CLINICAL STUDY

Insulin secretion and sensitivity in the prediction of type 1 diabetes in children with advanced β -cell autoimmunity

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Abstract

Objective: Reduced early insulin response has been shown to predict type 1 diabetes (T1D) in first-degree relatives of diabetic patients, while its role, as well as that of insulin resistance, has remained poorly defined in young children representing the general population. The predictive values of these markers and their relation to other risk factors of T1D were assessed in children with advanced β -cell autoimmunity, i.e. persistent positivity for two or more autoantibodies.

Design and methods: Intravenous glucose tolerance tests (IVGTTs) were carried out in 218 children with *HLA-DQB1*-conferred disease susceptibility and advanced β -cell autoimmunity. Baseline, metabolic and growth data were compared between children progressing to diabetes and those remaining unaffected. Hazard ratios for the disease predictors and the progression rate of T1D were assessed.

Results: Children developing T1D were younger at seroconversion, progressed more rapidly to advanced β -cell autoimmunity and had lower first-phase insulin response (FPIR) and homeostasis model assessment index for insulin resistance (HOMA-IR) than those remaining non-diabetic. The levels of HOMA-IR/FPIR, islet cell antibodies, insulin autoantibodies (IAA) and islet antigen 2 antibodies (IA-2A) were higher in progressors. BMI SDS, FPIR, age at IVGTT and levels of IAA and IA-2A were predictive markers for T1D.

Conclusions: Young age, higher BMI SDS, reduced FPIR and higher levels of IAA and IA-2A predicted T1D in young children with *HLA-DQB1*-conferred disease susceptibility and advanced β -cell autoimmunity. Disease risk estimates were successfully stratified by the assessment of metabolic status and BMI. The role of insulin resistance as an accelerator of the disease process was minor.

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Introduction

The prediabetic phase of type 1 diabetes (T1D) is characterised by destruction of the pancreatic β -cells leading to reduced insulin release and decreased glucose tolerance. Diabetes-associated autoantibodies can be used as predictive markers of T1D, and the risk assessment may be stratified by studying the insulin secretion and insulin sensitivity among prediabetic first-degree relatives (1, 2, 3, 4). The first-phase insulin response (FPIR) observed during an intravenous glucose tolerance test (IVGTT) provides information on the readily releasable insulin stored in the secretory granules located close to the β -cell membrane (5). A reduced FPIR represents the first marker of impaired

insulin secretion, and it may appear at an early stage of prediabetes (6, 7). It has been suggested that insulin resistance can modify the progression rate of preclinical diabetes (8, 9), implicating that increasing obesity in the childhood population might contribute to the rise in the incidence of T1D that has been observed over the last decades (10). We assessed the role of FPIR, homeostasis model assessment index for insulin resistance (HOMA-IR) and insulin release-adjusted insulin resistance (ratio of HOMA-IR to FPIR, i.e. relative insulin resistance) as the predictors of T1D, and their interactions with other risk indicators in young children with signs of advanced β -cell autoimmunity, recruited from the general population on the basis of *HLA-DQB1*-conferred disease susceptibility.

Figure 1 The DIPP study subcohort in the current analyses. IVGTT, intravenous glucose tolerance test.

Subjects and methods

Subjects and study design

The present study cohort comprises 218 participants in the Finnish Type 1 Diabetes Prediction and Prevention (DIPP) Study who were tested positive for more than one of four diabetes-associated autoantibodies (islet cell antibodies (ICA), autoantibodies against insulin (IAA),

GAD (GADA) and/or islet antigen 2 (IA-2A)) in at least two consecutive samples, taken with a minimum of 3 months apart and have undergone an IVGTT by January 1, 2006 (Fig. 1). Autoantibody analyses were performed as previously described (3). According to earlier analyses regarding the predictive value of autoantibodies and their combinations, the 5-year disease risk associated with multiple (≥ 2) autoantibody positivity varied between 16.7 and 69.6% (3).

