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Context: Characterization of slow progression to type 1 diabetes (T1D) may reveal novel means for prevention of T1D. Slow progressors might carry natural immunomodulators that delay β-cell destruction and mediate preservation of β-cell function.

Objective: To identify demographic, genetic, and immunological characteristics of slow progression from seroconversion to clinical T1D.

Design: HLA-susceptible children (n=7410) were observed from birth for islet cell (ICA), insulin (IAA), GAD_{65} (GADA), and islet antigen-2 autoantibodies (IA-2A), and for clinical T1D. Disease progression that lasted ≥7.26 years (slowest tertile) from initial seroconversion to diagnosis was considered slow. Autoantibody and genetic characteristics including 45 non-HLA single nucleotide polymorphisms (SNPs) predisposing to T1D were analyzed.

Results: By the end of 2015, altogether 1528 children (21%) had tested autoantibody positive, and 247 (16%) had progressed to T1D. The median delay from seroconversion to diagnosis was 8.7 years in slow (n=62, 25%) and 3.0 years in other progressors. Compared to other progressors, slow progressors were less often multipositive, had lower ICA and IAA titers, and lower frequency of IA-2A at seroconversion. Slow progressors were born more frequently in the fall, while other progressors were born more often in the spring. Compared to multipositive non-progressors, slow progressors were younger, had higher ICA titers, and higher frequency of IAA and multiple autoantibodies at seroconversion. We found no differences in the distributions of non-HLA SNPs between progressors.

Conclusions: We observed differences in autoantibody characteristics and the season of birth among progressors, but no characteristics present at seroconversion that were specifically predictive for slow progression.

We set out to characterize slow progression from seroconversion to overt type 1 diabetes. We found differences in autoantibodies and the season of birth, but no features specific for slow progression.

Introduction

Before the onset of clinical type 1 diabetes (T1D), autoantibodies against multiple β-cell antigens appear into the circulation as the first detectable sign of disease activity (1). The preclinical autoantibody profiles together with genetic factors can be used in disease prediction (2–3). Observations on individuals with islet autoantibodies have shown that the duration of this subclinical period is highly individual (4). It is plausible that critically timed environmental triggers are necessary for the initiation of the autoimmune response against the β-cells, but that individual genetic, immunological, or environmental mechanisms may modify the rate of the disease progression.

We have previously characterized rapid progressors to type 1 diabetes and observed that such children were younger, had higher autoantibody titers and tested more frequently positive for multiple autoantibodies at the time of seroconversion (5). The current study aims at describing the demographic, genetic, and immunological characteristics of slow disease progression in HLA-predisposed children recruited from the general population. Identifying the factors decisive for the progression rate might reveal insights into the pathomechanisms of the disease, lead to the discovery of natural immunomodulators and protective mechanisms behind the preservation of β-cell function, and provide a rationale for future interventions aimed at preventing or delaying the onset of clinical T1D.

Materials and Methods

Study participants and their samples

The Type 1 Diabetes Prediction and Prevention (DIPP) Study is a prospective birth cohort study carried out in three Finnish University Hospitals in Oulu, Tampere, and Turku. The aims of the study are to monitor the development of islet-specific autoimmunity in children with T1D-associated HLA risk genotypes recruited from the general population, and to identify strategies to delay or prevent clinical T1D in individuals at risk (6).

In the DIPP Study, the major disease risk-associated HLA genotypes have been assessed from cord blood. Study participants with eligible HLA-DR/DQ genotypes have been invited to follow-up, including immunological assessment, from the age of 3 months (6). The study has been carried out according to the principles of the Declaration of Helsinki. The local ethics committees of the participating hospitals have approved the study protocol. The legal

representatives of the newborn infants have given written informed consents for participation in the DIPP study.

The immunological follow-up includes regular venous blood sampling at clinical visits, starting from 3 months of age and continuing every 3 to 6 months until the age of 12–24 months and thereafter every 6–12 months (7). Children developing autoantibodies are invited to subsequent follow-up visits every 3 months in all three study centers.

The current study cohort comprised 7410 children (52.6% boys) who had participated in the follow-up for at least one year or had progressed to diabetes before the age of one year by the end of 2003 (Figure 1, Table 1). No cases of neonatal diabetes were observed. Participants with confirmed autoantibody positivity before T1D diagnosis were included in the detailed autoantibody analyses.

Genetic screening

Genotyping for the T1D risk-associated HLA-DR/DQ haplotypes was performed on cord blood samples by using PCR amplification, followed by time-resolved fluorometry with lanthanide-labeled sequence-specific oligonucleotide probes, as previously described (6,8). The eligible children carried either the high-risk *HLA-DQB1*02/*03:02* genotype or moderate-risk associated *HLA-DQB1*03:02/x* genotypes ($x \neq DQB1*02$, *03:01*, or *06:02*) (9,10).

Single nucleotide polymorphism (SNP) markers were analyzed from individuals testing positive for at least one biochemical autoantibody [autoantibodies to insulin (IAA), glutamic acid decarboxylase (GADA), and/or islet antigen 2 (IA-2A)] in addition to islet cell antibodies (ICA) and from their autoantibody negative controls (two controls per case) matched for sex, study center, and the closest birth date (11). SNP genotypes were analyzed by using the Sequenom platform (San Diego, California, USA; Genome Center of Eastern Finland, University of Eastern Finland, Kuopio, Finland). Genotyping was considered successful if the failure rate remained under 10% (12).

Single nucleotide polymorphisms of non-HLA genes predisposing to T1D were selected for analysis by using a web-based resource comprising information on the genetics and the genomics of immune-mediated diseases in man (*www.immunobase.org*, formerly *t1database.org*). Regarding the current study cohort, data were available for 45 non-HLA SNPs predisposing to T1D (13–22). The associations of the predisposing SNPs with age at seroconversion, age at diagnosis of T1D, time from single to multiple autoantibody positivity, delay from seroconversion to diagnosis among progressors, and disease-free time after seroconversion in all participants with confirmed seropositivity were analyzed.

