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Familial Clustering of Factor VIII and von Willebrand Factor Levels

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Summary

Recently, we found that high levels of clotting factor VIII (>150 IU/dl) are common and make an important contribution to thrombotic risk. The determinants of high factor VIII:C are unclear and might be partly genetic. Therefore, we tested the influence of age, blood group and von Willebrand factor (VWF) levels on factor VIII:C levels, and investigated whether factor VIII:C levels are genetically determined. We performed an analysis of 564 female relatives of hemophilia A patients, who had visited our center for genetic counseling. In univariate analysis, AB0 blood group, age and VWF antigen (VWF:Ag) levels all influenced factor VIII:C levels. After adjustment for the effect of VWF:Ag levels, both blood group and age still had an effect on factor VIII:C levels. In sister pairs, the Pearson correlation coefficient between factor VIII:C levels was 0.17 ($p = 0.024$) and this correlation remained positive (0.15, $p = 0.046$) after correction for the influence of VWF:Ag. In mother-daughter pairs, no correlation of factor VIII:C levels was found. The correlation of VWF:Ag levels in sisterpairs was 0.41 ($p < 0.001$) and in mother-daughter pairs 0.44 ($p < 0.001$), in line with the assumption that VWF:Ag levels are under control of autosomal genes. Familial influence on plasma factor VIII:C and VWF:Ag levels was investigated with a recently developed familial aggregation test. This test verifies whether familial aggregation of a particular parameter exists in a set of pedigrees. In 435 women from 168 families, factor VIII:C as well as VWF:Ag levels correlated significantly within families, which suggests a familial influence. The familial aggregation was more prominent for VWF:Ag levels than for factor VIII:C levels, possibly because the genetic effect on VWF:Ag levels is larger than on factor VIII:C levels.

Our results support the presence of a familial influence on factor VIII:C as well as on VWF:Ag levels.

Introduction

Several studies have indicated an association of the levels of clotting factor VIII:C and von Willebrand factor antigen (VWF:Ag) with arterial thrombosis (1-4). We have repeatedly found a low mortality of ischaemic heart disease in hemophiliacs (5, 6), whereas high factor VIII:C and VWF:Ag levels have been reported to be associated with ischaemic heart disease (3, 4). Higher risks of arterial thrombosis have also been reported for individuals with blood group non-0 (3, 7), in whom factor VIII:C and VWF:Ag concentrations are higher than in individuals with blood group 0 (8,9).

Recently, we investigated the roles of AB0 blood group, VWF:Ag and factor VIII:C levels in the occurrence of deep-vein thrombosis (10). Non-0 blood group and high concentrations of VWF:Ag and factor VIII:C increased the risk of deep-vein thrombosis. In multivariate analysis, the effect of non-0 blood group and VWF:Ag was fully explained by factor VIII:C, i.e. factor VIII:C appeared to be the final effector in promoting deep-vein thrombosis. The prevalence of factor VIII:C concentrations >150 IU/dl was 25% in thrombosis patients and 11% in healthy control subjects, which led to an adjusted odds ratio of 4.8 (95% CI 2.3-10.0) for venous thrombosis. We concluded that high levels of factor VIII:C are common and therefore make an important contribution to the risk of deep-vein thrombosis.

The large interindividual variation in both plasma factor VIII:C and VWF:Ag levels can be explained by blood group, age and several other factors (11-13). VWF:Ag is an important contributor to the factor VIII:C concentration, which is explained by VWF being the carrier protein for factor VIII. The non-bound factor VIII is particularly unstable in the circulation (14, 15). In the 1960s and 1970s, family studies indicated a genetic influence on the level of factor VIII:C (16, 17). More recently, Ørstavik et al. (18) found that the variance of factor VIII coagulation antigen (VIIICag) and factor VIII-related antigen (VIIIRag, i.e. VWF:Ag) levels was smaller within than between twin pairs, with a heritability estimate of 0.57 for factor VIIICag and 0.66 for factor VIIIRag. This points to genetic determinants of factor VIII:C levels, additional to VWF.

