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Inheritance of MHC class II genes in IDDM studied in population-based affected and control families

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Summary The transmission of HLA-DR and DQ was compared between 46 families with at least one child affected by insulin dependent diabetes mellitis (IDDM) and 43 healthy control families. In the patient families, there was an increased transmission of DR4 ($p < 0.025$) and DQB1*0302 ($p < 0.01$) from both parents to the index patient. There was an increased transmission of DQB1*0302 ($p < 0.03$) from the mothers only. The non-inherited maternal haplotypes showed a significantly decreased frequency ($p < 0.01$) of positively associated haplotypes (DR4-DQA1* 0301-DQB1*0302, DR3-DQA1*0501-DQB1*0201) compared to all parental haplotypes in the control families. In the control families neither transmission rates nor frequencies of non-inherited haplotypes differed from those expected in the control families. In conclusion, the observed reduction of IDDM-positively associated haplotypes in patient non-inherited maternal haplotypes, but not in non-inherited paternal haplotypes, suggests that tolerance during fetal life to maternal non-inherited HLA molecules may be important to diabetes development. [Diabetologia (1994) 37: 1105–1112]

Key words Transmission rates, non-inherited maternal haplotypes, non-inherited paternal haplotypes, HLA-DR, HLA-DQ.

Insulin-dependent diabetes mellitus (IDDM) is thought to be initiated by environmental factors among genetically predisposed individuals. This hypothesis is supported by a low concordance rate among monozygotic twins (30–50 %) [1, 2], and a low frequency (13 %) of affected first degree relatives among newly diagnosed IDDM patients [3]. The environmental factor(s) are yet to be identified. The genetic predisposition is in part conferred by HLA on human chromosome 6 since IDDM patients have an increased frequency of DR4-DQA1*0301-DQB1*0302 and DR3-DQA1*0501-DQB1*0201 haplotypes in the Caucasian population [4–8]. In a defined high-incidence area which we have studied [9], more than 90 % of IDDM patients had DQB1*0201, DQB1*0302 or both compared with 60 % in unrelated control subjects. These haplotypes therefore seem necessary, but not sufficient for IDDM, and it has been suggested that the DR4-DQA1*0301-DQB1*0302 haplotype acts in a dominant and the DR3-DQA1*0501-DQB1*0201 in a recessive fashion [10, 11]. Furthermore, the strong negative association between IDDM and DR2 and DQB1*0602 [6, 12–14] was confirmed [9]. The so called protective haplotype, DR2-DQA1*0102-DQB1*0602 acts in a dominant fashion since it is negatively associated with IDDM also in the presence of either DR4 or DQB1*0302 or both [9, 15].

Neither the mechanisms of the HLA association nor the mode of inheritance of IDDM-associated

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Abbreviations: HLA, Human Leucocyte antigen; NIMH, non-inherited maternal haplotype; NIPH, non-inherited paternal haplotype; MHC, major histocompatibility complex; RPE, relative predispositional effect; RFLP, restriction fragment length polymorphism; w/v, weight volume.

haplotypes are understood. Numerous studies have failed to identify a simple mode of inheritance of IDDM (for reviews see [16–18]). Using multiplex families or families with IDDM in two to three generations there is evidence suggesting that HLA Class II alleles are not only associated with but also genetically linked to IDDM [19, 20]. HLA haploidentical siblings have a similar risk to the general population for developing IDDM [21] while HLA identical siblings have an approximately 100 times higher risk [22]. In two generation multiplex families, DR4-positive affected fathers have been shown to transmit this allele more frequently to their offspring (diabetic or not) than did the mothers [23, 24]. These authors suggest that this distorted mode of inheritance may explain the higher risk of offspring of diabetic fathers (70 %) compared to that of diabectic mothers (30 %) of developing diabetes [3, 25, 26]. There are, however, reports that failed to detect an increased transmission of positively associated HLA-haplotypes from fathers compared with mothers [27–29]. In a previous investigation [9], ambiguities of haplotypes and selection bias were avoided in a case-control study of population-based IDDM probands and their families, and control families from the same high-incidence area in northern Sweden. The present study is distinct from some previous studies on the mode of inheritance of IDDM-associated HLA alleles and haplotypes in that comparisons were made with control families. The aim of this study was therefore to compare the transmission of HLA haplotypes between patient families and control families.

