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Blood Pressure and Renal Sodium Handling in Relation to Genetic Variation in the *DRD1* Promoter and *GRK4*

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Abstract—Activation of type-1 dopamine receptors (*DRD1*) reduces renal sodium reabsorption. In a family-based random sample of 611 untreated whites (women, 45.0%; mean age, 38.6 years), we measured blood pressure (BP). We used the endogenous lithium clearance to assess fractional sodium excretion (FE_{Na}) and proximal (RNa_{prox}) and distal (RNa_{dist}) tubular sodium reabsorption. We investigated multivariate-adjusted associations with the *DRD1* promoter (*A−48G*, *G−94A*, and *C−800T*) and *GRK4 (Ala142Val)*. The frequent *DRD1* haplotypes were *AGC* (48.2%), *GGT* (34.4%), and *AAC* (14.3%). While standardizing to mean sodium excretion (8.7 mmol/h) and adjusting for covariates and relatedness, RNa_{dist} was lower in *DRD1 −94GG* homozygotes than *−94A* allele carriers (effect size, −0.94%; $P=0.005$) with opposite findings for FE_{Na} (+0.084%; $P=0.014$). *AGC* carriers (−0.88%; $P=0.012$) and *AAC* carriers (+1.00%; $P=0.004$) had different RNa_{dist} compared to corresponding noncarriers. Furthermore, FE_{Na} was lower in *AAC* carriers than in noncarriers (−0.082%; $P=0.019$). The family-based analyses identified a significant between-family component in the variance of the renal phenotypes associated with the *DRD1* polymorphisms. Transmission of the *DRD1 AGC* haplotype was also associated with lower systolic (−3.54 mm Hg; $P=0.016$) and diastolic (−2.80 mm Hg; $P=0.0064$) BPs without significant between-family variance component. Plasma renin activity and urinary aldosterone excretion were not associated with *DRD1* variation. The GRK4 *Ala142Val* polymorphism did not contribute to the phenotypes under study. In conclusion, renal sodium handling and BP were associated with genetic variation in the *DRD1* promoter. The between-family variance component excluded population stratification for BP, but not for the renal phenotypes. **(*Hypertension*. 2008;51:1643-1650.)**

Key Words: blood pressure ■ clinical genetics ■ dopamine receptor gene ■ GRK4 ■ lithium clearance ■ population science ■ tubular transport

Dopamine reduces sodium reabsorption in the proximal renal tubules via activation of dopamine type-1 (DRD1) receptors, which leads to inhibition of sodium transporters, including the Na,H-exchanger and Na,K-ATPase.[1] The *DRD1* promoter harbors several single-nucleotide polymorphisms (SNPs),[2] which influence the expression of the gene. Dopamine exerts its actions via G protein–coupled receptors, which in turn are under control of G protein–coupled receptor kinases (GRKs).[1] Amino-acid changing polymorphisms in one particular member of this family, GRK4, cause hyperphosphorylation, desensitization, and internalization of the DRD1 receptor and enhance the expression of the angiotensin II type-1 receptor.[1] The genes encoding DRD1 and GRK4 localize to chromosomes 5q35.1[3] and 4p16.3,[4] respectively. The *GRK4* gene locus is embedded in a cluster on chromosome 4p16, which is associated with hypertension[5,6] and also includes α-adducin (*ADD1*). To our knowledge, there are no studies showing significant genome-wide linkage of hypertension with the *DRD1* locus, although a genome-scan meta-analysis[7] identified 5q as a suggestive region.

Measuring the clearance of endogenous lithium provides a way of estimating sodium handling in the proximal and postproximal nephron.[8,9] Expressing the renal clearance of endogenous lithium as a fraction of creatinine clearance provides a measure of tubular sodium reabsorption that is standardized for the glomerular filtration rate.[8,9] The fractional excretion of lithium (FE_{Li}) is a noninvasive marker of proximal tubular sodium handling and the proportion of sodium escaping reabsorption in the proximal segment of the nephron. FE_{Li} also allows the calculation of the fractional distal reabsorption of sodium (RNa_{dist}). To our knowledge, no prior study addressed the possible association of these renal

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measurements with genetic variation in the *DRD1* promoter and *GRK4*. We studied these associations in a family-based random sample of a white population.

Methods

Study Population

We invited 1507 participants of the Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO)[10,11] for a measurement of the clearance of endogenous lithium (for details, see the expanded Methods section available in the online data supplement at http://hyper.ahajournals.org). All participants or their parents provided informed consent to a protocol, which the University of Leuven Ethics Committee had approved.

