Arterial Properties in Relation to Genetic Variations in the Adducin Subunits in a White Population

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BACKGROUND

Adducin is a membrane skeleton protein, which consists of either α- and β- or α- and γ-subunits. We investigated whether arterial characteristics might be related to the genes encoding *ADD1* (*Gly460Trp-rs4961*), *ADD2* (*C1797T-rs4984*), and *ADD3* (*IVS11+386A>G-rs3731566*).

METHODS

We randomly recruited 1,126 Flemish subjects (mean age, 43.8 years; 50.3% women). Using a wall-tracking ultrasound system, we measured the properties of the carotid, femoral, and brachial arteries. We studied multivariate-adjusted phenotype–genotype associations, using a population- and family-based approach.

RESULTS

In single-gene analyses, brachial diameter was 0.15 mm ($P = 0.0022$) larger, and brachial distensibility and cross-sectional compliance were 1.55×10^{-3}/kPa ($P = 0.013$) and 0.017 mm^2/kPa ($P = 0.0029$) lower in *ADD3 AA* than *ADD3 GG* homozygotes with an additive effect of the *G* allele. In multiple-gene analyses, the association of brachial diameter and distensibility with the *ADD3 G* allele occurred only in *ADD1 GlyGly* homozygotes. Otherwise, the associations between the arterial phenotypes in the three vascular beds and the *ADD1* or *ADD2* polymorphisms were not significant. In family-based analyses, the multivariate-adjusted heritability was 0.52, 0.38, and 0.30 for brachial diameter, distensibility, and cross-sectional compliance, respectively ($P < 0.001$). There was no evidence for population stratification ($0.07 \le P \le 0.96$). Transmission of the mutated *ADD3 G* allele was associated with smaller brachial diameter in 342 informative offspring (-0.12 ± 0.04 mm; $P = 0.0085$) and in 209 offspring, who were *ADD1 GlyGly* homozygotes (-0.14 ± 0.06 mm; $P = 0.018$).

CONCLUSIONS

In *ADD1 GlyGly* homozygotes, the properties of the brachial artery are related to the *ADD3* (*A386G*) polymorphism, but the underlying mechanism needs further clarification.

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Adducin is a ubiquitously expressed membrane skeleton protein, which consists of either α- and β- or α- and γ-subunits. Mutation of the α-adducin gene (*ADD1*) is linked with increased Na^+,K^+-ATPase activity[1,2] and increased renal tubular sodium reabsorption.[3] Variation in the Na^+,K^+-ATPase activity and in the intracellular Na^+ concentration might influence the sodium-dependent transmembranous Ca^{2+} transport in vascular smooth muscle cells and via this mechanism might affect arterial tone.[4]

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In the participants of the Flemish Study on Environment, Genes and Health Outcomes, interaction between the genes encoding *ADD1* (*Gly460Trp* polymorphism), the angiotensin-converting enzyme, and the angiotensin II type-1 receptor influenced the distensibility, cross-sectional compliance, and intima-media thickness of the femoral artery.[5,6] In the same Flemish population[7] and in Polish and Russian subjects,[8] we noticed that blood pressure and the prevalence of hypertension were associated with the *C1797T* polymorphism in the β-adducin subunit (*ADD2*), particularly in postmenopausal women.[7] *T* allele carriers had significantly higher 24-h systolic blood pressure than *CC* homozygotes.[8] Moreover, Cwynar *et al.* reported interaction between the *ADD1 Gly460Trp* polymorphism and the *A386G* polymorphism in the γ-adducin subunit (*ADD3*).[9] Peripheral and central pulse pressures were higher in carriers of both the *ADD1 Trp* allele and the *ADD3 G* allele.[9]

To our knowledge, no previous study investigated whether arterial properties are related to genetic interactions between the three adducin subunits. We addressed this question in the participants of the Flemish Study on Environment, Genes and Health Outcomes with available ultrasonographically measured arterial phenotypes.

