Diabetes associated HLA genotypes affect birth weight in the general population.

H. E. Larsson et al.: Diabetes risk HLA genotypes affect birth weight.

H. Elding Larsson, K. Lynch, B. Lernmark, A. Nilsson, G. Hansson, P. Almgren, Å. Lernmark and S-A. Ivarsson for the DiPiS Study group[1].

Department of Clinical Sciences, University Hospital MAS, Lund University, Malmö, Sweden

[1]Members of the DiPiS Study Group are listed in the Acknowledgement

Address correspondence to:

Helena Elding Larsson

Department of Clinical Sciences - Pediatrics

University Hospital MAS

SE-205 02 Malmö

Sweden

helena.larsson@pediatrik.mas.lu.se

Word count:

Abstract: 246

Main text: 2880

Abstract

Aims/hypothesis. The aim was to test the hypothesis that diabetes risk HLA-genotypes, cord blood autoantibodies or both are associated with increased birth weight.

Methods. HLA-genotypes were determined in dried blood spots of cord blood from a total of 16 709 children born to healthy mothers in the Diabetes Prediction in Skåne (DiPiS) study, a population-based observational clinical investigation of newborn children. Children born to mothers with diabetes or gestational diabetes were excluded. Autoantibodies to glutamic acid decarboxylase (GAD65Ab) and insulinoma-associated protein 2 (IA-2Ab) were determined in standard radioligand binding assays. Birth weight (BW) was adjusted for gestational age and divided into quartiles. Upper quartile was defined as high relative birth weight (HrBW) and lower quartile as low relative birth weight (LrBW).

Results. Type 1 diabetes risk genotypes were strongly associated with relative birth weight (rBW) ($p=0.01$). The high risk HLA-DQ2/8, DQ8/0604 and DQ8/X genotypes were associated with HrBW (OR (95%CI) = 1.20 (1.08 – 1.33), $p=0.0006$). The type 1 diabetes negatively associated HLA-DQB1*0603 allele was also associated with HrBW ($p=0.025$), confirming a previous report on DQB1*0603-linked HLA-DR13. GAD65Ab were negatively associated with HrBW (OR (95%CI) = 0.72 (0.56 – 0.93), $p=0.01$). Regression analysis showed that the HLA associated increase in rBW was independent of confounding factors.

Conclusions/interpretation. HLA-genotypes may be associated with intrauterine growth independent of type 1 diabetes risk. The epidemiological observation that high BW is a risk factor for type 1 diabetes could possibly be a result of diabetes high risk HLA moderating intrauterine growth.

Abbreviations: BW, Birth Weight; DBS, Dried Blood Spots; DiPiS, Diabetes Prediction in Skåne; GAD65, Glutamic Acid Decarboxylase; GAD65Ab, Glutamic Acid Decarboxylase autoantibodies; HrBW, High relative Birth Weight; IA-2, Insulinoma-associated protein 2; IA-2Ab, Insulinoma-associated protein 2 autoantibodies; LGA, Large for Gestational Age; LrBW, Low relative Birth Weight; OR, odds ratio; rBW, relative Birth Weight; SGA, Small for Gestational Age.

Introduction

Insulin dependent diabetes mellitus, or type 1 diabetes mellitus, is a chronic disease caused by an autoimmune destruction of the pancreatic islet beta cells. The incidence of type 1 diabetes before 15 years of age is increasing [1, 2], especially for children diagnosed at an early age [3, 4, 5, 6, 7]. HLA susceptibility genes are strongly associated with type 1 diabetes [8, 9, 10, 11]. Still the concordance rate for identical twins to develop disease is below 50% [12, 13], which indicates that environmental factors are of importance.

It has been speculated that wealth-based over nutrition could be of importance for the current increase in type 1 diabetes incidence [14]. Children born large for gestational age (LGA) had a higher risk of type 1 diabetes later in childhood [15]. In one study the incidence of type 1 diabetes was found to increase almost linearly with birth weight (BW) [16].

HLA-DQ on chromosome 6 is the major genetic factor for type 1 diabetes. About 60 % of the genetic susceptibility is explained by HLA [8, 17]. HLA-DQB1*0302-A1*0301 (DQ8) and HLA-DQB1*0201-A1*0501(DQ2), confer to the highest risk for type 1 diabetes, especially when both are present in the genotype. Almost ninety percent of persons with type 1 diabetes have at least one of these haplotypes [9].

The autoimmune process destroying the beta cells is thought to occur at different rates. The pathogenesis seems to be marked by autoantibodies against glutamic acid decarboxylase (GAD65) [18], insulinoma-associated protein 2 (IA-2) [19, 20] and insulin (IAA) [21], alone or in combination. Up to 90 % of children with newly diagnosed type 1 diabetes have at least one of these autoantibodies at diagnosis and 85% of children developing diabetes do not have a first degree relative with the disease. In some children who later developed type 1 diabetes islet autoantibodies have been found retrospectively in cord blood [22], and it was therefore speculated that intrauterine factors might contribute to type 1 diabetes risk.

