

# Cow's Milk Consumption, HLA-DQB1 Genotype, and Type 1 Diabetes

## A Nested Case-Control Study of Siblings of Children With Diabetes

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The evidence for the putative role of cow's milk in the development of type 1 diabetes is controversial. We studied infant feeding patterns and childhood diet by structured questionnaire ( $n = 725$ ) and HLA-DQB1 genotype by a polymerase chain reaction-based method ( $n = 556$ ) in siblings of affected children and followed them for clinical type 1 diabetes. In a nested case-control design in a population who had both dietary and genetic data available, we selected as cases those siblings who progressed to clinical diabetes during the follow-up period ( $n = 33$ ). For each case, we chose as matched control subjects siblings who fulfilled the following criteria: same sex, age within 1 year, not from the same family, the start of the follow-up within 6 months of that of the respective case, and being at risk for type 1 diabetes at the time the case presented with that disease ( $n = 254$ ). The median follow-up time was 9.7 years (range 0.2–11.3). Early age at introduction of cow's milk supplements was not significantly associated with progression to clinical type 1 diabetes (relative risk adjusted for matching factors, maternal education, maternal and child's ages, childhood milk consumption, and genetic susceptibility markers was 1.60 [95% CI 0.5–5.1]). The estimated relative risk of childhood milk consumption for progression to type 1 diabetes was 5.37 (1.6–18.4) when adjusted for the matching and aforementioned sociodemographic factors, age at introduction of supplementary milk feeding, as well as for genetic susceptibility markers. In conclusion, our results provide support for the hypothesis that high consumption of cow's milk during childhood can be diabetogenic in siblings of children with type 1 diabetes. However, further studies are needed to assess the possible interaction between genetic disease susceptibility and dietary exposures in the development of this disease. *Diabetes* 49:912–917, 2000

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OR, odds ratio.

The putative diabetogenicity of cow's milk has been debated since the animal experiment by Elliott and Martin (1), showing that the risk of diabetes in BB rats could be decreased by feeding a semisynthetic amino acid diet instead of a milk protein supplementation diet from weaning. The findings from our Finnish case-control study were the first to suggest in humans a relationship between the early introduction of supplementary cow's milk feeding and an increased risk of type 1 diabetes (2) independent of the duration of breast-feeding (3). The results from case-control studies on the role of the consumption of cow's milk later in childhood as a possible risk factor of type 1 diabetes are conflicting (4–6). The only cohort study available suggests that consumption of cow's milk during childhood may be an important risk determinant among siblings of affected children (7). Geographical comparisons have shown a strong positive correlation between per capita cow's milk consumption and the risk of type 1 diabetes in children (8,9). Both increased humoral and cell-mediated immune responses to cow's milk proteins have been observed in newly diagnosed children with type 1 diabetes compared with those in control children (10). Cow's milk protein antibody levels were also found to be higher in affected children than in control siblings when matched for HLA-DQB1 risk alleles (11).

Data on a possible combined effect of cow's milk consumption and increased genetic predisposition to type 1 diabetes are scanty. The findings of 2 case-control studies indicate that there might be an interaction between increased genetic disease susceptibility based on HLA class II alleles and the early introduction of supplementary milk feeding during infancy on the risk of type 1 diabetes (12,13). In the present prospective follow-up of a cohort of siblings of affected children, we evaluated the effect of cow's milk exposure during infancy and later in childhood and of increased genetic susceptibility defined by HLA-DQB1 markers on the risk of developing type 1 diabetes.

### RESEARCH DESIGN AND METHODS

**Subjects.** In the nationwide Childhood Diabetes in Finland case-control study on genetic and environmental determinants of childhood type 1 diabetes (14), 801 families with a child presenting with clinical disease over a period from September 1986 to April 1989 were invited to participate. Detailed data were collected on infant feeding patterns and later childhood diet (3,15). Apart from the probands, all unaffected siblings between 3 and 19 years of age

were also asked to take part in the study. Among them, 82% participated in the dietary survey. In addition, 37 siblings younger than 3 years of age and 8 older than 19 years of age wanted to participate in the study by their own or their parents' initiative. Altogether, dietary data were available from 725 siblings, who form the source population of the present study. The median age of the siblings at the beginning of the study (at the time the index child developed diabetes) was 9.4 years (range 0.4–24.9).

The ethics committees of all participating hospitals approved the study protocol. Informed consent was obtained from the participating families.

