Short communication

The type 1 diabetes protective *HLA DQB1*0602* allele is less frequent in gestational diabetes mellitus

A. Papadopoulou[1,2], K. F. Lynch[1], N. Shaat[1], A. Nilsson[1], B. Lernmark[1], K. Berntorp[2], S.-A. Ivarsson[1,3], C.-D. Agardh[1,2], Å. Lernmark[1] on behalf of the DiPiS Study Group*

[1]Lund University/CRC, Department of Clinical Sciences, Unit of Diabetes and Celiac Disease, Entrance 72, Building 91, Plan 10, SE-20502 Malmö, Sweden

[2]Department of Endocrinology, Malmö University Hospital, Sweden

[3]Department of Pediatrics, Malmö University Hospital, Malmö, Sweden

*The DiPiS Study Group includes: A. Carlsson, E. Cederwall, B. Jönsson, C. M. Cilio, H. Larsson, K. Larsson, B. Lindberg, J. Neiderud, S. Resic-Lindehammer and S. Sjöblad.

Correspondence to:

A. Papadopoulou

e-mail: Anastasia.Papadopoulou@med.lu.se

Received 13 October 2008/Accepted 8 March 2009

Abstract

Aims/hypothesis We tested whether gestational diabetes mellitus (GDM) is associated with *HLA-DQ* genotype.

Methods A total of 764 mothers with non-autoimmune (GAD65, insulinoma-associated protein 2 [IA-2] and insulin autoantibody-negative) GDM were ascertained between September 2000 and

August 2004 in the population-based Diabetes Prediction in Skåne (DiPiS) study. *HLA-DQB1* genotypes were determined in these mothers and in 1191 randomly selected non-diabetic control mothers also negative for islet autoantibodies. The data were analysed in relation to maternal age, country of birth, number of pregnancies/siblings and pregnancy weight gain.

Results The frequency of type 1 diabetes high-risk *HLA-DQ* alleles (*DQB1*0201*, *DQB1*0302*) did not differ between GDM mothers and controls. In contrast, the low-risk *DQB1*0602* allele was less prevalent (OR 0.64, 95% CI = 0.51–0.80, p=0.0006) in GDM than in control mothers. The difference in *DQB1*0602* frequency between GDM mothers and controls remained after multiple logistic regression analysis correcting for maternal age, country of birth, number of pregnancies/siblings and weight gain during pregnancy (OR 0.67, 95% CI 0.51–0.88, p=0.009).

Conclusions/interpretation The negative association between mothers who have non-autoimmune GDM and *HLA-DQ*0602* suggest that this allele may protect not only from type 1 diabetes but also from GDM.

Keywords: Gestational diabetes mellitus, HLA

Abbreviations

DiPiS	Diabetes Prediction in Skåne
GDM	Gestational diabetes
IA-2	Insulinoma-associated protein 2
RU	Radioactivity Units

Introduction

Gestational diabetes (GDM) has an onset during pregnancy and complicates approximately 2% of all pregnancies in Scandinavia [1]. Genetic factors have been implicated in the pathogenesis of GDM, yet specific genes have not been identified [1]. HLA genes of the MHC have been related to GDM in several studies. Some have demonstrated a positive association between GDM and HLA class II alleles [2], while others have not [3]. If GDM is to be properly classified, it is necessary to

genetically characterise it and make distinctions from other types of diabetes. The aim of the present work was to investigate whether there is an association between GDM and *HLA-DQ* genotypes associated with type 1 diabetes.

Methods

Population Mothers and children were prospectively recruited from the Diabetes Prediction in Skåne (DiPiS study) [4]. Between September 2000 and August 2004, cord blood from newborns and venous blood from mothers were obtained as dried blood spots in the delivery room after informed consent had been obtained.

All pregnant women in Skåne were invited to undergo a 75 g OGTT at 25-28 weeks of pregnancy or at 10-12 weeks of pregnancy for those who had had GDM in earlier pregnancies or a parental history of diabetes. GDM was defined as a capillary 2 h glucose concentration ≥9.0 mmol/l. The midwife recorded the health status of the mother (diabetes or gestational diabetes) on the remittance for dried blood spots.

