

## **Growth and risk for islet autoimmunity and progression to type 1 diabetes in early childhood: The Environmental Determinants of Diabetes in the Young Study**

Running title: Growth and risk for islet autoimmunity and diabetes

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**Abstract**

Increased growth in early childhood has been suggested to increase the risk of type 1 diabetes. This study explored the relationship between weight, height and development of persistent islet autoimmunity and progression to type 1 diabetes during the first 4 years of life in 7,468 children at genetic risk of type 1 diabetes, followed in Finland, Germany, Sweden and US. Growth data collected every third month were used to estimate individual growth curves using mixed models. Cox proportional hazards models were used to evaluate the body size and risk of islet autoimmunity and T1D. In the overall cohort, development of islet autoimmunity (n=575) was related to weight z-scores at 12 months, (HR 1.16 per 1.14 kg in males or per 1.02 kg in females; 95%CI 1.06-1.27,  $p < 0.001$ , FDR=0.008), but not at 24 or 36 months. A similar relationship was seen between weight z-scores and development of multiple islet autoantibodies (1 year, HR 1.21 95%CI 1.08-1.35,  $p = 0.001$ , FDR=0.008; 2 years, HR 1.18 95% CI 1.06-1.32,  $p = 0.004$ , FDR=0.02). No association was found between weight or height and type 1 diabetes (n=169). In conclusion, greater weight in the first years of life was associated with an increased risk of development of islet autoimmunity.

## Introduction

Type 1 diabetes is one of the most common pediatric chronic diseases, in which a progressive autoimmune process destroys the beta-cells of pancreatic islets, resulting in loss of insulin secretion. Islet autoimmunity (IA) precedes the clinical onset of disease by months to years, and is detected by the presence of islet autoantibodies against glutamate decarboxylase (GADA), insulinoma-associated protein 2 (IA-2A) and insulin (IAA)(1). An estimated 70% of children with multiple islet autoantibodies progress to diabetes within ten years (2-4). The incidence of type 1 diabetes in children has increased 3%-5% annually, since the 1960s (5; 6). The cause of this secular trend remains unknown, but it is assumed that environmental factors trigger islet autoimmunity in genetically susceptible children who carry specific Human Leucocyte Antigen (HLA) DR and DQ genotypes (7; 8). Infant overfeeding and accelerated infant/toddler growth has been proposed as a potential trigger (9). According to the 'accelerator hypothesis', excessive weight gain and resulting insulin resistance accelerate beta-cell apoptosis and autoimmunity in presence of the susceptibility HLA genotypes (10; 11).

While several retrospective studies have reported associations between higher birth weight (12) or childhood height and weight (13-17) and type 1 diabetes, prospective studies of IA have found less evidence for the 'accelerator hypothesis'(18). The German BabyDiab study found no relationship between body mass index (BMI) or insulin resistance in 135 children developing IA(19); however, subsequently reported that an earlier infant BMI peak predicted IA(20). In the US DAISY study of children aged older than 2 years, an increased height velocity, but not weight or BMI velocity, predicted development of IA (n=143 children) (21). Interestingly, higher weight z-scores at age 2 and 4 years predicted development of IA (n=46) in a cohort of 548 Australian children followed from birth (22).

In this report, we tested the hypotheses that higher weight and, separately, height in the initial four years of life predict 1) the development of IA and 2) progression to type 1 diabetes. The Environmental Determinant of Diabetes in the Young (TEDDY) cohort (23) provided an opportunity to test the generalizability of evidence across four diverse populations.

## Methods

### *Design and Settings:*

TEDDY is a prospective cohort study funded by the National Institutes of Health with the primary goal of identifying environmental causes of type 1 diabetes. TEDDY includes six clinical research centers: three in the US (Colorado, Georgia/Florida, Washington) and three in Europe (Finland, Germany, and Sweden). Detailed study design and methods have been previously published (23). The study was approved by local Institutional Review Boards and is monitored by an External Evaluation Committee formed by the National Institutes of Health.

