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Original Article

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Dopamine D3 receptor gene polymorphisms, blood pressure and nephropathy in type 1 diabetic patients

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Abstract

Background. Dopamine modulates blood pressure in the kidney. The aim of this study was to investigate whether two previously known (−707 G/C, Ser9Gly) and one novel (Ala17Ala) polymorphism in the dopamine D3 receptor gene and/or their haplotypes are associated with blood pressure, diabetic nephropathy or renal variables in the study subjects.

Methods. A cross-sectional, case-control study with a total of 996 type 1 diabetic patients from the multicentre, nationwide FinnDiane Study. Patients were recruited consecutively and classified into four groups according to their renal status.

Results. The frequencies of the genotypes harbouring the minor allele were 33, 51 and 19% for the −707 G/C, Ser9Gly and Ala17Ala polymorphisms, respectively. Frequencies of the −707 G/C minor genotypes were 35 (normoalbuminuria), 32 (microalbuminuria), 28 (proteinuria) and 39% (end-stage renal disease) ($\chi^2 = 6.3$, df = 3, $P = 0.1$), of the Ser9Gly 52, 51, 46 and 57% ($\chi^2 = 6.3$, df = 3, $P = 0.1$) and of the Ala17Ala polymorphism 18, 19, 19 and 21% ($\chi^2 = 0.7$, df = 3, $P = 0.9$), respectively. Five haplotypes were identified, but no differences were seen between those with and without diabetic nephropathy. Furthermore, there were no differences in blood pressure levels nor in any renal variables between genotypes or haplotypes.

Conclusions. These results do not provide evidence for an involvement of the dopamine D3 receptor gene in blood pressure levels or in the pathogenesis of diabetic nephropathy in type 1 diabetic patients.

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Introduction

Diabetic nephropathy is characterized by elevated blood pressure, proteinuria and a relentless decline in renal function, and is considered one of the most devastating complications in diabetes. The pathogenetic mechanisms are still largely unknown, but there is substantial evidence for a genetic involvement in the pathogenesis, although no major gene loci or susceptibility genes have been identified.

Dopamine is important in modulating sodium excretion and blood pressure in the kidney, wherein it is synthesized independently of nervous activity [1]. Nitecapone, an inhibitor of the dopamine-metabolizing enzyme catechol-*O*-methyl transferase, has been shown to reverse hyperfiltration, focal glomerulosclerosis and albuminuria in diabetic rats [2]. Of the two known dopamine D2-like receptors, the dopamine D3 receptor is expressed in glomeruli, proximal tubules and arterioles of the rat kidney [3,4].

Several polymorphisms have been identified in the dopamine D3 receptor gene (DRD3) and, in human studies there is evidence for an involvement of this gene in schizophrenia [5]. However, in mice, disruption of the D3 receptor gene induced a renin-dependent form of hypertension [6]. This is interesting, as the renin–angiotensin–aldosterone system (RAAS) has been studied extensively in diabetic nephropathy and ACE inhibitors are known to reduce albumin excretion rate (AER) and to slow the progression of nephropathy. The human DRD3 gene locus on chromosome 3q13.3 has also been linked to hypertension in two different

study populations [7,8]. Therefore, this study was undertaken to elucidate whether polymorphisms in the DRD3 gene are associated with diabetic nephropathy or blood pressure levels in type 1 diabetic patients.

Subjects and methods

Study group

This study is part of the ongoing nationwide, multicentre study, the Finnish Diabetic Nephropathy (FinnDiane) Study, involving 996 patients recruited from 20 referral centres between 1994 and 1999. A detailed description of the study population has been published elsewhere [9]. Blood pressure was measured with a mercury sphygmomanometer twice during a routine check-up and the mean systolic and diastolic blood pressure value of the measurements was used. Diabetes diagnosis, antihypertensive medication (AHT) and cardiovascular disease (CVD), which include coronary heart disease, acute myocardial infarction and stroke, were obtained from medical records. Informed consent was obtained from all subjects participating in the study; the study protocol followed the principles expressed in the Declaration of Helsinki and was approved by all local ethics committees.

