Brief Genetics Report

The α-Adducin Gene Is Associated With Macrovascular Complications and Mortality in Patients With Type 2 Diabetes

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We examined the association between α-adducin 1 (*ADD1*) gene polymorphism (Gly460Trp) with macrovascular complications and mortality in type 2 diabetes in a Caucasian population aged ≥55 years. The study was part of the Rotterdam Study, a prospective population-based cohort study. *ADD1* polymorphism was determined in 6,471 participants, including 599 patients with type 2 diabetes at baseline. The prevalence of hypertension in type 2 diabetic patients was 2.57 times higher in *ADD1* TT carriers compared with GG carriers (95% CI 1.05–6.32, *P* = 0.03). Homozygous T carriers also had a higher mean common carotid intima media thickness (IMT) compared with GG carriers (mean difference 0.05 mm, *P* for trend = 0.03). In diabetic patients with hypertension, the risk of mortality was 1.83 times higher in homozygous T carriers compared with the GG genotype group (95% CI 1.07–3.16, *P* = 0.03). The increased risk was only present among TT carriers who did not use antidiabetes medication (hazard ratio 2.18 [95% CI 1.12–4.24], *P* = 0.02). The results of this population-based cohort study suggest that the *ADD1* gene contributes to the risk of hypertension and increases mean common carotid IMT in patients with type 2 diabetes. Furthermore, the study indicates that the *ADD1* polymorphism could be useful in identifying hypertensive type 2 diabetic patients with a high risk of mortality. *Diabetes* 55: 2922–2927, 2006

Adducin is a heterodimeric cytoskeleton protein composed of α-, β-, and γ-subunits (1). The α-subunit regulates the activity of transmembrane ion pumps and is encoded by the adducin 1 (*ADD1*) gene, located in chromosome 4q21 (2). A long series of parallel studies in the rat model and in humans indicated that a glycine (Gly) to tryptophan (Trp) substitution at amino acid position 460 in the protein (Gly460Trp) leads to higher activity of the Na^+-K^+ pump and, hence, increases renal tubular sodium reabsorption, which increases the risk of salt-sensitive hypertension, vascular pathology, and cardiovascular disease (3–6). The risk of mortality in patients with high systolic blood pressure was shown to be increased in the T-allele carriers of the α-adducin polymorphism compared with G-allele carriers (7).

Renal tubular absorption of sodium is also enhanced by insulin, which promotes sodium retention (8). Insulin-induced sodium retention links type 2 diabetes, insulin resistance, and salt sensitivity (9). Several clinical reports have shown that patients with insulin resistance or diabetes tend to have the salt-sensitive type of hypertension (10). The higher prevalence of hypertension in patients with type 2 diabetes is associated with a fourfold increase in mortality (9).

In the present study, we investigated the hypothesis that *ADD1* is a susceptibility gene for macrovascular complications in type 2 diabetic patients. We examined the association between *ADD1* (Gly460Trp) polymorphism with vascular complications among patients with diabetes in the Rotterdam Study, a large population-based cohort study. Moreover, we evaluated whether the *ADD1* polymorphism predicts survival in type 2 diabetes with and without hypertension.

RESEARCH DESIGN AND METHODS

Subjects were participants of the Rotterdam Study, a population-based cohort study designed to investigate the determinants of chronic diseases in the elderly. The design of the study has been described elsewhere (11). A total of 7,983 subjects aged ≥55 years who live in the Ommoord district of Rotterdam, the Netherlands, entered the study. Baseline data were collected between March 1990 and July 1993. The study was approved by the Medical Ethics Committee of Erasmus University. Written informed consent was obtained from all participants.

At the baseline examination, information about medical history, medication use, and smoking behavior was obtained using computerized questionnaires. Height and weight were measured, and BMI (in kilograms divided by the square of height in meters) was calculated. Blood pressure was measured (with the participant in the sitting position) at the right upper arm with the use of a random zero sphygmomanometer. The average of two measurements was used for the analysis. Hypertension was defined as a diastolic blood pressure 100 mmHg and/or a systolic blood pressure 160 mmHg and/or use of antihypertension medication indicated to treat high blood pressure (grades 2 and 3 of the 1999 World Health Organization criteria). Diabetes was defined as the use of blood glucose–lowering medication and/or random serum glucose level of ≥11.1 mmol/l (12). Total serum and HDL cholesterol levels were determined with an automated enzymatic procedure. Information on all-cause mortality was based on data from the municipality from baseline (1990–1993) until 1 January 2004.