Participants were originally recruited to the DIPP Study as newborn infants, based on screening for risk-associated *HLA-DQB1* genotypes defined by two risk-associated and two protective alleles. *HLA-DR/DQ* haplotypes relevant for risk determination were identified by using lanthanide-labelled sequence-specific oligonucleotides (11). As further analyses of *DRB1*, *DQB1* and *DQA1* loci revealed later that some of the children actually carried neutral or slightly protective *HLA-DQB1* genotypes (Table 1), risk estimates for genotype-associated disease were subsequently categorised into five groups (12). *INS* (rs689) and *PTPN22* (rs2476601) polymorphisms were genotyped by using the Sequenom (San Diego, CA, USA) platform (Genome Centre of Eastern Finland, University of Eastern Finland, Kuopio).

The majority (179; 82.1%) of the children studied participated in a randomised, double-blinded

Table 1 Baseline data and variables related to growth, progression of autoimmunity and metabolic factors in children developing type 1 diabetes (T1D) and remaining unaffected.

	All (n=218)	Non-diabetic (n=67)	T1D (n=151)	P value
Baseline data (n (%))				
Gender, male	129 (59.2)	39 (58.2)	90 (59.6)	0.847
Family history positive for T1D	27 (12.4)	6 (9.0)	21 (13.9)	0.306
Intervention study, participated	179 (82.1)	54 (80.6)	125 (82.8)	0.698
<i>HLA-DQB1</i> -associated disease risk				
Strongly increased	75 (34.4)	19 (28.4)	56 (37.1)	0.453
Moderately increased	120 (55.0)	40 (59.7)	80 (53.0)	
Neutral/slightly protective	23 (10.6)	8 (11.9)	15 (9.9)	
<i>PTPN22</i> gene, rs2476601 CC (vs CT/TT)	74 (34.1)	17 (25.4)	57 (38.0)	0.070
<i>INS</i> gene, rs689 AA (vs AT/TT)	170 (78.7)	51 (76.1)	119 (79.9)	0.534
Growth data (Median (range))				
Height-for-age (SDS) at IVGTT	0.6 (−3.1 to 4.0)	0.6 (−3.1 to 2.4)	0.6 (−1.2 to 4.0)	0.551
BMI (SDS) at IVGTT	0.3 (−2.2 to 5.5)	0.2 (−1.6 to 2.5)	0.4 (−2.2 to 5.5)	0.075
Delay (years) from:				
Seroconversion to multipositivity	0.0 (0.0 to 6.6)	0.3 (0.0 to 6.6)	0.0 (0.0 to 6.0)	0.003
Multipositivity to persistent multipositivity	0.3 (0.2 to 3.3)	0.3 (0.2 to 3.3)	0.3 (0.2 to 3.3)	0.036
Persistent multipositivity to IVGTT	0.2 (0.0 to 3.6)	0.3 (0.0 to 3.3)	0.2 (0.0 to 3.6)	0.020
Seroconversion to IVGTT	0.9 (0.2 to 7.4)	1.2 (0.4 to 7.4)	0.7 (0.2 to 6.3)	<0.001
Metabolic data				
Fasting glucose (mmol/l)	4.7 (2.6 to 6.3)	4.8 (3.7 to 6.0)	4.6 (2.6 to 6.3)	0.061
Fasting insulin (mU/l)	3.8 (0.1 to 13.4)	4.1 (1.1 to 9.9)	3.8 (0.1 to 13.4)	0.084
Maximal glucose level (mmol/l)	19.9 (14.0 to 27.6)	19.8 (14.2 to 26.3)	20.1 (14.0 to 27.6)	0.125
Maximal insulin level (mU/l)	20.2 (3.1 to 157.2)	31.3 (6.5 to 157.2)	17.7 (3.1 to 115.6)	<0.001
FPIR (mU/l)	36.0 (5.0 to 273.6)	58.7 (12.1 to 273.6)	30.6 (5.0 to 213.6)	<0.001
HOMA-IR (mmol × mU/l ²)	0.78 (0.02 to 3.27)	0.81 (0.22 to 2.64)	0.72 (0.02 to 3.27)	0.047
HOMA-IR/FPIR (mmol/l)	0.021 (0.003 to 0.139)	0.015 (0.003 to 0.044)	0.024 (0.003 to 0.139)	<0.001

