**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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**Abstract**

**Objective:** Natriuretic peptide (NP) concentrations are increased in cardiovascular diseases (CVD) but are associated with a lower diabetes risk. We investigated associations of N-terminal pro-B-type NP (NT-proBNP) and mid-regional pro-atrial NP (MR-proANP) with incident type 2 diabetes stratified by the presence of CVD.

**Research Design and Methods:** Based on the Biomarkers for Cardiovascular Risk Assessment in Europe-(BiomarCaRE) Consortium, we included 45,477 participants with NT-proBNP measurements (1,707 developed type 2 diabetes over 6.5 years of median follow-up; among these, 209 had CVD at baseline) and 11,537 participants with MR-proANP measurements (857 developed type 2 diabetes over 13.8 years of median follow-up; among these, 106 had CVD at baseline). The associations were estimated using multivariable Cox regression models.

**Results:** Both NPs were inversely associated with incident type 2 diabetes (hazard ratios [95%CI] per 1-standard deviation increase of log NP: 0.84 [0.79; 0.89] for NT-proBNP and 0.77 [0.71; 0.83] for MR-proANP). The inverse association between NT-proBNP and type 2 diabetes was significant in individuals without, but not in individuals with CVD (0.81 [0.76; 0.86] vs 1.04 [0.90; 1.19]; *P*-multiplicative interaction= 0.001). There was no significant difference in the association of MR-proANP with type 2 diabetes between individuals without and with CVD (0.75 [0.69; 0.82] vs 0.81 [0.66; 0.99]; *P*-multiplicative interaction= 0.236).

**Conclusions:** NT-proBNP and MR-proANP are inversely associated with incident type 2 diabetes. However, the inverse association of NT-proBNP seems to be modified by the presence of CVD. Further investigations are warranted to confirm our findings and to investigate the underlying mechanisms.

B-type natriuretic peptide (BNP) and atrial natriuretic peptide (ANP) are cardiac hormones that do not only exert physiological actions on cardiovascular homeostasis but also on glucose and lipid metabolism. Both natriuretic peptides (NPs) increase mitochondrial fat oxidative capacity in skeletal muscle and promote lipolysis, browning of white adipocytes, oxygen consumption, glucose uptake in adipose tissue and also modulate cytokine and adipokine responses (1, 2). Altogether, the aforementioned biological effects ameliorate insulin resistance and blood glucose control (3).

In previous epidemiological studies, higher concentrations of N-terminal pro-BNP (NT-proBNP) (4-9) and mid-regional pro-ANP (MR-proANP) (4, 10-12), the inactive fragments of BNP and ANP, respectively, were associated with a lower risk of type 2 diabetes. However, circulating concentrations of both NPs are elevated in cardiovascular diseases (CVDs) to compensate for cardiac pressure overload (2) and predict CVD prognosis (13). CVDs, such as heart failure (HF), myocardial infarction (MI) and stroke can give rise to abnormalities in glucose metabolism (14-16). For instance, HF invokes a compensatory neurohumoral response, which increases free fatty acids, thereby inhibiting muscular glucose uptake and insulin signaling (14). These effects, in turn, predispose to insulin resistance and type 2 diabetes. To date, it remains uncertain whether high NP concentrations are also associated with a lower risk of diabetes in individuals with CVD. Existing studies investigating the association of NPs with diabetes have only included non-diabetic individuals without prevalent CVD or did not report the association separately for individuals with and without prevalent CVD.

The present study aimed to investigate the prospective associations of NT-proBNP and MR-proANP with incident type 2 diabetes in several population-based studies from the multinational Biomarkers for Cardiovascular Risk Assessment in Europe (BiomarCaRE) Consortium (17). We specifically aimed to assess whether these associations differed by the presence of CVD. Additionally, to allow a more robust analysis, we applied two-sample Mendelian randomization (MR) approaches, using published data on genetic variants that are specific for either NT-proBNP or MR-proANP.

**RESEARCH DESIGN AND METHODS**

**Study design and population**

BiomarCaRE is based on the Monitoring of Trends and Determinants in Cardiovascular Diseases (MONICA) Risk Genetics Archiving and Monograph (MORGAM) Project (18), which has harmonized data from a large number of population-based cohorts. Our study complied with the Declaration of Helsinki. All participating cohorts were approved by local ethical review boards and written informed consent was obtained from all study participants.