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IMT, intima media thickness.

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TABLE 1
General characteristics of the total population stratified by *ADD1* genotype

	GG	GT	TT
n	4,018	2,140	313
Age (years)	69.57 ± 9.25	69.31 ± 8.90	68.95 ± 8.79
Male sex (%)	40.00	40.50	45.40
BMI (kg/m^2)	26.26 ± 3.71	26.37 ± 4.45	26.40 ± 3.92
Total cholesterol (mmol/l)	6.62 ± 1.23	6.59 ± 1.20	6.61 ± 1.19
HDL cholesterol (mmol/l)	1.34 ± 0.36	1.34 ± 0.37	1.34 ± 0.36
Systolic blood pressure (mmHg)	139.57 ± 22.18	138.98 ± 22.50	138.35 ± 20.97
Diastolic blood pressure (mmHg)	73.85 ± 11.39	73.47 ± 11.57	74.54 ± 11.10
Hypertension (%)	34.70	33.30	35.10
Using antihypertensive medication (%)	32.90	30.80	32.30
Current smoking (%)	21.80	23.80	25.80

Data are means ± SD unless otherwise indicated.

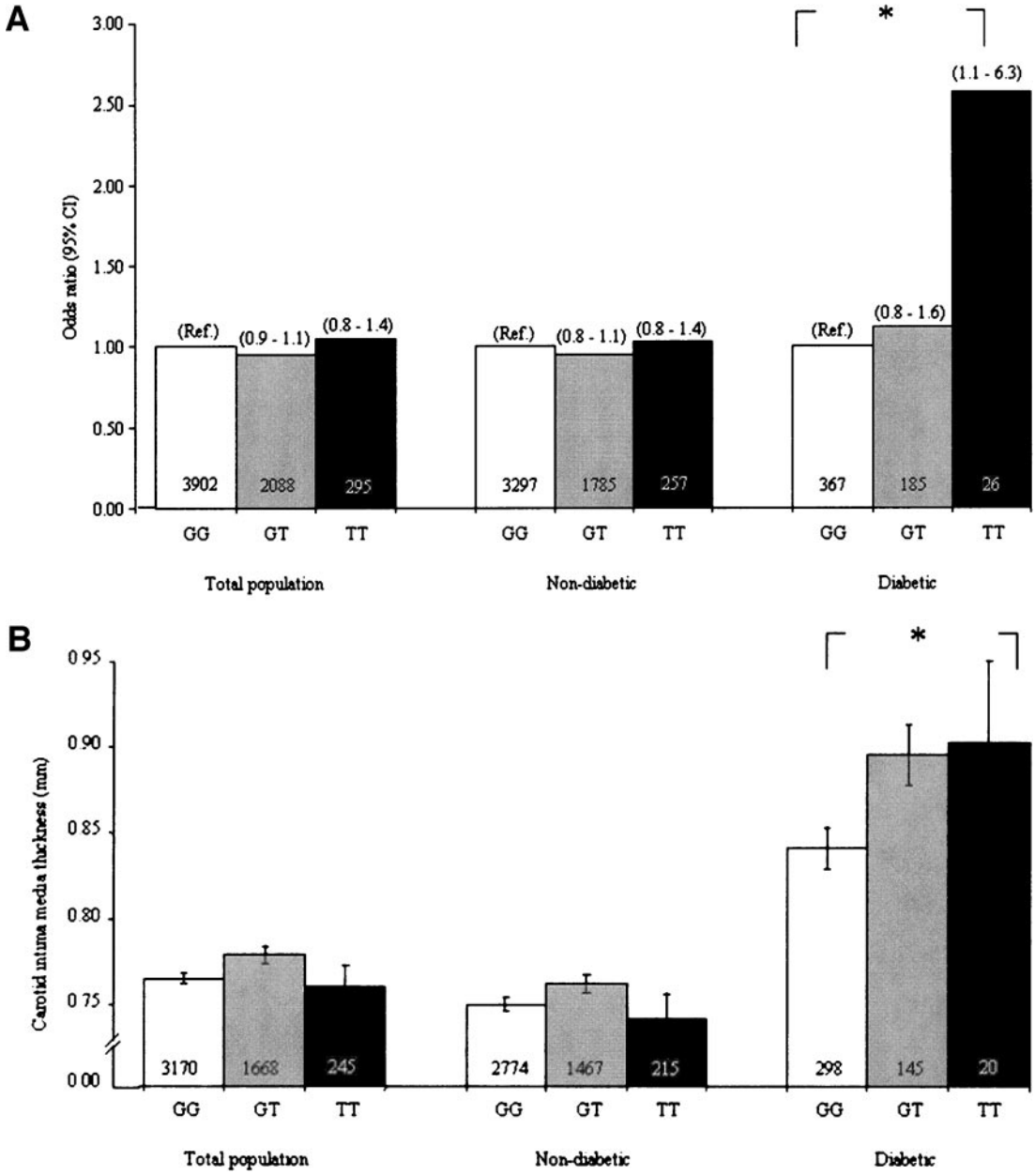

FIG. 1. *A*: Prevalence of hypertension stratified by *ADD1* genotypes in the total population and in nondiabetic and diabetic patients. Data are adjusted for age and sex. *P = 0.03. *B*: Carotid IMT in the total population and in nondiabetic and diabetic patients, stratified by *ADD1* genotype. Data are adjusted for age and sex. *P for trend = 0.03.

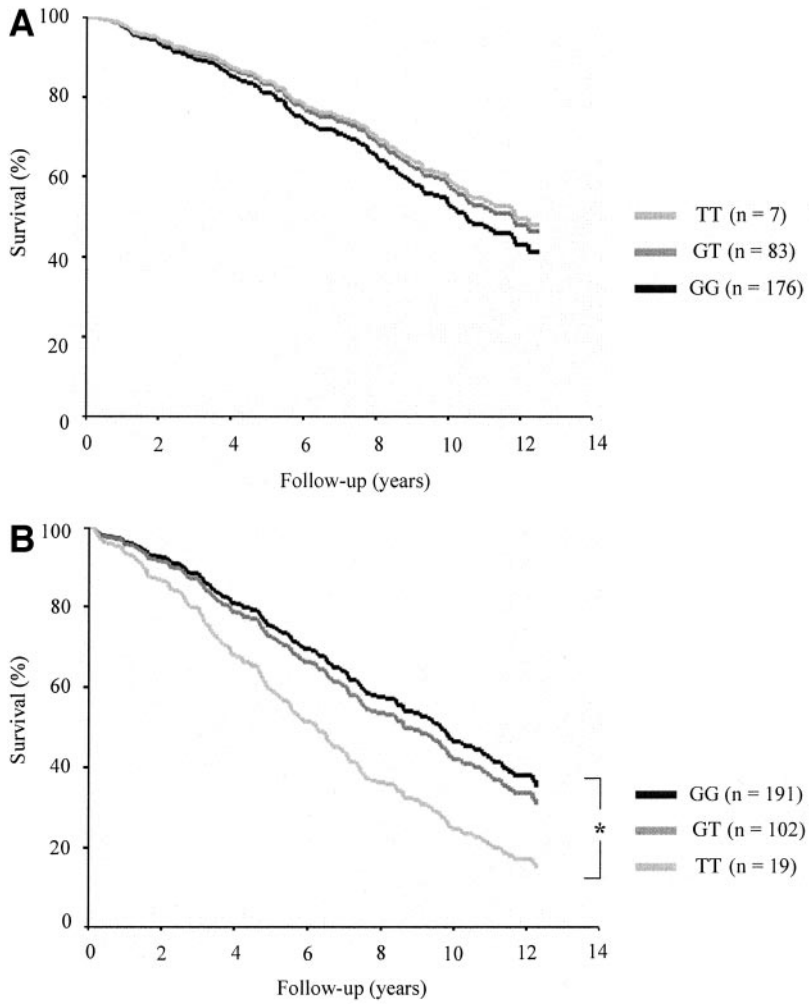

FIG. 2. Survival curves for diabetic patients without (*A*) and with (*B*) hypertension, stratified by *ADD1* genotype. Data are adjusted for age and sex. **P* = 0.03.

