# Effect of HLA Class I and Class II Alleles on Progression From Autoantibody Positivity to Overt Type 1 Diabetes in Children With Risk-Associated Class II Genotypes

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**OBJECTIVE—**Class II alleles define the main HLA effect on type 1 diabetes, but there is an independent effect of certain class I alleles. Class II and class I molecules are differently involved in the initiation and effector phases of the immune response, suggesting that class I alleles would be important determinants in the rate of β-cell destruction. To test this hypothesis we analyzed the role of HLA class I and class II gene polymorphisms in the progression from diabetes-associated autoimmunity to clinical disease.

**RESEARCH DESIGN AND METHODS—**The effect of HLA-DR-DQ haplotypes and a panel of class I HLA-A and -B alleles on the progression from autoantibody seroconversion to clinical diabetes was studied in 249 children persistently positive for at least one biochemical diabetes-associated autoantibody in addition to islet cell autoantibody.

**RESULTS—**The progression to clinical disease was separately analyzed after the appearance of the first and the second persistent biochemical autoantibody using Cox regression. Multivariate analysis demonstrated a significant protective effect of the A*03 allele (odds ratio [OR] 0.61, $P = 0.042$ after the first and OR 0.55, $P = 0.027$ after the second autoantibody), whereas the B*39 allele had a promoting effect after seroconversion for the second autoantibody (OR 2.4, $P = 0.014$). When children with the DR3/DR4 genotype were separately analyzed, HLA-B*39 had a strong effect (OR 6.6, $P = 0.004$ and OR 7.5, $P = 0.007$, after the appearance of the first and the second autoantibody, respectively). The protective effect of A*03 was seen only among children without the DR3/DR4 combination.

**CONCLUSIONS—**These results confirm that class I alleles affect the progression of diabetes-associated autoimmunity and demonstrate interactions between class I and class II alleles.
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The HLA gene region on the short arm of chromosome 6 is the most important of the multiple gene loci affecting susceptibility to type 1 diabetes. Disease onset is preceded by the presence of circulating autoantibodies as a marker of the ongoing autoimmune process, and the duration of this period is highly variable (1). There may be differences in the genetic and environmental factors affecting the initiation of the autoimmune response and the later course of β-cell autoimmunity, conceivably leading to clinical disease. This has also been suspected for genes within the HLA region where susceptibility to type 1 diabetes is mainly defined by alleles of class II DR and DQ genes, although evidence has also accumulated for the contribution of class I alleles in the A and B loci (2–5). It has been proposed that class II genes determine the initiation of autoimmunity, whereas class I genes define the progression of β-cell damage (6). This model is theoretically supported by the roles of class II and class I molecules in the immune response. This response is initiated when CD4+ T-cells recognize antigens in the context of class II HLA molecules, whereas cytotoxic CD8+ T-cells respond to antigenic peptides presented by the class I molecules.

To further explore this hypothesis we analyzed the effect of common class II haplotypes and a panel of class I alleles on the rate of progression to clinical diabetes in a follow-up group of children with established diabetes-associated autoimmunity. This cohort of 249 children was derived from the Finnish Diabetes Prediction and Prevention (DIPP) study. All the subjects had at least one persistently positive biochemically defined autoantibody in addition to islet cell autoantibodies (ICAs); of these, 136 (54.6%) developed type 1 diabetes during the follow-up period.

## RESEARCH DESIGN AND METHODS

The newborn infants were recruited to the DIPP study in three university hospitals in Finland: Turku, Oulu, and Tampere. After initial screening for HLA-DQ–associated genetic risk, the follow-up group was sampled at 3- to 12-month intervals and serum tested for ICA. Originally, newborns positive for HLA-DQB1*0302 and without DQB1*0301 or DQB1*0602/3 alleles were selected for the study, but later those with DQB1*0302/DQB1*0603 and boys with DQA1*05-DQB1*02 without DQA1*0201, DQB1*0301, or DQB1*0602/3 were also accepted (7). If ICAs were found to be positive, all samples available from that individual were tested for biochemically defined autoantibodies, i.e., insulin autoantibodies (IAA), and antibodies to the 65 kDa isoform of GAD (GADA) and to the protein tyrosine phosphatase related IA-2 molecule (IA-2A). All ICA-positive children who tested persistently positive (at least two consecutive positive samples taken at an interval of 3 months or longer) for at least one biochemically defined autoantibody besides ICA and whose sample was available for further genotyping ($N = 249$) were selected for the study. Of them, 195 were persistently positive for two or three biochemically defined

