The Heritability of HbA1c and Fasting Blood Glucose in Different Measurement Settings

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In an extended twin study we estimated the heritability of fasting HbA1c and blood glucose levels. Blood glucose was assessed in different settings (at home and in the clinic). We tested whether the genetic factors influencing fasting blood glucose levels overlapped with those influencing HbA1c and whether the same genetic factors were expressed across different settings. Fasting blood glucose was measured at home and during two visits to the clinic in 77 healthy families with same-sex twins and siblings, aged 20 to 45 years. HbA1c was measured during the first clinic visit. A 4-variate genetic structural equation model was used that estimated the heritability of each trait and the genetic correlations among traits. Heritability explained 75% of the variance in HbA1c. The heritability of fasting blood glucose was estimated at 66% at home and lower in the clinic (57% and 38%). Fasting blood glucose levels were significantly correlated across settings ($0.34 < r < 0.54$), mostly due to a common set of genes that explained between 53% and 95% of these correlations. Correlations between HbA1c and fasting blood glucoses were low ($0.11 < r < 0.23$) and genetic factors influencing HbA1c and fasting glucose were uncorrelated. These results suggest that in healthy adults the genes influencing HbA1c and fasting blood glucose reflect different aspects of the glucose metabolism. As a consequence these two glycemic parameters can not be used interchangeably in diagnostic procedures or in studies attempting to find genes for diabetes. Both contribute unique (genetic) information.

Keywords: heritability estimates, HbA1c, fasting blood glucose, twins, type 2 diabetes

Worldwide major efforts are ongoing to identify genetic variation underlying type 2 diabetes mellitus, one of the fastest growing threats to health (Sladek et al., 2007; Zeggini et al., 2007). To establish the diagnosis of type 2 diabetes in the early stage, clinicians often rely on measurement of fasting blood glucose (FBG) (A.D.A., 2006) although in some countries HbA1c levels are used instead (Bennett et al., 2007). During the course of the disease, type 2 diabetic patients may control their glucose homeostasis by measuring their fasting blood glucose, whereas health professionals mainly use HbA1c to monitor long-term glycaemia (McCarter et al., 2004; Tapp et al., 2006). Taken the heritability of type 2 diabetes (Medici et al., 1999) it is likely that both these indicators are themselves heritable.

Heritability of FBG has indeed been well-established, but the existing family and twin studies show large variation in the estimated contribution of genetic factors. A high heritability (77%) for FBG was reported in non diabetic first-degree relatives of type 2 diabetic patients (Mills et al., 2004). In contrast a mere heritability of 21% for the same variable was found in a large community based study of healthy families (Freeman et al., 2002). The lowest genetic influence on the variability of FBG was found by Schousboe (Schousboe et al., 2003). Heritability in adult nondiabetic females was only 12%, although somewhat higher estimates were found for males (38%). Other twin studies in Western European populations showed heritability's ranging from 38 to 67% (Baird et al., 2001; Katoh et al., 2005; Poulsen et al., 1999; Snieder et al., 1999; Souren et al., 2007).

A possible explanation for the discrepant heritability estimates for FBG is the potential influence of measurement setting on the relative contribution of genes and environment to FBG levels. Often the dietary state of the study participants is well-controlled, but blood glucose levels may be sensitive to many other behavioral factors like recent physical activity, psychological expectation, and degree of adaptation to blood letting procedures. These factors may be determined in part by the setting of blood letting. Collecting blood during a home visit, for instance, may lead to quite different behavioural antecedents than an active visit of the participant to a

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clinic. As part of an extended twin study addressing the genetic and environmental contribution to the variance of the beta-cell function in Dutch twin families, the first aim of the present study was to estimate the contribution of genes to the variance of FBG, obtained in different measurement settings, including a home visit and two visits to the clinic.

Despite its frequent use as an indicator of long-term glycaemic control and its established relation to diabetic complications (Tapp et al., 2006; McCarter et al., 2004) the genetics of HbA1c has been much less studied than that of FBG. Only two studies have reported heritability estimates. Snieder et al. (2001) found a heritability of 62% in healthy (only female) twins and an important contribution of age (14%). The heritability of HbA1c in nondiabetic first-degree relatives of type 2 diabetic patients was estimated at 55% (Mills et al., 2004). No heritability studies of HbA1c in male twins are known. A second aim of the present study was to estimate the heritability of HbA1c in both sexes.