Carotid ultrasonography. Carotid atherosclerosis was assessed by duplex scan ultrasonography of the carotid arteries, using a 7.5-MHz linear array transducer (Ultramark IV). Measurements of intima media thickness (IMT) were performed offline from the still images recorded on videotape. Details about this measurement have been published previously (13). Briefly, the interfaces of the far and near walls of the distal common carotid artery are marked over a length of 10 mm. We used the average of the measurements of three still images of both the left and right arteries. Common carotid IMT was determined as the mean IMT of near and far wall measurements of both the left and right arteries. Results from a reproducibility study of IMT measurements have been published elsewhere (14). The means ± SD in the common carotid IMT between paired measurements of sonographers, readers, and visits were −0.004 ± 0.10, 0.066 ± 0.07, and −0.013 ± 0.13 mm, respectively.
Genotyping. Genomic DNA was extracted from whole-blood samples obtained at the baseline examination, utilizing the salting-out method (15). Samples were genotyped for *ADD1* (Gly460Trp) polymorphism with a Taqman allelic discrimination assay-by-design (Applied Biosystems, Foster City, CA). Forward and reverse primer sequences were 5′-GAGAAGACAAGATGGCTGA ACTCT-3′ and 5′-GTCTTCGACTTGGGACTGCTT-3′, respectively. The minor groove binding probes were 5′-CATTCTGCCCTTCCTC-3′ (VIC) and 5′-ATTC TGCCATTCCTC-3′ (FAM). The assays utilized 5 ng genomic DNA and 2 μl reaction volumes. The amplification and extension protocol was as follows: an initial activation step of 10 min at 95°C preceded 40 cycles of denaturation at 95°C for 15 s and annealing and extension at 50°C for 60 s. Allele-specific fluorescence was then analyzed on an ABI prism 7900HT sequence detection system (version 2.1; Applied Biosystems, Foster City, CA).
Statistical analysis. Hardy-Weinberg equilibrium was tested with the χ^2 test. We used ANOVA (for continuous variables) and the χ^2 test (for categorical variables) to compare baseline characteristics of subjects between genotypes. Odds ratios (ORs) with 95% CIs were calculated by logistic-regression analyses, adjusted for sex and age. To investigate the association between IMT, systolic and diastolic blood pressure, and *ADD1* genotypes, ANCOVA was performed in the total population and in subjects with and without diabetes. Post hoc pairwise tests used a Bonfferoni correction for multiple comparisons. Cox's proportional hazards analysis was used to evaluate the contribution of *ADD1* polymorphism to overall mortality in patients with type 2 diabetes with and without hypertension after adjustment for sex and age. This association was also tested in patients with and without antidiabetes medication separately. All analyses were performed using the SPSS for Windows software package, version 11.0.

RESULTS

From 7,983 subjects, blood samples were available for 88.7%, of whom 6,471 (91.4%) were successfully genotyped for the *ADD1* polymorphism. Genotype frequencies were in Hardy-Weinberg equilibrium ($P = 0.20$). Table 1 summarizes the baseline characteristics of the participants. No significant differences were observed in baseline characteristics between the genotypes.

There was no significant difference in the prevalence of hypertension by *ADD1* genotype groups in the total population and in nondiabetic patients (Fig. 1*A*). In diabetic patients, the prevalence of hypertension in TT carriers was 2.57 times higher than in the GG carriers, after adjusting for sex and age (95% CI 1.05–6.32, $P = 0.03$) (Fig. 1*A*). After further adjustment for putative risk factors, this OR did not change materially but became borderline signifi-

