The Relation Between Insulin Resistance and Hemostasis: Pleiotropic Genes and Common Environment

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Risk factors for coronary heart disease (CHD), including prethrombotic changes in hemostasis, cluster with the insulin resistance (IR) syndrome. The aim of the present study was to investigate to what extent the relation between IR and hemostatic risk factors is due to shared genes or environmental factors. Multivariate genetic analysis was performed using a total of 314 (107 monozygotic and 207 dizygotic) twin pairs on IR assessed by HOMA, fibrinogen, plasminogen activator inhibitor (PAI-1), tissue plasminogen activator (tPA), factor VIII (FVIII), von Willebrand factor (vWF) and factor XIII B-subunit. The relationship between IR and the 6 hemostatic factors could best be explained by an independent pathway model consisting of 2 common genetic factors, one of which influenced IR and all hemostatic factors, and 3 common environmental factors, each representing the shared variance between IR and different aspects of the hemostatic system. Genetic correlations between IR and hemostatic proteins were larger than their environmental counterparts. Since IR and prethrombotic changes are features of both diabetes and CHD, the finding of one set of pleiotropic genes warrants the identification of these common pathways which may provide new avenues for treatment and prevention of both diabetes and CHD.

The etiology of coronary heart disease (CHD) is complex and consists of intertwined pathophysiological pathways with clustering of risk factors for CHD. This cluster of risk factors for CHD, which includes hyperinsulinemia, hyperglycemia, dyslipidemia, hypertension, central obesity and impaired fibrinolysis, is known as the insulin resistance (IR) syndrome (Reaven, 1988). Insulin resistance (IR), a reduced ability of insulin to stimulate glucose uptake is present in up to 25% of the normal population as well as in the majority of patients with impaired glucose tolerance or type 2 diabetes mellitus. The latter two groups are both at higher risk of developing atherosclerotic vascular disease (Hollenbeck & Reaven, 1987). However, studies in pursuit of the identification of causal pathways leading to CHD and investigating why diabetic subjects are at higher risk of developing CHD are largely inconclusive (Jarrett, 1984).

Reaven (1988) has suggested that the body's attempt to compensate for IR could set in motion a series of events that lead to the clustering of risk factors for CHD. However, diabetic subjects do not develop an increasing burden of atherosclerosis nor risk for CHD with longer duration of diabetes (Jarrett, 1984; Jarrett & Shipley, 1988). This suggests that increased risk of CHD may not be a direct consequence of the development of diabetes, but is rather due to an underlying set of risk factors predisposing to both diseases (Jarrett, 1984). This has led Stern (1995) to hypothesize that diabetes and cardiovascular disease have common genetic and environmental antecedents (i.e., that they stem from a "common soil"). IR provides one possible explanation in support of this hypothesis enhancing both diabetes and CHD risk.

Several studies have reported associations of IR with altered hemostatic function as characterized by elevated circulating levels of fibrinogen (Raynaud et al., 2000), plasminogen activator inhibitor (PAI-1) (Juhan-Vague & Vague, 1991; Potter van Loon et al., 1993), tissue plasminogen activator (tPA) (Meigs et al., 2000), von Willebrand factor (vWF) (Meigs et al., 2000), factor VIII (FVIII) (Juhan-Vague et al., 1993) and factor XIII B-subunit (Mansfield et al., 2000). Although altered hemostasis is shown to be a risk factor for the development of CHD (Folsom et al., 2001; Meade et al., 1986; Thompson et al., 1995), the mechanisms by which IR and hemostasis relate to each other is largely poorly understood (Meigs et al., 2000).

Although well-designed longitudinal studies are necessary to determine whether and how IR and hemostatic risk factors exert their risk concurrently in the development of CHD and diabetes, it is possible to use cross-sectional twin data to determine genetic and environmental correlations between the related risk factors for both diseases. If there are genes regulating both IR and the hemostatic risk factors simultaneously, this would support the existence of a predisposing underlying mechanism for the development of both hyperinsulinemia and altered hemostasis.

We have measured an array of hemostatic factors previously associated with increased risk of CHD in a large sample of twins (de Lange et al., 2001) and confirmed strong genetic influences also detected by a large family

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study (Souto et al., 2000). The hemostatic factors: fibrinogen, PAI-1, tPA, FVIII, vWF, activated factor XII (FXIIa), factor XIII activity (FXIII), and factor XIII A- & B-subunit were combined together with IR as measured by the HOMA index (Matthews et al., 1985) in a multivariate quantitative genetic analysis to investigate the extent to which the observed correlation (or covariance) between IR and hemostatic factors is caused by common genes and/or common environment.

Materials and Methods

Subjects

A total of 314 (107 monozygotic [MZ] and 207 dizygotic [DZ]) healthy Caucasian female twin pairs aged 18–72 from the St Thomas' UK Adult Twin Registry were available for this study. Twins from the registry were ascertained from the general population through national media campaigns in the United Kingdom. Participating twins were unaware of the specific hypotheses tested and informed consent was obtained from all subjects. The study was approved by the St Thomas' Hospital Research Ethics Committee. Zygosity was determined by standardized questionnaire and DNA fingerprinting was used for confirmation. Information on medication use, demographic variables, recalled birthweight and fasting time was obtained by standardized nurse administered questionnaire.