LGA and other altered growth rates appear to be associated with type 1 diabetes risk [15,16, 23, 24, 25, 26]. It is not established whether islet autoantibodies are associated with LGA or growth, or whether the relationship between high BW and type 1 diabetes is a result of the same genes being associated with both increased risk for type 1 diabetes and high BW. It has recently been reported that there is an association between HLA DR13 and mean BW [27]. We have extended this study to a large cohort of the general population to test the hypothesis that HLA genotypes that define type 1 diabetes risk, cord blood GAD65Ab and IA-2Ab, or both are associated with BW. Since diabetes in the mother is compromising foetal growth we have excluded all children born to mothers with diabetes or gestational diabetes. Hence, in contrast to previous investigations on the relationship between type 1 diabetes and BW [15, 16], we examined HLA genotypes in relation to BW with particular emphasis on type 1 diabetes risk genotypes.

Materials and methods

Population. The children in this study were included in DiPiS (Diabetes Prediction in Skåne), a prospective, population based study in Skåne, the most southern province of Sweden. The aim of DiPiS is to determine the positive predictive value for type 1 diabetes of genetic risk combined with islet cell autoantibody markers. The aim of DiPiS is also to identify factors before and after pregnancy that may trigger type 1 diabetes.

Blood samples were obtained from all mothers at delivery. Cord blood DNA was genotyped for HLA and for autoantibodies to GAD65 and IA-2. The parents fill in consent and questionnaire forms when the child is two months of age. The forms include family history of diabetes, birth weight and gestational age.

The Lund University Ethics Committee approved the DiPiS study.

HLA-Genotyping. HLA was analysed on dried blood spots (DBS) on filters as described [28, 29]. Filter samples (3 mm in diameter) of DBS were punched into 96-well PCR plates. HLA-DQB1 alleles were amplified with sequence specific primers. The product was transferred to DELFIA streptavidin-coated microtitration plates (Perkin Elmer Life Sciences, Boston, MA, USA) and diluted with DELFIA hybridisation buffer. After incubation and denaturation the single stranded DNA was hybridised with two sets of probes (Perkin Elmer Life Sciences). The first set contains Eu-DQB1*0602/3, Sm-DQB1*0603/4 and Tb-Control. The second one contains Eu-DQB1*0302, Sm-DQB1*0301 and Tb-DQB1*02. After washing and adding of DELFIA enhancement solution, the Eu and Sm fluorescence was counted in a Victor2 MultiLabel Counter (Perkin Elmer Life Sciences). The Tb signal to noise ratio was calculated with MultiCalc (Perkin Elmer Life Sciences). The samples positive for DQB1*02 were further analysed for DQA1*0201 and 05 alleles to separate DR3 from subjects with DR7. HLA-DQA1 typing was performed with the same technique as for DQB1-typing with some

modifications, using streptavidin-coated plates, dilution with DELFIA hybridisation buffer (Perkin Elmer Life Sciences) and the use of Sm-DQA1*05 and Tb-DQA1*0201 probes.

The HLA genotypes were divided into seven risk groups for type 1 diabetes (Table 4): 1) Very high risk having DQB1*0201-A1*0501 and DQB1*0302-A1*0301; 2) High risk having DQB1*0302-A1*0301 but not DQB1*0201-A1*0501; 3) Moderate risk having DQB1*0201-DQA1*0501 but not DQB1*0302-A1*0501; 4) Neutral HLA; 5) Low risk HLA; 6) Very low risk; and 7) No risk HLA.

GAD65 and IA-2 autoantibodies. Autoantibodies (Ab) to GAD65 or IA-2 were determined in a first combined screen, where eluates from DBS were incubated with labelled antigen, antibody-bound labelled antigen separated from free by Sepharose-Protein A (Amersham Biosciences, Uppsala, Sweden) and the radioactivity counted in a Beta Plate Reader (Perkin Elmer Life Sciences) as described elsewhere [30]. The combined screen referred to as COMB compared a positive reference with two negative samples to determine a cut off level that would require the sample to be analysed for both GAD65Ab and IA-2Ab in separate assays. Levels above COMB of 1.0 U/ml were considered high, and re-assayed individually for GAD65Ab and IA-2Ab [31, 32]. GAD65Ab above 35 U/ml and IA-2Ab above 6 U/ml were considered positive.

Statistical methods and datasets. All questionnaires and forms from the DiPiS-study and the results from HLA-typing and antibody-analyses were scanned into a database, BC/OS system, Biocomputing OS 2000 (Biocomputing Platforms Ltd Oy, Espoo, Finland).

In term newborns, relative BW (rBW) was calculated as Z scores for each gestational week and gender by the following formula:

Z score = (BW – mean [BW])/SD [BW]

Population means (SD) of BW were estimated internally using the study group. An ultra sound corrected standardized mean BW for each gestational age and gender were also calculated for all newborns using two polynomial equation formulas. These formulas were polynomial regressions fitted to 759 ultrasonically estimated foetal weights in 86 uncomplicated pregnancies and are used as base for the standard weights in Swedish maternity clinics [33, 34]. The SD was taken as 12% of the mean. LGA was defined as an ultra sound corrected rBW above 2 SD, and small for gestational age (SGA) as an ultra sound corrected rBW below 2 SD.