METHODS

Study population. The Flemish Study on Environment, Genes and Health Outcomes is part of the European Project on Genes in Hypertension[10] and is embedded in the InGenious HyperCare Network of Excellence. From August 1985 until July 2003, we recruited a random sample of families from a geographically defined area in Northern Belgium. The Ethics Committee of the University of Leuven approved the study. All participants or their parents gave informed written consent. The participation rate averaged 64.3%.

Of 1,306 participants, who underwent a vascular ultrasound examination,[11] 1,180 (90.3%) had high-quality images obtained at the common-carotid, femoral, and brachial arteries. We excluded 39 participants, because of missing genotypes, and seven because of incomplete information on important covariates. In addition, we detected eight cases of inconsistency in Mendelian segregation. Thus, the number of subjects analyzed totaled 1,126.

Clinical and biochemical measurements. For at least 3 h before the examination, the participants refrained from heavy exercise, smoking, and alcohol or caffeine-containing beverages. Trained nurses measured the subjects' anthropometric characteristics, heart rate and blood pressure. They administered a questionnaire to collect information about each participant's recent medical history, smoking and drinking habits, and intake of medications. Each subject's blood pressure was the average of five consecutive readings measured before the ultrasound examination after the subjects had rested in the sitting position for at least 5 min. Mean arterial pressure was diastolic pressure plus one-third of pulse pressure. Hypertension was defined as a blood pressure of at least 140 mm Hg systolic or 90 mm Hg diastolic, and/or the use of antihypertensive drugs. Body mass index was weight in kilograms divided by the square of height in meters.

Arterial measurements. Using a pulsed ultrasound wall-tracking system (Wall Track System; Pie Medical, Maastricht, The Netherlands), three trained researchers obtained vascular measurements at the common-carotid artery 2 cm proximal of the carotid bulb, at the femoral artery 1 cm proximal of the bifurcation into the profound and superficial branches, and at the right brachial artery 2 cm proximal of the antecubital fossa.

During the ultrasound examination, an automated oscillometric device (Dinamap 845; Critikon, Tampa, FL) recorded blood pressure at the upper arm at 5-min intervals. As for the conventional auscultatory measurements, cuff size was adjusted to the circumference of the upper arm.[10] Standard cuffs had an inflatable bladder of 12 × 24 cm. Larger cuffs had a 15 × 35 cm bladder. As described elsewhere,[12] the observers used applanation tonometry with a pencil-shaped probe (Millar Instruments, Houston, TX) and calibration to mean arterial pressure and diastolic blood pressure at the brachial artery to derive the local pulse pressure at the other arteries. We computed the distensibility (DC) and cross-sectional compliance (CC) from the diastolic cross-sectional area (A), the systolic increase in cross-sectional area (ΔA) and the local pulse pressure (PP) using the formula:[13] DC = (ΔA/A)/PP and CC = ΔA/PP. A and ΔA were calculated from diameter (D) and the change in diameter (ΔD) as $A = \pi \times (D/2)^2$ and $\Delta A = \pi \times ((D + \Delta D)/2)^2 - \pi \times (D/2)^2$, respectively. The intra-observer intra-session variability was <10% for the carotid measurements, <5% for the femoral and brachial diameter, and amounted from 10 to 15% for the femoral and brachial cross-sectional compliance and distensibility.[14] The intra-observer inter-session and inter-observer intra-session variability were of the same order of magnitude.[14]

Genotypes. We extracted DNA from white blood cells. For genotyping, we used a 5′ nuclease detection assay implemented on an ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA). Primers, probes, and PCR conditions for *ADD1 Gly460Trp* (rs4961),[15] *ADD2 C1797T* (rs4984),[7] and *ADD3 IVS11+386A>G* (rs3731566)[9] genotyping have already been described in detail elsewhere.