IVGTT, intravenous glucose tolerance test; PTPN22, protein tyrosine phosphatase non-receptor type 22; INS, insulin; FPIR, first-phase insulin response; HOMA-IR, homeostasis model assessment index for insulin resistance; HOMA-IR/FPIR, relative insulin resistance.

intervention trial of administration of intranasal insulin vs placebo. The intervention proved to have no effect on the progression to T1D (13). Cases with T1D were diagnosed by the World Health Organization (WHO) criteria (14) and ascertained from the patient records of the University Hospitals of Turku, Oulu, and Tampere, and from the National Diabetes Register (15). Follow-up for T1D continued until the end of October 2012. Local ethics committees had approved the protocol of the DIPPP study. Written informed consents were obtained from the guardians of the participants before the commencement of the study procedures.

Metabolic and growth data

The standardised Islet Cell Antibody Register Users Study (ICARUS) protocol was followed while performing the IVGTTs (1). Samples for plasma glucose and serum insulin assays were taken after overnight fasting. The fasting state (pre-infusion) samples were drawn 1 and 5 min before i.v. glucose infusion (20% glucose solution given 0.5 g glucose/kg, maximal dose 35 g; infusion time $3 \text{ min} \pm 15 \text{ s}$). Further samples were taken at 1, 3, 5 and 10 min after the infusion. Plasma glucose concentrations were measured by an enzymatic method (16) and serum intact insulin with an enzyme-linked two-site immunoassay (17). The intra-assay coefficient of variation was 12.9% for the insulin analyses and 2.8% for the glucose assay.

The FPIR was defined as the sum of post-infusion insulin concentrations at 1 and 3 min, while HOMA-IR represented the product of fasting glucose (mmol/l) and fasting insulin (mU/l) values divided by 22.5 (18). HOMA-IR:FPIR ratio (HOMA-IR:FPIR) represented the relative insulin resistance. Children's height and weight were measured at each follow-up visit. Values obtained (kilogram and centimetre) were adjusted for age and gender (19, 20), and were given as SDS for height-for-age and BMI. Children with a BMI $> +2$ SDS were identified as overweight and those with a BMI $> +3$ SDS as obese (20).

Statistical analyses

Predictive Analytics Software (PASW Statistic 20.0 release, Armonk, NY, USA) was used for the statistical analyses. As the majority of the continuous variables had skewed distributions, mainly non-parametric tests, e.g. Mann–Whitney *U* test, Kruskal–Wallis test, Wilcoxon signed-rank test and Spearman correlation analysis, were used. The χ^2 test was applied for binary variables and the Kaplan–Meier log-rank test was used for the analyses of the rate of disease progression. Analyses of covariance were performed whenever appropriate. Hazard ratios (HR) for the factors potentially predictive for T1D were assessed in an age-adjusted manner with the Cox regression models. CIs were set at 95% and $P < 0.05$ (two-tailed) was

considered statistically significant. One child with unclear severe hyperinsulinemia was excluded from the analyses.