### *Participants:*

The participants were identified at birth by screening for high-risk HLA genotypes (24). Starting in 2004, the TEDDY study included 8676 children with increased genetic risk of type 1 diabetes, who are to be followed from birth to 15 years of age. Height (length before 2 and standing height after 2 years of age) and weight of the child have been obtained at the TEDDY clinics by trained TEDDY personnel and blood samples drawn for measurements of islet autoantibodies every 3 months from 3 months up to 4 years of age and every 3-6 months after 4 years of age depending on autoantibody status. Children were excluded from these analyses (Figure 1) if: 1) their HLA eligibility could not be confirmed at a repeated genotyping at the age of 9 months; 2) their islet autoantibody results were indeterminate; 3) they had been followed for less than 12 months without developing IA or type 1 diabetes; or 4) they had more than five consecutive growth measures missing.

### *Main outcome measures:*

*Any islet autoimmunity* was defined as confirmed persistent presence of one or more of islet autoantibodies on two or more sequential clinic visits. Islet autoantibodies to GAD, IA-2 and

insulin were measured by radiobinding assays (25; 26) in two reference laboratories at the Barbara Davis Center for Childhood Diabetes, University of Colorado Denver and at the University of Bristol, United Kingdom. All positive samples and 5% of negative samples were re-tested in the other reference laboratory and deemed confirmed if concordant.

*Multiple islet autoantibodies* were defined as two or more islet autoantibodies at two consecutive clinic visits.

*Type 1 diabetes* was diagnosed using the American Diabetes Association criteria (27).

*Statistical analyses:*

The age at development of persistent confirmed IA was the age at the initial of two or more consecutive positive tests. In analyses of multiple islet autoantibodies, age of second persistent confirmed autoantibody was used. Only growth data up to the date of seroconversion to IA or to the date of diagnosis of type 1 diabetes were used. The analyses of progression to type 1 diabetes included only those children who developed persistent IA before the onset of type 1 diabetes.

Individual growth curves were developed for each child using mixed models with fixed and random effects of the height and weight on age for each gender. Best-fitting mixed models were used to produce estimates of mean growth curves and best linear unbiased predictors (BLUPS) for individual participants' growth curves for weight and height throughout childhood. Quadratic polynomials splines were applied to model the non-linear growth curve (weight and height) from birth to four years of age. The models were examined using several potential combinations of knots from the set (0.5, 1, 1.5, 2, 3). According to Akaike Information Criteria and Bayesian Information Criterion, four knots (at 0.5, 1, 2 and 3 year respectively) were used in the fitting of weight on age and five knots (at 0.5, 1, 1.5, 2 and 3 year respectively) were used in the fitting of height on age. Standardized growth measures (weight z-score and height z-score) were derived using the CDC standardized growth

charts(28) and SAS programs. These were best represented in 0-93 months by second-degree polynomials in fixed effects and first-degree in random effects. BLUPS of individual growth curves were assessed at each time points, including those when height and weight were missing, resulting in interpolation of ~18% of the height and weight measurements. Plots of the raw data and BLUP curves were very similar. ~~Stratified~~ Cox proportional hazards models were used to assess specific identified periods using fixed measures (birth to 12 months, birth to 24 months and birth to 36 months) and over time using repeated measures (time-varying covariate to four years and 3, 6, 9, 12 months prior to) for association with islet autoimmunity and type 1 diabetes. All models were adjusted for presence of the HLA-DR3/4 genotype (yes/no), presence of a family history of type 1 diabetes (mother, father or sibling), gender, birth size and country of residence. There was evidence of non-proportional hazards related to country of residence in the Cox models; and, therefore the country of residence was adjusted for by using the strata statement in SAS. The strata statement allowed fitting separate baseline hazard functions for each country in the Cox models. Weight models were adjusted for birth weight z-scores and height models for birth length z-scores (28). The heterogeneity by country was tested by assessing the interaction between country and body size (weight and height z-score) in the Cox regression models. Multiple comparisons were conducted using the false discovery rate ( $FDR \leq 0.05$  were considered significant).