Measurements

A venous blood sample was taken between 8.00 a.m. and 10.00 a.m. after an overnight fast. The blood was taken within 5 min from the co-twin into 0.13 trisodium citrate vacutainers (Becton Dickinson, Oxford, UK) and kept on ice for tests of fibrinolysis and at room temperature for tests of coagulation. Within 1 hour of collection, the samples were centrifuged at 2560g for 20 minutes to obtain platelet-poor plasma, frozen in aliquots in liquid nitrogen, and stored at –40°C until analysis. Biochemical assays were performed within six months of sample collection.

For a detailed description of the analysis of hemostatic factors see de Lange et al. (2001). Briefly, ELISAs were used for analysis of PAI-1 and tPA (Biopool, Umeå, Sweden), also for FVIII (Immuno, Vienna, Austria), vWF (in-house ELISA); for FXII an ELISA specific for the activated form of this factor was used (Axis-Shield, Dundee, Scotland). FXIII A- & B-subunit were analyzed using in-house sandwich ELISAs and FXIII activity with a 5(biotinamido) pentylamine incorporation assay (Ariëns et al., 1999). Fibrinogen was determined using functional clotting assay according to Clauss (1952).

Fasting insulin was measured by immunoassay from Abbott Laboratories Ltd. (Maidenhead, Berkshire, SL6 4XL, UK). Glucose was measured on an Ektachem 700 multichannel analyzer using an enzymatic colorimetric slide assay (Johnson & Johnson Clinical Diagnostic Systems, Amersham, Buckinghamshire, UK).

A total of 26 single twins were excluded from the analysis for various reasons. Twins who were not fasting for 8 h or more (1 MZ and 10 DZ twins) or had missing values for fasting glucose or insulin due to loss of sample (4 MZ and 6 DZ) were excluded. A twin with glucose level of > 6.8 mmol/l (1 DZ) and twins on oral anticoagulant treatment (2 DZ) or diabetic drugs (2 DZ) were also excluded. The singletons were kept in the analysis and contributed to the estimates of means and variance (5 MZ and 21 DZ twins). Some twins had missing values for one of the measurements of the hemostatic proteins due to loss of sample.

Statistical Analysis

A simple estimate of insulin resistance was obtained using the homeostasis model assessment (HOMA) of Matthews et al. (1985), where insulin resistance is calculated as (fasting insulin × glucose)/22.5. HOMA IR and all hemostatic factors were adjusted for age and seasonal variation prior to the analyses and log transformed to approximate normal distribution. These preliminary analyses were carried out in STATA (StataCorp).

Analytical Approach

Our analysis had several aims. First, we tested for the association between HOMA IR and the hemostatic proteins. Next, we estimated the relative influence of genetic and environmental factors on HOMA IR and hemostatic factors that showed a significant correlation with HOMA IR using univariate analysis. Finally, we examined how to best explain the correlation (or covariance) structure between HOMA IR and hemostatic factors using multivariate analysis.

Quantitative Genetic Model Fitting of Twin Data

Genetic model fitting techniques were used to obtain estimates of the genetic and environmental factors. These techniques make optimal use of the information available in the twin-co-twin covariance structure (Neale & Cardon, 1992). MZ twins share 100% of their genes whereas DZ twins share on average 50% of their genes, like normal siblings. Under the assumption that MZ and DZ twins share their environment to the same extent (i.e., equal environment assumption) a greater resemblance in MZ twins compared to DZ twins must be due to genes (Kyvik, 2000).

Model fitting is based on the comparison of the observed and expected variance-covariance matrices. The observed phenotypes are assumed to be linear functions of the underlying additive genetic variance (A), dominance genetic variance (D), shared or common environmental variance (C), and unique environmental variance (E), which also contains measurement error, so that the total (or phenotypic) variance (V_p) equals: A + D + C + E. Dividing each of these components by the total variance yields the different standardized components of variance, for example the heritability (h^2) which can be defined as the proportion of overall phenotypic variation that can be explained by additive genetic factors (Neale & Cardon, 1992).

Multivariate Analysis

Phenotypic correlations between HOMA IR and all 9 hemostatic factors were calculated and all variables showing a significant correlation ($p < 0.05$) were entered into the multivariate analysis. To test to what extent the phenotypic correlation between IR and the hemostatic factors can be explained by common genes or common environment, multivariate model fitting techniques were

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used to separate genetic from environmental effects (Neale & Cardon, 1992; Plomin et al., 1990).

