HbA_{1c} Levels Are Genetically Determined Even in Type 1 Diabetes

Evidence From Healthy and Diabetic Twins

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HbA_{1c}, a measure of blood glucose regulation, reflects glucose levels in the preceding months. In diabetes, HbA_{1c} levels predict the risk of microvascular complications. The aim of this study was to determine whether genetic factors could influence HbA_{1c} levels in normal subjects and type 1 diabetic patients. We performed a classical twin study of HbA_{1c} in healthy nondiabetic female twins and 42 monozygotic (MZ) and 47 dizygotic (DZ) pairs. Interclass correlations (r) were higher in MZ ($r = 0.77$) compared with DZ ($r = 0.53$) twin pairs, suggesting a substantial genetic effect; this was confirmed by quantitative genetic model fitting. Additive genetic effects (heritability) explained 62% (95% CI 47–75) of population variance in HbA_{1c}; the remainder was attributable to the influence of unique environment (23% [15–36]) and age (14% [5–28]). Multivariate modeling showed that genetic factors also have a substantial influence on fasting glucose levels (51%). However, HbA_{1c} heritability could not be explained by genes in common with fasting glucose. In the patients with type 1 diabetes, HbA_{1c} levels were correlated in 33 MZ twins concordant for diabetes ($r = 0.68$; $P < 0.001$) but also in 45 MZ twins discordant for the disease ($r = 0.52$; $P < 0.001$). These significant correlations for HbA_{1c} in both concordant and discordant pairs indicate a diabetes-independent familial effect. Thus, HbA_{1c} levels are largely genetically determined and independent of the genes influencing fasting glucose. Even in type 1 diabetes, familial (i.e., diabetes-independent) factors influence protein glycation, implying that familial factors may explain, in part, the risk for microvascular complications, as indicated by high HbA_{1c} levels. *Diabetes* 50: 2858–2863, 2001

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A, additive genetic components; D, dominant genetic components; C, shared environmental components; E, unique environmental components; DZ, dizygotic; MZ, monozygotic.

Diabetes is a major cause of excess mortality and morbidity. It is now the single most common cause of blindness and renal failure in middle age. Recent studies have emphasized the importance of blood glucose levels in predisposition to these microvascular complications (1–3). The index of blood glucose levels used in these seminal studies was HbA_{1c}, the levels of which were closely related to the frequency of diabetic microvascular complications (1–3). HbA_{1c} is a stable minor hemoglobin variant formed in vivo via post-translational modification by glucose, and it contains predominantly glycated NH_2-terminal β-chains (4). In early studies, HbA_{1c} was thought to be genetically determined, but wide differences in levels of HbA_{1c} between monozygotic (MZ) twins who were discordant (one twin affected) for diabetes suggested that levels reflected ambient blood glucose levels (5). Moreover, there was a strong relation between levels of HbA_{1c} and the average blood glucose levels over the previous 3 months (4). Despite the present wide acceptance of HbA_{1c} as the "gold standard" of blood glucose control, it has been recognized that levels may vary substantially between individuals, even those with similar blood glucose levels. Only one third or less of the variance in HbA_{1c} levels in nondiabetic subjects can be explained by differences in blood glucose levels (6). The cause of this variability in levels of HbA_{1c} is unclear, but a smaller difference in intraindividual than interindividual values suggests familial effects (7). In support of a familial effect, we noted that in one study, there was a correlation in HbA_{1c} levels between MZ twin pairs who were discordant for diabetes (5). We therefore decided to examine whether HbA_{1c} levels are influenced by genetic factors and, if so, whether genes in common with those controlling fasting glucose levels could explain such a genetic influence; to do this, we performed a twin study using data from MZ twins concordant and discordant for type 1 diabetes as well as healthy nondiabetic MZ and dizygotic (DZ) twins.

RESEARCH DESIGN AND METHODS

We studied two groups of twin pairs: *1*) healthy female nondiabetic MZ and DZ twins and *2*) MZ twins concordant and discordant for type 1 diabetes.

