Polymorphic Genes for Kinin Receptors, Nephropathy and Blood Pressure in Type 2 Diabetic Patients

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Key Words

Kinin receptors • Gene • Polymorphisms •
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Abstract

Background/Aims: There is evidence that hereditary predisposition contributes to the development of diabetic nephropathy and hypertension. Polymorphisms in the genes for bradykinin receptors (B_1R and B_2R) were found to be associated with decreased risk of the development of end-stage renal disease. This study examines whether B_1R G⁻⁶⁹⁹C and B_2R C¹⁸¹T polymorphisms are associated with microalbuminuria or overt nephropathy, or blood pressure variation in type 2 diabetic subjects. **Methods:** B_1R and B_2R polymorphisms were determined in 153 type 2 diabetic patients with microalbuminuria, 132 with overt nephropathy (macroalbuminuria or chronic renal failure), and 161 patients with normoalbuminuria despite diabetes duration longer than 10 years. **Results:** Distributions of the examined polymorphisms did not differ between patients with microalbuminuria or overt nephropathy, compared to normoalbuminuric control subjects. Patients carrying the B_2R T allele had lower DBP, compared with non-carriers: 83.6 ± 12.0 vs. $87.4 \pm$

12.1 mm Hg, $p < 0.05$. Among patients not receiving ACEI, both SBP and DBP was significantly lower in B_2R T allele carriers, compared to non-carriers (137.2 ± 20.3 vs. 146.5 ± 21.7 mm Hg, and 80.3 ± 11.9 vs. 85.8 ± 11.6 mm Hg, $p < 0.05$). **Conclusions:** Examined polymorphisms are not associated with the increased risk of incipient or overt nephropathy in type 2 diabetic patients. B_2R C¹⁸¹T polymorphism may contribute to blood pressure variation in these subjects.

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Introduction

Development of kidney disease in diabetic patients depends on the presence of hyperglycemia, together with several superimposing environmental and hereditary factors [1, 2]. On the other hand, predisposition to diabetic nephropathy is closely linked to hypertension [3, 4]. Also, raised blood pressure contributes substantially to faster rate of progression of renal disease, in diabetic as well as in non-diabetic subjects [5–8].

Recent studies provided evidence for a key role of the kallikrein-kinin system in blood pressure homeostasis [9]. Kinin peptides, acting via B_1 and B_2 receptors were shown

to have a protective role against salt-induced hypertension [10–15], and to express nephroprotective effect [16–18]. The B₂ receptor (B₂R) is a mediator of most physiological actions of bradykinins, including natriuresis, vasodilatation, increased production of eicosanoids and nitric oxide. The expression of the B₁ receptor (B₁R) is generally absent in normal conditions, but rapidly increases after tissue injury, under the stimulation of cytokines and growth factors [9, 19]. While angiotensin I-converting enzyme inhibitors (ACEI) are known as slowing the progression of diabetic nephropathy [20, 21], it has been demonstrated that ACEI action is, at least in part, a result of their ability to decrease degradation of kinins [22–24].

Thus, it has been postulated that sequence variants of the genes encoding for kinin receptors might determine different response of the kidney to various types of tissue injury, either as a B₂ receptor-mediated, blood pressure dependent effect, or under B₁ receptor-dependent mechanism of different reaction to cytokine stimulation. Recently, evidence for a relationship between sequence differences in the genes encoding for kinin receptors and the development of end-stage renal disease (ESRD) has surfaced from both case-control, and family-based association studies [25, 26]. In the present study, we report the results of association analysis between polymorphic markers in the genes encoding for kinin receptors: G⁻⁶⁹⁹→C substitution in the promoter region of the B₁R gene, and C¹⁸¹→T transition in exon 2 of the B₂R gene, with different stages of diabetic kidney disease, and blood pressure variation in patients with type 2 (non-insulin-dependent) diabetes mellitus.

Methods

Detailed method of collecting patients for the study was described elsewhere [27]. Briefly, 1,270 patients with type 2 diabetes were screened for microalbuminuria with dipstick MICRAL II test (Boehringer Mannheim), with subsequent verification of renal status according to repeated measurements of urinary albumin/creatinine ratio and careful review of medical files in all dipstick-positive, and in dipstick-negative patients with diabetes duration of at least 10 years (patients with previously documented persistent proteinuria or persistent raise in serum creatinine were included into the overt nephropathy group without further determination of microalbuminuria). Of the initial study 'screening group', we selected 132 patients with overt nephropathy, including 46 patients with chronic renal failure (among them 23 patients undergoing dialysis), 155 patients with microalbuminuria, and 163 normoalbuminuric subjects with known diabetes duration of at least 10 years. Hypertension was diagnosed, if patients were receiving antihypertensive treatment, or systolic or diastolic blood pressure taken at the time of microalbuminuria screening, or at time of examination of patients with previously determined

overt proteinuria, and during a subsequent visit in the clinic was ≥ 140 or 90 mm Hg (blood pressure was measured twice with a mercury sphygmomanometer, in the supine position, after at least 10 min of rest, and the results were averaged). All participants of the study were Caucasians, and gave written informed consent. The study protocol was approved by the appropriate Ethics Board.