Three types of multivariate models were tested as previously described (Sluyter et al., 2000): a triangular or Cholesky decomposition (Loehlin, 1996), independent pathway and common pathway models (Neale & Cardon, 1992; Neale et al., 1999). Although all these models decompose the variance into the components of variation: A, C, D and E, each represents different ways in which genes and the environment may affect the observed correlations between the measures. Briefly, the Cholesky model represents the most general way in which the variance-covariance structure of the data can be decomposed. That is, no specific hypotheses regarding the variance-covariance structure are tested (see Figure 1a). The independent pathway model is a submodel of the Cholesky decomposition, where it is assumed that one or more genetic or environmental common factors can explain the correlational (or covariance) structure of the data. Thus, this model predicts that one or more sets of pleiotropic genes in combination with common environmental factors are responsible for the relation between IR and hemostatic factors (see Figure 1b). Finally, the common pathway model constitutes a further simplification of the independent pathway model. Both genes and environment contribute to one or more latent (unmeasured) variables, which is or are responsible for the observed covariance between the outcome measures. For example, we would expect a common pathway model with one latent construct to fit if genes and environment contribute to the "true" unmeasured insulin resistance (i.e., the one latent construct), which subsequently induces correlations between IR as measured by HOMA and the hemostatic factors (see Figure 1c).

To ensure the identification of models with multiple common A and E factors (and of the common pathway model with 2 common factors) we used orthogonal rotations (i.e., uncorrelated common factors) with reference variables. For example, for the case of 2 common factors we first identified a variable that loaded high on one factor and low on the other. This reference variable was obtained from a varimax rotated exploratory factor model with the same number of common factors and the A or E correlation matrices derived from the Cholesky decomposition as input. Next, in our genetic modelling analyses the loading of the reference variable on the intended common factor was estimated, but the loading of the reference variable on the other common factor was fixed to zero. In a similar fashion 3 factor loadings were fixed to zero for models with 3 common factors (Sluyter et al., 2000). Identification of the model was confirmed by multiple fitting of the data to the final model using different starting values and similar results were obtained.

Model Fitting Procedure

Models were fitted to the raw data using normal theory maximum likelihood (Lange et al., 1976) allowing us to use the data from 26 singletons. The significance of A, C, and D was tested by removing them sequentially in specific submodels and comparing them with the full model. Standard likelihood-ratio tests between models were used to assess the importance of each variance component (A, C or D) on the fit of the model (Neale & Cardon, 1992; Neale et al., 1999). This eventually leads to a model that gives the most parsimonious fit to the data, from which estimates of the quantitative genetic and environmental parameters were obtained. All quantitative genetic model fitting was done

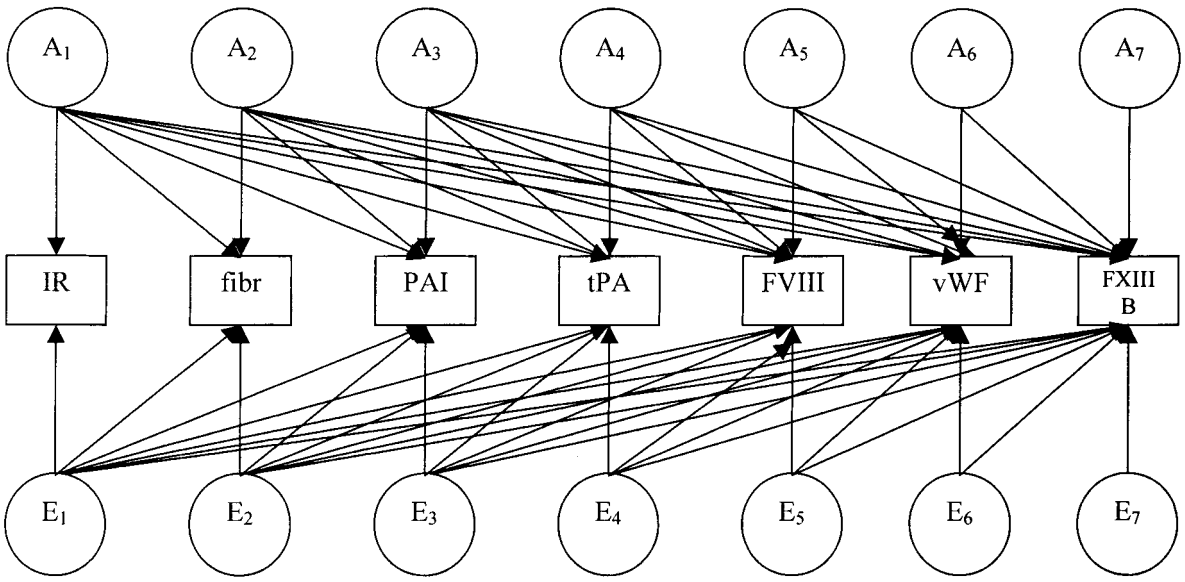

Figure 1a

Cholesky decomposition of the genetic and environmental factors for IR and hemostatic factors. A_n represents the additive genetic effect on subset of measures, for example A_1 represent the genetic factor loading on IR and all hemostatic factors. E_n represent the unique environmental factors. The effect of shared environmental influence is for reasons of clarity omitted.

