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The low-expression variant of *FABP4* is associated with cardiovascular disease in type 1 diabetes

FABP4 rs77878271 and CVD in T1D

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ABSTRACT

Fatty-acid binding protein 4 (FABP4) is implicated in the pathogenesis of cardiometabolic disorders. Pharmacological inhibition or genetic deletion of FABP4 improves cardiometabolic health and protects against atherosclerosis in preclinical models. As cardiovascular disease (CVD) is common in type 1 diabetes, we examined the role of FABP4 for the development of complications in type 1 diabetes, focusing on a functional, low-expression, variant (rs77878271) in the promoter of the *FABP4* gene. For this, we assessed the risk of CVD, stroke, coronary artery disease (CAD), end-stage kidney disease (ESKD), and mortality using Cox proportional-hazard models for the *FABP4* rs77878271 in 5,077 Finnish individuals with type 1 diabetes. The low-expression G-allele of rs77878271 increased the risk of CVD, independently of confounders. Findings were tested for replication in 852 Danish and 3,678 Finnish individuals with type 1 diabetes. In the meta-analysis, each G-allele increased the risk of stroke by 26% (p=0.04), CAD by 26% (p=0.006), and CVD by 17% (p=0.003). In Mendelian Randomization, a decrease in FABP4 increased CAD 2.4-fold. Hence**,** in contrast to the general population, the low-expression G-allele of rs77878271 increased CVD risk in type 1 diabetes, suggesting that genetically low FABP4 levels may be detrimental in the context of type 1 diabetes.

INTRODUCTION

Cardiovascular disease (CVD) impacts health and lifespan of individuals with type 1 diabetes, who are affected by CVD more frequently and develop more severe CVD at younger age (1). Individuals with type 1 diabetes carry many risk factors for CVD (2). Diabetes itself is a major risk factor, but in combination with kidney complications, this risk is increased tenfold (3,4). Furthermore, the recently observed increase in obesity in type 1 diabetes (5,6) might place even more individuals at risk of CVD. The mechanism how obesity results in increased morbidity and mortality remains unclear. However, obesity is associated with abnormal fatty acid metabolism and secretion of multiple adipokines, which have the potency to increase morbidity and mortality (7,8). One such adipokine is fatty acid-binding protein 4 (FABP4, adipocyte FABP or aP2), a fatty acid carrier protein mainly expressed in adipocytes and macrophages but also in venous and capillary endothelial cells (9), where it is required for fatty acid transport from the circulation to fatty-acid-consuming tissues such as the heart (10). In humans, higher circulating FABP4 levels associates with insulin resistance and the metabolic syndrome (11) and predict the development of CVD (12) as well as future cardiovascular morbidity and mortality in those with already manifested CVD (13). Furthermore, the *FABP4* gene was recently recognized as a shared risk factor in a joint genome-wide association study (GWAS) on coronary heart disease and type 2 diabetes in ~500,000 individuals (14).

Studies in preclinical models have demonstrated that inhibition of the FABP4 protein through genetic deletion (15,16) or pharmacological inhibition (17,18) protects against the harmful effects of obesity, insulin resistance and atherosclerosis. In humans, a functional single-nucleotide polymorphism (SNP), rs77878271 (A>G) in the promoter region of the *FABP4* gene, has been described to result in a clinical presentation that is strikingly similar to the phenotype of *FABP4*-deficient mice (19). The minor G-allele of this SNP impacts the *FABP4* promoter, resulting in reduced *FABP4* transcription in the adipose tissue and reduced circulating triglyceride concentrations combined with lower CVD risk and protection from obesity-induced type 2 diabetes (19,20). It also reduces *FABP4* transcription

in carotid plaques (20) and epicardial fat tissue 2-4-fold (21). In addition to its effects on transcription, *FABP4* rs77878271 was identified as the only cis-acting variant affecting circulating FABP4 protein concentrations in a large-scale GWAS of the plasma proteome with 21,758 participants (22).

While individuals with type 1 diabetes have high risk of CVD at an early age, there is increasing evidence that the pathophysiology is partially different from the general population (2). Furthermore, the role of FABP4 – a novel drug target for CVD – for the development of complications in type 1 diabetes has not yet been evaluated. Therefore, we aimed to examine the role of FABP4 in relation to the development of diabetic complications and mortality in type 1 diabetes, focusing on the low-expression variant of *FABP4* rs77878271. To this end, we genotyped rs77878271 and assessed the impact of this SNP on the risk of CVD, end-stage kidney disease (ESKD), and mortality in a cohort of nearly 6,000 individuals with type 1 diabetes.

RESEARCH DESIGN AND METHODS

FinnDiane study participants

The Finnish Diabetic Nephropathy (FinnDiane) study is an ongoing multicenter study aiming at identifying risk factors for late complications in type 1 diabetes. The diagnosis of type 1 diabetes was made by the attending physician at the time of onset according to national evidence-based clinical practice guidelines. Participants took part in the study by visiting their attending physician. During that visit, blood samples were drawn, urine samples collected, anthropometric data gathered by a trained nurse, and questionnaires regarding health, medications, physical activity, and eating habits were completed as previously described (23). In brief, serum lipids or lipoproteins were measured centrally as previously described (24) or locally at each center using accredited methods. Blood pressure was measured twice in the sitting position, and the average of these measurements was used in the analyses. BMI was calculated as weight (kg) divided by height (m) squared, and overweight was defined as BMI = 25.0-29.9 kg/m^2 and obesity as BMI $\geq$ 30.0 kg/m^2. Central obesity was defined as waist-to-hip ratio >0.85 for women and >0.90 for men. Staging of diabetic nephropathy was based on urinary albumin excretion rate (AER) or albumin-to-creatinine ratio (ACR) in two out of three timed overnight or 24h urine collections or in morning spot urine samples for ACR (**Supplemental Table 1**). ESKD was defined as ongoing dialysis or kidney transplant. “Any diabetic nephropathy” included microalbuminuria, macroalbuminuria, and ESKD, while “advanced diabetic nephropathy” macroalbuminuria and ESKD. The estimated glomerular filtration rate (eGFR) was calculated with the CKD-EPI formula (25). Furthermore, DNA samples for additional individuals with type 1 diabetes have been obtained in collaboration with the Finnish National Institute for Health and Welfare. For these, information was retrieved by carefully reviewing medical records from outpatient visits at the time of inclusion.

Study Design

Selection criteria. For this prospective study, all FinnDiane study participants with an available DNA sample (n=5,797) were genotyped for the *FABP4* rs77878271 polymorphism using TaqMan SNP Genotyping Assay (Applied Biosystems, Carlsbad, CA, USA). After removal of duplicate samples (n=66), 5,731 individuals remained. We excluded individuals with undetermined genotype (n=306, 5.6%), age below 16 years at baseline (n=105, 1.8%), diabetes onset age above 40 years or if there were any indications of another diabetes type including insulin treatment not initiated within one year (n=243, 4.2%). Thus, 5,077 individuals remained for the analyses.

Outcomes. We retrieved information on cardiovascular and cerebrovascular events from the Finnish Care Register for Health Care on December 31, 2014. We defined a CAD event as an International Classification of Diseases (ICD) code for myocardial infarction (ICD-10: I21-I23, ICD-9: 410-412) or surgical procedure code for coronary artery bypass surgery or balloon angioplasty. We defined stroke as an ICD code for ischemic or hemorrhagic stroke (ICD-10: I60–I64, ICD-9: 430-434). CVD was defined as incident CAD and/or stroke. Participants were censored at the study's data retrieval date (December 31, 2014) or death. Information on deaths was obtained from Statistics Finland until December 31, 2014 by complete linkage (100% coverage) using personal identification numbers (26). Mortality was defined as any cause of death excluding external causes of death such as injury, poisoning, violence, accidents, and self-harm (ICD-10 codes: S00–T98, V01–Y98).