Healthy twins. Healthy twin pairs were drawn from the St. Thomas' U.K. Adult Twin Registry in 1998. Twins from the registry are unselected, mainly female volunteers ascertained from the general population through national media campaigns in the U.K. (8). The 42 MZ and 47 DZ twin pairs (age range

21–75 years) selected for this study satisfied the following criteria: *1*) European origin, *2*) female sex, *3*) no family or personal history of diabetes, *4*) both twins of each pair available for study, *5*) similar age range in MZ and DZ twin pairs, and *6*) exclusion of frank diabetes at time of sampling by a random whole-blood glucose <10.0 mmol/l or a fasting blood glucose <6.1 mmol/l.

Diabetic twins. Diabetic twin pairs were selected from the British Diabetic Twin Study in 1989 (9). Twins from the registry were ascertained by referral through their physicians (9). We selected 33 MZ pairs concordant for type 1 diabetes (12 male and 21 female pairs) and 45 MZ pairs discordant for type 1 diabetes (22 male and 23 female pairs). They were eligible according to the following criteria: *1*) European origin, *2*) affected twins had type 1 diabetes, *3*) both twins of each pair were available for study, *4*) the range in age of pairs concordant for type 1 diabetes was similar to those discordant for the disease, *5*) neither twin was hypertensive, and *6*) neither twin had evidence of overt renal impairment or microalbuminuria (overnight albumin excretion <1.4 mg/mmol creatinine), because renal disease can influence HbA_{1c} levels (10). Initially, we ascertained 48 discordant diabetic twin pairs and 36 concordant diabetic twin pairs; 3 discordant pairs and 3 concordant pairs were excluded because of microalbuminuria in at least one twin of a pair, leaving 45 discordant and 33 concordant pairs. Type 1 diabetes was defined according to the National Diabetes Data Group criteria, and diabetes was excluded in the nondiabetic co-twins by a 75-g oral glucose tolerance test and random whole-blood glucose at testing <10.0 mmol/l (11). All diabetic twins had been treated from the time of diagnosis with insulin and were taking either highly purified porcine or human insulin at least twice daily. The duration (mean ± SD) of diabetes was as follows: in the concordant group, 21 ± 8 and 19 ± 8 years for the index twin and diabetic co-twin, respectively; and in the discordant group, 21 ± 9 years in the diabetic twin. The 45 nondiabetic twins were then followed from 1989 with periodic urine and blood tests and repeat oral glucose tolerance tests for 9 years until January 1999; 4 of the 45 twins developed diabetes (3 are on insulin treatment and 1 is on diet alone). We estimate that the risk of the remaining nondiabetic twins developing type 1 diabetes is now <2% (12,13). All subjects gave informed consent, and the respective hospital ethics committees approved the study.

Biochemical analyses and confirmation of zygosity

Healthy twins. Zygosity was determined by standardized questionnaire, and DNA fingerprinting was used for confirmation (14). Serum glucose was measured in whole blood on an Ektachem machine (Johnson & Johnson). A high-performance liquid chromatography method (BioRex 70 variant analyzer; Biorad) was used to measure HbA_{1c}, with a between-batch coefficient of variation of <2.5%.

Diabetic twins. Monozygosity was established in all twin pairs, using both clinical data and at least 22 blood groups, as described previously (15). The urinary albumin excretion rate was assessed on a fresh timed overnight urine sample on the day of study using radioimmunoassay (Beckman, High Wycombe, U.K.). Urinary albumin excretion, expressed as a function of urine creatinine, was raised if their ratio was >1.4 mg albumin/mmol creatinine. Serum and urinary creatinine were measured using a standard colorimetric method (Boehringer Mannheim). Blood glucose was estimated on venous whole blood (YSI, Yellow Springs, OH). The study of diabetic twins in 1989 used an electroendosmotic method (Corning, Medfield, PA) to measure HbA_{1c}, with a normal range in nondiabetic control subjects of 4.5–9.3% and a between-batch coefficient of variation of 5%. This method to measure HbA_{1c} is different from the one used in the healthy twins.