**Assessment of diet and sociodemographic characteristics.** Dietary and sociodemographic data were collected from the siblings by structured questionnaires at the time of diagnosis of type 1 diabetes in the index child (2). In the present study, the following infant feeding variables were used: the duration of overall (total, also includes the time period during which the child received other foods in addition to breast-feeding) breast-feeding, and the age at introduction of cow's milk products in infancy (infant formulas, cow's milk, other cow's milk products). The average combined amount of milk and sour milk consumed each day (in glasses [1 glass = 180 g]) over a 6-month period before entering the study was determined. The sociodemographic variables used in the present study were age and sex of the child, the length of education of the mother, and maternal age (at the time of the birth of the sibling).

**HLA-DQB1 genotyping.** Polymerase chain reaction–based HLA-DQB1 typing was successfully carried out in 556 siblings by means of a method that has been described in detail previously (16,17). The alleles associated with strong (DQB1\*0302) and weak (DQB1\*02) susceptibility to disease as well as strong (DQB1\*0602 or \*0603) and weak (DQB1\*0301) protection were typed. HLA-DQB1\*02/0302 heterozygosity confers the highest risk of type 1 diabetes, \*0302 alone in the absence of protective alleles confers moderate risk, and the remaining genotypes confer low or decreased risk (17). All siblings were invited to give a DNA sample regardless of the subsequent diabetes status of the child. Altogether, a somewhat larger proportion of those siblings who progressed to type 1 diabetes during the follow-up (35 of 39 [90%]) were typed compared with other siblings (521 of 686 [76%]).

**Outcome measure.** As an outcome measure, we used the presentation with clinical type 1 diabetes over the time period from the diagnosis of diabetes in the index child until 31 December 1997. Information on the newly diagnosed case of type 1 diabetes were obtained from the Central Drug Registry of the Finnish Social Insurance Institution as described earlier (18).

**Sampling of cases and control subjects.** Because the data on the HLA-DQB1 genotype were available for a larger proportion of those children who progressed to clinical diabetes during the follow-up compared with those who did not, it was not possible to analyze the data according to a full cohort design. Therefore, we adopted the following nested case-control strategy for sampling case and control subjects: For each sibling who developed type 1 diabetes during the follow-up period and who had dietary and HLA-DQB1 data available ( $n = 33$ ), we chose all of the possible control siblings from the source population who fulfilled the following criteria: same sex, age within  $\pm 1$  years (at the time of entry to the study), not from the same family, the start of the follow-up within 6 months of that of the respective case (recruitment lasted 32 months), and being at risk of diabetes (nondiabetic) at the time when the case presented with clinical disease. Altogether, we identified 254 eligible control subjects: the mean number of control subjects for each case was 7.7 (range 1–22). The median (range) age of the cases was 7.2 years (1.8–16.2) and that of the control subjects was 8.3 years (1.6–16.9). Median duration of follow-up since diagnosis of diabetes in the index child (the time at which dietary information also was collected) until diagnosis of diabetes or end of follow-up was 9.7 years (0.2–11.3).

**Statistical methods.** The relative risks of developing type 1 diabetes during the follow-up period associated with the potential risk factors of interest were estimated by the conditional logistic regression model. In this analysis, matching was retained and all of the selected risk factors as well as the sociodemographic variables (child's age, maternal age, and maternal length of education) were included, thus simultaneously adjusting for the possible confounding induced by these variables. Adjustment for child's age was included because in spite of matching, a small difference was observed in the median age of case and control subjects.

## RESULTS

The nondiabetic siblings who were typed did not differ from the untyped ones with respect to sex, length of maternal education, duration of total breast-feeding, age at introduction of milk supplements, and childhood milk and sour milk consumption (Table 1). The mean age was somewhat lower in the typed siblings than in the untyped ones (9.5 vs. 10.8

years). There was a smaller proportion of younger mothers among the typed siblings compared with the untyped ones.

A larger proportion of cases than control subjects had consumed 3 glasses of milk and sour milk daily before entering the study, whereas the proportions of cases and control subjects who had been breast-fed for at least 2 months or had received cow's milk supplement before the age of 2 months did not differ (Table 2). There was no correlation between the length of follow-up and amount of cow's milk consumed in the cases ( $r = 0.074$ ,  $P = 0.68$ ). The prevalences of the risk-conferring alleles HLA-DQB1\*02 and \*0302 were clearly higher in case than in control subjects. In all, 79% of the case and 30% of the control subjects carried a moderate- or high-risk HLA-DQB1 genotype. The proportion of mothers having a long education ( $> 13$  years) tended to be higher among cases than control subjects (Table 2). An equal proportion of case and control subjects lived in rural areas, and there was no difference in the average income of the families between case and control subjects (data not shown).