Study group From more than 35,000 deliveries, 902 mothers were classified as having GDM at least once. Of the GDM mothers, 70 gave birth to a child with the presence of autoantibodies to GAD65, insulinoma-associated protein 2 (IA-2) (>96th percentile of newborns) or insulin (>99th percentile of newborns). A further three children could not be analysed for all three autoantibodies. These 73 mothers with GDM were excluded. HLA genotypes were available in 764 (92%) of the remaining 829 mothers. As controls, 1191 additional unique mothers were randomly selected and included on the basis that they had no diabetes or autoantibodies during any of their pregnancies and could be analysed for HLA. The HLA distribution of the controls was compared with that of the newborn population. The Lund University Ethics Committee approved the DiPiS study.

HLA genotyping HLA-DQB1 genotypes were analysed and divided into five groups based on the association with type 1 diabetes, as previously described [4].

Autoantibody measurement Analysis of autoantibodies to GAD65 or IA-2 and antibodies against insulin were determined in cord blood of newborns [5]. Autoantibodies were determined in a first combined screen in which eluates from dried blood spots were incubated overnight in duplicate with labelled antigen. Antibody-bound antigen was precipitated with protein A–Sepharose and unbound antigen was removed by washing. The radioactivity of antibody-bound GAD65 and/or IA-2 was counted in a beta-counter. The combined screen (COMB) compared a positive reference with two negative reference samples in order to determine high levels. The diagnostic sensitivity for GAD65 autoantibodies using a cut-off at 31 RU/ml was 68% (1317/1950 consecutively diagnosed [during May 2005 to September 2008] type 1 diabetes patients from all over Sweden). In the IA-2 autoantibody assay using a cut-off of 5 RU/ml, the diagnostic sensitivity was 75% (1465/1950 patients). The GAD65 and IA-2 autoantibody assays showed mean inter-assay and intra-assay coefficients of variation of 14 and 8% respectively. Samples greater than the 99th percentile of COMB were individually analysed for each antibody. In children born after 2001 with high individual GAD65 or IA-2 autoantibodies (above the 99th percentile), the dried blood spot sample from the mother was also analysed for the two autoantibodies. The 99th percentile was defined on the entire population.

Insulin autoantibodies were analysed in cord blood serum samples, which were incubated in duplicate wells in 96-well plates. Antibody-bound and free-labelled insulin were separated with 40% protein A–Sepharose and the radioactivity was measured in a beta-counter. Results were expressed in arbitrary units and all samples above the 99th percentile were reanalysed in duplicate wells with 8 U/ml cold insulin to identify serum samples with non-specific binding. The data were expressed in relative units based on the degree of blocking ^{125}I-labelled insulin to the highest positive reference standard by cold insulin, and the inter- and intra-assay coefficients of variations were 6.8–7.8 and 5.2–7.8% respectively. Samples above the 99th percentile were considered to have high levels.

Statistical analysis Differences in proportions between categorical groups were examined for significance using the exact χ^2 test. Logistic regression on diabetes status was used to examine whether frequencies of type 1 diabetes-associated *HLA-DQB1* alleles differed between controls and GDM mothers. Our study was capable of detecting odds ratios below 0.7 and above 1.35 with 80% power. Maternal age, country of birth, number of pregnancies, number of siblings and weight gain during pregnancy were considered as confounders and were adjusted in a multiple logistic regression. Bonferroni correction was used in order to adjust for multiple comparisons.

Results

Maternal characteristics Mothers with GDM were older (p<0.0001), more often born outside Sweden (p<0.0001), had had fewer pregnancies (p=0.0002), had had more pregnancies before the screening period (p<0.0001) and gained less than 15 kg in body weight during at least one pregnancy (p<0.0001) than control mothers (see Electronic supplementary material [ESM] Table 1)

HLA distribution The frequency of *HLA-DQ* genotypes containing *DQB1*0302* or *DQA1*0501-B1*0201* together with either *DQB1*0604* or any other allele except those which were typed (**0301*,**0302*,**0201*,**0602*,**0603*,**0604*) did not differ between GDM and control mothers (ESM Table 2).