Investigation of heritability is complicated and places high demands on the group that is studied, with regards to structure and sample size. Therefore, an essential preliminary procedure is to test correlations between relatives.

With the current analysis, we had two aims. First, to quantify the determinants of the factor VIII:C levels, i.e. age, blood group and VWF, and to study to what extent the effect of blood group and age on factor VIII:C levels is influenced by VWF:Ag levels. Second, to investigate whether factor VIII:C levels are genetically determined.

These analyses require data on a large number of families. This may take many years to collect. We realized that factor VIII:C had been measured for many years in families for carrier testing in hemophilia A. This study was therefore performed within the database of families seen for genetic counseling for hemophilia A. The familial aggregation of plasma factor VIII:C and VWF:Ag levels of 435 women from 168 families was investigated with a recently developed statistical test that verifies the existence of familial aggregation (19).

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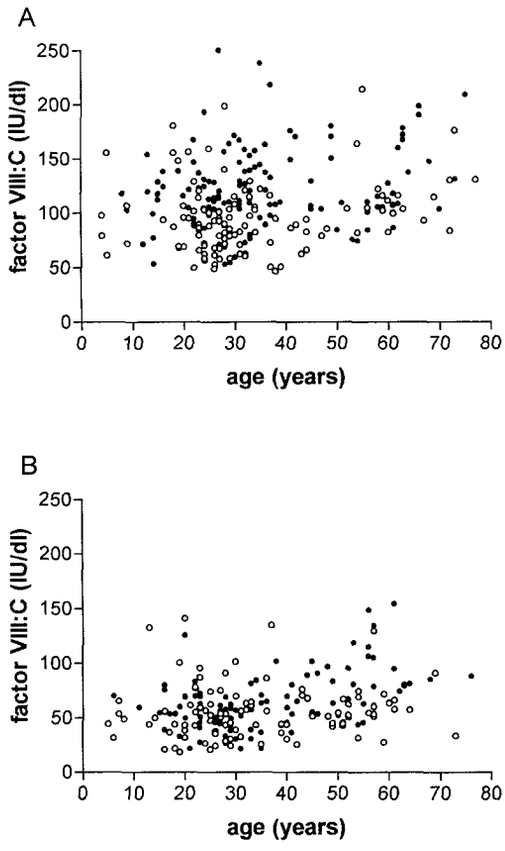

Fig. 1 Distribution of factor VIII:C levels for non-carriers (A) and carriers (B) in relation with age and blood group 0 (○) and non-0 (●)

Materials and Methods

Family Data

Female relatives of probands with documented hemophilia A who came to the Leiden hemophilia center for carriership testing between 1985 and 1995 were included. Carriership was investigated using segregation analysis of intra- and extragenic polymorphic markers of the factor VIII gene or of the deleterious mutation in the family (20). In this way it could be clearly established in 564 women from 172 families whether they were carriers of the diseased allele or not. All subjects were first- or second-degree relatives of the probands The pedigree size ranged from 2 (mother-daughter) to 8 persons.

Laboratory Assays

Blood was collected from the antecubital vein in 0.106 mM trisodium citrate. Factor VIII coagulant activity was measured by one-stage clotting assays with factor VIII deficient plasma. Before 1991, VWF:Ag was determined by Laurell immunoelectrophoresis. From 1991, VWF:Ag was measured by an ELISA using polyclonal antisera. Pooled normal plasma calibrated against an in-house reference, containing 92 IU/dl VWF:Ag and 93 IU/dl factor VIII:C (relative to the WHO international standard) was used as a reference.

Statistical Analysis

The distribution of both factor VIII:C and VWF:Ag was not normal, so they were logarithmically transformed for all statistical analyses. To investigate the association between factor VIII:C and VWF:Ag, the correlation coefficient (product moment) was used. To assess the effect of carriership, blood group and age on factor VIII:C levels, we used multiple linear regression. Carriership, as determined by DNA analysis, was entered into the regression models as a dichotomous variable (0 for non-carriers, 1 for carriers), age as a continuous variable (in years). Blood group was dichotomized into two groups (0 for blood group 0, 1 for non-0). To test whether the effect of age and blood group on factor VIII:C was independent of VWF:Ag level, VWF:Ag was added later in the regression model as a continuous variable. Potentially confounding effects of the different VWF:Ag assays were adjusted by multiple regression, which did not lead to any differences.