Statistical methods. For database management and statistical analyses, we used the SAS software (SAS Institute, Cary, NC), version 9.1. We compared means and proportions, using the large sample *z*-test and Fisher's exact test, respectively. We assessed Hardy–Weinberg proportions in unrelated founders, using an exact test based on Monte Carlo permutations. Our statistical methods also included single and multiple linear regressions. We searched for possible covariates of the arterial phenotypes, using a stepwise regression procedure with the *P* values for independent variables to enter and to stay in the model set at 0.15. As covariates,

Table 1 | Characteristics of participants

	Founders and unrelated participants (*n* = 430)	Offspring (*n* = 696)	*P*
Anthropometry			
Women (%)	219 (50.9)	347 (49.9)	0.76
Age (years)	51.5 ± 12.4	39.0 ± 15.1	<0.0001
Height (cm)	167.3 ± 9.1	169.4 ± 9.3	0.0002
Weight (kg)	71.5 ± 12.6	71.1 ± 14.2	0.60
Body mass index (kg/m^2)	25.5 ± 3.6	24.7 ± 4.2	0.0011
Systolic pressure (mm Hg)	130.5 ± 15.8	124.3 ± 14.4	<0.0001
Diastolic pressure (mm Hg)	81.8 ± 9.8	77.3 ± 11.0	<0.0001
Mean arterial pressure (mm Hg)	98.1 ± 10.5	93.0 ± 11.1	<0.0001
Heart rate (bpm)	62.5 ± 9.9	61.9 ± 9.4	0.34
Questionnaire data			
Hypertensives (*n* (%))	173 (40.2)	164 (23.6)	<0.0001
Antihypertensive treatment (*n* (%))	71 (16.5)	76 (10.9)	0.0082
Current smokers (*n* (%))	128 (29.8)	205 (29.4)	0.95
Alcohol intake ≥5 g/day (*n* (%))	132 (30.7)	365 (52.4)	<0.0001

Values are either mean ± s.d. or number (%) of subjects. *P* values are for the differences between founders and unrelated participants compared to offspring. The number of founders and unrelated subjects was 187 and 243, respectively.

we considered observer, sex, age, body mass index, heart rate, mean arterial pressure, and binary variables (0,1), coding for current smoking, alcohol intake, and use of antihypertensive drugs.

We performed both population- and family-based analyses. In the former approach, we applied a generalization of the standard linear model as implemented in the PROC MIXED procedure of the SAS package to test the associations between phenotypes and single-nucleotide polymorphisms (SNPs), while accounting for the nonindependence of phenotypes within families and adjusting for covariates. In the family-based analyses, we evaluated the within- and between-family components of phenotypic variability, using the orthogonal model proposed by Abecasis and colleagues.[16] We implemented the quantitative transmission disequilibrium test, using a mixed model with similar adjustments as in population-based analyses. The within-family component of phenotypic variance exhibits robustness to population stratification.

Table 2 | Arterial properties

Characteristics	Founders and unrelated participants ($n = 430$)	Offspring ($n = 696$)	P
Common carotid artery			
Diameter (mm)	7.35 ± 0.94	7.04 ± 0.89	<0.0001
Pulse pressure (mm Hg)	48.4 ± 13.5	47.2 ± 12.1	0.13
Distensibility (10^{-3}/kPa)	21.4 ± 10.2	28.9 ± 14.1	<0.0001
Cross-sectional compliance (mm^2/kPa)	0.89 ± 0.39	1.09 ± 0.45	<0.0001
Femoral artery			
Diameter (mm)	9.24 ± 1.39	9.16 ± 1.57	0.36
Pulse pressure (mm Hg)	53.0 ± 13.0	52.2 ± 12.9	0.33
Distensibility (10^{-3}/kPa)	9.0 ± 5.6	11.5 ± 7.3	<0.0001
Cross-sectional compliance (mm^2/kPa)	0.58 ± 0.35	0.73 ± 0.45	<0.0001
Brachial artery			
Diameter (mm)	4.28 ± 0.83	4.36 ± 0.85	0.10
Pulse pressure (mm Hg)	48.8 ± 10.8	48.3 ± 9.3	0.45
Distensibility (10^{-3}/kPa)	16.3 ± 11.4	10.8 ± 10.3	<0.0001
Cross-sectional compliance (mm^2/kPa)	0.21 ± 0.14	0.15 ± 0.13	<0.0001

Values are mean ± s.d. *P* values are for the differences between founders and unrelated participants compared to offspring.