Analytical approach

The aims of our analyses were twofold. First, to estimate the influence of genetic factors on HbA_{1c} levels and the extent to which that influence is dependent on glucose levels, we applied univariate and multivariate genetic model–fitting techniques in the healthy twins. Second, to examine the familial effect on HbA_{1c} levels and the dependence of HbA_{1c} on disease status, we used a multiple regression approach in the diabetic twins.

Quantitative genetic model fitting. The technique is based on the comparison of the covariances (or correlations) in MZ and DZ twin pairs and quantifies sources of individual differences by separation of the observed phenotypic variance into additive (A) or dominant (D) genetic components and shared (C) or unique (E) environmental components (16). The latter also contains measurement error. Dividing each of these components by the total variance yields the different standardized components of variance, such as heritability (h^2), which can be defined as the proportion of the total variance attributable to genetic variation. By incorporating age as a linear regression into the model, the influence of age on the phenotype can also be quantified (17). Estimates of genetic variation in the quantitative genetic model applied here represent the influence of the sum of several genes on the trait (i.e., the polygenic effect). The number of genes influencing the trait, their separate effects, and the mode of inheritance cannot be evaluated in the present twin design. Extension of the univariate HbA_{1c} model to a bivariate (or Cholesky) model (18,19), including both glucose and HbA_{1c}, also allows exploration of the extent to which the correlation between glucose and HbA_{1c} can be explained by common genes (i.e., the genetic correlation [r_g]) or a common environment (i.e., the environmental correlation [r_e]). In other words, this model enabled us to quantify which part of the variance components (genetic or environmental) was specific to HbA_{1c}, and which part was attributable to the influence of glucose (or age).

TABLE 1
Characteristics of healthy female twin pairs

	MZ	DZ
No. of pairs	42	47
Age (years)	53.9 ± 12.2	50.3 ± 15.6
Height (m)	1.60 ± 0.05	1.61 ± 0.07
Weight (kg)	65.9 ± 13.6	67.1 ± 11.9
BMI (kg/m^2)	25.6 ± 4.8	25.8 ± 4.1
Glucose (mmol/l)*	4.63 ± 0.49	4.47 ± 0.41
HbA_{1c} (%)	5.73 ± 0.41	5.67 ± 0.42

Data are mean ± SD. *Only for fasting twins (N_{MZ} = 38 and N_{DZ} = 41).

Figure 1 shows the Cholesky decomposition of the genetic and environmental factors for the two phenotypes (and age) included in the analysis. The observed phenotypes are shown in squares, and latent factors are shown in circles. The number of latent factors equals the number of variables. The first factor (A1, E1) contributes to both variables, and the second factor (A2, E2) reflects influences specific to HbA_{1c}. Factor loadings (cf. regression coefficients) of observed variables on the different latent factors are represented by the arrows.

Model-fitting procedure. For both univariate and multivariate model fitting analyses, a series of submodels nested within the full-parameter model were fitted to the variance-covariance matrices. The significance of variance components A, C, D, and age were assessed by testing the deterioration in model fit after each component was dropped from the full model, leading to a model in which the pattern of variances and covariances is explained by as few parameters as possible. Standard hierarchical χ^2 tests were used to select the best-fitting model (16). Before all data analyses, both HbA_{1c} and glucose values were log transformed to obtain normal distributions.

Statistical software. Data handling and preliminary analyses were done with STATA software (20). Univariate and multivariate quantitative genetic modeling were carried out using Mx software (21).

