4.2 (3.4; 5.3)	12.8 (7.8; 21.3)
Incident type 2 diabetes (%)	236 (4.6)	103 (4.5)	574 (7.6)	252 (9.7)	390 (1.8)	311 (3.3)
Person-years	79,849	17,025	97,448	39,696	91,902	128,734
Incidence rate of type 2 diabetes per 1000 person-years (95% CI)	2.96 (2.59; 3.36)	6.05 (4.94; 7.34)	5.89 (5.42; 6.39)	6.35 (5.59; 7.18)	4.24 (3.83; 4.69)	2.42 (2.15; 2.70)
NT-proBNP in pg/ml (antilog SD)	47.5 (2.6)	NA	40.9 (2.9) *	33.1 (2.7) ‡	44.3 (2.9)	34.5 (3.2)
MR-proANP in pmol/l (antilog SD)	NA	58.0 (1.6)	44.3 (1.6) †	66.0 (1.5) §	NA	NA
Age in years (SD)	47.2 (13.4)	52.5 (11.2)	47.6 (13.2)	54.8 (2.9)	55.0 (11.7)	48.3 (13.4)
Male (%)	2,439 (47.5)	1,076 (47.5)	3,739 (49.5)	2,596 (100.0)	10,090 (47.2)	4,622 (49.1)
Body mass index in kg/m^2 (SD)	26.5 (4.4)	27.0 (4.6)	26.6 (4.5)	26.2 (3.4)	27.9 (4.7)	26.9 (4.8)
Current smoking (%)	1,390 (27.1)	469 (20.7)	2,027 (26.8)	815 (31.4)	4,947 (23.2)	2,321 (24.6)
Systolic blood pressure in mmHg (SD)	128.8 (18.8)	121.2 (17.6)	135.4 (19.8)	133.8 (20.6)	140.0 (20.4)	129.3 (19.8)
Use of antihypertensive medication (%)	550 (10.7)	421 (18.6)	941 (12.5)	254 (9.8)	5,660 (26.5)	1,082 (11.5)

Total cholesterol in mmol/l (SD)	5.84 (1.11)	5.57 (1.00)	5.49 (1.05)	5.90 (1.01)	5.54 (1.07)	5.94 (1.26)
HDL in mmol/l (SD)	1.48 (0.44)	1.46 (0.38)	1.40 (0.36)	1.18 (0.32)	1.50 (0.38)	1.41 (0.41)
eGFR creatinine in ml/min/1.73m^2 (SD) ||	98.1 (16.7)	91.6 (14.9)	89.0 (20.0)	83.2 (20.5)	92.8 (14.6)	102.3 (17.2)
hsCRP in mg/l (antilog SD) ¶	1.19 (3.16)	1.09 (3.03)	1.21 (3.00)	1.70 (2.86)	1.62 (2.86)	1.01 (3.16)
Leptin in ng/ml (antilog SD) #	NA	9.87 (3.22)	9.30 (2.34)	5.26 (1.93)	NA	7.39 (2.39)
Adiponectin in µg/ml (antilog SD) **	NA	9.87 (1.75)	5.58 (1.88)	5.00 (1.88)	NA	NA
History of CVD (%)	328 (6.4)	128 (5.7)	545 (7.2)	163 (6.3)	589 (2.8)	413 (4.4)

Data are presented as frequency (percentage) for categorical variables and as mean (SD) for continuous variables. Continuous variables with skewed distributions are presented as geometric mean (antilog SD).

*Data were available and calculated in 7,240 participants.

†Data were available and calculated in 7,301 participants.

‡Data were available and calculated in 2,332 participants.

§Data were available and calculated in 1,971 participants.

||Data were available and calculated in 5,128 (KORA S3-S4); 2,264 (KORA F4); 7,271 (FINRISK); 1,807 (PRIME); 21,147 (Moli-sani); and 9,416 (Northern Sweden) participants.

¶Data were available and calculated in 5,063 (KORA S3-S4); 2,262 (KORA F4); 7,351 (FINRISK); 2,336 (PRIME); 21,077 (Moli-sani); and 9,351 (Northern Sweden) participants.

#Data were available and calculated in 2,263 (KORA F4); 6,801 (FINRISK); 2,011 (PRIME); and 9,231 (Northern Sweden) participants.

**Data were available and calculated in 537 (KORA F4); 7,210 (FINRISK) and 2,016 (PRIME) participants.

Abbreviations: CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; MR-proANP, mid-regional pro-atrial natriuretic peptide; NA, data not available; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SD, standard deviation.

Supplementary Table S6. Hazard ratios for incident type 2 diabetes by history of CVD, with individual adjustment for other biomarkers

	Adjustment model	With history of CVD		Without history of CVD	
		No. of events / person-years	HR [95%CI] per 1-SD	No. of events / person-years	HR [95%CI] per 1-SD
NT-proBNP	Model 2	209 / 18,249	1.04 [0.90; 1.19], *P* = 0.608	1,498 / 411,372	0.81 [0.76; 0.86], *P* < 0.001
	Model 2 + eGFR	196 / 17,307	1.02 [0.89; 1.18], *P* = 0.759	1,417 / 395,762	0.80 [0.75; 0.85], *P* < 0.001
	Model 2 + hsCRP	209 / 18,136	1.03 [0.90; 1.17], *P* = 0.717	1,492 / 407,728	0.79 [0.75; 0.84], *P* < 0.001
	Model 2 + leptin *	114 / 11,601	1.00 [0.83; 1.20], *P* = 0.993	947 / 230,569	0.80 [0.74; 0.86], *P* < 0.001
	Model 2 + adiponectin †	86 / 7,147	0.98 [0.79; 1.21], *P* = 0.858	612 / 108,814	0.80 [0.72; 0.87], *P* < 0.001
MR-proANP	Model 2	106 / 8,441	0.81 [0.66; 0.99], *P* = 0.042	751 / 132,765	0.75 [0.69; 0.82], *P* < 0.001
	Model 2 + eGFR	104 / 8,175	0.83 [0.68; 1.03], *P* = 0.087	732 / 130,218	0.75 [0.68; 0.82], *P* < 0.001
	Model 2 + hsCRP	102 / 8,131	0.81 [0.66; 1.00], *P* = 0.052	709 / 126,439	0.76 [0.70; 0.83], *P* < 0.001
	Model 2 + leptin	101 / 7,833	0.79 [0.64; 0.97], *P* = 0.027	697 / 116,047	0.75 [0.69; 0.82], *P* < 0.001
	Model 2 + adiponectin	99 / 8,097	0.77 [0.62; 0.95], *P* = 0.014	687 / 118,104	0.80 [0.73; 0.88], *P* < 0.001

The Cox models used age (continuous, in years) as time scale and were stratified by study cohort. The models were adjusted for sex (men/women), body mass index (continuous, in kg/m^2), current smoking (yes/no), systolic blood pressure (continuous, in mmHg), use of antihypertensive medication (yes/no), total and high-density lipoprotein cholesterol (continuous, in mmol/l), and history of

cardiovascular disease (yes/no). NT-proBNP and MR-proANP were log-transformed and (0,1)-standardized in the total study population to approximate normality and to evaluate the HRs per 1-SD increase. *Data were available and analyzed in FINRISK, PRIME Belfast and Northern Sweden. †Data were available and analyzed in FINRISK and PRIME Belfast. Abbreviations: CI, confidence interval; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HR, hazard ratio; hsCRP, high-sensitivity C-reactive protein; MR-proANP, mid-regional pro-atrial natriuretic peptide; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SD, standard deviation.

