Longitudinal Pattern of First-Phase Insulin Response Is Associated With Genetic Variants Outside the Class II HLA Region in Children With Multiple Autoantibodies

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A declining first-phase insulin response (FPIR) is associated with positivity for multiple islet autoantibodies, irrespective of class II HLA DR-DQ genotype. We examined the associations of FPIR with genetic variants outside the HLA DR-DQ region in the Finnish Type 1 Diabetes Prediction and Prevention (DIPP) study in children with and without multiple autoantibodies. Association between FPIR and class I alleles *A*24* and *B*39* and eight single nucleotide polymorphisms outside the HLA region were analyzed in 438 children who had one or more FPIR results available after seroconversion. Hierarchical linear mixed models were used to analyze repeated measurements of FPIR. In children with multiple autoantibodies, the change in FPIR over time was significantly different between those with various *PTPN2* (rs45450798), *FUT2* (rs601338), *CTSH* (rs3825932), and *IKZF4* (rs1701704) genotypes in at least one of the models. In general, children carrying susceptibility alleles for type 1 diabetes experienced a more rapid decline in insulin secretion compared with children without susceptibility alleles. The presence of the class I HLA *A*24* allele was also associated with a steeper decline of FPIR over time in children with multiple autoantibodies. Certain genetic variants outside the class II HLA region may have a significant impact on the longitudinal pattern of FPIR.

The first-phase insulin response (FPIR), a marker reflecting functional capacity of the β-cells in the pancreas, increases physiologically over time in children and adolescents (1). As a sign of deteriorating β-cell function, a decline in FPIR can, however, be observed several years before clinical type 1 diabetes (T1D) (1).

The class II HLA DR-DQ region has been shown to affect the appearance of islet-specific autoantibodies. Children with multiple autoantibodies have a high risk of progressing to clinical disease, and the presence of multiple autoantibodies seems to represent a point of no return (2). However, class II HLA does not have any effect on the progression rate from advanced islet autoimmunity to clinical diabetes (3), which in turn is influenced by some class I HLA alleles (4). Genetic variants outside the HLA region also affect the development of islet autoimmunity and/or progression to clinical diabetes (5–7).

We recently observed that the association between FPIR and class II HLA DR-DQ is secondary to the presence of multiple autoantibodies (8). The declining pattern of FPIR was associated with multiple autoantibodies irrespective of HLA class II risk group. However, it is possible that other genetic polymorphisms are specifically associated with the evolution of FPIR during progression from autoimmunity to clinical disease.

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Here, we studied the role of two class I HLA alleles and eight selected non-HLA gene polymorphisms in the development of insulin secretory capacity as measured by FPIR in children participating in the Finnish Type 1 Diabetes Prediction and Prevention (DIPP) study. Because the presence of multiple islet autoantibodies is strongly associated with β-cell failure, we analyzed separately children with and without multiple biochemical autoantibodies. The selected HLA class I alleles and non-HLA markers have previously been shown to associate with the progression rate from islet autoimmunity to clinical diabetes (4,5,9,10). However, it is not known how or whether these markers are associated with insulin response. The genetic variants of *INS* and *CTSH* genes were selected because of their known role in β-cell function (11,12).

RESEARCH DESIGN AND METHODS

The population-based DIPP study was launched in 1994 to screen for diabetes-associated risk by genotyping the major HLA DR-DQ haplotypes at birth (3). The study participants were followed regularly for the appearance of islet autoantibodies at 3–12-month intervals. Children who developed islet autoantibodies (islet cell antibodies and biochemical autoantibodies to insulin, GAD 65, and IA2) underwent an intravenous glucose tolerance test (IVGTT) (1), whereas autoantibodies to zinc transporter 8 were analyzed after IVGTT. β-Cell function was estimated by FPIR and change in FPIR (ΔFPIR) as described previously (8).

Genotyping Methods

HLA typing of major DR-DQ haplotypes was performed with a PCR-based lanthanide-labeled hybridization method using time-resolved fluorometry for detection (3). Genotyping using the Sequenom platform (San Diego, CA) of eight single nucleotide polymorphisms (SNPs), including *PTPN22* (rs2476601), *IFIH1* (rs1990760), *INS* (rs689), *IKZF4* (rs1701704), *ERBB3* (rs2292239), *CTSH* (rs3825932), *PTPN2* (rs45450798), and *FUT2* (rs6013380), was performed at the University of Eastern Finland (Kuopio, Finland) (5); *CTSH* (rs3825932) genotyping was performed using the Taqman SNP Genotyping Assay (Thermo Fisher Scientific, Waltham, MA). The assays of class I HLA alleles (*B*39*, *A*24*, and *B*39:06*) were analyzed on the DELFIA platform (4). SNPs in *ERBB3* and *IKZF4* polymorphisms were highly correlated (Fisher exact test $P < 0.0001$).

Autoantibody Analyses

Autoantibodies to insulin, GAD 65, IA2, and zinc transporter 8 were measured in serum samples by a radiobinding assay (13,14). Islet cell antibodies were measured by classical immunofluorescence method applied to sections of human pancreas, blood group O (15).

