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A Parent-Twin Study of Plasma Levels of Histidine-Rich Glycoprotein (HRG)

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Summary

Histidine-rich glycoprotein (HRG) is a non-enzymatic glycoprotein that acts as a modulator of several plasma proteins involved in coagulation and fibrinolysis. The contributions of genetic and environmental influences to inter-individual variation in plasma levels of HRG were studied in 160 Dutch families consisting of adolescent twin pairs and their parents. Results showed that 69% of the variance in plasma HRG concentrations could be accounted for by genetic factors. Heritability was the same in males and females and in parents and their offspring. There was no association between HRG levels of husband and wife and no evidence was found for the influence of shared family environment on the resemblance between relatives.

Introduction

Histidine-rich glycoprotein (HRG) is a single chain glycoprotein that is found in plasma at a concentration of 125 mg/l (1, 2) and in platelets at approximately 0.07% of plasma HRG concentrations (3). Plasma HRG is produced by the parenchymal cells of the liver (4, 5). The source of platelet HRG is uncertain, it may be synthesized by megakaryocytes (3). Since the purification of HRG in 1972 (6) many possible functions have been assigned to the protein and a diversity of biological interactions of HRG have been described. Plasma HRG is able to bind plasminogen (7), thrombospondin (8), and heparin (9). HRG binds to heme (10, 11) and several divalent metal ions also have affinity for HRG. Despite this knowledge, the physiological function of HRG remains unknown.

An important property of HRG is its specific affinity for plasminogen. Around 50% of circulating plasminogen is bound to HRG. When HRG levels are elevated the amount of free plasminogen is reduced and this may cause an inhibition of fibrinolysis since only non-bound plasminogen binds to fibrin (7). The ability of HRG to bind heparin and plasminogen suggests a procoagulant and antifibrinolytic effect, respectively, and may form a basis for the increased prevalence of elevated HRG levels in patients with venous thrombosis (12). In families with familial thrombosis familial elevation of HRG levels is seen and genetic influences on plasma HRG concentrations in these specific families have been suggested (13, 14, 15). The cDNA for HRG has been cloned and the gene for HRG has been assigned to chromosome 3 (16), but as yet the characteristics of the structural gene for HRG are unclear and very little is known about the determinants of individual differences in plasma HRG concentrations in the general population. In the present study we focus on the contribution of genetic and environmental factors to interindividual variation in plasma HRG levels in a random sample from the general population. Plasma HRG levels were measured in 160 adolescent twin pairs and their parents to examine the contributions of genetic and environmental factors. In a twin design the separation of genetic and environmental variance is possible because monozygotic (MZ) twins share 100% of their genetic make-up and dizygotic (DZ) twins share on average 50% of their additive genetic variance. If a trait is influenced by genetic factors, MZ twins should resemble each other to a greater extent than DZ twins. When twice the DZ correlation is greater than the MZ correlation, this may indicate that part of the resemblance between twins is induced by the shared family environment (17). Parents and offspring also share 50% of their additive genetic variance, as well as a common family environment. By including parents of twins in the design the presence of assortative mating (a correlation between HRG levels of husbands and wifes) and differences between generations in heritability can be examined.

Methods

Subjects

This study is part of a larger project in which cardiovascular risk factors were studied in 160 adolescent twin pairs and their parents. Addresses of twins (between 14–21 years of age) living in Amsterdam and neighboring cities were obtained from City Council population registries. Twins still living with both their biological parents were contacted by letter. A family was included in the study if the twins and both parents were willing to participate. In addition, a small number of families who heard of the study from other twins also volunteered. Zygosity was determinated by typing the following polymorphisms: ABO, MNS, P, Rhesus, Lutheran, Kell, Duffy, Kidd, Gm, Am and Km. Thirty-five twin pairs were also typed by DNA fingerprinting (18). Three series of triplets were included by discarding the data from the middle child. There were 35 MZ female pairs (average age 16.0, sd = 2.2), 35 MZ male pairs (16.6, sd = 1.8), 3.0 DZ female pairs (17.7, sd = 2.0), 31 DZ male pairs (17.2, sd = 1.7) and 29 DZ unlike sex pairs (16.4, sd = 1.8). Average age of fathers was 48.1 (sd = 6.3) and of mothers 45.6 (sd = 5.9).