Based on the idea that FBG and HbA1c are used interchangeably in the diagnosis and monitoring of diabetes it is expected that the genes influencing FBG and HbA1c should be largely overlapping. This has important consequences for large scale gene finding efforts that could then pool samples using either one of these quantitative endophenotypes for diabetes risk. As a third aim, the present study provides a test of the expectation that the genes influencing FBG and HbA1c are largely overlapping.

Materials and Methods

Between September 2004 and December 2006 the Netherlands Twin Register (Boomsma et al., 2006) invited 154 twin families by mail to participate in a study addressing various aspects of beta-cell function, according to the following inclusion criteria: Caucasian origin, good general health, aged 20 to 45 years, and having a sibling in the family of the same sex as the twin pair with a maximum age difference of 5 years. Exclusion criteria were diabetes mellitus, other relevant metabolic disorders, use of drugs that affect insulin secretion and/or insulin sensitivity, pregnancy and the first 6 months after childbirth. A minimum of two persons of one family (including one of the twins) was required. The twin-sibling design offers the opportunity to distinguish genetic and environmental sources of variation based on a comparison of the resemblance in family members of different genetic relatedness (Neale & Cardon, 1992). Including an additional sibling to the classical twin design significantly increases the power to detect the sources of variation (Posthuma & Boomsma, 2000).

The study protocol consisted of one home screening visit to exclude diabetes mellitus by a 75-g oral glucose tolerance test (OGTT) and one visit to the clinical research unit; a second visit to the research unit was optional. The procedures during the respective visits are outlined below. Fifty per cent of the invited families agreed to participate. Reasons for nonparticipation included the lack of time (45%), only one member of the family wanted to participate (16%), and fear of needles (13%).

The 77 twin families included consisted of: 51 MZ pairs (21 male) and 21 DZ pairs (8 male). There were 2 MZ (1male) and 3 DZ (2 male) incomplete twin pairs. Thirty-one siblings took part in this study (15 male). FBG results of the optional second visit to the clinic were obtained for 123 subjects (57 male) of 54 families, comprising 33 MZ pairs (14 male), 14 DZ pairs (7 male) and 7 incomplete twin pairs (3male) and 22 sibs (12 male). The two groups were comparable in zygosity, sex and BMI, but the group that also participated in the second visit was 1.5 years younger ($P = 0.043$). Twin zygosity was determined from DNA polymorphisms or, when DNA was not available, from survey questionnaires (Willemsen et al., 2005).

All subjects gave written informed consent. The study was approved by the local Ethics Committee and performed in accordance with the Declaration of Helsinki (2000).

Measurements

During the screening visit at home an OGTT was performed. At all test occasions participants were instructed to fast overnight during 12 hours prior to the visit and to refrain from heavy physical exercise, alcohol use and smoking. The OGTT was started between 8.00 and 10.00 am. Fasting and 2-h post-load capillary blood was obtained and analyzed by a glucose dehydrogenase method (HemoCue 201+, Ängelstrom, Sweden) for glucose level (FGBG-O). All measurements were below diabetic levels (FBG-O < 6.1, 2-h blood glucose < 11.1 mmol/l)

After a median period of 33 days participants arrived at the clinical research unit at 8:00 a.m. to undergo a meal test. First, weight (balance scale Seca, The Netherlands), height (LOG Harpender Stadiometer, Great Britain) and waist and hip circumference were measured. Second, subjects assumed a semi-recumbent position with their non-dominant hand resting in a heating box (50 °C) to obtain arterialized blood from a dorsal hand vein for measurement of among others fasting blood glucose and HbA1c. Whereas the test-tube for HbA1c determination was immediately transported to the clinical chemistry department (see below), fasting blood glucose was assessed at bedside using a glucose oxidase method (YSI 2300 Stat plus, Yellow Springs, OH, USA). At an interval of 5 minutes, a second blood sample was taken for baseline fasting glucose (FBG-M) and hormonal levels. After the initial blood sampling the meal-test was started.