Of 806 subjects willing to participate (53.5%), we excluded 195. Two subjects did not have all measurement required to compute the clearances, and 5 had a lithium concentration in serum ($\geq$1.0 μmol/L) or urine ($\geq$20 μmol/L) suggestive of external contamination. We excluded 170 subjects, because they were on medications, which affect blood pressure or the activity of the renin-angiotensin-aldosterone system, such as antihypertensive drugs (n=101), oral contraceptives (n=62), or hormonal replacement therapy (n=7). We removed 10 subjects, whose DNA failed to amplify and 8 with errors in Mendelian segregation. Thus, the number of subjects statistically analyzed totaled 611.

Clinical Measurements

Trained nurses administered a questionnaire to collect information about the participants' smoking and drinking habits, and intake of medications. Blood pressure was the average of 5 consecutive readings obtained at the examination center after the subjects had rested for at least 10 minutes in the sitting position. Mean arterial pressure was diastolic pressure plus one third of pulse pressure. Hypertension was a blood pressure of at least 140 mm Hg systolic or 90 mm Hg diastolic. Body mass index was weight in kilograms divided by the square of height in meters.

Renal Sodium Handling

The participants gave a venous blood sample and collected an exactly timed urine sample over 4 to 6 hours. We determined plasma renin activity (RIA-0180, DRG Instruments GmbH) and urinary aldosterone (DSL-8600 Active, Diagnostic Systems Laboratories Inc) by radioimmunoassay. Endogenous trace lithium was measured with an electrothermal atomic absorption spectrophotometer (model AAS 300) with a HGA-700 graphite furnace (Perkin-Elmer Inc).[12]

Clearances (C) were calculated as $C_x = U_x \times V/P_x$, where U_x and P_x are the urinary and plasma concentrations of the solute x, and V is the urine flow rate in milliliters per minute.[8,9] We computed the fractional excretion of sodium (FE_{Na}) and lithium (FE_{Li}) by dividing the sodium (C_{Na}) and lithium (C_{Li}) clearances by the creatinine clearance.[8,9] We expressed these ratios as a percentage. Fractional distal reabsorption of sodium (RNa_{dist}) was estimated as $[(FE_{Li}-FE_{Na})/FE_{Li}]\times 100$. RNa_{dist} is a measure of the amount sodium that escapes reabsorption in the proximal tubules and is reabsorbed in the postproximal tubules.[8,9] We defined the fractional proximal sodium reabsorption (RNa_{prox}) as $100-FE_{Li}$.[8,9]

Genotypes

We extracted genomic DNA from white blood cells, using standard kits (Qiagen). As described in the expanded Methods section, we genotyped *DRD1* for the promoter polymorphisms *A−48G*, *G−94A*, and *C−800T,* and *GRK4* for *C+583T (Ala142Val)*.

Statistical Analysis

We used SAS software (SAS Institute), version 9.1.3. We normalized the distributions of plasma renin activity and the urinary aldosterone excretion by a logarithmic transformation. We compared means and proportions, using the large sample *z*-test and Fisher exact test, respectively.

Our statistical methods also included single and multiple linear regressions. We included in our models covariates with known physiological relevance in relation to the phenotypes under study. We additionally searched for possible covariates of the phenotypes, using stepwise multiple regressions with the probability value for independent variables to enter and stay in the model set at 0.15. For analysis, we combined women and men, because the interaction terms between explanatory variables and sex were nonsignificant ($P>0.15$). We standardized FE_{Na}, RNa_{prox} and RNa_{dist} to the mean sodium excretion rate for the whole study population (8.7 mmol/h), because there is a linear correlation between these renal measurements and urinary sodium excretion.[9] We applied the formula $P_s = P_o - \beta(ENa_o - 8.7)$, where P_s is the standardized parameter, P_o the observed value, and β the regression coefficient relating the indexes of renal sodium handling to the observed sodium excretion rate (ENa_o).[9]

For analysis of single SNPs, we combined the least frequent homozygous group with the heterozygous subjects. We tested linkage disequilibrium and reconstructed haplotypes, using the SAS procedures PROC ALLELE and PROC HAPLOTYPE. 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 SNPs or haplotypes, while accounting for the nonindependence of phenotypes within families and adjusting for covariates.

In quantitative transmission disequilibrium tests (QTDT), we partitioned the phenotypic variance into between and within family components, using the orthogonal model as implemented in Abecasis' QTDT software, version 2.4, available at http://www.sph.umich.edu/csg/abecasis/QTDT).[13] The within-family component of phenotypic variance reflects the genetic effect and is robust to population stratification.[13]

Results

Characteristics of the Participants

The study population consisted of 573 relatives from 53 families and 38 unrelated individuals. Of the 53 families, 10, 31, and 12 spanned 1, 2, or 3 generations, respectively. Table 1 provides the characteristics of 459 offspring in comparison with the rest of the study population (114 founders and 38 unrelated subjects).

