

Diabetes-Associated HLA-DQ Genes and Birth Weight

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An association has recently been described between increased birth weight and increased risk of childhood-onset type 1 diabetes. Whether this relationship is explained by genes associated with both increased birth weight and increased risk of type 1 diabetes is unknown. In the present study, we tested the association between birth weight and HLA-DQ genotypes known to confer risk for type 1 diabetes among 969 nondiabetic children randomly selected from the Norwegian population. We found that HLA genotypes previously shown to confer risk for type 1 diabetes were associated with reduced birth weight (the mean difference in birth weight between the DQB1*0602/DQB1*0602 and DQ8/DQ2 genotypes was 354 g [95% CI 105–604]), which was opposite of that expected if HLA genes explained the birth weight–type 1 diabetes association. *Diabetes* 50: 2879–2882, 2001

A positive association between birth weight and risk of type 1 diabetes has recently been described in large epidemiological studies, even after exclusion of maternal diabetes (1,2). Together with other lines of evidence, such studies have been taken as evidence that environmental risk factors for type 1 diabetes may play a role in utero or early in life (3). However, we cannot exclude the possibility that the above-mentioned association (1,2) is explained by genes associated with both increased birth weight and increased risk of type 1 diabetes.

The most important genetic contribution to the risk of type 1 diabetes is encoded within the HLA complex on chromosome 6p (4), and different HLA-DQA1 and -DQB1 alleles confer either strongly increased or reduced risk (5). The insulin gene region (INS) on chromosome 11p is probably the second most important genetic contribution to variation in susceptibility to type 1 diabetes (4,6). The *class I* allele at *INS VNTR* that confers susceptibility to type 1 diabetes (6) has been associated with reduced birth weight (7), indicating that *INS VNTR* is unlikely to explain the observed association between birth weight and type 1 diabetes (1,2).

Genes in the HLA complex encode molecules involved

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in rejection of nonself tissue, and antibodies to fetal HLA molecules have been found in a large proportion of parous women (8). Previous studies have focused on maternal-fetal HLA compatibility and pregnancy outcome. Because there are indications that genes in the HLA complex are associated with fetal growth (9), we tested whether HLA-DQ genotypes previously found to confer risk for type 1 diabetes were associated with increased birth weight among Norwegian children randomly selected from the official population registry.

A total of 1,484 children born in Norway between 1982 and 1998 and residing in Vest-Agder county were contacted by mail in 1999 and received a questionnaire and mouth brushes for extraction of DNA and HLA typing. Of the 1,056 who returned the buccal cell samples, 1,008 were linked to the Medical Birth Registry of Norway, which stores computerized information on birth weight, gestational age, maternal age at delivery, maternal parity, and other perinatal factors for all births in Norway since 1967 (10). The majority of those children not linked were born abroad. Five children with type 1 diabetes were excluded. Those who were part of a twin pregnancy, whose mother had gestational diabetes or diabetes diagnosed up to the time of study, or who had missing data for birth weight were also excluded, leaving 969 children for analysis.

The general perinatal characteristics of the study sample were as expected for the general population of Norway for the given period (Table 1). We decided a priori to categorize HLA-DQ genotypes into five groups according to the degree of risk for type 1 diabetes, as determined in previous studies (11,12). The high-risk *DQA1*0301-DQB1*0302/DQA1*0501-DQB1*0201* (DQ8/DQ2) genotype, five other genotypes found by Rønningen (12) to confer significant risk of type 1 diabetes (*DQA1*0301-DQB1*0302/DQA1*0401-DQB1*0402*, *DQA1*0301-DQB1*0302/DQA1*0301-DQB1*0302*, *DQA1*0301-DQB1*0302/DQA1*0101-DQB1*0501*, *DQA1*0301-DQB1*0302/DQA1*0102-DQB1*0604*, or *DQA1*0501-DQB1*0201/DQA1*0501-DQB1*0201*), genotypes neutral with regard to type 1 diabetes susceptibility, genotypes with one copy of the strongly protective *DQB1*0602* allele (11), and genotypes with two copies of the *DQB1*0602* allele were compared (Fig. 1). The association was the opposite of that expected under the hypothesis that variation in the HLA-DQ genotype explains the previously observed positive association between birth weight and risk of type 1 diabetes (1,2). The overall association was statistically significant ($P = 0.04$; analysis of variance), and it was highly significant when we tested for trend by taking into consideration the ordering of the genotype categories (coded 1, 2, 3, 4, and 5) according to