To investigate the association between NT-proBNP and incident type 2 diabetes, we included 5 BiomarCaRE population-based cohorts comprising 45,477 initially non-diabetic participants with baseline measurements of NT-proBNP. The participating cohorts were the Cooperative Health Research in the Augsburg Region Study Survey 3 and 4 (KORA S3-S4), the 1997 survey of the FINRISK Study, the Prospective Epidemiological Study of Myocardial Infarction (PRIME) Belfast, the Moli-sani Study and the Northern Sweden MONICA Study. To investigate the association between MR-proANP and incident type 2 diabetes, we included 3 BiomarCaRE population-based cohorts involving 11,537 initially non-diabetic participants with baseline measurements of MR-proANP. The participating cohorts were the re-examination study of KORA S4 in 2006-2008 (KORA F4), FINRISK and PRIME Belfast. An overview of each participating cohort is provided in Supplementary Table S1. The exclusion criteria for analyzing NT-proBNP and MR-proANP are described in Supplementary Figure S1 and S2, respectively.

For each cohort, the following harmonized variables were available at baseline: age, sex, body mass index (BMI), systolic blood pressure, antihypertensive medication, smoking status, total and high-density lipoprotein (HDL) cholesterol, diabetes status and history of CVD. History of CVD was defined as having documented or self-reported history of either HF, MI, or stroke.

**Assessment of type 2 diabetes**

Prevalent diabetes was defined as a documented diagnosis of diabetes at baseline, either identified by record linkage or through self-report of the participants. In some cohorts, self-reported data were verified by medical chart review or through information obtained from the treating physician. Incident type 2 diabetes was defined as a new diagnosis of type 2 diabetes during follow-up, either identified by record linkage or through self-report of the participants without prevalent diabetes at baseline. Details on the assessment of type 2 diabetes and the general follow-up procedures in each cohort are provided in Supplementary Table S1.

**Laboratory measurements**

Baseline concentrations of NT-proBNP were measured using electrochemiluminescence immunoassay (ECLIA, Roche Diagnostics GmbH, Mannheim, Germany) on either the ELECSYS 2010 or the Cobas e411 system.Baseline concentrations of MR-proANP were measured using an immunoluminometric assay (BRAHMS, Hennigsdorf, Berlin, Germany) on the automated system BRAHMS KRYPTOR. The study-specific intra-assay and inter-assay coefficients of variation for each NP are described in Supplementary Table S2. Description of laboratory procedures in detail are provided in Supplementary Text S1.

**Statistical analysis**

Participant characteristics stratified by history of CVD were calculated separately for the study sample with baseline measurements of NT-proBNP and MR-proANP.

In our data, we distinguished between missing values, which were below the limit of detection (LOD), i.e. below the range to which the assay has been calibrated and missing values due to other reasons (e.g. samples were not available, sample volumes were inadequate, sample mix-up or a technical problem). Only missing values due to NP values below the LOD (N=2,725 for NT-proBNP and N=5 from the FINRISK study for MR-proANP) were imputed to the lower LOD (i.e. 5 pg/mL for NT-proBNP and 4.6 pmol/l for MR-proANP).

The associations of NT-proBNP and MR-proANP with incident type 2 diabetes were estimated by calculating hazard ratios (HRs) with 95% confidence intervals (95%CIs) in Cox proportional hazard (PH) models. Age (continuous, in years) was used as the time scale. The models were stratified by study cohort and were adjusted for sex (men/women) in model 1 and were further adjusted for BMI (continuous, in kg/m2), current smoking (yes/no), systolic blood pressure (continuous, in mmHg), use of antihypertensive medication (yes/no), total and HDL cholesterol (continuous, in mmol/l), and history of CVD (yes/no) in model 2. Additionally, we included history of CVD as a time-varying covariate in a sensitivity analysis of model 2. The distributions of NT-proBNP and MR-proANP were right-skewed (Supplementary Figure S3). Thus, both NPs were log-transformed and (0,1)-standardized in the overall study population to approximate normality and to evaluate the HRs per 1-SD increase. These associations were further investigated for non-linearity with restricted cubic spline regressions with three knots at 10th, 50th, and 90th percentile applied to model 2. The PH assumption was tested by plotting scaled Schoenfeld residuals against follow-up time for each covariate. No indication of non-proportionality was observed.

