Type 1 Diabetes Environmental Factors and Correspondence Analysis of HLA Class II Genes in the Yemenite Jewish Community in Israel

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OBJECTIVE — The Israeli Yemenite Jewish community has displayed an exceptionally rapid increase in the frequency of type 1 diabetes, having the highest rate of all Israeli ethnic groups. We studied the role of the environment, in relation to the nature and frequency of HLA class II genes, to evaluate its possible involvement in the development of diabetes.

RESEARCH DESIGN AND METHODS — We interviewed 196 elderly Yemenite women, who had immigrated to Israel as adults, in programmed encounters about signs and symptoms of type 1 diabetes, infant feeding customs, and infectious diseases in Yemen. We also performed HLA oligotyping of DRB1, DQA1, and DQB1 genes in 120 unrelated Yemenite Jews, including 44 type 1 diabetic patients and 76 healthy control subjects, and used these data in correspondence analysis comparing Yemenites with different Israeli ethnic groups.

RESULTS — Interviews indicated that early exposure to cow's milk was very common in Yemen. However, none of the women could recall classical presentations of diabetes. HLA oligotyping showed that gene frequencies of non-Asp-57 (of the HLA-DQB chain) in the patients (0.94) and control subjects (0.6) were similar to those of other populations with a known high incidence of type 1 diabetes. Correspondence analysis revealed that Yemenite Jews are genetically distinct from other ethnic groups in Israel.

CONCLUSIONS — The genetic distinctiveness of Yemenite Jews may explain their unusually high incidence of type 1 diabetes in Israel. Despite the presence of highly susceptible diabetogenic HLA class II genes in this community, early exposure to cow's milk did not cause phenotypic expression of diabetes in Yemen. This finding suggests that in this population, either cow's milk does not play a crucial role in triggering diabetes, or environmentally conferred protection, such as frequent infectious disease in Yemen, was dominant.

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The incidence of type 1 diabetes exhibits a marked geographic variation worldwide, probably resulting from different distributions of susceptible type 1 diabetes genes (1,2). The development of the disease has been associated with polymorphism within the peptide binding sites of the class II molecules HLA-DQ and -DR in the HLA immune response region of the short arm of chromosome 6 (3,4). The increasing interaction of these susceptible genes with the environment, leading to autoimmune β-cell destruction, may account for the steady rise in the frequency of type 1 diabetes in the last decades (5). In particular, early exposure to cow's milk has been linked by many investigators to initiation of β-cell autoimmunity in individuals at high genetic risk; however, the contribution of early exposure to the pathogenesis of type 1 diabetes is still controversial (6).

Israel, too, has witnessed a general increase in the incidence of type 1 diabetes over the last 15 years. There is, however, a notable variation among Israel's different ethnic groups, which has been attributed to differences in genetic or environmental factors (7). Among these groups, the Yemenites are unique, having the highest incidence of type 1 diabetes (18.5/100,000) compared with the overall Jewish population (5.7/100,000) (8).

The Yemenite Jews immigrated to Israel en masse between 1948 and 1951 from a distant Moslem country that was not only underdeveloped but also voluntarily isolated from the rest of the world (9). Yemen's Jews were doubly isolated—from their Moslem neighbors within the country and from the rest of the Jewish diaspora. However, during the same period, thousands of other immigrants arrived in Israel from all over the world, and all were exposed to a similar environment. The unusual rise in the incidence of type 1 diabetes among the Yemenites indicates that they may differ in genetic background from other Jews.