Study Participants

The 438 study children (268 [61.2%] males) with one or more FPIR results (133 [30.4%] who had progressed to T1D, 35 with a single biochemical, 65 with multiple biochemical autoantibodies who did not progress to T1D during the study period) had been categorized according to the biochemical autoantibody status (none/one or multiple [at least two] biochemical islet autoantibodies) at the time of the first IVGTT. The median age at the first IVGTT, which was performed at least 2 years before diagnosis in progressors, was 4.6 years. Diabetes was diagnosed according to World Health Organization criteria (16).

Statistical Analyses

ΔFPIR was calculated in children with and without multiple biochemical autoantibodies. Before data analysis, the response variable FPIR was log-transformed. Age-adjusted hierarchical linear models (8) applied to analyze the repeated measurements of FPIR included autoantibody status (0 or 1 autoantibody) in children without multiple autoantibodies, genotypes (three groups except for class I HLA genotypes, which were categorized into two groups), and interaction terms genotype by time and autoantibody group by time. The period of 0–5 years from the first IVGTT was examined.

Three types of models (additive, recessive, and dominant) were investigated for the SNP genotypes. In the additive model, all three groups were compared. In the recessive model, children homozygous for the risk allele were compared against those who were not homozygous for the risk allele (two groups). In the dominant model, children carrying the risk allele were compared with those who did not have a risk allele (two groups).

Statistical analyses were performed with JMP Pro version 11.2 and SAS 9.4 for Windows (SAS Institute, Cary, NC) software. $P < 0.05$ (two-tailed) was considered statistically significant.

Ethical Considerations

This study was conducted according to the guidelines of the Declaration of Helsinki II and was approved by local ethics committees. Written informed consent was obtained from all participants and/or their primary caregivers.

Data and Resource Availability

The data sets generated and analyzed during the current study are not publicly available because of privacy regulations. No applicable resources were generated or analyzed during the current study.

RESULTS

The median FPIR levels and ΔFPIR over the observation period are shown in children with and without multiple biochemical autoantibodies (Tables 1 and 2). FPIR increased over time in children without multiple autoantibodies (Table 2), whereas it declined in those with multiple autoantibodies (Table 1). When the hierarchical linear mixed models were used in children with multiple autoantibodies, modest associations were observed between the evolution of FPIR and three of the gene

Table 1—The median of the first FPIR and ΔFPIR over time according to different genotypes in 195 children with multiple (at least two) biochemical autoantibodies during follow-up

