Signs of β-Cell Autoimmunity in Nondiabetic Schoolchildren

A comparison between Russian Karelia with a low incidence of type 1 diabetes and Finland with a high incidence rate

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OBJECTIVE — We sought to study the prevalence of autoantibodies to various islet cell antigens in the background population of two neighboring countries with a sixfold difference in the incidence of type 1 diabetes.

RESEARCH DESIGN AND METHODS — Serum samples were obtained from 3,652 nondiabetic schoolchildren in Finland and from 1,988 schoolchildren in the adjacent Karelian Republic of Russia. The Karelian children were divided into three groups (Finns/Karelians, Russians, and others) based on the ethnic background of their mother. The samples were analyzed for islet cell antibodies (ICAs), insulin autoantibodies (IAAs), GAD antibodies (GADAs), and the tyrosine phosphatase-like insulinoma antigen 2 (IA-2A) protein and HLA class II genotypes.

RESULTS — The frequency of ICAs, IAAs, and GADAs did not differ significantly between the Karelian (3.5, 0.6, and 0.9%, respectively) and Finnish children (2.8, 0.9, and 0.5%, respectively). Similarly, the frequency of multiple (≥2) autoantibodies was similar in both countries (0.5 vs. 0.6%). The frequency of IA-2A was, however, four times higher in Finland (0.6 vs. 0.15% in Russian Karelia; $P = 0.03$). There were no significant differences in autoantibody prevalence among the three ethnic groups in Russian Karelia. There was a falling frequency of GADAs and of positivity for multiple autoantibodies along with decreasing HLA-conferred disease susceptibility among the Finnish schoolchildren.

CONCLUSIONS — These data indicate that β-cell autoimmunity among schoolchildren is as frequent in Russian Karelia as in Finland, although the incidence of clinical type 1 diabetes is six times higher in Finland. However, in contrast to this general trend, IA-2As were more common in Finland. Since IA-2As usually appear late in the preclinical process, this suggests that progressive β-cell autoimmunity is more rare in Russian Karelia.

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Abbreviations: DASP, Diabetes Autoantibody Standardization Program; GADA, GAD antibody; IAA, insulin autoantibody; IA-2A, tyrosine phosphatase-like insulinoma antigen 2; ICA, islet cell antibody; JDFU, Juvenile Diabetes Foundation units.

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

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Insulin-producing β-cells are destroyed by an immune-mediated process in type 1 diabetes (1,2). Clinical disease is preceded by a subclinical period, which is characterized by the emergence of circulating disease-associated autoantibodies. These autoantibodies include islet cell autoantibodies (ICAs) and insulin autoantibodies (IAAs), as well as the 65-kDa isoform of GAD antibodies (GADAs) and the tyrosine phosphatase-like insulinoma antigen 2 (IA-2A) molecule (3,4). The preclinical phase of type 1 diabetes may even last for >10 years. The emergence of multiple autoantibodies and particularly the appearance of IA-2As are associated with a progressive disease process and predict the development of overt type 1 diabetes (5).

Both environmental and genetic factors play a role in the pathogenesis of type 1 diabetes, and it is generally believed that environmental factors are needed to trigger and drive the disease process in genetically susceptible individuals (6–8). We have previously evaluated the interactions between environmental and genetic factors by comparing the incidence of type 1 diabetes in two populations that live close to each other in two neighboring countries: Finland and the Karelian Republic of Russia. These two populations share the same geophysical environment but differ remarkably in their standard of living. This border represents one of the most conspicuous socioeconomic gaps in the world with the mean gross national product ~33,000 U.S. dollars per capita in Finland compared with ~3,400 U.S. dollars per capita in Russian Karelia (9). We have recently reported (10) that the incidence of type 1 diabetes is about six times higher in Finland than in Russian Karelia. In the present study, we further evaluated the background of this intriguing difference in incidence rates by analyzing the frequency of subclinical β-cell autoimmunity, i.e., the prevalence of diabetes-associated autoantibodies, and HLA-risk genotypes in the background population in these two countries. To assess the effect of the ethnic background, these

analyses were carried out in Russian Karelia from separate children of Finnish-Karelian, Russian, or other ancestry.