## Results

TEDDY children (n=8676) were followed prospectively for a median 60 months (Q1-Q3: 29-79 months). Among the 7468 children with sufficient growth data, 575 (7.7%) developed persistent IA, 351 (4.7%) multiple IA and 169 (2.3%) have progressed to type 1 diabetes (Figure 1 and Table 1). Mean age at seroconversion to persistent IA was 31 (SD=21) months, multiple IA 34 (SD=20) months and for onset of type 1 diabetes 45 (SD=23) months. A total of 109 (19%) children developed islet autoimmunity before 12 months of age, and 265 (46%) before 24 months of age. Five children (3%) progressed to type 1 diabetes before 12 months of age and 44 (26%) before 24 months of age. Table 1 summarizes demographic characteristics of the study participants. The history of type 1 diabetes in a first degree relative and the HLA-DR3/4 genotype predicted development of IA and type 1 diabetes (Table 1).

### *Growth parameters and development of islet autoimmunity:*

In the overall cohort, there was a small increase in the risk of any persistent IA with greater toddler weight or height, adjusting for birth size, HLA-genotype, family history of type 1 diabetes, gender and country of residence, in Cox proportional hazards analysis. After correction for multiple analyses, weight z-score at 12 months (Hazard Ratio (HR) 1.16 per 1.14 kg Male/1.02 kg Female; 95%CI 1.06-1.27,  $p < 0.001$ , FDR=0.008) predicted IA (Figure 2/Table 2). Using the more stringent outcome defined as positive for multiple persistent islet autoantibodies, weight z-scores at 12 and 24 months were predictive in the overall cohort, when correction for multiple analyses was performed. Hazard Ratios for any IA and multiple IA were also examined in relation to weight-z scores or height z-scores at 3, 6, 8 or 12 months prior to development of IA (Figure 2/Table 2). Little evidence was found for any excess weight or height 3-12 months prior to development of any IA or multiple IA.



In a time varying model of growth up to 4 years of age, weight z-scores were not associated with development of any IA or multiple IA (Figure 2/Suppl. Table 2). There was no evidence of heterogeneity on the effect of weight or height z-score by country on any persistent IA (all p-values  $\geq 0.27$ ) or multiple persistent IA (all p-values  $\geq 0.38$ ).

A sensitivity analysis was carried out excluding children born to mothers with type 1 diabetes; this exclusion did not affect the results. Other factors identified related to increased weight during the first year of life were, being a girl and having the HLA DR3/3, while breastfeeding for more than 3 months was associated with lower weight. However, adjustment for these factors did not change the results.

*Growth parameters and progression to type 1 diabetes:*

Growth over time did not affect time to progression to type 1 diabetes in children with multiple IA (Figure 2/Table 2). The height and weight assessed in specific intervals (birth to 12, 24 and 36 months) was also not predictive of progression from multiple islet autoantibodies to type 1 diabetes (Figure 2/Suppl. Table 2).

## Discussion

In this large multi-country cohort of 7468 children at high genetic risk of type 1 diabetes we found a weak association between early weight z-scores and the development of any IA or multiple IA. The association was also found when controlling for confounding factors and correction for multiple analyses. No association between early growth and the progression to type 1 diabetes was found.

The study was performed to test the overload hypothesis, stating that increased weight could induce beta-cell autoimmunity in genetically predisposed children(9), and the accelerator hypothesis, proposing that insulin resistance due to weight gain accelerates the autoimmune process leading to type 1 diabetes (10; 11), but we could not conclusively confirm either hypothesis.

