Am J Hum Genet 37:89–101, 1985

Factor VIII and Factor IX in a Twin Population. Evidence for a Major Effect of *ABO* Locus on Factor VIII Level

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SUMMARY

In order to establish the relative importance of genetic factors on the variation in plasma concentration of coagulation factors VIII and IX, these parameters were determined in 74 monozygotic and 84 like-sexed dizygotic twin pairs. The twins belonged to two age groups: 33–39 years and 57–62 years. Factor VIII was determined as factor VIII coagulant antigen (VIIICAg) and as factor VIII-related antigen (VIIIRAg). Factor IX was determined as factor IX antigen (IXAg). A higher value for each coagulation factor was found in the older-age group compared to the younger group, whereas no difference was found between the sexes. A significant correlation was found between values for VIIIRAg and VIIICAg ($r = .56$). For VIIICAg, it could be demonstrated that the age effect was secondary to the age effect on VIIIRAg. The concentration of VIIICAg and VIIIRAg varied among ABO blood types, being lowest in type O individuals, higher in A_2 individuals, and highest in A_1 and B individuals. The effect of the *ABO* locus on VIIICAg was secondary to an effect on VIIIRAg. Analysis of variance revealed a significant genetic influence on the variance of VIIICAg and VIIIRAg with a heritability estimate of .57 for VIIICAg and .66 for VIIIRAg. This is in agreement with a previous hypothesis of an effect of several autosomal genes on factor VIII concentration. Thirty percent of the genetic variance of VIIIRAg was due to the effect of ABO blood type. The *ABO* locus is therefore a major locus for the determination of factor VIII concentration. No significant genetic effect on the variation in plasma concentration of IXAg could be detected.

Received March 21, 1984; revised August 28, 1984.

This work was supported by grants from the Norwegian Research Council for Science and the Humanities and the Norwegian Council on Cardiovascular Diseases, and grants HL06350 and HL32115 from the National Heart, Lung and Blood Institute (to H. R. and J. B. G.).

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INTRODUCTION

Patients with hemophilia A or B have a deficiency of coagulation factor VIII or IX activity. The plasma concentration of factors VIII and IX show a very wide range in individuals without a bleeding disorder. Several nongenetic factors are known to influence the level of these coagulation factors [1]. A genetic influence on the level of factor VIII has been indicated by family studies [1, 2]. These studies were in agreement with the hypothesis that the level of factor VIII in individuals without a bleeding disorder is determined by a series of genes on autosomes. A recent study from Sardinia, however, suggests that this variation is controlled by a series of normal isoalleles on the X chromosome [3]. Twin studies may give an estimate of the relative importance of genetic factors on the variation in concentration of a plasma protein by comparison of monozygotic (MZ) and dizygotic (DZ) twins. One previously reported twin study [1] failed to detect a genetic effect on factor VIII concentration. Another twin study [4] showed evidence of a significant genetic effect on factor VIII concentration but not on factor IX concentration. Since only a small number of twin pairs was included, a heritability estimate could not be established.

The family studies referred to above have been based on determination of the coagulant activity of the factors (VIIIC and IXC). Plasma factor VIII is a complex of two distinct proteins: a small factor VIII coagulant protein (VIIICAg) and a large carrier molecule, factor VIII-related protein (VIIIRAg), which are under separate genetic control [5]. Immunization of laboratory animals with purified factor VIII complex results in antibodies against VIIIRAg. Replacement therapy in hemophilia A patients, who are deficient in the coagulant activity of factor VIII, may result in the production of antibodies to VIIICAg. Here, we present the results of a twin study of the plasma concentration of VIIICAg, VIIIRAg, and factor IX protein (IXAg).

MATERIALS AND METHODS

Twins

Plasma were examined from 158 MZ or like-sexed DZ twin pairs. The twins were either 33–39 or 57–62 years old and belonged to a subgroup of pairs of known zygosity drawn from a population-based Norwegian twin panel [6] (table 1). Zygosity had been determined by testing for the blood group antigens A, A_1, B, C, c, D, E, e, M, N, S, s, P, Fy^a, Fy^b, Lu^a, Lu^b, Kell, Jk^a, and Jk^b and for the electrophoretic variants C3 and Hp. The probability of monozygosity was more than .99 for twin pairs concordant for each genetic marker. All twins had responded to a questionnaire that included questions on their health, but no specific questions about bleeding disorders. Included in the material was also plasma from 17 members of twin pairs, where plasma from the cotwin was not available. Values on these twins were included in the study of associations to genetic markers and for the calculation of the correlation between VIIIRAg and VIIICAg.