RESEARCH DESIGN AND METHODS

— Study subjects were recruited among schoolchildren in both countries. In Russian Karelia, serum samples were collected from 1,988 randomly selected schoolchildren in different regions from 1997 to 2001 (age range 6.2–18.3 years; mean age ± SD 11.6 ± 2.4 years). The series included 1,004 girls (50.5%) and 984 boys (49.5%). The prevalence of autoantibodies in Russian Karelia schoolchildren was estimated separately in three different ethnic groups: those of Finnish-Karelian ancestry (906), those of Russian ancestry (815), and others (267) based on questionnaire data. Ethnic group was defined according to the mother's ethnicity, since this criterion was used in our previous study (10) on the incidence of type 1 diabetes in Russian Karelia. In Finland, serum samples were collected in 1994 from 3,652 nondiabetic schoolchildren living in five communities (Haapajärvi, Ii, Oulainen, Yli-Ii, and ja Yli-Kiiminki) in the province of Northern Ostrobothnia (age range 7.0–18.0 years; mean 11.7 ± 2.6 years) including 1,867 girls (51.1%) and 1,785 boys (48.9%). The data on autoantibody frequencies in Finnish schoolchildren has been previously reported (11). All children obtained parental consent to take part in the study. The study was approved by the Ministry of Health in Russian Karelia and by the ethics committee of the medical faculty, University of Oulu in Finland. All serum samples were stored at −20°C until analyzed.

Diabetes-associated autoantibodies

All autoantibody measurements were carried out in the Research Laboratory, Department of Pediatrics, University of Oulu. Islet cell antibodies (ICAs) were measured with a standard immunofluorescence method using sections of frozen human group O pancreas. End point titers of each sample were converted to Juvenile Diabetes Foundation units (JDFU) relative to an international reference standard. The detection limit was 2.5 JDFU. The assay achieved 100% sensitivity and 98% specificity in the most relevant workshop for the standardization of the ICA assay (12). All samples initially positive for ICAs were retested for confirmation. IAAs were analyzed using a microassay modified from that described by Williams et al. (13). The limit for IAA positivity (1.56 relative units) was set at the 99th percentile in a separate series of 374 nondiabetic Finnish children and adolescents. This assay had a disease sensitivity of 58% and a specificity of 98% in the 2005 Diabetes Autoantibody Standardization Program (DASP) workshop.

Specific radioligand assays were used to quantify GADA and IA-2A levels as described (14,15). The cutoff limit for antibody positivity, which was set at the 99th percentile in >370 nondiabetic children and adolescents, was 5.36 relative units for GADA (n = 373) and 0.43 relative units for IA-2A (n = 374). The disease sensitivity of the GADA assay was 82% and the specificity 96%; corresponding characteristics of the IA-2A assay were 72 and 100%, respectively, in the 2005 DASP workshop.

All samples with IAA, GADA, and IA-2A levels between the 97th and 99.5th percentiles were reanalyzed to confirm their status. Multiple autoantibody positivity was defined as positivity for at least two antibodies of ICA, IAA, GADA, and IA-2A.

Both the Russian Karelian and Finnish samples were analyzed for IAA and IA-2A at the same time and in the same assay runs. Finnish samples were assayed for ICA and GADA 2 years earlier than the Russian Karelian samples. The same pancreas was used, however, as the substrate for both the Finnish and Russian Karelian samples, and the standard curve for the transformation of the ICA titers into JDFU remained unchanged when retested before starting the analysis of the Russian Karelian samples. The performance characteristics, specificity in particular, of our GADA assay has remained very stable over the last 3 years according to the DASP workshops. The disease sensitivity of the GADA assay was 80% and the disease specificity 96% in 2003, with corresponding values of 82 and 96%, respectively, in 2005. The possible drift-over time of the assays for molecular autoantibodies is continuously monitored based on both the standard curves and internal quality control samples run on each plate.

HLA analyses

HLA diabetes-related class II alleles were typed by PCR and microtiter well plate–based hybridization with lanthanide-labeled oligonucleotide probes as described (16). Samples from 1,977 schoolchildren (99%) from Russian Karelia and from 3,649 Finnish schoolchildren (99.9%) were available for genotyping. The HLA genotypes were categorized into four risk groups for type 1 diabetes: *1*) high risk (DQA1*05-DQB*02/*0302); *2*) moderate risk (DQB1*0302/x; x ≠ DQA1*05-DQB1*02, DQB1*0301, DQB1*0602 or DQB1*0603); *3*) low risk (DQA1*05-DQB1*02/y, DQA1*03-DQB1*02/y, DQB1*0301/*0302, DQB1*0302/*0603; y ≠ DQA1*0201-DQB1*02, DQB1*0302, DQB1*0301, DQB1*0602 or DQB1*0603); and *4*) decreased risk (all other genotypes).