Replication cohorts

Steno Diabetes Center Copenhagen (SDCC). We sought replication in 852 adult individuals with type 1 diabetes from the outpatient clinic at the SDCC in Denmark (27). Baseline examination took place 1993-2001 as the original cohort was recruited to study late diabetic complications (28). Follow-up data on endpoints were retrieved from Danish National Health Registry on December 31, 2016. Causes of deaths were obtained from the Danish National Death Registry (available until December

31, 2015), after tracing the participating individuals recruited at baseline. CAD was defined as nonfatal and fatal myocardial infarction (ICD-10 codes I21–I24) or a procedural code for coronary intervention (percutaneous arterial intervention or coronary bypass grafting; www.sst.dk). Stroke was defined as a nonfatal or fatal stroke or other manifestations of major cerebrovascular occlusive disease (ICD-10 codes I61–I66). In the present study, CVD was defined as a composite of the SDCC "CAD" and "stroke", instead of the original definition used (27). ESKD was defined as CKD stage 5 (ICD-10 code N18.5), eGFR <15 mL/min/1.73 m^2, a procedural code for initiation of permanent dialysis or kidney transplantation, or death because of kidney failure from CKD. Genotyping was performed with Illumina HumanCoreExome-12v1 Beadchip and imputation using 1000 Genomes (phase 3v5) reference panel as previously described (29). SNP rs77878271 was imputed (r^2=0.84) and dosages were converted to the most likely genotypes using 90% threshold for the genotype posterior probability.

FinnGen. Further replication was attempted in 3,678 Finnish individuals with type 1 diabetes from FinnGen (Finngen Data Freeze 7), a large nation-wide study of 309,312 individuals combining genomes and registry data. Type 1 diabetes was defined with ICD-10 code E10.[0-9], ICD-9 250[0-8]B as a hospital discharge diagnosis or cause of death, or ICD-10 E10 for eligibility of reimbursed medications. Details on data generation and genotype imputation have been previously described (30). The SNP rs77878271 was imputed (INFO=0.98, batch range: 0.97-1.00). All models were adjusted for four principal components of ancestry. Information on cardiovascular and cerebrovascular events by December 31, 2019 were retrieved from the hospital discharge registry, causes of death registry, and medication reimbursement registry, using the same ICD-codes as in FinnDiane.

Mendelian Randomization

To investigate causal effects of FABP4 on CVD, we applied two-sample Mendelian randomization (MR), which uses SNPs as instrumental variables (IV) for the exposure of interest (FABP4), and

summary data from different populations for the IV-exposure association and the IV-outcome association. We considered only the *FABP4* rs77878271 as IV since it is the only cis-acting variant with strong and independent effect on circulating FABP4 (22). SNP-exposure association was extracted from summary data from the SCALLOP consortium (22) including n=19,372 individuals of European ancestry. For SNP-outcomes associations, we used GWAS-studies on CAD in type 1 diabetes with summary data available in the GWAS catalogue. For CAD, two studies with totally n=8,426 individuals (31,32), including n=4,850 from FinnDiane (31), was available. No GWAS on stroke or the broader definition CVD in type 1 diabetes was available. Since we had one IV, we used the Wald Ratio implemented in the "TwoSampleMR" R-package. Associations from different studies were meta-analyzed prior performing MR.

Ethics approval and consent to participate

The Ethical Committee of Helsinki and Uusimaa Health District approved FinnDiane and FinnGen study protocols, and the local ethics committee approved the SDCC study protocol. FinnDiane and SDCC participants gave written informed consent before participation, and FinnGen participants provided informed consent for biobank research, based on the Finnish Biobank Act. Study-specific consents collected before the Finnish Biobank Act came into effect (September 2013) and start of FinnGen (August 2017) were transferred to the Finnish biobanks after approval by Fimea (Finnish Medicines Agency), National Supervisory Authority for Welfare and Health. Recruitment protocols followed the biobank protocols approved by Fimea. All studies were performed according to the Declaration of Helsinki.

Statistical analyses

We evaluated differences in continuous variables between genotypes of rs77878271 using ANOVA (normally distributed variables) or the Kruskal-Wallis test (non-normally distributed variables), and differences in categorical values using the Fisher's Exact test. We calculated the p-value for HWE

deviations using the Exact's tests of HWE (33). We evaluated *FABP4* rs77878271 associations with endpoints using Cox proportional-hazards models. To estimate "lifetime" effect, we used age as underlying timescale, which we calculated as years from birthdate to event date or death date/data registry retrieval date (**Supplemental Table 2**). To be able to adjust for confounders, we used follow-up years from the baseline examination as timescale, which was calculated as years from the baseline examination date until event date or death date/data registry retrieval date. In parallel with the Cox regression analyses, we visualized the results by estimating Kaplan-Meier curves using age as timescale and grouped by *FABP4* rs77878271 genotypes (AA/AG/GG). We used an additive genetic model for rs77878271 (AA=0, AG=1, GG=2, encoded as a continuous variable), unless stated otherwise. We used R software (version 4.0.2) for all analyses. We evaluated time-dependent effects using the survSplit() function ("survival" R package) to divide the time variable (age) into time groups (tgroup). We meta-analyzed individual results using an inverse variance fixed-effect model in the R package 'meta' and performed post-hoc power calculations using information in **Supplemental Table 2** and R package "survSNP". P-values below 0.05 were considered significant.

RESULTS

FABP4 rs77878271 and baseline clinical characteristics (FinnDiane)

Minor allele frequency (MAF) of *FABP4* rs77878271 was 6.0% in FinnDiane. At baseline, there was no difference in sex, age, diabetes duration, diabetes onset age, cardiovascular risk factors, laboratory measures, and microvascular complications between carriers of the *FABP4* rs77878271 genotypes AA, AG and GG (**Table 1**). There was, however, a difference in the use of angiotensin II receptor blockers (ARBs, p=0.0001) and lipid-lowering medication (p=0.004) among *FABP4* rs77878271 genotypes; a higher proportion of those with the low-expression GG genotype were on lipid-lowering therapy and ARBs. Since the rs77878271 G-allele has been associated with reduced triglyceride concentrations and lower total cholesterol and as the use of lipid-lowering medication was higher in those with the GG genotype in this study (**Table 1**), we also tested the association of rs77878271 with all available lipids after adjustment for age, sex, duration, BMI, and lipid lowering-medication but found no significant associations (**Supplemental Table 3**).

Since presence of diabetic nephropathy may impact clinical variables at baseline, we tested the association *FABP4* rs77878271 association with baseline variables separately in FinnDiane participants with diabetic nephropathy as well as in those without any sign of diabetic nephropathy. In the participants with any diabetic nephropathy, the low-expression G-allele was associated with a decrease of 0.17 mmol/mL in triglycerides (p=0.018, **Figure 1a**). On the other hand, in the participants with normal AER, the G-allele was also associated with worse glycemic control (HbA_{1c}, p=0.017, **Figure 1b**).