Plasma

The twins were bled in the laboratory or in their homes after an overnight fast. Blood was collected into evacuated blood-collecting tubes (Venoject) containing 1/10 vol 3.13% sodium citrate and was kept at 4°C for no more than 4 hrs before the separation of plasma. This was done by centrifugation at 2,000 *g* for 30 min at 4°C. The plasma samples were

TABLE 1

DISTRIBUTION OF AGE, SEX, AND ZYGOSITY IN 158 TWIN PAIRS

ZYGOSITY	33–39 YEARS		57–62 YEARS		TOTAL
	Males	Females	Males	Females	
MZ	19	18	16	21	74
DZ	15	21	23	25	84
Total	34	39	39	46	158

stored at −70°C until they were tested. A standard plasma was prepared by pooling equal volumes of plasma from 20 healthy individuals. This standard plasma was defined to contain 100% of each coagulation factor.

VIIICAg

VIIICAg was determined by a radioimmunoassay using a human antibody [7]. The samples were shipped frozen on dry ice from Oslo, and each sample was tested diluted 1/10 in duplicate at the University of North Carolina at Chapel Hill. For some of the twins, plasma was not available for VIIICAg assay.

VIIIRAg and IXAg

VIIIRAg and IXAg were determined by the "rocket" electroimmunoassay technique of Laurell in the laboratory in Oslo. The antiserum to factor VIII was prepared by immunization of rabbits with purified factor VIII (a gift from Lars Holmberg, Coagulation Laboratory, Allmänna Sjukhuset, Malmö, Sweden). This antiserum was monospecific as judged by double-immunodiffusion technique. Antiserum to factor IX was prepared as described [8, 9]. Each plasma sample was tested undiluted and diluted 1/2, in both cases in duplicate. The median of four values was chosen for further analysis. The detailed experimental conditions for the electroimmunoassay were as described [10]. The study was conducted blindly. Thus, the zygosity of the twins was unknown until all the samples had been analyzed.

Statistical Methods

For the studies of associations to genetic markers and for the calculation of the correlation between VIIIRAg and VIIICAg, a random sample of one member of each twin pair was used. This was done since the values on the two twins in a pair are not independent observations in genetic terms. F-test, linear regression, and z-scoring were performed as described in standard textbooks. For the estimation of genetic variance and its relative influence on total variance (heritability), a model-fitting approach was used [11].

The mean squares obtained from ordinary one-way analysis of variance for each twin type is interpreted as sums of the variance components. The mean squares within (MS_W) and between (MS_B) twin pairs are connected to the variance components within (V_W) and between (V_B) pairs as shown by Winer [12].

Mean squares	Degrees of freedom	Variance
MS_W	n	V_W
MS_B	n − 1	$V_W + 2V_B$,

where n is the number of twin pairs in each twin group.

TABLE 2

MODEL FOR GENETIC (V_G) AND ENVIRONMENTAL (V_{EW} and V_{EB}) VARIANCES FOR MEAN SQUARES OBTAINED FROM ANALYSIS OF VARIANCE IN MZ AND DZ TWIN PAIRS

Observed mean square	Variance	Model
MS_WMZ	V_W	V_{EW}
MS_BMZ	$V_W + 2\,V_B$	$V_{EW} + 2\,(V_G + V_{EB})$
MS_WDZ	V_W	$V_{EW} + \frac{1}{2}\,V_G$
MS_BDZ	$V_W + 2\,V_B$	$V_{EW} + 1\frac{1}{2}\,V_G + 2\,V_{EB}$

NOTE: MS_WMZ = mean square within MZ twin pairs, MS_BMZ = mean square between MZ twin pairs, MS_WDZ = mean square within DZ twin pairs, MS_BDZ = mean square between DZ twin pairs.

It is assumed that the observed total variance is the sum of variance components of additive genetic (V_G) and environmental (V_E) origin. The environmental variance may be divided into two parts: environmental variance within twin pairs (V_{EW}) and environmental variance between twin pairs (V_{EB}). V_{EW} represents environmental effects that are specific to each individual and will also include error variance. V_{EB} represents environmental effects that are common to the two members of a twin pair.

The variance within MZ twin pairs is the same as V_{EW} since environmental influences within families is the only variation source that can make MZ twin pairs different. The variance between MZ twin pairs on the other hand is the sum of V_G and V_{EB}. Furthermore, the variance within DZ twin pairs is due to both genotypic and within family environmental variance. Since DZ twins share half their genes, this variance is the sum of 1/2 V_G and V_{EW}. The variance between DZ pairs may be due to both genotypic and between pairs variance (table 2).

