

## Brief Genetics Report

# Genetic Liability of Type 1 Diabetes and the Onset Age Among 22,650 Young Finnish Twin Pairs

## A Nationwide Follow-Up Study

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Finland has the world's highest incidence of type 1 diabetes, and it is steadily increasing. We determined concordance rates and estimated heritability for type 1 diabetes in the Finnish Twin Cohort, a population-based twin cohort of 22,650 twin pairs. In addition, we studied age of onset in the first affected twin and discordance time between concordant twin pairs. Finnish twins born between 1958 and 1986 were followed for type 1 diabetes until 1998. We identified 228 twin pairs with type 1 diabetes: 44 monozygotic (MZ), 183 dizygotic (DZ), and 1 pair with unknown zygosity. The pairwise concordance for type 1 diabetes was 27.3% (95% CI 22.8–31.8) in MZ and 3.8% (2.7–4.9) in DZ twins. The probandwise concordance was 42.9% (26.7–59.2) and 7.4% (2.2–12.6), respectively. The longest discordance times were 6.9 years among concordant MZ twins and 23.6 years among DZ twins. The risk for type 1 diabetes was highest in cotwins of the index twins diagnosed at a very young age. The model with additive genetic and individual environmental effects was the best-fitting liability model, with 88% of phenotypic variance due to genetic factors and the remaining variance due to unshared environmental factors. In conclusion, these nationwide twin data demonstrated high genetic liability for type 1 diabetes. Early-onset diabetes increases the risk in cotwins. However, the majority of affected MZ twin pairs remain discordant for type 1 diabetes. *Diabetes* 52: 1052–1055, 2003

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ACE, additive genetic/shared environmental/unique environmental; AE, additive genetic/unique environmental; AIC, Akaike's information criteria; CDR, Central Drug Register; DZ, dizygotic; HDR, Hospital Discharge Register; MZ, monozygotic.

A recent study showed that Finland's record type 1 diabetes incidence increased predominantly in younger age-groups (1,2). The exact mechanism underlying the process leading to type 1 diabetes remains elusive, despite years of research. It is known that genes in the HLA region substantially influence the risk of type 1 diabetes and familial clustering (3,4). Several environmental factors have been proposed to contribute to its pathogenesis, but their causative role has not been established (5–7).

Twins provide a powerful tool to investigate relative importance of genetic and environmental factors on traits by comparing of concordance in monozygotic (MZ) and dizygotic (DZ) twins. Finland is one of the few countries with a population-based twin cohort (8) offering uniquely large, representative, and therefore unbiased data on type 1 diabetes among twins. The objective of this study was to determine concordance rates and to estimate heritability for type 1 diabetes in the Finnish population-based cohort. Furthermore, we examined risk for progression to diabetes in cotwins in relation to age at onset of the first affected twin (index twin) and the discordance time among concordant twin pairs.

Of 303 diabetic twins identified from different sources, 247 cases (in 228 pairs) had type 1 diabetes (116 females, 131 males), 28 had type 2 diabetes, 15 had secondary diabetes, and 13 had gestational diabetes. Of the type 1 diabetic twin pairs, 44 belonged to MZ pairs, 90 to opposite-sex DZ pairs, 93 to same-sex DZ pairs, and 1 to a same-sex pair of unknown zygosity, totaling 228 pairs (Table 1). Of the diabetic twin pairs, eight members were deceased (four diabetic and four nondiabetic); all of these belonged to DZ pairs. The cumulative incidence in 1998 of type 1 diabetes in this cohort was 0.54%.

Mean age of the members of this twin cohort at the end of follow-up in 1998 was 27.6 years (range 12.3–40.9). Mean diagnosis age for type 1 diabetes was 13.1 years (range 0.6–40.5, median 11.4). The follow-up time ranged from 0.1 to 40.9 years (mean 21.8, median 19.8).