SNP (*n*, % of total within the gene)†	Baseline FPIR (mU/L), median (95% CI)	Age at first IVGTT (years), median (IQR)	Time between last and first IVGTT (years), median (range)	ΔFPIR (mU/L/year)		Progressors, *n* (%)
				Median (95% CI)	*n*	
PTPN22 (193)	49.2 (44.6, 53.9)	3.5 (2.3, 5.5)	3.1 (0.8–14.5)	−3.3 (−4.5, −1.5)	151	128 (66)
AA (12, 6)	50.5 (32.2, 79.6)	2.4 (2.1, 4.7)	3.2 (1.1–10.0)	−0.7 (−5.9, 2.0)	10	8 (67)
A**G** (50, 26)	46.8 (41.2, 53.9)	2.9 (2.0, 5.0)	3.0 (0.8–8.5)	−4.0 (−5.6, −1.1)	42	37 (74)
GG (131, 68)	51.0 (44.1, 56.1)	3.6 (2.4, 5.5)	3.1 (1.0–14.5)	−3.4 (−5.4, −1.6)	99	83 (63)
IFIH1 (188)	47.6 (44.6, 53.5)	3.5 (2.3, 5.5)	3.1 (0.8–14.5)	−3.4 (−4.9, −1.6)	147	126 (67)
TT (70, 37)	52.3 (41.2, 67.3)	3.5 (2.2, 5.7)	2.6 (0.8–10.4)	−3.7 (−6.2, −1.3)	53	45 (64)
TC (90, 48)	50.3 (44.8, 55.0)	3.5 (2.4, 5.4)	3.4 (1.0–12.3)	−3.1 (−5.1, −0.7)	72	59 (66)
CC (28, 15)	40.1 (32.9, 51.8)	2.9 (2.2, 4.9)	4.3 (1.5–14.5)	−3.7 (−5.3, 1.4)	22	22 (79)
INS (195)	47.7 (44.6, 53.5)	3.5 (2.3, 5.5)	3.1 (0.8–14.5)	−3.4 (−4.5, −1.6)	153	130 (67)
AA (148, 76)	46.7 (42.7, 53.1)	3.5 (2.3, 5.4)	3.2 (0.8–14.5)	−3.4 (−5.1, −1.1)	115	100 (67)
AT (41, 21)	57.2 (43.7, 63.4)	3.4 (2.1, 5.1)	2.7 (1.0–11.3)	−3.7 (−7.2, −1.2)	33	26 (63)
TT (6, 3)	50.7 (39.8, 90.0)	5.6 (2.1, 6.0)	6.1 (2.1–10.0)	−1.3 (−18.3, 2.3)	5	4 (67)
IKZF4 (189)	47.4 (44.6, 53.5)	3.5 (2.3, 5.5)	3.1 (0.8–14.5)	−3.4 (−5.0, −1.6)	148	129 (68)
CC (20, 10)	48.5 (38.8, 76.9)	3.1 (2.3, 5.4)	3.1 (1.1–8.0)	−5.0 (−18.9, 1.4)	14	15 (75)
AC (75, 40)	53.9 (47.1, 63.3)	3.5 (2.2, 5.6)	3.4 (1.0–14.5)	−1.6 (−4.4, −0.4)	60	42 (56)
AA (94, 50)	43.8 (41.2, 47.4)	3.5 (2.3, 5.2)	2.9 (0.8–11.2)	−4.0 (−5.4, −2.3)	74	68 (72)
ERBB3 (193)	47.4 (44.1, 53.5)	3.5 (2.3, 5.5)	3.1 (0.8–14.5)	−3.4 (−4.5, −1.6)	151	129 (67)
AA (19, 10)	46.0 (38.6, 98.0)	3.0 (2.3, 5.4)	3.0 (1.0–8.0)	−3.3 (−18.9, 1.6)	13	13 (68)
CA (75, 39)	53.5 (47.7, 62.8)	3.5 (2.4, 5.6)	3.5 (1.0–14.5)	−1.9 (−6.2, −0.6)	61	46 (61)
CC (99, 51)	43.9 (41.6, 51.3)	3.5 (2.3, 5.5)	2.7 (0.8–11.2)	−3.7 (−5.3, −2.3)	77	70 (71)
CTSH (193)	47.3 (44.1, 53.5)	3.5 (2.3, 5.5)	3.1 (0.8–14.5)	−3.4 (−4.5, 1.6)	151	129 (67)
CC (75, 39)	47.0 (39.0, 53.9)	3.5 (2.4, 5.8)	2.5 (1.0–8.0)	−3.7 (−5.3, −1.1)	51	48 (64)
CT (87, 45)	47.0 (44.6, 60.2)	3.4 (2.3, 5.4)	3.1 (0.8–12.3)	−4.1 (−6.9, −1.7)	74	62 (71)
TT (31, 16)	50 (39.8, 71.8)	3.5 (2.2, 5.0)	4.1 (1.0–14.5)	−1.2 (−3.7, 2.3)	26	19 (61)
PTPN2 (192)	47.6 (44.6, 53.5)	3.5 (2.3, 5.5)	3.1 (0.8–14.5)	−3.4 (−4.9, −1.6)	150	128 (67)
CC (3, 1)	28.1 (26.2, 87.4)	2.8 (2.5, 4.4)	3.0 (2.1–4.0)	−5.5 (−5.8, −5.3)	2	3 (100)
GC (53, 28)	55.1 (46.8, 66.2)	3.5 (2.3, 6.5)	3.2 (1.0–12.3)	−1.3 (−6.9, −0.2)	42	36 (68)
GG (136, 71)	45.5 (42.7, 51.8)	3.4 (2.2, 5.1)	3.1 (0.8–14.5)	−3.4 (−5.0, −1.7)	106	89 (65)
FUT2 (169)	51.0 (46.0, 54.2)	3.5 (2.3, 5.5)	3.1 (0.8–14.5)	−3.0 (−4.4, −1.2)	131	109 (64)
AA (29, 17)	53.1 (40.3, 76.5)	3.5 (2.3, 5.8)	3.0 (0.8–10.4)	−4.4 (−8.4, −1.3)	25	21 (72)
GA (89, 53)	56.1 (47.4, 63.9)	3.6 (2.4, 5.7)	2.8 (1.0–11.3)	−2.8 (−5.3, −0.7)	64	56 (63)
GG (51, 30)	43.1 (41.2, 51.0)	3.5 (2.2, 4.9)	3.9 (1.0–14.5)	−1.4 (−3.7, 1.2)	42	32 (63)
Class I HLA alleles						
*A*24* (183)	47.7 (44.6, 53.5)	3.5 (2.3, 5.5)	3.1 (0.8–14.5)	−3.4 (−5.0, −1.7)	145	122 (67)
Present (32, 17)	41.1 (30.6, 47.4)	3.1 (2.1, 4.4)	2.3 (1.0–3.4)	−5.1 (−8.5, −3.3)	26	27 (84)
Absent (151, 83)	52.2 (46.5, 57.1)	3.5 (2.4, 5.7)	3.4 (0.8–14.5)	−2.6 (−4.5, −1.0)	119	95 (63)
*B*39* (187)	47.4 (44.6, 53.5)	3.5 (2.3, 5.5)	3.1 (0.8–14.5)	−3.4 (−4.9, −1.6)	148	125 (67)
Present (16, 9)	36.2 (27.9, 53.5)	3.3 (2.2, 4.0)	2.6 (1.0–6.1)	−3.9 (−8.5, 9.4)	12	10 (63)
3901 (15, 8)	32.0 (25.1, 53.5)	3.3 (2.2, 3.5)	3.0 (1.0–6.1)	−4.1 (−8.5, 24.1)	11	9 (60)
3906 (1, 1)	40.6	5.5	2.0	−3.4	1	1 (100)
Absent (171, 91)	49.5 (44.8, 54.0)	3.5 (2.3, 5.5)	3.1 (0.8–14.5)	−3.3 (−5.1, −1.5)	136	115 (67)

Major allele is marked in bold. †Within each SNP, alleles associated with T1D risk are presented first.

regions studied (*PTPN2* [rs45450798], *FUT2* [rs601338], and *CTSH* [rs3825932]) in the additive model ($P = 0.013$, $P = 0.020$, and $P = 0.0042$, respectively) (Table 3).