Assessment of diabetes-associated autoantibodies

The analyses of diabetes-associated autoantibodies were performed in the Research Laboratory, Department of Pediatrics, University of Oulu (Oulu, Finland). In the DIPP Study, the primary screening for β-cell autoimmunity was based on ICA from 1994 to 2003. The biochemical autoantibodies IAA, GADA, and IA-2A were analyzed from each child developing ICA positivity and/or T1D. All samples from all new participants have been analyzed for ICA and biochemical autoantibodies since 2003 and from the first one thousand children participating in the follow-up (from November 1994 to July 1997). For this study cohort, no data were available on zinc transporter 8 autoantibodies.

The analysis of ICA was performed using a standardized indirect immunofluorescence staining method, as previously described (23). The specific radiobinding assays used for the analysis of biochemical autoantibodies have been described in our earlier reports (7,24–26). According to the results from Diabetes Autoantibody Standardization Program (DASP) and the

Islet Autoantibody Standardization Program (IASP) in 2010–2015, the disease sensitivities of the IAA, GADA, and IA-2A radiobinding assays have been 36–62%, 64–88%, and 62–72%, respectively, while the corresponding disease specificities have been 94–98%, 94–99%, and 93–100%, respectively.

Definitions

The date of draw for the first autoantibody-positive sample was defined as the date of seroconversion to autoantibody positivity. Confirmed autoantibody positivity was defined as positivity for at least one autoantibody in at least two consecutive samples. If the child had initially only one positive sample, but later on tested autoantibody positive in at least two consecutive samples, the date of the first positive sample was defined as the date of seroconversion. Simultaneous positivity for two or more autoantibodies in the same sample was considered as multipositivity. Inverse seroconversions, i.e. disappearances of autoantibodies were not considered. Type 1 diabetes was diagnosed according to the WHO criteria (27).

Progression to T1D was considered slow in cases where the disease-free period from seroconversion to the diagnosis of T1D lasted for at least 7.26 years. The cut-off was set at the 75th percentile value of the delay from seroconversion to diagnosis among progressors. Although we have no biological evidence to support the selected cut-off (Figure 2), this definition provides a setting that enables us to investigate factors associated with a significantly longer delay from seroconversion to diagnosis than observed in the majority of the progressors in the DIPP Study cohort. In the whole study cohort, the median duration of prediabetic phase is 4.0 years. The multipositive non-progressors were defined as individuals with multiple autoantibodies, who remained unaffected by T1D by the end of 2015, and had been followed up for at least 7.26 years after seroconversion to autoantibody positivity.

Data management and statistical analyses

IBM SPSS predictive analytics software for Macintosh (version 25.0, Armonk, NY, USA) was used for multiple parametric and non-parametric statistical analyses. The confidence interval was set at 95% and the statistical significance at $P<0.05$ (two-sided). Cross-tabulation, the Pearson's χ^2 test, the Fisher's exact test, the Mann-Whitney U test, and the Kruskall-Wallis test were used to test statistical differences between the study subgroups. Categorical variables were analyzed by using the Pearson's χ^2 test and the Fisher's exact tests, when appropriate. Non-parametric variables were analyzed by using the Mann-Whitney U and the Kruskall-Wallis tests. While comparing autoantibody levels, only individuals who tested positive for the specific autoantibody reactivity were included in the analyses. In the SNP analyses, P values of the multiple comparisons were corrected for by applying the Benjamini-Hochberg step-up procedure using the cumulative minimum principle (R software for statistical computing for Macintosh 3.3.3) (28).

Results

Identifying study subgroups

The current study cohort was observed from birth over a median follow-up time of 16.2 years (range 0.9–21.1 years). Among these children, 1528 (20.6%) had confirmed positivity for at least one T1D-associated autoantibody, and 247 (16.2%) of the children with confirmed autoantibody positivity progressed to T1D by the end of 2015 (Table 1). The median age at seroconversion was 5.0 years (Table 1). Regarding the children with autoantibodies, we focused on three subgroups: the slow progressors, other progressors, and non-progressors who developed multiple autoantibodies during the follow-up. The median delay from seroconversion to diagnosis was 8.7

years (range 7.3–17.0) in slow progressors (n=62, 25.1% of the progressors), and 3.0 years (range 0.02–7.2) in other progressors (n=185, 74.9%) (Table 2, Figure 2).

Slow progressors vs. other progressors

Compared to other progressors, the slow progressors had lower titers of ICA and IAA, and lower frequency of IA-2A and multipositivity at seroconversion (Table 2). Considering progressors who were positive for a single autoantibody at seroconversion, the delay from single to multiple autoantibody positivity was longer in slow progressors compared to other progressors (Table 2). Slow progressors tended to have been born more often in the fall (from September to November) compared to other progressors (31 vs. 22%), while other progressors were born more frequently in the spring (from March to May; 15 vs. 31%; p=0.04) (Figure 3). We observed no significant differences between the two groups in sex distribution, age at seroconversion, prevalence of the high-risk *HLA-DQB1*02/*03:02* genotype, frequency of T1D-affected first-degree relatives at birth of the child, positivity for ICA, IAA, and GADA, or the titers of GADA and IA-2A at seroconversion (Table 2).

Slow progressors vs. multiple autoantibody-positive non-progressors

Since the majority of children with multiple autoantibodies develop clinical disease during childhood, the current multipositive non-progressors may still progress to T1D, although remarkably slowly (29). Thus, as expected, the two groups comprising slow progressors and multipositive non-progressors shared several common characteristics. However, there were also significant differences. Compared to the multipositive non-progressors, the slow progressors were younger, had higher titers of ICA, and higher frequencies of IAA and multiple autoantibodies at seroconversion (Table 2). The multipositive non-progressors tested more often GADA positive at seroconversion without simultaneous presence of IAA (Table 2).

