**–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 1 diabetes in offspring:
the importance of foetal or post partum
exposure to diabetogenic molecules.
Diabetologia 45:1340–1343**

To the Editor: Pani et al. [1] investigated children with Type 1 diabetes and their parents, and found that high risk HLA class II haplotypes were not only transmitted more frequently from both parents to the child with diabetes, but were also more common among the maternal non-transmitted haplotypes than paternal. They hypothesised that low exposure of the child to maternal non-transmitted haplotypes in retained maternal cells – microchimerism – might contribute to increased disease susceptibility. Maternal cells could persist into adult life [2] and contribute to the development of autoimmune disease in the offspring [3]. We have tried to replicate the findings of Pani et al. in a large United Kingdom population of families with Type 1 diabetes.

We analysed HLA *DQA1-DQB1* genotypes in 753 families from the United Kingdom with at least one child affected with Type 1 diabetes, diagnosed before age 21, and participating in the Bart's Oxford (BOX) study of childhood diabetes. The median age of the affected child at diagnosis was 10.5 years (range 0.7–20.9) and 57% were boys. HLA *DQA1-DQB1* genotyping was done by PCR-SSP. Haplotype frequencies were compared by Chi-square testing, and a *p* value of less than 0.05 was considered significant.

There was no difference in the frequency of non-transmitted *DQA1*0501-DQB1*0201* (*DQ2*) or *DQA1*0301-*

*DQB1*0302* (*DQ8*) haplotypes between fathers and mothers. In these families, *DQ2* was the non-transmitted haplotype in 14.3% of fathers and 12.9% of mothers, and *DQ8* was the non-transmitted haplotype in 9.6% fathers and 9.7% mothers (*p*>0.05 for all comparisons). There was an excess of non-transmitted *DQB1*0602* alleles in mothers compared to fathers (16.3% vs 10.6%, $\chi^2=10.527$; *p*=0.001).

The frequencies of non-transmitted *DQ2* and *DQ8* haplotypes in subsets of families stratified by the affected child's genotype are shown in Table 1. There was no difference between the frequency of non-transmitted paternal and maternal *DQ2* or *DQ8* haplotypes in affected children who were *DQ2* homozygous, *DQ8* homozygous or *DQ2/DQ8* heterozygotes. Similarly, when we examined the parents of affected children with *DQ2/X* or *DQ8/X* genotypes (where X represents a non-*DQ2*, non-*DQ8* haplotype) there was no difference in the numbers of non-transmitted haplotypes. Even in the subset of affected children with an *X/X* genotype there was no excess of *DQ2* or *DQ8* haplotypes as the non-transmitted maternal haplotype.

Based on our current understanding of microchimerism, the largest difference in frequency of non-transmitted parental haplotypes would be expected in families where the affected child has either a *DQ2/X* or *DQ8/X* genotype but is exposed to the complementary high risk maternal haplotype. In such families we see no maternal excess of non-transmitted haplotypes. In *DQ2/X* children, *DQ8* is present as non-transmitted haplotype in 12.7% of fathers and 6.4% of mothers, and for *DQ8/X* children, the *DQ2* haplotype is present as the non-transmitted haplotype in 16.2% of fathers and 12.1% of mothers (Table 1).

We found no evidence for an excess of non-transmitted maternal high-risk alleles when we excluded homozygous parents from the analysis. Similarly we saw no effect when we examined HLA *DRB1-DQA1-DQB1* haplotypes, and in particular there were no differences in the frequency of non-transmitted *DRB1*0401-DQA1*0301-DQB1*0302* haplotypes, the predominant *DRB1*04*-based risk haplotype in this United Kingdom population.

In conclusion, we see no evidence for a role of non-transmitted maternal HLA *DQA1-DQB1* haplotypes in increasing susceptibility to Type 1 diabetes in this large United Kingdom population of families with Type 1 diabetes. We did observe an excess of non-transmitted *DQB1*0602* alleles in mothers, but this requires confirmation. The difference between our findings and those of Pani et al. could perhaps be explained by dif-

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Table 1. Paternal and maternal non-transmitted HLA *DQA1-DQB1* haplotypes in 753 families

Genotype of affected offspring	Number of families	Number (%) of non-transmitted parental <i>DQ2</i> haplotypes		Number (%) of non-transmitted parental <i>DQ8</i> haplotypes	
		Fathers	Mothers	Fathers	Mothers
<i>DQ2/DQ8</i>	272	40 (15)	38 (14)	34 (13)	34 (13)
<i>DQ2/DQ2</i>	71	12 (17)	10 (14)	5 (7)	7 (10)
<i>DQ8/DQ8</i>	57	8 (14)	3 (5)	7 (12)	13 (23)
<i>DQ2/X</i>	110	14 (13)	15 (14)	14 (13)	7 (6)
<i>DQ8/X</i>	173	28 (16)	21 (12)	16 (9)	22 (13)
<i>X/X</i>	70	7 (10)	10 (14)	5 (7)	6 (9)
<i>DQ2/DQ2 + DQ8/DQ8</i>	128	20 (16)	13 (10)	12 (9)	20 (16)
<i>DQ2/X + DQ8/X</i>	283	41 (15)	36 (13)	30 (11)	29 (10)
All except <i>DQ2/DQ8</i>	481	69 (14)	59 (12)	47 (10)	55 (11)

X= non-*DQ2* and non-*DQ8* haplotypes

p values for all comparisons are non-significant (*p*>0.05)

ferences in the age at onset of diabetes in the two populations. In our study the median age at onset was young at 10.5 years and we find a high rate of transmission of high-risk haplotypes from both parents. For example, of 223 *DRB1*0401-DQB1*0302* haplotypes present in mothers, 194 (87%) were transmitted to the affected child, and of 210 in fathers, 170 (81%) were transmitted. The low overall frequency of high-risk haplotypes among those that are not transmitted mask their effect, and this might be more clearly seen in populations with an older age of onset in which a higher proportion of high-risk haplotypes could remain untransmitted.

