Evidence for Independent Heritability of the Glycation Gap (Glycosylation Gap) Fraction of HbA_{1c} in Nondiabetic Twins

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OBJECTIVE — HbA_{1c} (A1C) is substantially determined by genetic factors not shared in common with glucose. Fractions of the variance in A1C, the glycation gap (GG; previously called the glycosylation gap) and the hemoglobin glycosylation index, correlate with diabetes complications. We therefore tested whether GG (measured A1C − A1C predicted from glycated serum proteins [GSPs]) was genetically determined and whether it accounted for the heritability of A1C.

RESEARCH DESIGN AND METHODS — We conducted a classic twin study on A1C and GSP collected in 40 and 46 pairs of monozygotic and dizygotic healthy female twins, respectively. The predicted A1C was based on the regression line between A1C and GSP in a separate population spanning the pathophysiologic range.

RESULTS — GG was more strongly correlated between monozygotic ($r = 0.65$) than dizygotic ($r = 0.48$) twins, adjusted for age and BMI. The best-fitting quantitative genetic model adjusted for age and BMI showed that 69% of population variance in GG is heritable, while the remaining 31% is due to unique environmental influences. In contrast, GSP was similarly correlated between monozygotic ($r = 0.55$) and dizygotic ($r = 0.49$) twins, hence not genetically determined. GG was strongly correlated to A1C ($r = 0.48$), attributable mostly to genetic factors. About one-third of the heritability of A1C is shared with GG; the remainder is specific to A1C.

CONCLUSIONS — Heritability of the GG accounts for about one-third of the heritability of A1C. By implication, there are gene(s) that preferentially affect erythrocyte lifespan or glucose and/or nonenzymatic glycation or deglycation in the intracellular, rather than extracellular, compartment.

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Variation between different measures of glycemic control within subjects with diabetes is a common clinical finding (1–4). Yudkin et al. (5) and Gould et al. (6) described persistent differences between HbA_{1c} (A1C) and blood glucose in nondiabetic subjects and categorized these differences as "high glycator" and "low glycator" subsets. This observation has recently led to efforts to fractionate the variance in A1C to determine whether there are components that are more closely related to glycemic control and components that seem to remain constant despite variations in glycemic control. The strategy taken to fractionate the variance in A1C has been to examine the relationship between A1C and other measures of glycemic control, including, in one instance, glycated serum proteins (GSPs) using the measure fructosamine (i.e., resulting in a measure identified as the glycation gap [GG; previously called the glycosylation gap]) and in the other instance, by the mean of capillary blood glucose measured throughout the day (yielding a measure referred to as the hemoglobin glycation index [HGI]). Cohen et al. (7) reported that the GG is reproducible over time, despite variation in glycemic control reflected in A1C and GSPs. GG correlated with the development of diabetic nephropathy in a retrospective study. McCarter et al. (8) found that HGI was likewise reproducible over time and that retinopathy and nephropathy risk were predicted by the HGI determined on numerous extended capillary glucose profiles throughout the duration of the Diabetes Control and Complications Trial. Evidence from both healthy and diabetic twins indicates that A1C levels are genetically determined, which provides an independent line of evidence that A1C is in part determined by factors other than glycemic control (9). Given the two independent lines of evidence about A1C variance and heritability (7–9), a logical question that arises is whether the heritable components of A1C are associated preferentially with the GG fraction or the GSPs fraction of the A1C variance, as this would narrow the range of mechanisms involved and inform candidate gene studies. We therefore studied a cohort of healthy nondiabetic female monozygotic and dizygotic twins to determine the contributions of genetic and environmental factors to the GG and GSPs.

RESEARCH DESIGN AND METHODS

— We studied 86 healthy female nondiabetic monozygotic ($n = 40$) and dizygotic ($n = 46$) twin pairs (age range 21–76 years) from the St. Thomas' U.K. Adult Twin Registry. We used only female subjects to avoid a sex effect. The current twin sample and criteria for selection are the same as previously described except for the exclusion of two twin pairs