Subjects, materials and methods

Patient and control families. All children under the age of 16 years ($n = 75$) treated for IDDM at the Department of Pediatrics, University Hospital, Umeå, Sweden living within a 30-km radius of the clinic, having at least three members in the family, were asked to participate in a case-control family study, as previously described [9]. All IDDM children in this area are monitored at this pediatric clinic. The degree of ascertainment of families was 67 % (50 of 75), including 23 families from a previous investigation [30]. The transmission of HLA haplotypes could be followed in 46 families. In these families, 15 % (7 of 46) of the index patients had a first degree relative with IDDM compared with 8.3 % in a 10-year registry of all newly-diagnosed IDDM children throughout Sweden [3]. In four families (9 %), there were two affected siblings, which is comparable to the 4 % frequency in the registry [3]. Furthermore, 4 % of the index cases had a father or mother with the disease compared with 8.5 % in the entire country [3].

A total of 51 control families who had a child in the same school as a diabetic index child were studied. In none of these families did the index child have a first degree relative with diabetes. Of these 51 control families, 43 were successfully HLA typed.

Informed consent by participating patients and control subjects was obtained. The investigation was approved by the ethics committee at Umeå University, Sweden.

Experimental procedures. DNA was prepared from peripheral blood using standard methods as previously described [31]. Briefly, 20 μg of DNA was digested with either Taq1 or BamH1 according to the manufacturer's instructions (Boehringer Mannheim, Mannheim, Germany). Samples were electrophoresed in 1 % agarose, transferred in 0.4 mol/l NaOH onto Zeta-probe nylon membrane (Bio Rad Laboratories, Richmond, Calif., USA) by the method of Southern [32]. The Taq1 digested samples were first hybridized with a DRβ probe [33] and then, after a strip wash of boiling 1.5 mmol/l NaCl, 0.15 mmol/l sodium citrate and 0.1 % (w/v) SDS, with a DQα probe [34]. The BamH1 digests were hybridized with a DQβ probe [31]. Hybridizations were carried out at 42 °C for 16 h in 750 mmol/l NaCl, 75 mmol/l sodium citrate, 10 % dextran sulphate, 1 % SDS, 20 mmol/l sodium phosphate, 0.1 mg/ml carrier DNA, 50 % formamide, 0.2 % Ficoll 400, 0.2 % polyvinylpyrrolidone and 0.2 % bovine serum albumin (Pentax fraction V). The filters were washed at room temperature with 300 mmol/l NaCl, 30 mmol/l sodium citrate and 0.1 % SDS, and at 60 °C with 15 mmol/l NaCl, 1.5 mmol/l sodium citrate and 0.1 % SDS. The filters were exposed to X-OMAT film (Eastman Kodak Company, Rochester, NY, USA) for 3–5 days before developing.

RFLP-based genotypes. The assignment of DRB1, DRB3, DRB4, DQB1, DQA1 genotypes was made based on the RFLP patterns as previously described [9]. Haplotypes were confirmed by typing the first degree relatives of both patients and control subjects. Note that we cannot distinguish DR7 from DR9 when either occurs together with DQB1*0303 (DQ9), hence all DQB1*0303 (DQ9) haplotypes have been assigned as DQA1*0301. Individuals found to be positive for DQB1*0303 (DQ9) were assigned the DR 7/9 and DQA1*0301 or DQA1*0201 types. In addition we cannot distinguish the DQB1*0402 (DQ4)-DR8 haplotype from the DQB1*0302 (DQ8)-DR8 haplotype.

Statistical analysis

The differences of distribution of alleles between groups were tested by chi square analysis with Yates correction. Fisher's exact test was used if the expected number in any group was less than five. Resulting p values were not corrected for multiple comparisons, and $p < 0.05$ was considered significant.

