Association Between Physical Activity and Blood Pressure Is Modified by Variants in the G-Protein Coupled Receptor 10

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Abstract—Hypertension is strongly related to cardiovascular disease and all-cause mortality. Exercise reduces blood pressure but the response varies between individuals. The mechanisms by which physical activity energy expenditure (PAEE) modifies blood pressure are not fully defined but include modulation of sympathetic tone. Novel polymorphisms in the G-protein coupled receptor (GPR10) have been linked with high blood pressure. GPR10 may mediate the relationship between PAEE and blood pressure via central nervous mechanisms. We examined whether two GPR10 polymorphisms (G-62A and C914T) modify the association between PAEE and blood pressure in the MRC Ely study (N = 687). When stratified by the C914T genotype, there were between-group differences for body mass index (BMI) (P = 0.05), diastolic blood pressure (DBP) (P = 0.006), and systolic blood pressure (SBP) (P = 0.005). No differences were found between G-62A genotypes. The previously reported inverse relationship between PAEE and blood pressure was not observed in minor allele carriers for either polymorphism (A62 carriers: DBP β = 1.11, P = 0.52; SBP β = 1.66, P = 0.05. T914 carriers: SBP β = 3.27; P = 0.60) but was in common allele homozygotes (G62G: DBP β = -6.18 P = 0.00001; SBP β = -8.54 P = 0.0001. C914C: SBP β = -7.07; P = 0.00001). This corresponded to a significant interaction between PAEE and GPR10 polymorphisms on DBP (G-62A: P = 0.006) and SBP (G-62A: P = 0.008. C914T: P = 0.068). Significant interactions were observed between haplotype (derived from G-62A and C914T), PAEE, and blood pressure (DBP: P = 0.08; SBP: P = 0.023). The effect of physical activity on blood pressure is highly variable at population level. Knowledge of GPR10 genotype may define those who are least likely to benefit from physical activity. These findings may have relevance in the targeted treatment of hypertensive disease. (Hypertension. 2004;43:224-228.)

Key Words: exercise ■ epidemiology ■ blood pressure ■ genetics

There is a strong, continuous, and independent relationship between blood pressure and cardiovascular disease and all-cause mortality. Physical inactivity is strongly positively associated with hypertension, and intervention studies have demonstrated that increased physical activity is effective in the treatment of high blood pressure in a variety of populations. In view of this, physical activity is widely advocated in the treatment of hypertensive disease. Like all complex diseases, it is probable that a variety of environmental and genetic factors cause hypertension. These factors may act in an additive or multiplicative manner. Epidemiological studies have demonstrated that hypertension segregates within families and its prevalence differs between ethnicities. Common variants in a number of genes are thought to explain some of this difference. Because the protective effect of physical activity on blood pressure varies greatly from one person to the next, it is probable that this relationship is also modified by genotype.

We recently identified several variants within the G-protein coupled receptor (GPR10) gene. Of these, most had a very rare minor allele frequency (≤1%), and were therefore unsuitable for testing in our study. However, 2 single nucleotide polymorphisms (SNPs), C914T (P305L) and G-62A (non-coding), were more frequent (minor allele frequencies were 7% and 25%, respectively). In our earlier study we demonstrated using functional and epidemiological data that the C914T variant affects the binding of the ligand prolactin-releasing peptides (PrRPs) to GPR10, and that both C914T and G-62A are associated with diastolic and systolic blood pressure in a large white population. Although GPR10 and PrRP have not been extensively studied, physiological studies in mice have indicated a clear effect of PrRPs on blood pressure and appetite via hypothalamic processes. Thus far, however, the documented characterization of GPR10 in humans is limited to our study.