The matched odds ratios (ORs) (i.e., adjusted for the matching factors only by conditional logistic regression) for the development of type 1 diabetes associated with the dietary, genetic, and maternal sociodemographic variables are also presented in Table 2.

TABLE 1  
Distribution of sociodemographic and dietary factors in the nonaffected siblings by availability of information on HLA-DQB1 genotype

	Siblings typed for HLA-DQB1 alleles	Siblings not typed for HLA-DQB1 alleles
<b>n</b>	556	169
Boys	47	46
Maternal education (years)		
6–9	26	31
10–12	35	27
13–22	35	34
Missing data	4	8
Maternal age (years)		
17–22	26	35
23–29	52	41
30–44	22	23
Missing data	0	1
Duration of total breast-feeding (months)		
<2	12	14
$\geq 2$	82	78
Missing data	6	8
Age at introduction of milk supplements (months)		
<2	21	24
$\geq 2$	68	67
Missing data	11	9
Childhood milk and sour milk consumption (glasses/day)		
<3	33	36
$\geq 3$	66	64
Missing data	1	0

Data are %.

TABLE 2

Distribution of sociodemographic, dietary, and genetic factors by disease status in the siblings of affected children and matched ORs for development of type 1 diabetes

	Cases [n (%)]	Control subjects [n (%)]	Matched OR* (95% CI)
<b>n</b>	33	254	—
Boys	46 (15)	49 (124)	
Girls	54 (18)	51 (130)	
Maternal education (years)			
6–9	15 (5)	25 (64)	1.0
10–12	18 (6)	37 (95)	0.66 (0.2–2.4)
13–22	61 (20)	34 (86)	2.56 (0.9–7.5)
Missing data	6 (2)	4 (9)	
Maternal age (years)			
17–22	12 (4)	23 (59)	1.0
23–29	49 (16)	50 (127)	1.69 (0.5–5.4)
30–44	39 (13)	27 (68)	2.33 (0.7–8.1)
Duration of total breast-feeding (months)			
<2	12 (4)	12 (31)	1.0
2	85 (28)	83 (211)	1.11 (0.3–3.7)
Missing	3 (1)	5 (12)	—
Age at introduction of milk supplements (months)			
<2	24 (8)	21 (53)	1.85 (0.7–4.8)
2	64 (21)	68 (173)	1.0
Missing	12 (4)	11 (28)	—
Childhood milk and sour milk consumption (glasses/day)			
<3	15 (5)	35 (88)	1.0
3	85 (28)	65 (166)	3.24 (1.2–8.7)
HLA-DQB1 genotype determined risk of diabetes†			
Low/decreased	21 (7)	70 (178)	1.0
Moderate	33 (11)	21 (53)	4.43 (1.6–12.1)
High	46 (15)	9 (23)	10.8 (4.1–28.5)

\*Matched for age, sex, and start of follow-up. †The HLA-DQB1 genotypes were classified as conferring high risk (DQB1\*02/0302), moderate risk (DQB1\*0302/x, where x stands for alleles other than \*02, \*0301, \*0602, or \*0603), low risk (DQB1\*0301/0302, \*02/0301, \*02/y, where y stands for \*02 or neutral alleles, or \*0302/0602 or 0603), and decreased risk (other alleles).

In the model in Table 3, we simultaneously included age at introduction of supplementary milk feeding, childhood milk consumption, and HLA-DQB1 genotype risk classification together with maternal and child's ages and length of maternal education and the matching factors. Both high consumption of cow's milk during childhood and moderate- and high-risk genotypes were related to development of type 1 diabetes, whereas the age at introduction of supplementary milk feeding was not yet significantly associated with the devel-

opment of diabetes. The removal of the cases with the shortest follow-up did not change the results. When the cases with <2 years of follow-up were excluded, the respective OR for childhood milk consumption was 7.8 (95% CI 1.5–40.1).