Sixty-nine per cent of the participants returned to the clinic after a median period of 43 days at 8:00 a.m. for an optional (combined eu-and hyperglycaemic) clamp test. As before, two arterialized blood

samples were drawn from the dorsal hand vein for measurement of fasting glucose (FBG-C) and hormonal levels at a 5-minute interval. After this, the clamp test was started.

Laboratory Analysis

Analyses of HbA1c were performed at the VU University Medical Centre (department of Clinical Chemistry), Amsterdam, the Netherlands, using a DCCT standardized reversed-phase cation exchange chromatography (HA 8160 analyzer, Menarini, Florence, Italy). The HbA1c is detected by a dual-wavelength colorimetric (415-500). The intra-assay coefficient of variation (CV) is 0.6% at a mean of 4.9% and the inter-assay CV is 0.8 % at a mean of 5.5%. The HemoCue method has a CV of 1.5–2.5 % and correlates strongly with the YSI (r = .978; Stork et al., 2005). The YSI has a within run CV of 2 % and a day-to-day CV of 6% (Astles et al., 1996). The two repeated measurements of FBG at each of the clinic visits showed strong test-retest correlations across the 5-minute intervals (.90 < r < .93) and the mean value across the two measurements was used in all FBG analyses.

Data Analyses

Structural equation modelling was carried out in Mx (Neale et al., 2006). In a first step, a 4-variate unconstraint model was used to estimate means, variances and regression coefficients for covariates sex and age for each phenotype (HbA1c, FBG-O, FBG-M, FBG-C). Estimates of within trait and cross-traits correlations for MZ, DZ and twin-sib pairs were also obtained from this model. In the 4-variate analysis the following tests were carried out: (1) test of equality of means and variances for MZ and DZ twins and siblings, (2) equality of covariances for DZ twins and siblings, and (3) test of significance of age and sex regressions on the means. Likelihood-ratio tests were employed to identify the best model for the 4-variate data.

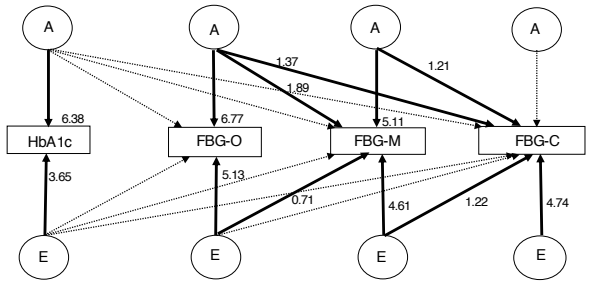

Figure 1

Genetic model for HbA1c and three FBG measures, with factor loadings of observed variables on the latent additive genetic and unique environmental factors.

Note: FBG-O = fasting blood glucose at home; FBG-M = fasting blood glucose pre-meal; FBG-C = fasting blood glucose pre-clamp. Bold line = significant; dotted line = nonsignificant.

MZ and DZ twin and twin-sib correlations, within person correlations between traits and cross-twin cross-trait correlations (e.g., between HbA1c level of the oldest twin and FBG-O level of the youngest twin) were estimated in the most parsimonious model. Next, a genetic triangular decomposition was fitted to the data (Fig. 1). An ACE model consisting of Additive genetic, Common environmental and unique Environmental factors were used. The raw data option in Mx was used and the influence of covariates sex and age was incorporated as fixed effects on the mean.

Results

FBG and HbA1c results on the OGTT and mixed meal test were obtained for 180 subjects (76 male) from 77 twin families, from which 51 MZ pairs and 60 DZ/sibling pairs (21 DZ) could be formed. FBG results on the optional clamp test were obtained for 123 subjects (57 male) from 54 twin families, from which 33 MZ pairs and 40 DZ/sibling pairs (14 DZ) could be formed.