Supplementary Table S7. Association between natriuretic peptides and incident type 2 diabetes in participants with complete data on both NT-proBNP and MR-proANP (N= 8,695)*

	No. of cases / person-years	HR [95%CI] per 1-SD increase	*P* for Multiplicative interaction	*P* for Additive interaction
NT-proBNP				
Overall	698 / 116,173	0.81 [0.74; 0.88], *P* < 0.001		
History of CVD			0.004	0.003
Yes	85 / 7,127	1.00 [0.81; 1.23], *P* = 0.986		
No	613 / 109,046	0.76 [0.70; 0.84], *P* < 0.001		
MR-proANP				
Overall	698 / 116,173	0.76 [0.70; 0.84], *P* < 0.001		
History of CVD			0.167	0.263
Yes	85 / 7,127	0.80 [0.64; 1.00.], *P* = 0.045		
No	613 / 109,046	0.75 [0.68; 0.83], *P* < 0.001		

The Cox models used age (continuous, in years) as time scale and were stratified by study cohort. The models were adjusted for sex (men/women), body mass index (continuous, in kg/m^2), current smoking (yes/no), systolic blood pressure (continuous, in mmHg), use of antihypertensive medication (yes/no), total and high-density lipoprotein cholesterol (continuous, in mmol/l), and history of cardiovascular disease (yes/no). NT-proBNP and MR-proANP were log-transformed and (0,1)-standardized in the total study population to approximate normality and to evaluate the HRs per 1-SD increase. *Data were available and analyzed in FINRISK and PRIME Belfast. †Interaction on additive scale was estimated with RERI [95%CI]. Abbreviations: BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; HR; hazard ratio; NT-proBNP, N-terminal pro-B-type natriuretic peptide; MR-proANP, mid-regional pro-atrial natriuretic peptide; RERI, relative excess risk due to interaction; SD, standard deviation.

Supplementary Table S8. Competing risk analysis of the association between natriuretic peptides and incident type 2 diabetes

	N cases	N death	sHR [95%CI] per 1-SD
NT-proBNP			
Overall	1,707	2,898	0.84 [0.79; 0.89], *P* < 0.001
History of CVD			
Yes	209	467	0.98 [0.86; 1.11], *P* = 0.721
No	1,498	2,431	0.81 [0.76; 0.87], *P* < 0.001
MR-proANP			
Overall	857	1,077	0.78 [0.72; 0.85], *P* < 0.001
History of CVD			
Yes	106	213	0.80 [0.66; 0.98], *P* = 0.027
No	751	864	0.77 [0.71; 0.84], *P* < 0.001

The Fine and Gray models were stratified by study cohort and were adjusted for age (continuous, in years), sex (men/women), body mass index (continuous, in kg/m^2), current smoking (yes/no), systolic blood pressure (continuous, in mmHg), use of antihypertensive medication (yes/no), total and high-density lipoprotein cholesterol (continuous, in mmol/l), and history of cardiovascular disease (yes/no). NT-proBNP and MR-proANP were log-transformed and (0,1)-standardized in the total study population to approximate normality and to evaluate the estimates per 1-SD increase. Abbreviations: BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; MR-proANP, mid-regional pro-atrial natriuretic peptide; N cases, number of incident type 2 diabetes cases during follow-up; N death, number of death without having diabetes during follow-up; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SD, standard deviation, sHR, subdistribution hazard ratio.

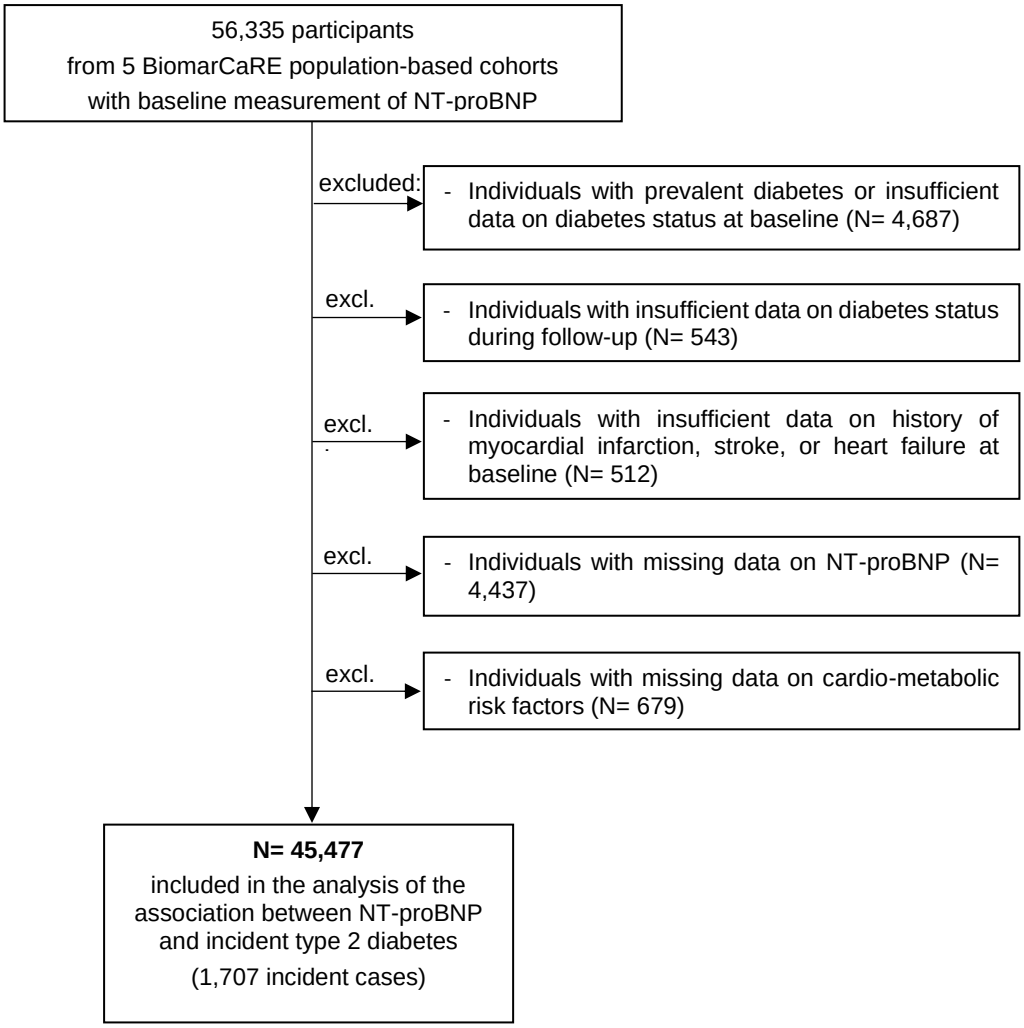
Supplementary Table S9. Independent SNPs associated with natriuretic peptides and type 2 diabetes