We also evaluated the possible modification of the effect of childhood milk consumption by HLA-DQB1–conferred genetic risk grouping and stratified the data jointly by these 2 factors. It was found that 67% (22 of 33) of the cases were both carrying a high- or moderate-risk genotype and had a high milk con-

TABLE 3

Adjusted relative ORs for development of type 1 diabetes associated with dietary factors and HLA-DQB1–defined disease risk

	OR* (95% CI)	<b>P</b>
Age at introduction of supplementary milk feeding (<2 vs. 2 months)	1.60 (0.5–5.1)	0.43
Childhood milk and sour milk consumption (3 vs. <3 glasses)	5.37 (1.6–18.4)	0.008
HLA-DQB1–defined risk of type 1 diabetes†		
Moderate vs. low/decreased	4.60 (1.5–13.8)	0.007
High vs. low/decreased	16.7 (4.8–57.7)	0.001

\*Adjusted for the matching factors and the length of maternal education, maternal and child's ages, and the other 2 variables in this table by conditional logistic regression. †The HLA-DQB1 genotypes were classified as conferring high risk (DQB1\*02/0302), moderate risk (DQB1\*0302/x, where x stands for alleles other than \*02, \*0301, \*0602, or \*0603), low risk (DQB1\*0301/0302, \*02/0301, \*02/y, where y stands for \*02 or neutral alleles, or \*0302/0602 or 0603), and decreased risk (other alleles).

TABLE 4

Distribution of childhood milk consumption among cases of type 1 diabetes ( $n = 33$ ) and their matched control subjects ( $n = 254$ ) stratified by HLA-DQB1 risk group and the adjusted ORs and 95% CI of type 1 diabetes associated with milk consumption

HLA-DQB1 risk group	Childhood milk consumption	Cases [ $n$ (%)]	Control subjects [ $n$ (%)]	Adjusted OR* for milk consumption (95% CI)
Low/decreased	<3 Glasses/day	1 (14)	55 (31)	1.0
	3 Glasses/day	6 (86)	123 (69)	2.36 (0.27–21.1)
	Total	7 (100)	178 (100)	
Moderate	<3 Glasses/day	1 (9)	23 (43)	1.0
	3 Glasses/day	10 (91)	30 (57)	11.1 (0.92–134)
	Total	11 (100)	53 (100)	
High	<3 Glasses/day	3 (20)	10 (43)	1.0
	3 Glasses/day	12 (80)	13 (57)	5.64 (0.87–36.7)
	Total	15 (100)	23 (100)	

\*Adjusted for the matching factors, the length of maternal education, and maternal and child's ages, and stratified for HLA-DQB1 risk grouping.

sumption, whereas this proportion among the control subjects was only 17% (43 of 254) (Table 4). A conditional logistic regression model was fitted in which the OR for milk consumption was allowed to be different in the 3 genetic risk groups. The results suggested that the relative risk associated with childhood milk consumption was higher in the groups at moderate and high genetic risk than in the low/decreased risk group. When combining high and moderate genetic risk groups, the OR for milk consumption in the high/moderate risk group was 6.34 (95% CI 1.69–23.8,  $P = 0.006$ ) and in the low/decreased risk group was 2.40 (0.27–21.2,  $P = 0.43$ ). However, the statistical evidence for this kind of effect modification was insufficient both in that case, in which the genetic risk was graded into 2 groups, and also when it was graded into 3 groups ( $P = 0.66$  and  $0.48$ , respectively, for interaction between milk consumption and genetic risk grouping). Similar analyses were performed for the modification of the diabetogenic effect of young age (<2 months) at introduction of supplementary milk by genetic disease susceptibility, but no consistent pattern for the group-specific estimates of relative risk were observed (Table 5). Neither did we observe any interaction between age at introduction of supplementary milk and childhood milk consumption (data not shown).

## DISCUSSION

Previous results concerning the diabetogenicity of cow's milk are controversial as are the conclusions drawn from such studies (19–22). Findings from a recent prospective cohort study from the same Childhood Diabetes in Finland project with 2-years-shorter follow-up suggested that a high level of consumption of cow's milk during childhood (>0.5 liters daily) increases the risk of type 1 diabetes 3-fold among siblings of affected children (7). In the present nested case-control study, we observed that when the HLA-DQB1 susceptibility markers of type 1 diabetes were also taken into account along with usual sociodemographic confounding factors and infant feeding variables, the relative risk associated with high consumption of cow's milk was 5.4.