FABP4 rs77878271 associations with clinical endpoints

To obtain age-specific cumulative incidences for each endpoint, we evaluated the effect of *FABP4* rs77878271 on the risk of CVD, stroke, ESKD, and death, using age as timescale. Kaplan-Meier curves for carriers of genotypes AA, AG, and GG demonstrated a difference in cumulative incidence

of CVD (**Figure 2**, p for CVD=0.0071) and CAD alone (p=0.023, **Supplemental Figure 1**), but not of stroke (p=0.097, **Supplemental Figure 1**). Probability of a CAD event by age 60 among participants with GG genotype was 41.1% (95%CI: 0.0-65.7) vs. 26.9% (95%CI: 21.1-32.2%) for AG and 24.9% for AA (95%CI: 22.9-26.8%), respectively. Probability of a CVD event for participants with rs77878271 GG, AG, and AA by age 60 was 54.0% (95%CI: 6.8-77.3), 36.0% (95%CI: 29.8-41.7%), and 31.1% (95%CI: 29.0-33.1%), respectively. There were no differences in ESKD and mortality (**Supplemental Figure 1).** We then calculated HRs for all outcomes (**Table 2**), using age as timescale. Each copy of the *FABP4* rs77878271 G-allele additively increased the risk of stroke by 30% (p=0.038), CAD by 27% (p=0.014), and CVD by 29% (p=0.003), and mortality by 22% (p=0.039) in the sex- and diabetes onset age-adjusted model. The *FABP4* rs77878271 was not associated with increased risk of ESKD.

Confounder-adjusted associations of FABP4 rs77878271 with clinical endpoints

To adjust for other confounders, we evaluated the effect of *FABP4* rs77878271 on the risk of incident stroke, CAD, CVD, ESKD, and mortality, using follow-up time from the baseline visit as timescale in a Cox proportional-hazards model. After controlling for sex, age, and diabetes duration at baseline, the *FABP4* rs77878271 G-allele additively increased risk of stroke by 37% (HR=1.37, p=0.048) and CVD by 31% (HR=1.31, p=0.02, **Figure 3, Model 1**). We adjusted the model further for use of lipid-lowering medication and ARBs (**Figure 3: Model 2**) since these differed among *FABP4* rs77878271 carriers at baseline (**Table 1**), after which similar but somewhat higher risk estimates were observed for stroke (HR=1.48, p=0.01), CVD (HR=1.36, p=0.006), and mortality (HR=1.28, p=0.02), but not for CAD or ESKD (**Figure 3: Model 2**). The associations with stroke and CVD remained even after addition of baseline nephropathy stage (**Figure 3: Model 3**) to model 1.

Replication analyses

We sought replication of our findings regarding stroke, CAD, CVD, and mortality in 852 adult Danish individuals with type 1 diabetes from the SDCC cohort (see **Supplemental Table 4** for clinical characteristics). Further replication was sought in 3,678 Finnish individuals with type 1 diabetes from FinnGen. The MAF of *FABP4* rs77878271 was 2.4% in SDCC and 6.1% in FinnGen, and the SNP was in HWE in both cohorts (**Supplemental Table 2**). *FABP4* rs77878271 was not associated with any of the individual outcomes in SDCC (**Supplemental Table 5**) or in FinnGen (**Figure 4**). Nevertheless, the magnitude and direction of the results for rs77878271 were mostly consistent with the results from FinnDiane. Furthermore, in the meta-analysis of SDCC and FinnDiane associations, the low-expression G-allele of rs77878271 increased the risk of stroke (p=0.04), CAD (p=0.006), and CVD (p=0.003) by 26%, 26%, and 17%, respectively, without significant heterogeneity (I^2=0-51%, p>0.05, **Figure 4**).

Mendelian Randomization

We investigated the causal effect of FABP4 on CAD using two-sample Mendelian Randomization. In line with our observational findings, the MR analysis revealed that one SD unit decrease in FABP4 causally increased the odds of CAD 2.4-fold (causal OR=2.40, 95% CI=1.18-5.08, p=0.016).

Sensitivity analyses

To understand the background behind our contradictory finding we performed additional sensitivity analyses. As the events started to increase at approximately 40 years of age, based on the Kaplan Meier curves (**Figure 1 and Supplemental Figure 1**), we evaluated the age-dependent effects by dividing the follow-up time (age) into two different time groups (tgroup, above and under 40 years) and calculating the HRs for each tgroup. These time-dependent analyses revealed that the rs77878271 low expression G-allele increased the risk of all endpoints except ESKD, exclusively when followed to ages above 40 years (**Figure 5**), but not when follow-up time was limited to ages below 40 years.

Next, we performed interaction analyses to evaluate if the association between FABP4 rs77878271 and the outcomes is potentially modified by other factors, such as diabetic nephropathy, obesity, diabetes onset age, diabetes duration and HbA_{1c} by adding these variables as an interaction term to model 2 and using time from baseline as timescale. As circulating FABP4 is suggested to be cleared through the kidneys (34,35), and since renal clearance can be reduced in kidney disease, we first examined whether the *FABP4* rs77878271 associations with clinical endpoints were modified by diabetic nephropathy. A significant interaction of *FABP4* rs77878271 with advanced nephropathy stage was identified for CAD (Interaction p=0.02). When stratified by advanced nephropathy vs. normal AER, the *FABP4* rs77878271 G-allele was associated with increased risk of CAD only among individuals with normal AER (p=0.009, **Figure 6**). Since obesity is suggested to interact with the *FABP4* rs77878271 SNP, we tested if obesity (BMI≥30.0) or overweight/obesity (BMI≥25.0) modified the associations with any of the endpoints. There was no interaction between rs77878271 and obesity or between the variant and overweight/obesity (**Supplemental Figure 2**) or between the variant and BMI analyzed as a continuous variable (**Supplemental Table 6**). However, in individuals without obesity (BMI<30kg/m^2), the G-allele increased the risk of CAD and CVD, whereas no association was present in those with obesity (BMI>30kg/m^2). The association between rs77878271 and CAD was modified by HbA_{1c}, and the association between the variant and ESKD was modified by diabetes duration and HbA_{1c} (**Supplemental Figure 3)**.

DISCUSSION

We found that the low-expression G-allele of *FABP4* rs77878271 is robustly associated with increased risk of CVD, suggesting that genetically determined low levels of FABP4 increase the risk of CVD in individuals with type 1 diabetes of both Finnish and Danish descent. Furthermore, Mendelian Randomization, including data from cohorts of other descent as well, demonstrated that lower circulating FABP4 causally increases the odds of CAD 2.4-fold. These findings are in striking contrast to findings in the general population, where the low-expression G-allele confers protection against CVD (19,20). To understand the background of this unexpected association, we performed several sensitivity analyses, including interaction analyses, where we tested the modifying effect of several CVD risk factors as well as analyzing rs77878271 as time-dependent variable. However, the paradoxical effect remained. Notably, a recent study in mice with streptozotocin-induced diabetes and genetic *Fabp4* deficiency showed, in contrast to prior expectations, that genetic blockage of *Fabp4* aggravates cardiac contractile dysfunction, instead of alleviating it (36). In nondiabetic mice, genetic *Fabp4* deficiency reduces the uptake of free fatty acids in the heart in combination with a compensatory, beneficial increase in glucose uptake to meet energy demands of the heart (10). In diabetes, such compensatory glucose uptake also occurs, but the energy yield from glucose is reduced as a consequence of insulin deficiency (36). This results in detrimental energy insufficiency in the diabetic heart because of reduced energy yield from glucose in combination with genetic *FABP4*-deficiency, which reduces the energy yield from free fatty acids (36).