RBW was divided into quartiles, where children in the lower quartile were defined to have a low relative BW (LrBW) and children in the upper quartile a high relative BW (HrBW). The association between BW and HLA genotype risk groups was examined using χ^2 tests. Multiple logistic regression analysis was used to test whether diabetes risk HLA genotypes were associated with HrBW or LrBW after adjusting for gender of the child, maternal age and maternal smoking.

Twins and triplets were excluded from the analysis, as their BW is known to be lower than the standard. All children born to mothers with diabetes including gestational diabetes were also excluded from the study since their children are known to have a high BW.

Results

Study group. In all, 16 709 children who were included in the DiPiS study between Sept 2000 and Dec 2003 were fully analysed. During this period, approximately 38 550 children were born in the province of Skåne (Figure 1). A total of 29 547 cord blood samples were obtained at the five maternity clinics. After excluding twins, triplets and children born to mothers with diabetes (n=2865), 26 682 children remained. Of these, 17 527 of the parents completed a questionnaire and consented to the study when their child was two months old [35, 36]. Reported BW from the questionnaire and gestational age recorded at birth were available on 16 709 children (62.6 % of the 26 682 eligible children). Since all parents did not fill out the questionnaire we next compared participating with non-participating children (Table 1), by using maternal and newborn information recorded at birth. Participation was higher with increasing age of the mother ($p< 0.001$) and with increasing gestational age ($p< 0.001$). There was no difference in frequency of diabetes high risk HLA or positive autoantibodies between the groups. Among the 16 709 newborn children, 51.3 % were boys and 48.0% were girls (gender missing in 0.7 %) (Table 2).

Birth Weight and Relative Birth Weight. The distribution of BW, internal Z-scores and ultra sound corrected BW (rBW) shown in Figure 2, panel A, B and C, respectively demonstrate that the mean ultra sound corrected rBW was +0.2 Z-score, indicating this population to be slightly heavier than the Swedish standard foetal weights for gestational age. A total of 5.1 % of the children were LGA while 1.7 % were SGA. The median (interquartile range) BW was 3610 g (3275-3950) and ultra sound corrected rBW 0.15 (-0.51-0.85) Z-score. Approximately 71% of children with HrBW had also a BW in the upper quartile and 69% of the children with LrBW had a BW in the lower quartile. Of the children with HrBW, 74 (0.5%) had a low actual BW. These children were born preterm at 24-36 gestational weeks and were LGA (Figure 2C). In the 16055 term newborns, the quartiles of the ultrasound corrected rBW and

internal rBW matched in 87.6% of children. The remaining 12.4% differed randomly by no more than one quartile.

HLA-DQB1 genotypes. HLA-DQB1 was first examined descriptively to determine and rank the DQB1 genotypes with the highest and lowest frequency of LrBW and HrBW (Table 3). Children with DQB1*0302 and either DQB1*02, DQB1*0603, DQB1*0604 or diabetes neutral DQX had the highest frequency of HrBW while children with DQB1*0302 and either DQB1*0301 or DQB1*0602 the lowest frequency (Table 3). Children with diabetes risk neutral DQX/DQX genotype also had a low frequency of HrBW. In contrast, LrBW was found highest in children with DQB1*0301/DQB1*0302. All children with DQB1*0603 included in their genotype were more likely to have a low frequency of LrBW.

Diabetes risk HLA genotypes. Children with DQB1*02 were typed for both DQA1*0201 and A1*0501 to specify the seven diabetes risk HLA genotype groups (Table 4). There was a strong association between the type 1 diabetes risk genotypes and quartiles of rBW (p=0.01). Children with the diabetes high risk HLA genotype DQB1*0201-A1*0501/DQB1*0302-DQA1*0301 (DQ2/DQ8), genotype DQB1*0302/DQB1*0604 (DQ8/6.4) and DQ8/X had an increased frequency of HrBW (28.0%)) compared to the rest of the population (24.5%, p=0.0007) (OR 1.20 (95%CI 1.08 – 1.33), p=0.0006). When compared to the neutral type 1 diabetes HLA risk group, children with DQ2/DQ8 had a significantly increased risk for HrBW (OR 1.32 (95% CI 1.09-1.60), p=0.004) as well as children with DQ8/6.4 and DQ8/X (OR 1.21 (1.06 – 1.38), p=0.005). Children with low risk HLA-DQB1*0603 were associated with increased frequency of HrBW (OR 1.13 (95%CI 1.02-1.28), p=0.025) and decreased frequency of LrBW compared to neutrals (OR 0.88 (95%CI 0.79 – 0.99), p=0.03). No other type 1 diabetes HLA risk group was associated with HrBW or LrBW.