Autoantibody status at seroconversion

As previously observed, the disease process beginning with IAA as the first detectable autoantibody shows a different pattern of β-cell autoimmunity compared to that beginning with GADA positivity (30). This phenomenon tended to hold true also in the current study cohort: most progressors (41.3%), both slow (45.2%) and the other progressors (40.0%), were IAA positive and GADA negative at seroconversion, and only 19.4% had GADA positivity without simultaneous IAA positivity at seroconversion (P=0.27). One third of the multipositive non-progressors (27.8%) were GADA positive and IAA negative at seroconversion, compared to the frequencies of 14.5% and 21.1% among the slow and the other progressors, respectively (P=0.04 compared to all progressors). Notably, 79.0% of all seroconverted non-progressors were single ICA positive at seroconversion (13). As expected, multipositive seroconversions were more common in slow (43.5%) and other progressors (63.2%) compared to seroconverted non-progressors (5.5%; P<0.001). However, our primary focus was on identifying the factors predictive for the pace of the disease process present at seroconversion, and we did not consider the possible loss of observed autoantibody signatures during the follow-up.

Non-HLA SNPs and progression rate

As the SNP data in the current study cohort were originally generated for other purposes, the coverage of the SNP data set was not optimal considering comprehensive analyses in the whole cohort. To survey whether the previously described T1D predisposing SNPs might associate with progression rate, we investigated their association with the delay from seroconversion to diagnosis, and their distributions between the groups of slow progressors, other progressors, and multipositive non-progressors (Table 3; 13). Although several non-HLA SNPs associated with

the delay from seroconversion to diagnosis, after correction for multiple testing by using the Benjamini-Hochberg step-up procedure, the differences in the distributions turned out to be statistically non-significant. For T1D-associated SNPs, the main focus was to find SNPs associated with the pace of disease progression rather than overall progression to T1D. However, considering the maturation of immunity, we found interesting that the predisposing *GPR183/EBI2* SNP seemed to associate with overall progression to T1D among all children tested in the study cohort, regardless of their autoantibody status (*GPR183*/*EBI2* CC, CT genotype, progressors 51 vs. non-progressors 41%; $P=0.008$, $P_c=0.06$). Also, the predisposing *PTPN22*, *INS* (rs689), and *PHTF1* SNPs associated with progression to T1D after correction for multiple comparisons (13).

Discussion

The progressive increase in the incidence rate of type 1 diabetes in most Western countries over the last decades emphasizes the need to generate innovative means for delaying or preventing T1D (31). Individuals with slow disease progression may possess protective mechanisms that postpone the transition from islet autoimmunity to clinical disease. Identifying such mechanisms might provide targets for immunomodulatory therapies aimed at slowing down the disease process.

In this prospective birth cohort study comprising 7410 children recruited from the general population based on HLA-conferred disease risk, we aimed at identifying individuals with slow progression to T1D. We observed differences in autoantibody characteristics and the season of birth among the progressors. However, no specific characteristics present at seroconversion predicted slow progression. The main challenge was to distinguish features related to slow progression from the ones predicting overall progression to T1D.

Compared to other progressors, the slow progressors tested less often positive for multiple autoantibodies and IA-2A, had lower titers of ICA and IAA at seroconversion, and progressed more slowly from single to multiple autoantibody positivity. We observed variation in the seasonality of birth among the progressors: slow progressors were often born in the fall, while other progressors were born more frequently in the spring. Compared to multiple autoantibody-positive non-progressors, slow progressors were younger, had higher ICA titers, and higher frequency of IAA and multiple autoantibodies at seroconversion. The multipositive non-progressors tested more often positive for GADA without the simultaneous presence of IAA at seroconversion.

The DAISY study reported that slow progressors had later onset of islet autoimmunity, were less likely to develop IAA positivity, had lower levels of autoantibodies, especially the initial levels of IAA, and progressed more slowly from single to multiple autoantibody positivity compared to rapid and moderate rate progressors (32). In the BABYDIAB study, later appearance of IA-2A positivity associated with slower disease progression, and some differences were reported in the distributions of non-HLA SNPs (33). A genetic risk score predicted progression rate of islet autoimmunity in the TEDDY cohort (34). Our results support previous observations, showing that lower initial levels of autoantibodies, including IAA, lower frequency of IA-2A positivity at seroconversion, and slower progression from single to multiple autoantibody positivity are characteristic of slow progression to clinical disease. Partly due to non-optimal data coverage and relatively small sample size, we found no differences between the progression groups in the distributions of the analyzed non-HLA alleles.

Since the majority of the multipositive children will eventually develop clinical diabetes, it seems plausible that the current autoantibody-positive non-progressors who later on developed multiple autoantibodies may still progress to T1D, although extremely slowly (29). In fact, compared to both progressors and single autoantibody-positive non-progressors, multipositive non-progressors had a distinct autoantibody profile at seroconversion. This non-progression or ultraslow progression associated pattern of autoantibody positivity starts at an older age with GADA-positive signature. As previously observed, islet autoimmunity initiating with IAA positivity is associated with younger age at diagnosis and homozygosity for the *HLA-DR4-DQ8* genotype, whereas GADA as the first autoantibody is associated with older age and *HLA-DR3-DQ2* homozygosity (30). IAA as the first autoantibody peaks during the second year of life, while GADA reactivity tends to appear later, and with a considerably lower and broader peak (35–37). Individuals testing positive for multiple autoantibodies but remaining unaffected for ≥10 years after the seroconversion are frequently GADA positive in their first sample positive for multiple autoantibodies (38). As the T1D-linked Coxsackievirus B1 infections have been associated with the appearance of IAA as the first autoantibody, but not with initial GADA positivity (39), the current observations support the idea that the triggers of the autoimmune process may be heterogeneous. Moreover, at least to some extent, the first autoantibody signature seems to predict the progression rate to clinical T1D. Comparisons between phenotypically variable progressors and multipositive non-progressors are valuable, since they may reveal protective mechanisms capable of slowing down the disease process.