Results

Metabolic data were available for 218 subjects (129 males, 59.2%), 151 (69.2%) of whom developed T1D (progressors) at the median age of 6.4 years (range 1.6–16.8 years; Table 1). The median follow-up time since IVGTT for the 67 subjects remaining non-diabetic (non-progressors) was 8.8 years (range 6.8–15.0 years), and their age at the end of the follow-up was 13.3 years (8.5–17.0 years). Progressors and non-progressors had similar distributions for baseline variables (Table 1), but progressors were younger at seroconversion and at all further time points regarding advanced seropositivity (Supplementary Table 1, see section on supplementary data given at the end of this article). Also, the delays from initial seroconversion to persistent multipositivity and IVGTT were shorter in progressors. As the age at IVGTT correlated significantly with metabolic markers (Spearman correlation coefficient, $r_s = 0.44$, $P < 0.001$ for FPIR; $r_s = 0.41$, $P < 0.001$ for HOMA-IR and $r_s = -0.14$, $P = 0.036$ for HOMA-IR/FPIR), age-adjustment was used in further analyses whenever applicable. Regarding autoantibodies, progressors had both higher numbers of positive autoantibodies and higher levels of ICA, IAA and IA-2A throughout the follow-up, except at the initial

Figure 2 First-phase insulin response (FPIR; A), homeostasis model assessment index for insulin resistance (HOMA-IR; B), relative insulin resistance (HOMA-IR:FPIR; C) and BMI SDS (D) values (with 95% CIs and medians) in children developing type 1 diabetes (T1D) and remaining unaffected. *P* values for Mann–Whitney *U* tests.

seroconversion. Levels of GADA were comparable between the two groups during the whole follow-up (Supplementary Table 1).

In general, FPIR and HOMA-IR were higher in non-progressors, while relative insulin resistance was higher in progressors (Table 1 and Fig. 2A, B, and C). Regarding HOMA-IR, the difference between the groups was small and the correlation between HOMA-IR and progression to T1D disappeared after adjustment for age ($r_s = -0.04$, $P = 0.57$). As expected, HOMA-IR was correlated with BMI SDS ($r_s = 0.17$, $P = 0.013$). To study the predictive value of metabolic markers and BMI SDS, children were grouped by the quartiles of these variables, and the estimates of the disease progression rates of the different quartiles were compared with the log-rank test in the Kaplan–Meier analyses (Fig. 3A, B, C, and D). For FPIR, progression rate increased by the decreasing insulin secretion capacity, and all but the two middle quartiles differed significantly from each other. Regarding progression rates related to HOMA-IR, HOMA-IR/FPIR and BMI SDS, mainly the highest quartiles differed from the other quartiles. The highest 5-year progression rates were observed for individuals belonging to

the lowest FPIR quartile (81.5%; CI 71.1–91.9%) and highest HOMA-IR/FPIR quartile (75.9%; CI 64.5–87.3%) and comparably, the lowest progression rates were recorded for those within the highest FPIR quartile (21.4%; CI 10.6–32.2%) and lowest HOMA-IR/FPIR (37.0%; CI 24.2–49.9%) quartile.

As obesity had been hypothesised to have an accelerating effect on the prediabetic disease process, we assessed whether increased BMI could explain the progression to T1D. In the current study cohort, there were 203 children with their height and weight data recorded both at seroconversion and at IVGTT. At seroconversion, the median BMI for the 65 non-progressors and 138 progressors were 0.2 SDS (range -1.7 to 2.2 SDS) and 0.5 SDS (range -1.8 to 5.5 SDS; $P = 0.004$) respectively. The median decrease in BMI during the follow-up from seroconversion to IVGTT was similar (0.2 SDS; $P = 0.703$) in both groups and did not correlate with progression to T1D ($r_s = -0.034$, $P = 0.704$). However, in the Kaplan–Meier analysis, the highest and the lowest BMI SDS quartile groups differed from each other only if the obese children ($n = 6$) were included in the analysis (Fig. 3D). Moreover, even if the BMI SDS was the main independent predictor for T1D in the Cox regression analysis (Table 2; HR 1.354, CI 1.137–1.613) in the whole cohort, this was not the case after excluding the obese children from the analysis. When the subcohort of the non-obese children ($n = 212$) was analysed, age at IVGTT, FPIR and IAA levels remained as significant predictors, while the ICA titre (HR = 1.001, CI 1.000–1.001; $P = 0.006$) replaced the IA-2A level as an independent predictor for T1D.