The TEDDY study is the largest prospective, longitudinal follow-up of children at risk of type 1 diabetes to date. As screening for islet autoimmunity is done every 3<sup>rd</sup> month, the time for seroconversion to IA is easy to define. At all visits height and weight are recorded, which makes it possible to evaluate growth before and after seroconversion. Therefore, TEDDY provides a unique opportunity to test the postulated overload and accelerator hypotheses. Potential confounders adjusted for or excluded using sensitivity analysis included: birth size, gender, family history of type 1 diabetes, breastfeeding and the HLA DR, DQ genotype. Further analyses may include non-HLA genetic markers that may be associated both with IA and infant/toddler growth and differ in allele frequencies between the participating countries in TEDDY.

In addition to breastfeeding, a number of infant/toddler dietary exposures have been linked to IA and type 1 diabetes (29-31). TEDDY has shown that some of the exposures vary significantly by country (32; 33). Some are also likely to increase or decrease weight gain during early childhood.

The children of this study are still young with a mean age at seroconversion to persistent IA of 31 months and at clinical onset of type 1 diabetes of 45 months. Insulin resistance as an accelerator for progression to type 1 diabetes may have a greater impact in older children (i.e., during puberty). As only a fraction of the children with IA in TEDDY have developed diabetes to date, the results may change in future analyses. The growth pattern in children with early seroconversion may also be different from the pattern of children seroconverting later in life. The TEDDY cohort will be followed to 15 years of age, which enable further analyses of growth and development of both IA and type 1 diabetes.

The TEDDY cohort was selected based on the presence of HLA genotypes with increased type 1 diabetes risk. As such, our findings may not be representative of all children developing IA and type 1 diabetes. In children with low-risk HLA genotypes, aberrations of growth may be of more significant importance for the development of type 1 diabetes, as previously described (34). It is also possible that the impact of growth on risk for type 1 diabetes may be present only in some cases and dependent of gene interactions and epigenetic factors. Since HLA genotypes associated with an increased type 1 diabetes risk are also associated with birth weight (35; 36), HLA-genotypes likely affect childhood growth. Nevertheless, increased early linear growth has been reported in children before the onset of type 1 diabetes, independent of HLA genotypes (15).

Childhood height and linear growth are strongly correlated to both maternal and paternal height (37; 38). We were not able to adjust the analyses for parental height. This may have an impact on our analyses of height data, while it would not affect weight data as much.

A number of previous studies have investigated growth in height and weight and development of type 1 diabetes, indicating that increased growth velocity may be a risk factor for type 1 diabetes (13-17). However, only few prospective investigations among children,

with the ability to separate the impact of growth on seroconversion to IA and progression to type 1 diabetes in IA positive children, have been published and results have been inconsistent. In the US DAISY study, an increased height growth velocity was associated with the risk of seroconversion (143/1714 children developed IA), while weight and BMI growth velocity had no effect (21). In the present study we could not confirm these findings. The reason for this may be that we used growth parameters from 3 months of age, while growth between 2 and 11 years of age was studied in DAISY. In an Australian study of first degree relatives to type 1 diabetes, increased weight z-score was reported as a predictor of islet autoimmunity, independent of dietary factors, in 46 children seroconverting at a low age (median 1.7 years) (22). In a study from Germany, using combined data from Babydiab and Babydiet, an early age of infant BMI peak was associated with development of IA in 135 children(20). Not being able to fully confirm those findings in our large multi-center study, we report findings similar to the Australian study. However, the weak association may indicate that growth is not the primary trigger of IA.

Insulin resistance has been suggested to accelerate the process from IA to clinically overt type 1 diabetes (39; 40). On the contrary, neither weight nor BMI velocity could be confirmed to increase the risk for progression to type 1 diabetes (n=21) in autoantibody positive children in the DAISY study, whereas height velocity independently increased the risk (21). In our current study, we were not able to study insulin resistance measured as HOMA-IR. Using weight z-score as an indicator of potential insulin resistance (41), we could not confirm previous studies indicating that insulin resistance accelerate the progression from persistent islet autoimmunity to type 1 diabetes (39; 40) (42). However, our children were young, and only in the DiME study, where HOMA-IR/FPIR predicted progression to type 1 diabetes in 77 autoantibody positive siblings of type 1 diabetes patients (42), the participants were at a comparably young age.

