Association of Nephrin Gene Polymorphisms With Type 2 Diabetes in a Japanese Population

The Funagata Study

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Nephrin is a major component of the glomerular filtration barrier, and its expression was at first thought to be specific to kidney glomerular podocytes (1). However, it was later found in nonrenal tissues, such as the pancreas (2–4), as well. Several studies (2,3,5) have shown the expression of nephrin in human pancreatic islet cells. Therefore, nephrin may play some roles in the pancreatic islet and, thus, may be involved in the pathophysiology leading to diabetes. We here examined the association of the nephrin gene polymorphisms with type 2 diabetes.

RESEARCH DESIGN AND METHODS — The associations of three single nucleotide polymorphisms (SNPs) (C294T, −61C/G, and C2289T) of the nephrin gene with diabetes were examined using two (first and second) sample sets (diabetes, $n = 72$ and 31, respectively; impaired glucose tolerance [IGT], $n = 75$ and 77, respectively; and normal glucose tolerance [NGT], $n = 227$ and 244, respectively) from the cohort population of the Funagata Study, a Japanese community-based study (6). The study conditions were the same as those in our previous report (7).

Three SNPs in the nephrin gene (C to T at nucleotide position 294 in exon 3 from the translation intitiation codon ATG (C294T) (JSNP database ID: IMS-JST 035656), C to G in intron 5 at nucleotide position 61 upstream from the intron 5/exon 6 splicing site (−61C/G) (IMS-JST021061), and C to T at nucleotide position 2,289 in exon 17 (C2289T) (IMS-JST000541) were analyzed. SNPs C294T and C2289T are silent. The genotyping was conducted with a fluorogenic PCR as described previously (8). The TaqMan primer sets used for the amplification were 5′-CCCCTGCAGGTGAATTCCA-3′ and 5′-GCACTCATACTCCGCGTCATC-3′ for SNP C294T, 5′-CCTGGATCCCAGAG GAGATCA-3′ and 5′-CAGGGTTATA GAGTCAGAGTCATCATCT-3′ for SNP −61C/G, and 5′-GACCCCACTGAGGT GAACGT-3′ and 5′-GAACATGCCCGG GAGGAT-3′ for SNP C2289T. TaqMan probes labeled with either FAM or VIC reporter molecules were: 5′-TGCACAT*T*GAG GCCT-3′ and 5′-CACAT*C*GAGGCCTG-3′ for SNP C294T, 5′-TCAGG*C*AGAA GAGG-3′ and 5′-TCAGG*G*AGAA GAGGT-3′ for SNP −61C/G, and 5′-TGGCATCAACAGTGC-3′ and 5′-ATTGGCATCGACAGTG-3′ for SNP C2289T. Linkage disequilibrium block constructions and haplotype analyses were performed as previously reported (9).

The association tests were conducted with standard allele positivity tables (Fisher's exact probability test) between SNP genotype frequency and case-control status in dominant and recessive genetic models. To assess the allelic association between each haplotype or each diplotype and the disease phenotype, we performed a Fisher's exact probability test with Bonferroni correction by collapsing a multicolumn table to a 2×2 table. $P < 0.05$ was accepted as significant.

RESULTS — The associations of the genotype of these SNPs with diabetes were analyzed using the first sample set. As shown in Table 1, all SNPs examined showed a significant association with diabetes. Furthermore, χ^2 tests for trend showed significant linear trends, namely, the frequency of subjects with the at-risk genotypes increased from the NGT to the IGT and to the diabetic groups. The association of SNP C2289T, which showed the most significant association among the SNPs examined, was further confirmed by analysis using the second sample set as well ($P = 0.0463$). We then conducted multiple logistic regression analysis to examine the independent association of SNP C2289T using the two sample sets combined ($n = 103$, 152, and 471, respectively for diabetes, IGT, and NGT). After adjustment for serum levels of triglyceride, BMI, and systolic blood pressure, which were significantly different among the study groups, the SNP still showed a significant association with IGT (odds ratio [OR] 0.60, $P = 0.0148$) and diabetes (OR 0.38, $P < 0.0001$).