The *DQB1*0602* allele was less prevalent (OR 0.64, 95% CI 0.51–0.80, p=0.0006) in GDM than in control mothers (Table 1). The frequency of the *DQB1*0301* allele was increased in GDM mothers compared with controls, but after adjusting for maternal age, country of birth, number of pregnancies/siblings and maternal weight gain there was no significant association between GDM and *DQB1*0301* (Table 2). Since this allele is present in several haplotypes (*DQA1*0302*, *DQA1*0501*), the positive association did not remain after correcting for the number of *DQB1*0301*-containing haplotypes. In contrast, the difference in *DQB1*0602* frequency between GDM mothers and controls remained after multiple logistic regression analysis (OR 0.67, 95% CI 0.51-0.88, p=0.009) (Table 2).

Discussion

The present 4 year study of more than 700 GDM mothers showed that *DQB1*0602* was negatively associated with GDM. As in a previous report [6], we interpret the result to mean that *DQB1*0602*-positive mothers would be less likely than *DQB1*0602*-negative mothers to develop GDM. In type 1 diabetes it is known that the *DQB1*0602* allele is protective, although this protection decreases with increasing age [7]. The loss of protection from *DQB1*0602* is important to the risk of developing mostly type 2, but also type 1 diabetes, in post-partum GDM mothers.

The strength of our study is that all participants were ascertained in one region of Sweden where we have access to all mothers giving birth and where there is a mandatory screening programme for GDM. The *HLA* distribution of controls was similar to that of 34,710 newborns analysed for *HLA*. The *HLA* distribution of controls can therefore be considered as representing the general population. Our study may therefore be viewed as population-based because we ascertained that approximately 2% of all mothers had GDM.

Adjusting for number of pregnancies, maternal age, country of birth and weight gain during pregnancy did not change the results for *DQB1*0602*. The provisional association between GDM and *DQB1*0301* did not remain after multiple logistic regression, supporting the idea that *DQB1*0301* may represent different haplotypes in addition to the confounding factors. In contrast, the *DQB1*0602* association was still significant after adjustment, supporting the idea that this particular allele is under-represented in women with GDM.

Previous studies have investigated the association between GDM and *HLA*. The same negative association between *DR2*, which is in linkage disequilibrium with *DQB1*0602*, and GDM has been reported in some studies [6, 8]. Most previous studies, however, have reported an association with the high-risk *HLA-DR3* and *-DR4*. A higher frequency of the type 1 diabetes high-risk *HLA* genotypes was observed in patients with GDM, but only in those women that were found to be positive for islet autoantibody at delivery [9]. When the GDM population was classified according to the presence or absence of islet cell autoantibodies, a higher frequency of *HLA-DR3/DR4* in the

autoimmune group and of *HLA-DR7-DQ2/γ*, *DR9-DQ9/γ* and *DR14-DQ5/γ* were observed in the non-autoimmune group [10]. In our study, we excluded antibody-positive women with GDM and antibody-positive control mothers in order to exclude women who would be considered at risk of developing type 1 diabetes in the future.

In conclusion, the frequency of type 1 diabetes high-risk *HLA-DQ* alleles (*DQB1*0201*, *B1*0302*) did not differ between GDM mothers and controls. The low-risk *DQB1*0602* allele was less prevalent. Maternal age, country of birth, pregnancy weight gain and number of pregnancies/siblings did not explain the difference in *DQB1*0602* frequency between GDM mothers and controls.

Acknowledgements

We thank B. Buveris-Svendburg, J. Gerardsson, G. Hansson, H. Rastkhani, I. Hansson and R. Håkansson for expert technical assistance. We also thank all the participating parents and their children. The study was supported by the Swedish Research Council (14064), the National Institutes of Health (DK26190), the Skåne County Council Funds for Research and Development, the Swedish Diabetes Association, the Childhood Diabetes Fund and UMAS Research Funds.

Duality of interest

The authors declare that there is no duality of interest associated with this study.