Greater weight in early childhood appears to predict a small increase in the risk of IA. The relevance of these findings for the future risk of diabetes will require longer follow-up of the cohort and evaluation of additional factors, such as infant feeding and genetic determinants of growth.

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**Conflict of Interest:** The authors declare that there are no conflicts of interest to report regarding this study.

**Guarantor statement:** Helena Elding Larsson and Marian Rewers are guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

**The TEDDY Study Group** (See online appendix)

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Figure legends:

Figure 1: Flow chart of the children included in The Environmental Determinants of Diabetes in the Young (TEDDY) study and the children where a full set of growth data was available for analysis. A total of 575 developed islet autoimmunity (IA), 351 multiple IA and 169 type 1 diabetes (T1D).

Figure 2: Hazard Ratios (HRs) and 95% confidence intervals for any islet autoimmunity (IA) and multiple IA (2+ IA) and T1D in relation to weight z-scores at 12, 24, and 36 months of age, change in weight z-scores 3, 6, 8 or 12 months prior to development of IA, 2+ IA and T1D and time varying growth up to 4 years of age (only events up to 4 years included). Only weight z-scores are showed since height z-scores were not significant.

Table 1. Characteristics for children developing persistent islet autoimmunity (IA) and Type 1 diabetes.

	Developed IA n=575	Did not develop IA n=6893	HR (95%CI)	Developed Type 1 diabetes n=169	Did not develop Type 1 diabetes n=7299	HR (95%CI)
Age at first Ab+ visit/diagnosis of Type 1 diabetes or most recent visit (months)	31 (SD=21)	61 (SD=26)		45 (SD=23)	64 (SD=26)	
Country						
Finland	25% (n=144)	22% (n=1513)		31% (n=52)	22% (n=1605)	
Germany	8% (n=46)	7% (n=462)		12% (n=21)	7% (n=487)	
Sweden	34% (n=194)	30% (n=2069)		27% (n=45)	30% (n=2218)	
US	33% (n=191)	41% (n=2849)		30% (n=51)	41% (n=2989)	
Family History of Type 1 diabetes						
Yes	22% (n=124)	10% (n=714)	2.36 (1.92-2.91) <sup>a</sup>	32% (n=54)	11% (n=784)	3.70 (2.62-5.22) <sup>a</sup>
High-risk HLA-DR, -DQ genotype						
DR3/4	50% (n=290)	38% (n=2642)	1.71 (1.45-2.01) <sup>a</sup>	55% (n=93)	39% (n=2839)	2.15 (1.58-2.92) <sup>a</sup>
Gender						
Female	46% (n=250)	49% (n=3407)	0.78 (0.66-0.92) <sup>a</sup>	46% (n=77)	49% (n=3580)	0.85 (0.63-1.15) <sup>a</sup>

Values are % (n) or mean (SD)