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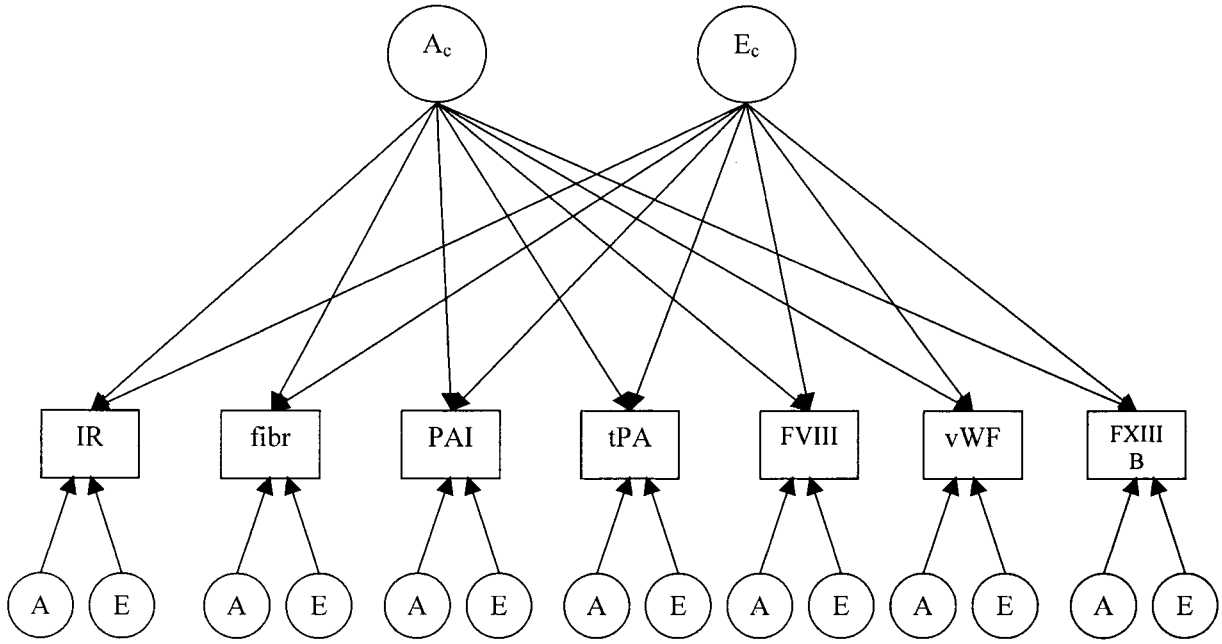

Figure 1b

Independent pathway, where one or more common genetic factors (A_c) and environmental factors (E_c) explain the relationship between IR and hemostatic factors. Apart from the common genetic and environmental factors, variation is explained by specific genetic (A) and environmental (E) factors.