To examine possible differences in the association of NPs with incident type 2 diabetes by CVD status at baseline, we conducted separate analyses for individuals with and without CVD history. As some individuals may experience more than one CVD at baseline, which was seen in our data, history of CVD was defined as the composite history of HF, MI and stroke. We also tested for multiplicative and additive interactions between NPs and CVD history in model 2. The multiplicative interaction was examined by incorporating both factors and their cross-product term in the same model. We calculated the additive interaction by estimating the relative excess risk due to interaction (RERI). RERI was calculated by comparing the joint and separate regression coefficients of NPs and CVD history from the same model using the following formula: RERI= . To test the null hypothesis that RERI=0 we computed the 95%CIs and *P-*valuesusing the delta method (19). To further evaluate whether other diabetes-related biomarkers might account for the observed associations, we additionally included the baseline measurement of estimated glomerular filtration rate (eGFR), high-sensitivity C-reactive protein (hsCRP), leptin and adiponectin individually in model 2. We log-transformed hsCRP, leptin and adiponectin to approximate normality. The distribution of eGFR was approximately normal and it was therefore not log-transformed. We further examined the potential interactions of NPs with BMI and sex in individuals with and without CVD history separately. False discovery rate (FDR) with the Benjamini–Hochberg method was used to correct for multiple testing. An interaction was considered statistically significant at FDR < 0.05.

As a sensitivity analysis, we examined the associations of NT-proBNP and MR-proANP with incident type 2 diabetes in the same individuals by including only participants with data on both NPs. We also calculated the HRs for each participating cohort and used Cochran’s Q to evaluate heterogeneity between cohorts. To account for death without experiencing diabetes as a competing event, we conducted competing risk analyses using Fine and Gray models.

Finally, we performed two-sample univariate Mendelian randomization (MR) analyses using results from published genome-wide association (GWA) studies to examine the associations between genetically predicted NPs and type 2 diabetes risk. We identified SNPs with effects specific to either NT-proBNP or MR-proANP at a *P*-value < 5E-8 as the genetic instrumental variables (IVs) from a published GWA study of European ancestry from Salo et al. (20). The association estimates of the IVs with type 2 diabetes were extracted from a meta-analysis of GWA studies by Xue et al (21) because of the data availability for populations of European ancestry and the large sample size (62,892 cases and 596,424 controls). The procedure for the MR analysis is provided in detail in Supplementary Text S2.

All statistical analyses were performed using R version 4.0.3 (22). *P*-values less than 0.05 were considered statistically significant.

**RESULTS**

**Participant characteristics**

Characteristics of the study participants stratified by CVD history for the study samples with data on NT-proBNP and MR-proANP, respectively, are presented in Table 1. At baseline, in comparison with participants without CVD, participants with CVD had higher concentrations of NT-proBNP and MR-proANP, were on average older and more frequently male, had a higher BMI and systolic blood pressure, were more likely to take antihypertensive medication, had lower concentrations of HDL cholesterol, adiponectin and eGFR, and had higher concentrations of leptin and hsCRP. Throughout the follow-up period, participants with CVD were more likely to develop type 2 diabetes than participants without CVD. Characteristics of the study participants in the quarters of baseline NT-proBNP and MR-proANP concentrations are shown in Supplementary Table S3 and S4, respectively. Characteristics for each participating cohort are provided in Supplementary Table S5.

**Associations of NT-proBNP and MR-proANP with incident type 2 diabetes**

During a median follow-up of 6.5 years (interquartile range (IQR) 9.9 years), among the 45,477 participants with NT-proBNP data, 1,707 developed type 2 diabetes. Of these, 209 had a history of CVD at baseline. Among the 11,537 participants with MR-proANP data, 857 developed type 2 diabetes during a median follow-up of 13.8 years (IQR 5.0 years). Of these, 106 had a history of CVD at baseline.

Both NT-proBNP and MR-proANP were inversely associated with incident type 2 diabetes in model 1 in the overall study population. The HRs [95%CIs] were 0.89 [0.84; 0.94] per 1-SD increase of log NT-proBNP and 0.79 [0.73; 0.86] per 1-SD increase of log MR-proANP. The associations remained significant after additional adjustment according to model 2 (HRs [95%CIs]: 0.84 [0.79; 0.89] per 1-SD increase of log NT-proBNP and 0.77 [0.71; 0.83] per 1-SD increase of log MR-proANP). The results were similar when we included history of CVD as a time-varying covariate (HR [95%CIs]: 0.84 [0.79; 0.89] per 1-SD increase of log NT-proBNP and 0.77 [0.71; 0.83] per 1-SD increase of log MR-proANP). The associations of NT-proBNP and MR-proANP with incident type 2 diabetes were also examined in each cohort without significant heterogeneity (Supplementary Figure S4 and S5).