Since the first description of FABP4 as a circulating protein (37), studies regarding serum FABP4 have emerged (38). Although serum FABP4 studies in type 1 diabetes have been scarce, higher FABP4 serum levels have been associated with elevated pre-eclampsia risk (39,40), higher BMI (41), worse glycemic control (42), and suggested to be involved in autoimmune destruction of β cells in type 1 diabetes (43). In this study, we did not find any association between the FABP4 low-expression G-allele and BMI, but in participants with normal AER, the low-expression G-allele was associated

with higher HbA_{1c} (**Figure 1**). In type 2 diabetes higher serum FABP4 levels have been linked to clinical outcomes related to worse cardiometabolic health (44). In addition, serum FABP4 has been proposed as a novel biomarker for kidney complications in type 2 diabetes due to its independent associations with diabetic nephropathy stage (45) and with kidney function decline (46). Whether lowering serum FABP4 concentrations in individuals with diabetes would result in lower risk of kidney complications is difficult to determine based on observational data. As the kidney is the key organ for the clearance of the circulating FABP4 protein, impairment of kidney function will naturally result in markedly increased serum FABP4 levels (35), complicating any conclusions regarding causality. In this study, using genetics, we did not detect an association between FABP4 — proxied by the low-expression G-allele — and ESKD in type 1 diabetes. However, our interaction analyses with diabetes duration revealed, that the effect of the low-expression G-allele on ESKD is evident only after long duration of diabetes (**Supplemental Figure 3**).

The cohorts in the present study differ from the general population in several aspects. By default, the prevalence of kidney disease is considerably higher in type 1 diabetes than in the general population (47). Still, even among participants with normal AER, who are more comparable to the general population, the rs77878271 G-allele was related to increased risk of CAD. The cohorts also differ besides prevalence of kidney disease. Lack of endogenous insulin production, which is the case in type 1 diabetes, could complicate the relationship between FABP4 and the risk of complications in diabetes. Furthermore, even though the obesity prevalence in type 1 diabetes has dramatically risen during the last decades (6), the prevalence of obesity in FinnDiane was still lower than in the general population (9.4%, **Supplemental Table 4**) and the interaction analysis with obesity, defined as a BMI above 30 kg/m^2, is likely underpowered due to the low prevalence of obesity and to the low MAF of the variant. As FABP4 is an adipokine implicated in obesity-induced disorders, and is also causally related to BMI (22), it is possible that the deleterious effects of FABP4 on cardiometabolic health would only be detectable in obese participants, which in the present study, is limited by number.

Despite strong experimental evidence linking the functional promoter polymorphism in *FABP4* gene to *FABP4* expression (21,19,20), it has also been suggested that the circulating FABP4 levels would not be genetically determined (48). However, in studies with individuals of Finnish descent (20), including this one, the rs77878271 G-allele frequency is significantly higher (6.0%), which may explain why the effect of this variant is more easily detectable. Furthermore, in a recent large-scale GWAS of the plasma proteome (22), the *FABP4* promoter rs77878271 G-allele was associated with lower levels of circulating FABP4 protein homogenously across cohorts ($p=6.4x10^{-14}$, $I^2=0\%$, heterogeneity p=0.49). Although the GWAS also included participants with diabetes, it is not granted, that the same association for this SNP and circulating FABP4 is present in individuals with type 1 diabetes.

Despite significant findings for this variant in the meta-analysis, without evidence of heterogeneity, the lack of replication remains a limitation. As we were not able to find statistical significance in the individual cohorts, we calculated the post-hoc power for the cohorts, which revealed that the power to detect the HR observed in FinnDiane (HR=1.29) was 18.2% in the SDCC, due to the lower MAF (2%) and the smaller sample size (**Supplemental Figure 4**). Although FinnGen provided a larger sample and higher MAF (6%), the power to detect an HR of 1.29 was only slightly higher (34.3%) because of lower CVD incidence rate (6.7%). Of note, replication was attained in the SDCC study for the original CAD definition without procedural codes (27), (HR=2.00, 95%CI=1.07-3.73, p=0.03), but after phenotype harmonization, the finding was no longer significant (p=0.17). However, the direction of effect was similar in all cohorts and the Mendelian Randomization, which also included samples not part of discovery or replication cohorts, provided further support for our findings. The need for additional confirmation in other cohorts is still needed. Finally, this study included only Nordic populations; thus, conclusions regarding other populations cannot be drawn. The strengths of this study is the carefully characterized large cohorts of type 1 diabetes and use of genetics to examine the relationship between FABP4 and outcomes. Genetically proxied FAPB4

levels represent a lifetime effect, and as genes cannot be changed by confounders such as social class, age, or kidney function, their impact on the outcome will be independent of these factors.

In conclusion, our results suggest that a certain level of *FABP4* expression seems to be required to maintain cardiovascular health in individuals with type 1 diabetes suggesting yet unexplored roles for this hormone in regulating the risk of diabetic cardiovascular complications.

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Author contributions. E.H.D, N.S., C.F., J.S., L.M.T., P.L., and P.-H. G. took part in the conception and design of the work. E.H.D, C.F., V.H.,N.M., and T.S.A. acquired the data and E.H.D. conducted the analysis for FinnDiane participants, N.U. for SDCC participants and N.M for FinnGen participants. E.H.D, C.F., J.S., L.M.T., N.S., N.U., T.S.A., V.H, P.L., P.R., N.M., and P.-H. G. interpreted the data and contributed to the discussion. EHD drafted the manuscript, which all authors revised critically for important intellectual content. All authors accepted the final version. P-H.G.

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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Duality of interest. P-H.G. reports receiving lecture honorariums from Astellas, Astra Zeneca, Bayer, Boehringer Ingelheim, Eli Lilly, Elo Water, Genzyme, Medscape, MSD, Mundipharma, Novartis, Novo Nordisk, PeerVoice, Sanofi, Sciarc and being an advisory board member of Astellas, Astra Zeneca, Bayer, Boehringer Ingelheim, Eli Lilly, Janssen, Medscape, MSD, Mundipharma, Novartis, Novo Nordisk, and Sanofi. PR has received honoraria to Steno Diabetes Center Copenhagen for lectures and consultancy from Astra Zeneca, Astellas, Boehringer Ingelheim, Bayer, Eli Lilly, Gilead, MSD, Mundipharma, Novo Nordisk, Sanofi, and Vifor. No other potential conflicts of interest relevant to this article were reported.

Data availability. The FinnGen data may be accessed through Finnish Biobanks' FinBB portal (www.finbb.fi). Any other datasets generated and/or analyzed for this study are not publicly available due to the local legislation and the consent of the study participants, which do not permit sharing individual-level phenotypic data.

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TABLES

Table 1 Clinical characteristics of FinnDiane participants by rs77878271 genotype at the baseline examination. For continuous variables, data are reported as mean (standard deviation) and p-values calculated using ANOVA unless otherwise noted. For categorical variables, data are reported as number (%) and p-values calculated with chi-squared or Fishers exact test (when the expected cell frequencies were less than 5).