We have recently shown an exceptionally high odds ratio for the universally susceptible HLA diabetogenic genotype DRB1*03011,*0402 and its related DQ alleles in Yemenite Jews with type 1 diabetes compared with nondiabetic Yemenite control subjects (10). In the present study, we further analyzed our

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A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Distribution of HLA-DQB genotypes and non-Asp-57 gene frequencies among Yemenite Jewish diabetic patients and unrelated control subjects*

Allele	*n* (%) of patients	*n* (%) of control subjects	Fisher's exact test (*P*)	Odds ratio	Odds ratio 95% CI
N/N	39 (88)	23 (36)	3.1×10^{-10}	18	6.3–51.4
N/A	5 (11)	45 (59)	1.4×10^{-7}	0.09	0.03–0.25
A/A	0	8 (10)	0.026	0.09	*
NAa	83 (94)	91 (60)	8.2×10^{-10}	11.1	4.3–29

N/N, non-Asp-57 homozygosity; N/A, non-Asp-57 heterozygosity; A/A, Asp-57 homozygosity; NAa, non-Asp-57 alleles. *Biased due to reliance on zero-cell adjustment in computing the odds ratio.

immunogenetic results in relation to various ethnic groups in Israel. In addition, we added structured interviews with elderly Yemenites who immigrated to Israel as adults in order to identify environmental factors that may explain the dramatic change in the disease expression before and after immigration.

RESEARCH DESIGN AND METHODS

Community inquiries

In this study, 196 elderly Yemenite women who immigrated to Israel as adults during the early 1950s were questioned in programmed interviews. Particular attention was paid to medical descriptions suggestive of type 1 diabetes in children and adolescents of the community (i.e., polyuria, polydipsia, and weight loss), other common childhood diseases, and vaccinations in Yemen. Information on neonatal and infant feeding customs in Yemen was also obtained, especially regarding exposure to cow's milk.

Molecular HLA class II analysis

Molecular HLA Class II analysis was performed in 120 unrelated individuals of unmixed Yemenite ancestry, including 44 patients with type 1 diabetes (20 males) and 76 healthy control subjects (47 males) according to the 11th International Histocompatibility Workshop protocol and as described previously (10,11). The relationship between the frequency of the non-Asp-57 gene in patients and control subjects and the incidence of type 1 diabetes was calculated with the method of Dorman et al. (2).

Correspondence analysis

Correspondence analysis is a statistical and graphic method for comparing the association among categorical variables (12), and can be used to examine the relationship between ethnic groups and alleles (13). For the present study, we assumed the independence of an assortment of alleles and performed the analysis on the basis of alleles rather than individuals. We used population-based multiple correspondence analysis (a variant of the correspondence analysis method) of distributions of allele families—that is, the major genetic description of the allele (e.g., *DRB1*0402*, *DRB1*0404*, and *DRB1*0405* are all members of the *DRB1*04* family). We mapped the ethnic groups for multiple dimensions in terms of their similarities in allelic distribution. We compared type 1 diabetic subjects from various Jewish and Arab communities in Israel based on the results of their HLA genotyping, including 44 Yemenites, 72 Ashkenazi, 33 Moroccans, and 36 Israeli Arabs.

The study protocol was approved by the Ethics Committee of Rabin Medical Center, and informed consent was obtained from all participants.

Statistical analysis

Fisher's exact tests were used to evaluate the statistical significance of the association between type 1 diabetes and Asp-57 alleles. Odds ratios for the development of type 1 diabetes and their 95% CI were determined when significant associations were found. In cases where correction was required for zero values, a value of 0.5 was used (as per the SAS software standard). For data analysis, we used JMP version 3.2.2 and SAS 6.09 for Unix (both products of the SAS Institute, Cary, NC) and Excel 7.0 (Microsoft, Redmond, WA).

RESULTS

Community inquiries

The 196 elderly Yemenites questioned gave detailed descriptions of common childhood diseases, such as pneumonia, gastroenteritis, and febrile convulsions. None were able to recall clinical symptoms characteristic of type 1 diabetes in children or adolescents in the community in Yemen. In addition, no vaccination program against common childhood diseases was reported in Yemen. All the elderly women described the traditional early introduction of creamy cow's milk to neonates and infants in Yemen as a supplement to breast-feeding during the first weeks of life.