SNP	Gene	Chr.	Position	Effect allele	Association estimates with natriuretic peptides *					Association estimates with type 2 diabetes †			
					Natriuretic peptide	Beta	SE	P-value	Sample size	Beta	SE	P-value	Sample size
rs198379	*NPPB*	1	11915467	C	NT-proBNP	0.257	0.016	2.30E-61	6,296	-0.020	0.008	1.26E-2	564,914
rs4845875	*MTHFR, NPPA*	1	11824133	C	MR-proANP	-0.156	0.020	3.37E-9	6,296	0.020	0.008	9.84E-3	597,478
rs3753584	*C1orf167, NPPA*	1	11864586	C	MR-proANP	0.275	0.038	4.19E-13	6,296	-0.021	0.011	5.39E-2	573,410

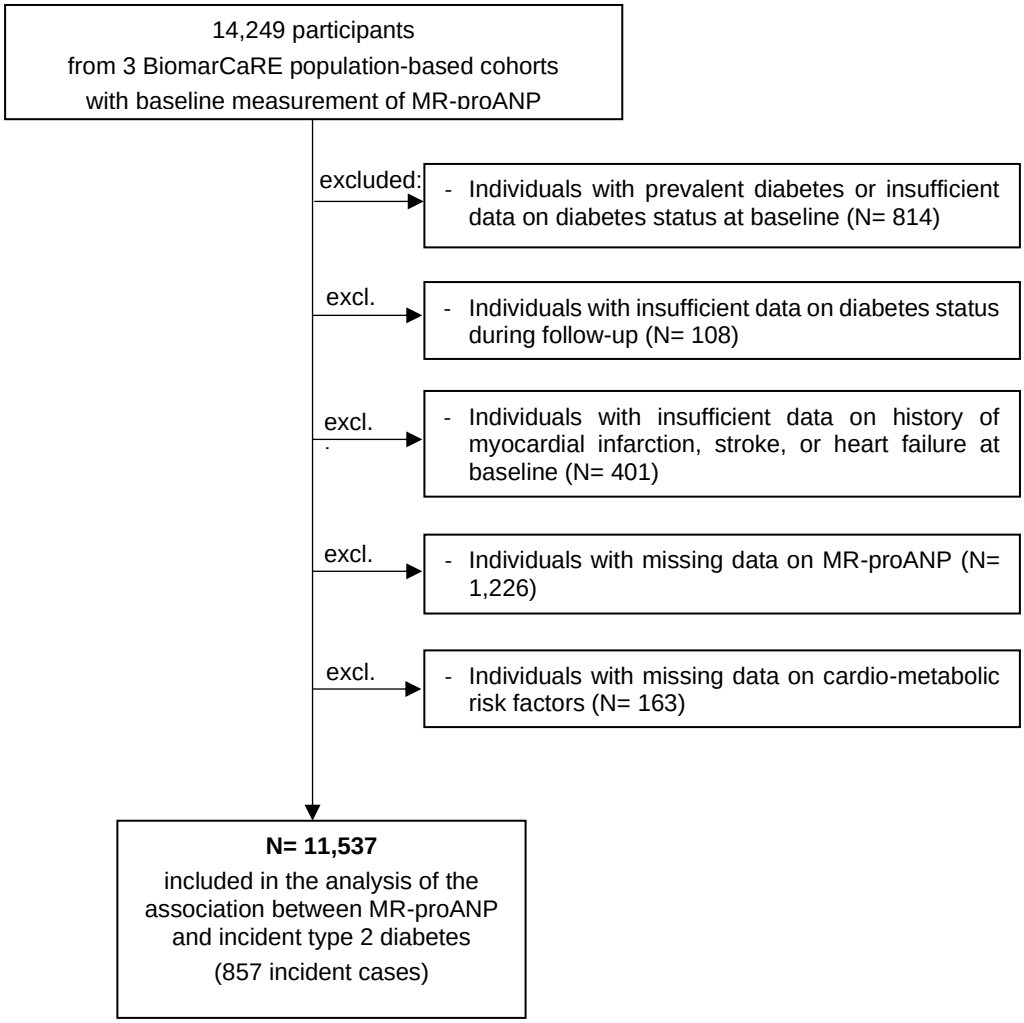
*Data source: Salo PP, et al. Genome-wide association study implicates atrial natriuretic peptide rather than B-type natriuretic peptide in the regulation of blood pressure in the general population. Circ Cardiovasc Genet. 2017;10(6):e001713.

†Data source: Xue A, et al. Genome-wide association analyses identify 143 risk variants and putative regulatory mechanisms for type 2 diabetes. Nat Commun. 2018;9(1):2941.