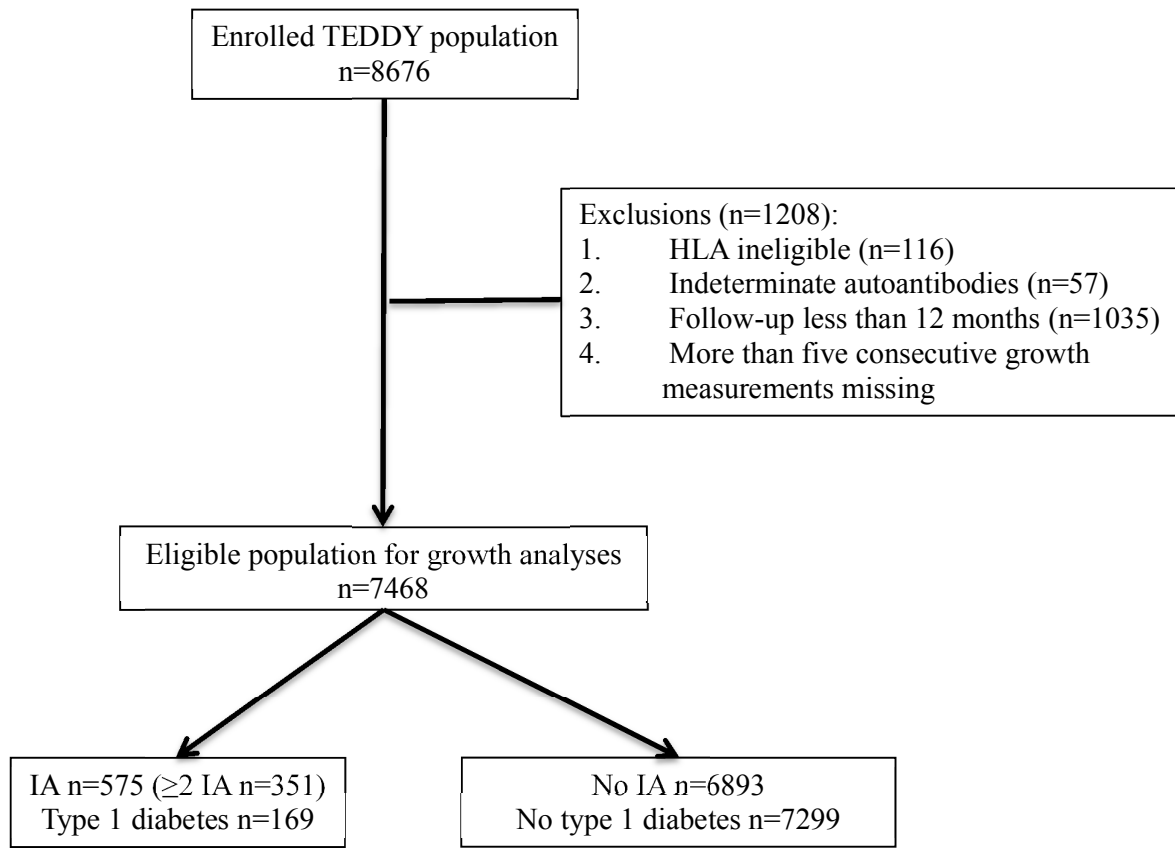
<sup>a</sup>HRs adjusted for relation to type 1 proband, HLA-DR\_DQ genotype, gender, age at first persistent confirmed Ab and stratified by country of residence.

Table 2. Hazard Ratios (HRs) for any islet autoimmunity (IA) and multiple IA (2+IA) and type 1 diabetes (T1D) in relation to a) weight z-scores or length/height z-scores at 12, 24, and 36 months of age, b) 3, 6, 9 and 12 months prior to IA, 2+IA or T1D, c) Time Varying Model: growth over time up to 4 years of age.