Molecular class II analysis

Results of this type analysis are shown in Table 1. Homozygosity for DQB1 Asp-57-positive was present only in the healthy control subjects and was significantly decreased among the patients. By contrast, we found a significant increase in non-Asp-57 homozygosity among the patients. The gene frequencies of non-Asp-57 in the patients (0.94) and control group (0.6) were similar to those found in other populations with a known high incidence of type 1 diabetes, such as Sardinians and Scandinavians. Plotting the obtained frequencies over the figure of Dorman et al. (2) yielded an expected incidence of ~22/100,000 (Fig. 1), which is slightly higher than the latest reported incidence of 18/100,000 (8).

Correspondence analysis

As depicted in Fig. 2, the multiple correspondence analysis showed the relationships of Yemenite, Ashkenazi, and North African Jews and Israeli Arabs in terms of DRB1, DQB1, and DQA1 families of alleles. Our preliminary results suggest that Yemenite Jews are genetically distinct from the other populations studied, including Israeli Arabs.

CONCLUSIONS — As we have previously reported (10), the Yemenite Jews carry highly susceptible HLA class II genes for the development of type 1 diabetes. However, the expression of these genes differs dramatically from before their immigration to Israel. Further analysis of HLA typing shows a high non-Asp-57 DQB1 gene frequency in both patients and control subjects, placing the Yemenites near the top of the list of susceptible populations, between Sardinians and Norwegians (2). It should be noted, though, that non-Asp-57 in the control subjects was linked with DRB1*0701,

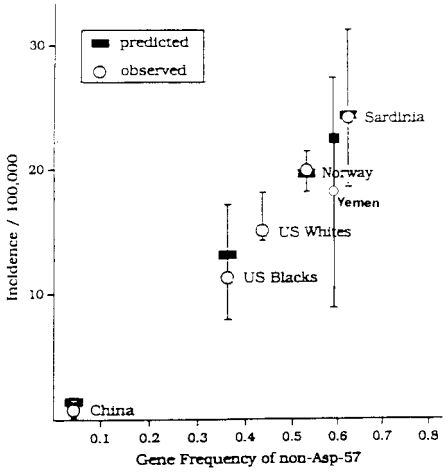

Figure 1—*Relationship of non-Asp-57 alleles in various ethnic populations to incidence of diabetes. Predicted incidences are based on U.S. whites as a point estimate. Observed incidences are provided with the 95% CI. This figure is adapted from Dorman et al. (2) with the kind permission of Prof. J.S. Dorman, with the addition of data for our Yemenite group.*

DQA1*0201, and DQB1*O2, a haplotype suspected to be associated with resistance to type 1 diabetes in this community (10). Thus, the nature of the association between non-Asp-57 and type 1 diabetes development in Israeli Jews of Yemenite origin remains unclear; larger numbers of diabetic patients, their healthy relatives, and control subjects need to be tested.

Considering the high frequency of type 1 diabetes susceptible genes among Yemenite Jews and the continuing increase in the incidence of the disease in this community, we speculate that if environmental conditions—such as low rate of mixed marriages and few effective preventive manipulations—remain unchanged, an additional increase in disease prevalence is to be expected, equal to or exceeding that observed in Sardinia and Finland. Indeed, the latest report of Israel's National Registry (1997–1998) indicates an incidence rate of 22/100,000 (Israel Type I Diabetes Registration Group, unpublished observation). This is compatible with the expected incidence predicted by Fig. 1 according to the frequency of the non-Asp-57 alleles in this population.

Unexpectedly, our community inquiry revealed an absence of type 1 diabetes in children of this community in Yemen, despite the traditional early introduction of cow's milk to neonates. This lack of β-cell autoimmune destruction could indicate either no triggering effect of cow's milk or an interference with protective effects possibly induced by frequent infections and the absence of a vaccination program in Yemen (14). The influence of infection has also been demonstrated in two animal models of type 1 diabetes—NOD mice and BB rats—in which differences in nutrition and housing conditions led to great differences in the incidence of diabetes (15–17).