The study sample included 105 (17.2%) untreated hypertensive patients. Of 275 women and 336 men, 65 (23.6%) and 105 (31.3%) were smokers; 121 women (44.0%) and 251 men (74.7%) reported intake of alcohol. In smokers, median tobacco use was 12 cigarettes per day (interquartile range, 7 to 20). In drinkers, the median alcohol consumption was 10 g per day (interquartile range, 4 to 20). Among women, 53 (19.3%) reported natural or surgical menopause. Compared to men, women had lower ($P\leq 0.01$) mean values of serum sodium (141.1 versus 143.1 mmol/L), urinary excretion of sodium (7.0 versus 10.2 mmol/h) and lithium (0.16 versus 0.20 μmol/h), and RNa_{dist} (93.6 versus 94.3%). Women and men had similar RNa_{prox} (80.7 versus 80.3%; $P=0.58$) and FE_{Na} (0.97 versus 0.98%; $P=0.87$).

Genotype and Haplotype Frequencies

Table 2 provides genotype and allele frequencies in the population sample for the 3 *DRD1* SNPs and the *GRK4 C +583T* (*Ala142Val*) polymorphism. The genotypic frequencies did not depart from Hardy-Weinberg proportions ($P\geq 0.17$). The 3 *DRD1* SNPs were in complete linkage disequilibrium. Lewontin disequilibrium coefficient D′ was >0.99 ($P<0.0001$). Among the 590 subjects, in whom the 3 *DRD1* SNPs were available, the haplotype frequencies were 457 (48.2%) for the combination of *−48A*, *−94G*, and *−800C* (*H1-AGC*), 406 (34.4%) for *−48G*,

Table 1. Characteristics of Informative Offspring and Other Participants

Characteristic	Offspring (n=459)	Founders and Unrelated Participants (n=152)	P
Anthropometry			
Women, n (%)	211 (46.0)	64 (42.1)	0.68
Age, y	35.5±14.5	48.0±12.1	<0.001
Body mass index, kg/m^2	24.3±4.45	26.1±3.79	<0.001
Systolic pressure, mm Hg	122.9±13.0	127.8±14.6	<0.001
Diastolic pressure, mm Hg	76.4±10.5	80.4±9.23	<0.001
Mean arterial pressure, mm Hg	91.9±10.4	96.2±9.22	<0.001
Blood biochemistry			
Serum sodium, mmol/L	142.2±3.23	142.2±3.37	0.80
Serum lithium, μmol/L	0.19±0.07	0.19±0.08	0.98
Serum creatinine, μmol/L	77.1±15.0	80.0±15.5	0.04
Plasma renin activity,* ng/mL per h	0.38 (0.25 to 0.57)	0.34 (0.22 to 0.54)	0.13
Urinary excretion rate			
Sodium, mmol/h	8.60±5.89	9.06±7.57	0.44
Lithium, μmol/h	0.18±0.11	0.19±0.13	0.26
Creatinine, mmol/h	0.50±0.31	0.52±0.45	0.58
Aldosterone,* nmol/24 hours	21.4 (13.5 to 33.1)	20.4 (14.1 to 28.2)	0.52
Measurements of renal handling			
Creatinine clearance, mL/min	107.0±50.7	105.0±72.6	0.71
RNa_{prox}, %	81.1±7.95	78.6±8.49	0.001
RNa_{dist}, %	93.9±3.71	94.4±3.00	0.13
FE_{Na}, %	0.96±0.35	1.02±0.45	0.06

Values are mean±SD or geometric mean (interquartile range). FE_{Na} indicates the fractional excretion of sodium. RNa_{prox} represents the fractional sodium reabsorption along the proximal tubules. RNa_{dist} is the calculated reabsorption of sodium in the postproximal tubules.

*Plasma renin activity and the urinary aldosterone excretion rate were available in 393 informative offspring and 144 other subjects and in 372 offspring and 137 other subjects, respectively.

−94G, and *−800T* (*H2-GGT*), 169 (14.3%) for *−48A*, *−94A*, and *−800C* (*H3-AAC*), 6 (0.5%) for *−48A*, *−94G*, and *−800T* (*H4-AGT*), and 3 (0.25%) for *−48G*, *−94G*, and *−800C* (*H5-GGC*).