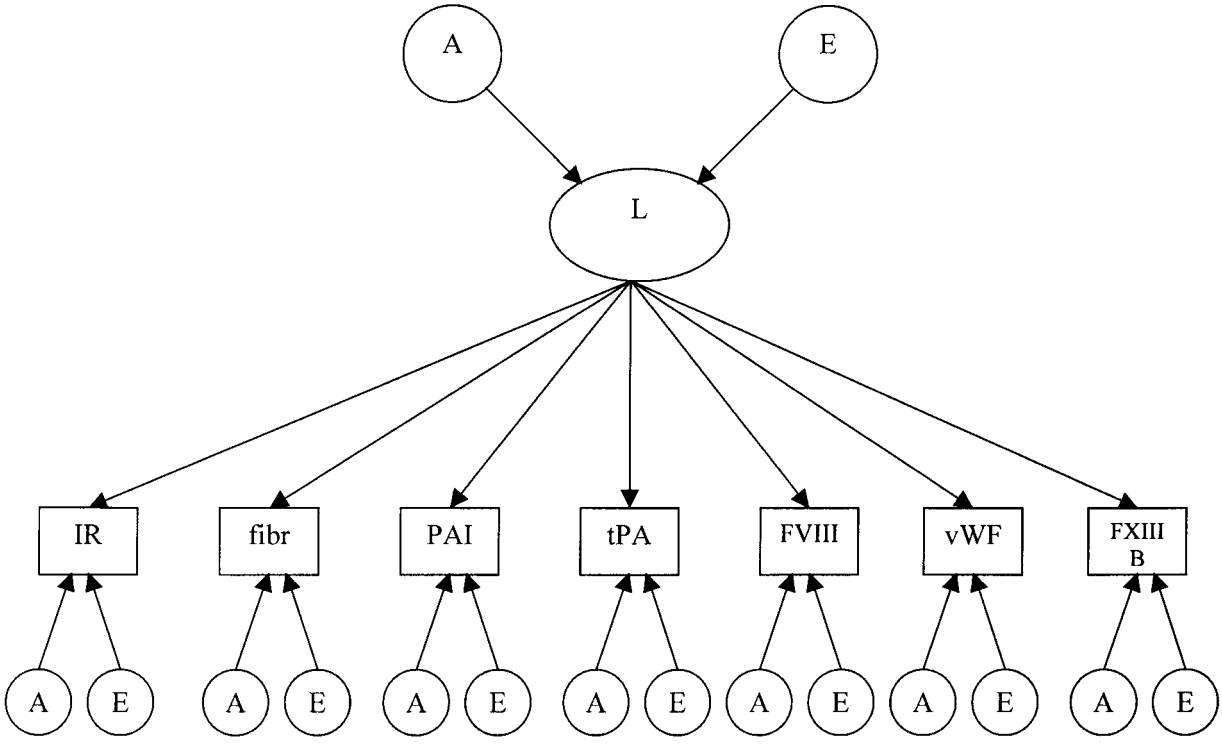

Figure 1c

Common pathway model where both genes and environment are assumed to contribute to one or more latent (unmeasured) variables (L) which is or are responsible for the correlation between IR and the hemostatic factors. Specific genetic (A) and unique environmental (E) influences are also incorporated in the model.

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using Mx, a computer program specifically designed for the analysis of twin and family data (Neale et al., 1999).

Results

The general characteristics of the MZ and DZ twins are shown in Table 1, which overall are very similar for the MZ and DZ twins. DZ twins were on average 5 years younger, less likely to be postmenopausal and had slightly lower fasting glucose levels than the MZ twins. However, we adjusted for age throughout and we did not detect a significant influence of menopausal status on the measures. Also, there was no significant difference in HOMA IR between MZ and DZ twins. FXIII B-subunit levels were higher in MZ twins than in DZ twins, which was unexplained but accounted for in subsequent model fitting.

Phenotypic correlations between HOMA IR and hemostatic factors were significant ($p < 0.05$) for fibrinogen (0.29), PAI-1 (0.36), tPA (0.29), FVIII (0.20), vWF (0.12), and FXIII B-subunit (0.26). Moreover, correlations amongst these hemostatic factors were quite substantial (ranging from 0.04 to 0.69). In contrast, activated FXII, FXIII activity and FXIII A-subunit were not significantly related to HOMA IR (see Table 2).

Table 3 presents the intraclass correlations for HOMA IR and hemostatic factors that showed a significant correlation with HOMA IR. All MZ correlations are higher than the DZ correlations and suggest a genetic influence on the individual variables, which was confirmed in univariate genetic analysis. Heritability of HOMA IR was estimated at 57% (95% CI: 46–67%). Estimates of the genetic and environmental influence on hemostatic factors were very similar to those previously reported, including a common environmental effect explaining 35% of the variation in FXIII B-subunit (see Table 3) (de Lange et al., 2001).

The best fitting Cholesky decomposition model was the AE model where the variance and covariance of the measures is explained by additive genetic and unique environmental influences alone. In the best fitting AE model a specific common environmental factor (C) influencing FXIII B-subunit was also included, based on the best fitting univariate model for this factor. The best fitting independent pathway model included, apart from the specific genetic and environmental factors (and a specific C for FXIII B-subunit), 2 common genetic and 3 common environmental factors. Subsequent models reducing the amount of common factors gave a worse fit to the data. We further tried to simplify this model by testing a common pathway model (see Figure 1c) with 3 and 2 common factors, respectively. These submodels, however, showed a fit that was significantly worse: $\chi^2(15) = 71.138$; $p < 0.001$ for 3A3E common pathway compared to 3A3E independent and $\chi^2(11) = 105.582$; $p < 0.001$ for 2A2E common pathway compared to 2A2E independent pathway.

Overall, the best fitting model explaining the relationship between IR and the 6 hemostatic factors was the independent pathway model consisting of 2 common genetic factors, one of which influenced IR and all hemostatic factors, and 3 common environmental factors, each representing the shared variance between IR and different aspects of the hemostatic system. Figure 2 shows the relative importance of the common genetic and environmental factors on IR and the hemostatic factors. IR, fibrinogen, PAI-1, tPA and FXIII B-subunit and to a lesser extent FVIII and vWF show high loadings on the first common genetic factor, implying that this factor is responsible for the genetic clustering of IR and all hemostatic factors. FVIII and vWF and to a lesser extent fibrinogen, but not IR, load high on the second common genetic factor. IR and both FVIII and vWF load high on the first common environmental factor, whereas IR, PAI-1 and tPA load on the second and IR, fibrinogen, FXIII B-subunit and FVIII on the third common environmental factor (see Table 4).

Apart from these common genetic and environmental influences, there are also specific genetic and environmental influences on IR and the hemostatic factors, except for FVIII, for which no specific influences were detected and all variance was accounted for by the common genetic and environmental factors. The genetic and environmental influence and their 95% confidence intervals from the best fitting independent pathway model for HOMA IR, fibrinogen, PAI-1, tPA, FVIII, vWF and FXIII B-subunit individually are given in Table 5.