Although no associations between the T1D-predisposing non-HLA SNPs and the pace of the disease progression were observed after correction for multiple testing, the finding that the predisposing *GPR183/EBI2* SNP tended to associate with overall progression to T1D was notable. This gene encodes the G protein-coupled receptor 183, a chemokine receptor (Ebstein-Barr virus-induced G protein-coupled receptor 2, EBI2), which regulates the migration of the immune cells in lymphoid tissues (reviewed in 40). Deficiencies in EBI2 or its ligand 7α,25-dihydroxycholesterol lead to defective humoral immune responses and impaired thymic negative selection in mice, and may affect central tolerance by controlling the migration rate of medullary thymocytes (40–41). EBI2-associated B cell responses may well be involved in the heterogeneity of the T1D pathogenesis, since in recent-onset T1D patients, two distinct profiles of insulitis (characterized by high vs. low frequencies of CD20-positive B cells) have been associated with significantly different age at diagnosis (42).

The variation observed in the seasonality of birth among the progressors implies that seasonal environmental factors, such as infections or nutritional factors, may play a role in the initiation of the β-cell autoimmunity, and in the determination of the progression rate to clinical disease. It has been suggested that early encounters with seasonal pathogens, especially with viruses, might trigger β-cell autoimmunity as early as *in utero* or in the perinatal period (43). Not surprisingly, certain enteroviruses that have been consistently associated with the initiation of the β-cell autoimmunity are among the strongest candidates for seasonal disease-associated pathogens (reviewed in 44). Considering that enteroviral infections peak in the late summer or early fall in Finland, the seasonality of birth observed among slow and other progressors suggests that enteroviral antigen presentation in the late pregnancy or early neonatal period may, in fact, slow down the disease process (National Infectious Diseases Register, National Institute for Health and Welfare, Finland). This might be explained by the fact that infants born in the spring may be exposed to such antigens at an older age when the protection of the maternal antibodies has vanished. In the DIPP study, the Coxsackievirus B1 infection-associated risk of

islet autoimmunity has been observed to be reduced in the presence of maternal anti-viral antibodies (45). The seasonality of birth in T1D patients has been reported in several populations, but the observations are inconsistent, probably reflecting the variations in the incidence of T1D, in the genetic background, in lifestyle, and in endemic seasonal pathogens of different populations (46).

In conclusion, we observed differences in autoantibody characteristics and seasonality of birth in relation to the progression rate to overt T1D. However, we found no specific characteristics present at seroconversion unambiguously predictive for slow progression. As slow progressors may represent individuals that possess protective mechanisms slowing down the progression to clinical disease, further investigations on the genetic, immunological, and environmental factors modifying the rate of disease progression are needed. They might provide insights into the pathomechanisms of T1D and reveal novel targets for future preventive interventions.

Contribution statement.

P.M.P. collected and researched the data and contributed to the study conception and design, data acquisition, analysis and interpretation, and drafted the first version of the article. H.S. contributed to data acquisition and to discussions, and edited the manuscript. M.K., J.L., A.-P.L., J.I., J.T. and R.V. generated data, contributed to discussion and revised the manuscript for important intellectual content. M.K. designed the study, is the guarantor of this work, had full access to all the data in the study, and takes responsibility for the data integrity and accuracy of the data analysis.

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Disclosure statement:
The authors have nothing to disclose.
Duality of Interest.
No conflicts of interest relevant to this article were reported.

Data availability.

The datasets generated and analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

References

1.   Knip M. Natural course of preclinical type diabetes. Horm Res 2002;57(Suppl 1):6–11
2.   Kimpimäki T, Kulmala P, Savola K, Kupila A, Korhonen S, Simell T, Ilonen J, Simell O, Knip M. Natural history of beta-cell autoimmunity in young children with increased genetic susceptibility to type 1 diabetes recruited from the general population. J Clin Endocrinol Metab 2002;87:4572–4579
3.   Kukko M, Kimpimäki T, Korhonen S, Kupila A, Simell S, Veijola R, Simell T, Ilonen J, Simell O, Knip M. Dynamics of diabetes-associated autoantibodies in young children with human leukocyte antigen-conferred risk of type 1 diabetes recruited from the general population. J Clin Endocrinol Metab 2005;90:2712–2717
4.   Knip M, Korhonen S, Kulmala P, Veijola R, Reunanen A, Raitakari OT, Viikari J, Åkerblom HK. Prediction of type 1 diabetes in the general population. Diabetes Care 2010;33:1206–1212
5.   Pöllänen PM, Lempainen J, Laine AP, Toppari J, Veijola R, Vähäsalo P, Ilonen J, Siljander H, Knip M. Characterisation of rapid progressors to type 1 diabetes among children with HLA-conferred disease susceptibility. Diabetologia 2017;60:1284–1293
6.   Kupila A, Muona P, Simell T, Arvilommi P, Savolainen H, Hämäläinen AM, Korhonen S, Kimpimäki T, Sjöroos M, Ilonen J, Knip M, Simell O; Juvenile Diabetes Research Foundation Centre for the Prevention of Type I Diabetes in Finland. Feasibility of genetic and immunological prediction of type I diabetes in a population-based birth cohort. Diabetologia 2001;44:290–297
7.   Siljander HT, Simell S, Hekkala A, Lähde J, Simell T, Vähäsalo P, Veijola R, Ilonen J, Simell O, Knip M. Predictive characteristics of diabetes-associated autoantibodies among children with HLA-conferred disease susceptibility in the general population. Diabetes 2009;58:2835–2842
8.   Sjöroos M, Iitiä A, Ilonen J, Reijonen H, Lövgren T. Triple-label hybridization assay for type-1 diabetes-related HLA-alleles. Biotechniques 1995;18:870–877
9.   Nejentsev S, Sjöroos M, Soukka T, Knip M, Simell O, Lövgren T, Ilonen J. Population-based genetic screening for the estimation of type 1 diabetes mellitus risk in Finland: selective genotyping of markers in the HLA-DQB1, HLA-DQA1 and HLA-DRB1 loci. Diabet Med 1999;16:985–992