From each patient, DNA was isolated from peripheral blood leucocytes. B₁R gene G⁻⁶⁹⁹→C substitution in the promoter region, as well as the B₂R gene C¹⁸¹→T transition in exon 2 were determined according to previously described protocols [26]. In 6 patients, genotypes were not determined due to technical problems with DNA quality.

Continuous variables were compared using either ANOVA and the post-hoc Sheffé test or Student's t test. A χ^2 test was used for comparisons of frequencies. Multiple linear regression was used to assess the association of various putative hypertension promoters to actual blood pressure values.

Results

Clinical characteristics of the study patients are given in table 1. Positive family history of hypertension in at least one parent was reported by 19.5% of the study patients, while positive history of type 2 diabetes – by 31.7% of patients.

Distributions of the examined B₁R G⁻⁶⁹⁹C and B₂R C¹⁸¹T genotypes in microalbuminuria and overt nephropathy groups did not differ from the control group of normoalbuminuric subjects (table 2). When both groups of microalbuminuric and overt proteinuric patients were analyzed jointly, still no deviation in genotype distributions was present (data not shown). Distributions of the examined polymorphisms were similar among type 2 diabetic patients stratified according to hypertension status: frequencies of the B₁R C allele carriers were 23.2% and 21.8%, B₂R T allele carriers – 15.5% and 12.0% for normotensive and hypertensive individuals, respectively. In the overall group of type 2 diabetic patients with determined B₁R and B₂R genotypes and included into the study groups (regardless of hypertension status), carriers of the B₂R T allele had significantly lower actual diastolic blood pressure (taken at the time of microalbuminuria screening), compared with non-carriers: 83.6 ± 12.0 vs. 87.4 ± 12.1 mm Hg, $p < 0.05$. Observed difference in systolic blood pressure: 145.6 ± 22.4 versus 150.3 ± 21.6 mm Hg (T allele carriers versus non-carriers, respectively), did not reach statistical significance. Among 250 patients not receiving ACEI, both systolic and diastolic blood pressure was significantly lower in 32 B₂R T allele carriers, compared to 218 non-carriers (137.2 ± 20.3 vs. 146.5 ± 21.7 mm Hg, and 80.3 ± 11.9 vs. 85.8 ± 11.6 mm Hg, $p < 0.05$). Not surprisingly, patients on

Table 1. Selected clinical characteristics of the study groups

	Normoalbuminuria diabetes duration ≥ 10 years	Micro- albuminuria	Overt proteinuria and CRF
n	163	155	132
Age at examination, years	64.3 ± 7.8	62.0 ± 8.2 ^a	62.0 ± 8.9
Age at diagnosis of diabetes, years	48.5 ± 9.4	49.7 ± 12.3	50.8 ± 10.3
Known diabetes duration, years	15.8 ± 5.5 ^b	11.2 ± 6.8	11.2 ± 7.0
Body mass index, kg/m ²	29.3 ± 4.5	29.9 ± 4.3	29.0 ± 4.3
HbA1c, %	8.9 ± 1.7	9.2 ± 1.7 ^c	8.4 ± 1.8 ^c
Hypertension, %	51.8	62.8	79.6 ^d
Retinopathy, %			
Any	54.2	49.7	61.6
Background	49.7	41.4	40.7
Proliferative/proliferative	4.5	8.3	20.9 ^e

CRF = Chronic renal failure.

^a p < 0.05, vs. normoalbuminuria; ^b p < 0.001, vs. microalbuminuria and overt proteinuria/CRF; ^c p < 0.01, vs. overt proteinuria/CRF; ^d p < 0.05, vs. microalbuminuria, p < 0.01, vs. normoalbuminuria; ^e p < 0.05, vs. microalbuminuria, p < 0.001, vs. normoalbuminuria.

Table 2. Frequencies of B1R G-699C and B2R C181T genotypes in the study groups

	Normoalbuminuria diabetes duration ≥ 10 years		Microalbuminuria		Proteinuria/CRF	
	%	n	%	n	%	n
B1R G-699C						
GG	78.3	126	78.5	120	73.9	96
GC	18.6	30	20.3	31	24.6	32
CC	3.1	5	1.3	2	1.5	2
B2R C181T						
CC	87.0	140	83.7	128	90.0	117
CT	13.0	21	15.7	24	10.0	13
TT	0	0	0.6	1	0	0

ACEI treatment had higher blood pressure values, compared to those without ACEI (156.4 ± 20.0 vs. 145.2 ± 22.0 mm Hg, and 89.6 ± 12.4 vs. 85.0 ± 11.9 mm Hg, SBP and DBP, respectively). The presence of the B₂R T allele was also independently associated with diastolic blood pressure, when accounted for renal status, body mass index, diabetes duration and glycemic control, (p < 0.01). There was no association between the systolic or diastolic blood pressure and the B₁R polymorphism (data not shown).

