AJH 2005; 18:1206–1210

Genetics

Association Between a Dopamine-4 Receptor Polymorphism and Blood Pressure

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Background: Dopamine receptor genes are candidates for hypertension susceptibility. Locally released dopamine increases renal sodium excretion, and defective renal dopamine receptor signaling has been shown to play a role in hypertension. Dopamine-4 receptors are expressed in juxtaglomerular and cortical collecting cells, where dopamine activation could alter sodium and water metabolism and affect blood pressure (BP). The dopamine-4 receptor (DRD4) gene has a 16 amino acid (48 base pairs [bp]) repeat polymorphism located in exon 3 where a G-protein binding area is encoded. The long allele (defined as at least one 7 to 10 repeat) has been associated with the personality trait Novelty Seeking and with substance abuse, but associations between dopamine-4 receptor polymorphisms and BP have not been reported.

Methods: We genotyped 479 female and 385 male subjects of white ethnicity at the DRD4 repeat polymorphism site and classified each subject as having either the long or short genotype.

Results: We found associations between the DRD4 long allele and increased systolic BP ($P = .031$), diastolic BP ($P = .034$), and a history of regular alcohol use ($P = .008$). Furthermore, for systolic BP ($P = .009$) and pulse pressure ($P = .002$), we found evidence for an interaction between dopamine-4 receptor alleles and age, indicating that the effects of dopamine-4 receptor variants on BP increase with age.

Conclusion: In this white population, the long variant of the DRD4 gene is associated with a 3–mm Hg higher systolic and 2–mm Hg higher diastolic BP. Am J Hypertens 2005;18:1206–1210 © 2005 American Journal of Hypertension, Ltd.

Key Words: Blood pressure, dopamine receptor, genetic polymorphism, alcohol, personality, hypertension.

Hypertension affects some 25% of adults in the United States and is a major risk factor for stroke, myocardial infarction, and renal insufficiency. Epidemiologic studies indicate that between 30% and 50% of the population variation in diastolic and systolic blood pressure (BP) is attributable to genetic factors; specific genes associated with BP or hypertension have been difficult to identify.[1] Rare Mendelian forms of hypertension result in distinctive abnormalities of renal sodium handling. Although the specific genetic disorders associated with these hypertensions are rare in the general hypertensive population, the principle that genetic variation affecting renal tubular sodium transport and pressure–natriuresis may underlie some fraction of essential hypertension suggests the possibility that common alleles for other genes involved in renal sodium handling could contribute to hypertension.

One of the most important regulators of sodium balance is renal dopamine. In normotensive subjects, the action of locally released dopamine is responsible for more than 50% of incremental sodium excretion during states of moderate sodium excess.[2] In essential hypertension the effect of dopamine on renal sodium excretion is blunted.[3–5] Most studies investigating the role of dopamine receptors in the control of renal sodium excretion and BP have focused on the dopamine-1 (D1) receptor, and both a D1 receptor gene polymorphism (A-48G)[6] and a variant in the G protein-coupled receptor kinase 4 gene (GRK4gA142V)[7] have been found to be associated with hypertension. Others have suggested that diminished renal dopamine pro-

Received August 16, 2004. First decision December 8, 2004. Accepted April 18, 2005.

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This work was supported by the Nancy Pritzker Depression Research Network (RMN, MB), the Michigan Society of Fellows, Rachel Upjohn Clinical Scholars Program and NRSA MH64299-01A1 (SS) and National Heart, Lung, and Blood Institute grant HL054512 (LG, AW).

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0895-7061/05/$30.00
doi:10.1016/j.amjhyper.2005.04.010

duction may play a role.[8] Recent evidence points to a potential role for dopamine-2–like (D2) receptors in BP regulation.[9–11] The site mediating the BP effects of both D1 and D2 receptors may well be the kidney, where D1 receptors inhibit renal tubular basolateral Na-K ATPase and luminal Na-H antiport and decrease tubular sodium reabsorption, whereas activation of D2 receptors stimulates Na-K ATPase. Thus, either the proposed defect in D1-inhibited or enhancement of D2-stimulated sodium reabsorption could promote hypertension.

