Brief Report

HLA-DPB1*0402 Protects Against Type 1A Diabetes Autoimmunity in the Highest Risk DR3-DQB1*0201/DR4-DQB1*0302 DAISY Population

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OBJECTIVE—A major goal in genetic studies of type 1A diabetes is prediction of anti-islet autoimmunity and subsequent diabetes in the general population, as >85% of patients do not have a first-degree relative with type 1A diabetes. Given prior association studies, we hypothesized that the strongest candidates for enhancing diabetes risk among DR3-DQB1*0201/DR4-DQB1*0302 individuals would be alleles of DP and DRB1*04 subtypes and, in particular, the absence of reportedly protective alleles DPB1*0402 and/or DRB1*0403.

RESEARCH DESIGN AND METHODS—We genotyped 457 DR3-DQB1*0201/DR4-DQB1*0302 Diabetes Autoimmunity Study of the Young (DAISY) children (358 general population and 99 siblings/offspring of type 1 diabetic patients) at the DPB1, DQB1, and DRB1 loci using linear arrays of immobilized sequence-specific oligonucleotides, with direct sequencing to differentiate DRB1*04 subtypes.

RESULTS—By survival curve analysis of DAISY children, the risk of persistently expressing anti-islet autoantibodies is ~55% for relatives (children with a parent or sibling with type 1 diabetes) in the absence of these two protective alleles vs. 0% (P = 0.02) with either protective allele, and the risk is 20 vs. 2% (P = 0.004) for general population children. Even when the population analyzed is limited to DR3-DQB1*0201/DR4-DQB1*0302 children with DRB1*0401 (the most common DRB1*04 subtype), DPB1*0402 influences development of anti-islet autoantibodies.

CONCLUSIONS—The ability to identify a major group of general population newborns with a 20% risk of anti-islet autoimmunity should enhance both studies of the environmental determinants of type 1A diabetes and the design of trials for the primary prevention of anti-islet autoimmunity. ***Diabetes*** **56: 2405–2409, 2007**

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DAISY, Diabetes Autoimmunity Study of the Young; MHC, major histocompatibility complex.

Type 1A (immune-mediated) diabetes occurs in the setting of genetic susceptibility. It has been long recognized that the polymorphisms of genes within the major histocompatibility complex (MHC) (and HLA alleles in particular) are major determinants of the disorder. Nevertheless, it is generally assumed that the positive predictive value of MHC alleles is relatively low given the complex genetics and potential multiple environmental factors hypothesized to contribute to diabetes risk. However, approximately one-half to one-third of U.S. children who develop type 1A diabetes before age 15 years have the highest risk DR/DQ genotype (HLA-DRB1*03-DQA1*0501-DQB1*0201/DRB1*04-DQA1*0301-DQB1*0302 [DR3-DQB1*0201/DR4-DQB1*0302]) (1,2). Nevertheless, the absolute risk associated with the DR3-DQB1*0201/DR4-DQB1*0302 genotype is low (~1 in 20), as 2.4% of newborns in Denver, Colorado, have this genotype (3). We are pursuing the hypothesis that additional major determinants of type 1A diabetes risk (in addition to DR and DQ alleles) are within or close to the MHC region (4).

Both HLA-DRB1*04 subtypes and DPB1 alleles have been reported to be associated with type 1A diabetes. DRB1*04 subtypes are well accepted as contributors to type 1A diabetes risk, and the DRB1*0403 subtype has been recognized as associated with protection against type 1A diabetes for more than 20 years (2,5–12). HLA-DPB1 alleles are not generally recognized as major contributors to type 1 diabetes (13). Several groups have reported a negative association of DPB1*0402 with type 1A diabetes (9,11,14–22). These studies have found that the frequency of the DPB1*0402 allele is lower in diabetic patients than in control groups (9,11,14–17,19,22,23) and that transmission of DPB1*0402 to type 1A diabetic patients is decreased (20,21). The relative risk for DPB1*0402 overall from these studies is in the range of 0.14–0.94, with a mean of 0.56 (9,11,14–22). Reported DRB1*0403 relative risk is ~0.3 (range 0.05–0.69) (2,5–12). We have carried out a combined study of risk analyzing both the presence of DRB1*0403 and/or DPB1*0402 concurrently; we are not aware of any similar combined studies. We have been following a large group of DR3-DQB1*0201/DR4-DQB1*0302 children; fixing the genotype at DQ allows us to analyze the effects of other genetic influences. A unique aspect of our analysis is that we have investigated the protective effect of DPB1*0402 in a prospective study (Diabetes Autoimmunity

Study of the Young [DAISY]), tracking the development of both anti-islet autoantibodies and type 1A diabetes in family members of type 1A diabetic patients and in the general population. In addition, for the general population, DAISY follows all children with the highest-risk DR3-DQB1*0201/DR4-DQB1*0302 genotype if families agree to participate. Thus, we were able to genotype 457 DAISY children with this high-risk DR3-DQB1*0201/DR4-DQB1*0302 genotype at the DPB1 and DRB1 loci.