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autoantibodies. Altogether, 201 of the children (80.7%) tested positive for IAA, 184 (73.1%) for GADA, and 176 (70.6%) for IA-2A. The lack of persistent autoantibody positivity was accepted only when the autoantibody was detected for the first time at the time of the diagnosis of diabetes because no further samples were available after the diagnosis.

The median age of the children at the time of seroconversion to positivity for the first biochemically defined autoantibody was 1.8 years (range 0.3–9.9) and 2.0 years (0.8–9.9) for the second biochemically defined autoantibody. Median follow-up time from the first biochemically defined autoantibody was 6.4 years (0.5–12.5) in the 113 children remaining nondiabetic and 2.8 years (0.0–10.8) in the 136 children progressing to type 1 diabetes. Median follow-up time from the appearance of the second biochemically defined autoantibody was 6.1 years (1.8–12.0) in the 78 children remaining unaffected and 2.5 years (0.0–5.8 years) in the 117 children progressing to type 1 diabetes. The diagnosis of type 1 diabetes was based on the World Health Organization criteria (8).

**Genotyping methods.** HLA class II typing was performed as described earlier (9,10) using a panel of lanthanide-labeled oligonucleotide probes. HLA-DQB1 alleles were analyzed as the first step and -DQA1 and -DRB1 alleles thereafter as needed for the haplotype deduction. The typing protocol defined the presence of common European HLA-DR-DQ haplotypes with special reference to those associated with diabetes risk (11). The high-risk genotypes formed by HLA-(DR3)-DQA1*05-DQB1*02 and HLA-DRB1*0401-DQB1*0302 or HLA-DRB1*0404-DQB1*0302 haplotypes were combined and named the DR3/DR4 genotype. All 249 children were successfully typed for the presence of class II haplotypes. The common HLA-A and -B alleles in the Finnish population were typed using allele-specific amplification and detection of amplification products on agarose gels (12). The defined alleles include those commonly detected in type 1 diabetes–associated HLA-DR3 and -DR4 positive haplotypes (5,13). The presence of HLA-A*01, -A*02, -A*03, -A*24, -A*28, -A*32, -B*08, -B*27, -B*35, -B*39, -B*56, -B*60, and -B*62 alleles could be defined in most cases, but the typing did not give a definite result in a subset of samples varying from 38 (15.3%) for -A*32 to 57 (22.1%) for -B*56.

**Statistical analysis.** The effect of various HLA alleles, haplotypes, and genotypes on the progression to clinical disease was tested applying Cox regression analysis. The PASW 18.0 statistical software (SPSS Inc., Chicago, IL) was used for the analyses.

## RESULTS

We started by using the Cox regression univariate analysis to test the effect of all typed class I alleles and class II haplotypes on the progression to type 1 diabetes after the appearance of the first biochemically defined autoantibody as well as after the appearance of at least two biochemically defined autoantibodies. Those class I alleles with a significant effect ($P < 0.05$) in the analysis carried out after the appearance of either one or two persistently positive biochemical autoantibodies were selected for multivariate analysis together with the HLA-DR3/DR4 genotype. This genotype showed a significant association in the univariate analysis ($P = 0.010$) with progress to disease when tested after the appearance of the first biochemical autoantibody. When the effect of the HLA-DR-DQ haplotypes was tested, (DR3)-DQA1*05-DQB1*02 was found to be associated with progression to type 1 diabetes ($P = 0.015$) and (DR1/10)-DQB1*0501 with protection against overt disease ($P = 0.03$). Similar associations were also found when these two haplotypes were associated with DRB1*0401-DQB1*0302; these haplotype associations were most probably secondary to the DR3/DR4 genotype association because of the selection criteria used for the majority of the study participants. (DR3)-DQA1*05-DQB1*02 was found in the DR3/DR4 genotype in 80 of 88 (90.9%) participants, whereas (DR1/10)-DQB1*0501 is the major "neutral" haplotype in the Finnish population, thus representing those children lacking DR3/DR4.