In general, children carrying susceptibility alleles had a more rapid decline in insulin secretion compared with those who did not carry a susceptibility allele. Children homozygous for the diabetes-associated risk allele in *IKZF4* and *PTPN2* genes had a steeper decline of FPIR than those who were not homozygous for the risk allele in these genes (recessive model $P = 0.026$ and $P = 0.0035$, respectively) (Table 3). Children carrying the T1D-associated risk allele in *FUT2* and *CTSH* genes experienced also a steeper decline of FPIR than those without the risk allele in these genes (dominant model $P = 0.0098$ and $P = 0.0011$, respectively) (Table 3). In an analysis where risk scores were calculated on the basis of T1D risk in four SNPs that were significant in the model, there were no clearly additive effects (data not shown).

The class I HLA *A*24* allele was also associated with the evolution of FPIR in children with multiple autoantibodies ($P = 0.037$) (Table 3) so that the presence of the *A*24* allele was associated with a steeper coefficient

Table 2—The median of the first FPIR and ΔFPIR over time according to different genotypes from 243 children with zero or one biochemical autoantibody at the time of the first IVGTT

SNP (*n*, % of total within the gene)†	Baseline FPIR (mU/L), median (95% CI)	Age at the first IVGTT (years), median (IQR)	Time between last and first IVGTT (years), median (range)	ΔFPIR (mU/L/year)		Progressors, *n* (%)
				Median (95% CI)	*n*	
PTPN22 (237)	77.6 (72.5, 87.8)	6.1 (3.6, 8.1)	2.3 (0.4–11.0)	4.2 (2.6, 10.8)	88	3 (1)
AA (3, 1)	74.3 (39.8, 281.2)	6.1 (5.7, 7.5)	NA (0)	NA	0	0
A**G** (48, 20)	73.0 (62.0, 91.9)	4.6 (3.6, 7.5)	2.1 (0.4–6.6)	7.8 (2.9, 15.0)	20	0
GG (186, 79)	80.1 (74.3, 89.8)	6.4 (3.6, 8.2)	2.5 (0.6–11.0)	3.8 (−0.3, 11.2)	68	3 (2)
IFIH1 (128)	75.7 (67.4, 88.8)	5.1 (3.0, 7.8)	2.3 (0.4–11.0)	3.7 (1.5, 11.2)	68	3 (2)
TT (45, 35)	69.0 (57.2, 82.7)	3.8 (2.9, 7.2)	2.5 (0.6–7.4)	−0.3 (−2.2, 12.7)	19	3 (7)
C**T** (60, 47)	77.2 (63.0, 107.9)	6.2 (3.5, 8.5)	2.5 (0.4–11.0)	8.0 (3.1, 15.1)	33	0
CC (23, 18)	80.2 (66.0, 115.3)	5.6 (2.5, 6.8)	2.0 (0.8–7.4)	1.5 (−12.3, 15.0)	16	0
INS (239)	77.6 (72.0, 87.1)	6.0 (3.6, 8.1)	2.3 (0.4–11.0)	4.0 (2.6, 10.8)	88	3 (1)
AA (151, 63)	76.0 (68.4, 87.8)	6.3 (3.6, 8.2)	2.2 (0.4–7.7)	3.4 (1.6, 10.8)	64	3 (2)
AT (79, 33)	82.7 (69.4, 102.1)	5.3 (3.7, 7.5)	2.9 (0.8–11.0)	6.0 (−1.5, 13.0)	23	0
TT (9, 4)	117.8 (53.1, 125.5)	6.4 (3.6, 8.3)	6.4	12.6	1	0
IKZF4 (125)	75.2 (66.4, 87.1)	5.3 (3.0, 7.8)	2.3 (0.4–11.0)	3.9 (1.6, 11.2)	68	2 (2)
CC (14, 11)	69.8 (50.5, 119.8)	3.9 (2.3, 7.6)	2.1 (0.4–4.3)	−1.0 (−9.0, 18.1)	7	0
AC (51, 41)	75.2 (61.4, 98.0)	6.9 (3.6, 8.5)	2.3 (0.6–7.4)	6.2 (−0.3, 15.0)	26	2 (4)
AA (60, 48)	77.0 (66.0, 100.0)	4.6 (3.0, 6.8)	2.6 (0.8–11.0)	3.8 (−1.0, 11.2)	35	0
ERBB3 (236)	77.4 (71.5, 86.8)	6.1 (3.6, 8.1)	2.3 (0.4–11.0)	4.0 (2.6, 10.8)	86	2 (1)
AA (22, 9)	92.4 (64.3, 140.9)	6.3 (2.5, 8.9)	2.4 (0.4–7.7)	5.7 (−9.0, 30.0)	6	0