Dopamine-4 (D4) receptors (along with dopamine-3 receptors) are part of the D2-like family of dopamine receptors.[11] It has been observed that D4 knockout mice show an increase in fear, unconditioned anxiety, and sensitivity to alcohol[12,13]; cardiovascular profiles of these mice have not been reported. The most intensively studied D4 receptor polymorphism is a 48 bp repeat located in exon 3 of the D4 receptor gene (DRD4). This variant codes for a 16– amino acid sequence located in the third intracellular loop of the D4 receptor protein, a region that is thought to interact with G-proteins and influence intracellular levels of cAMP.[14] The number of repeats at the DRD4 site varies from 2 to 10, but in populations of white ethnicity the 4 and 7 repeat lengths are the most common. Despite much investigation, it is still not clear what functional effect, if any, this polymorphism produces.[15,16]

Extensive study has been given to DRD4 in human behavioral genetics. The presence of a long allele (7, 8, or 10 repeat) at the DRD4 site has been strongly associated with attention deficit hyperactivity disorder.[17] In addition, there are conflicting, but predominantly negative, reports concerning the association of the D4 receptor long allele and both increased scores for the personality trait Novelty Seeking[18] and alcohol dependence.[15,17,19] However, no studies investigating the role of D4 variants in BP have been reported. In this study we explored the association between DRD4 and BP; we also examined putative associations between DRD4 and both personality traits and alcohol use.

Methods and Materials

Study Population

The present analysis includes 479 female and 385 male subjects from 286 families participating in the National Heart, Lung, and Blood Institute Family Blood Pressure Program at the site in Tecumseh, MI. Of the subjects, 99% were of non-Hispanic white ethnicity. Family eligibility in this study was dependent on the availability of a proband between 25 and 40 years old with systolic BP in the upper 15% of the BP distribution in earlier rounds of examinations and a sibling willing to participate.[20] Because we used BP as a quantitative phenotype in this study, treatment with antihypertensive medications excluded subjects from this analysis. In addition, subjects with elevated serum creatinine (>1.5 mg %), diabetes mellitus, pregnancy, or serious medical illness were excluded. When available, parents and additional siblings of included probands were studied irrespective of BP. All participants read and signed an informed consent document approved by the University of Michigan Insitutional Review Board for Human Subject Research.

Genotyping

The D4 receptor variant was amplified using primers D4-42 (5′-AGGACCCTCATGGCCTTG-3′) and D4-3 (5′-GCGACTACGTGGTCTACTCG-3′). A PTC 100 thermal cycler (MJ Research, Watertown, MA) was used for DNA amplification. Amplification reactions were performed in a total volume of 20 μL, containing approximately 50 ng of genomic template, 1 μmol/L of each primer, 200 μmol/L dNTP, 2 μL 10X Opti-Prime Buffer #6 (Stratagene, La Jolla, CA), and 1 U of *Taq* polymerase. The polymerase chain reaction cycling conditions consisted of an initial denaturation for 2 min at 94°C followed by 35 cycles of 94°C for 1 min, 60°C for 2 min and 72°C for 2 min and a final extension at 72°C for 4 min. The polymerase chain reaction products were electrophoresed on a 2% agarose gel and visualized under ultraviolet light using the GelStar nucleic acid gel stain (BioWhittaker Molecular Applications, Rockland, ME).

In accordance with previous studies, subjects were grouped on the basis of the presence of a long allele (7, 8, or 10 repeats).[15]

Blood Pressure Assessment

Blood pressure measurements were made with a standardized protocol by a single observer (LG) previously trained and certified in BP measurement technique. Two manual measurements were obtained for each subject with a standard mercury manometer, and the average systolic and diastolic BP were used in this study. Pulse pressure is the difference between systolic and diastolic pressure.

Questionnaire

Personality traits were assessed through the NEO Personality Inventory (NEO-PI). This inventory, consisting of 181 questions, assesses subjects on five global personality domains and breaks down three of these domains (Neuroticism, Extraversion, and Agreeableness) into six facets each. The NEO-PI is a well established inventory constructed through factor analytic strategies and provides high test–retest reliability and longitudinal stability.[21] A weighted Novelty Seeking score was calculated from NEO-PI domain scores using the formula described by Benjamin et al.[22]

Data necessary to establish diagnoses of alcohol abuse and dependence were not collected in this sample. However, subjects were asked: “Have you ever regularly consumed alcohol in your life?” The presence of association between D4 receptor genotypes and subjects’ response to this question were also explored.