Most of these data were confirmed when ultra sound corrected Z-scores were used. First, children with the diabetes high risk HLA genotype DQB1*0201-A1*0501/DQB1*0302-

DQA1*0301 (DQ2/DQ8) had an increased frequency of HrBW (29.2% compared to expected 25%). This group differed significantly from all other genotype groups (p=0.01). Second, when compared to the neutral type 1 diabetes HLA risk group, children with DQ2/DQ8 had a significantly increased risk for HrBW (OR 1.30 (95% CI 1.08-1.56), p=0.006). The DQ8/0604 or DQ8/X were not significant. Children with low risk HLA-DQB1*0603 were associated with HrBW (OR of 1.12 (1.01-1.26), p=0.039) compared to neutrals. However, this association was more due to a strong protection from LrBW (OR 0.83 (0.75-0.93), p=0.002), as the frequency of HrBW among DQB1*0603-positive children did not differ from the frequency of children in the normal standard weight groups (2nd and 3rd quartile).

GAD65 and IA-2 autoantibodies.

In all, 477 (3.0%) term children had autoantibodies to GAD65 or IA-2. Of these, 413 had GAD65Ab, 14 had IA-2Ab and 50 had both. Only GAD65Ab were associated with relative birth weight. Children with antibodies to GAD65 were less likely to have HrBW (OR= 0.78 (95%CI 0.62 – 0.98), p=0.03). This association remained significant after adjusting for confounding factors (Table 5).

Discussion

In this study we analysed a large group of newborn children from the Skåne province of Sweden to uncover that children with the HLA-DQ2/DQ8 and DQ8/0604 and DQ8/X genotypes had a higher frequency of HrBW. We confirmed that children with the DQB1*0603 allele, strongly linked to DR13 [37] and known to be protective of type 1 diabetes, also had a higher frequency of HrBW. The data therefore confirm the previous correlation between DR13 and mean BW [27], but suggest that the effect could be either from the DQ, DR or both alleles. The effects on BW were small and therefore not likely to be seen in study populations of a relatively little size. For example, in a previous study it was reported that the HLA-DQ2/DQ8-genotype had a lower BW compared to DQB1*0602/DQB1*0602, but this conclusion was based on only 969 children [38].

The development of type 1 diabetes is thought to be a result of genetic susceptibility in combination with environmental factors. Numerous triggers for type 1 diabetes have been proposed. These include viral infections [39, 40, 41], dietary factors (cows milk, nitrosamines, high protein intake) [42, 43], neonatal jaundice and ABO-incompatibility [44], neonatal respiratory disease [44], early supplementary milk-based feeding, short time of breast-feeding [42, 45] and stress events such as severe life events [42, 46, 47]. Several studies have found that children who later develop diabetes have a higher BW than controls, and also have an increased linear growth in childhood [15, 16, 42, 48, 49]. Our finding that HLA genetic factors, which are associated with susceptibility for type 1 diabetes, are related to intrauterine growth might in fact contribute to the increased BW in type 1 diabetes children.

We also showed that other, non-diabetes associated HLA-genotypes, could be ranked in order according to frequency of children with HrBW and LrBW. The HLA-types with highest frequency of children with LrBW had a low frequency of children with HrBW and the opposite. The conclusion of this is that HLA-genotypes seem to have a modulating effect on

BW. This direct correlation between HLA-DQ subtypes and BW might explain the previously reported increase in BW in children who later develop type 1 diabetes. The mechanism may include different ways to respond to intrauterine infections, possibly by HLA-mediated alterations in the immune system. It could also represent an altered metabolic control in mothers with diabetes susceptible genotypes, which perhaps give a subclinical hyperglycaemia during pregnancy and thus an increased BW. In the present study, all children born to mothers with diabetes and gestational diabetes were excluded, since these children are known to be heavier at birth. Children born to fathers with diabetes were not excluded, since this should not affect the BW. Further we are not able to foresee if any of those fathers or mothers will get diabetes in the future.

There was a decrease in rBW with positive GAD65 autoantibodies in cord blood. This effect was independent of HLA and is of interest since it was recently reported that offspring who were GAD or IA-2 autoantibody positive at birth had significantly lower risks for developing multiple islet autoantibodies and diabetes than offspring who were islet autoantibody negative at birth [50]. Further studies will therefore be needed to further analyse the possible effects of islet autoantibodies on BW since the number of children with autoantibodies in our study was small.

The cohort studied was representative for the entire population, but some differences between the mothers and children participating in DiPiS and those not participating were found. Older mothers were more likely to participate whereas a lower participation was evident when the child was born preterm. Our study contains a larger percentage of children born LGA (5.1 %) and a slightly lower percentage of children born SGA (1.7 %), than expected from the 1996 reference material [33, 34]. Comparing this observation with a statistical report from the Swedish Medical Birth Registry, made by the National Board of Health and Welfare [51],

confirmed that this was equivalent to a national trend of increasing BW and amount of children born LGA.

In conclusion, this large population of children born to healthy mothers showed an association between HrBW and the diabetes high risk HLA-DQ2/8, DQ8/0604, DQ8/X as well as the low risk HLA-DQB1*0603. We speculate that previous observations of an association between high BW and type 1 diabetes are secondary to the primary association between HLA and BW, and that high BW may not be a risk factor for type 1 diabetes but an effect of HLA.