Phenotype-Genotype Associations for SNPs

Both before and after adjustment for sex, age, body mass index, and mean arterial pressure (Table 3), RNa_{dist} was significantly lower in *−94GG* homozygotes than *−94A* allele carriers (adjusted effect size, −0.94%; 95% confidence interval [CI], −1.60 to −0.29%; $P=0.005$), whereas the opposite was the case for FE_{Na} (+0.084%; CI, +0.017 to +0.151%; $P=0.014$). FE_{Na} also tended to be lower in *DRD1 −800CC* homozygotes than *−800T* allele carriers (−0.055%; CI, −0.116 to +0.005; $P=0.071$). None of the other genotypic differences in the indexes of renal sodium handling

Table 2. Genotypic and Allelic Frequencies for SNPs in the *DRD1* and the *GRK4* Genes

	No. of Subjects With Genotype			No. of Alleles		
Gene						
DRD1	*−48AA*	*−48AG*	*−48GG*	*A*	*G*	*P*
	253 (41.4%)	296 (48.4%)	62 (10.2%)	802 (65.6%)	420 (34.4%)	0.17
DRD1	*−94GG*	*−94GA*	*−94AA*	*G*	*A*	
	456 (74.6%)	134 (21.9%)	21 (3.4%)	1046 (85.6%)	176 (14.4%)	0.37
DRD1†	*−800TT*	*−800TC*	*−800CC*	*T*	*C*	
	62 (10.5%)	282 (47.8%)	246 (41.7%)	406 (34.4%)	774 (65.6%)	0.20
GRK4†	*+583CC*	*+583CT*	*+583TT*	*C*	*T*	
	239 (41.0%)	288 (49.4%)	56 (9.6%)	766 (65.7%)	400 (34.3%)	0.61

**P* value for the Hardy-Weinberg proportions in the founder generation.

†The No. of subjects genotyped for *DRD1 C−800T* and *GRK4 C+583T* (*Ala142Val*) totaled 590 and 583, respectively.

Table 3. Blood Pressure and Renal Sodium Handling in Relation to SNPs in Multivariate-Adjusted Analyses

DRD1 A−48G	*AA* (n=253)	*AG+GG* (n=358)	*P*
Systolic pressure, mm Hg	123.8±0.99	124.2±0.89	0.65
Diastolic pressure, mm Hg	77.6±0.71	78.0±0.64	0.62
RNa_{prox}, %	80.2±0.67	80.5±0.61	0.60
RNa_{dist}, %	94.3±0.27	94.0±0.24	0.26
FE_{Na}, %	0.95±0.023	0.99±0.019	0.17
DRD1 G−94A	*GG* (n=456)	*GA+AA* (n=155)	*P*
Systolic pressure, mm Hg	123.8±0.86	124.8±1.20	0.43
Diastolic pressure, mm Hg	77.6±0.62	78.5±0.85	0.24
RNa_{prox}, %	80.6±0.59	79.9±0.80	0.35
RNa_{dist}, %	93.9±0.22	94.9±0.32	0.005
FE_{Na}, %	0.99±0.017	0.91±0.029	0.014
DRD1 C−800T	*TT+TC* (n=347)	*CC* (n=243)	*P*
Systolic pressure, mm Hg	124.3±0.91	123.7±1.01	0.57
Diastolic pressure, mm Hg	77.9±0.66	77.8±0.73	0.91
RNa_{prox}, %	80.2±0.61	80.0±0.67	0.90
RNa_{dist}, %	94.2±0.23	94.4±0.26	0.40
FE_{Na}, %	1.00±0.020	0.94±0.024	0.07
GRK4 C+583T	*CC* (n=239)	*CT+TT* (n=344)	*P*
Systolic pressure, mm Hg	123.6±1.00	123.9±0.91	0.80
Diastolic pressure, mm Hg	77.8±0.73	77.6±0.67	0.76
RNa_{prox}, %	80.2±0.69	80.5±0.63	0.60
RNa_{dist}, %	94.0±0.28	94.1±0.25	0.64
FE_{Na}, %	0.97±0.023	0.99±0.019	0.51

FE_{Na} indicates the fractional excretion of sodium. RNa_{prox} represents the fractional sodium reabsorption along the proximal tubules. RNa_{dist} is the calculated reabsorption of sodium in the postproximal tubules. Values are mean±SE and account for family clusters. Covariates considered in these analyses were sex, the linear and squared terms of age, and body mass index for blood pressure; and sex, age, body mass index, and mean arterial pressure for the renal measurements, which were also standardized to the mean sodium excretion rate (8.7 mmol/h).

reached statistical significance in relation to *DRD1* or *GRK4* (Table 3). For the *DRD1* and *GRK4* SNPs under study, the genotypic differences in systolic and diastolic blood pressures (Table 3; $P \geq 0.24$), plasma renin activity ($P \geq 0.37$) and the aldosterone excretion rate ($P \geq 0.21$) were not significant. Covariates considered in these analyses were sex, the linear and squared terms of age, and body mass index for blood pressure; sex, age, body mass index, and mean arterial pressure for plasma renin activity and the urinary aldosterone excretion rate. For plasma renin activity, we additionally entered the time of day of the blood collection into the multivariate model. The gene-gene interaction terms between the *DRD1* and *GRK4* SNPs did not improve the multivariate-adjusted models for any phenotype ($P \geq 0.21$).

