Birth weight in newborn infants with different diabetes-associated HLA genotypes in three neighbouring countries: Finland, Estonia and Russian Karelia

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†The full DIABIMMUNE Study Group is listed in the Appendix at the end of the article.

Abstract

Background Human leukocyte antigen (HLA) genotypes associated with increased risk for type 1 diabetes mellitus (T1D) have been reported to be associated with increased birth weight. We set out to investigate the association between HLA haplotypes conferring risk for T1D and birth weight and search for possible differences in the strength of these associations among populations with contrasting incidence of T1D.

Methods As a part of the EU-funded DIABIMMUNE study, genotyping for the HLA haplotypes associated with T1D was performed in 8369 newborn infants from Estonia, Finland and Russian Karelia. Infants born before 35 gestational weeks, from mothers with diabetes, and multiple pregnancies were excluded. Relative birth weight, expressed in standard deviation scores, was estimated for each gestational week, sex and country. The standard deviation scores were calculated internally using the actual population studied. According to their HLA haplotypes, participants were divided into risk groups, and the distribution of birth weight between quartiles was analysed.

Results We did not find any direct association between various HLA risk-associated genotypes (HLA DR3-DQ2/DR4-DQ8, DR3-DQ2/X or DR4-DQ8/X) and birth weight. We observed a significant relationship between increased relative birth weight and the protective HLA-DR2-DQ6 and DR13-DQ6 haplotypes. This association was significant only when these haplotypes were found together with the DR4-DQ8 haplotype.

Conclusions The previously reported association between HLA-risk haplotypes for T1D and an increased birth weight was not confirmed. This suggests that the mechanisms behind the association between high birth weight and risk for T1D may be not directly HLA related. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords type 1 diabetes mellitus; birth weight; HLA association; environment

Introduction

The incidence of type 1 diabetes (T1D) is increasing rapidly all around the world by about 4% annually [1]. The reasons for such an increase are largely unknown, but environmental changes must be the most likely explanation [2]. Some studies have proposed that external exposures such as viral infections, early dietary introduction of foreign complex proteins, vitamin D deficiency, high standard of hygiene and several others could be important factors already in early life [3–10]. Recently, two large meta-analyses showed
an almost linear correlation between increasing birth weight and the subsequent risk for development of T1D, indicating that environmental determinants predisposing to T1D are important already in intrauterine life [11,12]. There is no apparent explanation for the mechanism(s) behind the association of higher birth weight with T1D risk. It has been proposed that because most patients with T1D have particular human leucocyte antigen (HLA) haplotypes, those conferring risk for T1D could themselves, at least partly, explain the association of an increased birth weight and T1D [13]. A Swedish study [14] reported a significant association between high birth weight and the haplotypes conferring the highest risk for T1D, whereas some other studies have failed to observe such an association [13,15–17]. Furthermore, Stene et al., [13] found a strong association between high birth weight and protective HLA alleles (such as DQB1*0602). The variable and conflicting study results may be explained by differences in T1D incidence in the background population and in study cohort sizes but also by different methodologies applied in these studies.

So far, only one study has been conducted to investigate and compare the possible association between HLA-risk genotypes for T1D and birth weight in different countries, that is, Finland, Germany, Sweden and the USA [17]. That study did not reveal any significant differences between risk groups, in these countries with different genetic background and climatic conditions, but quite similar socio-economic level. We studied, as a part of the international DIABIMMUNE study, birth weight parameters in newborn infants with different HLA class II genotypes in three neighbouring countries with similar climatic conditions but with different socio-economic level, environmental background and a significant difference in the incidence of T1D to obtain an additional insight into the possible impact of various factors. We hypothesized that if an association between increased birth weight and diabetes-associated HLA genotypes exists, it would be most conspicuous in the country where environmental exposures most strongly influence genetically predisposed subjects causing the highest incidence of T1D in the world, that is, Finland.