The relative predispositional effect (RPE) method as previously described [35] was used to rank alleles differing between groups. An attempt was made to combine groups so that the expected values were greater than 5. Fisher's exact test was used to compare the distributions of HLA alleles between patients and control subjects before groups of alleles were combined.

The frequency of transmission of alleles was expressed as the number of times the specificity was inherited, divided by the number of times it could have been inherited by the offspring. This frequency was then compared with an expected rate of 50 %.

We tested if the combination of parental haplotypes occurred in a non-random fashion. The frequency of parental haplotypes was used to calculate the expected number of children with each genotype, assuming no transmission distortion, as described [36], except that differing haplotype frequencies among fathers and mothers were taken into account. In order to rank which combination differed the most from expected, the RPE analysis was used [35]. The p value of the groups was obtained by two-tailed Z-test.

Table 1. Transmission rates compared with the expected 50 % transmission rate

Transmission from: either parent to their children in the control families

Allele	From parents		*p* value
	n	%	
DR2	32	47	NS
DR3	23	59	NS
DR4	45	51	NS
DQB1*0201	33	61	NS
DQB1*0302	38	46	NS
DQB1*0602	32	47	NS

Transmission from: either parent to index patients in the patient families

Allele	From parents		*p* value
	n	%	
DR2	2	8	0.005
DR3	20	71	NS
DR4	47	73	0.025
DQB1*0201	22	73	NS
DQB1*0302	41	75	0.025
DQB1*0602	0	0	0.0005

Transmission from: either parent to healthy siblings in the patient families

Allele	From parents		*p* value
	n	%	
DR2	23	64	NS
DR3	13	43	NS
DR4	39	53	NS
DQB1*0201	13	37	NS
DQB1*0302	34	50	NS
DQB1*0602	20	62	NS

Transmission from: mother to index patients in the patient families

Allele	From mothers		*p* value
	n	%	
DR2	1	7	0.02
DR3	12	80	NS
DR4	21	78	NS
DQB1*0201	14	82	NS
DQB1*0302	19	83	0.05
DQB1*0602	0	0	0.04

Transmission from: father to index patients in the patient families

Allele	From fathers		*p* value
	n	%	
DR2	1	9	NS
DR3	8	62	NS
DR4	26	70	NS
DQB1*0201	8	62	NS
DQB1*0302	22	69	NS
DQB1*0602	0	0	NS

Transmission from: either parent to all children in the patient families

Allele	From parents		*p* value
	n	%	
DR2	25	40	NS
DR3	33	57	NS
DR4	86	63	0.05
DQB1*0201	35	54	NS
DQB1*0302	75	61	NS
DQB1*0602	20	37	NS

Table 1. Continued

Transmission from: either parent to healthy siblings in the patient families

Allele	From mothers		*p* value
	n	%	
DR2	23	64	NS
DR3	13	43	NS
DR4	39	53	NS
DQB1*0201	13	37	NS
DQB1*0302	34	50	NS
DQB1*0602	20	62	NS

In an analysis of non-inherited parental haplotypes those haplotypes that were not inherited by the index child from one or the other parent were compared with all haplotypes among the control subjects; i.e., a pool of control haplotypes. The allele frequencies of the control pool were used to calculate the expected number of alleles in each group for the non-inherited haplotypes. The observed and expected haplotypes were then compared in a 2 by 3, or 2 by 4 contingency table using chi square analysis.

Results

Parental HLA allele frequency of DRB1, DQA1 and DQB1 alleles among mothers and fathers in diabetic families did not differ. Unexpectedly however, the frequency of DR7 or 9 was higher among control fathers (17 %) than among control mothers (1 %, $p < 0.0005$), and the frequency of DR6 was lower among fathers (5 %) than mothers (18 %, $p < 0.02$). DQB1*0603, found on many DR6 haplotypes [37], consequently also had a lower frequency among control fathers (2 %) than mothers (14 %, $p < 0.01$). DQB1*0303 was not detected among the control mothers but among 9 % ($p < 0.003$) of fathers. Both DQA1*0103 and DQA1*0101 showed a higher frequency among mothers (16 % and 19 %, respectively) than fathers (2 %, $p < 0.03$ and 6 % $p < 0.02$, respectively). While these differences in allele frequencies remain unexplained they will not affect our analysis of the mode of transmission. In contrast to the control parents, there was no difference in the frequency of DRB1, DQA1 and DQB1 alleles between mothers and fathers of the IDDM probands.

