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The 5′ insulin gene polymorphism and the genetics of vascular complications in Type 1 (insulin-dependent) diabetes mellitus

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Summary. Recent data suggest genetic contributions to the microvascular complications of Type 1 (insulin-dependent) diabetes mellitus. Most research has focused on the HLA region, and the potential role of other genetic loci has not been adequately explored. We examined the possible relationship between DNA polymorphisms in the region 5′ to the insulin gene on chromosome 11 and diabetic nephropathy. This was done by comparison of those diabetic patients homozygous for class 1 alleles at the 5′ insulin gene polymorphism locus to 1/3 heterozygotes in a well-characterized series of 324 insulin-requiring diabetic patients from the Wisconsin Epidemiologic Study of Diabetic Retinopathy. Proteinuria (defined as ≥ 0.3 g protein/l urine), was used as suggestive evidence for diabetic nephropathy. Hypertension, a frequent associated finding in diabetic patients with nephropathy, was defined as a blood pressure greater than 140/90 or a history of previous treatment of hypertension. The two genotypically defined groups did not differ from each other in regard to sex ratio, age at diagnosis, age at examination, duration of diabetes, body mass, HbA_{1c} or C-peptide. The 1/1 group had a higher prevalence of proteinuria, 29% as compared to 16.2% in other genotypes ($p < 0.05$). There was no significant difference in the frequency of hypertension between the two genotypic groups. This finding suggests that the 5′ insulin gene polymorphism may be associated with risk for nephropathy, but the pathophysiologic mechanism remains unclear.

Key words: Type 1 (insulin-dependent) diabetes mellitus, diabetic nephropathy, 5′ insulin gene polymorphism, heredity.

During the past 20 years, many studies have demonstrated the importance of genetic factors in susceptibility to Type 1 (insulin-dependent) diabetes mellitus [1]. In recent years, attention has started to also focus on the contribution of genetic loci to the natural history of Type 1 diabetes, including the occurrence and severity of diabetic complications [1, 2]. In particular, several studies have suggested an increased incidence of diabetic retinopathy in Type 1 diabetic patients who carry the HLA-DR4 allele [1–4]. Until now however, there has been relatively little attention paid to the potential role of non-HLA loci in diabetes natural history and complications. We wish to report the results of our investigations of the 5′ insulin gene polymorphism and its role in one of the vascular complications of Type 1 diabetes, diabetic nephropathy.

The 5′ insulin gene polymorphism is a region of DNA length variability immediately 5′ to the insulin gene itself [5]. Although the function of this region remains unknown, a number of case-control studies have detected an association between small insertions, called class 1 alleles, and Type 1 diabetes [6–8]. The present study was undertaken to assess the possible role of the 5′ insulin gene polymorphism in specific complications of Type 1 diabetes.

Subjects and methods

Study sample

A cross-sectional population-based cohort of individuals with Type 1 diabetes, with an onset of disease prior to 30 years of age, was recruited between 1 July 1979 and 30 June 1980 as part of the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR1), as reported previously [9, 10]. All subjects consented to participate following explanation of the purpose of the study. The study protocol was approved by the institutional review board at the University of Wisconsin. In a follow-up study of genetic and autoimmune factors involved in diabetic retinopathy, 440 randomly selected members of the original 996-member cohort were re-investigated between 1984 and 1986 (WESDR2). In addition to a thorough ophthalmological examination to assess the presence and severity of diabetic retinopathy, venous blood was obtained from all subjects for the study of a number of genetic markers, including HLA-A, B, C and DR alleles and the 5′ insulin gene polymorphism. Unequivocal

determination of the 5′ insulin gene polymorphism DNA fragment size, as well as blood pressure data from WESDR1 and WESDR2, was available in 324 of the original 440 individuals studied. The group of 324 does not differ from other members of the original cohort in terms of age at diagnosis, age at examination, systolic or diastolic blood pressure, history of hypertension, sex, degree of diabetic retinopathy, presence of proteinuria or family history of diabetes (all p-values for comparisons 0.10 or greater by chi-square or t-test). This group of 324 Caucasian Type 1 diabetic subjects was used for the present analysis.

Proteinuria was defined as ≥ 0.3 g protein/l urine and its presence was used as suggestive evidence for nephropathic changes. Hypertension was defined as a blood pressure greater than 140/90 at the time of examination or a history of previous treatment for hypertension.