Phenotype-Genotype Associations for Haplotypes

Both before and after adjustment (Table 4), *H1-AGC* carriers (adjusted effect size, −0.88%; CI, −1.55 to −0.21%; $P=0.01$) and *H3-AAC* carriers (+1.00%; CI, +0.31 to +1.61%; $P=0.004$) had different RNa_{dist} compared to the corresponding noncarriers. Furthermore, FE_{Na} was lower in *H3-AAC* carriers than in noncarriers (−0.082%; CI, −0.150 to −0.013%; $P=0.019$). None of the other associations between the indexes of renal sodium handling and the *DRD1* haplotypes reached significance (Table 4). The number of subjects carrying 2 copies of the same haplotype was 145 (25.5%) for H1-AGC, 62 (10.9%) for H2-GGT, and 21 (3.7%) for H3-AAC (Figure). We noticed a dose-effect, depending on the number of haplotypes, for *H1-AGC* in relation to RNa_{dist} ($P=0.011$), and for *H3-AAC* in relation to RNa_{dist} ($P=0.003$) and FE_{Na} ($P=0.016$).

Table 4. Blood Pressure and Renal Sodium Handling in Relation to Common *DRD1* Haplotypes in Multivariate-Adjusted Analyses

DRD1 Haplotype 1 (*AGC*)	Carriers (n=457)	Noncarriers (n=133)	*P*
Systolic pressure, mm Hg	124.1±0.84	123.8±1.21	0.80
Diastolic pressure, mm Hg	77.8±0.61	77.9±0.87	0.88
RNa_{prox}, %	80.3±0.59	79.5±0.81	0.34
RNa_{dist}, %	94.0±0.21	95.0±0.32	0.01
FE_{Na}, %	0.99±0.017	0.94±0.030	0.19
DRD1 Haplotype 2 (*GGT*)	Carriers (n=344)	Noncarriers (n=246)	*P*
Systolic pressure, mm Hg	124.2±0.89	123.8±0.98	0.63
Diastolic pressure, mm Hg	78.0±0.64	77.6±0.71	0.60
RNa_{prox}, %	80.5±0.61	80.2±0.67	0.67
RNa_{dist}, %	94.0±0.24	94.3±0.27	0.28
FE_{Na}, %	0.99±0.019	0.95±0.023	0.16
DRD1 Haplotype 3 (*AAC*)	Carriers (n=149)	Noncarriers (n=441)	*P*
Systolic pressure, mm Hg	124.8±1.20	123.8±0.86	0.43
Diastolic pressure, mm Hg	78.6±0.85	77.6±0.62	0.24
RNa_{prox}, %	79.6±0.80	80.3±0.58	0.34
RNa_{dist}, %	95.0±0.32	94.0±0.21	0.004
FE_{Na}, %	0.91±0.020	0.99±0.017	0.019

FE_{Na} indicates the fractional excretion of sodium. RNa_{prox} represents the fractional sodium reabsorption along the proximal tubules. RNa_{dist} is the calculated reabsorption of sodium in the postproximal tubules. Values are mean±SE and account for family clusters. Covariates considered in these analyses were sex, the linear and squared terms of age, and body mass index for blood pressure; and sex, age, body mass index, and mean arterial pressure for the renal measurements, which were also standardized to the mean sodium excretion rate (8.7 mmol/h).

For the 3 most frequent *DRD1* haplotypes, systolic and diastolic blood pressures (Table 4; $P \geq 0.20$), plasma renin activity ($P \geq 0.24$), and the aldosterone excretion rate ($P \geq 0.09$) were similar in carriers and noncarriers. Sensitivity analyses, in which we additionally adjusted for smoking and drinking alcohol produced results, which were not materially different from those shown for the *DRD1* and *GRK4* SNPs in Table 3 and from those for the *DRD1* haplotypes in Table 4