Some case-control studies, but not all, have shown that early introduction of cow's milk during infancy may be diabetogenic (21,22). The statistical power of the available cohort study (7) and another nested case-control study (23) addressing the effect of the early introduction of cow's milk feeding on the development of type 1 diabetes and/or on the seroconversion to positivity of diabetes-specific autoantibodies was too weak to detect a relative risk of the same magnitude as that observed in case-control studies.

TABLE 5

Distribution of age at introduction of supplementary milk feeding among cases of type 1 diabetes ( $n = 29$ ) and their matched control subjects ( $n = 226$ ) stratified by HLA-DQB1 risk group and the adjusted ORs and 95% CI of type 1 diabetes associated with age at introduction of supplementary milk feeding

HLA-DQB1 risk group	Age at introduction of supplementary milk feeding	Cases [ $n$ (%)]	Control subjects [ $n$ (%)]	Adjusted OR* for age at introduction of supplementary milk feeding (95% CI)
Low/decreased	<2 months	2 (40)	37 (24)	1.0
	2 months	3 (60)	119 (76)	2.74 (0.40–18.8)
	Total	5 (100)	156 (100)	
Moderate	<2 months	2 (20)	12 (26)	1.0
	2 months	8 (80)	35 (74)	1.28 (0.17–9.5)
	Total	10 (100)	47 (100)	
High	<2 months	4 (29)	4 (17)	1.0
	2 months	10 (71)	19 (83)	2.36 (0.40–13.9)
	Total	14 (100)	23 (100)	

\*Adjusted for the matching factors, the length of maternal education, and maternal and child's ages, and stratified for HLA-DQB1 risk grouping.

Two previous small case-control studies addressed the issue of putative interaction between increased genetic susceptibility and an early age at introduction of supplementary feeding/short duration of exclusive breast-feeding on the risk of type 1 diabetes, but did not find any significant interaction (12,13). In our prospective subject series, the relative risk of an early age at introduction of cow's milk for contracting type 1 diabetes remained nonsignificant, even after the HLA-DQB1-defined genetic risk was taken into account. Still, the number of progressors was too small in the present study to exclude a diabetogenic effect of an early exposure to cow's milk or an interaction between that and genetic disease susceptibility.

The highest risk for type 1 diabetes is associated with heterozygosity for DQB1\*02 and \*0302, whereas DQB1\*0302 alone in the absence of protective alleles indicates a moderate risk (17). The results in the present series of initially unaffected siblings were consistent with such a risk grouping. The relative risks conferred by the susceptibility genotypes were further increased when childhood milk consumption was included in the model. The DQB1\*02 allele has been reported to be associated with bovine serum albumin IgG antibodies in children with type 1 diabetes and in their siblings (11), and also with GAD autoantibodies (24), the latter suggesting that children with this allele could be more susceptible to autoimmunity in general. On the other hand, the DQB1\*0302 allele has been associated with insulin autoantibodies in Swedish and Finnish children with type 1 diabetes (24,25). Both high and moderate genetic risk groupings in the present study include the allele DQB1\*0302. Our results suggest that the relative risk associated with childhood milk consumption may be higher in those carrying the high and moderate genetic risk genotypes compared with those with lower or decreased genetic risk (OR 6.3 vs. 2.4, respectively). This is interesting because of the recent hypothesis that bovine insulin could be an initiator of  $\beta$ -cell destruction (26). The DQB1\*0302 allele is more common in subjects with type 1 diabetes in the Nordic countries than elsewhere in Europe (27).

The major virtues of our study are a well-defined study population and a high participation rate. In addition, no selection bias was seen for the HLA-DQB1 typing. In the present study, the collection of dietary data before the development of type 1 diabetes excluded the possibility of a differential bias in the selection of subjects or in the reporting of dietary habits. The amount of milk consumed by the present-study subjects agrees well with another Finnish survey on the diet of children and adolescents performed in various parts in Finland in 1980 (28).

A major limitation of the present study is the small number of cases progressing to clinical disease, causing imprecision in the relative risk estimates, especially when trying to evaluate whether the effect of milk consumption depends on genetic susceptibility.

In conclusion, our results provide support for the hypothesis that high consumption of cow's milk during childhood can be diabetogenic in siblings of children with type 1 diabetes. However, further studies are needed to assess the possible interaction between genetic disease susceptibility and dietary exposures in the development of this disease.

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#### APPENDIX

##### The Childhood Diabetes in Finland Study Group.

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