We observed a significant interaction between NT-proBNP and history of CVD on both multiplicative and additive scales with respect to incident type 2 diabetes (*P-*value for interaction on multiplicative scale= 0.001 and on additive scale= 0.015; Table 2). When stratified by CVD history, NT-proBNP was significantly inversely associated with incident type 2 diabetes in participants without, but not in participants with CVD history. The association between NT-proBNP and incident type 2 diabetes in participants without CVD was approximately linear (Figure 1A). The multivariable HRs [95%CIs] per 1-SD increase were 0.81 [0.76; 0.86] and 1.04 [0.90; 1.19] in participants without and with CVD history, respectively. These results were consistent after further adjustment for eGFR, hsCRP, leptin and adiponectin (Supplementary Table S6). In our subgroup analyses, we observed a significant interaction of NT-proBNP with BMI and sex in participants without CVD history, with a stronger association in women than in men and in obese than in non-obese participants (Supplementary Figure S6).

For MR-proANP, we did not observe a significant difference in the association with incident type 2 diabetes between individuals with and without CVD history (*P-*value for interaction on multiplicative scale= 0.236 and on additive scale= 0.441; Table 2). The multivariable HRs [95%CIs] per 1-SD increase were 0.81 [0.66; 0.99] and 0.75 [0.69; 0.82] in participants with and without CVD history, respectively. Inspection of restricted cubic splines indicated an inverse linear relationship between MR-proANP and incident type 2 diabetes in participants with and without CVD history (Figure 1B). Further individual adjustment for eGFR, hsCRP, leptin and adiponectin only marginally affected the association (Supplementary Table S6). No significant differences in the association between MR-proANP and incident type 2 diabetes were observed across BMI and sex categories (Supplementary Figure S7).

In a sensitivity analysis, including only participants with complete data on both NT-proBNP and MR-proANP (N=8,695), the results were consistent. We only observed a significant difference by CVD history in the association between NT-proBNP and incident type 2 diabetes (Supplementary Table S7). Our competing risk analyses yielded similar results (Supplementary Table S8).

**Two-sample MR analyses on the associations of genetically predicted NT-proBNP and MR-proANP with type 2 diabetes risk**

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 genetic IVs. The genetic associations with each NP and with type 2 diabetes were extracted from the previously mentioned GWA studies (20, 21) and are provided in Supplementary Table S9.

In line with the results from the survival analysis, our MR analyses showed that genetically predicted NT-proBNP and MR-proANP were inversely associated with type 2 diabetes risk. The odds ratios [95%CIs] were 0.93 [0.87; 0.98] for NT-proBNP and 0.91 [0.86; 0.97] for MR-proANP. Sensitivity analyses using the likelihood-based and the weighted mode-based methods yielded similar results (Table 3). We did not observe significant heterogeneity between the two IVs for MR-proANP with respect to the association with type 2 diabetes (Table 3 and Supplementary Figure S8).

**CONCLUSIONS**

Our results show that higher circulating concentrations of NT-proBNP and MR-proANP were significantly associated with a lower incidence of type 2 diabetes. We were able to show for the first time that the association between NT-proBNP and incident type 2 diabetes was modified by the presence of CVD, while there was no significant difference in the inverse association of MR-proANP with incident type 2 diabetes between individuals with and without CVD history. We only observed an inverse association of NT-proBNP with incident type 2 diabetes in individuals without, but not in individuals with CVD history. Further adjustment for eGFR, hsCRP, leptin and adiponectin did not substantially alter the results. In addition, our MR analyses yielded significant associations of genetically predicted NT-proBNP and MR-proANP with the risk of type 2 diabetes.

Our findings support the growing evidence associating high concentrations of NT-proBNP and MR-proANP with a lower risk of type 2 diabetes in individuals without CVD at baseline (5-12) and provide further information regarding these associations in individuals with CVD. Furthermore, our MR analyses corroborate findings from previous studies (5, 23) suggesting a potential causal relationship between higher concentrations of NT-proBNP and a lower risk of type 2 diabetes in individuals without prevalent diabetes and CVD and additionally provide the same evidence for MR-proANP.

The underlying mechanisms whereby higher concentrations of BNP and ANP are associated with a lower risk of type 2 diabetes are not fully understood. In adipose tissue, ANP stimulates lipolysis via cGMP-mediated phosphorylation thereby inhibits visceral adipocyte hypertrophy (6), while BNP induces browning of white adipose tissue. ANP also inhibits leptin release and both BNP and ANP reduce secretion of pro-inflammatory cytokines from adipose tissue and enhance adiponectin secretion via the activation of NP receptor A (NPR-A), which suppresses low-grade inflammation of the adipose tissue (24). Thus, BNP and ANP may counteract insulin resistance. However, in our study, the inverse association of NT-proBNP and MR-proANP concentrations with incident type 2 diabetes remained stable after further adjustment for hsCRP, leptin, and adiponectin.