RESEARCH DESIGN AND METHODS

We HLA typed ~33,000 individuals with cord blood in DAISY. Subgroups of general population DAISY children (newborn cohort [NEC]) were enrolled for prospective follow-up of development of anti-islet autoantibodies and diabetes. All children with the high-risk genotype (DR3-DQB1*0201/DR4-DQB1*0302) and a subset of those with the moderate risk (DR4-DQB1*0302/DR4-DQB1*0302, DR3-DQB1*0201/DR3-DQB1*0201, and DR4-DQB1*0302/X, where X is not DR4-DQB1*0302, DR3, or DQA1*0102-DQB1*0602) and average risk (all other) genotypes were invited to participate in the study. Relatives (sibling-offspring cohort [SOC]) were entered into follow-up independent of HLA genotypes. Insulin, GAD65, and IA-2 autoantibody levels were measured at follow-up visits at 9, 15, and 24 months of age and annually thereafter in children with normal autoantibody levels (24). Samples were obtained with informed parental consent and institutional review board oversight at the University of Colorado at Denver and Health Sciences Center (3,24). In the DR3-DQB1*0201/DR4-DQB1*0302 NEC group (general population, 358 individuals analyzed), 20 individuals have become persistently positive for anti-islet autoantibodies to date, 8 have progressed to type 1A diabetes, and 338 remain unaffected. In the DR3-DQB1*0201/DR4-DQB1*0302 SOC group (relatives, 99 individuals analyzed), 27 individuals have become persistently positive for anti-islet autoantibodies, 15 have progressed to type 1A diabetes, and 72 remain unaffected.

HLA genotyping. We performed DRB1, DQB1, and DPB1 genotyping using linear arrays of immobilized sequence-specific oligonucleotides similar to previously described methodology (25), with direct sequencing of DRB1 exon 2 to differentiate DRB1*04 subtypes.

Statistical analysis. We performed survival curve analysis for progression to persistent anti-islet autoantibody positivity or diabetes with PRISM software, using the log-rank test and an α level for significance set at 0.05.

RESULTS

In this study, we examined children from the highest risk group of DAISY, DR3-DQB1*0201/DR4-DQB1*0302 heterozygotes, as this was the largest cohort followed with a fixed DQ genotype. We performed survival curve analysis to identify the effects of both DPB1*0402 and DRB1*0403 on progression to anti-islet autoantibody positivity (Fig. 1*A* and *C*) and diabetes (Fig. 1*B* and *D*). The NEC children (general population cohort) with either DRB1*0403 or DPB1*0402 had, by survival curve analysis, a 2 ± 2% (SEM) risk of developing persistent anti-islet autoantibodies by age 12 years (Fig. 1*A*) and a 0% risk for developing diabetes (Fig. 1*B*). In contrast, in NEC children with neither DRB1*0403 nor DPB1*0402, 19 ± 5% expressed anti-islet autoantibodies by age 12 years ($P = 0.004$, two tailed) (Fig. 1*A*), whereas 12 ± 2% developed diabetes ($P = 0.03$) (Fig. 1*B*).

The SOC children (relatives of an individual with type 1A diabetes) with either the DRB1*0403 or DPB1*0402 alleles had a 0% risk of developing persistent anti-islet autoantibodies by age 15 years (Fig. 1*C*) and also a 0% risk of developing diabetes (Fig. 1*D*). In SOC children with neither DRB1*0403 nor DPB1*0402, 37 ± 6% expressed anti-islet autoantibodies by age 10 years—56 ± 14% by age 15 years ($P = 0.02$) (Fig. 1*C*)—with 30 ± 7% developing diabetes by age 10 years ($P = 0.1$) (Fig. 1*D*).