Genetic and environmental variance components with their standard errors were estimated by the method of weighted least squares [11]. The goodness of fit of the model was evaluated after calculation of the weighted sum of squared deviations of the expected mean squares from the observed values. This statistic follows the chi-square distribution. A simple presentation of this model has been presented by Sundet et al. [13].

RESULTS

Distribution of Values

The distribution of the plasma concentration of VIIIRAg, VIIICAg, and IXAg in the total material is demonstrated in figures 1, 2, and 3. The distributions of VIIICAg and VIIIRAg differed significantly from normality. Normality was obtained after logarithmic transformation and the natural logarithm was used for all statistical analyses.

Thirteen individuals had VIIIRAg values below 40%. The health questionnaire had been returned by eight of these individuals. None of them reported symptoms of any serious disorder or bleeding episodes. Four individuals, including the individual with a plasma concentration of VIIIRAg of only 19%, had undergone surgery uneventfully.

The older-age group had significantly higher values for all three parameters than the younger-age group (table 3). This age effect was seen in both males and females, and no difference was found between the sexes.

The relationship between the concentration of VIIIRAg and VIIICAg was determined using Pearson's coefficient of correlation and was .56 ($P < .0001$). The effect of age on VIIICAg could be an effect that was secondary to the effect on VIIIRAg. The values for VIIICAg were therefore corrected for the correlation with VIIIRAg ($VIIICAg_{corr}$) by linear regression: ln $VIIICAg_{corr}$ = ln VIIICAg + 0.696 (mean ln VIIIRAg − ln VIIIRAg).

After this correction, an age effect was no longer present (table 4). Similarly, the values for VIIIRAg were corrected for the correlation with VIIICAg ($VIIIRAg_{corr}$): ln $VIIIRAg_{corr}$ = ln VIIIRAg + 0.461 (mean ln VIIICAg − ln VIIICAg).

After this correction, an effect of age on VIIIRAg could still be found. Thus, it seems that the effect of age on FVIII concentration is mainly an effect on VIIIRAg concentration.

Association to genetic markers. The previously described association between plasma concentration of factor VIII and ABO blood type [2, 4, 14] was confirmed in the present study for both VIIICAg and VIIIRAg (table 5). Individuals with blood type O had a significant lower plasma concentration of factor VIII than individuals with blood type A_1 or B. Individuals with blood type A_2 had a concentration that was intermediate between individuals with blood type O and blood type A_1.

A difference as described here could be due to a stratification, that is, the existence of a subpopulation with a high frequency of blood group O and a lower