To investigate the genetic influence on factor VIII:C and VWF:Ag levels, the residuals of the multiple regression models were used. Residuals are the differences between the observed level Y_i for person i and the predicted value μ_i obtained from the multiple regression model. As a first test for familial effects, correlations between the residuals (obtained from the multiple regression model) of mother-daughter pairs and sister pairs were calculated. Between all mother-daughter pairs, for which $Y_i-\mu_i$ and $Y_j-\mu_j$ are the residuals of mother i and daughter j with variance σ^2, the correlation will be:

$$\sum_{pairs} \frac{(Y_i-\mu_i)(Y_j-\mu_j)}{\sigma^2} / \text{number of pairs}$$

Familial aggregation of factor VIII:C and VWF:Ag levels was studied using a recently developed method (19), that tests the null hypothesis of no correlation within randomly chosen pedigrees. Since we adjust for carriership by multiple regression in these families, this test can be used. Within pedigrees, the correlation of the genetic effects is assumed to have the following natural correlation structure R: parent-offspring and individuals within a sibship: correlation 1/2, grandmother-granddaughter and aunt-niece: correlation 1/4, etc. To combine all information about correlations within a pedigree, Q can be applied as the weighted sum of correlations between pairs of relatives within a pedigree. Its mathematical formula reads:

$$Q = \sum_{i=1}^{n} \sum_{j=1}^{n} \frac{(Y_i-\mu_i)(Y_j-\mu_j)R_{ij}}{\sigma_2}$$

R_{ij} is the natural correlation between person i and j of the same pedigree (1/2, 1/4 etc.). $R_{ij} = 0$ if person i and j are not related. The value of Q is determined mainly by sibship and parent-child relations, but the other relationships also contribute to the value of Q. The test for familial aggregation is positive when the calculated Q is significantly larger than the expected Q value under the null hypothesis of no aggregation. See reference 19 for details on the calculation of the associated p-value.

Results

We included 564 women from 172 families, who were all tested for carriership for hemophilia A. Because of the possible effect of blood group on factor VIII:C levels, all analyses were restricted to individuals in which blood group was measured. 129 females were therefore excluded: In 4 families, containing 25 subjects, blood group was not

Table 1 Increase in factor VIII:C# (IU/dl) and in VWF:Ag levels (IU/dl) in relation to age and blood group

	Factor VIII:C (95% CI)	Factor VIII:C (95% CI) corrected for VWF:Ag	VWF:Ag (95% CI)
Age (10 years)	5.6** (3.5–7.9)	3.1* (1.2–5.1)	5.8** (3.4–8.2)
Blood group (0 vs non-0)	22.4** (15.1–31.2)	9.3* (2.8–16.2)	31.5** (22.4–41.3)

* $p < 0.01$, ** $p < 0.001$

Factor VIII:C levels adjusted for carriership

analyzed, and in 37 families blood group was analyzed in one member only, leaving out 104 persons. Of those 37 families, we included the one member with known blood group as a reference in the familial aggregation test. So 435 subjects from 168 families were eligible for analyses. The mean age was 35 years (range 3-84). 193 women were carriers of hemophilia A and 242 were non-carriers.

Factor VIII:C levels were strongly correlated (r = 0.58) with VWF:Ag levels. Factor VIII:C levels were higher in the 227 subjects with blood group non-0 than in the 208 persons with blood group 0 in both carriers and non-carriers (Fig 1). As shown in Table 1, after correction for carriership, factor VIII:C concentrations in subjects with blood group non-0 were 22.4 IU/dl (95% CI 15.1-31.2) higher than those with blood group 0. For VWF:Ag levels this difference was 31.5 IU/dl (22.4-41.3). Both factor VIII:C and VWF:Ag levels increased with age. For every successive 10 years of age, the factor VIII:C level increased 5.6 IU/dl and the VWF:Ag level 5.8 IU/dl. To see whether the effect of blood group and age on factor VIII:C concentration depended on VWF:Ag level, we used multiple linear regression. After adjustment for the influence of VWF:Ag level, the regression coefficient for blood group decreased to 9.3 IU/dl (2.8-16.2) and for age to 3.1 IU/dl (1.2-5.1) suggesting a VWF:Ag-mediated effect of these variables on factor VIII:C level (Fig 2). Nevertheless, blood group and age still had an effect on factor VIII:C, independent of VWF:Ag.