TABLE 1

Perinatal characteristics in a random sample of 969 children born between 1982 and 1998 and residing in Vest-Agder county, Norway, in 1999

Male sex	467 (48.2)
First-born*	357 (37.1)
Second-born	347 (36.1)
Third-born or later	257 (26.7)
Cesarean delivery	82 (8.5)
Maternal smoking during most of pregnancy†	192 (20.0)
Maternal smoking during parts of pregnancy	99 (10.3)
No maternal smoking during pregnancy	669 (69.7)
Mean birth weight (g)	3,576 ± 548.6
Mean gestational age (weeks)‡	40.3 ± 1.91
Mean maternal age at birth (years)	27.3 ± 4.81

Data are *n* (%) or means ± SD. *Birth order defined as number of previous births reported by mother at birth of the index child plus one. Eight children had missing data for birth order. †Information obtained from maternal recall in 1999. Nine children had missing data for maternal smoking in pregnancy. ‡A total of 70 (7.2%) had missing data on gestational age.

risk of type 1 diabetes determined in previous studies ($P = 0.004$; linear regression test for trend). Subjects homozygous for the DQB1*0602 allele, previously shown to confer protection against type 1 diabetes (11), had the highest birth weights, whereas subjects carrying the DQ8/DQ2 genotype, previously found to confer high risk for type 1 diabetes, had the lowest birth weights (mean difference

354 g [95% CI 105–604]). The strength of association was slightly reduced, but the conclusion remained unchanged after adjustment for gestational age, maternal smoking during pregnancy, maternal age at birth, birth order, cesarean section, maternal education, sex, and age (Table 2). The association was similar at different levels of the possible confounders adjusted for in Table 2 (data not shown). There was no significant association between HLA-DQ genotype and gestational age (Table 2).

Our data suggest that HLA genotype cannot explain the positive association between birth weight and risk of type 1 diabetes observed in previous studies (1,2). Our data supports previous indications that HLA genes or HLA-linked genes may be associated with variation in fetal growth (9), although the mechanisms that may be operating are unknown. We did not obtain DNA from the mothers, but maternal genotype is of less importance in regard to our research question, which is whether there were any associations between diabetes-associated HLA-DQ genes and birth weight among children. We did not have data on ethnicity in our study. Although it is theoretically possible that our results could be confounded by the presence of ethnic minorities with different birth weights and different frequencies of HLA-DQ alleles compared with ethnic Norwegians, this is unlikely because non-Western ethnic groups comprise a very small proportion of the Norwegian population. The six major non-

