Effect of the *PTPN22* and *INS* Risk Genotypes on the Progression to Clinical Type 1 Diabetes After the Initiation of β-Cell Autoimmunity

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We set out to analyze the role of two major non-HLA gene polymorphisms associated with type 1 diabetes (T1D), *PTPN22* 1858C/T and insulin gene *INS*−23 A/T in progression to clinical T1D after the appearance of β-cell autoimmunity. The study population comprised 249 children with HLA-associated T1D susceptibility. All subjects were persistently positive for at least one of the T1D-associated biochemically defined autoantibodies (insulin autoantibody, GAD antibody, or IA-2 antibody), and 136 subjects presented with T1D over a median follow-up of 4.3 years (range 0.0–12.5) after the appearance of the first autoantibody. The *PTPN22* 1858T allele was strongly associated with progression to T1D after the appearance of the first biochemically defined β-cell autoantibody (hazard ratio 1.68 [95% CI 1.09–2.60], P = 0.02 Cox regression analysis, multivariate test), and the effect remained similar when analyzed after the appearance of the second autoantibody (P = 0.013), whereas *INS*−23 HphI AA genotype was not associated with progression to clinical diabetes after the appearance of the first or second autoantibody (P = 0.38 and P = 0.88, respectively). The effect of the *INS* risk genotype seems to be limited to the induction and early phases of β-cell autoimmunity, but the *PTPN22* 1858T allele instead affects the initiation and late progression phase of diabetes-associated autoimmunity. ***Diabetes* 61:963–966, 2012**

Type 1 diabetes (T1D) is an autoimmune disease resulting from the destruction of the insulin-producing pancreatic β-cells. The etiology of the disease is not completely understood, but the strong inherited effect of multiple gene loci is a crucial component (1). The HLA gene region on the short arm of chromosome 6 is the most important gene locus affecting susceptibility to T1D. A number of other susceptibility loci have been described outside the HLA region. Of these, the effect of the polymorphisms in the promoter region of the *INS* gene and the *PTPN22* gene that encode the lymphocyte tyrosine phosphatase that regulates T-cell activation on the increased appearance of β-cell autoimmunity and T1D have been confirmed in several populations, and their effect is the strongest one among the non-HLA genes (2).

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The diagnosis of T1D is preceded by an autoimmune process that damages the pancreatic β-cells, and circulating autoantibodies can be detected as a marker of the ongoing process. This process is asymptomatic, and the duration of this period varies widely (3). The significance of the various genetic and environmental risk factors in the different stages of the autoimmune disease process is not fully understood. It has been proposed that HLA class II genes determine the initiation of β-cell autoimmunity, whereas other factors (e.g., HLA class I genes) affect the progression of β-cell destruction (4). In support of this hypothesis, we recently reported an enhancing effect of the HLA-B*39 allele and a protecting effect of the *A03 allele on the progression to clinical T1D after the appearance of humoral signs of β-cell autoimmunity (5). Moreover, this effect of the HLA class I genes was affected by the HLA class II genotype, although the high-risk class II genotype did not directly affect the progression to the disease after establishment of advanced β-cell autoimmunity.

Data on the effect of the genetic risk factors outside the HLA region in the different stages of β-cell autoimmunity are limited (6). Thus, in the current study, we analyze the role of the two major non-HLA gene polymorphisms associated with T1D, *PTPN22* 1858C/T and *INS*−23 A/T in progression from islet autoimmunity to clinical T1D.

RESEARCH DESIGN AND METHODS

The study subjects were participants in the Finnish Diabetes Prediction and Prevention (DIPP) study and carried HLA-DQB1 genotypes associated with an increased risk for T1D (HLA-DQB1*0302, the HLA-DQB1*0302/DQA1*05-DQB1*02 combination, or DQA1*05-DQB1*02 alone [a subgroup of boys born in Turku] without the presence of the protective alleles [HLA-DQB1*0301 or HLA-DQB1*0602]). According to the study protocol, subjects were prospectively followed up at 3- to 12-month intervals for the appearance of serologic signs of T1D-associated autoantibodies: islet cell antibodies (ICA), and when ICA were detected, for the signs of insulin autoantibodies (IAA), antibodies to the 65 kD isoform of GAD (GADA), and antibodies to the protein tyrosine phosphatase-related IA-2 molecule (IA-2A) (7). All subjects who tested persistently positive for ICA and were positive for at least one of the biochemically defined autoantibodies (IAA, GADA or IA-2A) by May 2004 and whose DNA sample was available for genotyping were included in the current study. In addition, one subject positive for IAA but not ICA and presenting with clinical T1D by May 2004 was added to the study population. The local ethical committees approved the study protocol, and informed consent was obtained from the parents of the study participants.