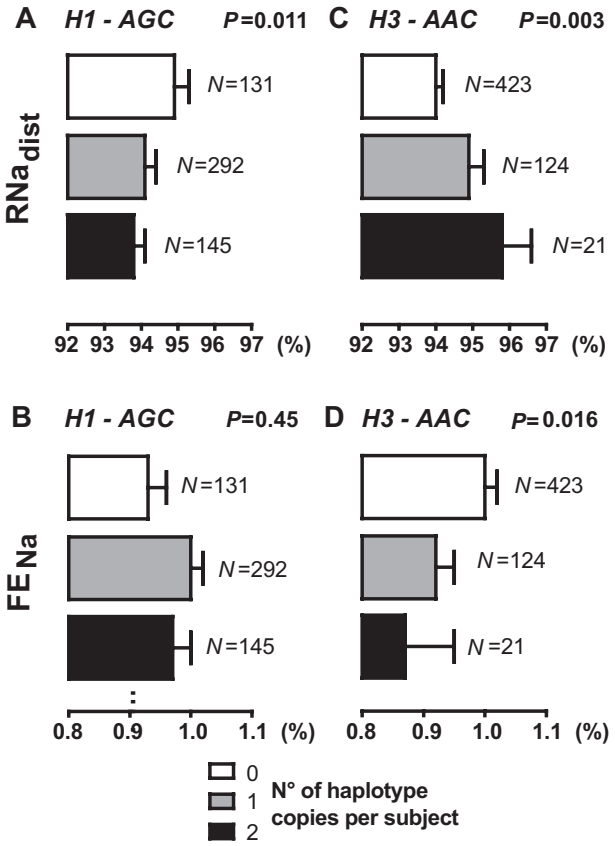

Figure. Association of the fractional distal sodium reabsorption (RNa_{dist}; A and C) and the fractional excretion of sodium (FE_{Na}; B and D) with the *H1-AGC* haplotype (A and B) and *H3-AAC* haplotype (C and D) by number of *DRD1* haplotype copies. Values are means (SE) standardized to a sodium excretion of 8.7 mmol/h and adjusted for nonindependence within families, sex, age, body mass index, and mean arterial pressure. Probability values denote the significance of linear trend. The number of subjects contributing to each mean is given.

and the Figure. Finally, we did not find any interaction between the *DRD1* haplotypes and the *GRK4* SNP in relation to the phenotypes ($P \geq 0.05$).

QTDT Analysis

We adjusted the QTDT analyses as described above. Transmission of *DRD1* or *GRK4* alleles from parents to informative offspring was not associated with any difference ($P \geq 0.19$) in the indexes of renal sodium handling (number of informative offspring ranging from 112 to 208), blood pressure (112−208), plasma renin activity (100−177), or the aldosterone excretion rate (98−165). For the *DRD1* −94G allele in relation to RNa_{prox} ($P=0.042$) and RNa_{dist} ($P=0.0026$), the between-family component of variance was significant.

Table 5 summarizes the between-family and within-family effect sizes associated with the *DRD1* haplotypes. Transmission of *H1-AGC* was associated with lower systolic (−3.54 mm Hg; $P=0.016$) and diastolic (−2.80 mm Hg; $P=0.019$) blood pressures without evidence for a significant between-family component in the variance ($P \geq 0.28$). On the other hand, RNa_{prox} and RNa_{dist} were not associated with transmission of the *DRD1* haplotypes ($P \geq 0.26$), but the between-family effect sizes were significant for RNa_{prox} in relation to *H1-AAC* ($P=0.009$) and for RNa_{dist} in relation to *H1-AGC* ($P=0.012$) and *H3-AAC* ($P=0.0009$). The within-family ($P \geq 0.40$) and between-family ($P \geq 0.08$) effect sizes for plasma renin activity and the aldosterone excretion rate in relation to the *DRD1* haplotypes were not significant.

Discussion

The key finding of our study was that RNa_{dist} was significantly lower in *DRD1 −94GG* homozygotes than *−94A* allele carriers, whereas the opposite was true for FE_{Na}. RNa_{dist} varied with the *DRD1 H1-AGC* and *H3-AAC* haplotypes. FE_{Na} was lower in *H3-AAC* carriers than in noncarriers. Furthermore, transmission of the *H1-AGC* haplotype was associated with lower systolic and diastolic blood pressure. We did not find any significant interaction of the *DRD1* SNPs or haplotypes with the *GRK4* SNP in relation to the studied phenotypes.

In the family-based analyses, we partitioned association effects into between-family and within-family components. The within-family effects are free of confounding by population-substructure effects, regardless of the composition of families.[13] The between-family effects estimate to what extent associations are attributable to population stratification or admixture. In the QTDT analyses, we noticed significant between-family effects for the renal phenotypes, but not for blood pressure. This suggests that in the population-based analyses, in which we also accounted for relatedness, population stratification might have contributed to the association of the renal phenotypes with the *DRD1* polymorphisms, whereas this was not the case for blood pressure in the family-based analyses.