Analysis of inherited alleles. We tested if the transmission rates of HLA haplotypes were different between fathers and mothers. In control families, the expected and observed transmission rates did not differ since they all were consistent with an expected rate of 50 % (Table 1). As anticipated, several differences from the 50 % expected rate were observed for the transmission of HLA alleles and haplotypes to the index patients. In diabetic families DR4 (73 %) and DQB1*0302 (75 %) were transmitted more frequently than expected ($p < 0.025$). These two alleles often occur on the same haplotype due to the strong link-

Table 2. Analysis of combinations of maternal and paternal haplotypes

Children	Overall p	To remove		p of group to remove	Rank
Control child	0.001	neutral/neutral	↑	0.001	1
	0.001	negative/neutral	↓	0.002	2
	0.01	positive/negative	↓	0.01	3
Index patients	0.001	positive/positive	↑	0.02	1
	0.005	positive/neutral	↑	0.002	2
	0.025	neutral/neutral	↑	0.005	3

Increased, ↑, and decreased, ↓, frequency among children compared with expected frequency based on parental haplo-frequencies. Positive haplotype group contains, DR3 and DR4 haplotypes, negative DR2 and neutral remaining haplotypes. Group to remove is the one that has the highest contribution to overall chi-square. Siblings to index patients showed no significant overall p

age-disequilibrium between DRB and DQB. DR3 and DQB1*0201, that also often occur on the same haplotype, showed similar transmission rates to DR4 and DQB1*0302 but were not significantly increased compared to expected rates. There were 26 parents with DR2; only 2 (8 %) index patients received this allele ($p < 0.005$). Similarly, DQB1*0602 was not transmitted at all from a total of 22 parents to 22 index patients ($p < 0.0005$ when compared with 50 % transmission frequency). Transmission rates to healthy siblings in the diabetic families did not differ from the expected 50 % rate for any of the alleles. This is illustrated by the transmission of DR2 to healthy siblings that was 26 of 36 of (64 %), which does not differ from the 50 % rate ($p > 0.05$) but differs from the transmission rate to index patients ($p < 0.0005$).

We next tested whether these distortions in transmission rates differed between parents. As can be seen from the data in Table 1, the results were explained mainly by distorted transmission rates from the mothers. The transmission of DQB1*0302 from mother to index patients was 83 % ($n = 19$, $p < 0.05$, Table 1) which is significantly higher than the expected 50 %. In addition, although there were DR2-positive mothers, DR2 was only transmitted to one (7 %) of the index patients, ($p < 0.02$ when compared with the expected 7.5 or 50 %). DQB1*0602 was not transmitted to any of the index patients from the 13 DQB1*0602 positive mothers ($p < 0.04$ when compared with the expected 50 %).

Our analysis demonstrates further that HLA haplotype transmission rates from fathers to index patients did not differ from the expected 50 %. However, the transmission rates from fathers to index patients were not statistically different from that of the mothers to index patient (data not shown). Finally, when the transmission rates from both parents to index patients and their siblings were combined, the transmission rate of DR4 (86 of 137, 63 %) was found to be increased compared with the expected rate of 50 % ($p < 0.05$). These results indicate that the DR4 and DQB1*0302 alleles tended to be transmitted to the index patients more often from the mother than from the father. Since these alleles often occur on the same haplotype, we next investigated the presence of positively, negatively and neutrally associated haplotypes in the offspring.

