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RESEARCH LETTER

Blood pressure and metabolic phenotypes in relation to the *ADRB1 Arg389Gly* and *ADRA2B I/D* polymorphisms in a White population

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Previous studies of cases and controls or selected patients demonstrated association of the *ADRB1 Arg389Gly* and *ADRA2B I/D* polymorphisms with blood pressure, measures of obesity, and/or serum lipids. In multivariate-adjusted analyses of 1881 subjects randomly recruited from a White population, *ADRB1 ArgArg* homozygotes, compared with *Gly* allele carriers, had higher diastolic blood pressure (79.4 vs 78.4 mm Hg; $P = 0.012$), and higher serum high-density lipoprotein (HDL) cholesterol (1.33 vs 1.29 mmol l^{-1}; $P = 0.020$), whereas none of the other cardiovascular or metabolic phenotypes reached significance in relation to the two polymorphisms. Our family-based design excluded population stratification as a possible explanation for the significant association of the *ADRB1 Arg389Gly* polymorphism with blood pressure and HDL cholesterol.

The β1 (*ADRB1*) and α2B (*ADRAB2*) adrenergic receptors are important in the regulation of blood pressure (BP), cardiovascular function and lipid metabolism. Their genes locate to chromosomes 10q24–26 and 2p13–q13, respectively. The *ADRB1 Arg389Gly* polymorphism leads to the substitution of the amino acid arginine by glycine near to the intracellular carboxyl terminal, which is involved in G-protein binding.[1] The *ADRA2B* insertion/deletion (Ins + 910Del (*I/D*)) polymorphism involves the deletion of three glutamic acids from a repeat element in the third intracellular loop of the protein.[2] *ADRB1 Arg* allele carriers have increased adenyl cyclase activity in response to agonists, such as isoproterenol, epinephrine and norepinephrine.[1] The *ADRA2B* deletion results in a loss of agonist-promoted desensitization.[3]

Case–control studies[1] and continuous trait analyses in patients with coronary heart disease[4] demonstrated association of the *ADRB1 Arg389Gly* polymorphism with hypertension[1] or with systolic[1] or diastolic[1,4] BP. However, none of these studies involved families randomly recruited from a general population. Furthermore, we previously demonstrated that in Chinese men the *ADRA2B I* allele was associated with higher BP, but with lower body mass index and waist-to-hip ratio, and with less insulin resistance.[5] Several studies in diabetic[6] and non-diabetic[2,7] patients reported association of the *ADRA2B I/D* polymorphism with metabolic phenotypes. However, with the exception of our small Chinese Study ($n = 481$),[5] none of these previous reports was *ADRA2B* involved a family-based population sample or assessed the population stratification as a possible explanation of significant phenotype–genotype associations.

In the present study, we investigated in a family-based cohort possible association of the *ADRB1 Arg389Gly* and *ADRA2B I/D* polymorphisms with BP and metabolic phenotypes, analysed as continuous traits. For analysis, we applied a variance decomposition method, which is robust to population stratification or admixture. The University of Leuven Ethics Committee approved the Flemish Study on Environment, Genes and Health Outcomes.[8] From August 1985 until December 2006, we randomly recruited 2165 subjects from a geographically defined area in Northern Belgium.[8] The participation rate averaged 64.3%. All participants gave informed written consent. We excluded 284 subjects from analysis, because their DNA could not be extracted or genotyped ($n = 42$), because of Mendelian inconsistency ($n = 22$), because participants were younger than 18 years ($n = 200$), or because of important co-variables had not been measured ($n = 20$). Thus, the number of subjects statistically analysed totalled 1881.

At the enrolment home visit, trained nurses measured BP and anthropometric characteristics. They also administered a questionnaire to collect information about each subject's medical history, smoking and drinking habits, and intake of medications. BP was the average of five consecutive readings. Body mass index was weight in kilograms divided by the square of height in metres. The waist-to-hip ratio, determined by means of a tape measure, was the smallest circumference at the waist divided by the largest circumference at the hip. We measured serum total and high-density lipoprotein (HDL) cholesterol and genotyped the *ADRAB1 Arg389Gly* and *ADRA2B I/D* polymorphisms (genotyping methods available as Supplementary Information). Hypertension was a BP of at least 140 mm Hg systolic or 90 mm Hg diastolic or use of anti-hypertensive drugs.

