Letters

Comments

**–to: Pani MA, Van Autreve J,
Van Der Auwera BJ, Gorus FK,
Badenhoop K (2002) Non-transmitted
maternal HLA DQ2 or DQ8 alleles
and risk of Type I diabetes in offspring:
the importance of foetal or post partum
exposure to diabetogenic molecules.
Diabetologia 45:1340–1343**

Keywords Type 1 diabetes, HLA, genetic susceptibility, non-inherited alleles, microchimerism.

To the Editor: Recently, it has been suggested that mothers of patients with Type 1 diabetes carry HLA *DQ2* or *DQ8* disease risk alleles as non-transmitted alleles more often than fathers. Thereby, non-inherited maternal HLA risk alleles could contribute to disease susceptibility in the offspring [1]. The same phenomenon was described in rheumatoid arthritis suggesting that the close contact of the foetus with certain maternal antigen patterns could contribute to the development of autoimmune diseases later in life [2]. A subsequent study could not, however, confirm this [3].

It is conceivable that exposure to maternal antigens in utero has long-term effects on immune responses in the offspring. For example, an improved survival of allografts from HLA haploidentical sibling donors expressing maternal HLA antigens not inherited by the recipient has been described [4]. Similarly, microchimerism for maternal cells has been shown to persist into adult life in healthy subjects [5]. Moreover, the proportion of subjects carrying maternal cells was found to be increased among children with juvenile dermatomyositis as compared to control children [6]. Accordingly, it has been proposed that microchimerism for maternal cells carrying non-inherited HLA molecules could play a role in the initiation of diabetes-related autoimmunity [1].

The aim of this study was to confirm or reject the hypothesis that non-transmitted parental HLA haplotypes could influence Type 1 diabetes susceptibility in the offspring.

We analysed HLA *DQA1-DQB1* genotypes in 622 Finnish nuclear families with one child affected by Type 1 diabetes and non-diabetic parents. All patients had classic clinical onset of Type 1 diabetes and were diagnosed according to the World Health Organization criteria before the age of 15 years (mean

age was 7.9 ± 3.9 years). There were 340 boys (54.7%) and 282 girls (45.3%) among the affected subjects. HLA *DQA1-DQB1* genotyping and *DRB1*04* subtyping were done by using a PCR-based lanthanide-labelled oligonucleotide hybridisation method with time-resolved fluorimetry detection as described previously [7]. Chi-square statistics was used for comparisons of frequencies of haplotypes and genotypes analysed. A *p* value of 0.05 or less was considered to be significant. The local Ethics Committees approved the study and informed consent was obtained from participating subjects and/or their parents.

To analyse haplotype transmissions we used genotype definitions for family subsets identical to those in [1]. Including all affected offsprings, no differences were seen in the frequencies of non-transmitted *DQ2* or *DQ8* risk haplotypes between fathers and mothers (7.1 vs. 8.2% and 11.4 vs. 9.3%, respectively). Similarly, there was no parent of origin difference in frequencies of non-transmitted protective [(*DR5*)-*DQA1*05-DQB1*0301*, (*DR7*)-*DQA1*0201-DQB1*0303*, (*DR14*)-*DQB1*0503*, (*DR15*)-*DQB1*0602*, (*DR1301*)-*DQB1*0603*] or neutral [(*DR7*)-*DQA1*0201-DQB1*02*, (*DR4*)-*DQA1*03-DQB1*0301*, (*DR9*)-*DQA1*03-DQB1*0303*, (*DR8*)-*DQB1*04*, (*DR1*)-*DQB1*0501*, (*DR1302*)-*DQB1*0604*] haplotypes.