FABP4 rs77878271	**AA**	**AG**	**GG**	**P**
Number (%)	4,490 (88.4)	564 (11.1)	23 (0.5)	
Men, n (%)	2,336 (52.0)	310 (55.0)	15 (65.2)	0.19
Age, years	39.2 (12.3)	39.0 (12.1)	42.2 (11.4)	0.49
Diabetes duration, years	23.1 (12.6)	23.1 (12.3)	23.8 (12.0)	0.96
Diabetes onset age, years	14.0 (9.0-22.4)	14.1 (9.5-21.6)	17.9 (9.9-23.1)	0.57*
Hypertension, n (%)	2,339 (56.5)	305 (59.2)	14 (63.6)	0.42
Systolic blood pressure, mmHg	134 (19)	135 (19)	133 (21)	0.82
Diastolic blood pressure, mmHg	80 (10)	79 (10)	79 (10)	0.94
Ever smoked, n (%)	1,925 (47.9)	220 (43.5)	11 (52.4)	0.14
HbA_{1c}, %	8.5 (1.5)	8.5 (1.5)	8.7 (1.0)	0.51
HbA_{1c}, mmol/mol	69 (16)	70 (16)	71 (11)	0.51
eGFR, ml/min/1.73m^2	88.6 (69.8, 105.7)	88.8 (66.1,107.1)	87.3 (78.8,112.4)	0.96*
BMI, kg/m2	25.1 (3.7)	25.2 (3.8)	25.1 (3.6)	0.68
BMI category:				
Normal weight, n (%)	2,235 (52.6)	272 (51.5)	10 (45.5)	0.71
Overweight, n (%)	1,561 (36.7)	197 (37.3)	10 (45.5)	0.65
Obesity, n (%)	397 (9.3)	52 (9.8)	2 (9.1)	0.90
Waist-to-hip ratio	0.87 (0.08)	0.88 (0.08)	0.88 (0.10)	0.17

Central obesity[†], n (%)	1,619 (42.0)	227 (47.7)	10 (50.0)	0.047
Antihypertensive medication, n (%)	41.7 (1755)	42.2 (224)	54.5 (12)	0.47
RAAS-blockers (ACEi+ARBs), n (%)	1,441 (33.2)	170 (31.3)	7 (33.3)	0.67
ACEIs, n (%)	1,173 (27.1)	153 (28.2)	4 (19.0)	0.63
ARBs, n (%)	333 (7.7)	18 (3.3)	3 (14.3)	0.0001
Lipid-lowering medication, n (%)	657 (15.2)	71 (13.1)	9 (40.9)	0.004
Lipids:				
Total cholesterol, mmol/l	4.9 (1.0)	4.9 (1.0)	4.7 (1.0)	0.52
HDL cholesterol, mmol/l	1.4 (0.4)	1.4 (0.4)	1.3 (0.3)	0.58
LDL cholesterol, mmol/l	3.0 (0.9)	3.0 (0.9)	2.8 (0.9)	0.48
Triglycerides, mmol/l	1.0 (0.8, 1.5)	1.0 (0.8, 1.4)	1.1 (0.9, 1.4)	0.80[*]
Apolipoprotein A-I, g/l	138.5 (22.8)	137.6 (22.4)	135.4 (16.5)	0.58
Apolipoprotein B, g/l	87.2 (22.8)	87.4 (23.4)	85.3 (25.3)	0.92
Diabetic nephropathy stage:				
Normal AER, n (%)	2,676 (63.0)	319 (61.1)	12 (52.2)	0.40
Microalbuminuria, n (%)	532 (12.5)	72 (13.8)	4 (17.4)	0.46
Macroalbuminuria, n (%)	675 (15.9)	81(15.5)	2 (8.7)	0.75
ESKD, n (%)	365 (8.6)	50 (9.6)	5 (21.7)	0.07

[*]Continuous data presented as median with 1st and 3rd quartiles, and p-value from Kruskal-Wallis test.

[†]Central obesity defined as waist-to-hip ratio>0.85 for women and waist-to-hip ratio >0.90 for men.

ESKD=End-stage kidney disease, AHT=antihypertensive medication, BMI=Body Mass Index. RAAS= Renin-Angiotensin-Aldosterone System, ACEi= Angiotensin-converting enzyme inhibitors, ARBs= Angiotensin II receptor blockers.

Table 2. *FABP4* rs77878271 and risk of stroke, CAD, CVD, ESRD and mortality, using age as the time scale. Unadjusted HRs and adjusted HRs (for diabetes onset age and sex) from Cox proportional hazard-models. Number of events is 444 (Stroke), 785 (CAD), 1047 (CVD), 782 (ESKD), and 793 (Mortality)

Outcome	**Model**	**HR (95%CI)**	**p-value**
Stroke	unadjusted	1.31 (1.02-1.67)	0.032
	adjusted	1.30 (1.01-1.66)	0.038
CAD	unadjusted	1.27 (1.05-1.53)	0.012
	adjusted	1.27 (1.05-1.54)	0.014
CVD	unadjusted	1.29 (1.10-1.52)	0.002
	adjusted	1.29 (1.09-1.52)	0.003
ESKD	unadjusted	1.16 (0.95-1.41)	0.140
	adjusted	1.14 (0.94-1.39)	0.183
Mortality	unadjusted	1.23 (1.02-1.49)	0.032
	adjusted	1.22 (1.01-1.48)	0.039

CAD=Coronary artery disease, CVD=Cardiovascular disease, ESKD=End-stage kidney disease

FIGURE LEGENDS

Figure 1 Baseline association of rs77878271 G-allele with A) HbA_{1c} and B) triglycerides, stratified by presence of diabetic nephropathy (DN). DN was defined as microalbuminuria, macroalbuminuria or ESKD. Betas are unadjusted and calculated for baseline measurements using linear regression.

Figure 2 Cumulative events for CVD followed from birth, stratified by FABP4 rs78778271 genotypes. CVD= Cardiovascular disease. P-value by Log-rank test.

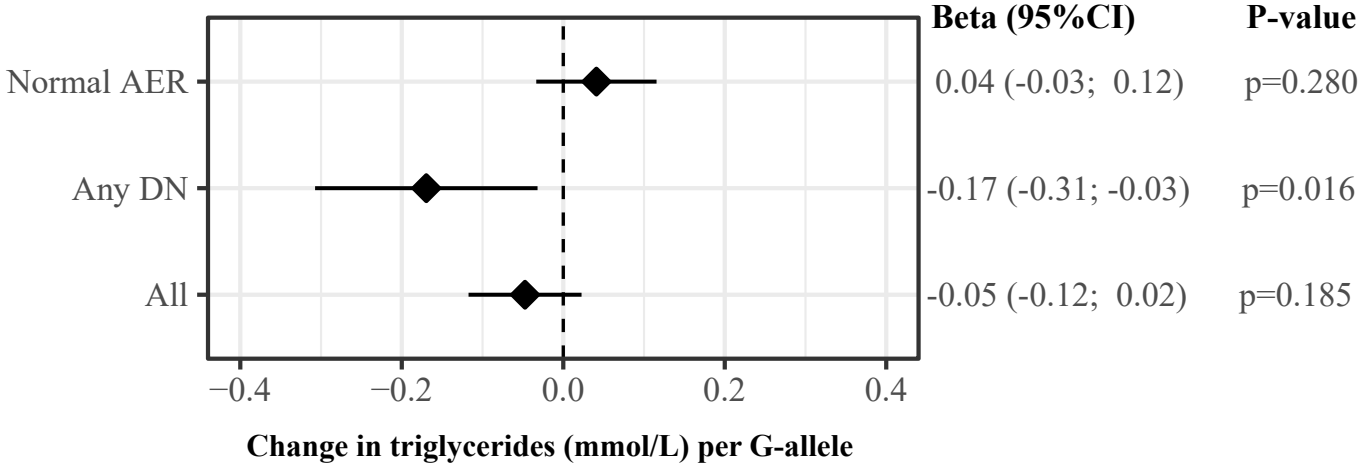
Figure 3 SNP rs77878271 in FABP4 and risk of incident stroke, CAD, CVD, ESRD and mortality followed from baseline examination. Unadjusted and adjusted HRs. Model 1: adjusted for age, sex, diabetes duration. Model 2: Model 1+ lipid lowering medication and ARBs medication**.** Model 3: Model 1+ nephropathy stage (normo-, micro-, macroalbuminuria or ESKD). X-axis is log-scaled.

Figure 4. Meta-analysis of results in the SDCC and FinnDiane studies for FABP4 rs77878271. CAD=Coronary artery disease. CVD= Cardiovascular disease.