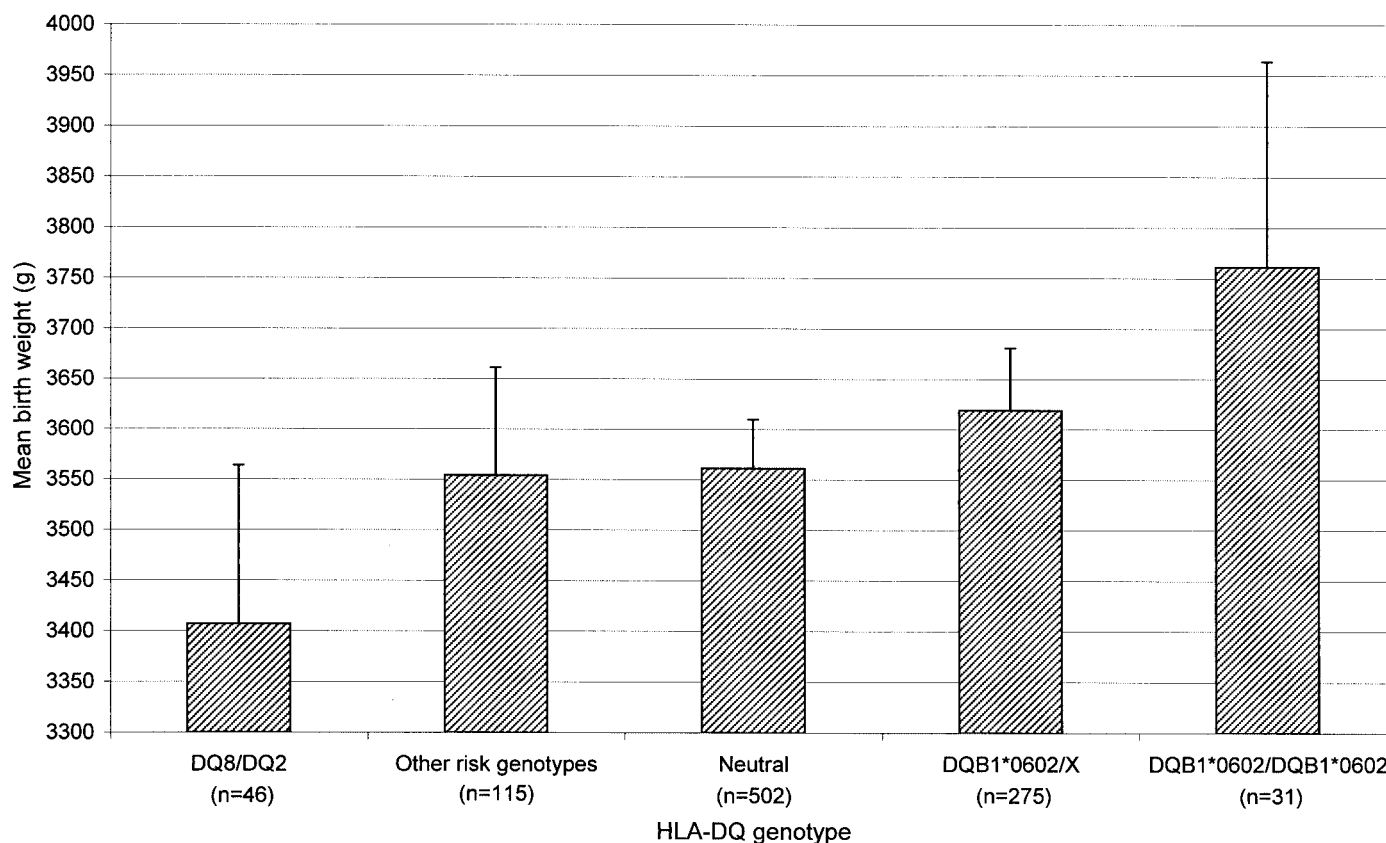


FIG. 1. Birth weight and HLA-DQ genotype in relation to susceptibility to type 1 diabetes as determined from previous studies (11,12). DQ8/DQ2: DQA1*0301-DQB1*0302/DQA1*0501-DQB1*0201; other diabetes risk genotypes: DQA1*0301-DQB1*0302/DQA1*0401-DQB1*0402, DQA1*0301-DQB1*0302/DQA1*0301-DQB1*0302, DQA1*0301-DQB1*0302/DQA1*0101-DQB1*0501, DQA1*0301-DQB1*0302/DQA1*0102-DQB1*0604, or DQA1*0501-DQB1*0201/DQA1*0501-DQB1*0201 (12); neutral, remaining HLA-DQ genotypes. P value for trend = 0.004. Error bars show upper 95% confidence limit.