One of the primary mechanisms through which physical activity affects blood pressure regulation is through modulation of sympathetic nervous function. It is therefore biologically plausible that GPR10 and PrRP may be components in...
Methods

This study is based on a subsample of the MRC Ely Study, an ongoing investigation into the cause and pathogenesis of type 2 diabetes and related endpoints in 1122 men and women from Cambridgeshire, UK. The Ely cohort is a randomly selected, population-based sample. In the present study, only participants for whom data were available for PAEE, blood pressure, blood pressure medication, GPR10 genotype, body composition, and anthropometry were included. Missing data were attributable to abnormal ECG and, hence, exclusion from the exercise stress test, incomplete heart rate data because of equipment malfunction, inaccessible blood pressure medication data, genotyping failure, incomplete exercise stress test data, or refusal to perform exercise stress test. In the present subsample, the means and distributions for blood pressure, body mass index (BMI), and physical activity level compare well against other representative samples from the United Kingdom, suggesting that data omission in the present study was random. The Cambridgeshire Local Research Ethics Committee approved the study, and all participants provided written informed consent.

After an overnight fast, valid anthropometric and clinical data were obtained without knowledge of genotype in 687 participants without known diabetes at baseline. Participants provided information regarding the use of anti-hypertensive therapies. Blood pressure was measured with the participant seated by trained personnel using an Accutorr automated sphygmomanometer (Datascope, Cambridge, UK). Resting and PAEE expenditure were measured objectively using indirect calorimetry and continuous heart rate monitoring over an Accutorr automated sphygmomanometer (Datascope, Cambridge, UK). The Ely cohort is a randomly selected, population-based, on-going cohort study in which ongoing investigation into the cause and pathogenesis of type 2 diabetes and related endpoints in 1122 men and women from Cambridgeshire, UK. The Ely cohort is a randomly selected, population-based sample. In the present study, only participants for whom data were available for PAEE, blood pressure, blood pressure medication, GPR10 genotype, body composition, and anthropometry were included. Missing data were attributable to abnormal ECG and, hence, exclusion from the exercise stress test, incomplete heart rate data because of equipment malfunction, inaccessible blood pressure medication data, genotyping failure, incomplete exercise stress test data, or refusal to perform exercise stress test. In the present subsample, the means and distributions for blood pressure, body mass index (BMI), and physical activity level compare well against other representative samples from the United Kingdom, suggesting that data omission in the present study was random. The Cambridgeshire Local Research Ethics Committee approved the study, and all participants provided written informed consent.

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Genomic DNA was amplified by polymerase chain reaction (PCR) after isolation from whole blood using a QIAamp blood kit (Qiagen). PCR was performed using BioTaq (Bioline) and performed as recommended by the manufacturer. Thirty-five cycles (30 seconds at 96°C, 40 seconds at 60°C) were performed using a PTC-225 Peltier Thermal Cycler (MJ Research). After digestion, gel electrophoresis was performed using 3% (wt/vol) agarose gels (Gibco BRL) containing ethidium bromide, and the pattern of bands were visualized and recorded after exposure of the gel to ultraviolet radiation. As described previously, the G-62A and C914T polymorphisms were identified through sequencing of an extreme phenotype cohort. Although other GPR10 polymorphisms have previously been described, their rare minor allele frequencies render them unsuitable for testing for interaction studies. Thus, analyses were only performed on the G-62A and C914T polymorphisms. Sequencing was performed using BigDye terminator chemistry (Perkin-Elmer) and electrophoresis on an ABI 377 automated DNA sequencer (Perkin-Elmer). G-62A was genotyped by PCR amplification of the fragment containing this polymorphism, and digestion was performed with the restriction enzyme BstNI (New England Biolabs) in accordance with the manufacturer’s protocol. Individual haplotype combinations were estimated from individual genotype information using the program Snpaph (http://www-gene-cimr.cam.ac.uk/clayton/software). All interaction modeling was undertaken via generalized linear regression analyses using the SPSS software package (release 11.0). Statistical significance was set at 0.05. All interaction analyses were adjusted for BMI, age, blood pressure medication, and gender. PAEE was analyzed in its continuous form, except for the comparison between genotypes of blood pressure at