Procedure

Fasting blood samples were taken between 8:30 and 10:30 am. Blood was collected under standardized conditions by venipuncture, using Becton-Dickinson Vacutainers containing sodium-EDTA. Plasma was separated from cells after centrifugation at 3,000 rpm for 10 min at 4° C. The samples were stored at −20° C and thawed immediately before use. Plasma HRG levels were measured by radial immunodiffusion (19) using 1% agarose plates with 7.5 mM veronal buffer (5,5-diethylbarbituricacid) pH 8.6 and 0.4% home-made rabbit anti HRG-antiserum. Polyclonal rabbit anti HRG antibodies were raised against purified human HRG kindly provided by Dr. Heimburger (Behringwerke, FRG). Before measuring, plasma was diluted four times in phosphate buffered saline pH 7,4 using an automated diluter (Hamilton Bonaduz AG, Switzerland).

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Five μl of the diluted sample was placed in a well. Sharply delineated precipitation rings were observed. HRG concentrations were calculated from a standard dilution series of pooled plasma obtained from 20 healthy volunteers. HRG levels were expressed as a percentage of pooled plasma, taking pooled plasma as 100%. All measures were carried out in duplicate and averaged. The inter-assay precision (coefficient of variation) of the duplicate measurements was 10%. Family members were never measured on the same agarose plate in order to exclude the possibility that resemblance between family members is influenced by interassay fluctuations.

Statistical Analysis

The effects of sex, generation and zygosity on mean HRG levels, on variances and correlations between relatives were assessed by likelihood-ratio χ^2 tests. These tests were used to compare the fit of a model that constrained parameter estimates to be equal across sexes or generations to one which allowed them to vary, while taking into account the dependency that exists between observations from family members (for a detailed description of the statistical procedures see [20]).

Genetic model fitting was carried out on variance-covariance matrices of the 5 different family groupings (i.e. families of MZ male and female twins and families of DZ male, female and opposite-sex twins). Genetic models specified variation in phenotype to be due to genotype and environment. Sources of variation considered were G, additive genetic variation (i.e. the sum of the average effects of the individual alleles at all loci); C, common environment shared by family members living in the same household and E, a random environmental deviation that is not shared by family members. Their influence on the phenotype is given by parameters h, c, and e that are equivalent to the standardized regression coefficients of the phenotype on G, C and E, respectively. The proportion of variance due to each source is the square of these parameters. To account for possible sex or generation differences in genetic architecture, three different genetic models were examined:

A. full model in which estimates for h, c, and e are allowed to differ in magnitude between males and females, or between parents and offspring;
B. scalar model in which parameters h, c, and e in one sex or generation are a constant multiple of the parameters in the other sex or generation. The model allows the total variances for each group to be different, but the relative importance of genetic (heritability) and environmental influences is constrained to be equal across sexes or generations (17);
C. constrained model in which 1 or all parameter estimates for h, c and e are constrained to be equal in magnitude across sexes or generations.

Parameters h, c and e were estimated by maximum likelihood, using the computer program LISREL7 (21). Goodness-of-fit was assessed by likelihood-ratio χ^2 tests. The overall χ^2 tests the agreement between the observed and the predicted variances and covariances in the 5 family groupings. A large χ^2 (and a low probability) indicates a poor fit, while a small χ^2 (accompanied by a high p-value) indicates that the data are consistent with the model. Submodels were compared by hierarchic χ^2 tests, in which the χ^2 for the full model is subtracted from that for a reduced model. The degrees of freedom (df) for the this test are equal to the difference between the df for the full and the submodel (17). The scalar model (B) is a submodel of the full model (A) and the constrained model (C) is nested under (B).

Results

Table 1A lists means and standard deviations in HRG levels for fathers, mothers, sons and daughters. In Table 1B and 1C the results of testing for sex and generation differences in means and standard deviations are presented. Both means and standard deviations differ significantly between sexes and generations. Parents have higher HRG levels than their children and in both generations males have higher HRG levels than females. Parents also are more variable than their children, but whereas fathers are more variable than mothers, in the generation of the children girls are more variable than boys.