Materials and methods

Material

Subjects
As part of the international DIABIMMUNE study, testing the hygiene hypothesis in T1D and other immune-mediated diseases, HLA DR-DQ alleles conferring risk or protection against T1D were analysed from cord blood samples from children born in the three following neighbouring countries: Estonia, Finland and Russian Karelia between September 2008 and August 2010 whenever it was technologically possible, and parents were given written informed consent. The infants included in the study were born in the maternity hospital in Espoo, Finland (n = 3103), in the maternity hospitals in Tartu and Põlva, Estonia (n = 2713) and in two delivery hospitals in the Petrozavodsk, Karelian Republic of Russia (n = 2553). A questionnaire asking about the course of pregnancy, gestational age, delivery, child condition at birth, birth weight and length was completed by the study nurse during the first 3 days after delivery. At the same time, a questionnaire on family history was completed by the parents. For the subsequent analysis, infants born by mothers with any type of diabetes, from multiple pregnancies, or born preterm (gestational age < 35 weeks) were excluded. Late-preterm infants (35–36 gestational weeks) were included into the analysis in order to maximize the number of study subjects and to increase the statistical power of the study. Overall, the data of 7931 newborns, 2931 from Finland, 2521 from Estonia and 2479 from Russia were analysed. The local Ethics committees in all three countries approved the study.

Methods

General characteristics
The mean birth weight in grammes, gestational age, distribution by sex and mean maternal age at birth were calculated for all HLA-typed newborn infants in the total study cohort as well as for the individual national cohorts. Relative birth weight was expressed as a standard deviation score (SDS): SDS = subjects’ weight minus average birth weight (adjusted for sex, gestational age and country of origin) divided by the standard deviation (SD) that was also calculated for every gestational week, sex and country of origin using the study population internal data.

HLA analysis
The newborn infants were initially analysed for the presence of HLA DQB1*02, DQB1*0301, DQB1*0302 and DQB1*0602/3 alleles using a homogeneous screening assay [18]. Depending on the initial result, additional analyses included low resolution DQB1 full-house typing, DQA1 typing with three allele-specific probes and DR4 subtyping [19]. Samples positive for the DQA1*05-DQB1*02 combination (the DR3-DQ2 haplotype) and/or those with DQB1*0302/4 without the presence of DRB1*0403/6 (DR4-DQ8) were selected for the study if no protective haplotype (i.e. DQB1*0602/3, DQB1*0503 or the combinations of DQA1*05-DQB1*0301 or DQA1*0201-DQB1*0303) was present. The original purpose of this screening was to identify children with increased risk for T1D for further follow-up in the DIABIMMUNE study. Therefore, if the initial HLA analyses showed definitely protective HLA alleles, no further analysis of such alleles was performed. For example, after the identification of HLA DQB1*0602/3, further analysis to define whether the allele was DQB1*0602 or DQB1*0603 was not performed. Similarly, if the original analysis did not find a potential risk genotype no further analyses were performed.

The subjects were divided into four groups based on their HLA-associated risk for T1D. The subjects with the DR3-DQ2/DR4-DQ8 genotype were categorized as the group with very high risk for T1D, those carrying
the DR4-DQ8/X genotype (X = non-protective allele or haplotype) as a group with high risk and those with the DR3-DQ2/X genotype as a group with moderate risk for T1D. All other allele combinations were considered as neutral or protective for T1D [20] (Table 1).

**Statistical analysis**

The children were divided into quartiles according to relative birth weight and the distribution of various quartiles in each HLA-risk group was compared in the whole study group as well as for each country separately. The Chi-square test was used for statistical comparison between the groups and quartiles. In order to find factors influencing the birth weight SDS scores, a multiple linear regression analysis was also performed. As the country of origin, gender and gestational age were already taken into account during SDS calculation, HLA-risk class (very high, high, moderate, neutral or protective), maternal age and route of delivery were included into the multiple linear regression analysis.