		Any IA				2+ IA				2+IA to T1D				
		HR	95 % CI	P-value	FDR*	HR	95 %CI	P-value	FDR*	HR	95 %CI	P-value	FDR*	
a)	<b>Weight</b>	12 mo or age	<b>1.16</b>	<b>1.06-1.27</b>	<b>0.001</b>	<b>0.008</b>	<b>1.21</b>	<b>1.08-1.35</b>	<b>0.001</b>	<b>0.008</b>	0.98	0.81-1.18	0.81	0.81
		24 mo of age	1.10	1.01-1.20	0.03	0.091	<b>1.18</b>	<b>1.06-1.32</b>	<b>0.004</b>	<b>0.021</b>	0.96	0.80-1.16	0.69	0.69
		36 mo of age	1.07	0.98-1.17	0.15	0.17	1.13	1.01-1.28	0.04	0.091	0.97	0.80-1.18	0.79	0.79
	<b>Length/Height</b>	12 mo of age	1.12	1.01-1.24	0.03	0.16	1.15	1.01-1.31	0.03	0.16	0.93	0.75-1.16	0.53	0.69
		24 mo of age	1.07	0.97-1.18	0.20	0.39	1.11	0.98-1.26	0.12	0.39	0.96	0.79-1.18	0.69	0.69
		36 mo of age	1.07	0.97-1.18	0.18	0.39	1.15	1.02-1.30	0.02	0.16	0.92	0.74-1.15	0.46	0.69
b)	<b>Weight</b>	3 mo prior	1.13	1.02-1.25	0.02	0.08	1.11	0.97-1.26	0.13	0.17				
		6 mo prior	1.06	0.95-1.18	0.29	0.29	1.14	1.00-1.30	0.05	0.096				
		9 mo prior	1.08	0.97-1.21	0.16	0.17	1.14	0.99-1.30	0.06	0.096				
		12 mo prior	1.12	0.99-1.26	0.06	0.096	1.13	0.98-1.30	0.11	0.16				
	<b>Length/Height</b>	3 mo prior	1.03	0.92-1.15	0.66	0.70	1.09	0.94-1.26	0.24	0.39				
		6 mo prior	1.06	0.95-1.19	0.32	0.39	1.09	0.94-1.26	0.25	0.39				
		9 mo prior	0.98	0.87-1.11	0.80	0.80	1.07	0.92-1.25	0.37	0.42				
		12 mo prior	1.07	0.94-1.22	0.32	0.39	1.11	0.95-1.31	0.19	0.39				
c)	<b>Weight</b>	up to 4 years	1.11	1.01-1.23	0.04	0.09	1.10	0.96-1.25	0.16	0.17	0.79	0.61-1.04	0.09	0.15
	<b>Length/Height</b>	up to 4 years	1.08	0.97-1.21	0.17	0.39	1.08	0.94-1.25	0.28	0.39	0.80	0.59-1.07	0.13	0.52

HRs adjusted for birth weight z-score (for weight z-score models) and birth length z-scores (for length/height z-score models), relation to type 1 proband, HLA-DR\_DQ genotype and gender, and stratified by country of residence.

\*FDR = False Discovery Rate



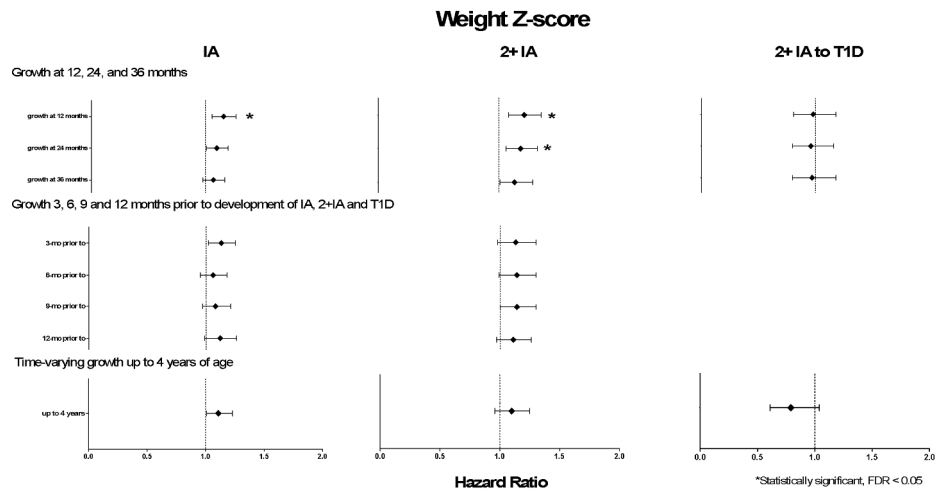


Figure 2  
232x119mm (300 x 300 DPI)

Online appendix:

## **Appendix**

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