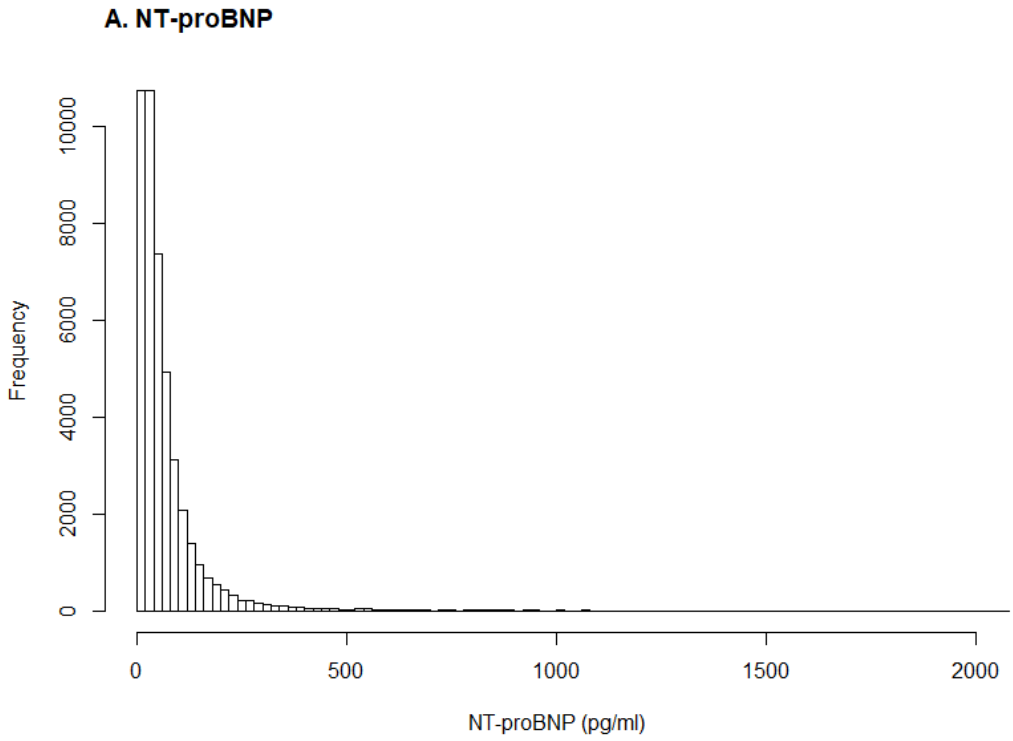
Abbreviations: Chr., chromosome; C1orf167, chromosome 1 open reading frame 167; MR-proANP, mid-regional pro-atrial natriuretic peptide; MTHFR, methylenetetrahydrofolate reductase; NPPA, natriuretic peptide precursor A; NPPB, natriuretic peptide precursor B; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SE, standard error; SNP, single nucleotide polymorphism.

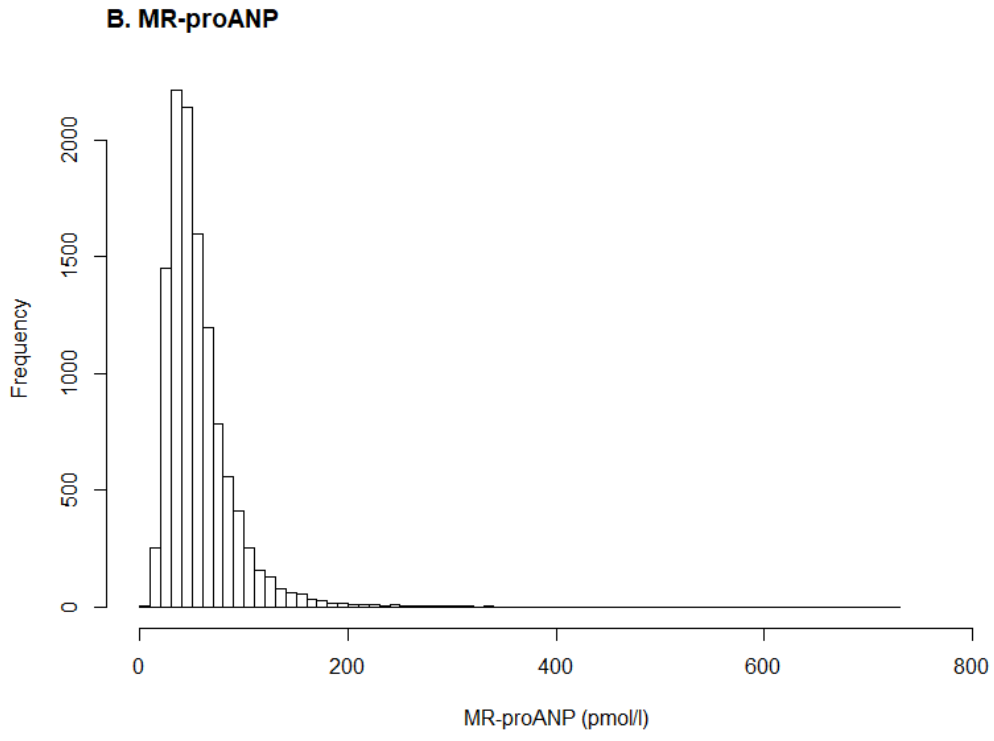


Supplementary Figure S1. Exclusion criteria for analyzing the association between NT-proBNP and incident type 2 diabetes. Abbreviations: BiomarCaRE, Biomarkers for Cardiovascular Risk Assessment in Europe; NT-proBNP, N-terminal pro-B-type natriuretic peptide.



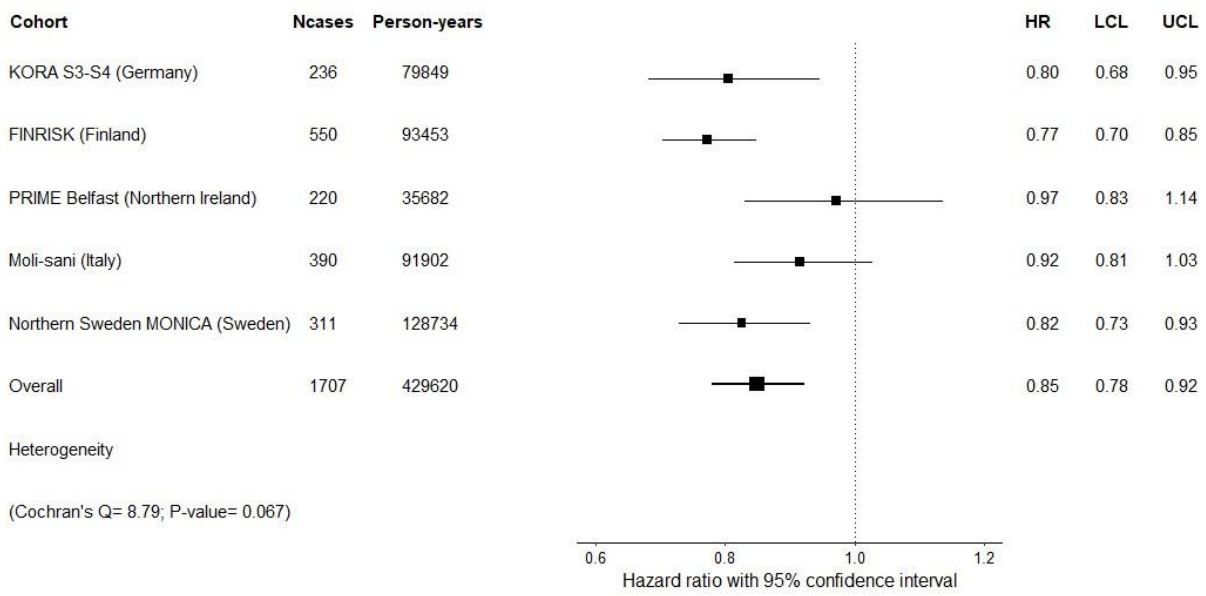
Supplementary Figure S2. Exclusion criteria for analyzing the association between MR-proANP and incident type 2 diabetes. Abbreviations: BiomarCaRE, Biomarkers for Cardiovascular Risk Assessment in Europe; MR-proANP, mid-regional pro-atrial natriuretic peptide.