Of the pairs, 19 were concordant and 209 pairs were discordant for type 1 diabetes. The crude probandwise concordance rate for type 1 diabetes was 42.9% (95% CI 26.7–59.2%) in MZ and 7.4% (2.2–12.6%) in DZ twins. The

TABLE 1  
Number of concordant and discordant twin pairs and crude concordance rates for type 1 diabetes in Finnish Young Twin Cohort

	Zygoty	Concordant pairs	Discordant pairs	Concordance rate (%)		<i>n</i>
				Probandwise	Pairwise	
Sex						
Male	MZ	7	19	42.4	27.0	33
	DZ	2	47	7.8	4.1	51
Female	MZ	5	13	43.5	27.8	23
	DZ	1	43	4.4	2.3	45
Opposite sex	DZ	4	86	8.5	4.4	94
Age at onset in index twin ≤10 years	MZ	7	7	66.7	50.0	21
	DZ	6	76	13.6	7.3	88
Age at onset in index twin >10 years	MZ	5	25	28.6	16.7	35
	DZ	1	100	2.0	1.0	102
Total*	MZ	12	32	42.9 (26.7–59.2)	27.3 (22.9–31.8)	56
	DZ	7	176	7.4 (2.2–12.6)	3.8 (2.7–4.9)	190

Data are *n* unless otherwise indicated. \*Plus one discordant pair with unknown zygoty.

crude pairwise concordance rate was 27.3% (22.8–31.8%) in MZ and 3.8% (2.7–4.9%) in DZ twins (Table 1).

In the survival analysis, the 10-year progression rate for the MZ cotwin was 32.8% (95% CI 21.3–42.6%) and for the DZ cotwin 3.2% (0.49–5.8%) ( $P < 0.001$ , log-rank test) (Fig. 1). Median discordance time was 2.0 years (range 0–6.9) among the concordant MZ and 6.0 years (range 1.5–23.6) among the concordant DZ twin pairs.

If the MZ index twin had been diagnosed at an age of 10 years or under ( $n = 14$ , Table 1), progression to diabetes in cotwins was 50% (95% CI 35.0–61.6%) within 7 years compared with 23% (6.9–36.8%) for age >10 years ( $n = 30$ ) (Table 1 and Fig. 2). Even though not statistically significant ( $P = 0.06$ , log-rank test), may be due to small numbers, the risk was found to be higher in the early-onset group. Among the concordant DZ pairs, the index twin had

early-onset diabetes in six of seven pairs. Correlation with age at onset among the concordant MZ pairs was very high, at 0.95 ( $P < 0.001$ ), but modest among the concordant DZ pairs, at 0.38 ( $P = 0.41$ ) (Fig. 3).

The best fitting liability model was the additive genetic/unique environmental (AE) model, which gave a good fit to the data ( $P = 0.60$ , Akaike's information criteria [AIC] =  $-5.23$ ). The estimates yielded suggested that 88% of phenotypic variance in the liability to type 1 diabetes was due to additive genetic (A) effects (95% CI 78–94%) and 12% to unique environmental (E) effects (6–22%). A model also including shared environmental effects (additive genetic/shared environmental/unique environmental [ACE]) fitted second best (AIC =  $-3.23$ ,  $P = 0.43$ ), approximately the same estimates as in the AE model.

Different twin studies have reported the pairwise concordance for type 1 diabetes between 13 and 52% in MZ