RESULTS

We divided study participants into founders ($n = 187$) and unrelated subjects ($n = 243$) as compared with offspring ($n = 696$). Subjects in the founders group (mean age 51.5, range 12.6–81.5 years) were older compared with offspring (39.0; range 10.9–81.0 years). Table 1 summarizes their demographic characteristics. In contrast to founders, more offspring reported alcohol intake. In drinkers, the median alcohol consumption was 10 g per day (interquantile range, 4–20). In smokers, median tobacco use was 14 cigarettes per day (interquantile range, 8–20). Table 2 lists the arterial properties by generation and vascular territory. Brachial distensibility and cross-sectional compliance were higher in founders than in offspring whereas the opposite was the case for the corresponding common-carotid and femoral properties.

Table 3 summarizes the genotype and allele frequencies for the adducin genes in the whole study population. In the founder generation ($0.41 < P < 0.67$), the genotype frequencies complied with Hardy–Weinberg proportions.

Population-based analyses

In stepwise multiple regression, in line with our previous publications,[11] we identified the following covariates as significant

Table 3 | Allele and genotype frequencies

Gene	Allele		Genotype		
ADD1	*Gly*	*Trp*	*GlyGly*	*GlyTrp*	*TrpTrp*
	1,722 (76.53)	530 (23.5)	666 (59.2)	390 (34.6)	70 (6.2)
ADD2	*C*	*T*	*CC*	*CT*	*TT*
	2,000 (88.8)	252 (11.2)	888 (78.9)	224 (19.9)	14 (1.2)
ADD3	*A*	*G*	*AA*	*AG*	*GG*
	1,297 (57.6)	955 (42.4)	388 (34.4)	521 (46.3)	217 (19.3)

Values indicate number of alleles or genotypes (%).

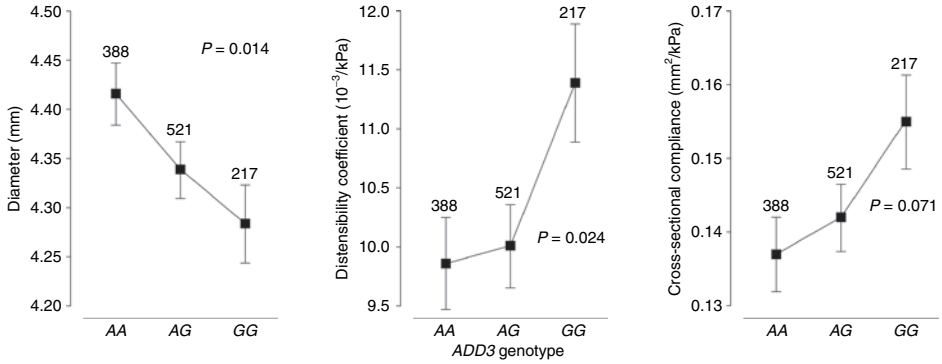

Figure 1 | Diameter, distensibility, and cross-sectional compliance of the brachial artery in relation to the *ADD3 A386G* polymorphism. The analyses were adjusted for observer, sex, age, body mass index, mean arterial pressure, heart rate, smoking, alcohol intake, and the use of antihypertensive drugs and account for family clusters. Values are least square means ± s.e. The number of subjects contributing to each plotted point is given. *P* values are for linear trend across the *ADD3* genotypes.