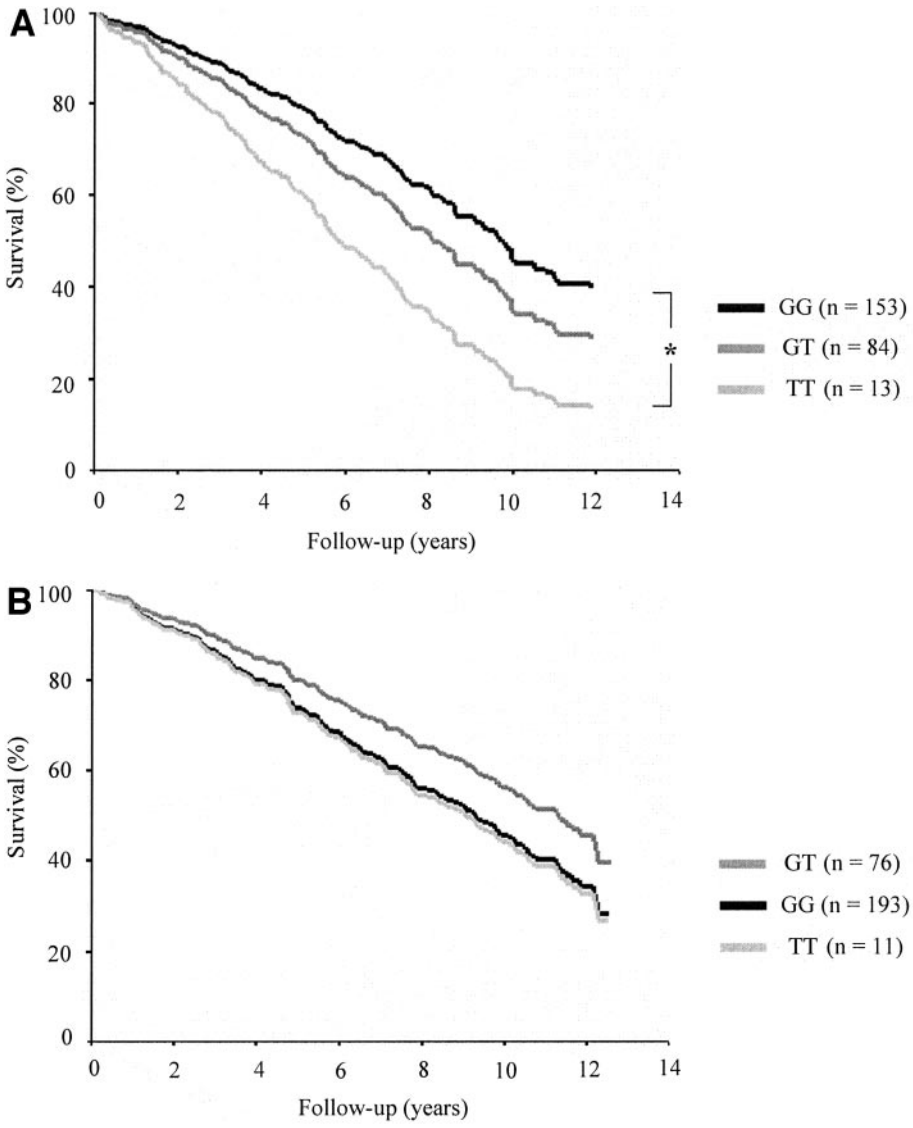

FIG. 3. Survival curves for diabetic patients without (*A*) and with (*B*) antidiabetes medication, stratified by *ADD1* genotype. Data are adjusted for age and sex. **P* = 0.02.

cant (OR 2.39 [95% CI 0.95–6.05], $P = 0.06$). There were no significant differences between the genotype groups in systolic and diastolic blood pressure in the total population and in diabetic patients. To examine whether this effect is modified by antihypertensive medication, we compared the effect of the *ADD1* polymorphism on systolic and diastolic blood pressure in those who did not use antihypertensive medication. No significant differences were observed between the genotype groups, although in diabetic patients there was a tendency to higher systolic and diastolic blood pressure in TT carriers. On average, systolic blood pressure was 9.94 mmHg (95% CI −6.48 to 26.36, $P = 0.23$) higher in TT carriers than GG carriers, and this difference was 5.41 mmHg (−0.65 to 14.46, $P = 0.24$) for diastolic blood pressure in those who did not use antihypertensive medication.

There was an increase in the IMT in diabetic patients with hypertension compared with those without hypertension (mean difference 0.03 mm, $P = 0.05$). No significant association was observed in the total population and in nondiabetic patients between the T-allele of the *ADD1* polymorphism and the mean common carotid IMT. In diabetic patients, however, those homozygous for the T-alleles had a higher mean common carotid IMT compared with GG carriers (mean difference 0.05 mm, *P* for trend = 0.03) (Fig. 1*B*). After further adjustment for putative risk factors, this significant difference did not change (*P* for trend = 0.05).