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Abbreviations: GG, glycation gap; GSP, glycated serum protein; HGI, hemoglobin glycation index.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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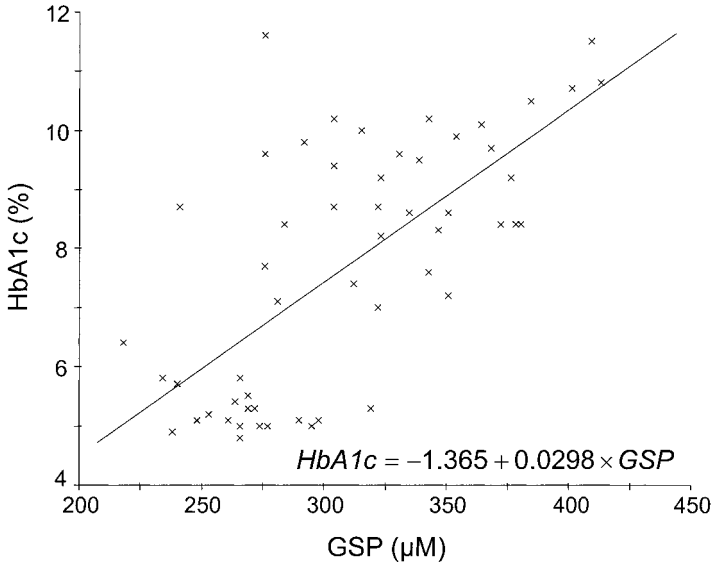

Figure 1—*Reference line for predicting A1C from GSP. The sample consisted of 56 subjects with mean (±SD) age 44 ± 18.7 years; 24 (43%) were female, and 19 (34%) were individuals with diabetes.* $r^2 = 0.456$ *in this reference population.*

Table 1—*Summary statistics of primary measures in twin study population*

Monozygotic twins ($n = 80$)	
Age (years)	54.0 ± 12.5 (22.5, 75.7)
Height (cm)	160 ± 5 (148, 170)
BMI (kg/m^2)	25.7 ± 4.8 (18.4, 41.2)
A1C (%)	5.7 ± 0.4 (4.5, 7.1)
GSP (μmol/l)	221 ± 19 (178, 262)
GG	0.62 ± 0.64 (−0.96, 2.0)
Dizygotic twins ($n = 92$)	
Age (years)	49.9 ± 15.5 (21.3, 74.1)
Height (cm)	161 ± 6 (147, 174)
BMI (kg/m^2)	25.8 ± 4.1 (17.4, 39.0)
A1C (%)	5.6 ± 0.4 (4.7, 6.6)
GSP (μmol/l)	223 ± 21 (162, 269)
GG	0.49 ± 0.74 (−1.05, 2.52)

Data are means ± SD (minimum, maximum).

in whom GSP was determined on only one twin of the pair and one twin pair in whom the GSP was a clear outlier in one of the twins (>6 SDs above twin group mean) (9). Samples were not available for GSP measurements on the diabetic twin subjects included in that study. Exclusion of frank diabetes at the time of sampling was made by a random whole blood glucose <10.0 mmol/l (180 mg/dl) or a fasting glucose <6.1 mmol/l (110 mg/dl).

The separate reference population (Fig. 1) was selected consecutively from patients or their partners attending a hospital clinic because they *1*) were either normal or had type 1 diabetes, *2*) were aged 21–76 years, *3*) had normal serum creatinine without proteinuria, and *4*) had no current condition other than diabetes. All subjects gave informed consent, and the St. Thomas' Hospital and St. Bartholomew's Hospital ethics committees approved the study. Both diabetic and nondiabetic subjects were included in the reference population to better describe the relationship between A1C and GSP, which spans the normal and hyperglycemic range. In previous studies, there had been no sex difference in the A1C-GSP relationship (7).

Biochemical analyses and confirmation of zygosity

Zygosity was determined by standardized questionnaire and confirmed by DNA fingerprinting. A1C was measured as previously reported (9) with interassay coefficient of variation (CV) <2.5%. GSP was determined by a nonseparation fluorescence–quenching assay with intra- and interassay CVs of 2.1 and 2.5%, respectively (10).

Analytical approach

The aims of our statistical analyses were to estimate the relative influence of genetic and environmental factors on the GG and the extent to which genetic factors were shared with those for A1C levels.

Quantitative genetic model fitting

We compared covariances (or correlations) in monozygotic and dizygotic twin pairs and quantified sources of individual differences by separation of observed phenotypic variance into additive (A) or dominant (D) genetic components and shared (C) or unique environmental (E) components (11). The latter also contains measurement error. Dividing each of these components by the total variance yields the different standardized components of variance (e.g., heritability).

Models were fitted to monozygotic and dizygotic variance/covariance matrices by the maximum-likelihood method. The significance of components A, C, and D was assessed by testing deterioration in model fit after each component was dropped from the full model (ACE or ADE), leading to the most parsimonious model in which the pattern of variance/covariance are explained by as few parameters as possible. Standard hierarchic χ^2 tests were used to select the best-fitting model in combination with Akaike's Information Criterion (AIC = χ^2 − 2 df) (11). The model with the lowest AIC reflects the best balance of goodness of fit and parsimony. Extension of these models to the bivariate case, including both GG and A1C, allows additional exploration of the extent to which the correlation between GG and A1C can be explained by common genes (i.e., the genetic correlation [r_g]) or 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 GG and which part was due to the influence of A1C.