CA (110, 47)	77.6 (69.3, 89.1)	6.8 (4.5, 8.1)	2.2 (0.6–7.4)	6.1 (2.6, 12.8)	37	2 (2)
CC (104, 44)	75.4 (67.4, 91.9)	5.0 (3.1, 7.7)	2.3 (0.8–11.0)	3.7 (−0.5, 11.2)	43	0
CTSH (241)	77.6 (72.5, 87.8)	6.1 (3.6, 8.1)	2.3 (0.4–11.0)	4.1 (2.6, 10.8)	89	3 (12)
CC (89, 37)	74.3 (65.3, 92.9)	6.0 (4.1, 8.1)	2.2 (0.6–6.6)	4.5 (1.6, 11.2)	34	1 (1)
CT (114, 47)	80.1 (71.5, 93.1)	6.2 (3.2, 8.1)	2.5 (0.4–7.7)	6.9 (−0.5, 16.9)	42	2 (2)
TT (38, 16)	88.1 (67.4, 98.0)	6.2 (4.4, 8.0)	2.0 (0.8–11.0)	2.7 (−12.3, 12.7)	13	0
PTPN2 (238)	77.6 (72.0, 87.1)	6.1 (3.6, 8.1)	2.3 (0.4–11.0)	4.1 (2.6, 10.8)	89	3 (1)
CC (5, 2)	88.8 (69.3, 131.5)	6.3 (4.2, 8.8)	3.1 (1.8–4.3)	5.9 (−1.0, 12.8)	2	0
GC (67, 28)	74.3 (65.0, 86.8)	6.1 (4.0, 8.3)	1.9 (0.4–11.0)	2.7 (−1.7, 14.9)	26	2 (3)
GG (166, 70)	80.2 (72.5, 93.1)	6.1 (3.5, 8.0)	2.5 (0.8–7.7)	5.2 (2.7, 11.3)	61	1 (1)
FUT2 (27)	97.0 (64.3, 131.5)	7.6 (2.3, 15.1)	2.9 (0.6–5.9)	8.9 (−2.5, 26.3)	14	2 (7)
AA (3, 11)	82.7 (37.6, 131.5)	4.5 (2.9, 9.1)	3.5 (3.3–3.7)	−1.5 (−2.5, −0.5)	2	1 (33)
GA (19, 70)	97.0 (60.0, 128.6)	7.8 (3.0, 8.5)	2.9 (0.6–5.9)	12.7 (−5.0, 26.3)	10	1 (5)
GG (5, 19)	179.6 (55.8, 362.4)	7.6 (4.9, 12.3)	1.7 (1.4–2.0)	−8.1 (−71.0, 54.9)	2	0
Class I HLA alleles						
*A*24* (233)	77.6 (72.5, 87.1)	6.1 (3.7, 8.1)	2.3 (0.4–11.0)	4.2 (2.6, 10.8)	84	2 (1)
Present (48, 20)	76.7 (65.3, 117.8)	4.9 (2.9, 7.8)	2.1 (1.1–11.0)	11.2 (1.6, 26.0)	16	1 (2)
Absent (185, 76)	78.2 (72.0, 87.1)	6.3 (4.0, 8.2)	2.3 (0.4–7.4)	4.0 (2.3, 9.6)	68	1 (1)
*B*39* (232)	77.4 (72.0, 86.8)	6.1 (3.6, 8.1)	2.3 (0.4–11.0)	5.2 (2.8, 11.3)	85	2 (1)
Present (19, 8)	98.0 (54.1, 144.6)	5.0 (2.9, 8.3)	2.2 (1.1–11.0)	12.9 (−1.7, 187.8)	7	1 (5)
3901 (16, 7)	80.5 (43.9, 130.0)	4.8 (2.5, 8.1)	3.0 (1.2–11.0)	7.2 (−1.7, 46.2)	6	1 (6)
3906 (3, 1)	148.0 (57.2, 186. 8)	7.7 (3.5, 9.8)	1.1	187.8	1	0
Absent (213, 92)	77.2 (72.0, 85.7)	6.1 (3.7, 8.1)	2.4 (0.4–7.7)	4.8 (2.8, 11.2)	78	1 (0)

Major allele is marked in bold. NA, not available. †Within each SNP, alleles associated with T1D risk are presented first.

estimate of FPIR (−0.00037, SE 0.000098, $P = 0.0002$) (Table 3). In children without multiple autoantibodies, the FPIR increased over time independent on *A*24* allele status (Table 2). Furthermore, in children without multiple autoantibodies, *ERBB3* (rs2292239) showed a significant association with FPIR in the recessive model ($P = 0.0075$) (Table 4).

DISCUSSION

In this study, we identified novel associations between FPIR and genetic variants known to affect T1D. In children with multiple autoantibodies, the change in FPIR over time was different between those categorized by their *PTPN2* (rs45450798), *FUT2* (rs601338), *CTSH* (rs3825932), and *IKZF4* (rs1701704) genotypes. Children carrying disease susceptibility alleles had a more rapid decline in insulin secretion over time compared with those who did not carry the allele associated with susceptibility for T1D.