Table 1 shows the result of the multivariate analyses including HLA-A*03, *A24 and *B39 alleles and the DR3/DR4 genotype adjusted for the age at seroconversion for the first or second biochemical autoantibody. HLA-A*03 was associated with protection against disease in both analyses but HLA-B*39 with progression of autoimmunity only in the analysis after the appearance of the second autoantibody. HLA-DR3/DR4 had a tendency to associate with progressing autoimmunity after the appearance of the first autoantibody, but no effect was detected in the analysis after seroconversion for two autoantibodies.

TABLE 1
Cox regression analysis of HLA effects on progression to clinical diabetes in children with diabetes-associated autoantibodies

| | | Multivariate analysis |
|---|---|---|
| Follow-up from appearance of first biochemically defined autoantibody* | | |
| HLA-A*03 | 0.042 | 0.611 (0.380–0.982) |
| HLA-A*24 | 0.191 | 1.422 (0.839–2.411) |
| HLA-B*39 | 0.159 | 1.549 (0.843–2.844) |
| HLA-DR3/4 | 0.070 | 1.482 (0.968–2.269) |
| Follow-up from appearance of second biochemically defined autoantibody† | | |
| HLA-A*03 | 0.027 | 0.552 (0.326–0.933) |
| HLA-A*24 | 0.598 | 1.176 (0.644–2.146) |
| HLA-B*39 | 0.014 | 2.401 (1.196–4.823) |
| HLA-DR3/4 | 0.809 | 1.063 (0.647–1.746) |

Data are *P* values and hazard ratio for diabetes progression (95% CI). *Stratified according to age at appearance of first biochemically defined autoantibody. †Stratified according to age at appearance of second biochemically defined autoantibody. DR3/4, (DR3)-DQA1*05-DQB1*02/DRB1*0401/4-DQB1*0302.

We also performed the same analysis with class I alleles after dividing the children according to the presence of the HLA-DR3/DR4 genotype (Table 2). This revealed a strong association of HLA-B*39 with progression to diabetes when associated with the HLA-DR3/DR4 genotype, but not in children with other class II genotypes. The rate of disease development is also shown in the Kaplan-Meier curve in Fig. 1, and the number of children in follow-up at each time point is also given.

## DISCUSSION

Our results confirm the strong effect of two class I alleles on the progression of type 1 diabetes–associated autoimmunity. The possibility that other linked genes in the major histocompatibility complex block are behind these findings cannot be excluded, but the binding of autoimmune epitopes efficiently presented to cytotoxic CD8 cells by these class I alleles is an attractive hypothesis for the operative mechanism.

Although it is clearly demonstrated that class II HLA genotypes associated with type 1 diabetes are also linked to positivity for multiple autoantibodies predicting the development of clinical disease (7,14,15), their additional role in affecting the progression of β-cell autoimmunity cannot be excluded. Two studies based on the Diabetes Prevention Trial–Type 1 (DPT-1) series have set out to analyze the effect of class II HLA alleles on the progression to type 1 diabetes among autoantibody-positive first-degree relatives. Redondo et al. (16) concluded that the strong class II genotype effects observed were mainly due to differences in the initial autoantibody profiles of the study subjects. The other DPT-1 study selected only subjects with ICA and IAA with initially preserved β-cell function and reported that the DQB1*0302 allele was associated with progression while DQB1*0301 was related to protection against type 1 diabetes (17). The DPT-1