For statistical analysis, we used SAS software, version 9.1.3 (SAS Institute, Cary, NC, USA). We compared means and proportions by the standard normal *z*-test and the χ^2-statistic, respectively. We searched for possible covariates of the phenotypes under study, using stepwise multiple regression analysis with the *P*-value for independent variables to enter and stay in the models set at 0.15. In population-based analyses, we tested phenotype–genotype associations, using the PROC MIXED procedure of the SAS package, while adjusting for co-variables and family clusters. We tested for heterogeneity, using appropriate interaction terms. In family-based analyses, we evaluated the within- and between-family components of phenotypic variance, using the orthogonal model proposed by Abecasis *et al*.[9]

The study population consisted of 1802 relatives from 175 families and 79 unrelated subjects. Mean values were 45.5 years for age and 124.7 and 76.9 mm Hg for systolic and diastolic BP, respectively. The study sample included 525 (27.9%) hypertensive patients, of whom 281 were on anti-hypertensive drugs. Women compared to men had lower BP and less frequently reported smoking and alcohol consumption (Supplementary Table). The frequencies of the *ADRB1* genotypes (*ArgArg* 56.2%, *ArgGly* 36.9% and *GlyGly* 6.9%; $P=0.66$) and the *ADRA2B* genotypes (*II* 45.7%, *ID* 41.7%, and *DD* 12.5%; $P=0.05$) did not deviate from Hardy–Weinberg proportions. Table 1 shows the multivariate-adjusted phenotype–genotype associations for the two polymorphisms in the population-based analyses, which accounted for family clusters. Both before and after adjustment (Table 1) for co-variables, *ADRB1 Arg* homozygotes, compared with *Gly* allele carriers, had higher diastolic BP (79.4 vs 78.4 mm Hg; $P=0.012$) and higher serum HDL cholesterol (1.33 vs 1.29 mmol l^{-1}; $P=0.020$). Otherwise, none of the phenotype–genotype associations for both genes under study reached statistical significance. Furthermore, a sensitivity analysis by sex did not reveal significant heterogeneity. Considering both single-nucleotide polymorphisms (SNPs) together, with or without their interaction, did not significantly improve the fit of the mixed models. This was also the case, when we applied an additive model for the *ADRA2B I/D* polymorphism ($P\geqslant 0.24$).

Our family-based analyses included 175 pedigrees, of which 72 spanned more than two generations. We adjusted the family-based analyses as in Table 1. For both the *ADRB1 Arg389Gly* and *ADRA2B I/D* polymorphisms, the orthogonal model did not reveal significant population stratification for any phenotype–genotype association ($P\geqslant 0.23$ and $P\geqslant 0.32$, respectively). The effect size (± s.e.) of the transmission of the *ADRB1 Gly* allele from parents to informative offspring was −0.33 mm Hg ($n=906$; $P=0.64$) for diastolic BP and −0.045 mmol l^{-1} ($n=906$; $P=0.07$) for HDL cholesterol.

The key finding of our study was that in the absence of population stratification diastolic BP and serum HDL cholesterol were weakly but significantly lower in *ADRB1 Gly* allele carriers than in *ArgArg* homozygotes. However, we could not replicate the association of BP and the metabolic phenotypes with *ADRA2B I/D* polymorphism.

Several tissues, including adipocytes, cardiac myocytes and kidney cells, express *ADRB1*. Its activation increases BP, stimulates lipolysis,[10] enhances myocardial contractility,[1] and stimulates the release of renin.[1] In transfected Chinese hamster fibroblasts, the *ADRB1 Arg389Gly* variant affects the receptor's ability to bind the Gs molecule.[11] The SNP is a 'loss-of-function' polymorphism with the *ArgArg* homozygotes exhibiting a three- to fourfold higher isoprenaline-stimulated adenylyl cyclase