To test for correlations of factor VIII:C and VWF:Ag levels within mother-daughter and sister pairs, we analyzed all combinations of these familial relationships. As shown in Table 2, after adjustment for blood group and age, correlations for factor VIII:C levels between mother and daughter were very weak (r = 0.08). In sister pairs this correlation was 0.17 (p = 0.024) and remained significant (r = 0.15, p = 0.042) after correction for the influence of VWF:Ag level. VWF:Ag levels showed a strong association in mother-daughter pairs (r = 0.44, p <0.001) and sister pairs (r = 0.41, p <0.001).

Table 3 shows the results of the familial aggregation test for factor VIII:C and VWF:Ag levels. In this test, the null hypothesis of no correlation was rejected for both factor VIII:C and VWF:Ag levels. For factor VIII:C Q was 469 (p = 0.028), whereas the expected value of no correlation was 429. For VWF:Ag level Q was 606 (p <0.001). After correction for VWF:Ag level, Q for factor VIII:C levels was 464 (p = 0.04) and was thus not essentially affected, suggesting that familial influence on factor VIII:C levels is not regulated through VWF:Ag levels alone.

Discussion

In this study we used a new test, the familial aggregation test, specifically developed for testing correlations between family members, which uses all possible combinations in families and provides more information than the analysis of variance between pairs of relatives. In the familial aggregation test, both factor VIII:C and VWF:Ag levels were positively correlated within families, suggesting a genetic influence.

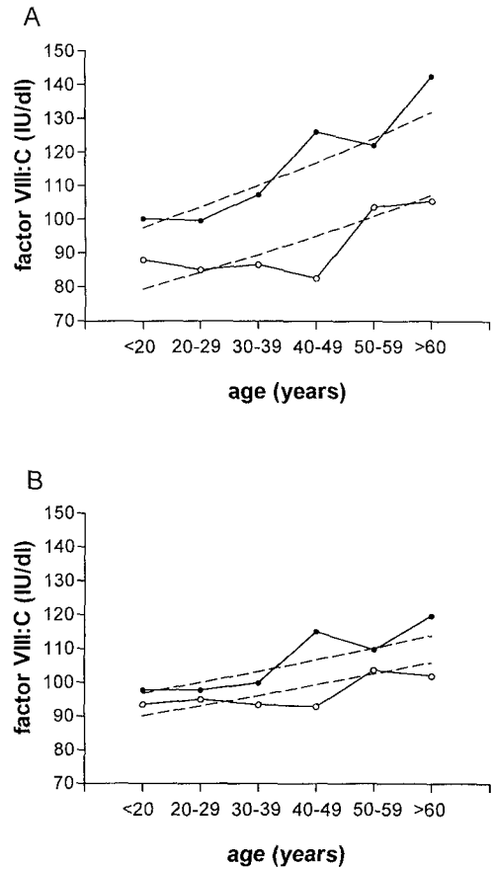

Fig. 2 Mean (○, ●) and predicted (---) factor VIII:C levels for age and blood group. Factor VIII:C levels were adjusted for carriership (in A) and for carriership as well as for the influence of VWF:Ag levels (in B). Predicted values for age and blood group were calculated by multiple regression. The difference in factor VIII:C levels between blood group 0 and non-0 becomes smaller after the correction for the influence of VWF:Ag levels, but is still present

We found a positive correlation of factor VIII:C levels in sister pairs only and not in mother-daughter pairs. For X-linked determinants it can be predicted that factor VIII:C levels should give higher correlations in sisters pairs than in mother-daughter pairs, because sisters inherit the same X-chromosome from their father. This means that on average 50% of the sisters will have the same combination of X-chromosomes, in contrast to 25% for autosomes. These findings suggest a control of factor VIII:C variation by X-linked alleles, possibly the factor VIII gene itself. Filippi et al. (21) suggested a primary role of X-linked genetic determinants in the variation of factor VIII:C levels within families. They found a positive correlation within groups of male pairs, only if they had identical X-alleles.