Assays

The assays for measurement of serum and urinary creatinine levels, urinary albumin concentration, C-peptide concentrations, HbA_{1c} and cholesterol levels have been described previously [9]. The urinary sodium (Na^+) and potassium (K^+) were measured from one 24-h urine collection by using a standardized ion-selective electrode technique (normal range 130–240 and 60–90 mmol/l, respectively).

Genotyping

Genotyping was performed by an investigator unaware of the phenotypes using regular polymerase chain reaction (PCR). The reverse primers in each PCR reaction were biotinylated and the genotypes were determined using a solid-phase mini-sequencing method. The Ser9Gly and the Ala17Ala sites located on the exon 1 were comprised in the same PCR reaction using the forward primer 5′-TTTCTGTCTCCT CACAGGAA-3′ and reverse primer 5′-CAGCAGGCCATT GCCGAA-3′. Large sections with an emphasis on areas with no previously known polymorphisms in the DRD3 gene coding region, promoter and 3′ end were systematically sequenced by using standard sequencing procedures in 12 healthy control patients. Only one previously unknown A to G substitution in the codon 17 (Ala17Ala) at base position 278 bp (GenBank accession U25441) was discovered. The forward mini-sequencing primers in the Ser9Gly and Ala17Ala variants were 5′-CATCTCTGAGTCAGCTGA GT-3′ and 5′-CTGAACTACACCTGTGGGGC-3′, respectively. The PCR primers in the −707 G/C polymorphism have been described previously [10], wherein the 5′-TGGGAA GAATCTGGAGCTCA-3′ mini-sequencing primer was used for genotyping in this study. The haplotypes were determined using the PHASE computer program [11].

Statistical analysis

Descriptive data are expressed as mean ± SEM unless otherwise stated. Categorical variables were compared using the χ^2 test. Normally distributed continuous variables were tested with Student's *t*-test, while non-normally distributed variables were logarithmically transformed before analysis. Analyses were carried out using a STATISTICA 4.1 statistical package (Tulsa, OK). A *P*-value <0.05 was considered statistically significant. Linkage disequilibrium (LD) analyses between the markers were performed using the 2 by 2 program, version 1.50 [12].

Results

The clinical characteristics of the four patient groups are shown in Table 1. All four groups were in Hardy–Weinberg equilibrium regarding all polymorphisms. The frequencies of the minor alleles were 18, 30 and 10% for the −707 G/C, Ser9Gly and Ala17Ala, respectively. No differences in the genotypes or allele frequencies were observed between the four groups (Table 2). The cohorts were sufficiently large to yield 80% power to detect a 11% deviation of mutant genotype frequency with $P < 0.05$ and had 99.5% power to detect a 13% deviation between the four groups with one marker. The −707 G/C and Ala17Ala were in tight LD with the Ser9Gly ($D = 0.13$, $D' = 1.00$ and $D = 0.07$, $D' = 0.98$, respectively, $P < 0.0001$).

No differences in blood pressure levels, AER, creatinine clearance, urinary Na^+/K^+-ratio or in the prevalence of AHT and CVD were seen in any of the polymorphisms comparing patients with the wild genotype to patients with one or two mutant alleles. Neither were there any associations between the genotypes and endpoints like initiation of AHT, renal replacement therapy or onset of diabetic nephropathy. We further analysed whether any haplotype combination would associate with the renal status but no differences between the groups were seen (Table 3).

Discussion

In this study we found no association between the three studied polymorphisms in the dopamine D3 receptor and diabetic nephropathy, blood pressure levels, cardiovascular disease or any renal variable in patients with type 1 diabetes.