We analyzed the effect of DPB1*0402 independently, revealing a similar trend. NEC children with DPB1*0402 had a 3 ± 2% risk of developing anti-islet autoantibodies by age 12 years (Fig. 2*A*), with 0% of children developing diabetes (Fig. 2*B*). Comparatively, NEC children without DPB1*0402 had an 18 ± 5% risk for developing anti-islet autoantibodies by age 12 years ($P = 0.02$) (Fig. 2*A*) and an 11 ± 6% risk of developing diabetes ($P = 0.08$) (Fig. 2*B*). In the presence of the DPB1*0402 allele, SOC children had a low risk for developing anti-islet autoantibodies (0% by age 15 years). However, in the absence of DPB1*0402, SOC children had a 35 ± 6% risk by age 10 years—49 ± 11% by age 15 years ($P = 0.09$) (Fig. 2*C*)—but as expected, there were few relatives with the DPB1*0402 allele.

When we limited the analysis to NEC children with the most common DRB1*04 subtype, DRB1*0401 (Fig. 3*A* and *B*), and performed survival curve analysis, children with DPB1*0402 had a lower risk (0% by age 12 years) of developing anti-islet autoantibodies than children without DPB1*0402 (22 ± 8% by age 10 years, 29 ± 10% by age 12 years) ($P = 0.06$) (Fig. 3*A*).

DISCUSSION

Given prior data demonstrating protection by these alleles, we evaluated a single specific hypothesis in this study: that the presence of either of these protective alleles would decrease development of anti-islet autoimmunity. These alleles are associated with "protection" in both the general population ($P = 0.004$) and relatives of patients with type 1A diabetes ($P = 0.02$). As would be expected, development of each of the autoantibodies analyzed separately is markedly decreased (combined NEC and SOC analysis: insulin autoantibodies 11.9–2.4% ($P = 0.02$), GAD 10.7–2.4% ($P = 0.03$), and IA-2 14.4–2.4% ($P = 0.01$) in the presence of DPB1*0402.

Thus far, 31% of DR3-DQB1*0201/DR4-DQB1*0302 relatives (with neither DPB1*0402 nor DRB1*0403) and 8% of the general population cohort have developed persistent anti-islet autoantibodies. To date, 56% (15 of 27) of the autoantibody-positive relatives have progressed to diabetes. Of the 12 autoantibody-positive relatives who have not yet developed diabetes, 6 (50%) express two or more biochemical autoantibodies (an indicator of a high risk of progression). Of the autoantibody-positive general population children, 42% (8 of 19) have already progressed to diabetes and 64% (7 of 11) of the remaining autoantibody-positive children express two or more biochemical autoantibodies.

As seen in Fig. 1*A* and *B*, the difference in risk of developing persistent anti-islet autoantibodies between relatives and the general population (DR3-DQB1*0201/DR4-DQB1*0302, neither DPB1*0402 nor DRB1*0403) ($P < 0.0001$, hazard ratio [HR] 3.4, by survival curve analysis) indicates that other unidentified factors (genetic and/or environmental) contribute to the development of anti-islet autoimmunity in this HLA-identified high-risk group. In addition, siblings have a higher risk than offspring, as DR3-DQB1*0201/DR4-DQB1*0302 (neither DPB1*0402 nor DRB1*0403) siblings have a 49% risk of developing persistent anti-islet autoantibodies by age 10 years, while offspring have only a 27% risk ($P = 0.01$, HR 2.8) by survival curve analysis (data not shown).

The risk for the general population to express anti-islet autoimmunity, given high-risk alleles of HLA-DR, -DQ, and -DP (DR3-DQB1*0201/DR4-DQB1*0302, neither DRB1*0403 nor DPB1*0402), is impressive at 19 ± 5% (Fig. 1*A*). This genetic subgroup represents 231 of 1,243 (19%) of all general population children in follow-up, 19 of 38 (50%) of all case