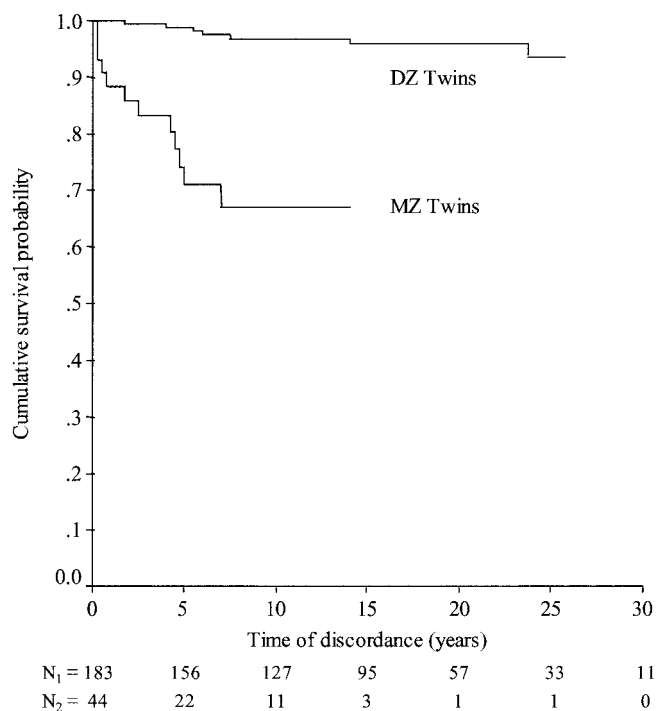


FIG. 1. Kaplan-Meier diabetes-free survival curves in cotwins of type 1 diabetic twins by zygosity.  $N_1$  and  $N_2$  are the numbers of subjects at risk at each time point for DZ twins ( $N_1$ ) and for MZ twins ( $N_2$ ).

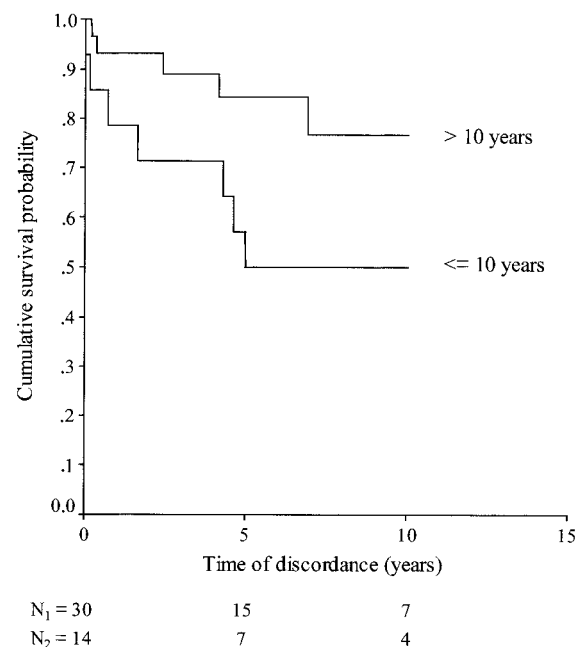


FIG. 2. Kaplan-Meier diabetes-free survival curves in MZ cotwins by age at diagnosis in the index twin.  $N_1$  and  $N_2$  are the numbers of subjects at risk at each time point for each diagnosis age groups,  $N_1 = >10$  years and  $N_2 = \leq 10$  years.