TABLE 2
Cox regression analysis of HLA class I effects on progression to clinical diabetes in children with diabetes-associated autoantibodies when categorized according to presence of DR3/DR4 class II combination

| | Multivariate analysis | | | |
|---|---|---|---|---|
| Follow-up from appearance of first biochemically defined autoantibody* | DR3/4 not present | | DR3/4 present | |
| HLA-A*03 | 0.015 | 0.490 (0.276–0.870) | 0.741 | 1.142 (0.518–2.518) |
| HLA-A*24 | 0.096 | 1.643 (0.915–2.948) | 0.142 | 0.372 (0.099–1.391) |
| HLA-B*39 | 0.660 | 1.208 (0.520–2.805) | 0.004 | 6.564 (1.801–23.926) |
| Follow-up from appearance of second biochemically defined autoantibody† | Multivariate analysis | | | |
| | DR3/4 not present | | DR3/4 present | |
| HLA-A*03 | 0.003 | 0.388 (0.208–0.724) | 0.554 | 1.310 (0.535–3.206) |
| HLA-A*24 | 0.252 | 1.455 (0.766–2.765) | 0.117 | 0.297 (0.065–1.356) |
| HLA-B*39 | 0.013 | 3.160 (1.279–7.806) | 0.007 | 7.454 (1.740–31.928) |

Data are *P* values and hazard ratio for diabetes progression (95% CI). *Stratified according to age at appearance of first biochemically defined autoantibody. †Stratified according to age at appearance of second biochemically defined autoantibody.

cohort comprised older individuals than our subjects, and the timing of seroconversion could not be defined in the DPT-1 study.

In the present survey, we were able to start the analysis from the initial detection of autoantibodies in children closely monitored from birth onward. After performing the analysis from the appearance of the second instead of the first biochemical antibody, the original tendency of the DR3/DR4 effect disappeared, indicating that the possible effect might reflect expansion of the autoimmune response to a more advanced type with more autoantibody specificities associated with a higher risk.

The strong linkage disequilibrium between alleles in class I and class II HLA loci emphasizes the importance of stratification for class II alleles when looking for class I effects. The major finding in our study—the strong effect of B*39 on the progression from β-cell autoimmunity to clinical disease—was only seen in subjects carrying the combination of both major class II risk haplotypes, the DR3/DR4 genotype. We have earlier observed HLA-B*39 to be common in type 1 diabetes–associated DRB1*0404 haplotypes in Finland (5), and we have also been able to demonstrate its predisposing effect on type 1 diabetes risk in DRB1*0404-DQB1*0302–positive subjects in Estonia and Russia (18). The presence of the HLA-B*39 allele in the DRB1*08-DQB1*0402 haplotype has also been found to be a risk factor for type 1 diabetes (3). Extensive single nucleotide polymorphism analysis has recently confirmed the independent effect of class I alleles and especially of the B*39 allele on type 1 diabetes risk (2). In this study, HLA-B*39 was also, in most cases, found in DRB1*0404-positive haplotypes, but interestingly the combination