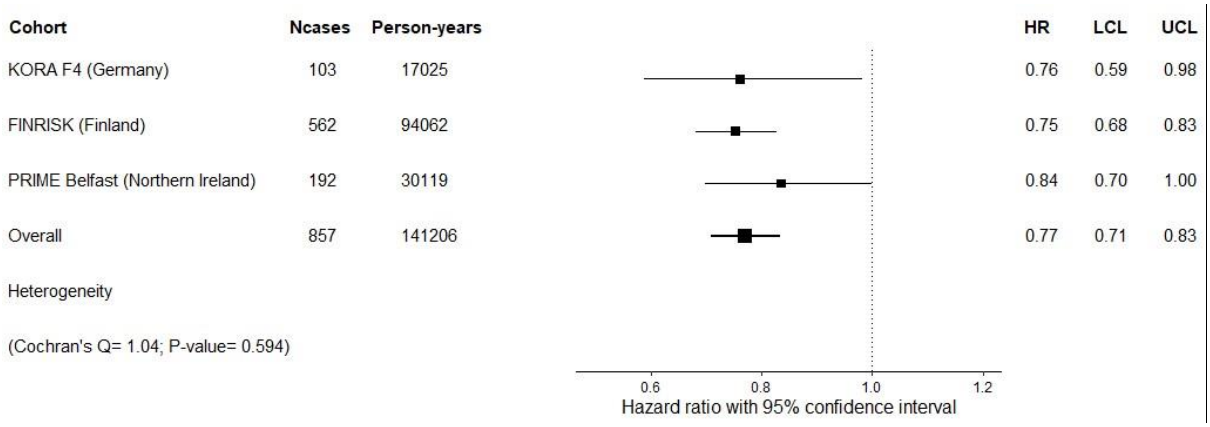


Supplementary Figure S3. The distribution (frequency histogram) of NT-proBNP (A) and MR-proANP (B) in the study population. For NT-proBNP, mean value: 81 pg/ml (SD 243 pg/ml); median value: 43 pg/ml (IQR 61 pg/ml). For MR-proANP, mean

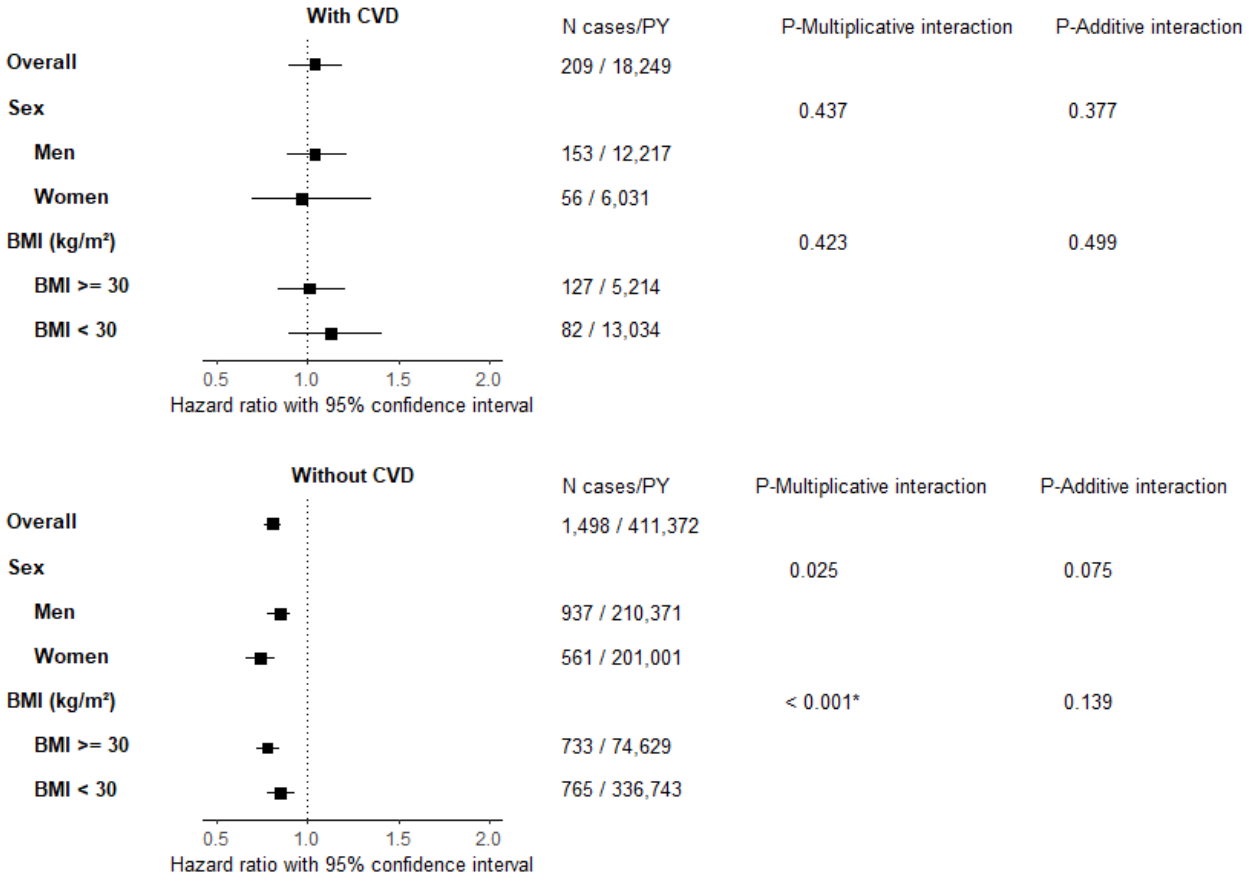
value: 57 pmol/l (SD 36 pmol/l; median 49 pmol/l, IQR 33 pmol/l. Abbreviations: IQR, interquartile range; MR-proANP, mid-regional pro-atrial natriuretic peptide; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SD, standard deviation.