Figure 3 Progression to T1D according to the quartiles of first-phase insulin response (FPIR; A), homeostasis model assessment index for insulin resistance (HOMA-IR; B), relative insulin resistance (HOMA-IR/FPIR; C) and BMI SDS (D) in children developing type 1 diabetes (T1D) and remaining unaffected. The 1st quartile: continuous line; 2nd quartile: dotted line; 3rd quartile: sparse broken line; 4th quartile: dense broken line. IVGTT, intravenous glucose tolerance test. P values for Kaplan–Meier log-rank tests. * $P < 0.001$, † $P = 0.002$, ‡ $P = 0.01$, # $P = 0.025–0.047$.

Discussion

We studied the prognostic value of metabolic data obtained soon after the confirmation of advanced β -cell autoimmunity in young children from the general population, carrying *HLA-DQB1*-conferred susceptibility to T1D. Our main aims were to test the hypothesis that obesity-related insulin resistance might modify the disease process, to confirm the role of a reduced FPIR as an important risk factor for rapid progression to T1D, and to assess the interactions and significance of the various disease risk factors present at the time of the IVGTT in children from the general population.

Although all children in the current study cohort had fulfilled the criteria of advanced β -cell autoimmunity before IVGTT, their progression rate to T1D was highly variable. In these children, the baseline factors, such as gender, *HLA-DQB1* genotypes, insulin and *PTPN22* gene polymorphisms, and the family history for T1D, could not identify individuals with rapid disease progression. This may be due to the inclusion criteria used, resulting in a relatively homogeneous study population, but may also reflect the idea that factors contributing to the initiation of the disease process might be different from those driving

Table 2 Factors potentially predictive for type 1 diabetes (T1D) in the Cox univariate and multivariate analyses.

Variables in the univariate analyses	Hazard ratio with CI	P value
Background data		
Gender male vs female*	0.965 (0.696–1.337)	0.829
Family history for T1D positive vs negative*	0.739 (0.465–1.174)	0.200
<i>HLA-DQB1</i> genotype by decreasing disease risk class (see Table 2)*	0.876 (0.671–1.143)	0.330
<i>INS</i> genotype rs689 AA vs AT/TT*	0.806 (0.538–1.207)	0.296
<i>PTPN22</i> genotype rs2476601 CC vs CT/TT*	0.719 (0.516–1.001)	0.051
Intervention study, participated vs refused	0.928 (0.608–1.416)	0.728
Time-related variables		
Age at seroconversion (years)	0.837 (0.748–0.936)	0.001
Age at persistent multipositivity (years)	0.795 (0.716–0.884)	<0.001
Age at IVGTT (years)*	0.778 (0.701–0.862)	<0.001
Delay from seroconversion to IVGTT (years)*	0.747 (0.621–0.897)	0.001
Data on autoantibodies		
Autoantibody levels at IVGTT		
ICA (JDFU)*	1.000 (1.000–1.001)	0.001
IAA (RU)*	1.012 (1.008–1.016)	<0.001
GADA (RU)*	1.000 (0.997–1.003)	0.732
IA-2A (RU)*	1.007 (1.004–1.010)	<0.001
Number of positive autoantibodies		
At seroconversion	1.383 (1.127–1.696)	0.001
At seroconversion for multiple autoantibodies	1.622 (1.306–2.014)	<0.001
At persistent multipositivity	1.669 (1.328–2.098)	<0.001
At IVGTT*	1.643 (1.343–2.011)	<0.001
Metabolic and growth related data		
FPIR (mU/l)*	0.978 (0.970–0.985)	<0.001
HOMA-IR (mmol × mU/l ²)*	0.719 (0.484–1.069)	0.103
HOMA-IR/FPIR (μmol/l)*	1.019 (1.013–1.026)	<0.001
Height-for-age (SDS)	1.124 (0.967–1.307)	0.124
BMI (SDS)*	1.280 (1.092–1.501)	0.002
Independent predictors of T1D (multivariate model)		
BMI (SDS)	1.354 (1.137–1.613)	0.001
Age at IVGTT (years)	0.872 (0.780–0.974)	0.015
FPIR (mU/l)	0.985 (0.978–0.993)	<0.001
IAA at IVGTT (RU)	1.010 (1.005–1.014)	<0.001
IA-2A at IVGTT (RU)	1.005 (1.002–1.009)	0.003

