TNFa Microsatellite Polymorphism Modulates the Risk of IDDM in Caucasians With the High-Risk Genotype HLA DQA1*0501-DQB1*0201/DQA1*0301-DQB1*0302

Payman Hanifi Moghaddam, Aeilko H. Zwinderman, Peter de Knijff, Bart O. Roep, Ron F. Schipper, Bart Van der Auwera, Albert Naipal, Frans Gorus, Frans Schuit, Marius J. Giphart, and the Belgian Diabetes Registry

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IDDM is a genetically controlled autoimmune disease. In particular, the loci of the HLA region on chromosome 6p are associated with the genetic risk for developing IDDM. DQA1*0501-DQB1*0201/DQA1*0301-DQB1*0302 is the most prevalent susceptibility genotype in Caucasians, carrying a relative risk between 20 and 50 (1). However, the frequency of this high-risk genotype in the population is 10–20 times higher than the prevalence of IDDM associated with this genotype, suggesting that additional protective genes (HLA or non-HLA) and/or environmental factors can influence susceptibility to the disease (1). Recently, it has been shown that DRB1*0403 protects against IDDM in Caucasians with the high-risk heterozygous DR3-DQ2/DR4-DQ8 genotype (2), but it accounts for only ~10% of the protection. Thus, DRB alleles alone cannot explain either protection or susceptibility for IDDM.

To define additional markers for genetic susceptibility to or protection against IDDM in the HLA-DQ high-risk genotype group, 120 diabetic patients and 83 nondiabetic control subjects, all sharing the DQA1*0501-DQB1*0201/DQA1*0301-DQB1*0302 genotype, were studied with eight microsatellite loci spanning the entire major histocompatibility complex (MHC) region and a region 2 cM telomeric of MHC. All subjects were unrelated European Caucasians and were recruited by the Belgian Diabetes Registry (2,3). HLA-typing and amplification of microsatellite markers were performed as described previously (3–5). Primer sequences for the markers HLA-F, D6S265, TNFa, D6S273, D6S1014, DQCar, TAP1, and D6S291 are available from the Genome Data Bank and were used as described previously (5). Microsatellite analysis was performed on an automated laser fluorescence-DNA sequencer (Pharmacia Biotech), using fluorescence-based semi-automated DNA-sizing technology (5).

The allele frequencies and homozygosity patterns of the markers were compared between patients and control subjects using Pearson's χ^2 statistics. The P values were adjusted for multiple testing using the method of Edwards (6). Selection of the markers discriminating the most between patients and control subjects was done with stepwise logistic regression analysis. Based on the current findings, the probability of developing IDDM was calculated with Bayes' theorem, assuming an 8% absolute risk of developing IDDM before 40 years of age in the population carrying the highest HLA-DQ–linked risk.

No significant difference was observed between the frequencies of alleles of any of the eight microsatellite markers in the diabetic patients, compared with the control subjects.

For TNFa, 47% of the control subjects and only 23% of the diabetic patients were homozygous (odds ratio [OR], 0.32; 95% CI 0.17–0.60; $P < 0.0003$; Table 1). There was no indication that age at clinical onset interacted with TNFa or the other markers with respect to the case-control classification. Within the TNFa marker, allele 99 displayed a significant difference between diabetic patients and control subjects. Heterozygosity for allele 99 was observed in 62% of the patients and 35% of the control subjects ($P < 0.0005$). A detailed linkage disequilibrium (LD) analysis indicated a strong LD between TNFa*99 and HLA-DRB1*0301. No LD was observed between TNFa*99 and DRB1*04. Furthermore, we did not find any significant difference in homozygosity of TNFa within DRB1*04 subtypes.

Stepwise logistic regression analysis identified the heterozygous presence of allele 99 and 97 as the most predictive for IDDM, with minor contributions of alleles 105 and 117. Heterozygosity for alleles 97, 99, or 117 was observed in 44% of the control subjects and 75% of the patients (OR, 3.84; 95% CI 2.08–7.09; $P < 0.0001$). Backward stepwise logistic regression on all alleles resulted in the selection of the TNFa*99 allele and DRB1*0403 allele as most predictive for IDDM: the OR for TNFa*99 was 3.37 ($P < 0.0007$), and the OR for DRB1*0403 was 0.09 ($P < 0.04$).