RESULTS

Healthy twins. Table 1 shows the characteristics of the healthy female twin pairs. Mean HbA_{1c} values were similar in MZ and DZ twins, as were all other characteristics. HbA_{1c} was significantly correlated with age (r = 0.38; P < 0.001). Twin correlations for HbA_{1c} levels were significantly (P < 0.02) higher in MZ (r = 0.77; SE 0.064) compared with DZ (r = 0.53; SE 0.106) twin pairs, suggesting a substantial genetic effect, and were confirmed by univariate genetic model fitting (Table 2). In the best-fitting model, additive genetic effects (heritability) explained 62% (95% CI 47–75) of the population variance in HbA_{1c} levels. The remaining part was attributable to the influence of unique environment (23% [15–36]) and age (14% [5–28]). Variance components C and D did not contribute significantly and were thus excluded from the model (Table 2).

For the multivariate analysis, including both HbA_{1c} and fasting glucose, we only used pairs in which both twins were fasting, which reduced the sample size to 38 MZ and 41 DZ pairs. The phenotypic correlation between HbA_{1c} and fasting glucose was 0.31 (P < 0.001). Correlations with age were still significant for both HbA_{1c} (r = 0.26; P = 0.001) and fasting glucose (r = 0.28; P < 0.001). Results of the multivariate analysis are shown in Table 3 and Fig. 2.