In the total population, the risk of mortality was 1.23 times higher in those homozygous for the T-alleles (95% CI 1.00–1.76, $P = 0.05$) compared with the GG carriers. This increased risk was 1.50 times increased in diabetic patients (0.93–2.42, $P = 0.10$). No significant association with mortality was observed in diabetic patients without hypertension for the TT genotype (hazard ratio 0.83 [95% CI 0.26–2.63], $P = 0.75$) and for the GT genotype (0.87 [0.61–1.25], $P = 0.45$) (Fig. 2*A*). However, the risk of mortality in diabetic patients with hypertension was 1.83 times increased in homozygous T carriers compared with the GG genotype group (95% CI 1.07–3.16, $P = 0.03$) (Fig. 2*B*). In a further analysis in diabetic patients who did not use antidiabetes medication, the risk of mortality was 2.18 (1.12–4.24, $P = 0.02$) for TT carriers and was 1.35 (0.96–1.90, $P = 0.08$) for GT carriers (Fig. 3*A*). In diabetic

patients who used antidiabetes medication, the risk of mortality was 1.05 (0.49–2.25, $P = 0.91$) for the TT genotype and 0.73 (0.51–1.05, $P = 0.09$) for the GT genotype (Fig. 3*B*).

DISCUSSION

In this population-based cohort study, we observed a significant increased risk of hypertension and an increased mean common carotid IMT in homozygous T carriers of the *ADD1* polymorphism among type 2 diabetic patients. Furthermore, we found an increased risk of mortality associated with the T-allele among type 2 diabetic patients with hypertension. This association was more prominent in those who did not use antidiabetes medication.

This is the first study to address the role of *ADD1* polymorphism in relation to hypertension in type 2 diabetes. A possible mechanism by which the *ADD1* gene is involved in hypertension and atherosclerosis in type 2 diabetic patients is through the Na^+-K^+ pump. A higher Na^+-K^+ pump activity and an impaired Na^+-K^+ pump endocytosis in renal tubular cells is present in carriers of the T-allele (16). In addition, a significant increase in the urinary excretion of nitric oxide (NO) metabolites was observed in carriers of the T-allele (17), which may contribute to abnormal renal sodium handling (18). Moreover, there is a strong biological link between insulin and increased renal tubular sodium reabsorption and salt sensitivity (19). There is considerable evidence that hypertension and diabetes are associated with salt sensitivity (20), endothelial dysfunction, and reduced NO production and/or bioavailability (18,19). These findings prompted us to investigate the role of *ADD1* as a susceptibility gene leading to hypertension through a defective regulation of sodium (21).

A significant association was found between the *ADD1* polymorphism and the mean common carotid IMT in type 2 diabetic patients. However, this may be explained by increased risk of atherosclerosis. The *ADD1* gene polymorphism may play a role through sodium retention, which may lead to chronic expansion of the extracellular fluid volume. These mechanisms can induce compensatory structural changes in the wall of large muscular arteries, particularly in diabetic patients (22).

Several studies have reported an association between salt sensitivity, or high sodium intake, and an increased risk of cardiovascular events and mortality (23,24). Moreover, the risk of total mortality and cardiovascular mortality and cardiovascular events was shown to be increased in T-allele carriers with high systolic blood pressure (7). The results of these studies are in line with our finding that type 2 diabetic patients with the homozygous T-allele and with hypertension had a higher risk of mortality than the GG carriers. Considering the low frequency of the TT genotype (4.3%), however, the number of events among the TT genotype is low (range 7–19), while the association we find is very consistent over different risk factors. More importantly, our study shows that treatment of type 2 diabetes may prevent *ADD1*-induced mortality in diabetic patients. In our study sample, of the diabetic patients with hypertension, 34.1% were treated with diuretics.

Admixture could be a possible confounder when both allele frequency and disease frequency differ between the populations. However, 98% of our population is Caucasian, as is defined by the ethnicity of the grandparents of the subjects. Additionally, selection bias is unlikely, since our study was population based and loss to follow-up was negligible.

In conclusion, this population-based cohort study shows an association between the *ADD1* gene polymorphism, hypertension, and increased mean common carotid IMT in type 2 diabetes. Furthermore, our results suggest that the *ADD1* polymorphism predicts a high risk of mortality in patients with type 2 diabetes. These findings indicate the need for further studies to evaluate the progression of macrovascular complications in type 2 diabetic patients who carry the *ADD1* T-allele.

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