The absolute risk of developing IDDM before 40 years of age in subjects with the high-risk HLA-DQ genotype is 8%. We investigated the posterior probability of IDDM given het-

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From the Department of Immunohematology and Blood Bank (P.H.M., B.O.R., R.F.S., A.N., M.J.G.), University Hospital, Leiden, The Netherlands; the Departments of Medical Statistics (A.H.Z.) and Human Genetics (P.K.), Leiden University, Leiden, The Netherlands; and the Departments of Biochemistry (B.V.A., F.S.) and Metabolism and Endocrinology (F.G.), Free University Brussels, Brussels, Belgium.

Address correspondence and reprint requests to Dr. M.J. Giphart, Department of Immunohematology and Blood Bank, Building 1, E3Q, University Hospital, P.O. Box 9600, 2300 RC Leiden, The Netherlands.

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LD, linkage disequilibrium; MHC, major histocompatibility complex; OR, odds ratio; PPV, positive predictive value.

TABLE 1
Analysis of homozygosity of microsatellite loci in controls versus patients

MS Locus	Controls (%)	Patients (%)	*P*-uncorr	*P*-corr	OR (95% CI)
HLA-F	28	29	0.94	NS	
D6S265	22	28	0.38	NS	
TNFa	47	23	0.0003	0.007	0.32 (0.17–0.60)
D6S273	9	7	0.61	NS	
D6S1014	19	23	0.58	NS	
DQCar	5	0	0.026	NS	
TAP1	66	70	0.68	NS	
D6S291	26	26	0.98	NS	

P value of Pearson χ^2 test, or Fisher's exact test.

erozygosity on the TNFa microsatellite marker locus. The positive predictive value (PPV) of heterozygosity on the TNFa locus was 11.4% and for allele TNFa*99 13.4%, while their negative predictive values were 96.0 and 95.2%, respectively. The PPV of the heterozygous presence of TNFa allele 99 and the absence of DRB1*0403 allele was 13.7%. Thus, the PPV was increased from 8% baseline to 13.7% (67% increase), taking the described heterozygosity of TNFa*99 into account.

Remarkably, the prevalence of homozygosity in the TNFa locus was significantly higher in the control subjects, compared with the diabetic patients. LD analysis showed that TNFa*99 allele is in strong LD with DRB1*0301, which was observed in both control and diabetic subjects, but the absence of allele 99 of TNFa on the other haplotype (DQ8-DR4) in patients is associated with susceptibility to IDDM. A study of TNF locus microsatellites (7) demonstrated a higher frequency of the TNFa-2 (allele 99) in DR3/DR4 heterozygous diabetic patients than in control subjects. Since this marker has been associated with higher TNFa production independent of class II alleles, a direct role of the TNF locus in susceptibility to IDDM can be suggested at least in DR4$^+$ individuals (7). In the absence of a pathophysiological correlation to this marker study, it is presently unknown if homozygosity at the TNFa locus is associated with protection against IDDM and, conversely, if heterozygosity of the same locus encodes susceptibility to IDDM.

Our results support the hypothesis that two (or more) susceptibility loci in the MHC (one in the HLA class II and another in the central MHC region) act epistatically to increase susceptibility to IDDM. Our results also suggest different effects on the haplotype or genotype level. The presence or absence of allele 99 on one haplotype (DQ2-DR3-TNFa*99/DQ8-DR4-TNFa*99 or DQ2-DR3-TNFa*99/DQ8-DR4-TNFa*X) may indicate cis-complementation effects. In addition, it is remarkable that heterozygosity of TNFa and heterozygosity for DQ is associated with the highest risk for IDDM. This may be interpreted as trans-complementation and/or gene dosage effect of the TNF gene. The phenomenon, termed "dosage effect" (8), is particularly remarkable for the DR3 haplotype (9). In conclusion, homozygosity of TNFa locus is an independent effect, and allele TNFa*99 is not in LD with DRB1*04 alleles. Furthermore, disease-associated genetic markers can be used to define high-risk individuals.

We have shown that a combination of DRB1 subtyping and genotype analysis of the TNFa microsatellite locus results in a considerably higher predictive value, compared with that of the DQ2/DQ8 heterozygous genotype alone. Consequently, genotype analysis of TNFa microsatellite locus contributes to the refinement of IDDM risk assessment in carriers of the HLA-DQ highest risk genotype, which may be relevant to future therapeutic strategies.

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