Statistical analysis

χ^2 test and Fisher's exact test were used to compare the frequency of autoantibodies, and Mann-Whitney U test was used in the analysis of nonparametric data (SPSS version 10.1; SPSS, Chicago, IL). A P value <0.05 was considered statistically significant. P values were not corrected for the number of comparisons. The 95% CIs were calculated with the exact method.

RESULTS

— Altogether, 90 of the 1,988 (4.5%) nondiabetic schoolchildren in Russian Karelia (95% CI 3.7–5.5) tested positive for at least one of the four autoantibodies analyzed compared with 141 of the 3,652 (3.9%) schoolchildren in Finland (3.3–4.5). Eighty-one (4.1%) children (3.3–5.0) in Russian Karelia were positive for a single autoantibody and nine (0.5%) children (0.2–0.9) for multiple autoantibodies (Fig. 1). The corresponding figures in Finland were 120 (3.3%) children (2.7–3.9) with a single autoantibody and 21 (0.6%) children (0.4–0.9) with multiple autoantibodies showing no significant difference between the countries. The individual frequencies of ICAs, IAAs, and GADAs did not differ significantly between the countries, but IA-2As were four times more frequent in Finland (0.57 vs. 0.15%; P = 0.03). The frequency of single autoantibody specificities did not differ between the three ethnic groups (Finnish-Karelian, Russian, or other ancestry) in Russian Karelia (Table 1). The proportion of children positive for multiple autoantibodies tended to be higher among those of Finnish-Karelian ancestry compared with those of Russian ancestry (P = 0.07). Finnish ICA-positive schoolchildren had higher ICA titers (median 6 JDFU [range 4–514]) than schoolchildren in Russian Karelia (5 JDFU [4–1,027]; P = 0.002). The same was true for the GADA levels

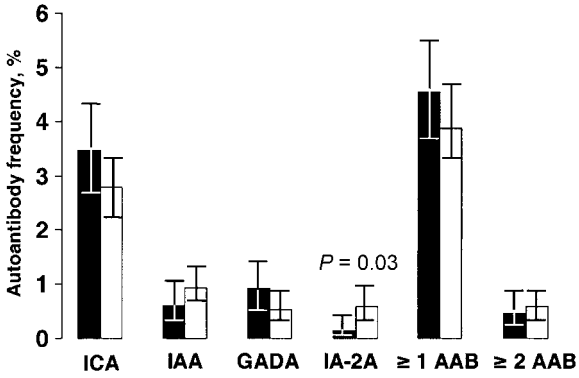

Figure 1—*The frequency of ICAs, IAAs, GADAs, IA-2As, and at least one (≥1) and at least two (≥2) autoantibodies (AAB) among 1,988 schoolchildren from Russian Karelia (■) and 3,652 schoolchildren from Finland (□). Error bars represent the 95% CIs.*

among GADA-positive subjects (41.7 relative units [range 6.64–113.6] in Finland vs. median 10.3 relative units [5.4–215.7] in Russian Karelia; $P = 0.038$). There were no significant differences between Finnish and Karelian schoolchildren in terms of IAA and IA-2A levels. The autoantibody levels in the 9 Karelian and 21 Finnish schoolchildren with multiple autoantibodies are shown in Fig. 2. The former group had significantly lower IA-2A levels than the latter.

There was no differences in the frequency of the high-risk genotype between the two populations (2.2% in Finnish children and 1.7% in Karelian children, difference 0.5% [95% CI −0.3 to 1.3]; $P = 0.271$), but the Finnish children more often carried moderate-risk genotypes (11.5 vs. 9.4%, difference 2.1% [0.5–3.8]; $P = 0.016$) and low-risk genotypes (15.1 vs. 13.2%, difference 2.0% [0.1–3.9]; $P = 0.048$). The Russian Karelian children had genotypes conferring decreased risk somewhat more frequently than the Finnish children (75.7 vs. 71.1%, difference 4.6% [2.2–7.0]; $P = 0.003$). The children with the high-risk HLA genotype most frequently tested positive for each autoantibody reactivity except for IAAs both in Russian Karelia and in Finland (Fig. 3).

