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Short communication

Non-transmitted maternal *HLA DQ2* or *DQ8* alleles and risk of Type I diabetes in offspring: the importance of fetal or post partum exposure to diabetogenic molecules

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Abstract

Aims/hypothesis. Type I (insulin-dependent) diabetes mellitus results from an immune-mediated destruction of pancreatic beta cells for which HLA haplotypes *DR3-DQ2* and *DR4-DQ8* represent the strongest genetic risk markers. Mothers of patients with rheumatoid arthritis carry more frequently the HLA *DR4-DQ8* haplotype as non-transmitted haplotype than mothers of healthy control subjects. As maternal cells have been shown to persist in their offspring up to 30 years after birth, we investigated whether the association of HLA *DR3-DQ2* and *DR4-DQ8* with Type I diabetes is purely a genetic effect acting through inheritance or whether it can also act as an environmental factor, for example through foetal exposure in utero to maternal circulating cells.
Methods. We analysed the non-transmitted parental HLA *DQ* alleles of 464 families (1367 subjects) with a Type I diabetic offspring. HLA *DQ* alleles were assessed using sequence-specific primers and allele-specific oligonucleotides hybridisation. A chi-square test was done to compare allele and transmission frequencies in the respective subsets of families.
Results. The non-transmitted HLA *DR3-DQ2* and *DR4-DQ8* were more frequent in mothers than in fathers of all non-*DQ2/DQ8* heterozygous diabetic offspring (p=0.0001) as well as in offspring not carrying any HLA high-risk allele (p=0.0243). In patients with either risk allele alone, more maternal than paternal non-transmitted risk alleles complemented the constellation to *DQ2/DQ8* (p<0.0099).
Conclusion/interpretation. HLA high risk alleles were more frequent among maternal non-inherited (but possibly exposed) alleles than among paternal non-inherited alleles. These results indicate that HLA *DR-DQ* is an environmental risk factor for Type I diabetes. [Diabetologia (2002) 45:1340–1343]

Keywords Genetic susceptibility, microchimerism, HLA, maternal transmission, beta-cell autoimmunity.

Type I (insulin-dependent) diabetes mellitus results from an immune-mediated destruction of pancreatic beta cells with strongest susceptibility conferred by HLA region genes identified in 1974 [1]. Whether particular epitopes of HLA *DR-DQ* haplotypes fully explain this strong association or whether linked genes play a role is not resolved because at least one non-HLA gene on chromosome 6p is involved in susceptibility [2]. HLA *DR* and *-DQ* genes encode antigen-presenting molecules that initiate T-lymphocyte proliferation after having bound "foreign" peptides. T-lymphocytes are the key elements of the insulitis leading to selective beta-cell loss – a process that can occur as early as during embryonic-foetal development [3].

Several groups have accumulated much evidence showing that HLA *DR4-DQ8* and *DR3-DQ2* represent the main predisposing haplotypes [4, 5]. This

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Abbreviations: TDT, Transmission disequilibrium test

was shown by both case-control and family studies, in which haplotype distributions of patients were compared with the non-transmitted haplotypes of their parents or with the haplotype frequencies of unrelated control subjects. To elucidate the parental origin of diabetogenic risk alleles we examined whether in Type I diabetic offspring the frequencies of maternal differ from paternal non-transmitted HLA *DQ* alleles.

Subjects and methods

The study was carried out in accordance with the declaration of Helsinki. Families of Caucasian origin were recruited at the diabetic outpatient clinic at the University Hospital Frankfurt am Main, Germany, and by the Belgian Diabetes Registry (206 trios) and informed consent was obtained. Altogether, we studied 1367 subjects (464 offspring affected with Type I diabetes that were derived from 439 complete pedigrees and 25 mother-child pairs). All parents were non-diabetic. HLA DQ alleles had been assigned by standard methods using sequence-specific primers [6] for the German and allele-specific oligonucleotide (ASO) hybridization for the Belgian families [7]. HLA *DQ2* (*DQA1*0501-DQB1*0201*) and HLA *DQ8* (*DQA1*0301-DQB1*0302*) were considered as high risk alleles. Preferential transmission to all 464 affected offspring was detected by transmission disequilibrium testing (TDT) [8], which compares the observed transmission frequencies by heterozygous parents with the expected frequency of 50%. The families were assigned to subsets according to the offsprings' HLA *DQ* genotype: *DQ2/DQ8* heterozygous – a; *DQ2* and *DQ8* homozygous – b; genotype *DQ2*/x or *DQ8*/x – c; genotype *DQ*x/x – d (x: not *D2* or *DQ8*). The analyses were done for HLA DQ2 and DQ8 combined. For subsets b and c both risk alleles were also considered individually. Chi-square testing with Yates' correction was applied to compare the frequencies of maternal and paternal non-transmitted HLA DQ alleles excluding the 25 mother-child pairs. A p value of less than 0.05 was considered significant.