10.   Hermann R, Turpeinen H, Laine AP, Veijola R, Knip M, Simell O, Sipilä I, Åkerblom HK, Ilonen J. HLA DR-DQ-encoded genetic determinants of childhood-onset type 1 diabetes in Finland: an analysis of 622 nuclear families. Tissue Antigens 2003;62:162–169

11.   Lempainen J, Laine AP, Hammais A, Toppari J, Simell O, Veijola R, Knip M, Ilonen J. Non-HLA gene effects on the disease process of type 1 diabetes: From HLA susceptibility to overt disease. J Autoimmun 2015;61:45–53

12.   Laine AP, Knip M, Ilonen J; Finnish Pediatric Diabetes Register. Transmission disequilibrium analysis of 31 type 1 diabetes susceptibility loci in Finnish families. Tissue Antigens 2013;82:35–42

13.   Pöllänen PM, Lempainen J, Laine AP, Toppari J, Veijola R, llonen J, Siljander H, Knip M. Data from: Characteristics of slow progression to type 1 diabetes in children with increased HLA-conferred disease risk. figshare 209. Deposited June 28 2019. https://doi.org/10.6084/m9.figshare.8088623.v2

14.   Barrett JC, Clayton DG, Concannon P, Akolkar B, Cooper JD, Erlich HA, Julier C, Morahan G, Nerup J, Nierras C, Plagnol V, Pociot F, Schuilenburg H, Smyth DJ, Stevens H, Todd JA, Walker NM, Rich SS; Type 1 Diabetes Genetics Consortium. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. Nat Genet 2009;41:703–707

15.   Smyth DJ, Plagnol V, Walker NM, Cooper JD, Downes K, Yang JH, Howson JM, Stevens H, McManus R, Wijmenga C, Heap GA, Dubois PC, Clayton DG, Hunt KA, van Heel DA, Todd JA. Shared and distinct genetic variants in type 1 diabetes and celiac disease. N Engl J Med 2008;359:2767–2777

16.   Todd JA, Walker NM, Cooper JD, Smyth DJ, Downes K, Plagnol V, Bailey R, Nejentsev S, Field SF, Payne F, Lowe CE, Szeszko JS, Hafler JP, Zeitels L, Yang JH, Vella A, Nutland S, Stevens HE, Schuilenburg H, Coleman G, Maisuria M, Meadows W, Smink LJ, Healy B, Burren OS, Lam AA, Ovington NR, Allen J, Adlem E, Leung HT, Wallace C, Howson JM, Guja C, Ionescu-Tîrgovişte C; Genetics of Type 1 Diabetes in Finland, Simmonds MJ, Heward JM, Gough SC; Wellcome Trust Case Control Consortium, Dunger DB, Wicker LS, Clayton DG. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. Nat Genet 2007;39:857–864

17.   Heinig M, Petretto E, Wallace C, Bottolo L, Rotival M, Lu H, Li Y, Sarwar R, Langley SR, Bauerfeind A, Hummel O, Lee YA, Paskas S, Rintisch C, Saar K, Cooper J, Buchan R, Gray EE, Cyster JG; Cardiogenics Consortium, Erdmann J, Hengstenberg C, Maouche S, Ouwehand WH, Rice CM, Samani NJ, Schunkert H, Goodall AH, Schulz H, Roider HG, Vingron M, Blankenberg S, Münzel T, Zeller T, Szymczak S, Ziegler A, Tiret L, Smyth DJ, Pravenec M, Aitman TJ, Cambien F, Clayton D, Todd JA, Hubner N, Cook SA. A trans-acting locus regulates an anti-viral expression network and type 1 diabetes risk. Nature 2010;467:460-464

18.   Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447:661–678

19.   Cooper JD, Smyth DJ, Smiles AM, Plagnol V, Walker NM, Allen JE, Downes K, Barrett JC, Healy BC, Mychaleckyj JC, Warram JH, Todd JA. Meta-analysis of genome-wide association study data identifies additional type 1 diabetes risk loci. Nat Genet 2008;40:1399–1401

20.   Smyth DJ, Cooper JD, Howson JM, Clarke P, Downes K, Mistry T, Stevens H, Walker NM, Todd JA. FUT2 nonsecretor status links type 1 diabetes susceptibility and resistance to infection. Diabetes 2011;60:3081–3084

21.   Kisand K, Uibo R. LADA and T1D in Estonian population – two different genetic risk profiles. Gene 2012;497:285–291

22.   Achenbach P, Lampasona V, Landherr U, Koczwara K, Krause S, Grallert H, Winkler C, Pflüger M, Illig T, Bonifacio E, Ziegler AG. Autoantibodies to zinc transporter 8 and SLC30A8 genotype stratify type 1 diabetes risk. Diabetologia 2009;52:1881–1888

23.   Bottazzo GF, Florin-Christensen A, Doniach D. Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. Lancet 1974;2:1279–1283

24.   Williams AJ, Bingley PJ, Bonifacio E, Palmer JP, Gale EA. A novel micro-assay for insulin autoantibodies. J Autoimmun 1997;10:473–478

25.   Savola K, Bonifacio E, Sabbah E, Kulmala P, Vähäsalo P, Karjalainen J, Tuomilehto-Wolf E, Meriläinen J, Åkerblom HK, Knip M. IA-2 antibodies — a sensitive marker of IDDM with clinical onset in childhood and adolescence. Childhood Diabetes in Finland Study Group. Diabetologia 1998;41:424–429

26.   Savola K, Sabbah E, Kulmala P, Vähäsalo P, Ilonen J, Knip M. Autoantibodies associated with Type I diabetes mellitus persist after diagnosis in children. Diabetologia 1998;41:1293–1297

27.   World Health Organization (WHO), Department of Noncommunicable Disease Surveillance. Definition, diagnosis and classification of diabetes mellitus and its complications. Report of a WHO consultation. Part 1: Diagnosis and Classification of Diabetes Mellitus. Geneva, Switzerland 1999; WHO/NCD/NCS/99.2 59 p.