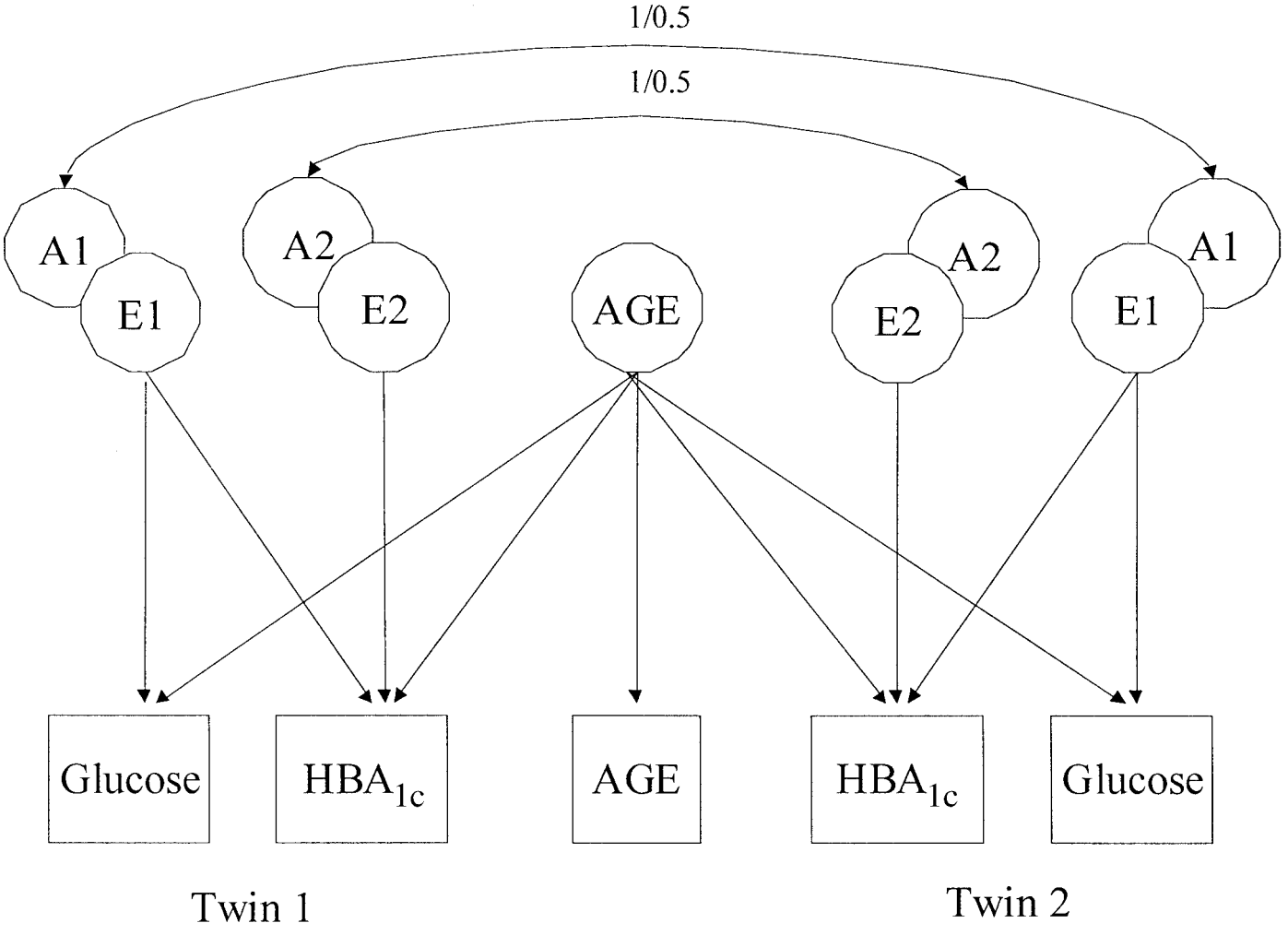

FIG. 1. Path diagram showing the influence of age and variance factors A and E on glucose and HbA$_{1c}$ (Cholesky decomposition). The observed phenotypes are shown in squares, and latent factors are shown in circles. Factor loadings of observed variables on the different latent factors are represented by the arrows. The correlation between A variance factors is 1 and 0.5 for MZ and DZ pairs, respectively. For clarity, C and D latent factors are not included in the model, and arrows loading on the latent A factors are omitted.

Table 3 shows variance component estimates for the best-fitting model. Estimates for HbA$_{1c}$ changed only slightly compared with the univariate analysis because of the smaller number of twin pairs. The heritability for fasting glucose was estimated at 51% (29–67).

The amount of overlap in genetic and environmental influences on glucose and HbA$_{1c}$ was small, as indicated by low genetic (r_g = 0.16) and environmental (r_e = 0.36) correlations. The same result is reflected in a different way in Fig. 2, which shows sources of individual differences in HbA$_{1c}$ levels (expressed as a percentage of the total population variance) based on the best-fitting multivariate model. It illustrates that almost all of the variance in HbA$_{1c}$ could be explained by genetic and environmental factors specifically influencing HbA$_{1c}$. Only a very small part of the total variance in HbA$_{1c}$ could be attributed to genes in common with fasting glucose (1.6%).

Diabetic twins. Table 4 shows characteristics of MZ twin pairs concordant and discordant for type 1 diabetes. There were no differences in mean HbA$_{1c}$ between men and women and no differences in mean age between men and women or diabetic and nondiabetic twins. As expected, HbA$_{1c}$ levels were significantly higher in diabetic than nondiabetic twins (χ^2[1] = 64.41; $P < 0.0001$).

TABLE 2
Quantitative genetic univariate model–fitting results for HbA$_{1c}$ levels in healthy twins

Models	A^2 (95%CI)	C^2/D^2 (95%CI)	E^2 (95% CI)	Age^2 (95% CI)	χ^2	df	$\Delta\chi^2$	vs.	Δdf	P
A-D-E	0.62 (0.00–0.75)	0.00 (0.00–0.68)	0.23 (0.15–0.36)	0.14 (0.05–0.28)	6.61	7	—		—	—
A-C-E	0.50 (0.09–0.75)	0.13 (0.00–0.47)	0.24 (0.15–0.38)	0.14 (0.05–0.28)	6.26	7	—		—	—
A-C-E no age	0.50 (0.08–0.84)	0.28 (0.00–0.62)	0.24 (0.15–0.39)	—	23.29	8	17.03	A-C-E	1	<0.001
A-E	0.62 (0.47–0.75)	—	0.23 (0.15–0.36)	0.14 (0.05–0.28)	6.61	8	0.35	A-C-E	1	0.55
C-E	—	0.49 (0.34–0.63)	0.36 (0.26–0.51)	0.14 (0.04–0.28)	12.04	8	5.78	A-C-E	1	0.016

Note: AE is the best fitting model. Age^2, variance component due to age; $\Delta\chi^2 = \chi^2$ (submodel) $- \chi^2$ (full model); Δdf = df (submodel) $-$ df (full model); vs., versus and indicates with which full model the submodel is compared.