Another potential mechanism for a lower risk of type 2 diabetes is via the direct effects of BNP and ANP on the oxidative metabolism of skeletal muscles (1). NPR-A is upregulated in the muscle of exercise-trained individuals, suggesting that some of the metabolic adaptations of skeletal muscle in response to chronic exercise may be mediated by NPs (1, 24). In healthy individuals, increased NT-proBNP concentrations are also associated with prolonged physical activity (25). In contrast, in obesity, the concentrations of NPs are reduced (26), possibly due to the deleterious effects of cardiac ectopic fat and the upregulation of the NP receptor C (NPR-C) in adipose tissue that increases NPs clearance (24). Moreover, evidence from previous epidemiological studies suggests that NPs are inversely associated with insulin resistance and obesity (26, 27). Intriguingly, we observed a stronger association of NT-proBNP and incident type 2 diabetes in obese than in non-obese participants without CVD history. Of note, a recent study indicates that the inverse association between NT-proBNP and obesity could be modified by sex, with a more pronounced association in women than in men (28), which could be explained by the sex differences in body composition and fat distribution. This observation is in line with our findings and a previous study (9) reporting a stronger inverse association between NT-proBNP and incident type 2 diabetes in women than in men without CVD history, which could possibly be further explained by sex hormones, especially testosterone. Testosterone suppresses NT-proBNP production (29) and in turn may partially account for higher circulating NT-proBNP concentrations in women than in men (24). Interestingly, in men testosterone was inversely associated with type 2 diabetes, while in women this association was positive in cross-sectional settings (30). This evidence is similar in prospective studies, however, the positive association in women was no longer significant after controlling for diabetes risk factors (30, 31). The apparent sex-specific associations between testosterone and type 2 diabetes may be driven by the extreme spectrum of testosterone concentrations; for instance, abnormally high testosterone concentrations (hyperandrogenism) in women and abnormally low testosterone concentrations (hypogonadism) in men are associated with a higher risk of type 2 diabetes (32). However, in the previous study reporting sex-specific associations between NT-proBNP and incident type 2 diabetes (9), the sex differences were still observed after adjustment for testosterone and other sex hormones, suggesting other possible explanations.

Furthermore, previous studies have shown that elevated blood pressure is associated with increased NP concentrations, which reflects a compensatory response to restrain blood pressure (2, 26). Cardiovascular and metabolic regulations are tightly linked, therefore lowering blood pressure may lower type 2 diabetes risk (2, 33). However, some classes of antihypertensive medication may exhibit differential effects on type 2 diabetes risk. Thiazides and β-blockers tend to increase diabetes risk, while neprilysin blockers, angiotensin receptor blockers, angiotensin converting enzyme inhibitors and calcium channel blockers decrease the risk (34, 35). Due to lack of data on specific antihypertensive medications, we were unable to examine the differential effects of antihypertensive medication classes on the association between NPs and type 2 diabetes.

Differences between BNP and ANP in their cardio-metabolic effects have not been widely studied. Within the heart, ANP is considered to be mainly secreted from the atria, while BNP is mainly from the ventricles (36). Although both NPs are known to lower blood pressure, data from animal models (37) and a recent GWA study (20) implicate ANP rather than BNP as a strong blood pressure–lowering hormone. However, in individuals with left ventricular dysfunction, BNP concentrations are markedly increased as compared with ANP, suggesting BNP rather than ANP as an emergency defense against ventricular overload (36). Elevated BNP concentrations are also strongly correlated with the severity of CVD (38), which is associated with a higher risk of type 2 diabetes (14, 39, 40). This could have attenuated the inverse association of NT-proBNP with incident type 2 diabetes in individuals with CVD and thus, could explain the difference in the association between individuals with and without CVD seen in our study. Unfortunately, due to lack of relevant data, we were unable to further examine whether the observed associations could be influenced by the severity of CVD. Furthermore, as NT-proBNP concentrations are higher in individuals with CVD compared to individuals without, one could speculate that there is a plateau effect and that the inverse association between NT-proBNP and incident type 2 diabetes is only seen in the lower concentration range. However, our results did not support this hypothesis. An inverse association between NT-proBNP and incident type 2 diabetes in individuals without CVD was also observed within the concentration range seen in individuals with CVD, although with some uncertainty due to the wide 95%CIs at the upper end of NT-proBNP concentrations (Figure 1A). Of note, a non-significant association is not evidence for no association. Indeed, based on the 95%CI from the present analysis, we cannot rule out a small effect of NT-proBNP on type 2 diabetes risk in individuals with CVD. Furthermore, our interaction analyses for MR-proANP may have been underpowered to detect differences between individuals with and without CVD history. More studies with larger study populations, particularly with a large number of incident type 2 diabetes cases in individuals with CVD history are needed to confirm our findings.