Homozygosity for the risk alleles in the *IKZF4* and *PTPN2* genes was associated with a steeper decline of FPIR compared with nonhomozygosity. *IKZF4* encodes

Table 3—FPIR as analyzed by a hierarchical linear mixed model adjusted for age from 195 children with multiple autoantibodies at the time of the first IVGTT

SNP†	Gene (*n*)	Model *P* value			Coefficient estimate (SE) of FPIR in each genotype	*P* value of individual coefficient estimate‡	Comparison of the coefficient estimates between genotypes	*P* value
		Additive	Recessive	Dominant				
rs2476601	*PTPN22* (193)	0.36	0.84	0.22	AA −0.00020 (0.000129)	0.13	AA vs. AG	0.48
					AG −0.00010 (0.000062)	0.13	AA vs. GG	0.98
					GG −0.00020 (0.000040)	<0.0001	AG vs. GG	0.16
rs1990760	*IFIH1* (188)	0.53	0.65	0.41	TT −0.00019 (0.000059)	0.0034	TT vs. CT	0.45
					CT −0.00014 (0.000045)	0.0027	TT vs. CC	0.72
					CC −0.00023 (0.000077)	0.0013	CT vs. CC	0.31
rs689	*INS* (195)	0.53	0.36	0.32	AA −0.00019 (0.000037)	<0.0001	AA vs. AT	0.58
					AT −0.00015 (0.000073)	0.044	AA vs. TT	0.29
					TT −0.00004 (0.000141)	0.78	AT vs. TT	0.50
rs1701704	*IKZF4* (189)	0.068	0.026	0.99	CC −0.00041 (0.000112)	0.00030	CC vs. AC	0.021
					AC −0.00013 (0.000048)	0.0085	CC vs. AA	0.050
					AA −0.00017 (0.000047)	0.0003	AC vs. AA	0.51
rs2292239	*ERBB3* (193)	0.49	0.24	0.94	AA −0.00030 (0.000118)	0.01	AA vs. AC	0.23
					AC −0.00015 (0.000048)	0.0014	AA vs. CC	0.29
					CC −0.00017 (0.000047)	0.0004	AC vs. CC	0.79
rs3825932	*CTSH* (193)	0.0042	0.42	0.0011	CC −0.00022 (0.000061)	0.0004	CC vs. CT	0.80
					CT −0.00024 (0.000044)	<0.0001	CC vs. TT	0.0078
					TT 0.000031 (0.000070)	0.65	CT vs. TT	0.0013
rs45450798	*PTPN2* (192)	0.013	0.0035	0.99	CC −0.00107 (0.000308)	0.0006	CC vs. CG	0.0031
					CG −0.00014 (0.000060)	0.0205	CC vs. GG	0.0040
					GG −0.00017 (0.000039)	<0.0001	CG vs. GG	0.64
rs601338	*FUT2* (169)	0.020	0.054	0.0098	AA −0.00031 (0.000081)	0.0001	AA vs. AG	0.26
					AG −0.00020 (0.000050)	<0.0001	AA vs. GG	0.0085
					GG −0.00005 (0.000057)	0.36	AA vs. GG	0.045

Class I HLA alleles

Allele (*n*)	Status (*n*)	Model *P* value	Coefficient estimate (SE)	*P* value of individual coefficient estimate‡
*A*24* (183)	Present (32)	0.037	−0.00037 (0.000098)	0.0002
	Absent (151)		−0.00015 (0.000035)	<0.0001
*B*3901* (186)	Present (15)	0.10	0.000049 (0.000139)	0.73
	Absent (171)		−0.00018 (0.000034)	<0.0001

†Within each SNP, alleles associated with T1D risk are presented first. ‡*P* value of individual coefficient indicates whether genotype influences FPIR (null hypothesis is that coefficient estimate equals 0).

Table 4—FPIR as analyzed by a hierarchical linear mixed model adjusted for age and the number of autoantibodies from 243 children without multiple autoantibodies

SNP†	Gene (*n*)	Model *P* value			Coefficient estimate (SE) of FPIR in each genotype	*P* value of individual coefficient estimate‡	Comparison of the coefficient estimates between genotypes	*P* value
		Additive	Recessive	Dominant				
rs2476601	*PTPN22* (237)	0.96	NA	0.97	AA NA		AA vs. AG	
					AG 0.000198 (0.000107)	0.064	AA vs. GG	
					GG 0.000192 (0.000054)	0.0005	AG vs. GG	0.96
rs1990760	*IFIH1* (128)	0.25	0.15	0.94	TT 0.000068 (0.000083)	0.21	CC vs. CT	0.40
					CT 0.000242 (0.000074)	0.0015	CC vs. TT	0.64
					CC 0.000131 (0.000104)	0.41	CT vs. TT	0.10
rs689	*INS* (239)	0.20	0.54	0.20	AA 0.000193 (0.000061)	0.0016	AA vs. AT	0.31
					AT 0.000097 (0.000084)	0.25	AA vs. TT	0.18
					TT 0.000521 (0.000229)	0.024	AT vs. TT	0.09
rs1701704	*IKZF4* (125)	0.62	0.43	0.99	CC 0.000031 (0.000165)	0.33	CC vs. AC	0.33
					AC 0.000206 (0.000072)	0.38	CC vs. AA	0.38
					AA 0.000187 (0.000069)	0.0072	AC vs. AA	0.84
rs2292239	*ERBB3* (236)	0.024	0.0075	0.56	AA 0.000620 (0.000158)	<0.0001	AA vs. AC	0.0079
					AC 0.000176 (0.000065)	0.0003	AA vs. CC	0.0099
					CC 0.000190 (0.000069)	0.0001	AC vs. CC	0.87
rs3825932	*CTSH* (241)	0.15	0.33	0.20	CC 0.000121 (0.000077)	0.12	CC vs. CT	0.15
					CT 0.000257 (0.000063)	<0.0001	CC vs. TT	0.55
					TT 0.000036 (0.000122)	0.77	CT vs. TT	0.098
rs45450798	*PTPN2* (238)	0.52	0.62	0.26	CC 0.000086 (0.000232)	0.14	CC vs. CG	0.96
					CG 0.000100 (0.000101)	0.32	CC vs. GG	0.60
					GG 0.000213 (0.000055)	0.0002	CG vs. GG	0.29
rs601338	*FUT2* (27)	0.43	0.15	0.91	AA 0.000236 (0.000296)	0.43	AA vs. AG	0.19
					AG 0.000576 (0.000193)	0.0063	AA vs. GG	0.60
					GG 0.000507 (0.000499)	0.32	AG vs. GG	0.89