Statistical Analysis

The presence of association was determined using the QTDT Program version 2.1 (available at http://www.sph.umich.edu/statgen/abecasis/QTDT/). QTDT was used to fit a variance components model to account for familial resemblance because of kinship and linkage. However, rather than modeling allelic effects based on allelic transmission, we tested the overall additive genetic effect of each polymorphism.[23,24] The presence of a statistical interaction between D4 receptor genotypes and age for diastolic and systolic BP was determined through a general linear model in SPSS 10.0 (SPSS Inc., Chicago, IL). Familial resemblance was not considered in testing for interactions. A concern in this type of study is the potential presence of population stratification that could result in false associations.[25] To assess whether the reported results were caused by stratification, STRUCTURE, a program designed to infer population structure using genotypes from numerous unlinked markers as genomic controls, was implemented.[26]

Results

We determined DRD4 genotypes at the D4 receptor repeat polymorphism for 864 subjects examined at the Tecumseh, MI, site of the National Heart, Lung, and Blood Institute Family Blood Pressure Program.[20] The allele frequencies (adjusted for familial correlation) were consistent with previously reported frequencies for white populations[27] (Table 1).

In an analysis controlling for familial correlations, we found an association between the presence of a D4 receptor long allele and higher systolic ($P = .031$) and diastolic BP ($P = .034$) (Table 2) but not pulse pressure ($P = .179$) (Table 2). In addition, there were significant interactions between D4 receptor genotype for age and systolic BP ($P = .009$) and age and pulse pressure ($P = .0002$). Figure 1 shows the interaction of age and mean systolic BP. For diastolic BP, there was no evidence of an interaction between genotype and age ($P = .824$).

In this study, subjects with the long allele had a higher frequency of self-reported regular alcohol use at some point during their lives compared with self-reported lifetime abstainers ($P = .008$). We could not distinguish between alcohol use and abuse using our available data. There was no correlation between reported lifetime history of alcohol use and systolic BP, diastolic BP, or pulse pressure in either male or female subjects in this sample. The D4 receptor variants showed no associations with any of the five NEO-PI personality traits or with a weighted Novelty Seeking personality trait (Table 2).

Weak evidence for clustering into two groups was found ($P = .02$) (B. Thiel and N.J. Schork, unpublished observations, 2003). However, there were no significant differences in D4 receptor allele frequency, systolic or diastolic BP, alcohol use, or any of the personality traits between the two clusters. Thus these reported results are unlikely to be the product of population stratification.

Table 1. Dopamine receptor 4 allele frequencies

Allele*	Frequency
2	0.08
3	0.04
4	0.68
5	0.01
6	<0.01
7	0.17
8	<0.01
10	<0.01

* Data are numbers of repeats.

Discussion

The results of this study indicate that the D4 receptor length polymorphism in exon 3 accounts for a small but significant proportion of the population variation in both systolic and diastolic BP in our sample of individuals of white ethnicity. In addition, we find evidence that the effect of D4 receptor genotype on systolic BP and pulse pressure is strongest in subjects >60 years of age.

The mechanisms mediating an association between the D4 length polymorphism and high BP are unknown, as there is limited information available concerning the potential role of D4 receptors in cardiovascular or renal function. Clozapine, an "atypical" antipsychotic agent with a relatively selective D4 affinity, has been reported to cause both hypotension and hypertension as well as substantial increases in heart rate, perhaps through stimulation of D4 receptors expressed in the heart.[28] Experimental studies have identified several renal physiologic effects associated with D4 receptor activation. In the cortical collecting tubule, D4 receptors antagonize the effects of V_2vasopressin receptors and aldosterone, thereby decreasing sodium and water reabsorption.[29] There is also a reported interaction between D4 and angiotensin II type 1 receptors, which could affect renal sodium reabsorption.[30] Along with proximal and distal tubules, D4 receptors are found prejunctionally in renal nerves that are widely distributed in periadventitial tissues of segmental, arcuate, interlobar, interlobular, afferent, and efferent arterioles and the glomerular capillary tuft.[31] The expression of D4 receptors on juxtaglomerular cell suggests a possible role in the control of renin release, although a D4 receptor mutation model in mice does not demonstrate alteration of renal function or circulating renin levels.[32]