Acknowledgements

References

1. Diabetes Epidemiology Research International Group (1990) Secular trends in incidence of childhood IDDM in 10 countries. Diabetes 39:858-64

2. Karvonen M, Tuomilehto J, Libman I et al. (1993) A review of the recent epidemiological data on the worldwide incidence of type 1 (insulin-dependent) diabetes mellitus. World Health Organization DIAMOND Project Group. Diabetologia 36:883-92

3. Dahlquist G and Mustonen L (1994) Childhood onset diabetes-time trends and climatological factors. Int J Epidemiol 23:1234-41

4. Gardner SG, Bingley PJ, Sawtell PA et al. (1997) Rising incidence of insulin dependent diabetes in children aged under 5 years in the Oxford region: time trend analysis. The Bart's-Oxford Study Group. BMJ 315:713-7

5. Svensson J, Carstensen B, Molbak A et al. (2002) Increased risk of childhood type 1 diabetes in children born after 1985. Diabetes Care 25:2197-201

6. Tuomilehto J, Virtala E, Karvonen M et al. (1995) Increase in incidence of insulin-dependent diabetes mellitus among children in Finland. Int J Epidemiol 24:984-92

7. EURODIAB Ace Study Group and EURODIAB Ace Substudy 2 Study Group (1998) Familial risk of type I diabetes in European children. Diabetologia 41:1151-6

8. Todd JA (1995) Genetic analysis of type 1 diabetes using whole genome approaches. Proc Natl Acad Sci U S A 92:8560-5

9. Redondo MJ, Fain PR and Eisenbarth GS (2001) Genetics of type 1A diabetes. Recent Prog Horm Res 56:69-89

10. Noble JA, Valdes AM, Cook M et al. (1996) The role of HLA class II genes in insulin-dependent diabetes mellitus: molecular analysis of 180 Caucasian, multiplex families. Am J Hum Genet 59:1134-48

11. Kockum I, Sanjeevi CB, Eastman S et al. (1995) Population analysis of protection by HLA-DR and DQ genes from insulin-dependent diabetes mellitus in Swedish children with insulin-dependent diabetes and controls. Eur J Immunogenet 22:443-65

12. Barnett AH, Eff C, Leslie RD et al. (1981) Diabetes in identical twins. A study of 200 pairs. Diabetologia 20:87-93

13. Kaprio J, Tuomilehto J, Koskenvuo M et al. (1992) Concordance for type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland. Diabetologia 35:1060-7

14. Patterson CC, Dahlquist G, Soltesz G et al. (2001) Is childhood-onset type I diabetes a wealth-related disease? An ecological analysis of European incidence rates. Diabetologia 44 Suppl 3:B9-16

15. Dahlquist G, Bennich SS and Kallen B (1996) Intrauterine growth pattern and risk of childhood onset insulin dependent (type I) diabetes: population based case-control study. BMJ 313:1174-7

16. Stene LC, Magnus P, Lie RT et al. (2001) Birth weight and childhood onset type 1 diabetes: population based cohort study. BMJ 322:889-92

17. Verge CF, Gianani R, Kawasaki E et al. (1996) Prediction of type I diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-2 autoantibodies. Diabetes 45:926-33

18. Baekkeskov S, Aanstoot HJ, Christgau S et al. (1990) Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. Nature 347:151-6

19. Solimena M, Dirkx R, Jr., Hermel JM et al. (1996) ICA 512, an autoantigen of type I diabetes, is an intrinsic membrane protein of neurosecretory granules. EMBO J 15:2102-14

20. Saeki K, Zhu M, Kubosaki A et al. (2002) Targeted disruption of the protein tyrosine phosphatase-like molecule IA-2 results in alterations in glucose tolerance tests and insulin secretion. Diabetes 51:1842-50

21. Atkinson MA and Eisenbarth GS (2001) Type 1 diabetes: new perspectives on disease pathogenesis and treatment. Lancet 358:221-9

22. Lindberg B, Ivarsson SA, Landin-Olsson M et al. (1999) Islet autoantibodies in cord blood from children who developed type I (insulin-dependent) diabetes mellitus before 15 years of age. Diabetologia 42:181-7

23. Hypponen E, Virtanen SM, Kenward MG et al. (2000) Obesity, increased linear growth, and risk of type 1 diabetes in children. Diabetes Care 23:1755-60

24. Baum JD, Ounsted M and Smith MA (1975) Letter: Weight gain in infancy and subsequent development of diabetes mellitus in childhood. Lancet 2:866

25. Johansson C, Samuelsson U and Ludvigsson J (1994) A high weight gain early in life is associated with an increased risk of type 1 (insulin-dependent) diabetes mellitus. Diabetologia 37:91-4

26. Bruining GJ (2000) Association between infant growth before onset of juvenile type-1 diabetes and autoantibodies to IA-2. Netherlands Kolibrie study group of childhood diabetes. Lancet 356:655-6

27. Aroviita P, Partanen J, Sistonen P et al. (2004) High birth weight is associated with human leukocyte antigen (HLA) DRB1*13 in full-term infants. Eur J Immunogenet 31:21-6