Data handling and preliminary analyses were done with Stata. Quantitative genetic modeling was carried out using Mx software (12). GG was calculated as $A1C_{meas} - A1C_{pred}$, where $A1C_{pred}$ is calculated from GSP based on the reference population regression (A1C = −1.365 + 0.0298 × GSP).

Before genetic modeling, A1C was log transformed to obtain a normal distribution. All correlation coefficients shown in the RESULTS section are significant at the $P < 0.05$ level unless otherwise indicated.

RESULTS

— Mean GSP, GG, age, height, and BMI (Table 1) did not significantly differ between the monozygotic and dizygotic twins. A1C was previously reported and did not differ between groups (9). The GG was significantly correlated with age ($r = 0.22$) and BMI ($r = 0.21$). GSPs were not associated with age or BMI. The GG was significantly corre-

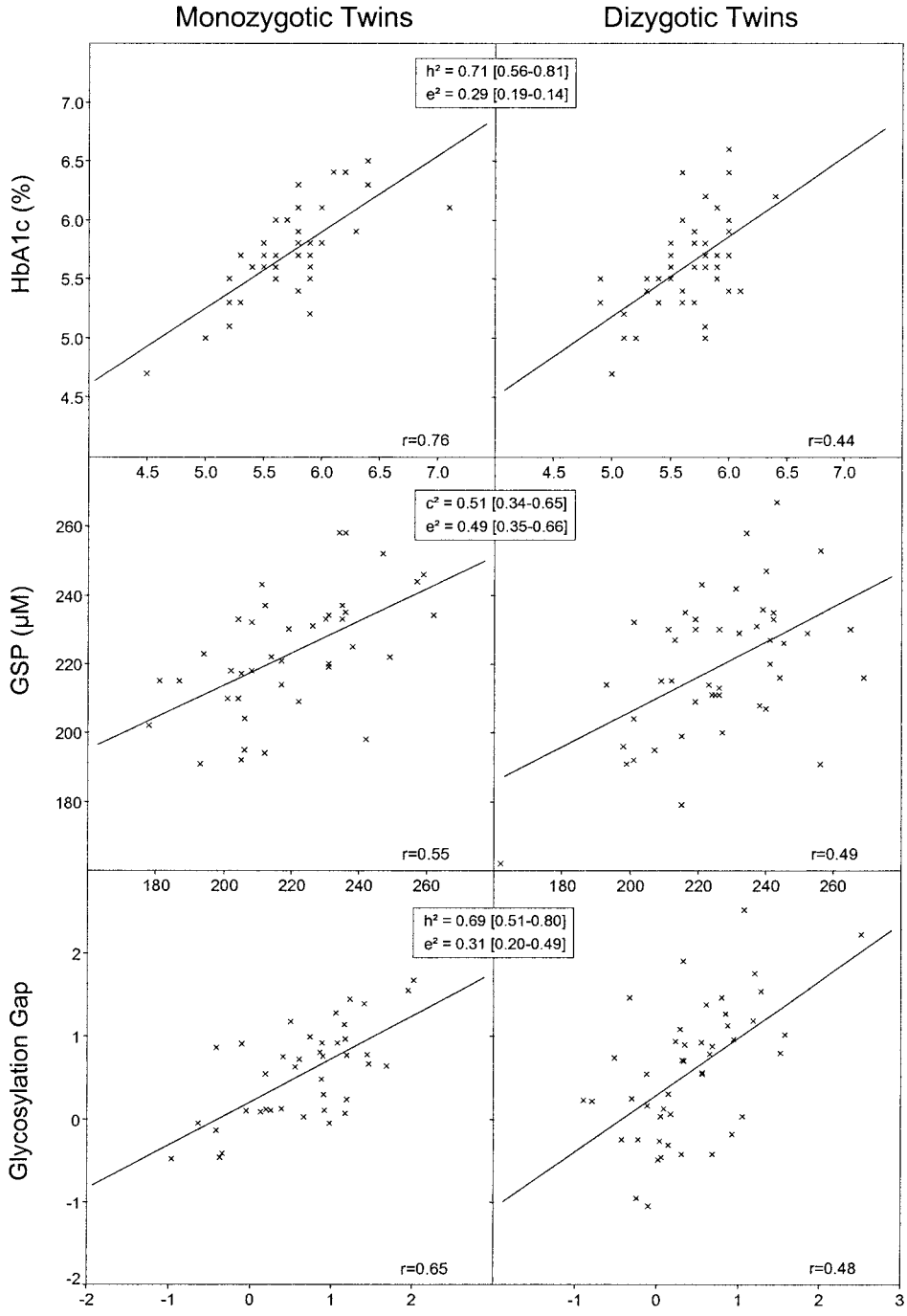

Figure 2—*Correlation between monozygotic twin pairs and between dizygotic twin pairs for A1C, GSP, and GG. Correlations for A1C are modified from Snieder et al. (9) for the subjects available for this study. All variables were adjusted for age and BMI.*

lated with log-transformed A1C (r = 0.54), as would be expected from the manner in which it is calculated. The GG showed a strong negative correlation with GSP (r = −0.83) in these nondiabetic subjects. Twin correlations for GG were highly significant ($P < 0.001$ for both monozygotic and dizygotic pairs; Fig. 2). When adjusted for age and BMI, a clear difference between monozygotic and dizygotic twin pair correlations was evident (r = 0.65 vs. 0.48, respectively, $P <$ 0.05). In the best-fitting quantitative genetic model adjusted for age and BMI, GG showed a heritability estimate of 69% (95% CI 51–80) with the remaining variance (31% [20–49]) explained by unique environmental influences. In contrast, the twin correlations for GSP were substantial for both monozygotic (r = 0.55) and dizygotic (r = 