The role of the *DRD1* receptor in the regulation of blood pressure is well established.[1] Stimulation of *DRD1* by fenoldopam, a D1-like agonist, decreases blood pressure,[14] whereas inhibition of *DRD1* by ecopipam, a long-acting *DRD1* antagonist, has the opposite effect.[15] Furthermore, *DRD1* knock-out mice have an elevated blood pressure.[16] However, previous studies[2,17,18] on the possible influence of variation in *DRD1* on blood pressure yielded inconsistent results. Beige and coworkers genotyped the *A−48G* and *G−94A* polymorphisms in 493 hypertensive patients and 209 normotensive controls,[2] but these 2 SNPs were not associated with hypertension or blood pressure. In a study involving 407 black and 505 white normotensive twins with mean age of 17.4 years, Lu and colleagues[17] found that *−48G* allele carriers, compared with *−48AA* homozygotes, had a significantly lower diastolic blood pressure at rest (58.7 versus 59.6 mm Hg, $P=0.032$) and during a car driving simulation test (66.3 versus 67.5 mm Hg, $P=0.046$). However, the sib-pair transmission disequilibrium test, involving 39 informative twin pairs for *A−48G*, did not reveal any significant association with systolic or diastolic blood pressure.[17] In a Japanese study[18] of 131 hypertensive patients and 136 normotensive controls, the allele frequencies of *A−48G* substantially deviated from those observed in whites, amounting to 0.92/0.08 in the normotensive controls and 0.84/0.16 in the hypertensive patients. In this study,[18] the *DRD1 −48G* allele was more frequent in hypertensive patients than normoten-

Table 5. Between-Family and Within-Families Effect Sizes Associated with *DRD1* Haplotypes in the Quantitative Transmission Disequilibrium Test

			Effect Size±SE			
			Between-Family		Within-Family	
Phenotype	Haplotype	No.	Estimate	*P*	Estimate	*P*
Systolic pressure, mm Hg	*H1-AGC*	206	+1.15±1.07	0.28	−3.54±1.46	0.016
	H2-GGT	208	−0.39±1.09	0.72	+1.84±1.43	0.20
	H3-AAC	108	−1.21±1.40	0.39	+3.30±1.99	0.10
Diastolic pressure, mm Hg	*H1-AGC*	206	+1.31±0.78	0.09	−2.80±1.02	0.0064
	H2-GGT	208	−0.96±0.80	0.23	+1.39±1.02	0.17
	H3-AAC	108	−0.87±1.03	0.40	+2.41±1.33	0.068
RNa_{prox}, %	*H1-AGC*	206	+1.17±0.32	0.11	+0.61±0.94	0.52
	H2-GGT	208	−0.07±0.73	0.93	+0.17±0.93	0.86
	H3-AAC	108	−2.45±0.93	0.009	−1.16±1.23	0.34
RNa_{dist}, %	*H1-AGC*	206	−0.80±0.32	0.012	−0.30±0.46	0.52
	H2-GGT	208	−0.02±0.03	0.96	−0.06±0.46	0.90
	H3-AAC	108	+1.36±0.40	0.0009	+0.67±0.50	0.26

RNa_{dist} is the calculated reabsorption of sodium in the postproximal tubules, standardized to the mean sodium excretion rate (8.7 mmol/hour). No. is the number of informative offspring. All traits were adjusted for sex, age, and body mass index. Additional covariates were age^2 for blood pressure and mean arterial pressure for RNa_{dist}. The within-family component of phenotypic variance reflects the genetic effect and is robust to population stratification.

sive controls.[18] Moreover, among untreated hypertensive patients, *−48G* allele carriers had a higher diastolic blood pressure than *−48AA* homozygotes.[18]

In the current study, we observed that RNa_{dist}, but not RNa_{prox}, was associated with variation in the *DRD1* gene. Most experimental and clinical studies suggest that DRD1 primarily affects proximal tubular sodium reabsorption.[1] Selective DRD1 stimulation induces natriuresis via inhibition of sodium reabsorption in the proximal convoluted tubules.[19,20] However, the kidney expresses DRD1 not only in the proximal tubules, but also in the medullary ascending limb of Henle, and in the medullary and cortical collecting ducts.[21,22] In the current study population, we previously demonstrated an inverse relation between RNa_{prox} and mean arterial pressure.[9] In line with these findings,[9] the current analyses of the *DRD*1 haplotypes *H1-AGC* and *H3-AAC* showed opposite trends in blood pressure and RNA_{prox}. It is therefore possible that, along with the variation in the *DRD1* gene, pressure-natriuresis can compensate for the proximal genetic effects on sodium reabsorption. The alternative hypothesis is that variation in the *DRD1* gene might have extrarenal effects on blood pressure with secondary changes in proximal tubular sodium handling. Finally, in humans, juxtaglomerular cells do not express DRD1,[23] and selective D1-like receptor stimulation has no significant effects on plasma renin activity.[24] In keeping with our current findings, in the aforementioned Japanese study,[18] plasma renin activity and the plasma aldosterone concentration were not associated with the *DRD1* genotype in 90 untreated hypertensive patients.