Correlations between twins, spouses, and parents and their offspring are summarized in Table 2A. It can be seen that MZ correlations are larger than DZ and parent-offspring correlations. Table 2B gives the results of significance testing of familial correlations, by comparing submodels in which correlations are constrained to be zero or in which correlations are constrained to be equal to other correlations. The correlation between husband and wife is low (r = 0.12) and not different from zero (χ^2 difference between the last model in Table 1C in which the spouse correlation is free and the model in Table 2B in which it is constrained to be zero is 2.38 which is a non-significant increase in χ^2). Constraining the 2 MZ correlations to be equal to one another or the 3 DZ correlations to be same (i.e. specifying them to be equal for males and females) does not lead to significant increases in χ^2. However, the test of differences between the MZ and DZ correlations gives a highly significant increase in χ^2, indicating that MZ twins resemble each other more than DZ twins. Parent-offspring correlations can be constrained to be the same (i.e. identical correlations between father and son, mother and son, father and daughter, and mother and daughter) and, finally, the DZ and the parent-offspring correlations can also be equated to each other without a decrease in fit. Estimating one MZ correlation and one correlation for DZ twins and parent and offspring, while constraining the spouse correlation to be zero gives maximum likelihood estimates of 0.69 for the MZ correlation and of 0.34 for the DZ and parent-offspring correlations. These correlations suggest a simple genetic model for inter-individual variation in HRG levels without sex or intergenerational differences in heritabilities. As the DZ and parent-offspring correlations are exactly half the MZ correlation, there also does not seem to be an effect of shared environment.

Fifteen mothers of twins, and 13 MZ and 11 DZ female twins used oral contraceptives. Although this influences HRG levels, it did not influence familial resemblances very much. When women using contraceptives are excluded from the sample twin correlations are 0.69 for MZF (was 0.72), 0.42 for DZF (was 0.45) and 0.42 for DOS (was 0.40). Genetic analyses were therefore carried out using data from all families.

Table 3 presents the results of the genetic model fitting analyses. Models in Table 3A that include a genetic component and allow for sex and generational differences in the size of the parameter estimates show a reasonably good fit to the data. Compared to the full G, C, E model a G, E model in which familial resemblance is completely accounted for by additive genetic factors, can be accepted, as there is a non-significant

Table 1 Maximum likelihood estimates of HRG means and standard deviations (expressed as a percentage of pooled plasma) for fathers, mothers, sons and daughters; χ^2 statistics and corresponding probabilities for models of sex and generation differences in means and standard deviations

A	Fathers	Mothers	Sons	Daughters
Mean	127.2	120.3	112.5	106.1
SD	32.4	28.8	24.7	30.6

	χ^2	df	p
B Test of means			
No differences	136.20	61	0.000
Sex differences	133.82	60	0.000
Generation differences	74.66	60	0.096
Generation and sex differences	65.76*	58	0.226
C Test of standard deviations			
No differences	52.59	39	0.072
Sex differences	52.58	38	0.058
Generation differences	48.41	38	0.120
Generation and sex differences	39.96*	36	0.299

* preferred model.

Table 2 Maximum likelihood estimates of familial correlations for HRG levels in twins spouses and offspring; χ^2 statistics and corresponding probabilities for different models testing equality of correlations

A	MZM	MZF	DZM	DZF	DOS	All MZ	All DZ
Correlation	0.69	0.72	0.49	0.45	0.40	0.70	0.43
	Spouses	Fa-Son	Fa-Dau	Mo-Son	Mo-Dau	All Parent-Child	
Correlation	0.12	0.48	0.34	0.34	0.37	0.34	

B Significance tests	χ^2	df	p
Spouse correlation zero	42.34	37	0.251
MZ correlations equal	42.47	38	0.285
DZ correlations equal	42.69	40	0.356
MZ = DZ correlation	50.90**	41	0.138
Parent-child correlations equal	45.14	43	0.383
DZ = Parent-child correlation	46.65*	44	0.364

MZM = Monozygotic Males, MZF = Monozygotic Females, DZM = Dizygotic Males, DZF = Dizygotic Females, DOS = Dizygotic Opposite-Sex Twins.
Fa = Father, Dau = Daughter, Mo = Mother.
* Preferred model.
** Significant increase in χ^2.