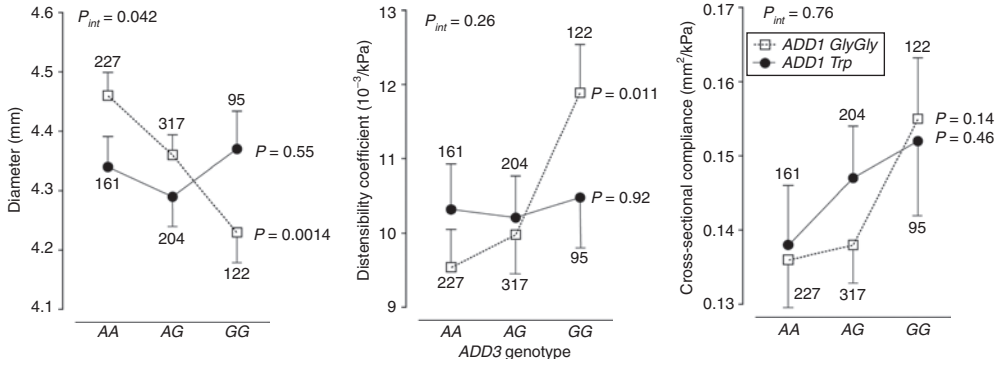

Figure 2 | Diameter, distensibility, and cross-sectional compliance of brachial artery in relation to the *ADD1 Gly460Trp* and *ADD3 A386G* polymorphisms. P_{int} indicates the significance of the two-way interaction between *ADD1* and *ADD3*. For further explanation, see **Figure 1**.

determinants of one or more of the arterial phenotypes in the three vascular beds under study: sex, age, body mass index, mean arterial pressure, heart rate, smoking, daily alcohol intake in excess of 5 g, and use of antihypertensive drugs. We adjusted all phenotype–genotype associations for these covariates, and in addition for observer.

In single-gene analyses (**Figure 1**), brachial diameter was 0.15 mm (95% confidence interval (CI), 0.05–0.24; $P = 0.0022$) larger, and brachial distensibility and cross-sectional compliance were 1.55×10^{-3}/kPa (CI, 0.33–2.77; $P = 0.013$) and 0.017 mm^2/kPa (CI, 0.002–0.032; $P = 0.0029$) lower in *ADD3 AA* homozygotes than in their *GG* counterparts. As shown by the *P* values for linear trend, the *G* allele had an additive effect in relation to these phenotypes. In single-gene analyses, we did not find any other significant association between the arterial phenotypes under study in the three vascular beds and the *ADD1* ($P \geq 0.26$) or *ADD2* ($P \geq 0.27$) polymorphisms.

In multiple-gene analyses, there was a significant gene–gene interaction between *ADD1* and *ADD3* in relation to the diameter of the brachial artery ($P = 0.042$; **Figure 2**). In carriers of the wild-type *ADD1*, brachial diameter was 0.23 mm (CI, 0.11–0.34; $P = 0.0001$) larger in *ADD3 AA* homozygotes than in *GG* homozygotes with a significant *P* value for linear trend ($P = 0.0014$). This was not the case in *ADD1 Trp* allele carriers (*P* for linear trend, 0.55). Furthermore, in carriers of the wild-type *ADD1*, brachial distensibility was 2.54×10^{-3}/kPa (CI, 0.95–4.13; $P = 0.0018$) lower in *ADD3 AA* homozygotes than in their *GG* counterparts with a significant *P* value for trend ($P = 0.011$). Although in *ADD1 Trp* allele carriers brachial distensibility did not change with the *ADD3* genotype ($P = 0.92$), the *P* value for the *ADD1*-by-*ADD3* gene–gene interaction was only 0.26. We did not find any significant gene–gene interaction between carotid and femoral arterial properties and the adducin subunits polymorphisms. In a sensitivity analysis, from which we excluded 147 patients on antihypertensive drugs, we reproduced our results for the properties of the brachial artery (**Figure 3**).