In Finland, the high-risk children had significantly more often GADAs than the children carrying genotypes conferring low or decreased genetic risk. In addition, those with moderate-risk genotypes more often tested positive for GADAs than those with genotypes conferring decreased risk. Positivity for multiple autoantibodies was observed significantly more frequently among Finnish children with the high-risk HLA genotype than among those with genotypes conferring low or decreased risk for type 1 diabetes. Finnish children with protective genotypes tested positive for multiple autoantibodies less frequently than those with moderate-risk genotypes. Similar trends were seen among the children from Russian Karelia in terms of positivity for GADAs and multiple autoantibodies, but the differences remained statistically nonsignificant due to the smaller number of children studied.

CONCLUSIONS — Type 1 diabetes is characterized by autoimmune responses directed against multiple autoantigens. A number of studies on diabetes autoimmune markers have documented that the presence of two or more autoantibodies is associated with progression to overt diabetes over the next decade in a majority of first-degree relatives of affected patients, whereas expression of a single autoantibody appears to represent "harmless" β-cell autoimmunity in most cases (17–19). The prevalence of diabetes-associated autoantibodies in the background population has been reported (12,20,21) to vary widely between countries, but this variation may be at least partly due to methodological differences. In the present survey, all autoantibody assays were performed in the same laboratory. The aim of the study was to establish the prevalence of β-cell autoantibodies among schoolchildren from two adjacent countries with a sixfold gradient in the incidence of type 1 diabetes (10). The cumulative incidence of type 1 diabetes from a mean age of 12–20 years can be estimated to be close to 0.35–0.4%, according to available Finnish incidence data (A. Reunanen, personal communication). We do not have access to similar information in Russian Karelia, but based on the observed incidence among children under the age of 15 years (10), cumulative incidence from the age of 12 to 20 years could be estimated to be five to six times lower than in the Finnish population, i.e., 0.06–0.07%.

Our observations on a similar frequency of disease-associated autoantibodies in Finnish and Karelian schoolchildren is consistent with the study by Marciulionyte et al. (22) who reported no difference in the overall pattern of humoral β-cell autoimmunity between children from the U.K. and Lithuania in spite of a two- to threefold difference in the rate of childhood type 1 diabetes. These observations indicate that the prevalence of subclinical β-cell autoimmunity in the background population

Table 1—*Frequency of autoantibodies in schoolchildren in Russian Karelia by ethnicity of the mother*

	Finnish-Karelians ($n = 906$)		Russians ($n = 815$)		Others ($n = 267$)	
	n (%)	95% CI	*n* (%)	95% CI	*n* (%)	95% CI
ICA	31 (3.4)	2.3–4.8	32 (3.9)	2.7–5.5	6 (2.3)	0.8–4.8
IAA	7 (0.8)	0.3–1.6	3 (0.4)	0.1–1.1	2 (0.8)	0.1–2.7
GADA	9 (1.0)	0.5–1.9	5 (0.6)	0.2–1.4	4 (1.5)	0.4–3.8
IA-2A	1 (0.1)	0.0–0.6	2 (0.3)	0.0–0.9	0 (0.0)	0.0–1.4
Any autoantibody	39 (4.3)	3.1–5.8	40 (4.9)	3.5–6.6	11 (4.1)	2.1–7.3
Multiple autoantibodies	7 (0.8)	0.3–1.6	1 (0.1)	0.03–0.7	1 (0.4)	0.0–2.1