5′Insulin gene polymorphism

Leucocytes were separated from 10 ml of venous blood using a Histopaque-1077 (Sigma Chemical Co., St. Louis, Mo., USA) gradient or a modified ammonium chloride/ammonium bicarbonate buffer system [11, 12]. DNA was extracted using the method of Denaro et al. [13], and digested with the restriction enzyme Bgl I according to manufacturer's instructions. The samples were subjected to electrophoresis in 0.9% agarose gels and the DNA transferred to nitrocellulose filters by Southern's technique [14]. Prehybridization and hybridization of the DNA-containing filters were performed by the method described by Wahl et al. [15]. Hybridization was performed using a ^{32}P-human insulin probe, which was prepared from an Hinc/II-Bgl I fragment of the plasmid phins 214 grown in the bacteria HB101. Phins 214 contains a 1650 base pair fragment which includes the human insulin gene and 58 5′ and 117 3′ flanking nucleotides [5]. The probe was kindly provided by Dr. G. Bell. Classification of the DNA polymorphism is based on restriction endonuclease fragment size. Fragments of 2800 ± 300 base pairs are termed class 1 (small) alleles; those of 3500 ± 300 base pairs are class 2 (intermediate) alleles, and those greater than 3900 base pairs are class 3 (large) alleles.

Statistical analysis

All comparisons were tested for statistical significance using standard methods. Given the ordered nature of the variables, the log-likelihood ratio test for contingency tables was applied, with the statistic G being distributed as a chi-square statistic, with degrees of freedom (r-1) (c-1), where r and c are the numbers of the rows and columns respectively [16, 17].

Results

The relationship between proteinuria and the 5′ insulin polymorphism was evaluated for both the initial patient examination in 1980–1982 (WESDR1) and the follow-up visit in 1984–1986 (WESDR2). Those individuals homozygous for class 1 alleles were compared to those who were either heterozygous (1/3) or homozygous for class 3 alleles. At initial examination in WESDR1 (Table 1), individuals homozygous for class 1 were significantly more likely to have proteinuria ($p < 0.05$). By the time of the re-evaluation in WESDR2 (Table 2), the increased risk for proteinuria associated with the homozygosity for class 1 alleles was more evident, with 29% of those with a 1/1 genotype having proteinuria (0.3 g/l or greater), while only 16% of those with a class 3 allele had proteinuria ($p < 0.05$). Alternatively, of the individuals with proteinuria, 75% had a 1/1 genotype, while this was true of only 58% of individuals with traces of or no proteinuria.

Although most individuals showed no change in degree of proteinuria between WESDR1 and 2, those who did were more likely to have worsening rather than improvement in their proteinuria. To determine if the 5′ insulin polymorphism played a role in progression of nephropathy, change in status between the two examinations was assessed for the two insulin gene polymorphism groups (Table 3). Of those individuals homozygous for class 1 alleles, 18% had worsening of their proteinuria, whereas only 11% of those with a class 3 allele had progression of their proteinuria. Although the difference does not reach statistical significance, the trend seen in Table 3 is also consistent with the two cross-sectional observations of WESDR1 and WESDR2.

Table 1. Proteinuria prevalence: Wisconsin Epidemiologic Study of Diabetic Retinopathy 1 (1979–1980)

5′ insulin gene polymorphism	Proteinuria severity			
	None or trace	0.3–1.0 g/l	3.0 + g/l	Total
1/1	155 (77%)	38 (19%)	9 (4%)	202
Any 3	100 (88%)	12 (10%)	2 (2%)	114

$G^2 = 6.15$, 2 degrees of freedom, $p < 0.05$

[a] G^2 is the log-likelihood chi-square statistic

Table 2. Proteinuria prevalence: Wisconsin Epidemiologic Study of Diabetic Retinopathy 2 (1984–1986)

5′ insulin gene polymorphism	Proteinuria severity			
	None or trace	0.3–1.0 g/l	3.0 + g/l	Total
1/1	137 (71%)	46 (24%)	10 (5%)	193
Any 3	98 (84%)	16 (14%)	3 (2%)	117

$G^2 = 6.80$, 2 degrees of freedom, $p < 0.05$

G^2 is the log-likelihood chi-square statistic

Table 3. Progression of proteinuria: Wisconsin Epidemiologic Study of Diabetic Retinopathy 1 (1979–1980)---› 2 (1984–1986)

5′ insulin gene polymorphism	No change	Worse	Total
1/1	157 (81%)	35 (18%)	192
Any 3	99 (89%)	13 (11%)	112

$G^2 = 2.42$, 1 degree of freedom, $p = 0.12$

G^2 is the log-likelihood chi-square statistic

Table 4. Hypertension and the insulin gene polymorphism

Wisconsin Epidemiologic Study of Diabetic Retinopathy 1 (1979–1980)			
	Normal blood pressure	Hypertensive	Total
1/1	160 (78%)	45 (22%)	205
Any 3	99 (83%)	20 (17%)	119