Table 1 lists the mean values of the glycaemic parameters separately for men and women. There was a significant sex effect on FBG in the hospital settings (p < .001), with men having higher values than women. Age had a significant positive influence on HbA1c (β = 0.015 ; p .003) and FBG-C (β = 0.014 ; p .032)

The phenotypic correlations between the FBG levels in different settings were significant, albeit to a modest extent: r = 0.49 (CI = 0.35–0.61) between FBG-O and FBG-M, r = .34 (CI = 0.16–0.49) between FBG-O and FBG-C and r = .54 (CI = 0.39–0.67) between FBG levels in the two clinical settings. In contrast, the correlations for HbA1c with FBG-O (r = .11, CI = –0.06–0.23), FBG-M (r = .15, CI = –0.02–0.31) and FBG-C (r = .23, CI = 0.05–0.41) were low and achieved significance only for FBG-C.

Table 2 shows the MZ and DZ/Sibling correlations with the 95% confidence intervals on the diagonal. MZ twin pairs resembled each other more strongly than the same-sex DZ twin and sibling pairs for all indicators of glycaemia, except for the FBG-C. The lower part of Table 2 gives the cross-trait cross-twin correlations. For the various FBG measurements these cross-trait correlations were generally higher in MZ

Table 1

Maximum Likelihood Estimates of Means and Standard Deviations (*SD*)

Variable	Mean male	Mean female	*SD*
Age (years)	30.32	30.84	4.63
HbA1c (%)	5.29	5.20	0.25
FBG-O (mmol/l)	4.71	4.56	0.45
FBG-M (mmol/l)	4.53	4.27	0.37
FBG-C (mmol/l)	4.61	4.29	0.31

Note: FBG-O, fasting blood glucose at home; FBG-M, fasting blood glucose before meal; FBG-C, fasting blood glucose before clamp

Table 2

MZ and DZ / Sibling Correlations and Cross-Trait Correlations (95% Confidence Interval)

	HbA1c	FBG-O	FBG-M	FBG-C
MZ				
HbA1c	0.75 (0.61 to 0.84)			
FBG-O	0.14 (–0.05 to 0.32)	0.63 (0.42 to 0.76)		
FBG-M	0.12 (–0.06 to 0.30)	0.36 (0.19 to 0.51)	0.56 (0.37 to 0.70)	
FBG-C	0.16 (–0.04 to 0.35)	0.27 (0.06 to 0.46)	0.24 (0.02 to 0.44)	0.35 (0.05 to 0.58)
DZ /sibling				
HbA1c	0.47 (0.22 to 0.65)			
FBG-O	0.08 (–0.12 to 0.65)	0.53 (0.31 to 0.69)		
FBG-M	0.07 (–0.13 to 0.26)	0.31 (0.12 to 0.47)	0.37 (0.12 to0.56)	
FBG-C	0.18 (–0.03 to 0.37)	0.26 (0.05 to 0.45)	0.32 (0.10 to0.56)	0.39 (0.11 to 0.60)

Note: FBG-O, fasting blood glucose at home; FBG-M, fasting blood glucose before meal; FBG-C, fasting blood glucose before clamp. Twin correlations on the diagonal; cross-trait cross-twin correlations off-diagonal.

Table 3

Model Fitting Results For Multivariate Analyses of FBG in Different Settings and HbA1c

Test	Model	–2LL	*df*	vs.	Δ*df*	ΔX^2	*P*
1	ACE	4.325.946	623				
2	CE	4.340.964	633	1	10	15.018	0.131
3	AE	4.333.074	633	1	10	7.128	0.713
4	E	4.455.775	643	1	20	129.829	<0.001
5	**AE, no nonsignificant parameters**	**4.340.422**	**640**	**3**	**7**	**7.348**	**0.394**

Note: FBG = fasting blood glucose; –2LL= –2 log likelihood; df = degrees of freedom; vs.= compared to model; A = additive genetic influences; C = shared environmental; E = non-shared environment. Δ = difference; Significant age and sex covariates are included in all models. Preferred model in bold.

pairs than in DZ pairs, suggesting that genetic factors contribute to the correlation between FBG in the three different settings. No shared genetic contribution is evident for HbA1c and FBG in any setting.