The interaction observed between D4 receptor genotypes and age is of interest because systolic and pulse pressures rise continuously from middle age onward. It seems likely that the effects of genes affecting the level of BP differ over time, either because they are active during a critical period in the evolution of hypertension or be-

Table 2. Blood pressure and psychological trait scores as a function of dopamine receptor 4 genotype

Trait	Genotype	*N*	Mean	SEM	*P*
Systolic BP	Short/short	587	120.59	0.67	.031
	long/-	275	123.69	1.11	
Diastolic BP	Short/short	587	76.01	0.40	.034
	long/-	275	77.81	0.61	
Pulse pressure	Short/short	587	44.58	0.51	.179
	long/-	275	45.88	0.89	
Neuroticism	short/short	269	82.86	1.21	NS
	long/	128	85.81	1.82	
Extraversion	short/short	269	109.20	1.12	NS
	long/-	128	107.06	1.33	
Openness	short/short	268	103.98	0.97	NS
	long/	128	104.21	1.63	
Agreeableness	short/short	271	47.22	0.41	NS
	long/-	130	47.49	0.53	
Conscientiousness	short/short	271	48.87	0.49	NS
	long/-	129	47.16	0.74	
Novelty seeking (weighted)	short/short	267	19.92	0.42	NS
	long/-	127	20.78	0.69	
Alcohol use	short/short	406	0.34	0.02	.008
	long/-	182	0.50	0.04	

BP = blood pressure.

cause they contribute to age-related target organ effects caused by hypertension.[33]

If D4 receptor genotypes affect renal sodium handling, they may contribute to the increasing prevalence of salt sensitivity of BP with the aging process.

In addition to the relationship to BP, we also found an association between D4 receptor long alleles and a self-reported history of regular alcohol use. Most studies that have investigated D4 receptor variants and alcohol have focused on alcohol dependence or abuse. Although some studies have found an association between D4 receptor long alleles and increased incidence or severity of these traits, most findings have been negative.[15] Given our results, it may be fruitful to investigate the role of D4 receptor in the initiation of alcohol use rather than the escalation of alcohol use to the level of abuse and dependence. There is no association in this sample between D4 receptor and personality traits, a finding consistent with the results of two recent meta-analyses.[34,35]

In summary, our findings support a role for a common D4 polymorphism in the control of systolic BP in individuals >60 years of age. Investigation of the actions of D4 receptors in cardiovascular—renal physiology may yield insight into a mechanism of hypertension in human beings.

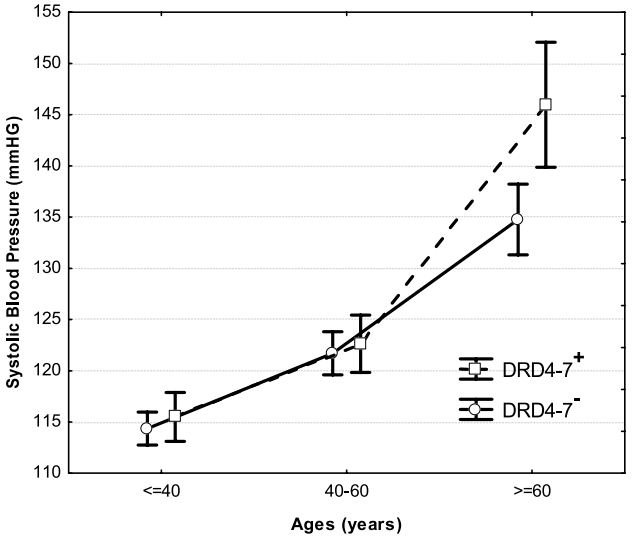

FIG. 1. Interaction between systolic blood pressure and age in subjects with long (DRD4-7+) and short (DRD4-7−) genotypes (mean ± 95% confidence intervals). DRD4 = dopamine-4 receptor.