Supplementary Figure S4. Meta-analysis of the association between NT-proBNP and incident type 2 diabetes across participating BiomarCaRE cohorts. Hazard ratios for each study cohort were computed using Cox models. The models used age (continuous, in years) as time scale and were adjusted for sex (men/women), body mass index (continuous, in kg/m2), current smoking (yes/no), systolic blood pressure (continuous, in mmHg), use of antihypertensive medication (yes/no), total and high-density lipoprotein cholesterol (continuous, in mmol/l). NT-proBNP was log-transformed and (0,1)-standardized to approximate normality in the total study population and to evaluate the hazard ratios per 1-standard deviation increase. Overall estimate was calculated using DerSimonian-Laird random-effects model. Black squares represent hazard ratios and bars represent 95% confidence intervals per 1-standard deviation increment of log NT-proBNP. Abbreviations: BiomarCaRE, Biomarkers for Cardiovascular Risk Assessment in Europe; HR, hazard ratio; LCL, lower confidence limit; Ncases, number of incident type 2 diabetes cases; NT-proBNP, N-terminal pro-B-type natriuretic peptide; UCL, upper confidence limit.

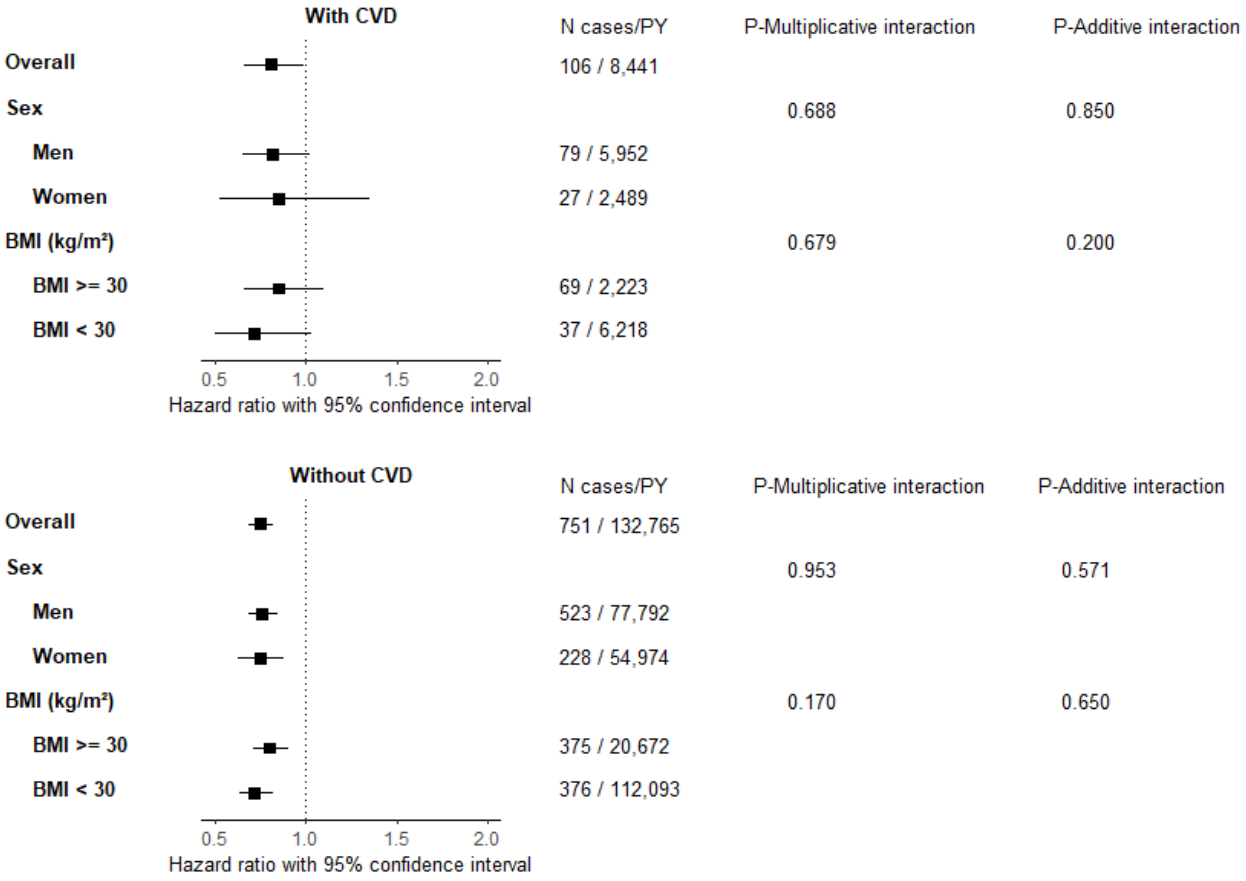


Supplementary Figure S5. Meta-analysis of the association between MR-proANP and incident type 2 diabetes across participating BiomarCaRE cohorts. Hazard ratios for each study cohort were computed using Cox models. The models used age (continuous, in years) as time scale and were adjusted for sex (men/women), body mass index (continuous, in kg/m2), current smoking (yes/no), systolic blood pressure (continuous, in mmHg), use of antihypertensive medication (yes/no), total and high-density lipoprotein cholesterol (continuous, in mmol/l). MR-proANP was log-transformed and (0,1)-standardized in the total study population to approximate normality and to evaluate the hazard ratios per 1-standard deviation increase. Overall estimate was calculated using DerSimonian-Laird random-effects model. Black squares represent hazard ratios and bars represent 95% confidence intervals per 1-standard deviation increment of log MR-proANP. Abbreviations: BiomarCaRE, Biomarkers for Cardiovascular Risk Assessment in Europe; HR, hazard ratio; LCL, lower confidence limit; MR-proANP, mid-regional pro-atrial natriuretic peptide; Ncases, number of incident type 2 diabetes cases; UCL, upper confidence limit.



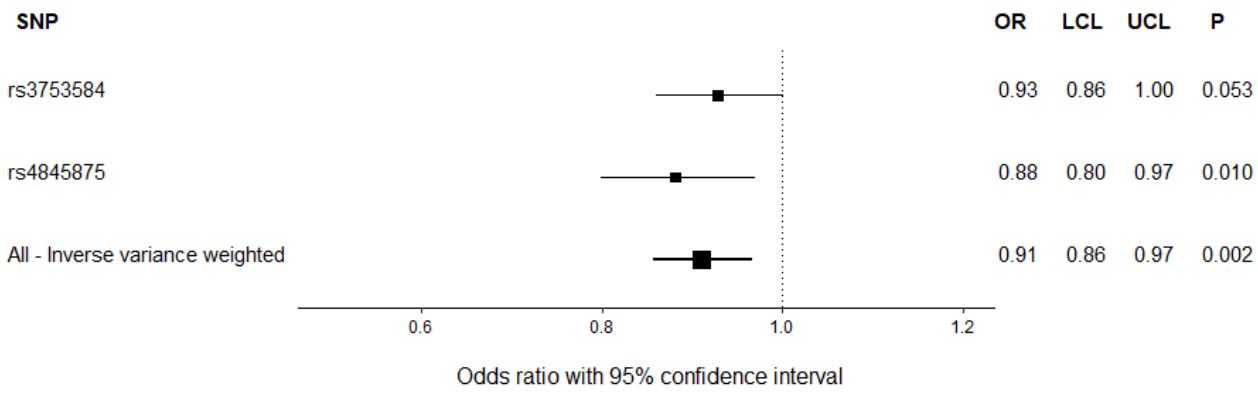
Supplementary Figure S6. Subgroup analysis of the association between NT-proBNP and incident type 2 diabetes in individuals with and without history of CVD. Hazard ratios were computed using Cox models. The Cox models used age (continuous, in years) as time scale and were stratified by study cohort. The models were adjusted for sex (men/women), body mass index (continuous, in kg/m^2), current smoking (yes/no), systolic blood pressure (continuous, in mmHg), use of antihypertensive medication (yes/no), total and high-density lipoprotein cholesterol (continuous, in mmol/l), and history of cardiovascular disease (yes/no). NT-proBNP was log-transformed and (0,1)-standardized in the total study population to approximate normality and to evaluate the hazard ratios per 1-standard deviation increase. Additive interaction was estimated by calculating RERI (relative excess risk due to interaction). The *P*-values for the additive interaction (RERI) were calculated based on the delta method by Hosmer and Lemeshow. *False discovery rate adjusted *P*-values < 0.05 using Benjamini-Hochberg method. Black squares represent hazard ratios and bars represent 95% confidence intervals per 1-standard deviation increment

