

Linkage and Association Between a *CD4* Gene Polymorphism and IDDM in Danish IDDM Patients

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CD4⁺ T-cells play a central role in IDDM pathogenesis (1). Hence, gene polymorphisms that modulate the CD4⁺ T-cell response to antigen presentation may confer susceptibility to IDDM. The CD4 receptor augments the CD4⁺ T-cell receptor-mediated response to antigen presentation by interacting with major histocompatibility complex (MHC) class II molecules on antigen-presenting cells and the T-cell receptor, and by enhancing the antigen response through the CD4-associated tyrosine-protein kinase p56^{lck} (2).

The *CD4* receptor gene maps to chromosome 12p12 in humans and to chromosome 6 in mice (3,4) close to the diabetes-linked locus *Idd6* in the nonobese diabetic (NOD) mouse (5). Furthermore, a chromosome 6 locus conferring susceptibility to diabetes was found in the nonobese, nondiabetic (NON) mouse (6). Maximal linkage was found <6 cM from the *CD4* gene.

Random marker genome studies in humans have not linked loci on chromosome 12p to IDDM (7,8). However, in a Belgian case-control study an association between IDDM and the *CD4*A4/A4* genotype of a pentanucleotide repeat polymorphism, 1250 bp upstream to the first exon, was demonstrated, suggesting the existence of an IDDM susceptibility locus in the *CD4* gene or in a closely linked gene (9).

Thus, several lines of evidence qualify the *CD4* gene as a candidate gene in IDDM. The aim of the present study was therefore to investigate if the *CD4*A4* allele is associated with and/or linked to IDDM in the Danish population.

The study material comprised two types of IDDM multiplex families of Danish Caucasian origin: 115 sibpair families with 237 affected (110 female subjects, 129 affected sibpairs) and 91 unaffected (46 female subjects) offspring, and 105 parent-offspring families with a total of 105 affected (55 female subjects) and 112 unaffected (55 female subjects) offspring. Mean

and median ages at onset of diabetes in the combined material were 11.2 and 9.9 years, respectively. *P* values < 0.05 were considered statistically significant in all tests performed.

Genotyping for the *CD4* gene pentanucleotide polymorphism was performed as previously described by Zamani et al. (9) or by a modified method by Sandberg-Wollheim et al. (10), using identical primer and cycling conditions (9). The nomenclature of the alleles was as detailed in the work by Zamani et al. (9).

TRANSMISSION DISEQUILIBRIUM STUDY

The *CD4* alleles were found to be in the Hardy-Weinberg equilibrium. The *CD4*A4* allele transmission was analyzed by the transmission disequilibrium test (TDT) (11). A distorted transmission of the *CD4*A4* allele to all IDDM offspring in the combined family material was found (Table 1; 58% transmitted, $\chi^2 = 7.16$ [1 df]; *P* = 0.0075). Random transmission of the *CD4*A4* allele in unaffected offspring was observed (Table 1; 49% transmitted). The *CD4*A4* allele transmission from CD4**A4* heterozygous parents (1) to the index case (i.e., the first affected offspring, *n* = 220) in all families of the combined family material, (2) to all affected offspring in the sibpair families, and (3) to the IDDM offspring in the parent-offspring families was investigated. The transmission of the *CD4*A4* allele to the IDDM subjects was 57, 58, and 58% in the three subsets, respectively. In the two first groups, the TDT analysis reached statistical significance (Table 1; $\chi^2 = 3.94$ [1 df], *P* = 0.047; $\chi^2 = 4.98$ [1 df], *P* = 0.026; $\chi^2 = 2.18$ [1 df], *P* = 0.14, respectively). However, the transmission pattern to the IDDM offspring in the parent-offspring families was identical to the transmission pattern in the sibpair families, but the number of observed transmissions was lower.