Acknowledgments

We thank all the GenNet families who participated in the studies and Sandra Villafuerte for assistance with analyses.

References

1. Vinck WJ, Fagard RH, Loos R, Vlietinck R: The impact of genetic and environmental influences on blood pressure variance across age-groups. J Hypertens 2001;19:1007–1013.
2. Jose PA, Eisner GM, Felder RA: Role of dopamine receptors in the kidney in the regulation of blood pressure. Curr Opin Nephrol Hypertens 2002;11:87–92.
3. Hussain T, Kansra V, Lokhandwala MF: Renal dopamine receptor signaling mechanisms in spontaneously hypertensive and Fischer 344 old rats. Clin Exp Hypertens 1999;21:25–36.
4. Jose PA, Eisner GM, Felder RA: Renal dopamine receptors in health and hypertension. Pharmacol Ther 1998;80:149–182.

5. Tsuchida H, Imai G, Shima Y, Satoh T, Owada S: Mechanism of sodium load-induced hypertension in non-insulin dependent diabetes mellitus model rats: defective dopaminergic system to inhibit Na-K-ATPase activity in renal epithelial cells. Hypertens Res 2001; 24:127–135.
6. Sato M, Soma M, Nakayama T, Kanmatsuse K: Dopamine D1 receptor gene polymorphism is associated with essential hypertension. Hypertension 2000;36:183–186.
7. Felder RA, Sanada H, Xu J, Yu PY, Wang Z, Watanabe H, Ascico LD, Wang W, Zheng S, Yamaguchi I, Williams S, Gainer J, Brown NJ, Hazen-Martin D, Wong LJC, Robillard JE, Carey RC, Eisner GM, Jose PA: G protein-coupled receptor kinase 4 gene variants in human essential hypertension. Proc Natl Acad Sci USA 2002;99: 3872–3877.
8. Shikuma R, Yoshimura M, Kambra S, Yamazaki H, Takashina R, Takahashi H, Takeda K, Ijichi H: Dopaminergic modulation of salt-sensitivity in patients with essential hypertension. Life Sci 1986;38:915–921.
9. Rosmond R, Rankinen T, Chagnon M, Perusse L, Changnon YC, Bouchard C, Bjorntorp P: Polymorphism in exon 6 of the dopamine D(2) receptor gene (DRD2) is associated with elevated blood pressure and personality disorders in men. J Hum Hypertens 2001;15: 553–558.
10. Thomas GN, Tomlinson B, Critchley JA: Modulation of blood pressure and obesity with the dopamine D2 receptor gene TaqI polymorphism. Hypertension 2002;36:177–182.
11. Hussain T, Lokhandwala MF: Renal dopamine receptors and hypertension. Exp Biol Med 2003;228:134–142.
12. Falzone TL, Gelman DM, Young JI, Grandy DK, Low MJ, Rubinstein M: Absence of dopamine D4 receptors results in enhanced reactivity to unconditioned, but not conditioned, fear. Eur J Neurosci 2002;15:158–164.
13. Rubinstein M, Phillips TJ, Bunzow JR, Falzone TL, Dziewczapolski G, Zhang G, Fang Y, Larson JL, McDougall JA, Chester JA, Saez C, Pugsley TA, Gershanik O, Low MJ, Grandy DK: Mice lacking dopamine D4 receptors are supersensitive to ethanol, cocaine, and methamphetamine. Cell 1997;90:991–1001.
14. Van Tol HHM, Wu CM, Guan H-C, Ohara K, Bunzow JR, Civelli O, Kennedy J, Seeman P, Niznik HB, Jovanovic V: Multiple dopamine D4 receptor variants in the human population. Nature 1992; 358:149–152.
15. Lusher JM, Chandler C, Ball D: Dopamine D4 receptor gene (DRD4) is associated with Novelty Seeking (NS) and substance abuse: the saga continues. Mol Psychiatry 2001;6:497–499.
16. Oak JN, Oldenhof J, Van Tol HH: The dopamine D(4) receptor: one decade of research. Eur J Pharmacol 2000;405:303–327.