0.49) twins when adjusted for age and BMI and did not differ between them. A model including shared environment (51% [95% CI 34–65]) and unique environment (49% [35–66]) offered the best explanation for the familial resemblance in GSP, as evidenced by these twin correlations (ACE vs. CE, P = 0.48); however, a model including additive genetic effects instead of shared envi-

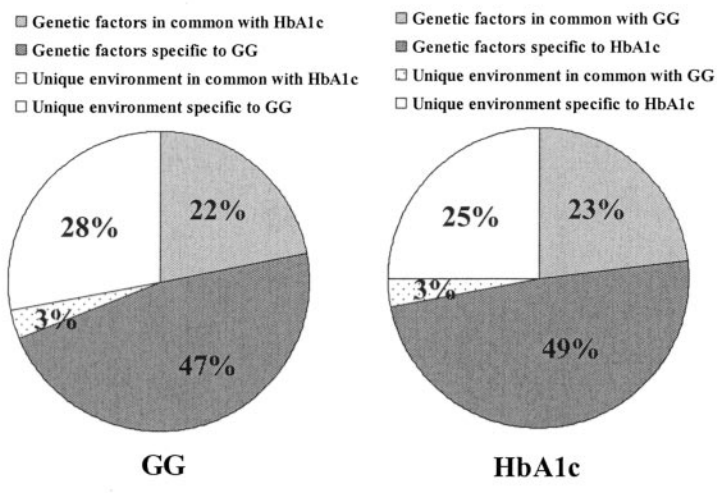

Figure 3—*Genetic and environmental components of GG and A1C variance.*

ronment could not be entirely excluded (ACE vs. AE, $P = 0.14$). We did not test for evidence of genetic factors in common between A1C and GSPs, since GSP does not show any heritability in our data and A1C and GSP are not correlated ($r = 0.02$, P = NS) within this narrow nondiabetic range of glycemic control despite there being a substantial correlation in GSP between twins, consistent with GSP being determined by glucose, independent of genetic factors. There was a single healthy twin with A1C 7.1%, despite all other measures being normal; analyses were not affected whether this twin was included or excluded.

We then examined the genetic versus environmental origin of the correlation between GG and A1C by multivariate analysis (Fig. 3, variance components of GG and A1C). In the best-fitting model, the genetic correlation (r_g) was 0.57 (95% CI 0.36–0.73) and the environmental correlation (r_e) was 0.31 (0.04–0.54). The heritability of GG, after adjusting for age and BMI, was 0.69 in the best-fitting AE model, of which 0.22 was in common with A1C and 0.47 was specific to GG. This means that about one-third of the heritability of GG can be attributed to genes that also influence A1C, but most of the heritability is specific to GG. Expressed another way, for A1C, the heritability is 0.72, of which 0.49 is specific and 0.23 shared with GG; again, ~32% of the heritability of A1C is attributable to GG within the glycemic range represented in the study population.