Combination of maternal and paternal haplotypes. The parental gene frequencies were used to calculate the expected genotype frequencies among the offspring assuming random transmission (Table 2). A comparison of genotypes among control parents with those among index patients [9], leads to the combination of genotypes into three groups: positively associated haplotypes (DR3-DQA1*0501-DQB1*0201, DR4-DQA1*0301-DQB1*0302), negatively associated haplotypes (DR2-DQA1*0102-DQB1*0602) and neutral haplotypes (all remaining haplotypes). A chi-square test was used to test the null hypothesis that the observed genotypes in the offspring did not differ from those expected assuming random pairing of parental haplotypes. This test provided the overall p value in Table 2. The group to be removed is the group that differs the most from the expected value. The data in Table 2 show that the combination of haplotypes differed from that expected, based on the parental haplo-frequencies both among control children and index patients ($p < 0.001$). Among control subjects, the group deviating the most from expected values were individuals homozygous for neutral haplotypes ($p < 0.001$) which were increased compared to the expected rate. This was followed by negative/neutral heterozygotes ($p < 0.001$) and positive/negative heterozygotes ($p < 0.002$), both of which were reduced compared with expected. Index patients homozygous for the positively associated haplotypes were increased compared with the expected frequency ($p < 0.01$). Positively/neutrally and neutrally/neutrally associated haplotypes ($p < 0.001$ and $p < 0.005$, respectively) were also increased compared with expected values. There were no differences between observed and expected genotypes among either siblings to patients or patients and their siblings combined (data not shown). Thus, in both control and patient families, it appears that random combination of parental haplotypes does not occur. Families with IDDM have an increase of combinations causing disease while in control families the "neutral/neutral" genotype is inher-

Table 3. Non-inherited haplotypes

	Observed no.	Expected no.	chi square *(p)*
Control families			
NIPH			
Positive haplotype	12	12.4	0.01
Negative haplotype	9	13.3	1.14
Neutral haplotype	19	14.3	1.56
Total	40	40	2.97 NS
NIMH			
Positive haplotype	12	13.5	0.16
Negative haplotype	10	12.8	0.61
Neutral haplotype	19	14.8	1.22
Total	41	41	1.98 NS
Control Families with DR3 or DR4			
NIPH			
Positive haplotype	12	8.05	1.94
Negative haplotype	7	8.67	0.32
Neutral haplotype	7	9.29	0.56
Total	26	26	2.82 NS
NIMH			
Positive haplotype	11	8.53	0.72
Negative haplotype	4	8.11	2.08
Neutral haplotype	11	9.36	0.29
Total	26	26	3.09 NS
Patient families			
NIPH			
Positive haplotype	13	14.1	0.09
Negative haplotype	10	14.1	1.19
Neutral haplotype	22	16.8	1.60
Total	44	44	2.88 NS
NIMH			
Positive haplotype	5	14.1	5.87
Negative haplotype	14	14.1	0.00
Neutral haplotype	26	16.8	5.03
Total	45	45	10.9 < 0.01
NIMH			
DR3-DQB1*0201	2	4.61	1.48
DR4-DQB1*0302	3	9.49	4.44
Negative haplotype	14	14.1	0.00
Neutral haplotype	26	16.8	5.03
Total	45	45	10.9 < 0.025

Table 4. Non-inherited maternal haplotypes (NIMH) among DR3 or DR4 positive children

NIMH	Control subjects		Patients		Odds ratio	*p*
	n	%	*n*	%		
DR2	5	22	14	33	0.56	NS
DR3	3	13	2	5	3.03	NS
DR4	5	22	4	10	2.63	NS
Neutral	10	43	22	52	0.70	NS
Positive	8	35	6	14	3.23	0.05
DR2 or neutral	15	65	36	86	3.12	0.05

ited more often than expected. This possibility was further analysed by comparing the haplotypes that were not inherited by the offspring in the diabetic and the control families.

Non-inherited paternal and maternal haplotypes. The HLA haplotypes were divided into three groups to analyse non-inherited maternal haplotype (NIMH) and non-inherited paternal haptotype (NIPH): 1) haplotypes positively associated with IDDM (DR3-DQA1*0501-DQB1*0201 and DR4-DQA1*0301-DQB1*0302), 2) haplotypes negatively associated (DR2-DQA1*0102-DQB1*0602) and 3) haplotypes neutrally associated representing all remaining haplotypes. Those haplotypes that were not inherited by index children in the control families were first compared with the remaining group of control haplotypes (Table 3). Neither NIPH nor NIMH showed a difference in haplotype frequencies from those expected. The same analysis was repeated for those control families that contained either DR3 or DR4 or both. Again, neither NIPH nor NIMH differed from the expected frequencies.