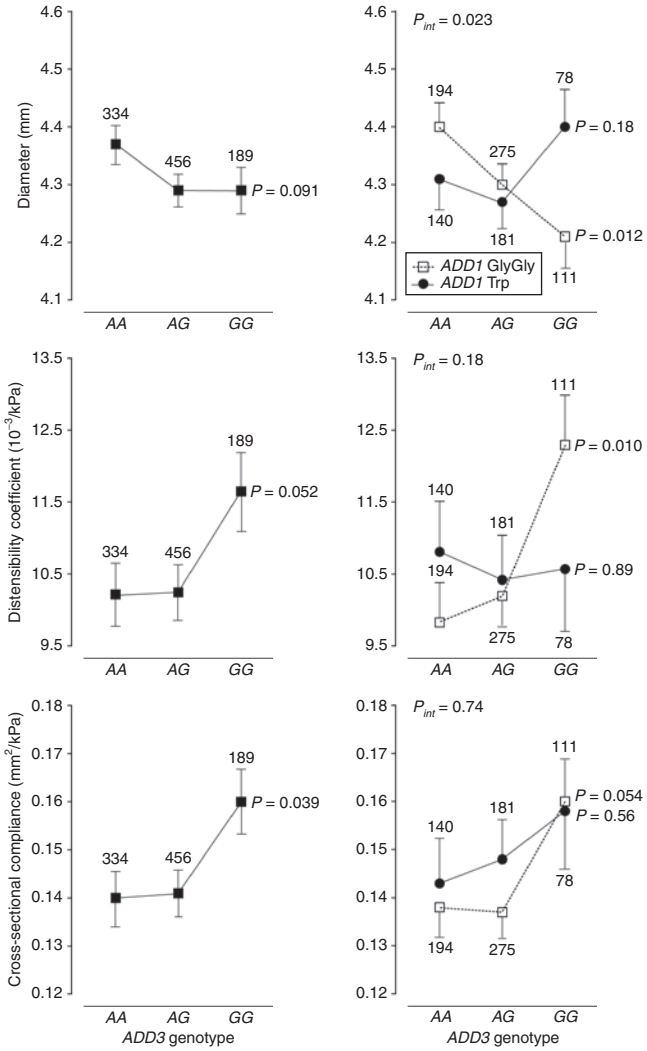

Figure 3 | Diameter, distensibility, and cross-sectional compliance of brachial artery in relation to the *ADD1 Gly460Trp* and *ADD3 A386G* polymorphisms in 979 untreated subjects. For further explanation, see **Figure 1**.

Family-based analyses

Our study sample consisted of 58 pedigrees, of which 27 spanned more than two generations, and additionally of 273 unrelated subjects. The number of offspring per pedigree was <3 in 24 pedigrees, ranged from 3 to 8 in 30 families, and amounted to >8 in 4 pedigrees.

The multivariate-adjusted heritability estimates, as reported by the Abecasis software,[16] were 0.52 for the brachial diameter,

0.38 for distensibility, and 0.30 for cross-sectional compliance ($P < 0.001$ for all). Abecasis' orthogonal model did not reveal population stratification ($0.07 \leq P \leq 0.96$). In 342 informative offspring, transmission of the mutated *ADD3 G* allele was associated with a significant decrease in brachial diameter (-0.12 ± 0.04 mm; $\chi^2 = 6.99$; $P = 0.0085$). We observed a similar trend in 209 informative offspring, who were homozygous for the *ADD1 Gly* allele (-0.14 ± 0.06 mm; $\chi^2 = 5.72$; $P = 0.018$). For brachial distensibility or cross-sectional compliance, these estimates were not significant ($0.28 \leq P \leq 67$).

DISCUSSION

The key finding of our study was that brachial diameter decreased, while brachial distensibility increased with the *ADD3 G* allele, and that these associations were confined to *ADD1 GlyGly* homozygotes. In the family-based analyses, we did not find any evidence for population stratification. Transmission of the *ADD3 G* allele was associated with smaller brachial diameter in all informative offspring as well as in offspring homozygous for *ADD1 Gly* allele. *ADD1* and *ADD2,* alone or in combination with each other, were not associated with the arterial properties in the three arterial beds. This was also true for *ADD1* and *ADD3* in relation to the carotid and femoral phenotypes.