TABLE 3
Estimates of variance components in the best fitting multivariate model for fasting healthy female twins

	h^2 (95% CI)	E^2 (95% CI)	Age^2 (95% CI)
Glucose	0.51 (0.29–0.67)	0.43 (0.28–0.65)	0.06 (0.005–0.17)
HbA_{1c}	0.63 (0.45–0.76)	0.31 (0.20–0.48)	0.06 (0.005–0.18)

h^2, genetic variance component (heritability); age^2, variance component due to age.

Correlations for HbA_{1c} in MZ twin pairs concordant and discordant for type 1 diabetes are shown in Table 5. Figure 3 shows that the scatterplot of log transformed HbA_{1c} values for discordant pairs (Fig. 3*B*) is shifted downwards compared with the concordant plot (Fig. 3*A*) because of the lower HbA_{1c} levels in the healthy twin (twin 2). However, the positive slope of the regression line reflecting the strength of the relation between the twins is similar for concordant and discordant pairs. Significant correlations for HbA_{1c} in both concordant and discordant pairs indicate a diabetes-independent familial effect.

Of the 45 nondiabetic twins from the discordant pairs, 4 subsequently developed diabetes, but their mean HbA_{1c} levels (6.9 ± 2.6%) were similar to that of the 41 twins who remained nondiabetic (6.6 ± 1.1%).

DISCUSSION

We performed a twin study using data from both normal subjects and patients with type 1 diabetes to determine the heritability of HbA_{1c} levels and that heritability's dependence on fasting glucose levels. Genetic effects explained 62% of the population variance in HbA_{1c}; the remainder was attributable to the influence of unique environment (23%) and age (14%). Although genetic factors have a substantial influence on fasting glucose levels (51%) in healthy twins, the HbA_{1c} heritability could not be explained by genes in common with fasting glucose because they explained only 1.6% of the total variance in HbA_{1c}. The amount of overlap in genetic determinants of HbA_{1c} and fasting glucose in diabetic individuals could not be studied in our data and may possibly be different.

For the bivariate analysis, only fasting twin pairs were included, whereas all twin pairs were used in the univariate analysis. However, heritability estimates of HbA_{1c} in the univariate and bivariate analysis were very similar (62 and 63%). We therefore think it unlikely that the possible inclusion of undiagnosed diabetic patients in the univariate analysis might have biased the results.

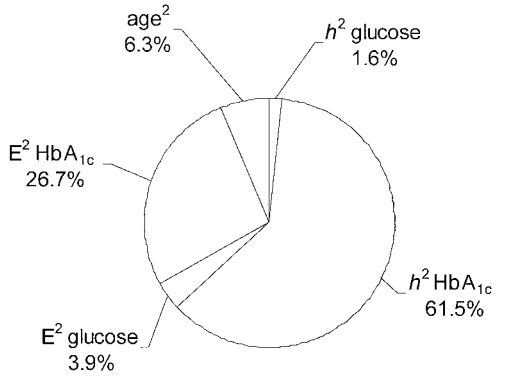

FIG. 2. Sources of variance in HbA_{1c} (expressed as percentage of total HbA_{1c} variance) based on the best fitting multivariate model. h^2 HbA_{1c}, genetic variance component (heritability) specifically influencing HbA_{1c}; h^2 glucose, genetic influence on HbA_{1c} due to glucose; E^2 HbA_{1c}, unique environmental variance component specifically influencing HbA_{1c}; E^2 glucose, unique environmental influence on HbA_{1c} due to glucose; age^2, variance component due to age.