From the factor loadings it is possible to compute the proportion of variance explained by a common factor, which can be used to calculate the breakdown of the phenotypic correlation into a genetic and an environmental portion. Table 6 shows the breakdown of phenotypic correlations between HOMA IR and hemostatic proteins into

Table 1

General Characteristics and Means (SD) of Insulin, Glucose, HOMA IR and Hemostatic Proteins in MZ and DZ Twins

	MZ twins	DZ twins
number of pairs	107	207
age (years)	50.2 (14.3)	45.1 (12.0)
body mass index (kg/m²)	25.4 (4.5)	25.0 (4.7)
postmenopausal *n* (%)	121 (58%)	154 (39%)
current HRT use *n* (%)	45 (22%)	58 (15%)
current OCP use *n* (%)	23 (11%)	51 (13%)
current smoker *n* (%)	38 (18%)	95 (24%)
fasting insulin (μU/mL)	6.92 (4.49)	6.84 (3.97)
fasting glucose (mmol/L)	4.54 (0.54)	4.40 (0.45)
insulin resistance (HOMA)	1.42 (0.97)	1.36 (0.89)
fibrinogen (g/L)	3.13 (0.68)	3.07 (0.74)
PAI-1 (ng/mL)	9.96 (8.86)	10.36 (10.09)
tPA (ng/mL)	6.93 (3.68)	6.50 (3.54)
FVIII (IU/mL)	0.86 (0.32)	0.85 (0.3)
von Willebrand factor (IU/mL)	1.13 (0.4)	1.13 (0.41)
FXIIa (ng/mL)	2.62 (0.89)	2.56 (1.00)
FXIII activity (%)*	111.8 (31.9)	103.4 (30.4)
FXIII A-subunit (u/mL)†	1.09 (0.32)	1.07 (0.30)
FXIII B-subunit (u/mL)†	1.07 (0.35)	1.00 (0.31)

Note: HRT: hormone replacement therapy
OCP: oral contraceptive pill
HOMA index = fasting insulin x glucose / 22.5
*Concentrations expressed as % of pooled normal plasma
†Concentrations expressed as units/mL of pooled normal plasma

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Table 2

Phenotypic Correlation Between IR, Fibrinogen, PAI-1, tPA, FVIII, vWF, FXIIa, FXIII act, FXIII A- & B-subunit

	IR	Fibr	PAI-1	tPA	FVIII	vWF	FXIIa	FXIII act	FXIII A	FXIII B
IR	—									
Fibr	**0.29**	—								
PAI-1	**0.36**	**0.11**	—							
tPA	**0.29**	**0.15**	**0.50**	—						
FVIII	**0.20**	**0.29**	**0.12**	**0.15**	—					
vWF	**0.12**	**0.21**	0.04	0.07	**0.69**	—				
FXIIa	0.07	–0.04	0.09	0.08	0.00	0.01	—			
FXIII act	0.06	**0.20**	0.08	**0.12**	0.05	0.07	**0.14**	—		
FXIII A	0.06	**0.13**	0.08	0.05	–0.02	0.00	**0.11**	**0.18**	—	
FXIII B	**0.26**	**0.28**	**0.16**	**0.12**	0.06	**0.10**	**0.17**	**0.20**	**0.58**	—

Note: $p < 0.05$ printed in **bold**

Table 3

Intraclass Correlations of MZ and DZ twins, Number of Complete Pairs (N) and Univariate Model Fitting Results

	MZ	(N)	DZ	(N)	a^2	c^2	e^2
IR	0.58	(102)	0.33	(186)	0.57	—	0.43
Fibrinogen	0.53	(102)	0.23	(185)	0.55	—	0.45
PAI-1	0.51	(101)	0.30	(184)	0.52	—	0.48
tPA	0.63	(96)	0.39	(181)	0.68	—	0.32
FVIII	0.68	(102)	0.36	(185)	0.68	—	0.32
vWF	0.76	(102)	0.43	(185)	0.78	—	0.22
FXIII B-subunit	0.70	(99)	0.55	(185)	0.37	0.35	0.28

Note: a^2 = heritability, c^2 = shared environmental component, e^2 = unique environmental variance component