**Results**

**General characteristics of the subjects**

The general characteristics of the subjects are presented in Table 2. The mean birth weight in Russian Karelia was significantly lower than in Estonia (p < 0.001) and Finland (p < 0.001). The mean age of the mothers at delivery and mean duration of pregnancy was also significantly lower in Russian Karelia than in Finland and Estonia (both p < 0.001). Estonian mothers were younger than the Finnish ones, and the duration of pregnancy was shorter in Estonia than in Finland (p < 0.001).

**Birth weight SDS**

The division of newborn infants belonging to various HLA-risk genotype classes into the lowest and highest quartile of relative birth weight (SDS) is shown in Table 4. There were no significant differences between any risk groups toward low or high relative birth weight when analysed either in the total study population or separately in the Finnish, Estonian or Russian Karelian cohorts. Multiple linear regression analysis showed that maternal age as well as delivery by caesarean section were both directly associated with birth weight SDS. At the same time, HLA-risk class did not significantly influence birth weight SDS.

Because all the subjects studied were analysed for the presence of DQB1 *02, *0301, *0302 and *0602/3, we also analysed the distribution of subjects between the four birth weight SDS quartiles for all genotypes formed by these alleles. In this analysis, we observed a statistically significant shift toward higher relative birth weight for the neutral/protective HLA-DQB1 *0302/DQB1*0602/3 genotype. For this group, the protective alleles DQB1*0602 and DQB1*0302, *0503, *0602, *0603 and not a combination of DQB1*0303/DQA1*0201 or DQB1*0301/DQA1*05, but homozygosity is possible.

Y is not DQB1*0302, *0503, *0602, *0603 and not a combination of DQB1*0303/DQA1*0201 or DQB1*0301/DQA1*05, but homozygosity is possible.

*DQB1*02/DQA1*0501.

<table>
<thead>
<tr>
<th>Table 1. HLA-risk classes for type 1 diabetes according to their genotype</th>
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</thead>
<tbody>
<tr>
<td><strong>Diabetes risk</strong></td>
</tr>
<tr>
<td>Very high: DR3-DQ2/DR4-DQ8</td>
</tr>
<tr>
<td>High: DR4-DQ8/X</td>
</tr>
<tr>
<td>Moderate: DR3-DQ2/X</td>
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<tr>
<td>Neutral or protective</td>
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</table>

**Table 2. General clinical characteristics (mean and 95% CI) of the infants included in the study**

<table>
<thead>
<tr>
<th>Finland</th>
<th>Estonia</th>
<th>Russian Karelia</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 2931; 1402 girls</td>
<td>n = 2521; 1231 girls</td>
<td>n = 2479; 1191 girls</td>
<td>n = 7931; 3824 girls</td>
</tr>
<tr>
<td>Mean birth weight (g)</td>
<td>3599 (3582–3616)*</td>
<td>3627 (3608–3646)*</td>
<td>3452 (3434–3469)</td>
</tr>
<tr>
<td>Girls</td>
<td>3627 (3608–3646)*</td>
<td>3547 (3521–3573)</td>
<td>3375 (3349–3397)</td>
</tr>
<tr>
<td>Boys</td>
<td>3615 (3627–3675)</td>
<td>3704 (3677–3730)</td>
<td>3525 (3500–3549)</td>
</tr>
<tr>
<td>Mean duration of pregnancy (days)</td>
<td>281.2 (280.9–281.6)**</td>
<td>280.2 (279.8–280.5)**</td>
<td>277.3 (277.0–277.6)**</td>
</tr>
<tr>
<td>Mean age of mother at delivery (years)</td>
<td>31.4 (31.3–31.6)**</td>
<td>28.8 (28.5–29.0)**</td>
<td>27.6 (27.4–27.8)**</td>
</tr>
</tbody>
</table>

*p < 0.001 – compared with Russian Karelia. **p < 0.001 – compared with the other two countries.
and DQB1*0603 in cross-combination with the risk allele DQB1*0302 were associated with increased birth weight. In this group, the distribution of children between quartiles was as follows: 18% in the first quartile (the total number of newborns n = 66), 53% in second and third quartiles (n = 199), and 29% (n = 109) in the fourth quartile. The difference between first and fourth quartiles was 11% and it was statistically significant (p = 0.003; OR = 0.52 (CI = 0.36–0.75)). Infants with this genotype constitute 4.7% of all study subjects and 5.8% of the children with neutral/protective genotypes. The distribution of newborn infants with these genotypes over the quartiles was similar in all three national groups. The combination of DQB1*0302 or DQB1*0602 and *0603 with any other allele did not show significant associations with increased birth weight (data not shown).