CONCLUSIONS — This twin study establishes that the GG is 69% heritable in nondiabetic subjects; the remainder (31%) being due to unique environment. Furthermore, the GG identifies ~32% of the heritability of A1C. In contrast, GSP is not significantly inherited and not associated with A1C; hence, we did not test for genetic factors in common between A1C and GSPs. According to the present model, there is substantial heritability specific to the GG. By inference, the correlation between GSP and GG must reflect shared environmental factors.

There are two key implications of the present findings. First, they demonstrate a contrast between GSPs, which are not inherited, and A1C, which is inherited. This observation limits the range of candidate mechanisms that could account for the heritability of A1C (9). Second, these findings demonstrate that a measure, the GG, which captures the variation of A1C in a population beyond that which is attributable to blood glucose variation, also captures a proportion of the heritability of A1C based on quantitative genetic modeling. This second observation could not have been predicted given that the GG is derived from both A1C and GSPs, and the former is inherited while the latter is not. The present work illustrates how measures of A1C variance from blood glucose control, including the GG (7) and Chalew and colleagues' (8) "hemoglobin glycosylation index," are compatible with each other and internally consistent. They demonstrate that in normal individuals, these measures capture different aspects of the same clinically important biological phenomenon, namely that an A1C of a given value may not necessarily have the same clinical meaning in two different diabetic individuals, even if a reduction of A1C by one percentage point does.

A1C is a measure of glycemic control determined in the intraerythrocyte space, while glycation of serum proteins reflects a process in the extracellular compartment. By inference, the GG reflects the variance in A1C determined by processes in both the extra- and intracellular compartments compared with those unique to the extracellular compartment. Our present results implicate genetically determined mechanisms active in the intracellular erythrocyte compartment in the determination of A1C levels. This could occur through the modification of glycation or deglycation or intra–red cell glucose concentration. Jacquez (13) argued that the capacity of the erythrocyte for glucose transport is 12,000 times the rate of erythrocyte glucose metabolism, and this may play some role in total body glucose transfer, which makes variation in glycolytic enzymes less likely to affect hemoglobin exposure to glucose. We cannot exclude the possibility that heritable differences in erythrocyte lifespan may contribute as well and are pursuing that line of investigation (14).

It was practical to readily accomplish this study because sera were available from earlier nondiabetic twin studies. The supplemental data required to complete the analysis included establishing the reference line between the A1C and GSPs in subjects who spanned the broad pathophysiologic range of glycemic control, including reference population subjects both with and without diabetes using the same assays that were used in the twin subjects. One of the limitations of this study is that we did not have parallel samples for GSP assay in diabetic twins, a far more scarce population. Given that the experimental population was only nondiabetic subjects, one might ask why it was necessary to include both diabetic and nondiabetic subjects in the reference population. The previous data indicate that the underlying relationship between A1C and GSP is most evident and stable when a broad range of glycemic control is examined. The narrowness of glycemic control represented in the nondiabetic twin population is the most likely explanation for our failure to detect a significant correlation of A1C to GSP within that group. It is possible that this fraction of the variance shared in common between GG and A1C is most apparent when the range of glycemia over which these effects are examined is constrained to normoglycemia.

The fraction of the variance in A1C captured in GG may therefore be less when examined outside the glycemic range represented in this study. The lack of correlation found here between A1C and GSP might be predicted from the findings of Rohlfing et al. (15) in which there was a much reduced correlation between GHb and the average of a series of fasting plasma glucose determinations in nondiabetic subjects. There was also a narrower range of GHb results in the Rohlfing et al. study, leading to the conclusion that there was no more than minimal biologic variation in that population.

Our results do not distinguish whether the processes invoked in erythrocytes to explain the GG are unique compared with other intracellular compartments where they could influence the risk of diabetes complications. One important consideration is that glucose entry into erythrocytes is mediated by the glucose transporter GLUT1, as is the preponderance of glucose entry into endothelial cells, the major target tissue of diabetes complications, implying that processes related to GLUT1-mediated transport could be a candidate (16). Gould et al. (6) reported an elevation in 2,3 diphosphoglycerate in high glycators compared with low glycators, as well as a positive correlation of A1C with intracellular pH and a negative correlation of A1C with plasma amino acids. One candidate enzyme that might explain genetically determined differential glycation is fructosamine 3-kinase, which reverses the Amadori reaction of nonenzymatic glycation (17,18); however, this enzyme cannot account for our present results, as it appears to be specific for deglycation of glucosyl-lysine and not for reversal of the NH_2-terminal glycation found in A1C. In summary, the GG is substantially inherited and accounts for one-third of the heritability of A1C in nondiabetic subjects. The fact that heritability of A1C is only partially shared with the GG provides evidence that multiple molecular mechanisms contribute to the heritability of A1C.

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