A total of 249 subjects fulfilled the inclusion criteria and were accordingly included in the study population; of these, 201 (80.7%) tested positive for IAA, 184 (73.1%) for GADA, and 176 (70.6%) for IA-2A. The median age of the children was 1.8 years (range 0.3–12.0) at the time of seroconversion to positivity for the first biochemically defined autoantibody and 2.0 years (0.8–9.9) for the second biochemically defined autoantibody. Median follow-up from the appearance of the first biochemically defined autoantibody was 6.4 years (0.5–12.5) in the 113 children remaining clinically healthy and 2.8 years (0.0–10.8) among the 136 children who progressed to clinical T1D. The median follow-up times were 6.1 years (1.8–12.0) after the second biochemically defined autoantibody and in the 78 children remaining unaffected and 2.5 years (0.0–10.8)

in the 117 children presenting with T1D. All subjects with clinical T1D were diagnosed according to the World Health Organization criteria. At the time of study recruitment, 32 of the 249 study subjects had a first-degree relative with T1D.

Genotyping methods. HLA genotyping was described in our earlier study (5). The genotyping methods for the *PTPN22* 1858C/T (rs2476601) and *INS*−23 A/T (rs689) polymorphisms were based on the use of lanthanide-labeled probes and have been described earlier (8,9). The *PTPN22* 1858C/T and *INS*−23 A/T genotypes could be defined in 241 (96.8%) and 240 (96.4%) children, respectively.

Statistical analysis. Cox regression analysis and Kaplan-Meier analysis with log-rank test were used to compare progression to clinical disease in subjects with various genotypes. The analysis defined time of entry as the time of the appearance of the first or second biochemically defined autoantibody. The effect of the analyzed variants on the progression to clinical T1D was analyzed until the diagnosis of T1D (uncensored) or the end of the follow-up (censored). All statistical analyses were performed with SPSS 19.0 software (IBM SPSS Inc., Chicago, IL).

RESULTS

The effect of the *PTPN22* 1858T allele and *INS*−23 HphI AA genotype on the progression to clinical T1D after the appearance of humoral β-cell autoimmunity was analyzed. We observed a strong effect of the *PTPN22* 1858T allele on the progression to clinical T1D after the first signs of humoral β-cell autoimmunity (Table 1, Fig. 1). After the appearance of the first biochemically defined autoantibody, 58 subjects (66.7%) with the TT or CT genotype presented with T1D compared with 71 (46.1%) with the CC genotype (hazard ratio [HR] 1.63 [95% CI 1.15–2.30], $P = 0.006$, Cox regression analysis, univariate test for the effect of *PTPN22* TT/CT vs. CC genotypes). Cumulative risk of T1D at 10 years of follow-up was 78.2% for subjects carrying the T allele and 53.7% for subjects with CC genotype ($P = 0.005$, Kaplan-Meier analysis, log-rank test).

TABLE 1
The effect of the *PTPN22* 1858C/T, *INS*−23 HphI A/T, HLA DR3/4, HLA-A*03, and *B39 genotypes, sex, and presence of first-degree relative with T1D on the progression to clinical T1D after the appearance of the first and second biochemically defined autoantibody (Cox regression analysis, univariate test)

Follow-up from the appearance of the first biochemically defined autoantibody			
Variable	*n/N*	*P*	HR (95% CI)†
PTPN22 1858 TT/CT	87/241	0.006	1.63 (1.15–2.30)
INS−23 AA	195/240	0.27	1.27 (0.83–1.93)
HLA-A*03	70/197	0.003	0.52 (0.34–0.81)
HLA-B*39	28/208	0.045	1.68 (1.01–2.79)
HLA-DR3/4	80/249	0.010	1.58 (1.12–2.22)
Female sex	107/249	0.041	1.42 (1.01–1.99)
1st-degree relative with T1D	32/249	0.11	1.44 (0.92–2.25)
Follow-up from the appearance of the second biochemically defined autoantibody			
Variable	*n/N*	*P*	HR (95% CI)†
PTPN22 1858 TT/CT	73/187	0.003	1.77 (1.22–2.58)
INS−23 AA	143/186	0.78	1.06 (0.68–1.66)
HLA-A*03	56/151	0.006	0.52 (0.38–0.83)
HLA-B*39	21/159	0.002	2.32 (1.35–4.00)
HLA-DR3/4	66/195	0.14	1.33 (0.91–1.93)
Female sex	82/195	0.013	1.59 (1.10–2.29)
1st-degree relative with T1D	30/195	0.11	1.46 (0.91–2.32)

DR3/4 = (DR3)-DQA1*05-DQB1*02/DRB1*0401/4-DQB1*0302. †For diabetes progression.