Results

Transmission of HLA DQ2 and DQ8. Both, HLA *DQ2* (264 t vs 100 nt; $p=8.3\times10^{-18}$) and HLA *DQ8* (276 t vs 66 nt; $p=7.0\times10^{-30}$) were preferentially transmitted to Type I diabetic offspring (Table 1).

Non-transmitted HLA DQ2 and DQ8 alleles – all patients. Considering all affected offspring, the non-transmitted DQ8 allele was more abundant ($p<0.04$) in mothers (13.6%) than in fathers (8.9%). We then wondered whether this observation might have been due to differences in the proportion of *DQ2/DQ8* heterozygous mothers or fathers. However, only 41 of 464 (8.8%) mothers and 32 of 439 fathers (7.3%) were typed *DQ2/DQ8* and transmission frequencies of *DQ8* did not differ between mothers (46.3%) and fathers (40.6%), thus not accounting for the differences in non-transmission rates.

Non-transmitted HLA DQ2 and DQ8 alleles – subset analyses. Subsequently we compared maternal and paternal non-transmitted HLA DQ alleles in the subsets stratified for the offsprings' HLA *DQ* genotype. Considering the 143 *DQ2/DQ8* heterozygous offspring (group a; Table 2) the frequencies of maternal and paternal non-transmitted HLA *DQ2* and *DQ8* did not differ ($p=0.3144$). However, mothers of the 63 HLA *DQ2* and *DQ8* homozygous children (group b) were more often carriers of a non-transmitted HLA *DQ2* or *DQ8* than fathers (34.9% vs 17.5% – $p=0.0427$). Similarly, a maternal non-transmitted HLA *DQ* high risk haplotype (40.2%) was more frequent than a paternal one (27.6%) in the subset of those 174 patients with genotype *DQ2*/x and *DQ8*/x ($p=0.0343$). Only 59 patients did not inherit a susceptibility allele from either parent, of which 18 mothers (12 *DQ2*, 6 *DQ8*) but only 7 fathers (6 *DQ2*, 1 *DQ8*) carried a non-transmitted diabetogenic risk allele ($p=0.0243$).

As heterozygosity for *DR3-DQ2/DR4-DQ8* confers the highest risk for Type I diabetes, we investigated whether *DQ2* and *DQ8* homozygous patients (group b) and those with either risk allele alone (group c) might have encountered a complementary non-inherited susceptibility combination through their mothers. Although 14.3% of the mothers of HLA *DQ2* and *DQ8* homozy-

Table 1 Transmission analysis of HLA *DQ2* and *DQ8*

	DQ2			*DQ8*		
	T	NT	$p_{(TDT)}$	T	NT	$p_{(TDT)}$
All parents to all offspring	264	100	8.3×10^{-18}	276	66	7.0×10^{-30}
To affected sons from either parent	141	49	2.5×10^{-11}	117	35	2.9×10^{-11}
To affected daughters from either parent	123	51	4.8×10^{-8}	159	31	1.6×10^{-20}
$P_{(\chi^2)}$	NS			NS		
From fathers to affected offspring	123	40	8.0×10^{-11}	156	24	7.7×10^{-23}
From mothers to affected offspring	141	60	1.1×10^{-8}	120	42	8.9×10^{-10}
$P_{(\chi^2)}$	NS			<0.005		

Table 2 Maternal and paternal non-transmitted diabetogenic HLA *DQ* alleles (*DQ2* or *DQ8*) in 439 families with a Type I diabetic patient (*DQ2* is *DQA1*05-DQB1*02*; *DQ8* is *DQA1*03-DQB1*0302*)

HLA *DQ* genotype of affected offspring	Number of patients	Number of DQ2 or DQ8 as not transmitted parental allele	$p(\chi^2)$
a) *DQ2/DQ8*	143	Mothers: 33 (23.1%) Fathers: 41 (28.7%)	0.3144
b) *DQ2/DQ2* and *DQ8/DQ8*	63	Mothers: 22 (34.9%) Fathers: 11 (17.5%)	0.0427
c) *DQ2/x* and *DQ8/x*	174	Mothers: 70 (40.2%) Fathers: 48 (27.6%)	0.0343
d) *DQx/DQx*	59	Mothers: 18 (30.5%) Fathers: 7 (11.9%)	0.0243
e) All except *DQ2/DQ8* hetero-zygous offspring	296*	Mothers: 110 (37.2%) Fathers: 66 (22.3%)	0.0001