TABLE 4
Characteristics of MZ twin pairs concordant and discordant for type 1 diabetes

			HbA_{1c} (%)	
	N	Age	Twin 1	Twin 2
MZ concordant				
Men	12	36.7 ± 10.4	10.6 ± 2.2	10.6 ± 2.3
Women	21	35.2 ± 8.3	10.4 ± 2.7	10.3 ± 2.9
MZ discordant*				
Men	22	40.2 ± 12.1	10.2 ± 2.0	6.7 ± 1.0
Women	23	41.9 ± 13.7	9.1 ± 1.5	6.5 ± 1.3

Data are means ± SD. *N* = number of twin pairs. *Twin 1 is the diabetic twin and twin 2 is the healthy twin.

MZ twins concordant and discordant for type 1 diabetes showed substantial HbA_{1c} correlations of similar size, despite the majority of these twins living apart, attending different physicians, and receiving different insulin treatment regimens. The similarity of the correlations in concordant and discordant twin pairs indicate that a considerable portion of the variance of HbA_{1c} levels in patients with type 1 diabetes must be due to shared familial factors, which are not diabetes-dependent. Given the results in the healthy twins, it is likely that most of this familial effect is attributable to genetic factors rather than shared environment. However, because no diabetic DZ twins were available for study, these familial sources of variance cannot be separately estimated. Our sample of healthy MZ and DZ twins was limited to women, and future twin studies are needed to confirm these results for men. However, results for male and female diabetic twin pairs were very similar in the present study.

Our data emphasizes the importance of familial factors as determinants of HbA_{1c} variation in type 1 diabetic patients. However, this by no means implies that type 1 diabetes is unimportant in determining HbA_{1c} levels, as is illustrated by the large difference in HbA_{1c} levels between diabetic and healthy twins shown in Table 4 and Fig. 3.

Genetic factors could influence glycation of proteins by glucose-dependent or glucose-independent mechanisms. Glucose metabolism is, in part, genetically determined, and our study is in line with other twin and family studies that have shown substantial heritability of fasting glucose and postload glucose levels (22). Our heritability estimate of 51% for fasting glucose is comparable with another recent twin study that reported a heritability of 50% (19). However, fasting glucose plays only a small role in determining HbA_{1c} levels in normal subjects, just as it does not

TABLE 5
Twin correlations in MZ pairs concordant and discordant for type 1 diabetes

	Concordant	*P*	Discordant	*P*
Men	0.52	0.085	0.54	0.010
Women	0.74	<0.001	0.50	0.014
Combined	0.68	<0.001	0.52	<0.001