28.   Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society. Series B (Methodological) 1995;57:289-300

29.   Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, Winkler C, Ilonen J, Veijola R, Knip M, Bonifacio E, Eisenbarth GS. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. JAMA 2013;309:2473–2479

30.   Ilonen J, Hammais A, Laine AP, Lempainen J, Vaarala O, Veijola R, Simell O, Knip M. Patterns of beta-cell autoantibody appearance and genetic associations during the first years of life. Diabetes 2013;62:3636–3640

31.   Patterson CC, Dahlquist GG, Gyürüs E, Green A, Soltész G; EURODIAB Study Group. Incidence trends for childhood type 1 diabetes in Europe during 1989–2003 and predicted new cases 2005-20: a multicenter prospective registration study. Lancet 2009;373:2027–2033

32.   Steck AK, Dong F, Waugh K, Forhnert BI, Yu L, Norris JM, Rewers MJ. Predictors of slow progression to diabetes in children with multiple islet autoantibodies. J Autoimmun 2016;72:113–117

33.   Achenbach P, Hummel M, Thümer L, Boerschmann H, Höfelmann D, Ziegler AG. Characteristics of rapid vs slow progression to type 1 diabetes in multiple islet autoantibody-positive children. Diabetologia 2013;56:1615–1622

34.   Beyerlein A, Bonifacio E, Vehik K, Hippich M, Winkler C, Frohnet BI, Steck AK, Hagopian WA, Krischer JP, Lernmark Å, Rewers MJ, She JX, Toppari J, Akolkar B, Rich SS, Ziegler AG; TEDDY Study Group. Progression from islet autoimmunity to clinical type 1 diabetes is influenced by genetic factors: results from the prospective TEDDY study. J Med Genet 2018; pii: jmedgenet-2018-105532. doi: 10.1136/jmedgenet-2018-105532

35.   Ilonen J, Lempainen J, Hammais A, Laine AP, Härkönen T, Toppari J, Veijola R, Knip M; 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

36.   Krischer JP, Lynch KF, Schatz DA, Ilonen J, Lernmark Å, Hagopian WA, Rewers MJ, She JX, Simell OG, Toppari J, Ziegler AG, Akolkar B, Bonifacio E; TEDDY Study Group. The 6 year incidence of diabetes-associated autoantibodies in genetically at-risk children: the TEDDY study. Diabetologia 2015;58:980–987

37.   Giannopoulou EZ, Winkler C, Chmiel R, Matzke C, Scholz M, Beyerlein A, Achenbach P, Bonifacio E, Ziegler AG. Islet autoantibody phenotypes and incidence in children at increased risk for type 1 diabetes. Diabetologia 2015;58:2317–2323

38.   Long AE, Wilson IV, Becker DJ, Libman IM, Arena VC, Wong FS, Steck AK, Rewers MJ, Yu L, Achenbach P, Casas R, Ludvigsson J, Williams AJK, Gillespie KM. Characteristics of slow progression to diabetes in multiple islet autoantibody-positive individuals from five longitudinal cohorts: the SNAIL study. Diabetologia 2018;61:1484–1490

39.   Sioofy-Khojine AB, Lehtonen J, Nurminen N, Laitinen OH, Oikarinen S, Huhtala H, Pakkanen O, Ruokoranta T, Hankaniemi MM, Toppari J, Vähä-Mäkilä M, Ilonen J, Veijola R, Knip M, Hyöty H. Coxsackievirus B1 infections are associated with the initiation of insulin-driven autoimmunity that progresses to type 1 diabetes. Diabetologia 2018;61:1193–1202

40.   Cyster JG, Dang EV, Reboldi A, Yi T. 25-Hydroxycholesterols in innate and adaptive immunity. Nat Rev Immunol 2014;14:731-743

41.   Sanghee K, Thyagarajan HM, Hu Z, Lancaster JN, Ehrlich LIR. EBI2 contributes to the induction of thymic central tolerance in mice by promoting rapid motility of medullary thymocytes. Eur J Immunol 2017;47:1906-1917

42.   Arif S, Leete P, Nguyen V, Marks K, Nor NM, Estoninho M, Kronenberg-Versteeg D, Bingley PJ, Todd JA, Guy C, Dunger DB, Powrie J, Willcox A, Foulis AK, Richardson SJ, de Rinaldis E, Morgan NG, Lorenc A, Peakman M. Blood and islet phenotypes indicate immunological heterogeneity in type 1 diabetes. Diabetes 2014;63:3835-3845

43.   McKinney PA, Parslow R, Gurney K, Law G, Bodansky HJ, Williams DR. Antenatal risk factors for childhood diabetes mellitus; a case-control study of medical record data in Yorkshire, UK. Diabetologia 1997;40:933–939

44.   Hyöty H, Leon F, Knip M. Developing a vaccine for type 1 diabetes by targeting coxsackievirus B. Expert Rev Vaccines 2018;17:1071–1083

45.   Laitinen OH, Honkanen H, Pakkanen O, Oikarinen S, Hankaniemi MM, Huhtala H, Ruokoranta T, Lecouturier V, André P, Harju R, Virtanen SM, Lehtonen J, Almond JW, Simell T, Simell O, Ilonen J, Veijola R, Knip M, Hyöty H. Coxsackievirus B1 is associated with induction of β-cell autoimmunity that portends type 1 diabetes. Diabetes 2014;63:446–455

46.   Laron Z, Lewy L, Wilderman I, Casu A, Willis J, Redondo MJ, Libman I, White N, Craig M. Seasonality of month of birth of children and adolescents with type 1 diabetes mellitus in homogenous and heterogeneous populations. Isr Med Assoc J 2005;7:381–384

Figure 1 The DIPP Study cohort.