**Discussion**

In our study, we did not find any direct association between HLA-risk genotypes (DR3-DQ2/DR4-DQ8, DR3-DQ2/X, DR4-DQ8/X) and birth weight. However, we observed that a relatively high birth weight was associated with the protective alleles HLA-DQB1*0602 and DQB1*0603, when combined with the strong risk allele DQB1*0302. This finding is partially consistent with previous studies, where an association of all these haplotypes with high birth weight has been reported [13,14,16]. The principal difference from previous reports was that in our study, only the cross-combinations of these haplotypes with each other were seen to be associated with a significantly increased birth weight, whereas no association with increased birth weight was observed if these haplotypes were combined with other haplotypes.

Most previous studies aimed at finding possible associations between HLA-risk haplotypes for T1D and birth weight have been conducted in one particular country. In our study, we set out to investigate whether such an association exists in three adjacent countries with similar climate and geophysical conditions but multi-fold differences in the incidence of T1D and conspicuous differences in socio-economic level and living environment. Because environmental factors are most likely the main factors responsible for the increase seen in T1D incidence over the last decades in developed countries, we hypothesized that the impact of the HLA haplotype on birth weight would be strongest in the country with the highest incidence of T1D, that is, in Finland. Some observations speak in favour of this hypothesis. It has for example, been reported that some environmental factors such as maternal smoking and intrauterine infections may influence foetal growth modulated by HLA genotypes [22,23]. Various HLA genotypes may influence for example, the microbial colonization of the newborn infant [24]. Therefore, differences in such factors between the countries studied here could potentially influence the association between HLA genotype and birth weight. However, the distribution of birth weight over quartiles in different HLA-risk classes or their combinations was not significantly different in any of the countries studied or in the whole study cohort. Even in groups with a non-homogeneous
distribution of birth weight over quartiles, such as in the case of HLA \( DQB1^{*}0302/DRB1^{*}0602/3 \), no significant differences was seen between Finland, Estonia and Russian Karelia.

One reason why we did not detect any association between common HLA-risk genotypes and birth weight might be the relatively small number of subjects in each country for the detection of such an association as that one seen in the Swedish study comprising 16,709 newborn infants [14]. The second reason might be slightly different classifications applied for HLA-risk categories. For example, in our study, the DR4 in \( DQB1^{*}0302 \) positive subjects was additionally subtyped to find the protective allele \( DRB1^{*}0403 \) in order to exclude those subjects from the high-risk group. However, after applying exactly the same classification into HLA-risk groups as that one used by Larsson et al. [14], the distribution of children over quartiles remained the same as in our initial analysis (data not shown). The third reason might be the difference in how birth weight SDS was calculated. In the Swedish study [14], the birth weight SDS was based on the mean birth weight derived from national standardized intrauterine growth curve. In our study, such a standardized curve was available only for the Finnish cohort [25]. The comparison of Finnish birth weight data (mean birth weight by gestational week and sex and their standard deviation) with the Finnish national reference growth curve showed that at least this national cohort represented the general population very well (data not shown).

One might also expect an effect opposite to our hypothesis. Namely, in Finland, where the incidence of T1D is the highest, the effect of HLA on birth weight may remain unrecognized because of other more powerful risk factors affecting birth weight. Under such circumstances, an effect of HLA on birth weight could be expected to be seen in countries with lower prevalence of T1D. However, both in Estonia and Russian Karelia, no such differences in birth weight distribution were observed.