28. Sjoroos M, Iitia A, Ilonen J et al. (1995) Triple-label hybridization assay for type-1 diabetes-related HLA alleles. Biotechniques 18:870-7

29. Ilonen J, Reijonen H, Herva E et al. (1996) 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 19:795-800

30. Hampe CS, Hammerle LP, Bekris L et al. (2000) Recognition of glutamic acid decarboxylase (GAD) by autoantibodies from different GAD antibody-positive phenotypes. J Clin Endocrinol Metab 85:4671-9

31. Grubin CE, Daniels T, Toivola B et al. (1994) A novel radioligand binding assay to determine diagnostic accuracy of isoform-specific glutamic acid decarboxylase antibodies in childhood IDDM. Diabetologia 37:344-50

32. Verge CF, Stenger D, Bonifacio E et al. (1998) Combined use of autoantibodies (IA-2 autoantibody, GAD autoantibody, insulin autoantibody, cytoplasmic islet cell antibodies) in type 1 diabetes: Combinatorial Islet Autoantibody Workshop. Diabetes 47:1857-66

33. Marsal K, Persson PH, Larsen T et al. (1996) Intrauterine growth curves based on ultrasonically estimated foetal weights. Acta Paediatr 85:843-8

34. Persson PH and Weldner BM (1986) Intra-uterine weight curves obtained by ultrasound. Acta Obstet Gynecol Scand 65:169-73

35. Larsson K, Elding-Larsson H, Cederwall E et al. (2004) Genetic and perinatal factors as risk for childhood type 1 diabetes. Diabetes Metab Res Rev 20(6):429-37

36. Lernmark B, Elding Larsson H, Hansson G et al. (2004) Parent responses to participation in genetic screening for diabetes risk. Pediatric Diabetes 5(4)174-81

37. Hermann R, Turpeinen H, Laine AP et al. (2003) HLA DR-DQ-encoded genetic determinants of childhood-onset type 1 diabetes in Finland: an analysis of 622 nuclear families. Tissue Antigens 62:162-9

38. Stene LC, Magnus P, Ronningen KS et al. (2001) Diabetes-associated HLA-DQ genes and birth weight. Diabetes 50:2879-82

39. Dahlquist G, Frisk G, Ivarsson SA et al. (1995) Indications that maternal coxsackie B virus infection during pregnancy is a risk factor for childhood-onset IDDM. Diabetologia 38:1371-3

40. Dahlquist GG, Ivarsson S, Lindberg B et al. (1995) Maternal enteroviral infection during pregnancy as a risk factor for childhood IDDM. A population-based case-control study. Diabetes 44:408-13

41. Hyoty H, Hiltunen M, Knip M et al. (1995) A prospective study of the role of coxsackie B and other enterovirus infections in the pathogenesis of IDDM. Childhood Diabetes in Finland (DiMe) Study Group. Diabetes 44:652-7

42. Dahlquist G, Blom L and Lonnberg G (1991) The Swedish Childhood Diabetes Study-a multivariate analysis of risk determinants for diabetes in different age groups. Diabetologia 34:757-62

43. Dahlquist GG, Blom LG, Persson LA et al. (1990) Dietary factors and the risk of developing insulin dependent diabetes in childhood. BMJ 300:1302-6

44. Dahlquist GG, Patterson C and Soltesz G (1999) Perinatal risk factors for childhood type 1 diabetes in Europe. The EURODIAB Substudy 2 Study Group. Diabetes Care 22:1698-702

45. Virtanen SM, Rasanen L, Aro A et al. (1992) Feeding in infancy and the risk of type 1 diabetes mellitus in Finnish children. The 'Childhood Diabetes in Finland' Study Group. Diabet Med 9:815-9

46. Thernlund GM, Dahlquist G, Hansson K et al. (1995) Psychological stress and the onset of IDDM in children. Diabetes Care 18:1323-9

47. Robinson N and Fuller JH (1985) Role of life events and difficulties in the onset of diabetes mellitus. J Psychosom Res 29:583-91

48. Blom L, Persson LA and Dahlquist G (1992) A high linear growth is associated with an increased risk of childhood diabetes mellitus. Diabetologia 35:528-33

49. EURODIAB Substudy 2 Study Group (2002) Rapid early growth is associated with increased risk of childhood type 1 diabetes in various European populations. Diabetes Care 25:1755-60

50. Koczwara K, Bonifacio E, Ziegler AG (2004) Transmission of maternal islet antibodies and risk of autoimmune diabetes in offspring of mothers with type 1 diabetes. Diabetes 53:1-4

51. National Board of Health and Welfare (2002) Fakta om mammor, förlossningar, nyfödda barn - Medicinska födelseregistret 1973-2000

Table 1 Factors in mother and newborn associated with participation in the study. Children included in the study are compared with children with missing birth weight born of healthy mothers. Odds ratios of participation calculated in a multiple logistic regression.