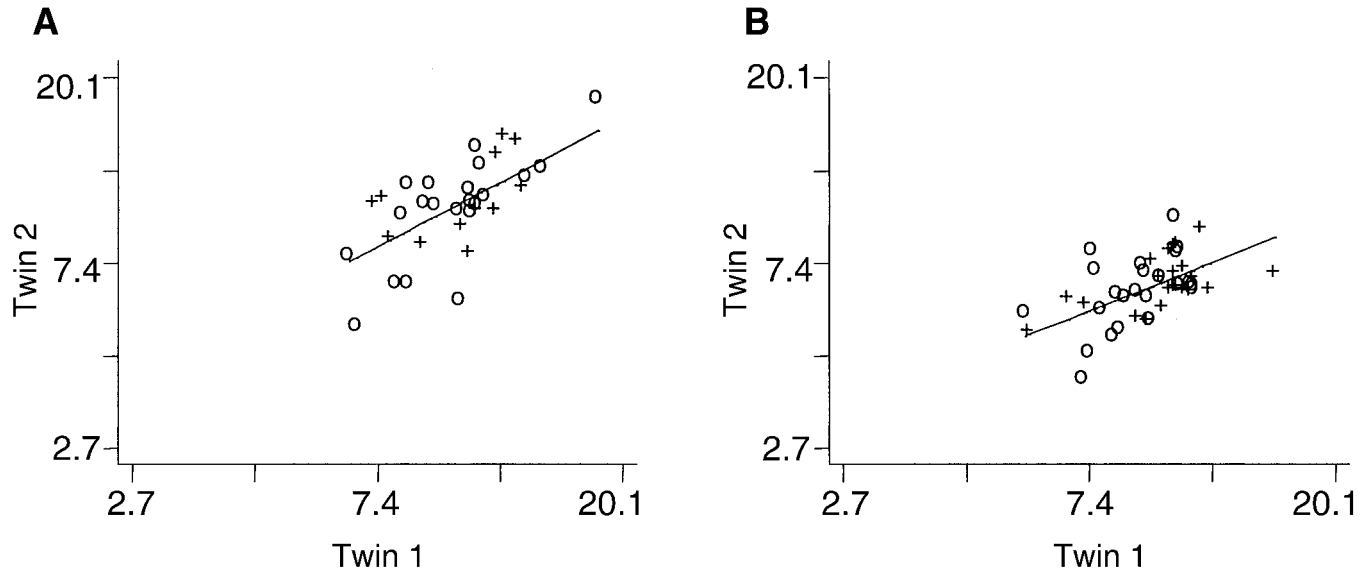

FIG. 3. *A*: Scatterplot of HbA$_{1c}$ values on a logarithmic scale for male (+) and female (○) MZ pairs concordant for type 1 diabetes. *B*: Scatterplot of HbA$_{1c}$ values on a logarithmic scale for male (+) and female (○) MZ pairs discordant for type 1 diabetes (twin 1 is diabetic and twin 2 is healthy).

precisely predict HbA$_{1c}$ levels in diabetic patients (6,23). Therefore, postprandial glucose may play a more important role in determining HbA$_{1c}$ levels. Although glycation of proteins is nonenzymatically determined, it is possible that genetic factors influence events upstream or downstream of the glycation process. What is surprising is that diabetes-independent familial factors appear to influence HbA$_{1c}$ levels in type 1 diabetic patients. These diabetes-independent factors are likely to affect the levels of both blood glucose and HbA$_{1c}$ and, hence, the risk of developing diabetic microvascular complications.

Alternatively, genetic factors could influence glycation of proteins by glucose-independent mechanisms. Thus, levels of HbA$_{1c}$ can be influenced by rates of hemoglobin glycation, red cell survival, oxygen tension, 2,3-diphosphoglycerate levels, intra-erythrocyte pH, and erythrocyte glucose permeability (24–26). Certainly, HbA$_{1c}$ reproducibility is improved by testing blood samples taken within 4 months (i.e., within the normal erythrocyte life span) (24). We studied levels of glycated protein in our normal twins (data not shown), but the assay was not sensitive enough to the variation in the normal range of these healthy individuals. Protein glycation is widespread, and the physical properties of proteins are closely related to glycation, which can influence their folding, trafficking, packing, stabilization, protease protection, quaternary structure, and organization. Glycation of proteins is probably important in leading to diabetic microvascular complications, and inhibition of protein glycation can prevent their development (27). These diabetic microvascular complications tend to cluster in families, partly because of genes that remain unidentified (28). Studies seeking these genes have focused on determinants of blood pressure and tissue oxidation; our present study suggests that genes influencing levels of protein glycation and/or glucose metabolism could also be important.

Clinically, it will be important to establish the extent to which HbA$_{1c}$ levels reflect genetically determined protein glycation as distinct from genetically determined glucose metabolism, which could account for the occasional anomalies between HbA$_{1c}$ levels and blood glucose levels, microvascular complications, or both (1,2,26). Our present observations explain the tendency for HbA$_{1c}$ to "track" at certain levels in particular individuals. Because much of the variation in HbA$_{1c}$ levels between individuals is inherited, elevated HbA$_{1c}$ levels may indicate an increased familial risk of diabetic microvascular disease.

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