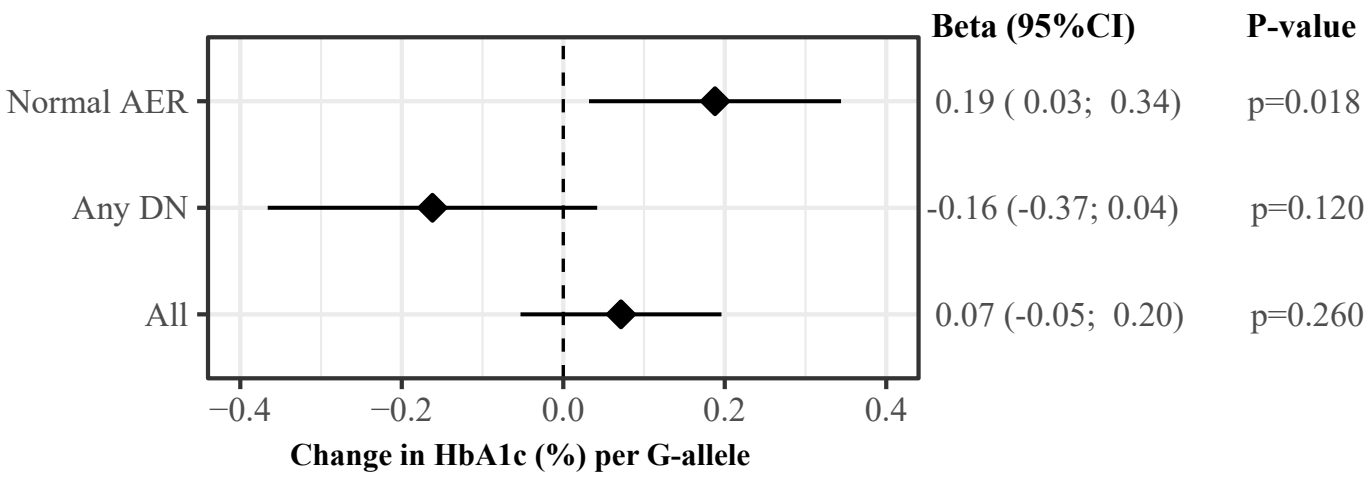
Figure 6. Time (age)-dependent effect of rs77878271 on endpoints in the FinnDiane study. Tgroup= time group. Analyses adjusted for age at diabetes onset and, sex. X-axis is log-scaled.

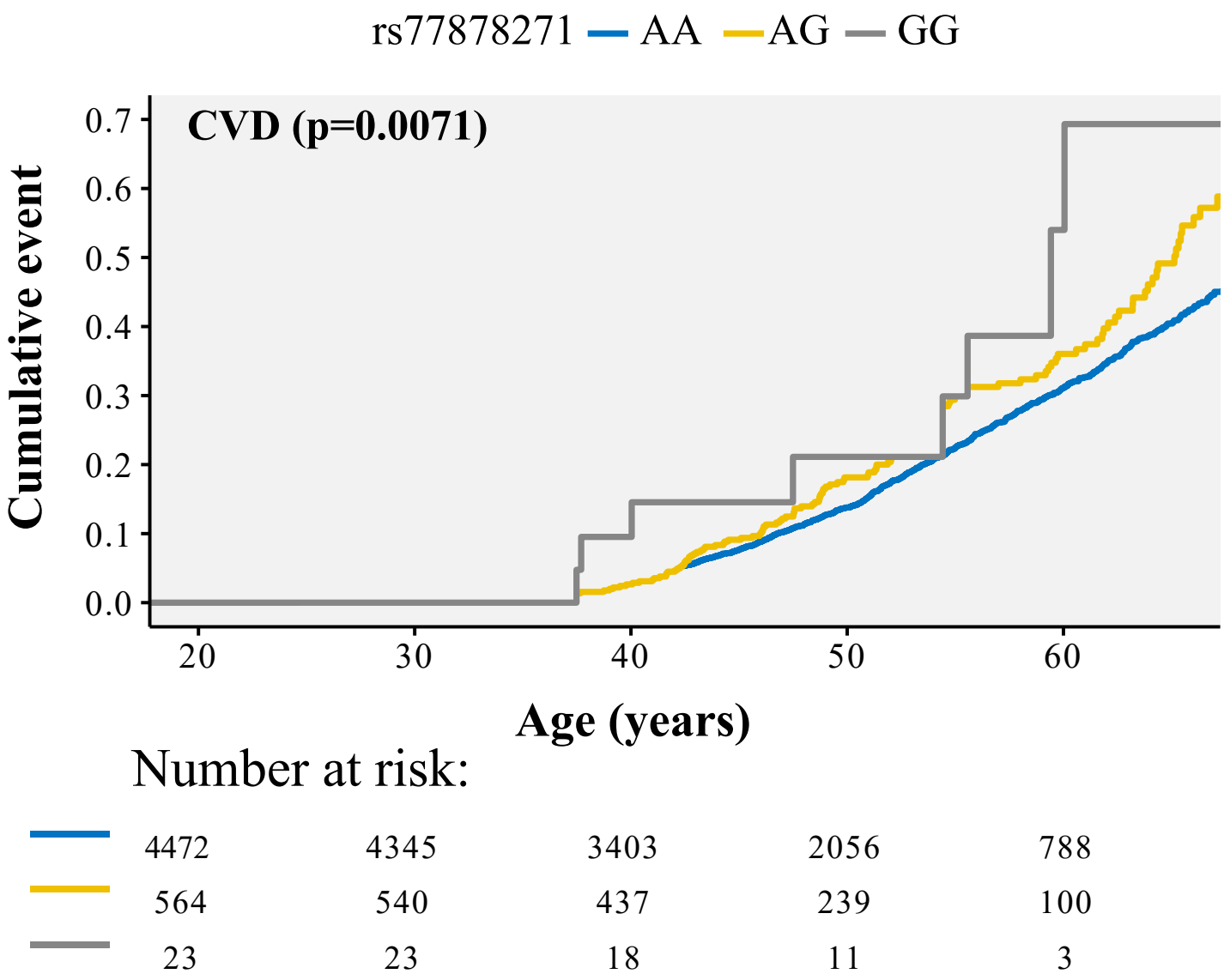
Figure 6. Effect of rs77878271 on outcomes stratified by diabetic nephropathy (DN). Any DN is defined as microalbuminuria, macroalbuminuria and ESRD. Advanced DN is defined as macroalbuminuria and ESKD. X-axis is log-scaled.

