Brief Genetics Reports

Genetic Variation of the Heparan Sulfate Proteoglycan Gene (Perlecan Gene)

Association With Urinary Albumin Excretion in IDDM Patients

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Both in patients with IDDM (1) and in healthy control subjects (2,3), increased urinary albumin excretion rate (AER) is associated with high relative morbidity and mortality. In IDDM patients, genetic susceptibility factors are most likely contributing to an increased AER (4,5) resulting in a cumulative incidence of nephropathy (AER >300 mg/24 h) of ~30% (6). So far, candidate genes, proposed as susceptibility markers linked to abnormal albuminuria, have been identified because of the knowledge of pathophysiological events related to diabetic nephropathy. Heparan sulfate proteoglycan (HSPG, i.e., Perlecan) constitutes an integrated part of the glomerular basement membrane. It consists of a central core protein to which anionic sulfated polysaccharide chains (heparan sulfate [HS]) are linked (7), thus contributing to the negative charge of the glomerular filtration barrier and thereby indirectly to the composition of the glomerular filtration product (8,9). In IDDM patients, it has been demonstrated that increased AER is reduced by the administration of heparin (10) most likely because of a stimulating effect on the HS synthesis (11).

The aim of the present study was for the first time to identify and characterize polymorphisms within the HSPG gene (HSPG2) and subsequently evaluate a possible association between such markers and nephropathy in a case-control study design comprising large groups of Caucasian IDDM patients with and without diabetic nephropathy from Denmark and U.K. The Danish population comprised 260 patients with IDDM recruited from the outpatient clinic at the Steno Diabetes Center. Of the patients, 170 had diabetic nephropathy (DK_N), defined as persistent albuminuria (AER >300 mg/24 h) in more than two consecutive 24-h urine collections. They had no clinical or biochemical evidence of ongoing nondiabetic kidney or renal tract disease, and AER was <30 mg/24 h within the first 5 years from the onset of diabetes. Ninety diabetic patients were normoalbuminuric (DK_0) (AER <30 mg/24 h), with a diabetes duration of >20 years. The U.K. population comprised 397 IDDM patients collected from a number of centers in the U.K. Of the patients, 247 had diabetic nephropathy (UK_N), defined as the presence of persistent "Albustix" (Bayer Diagnostics, Basingstoke, U.K.), positive proteinuria on three separate occasions within 6 months, hypertension, and retinopathy. Urinary tract infection, cardiac failure, or other nondiabetic renal disease was not observed. Of the patients, 150 were nonnephropathic (UK_0) with long duration of diabetes (>20 years). Some of this cohort may have microalbuminuria (AER in the range of 20–200 μg/min). However, the positive predictive value of microalbuminuria and progression to overt nephropathy after such long duration of disease is extremely low (12).

Genomic DNA extracted from leukocytes were screened for restriction fragment length polymorphisms (RFLPs) using 15 different endonucleases. In polymerase chain reaction (PCR)-based assays, three diallelic polymorphic restriction sites were revealed: two *Bam*HI sites and one *Taq* I site. Allele- and genotype frequencies were compared using Fisher's exact test, or for 3 × 2 contingency tables, the χ^2 test was used. *P* values (two-sided) of <0.05 were accepted as significant. Odds ratios were calculated for alleles and corresponding genotypes.

When comparing the groups DK_N and DK_0, a significant difference was found for one of the *Bam*HI sites, allelic frequencies ($P = 0.02$), and genotype frequencies ($P = 0.07$) (Table 1). The frequency of the genotypes in the Danish background population ($n = 89$) was 62% (250 bp homozygous), 31% (heterozygous), and 7% (150 + 100 bp homozygous). To examine the possibility of a type 1 error, the U.K. material was analyzed. An identical distribution of alleles and genotypes was revealed, i.e., a significant difference in allele frequency ($P = 0.04$) and a trend for difference in genotypes ($P = 0.07$) (Table 1). Because no heterogeneity was observed between

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Additional information can be found in the on-line appendix at www.diabetes.org/diabetes/appendix.htm.

AER, albumin excretion rate; DK_0, subjects in Denmark without nephropathy; DK_N, subjects in Denmark with nephropathy; HS, heparan sulfate; HSPG, heparan sulfate proteoglycan; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; UK_0, subjects in the U.K. without nephropathy; UK_N, subjects in the U.K. with nephropathy.

TABLE 1
*Bam*HI HSPG2 allelic and genotypic frequencies in IDDM patients with nephropathy and without nephropathy

	DK_O	DK_N	*P* value	UK_O	UK_N	*P* value
n	90	170	—	150	247	—
Alleles (bp)						
250	131 (0.73)	278 (0.82)	—	225 (0.75)	403 (0.81)	—
(150 + 100)	49 (0.27)	62 (0.18)	0.02	75 (0.25)	91 (0.19)	0.035
Genotypes (bp)						
Homozygous (250)	50 (56)	115 (68)	—	82 (55)	161 (65)	—
Heterozygous (250 [150 + 100])	31 (34)	48 (28)	0.07	61 (40)	81 (33)	0.065
Homozygous (150 + 100)	9 (10)	7 (4)	—	7 (5)	5 (2)	—

For alleles, data are *n* (frequency). For genotypes, Data are *n* (%). The primers used for this assay were 5′-CATGTCCCATGCCCCACGTGTGCT-3′ and 5′-ATTGTAGCTGTGGCAGGCAAACTC-3′.

the subgroup data sets from Denmark and the U.K., respectively, the data were combined. This revealed significant differences both in allele frequency ($P < 0.002$) and genotype frequency ($P < 0.005$). The results indicate that diabetic patients possessing the 250-bp allele have a risk of nephropathy of 2.4 times (95% CI 1.7–3.2) those not possessing the 250-bp allele. The cohorts were sufficiently large to yield 80% power to detect a 7% deviation in carrier rate of the 250-bp allele with $P < 0.05$.

Within the HSPG2 gene located on chromosome 1p36.1→p35, the present approach allowed us to estimate the intron-exon organization of the first part of the gene. The polymorphic *Bam*HI restriction site was mapped to domain I, intron 6. Domain I, near the amino terminus, appears unique for the proteoglycan, since it shares no significant homology with any other proteins. Domain I contains the putative attachment sites for the HS side chains (13), thereby being of importance for the presentation of anionic molecules in the glomerular basement membrane. Domain I is therefore an obvious candidate region in the search for defects associated with albuminuria, and the *Bam*HI RFLP may be a marker of this. Within this region, we sequenced a 241-bp region in which the polymorphic *Bam*HI site was identified in position 97–102 caused by a T-to-G transversion. The result was reproduced sequencing DNA from two individuals homozygous for the 250-bp allele, two individuals homozygous for the 150 + 100-bp allele, and five randomly chosen unrelated individuals. No other polymorphisms were identified.

In conclusion, this is the first report that demonstrates an association of diabetic nephropathy and a candidate gene involved in the synthesis of glomerular structure proteins. We have demonstrated a significant association of a *Bam*HI HSPG2 polymorphism with diabetic nephropathy confirmed in two independent case-control studies. However, other study populations should be investigated, and intrafamilial studies (e.g., using the transmission disequilibrium test [TDT] [14]) are required to confirm or refute these data.

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