There have been also other studies looking for possible associations between HLA-risk group and birth weight [13,15,16,26]. Järvinen et al. [16] demonstrated that only one population-specific relatively rare \( DQB1^{*}0302-DR4 \) haplotype with the \( B56 \) allele conferring increased risk for T1D was associated with higher birth weight, whereas other HLA haplotypes conferring risk for T1D were associated with only a modest and non-significant trend toward higher birth weight. In a German study [26], the association of HLA \( DQB1^{*}0302-DR4 \) with high birth weight was demonstrated only in offspring of diabetic mothers. Our results are more similar to those reported by Stene et al. [13] and Locatelli et al. [15] who did not find any significant associations between a high birth weight and HLA haplotypes conferring increased risk for T1D. Our results also agree with the outcome of the recent study by Sterner et al. [17], where the populations of four countries with similar socioeconomic level but with different climatic conditions and genetic background were compared, and no difference between groups with different HLA-risk genotypes was seen.

We observed a positive association between the HLA genotype and increased birth weight for the HLA \( DQB1^{*}0302/DRB1^{*}0602/3 \) combination. Stene et al. [13] demonstrated a positive association between high birth weight and the protective HLA \( DQB1^{*}0602 \) allele. Other authors have noticed some associations between an increased birth weight and the protective HLA \( DQB1^{*}0603 \) allele [14] and the predisposing allele HLA \( DQB1^{*}0302 \) allele [14,16,26]. We suggest that these haplotypes have an additive effect on birth weight and when combined, the effect on birth weight is most obvious. If those haplotypes are present separately, the additive effect vanishes and in our study, we did not see any impact on birth weight.

Our study has a couple of limitations. First is the selection bias. Because of technical difficulties in emergency situations, the cord blood samples were not obtained from a few children born by caesarean section. We were not able to find out the exact number of such missed subjects. Therefore, the data of these children who are often extremely large or because of their prematurity very small are missing in the study. The second limitation is a possible information bias. Namely, the gestational age of some infants might be misclassified. One sign of such a possible misclassification is the fact that the duration of pregnancy was statistically different between the countries. This might be caused by the differences in the methodology of estimation of pregnancy duration between the countries. In our SDS calculation, however, we have taken into consideration the country of origin and therefore should eliminate the influence of possible misclassification of gestational age. The third limitation is that we were not able to identify exactly the number of previous deliveries by the mother. We asked about the number of siblings but not about the number of previous deliveries. Therefore, we could not adjust the birth weight data for birth order. It is known that the birth weight is increasing along with birth order in singleton pregnancies [27]. However, many studies on HLA associations with birth weight have shown that adjustment for birth order did not influence the results [14,15,26]; and therefore, we may assume that the same is true for the current study. In addition, birth weight is influenced by many biological, medical and socioeconomic factors, information of which was not available and thus could not be adjusted for neither in previous studies nor in our study. Furthermore, the increased HLA risk and high birth weight may act in synergy in the disease process leading to T1D, which can be seen only in longitudinal studies.

To conclude, in this study, we were not able to confirm the previously reported observation of an association between common HLA-risk haplotypes for T1D and increased birth weight. However, we found a significant association between increased relative birth weight and some protective alleles in cross-combination with the \( DQB1^{*}0302 \) risk allele. Further comprehensive epidemiological studies are needed to assess to which extent HLA haplotypes conferring susceptibility to T1D are contributing to the increased birth weight associated with enhanced disease risk and the role of different environmental factors in this process. Our observations suggest
that the mechanisms behind the association between high birth weight and risk for TID could be others than HLA genotypes.

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Contribution statement

AP conceived the study along with VT and MK, participated in its design and coordination and drafted the manuscript. PK designed and performed all statistical analyses. JI was responsible for HLA testing, determined the HLA subgroups and helped to draft the manuscript. All authors read and approved the final manuscript.

Collaborators (42)


Conflict of interest

The authors declare that there is no conflict of interest associated with this manuscript.

Appendix

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