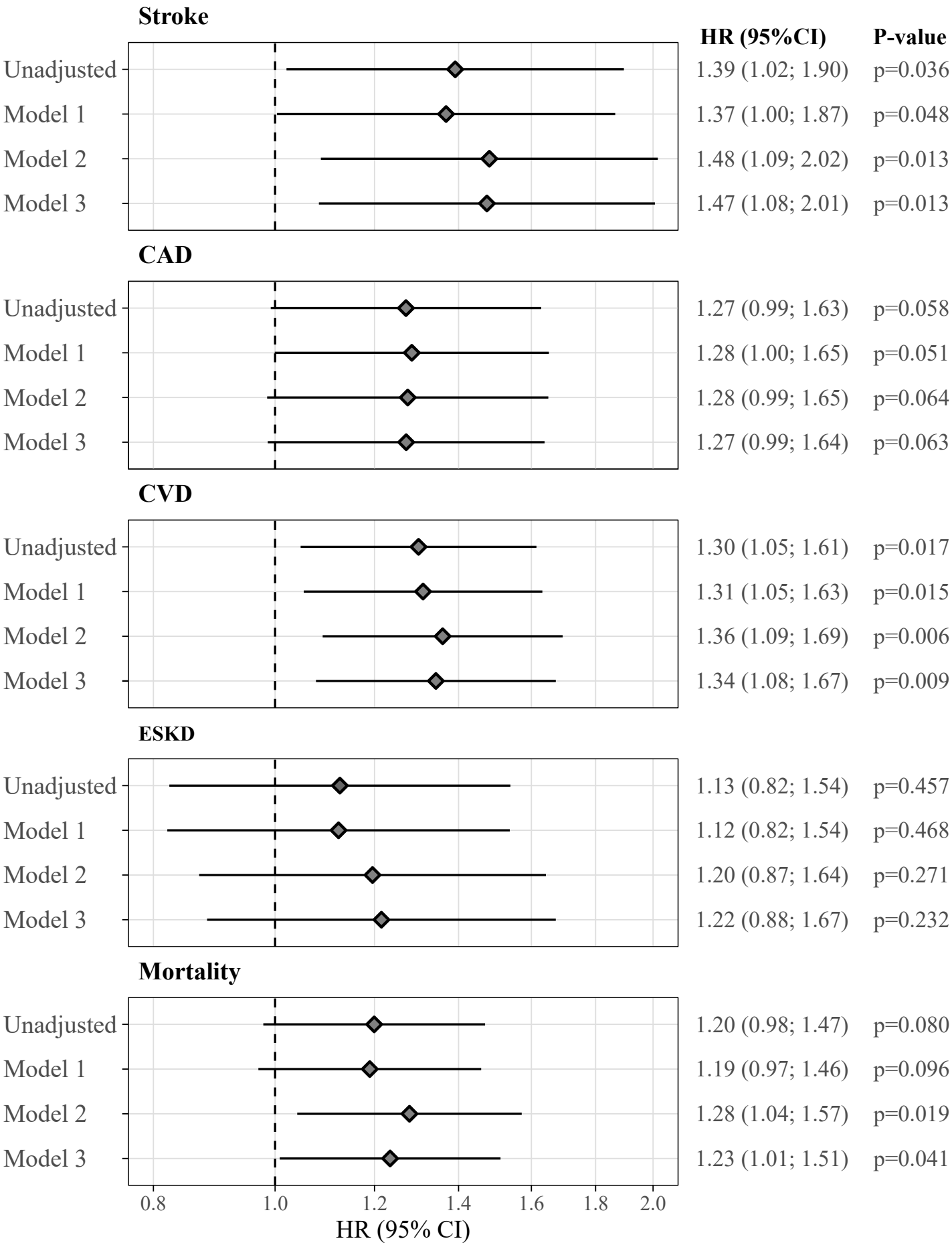
A

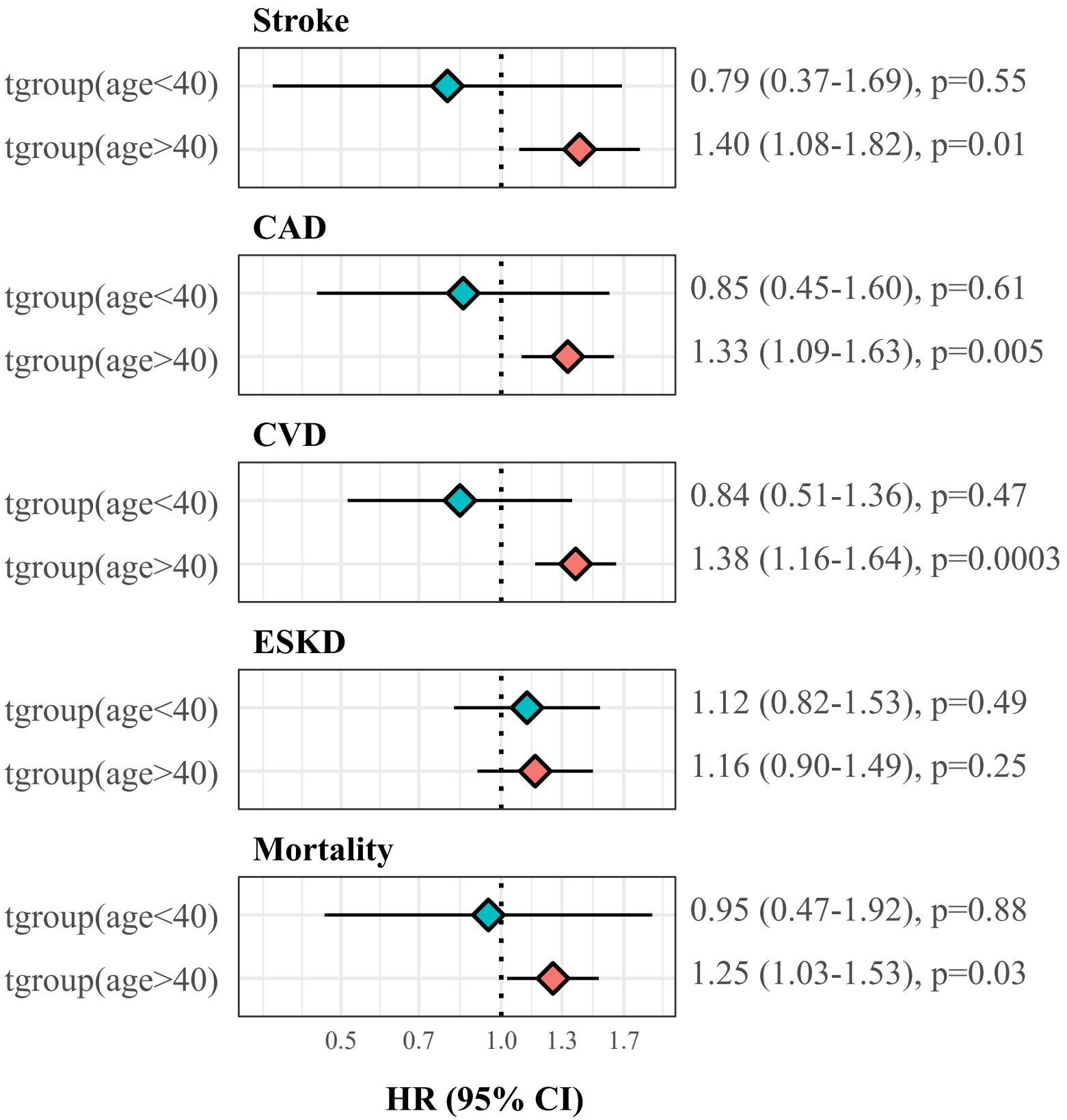

Beta (95%CI)
P-value
Normal AER
0.04 (-0.03; 0.12)
p=0.280
Any DN
-0.17 (-0.31; -0.03)
p=0.016
All
-0.05 (-0.12; 0.02)
p=0.185
−0.4
−0.2
0.0
0.2
0.4
Change in triglycerides (mmol/L) per G-allele