*Equals the sum of subsets b, c and d

gous patients, but only 7.9% of their fathers carried a non-transmitted complementary HLA risk allele (i.e. *DQ8* in the case of a *DQ2* homozygous offspring and vice versa), the difference was not significant (p=0.3951). However, a complementary HLA high risk allele was found in 15.5% of the mothers as opposed to 6.3% in the fathers of those patients carrying either *DQ2* or *DQ8* alone (p<0.0099). When considering all 296 patients that were not *DQ2/DQ8* heterozygous, 110 mothers (30.5%) but only 66 fathers (22.3%) carried a non-transmitted *DQ2* or *DQ8* allele (p=0.0001) 37.2%.

Altogether, a high risk HLA *DQ* allele was present in either transmitted or maternally non-transmitted form in 420 (90.5%) of all the patients studied.

Discussion

Although the inherited susceptibility to Type I diabetes are conferred by HLA *DR-DQ* genes, the largest proportion of patients suffers from a sporadic disease. We therefore focused on whether such genetic factors differ in mothers of patients from those present in fathers. We show that mothers of Type I diabetic patients, except those who are *DQ2/DQ8* heterozygous, carry significantly more often HLA *DQ2* or *DQ8* as non-transmitted allele than fathers. However, there was no such difference in those families with a *DQ2/DQ8* heterozygous offspring. Mothers of patients with Type I diabetes had been reported to have less non-transmitted HLA *DR-DQ* high risk alleles than parents of healthy children [9]. HLA *DR4-DQ8* is more frequent as maternal non-transmitted haplotype in patients with rheumatoid arthritis than in control subjects. This observation can be explained by a non-genetic effect of *DR4-DQ8* on the formation of the T-cell repertoire [10]. These non-inherited maternal HLA antigens have also been implicated in the tolerance of organ transplants [11]. The patient's exposure to the maternal non-inherited HLA *DR-DQ* haplotype could contribute to disease susceptibility by a yet unknown mechanism. If non-transmitted HLA *DQ* alleles increase the risk for Type I diabetes by complementing the patient's genetic risk, a functional mechanism must be involved in which either two different HLA molecules present the antigenic peptide better than one or two antigen receptors increase the likelihood of a pathogenic process leading to beta-cell autoimmunity. This could either happen in utero or post partum. Several studies have shown that maternal cells persist in their children and can be found in the offspring's peripheral blood, lymph nodes and other tissues up to 30 years post partum. Such 'microchimerism' has been shown to be more common in the autoimmune diseases systemic sclerosis, Sjögren's syndrome, and primary biliary cirrhosis [12, 13]. Persistent maternal microchimerism has been described in juvenile myopathy [14] and was recently reported to occur more frequently in patients than in healthy control subjects [15]. Such microchimerism has been found to be associated with HLA *DR3-DQ2* and can increase the risk for an autoimmune disease both in the mother and the infant depending on the HLA *DR-DQ* compatibility in either direction. Persisting foetal cells were found in the thyroid tissues of mothers with Graves' disease [16] and some evidence suggests that these cells are more frequent in mothers with thyroid disorders compared to subjects with clinically and histologically normal thyroids [17]. Although microchimerism has so far not been reported for Type I diabetes, our observations are compatible with maternal microchimerism contributing to Type I diabetes susceptibility. *DR3-DQ2/DR4-DQ8* heterozygosity confers an excess risk to Type I diabetes that is not explained by the individual haplotypes. This heterozygous state can extend to subjects harbouring microchimers with the complementary HLA *DQ* allele. Thus maternal cells carrying high risk HLA *DR-DQ* haplotypes would persist in the child's circulation or lymph node ready to present antigenic peptides to T-lymphocytes. In this

case, the destruction of beta cells could result from a graft versus host reaction rather than from a true autoimmune process. Whether persisting maternal immunogenic cells could also help to shape the thymic repertoire, has yet to be elucidated.

In conclusion, we have detected an additional susceptibility factor for Type I diabetes that is conferred by mothers through non-transmitted HLA *DQ2* and *DQ8* alleles. Thus these antigen presenting molecules are not only genetic but also environmental factors that are present in either constellation in 91% of patients. These results contribute to the understanding of the HLA association with Type I diabetes. Structural features of HLA *DQ* such as the P9 pocket and its functional sequelae on binding of immunodominant peptides of proteins such as insulin [18] should direct further research to predict and prevent Type I diabetes. It remains to be elucidated by which mechanism the intrauterine exposure to maternal HLA molecules or the persistence of maternal cells (microchimerism) could influence the offspring's susceptibility to Type I diabetes.

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