Factor	OR (95%CI)[a]	p-value
Increasing maternal age (years)	**1.04 (1.04 – 1.05)**	**<0.001**
Increasing gestational age (weeks)	**1.04 (1.03 – 1.06)**	**<0.001**
Gender of child: Girl	**0.99 (0.94 – 1.05)**	**0.75**
GAD65Ab or IA-2Ab	**1.07 (0.91 – 1.25)**	**0.44**
HLA-DQB1 T1DM risk		
Moderate - High risk of T1DM	**1.06 (0.99 – 1.13)**	**0.09**

a = odds ratio >1 indicates more likely to participate

Table 2 Characteristics of the children (n=16 709) participating in the study.

	Number of children (%)
Newborn:	
Female	**8019 (48.0)**
Gestational age <37 weeks	**654 (3.9)**
Birth weight <2500 grams	**364 (2.2)**
Birth weight >4500 grams	**816 (4.9)**
Small for gestational age (<-2 SD)	**277 (1.7)**
Large for gestational age (>+2 SD)	**838 (5.0)**
Maternal factors:	
<25 years of age	**1522 (9.1)**
>40 years of age	**277 (1.7)**
Smoked during pregnancy	**1597 (9.6)**

Table 3 Percent of different HLA-DQB1 genotypes (% (95%CI)) with low or high relative birth weight.

Rank Order[a]	Low relative Birth Weight HLA DQB1 Genotype		Low relative Birth Weight Percent of genotype	High relative Birth Weight HLA DQB1 Genotype		High relative Birth Weight Percent of genotype
	Haplo1	Haplo2	% (95%CI)	Haplo1	Haplo2	%(95%CI)
1	0301	0302	29.9 (26.5 – 33.3)	0603	0302	28.3 (23.6 – 33.0)
2	0301	0604	27.3 (21.9 – 32.7)	0302	X	27.6 (25.2 – 30.0)
3	02	X	26.2 (24.3 – 28.1)	02	0302	27.5 (24.6 – 30.4)
4	0301	X	26.0 (23.9 – 28.1)	0302	0604	27.0 (20.8 – 33.2)
5	0302	0604	26.0 (19.9 – 32.1)	0603	02	26.6 (22.7 – 30.5)
6	0301	02	25.6 (23.0 – 28.2)	602/603/604[b]		26.2 (23.8 – 28.6)
7	0602	0301	25.6 (22.6 – 28.6)	0301	0604	25.4 (20.1 – 30.7)
8	X	X	25.1 (21.6 – 28.6)	0604	X	25.1 (20.8 – 29.4)
9	0602	X	24.7 (22.5 – 26.9)	0602	X	25.1 (22.9 – 27.3)
10	602/603/604[b]		24.7 (22.4 – 27.0)	02	X	24.8 (23.0 – 26.6)
11	0604	X	24.6 (20.3 – 28.9)	0602	0301	24.6 (21.6 – 27.6)
12	0302	X	24.6 (22.3 – 26.9)	0602	02	24.5 (21.8 – 27.2)
13	0603	02	24.4 (20.6 – 28.2)	0301	02	24.3 (21.7 – 26.9)
14	0602	02	24.1 (21.4 – 26.8)	0301	X	24.2 (22.1 – 26.3)
15	0602	0302	23.4 (20.2 – 26.6)	02	0604	24.2 (19.7 – 28.7)
16	02	0302	22.9 (20.1 – 25.7)	0603	0301	23.9 (19.4 – 28.4)
17	0603	0301	22.0 (17.7 – 26.3)	X	X	23.2 (19.7 – 26.7)
18	02	0604	21.4 (17.0 – 25.8)	0602	0302	22.4 (19.3 – 25.5)
19	0603	0302	20.0 (15.8 – 24.2)	0301	0302	21.9 (18.9 – 24.9)

[a] Ranked from highest frequency to lowest frequency
[b] genotypes DQB1* 0603/0602, 0603/0604, 0603/X or 0602/0604
X is not DQB1 02, 0301, 0302, 0602, 0603, 0604 but includes potential homozygozity

Table 4 Percent of different HLA type 1 diabetes genotypes and risk groups having a relative birth weight in the lower (LrBW), second, third and upper (HrBW) quartile of all term children (n=16 055).