Class I HLA alleles

Allele (*n*)	Status (*n*)	Model *P* value	Coefficient estimate (SE)	*P* value of individual coefficient estimate‡
*A*24* (233)	Absent (185)	0.11	0.000187 (0.000054)	<0.0008
	Present (48)		0.000327 (0.000103)	0.0011
*B*3901* (229)	Absent (16)	0.28	0.000222 (0.000051)	<0.0001
	Present (213)		0.000060 (0.000145)	0.68

NA, not available. †Within each SNP, alleles associated with T1D risk are presented first. ‡*P* value of individual coefficient indicates whether genotype influences FPIR (null hypothesis is that coefficient estimate equals 0).

for Eos, which is known to play an important role in lymphoid development (17). A decreased tyrosine phosphatase expression associated with the *PTPN2* variant has been shown to sensitize β-cells to cytokine-induced apoptosis (18).

Children with multiple autoantibodies carrying at least one risk allele in the *CTSH* and *FUT2* genes were characterized by a steeper decline of FPIR compared with those who did not carry a risk allele. In recently diagnosed children, however, it was, the CT genotype of *CTSH* that was associated with the lowest dose of insulin, and the children with the CT genotype were most often in remission 12 months after onset compared with those with other genotypes (11). Interestingly, in healthy adults, the *CTSH* genotype affected β-cell function in the oral glucose tolerance test but showed no effect on FPIR (11).

Fructosyltransferase 2 enzyme in the Golgi apparatus is involved in the creation of a precursor of the H antigen, which is needed in the synthesis of A and B antigens found in secretions. Individuals carrying the major allele G are called secretors, and they have a functional *FUT2* gene (19). In the current study, we observed a difference between children carrying the AA or AG genotype versus the GG genotype. The mechanisms underlying the association between *FUT2* and FPIR are not known but could be related by the observation that the secretor status has been associated with composition of the human microbiome (20), although this is controversial (21).

IFIH1, *PTPN22*, and *INS* did not show any association with FPIR in this study, which could partly be explained by the observation that they all have been found in the DIPP study to have their main effect on the development of islet autoimmunity (5). It is not known whether associations between insulin secretion and various genotypes would be different in children without or before the appearance of islet autoantibodies. In autoantibody-positive children carrying both *INS* risk alleles but without class II HLA risk, the increase of FPIR was slower than in children who carried one or no *INS* risk alleles (12). Some effect of these genes could potentially be seen in subgroups; for example, the association of caesarean section with the development of T1D was reported to be affected by the *IFIH1* genotype (22).

Hyperexpression of class I HLA antigens is often seen in pancreatic islets from patients with T1D (23). In this study, the presence of the class I HLA *A*24* allele was associated with a steeper decline of FPIR in children with multiple autoantibodies. The presence of the *A*24* allele has previously been reported to predict rapid progression to clinical disease in autoantibody-positive relatives of patients with T1D (24).

The unique possibility to analyze young, genetically predisposed children followed intensively over a relatively long period is a strength of this study. A weakness is the low number of observations within some genotypes, which reduces the statistical power. We did not analyze FPIR and its changes over time in relation to the initiating autoantibody (5,9). Although the overall effect of the genetic markers studied on FPIR is modest, it is conceivable that quite a variation in the β-cell mass exists. A wide range of the estimated β-cell mass was observed in adults, even in subjects with low FPIR and multiple autoantibodies (25).

In conclusion, our results show that certain genetic variants outside the class II HLA region can have a significant impact on the longitudinal pattern of FPIR. In children with multiple autoantibodies, the diabetes risk alleles were associated with more rapid loss in β-cell secretory capacity. The underlying mechanisms are still unknown.