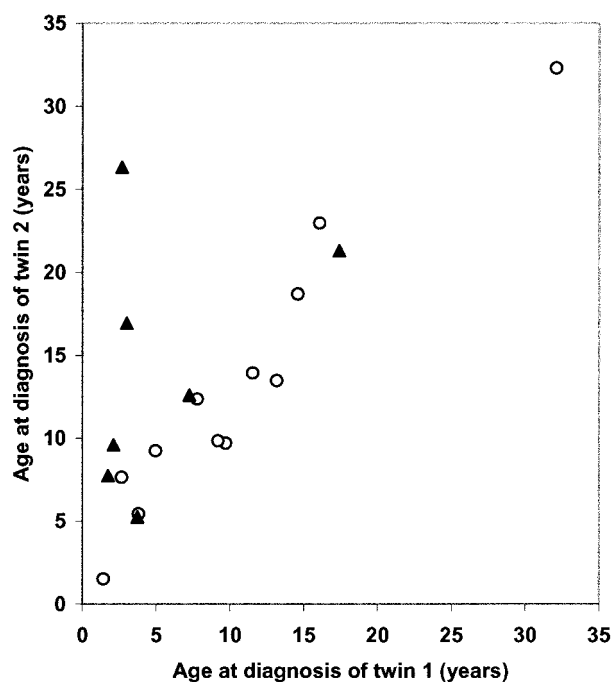


FIG. 3. Age at diagnosis of MZ (○) and DZ (▲) twin pairs concordant for type 1 diabetes. Intrapair correlation between MZ twins was 0.95 ( $P < 0.001$ ) and between DZ twins 0.38 ( $P = 0.41$ ).

twins (9–17) and 2.5 and 14% in DZ twins (9–11). Many twin studies have ascertained diabetic twin pairs by advertising or by opportunistically sampling pairs from diabetes clinics, which can easily lead to overestimation of concordance rates, unlike our study in that it is based on a large nationwide and population-based cohort (18).

The case ascertainment of diabetic subjects approached 100% because multiple data sources covering the entire country existed, and they can be linked with the national ID number assigned to every Finnish resident. The cumulative incidence of type 1 diabetes in this young twin cohort was 0.54%, consistent with the overall cumulative incidence of the disease in the Finnish population, estimated to be 0.5–0.6% at the age of 35 years. Our study thus provides concordance estimates in twins for type 1 diabetes that are free of both ascertainment and selection bias.

The vast majority of the diabetic twin pairs were discordant for type 1 diabetes. The concordance rates of MZ twins were substantially higher in the Finnish Young Twin Cohort (probandwise 43%, pairwise 27%) than in the Old Twin Cohort of same-sex twin pairs born before 1958 (probandwise 23%, pairwise 13%) (9) and actually doubled in the successive birth cohorts. The concordance rate in DZ twins, meanwhile, did not change very much. However, the concordance in the Old Twin Cohort was probably underestimated. When the Old Twin Cohort was established in the mid-1970s, the twins had to be alive in 1974 (8). Since in the first part of the 20th century, type 1 diabetes was associated with high mortality, many diabetic twin pairs were probably missed. This selection bias due to mortality was obviously larger in pairs concordant for diabetes.

The period of birth of our twins (1958–1985) was comparable with that in the Danish Twin study (1953–1982), which was also population based (10). The crude

probandwise concordance rate for Danish MZ twins (53%) was higher than ours (43%), although prevalence of type 1 diabetes is lower in the Danish population. Heritability for type 1 diabetes observed by Kyvik et al. (10) was 72%, which is of the same magnitude as that in the Finnish Old Cohort (73.6%) (9).

Our results confirm the influence of young age at onset in the index twin on the increased risk of cotwin and on shortened discordance time. In the series of British and U.S. MZ pairs, the risk pattern was very similar to ours. When the index twin was diagnosed with diabetes at a very young age, ~50% of the cotwins developed diabetes within a few years after the index twin's diagnosis (11,14,16). All of these data thus suggest that the process leading to  $\beta$ -cell destruction and type 1 diabetes seems to occur within a relatively short interval.

The first suggestive evidence for HLA type differences between concordant and discordant MZ twins was revealed by Johnston et al. (19), who found that prevalence of HLA-DR3, DR4 heterozygosity was higher in concordant than in discordant pairs of all onset ages. Furthermore, HLA type differences related to age at onset have been found (20). An increased frequency has recently been reported of diabetes-associated insulin gene genotypes in concordant twins (21). It is thus possible that concordance depends at least to some extent on genetic factors. The statistical modeling of our data suggested that the majority of the liability to type 1 diabetes was due to genetic effects, in keeping with our previous findings (9) and data from Denmark (10). The contribution of environmental factors seems to be ~20%. Our results also suggest that the importance of genetic effects has probably increased over time, possibly indicating an increasing expression of the liability genes of type 1 diabetes. However, this interpretation should be made with caution because of possible selection bias due to mortality in the Old Twin Cohort.