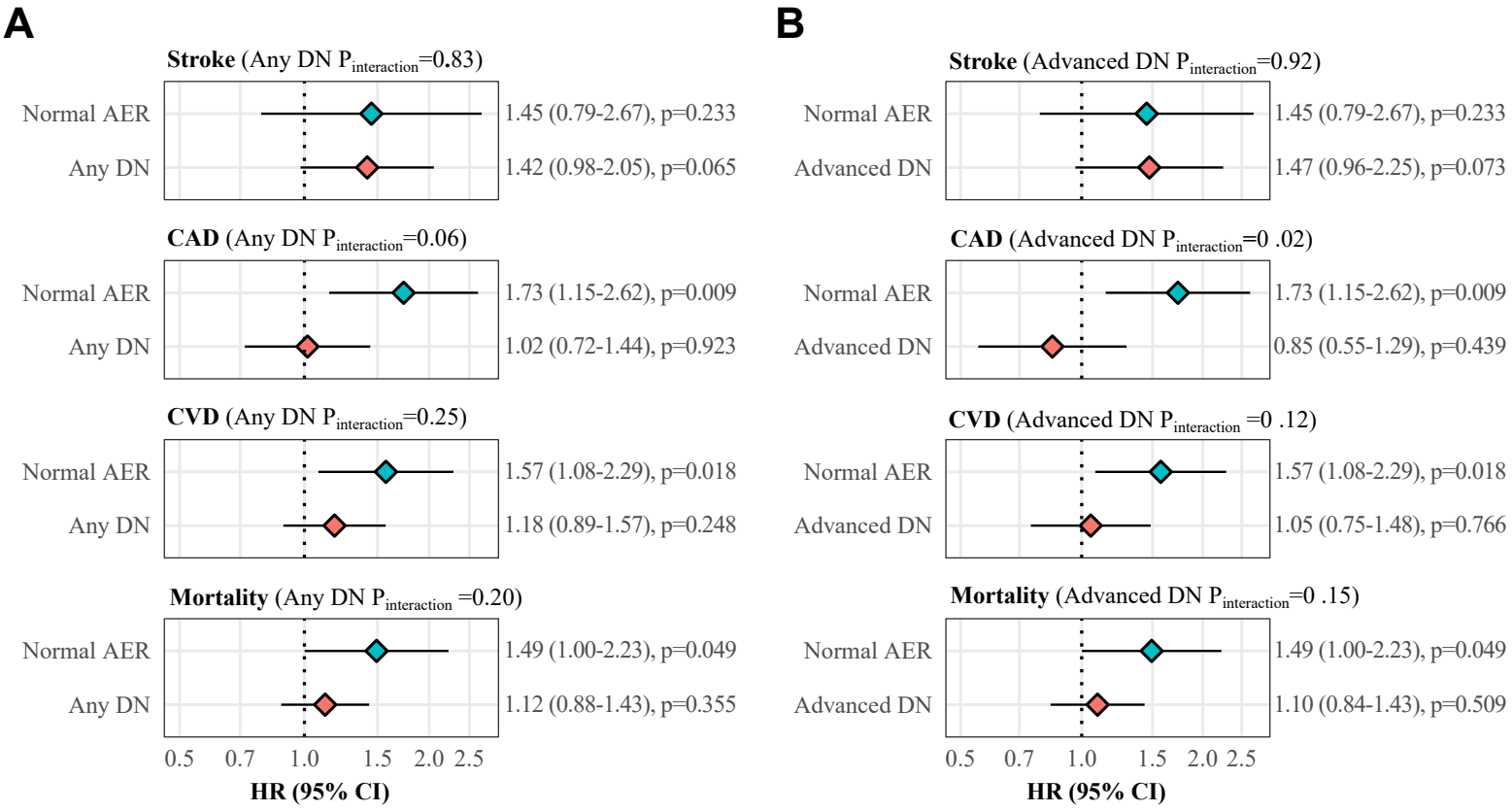
B


Beta (95%CI)
P-value
Normal AER
0.19 (0.03; 0.34)
p=0.018
Any DN
-0.16 (-0.37; 0.04)
p=0.120
All
0.07 (-0.05; 0.20)
p=0.260
−0.4
−0.2
0.0
0.2
0.4
Change in HbA1c (%) per G-allele


rs77878271 AA AG GG
CVD (p=0.0071)
Cumulative event
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
20
30
40
50
60
Age (years)
Number at risk:
4472 4345 3403 2056 788
564 540 437 239 100
23 23 18 11 3


Stroke
HR (95%CI)
P-value
Unadjusted
1.39 (1.02; 1.90)
p=0.036
Model 1
1.37 (1.00; 1.87)
p=0.048
Model 2
1.48 (1.09; 2.02)
p=0.013
Model 3
1.47 (1.08; 2.01)
p=0.013
CAD
Unadjusted
1.27 (0.99; 1.63)
p=0.058
Model 1
1.28 (1.00; 1.65)
p=0.051
Model 2
1.28 (0.99; 1.65)
p=0.064
Model 3
1.27 (0.99; 1.64)
p=0.063
CVD
Unadjusted
1.30 (1.05; 1.61)
p=0.017
Model 1
1.31 (1.05; 1.63)
p=0.015
Model 2
1.36 (1.09; 1.69)
p=0.006
Model 3
1.34 (1.08; 1.67)
p=0.009
ESKD
Unadjusted
1.13 (0.82; 1.54)
p=0.457
Model 1
1.12 (0.82; 1.54)
p=0.468
Model 2
1.20 (0.87; 1.64)
p=0.271
Model 3
1.22 (0.88; 1.67)
p=0.232
Mortality
Unadjusted
1.20 (0.98; 1.47)
p=0.080
Model 1
1.19 (0.97; 1.46)
p=0.096
Model 2
1.28 (1.04; 1.57)
p=0.019
Model 3
1.23 (1.01; 1.51)
p=0.041
0.8
1.0
1.2
1.4
1.6
1.8
2.0
HR (95% CI)


Stroke
tgroup(age<40)
tgroup(age>40)
0.79 (0.37-1.69), p=0.55
1.40 (1.08-1.82), p=0.01
CAD
tgroup(age<40)
tgroup(age>40)
0.85 (0.45-1.60), p=0.61
1.33 (1.09-1.63), p=0.005
CVD
tgroup(age<40)
tgroup(age>40)
0.84 (0.51-1.36), p=0.47
1.38 (1.16-1.64), p=0.0003
ESKD
tgroup(age<40)
tgroup(age>40)
1.12 (0.82-1.53), p=0.49
1.16 (0.90-1.49), p=0.25
Mortality
tgroup(age<40)
tgroup(age>40)
0.95 (0.47-1.92), p=0.88
1.25 (1.03-1.53), p=0.03
0.5
0.7
1.0
1.3
1.7
HR (95% CI)


A
Stroke (Any DN P interaction=0.83)
Normal AER
Any DN
1.45 (0.79-2.67), p=0.233
1.42 (0.98-2.05), p=0.065
CAD (Any DN P interaction=0.06)
Normal AER
Any DN
1.73 (1.15-2.62), p=0.009
1.02 (0.72-1.44), p=0.923
CVD (Any DN P interaction=0.25)
Normal AER
Any DN
1.57 (1.08-2.29), p=0.018
1.18 (0.89-1.57), p=0.248
Mortality (Any DN P interaction =0.20)
Normal AER
Any DN
1.49 (1.00-2.23), p=0.049
1.12 (0.88-1.43), p=0.355
0.5 0.7 1.0 1.5 2.0 2.5
HR (95% CI)
B
Stroke (Advanced DN P interaction=0.92)
Normal AER
Advanced DN
1.45 (0.79-2.67), p=0.233
1.47 (0.96-2.25), p=0.073
CAD (Advanced DN P interaction=0 .02)
Normal AER
Advanced DN
1.73 (1.15-2.62), p=0.009
0.85 (0.55-1.29), p=0.439
CVD (Advanced DN P interaction =0 .12)
Normal AER
Advanced DN
1.57 (1.08-2.29), p=0.018
1.05 (0.75-1.48), p=0.766
Mortality (Advanced DN P interaction=0 .15)
Normal AER
Advanced DN
1.49 (1.00-2.23), p=0.049
1.10 (0.84-1.43), p=0.509
0.5 0.7 1.0 1.5 2.0 2.5
HR (95% CI)