Table 3 shows the model fitting results, starting with the full ACE model and ending in the most parsimonious AE model. Figure 1 illustrates this final model and presents the factor loadings of the observed variables on the different latent factors. This model resulted in heritability estimates of 66% (CI = 50–77%) for FBG obtained during the test at home, 57% (CI = 40–71%) for FBG determined before the meal test, and 38% (CI = 11–58%) for FBG measured during the pre-clamp baseline condition. Heritability of HbA1c was 75% (CI = 62–84%). The model showed that correlation of FBG across the three different settings was due to shared genetic as well as unique environmental influences (bold arrows). However, the contribution of the genetic factors was most striking, accounting for 78% of the covariance between FBG-O and FBG-M, 95% for FBG-O and FBG-C and 53% for FBG-M and FBG-C respectively. In keeping with the low phenotypic correlations, no significant genetic or environmental correlations were found between HbA1c and the FBG in any of the three settings.

Discussion

The present study shows substantial contribution of genetic influences to the variance in fasting blood glucose levels although heritability estimates varied across different measurement settings. The highest heritability estimate (66%) was found in the most natural environment, when FBG was assessed at home. Comparable heritability (57%) was found in the clinic during the pre-meal test. The lowest heritability (38%) was found on the final and most demanding test day, obtained pre-clamp when subjects knew they had to undergo invasive tests during the whole day. These different heritability estimates across settings may account for part of the discrepancy in heritability estimates found in the literature.

Inspection of the estimates of the variance components showed that the lower heritability of FBG on the pre-clamp assessment was mainly caused by differences in genetic variances in the three settings, whereas estimates of environmental variances were largely similar. We cannot rule out, however, that these 'setting' differences simply reflect the substantial day-to-day variation reported for FBG (Balion et al., 2007). Such day-to-day variation is also evident in the modest phenotypic correlations between the repeated measurements of FBG across the three different settings ($.34 < r < .54$). Importantly, the stable part of the

individual differences in FBG across settings could be largely attributed to common genetic factors that influence FBG irrespective of the setting of the blood collection. Large collaborative gene finding efforts that pool FBG samples across many different studies and countries have tacitly assumed that the exact setting in which FBG was obtained (clinic vs. home; OGTT , meal or clamp studies) should not matter. The data from the present study confirm that such gene-finding efforts may safely pool samples from different settings.

Heritability of HbA1c was estimated at 75%, which is higher than reported by two previous studies (Mills et al., 2004; Snieder et al., 2001). Because FBG and HbA1c are used interchangeably in the diagnosis and monitoring of diabetes it was expected that the genes influencing FBG and HbA1c should be largely overlapping. This expectation was not confirmed. Phenotypic correlations between HbA1c and FBG were either small or non-significant and no evidence was found for common genetic factors influencing FBG and HbA1c.

The small correlation among FBG and HbA1c and the lack of common genetic influences are in line with the study of Monnier (2003) that showed only modest contribution of fasting glucose levels to the variance of HbA1C. On the contrary, the correlation between mean blood glucose (measured by continuous glucose monitoring over the preceeding 12 weeks) and HbA1C is much higher reaching up to a correlation of 0.9 (Nathan et al., 2007). This suggests that non-fasting glucose levels are important determinants of HbA1C, allowing genetic factors influencing dietary habits, and behavioral and physical activity patterns to enter into the heritability of Hb1AC In addition, non-glucose related factors may contribute to the heritability of HbA1c as there are substantial individual differences in glycation rate and intra-erytrocyt metabolism(Cohen et al., 2006; Gould et al., 1997; Hempe et al., 2002). Importantly, a recent Japanese study (Inoue et al., 2007) suggests that both HbA1C and FBG contribute information on diabetes risk. HbA1c and fasting plasma glucose independently predicted the progression to diabetes in a healthy population, particularly when the FBG was ≥ 5.55 mmol/l.

In summary, the results of the present study suggest that in healthy adults the genes influencing FBG in different settings are largely overlapping. HbA1c and FBG, however, reflect different aspects of the genetics of glucose metabolism. As a consequence, these two glycemic parameters cannot be used interchangeably in diagnostic procedures or in studies attempting to find genes for diabetes. Both contribute unique (genetic) information.

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