References

1. Shaat N, Groop L (2007) Genetics of gestational diabetes mellitus. Curr Med Chem 14:569-583
2. Freinkel N, Metzger BE, Phelps RL et al (1985) Gestational diabetes mellitus. Heterogeneity of maternal age, weight, insulin secretion, HLA antigens, and islet cell antibodies and the impact of maternal metabolism on pancreatic B cell and somatic development in the offspring. Diabetes 34(Suppl 2):1-7

3. Vambergue A, Fajardy I, Bianchi F et al (1997) Gestational diabetes mellitus and HLA class II (-DQ, -DR) association: The Digest Study. Eur J Immunogenet 24:385-394
4. Larsson HE, Lynch K, Lernmark B et al (2005) Diabetes-associated HLA genotypes affect birthweight in the general population. Diabetologia 48:1484-1491
5. Lynch KF, Lernmark B, Merlo J, Cilio CM, Ivarsson SA, Lernmark Å for the Diabetes Prediction in Skåne (DiPiS) Study Group (2008) Cord blood islet autoantibodies and seasonal association with the type 1 diabetes high-risk genotype. J Perinatol 28:211-217
6. Song D, Liu Y, Han Y et al (2002) Study on the gestational diabetes mellitus and histocompatibility human leukocyte antigen DRB allele polymorphism. Zhonghua Fu Chan Ke Za Zhi 37:284-286
7. Graham J, Kockum I, Sanjeevi CB et al (1999) Negative association between type 1 diabetes and HLA DQB1*0602-DQA1*0102 is attenuated with age at onset. Swedish Childhood Diabetes Study Group. Eur J Immunogenet 26:117-127
8. Damm P, Kuhl C, Buschard K et al (1994) Prevalence and predictive value of islet cell antibodies and insulin autoantibodies in women with gestational diabetes. Diabet Med 11:558-563
9. Shaat N, Ekelund M, Lernmark Å et al (2004) Genotypic and phenotypic differences between Arabian and Scandinavian women with gestational diabetes mellitus. Diabetologia 47:878-884
10. Törn C, Gupta M, Sanjeevi CB, Åberg A, Frid A, Landin-Olsson M (2004) Different HLA-DR-DQ and MHC class I chain-related gene A (MICA) genotypes in autoimmune and non-autoimmune gestational diabetes in a Swedish population. Hum Immunol 65:1443-1450

Table 1 Comparison of the distribution of *HLA-DQB1* alleles associated with type 1 diabetes in mothers with GDM and control mothers

Presence of type 1 diabetes-associated *HLA-DQB1* allele	Controls (*n*=1191) with allele, *n* (% of total)	GDM mothers (*n*=764) with allele, *n* (% total)	Controls vs GDM mothers		
			OR	95% CI	*p* value[a]
**0201*	425 (35.7)	265 (34.7)	0.96	0.79–1.16	>0.05
**0301*	341 (28.6)	274 (35.9)	1.39	1.15–1.69	0.0048
**0302*	333 (28.0)	206 (27.0)	0.95	0.78–1.17	>0.05
**0602*	301 (25.3)	136 (17.8)	0.64	0.51–0.80	0.0006
**0603*	193 (16.2)	111 (14.5)	0.88	0.68–1.13	>0.05
None of the above	67 (5.6)	57 (7.5)	1.35	0.94–1.95	>0.05

[a]After Bonferroni adjustment for multiple comparisons

Table 2 Association between *HLA-DQ* genotypes and mothers with GDM after adjusting for number of pregnancies/siblings, age of mother in 2000, country of birth and pregnancy weight gain

Predictors: *HLA-DQB1* genotype	Mothers with GDM		
	OR	95% CI	*p* value[a]
**0301*	1.27	1.02–1.59	>0.05
**0602*	0.67	0.51–0.88	0.009
**0301/0602*	0.89	0.54–1.48	>0.05
All other genotypes[b]	1.00	Ref[c]	Ref[c]

[a]After Bonferroni adjustment for multiple comparisons
[b]All genotypes that do not contain **0301* or **0602*
[c]Reference value

Electronic supplementary material

ESM Table 1 Characteristics of mothers who did not have diabetes and mothers who developed GDM between September 2000 and August 2004