Unadjusted Mean Anthropometric and Energy Expenditure Characteristics Stratified by GPR10 Genotypes: The MRC Ely Study

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>G-62A n (m/f)</th>
<th>Age (y)</th>
<th>BMI (kg/m²)</th>
<th>DBP (mm Hg)</th>
<th>SBP (mm Hg)</th>
<th>PAEE (TEE/BMR)</th>
<th>G914T n (m/f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG (161/219)</td>
<td>57.2 (7.47)</td>
<td>26.6 (4.41)</td>
<td>75.9 (10.1)</td>
<td>125.8 (15.7)</td>
<td>1.86 (0.37)</td>
<td>0.06 (0.37)</td>
<td></td>
</tr>
<tr>
<td>GA (120/148)</td>
<td>57.5 (7.45)</td>
<td>26.5 (4.13)</td>
<td>77.5 (10.9)</td>
<td>127.7 (15.7)</td>
<td>1.84 (0.36)</td>
<td>1.83 (0.37)</td>
<td></td>
</tr>
<tr>
<td>AA (14/27)</td>
<td>58.7 (8.57)</td>
<td>26.7 (3.12)</td>
<td>77.2 (8.66)</td>
<td>130.3 (16.2)</td>
<td>1.83 (0.37)</td>
<td>1.83 (0.37)</td>
<td></td>
</tr>
</tbody>
</table>

Data shown are mean (SD); n = 687. m indicates male; f, female; BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; PAEE, physical activity energy expenditure.

ANOVA for difference between genotypes: *P < 0.05; †P < 0.01.

Results

All analyses were initially undertaken stratified by gender. However, because the direction of association was the same for men and women, data were combined. Men and women had similar BMIs; however, men were older (P < 0.05), had higher DBP (P < 0.001) and SBP (P < 0.001), and were more active (P < 0.001). The Table shows participant characteristics stratified by GPR10 genotypes. In this population, minor allele homozygotes for G-62A were present at ~6%, whereas 38.7% carried the homozygous genotype, and 55.3% carried both copies of the common allele. For C914T, only 2 people (0.3%) carried the homozygous T914T genotype, whereas 7.2% and 92.4% carried the C914T and C914C genotypes, respectively. The G-62A and C914T polymorphisms were in linkage disequilibrium (r² = 0.014, D’ = 1; P < 0.00001) and did not deviate significantly from Hardy-Weinberg predictions (P > 0.05).

When stratified by the G-62A genotype, the unadjusted means for age, PAEE, SBP, and DBP did not differ. However, when stratified by the C914T genotype, there were between-group differences for BMI (P < 0.05), DBP (P = 0.006), and SBP (P < 0.005) (Table 1). When genotype was excluded from the model, PAEE and blood pressure were strongly inversely related (DBP β = 4.38, P < 0.0001; SBP β = 6.13, P < 0.0001) (Figure 1). The interaction between the G-62A genotypes and PAEE on DBP and SBP was initially evaluated in a codominant model for the G-62A variant, because this is the most conservative approach. In this model interactions were observed for SBP (P = 0.009) and DBP (P = 0.01). Because the associations were similar for A62 allele heterozygotes and homozygotes, and because of the low frequency of A62 homozygotes, all subsequent interac-
tion analyses between PAEE and G-62A were conducted in models comparing G-62 allele homozygotes with A62 allele carriers. Because of the very low frequency of minor allele homozygotes for the C914T polymorphism, all interaction analyses involving this genotype were conducted with the C914T genotype combined with T allele homozygotes. Minor allele homozygotes were not combined for either SNP when constructing haplotype combinations.