The effect of the T allele marking disease risk remained strong also after the appearance of the second biochemically defined autoantibody. Here, 53 of the subjects (72.6%) carrying the T allele presented with T1D, whereas 57 (50.0%) with the CC genotype progressed to clinical T1D ($P = 0.003$, Cox regression analysis; Table 1). In contrast, no effect of the T1D-risk associated *INS*−23 HphI AA genotype on progression to clinical T1D after the appearance of β-cell autoimmunity was observed in Cox regression analysis (Table 1) or in Kaplan-Meier survival analysis (data not shown). A total of 102 of the subjects (55.1%) with the AA genotype presented with T1D after the appearance of the first biochemically defined autoantibody compared with 28 of the subjects (50.9%) with the AT or TT genotype ($P = 0.27$), and the result remained similar also after the appearance of the second biochemically defined autoantibody (85 [59.4%] vs. 26 [60.5%], respectively; $P = 0.78$).

The effect of the major HLA class II risk genotypes, the two HLA class I alleles, which in the same series affected progression of β-cell autoimmunity to clinical disease, HLA-A*03 and *B39 (5), and sex, presence of first-degree relative with T1D, and the age at autoantibody seroconversion were then included in the analysis. After these adjustments, the strong effect of the *PTPN22* T allele and the absent effect of the *INS* genotype on the disease progression remained. After the appearance of the first and second biochemically defined autoantibody, the *PTPN22* T disease-risk allele was associated with enhanced progression to clinical disease ($P = 0.02$ and $P = 0.013$, respectively), whereas no effect of the *INS* genotype on the appearance of clinical T1D was observed ($P = 0.38$ and $P = 0.88$, follow-up after the appearance of the first and second autoantibody, respectively; Table 2).

DISCUSSION

This study describes the role of the two major non-HLA gene polymorphisms associated with T1D, *PTPN22* 1858C/T and *INS*−23 A/T, in progression to clinical T1D after the appearance of β-cell autoimmunity. The effect of these polymorphisms on the T1D risk has been repeatedly confirmed (2). However, data describing their effect on the disease risk in the different stages of the disease process is scarce. Butty et al. (6) reported results on the effect of the various genetic factors, including *PTPN22* C1858T (R620W) and *INS*−23 HphI A/T polymorphisms, in the progression to clinical T1D among at-risk relatives of T1D patients with autoimmune manifestations in the Diabetes Prevention Trial–Type 1 (DPT-1) trial. They observed no differences in the *PTPN22* and *INS* allele frequencies between the nonprogressors who remained clinically healthy and progressors who presented with clinical T1D in their study cohort. Neither did the recent report on the Diabetes Autoimmunity Study in the Young (DAISY) follow-up cohort see any significant effect in progression from persistent autoimmunity to clinical diabetes in the smaller cohort of 112 subjects with persistent autoimmunity and in 47 diagnosed with T1D, although they observed a significant effect of the *PTPN22* risk genotypes on the progression to persistent islet autoimmunity (10).

There are obvious differences between the study cohort in the DPT-1 trial and in our study. The DPT-1 cohort comprised ICA- and IAA-positive relatives of T1D patients, and the presence of other autoantibodies was not taken into account. The subjects were older at screening (mean age, 9.5 years), and the exact autoantibody seroconversion

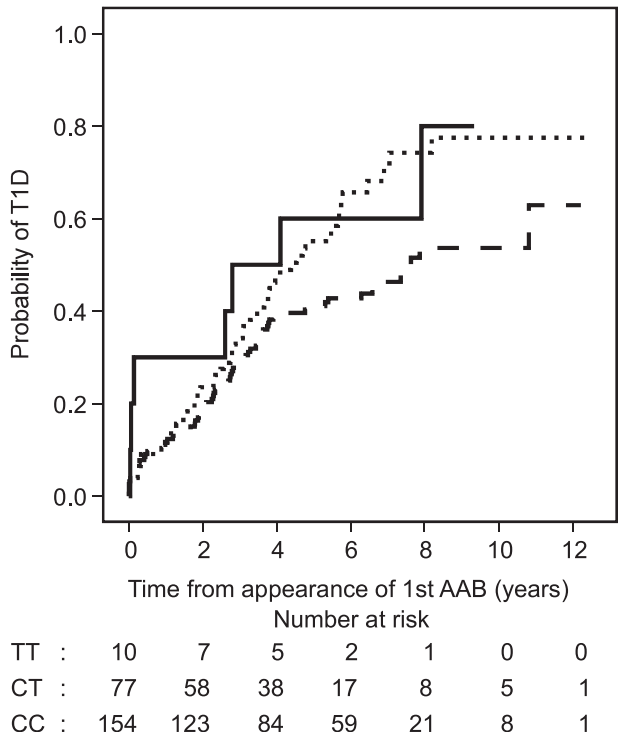

FIG. 1. Kaplan-Meier survival analysis shows the effects of the *PTPN22* 1858TT (solid line), 1858CT (dotted line), and 1858CC (dashed line) genotypes on the progression to clinical T1D after the appearance of the first biochemically defined autoantibody (AAB).