INS, insulin; PTPN22, protein tyrosine phosphatase non-receptor type 22; IVGTT, intravenous glucose tolerance test; ICA, islet cell antibodies; JDFU, Juvenile Diabetes Research Foundation Unit; IAA, insulin autoantibodies; RU, relative unit; GADA, autoantibodies to GAD; IA-2A, autoantibodies to islet antigen 2; FPIR, first-phase insulin response; HOMA-IR, homeostasis model assessment index for insulin resistance; HOMA-IR/FPIR, relative insulin resistance. * indicates variables in multivariate analysis.

the process towards overt disease. However, as previously shown (2, 3, 6, 21, 22), several of the factors associated with the initiation of β -cell autoimmunity do contribute to the phenomena associated with the progression rate, such as age at initial seroconversion, the pace of progression towards persistent multipositivity and autoantibody levels. The current study confirmed the prior observations that progression to T1D is related to young age at seroconversion, early progression to multipositivity, higher numbers of detectable autoantibodies and to higher levels of ICA, IAA and IA-2A (1, 2, 3).

Regarding IVGTT and metabolic data, the young and relatively variable age of the participants complicated the analyses. First of all, for HOMA-IR or HOMA-IR/FPIR scarcely any comparable age-adjusted data from a healthy, non-obese reference population was available, and second, as FPIR, HOMA-IR and HOMA-IR/FPIR correlated strongly with the age at IVGTT, age adjustments were required in the analyses. However,

we confirmed that a low FPIR (< 24 mU/l) identifies the future cases of T1D with high accuracy (5-year progression rate > 80%), while children with reasonably high insulin secretion (FPIR > 59 mU/l) remain unaffected for several years (5-year progression rate 21%), although they already have signs of advanced β -cell autoimmunity.

The observation that HOMA-IR was remarkably low in the current cohort, and especially that it was slightly higher in non-progressors, implicates that the role of insulin resistance may be modest in the prediabetic process in young, mainly non-obese children. However, there were indications that both obesity-related insulin resistance and insulin resistance occurring in parallel with a reduced insulin secretion may modify the disease risk. These include the findings that HOMA-IR/FPIR was higher in progressors, it correlated positively with BMI SDS and the fact that in the Kaplan–Meier analyses assessing the rates of disease progression, the difference

between the two highest BMI quartiles disappeared after excluding the obese children from the analysis, as did the significance of BMI as an independent predictor of T1D in the Cox regression analysis.

Our current findings are in line with the results of the British study on identical twins (23) and the data from the European Nicotinamide Diabetes Intervention Trial (ENDIT; (24)). In both studies, HOMA-IR was an independent predictor of T1D only in subjects with reduced FPIR, not in the whole study cohorts. The observation that both in the Diabetes Prevention Trial-Type 1 (DPT1) Study and in the Childhood Diabetes in Finland Study (DiMe) HOMA-IR and FPIR/HOMA-IR were predictive indicators for T1D (2, 4) might be explained by the older age of the study participants. In all, it seems that even if a low-grade insulin resistance is unable to shift glucose metabolism towards diabetes in individuals with normal FPIR, it might do so in individuals with reduced insulin release.