Table 3 Genetic model fitting; χ^2 statistics and probabilities for different genetic/environmental models of familial resemblance

	χ^2	df	p
A Sex and Gener Differences in G, E, C	40.03	38	0.380
Sex and Gener Differences in G, E	45.98	42	0.311
Sex and Gener Differences in C, E	53.27	42	0.114
B Scalar Model, G, E, C	44.38	44	0.456
Scalar Model, G, E	46.66*	45	0.404
Scalar Model, C, E	76.72	45	0.002
C No Sex or Gener Differences in G, E, C	59.18	47	0.110
No Sex or Gener Differences in G, E	62.14	48	0.083
No Sex or Gener Differences in C, E	95.47	48	0.000

G represents additive genetic influences, C common environment shared by family members and E individual specific environmental influences.
* Preferred model.

increase in χ^2. Heritabilities under this model are between 55% and 70%. However, when a C, E model is analyzed in which familial resemblance is entirely due to the shared family environment, this leads to a significant increase in χ^2 (χ^2 difference is 15.24 with 4 df, $p = 0.004$). Constraining heritabilities to be the same in both sexes and across generations while allowing for differences in variances (scalar model 3B) provides the best fitting model and gives most parsimonious account of the data. This model gives a heritability estimate for HRG levels of 69%. Models that do not take into account the differences in total variances between sexes and generations do not fit the data, although it may be seen in Table 3C that a model including a genetic component always gives a better explanation of the data than a purely environmental one.

Discussion

This is the first study to examine the inheritance of plasma HRG levels in an unselected twin sample from the general population. Evidence was found for a simple additive genetic model of inheritance. Correlations between MZ ($r = 0.70$) and DZ twins ($r = 0.43$) and parents and their *offspring* ($r = 0.34$), as well as the model fitting results indicate that familial resemblance in HRG concentrations can be accounted for by genetic factors. There is no influence of the shared family environment, despite the fact that our sample consists entirely of parents and children living in the same household and to a large extent sharing the same diet (22). The amount of interindividual variance that is due to non-genetic factors (31%) is made up only of environmental influences that are unique to each individual. Genetic factors explain 69% of the variance in plasma HRG levels. The genetic heritability is the same in males and females and in parents and offspring. Twin studies sometimes have been criticized for giving heritability estimates that are higher than the estimates obtained from other family groupings. In our study the resemblances between parents and offspring who share 50% of their genetic material agree very well with the resemblances between DZ twins who also have on average 50% of their genes in common. Comparing parent-offspring and DZ twin correlations may a first suggestion about age-dependent genetic effects. Significant parent-offspring resemblance implies significant heritabilities both in adolescence and adulthood and a substantial genetic correlation across time. We found that the parent-child and the DZ correlations for HRG levels do not differ significantly from each other. This implies that there must be substantial stability from adolescence to adulthood in the genetic factors that influence variation in HRG levels, i.e., that the same genes are expressed at different ages.

Other haemostatic factors show a pattern of genetic inheritance that very closely resembles these results for HRG. Hamsten et al. (23) obtained a heritability of 51% for plasma fibrinogen concentrations in a family study. Iselius (24) reports a heritability of 41% for factor VIII using the same family material. No evidence for shared environmental factors or for intergenerational differences in heritability was found for either fibrinogen or factor VIII.

It is clear that the familial elevation of HRG levels that is seen for example in some families with familial thrombosis cannot be attributed to common environmental influences shared by family members, but has to be attributed to genetic influences shared by parents and children. The substantial heritability that was found justifies the search for quantitative trait loci, which is possible with sibling (25) or twin data (26).

From our study we cannot draw any conclusions regarding the association between elevated HRG levels and familial thrombosis. Linkage disequilibrium may be involved (27) but several other mechanisms also are possible. It may be that an elevated

HRG level favors the development of thrombosis. This would imply a causal mechanism where the genes that influence HRG levels do not themselves directly predispose to thrombosis. Another possible explanation for the association between elevated HRG and thrombosis is pleiotropy, where the genes that influence HRG levels also directly influence thrombosis predisposition. These last two mechanisms can be distinguished from each other with cross-sectional twin data or data from other genetically informative constellations of relatives (17, 28, 29) if information on HRG levels and thrombosis status is available for all subjects.

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