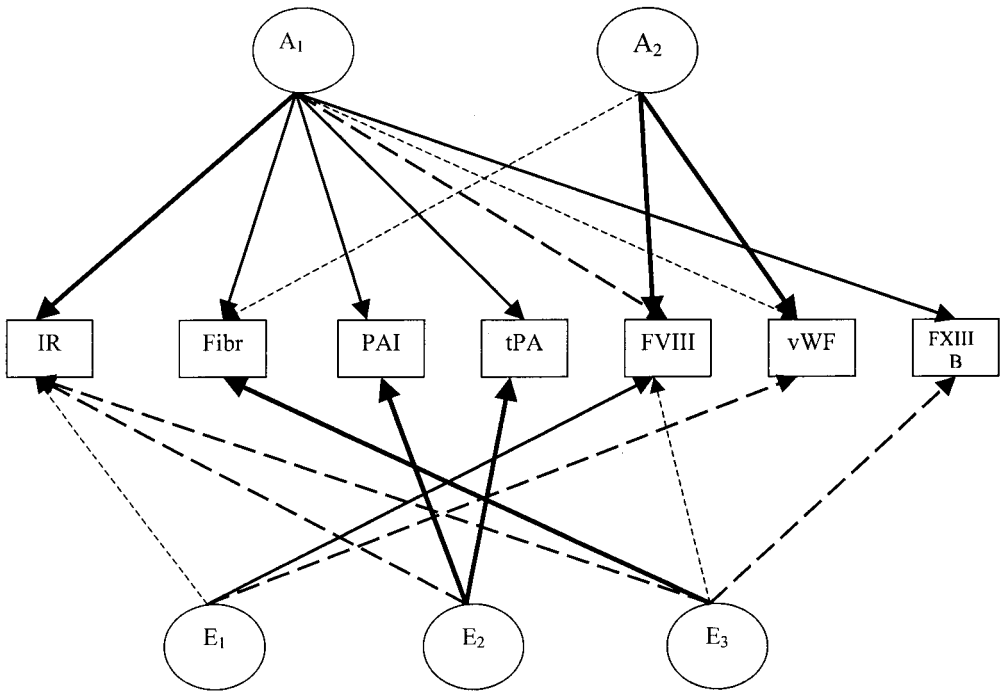

Figure 2

Graphical display of relative genetic and environmental factor loadings on IR and hemostatic factors. A_1 is the first common genetic factor explaining all measures, A_2 is the second common genetic factor. E_1, E_2, and E_3 are the common environmental factors. Black solid lines represent high loadings, long dashed lines represent moderate loadings and light dashed lines represent smaller loadings. For reasons of clarity the specific genetic and environmental influences on IR and hemostatic factors are omitted.

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Table 4

Standardized Solution (Total Variance of Each Variable Standardized to 1) of the Best Fitting AE Independent Pathway Model

	First common factor		Second common factor		Third common factor	Specific factors		
	A	E	A	E	E	A	C	E
IR	0.49	0.11	0.02	0.22	0.22	0.58	—	0.56
fibr	0.41	0.03	0.16	–0.09	0.48	0.59	—	0.47
PAI-1	0.46	0.00*	0.00*	0.64	–0.03	0.50	—	0.35
tPA	0.42	0.03	0.02	0.45	0.00*	0.68	—	0.39
FVIII	0.19	0.53	0.81	0.06	0.16	0.00	—	0.00
vWf	0.15	0.22	0.66	0.00	0.09	0.56	—	0.41
FXIII B	0.40	–0.02	–0.03	0.00*	0.21	0.54	0.52	0.48

Note: *these factor loadings were fixed at zero.

Table 5

Estimates of Variance Explained by Genes (a^2) and Environment (c^2, e^2) with 95% Confidence Intervals from the Best Fitting AE Independent Pathway Model

	a^2	(95% CI)	c^2	(95% CI)	e^2	(95% CI)
Insulin resistance	0.57	(0.46–0.67)	—	—	0.43	(0.33–0.54)
Fibrinogen	0.53	(0.41–0.64)	—	—	0.47	(0.36–0.59)
PAI-1	0.46	(0.33–0.58)	—	—	0.54	(0.42–0.67)
tPA	0.64	(0.53–0.73)	—	—	0.36	(0.27–0.47)
FVIII	0.69	(0.60–0.76)	—	—	0.31	(0.24–0.40)
vWF	0.77	(0.70–0.83)	—	—	0.23	(0.17–0.30)
FXIII B-subunit	0.45	(0.22–0.68)	0.27	(0.06–0.46)	0.28	(0.20–0.37)

Table 6

Breakdown of Phenotypic Correlation Between IR and the Hemostatic Factors, Fibrinogen, PAI-1, tPA, FVIII, vWF and FXIII B-subunit into Genetic and Environmental Influenced Portions (G/E)

	IR	Fibr	PAI-1	tPA	FVIII	vWF
IR	—					
Fibr	**0.20/0.09**	—				
PAI-1	**0.23/0.13**	**0.19**/–0.07	—			
tPA	**0.21/0.10**	**0.18**/–0.04	**0.19/0.29**	—		
FVIII	**0.11/0.11**	**0.21/0.09**	0.09/0.03	0.10/0.04	—	
vWF	0.09/0.04	**0.17**/0.05	0.07/–0.003	0.08/0.007	**0.56/0.13**	—
FXIII B	**0.20**/0.04	**0.16/0.10**	**0.18**/–0.01	**0.17**/–0.001	0.05/0.02	0.04/0.01

Note: Significant genetic and/or environmental portions of the phenotypic correlations ($p < 0.05$) are printed in **bold**.

genetic and environmental components for the best fitting independent pathway model. For most variables the genetic contributions to the correlations between IR and hemostatic proteins were larger than the environmental contributions.

Discussion

Our study observed substantial phenotypic correlations between IR and levels of fibrinogen, PAI-1, tPA, FVIII, vWF and FXIII B-subunit that were best explained by a model including 2 common genetic factors and 3 common environmental factors with one set of pleiotropic genes responsible for the genetic relation between IR and hemostatic factors (see Figure 2). These genetic influences explained a larger chunk of the correlation between IR and hemostatic factors than environmental factors. We were able to exclude an alternative (common pathway) model, in which one or more underlying defects (e.g., insulin resistance) are influenced by genes and environment and subsequently lead to clustering of HOMA IR and levels of hemostatic proteins. The findings suggest that a more complex combination of common genetic and environmental mechanisms underlie the co-occurrence of these risk factors for diabetes and CHD as suggested in the "common soil" hypothesis (Stern, 1995).