The strengths of the current study include the prospective, population-based design, the large sample size, and the thorough adjustment for different cardio-metabolic risk factors. Since 1998, we have harmonized data from population-based cohort studies in the MORGAM Data Centre in Helsinki, providing the best possible exposure and covariate allignment as well as endpoint validation. Standardized epidemiological and laboratory procedures based on individual level data also allow for the best possible data analyses.

Our study has some limitations that merit consideration. Although the assessment of type 2 diabetes incidence was systematic and detailed, it was mainly based on medical reviews and for a small number of participants on self-report, which may have led to misclassification of incident cases. However, since we expect people under regular review by their physician for CVD to have more opportunities for the detection of diabetes, any bias introduced by these means of ascertainment could not explain the lower risk of diabetes seen in persons with elevated NP concentrations. History of CVD was based on medical review or self-report, therefore it is possible that some of the individuals classified as having no CVD could have had underlying undiagnosed CVD. We had only a single measurement of NT-proBNP and MR-proANP at baseline and, therefore, intra-individual variation could not be taken into account. This could have led to misclassification of participants and biased the estimates towards the null. Harmonized data on other known cardio-metabolic risk factors, such as physical activity and diet were lacking in the present study, which could have led to some degree of residual confounding. Furthermore, the exclusion of eligible individuals due to missing values of the NPs or of cardio-metabolic risk factors (10.1% in the sub-sample for NT-proBNP and 10.7% in the sub-sample for MR-proANP) decreased the statistical power of the analyses and could have led to biased association estimates if the data were not missing completely at random. Finally, due to a limited number of genetic IVs, we were unable to perform more robust sensitivity analyses for our MR.

In conclusion, our findings suggest that NT-proBNP and MR-proANP are inversely associated with incident type 2 diabetes. However, the inverse association of NT-proBNP seems to be modified by the presence of CVD. Future studies are needed to examine the underlying mechanisms, the differences in the metabolic actions between both NPs and their potential as targets for therapeutic interventions of type 2 diabetes.

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# **Duality of Interest**

VS has received honoraria for consulting from Novo Nordisk and Sanofi. He also has ongoing research collaboration with Bayer AG (All unrelated to the present study). JJ served as advisor for Bayer and Novo-Nordisk and is co-founder of Eternygen GmbH. SS reports speaker’s honoraria from Actelion Ltd and participation in advisory boards for Actelion LTD and MSD. Other authors have nothing to disclose related to the content of this manuscript.

# **Author Contributions**

CS and BT conceptualized the current study design. CS drafted the manuscript and conducted the statistical analyses. UM and JR provided statistical analysis advice. VS, FK, LI, SS, JJ, PJ, DW, WK, KK, JR, SB, TZ, CH, AP and BT collected and researched data. CS, BT, UM, CH, FK and AP contributed to data interpretation. All authors contributed to and critically reviewed the manuscript and approved the final manuscript. CS is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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**Table 1. Participant characteristics in the total study population and stratified by history of CVD**

