

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

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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, TNF α , D6S273, D6S1014, DQC α , 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 TNF α , 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 TNF α or the other markers with respect to the case-control classification. Within the TNF α 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 TNF α *99 and HLA-DRB1*0301. No LD was observed between TNF α *99 and DRB1*04. Furthermore, we did not find any significant difference in homozygosity of TNF α 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 TNF α *99 allele and DRB1*0403 allele as most predictive for IDDM: the OR for TNF α *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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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	
TNF α	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 TNF α microsatellite marker locus. The positive predictive value (PPV) of heterozygosity on the TNF α locus was 11.4% and for allele TNF α *99 13.4%, while their negative predictive values were 96.0 and 95.2%, respectively. The PPV of the heterozygous presence of TNF α 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 TNF α *99 into account.

Remarkably, the prevalence of homozygosity in the TNF α locus was significantly higher in the control subjects, compared with the diabetic patients. LD analysis showed that TNF α *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 TNF α 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 TNF α -2 (allele 99) in DR3/DR4 heterozygous diabetic patients than in control subjects. Since this marker has been associated with higher TNF α 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 TNF α 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-TNF α *99/DQ8-DR4-TNF α *99 or DQ2-DR3-TNF α *99/DQ8-DR4-TNF α *X) may indicate cis-complementation effects. In addition, it is remarkable that heterozygosity of TNF α 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 TNF α locus is an independent effect, and allele TNF α *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 TNF α microsatellite locus results in a considerably higher predictive value, compared with that of the DQ2/DQ8 heterozygous genotype alone. Consequently, genotype analysis of TNF α 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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REFERENCES

1. She JX: Susceptibility to type I diabetes: HLA-DQ and DR revisited. *Immunol Today* 17:329–336, 1996
2. Van der Auwera B, Waeyenberge CV, Schuit F, Heimberg H, Vandewalle C, Gorus F, Flament J, the Belgian Diabetes Registry: DRB1*0403 protects against IDDM in Caucasians with the high risk heterozygous DQA1*0301-DQB1*0302/DQA1*0501-DQB1*0201 genotype. *Diabetes* 44:527–530, 1995
3. Vandewalle CL, Decraene T, Schuit FC, De Leeuw IH, Pipeleers DG, Gorus FK, the Belgian Diabetes Registry: Insulin autoantibodies and high titre islet cell antibodies are preferentially associated with the HLA DQA1*0301-DQB1*0302 haplotype at clinical type 1 (insulin-dependent) diabetes mellitus before age 10 years, but not at onset between age 10 and 40 years: the Belgian Diabetes Registry. *Diabetologia* 36:1155–1162, 1993
4. Ziegler JS, Su Y, Corcoran KP, Nie L, Mayrand PE, Hoff LB, McBride LJ, Kronick MN, Diehl SR: Application of automated DNA sizing technology for genotyping microsatellite loci. *Genomics* 14:1026–1031, 1992
5. Dib C, Fauré S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Marc S, Hazan J, Seboun E, Lathrop M, Gyapay G, Morissette J, Weissenbach J: A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 380:152–154, 1996
6. Edwards JH: HLA and diseases: the detection of associations. *J Immunogenet* 1:249–257, 1974
7. Pociot F, Briant L, Jongeneel CV, Molvig J, Worsaae H, Abbal M, Thomsen M, Nerup J: Association of tumor necrosis factor (TNF) and class II major histocompatibility complex alleles with the secretion of TNF-alpha and TNF-beta by human mononuclear cells: a possible link to insulin-dependent diabetes mellitus. *Eur J Immunol* 23:224–231, 1993
8. Khalil I, Deschamps I, Lepage V, Al-Daccak R, Degos L, Hors J: Dose effect of cis- and trans-encoded HLA-DQ alpha beta heterodimers in IDDM susceptibility. *Diabetes* 41:378–384, 1992
9. Huang HS, Peng JT, She JX: HLA encoded susceptibility to insulin dependent diabetes mellitus is determined by DR and DQ genes as well as their linkage disequilibria in a Chinese population. *Hum Immunol* 44:210–219, 1995