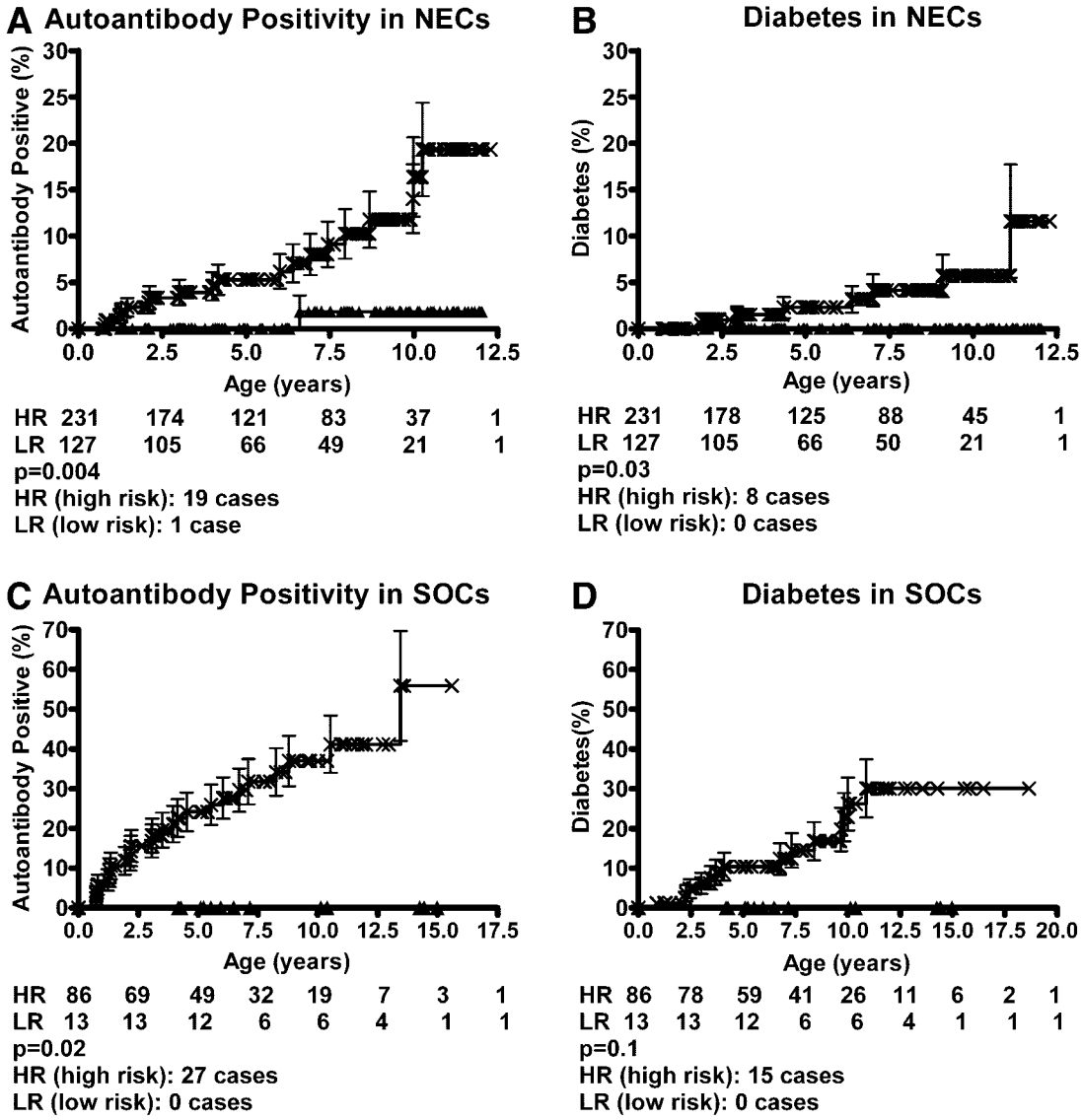

FIG. 1. Protective effects of DPB1*0402 and DRB1*0403 in DR3-DQB1*0201/DR4-DQB1*0302 NECs and SOCs. *A*: Anti-islet autoantibody development in NECs (newborn/general population cohort). *B*: Progression to diabetes in NECs. *C*: Anti-islet autoantibody development in SOCs (sibling/offspring cohort). *D*: Progression to diabetes in SOCs. ×, no protective alleles (no DPB1*0402 or DRB1*0403); ▲, DRB1*0403 and/or DPB1*0402.

subjects who have developed persistent autoantibodies in the general population cohort of DAISY, and 8 of 12 (67%) of those who have progressed to diabetes. It should be noted that the general population cohort of DAISY was selected based on HLA type (see RESEARCH DESIGN AND METHODS). For high-risk relatives, the risk is 56 ± 14% after fixing specific alleles of HLA-DR, -DQ, and -DP (Fig. 1*C*). This subgroup represents 86 of 971 (9%) of relatives followed but 27 of 69 (39%) of SOC autoantibody-positive children and 15 of 36 (42%) of all those who have progressed to diabetes. Though DAISY has HLA typed >33,000 individuals, the number entered into follow-up with the highest risk genotype is much smaller ($n = 457$) and those who have developed autoimmunity is an even smaller subset. We believe that with a larger series or longer follow-up, some SOCs and NECs with the protective DPB1*0402 or DRB1*0403 alleles will progress to diabetes.