|  |  |  |
| --- | --- | --- |
|  | **Study population with NT-proBNP measurement** | **Study population with MR-proANP measurement** |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Overall** | **With CVD \*** | **Without CVD** | **Overall** | **With CVD †** | **Without CVD** |
| N | 45,477 | 1,995 | 43,482 | 11,537 | 783 | 10,754 |
| Incident type 2 diabetes (%) | 1,707 (3.8) | 209 (10.5) | 1,498 (3.4) | 857 (7.4) | 106 (13.5) | 751 (7.0) |
| NT-proBNP in pg/ml (antilog SD) | 41.3 (3.0) | 122.7 (3.6) | 39.3 (2.9) | 39.3 (2.9) ‡ | 107.8 (3.4) ‡ | 36.2 (2.8) ‡ |
| MR-proANP in pmol/l (antilog SD) | 47.9 (1.6) ‡ | 76.7 (1.8) ‡ | 46.1 (1.6) ‡ | 49.9 (1.6) | 82.3 (1.8) | 47.9 (1.6) |
| Study cohort (%) |  |  |  |  |  |  |
| KORA S3-S4 | 5,130 (11.3) | 328 (16.4) | 4,802 (11.0) | NA | NA | NA |
| KORA F4 | NA | NA | NA | 2,265 (19.6) | 128 (16.3) | 2,137 (19.9) |
| FINRISK | 7,240 (15.9) | 518 (26.0) | 6,722 (15.5) | 7,301 (63.3) | 532 (67.9) | 6,769 (62.9) |
| PRIME Belfast | 2,332 (5.1) | 147 (7.4) | 2,185 (5.0) | 1,971 (17.1) | 123 (15.7) | 1,848 (17.2) |
| Moli-sani | 21,357 (47.0) | 589 (29.5) | 20,768 (47.8) | NA | NA | NA |
| Northern Sweden | 9,418 (20.7) | 413 (20.7) | 9,005 (20.7) | NA | NA | NA |
| Age in years (SD) | 51.5 (12.7) | 62.7 (10.1) | 51.0 (12.6) | 49.9 (12.0) | 60.9 (9.7) | 49.1 (11.8) |
| Male (%) | 23,045 (50.7) | 1,386 (69.5) | 21,659 (49.8) | 6,677 (57.9) | 552 (70.5) | 6,125 (57.0) |
| Body mass index in kg/m2 (SD) | 27.2 (4.6) | 28.7 (4.6) | 27.2 (4.6) | 26.6 (4.3) | 28.4 (4.6) | 26.5 (4.3) |
| Current smoking (%) | 11,333 (24.9) | 367 (18.4) | 10,966 (25.2) | 2,998 (26.0) | 166 (21.2) | 2,832 (26.3) |
| Systolic blood pressure in mmHg (SD) | 135.5 (20.6) | 142.2 (22.4) | 135.2 (20.5) | 132.5 (20.3) | 140.3 (22.4) | 132.0 (20.0) |
| Use of antihypertensive medication (%) | 8,424 (18.5) | 964 (48.3) | 7,460 (17.2) | 1,533 (13.3) | 331 (42.3) | 1,202 (11.2) |
| Total cholesterol in mmol/l (SD) | 5.67 (1.13) | 5.59 (1.20) | 5.67 (1.13) | 5.58 (1.05) | 5.61 (1.01) | 5.58 (1.05) |
| HDL cholesterol in mmol/l (SD) | 1.45 (0.40) | 1.31 (0.38) | 1.45 (0.40) | 1.38 (0.37) | 1.26 (0.37) | 1.39 (0.37) |
| eGFR in ml/min/1.73m2 (SD) § | 94.5 (17.3) | 82.8 (18.7) | 95.1 (17.0) | 88.6 (19.3) | 78.1 (18.9) | 89.4 (19.1) |
| hsCRP in mg/l (antilog SD) || | 1.35 (3.03) | 1.99 (3.00) | 1.34 (3.03) | 1.26 (3.03) | 1.97 (3.00) | 1.22 (3.00) |
| Leptin in ng/ml (antilog SD) ¶ | 7.77 (2.36) | 9.30 (2.36) | 7.69 (2.36) | 8.67 (2.56) | 10.59 (2.59) | 8.58 (2.53) |
| Adiponectin in μg/ml (antilog SD) # | 5.42 (1.88) | 4.90 (1.95) | 5.47 (1.88) | 5.64 (1.90) | 5.31 (1.99) | 5.64 (1.90) |

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).

\* Among 1,995 participants with CVD history and NT-proBNP measurement, 570 had HF (120 with MI, 28 with stroke, 16 with MI and stroke), 1,114 had MI (120 with HF, 65 with stroke, and 16 with HF and stroke) and 556 with stroke (28 with HF, 65 with MI and 16 with HF and MI).

† Among 783 participants with CVD history and MR-proANP measurement, 315 had HF (88 with MI, 34 with stroke, 10 with MI and stroke), 411 had MI (88 with HF, 19 with stroke and 10 with HF and stroke) and 218 with stroke (34 with HF, 19 with MI and 10 with HF and MI).

‡ Data were available and calculated in 8,695 (612 with and 8,083 without a history of CVD).

§ Data were available and calculated in 44,219 (1,922 with and 42,297 without a history of CVD) participants with NT-proBNP measurement and 11,341 (764 with and 10,577 without a history of CVD) participants with MR-proANP measurement.

|| Data were available and calculated in 45,032 (1,981 with and 43,051 without a history of CVD) participants with NT-proBNP measurement and 11,073 (758 with and 10,315 without a history of CVD) participants with MR-proANP measurement.