age could not be defined. HLA-associated risk was also lower in the DPT-1 study because only the presence of the protective DQB1*0602 allele was excluded. It is thus conceivable that a subgroup with high disease risk and rapid progression to clinical disease in early childhood was less well represented in the DPT-1 than in our DIPP follow-up cohort. The setting of the DAISY study, with follow-up from birth on, was closer to our study. An apparent difference is the higher power of our study owing to higher numbers in the follow-up cohort and probably a more homogeneous population. In addition, we point out that the frequency of the risk-associated *PTPN22* minor T allele is clearly more common among both the Finnish T1D patients and the background population than in the respective U.S. populations, thus further increasing the power of the DIPP study in this context (11).

The results indicate differences in the rate of progression between the various susceptibility genes in the process leading to the development of β-cell autoimmunity and eventually to loss of insulin-secreting β-cells. The *INS* disease-risk genotype is associated with lower levels of insulin expression in the thymus, leading to ineffective deletion of insulin-specific T cells (12–14). In addition, the *INS* disease-risk genotype has been associated with IAA (15,16), which is usually the first autoantibody reactivity to be detected, especially among young children (17,18). Thus, in accordance with our findings, the *INS* T1D-risk genotype is likely to affect the initial breakdown of insulin-specific immunologic tolerance rather than to affect the immunoregulation leading to β-cell destruction. The *PTPN22* disease allele is shown to alter T cell and to some extent also B cell function, but exactly how it affects the process leading to β-cell destruction is not known (19–21). A major hypothesis suggests that poor T-cell receptor–mediated

TABLE 2
The effect of the *PTPN22* 1858C/T and *INS*−23 HphI A/T genotypes on the progression to clinical T1D after the appearance of the first and second biochemically defined autoantibody (Cox regression analysis, multivariate test)

Follow-up from the appearance of the first biochemically defined autoantibody#			
Variable	*N*	*P*	HR (95% CI)†
PTPN22 1858 TT/CT	187	0.02	1.68 (1.09–2.60)
INS−23 AA	187	0.38	1.25 (0.76–2.06)
Follow-up from the appearance of the second biochemically defined autoantibody‡			
Variable	*N*	*P*	HR (95% CI)†
PTPN22 1858 TT/CT	142	0.013	1.84 (1.14–2.98)
INS−23 AA	142	0.88	0.96 (0.56–1.66)

#Adjusted for the effect of HLA DR3/4 [(DR3)-DQA1*05-DQB1*02/DRB1*0401/4-DQB1*0302], HLA-A*03 and *B39, sex, presence of first-degree relative with T1D, and the age at the appearance of the first biochemically defined autoantibody. †For diabetes progression. ‡Adjusted for the effect of HLA DR3/4, HLA-A*03 and *B39, sex, presence of first-degree relative with T1D, and the age at the appearance of the second biochemically defined autoantibody.

signaling in the thymus is associated with defective negative selection and maintenance of an autoreactive T-cell repertoire. Alternative mechanisms might be related to the function of high levels of Lyp-expressing dendritic cells, which are important in antigen presentation and activation of T helper cells or further, that the decreased function of naturally occurring self-reactive regulatory T cells allows the generation of autoreactive T-effector cells (22). The last alternative might best fit our finding of more rapid course of β-cell destruction after establishment of islet-specific autoimmunity. In line with this, the 1858T allele has been associated with a diminished residual β-cell function and metabolic control or with increased residual β-cell stress as measured by elevated proinsulin/C-peptide level at the time of T1D diagnosis and during the early period of insulin treatment (23,24). These findings, together with the results observed in this study, suggest that the *PTPN22*+1858T allele has a role in the β-cell destruction leading to clinical T1D in addition to the initiation of β-cell autoimmunity.

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No potential conflicts of interest relevant to this article were reported.

J.L. acquired and reviewed research data used in the analysis, undertook statistical analysis and interpretation of the results, and drafted the manuscript. R.H. and R.V. were involved in the interpretation of the results and critically reviewed the manuscript. O.S. and M.K. were two of the principal investigators, designed the DIPP study, were involved in the interpretation of the results, and critically reviewed the manuscript. J.I. was one of the principal investigators, was involved in the interpretation of the results, designed the DIPP study, provided the genotyping results of the research subjects, and critically reviewed the manuscript. J.L. is the guarantor of this work and, as such, had full access to all of the data in the study

and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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