Discussion

Recently, two studies revealed an association between the B₁R -699C allele and decreased risk of ESRD, which was demonstrated as lower C allele frequency, or diminished C allele transmission from heterozygous parents to patients affected with ESRD [25, 26]. In these studies, proportion of patients with diabetic nephropathy was 10.5 and 19%, respectively, thus giving insufficient numbers of subjects to assess the magnitude of impact of the C allele on the risk of ESRD in diabetic patients. In the present study, aimed on type 2 diabetic patients with microalbuminuria or overt nephropathy, no association similar to that previously observed in ESRD patients was

demonstrated. However, the relatively small number of type 2 diabetic patients with chronic renal failure included into the overt nephropathy group did not allow us to completely exclude any minor impact of B₁R polymorphism on progression to most advanced stages of diabetic nephropathy.

In case of B₂R exon 2 polymorphism, previous reports demonstrated significantly diminished transmission of the ¹⁸¹T allele to offspring affected with ESRD [26], with only minor decrease in its frequency in ESRD patients compared to healthy control subjects [25]. The present analysis revealed no differences in the distributions of this polymorphism in type 2 diabetic patients stratified according to the stage of diabetic nephropathy, or the presence or absence of hypertension. On the other hand, carriers of the ¹⁸¹T 'protective' allele had lower actual blood pressure values, taken at the time of examination, compared to non-carriers, and the effect was even more profound, when patients with possibly confounding impact of treatment with ACEI were excluded from the analysis. However, several limitations of this analysis should be mentioned: Firstly, the effect of response to antihypertensive treatment other than ACEI, different in ¹⁸¹T allele carriers and non-carriers, however unlikely, cannot be completely ruled out. Secondly, biased sampling of patients for the analysis might have occurred. Such selection bias, for instance associated with the exclusion of normoalbuminuric patients with type 2 diabetes duration shorter than 10 years from the study group, or resulting from the 'cardiovascular survival effect' of the examined polymorphism should also be considered. Lastly, only one blood pressure measurement performed in controlled conditions was available for most of our patients, which contributed to a large variation of blood pressure levels in our examined group. Anyhow, such issues can only be completely resolved by a prospective, observational study, focused on repeated blood pressure measurements in an unselected population of diabetic patients, with careful consideration of antihypertensive treatment and survival.

By which mechanisms would molecular variants of the genes encoding for kinin receptors modify the risk of ESRD, or other 'cardiovascular phenotypes' (e.g. blood pressure values), is yet unclear. Previous functional experiments have determined that the presence of the B₁R promoter -⁶⁹⁹C allele is associated with higher transcriptional promoter activity [25]. Whether this sequence difference may also cause a different magnitude of the B₁R overexpression in response to tissue injury or in recently observed B₁R upregulation in animal models of hyperten-

sion or B₂R knockout [28] remains to be determined. Interestingly, it was demonstrated that in the absence of the B₂ receptor, the B₁R assumes some of the hemodynamic properties of the B₂R [28].

Functional significance of the B₂R Arg¹⁴→Cys exchange in the N-terminal extracellular domain caused by the C¹⁸¹T substitution is still unclear. The N-terminal domain can be truncated without the apparent loss of function by the receptor [29], which, however, does not exclude any functional alteration associated with Arg→Cys substitution. Moreover, the C¹⁸¹T polymorphism was shown to selectively influence the bradykinin-induced contractility of the human umbilical vein, which may, for instance, suggest an altered affinity of the polymorphic extracellular domain of the receptor [30].

Genes encoding for bradykinin receptors were also studied as candidates that might influence the risk of development of hypertension. The B₂R promoter -⁵⁸C allele was found to be associated with hypertension in populations of Japanese and African-Americans [31, 32], but no positive association with other examined sequence differences in bradykinin receptor genes was detected. On the other hand, in a study based on Caucasian tissue donors, this B₂R promoter polymorphism exerted no effect on contractile response of the umbilical vein to kinins [30], even though previous experiments have suggested its influence on transcriptional activity [33, 34]. Interestingly, in a recent study of 49 type 1 and 112 type 2 diabetic patients, a polymorphic variant located in exon 1 of the B₂R gene was associated with increased urinary albumin to creatinine ratio and serum creatinine levels [35].

In summary, our study demonstrated no relationship between polymorphisms in the genes encoding for kinin receptors: B₁R G-⁶⁹⁹C and B₂R C¹⁸¹T, and the presence of incipient or overt nephropathy in Caucasian type 2 diabetic patients. However, in our examined population of type 2 diabetic patients, the B₂R ¹⁸¹T allele was associated with significantly lower systolic and diastolic blood pressure. Certainly, our results obtained in a relatively small group should be replicated in larger samples of diabetic patients, and, moreover, cannot be generalized to a population of non-diabetic patients. Also, further studies of several markers from the bradykinin receptors genes, with estimations of linkage disequilibrium between the examined loci, as well as combining the examined polymorphisms into haplotypes should on one hand give us more power to detect marker-phenotype association, while on the other, could lead us to determination of target loci for subsequent functional experiments.

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