Haplotype analysis using these SNPs revealed a linkage disequilibrium block with the six haplotypes. Haplotype 1, which is composed of the non–

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Abbreviations: IGT, impaired glucose tolerance; MEC, microvascular endotherial cell; NGT, normal glucose tolerance; SNP, single nucleotide polymorphism.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Table 1—*Association of SNPs in the nephrin gene with diabetes*

SNP	Genotypes	NGT	IGT	*P*	Diabetes	*P*	*P* for trend
n		227 or 225	75		72		
C294T	At risk (TT + CC)	39 (17.3)	16 (21.3)	0.4909	25 (34.7)	0.0028*	0.0026*
Silent, exon 3	Non–at risk (CC)	186 (82.7)	59 (78.7)		47 (65.3)		
−61C/G	At risk (GG + CG)	21 (9.3)	10 (13.5)	0.3783	14 (19.4)	0.0336†	0.0215†
Intron 5	Non–at risk (CC)	204 (90.7)	64 (86.5)		58 (80.6)		
C2289T	At risk (TT + CT)	42 (18.5)	21 (28.0)	0.1004	28 (38.9)	0.0007*	0.0003*
Silent, exon 17	Non–at risk (CC)	185 (81.5)	54 (72.0)		44 (61.1)		

Data are *n* (%). Frequency of the at-risk genotypes in the IGT and diabetes groups was compared with that in the NGT group. Significance of the linear trend, i.e., the frequency of diabetic subject increases from the NGT to the IGT and to the diabetes groups, was evaluated by χ^2 tests for trend (*P* for trend). *$P < 0.01$ and †$P < 0.05$.

at-risk (or protective) alleles of each SNP examined, showed a significant association with diabetes (OR 0.42, $P = 0.0008$) ($P = 0.0047$, after the Bonferroni correction). Furthermore, diplotype 1/1 (combination of two haplotype 1s) was shown as significantly associated with diabetes as well (OR 0.35, $P = 0.0005$) ($P = 0.0040$, after the Bonferroni correction).

We then examined whether some clinical traits are different between the at-risk and the non–at-risk genotype groups with regard to SNP C2289T, since such clinical traits may represent intermediate phenotypes that link the nephrin gene to diabetes. The NGT group (of the two sample sets combined) alone was used for this analysis, since clinical traits in the diabetes and IGT groups seem to be, at least in part, influenced by their diabetic conditions and may thus not represent their status predisposed by the genetic factors relevant to diabetes. The fasting serum insulin levels in the non–at-risk genotype group were significantly higher than those in the at-risk genotype group (4.6 ± 3.0 vs. 3.9 ± 2.4 μU/ml, $P = 0.035$). No other traits examined were significantly different between them.

CONCLUSIONS — Several studies have shown the expression of nephrin in human pancreatic β-cells (2,3). However, very recently its expression was reported in human islet microvascular endotherial cells (MECs) rather than in human pancreatic β-cells (5). Islet MECs are known to have important roles in fine-tuning blood glucose sensing and regulation as well as in the facilitation of rapid insulin release (10–13). Although it is controversial which cells in the pancreatic islet express nephrin, the expression of nephrin in the pancreatic islet seems to be definitive (2–5). In either case, the impaired function of nephrin may have some influence on the function of pancreatic β-cells directly and/or through islet MECs. The fasting serum insulin levels were significantly different between the at-risk and the non–at-risk genotype groups of the nephrin gene, which may support the idea mentioned above.

The nephrin gene has two alternatively used exons at its 5′ region (exons 1A and 1B); from these, exon 1B and its immediate surrounding sequence were shown by analyses of the expression of promoter-reporter gene constructs in transgenic mice to be necessary for the expression of the gene in the pancreas and the spinal cord but not in the kidney and the brain (4). Therefore, the expression of the nephrin gene seems to be tissue-specifically regulated; thus, variations of the nephrin gene such as those examined here may have some functional influence in the pancreas but not in the kidney.

In conclusion, the SNPs of the nephrin gene were associated with diabetes, suggesting that nephrin may play an important role in the pathophysiology of diabetes.

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