In the population-based analyses, we noticed that FE_{Na} differed across the *DRD1* SNPs and haplotypes. Although there is high intraindividual variability in sodium excretion, means are accurate to reflect the average salt intake of a group.[25] On the assumption that our participants were in dietary balance, our findings suggest that genetic variability in *DRD1* might influence salt intake. In previous studies, we also noticed that the 24-hour urinary sodium excretion differed according to the combination of *ADD1* (*Gly460Trp*) and *ACE* (*I/D*) genotypes[10] or according to the β-adducin (*ADD2 C1797T*) polymorphism.[26] Like *DRD1*, *ADD1*, *ADD2*, and *ACE* are involved in sodium homeostasis. Genetic factors stimulating sodium retention lead to inhibition of the renin system and to a decreased systemic or local generation of angiotensin II, a major endocrine and paracrine factor driving salt appetite. Moreover, under certain conditions,[27,28] dopamine receptors participate in the regulation of salt appetite, although the evidence favors *DRD2* over *DRD1*.[28]

GRK4 is expressed in the nephron segments of the kidney, where sodium transport is regulated by dopamine and angiotensin II.[1] The allelic variant *C+583T* (GCC to GTC) results in amino acid substitution Ala142Val, and is associated with a constitutive increase in GRK4 kinase activity in proximal tubular cells from humans with essential hypertension.[29] This variant in the γ isoform of GRK4, when expressed heterologously in Chinese hamster ovary cells[29] but not in HEK-293 cells,[30] increased the phosphorylation of DRD1 and impaired its ability to stimulate cAMP production. Reducing the activity of GRK4 with heparin or GRK4 antisense oligonucleotides in proximal tubular cells from normotensive humans with the wild-type GRK4 did not affect DRD1 function, but it restored DRD1 responsiveness in proximal tubular cells from hypertensive patients carrying *GRK4* mutants.[29,31] GRK4 does not cause desensitization of the angiotensin II type-1 receptor,[32] but might actually increase its expression.[1]

Evidence for an association between blood pressure and genetic variation in *GRK4* comes from a study in 503 Chinese hypertensive patients and 490 sex- and age-matched con-

trols[33] and from a twin study of 934 black (44.2%) and white (55.8) normotensive American adolescents.[34] In a study of 184 newly diagnosed and untreated hypertensive Japanese,[35] mutation of *GRK4* reduced the natriuretic response to dopaminergic stimulation. We did not find any association of blood pressure and renal sodium handling with *GRK4* or the interaction between *GRK4* and *DRD*1. There are major ethnic differences in the *GRK4* allele frequencies.[36] Previous studies included Asians[33,35] and black[34] subjects. Furthermore, the phenotype-genotype associations with *GRK4* might not occur in the absence of dopaminergic stimulation or at a very high sodium intake, as observed in our study (209 mmol/d).

The present study has potential limitations. First, salt intake is an important determinant of renal sodium handling. In population studies, salt intake is highly variable. We therefore standardized our analyses to the mean sodium excretion rate. However, analyses not standardized or not adjusted for sodium excretion showed consistent results (see supplemental information available online at http://hyper.ahajournals.org). Second, measurement of the clearances of sodium and endogenous lithium only allows differentiating between proximal and postproximal renal tubular sodium reabsorption. Third, of 3 known amino-acid changing SNPs in *GRK4*,[1] we only genotyped *Ala142Val*, but not *Arg65Leu* and *Ala486Val*. However, in Whites, *Arg65Leu* and *Ala142Val* are in one contiguous haplotype block (pairwise $D'=1$).[36] In all ethnicities, there is high linkage disequilibrium in *GRK4* between noncontiguous SNPs.[36] In Chinese, among 33 SNPs in 11 candidate genes, *Ala142Val* was associated with hypertension.[33] In Japanese, *Ala142Val* was as single SNP 78.4% predictive of salt-sensitive hypertension, whereas the other 2 *GRK4* SNPs only added 16.0% to the genetic model.[35] Experimental studies showed that the *Ala142Val* variant is functional and leads to a constitutive increase in GRK4 kinase activity.[29] Finally, a drawback of the QTDT analysis, which is robust to population stratification,[13] is a sizable reduction in sample size because of the exclusion of noninformative allelic transmission from parents to offspring and hence a reduction in statistical power.

Perspectives

To our knowledge, our study is the first to investigate blood pressure and renal sodium handling in relation to *DRD1* and *GRK4* polymorphisms in a family-based random sample of a white population. We found association of RNa_{dist}, FE_{Na}, and blood pressure with genetic variation in the *DRD1* promoter without significant interaction between the *DRD1* and *GRK4* polymorphisms. We also noticed opposite trends in the phenotype-genotype associations between the *DRD1 H1-AGC* and *H3-AAC* haplotypes. The functional relevance of these haplotypes might therefore be conditional on the presence or absence of the *−94G* allele. Pending confirmation by further epidemiological and experimental research, our current findings suggest that interference with dopaminergic signaling by modification of DRD1 function[37] might be a way of intervening with sodium homeostasis and blood pressure.

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Disclosures

None.

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