Figure 2 shows the interaction between PAEE and G-62A genotype (GG vs GA+AA) on DBP (lower panel) and SBP (upper panel). When stratified by above and below the sex-specific median for PAEE, no statistical difference in DBP or SBP was observed between G-62 homozygotes and A62 allele carriers (DBP: 79.2 versus 79.4 mm Hg, \( P = 0.862 \); SBP: 131.1 versus 131.7 mm Hg, \( P = 0.745 \)). However, in the high PAEE group, DBP and SBP were higher in A allele carriers, as compared with G allele homozygotes (DBP: 79.3 versus 74.0 mm Hg, \( P = 0.001 \); SBP: 126.7 versus 122.0 mm Hg, \( P = 0.002 \)). In Figure 2, it is evident that the anticipated inverse relationship between PAEE and blood pressure observed in the cohort as a whole (Figure 1) is not significant in carriers of the minor allele (SBP: \( \beta = 1.66, P = 0.52 \); DBP: \( \beta = 1.11, P = 0.52 \)). In contrast, for individuals homozygous for the common G-62 allele, PAEE is strongly inversely associated with blood pressure (SBP: \( \beta = 8.54, P = 0.0001 \); DBP: \( \beta = 6.18, P = 0.00001 \)). The interaction between the G-62A polymorphism and PAEE was significant for SBP (\( P = 0.008 \)) and DBP (\( P = 0.06 \)).

In models testing the interaction between PAEE and the C914T genotype, a borderline interaction was observed on SBP (\( P = 0.068 \), but no interaction was detectable on DBP (\( P = 0.230 \)). The slope of the relationship between PAEE and SBP in C allele homozygotes was inverse and highly significant (\( \beta = 7.07, P = 0.000015 \)), whereas there was no significant association between PAEE and SBP in carriers of the minor T914 allele (\( \beta = 3.27, P = 0.599 \)). Given that both GPR10 SNPs may modify the association between PAEE and SBP and are in linkage disequilibrium, we then assessed whether the haplotypes derived from the G-62A and C914T genotypes interact with PAEE to modify blood pressure. Because the minor T914 allele is present at a low frequency in this population, we were only able to reconstruct 6 haplotype combinations. However, one of these combinations (G62G/T914T) contained only 2 individuals; therefore, this combination was excluded from further analyses. The number of individuals carrying each haplotype was as follows: G62G/C914C n = 342; G-62A/C914C n = 253; A62A/C914C n = 41; G62G/C914T n = 36; and G-62A/C914T n = 13. In interaction analyses (GPR10 haplotype by PAEE) adjusted for age, sex, BMI, and blood pressure medication, a significant interaction was observed on SBP (\( P = 0.02 \)), and a borderline interaction was observed on DBP (\( P = 0.08 \)). PAEE was inversely related with SBP and DBP in all haplotype combinations, with the exception of the haplotype G-62A/C914T (Figure 3).

**Discussion**

In the present study, resting blood pressure was inversely related to physical activity. This relationship was also inverse in people who carried both copies of the major alleles for the G-62A and the C914T GPR10 polymorphisms. However, in people who carried the minor alleles of these polymorphisms, there was no significant association between PAEE and blood pressure, and the between-genotype differences in slopes were significant for both SNPs. These interactions were consistent across men and women and for DBP and SBP in the G-62A genotype; however, for the C914T variant, the interaction on SBP was of only borderline significance and no
interaction was observed for DBP. Using haplotypes derived from the G-62A and C914T variants, a significant interaction with PAEE was observed on SBP, and an interaction of borderline significance was observed on DBP. These observations indicate that physical activity is only significantly inversely associated with blood pressure in certain GPR10 haplotypes.

The ability to detect gene-environment interactions is highly dependent not only on the magnitude of effect and sample size but also on the precision with which the environmental exposure is measured.\(^{14,15}\) The detection of the interactions in this study was aided by objective assessment of physical activity using heart rate monitoring with individual calibration, a method previously validated against the gold standard techniques of doubly labeled water and whole-body calorimetry.\(^{16,17}\) The sample size in this study is large compared with other studies in which physical activity has been measured objectively. In the case of the G-62A polymorphism, the minor allele is present at a high frequency in this cohort. However, the minor allele of the C914T variant is much less frequent, which limits statistical power to detect interactions involving this variant, either alone or in combination with the G-62A polymorphism as a haplotype. Aside from the G-62A and C914T polymorphisms, several other GPR10 polymorphisms have been identified. However, these variants have very rare minor alleles (\(\sim 1\%\)) and are often in tight linkage with the variants analyzed in the present study, thus offering virtually no additional information, and are unsuitable for testing in interaction analyses.