In patient families, the non-inherited haplotype frequencies were compared with the expected frequencies, that were based on the frequencies in the parents of control families (Table 3). NIPH in the patient families did not show a haplo-frequency that differed from those in the control group. However, NIMH did show a lower (5 of 45) frequency of positively associated haplotypes compared with that expected based on the control parents (14 of 45) ($p < 0.01$). Since the positively associated haplotype showed a difference, we analysed whether this was due to DR3-DQA1*0501-DQB1*0201 or DR4-DQA1*0301-DQB1*0302. This analysis revealed that the distortion between the observed and expected frequencies of NIMH was mainly due to a reduction of DR4-DQA1*0301-DQB1*0302 among NIMH. After removal of the DR4-DQA1*0301-DQB1*0302 haplotypes, the observed and expected NIMH values did not differ.

The NIMH was further analysed by comparing the 23 index control subjects with the 42 index patients, all being positive for DR3, DR4 or both (Table 4). The positively associated haplotypes, DR3 and DR4, were decreased among index patient NIMH compared to those of index control subjects ($p < 0.05$). Therefore, the observed decrease of positively associated haplotypes in the NIMH of the index patients was not an effect of DR3 and DR4, but specific for the diabetic child.

Discussion

In contrast to the control families, who showed the expected mode of inheritance, we observed an increased transmission rate of the diabetes associated HLA haplotypes, DR4-DQA1*0301-DQB1*0302 and DR3-DQA1*0501-DQB1*0201, from both parents to the index patients. This increase in transmission rate is reflected in a reduced frequency of positively associated haplotypes among the NIMH. In an HLA- associated disease, the transmission rate of a positively associated HLA allele to affected children

is expected to be higher than 50 %. If the penetrance was 100 %, i.e. all individuals with a susceptible allele would develop the disease, one would also expect the transmission rates to the healthy siblings of the index patients to be lower than 50 %. However, if the penetrance is low, transmission rates would be closer to 50 %. The observed increased transmission of DR4-DQA1*0301-DQB1*0302 and reduced transmission of DR2-DQA1*0102-DQB1*0602 seems to be most pronounced among mothers, although we did not observe a significant difference between transmission rates from mothers and fathers.

We observed a significant difference in the frequency of DR7 and/or 9 between fathers and mothers in the control families. This unexpected finding was probably due to chance variation, but needs to be verified in other healthy families. Despite this we did not observe any distortion in segregation ratios in the control families which is consistent with previous reports [29, 38–41]. The only haplotype inherited more frequently than expected, was C2 – B18 – DR2 [39]. There were no significant differences in transmission rates from mother or father [29, 38–41]. Hence, there does not seem to be a segregation distortion in families without IDDM.

In our study, we did not observe an increased transmission of DR3. There are reports that DR3 haplotypes are transmitted at a higher frequency than 50 % to index patients in families with IDDM [23, 24]. Deschamps et al. [24] observed an increase of maternal DR3 and paternal DR4 but only analysed DR3/4 heterozygotes. When we combined several family studies [23, 24, 28, 38, 42, 43], we observed that both mothers and fathers transmit DR3 to IDDM children at a 75 % rate, which was significantly higher than the expected rate of 50 % ($p < 0.00005$). We therefore conclude that there are no differences between paternal and maternal transmission of the DR3 haplotype.

Exclusion of probands in families with IDDM resulted in a 63 % transmission rate of the A1-B8 haplotype [44]. Using previously published data [28], we could recalculate that the transmission rate of DR3 from mothers to healthy offspring is 82 % ($p < 0.005$ vs 50 %) and that this differs significantly from the 48 % rate of transmission from fathers. Combining all the reported data on transmission of DR3 to siblings in diabetic families leads us to conclude that there is no significant deviation from the 50 % transmission rate of DR3 from parents, nor is there a difference in transmission rates from mothers or fathers. This is consistent with our findings.