$G^2 = 1.27$, 1 degree of freedom, $p = 0.26$

Wisconsin Epidemiologic Study of Diabetic Retinopathy 2 (1984–1986)			
	Normal blood pressure	Hypertension	Total
1/1	156 (77%)	47 (23%)	203
Any 3	96 (79%)	25 (21%)	121

$G^2 = 0.27$, 1 degree of freedom, $p = 0.60$

G^2 is the log-likelihood chi-square statistic

In an effort to assess whether the 5′ insulin gene polymorphism was associated primarily with renal disease or secondarily via hypertensive damage to the kidney, the relationship between hypertension and the insulin gene polymorphism was also examined (Table 4). There was no difference in the genotype frequencies in those with or without hypertension, either at initial examination or at the follow-up 4 years later.

Discussion

The aetiology of nephropathy in Type 1 diabetes remains unclear. One possibility which has been considered is that diabetic nephropathy is solely the result of the metabolic derangements which occur in diabetes. Supporting this idea is the observation that clinical renal disease is uncommon before 10 years duration of diabetes, with a peak incidence not occurring until between 15 and 20 years after disease onset [18–20]. Not all patients with long duration of diabetes develop nephropathy, however, suggesting that factors other than strictly metabolic derangements must also be implicated [19, 21]. A possibility which must be entertained is that at least some of these additional risk factors may be genetic.

Supporting the concept that genetic factors are likely to be important in the development of diabetic nephropathy is the recent report by Seaquist et al. [22] regarding the occurrence of nephropathy in diabetic sibling pairs. Only 17% of Type 1 diabetic siblings of diabetic probands without nephropathy, but 83% of Type 1 diabetic siblings of diabetic probands with nephropathy, had evidence of nephropathy themselves. While a common environment could explain some of the increased risk in siblings, shared genetic factors must also be considered as an explanation. In contrast to diabetic retinopathy, where numerous studies have addressed the possibility of genetic predisposition, few studies have evaluated the role of genetic factors in diabetic nephropathy. The one candidate locus which has been studied previously is HLA, with no clearly identified association to nephropathy [2, 23].

The results of the present study suggest an association of class 1 alleles of the 5′ insulin gene polymorphism with nephropathy, as indicated by the presence of proteinuria. The progression of nephropathy in Type 1 diabetic patients also appears to be influenced by the insulin gene polymorphism, as individuals carrying the class 1 allele were more likely to show worsening of their proteinuria between WESDR1 and 2 than were those with class 3 alleles. Although it was not possible to directly document the presence of diabetic nephropathy with 24-h urine collections in this population-based study, the finding of proteinuria in random urine specimens is highly suggestive of renal disease. It is well-established that proteinuria is a reliable indicator of early renal disease, and that diabetic subjects with microalbuminuria are 20 times more likely to progress to clinical nephropathy than those without it [24, 25].

Given the apparent association of the 5′ insulin gene polymorphism with nephropathy, several mechanistic explanations might be invoked. The first is that the nephropathy might be mediated through an atherogenic gene linked to the insulin gene locus. This initially seems plausible, given the previous reports of an association of the 5′ insulin gene polymorphic region and atherosclerosis [26–29]. This possibility can be excluded, however, since the association with nephropathy found in the current study is with the class 1 allele of the 5′ insulin gene polymorphic locus, in contrast to the class 3 association previously reported for both atherosclerosis and hypertriglyceridaemia [26–29].

Another potential mechanism is that nephropathy might occur as a consequence of hypertensive damage to the kidney. Hypertension has been demonstrated to be an independent risk factor for the development of diabetic nephropathy, rather than simply a secondary complication of pre-existing renal damage [19, 24, 30, 31]. In addition, it is clear that this predisposing hypertension has genetic components [30, 32]. In the present study, this possibility was examined, as shown in Table 4. Our results suggest that the class 1 alleles of the 5′ insulin gene polymorphism are not associated with a predilection to hypertension among diabetic subjects. Therefore, the increased risk for nephropathy associated with the class 1 allele must be mediated through a different mechanism, unrelated to the pathophysiology of hypertension.

Genetic predisposition to diabetic complications may be mediated by genes which themselves predispose to the development of diabetes, or by genes which are unrelated to disease aetiology. In the case of the 5′ insulin gene polymorphism, the latter possibility appears more probable, although controversy remains. Several population studies have identified an association of the class 1 alleles with Type 1 diabetes [33–35]. Results of family linkage studies, however, are inconclusive [35–40]. The results of our present study suggest that this region is important in at least one of the complications associated with Type 1 diabetes. Further studies are needed to confirm this association with nephropathy, following which it will be necessary to study the exact mechanism(s) by which the 5′ insulin gene polymorphic region contributes to this complication.

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