Thus far, the documentation of studies examining the effect of GPR10 polymorphisms in humans is limited. In a recent study,\(^{10}\) we demonstrated that within a population cohort of 1085 whites in the United Kingdom, polymorphisms in GPR10 were associated with high blood pressure. The precise mechanisms underlying this effect remain unclear. Chen et al\(^{18}\) have proposed that GPR10 and its ligand PrRP are components in a physiological stress response process driven by the hypothalamus and that the effects on blood pressure that have been observed after intracerebroventricular infusion of PrRP result from activation of the autonomic nervous system by GPR10.

One of the primary mechanisms through which physical activity is thought to affect blood pressure is through the central command process. This involves neural signaling via motor centers in the brain that promote the contraction of skeletal muscle, while in a similar manner brain stem centers are activated that control autonomic neural outflow directed toward the cardiovascular system.\(^{19}\) It is through these central processes that GPR10 may act in modifying the effect of physical activity on blood pressure.

Physical inactivity is strongly associated with high blood pressure and has been shown to increase all-cause mortality risk. In the present study, variants in the GPR10 gene modified the relationship between activity level and blood pressure, such that there is no association between physical activity and blood pressure in carriers of the minor alleles for these polymorphisms. Furthermore, it appears that in one haplotype derived from the G-62A and C914T variants (ie, G-62A/C914T), PAEE and blood pressure are not inversely related. Because the minor allele in the G-62A polymorphism is present in almost half of the population, and because the between-genotype difference in the regression slopes for physical activity and blood pressure is large, the G-62A genotype may explain a large proportion of the between-individual variation in responsiveness when exercise is prescribed as treatment for hypertension. However, it is presently unknown whether G-62A is located in an important coding motif within the promoter, and there is no evidence to link the G-62A polymorphism with functional changes in GPR10 expression or translation. In contrast, we have previously demonstrated that the C914T variant, which codes for a nonconservative amino acid change of proline for lysine at codon 305 and has a much rarer minor allele, functionally affects the intracellular signaling of the GPR10 ligand prolactin-releasing peptide.\(^{10}\) Therefore, it is possible that the interaction we observed between G-62A and PAEE is attributable to some extent to linkage with the C914T variant. However, one cannot discount the possibility that G-62A has its own functional properties that are yet unknown.

**Perspectives**

The identification of genes that increase the susceptibility of subsections of the population to the detrimental effects of sedentary living may have usefulness as part of targeted preventive strategy. The findings of the present study are cross-sectional and are thus limited in the extent to which one can infer causality. Nonetheless, it is plausible that carriers of the A62 allele will not incur a reduction in blood pressure after exercise intervention to the same extent as carriers of the G62G genotype. This may partially explain why exercise is an inadequate intervention for the control of blood pressure in some individuals.\(^{20}\) These findings should not be interpreted...
as meaning that in carriers of the A62 allele physical activity is not important per se, because many other benefits are associated with this behavior. However, understanding the biological mechanisms that mediate the effect of the GPR10 gene on blood pressure, in addition to comprehending how GPR10 genotypes modify the protective effects of physical activity on disease, may facilitate the identification of those individuals who will benefit most from progressing more rapidly to other forms of anti-hypertensive therapy. Moreover, the experience of adhering to an anti-hypertensive therapy, such as exercise, without experiencing a detectable reduction in blood pressure may be demotivating for the patient. Circumvention of experiences such as this may be important if subsequent adherence to other therapies is to be achieved.

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References
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