Thereafter, we compared non-transmitted HLA haplotype frequencies in subsets of families stratified according to the offspring's HLA genotype (Table 1.) The frequencies of non-transmitted HLA *DQ2* and *DQ8* haplotypes showed no differences between mothers and fathers of *DQ2*- (4/67 vs 7/67) or *DQ8*-homozygous (10/67 vs 8/67) children with Type 1 diabetes. Similarly, no differences were observed in the frequencies of non-inherited risk haplotypes in parents with *DQ2/DQ8* heterozygous offspring. The frequencies of maternal and paternal non-inherited *DQ2* and *DQ8* haplotypes in children with *DQ2/x* and *DQ8/x* ($x \neq DQ2$ or *DQ8*) genotypes were as follows: 7.7% vs 8.7% and 9.0% vs 10.8%, the differences being non-significant. In addition, the distribution of non-transmitted *DQ2* and *DQ8* haplotypes was similar in the two parental groups of the 86 families where the affected offspring carried no risk haplotypes (x/x genotype group in Table 1). Moreover, no parental differences were seen in the frequencies of non-transmitted neutral or protective haplotypes in any of the family subsets.

It was also implicated that a complementary non-inherited HLA haplotype in patients with *DQ2/DQ2* and *DQ8/DQ8* or *DQ2/x* and *DQ8/x* genotypes (i.e. *DQ8* in the case of *DQ2*-positive offspring and vice versa) might confer an additional risk of diabetes [1]. We did the same analyses and found no support for such an effect (Table 1).

In conclusion, we observed no evidence indicating the possible contribution of non-inherited parental HLA haplotypes to the risk of Type 1 diabetes in the offspring in the Finnish population. An earlier study comprising a much smaller family series suggested that non-inherited maternal HLA risk alleles are decreased in mothers of children with Type 1 diabetes [8]. The discrepancies between the three studies most probably originate from chance, and marginally significant *p* values might represent type I statistical errors. The lowest *p* value in the report of Pani et al. [1] was observed after combining groups

Table 1. Maternal and paternal non-transmitted HLA *DQA1-DQB1* risk haplotypes in 622 nuclear families with one family member affected by Type 1 diabetes

HLA DQ genotype of affected offspring	Number of families	Number (%) of non-transmitted parental DQ2 or DQ8 haplotypes		<i>p</i> value
		Fathers	Mothers	
<i>DQ2/DQ8</i>	146	27 (18.5%)	24 (16.4%)	NS ^a
<i>DQ2/DQ2+DQ8/DQ8</i>	67	14 (20.9%)	15 (22.4%)	NS
<i>DQ2/x^b + DQ8/x</i>	323	63 (19.5%)	54 (16.7%)	NS
<i>x/x</i>	86	11 (12.8%)	16 (18.6%)	NS
All except <i>DQ2/DQ8</i>	476	88 (18.5%)	85 (17.9%)	NS

HLA DQ genotype of affected offspring	Number of families	Number (%) of non-transmitted complementary ^c parental DQ2 or DQ8 haplotypes		<i>p</i> value
		Fathers	Mothers	
<i>DQ2/DQ2+DQ8/DQ8</i>	67	5 (7.5%)	6 (9.0%)	NS
<i>DQ2/x + DQ8/x</i>	323	28 (8.7%)	25 (7.7%)	NS

^a NS, not significant; ^b *x* ≠ *DQ2* or *DQ8* haplotype

^c Complementary parental DQ2 or DQ8 haplotypes = DQ8 in the case of a DQ2-positive offspring and vice versa

showing significant results, whereas families with offspring heterozygous for the high risk *DQ2/DQ8* genotype with no observed statistically significant difference were excluded. We wish to emphasise that our study had more than a 99% power to detect the differences in allele frequencies in the range reported earlier.

Of note, HLA *DQ2* (*DQA1*0501-DQB1*0201*) and *DQ8* (*DQA1*0301-DQB1*0302*) are the major diabetes susceptibility haplotypes common for the Belgian, German and Finnish populations. Moreover, in the vast majority of patients with Type 1 diabetes *DQ8* is seen together with either the *DRB1*0401* allele or to a lesser extent with the *DRB1*0404* allele, and *DQ2* occurs most often on the HLA B8-DR3 haplotype.

A population-specific effect of non-inherited maternal HLA haplotypes cannot of course be excluded, although we find that unlikely.

R. Hermann, R. Veijola, I. Sipilä, M. Knip, H. K. Åkerblom, O. Simell, J. Ilonen
Department of Virology, University of Turku, Turku, Finland

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Corresponding author: Dr. R. Hermann, Department of Virology, University of Turku, Kiinamyllynkatu 13, 20520 Turku, Finland
e-mail: robert.hermann@utu.fi