Table 1 Phenotype–genotype associations in population-based analyses

	ADRB1 Arg389Gly		P	*ADRA2B I/D*		P
	GlyArg+GlyGly (n = *808*)	*ArgArg* (n = *1073*)		*ID+DD* (n = *1026*)	*II* (n = *855*)	
Clinical features						
Systolic pressure (mm Hg)[a]	128.3 ± 0.9	128.9 ± 0.8	0.31	128.7 ± 0.8	128.6 ± 0.8	0.83
Diastolic pressure (mm Hg)[a]	78.4 ± 0.6	79.4 ± 0.6	0.01	79.0 ± 0.6	78.9 ± 0.6	0.90
Pulse rate (b.p.m.)	66.6 ± 0.6	66.8 ± 0.6	0.58	66.4 ± 0.6	67.1 ± 0.6	0.12
Body mass index (kg m^{-2})[b]	26.5 ± 0.2	26.5 ± 0.3	0.72	26.4 ± 0.2	26.7 ± 0.2	0.12
Body weight (kg)	75.5 ± 0.8	75.9 ± 0.8	0.39	75.5 ± 0.8	76.1 ± 0.8	0.25
Waist-to-hip ratio[c]	0.854 ± 0.004	0.851 ± 0.004	0.24	0.852 ± 0.004	0.854 ± 0.004	0.44
Serum cholesterol (mmol l^{-1})[d]						
Total	5.49 ± 0.07	5.45 ± 0.07	0.46	5.44 ± 0.07	5.51 ± 0.07	0.16
HDL	1.29 ± 0.02	1.33 ± 0.02	0.02	1.33 ± 0.02	1.31 ± 0.02	0.62

Abbreviation: HDL, high-density lipoprotein.
Values are least-squared means ± s.e. On the basis of the results of stepwise multiple regression, all models were adjusted for sex, age, smoking and alcohol consumption, and use of diuretics, β-blockers and female sex hormones. We additionally adjusted blood pressure, pulse rate and serum cholesterol for the quadratic term of age and serum cholesterol also for the use of lipid-lowering drugs.
[a]Average of five blood pressure readings obtained at one home visit.
[b]The body mass index is weight in kilograms divided by the square of height in metres.
[c]The waist-to-hip ratio is the smallest circumference at the waist divided by the largest circumference at the hip level.
[d]To convert values for total and HDL cholesterol to milligrams per 100 ml, divided by 0.02586.

activity than the *Gly389* variant due to a better coupling of the *Arg389* to Gs protein than the *Gly389* variant.[11] On the basis of these experimental evidences, we speculated that *ADRB1 ArgArg* homozygotes might be more responsive to sympathetically mediated increase in peripheral resistance. If these hypotheses were true, it might explain the slight increase in diastolic BP as observed in the present study, peripheral arterial resistance being the main determinant of diastolic BP. In line with our current findings, the *Arg389Gly* polymorphism in *ADRB1* might be a potential pharmacogenetic target. Indeed, *ADRB1 ArgArg* homozygotes are more sensitive to β-blockers, or to β1-adrenergic agonist activity.[12] *ADRB1* stimulation activates lipolysis.[1] We hypothesize that facilitated signal transduction after stimulation of the β1-adrenergic receptor[1] might explain the higher serum HDL cholesterol level in *ADRB1 ArgArg* homozygotes.

Although functional, the evidence linking the *ADRA2B I/D* polymorphism with BP and metabolic phenotypes remains equivocal. In our previous family-based study in Chinese,[5] we found associations only in men, but not in women. Other studies of the *ADRA2B I/D* polymorphism mainly involved selected groups of patients.[2,7] In addition to selection, small sample size and the possibility of undetected population stratification limit the interpretation of most *ADRA2B* study in humans.

In conclusions, our study showed a weak but statistically significant association of diastolic BP and HDL cholesterol with the *ADRB1 Arg389Gly* polymorphism in the absence of population stratification, whereas the *ADRA2B* polymorphism does probably not contribute much to the variation of BP and metabolic phenotypes in unselected White Europeans.

What is known about this topic

- Case–control studies and continuous trait analyses in patients with coronary heart disease demonstrated association of the *ADRB1 Arg389Gly* polymorphism with hypertension or blood pressure
- Several studies in diabetic and non-diabetic patients reported association of the *ADRA2B I/D* polymorphism with blood pressure or metabolic phenotypes
- The sample size ranged from 40 to 935 (median 223) and from 166 to 1589 (median 358) in the *ADRB1* and *ADRA2B* studies, respectively. With the exception of one, none of the previous studies was population based

What this study adds

- We investigated 1881 subjects, randomly recruited from a White population
- Our family-based design excluded population stratification for phenotype–genotype associations, involving blood pressure and metabolic phenotypes
- Our study confirmed association of diastolic BP and HDL cholesterol with the *ADRB1 Arg389Gly* polymorphism in the absence of population stratification. It did not support a contribution of the *ADRA2B* polymorphism to variation in blood pressure or metabolic phenotypes in an unselected population

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Conflict of interest

None of the authors has a conflict of interest with regard to the data presented in this paper.

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Supplementary Information accompanies the paper on the Journal of Human Hypertension website (http://www.nature.com/jhh)