17. Faraone SV, Doyle AE, Mick E, Biederman : Meta-analysis of the association between the 7-repeat allele of the dopamine D(4) receptor gene and attention deficit hyperactivity disorder. Am J Psychiatry 2001;158:1052–1057.
18. Ebstein RP, Novick O, Umansky R, Priel B, Osher Y, Blaine D, Bennett ER, Nemanov L, Katz M, Belmaker RH: Dopamine D4 receptor (D4DR) exon III polymorphism associated with the human personality trait of Novelty Seeking. Nat Genet 1996;12:78–80.
19. Gelernter J, Kranzler H, Coccaro E, Siever L, New A, Mulgrew CL: D4 dopamine-receptor (DRD4) alleles and novelty seeking in substance-dependent, personality-disorder, and control subjects. Am J Hum Genet 1997;61:1144–1152.
20. Thiel BA, Chakravarti A, Cooper RS, Luke A, Lewis S, Lynn A, Tiwari H, Schork NJ, Weder AB: A genome-wide linkage analysis investigating the determinants of blood pressure in whites and African Americans. Am J Hypertens 2003;16:151–153.
21. Costa PT Jr, McCrae RR: Stability and change in personality assessment: the revised NEO Personality Inventory in the year 2000. J Pers Assess 1997;68:86–94.
22. Benjamin J, Li L, Patterson C, Greenberg BD, Murphy DL, Hamer DH: Population and familial association between the D4 dopamine receptor gene and measures of Novelty Seeking. Nat Genet 1996; 12:81–84.
23. Abecasis GR, Cardon LR, Cookson WO: A general test of association for quantitative traits in nuclear families. Am J Hum Genet 2000;66:279–292.
24. Abecasis GR, Cookson WO, Cardon LR: Pedigree tests of transmission disequilibrium. Eur J Hum Genet 2000;8:545–551.
25. Ding YC, Chi HC, Grady DL, Morishima A, Kidd JR, Kidd KK, Flodman P, Spence MA, Schuck S, Swanson JM, Zhang Y-P, Moyzis RK: Evidence of positive selection acting at the human dopamine receptor D4 gene locus. Proc Natl Acad Sci USA 2002; 99:309–314.
26. Lander ES, Schork NJ: Genetic dissection of complex traits. Science 1994;265:2037–2048.
27. Pritchard JK, Rosenberg NA: Use of unlinked genetic markers to detect population stratification in association studies. Am J Hum Genet 1999;65:220–228.
28. O'Malley KL, Harmon S, Tang L, Todd RD: The rat dopamine D4 receptor: sequence, gene, structure, and demonstration of expression in the cardiovascular system. New Biol 1992;4:137–146.
29. Li L, Schafer JA: Dopamine inhitis vasopressin-dependent camp production in the rat cortical collecting duct. Am J Physiol 1998;275 (Renal Physiol 44) :F62–F67.
30. Bek M, Zheng S, Asico LD: D4 dopamine receptor regulates blood pressure via V1 and AT 1 receptors (abstract). Am J Hypertens 2001;14:157A.
31. Ricci A, Marchal-Victorion S, Bronzetti E, Parini A, Amenta F, Tayebati SK: Dopamine D4 receptor expression in rat kidney; evidence for pre- and postjunctional localization. J Histochem Cytochem 2002;50:1091–1096.
32. Sanada H, Yao L, Jose PA, Carey RM, Felder RA: Dopamine D3 receptors in rat juxtaglomerular cells. Clin Exp Hypertens 1997:19: 93–105.
33. Schork NJ, Jokelainen P, Grant EJ, Schork MA, Weder AB: Relationship of growth and blood pressure in inbred rats. Am J Physiol (Regul Integrat Comp Physiol) 1994;R702–R708.
34. Kluger AN, Siegfried Z, Ebstein RP: A meta-analysis of the association between DRD4 polymorphism and novelty seeking. Mol Psychiatry 2002;7:712–717.
35. Schinka JA, Letsch EA, Crawford FC: DRD4 and novelty seeking: results of meta-analyses. Am J Med Genet 2002;114:643–648.