of log natriuretic peptide. Abbreviations: BMI, body mass index; CVD, cardiovascular disease; Ncases, number of incident type 2 diabetes cases; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PY, person-year.



Supplementary Figure S7. Subgroup analysis of the association between MR-proANP and incident type 2 diabetes in individuals with and without history of CVD. Hazard ratios were computed using Cox models. The Cox models used age (continuous, in years) as time scale and were stratified by study cohort. The models were adjusted for sex (men/women), body mass index (continuous, in kg/m^2), current smoking (yes/no), systolic blood pressure (continuous, in mmHg), use of antihypertensive medication (yes/no), total and high-density lipoprotein cholesterol (continuous, in mmol/l), and history of cardiovascular disease (yes/no). MR-proANP was log-transformed and (0,1)-standardized in the total study population to approximate normality and to evaluate the hazard ratios per 1-standard deviation increase. Additive interaction was estimated by calculating RERI (relative excess risk due to interaction). The *P*-values for the additive interaction (RERI) were calculated based on the delta method by Hosmer and Lemeshow. *False discovery rate adjusted *P*-values < 0.05 using Benjamini-Hochberg method. Black squares represent hazard ratios and bars represent 95% confidence intervals per 1-standard deviation increment

of log natriuretic peptide. Abbreviations: BMI, body mass index; CVD, cardiovascular disease; Ncases, number of incident type 2 diabetes cases; MR-proANP, mid-regional pro-atrial natriuretic peptide; PY, person-years.



Supplementary Figure S8. Forest plot of the Mendelian randomization estimates of each SNP associated with MR-proANP for the risk of type 2 diabetes. Mendelian randomization estimates were computed using Wald ratio and are reported on the odds ratio scale. A fixed- effects model using inverse variance weighted meta-analysis was used to estimate the overall Wald ratio. Black squares represent odds ratios and bars represent 95% confidence intervals. Abbreviations: OR, odds ratio; P; *P*-values; LCL, lower confidence limit; UCL, upper confidence limit; SNP, single nucleotide polymorphism.

Supplementary Text S1. Laboratory measurements for other biomarkers used in the analyses

Baseline concentrations of NT-proBNP were measured from serum, except for a small percentage (< 7.5%) of the Northern Sweden Study that were measured from EDTA plasma. The data were measured centrally in the MORGAM/BiomarCaRE core laboratory in Mainz, Germany, for FINRISK and PRIME Belfast (in 2008) and in Hamburg, Germany, for the other participating cohorts (in 2014-2018) using electrochemiluminescence immunoassay (ECLIA, Roche Diagnostics GmbH, Mannheim, Germany) on either the ELECSYS 2010 or the Cobas e411 system. The analytical range of NT-proBNP for all cohorts was 5 to 35,000 pg/ml. Baseline concentrations of MR-proANP were only available for KORA-F4, FINRISK, and PRIME Belfast. The data were measured centrally in the MORGAM/BiomarCaRE core laboratory for FINRISK and PRIME Belfast (in 2008) and locally for KORA F4 (in 2016-2017). The baseline concentrations of MR-proANP for FINRISK, PRIME Belfast and KORA F4 were measured from EDTA plasma using an immunoluminometric assay (BRAHMS, Hennigsdorf, Berlin, Germany) on the automated system BRAHMS KRYPTOR. The analytical range of MR-proANP was 4.6 to 1,810 pmol/l for FINRISK, 0 to 1,000 pmol/l for PRIME Belfast and 2.1 to 1,000 for KORA F4.

Baseline concentrations of high-sensitivity C-reactive protein (hsCRP) were available in all participating cohorts and measured from serum, except for a small percentage (< 7.5%) of the Northern Sweden Monitoring of Trends and Determinants in Cardiovascular Diseases (MONICA) Study that were measured from EDTA plasma. The data were centrally measured in MONICA Risk Genetics Archiving and Monograph MORGAM / Biomarkers for Cardiovascular Risk Assessment in Europe (BiomarCaRE) core laboratory for all included cohorts, except for the Cooperative Health Research in the Augsburg Region Study Survey 3 and 4 (KORA S3-S4) and the re-examination study of KORA S4 in 2006-2008 (KORA F4). The measurements were carried out using latex immunoassay CRP16 (Turbidimetric/ Immunoturbidimetric, Architect c8000 Abbott, Wiesbaden, Germany) for the FINRISK Study and the Prospective Epidemiological Study of Myocardial Infarction (PRIME) Belfast and latex immunoassay (Abbott, Architect i2000) for the Northern Sweden and the Moli-sani Study. In both KORA S3-S4 and KORA F4, hsCRP was measured from

plasma using a latex-enhanced immunonephelometry (BN II, Siemens, Erlangen, Germany).

Baseline levels of leptin were only available for FINRISK, PRIME, Northern Sweden and KORA-F4. Leptin was measured from serum with an enzyme immunoassay technique using the Quantikine ELISA Kit (R&D Systems, Minneapolis, MN, USA) for FINRISK and PRIME and using the Mercodia ELISA (Mercodia AB, Uppsala, Sweden) for KORA F4. In Northern Sweden, leptin was measured from plasma with a double-antibody radioimmunoassay method (Millipore Corporation, Billerica, MA, USA).