Figure 2 Delay from seroconversion to diagnosis in relation to age at seroconversion in all participants with confirmed seroconversion (Spearman's correlation coefficient 0.051, *P*=0.43).

Figure 3 Season of birth in slow and other progressors.

Figure 3 Season of birth in slow and other progressors. *Slow vs. other progressors. **Slow progressors vs. multipositive non-progressors, Other progressors vs. multipositive non-progressors

Table 1 Background variables and data on islet autoimmunity in the current study cohort (N_{All}=7410). JDFU=Juvenile Diabetes Foundation units, RU=Relative units.

	N (%)
Sex	
Boys	3895 (52.6)
HLA genotype	
High-risk HLA genotype *DQB1*02/*0302*	1575 (21.3)
Moderate-risk HLA genotypes *DQB1*0302/x* [a]	5835 (78.7)
First-degree relatives affected by type 1 diabetes	177 (2.4)
Seroconversion to autoantibody positivity	
≥1 positive autoantibodies in ≥2 consecutive samples	1528 (20.6)
ICA	1214 (16.4)
IAA	301 (4.1)
GADA	257 (3.5)
IA-2A	83 (1.1)
Multipositivity at seroconversion	214 (2.9)
Multipositivity in subsequent samples	272 (3.7)
Progression to clinical type 1 diabetes	
During the follow-up	247 (16.2)
Disease-free for ≥ 7.26 years from seroconversion	62 (25.1% of the progressors)
	Median (range)
Follow-up time, years	16.2 (0.9–21.1)
Delay from seroconversion to diagnosis, years	4.0 (0.02–17.0)
Age at seroconversion, years	5.0 (0.2–15.1)
Age at diagnosis, years	7.6 (0.9–18.0)
Time from single to multiple autoantibody positivity, years	1.0 (0.2–10.5)
Autoantibody titers at seroconversion	
ICA, JDFU (n=1214)	5 (2.5–668)
IAA, RU (n=301)	7.9 (3.5–148.7)
GADA, RU (n=262)	19.5 (5.4–342.1)
IA-2A, RU (n=84)	13.0 (0.4–121.0)

[a] x ≠ DQB1*02, *03:01, *06:02

Table 2 Clinical characteristics for the slow progressors, other progressors, and multiple autoantibody positive non-progressors.

[a] Slow progressors vs. other progressors

[b] Slow progressors vs. multiple autoantibody-positive non-progressors

[c] Other progressors vs. multiple autoantibody-positive non-progressors

	PROGRESSORS			NON-PROGRESSORS		
	Slow progressors (n=6	Other progressors (n=185)	*P* [a]	Multipositive non-progresso (n=198)	*P* [b]	*P* [c]
	N (%)					
HLA genotype						
High risk HLA genotype	24 (39)	78 (42)	0.63	57 (29)	0.14	0.00
Sex						
Boys	35 (57)	107 (58)	0.85	123 (62)	0.43	0.39
Affected first-degree relatives	4 (7)	24 (13)	0.16	14 (7)	1.00	0.05
AAB positivity at seroconversion,						
ICA	30 (48)	112 (61)	0.09	110 (56)	0.32	0.32
IAA	44 (71)	126 (68)	0.67	68 (34)	<0.0	<0.0
GADA	25 (40)	91 (49)	0.23	71 (36)	0.53	0.008
IA-2A	5 (8)	50 (27)	0.002	18 (9)	0.80	<0.0

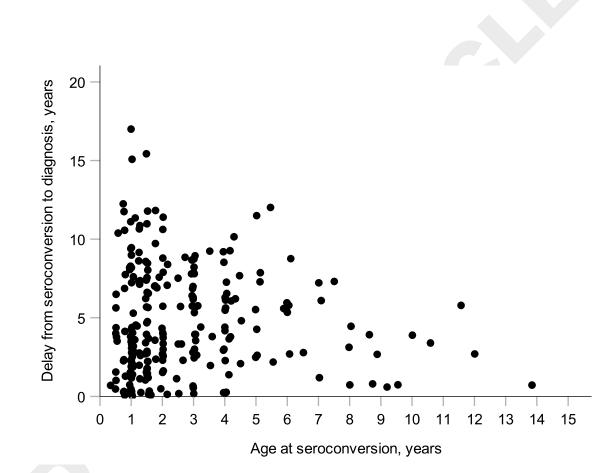|						
IAA positive without GADA	28 (45)	74 (40)	0.48	52 (26)	0.00	0.004
GADA positive without IAA	9 (15)	39 (21)	0.26	55 (28)	0.03	0.13
IAA and GADA	16 (26)	52 (28)	0.73	16 (8)	<0.0	<0.0
Multipositivity	27 (44)	117 (63)	0.006	51 (26)	0.00	<0.0
Multiple biochemical autoantibodies	17 (27)	74 (40)	0.08	22 (11)	0.00	<0.0
Multipositivity during follow-up	62 (100)	182 (98)	0.31	198 (100)		
	Median (range)					
AAB levels at seroconversion						
ICA, JDFU	7 (4–131)	15 (3–668)	0.001	5 (3–110)	0.00	<0.00
IAA, RU	7.1 (3.5–40.3)	10.2 (3.6–81.0)	0.003	7.0 (3.5–148.7)	0.78	<0.00
GADA, RU	21.0 (5.9–135.3)	32.9 (5.6–310.4)	0.13	16.9 (5.5–342.1)	0.52	0.009
IA-2A, RU	9.8 (0.4–57.3)	22.9 (0.6–121.0)	0.20	2.7 (0.5–70.4)	0.88	0.003
Age at seroconversion, years	1.5 (0.6–7.5)	2.0 (0.3–13.8)	0.29	3.5 (0.2–13.1)	<0.0	<0.0
Age at diagnosis, years	11.4 (8.6–18.0)	5.5 (0.9–17.4)				
Delay from seroconversion to diagnosis, years	8.7 (7.3–17.0)	3.0 (0.02–7.2)				
Time from single to multiple autoantibody positivity, years	1.5 (0.2–8.2)	0.5 (0.2–6.0)	0.01	1.8 (0.2–10.5)	0.13	<0.0
Follow-up time from seroconversion, years	8.7 (7.3–17.0)	3.0 (0.02–7.2)		12.8 (7.4–20.1)		