At the same time, we cannot ignore the dramatic change in body size in the Yemenite community in Israel over the last 20 years, from an average weight and height in adult males of 36 kg and 160 cm, respectively, in 1950 to 63 kg and 171 cm, respectively, in 1971 (18). The relationship between body mass and β-cell autoimmunity is supported by the recently reported association of islet autoimmunity and β-cell function abnormality with obesity and insulin resistance (19).

The results of molecular HLA class II typing of the Yemenite Jews differ substantially from those obtained in other Jewish communities in Israel (S.I., O.J.K., N.W., E.S., P.V., C.B., unpublished observation). Using these results for correspondence analysis (Fig. 2), our preliminary study shows that type 1 diabetic Jews of Yemenite origin are distinct by class II genes from every other ethnic group we investigated. We used the allele family–based taxonomy in our analytic approach. Based on the assumption that allelic subclasses developed later from major alleles, this method allowed us to investigate different degrees of genetic distinctiveness. In this analysis, Yemenite Jews remained genetically distinct, suggesting a divergence from the other populations in the distant past. Although similar models have been previously constructed on the basis of genetic analysis of healthy subjects, we assumed that the major histocompatibility complex region, being only one of several genes responsible for type 1 diabetes, and one that in the past has not associated with the disease, could represent a population genomic inheritance (13). Our results support the finding of Bonne-Tamir et al. (20) that Yemenite Jews differ by gene clustering from all other Jews. The unique genetic background of this ethnic group is supported by its unusually high incidence of autosomal dominant benign neutropenia (21), α-thalassemia with specific deletion in the α-globin gene (22), and phenylketonuria with specific mutation in the phenylalanine hydroxylase gene (23), as well as an exceptionally low incidence of familial Mediterranean fever (24), glucose-6-phosphate dehydrogenase deficiency (25), and cystic fibrosis (26).

In summary, the extraordinarily high frequency of genes associated with type 1 diabetes in Yemenite Jews living in Israel might explain the high incidence of the disease in this community; however, the different expression of the genetic potential during a short period of time suggests

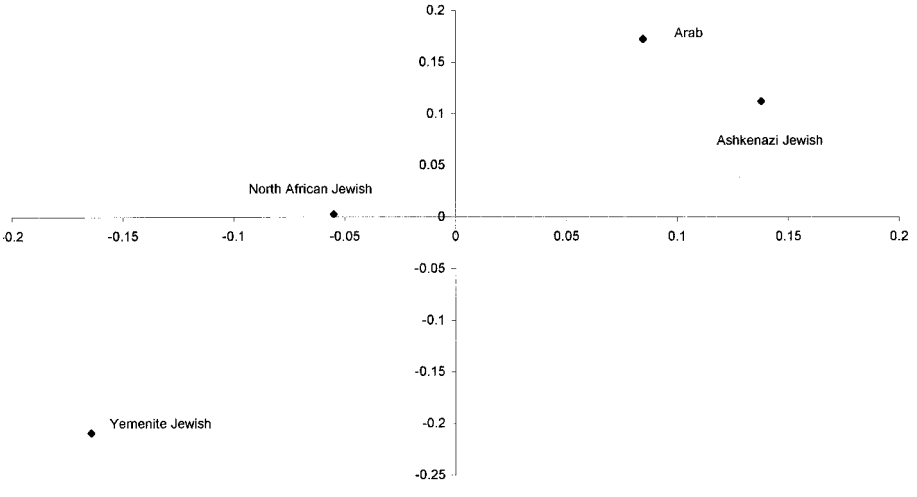

Figure 2—*Relationships of the genetic inheritance patterns of the various diabetic groups, as shown by multiple correspondence analysis of DRB, DQA, and DQB allele family taxonomy. Axes represent degree of relationship obtained from the analyses, such that proximity and similar location indicate closeness.*

an acute involvement of environmental factors. As the early introduction of cow's milk to neonates in this community in Yemen did not induce β-cell autoimmunity, different etiologic factors should be sought.

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