A. P. Lambert, K. M. Gillespie, P. J. Bingley, E. A. M. Gale
Diabetes and Metabolism, Medical School Unit, Southmead Hospital, Bristol, UK

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Authors' reply

To the Editor: Hermann et al. question the influence of non-transmitted maternal *HLA DQ* risk alleles in Type 1 diabetes as shown in our recent study [1] and as earlier shown in mothers of *HLA DR4*-negative patients with rheumatoid arthritis [2]. Although this observation in rheumatoid arthritis could not be confirmed in a study from the United Kingdom [3], a more recent analysis of rheumatoid arthritis families clearly confirms the association of maternal non-inherited *HLA DR4* and rheumatoid arthritis susceptibility in two independent populations [4].

Herman et al. report their findings of 622 nuclear families with Type 1 diabetic children from Finland without significant differences between non-transmitted maternal and paternal *HLA DR-DQ* risk haplotypes. Although their data outline is similar to ours there are different frequencies of *HLA DQ* allele risk constellations: *HLA* genotypes *DQ2/x* and *DQ8/x* (51.93% in the Finnish and 39.64% in our population), *DQ2/DQ2* and *DQ8/DQ8* (10.77% vs 14.35%) and *DQ2/DQ8* (23.47% vs 32.57%). This could reflect a different background of genetic susceptibility. Nevertheless, more mothers are carrying a non-transmitted *HLA DQ2* or *DQ8* (18.6% vs 12.8% in fathers) in the group of patients with genotype *DQx/x*, similar to our observations, although not significant in the Finnish population.

In addition there are differences in the genotyping strategy between the two studies that could explain differences in their and our observations: If parent-child transmissions are screened for selective *DQB1* alleles or certain *DQA1-B1* combinations without including all possible variants, those alleles could be underdetected that are not transmitted. The full *DQA1-B1* typing enhances this resolution [5].

Lambert et al. analysed the *HLA DQA1-DQB1* genotypes in patients with Type 1 diabetes onset before age 21 and find no excess of non-transmitted maternal *DQ* risk haplotypes. However they observe a higher frequency of maternal non-transmit-

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Corresponding author: Dr. A. P. Lambert, Diabetes and Metabolism, Medical School Unit, Southmead Hospital, Bristol, UK
E-mail: A.P.Lambert@bristol.ac.uk

ted *DQB1*0602* (16.3%) in comparison to this paternal non-transmitted allele (10.6%). This is of interest since the *DQB1*0602*'s frequency in the general population (19% [5]) is higher. This finding, if confirmed, suggests that mothers could confer more protection from Type 1 diabetes in offspring than fathers.

To test for data consistency we examined 26 additional families including patients with Type 1 diabetes thereby expanding our combined Belgian-German study population to 465 families (Table 1): More mothers carry *HLA DQ* risk alleles as non-transmitted in families with *DQ2/x* and *DQ8/x* patients and in *DQx/x* patients ($p=0.006$ and $p=0.048$), whereas the difference in *DQ2/DQ2* and *DQ8/DQ8* offspring shows merely a trend ($p=0.0747$). Mothers of all patients (except the *DQ2/DQ8* heterozygous ones) carry more often a non-transmitted *DQ* risk allele (35.9%) than fathers (21.6%, $p=8.5 \times 10^{-4}$). Thus we confirm our earlier findings and we are confident in the consistency of our data.

The inherited form of Type 1 diabetes susceptibility conferred by *HLA DQ* remains the single most important risk factor. Only 18 patients out of 465 (3.9%) who have the genotype *HLA DQx/DQx* have a mother with a non-transmitted risk allele, whereas 86% of patients have at least one inherited susceptible *HLA DQ*. Therefore the contribution of non-transmitted alleles to disease susceptibility is small. However, it shows a potential of *HLA DQ* to act in a non-genetic, but "environmental" fashion. Whether this mode of action is due to maternal microchimerism or caused by an in utero acquired infection or antigen presentation remains to be examined. We believe the main reason for the discrepancy between Hermann's et al. and our findings is due to the different populations studied. There are major differences between the Finnish, Belgian and German populations, most of which are reflected by a different incidence of the disease but also by particular predisposing haplotypes unique to the Finnish population [6, 7]. Also, disease heterogeneity between and within the two populations could result in a different selection of families [8].

The discrepancy of our to the United Kingdom or Finnish findings could result from such heterogeneous variables as age of onset (median of 7 years in the Belgian and 6 years in those German families with an age at manifestation recorded) but