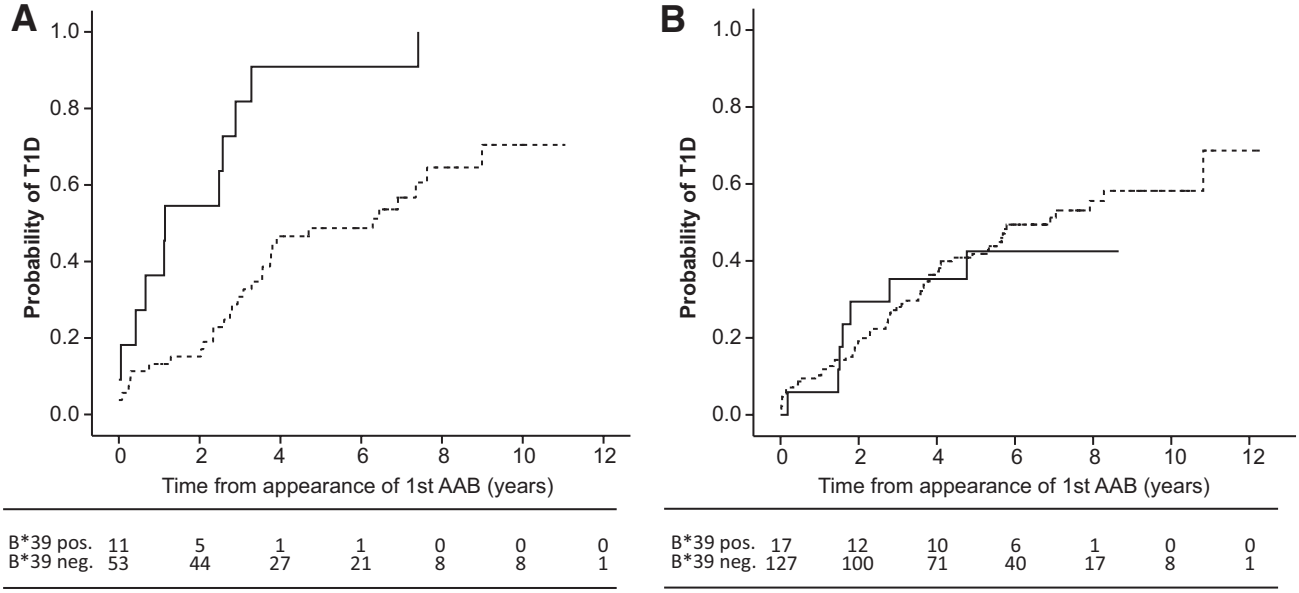


**FIG. 1. The effect of the HLA-B*39 allele on the progression to type 1 diabetes after seroconversion to persistent positivity for ICA and at least one biochemically-characterized autoantibody in children with the HLA-DR3/DR4 combination (*A*), and children with other class II genotypes (*B*). HLA-B*39–positive children indicated by solid line and B*39-negative by dashed line. Kaplan-Meier analysis demonstrated a highly significant difference between the B*39 positive ($n$ = 11) and B*39–negative ($n$ = 53) groups among children carrying the DR3/DR4 combination ($P$ = 0.00007, log-rank test), but no difference was seen between the HLA-B*39–positive ($n$ = 17) and –negative ($n$ = 127) children who did not carry the high-risk HLA class II combination ($P$ = 0.768, log-rank test). The panels below the figure show the number of subjects followed at each time point. AAB, autoantibody; T1D, type 1 diabetes.**

with DR3-positive haplotype was needed because the B*39 effect could not be seen in other genotypes with DRB1*0404. Among DR3/DR4 heterozygotes, 7 of 11 B*39-positive samples were DRB1*0404 positive. HLA-B*39 might of course also be associated with the other haplotype in these cases, but in a Finnish family trio analysis we detected B*39 in only 0.9% of 326 (DR3)-DQA1*05-DQB1*02 haplotypes transmitted to the diabetic child (unpublished data, J.I.). This difference in B*39 effect between DR3/DR4 and other genotypes may suggest some specific interactions between class II and class I.

The genetic constitution of our study population, the Finnish population in general, and especially the follow-up cohort derived through HLA DQ-based genetic screening (7) must also be taken into account when interpreting the results. Almost all children included in the current analysis carried the DR4-DQB1*0302 haplotype with either DRB1*0401 or DRB1*0404. We are thus unable to distinguish between the findings associated with either DR3/DR4 genotype or DR3 positivity. Because of the genetic screening criteria applied for the study group, we were unable to analyze the possible further effects of protective or neutral class II genotypes on the progression of β-cell autoimmunity.

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K.L. and Z.G. researched data and wrote the manuscript. M.K. researched data and contributed to discussion. H.S. reviewed the manuscript and contributed to discussion. J.L. researched data and edited the manuscript. R.H., R.V., and O.S. reviewed and edited the manuscript and contributed to discussion. M.K. researched data, reviewed the manuscript, and contributed to discussion. J.I. researched data and wrote the manuscript.

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