	Maternal diabetes group		
	Controls (*n*=1,191)	GDM (*n*=764)	
Characteristics of the mothers	*n* (%)	*n* (%)	*p* value[a]
Age (years) in year 2000			
<25	183 (15.4)	113 (14.8)	
26–34	847 (71.1)	482 (63.1)	
≥35	161 (13.5)	169 (22.1)	<0.0001
Number of other pregnancies reported during the study period			
0	934 (78.4)	655 (85.7)	
≥1	257 (21.6)	109 (14.3)	0.0002
Number of siblings before year 2000			
0	422 (35.4)	324 (42.4)	
1	251 (21.1)	139 (18.2)	
≥2	125 (10.5)	112 (14.7)	
Not available	393 (33.0)	189 (24.7)	<0.0001
Country of birth			
Sweden	746 (62.6)	426 (55.8)	
Outside Sweden	46 (3.9)	131 (17.1)	
Not available	399 (33.5)	207 (27.1)	<0.0001
Gained >15 kg during pregnancy			
No	480 (40.3)	404 (52.9)	
Yes	310 (26.0)	154 (20.1)	
Not available	401 (33.7)	206 (27.0)	<0.0001

[a]The *p* values denote significant differences between controls and GDM for all participants for each characteristic of the mothers

ESM Table 2 Frequency of type 1 diabetes risk *HLA-DQB1* genotypes in mothers with GDM and control mothers

Association of type 1 diabetes with *HLA-DQ*	*HLA-DQB1* Genotype		Population[a] (*n*=34,710)	Controls (*n*=1,191)	GDM (*n*=764)
			n (% of total)	*n* (% of total)	*n* (% of total)
Strongly positive	*02*[b]	*0302*	1,258 (3.6)	43 (3.6)	33 (4.3)
	0302	*0604*	435 (1.3)	15 (1.3)	12 (1.6)
	0302	*X*	2,841 (8.2)	103 (8.6)	69 (9.0)
	02[b]	*0604*	472 (1.4)	15 (1.3)	11 (1.4)
	02[b]	*X*	2,460 (7.1)	62 (5.2)	51 (6.7)
	Total		7,466 (21.5)	238 (20.0)	176 (23.0)
No specific association	*02*[c]	*0604*	224 (0.6)	9 (0.8)	8 (1.0)
	02[c]	*X*, *02*[b]	2,265 (6.5)	93 (7.8)	41 (5.4)
	02[c]	*0302*	687 (2.0)	20 (1.7)	10 (1.3)
	0604	*X*	859 (2.5)	31 (2.6)	15 (2.0)
	X	*X*	1,327 (3.8)	36 (3.0)	42 (5.5)
	Total		5,362 (15.4)	189 (15.9)	116 (15.2)
No association (*DQB1*0301*)	*0301*	*0302*	1,625 (4.6)	67 (5.6)	41 (5.4)
	0301	*02*	2,345 (6.8)	62 (5.2)	64 (8.4)
	0301	*0604*	548 (1.6)	21 (1.8)	14 (1.8)
	0301	*X*	3,979 (11.5)	120 (10.1)	106 (13.9)
	Total		8,497(24.5)	270 (22.6)	225 (29.5)
Weakly negative (*DQB1*0603*)	*0603*	*0302*	690 (2.0)	20 (1.7)	10 (1.3)
	0603	*02*	1,035 (3.0)	41 (3.4)	14 (1.8)
	0603	*0301*	811 (2.3)	21 (1.8)	22 (2.9)
	0603/0602/0604/X[d]		2,827 (8.1)	111 (9.3)	65 (8.5)
	Total		5,363 (15.5)	193 (16.2)	111 (14.5)
Strongly negative (*DQB1*0602*)	*0602*	*0302*	1,347 (3.9)	65 (5.5)	31 (4.1)
	0602	*02*	1,976 (5.5)	80 (6.7)	33 (4.3)
	0602	*0301*	1,653 (4.6)	50 (4.2)	27 (2.5)
	0602	*X*	3,046 (8.8)	106 (8.9)	45 (5.9)
	Total		8,022 (23.1)	301 (25.3)	136 (17.8)

[a]Population includes all infants born between September 2000 and August 2004 that were typed for *HLA*

[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* or *0604* unless it makes the genotype homozygous