Baseline levels of adiponectin were only available for FINRISK, PRIME and KORA-F4. Adiponectin was measured using the Quantikine ELISA Kit (R&D Systems) from plasma for FINRISK and PRIME and from serum for KORA F4. Estimated glomerular filtration rate (eGFR) in all participating cohorts was estimated using the CKD-EPI formula with creatinine (1).

Supplementary Text S2. Procedure for the univariate Mendelian randomization analysis

Selection of genetic variants

There are three main assumptions for a valid instrumental variable in Mendelian randomization (MR) analysis: the instrumental variable (IV) must be associated with the exposure (IV1 assumption), the IV should not influence the outcome through some pathways other than the exposure (IV2 assumption) and the IV should not be associated with confounders of the exposure-outcome association (IV3 assumption). The IV1 assumption is the only one of the three assumptions that can be directly tested (2).

In the current study the IV1 assumption was satisfied by including only single nucleotide polymorphisms (SNPs) that are associated either with N-terminal pro-B-type natriuretic peptide (NT-proBNP) or mid-regional pro-atrial natriuretic peptide (MR-proANP) at a *P*-value < 5E-8.

Violations of the IV2 assumption can occur in case of horizontal pleiotropy (that is the scenario where a genetic variant is associated with variables on different causal pathways to the outcome) or in case of linkage disequilibrium (LD) between the included SNPs (2, 3). We tried to meet the IV2 assumption by including only SNPs that are specific to either NT-proBNP or MR-proANP. We then performed the clumping procedure, which implies selecting only the SNPs that are not in LD with each other, using the r^2 cut-off of 0.1 to obtain independent SNPs.

Furthermore, violations of IV3 assumption can occur in case of population stratification in addition to horizontal pleiotropy and LD. The stratification of population can occur when the study sample includes subgroups with different genetic ancestries and thus different allele frequencies (2). We tried to overcome this issue by focusing our MR analysis on homogeneous ancestry groups.

Data sources

We identified SNPs with effects specific to either NT-proBNP or MR-proANP at a *P*-value < 5E-8 from a published genome-wide association (GWA) study of European

ancestry without both diabetes and cardiovascular diseases from Salo et al. (4). The study consists of 4,932 GWA study samples and 1,373 replication samples from the FINRISK 1997 study. We extracted at least information on the SNPs beta estimates, standard errors and effect alleles. To ensure that the selected SNPs are independent from each other we clumped the selected SNPs using the cutoff LD-r^2 of 0.1 to prune the final list of IVs.

After the clumping procedure we included one SNP located in the *natriuretic peptide precursor B* (*NPPB*) gene (rs198379) for NT-proBNP and two independent SNPs in the *natriuretic peptide precursor A* (*NPPA*) gene for MR-proANP (rs4845875 and rs3753584) as the IVs. The LD (for European ancestry haplotypes) of rs4845875 and rs3753584 is D´= 0.29 and r^2= 0.02 (4).

The estimates of the genetic associations between IVs and the risk of type 2 diabetes were obtained from a meta-analysis of GWA studies of European ancestry on type 2 diabetes by Xue et al (5) comprising 62,892 type 2 diabetes cases and 596,424 controls. We extracted at least the same information as with the exposure data.

Primary analysis of the Mendelian randomization

Before performing the analyses we made sure that the SNP effect estimates on the natriuretic peptides and on type 2 diabetes correspond to the same effect alleles. We did not identify any ambiguous palindromic SNPs (SNPs with A/T or G/C alleles).

We used the Wald ratio to compute the MR estimates (6). For an easier interpretation, we report the MR estimates with 95% confidence intervals on the odds ratio (OR) scale. We used fixed- instead of random-effects model using inverse variance weighted (IVW) meta-analysis to combine the Wald ratio estimates when more than one IV was included. *P*-values < 0.05 were considered statistically significant.

Sensitivity analyses

We used different methods to assess the robustness of our results in the sensitivity analyses. We computed the MR estimates using the likelihood-based method (7) that allows for correlation between the genetic association estimates with the exposure

and outcome, which is ignored in the IVW method and the weighted mode-based method that is more robust to outliers than IVW (3, 8). Due to a very few number of IVs, we were not able to perform other robust methods, including MR-Egger regression model (9).

To test the presence of heterogeneity as a measure of possible pleiotropic bias (3) we computed Cochran´s Q and I^2 statistics (10, 11). We used a forest plot to describe the Wald ratio estimates of each IV for MR-proANP with respect to the association with the risk of type 2 diabetes.

Our MR analyses were performed in the statistical software R version 4.0.3 (12) using “MendelianRandomization” R-package version 0.5.0 (13).

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Appendix

The BiomarCaRE Consortium website: http://www.biomarcare.eu/

Sites and key personnel of contributing BiomarCaRE centers:

BiomarCaRE center	Investigators
Kooperative Gesundheits-forschung in der Region Augsburg (KORA)	Barbara Thorand (Principal Investigator), Annette Peters (former Principal Investigator), Angela Döring (former Principal Investigator), Christa Meisinger, Margit Heier, Andrea Schneider.
FINRISK	Veikko Salomaa (Principal Investigator), Anne Juolevi, Erkki Vartiainen, Pekka Jousilahti, Tiina Laatikainen, Kennet Harald.
Prospective Epidemiological Study of Myocardial Infarction (PRIME) Belfast	Frank Kee (Principal Investigator), Alun Evans (former Principal Investigator), John Yarnell, Angela Scott, Evelyn Gardner (up to 2008).
Moli-sani Study	Licia Iacoviello (Principal Investigator), Simona Costanzo, Augusto Di Castelnuovo, Amalia De Curtis, Marialaura Bonaccio, Maria Benedetta Donati, Giovanni de Gaetano.
Northern Sweden MONICA	Stefan Söderberg (Principal investigator), Mats Eliasson (Principal Investigator), Per Ivarsson, Gunborg Rönnberg, Åsa Johansson, Karin Ruikka, Robert Lundqvist; Former key personnel: Per-Gunnar Wiklund (former Principal Investigator), Birgitta Stegmayr (former Principal Investigator), Salmir Nasic, Vivan Lundberg, Elsy Jägare-Westerberg, Torbjörn Messner.
BiomarCaRE Laboratory	Tanja Zeller (Head), Stefan Blankenberg, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.
MORGAM Data Centre	Kari Kuulasmaa (Head), Ari Haukijärvi, Teemu Niiranen, Tarja Palosaari, Jaakko Reinikainen, National Institute for Health and Welfare (THL), Helsinki, Finland
MORGAM/BiomarCaRE steering committee	Stefan Blankenberg, Alun Evans, Licia Iacoviello, Frank Kee, Wolfgang Koenig, Kari Kuulasmaa, Teemu Niiranen, Markus Perola, Veikko Salomaa, Renate Schnabel, Hugh Tunstall-Pedoe, Giovanni Veronesi, Tanja Zeller.