Adducin functions within the cell as a tissue-specific heterodimer, which consists of either α- and β-subunits or α- and γ-subunits. This provides the physiologic and biochemical basis for studying the interaction among the three adducin genes, which map to different chromosomes.[3] To our knowledge, no prior study investigated the genetic interactions between the three adducin subunits in relation to arterial properties. In the present population, we previously demonstrated that interaction between the *ADD1*, the angiotensin-converting enzyme (*ACE I/D)*, and the angiotensin II type-1 receptor (*AT1R C1166A*) polymorphisms modulated the properties of the femoral artery. Indeed, in the presence of *the ADD1 460Trp* allele, femoral intima-media thickness was higher in *ACE D* carriers than *II* homozygotes.[5] Moreover, in *ACE DD* homozygotes, carriers of mutated *ADD1* had higher femoral distensibility and cross-sectional compliance than those with the wild-type *ADD1*.[6]

Our epidemiologic study only allows speculation about the reasons why there might be an association between the properties of the brachial artery, a small muscular artery, and the *ADD3 A386G* polymorphism. One possible mechanism is that the polymorphism might affect the neurogenic tone of vascular smooth muscle cells. Indeed, in rat models, increased blood pressure was associated with a decrease in the hypothalamic levels of γ-adducin mRNA and protein.[17,18] Furthermore, inhibition of γ-adducin by intracellular delivery of γ-adducin-specific antibodies increased the neuronal firing rate possibly via regulation of Na^+,-K^+-ATPase.[17] On the other hand, given that the phenotypes representative of two other arterial segments did not shown any association with the adducin SNPs, this suggests that local mechanisms in the brachial artery, rather than the central nervous system, might underlie the demonstrated findings.

We did not find any association between the polymorphisms under study and the properties of the elastic carotid artery. The central elastic arteries, such as the carotid artery, and the more peripheral muscular conduit vessels, including the brachial and femoral arteries, have different properties.[19] Going from the central to the peripheral arteries, the collagen/elastin ratio reverses, vascular smooth muscle cells become the predominant component of the arterial wall, and the phenotype of the vascular smooth muscle cells changes.[19] Thus, the effects of genetic variation in adducin subunits on different arteries must be complex and may depend on the vessel wall component that is involved.

Our study should be interpreted within the context of its limitations. First, our epidemiologic study demonstrated association of arterial properties with variation in *ADD3*, but did not provide direct information on the mechanisms underlying these phenotype–genotype associations. Second, we did not use the tagging SNP strategy to investigate genotype–phenotype associations. However, we have extensively investigated nucleotide and haplotype variation in both *ADD1* and *ADD3*, but in experimental studies the additional common SNPs did not enhance the phenotype–genotype association, over and beyond the single SNPs *ADD1 rs4961* and *ADD3 rs3731566*. We will publish these results elsewhere. Therefore, we focused on the *Gly460Trp* polymorphism of the *ADD1* gene, because it is functional, increases Na^+,K^+-ATPase activity,[1,2] and is associated with various cardiovascular[5,6,9] and renal phenotypes.[3] The *A* to *G* substitution in *ADD3* is located in intron 11 (*IVS11 +386A>G* –rs3731566).[3] Neither previous publications nor genome browser databases provided any suggestion about the functional role of the *ADD3 A386G* polymorphism. A nucleotide variation analysis is needed to elucidate the pattern of linkage disequilibrium of the entire *ADD3* locus and to establish whether this intronic common polymorphism is linked with the "causal" SNP or is the regulatory variant "*per se*." According to the latter hypothesis, preliminary data showed that *ADD3* mRNA level was significantly enhanced in *GG* homozygotes compared with the other genotypes in 39 kidney cortex samples from human donors (L.C., unpublished data). Third, arterial measurements are quantitative traits prone to measurement error. However, the repeatability and reproducibility of the arterial phenotypes collected in the present study is high.[14] We adjusted for observer bias. Moreover, for the brachial diameter in relation to the *ADD3* and *ADD1* polymorphisms, there was consistency between the population-based and the family-based analyses. We did not find any evidence for population stratification. Fourth, we did not adjust significance levels for multiple testing. However, arterial diameter, distensibility, and cross-sectional compliance are correlated phenotypes. In such case, multiple testing is not indicated, because each test does not provide a completely independent opportunity for type I error.[20]

In conclusion, the properties of the brachial artery are related to the *ADD3* (*A386G*) polymorphism, particularly in *ADD1 GlyGly* homozygotes. Further clinical observations and experimental studies should confirm the present observation and eventually clarify the underlying mechanism.

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