TABLE 2

Crude and adjusted linear regression coefficients for birth weight with diabetes-associated HLA-DQ genotypes as dependent variable, as well as gestational age, in a random sample of 969 children born between 1982 and 1998 and residing in Vest-Agder county, Norway, in 1999

HLA-DQ genotype*	Birth weight (g)			Gestational age (weeks)
	Crude regression coefficient	Adjusted regression coefficient, model 1†	Adjusted regression coefficient, model 2‡	Crude mean
DQ8/DQ2	0 (reference)	0	0	40.1 ± 1.71
Other diabetes risk genotypes	147 (-40.3 to 334.2)	133.6 (-35.7 to 302.8)	130.3 (-50.5 to 311.1)	40.2 ± 2.35
“Neutral”	154.0 (-11.3 to 319.4)	132.3 (-16.7 to 281.3)	105.9 (-53.7 to 265.4)	40.2 ± 1.92
DQB1*0602/X	211.8 (40.9–382.8)	167.8 (13.3–322.3)	169.2 (4.3–334.1)	40.4 ± 1.75
DQB1*0602/DQB1*0602	354.3 (105.0–603.7)	279.7 (45.9–513.5)	291.7 (52.8–530.7)	40.0 ± 1.60
Linear regression test for trend§	<i>P</i> = 0.004	<i>P</i> = 0.02	<i>P</i> = 0.02	<i>P</i> = 0.31

*DQ8/DQ2: DQA1*0301-DQB1*0302/DQA1*0501-DQB1*0201; other diabetes risk genotypes: DQA1*0301-DQB1*0302/DQA1*0401-DQB1*0402, DQA1*0301-DQB1*0302/DQA1*0301-DQB1*0302, DQA1*0301-DQB1*0302/DQA1*0101-DQB1*0501, DQA1*0301-DQB1*0302/DQA1*0102-DQB1*0604, or DQA1*0501-DQB1*0201/DQA1*0501-DQB1*0201 (12); DQB1*0602/X, X not DQB1*0602; neutral, remaining HLA-DQ genotypes. †Model 1, adjusted for gestational age (*n* = 899); ‡model 2, adjusted for maternal smoking, maternal education, maternal age at delivery, birth order, age, cesarean section, and sex (*n* = 948); §*P* value for HLA-DQ genotype entered as a continuous variable coded 1, 2, 3, 4, and 5 for the five genotype categories.

Western immigrants to Norway comprised ~3.2% of the Norwegian population (13), probably less in Vest-Agder county. We tested associations using two-sided tests, which are designed to detect deviations from the null hypothesis in either direction. It is probably premature to conclude that there is a causal link between HLA-DQ genes and birth weight, but our results strongly suggest that HLA-DQ genotypes previously shown to confer increased risk for type 1 diabetes are not associated with increased birth weight. Although contributions from other genes cannot be excluded, our data, together with the previously observed association between INS VNTR and birth weight (7), suggest that nongenetic factors are more likely explanations of the previously observed association between birth weight and risk of type 1 diabetes (1,2). Such factors are as yet unknown, but it could be speculated that increased growth in utero or in early childhood mediated by increased β -cell secretory activity could be part of the explanation. Future studies should include subjects with type 1 diabetes and assess the association between birth weight and type 1 diabetes after adjusting for HLA genotype.

RESEARCH DESIGN AND METHODS

Subjects were asked to take a buccal cell sample using two cytology brushes (Medscan Medical, Malmö, Sweden) and return them by mail. DNA was extracted using a method similar to that described by Parad (14). The validity of the mouth brush method had been tested previously (15,16). The *HLA-DQA1* and *-DQB1* loci were typed using polymerase chain reaction with biotinylated primers and reverse hybridization with sequence-specific oligonucleotide probes, as previously described (17). The *DQA1* locus was only typed for the *DQA1*0201*, *DQA1*03*, and *DQA1*0501* alleles, whereas other alleles were inferred from known linkage disequilibria with *DQB1* alleles (18). To ensure complete typing of the *DQB1* locus, nine probes were used in addition to those described by Cinek et al. (17). The designations and sequences of these probes are available from the authors.

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