To conclude, the role of insulin resistance as an accelerator of the prediabetic disease process of T1D seems to be minor. However, the assessment of the metabolic status provides important information regarding the stratification of the disease risk in prediabetic children. This information can be applied in intervention trials aimed at preventing T1D: for the identification of the optimal trial participants, for the assessment of the metabolic changes during and after the treatment and as guidance in the interpretation of the study results.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-13-0206>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

H T Siljander analysed the data, wrote the first version of the manuscript and edited the manuscript. R Hermann was involved in the genetic analyses. H T Siljander, A Hekkala, J Lähde, L Tanner, P Keskinen and R Veijola were involved in the clinical follow-up of the study participants. J Ilonen, O Simell and M Knip are the Principal Investigators of the DIPP study. J Ilonen was responsible for the genetic analyses and M Knip and R Veijola for the autoantibody analyses. M Knip planned the study and contributed to the discussion. All authors reviewed and approved the manuscript. M Knip is the guarantor of this work and, as such, had full access to all 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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References

- Bingley PJ. Interactions of age, islet cell antibodies, insulin autoantibodies, and first-phase insulin response in predicting risk of progression to IDDM in ICA+ relatives: the ICARUS data set. Islet Cell Antibody Register Users Study. *Diabetes* 1996 **45** 1720–1728. (doi:10.2337/diabetes.45.12.1720)
- Mrena S, Virtanen SM, Laippala P, Kulmala P, Hännilä ML, Åkerblom HK & Knip M. Models for predicting type 1 diabetes in siblings of affected children. *Diabetes Care* 2006 **29** 662–667. (doi:10.2337/diacare.29.03.06.dc05-0774)
- 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. (doi:10.2337/db08-1305)
- Xu P, Wu Y, Zhu Y, Dagne G, Johnson G, Cuthbertson D, Krischer JP, Sosenko JM, Skyler JS & Diabetes Prevention Trial-Type 1 (DPT-1) Study Group. Prognostic performance of metabolic indexes in predicting onset of type 1 diabetes. *Diabetes Care* 2010 **33** 2508–2513. (doi:10.2337/dc10-0802)
- Daniel S, Noda M, Straub SG & Sharp GWG. Identification of the docked granule pool responsible for the first phase of glucose-stimulated insulin secretion. *Diabetes* 1999 **48** 1–5. (doi:10.2337/diabetes.48.9.1686)
- Veijola R, Vähäsalo P, Tuomilehto-Wolf E, Reijonen H, Kulmala P, Ilonen J, Åkerblom HK & Knip M. Human leukocyte antigen identity and DQ risk alleles in autoantibody-positive siblings of children with IDDM are associated with reduced early insulin response. *Diabetes* 1995 **44** 1021–1028. (doi:10.2337/diabetes.44.9.1021)
- Keskinen P, Korhonen S, Kupila A, Veijola R, Erkkilä S, Savolainen H, Arvilommi P, Simell T, Ilonen J, Knip M *et al.* First-phase insulin response in young healthy children at genetic and immunological risk for type I diabetes. *Diabetologia* 2002 **45** 1639–1648. (doi:10.1007/s00125-002-0981-8)
- Fourlanos S, Narendran P, Byrnes GB, Colman PG & Harrison LC. Insulin resistance is a risk factor for progression to type 1 diabetes. *Diabetologia* 2004 **47** 1661–1667. (doi:10.1007/s00125-004-1507-3)
- Xu P, Cuthbertson D, Greenbaum C, Palmer JP, Krischer JP & Diabetes Prevention Trial-Type 1 Study Group. Role of insulin resistance in predicting progression to type 1 diabetes. *Diabetes Care* 2007 **30** 2314–2320. (doi:10.2337/dc06-2389)
- Wilkin TJ. The accelerator hypothesis: weight gain as the missing link between type I and type II diabetes. *Diabetologia* 2001 **44** 914–922. (doi:10.1007/s001250100548)
- Ilonen J, Reijonen H, Herva E, Sjöroos M, Iitiä A, Lövgren T, Veijola R, Knip M & Åkerblom HK. Rapid HLA-DQB1 genotyping for four alleles in the assessment of risk for IDDM in the Finnish population. The Childhood Diabetes in Finland (DiMe) Study Group. *Diabetes Care* 1996 **19** 795–800. (doi:10.2337/diacare.19.8.795)
- Hekkala A, Ilonen J, Knip M, Veijola R & Finnish Paediatric Diabetes Register Family history of diabetes and distribution of class II HLA genotypes in children with newly diagnosed type 1 diabetes: effect on diabetic ketoacidosis. *European Journal of Endocrinology* 2011 **165** 813–817. (doi:10.1530/EJE-11-0376)