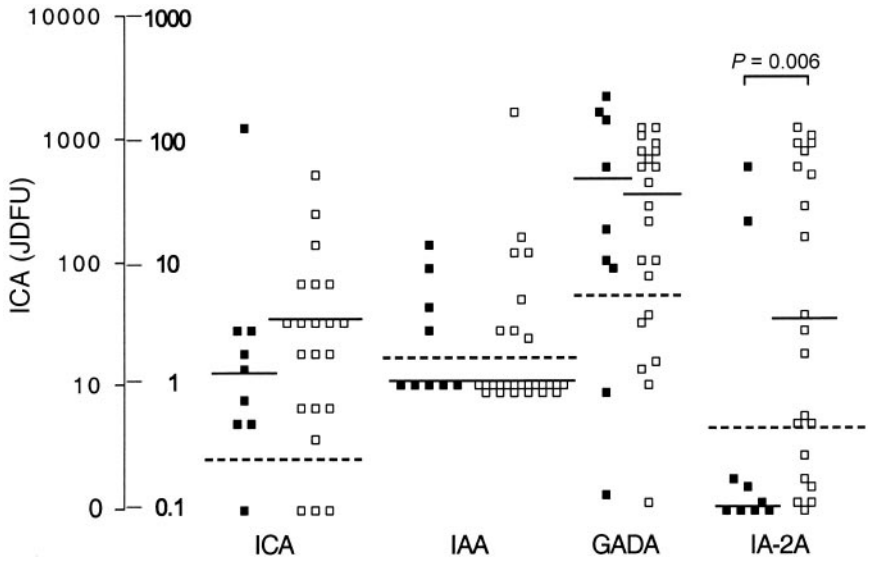

Figure 2—*The levels of ICAs, IAAs, GADAs and IA-2As in 9 schoolchildren from Russian Karelia (■) and 21 Finnish schoolchildren (□) with multiple (two or more) diabetes-associated autoantibodies. The solid line represents the median, whereas the broken line marks the cutoff limit for autoantibody positivity. ICAs are measured in JDFU, and IAAs, GADAs, and IA-2As are measured in relative units.*

is of the same magnitude in two populations with a conspicuous difference in the incidence of diabetes. Marciulionyte et al. (22) observed, however, that the prevalence of IA-2As was 10-fold lower among the Lithuanian schoolchildren (0.2 vs. 2.4%). In our study, a significant difference was similarly seen in the frequency of IA-2As between schoolchildren in Russian Karelia and Finland.

IA-2As have been observed to be the last appearing autoantibody reactivity in most cases during the pre-diabetic process before clinical manifestation of diabetes (23). Finnish data have shown that IA-2As are associated with the highest predictive value for progression to clinical type 1 diabetes among individual autoantibody reactivities both in siblings of affected children (24) and in young children with HLA-conferred susceptibility recruited from the general population (19). The decreased prevalence of IA-2As among schoolchildren in Russian Karelia indicates that progressive β-cell autoimmunity is less common in Russian Karelia than in Finland. This difference may either be due to a reduced exposure to a driving exogenous diabetogenic factor or the presence of protective factor(s) in Russian Karelia. In addition, our study indicates that there are no significant differences in the prevalences of single autoantibody reactivities between the three ethnic groups in Russian Karelia, whereas we observed that multiple autoantibodies tended to be more frequent among those of Finnish-Karelian ancestry. In our previous study (10), the annual incidence rate was about two times higher among the children with Finnish-Karelian background (11.1 of 100,000 children aged <15 years) than in children with a Russian or another ethnic background (6.7 and 5.0 of 100,000 children, respectively) in Karelia, but these differences remained nonsignificant.

To our knowledge, this is the first survey generating data on genotype-specific autoantibody frequencies based on an extensive unselected cohort of schoolchildren representing the general population. The data show that the high-risk HLA genotype was associated with the highest frequency of each autoantibody reactivity and of positivity for multiple autoantibodies in both Russian Karelia and Finland. IAA positivity represented the only exception, but this may be due to the low overall frequency of IAAs. The various autoantibodies showed a falling frequency along with decreasing HLA-conferred disease susceptibility with the exception of ICAs. The most apparent gradients were observed for GADAs and multiple autoantibody positivity, and as a matter of fact, these gradients were statistically significant among the Finnish schoolchildren. Despite a similar profile, the gradients remained nonsignificant among the schoolchildren from Russian Karelia, most likely as a consequence of the smaller cohort size. When comparing with a study on a sib cohort from the Finnish Diabetes Prediction and Prevention study, the profile of positivity for multiple autoantibodies in relation to HLA genotypes is quite similar (25), although the difference between high- and moderate-risk genotypes is more conspicuous in the schoolchildren. This might indicate that the difference increases with rising age, since the mean age in the present study population is about two times older than that in the Finnish Diabetes Prediction Prevention study sib cohort (11.7 vs. 6.2 years).