Type 1 diabetes among children aged  $\leq 15$  years has increased worldwide during the past decades (22). In light of population genetics, the rate of increase in the incidence of type 1 diabetes is too rapid to be caused by changes in the population gene pool. Despite harmful effects of diabetes-associated alleles, they are common in many populations. Environmental risk factors may directly trigger the process leading to type 1 diabetes or may interact with diabetes susceptibility genes that modify the penetrance. Heritability was found to be higher than that discovered before. If the increase in heritability is real, it should be at least partly interpreted as a changed penetrance of the diabetes susceptibility genes.

## RESEARCH DESIGN AND METHODS

The Finnish Twin Cohort was established in 1974; in its first phase, it was comprised of twin pairs born before 1958 (The Old Twin Cohort) (8). In 1987, the twin study was expanded to all twin births from 1958 to 1986, comprising 22,650 twin pairs (The Young Twin Cohort) (18). The cohorts were compiled from the Central Population Registry of all Finnish residents. To identify diabetic twins, The Young Twin Cohort data were linked to the National Hospital Discharge Register (HDR) for the years 1970–1998, to the nationwide Finnish Diabetes Register for Children and Young Adults for the years 1965–1998, and to the Social Insurance Institution Central Drug Register (CDR) started in 1964. Linkage was done by means of the national ID code assigned to every resident of Finland. The CDR covers all patients receiving free-of-charge medication for chronic diseases including insulin for type 1 diabetes and also comprises patients diagnosed before 1964. This register is virtually complete regarding type 1 diabetes (23). The HDR lists discharges of

all hospital patients and includes each patient's ID code, dates of hospitalization admission and discharge, and up to four diagnoses. The HDR has covered all hospital discharges nationwide since 1972. The record linkage yielded a total of 303 cases with any type of diabetes.

Copies of original medical records for all diabetic subjects were reviewed in order to verify type of diabetes and to define the date of manifestation. Questionnaires were mailed to all diabetic twin pairs with questions on zygosity, diabetes type, date of onset, start of insulin therapy, and history of diabetes among family members.

Determination of zygosity was based on responses from the questionnaires, and the validity of this method of zygosity determination was ascertained (24). In addition, zygosity in the present study was confirmed by DNA analysis of 10 highly informative polymorphic microsatellite loci (D3S1358, vWA, FGA, AMEL, THO1, TPOX, CSF1PO, D5S818, D13S317, and D7S820) in 14 of 15 same-sex concordant pairs and was consistently confirmed when any doubt arose as to zygosity ( $n = 5$ ). In the case when neither member of a same-sex pair answered the questionnaire ( $n = 24$ ), all other available data sources (parents, diabetes nurses, and medical records) were used. Only one diabetic pair with both members having moved abroad and both parents being deceased remained unclassified.

Concordance was estimated through both crude pairwise and probandwise concordance rates (25) and with Kaplan-Meier survival analyses. Furthermore, in the survival analyses the cotwins were divided into two groups: those in which the index twin was diagnosed at 10 years or younger ( $n = 14$  MZ and 82 DZ) and those in which the index twin was older than 10 ( $n = 30$  MZ and 101 DZ). The log-rank test served to test risk difference between groups.

The contribution of genetic factors to susceptibility to type 1 diabetes was estimated by the method of analysis based on structural equation models. A polygenic, multifactorial liability model was fitted to the data. It assumes that there is normally distributed liability to disease. This analysis method permits formulation of models with differing components of variance: additive genetic (A), genetic variance due to dominance (D), environmental effects common to the twins (C), or unique environmental (E) sources of variation in this underlying liability to disease. The superiority of alternative hierarchically nested models was assessed by a likelihood ratio test (26). The model with the lowest AIC value indicates the best goodness of fit and the parsimony. Analyses were carried out with the Mx-software package (27) developed especially for twin analyses.

This study was approved by the ethical committee of National Public Health Institute of Finland.

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