- 13 Nääntö-Salonen K, Kupila A, Simell S, Siljander H, Salonsaari T, Hekkälä A, Korhonen S, Erkkola R, Sipilä J, Haavisto L *et al.* Nasal insulin in prevention of type 1 diabetes in autoantibody-positive children with HLA-conferred disease susceptibility and recruited from the general population. *Lancet* 2008 **372** 1746–1755. (doi:10.1016/S0140-6736(08)61309-4)
- 14 World Health Organization. Definition, Diagnosis, and Classification of Diabetes Mellitus and its Complications: Report of a WHO Consultation. In *World Health Organization publications*, pp 66. Geneva, World Health Organization, 1999.
- 15 Parkkola A, Härkönen T, Ryhänen SJ, Ilonen J, Knip M & Finnish Pediatric Diabetes Register. Extended family history of type 1 diabetes and phenotype and genotype of newly diagnosed children. *Diabetes Care* 2013 **36** 348–354. (doi:10.2337/dc12-0445)
- 16 Beach EF & Turner JJ. An enzymatic method for glucose determination in body fluids. *Clinical Chemistry* 1958 **4** 462–475.
- 17 Andersen L, Dinesen B, Jørgensen PN, Poulsen F & Røder M. Enzyme immunoassay for intact human insulin in serum and plasma. *Clinical Chemistry* 1993 **39** 578–582.
- 18 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF & Turner RC. Homeostasis model assessment: insulin resistance and β -cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985 **28** 412–419. (doi:10.1007/BF00280883)
- 19 WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development. In *World Health Organization publications*, pp 312. Web site: <http://www.who.int/childgrowth/publications/en>. Geneva: World Health Organization, 2006.
- 20 De Onis M, Onyango A, Borghi E, Siyam A, Nishida C & Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. *Bulletin of the World Health Organization* 2007 **85** 661–668. (doi:10.2471/BLT.07.043497)
- 21 Hermann R, Lipponen K, Kiviniemi M, Kakko T, Veijola R, Simell O, Knip M & Ilonen J. Lymphoid tyrosine phosphatase (LYP/PTPN22) Arg620Trp variant regulates insulin autoimmunity and progression to type 1 diabetes. *Diabetologia* 2006 **49** 1198–1208. (doi:10.1007/s00125-006-0225-4)
- 22 Hermann R, Laine AP, Veijola R, Vahlberg T, Simell S, Lähde J, Simell O, Knip M & Ilonen J. The effect of HLA class II, insulin and CTLA4 gene regions on the development of humoral β cell autoimmunity. *Diabetologia* 2005 **48** 1766–1775. (doi:10.1007/s00125-005-1844-x)
- 23 Hawa MI, Bonfanti R, Valeri C, Delli Castelli M, Beyan H & Leslie RDG. No evidence for genetically determined alteration in insulin secretion or sensitivity predisposing to type 1 diabetes: a study of identical twins. *Diabetes Care* 2005 **28** 1415–1418. (doi:10.2337/diacare.28.6.1415)
- 24 Bingley PJ, Mahon JL, Gale EA & the European Nicotinamide Diabetes Intervention Trial (ENDIT) Group. Insulin resistance and progression to type 1 diabetes in the European Nicotinamide Diabetes Intervention Trial (ENDIT). *Diabetes Care* 2008 **31** 146–150. (doi:10.2337/dc07-0103)

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