No children with DRB1*0403 and only 1 child (of 140) with DPB1*0402 has developed persistent anti-islet autoantibodies. This child has expressed anti-islet autoantibodies on 10 consecutive occasions (first autoantibody positive at age 6.6 years), currently expresses all three biochemical autoantibodies (age 9.6 years), and we believe is at high risk for progression to diabetes.

From the current study, we cannot be sure that DRB1*0403 and/or DPB1*0402 are the true causative genetic polymorphisms related to decreased risk. As with all studies of the MHC and its multiple immunologically important genes, these alleles may be in linkage disequilibrium with other causative variants.

We believe there might be polymorphisms of other genes linked to or within the MHC, in addition to HLA-DP and -DR, that will allow even greater risk to be identified for general population children. Further high-density analysis of thousands of single nucleotide polymorphisms within and surrounding the MHC may allow definition of additional polymorphisms associated with high risk. In addition, genes outside the MHC can have a measurable influence, and we believe the genetic prediction of type 1A diabetes is likely to advance dramatically in terms of both

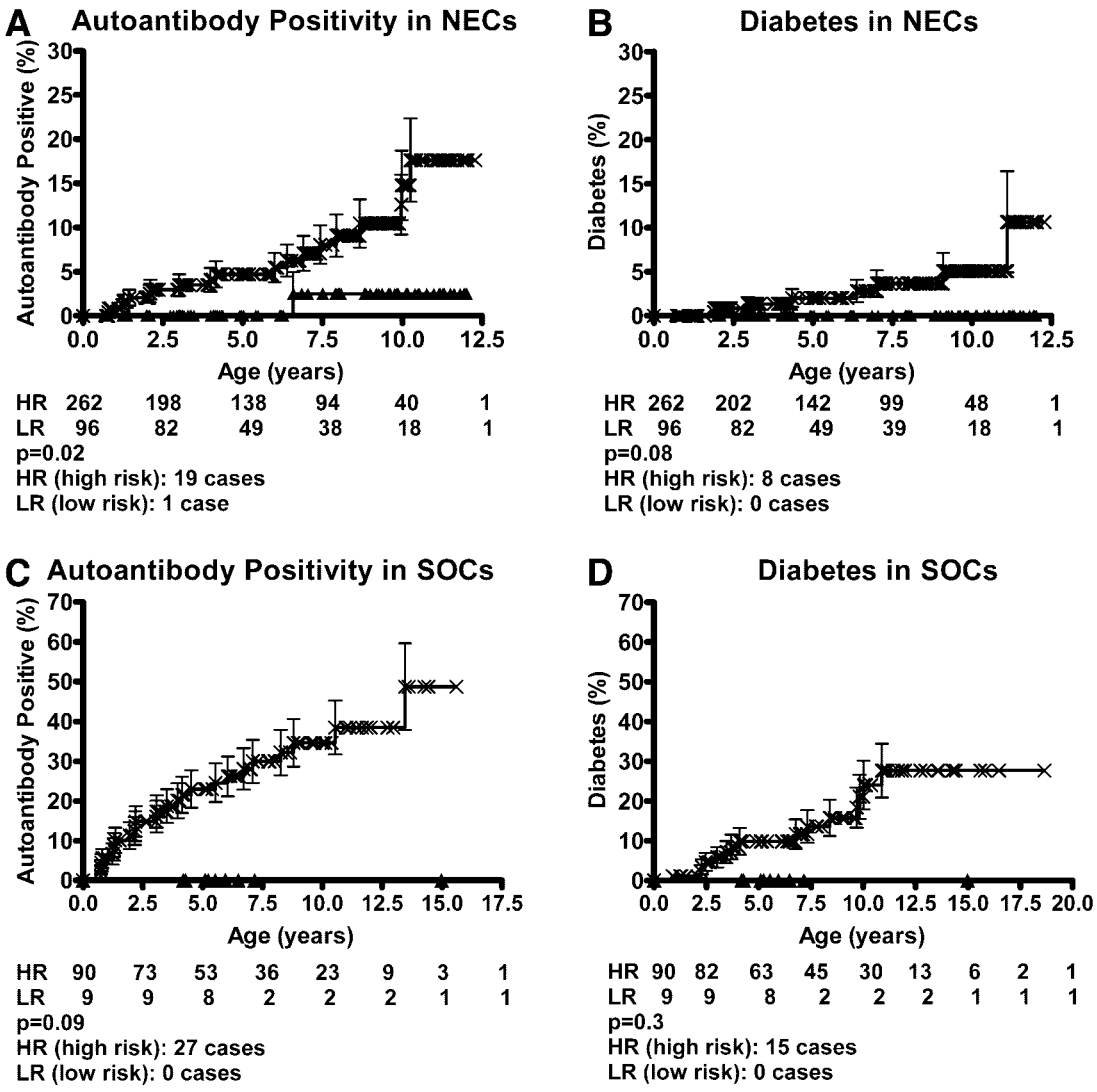