¶ Data were available and calculated in 17,927 (1,038 with and 16,889 without a history of CVD) participants with NT-proBNP measurement and 10,311 (734 with and 9,577 without a history of CVD) participants with MR-proANP measurement.

# Data were available and calculated in 8,669 (611 with and 8,058 without a history of CVD) participants with NT-proBNP measurement and 9,673 (738 with and 8,935 without a history of CVD) participants with MR-proANP measurement.

Abbreviations: CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HF, heart failure; hsCRP, high-sensitivity C-reactive protein; KORA, the Cooperative Health Research in the Augsburg Region Study; MI, myocardial infarction; MR-proANP, mid-regional pro-atrial natriuretic peptide; NA, data not available; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PRIME, the Prospective Epidemiological Study of Myocardial Infarction; SD, standard deviation.

**Table 2.** **Association between natriuretic peptides and incident type 2 diabetes**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **No. of cases / person-years** | **HR [95%CI] per 1-SD increase** | **Multiplicative interaction** | **Additive interaction \*** |
| **NT-proBNP** |  |  |  |  |
| Overall | 1,707 / 429,620 | 0.84 [0.79; 0.89], *P* < 0.001 |  |  |
| History of CVD |  |  | 1.25 [1.09; 1.43]; *P* = 0.001 † | 0.20 [0.04; 0.37], *P =* 0.015 † |
| Yes | 209 / 18,249 | 1.04 [0.90; 1.19], *P* = 0.608 |  |  |
| No | 1,498 / 411,372 | 0.81 [0.76; 0.86], *P* < 0.001 |  |  |
| **MR-proANP** |  |  |  |  |
| Overall | 857 / 141,206 | 0.77 [0.71; 0.83], *P* < 0.001 |  |  |
| History of CVD |  |  | 1.12 [0.93; 1.36], *P* = 0.236 | 0.08 [-0.12; 0.27], *P* = 0.441 |
| Yes | 106 / 8,441 | 0.81 [0.66; 0.99], *P* = 0.042 |  |  |
| No | 751 / 132,765 | 0.75 [0.69; 0.82], *P* < 0.001 |  |  |

The Cox models used age (continuous, in years) as time scale and were stratified by study cohort 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), 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. \*Interaction on additive scale was estimated with RERI [95%CI]. †False discovery rate adjusted *P*-values < 0.05 using Benjamini-Hochberg method. 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.

**Table 3.** **Mendelian randomization results of the association between genetically predicted natriuretic peptides and the risk of type 2 diabetes**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Phenotype** | **No. of IV** | **Methods** | **Mendelian randomization estimates on odds ratio scale [95%CI]** | **Cochran’s Q** | ***P* for Cochran’s Q** | **I2** |
| NT-proBNP | 1 (rs198379) | Wald ratio | 0.93 [0.87; 0.98]; *P* = 0.012 | -- | -- | -- |
|  |  | Maximum likelihood | 0.93 [0.87; 0.98]; *P* = 0.013 | -- | -- | -- |
| MR-proANP | 2 (rs4845875 & | IVW fixed-effect model | 0.91 [0.86; 0.97]; *P* = 0.002 | 0.701 | 0.402 | 0% |
|  | rs3753584) | Maximum likelihood | 0.91 [0.85; 0.97]; *P* = 0.003 | 0.649 | 0.421 | -- |
|  |  | Weighted mode | 0.92 [0.86; 0.98]; *P* = 0.016 | -- | -- | -- |

Cochran’s Q and I2 statistics to test for heterogeneity were calculated when more than one IV was included in the analysis. Abbreviations: CI, confidence interval; IV, instrumental variable; IVW, inverse-variance weighted; MR-proANP, mid-regional pro-atrial natriuretic peptide; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

**Legends for Figure**

**Figure 1.** **Shape of the association between natriuretic peptides and incident type 2 diabetes in the total study population and stratified by history of CVD.** The linearity was assessed with restricted cubic splines with three knots at 10th, 50th, and 90th percentile. Data and the smoothed splines are fitted using Cox models. The models used age as time scale and were stratified by study cohort . The models 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) 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 hazard ratios per 1-standard deviation increase. Shaded areas around the curves depict 95%CI. Kernel density plots are imposed along the *x* axis, with vertical dotted lines depicting (from the left) the 25th, 50th, and 75th percentiles of the population. Abbreviations: CI, confidence interval; CVD, cardiovascular disease; MR-proANP, mid-regional pro-atrial natriuretic peptides; NT-proBNP, N-terminal pro-B-type natriuretic peptide.