Diabetes Risk[a]	HLA DQB1 Genotype			Relative Birth Weight Percent of genotype in each quartile			
	Haplo 1	Haplo 2	n	1st %	2nd %	3rd %	4th %
All term children			16055	25.0	25.0	25.0	25.0
Very high	02[b]	0302	594	22.6	22.4	25.7	29.3
				22.6	**22.4**	**25.7**	**29.3**
High	0302	0604	196	26.0	20.9	26.0	27.0
High	0302	X	1328	24.6	24.1	23.7	27.6
				24.8	**23.7**	**24.0**	**27.5**
Moderate	02[b]	0604	221	18.6	24.9	30.3	26.2
Moderate	02[b]	X	1121	27.2	25.4	22.9	24.5
				25.8	**25.3**	**24.1**	**24.8**
Neutral	02[c]	0604	120	26.7	28.3	24.2	20.8
Neutral	02[c]	X	611	26.4	24.2	23.2	26.2
Neutral	02[c]	02[b]	411	23.1	28.0	25.5	23.4
Neutral	02[c]	0302	301	23.6	25.6	27.2	23.6
Neutral	0301	02	1069	25.6	25.4	24.6	24.3
Neutral	0301	0302	712	29.9	24.2	24.0	21.9
Neutral	0604	X	382	24.6	25.7	24.6	25.1
Neutral	X	X	574	25.1	23.0	28.8	23.2
				25.9	**25.1**	**25.2**	**23.8**
Low	0301	0604	260	27.3	26.9	20.4	25.4
Low	0301	X	1672	26.0	26.7	23.1	24.2
				26.1	**26.7**	**22.8**	**24.4**
Very Low	0603	0302	346	20.0	25.4	26.3	28.3
Very Low	0603	02	500	24.4	24.0	25.0	26.6
Very Low	0603	0301	351	22.0	23.9	30.2	23.9
Very Low	0602/0603/0604[d]		1337	24.7	24.4	24.7	26.2
				23.6	**24.4**	**25.7**	**26.3**
No risk	0602	0302	692	23.4	26.6	27.6	22.4
No risk	0602	02	960	24.1	24.7	26.8	24.5
No risk	0602	0301	802	25.6	24.6	25.3	24.6
No risk	0602	X	1495	24.7	24.8	25.5	25.1
				24.7	**24.9**	**26.3**	**24.1**

Overall chi-square test on bold numbers show significant difference between diabetes risk groups (p= 0.01)
[a] Inclusion criteria used to identify newborns most at risk for developing type 1 diabetes.
[b] DQB1*02- DQA1*0501
[c] DQB1*02-DQA1*0201
[d] genotypes DQB1* 0603/0602, 0603/0604, 0603/X or 0602/0604
X is not DQB1 02, 0301, 0302, 0602, 0603, 0604 but includes potential homozygozity.

Table 5 Multiple logistic regression of HLA type 1 diabetes (T1DM) risk genotypes and autoantibodies on low and high relative birth weight, respectively.

Factor	Low relative Birth Weight		High relative Birth Weight	
	OR (95%CI)[a]	p-value	OR (95%CI)[a]	p-value
HLA T1DM risk group: [b]				
Very High risk	0.83 (0.67 – 1.02)	0.072	1.32 (1.09 – 1.61)	0.005
High risk	0.93 (0.81 – 1.07)	0.299	1.23 (1.07 – 1.41)	0.003
Moderate risk	0.97 (0.84 – 1.12)	0.662	1.10 (0.95 – 1.27)	0.198
Low risk	0.99 (0.87 – 1.12)	0.822	1.04 (0.92 – 1.19)	0.527
Very Low	0.88 (0.78 – 0.98)	0.026	1.14 (1.01 – 1.28)	0.029
No risk	0.94 (0.85 – 1.04)	0.215	1.01 (0.91 – 1.12)	0.895
Autoantibodies				
GAD65Ab	1.23 (0.98 – 1.54)	0.072	0.72 (0.56 – 0.93)	0.010
IA-2Ab	1.10 (0.62 – 1.94)	0.738	1.33 (0.72 – 2.42)	0.347

[a] The results are corrected in a multiple logistic regression for gender of child, maternal and gestational age, and number of cigarettes taken a day by mother.

[b] The HLA reference group is Neutral.

Legends to the Figures.

Figure 1 Children born in the region of Skåne in Sweden between September 2000 and December 2003, and the frequency of participation and exclusion in the Diabetes Prediction in Skåne (DiPiS) study.

Figure 2
Panel A Distribution of birth weight in relation to gestational age in 16 709 children born to healthy mothers. The line represents the mean birth weight of boys and girls together as estimated by polynomial regression.

Panel B Distribution of relative birth weight using internal Z-scores (only including gestational ages 37 weeks or longer) in relation to gestational age in 16 055 children born to healthy mothers. The standard intrauterine growth curve (mean ± 3SD) is shown.

Panel C Distribution of relative birth weight using ultra sound Z-scores in relation to gestational age in 16 709 children born to healthy mothers. The standard intrauterine growth curve (mean ± 3SD) is shown.

```
                    ┌─────────────────┐
                    │     38 550      │
                    │ Newborn children│
                    └─────────────────┘
                     ↙               ↘
        ┌─────────────────┐      ┌─────────────────┐
        │     9 003       │      │     29 547      │
        │Missing cord blood│     │   Cord blood    │
        └─────────────────┘      └─────────────────┘
                                  ↙               ↘
                   ┌─────────────────┐      ┌─────────────────────┐
                   │     26 682      │      │       2 865         │
                   │ Newborns born to│      │  Twins, triplets,   │
                   │ healthy mothers │      │ mother with diabetes│
                   └─────────────────┘      └─────────────────────┘
                   ↙        ↓        ↘
    ┌─────────────────┐     │     ┌─────────────────────┐
    │     9 155       │     │     │        818          │
    │No answer,excluded│    │     │ Answered but missing│
    │(moved or died etc)│   │     │ BW, gestational age │
    └─────────────────┘     │     │      or HLA         │
                            │     └─────────────────────┘
                            ↓
                   ┌─────────────────┐
                   │     16 709      │
                   │    Children     │
                   └─────────────────┘
```