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The first common genetic factor influences IR and all hemostatic factors, suggesting that one set of genes causes the grouping of IR and these hemostatic factors. The second common genetic factor influences FVIII and vWF and to a lesser extent fibrinogen, but not IR. None of the common environmental factors seem to group all of the measures but each captures shared variance between IR and different aspects of the hemostatic system. The first common environmental factor clusters FVIII and vWF with only a small effect on IR. The second factor influences the cluster of IR and 2 proteins involved in fibrinolysis, PAI-1 and tPA. The third factor has a joint effect on IR and fibrinogen, FXIII B-subunit and a small effect on FVIII. Apart from these common genetic and environmental influences, there were also significant effects of specific genetic and environmental influences on IR and hemostatic levels.

The finding that a substantial degree of genetic pleiotropy underpins the association between IR and thrombotic CHD risk provides support for the view that diabetes and CHD may to some extent be regarded as the same disorder. Our results raise the possibility that genetic regulatory proteins such as transcription factors or RNA binding proteins could be responsible for these associations. Evidence to support this view comes from the use of insulin resistance lowering agents such as metformin or the thiazolidinediones which effect multiple components of the IRS risk cluster (Forman et al., 1996). The thiazolidinediones are PPARγ activators which are members of a family of transcriptional activators that, when stimulated, have beneficial effects on IR, glycemic control and some other features of the IRS. These pharmacological findings lend further support to the view that abnormalities of transcriptional activation may underpin the genetic association between IR and clustering of vascular risk.

The additive genetic effect (heritability) in our study was 57% for IR, which is higher than the estimate of 37% Lehtovirta et al. (2000) obtained based on "the gold standard" direct measure of insulin sensitivity using the euglycaemic clamp but in a smaller twin sample. Other studies using the HOMA approximation for IR found heritabilities of 66% and 39% (Hong et al., 1997; Narkiewicz et al., 1997).

Our results are in line with previous findings of associations of IR with altered hemostasis due to elevated levels of fibrinogen (Raynaud et al., 2000), PAI-1 (Juhan-Vague & Vague, 1991; Potter van Loon et al., 1993), tPA (Meigs et al., 2000), vWF (Meigs et al., 2000), FVIII (Juhan-Vague et al., 1993) and FXIII B-subunit (Kohler et al., 1998; Kohler et al., 1999). The emphasis of most previous studies has been on the independence of risk factors for CHD using multiple regression and fail to take account of the way related factors may alter risk in concert (Cesari & Rossi, 1999). Even a risk factor that is involved in pathogenesis in an important way may disappear from a multivariate statistical model if the model contains other variables in the same causal pathway, especially if these other variables can be measured with greater precision than the risk factor of interest (Stern, 1995). This together with our results stresses the importance of recognizing the interrelated pathways which will lead to a better understanding of the pathophysiology of CHD.

The influence of potential confounders may impose some limitations on our results. Therefore, we tested the effect of birthweight in our data, but little or no effect of birthweight on IR and hemostatic factors, nor on heritability estimates were observed. We also looked at the possible effect of oral contraceptive pill (OCP) use on levels of IR and hemostatic factors. A small but significant influence was detected on IR, PAI-1 and tPA, explaining only 0.7%, 3.1% and 4.5% of the variance, respectively. Since there is no major difference between proportion of OCP users in MZ and DZ twins, the heritability is very unlikely to be significantly affected (Hammond et al., 2000).

The conclusion that pleiotropic genes influence IR and hemostatic levels may help to understand the relative unsuccessful search for genes influencing CHD. Although studies have reported associations between both the levels of hemostatic factors and the risk for CHD and between hemostatic levels and certain polymorphisms, the relationship between hemostatic polymorphisms and CHD is still not clear (see for review Lane & Grant, 2000). This could be due to relatively small effects of genes on hemostatic risk factors and/or, as this study emphasizes, there may be different sets of genes contributing simultaneously to many risk factors. Many genes, each with a small effect, are more difficult to detect and make it necessary to study larger sample sizes. It may also be that there are gene–gene and gene–environment interactions which affect the relative risk of certain groups but not others, obscuring the relationship between genes and disease. Future multivariate analysis of candidate genes for these CHD risk factors is promising and allows for detection of quantitative trait loci (QTL) explaining small to medium proportion of the genetic variance rather than analyzing the effect on one risk factor at a time (Boomsma & Dolan, 2000). New techniques can be used to detect the interaction between genotype and environment with realistic sample sizes, which will lead to a better understanding of the pathological pathways resulting in CHD (Van den Oord, 1999).

The considerable genetic and environmental overlap between insulin resistance and hemostatic risk factors for CHD may provide new avenues for the prevention and treatment of both type 2 diabetes and CHD. The focus of current treatments aimed at individual risk factors may be improved in the future by strategies that modify or utilize the "common soil" of the multiple risk factors.

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