Table 3 Predisposing SNPs associated with the delay from seroconversion to diagnosis of type 1 diabetes in progressors. NS=non-significant, P_c=corrected P value after the Benjamini-Hochberg corrections for multiple comparisons.
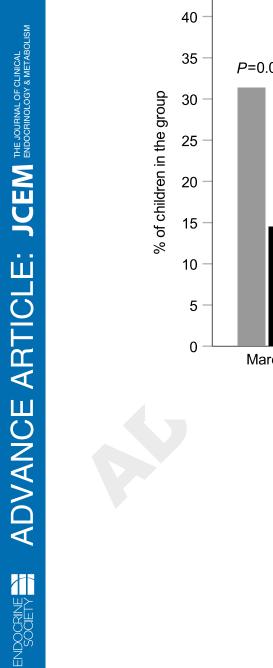
Gene	SNP	Genotype (n)	Median (range)	P value	P_c
RGS1	rs2816316	TT (148)	4.6 (0.02–17.0)	0.03	NS
		GT, GG (55)	3.5 (0.12–11.8)		
FUT2	rs601338	AA, AG (133)	4.8 (0.1–17.0)	0.04	NS
		GG (69)	3.1 (0.02–12.3)		
LOC646538	rs630115	GG (108)	4.6 (0.1–15.1)	0.01	NS
		AA, AG (118)	3.4 (0.02–17.0)		
IGF2BP2	rs4402960	GT, GG (219)	4.3 (0.02–17.0)	0.04	NS
		TT (18)	2.8 (0.2–7.6)		
High risk HLA genotype					
SH2B3	rs3184504	TT (12)	2.4 (0.1–11.0)	0.04	NS
		CT, CC (75)	4.1 (0.1–12.0)		
CTSH	rs3825932	TT (16)	2.3 (0.2–8.0)	0.01	NS
		CT, CC (81)	4.1 (0.1–12.0)		
SLC30A8	rs13266634	TT (8)	7.5 (0.3–10.6)	0.04	NS
		CT, CC (91)	3.5 (0.1–12.0)		
FUT2	rs601338	AA, AG (56)	4.7 (0.3–12.0)	0.02	NS
		GG (31)	2.7 (0.1–11.4)		
Moderate risk HLA genotype					
ERBB3	rs2292239	AA (15)	2.4 (0.1–15.4)	0.007	NS
		AC, CC (120)	4.7 (0.02–17.0)		
IL2RA	rs11594656	TT, AT (112)	4.4 (0.1–17.0)	0.04	NS
		AA (3)	1.2 (0.2–3.4)		
GIMAP5	rs6965571	GG (100)	5.1 (0.1–15.4)	0.03	NS
		AG, AA (36)	2.6 (0.02–17.0)		
SLC30A8	rs13266634	CC, TT (76)	3.9 (0.1–15.4)	0.03	NS
		CT (63)	5.7 (0.02–17.0)		
Boys					
LOC646538	rs630115	GG (61)	5.6 (0.3–15.1)	0.004	NS
		AG, AA (68)	3.3 (0.1–17.0)		
IGF2BP2	rs4402960	GT, GG (126)	4.5 (0.1–17.0)	0.02	NS
		TT (8)	2.7 (0.2–4.1)		
NRP1	rs2666236	AA, AG (76)	5.3 (0.1–17.0)	0.04	NS

		GG (40)	3.1 (0.1−12.3)		
Girls					
KIAA0350-CLEC16A	rs2903692	GG (35)	3.0 (0.1−10.6)	0.05	NS
		AG, AA (49)	5.3 (0.02−11.4)		
AFF3	rs9653442	CC (16)	2.3 (0.1−8.0)	0.02	NS
		CT, TT (71)	4.4 (0.02−11.8)		
INS	rs689	TT (2)	9.3 (8.0−10.7)	0.04	NS
		AA, AT (101)	3.6 (0.02−11.8)		

HLA screening
by July 31, 2003
n=75,813

No HLA-conferred
disease risk
n=64,779 (85.4%)

HLA-conferred
disease susceptibility
n=11,034 (14.6%)

Did not participate
in follow-up
n=2732 (24.8%)

Participated in
clinical follow-up
n=8302 (75.2%)

Follow-up not completed:
Excluded from the analysis
n=892 (10.7%)

Comparable follow-up data and
autoantibody samples available
n=7410 (89.3%)

No antibody positivity
during the follow-up
n=5751 (77.6%)

Tested positive for ≥1 autoantibody in at least
one sample during the follow-up
n=1659 (22.4%)

Developed clinical type 1 diabetes
without known autoantibody positivity
n=14 (0.2%)

First autoantibody-positive sample
at the diagnosis of T1D
n=14 (0.2%)

Autoantibody positivity
before the diagnosis of T1D
n=1645 (22.2%)

Tested positive for ≥1 autoantibody in at least
two consecutive samples during the follow-up
n=1528 (20.6%)

Unaffected by type 1 diabetes
by December 31, 2015
n=1281 (83.8%)

Progressed to type 1 diabetes
by December 31, 2015
n=247 (16.2%)

Other progressors
Delay from seroconversion to diagnosis <7.26 years
n=185 (74.9%)

Slow progressors
Delay from seroconversion to diagnosis ≥7.26 years
n=62 (25.1%)