VWF:Ag levels were strongly correlated in both mother-daughter and sister pairs, which is in agreement with previous publications that VWF:Ag levels are under control of autosomal genes (16, 22).

Table 2 Correlation coefficients of factor VIII:C and VWF:Ag levels between mother-daughter and sister pairs#

	No. of pairs	Factor VIII:C	Factor VIII:C corrected for VWF·AG	VWF:Ag
Mother vs daughter	213	0.8 (p = 0.26)	0.07 (p = 0.33)	0.44 (p <0.001)
Sister vs sister	174	0.17 (p = 0.024)	0.15 (p = 0.046)	0 41 (p <0.001)

Factor VIII:C levels adjusted for carriership, age and blood group; VWF:Ag levels adjusted for age and blood group

Table 3 Familial aggregation of factor VIII C and VWF Ag levels in 168 families[#]

	Factor VIII C	Factor VIII C corrected for VWF Ag	VWF Ag
Q	469	464	606
Exp (Q)	429	428	429
p value	0 028	0 040	<0 001

[#] Factor VIII C levels adjusted for carriership age and blood group VWF Ag levels adjusted for age and blood group

Furthermore, in the Q test familial aggregation was prominent, indicating that VWF Ag levels among women were stongly influenced by familial factors

VWF Ag levels showed higher correlations in sister pairs than factor VIII C levels Assuming X-linked regulation of factor VIII C levels, one would expect the opposite One possible explanation is that the genetic influence on VWF Ag levels is larger than on factor VIII C levels This hypothesis is supported by the higher Q we found for VWF Ag levels in the familial aggregation test Another possibility is that the adjustment of factor VIII C levels for the influence of VWF Ag levels is not correct, e g because we consider the interaction between factor VIII and VWF to be similar in all individuals It is possible however, that different binding affinities between VWF and factor VIII result in a variation of factor VIII levels in plasma Such different affinities might be due to variations in either the factor VIII or VWF protein

Both factor VIII and VWF Ag levels are also influenced by blood group and age Ørstavik and coworkers (18) found that the effect of blood group and age on factor VIII level was secondary to the effect on VWF Ag In our data, factor VIII C and VWF Ag levels increased with age, an effect that persisted for factor VIII C after adjustment for the influence of VWF Ag levels This small independent effect of age on factor VIII C may be mediated through several other variables, such as body mass index, or the concentration of triglycerides and fibrinogen, that also increase with age and are positively correlated with the factor VIII C concentration (11 13) It is also possible that age has a more direct influence on factor VIII C levels by increasing the activity of transcription factors binding to the promotor of the factor VIII gene Individuals with blood group non-0 had higher levels of factor VIII C and VWF Ag than subjects with blood group 0 Most of the variation in factor VIII C levels caused by the effect of blood group was mediated through VWF Ag However, using multiple regression analysis, blood group still resulted in a predicted change in factor VIII C concentration of 9 3 IU/dl after the correction for the effect of VWF Ag levels In our previous study, an effect of blood group on factor VIII C of 7 6 IU/dl was noticed (10) How AB0 blood group influences plasma concentrations of VWF Ag and factor VIII C is unknown Blood group A, B, and H(0) oligosaccharide structures have been identified on the factor VIII/vWF complex and on purified VWF (23, 24) It is possible that blood group determinants not only influence the plasma concentration of VWF Ag but also the activity of factor VIII C, e g by affecting its secretion, activity or degradation (8) The direct influence of blood group as well as age on factor VIII C levels differs from the findings by Ørstavik et al (18) and may be explained by the fact that in their study factor VIII C antigen was measured instead of factor VIII clotting activity To conclude, VWF Ag levels and factor VIII C levels seem to be genetically determined Factor VIII C levels are influenced by age, blood group and other familial factors, even after correction for the effects acting via VWF Ag levels

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