The possible interaction between *IDDM1* and the *CD4* gene polymorphism was evaluated by TDT analysis of the *CD4*A4* allele transmission pattern to HLA "high" risk (HLA-DR3/4 heterozygous) and HLA "non-high" risk (non-HLA-DR3/4 heterozygous) IDDM subjects. No difference in transmission pattern of the *CD4*A4* allele was found between the two groups (Table 1).

To exclude the possibility that only a subset of the affected offspring was causing the linkage and intrafamilial association found between the *CD4*A4* allele and IDDM, and that the rest of the material displayed random transmission, stratification based on 1) sex of the affected offspring, 2) age at onset above or below the median age at onset (9.9 years), and 3) paternal or maternal transmission, was carried out. No differences between the groups were found (data not shown).

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ESPA, extended sibpair analysis; IBD, identical-by-descent; TDT, transmission disequilibrium test.

TABLE 1
CD4*A4 allele transmission

Transmission	n	Transmitted	Nontransmitted	P _{tdt}
All IDDM offspring	342	164	119	0.0075
All unaffected offspring	203	89	92	NS
All index cases in the combined families	220	106	79	0.047
All IDDM offspring in the sibpair families	237	112	81	0.026
IDDM offspring in the parent-offspring families	105	52	38	0.14
HLA DR3/4 heterozygous IDDM offspring*	139	70	50	0.067
HLA non-DR3/4 heterozygous IDDM offspring	203	94	69	0.050

Data are n or P values. *Comparison of transmission of the CD4*A4 allele with HLA non-DR3/4 heterozygous IDDM offspring (P = 0.99).

AFFECTED SIBPAIR ANALYSIS

Affected sibpair analysis was performed by the extended affected sibpair analysis (ESPA) computer program (12). Only families with both parents available for genotyping (122 sibpairs) were included in the analysis. The frequency of allele sharing was 49.1% (78 of 159 informative alleles, 83 alleles uninformative; NS). Hence, no deviation from the expected 50% allele sharing was demonstrated.

CONTRIBUTION OF THE CD4 GENE POLYMORPHISM TO FAMILIAL CLUSTERING

The degree of familial clustering (λ_s) is estimated from the ratio of the risk to siblings of patients and the population prevalence (13). In the Danish population, λ_s is 6:0.4% = 15. The contribution of a single locus to the total λ_s can be estimated from the ratio of the expected proportion of affected sibpairs sharing zero alleles identical-by-descent (IBD) as well as the observed proportion. In the present material, 38 sibpairs were completely informative (i.e., both parents were heterozygous and at least three different alleles were present in the parental generation) for the IBD analysis. IBD 0, 1, and 2 were 8, 20, and 10, respectively. Thus λ_s for the CD4 polymorphism is 1.19.

DISCUSSION

The TDT analysis demonstrated evidence for linkage and intrafamilial association between IDDM and the CD4*A4 allele of a CD4 receptor gene polymorphism in Danish IDDM patients. The CD4*A4 allele displayed a distorted transmission to the IDDM offspring, whereas random transmission of this allele was observed in unaffected offspring. The skewed transmission was found both in the families with multiple affected siblings and in the families when transmission to only one IDDM subject per family was observed, suggesting linkage and intrafamilial association, respectively. The contribution of the CD4 polymorphism to familial clustering, λ_s , was found to be discrete. However, it should be noted that the transmission frequency of the CD4*A4 allele from heterozygous parents to affected offspring in all subsets of the present material was 57–58%. This transmission distortion is close to the transmission pattern found for IDDM2 (11). The ESPA analysis revealed no evidence of linkage between the polymorphism and IDDM. This may be due to the limited power of this type of analysis as compared with the power of the TDT (11), which may also in part explain the failure of the random marker studies (7,8) to demonstrate linkage between IDDM and this locus in humans.

The observation of the linkage and intrafamilial association between the CD4 locus and IDDM in the Danish population combined with the association found in the Belgian population (9) suggests that this or other polymorphisms in or close to the CD4 gene may be of etiological importance in contributing to IDDM susceptibility.

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