There are experimental data supporting a role for the dopamine system in the kidney [1], where the DRD3 receptor is expressed [3]. Disruption of the receptor in mice has resulted in an elevation of blood pressure levels with ~20 mmHg [6]. Renal renin activity was also higher in homozygous than in wild-type mice, and interestingly, blockade of the angiotensin II receptor 1 resulted in a more prolonged blood pressure decrease. A polymorphism in the DRD3 gene could theoretically influence the RAAS, a key player in both diabetic nephropathy and hypertension. There was, however,

Table 1. Clinical characteristics of 996 type 1 diabetic patients included in the study

	Normo $n=321$	Micro $n=166$	Prot $n=325$	ESRD $n=184$
M/F (%)	41/59	64/36	59/41	59/41
Age (years)	40.4 ± 0.5	38.1 ± 0.9	40.3 ± 0.5	44.0 ± 0.6
Duration of diabetes (years)	26.7 ± 0.4	25.1 ± 0.7	28.0 ± 0.4	32.0 ± 0.6
BMI (kg/m^2)	24.9 ± 0.2	25.7 ± 0.3	25.9 ± 0.2	23.8 ± 0.3
Waist-to-hip ratio male	0.90 ± 0.01	0.91 ± 0.01	0.94 ± 0.01	0.95 ± 0.01
Female	0.81 ± 0.01	0.82 ± 0.01	0.83 ± 0.01	0.87 ± 0.01
Antihypertensive therapy (%)	47 (15)	99 (62)	304 (96)	154 (91)
Cardiovascular disease (%)	10 (3)	6 (4)	42 (13)	48 (27)
Acute myocardial infarction (%)	5 (2)	4 (2)	21 (7)	23 (13)
Coronary heart disease (%)	9 (3)	6 (4)	33 (11)	37 (21)
Stroke (%)	2 (1)	0 (0)	17 (5)	18 (10)
Retinal laser treatment (%)	92 (29)	84 (51)	260 (81)	179 (99)
Systolic blood pressure (mmHg)	132 ± 1	138 ± 1	144 ± 1	154 ± 2
Diastolic blood pressure (mmHg)	79 ± 1	82 ± 1	83 ± 1	87 ± 1
HbA_{1c} (%)	8.0 ± 0.1	8.7 ± 0.1	8.9 ± 0.1	8.4 ± 0.1
Albumin excretion rate				
μg/min[a]	5 (1–17)	38 (3–198)	–	–
mg/24 h[a]	–	–	1063 (10–16 600)	–
Creatinine clearance ($ml \times s^{-1} \times 1.73\,m^{-2}$)	1.62 ± 0.02	1.51 ± 0.04	1.02 ± 0.04	0.81 ± 0.07
Urinary sodium/potassium ratio	1.82 ± 0.05	1.92 ± 0.07	2.03 ± 0.06	1.83 ± 0.12

Data are means ± SEM, median (range) or *n* (%). Normo, normal albumin excretion rate; Micro, microalbuminuria; Prot, proteinuria; ESRD, end-stage renal disease.
[a]Some patients with previously abnormal AER had responded to antihypertensive treatment and showed a regression of AER at the time of investigation.

Table 2. Distribution of genotypes and minor allele frequencies of the DRD3 polymorphisms

Polymorphism	Normo	Micro	Prot	ESRD	*P*
−707 G/C: GG	210 (65)	113 (68)	233 (72)	113 (61)	
GC	102 (32)	46 (28)	82 (25)	62 (34)	
CC	9 (3)	7 (4)	10 (3)	9 (5)	NS
C	0.19	0.18	0.16	0.22	NS
Ser9Gly: AA	153 (48)	81 (49)	174 (54)	80 (43)	
AG	140 (44)	70 (42)	123 (38)	80 (44)	
GG	28 (9)	15 (9)	28 (9)	24 (13)	NS
G	0.31	0.30	0.28	0.35	NS
Ala17Ala: AA	263 (82)	134 (81)	263 (81)	145 (79)	
AG	53 (17)	29 (17)	59 (18)	38 (21)	
GG	5 (2)	3 (2)	3 (1)	1 (1)	NS
G	0.10	0.11	0.10	0.11	NS

Genotype data are *n* (%). Normo, normal albumin excretion rate; Micro, microalbuminuria; Prot, proteinuria; ESRD, end-stage renal disease.