The conspicuous difference in socioeconomic status between Finland and Russian Karelia does have a profound effect on the lifestyle and living conditions in the two countries, such as effects on early feeding and the frequency of early gastrointestinal infections. There are also differences in childhood vaccination practices between the two countries. Studies in progress are aimed at delineating the consequences of these differences in the living conditions on the maturation and programming of the immune system in young children.

In summary, the present data suggest that β-cell autoimmunity is induced as frequently in schoolchildren from the low-incidence Russian Karelia as in peers from the high-incidence Finland, but progressive β-cell autoimmunity is less common in Russian Karelia, eventually resulting in a sixfold difference in the incidence rate of clinical type 1 diabetes. It is an open issue whether the increase seen in the incidence of type 1 diabetes among children aged <15 years in most developed countries after World War II (26) is due to a more frequent disease initiation or a more rapid disease progression (27). Both the present observation and the consistent finding in the Lithuania-U.K. comparison (22) favor the latter alternative. The need remains, however, to define whether the more rapid disease progression is a consequence of an increased exposure to one or more driving exogenous diabetogenic factors or to the lack of protective factors in the affluent Western society.

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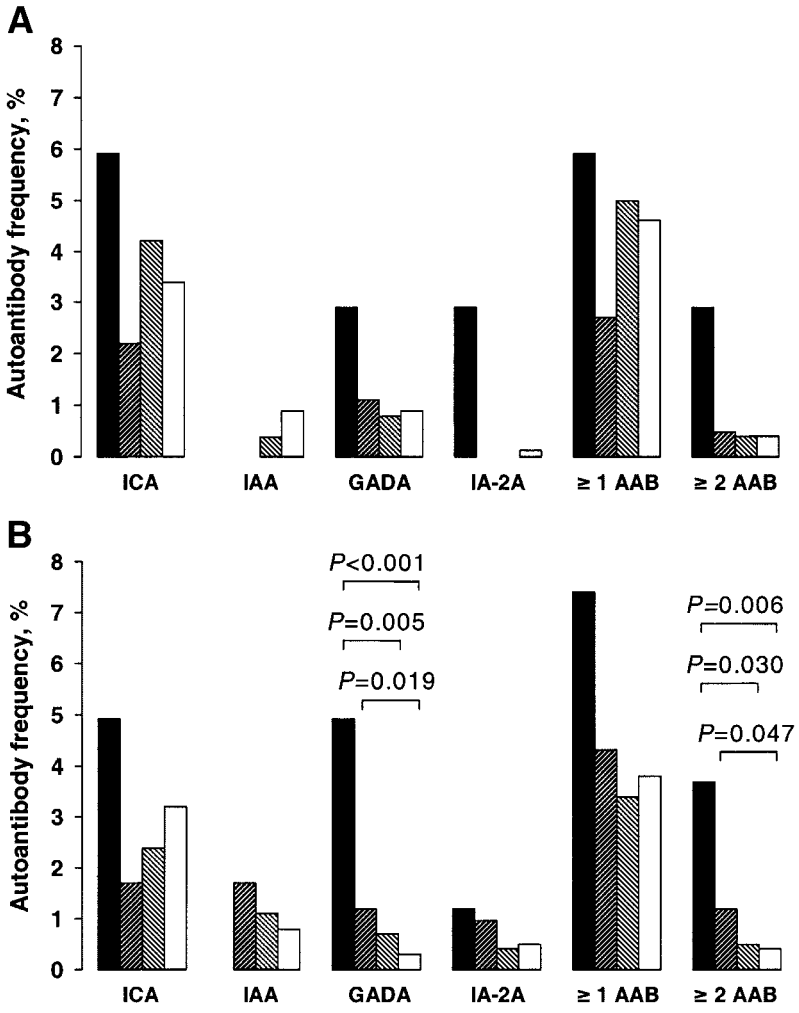

Figure 3—*The frequency of ICAs, IAAs, GADAs, IA-2As, and at least one (≥1) and at least two (≥2) autoantibodies (AAB) in 1,977 schoolchildren from Russian Karelia* (A) *and 3,649 schoolchildren from Finland* (B) *in relation to HLA-conferred susceptibility to type 1 diabetes (high [■], moderate [▨], low- [▧], and decreased-risk [□]genotypes). Comparison of all four genotypes among Finnish schoolchildren:* $P < 0.001$ *for both GADA and multiple autoantibodies.*

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