We did not observe a difference in paternal and maternal transmission of DR4, either in our families or when we combined the previously reported studies [23, 24, 28, 38, 42, 43].

There is a report of increased transmission rates of DR4 from father than from mother to siblings of IDDM patients [23]. We could not substantiate this in our families, nor did we observe any deviations in transmission of DR4 haplotypes from either parent to siblings to IDDM patients.

We therefore conclude that the increased transmission rates of DR3 and DR4 associated haplotypes to IDDM children reflect the positive association of these haplotypes with IDDM. The lack of reduction in transmission rates to siblings of patients could be explained by the fact that additional genetic factors are required to develop IDDM.

To our knowledge there are no previous reports on the reduced transmission rates of DR2-DQB1*0602 haplotypes in families with members suffering from IDDM. The reduction seems to be mainly due to a reduced transmission by the mother and when we used published pedigrees in two studies of multiplex families [42, 43], a reduced transmission of DR2 was observed, although there was no transmission from the fathers. In all three studies, transmission of DR2 to healthy siblings did not differ from expected rates. Again, this reduced transmission of DR2-DQB1*0602 reflects the negative association of this haplotype with IDDM.

Non-inherited maternal HLA antigens (NIMA) have been suggested to play a role in development of autoimmune diseases among individuals who do not carry susceptible HLA [45]. The susceptible HLA antigen is proposed to shape the T-cell repertoire in such a way that the disease is developed if the antigen is present in the patient or if it is present on the NIMH. In our study, DR3 or DR4 was not increased among NIMH since these positively associated alleles were reduced among NIMH compared with control haplotypes. We conclude, therefore, that IDDM NIMH more often represent alleles that are protective or neutral with respect to the disease. A similar increase of negatively associated alleles among NIMH was observed for DR6 in rheumatoid arthritis [45] and for DR12 and DR8 in childhood onset myasthenia gravis [46]. These observations of negatively associated alleles being increased among NIMH are consistent with our finding. In IDDM this appears to be due to a decrease in DR4-DQA1*0301-DQB1*0302 haplotypes among patients NIMH. This effect appears specific to families with diabetes since DR3 and DR4 as NIMH in control families who are DR3 or DR4 did not differ from the remaining control group. The reduction of positively associated haplotypes in the patient NIMH is consistent with a higher transmission of positively associated haplotypes to IDDM children. Since we did not observe any difference in transmission rates between mothers and fathers, and NIPH did not differ from expected transmission rates, an alternative explanation is that NIMH play a role during pregnancy.

The possible role of NIMH in the maturation of the immune system is not known. It is speculated

that NIMH could serve as an allotypic immune stimulus. Since the diabetes risk alleles (DR4 and or DR3 haplotypes) are reduced among NIMH, the consequence is less allotypic stimulation against diabetes-associated haplotypes. This may influence the development of IDDM later in life due to a lack of allotype stimulation. In other studies, it has been shown that patients receiving multiple blood transfusions become highly sensitized and develop antibodies to almost all HLA alloantigens. However, many of these patients do not form antibodies to NIMH [47]. Subsequent repetitive antigen challenge may break this tolerance [48]. Tolerance to NIMH may in part be mediated by antiidiotypic antibodies [49]. This B-cell tolerance does not appear to be mediated by clonal deletion of T cells by exposure to the maternal cells neonatally or in utero [50]. The absence of a diabetes-associated DR4-DQA1*0301-DQB1*0302 in the NIMH during development may therefore predispose to autoimmunity by the absence of otherwise protective antiidiotypes against DR4-DQA1*0301-DQB1*0302.

In conclusion, we have observed a distortion in transmission of HLA haplotypes to our index patients, which can be explained by the association of these haplotypes with IDDM. The distorted transmission from mothers was more pronounced than from fathers, and especially NIMH differed from expected, while NIPH did not. We speculate therefore that sensitization or induction of tolerance to maternal non-inherited HLA antigens during fetal life may be important to the development of diabetes.

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