Table 3. Haplotype distribution of the −707 G/C, Ser9Gly and Ala17Ala (A/G) polymorphisms in 996 type 1 diabetic patients

Haplotypes	Allele combination	Normo ($n=642$)	Micro ($n=332$)	Prot ($n=650$)	ESRD ($n=368$)	*P*
H1	G-Ser-A	446 (69.5)	232 (70.0)	470 (72.3)	238 (64.7)	NS
H2	C-Gly-A	120 (18.7)	60 (18.1)	102 (15.7)	80 (21.7)	NS
H3	G-Gly-G	63 (9.8)	35 (10.5)	64 (9.9)	38 (10.3)	NS
H4	G-Gly-A	13 (2.0)	5 (1.5)	13 (2.0)	10 (2.7)	NS
H5	G-Ser-G	0	0	1 (0.2)	2 (0.5)	NS

Data are *n* (%). NS, non-significant; Normo, normoalbuminura; Micro, microalbuminuria; Prot, proteinuria; ESRD, end-stage renal disease.

no association with blood pressure levels or with any stage of nephropathy between the genotypes. In order to eliminate a possible bias introduced by the use of antihypertensive medication, the analyses were performed separately excluding normoalbuminuric and microalbuminuric patients taking ACE inhibitors and ATII receptor blockers. However, the results did not change. Interestingly, inhibition of Na^+-K^+-ATPase activity has been shown recently to be mediated by the D2-like receptor [13], but we did not observe an impact of the DRD3 gene on the urinary Na^+-K^+-ratio.

It has been suggested that information from as few as two or three single nucleotide polymorphism (SNPs) can identify a haplotype block in a chromosomal region. Thus, haplotype analysis is a powerful method to analyse close-by SNPs and can detect associations that are missed if the polymorphisms will be analysed separately only. However, such an approach did not change the results in this study.

There are no data available on any polymorphism having an impact on the function of the DRD3 gene. This is probably due to the fact that, in comparison with other dopamine receptors, the DRD3 receptor has a low abundance and there is an absence of selective ligands [14].

There are several reasons why we chose these particular polymorphisms for the present association study. The Ser9Gly polymorphism leads to an amino acid substitution and, although the functionality is not established, a meta-analysis supports an association with schizophrenia [5], thus suggesting that this polymorphism could be in itself or in LD with a functional polymorphism. The novel Ala17Ala polymorphism has not been reported previously and, although it does not change the amino acid, it could reflect a LD with a still unknown polymorphism. From the 5′ end of the gene, we genotyped only the –707 G/C polymorphism, as it is in almost complete LD with the other polymorphisms identified in this region [10].

Although we cannot entirely exclude a role for the DRD3 gene in the regulation of blood pressure levels or the pathogenesis of diabetic nephropathy, the present study provides rather strong evidence against a major impact of the gene in these traits. Our study had sufficient statistical power to detect a significant difference in the genotypes between the groups. We analysed the data both separately by dividing the patients into four groups according to their AER and by pooling together those with an abnormal AER. An association between blood pressure and genotypes was assessed from actual blood pressure measurements and from the use of antihypertensive medication, but no differences were seen between the genotypes. Recently, focus has been put on the duration of diabetes to the onset of nephropathy [15]. However, no differences in the genotype distributions or haplotypes between the groups were seen when the patients were divided into medians and quartiles according to the duration of diabetes.

Our results do not, however, exclude a possible role of other dopamine receptors or the dopamine system in the development of diabetic nephropathy or hypertension in general. Disruption of the type 2 dopamine receptor gene (DRD2) has resulted in a sodium-dependent increase in blood pressure in mice [16], which is interesting, as an association between a polymorphism in the coding region of this gene and elevated blood pressure has been reported in two studies [17,18]. On the other hand, possible involvement of D1-like receptors is supported by a study in DRD1 knockout mice, showing impaired renal sodium transport and hypertension [19]. A polymorphism in this gene has also been associated with hypertension in humans [20]. A recent study has reported increased sympathetic tone and elevated blood pressure in mice lacking the DRD5 receptor [21], but no polymorphisms have so far been studied regarding hypertension or kidney function in humans.

In conclusion, this study does not provide evidence for a role of the dopamine D3 receptor gene in blood pressure regulation or in the pathogenesis of diabetic nephropathy in type 1 diabetic patients.

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