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References

1. Koskinen MK, Helminen O, Matomäki J, et al. Reduced β-cell function in early preclinical type 1 diabetes. Eur J Endocrinol 2016;174:251–259

2. Insel RA, Dunne JL, Atkinson MA, et al. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. Diabetes Care 2015;38:1964–1974

3. Ilonen J, Kiviniemi M, Lempainen J, et al.; Finnish Pediatric Diabetes Register. Genetic susceptibility to type 1 diabetes in childhood - estimation of HLA class II associated disease risk and class II effect in various phases of islet autoimmunity. Pediatr Diabetes 2016;17(Suppl. 22):8–16

4. Mikk ML, Heikkinen T, El-Amir MI, et al.; Finnish Paediatric Diabetes Register. The association of the HLA-A*24:02, B*39:01 and B*39:06 alleles with type 1 diabetes is restricted to specific HLA-DR/DQ haplotypes in Finns. HLA 2017; 89:215–224

5. Lempainen J, Laine AP, Hammais A, et al. Non-HLA gene effects on the disease process of type 1 diabetes: from HLA susceptibility to overt disease. J Autoimmun 2015;61:45–53

6. Krischer JP, Lynch KF, Lernmark Å, et al.; TEDDY Study Group. Genetic and environmental interactions modify the risk of diabetes-related autoimmunity by 6 years of age: the TEDDY study. Diabetes Care 2017;40:1194–1202

7. Sharma A, Liu X, Hadley D, et al.; TEDDY Study Group. Identification of non-HLA genes associated with development of islet autoimmunity and type 1 diabetes in the prospective TEDDY cohort. J Autoimmun 2018;89:90–100

8. Koskinen MK, Lempainen J, Löyttyniemi E, et al. Class II HLA genotype association with first-phase insulin response is explained by islet autoantibodies. J Clin Endocrinol Metab 2018;103:2870–2878

9. Ilonen J, Lempainen J, Hammais A, et al.; Finnish Pediatric Diabetes Register. Primary islet autoantibody at initial seroconversion and autoantibodies at diagnosis of type 1 diabetes as markers of disease heterogeneity. Pediatr Diabetes 2018;19:284–292
10. Winkler C, Lauber C, Adler K, et al. An interferon-induced helicase (IFIH1) gene polymorphism associates with different rates of progression from autoimmunity to type 1 diabetes. Diabetes 2011;60:685–690
11. Fløyel T, Brorsson C, Nielsen LB, et al. CTSH regulates β-cell function and disease progression in newly diagnosed type 1 diabetes patients. Proc Natl Acad Sci U S A 2014;111:10305–10310
12. Halminen M, Veijola R, Reijonen H, Ilonen J, Åkerblom HK, Knip M. Effect of polymorphism in the insulin gene region on IDDM susceptibility and insulin secretion. The Childhood Diabetes in Finland (DiMe) Study Group. Eur J Clin Invest 1996;26:847–852
13. Siljander HT, Simell S, Hekkala A, et al. Predictive characteristics of diabetes-associated autoantibodies among children with HLA-conferred disease susceptibility in the general population. Diabetes 2009;58:2835–2842
14. Salonen KM, Ryhänen S, Härkönen T, Ilonen J, Knip M; Finnish Pediatric Diabetes Register. Autoantibodies against zinc transporter 8 are related to age, metabolic state and HLA DR genotype in children with newly diagnosed type 1 diabetes. Diabetes Metab Res Rev 2013;29:646–654
15. Bottazzo GF, Florin-Christensen A, Doniach D. Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. Lancet 1974;2: 1279–1283
16. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15:539–553
17. Sharma MD, Huang L, Choi JH, et al. An inherently bifunctional subset of Foxp3+ T helper cells is controlled by the transcription factor eos. Immunity 2013; 38:998–1012
18. Moore F, Colli ML, Cnop M, et al. PTPN2, a candidate gene for type 1 diabetes, modulates interferon-gamma-induced pancreatic β-cell apoptosis. Diabetes 2009;58:1283–1291
19. Smyth DJ, Cooper JD, Howson JM, et al. FUT2 nonsecretor status links type 1 diabetes susceptibility and resistance to infection. Diabetes 2011;60:3081–3084
20. Wacklin P, Mäkivuokko H, Alakulppi N, et al. Secretor genotype (FUT2 gene) is strongly associated with the composition of Bifidobacteria in the human intestine. PLoS One 2011;6:e20113
21. Turpin W, Bedrani L, Espin-Garcia O, et al. FUT2 genotype and secretory status are not associated with fecal microbial composition and inferred function in healthy subjects. Gut Microbes 2018;9:357–368
22. Bonifacio E, Warncke K, Winkler C, Wallner M, Ziegler AG. Cesarean section and interferon-induced helicase gene polymorphisms combine to increase childhood type 1 diabetes risk. Diabetes 2011;60:3300–3306
23. Richardson SJ, Rodriguez-Calvo T, Gerling IC, et al. Islet cell hyperexpression of HLA class I antigens: a defining feature in type 1 diabetes. Diabetologia 2016; 59:2448–2458
24. Mbunwe E, Van der Auwera BJ, Vermeulen I, et al.; Belgian Diabetes Registry. HLA-A*24 is an independent predictor of 5-year progression to diabetes in autoantibody-positive first-degree relatives of type 1 diabetic patients. Diabetes 2013;62:1345–1350
25. Hao W, Woodwyk A, Beam C, Bahnson HT, Palmer JP, Greenbaum CJ. Assessment of β cell mass and function by AIRmax and intravenous glucose in high-risk subjects for type 1 diabetes [published correction appears in J Clin Endocrinol Metab 2018;103:1653]. J Clin Endocrinol Metab 2017;102:4428–4434