FIG. 2. Protective effects of DPB1*0402 alone in DR3-DQB1*0201/DR4-DQB1*0302 NECs and SOCs. *A*: Anti-islet autoantibody development in NECs (newborn/general population cohort). *B*: Progression to diabetes in NECs. *C*: Anti-islet autoantibody development in SOCs (sibling/offspring cohort). *D*: Progression to diabetes in SOCs. ×, no protective allele (no DPB1*0402); ▲, DPB1*0402.

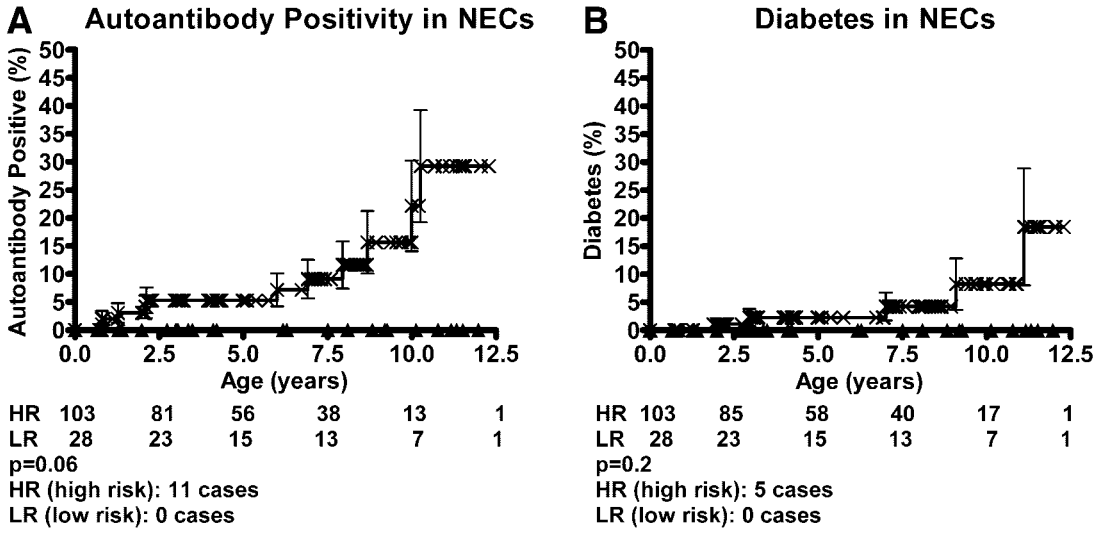

FIG. 3. Protective effects of DPB1*0402 in DR3-DQB1*0201/DR4-DQB1*0302 NECs with the DRB1*0401 allele. *A*: Anti-islet autoantibody development. *B*: Progression to diabetes. ×, no protective allele (no DPB1*0402); ▲, DPB1*0402.

sensitivity and specificity in the near future. Advances in genetic prediction should greatly facilitate the search for environmental factors contributing to type 1A diabetes risk. Given the risk for development of anti-islet autoantibodies associated with the DR3-DQB1*0201/DR4-DQB1*0302 genotype, we believe such environmental factors (increasing or decreasing risk) are likely to be ubiquitous, with most of the population exposed. By identifying extreme genetic risk, targeted studies will hopefully aid in elucidation of such factors and allow trials for type 1A diabetes prevention for individuals with specific genetic susceptibility.

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