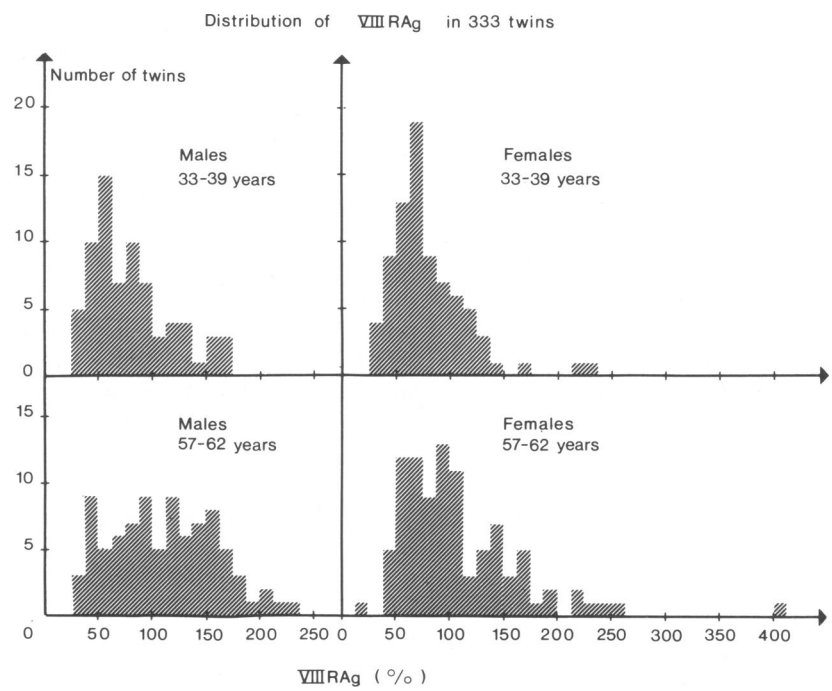

FIG. 1.—Distribution of plasma concentration of VIIIRAg according to age and sex

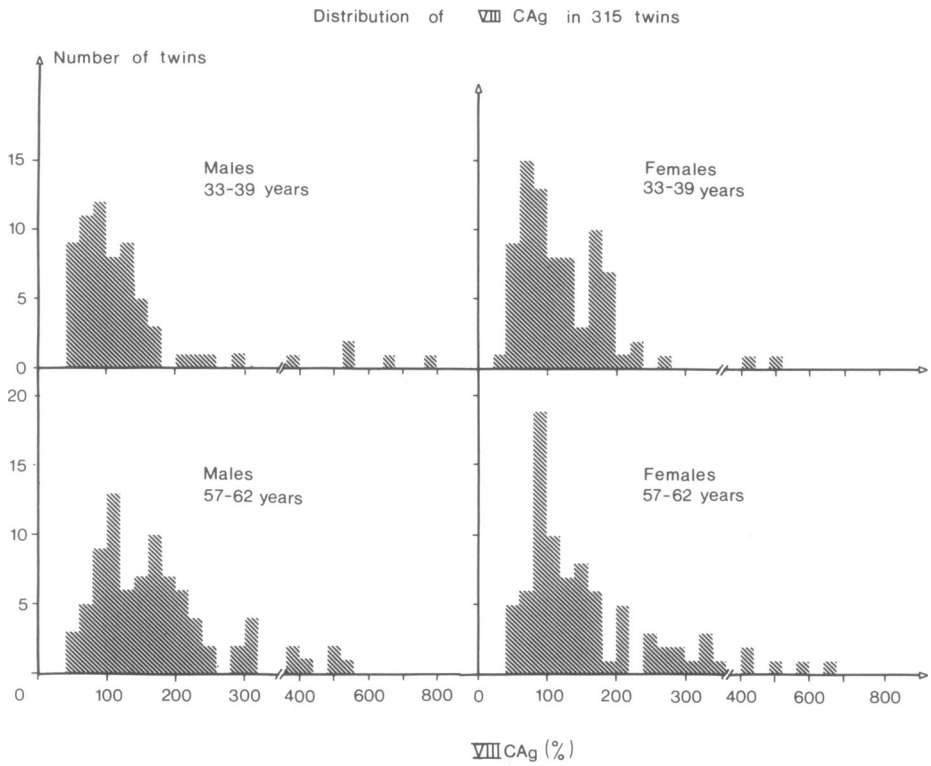

FIG. 2.—Distribution of plasma concentration of VIIICAg according to age and sex

concentration of factor VIII. If this were the case, one would not expect to find the differences between type O and type A individuals in different populations or within families. The same difference in VIIIRAg concentration was, however, also found within families, since it was present in 18 pairs of fraternal twins where one twin was blood type O (mean ln VIIIRAg concentration 4.28) and the other was type A_1 or B (mean ln VIIIRAg concentration 4.67).

Like the effect of age, the effect of ABO blood type on VIIICAg could be secondary to an effect on VIIIRAg. When VIIICAg values that had been corrected for VIIIRAg effect were used, an association to ABO blood type was no longer present. When, similarly, VIIIRAg values were corrected for VIIICAg effect, a significant effect on ABO blood type on VIIIRAg concentration was still present (table 5).

No other significant association was found among plasma concentrations of VIIICAg, VIIIRAg, or IXAg and any of the other markers used for zygosity testing.

Estimation of Genetic Variance by Model-fitting

VIIIRAg and VIIICAg. The mean squares for VIIIRAg and VIIICAg are shown in table 6. A model including only V_{EW} or both V_{EW} and V_{EB} could be discarded.

A model including V_G, V_{EW}, and V_{EB} gave a negative estimate of V_{EB}. A model including V_G and V_{EW} gave the best fit. Very similar estimates of heritability were obtained when the analyses were performed separately for young and old twins. The estimates given in table 7 were therefore determined on the total material after correction for age effect by z-scoring.

We found a major effect of ABO blood type on factor VIII concentration. We therefore calculated the fraction of the genetic variance that was due to the variation in ABO blood type, again using analysis of variance. As can be seen from table 8, 30% of the genetic variance of VIIIRAg and 12% of the genetic variance of VIIICAg could be explained by ABO blood type.

Since the effect of ABO blood type on VIIICAg was secondary to an effect on VIIIRAg, the genetic variance due to ABO blood type was also determined on VIIICAg values that had been corrected for effect on VIIIRAg. As expected, a genetic variance due to ABO blood type could no longer be detected (table 8).

Correlation of VIIICAg between DZ Twin Pairs

Mather and Links [15] showed that for sex-linked variables the sister-sister correlations are higher than the brother-brother correlations. Filippi et al. [3] found that the variation of VIIIC in normal individuals was largely controlled by a series of X-linked isoalleles. We therefore compared the intraclass correlation

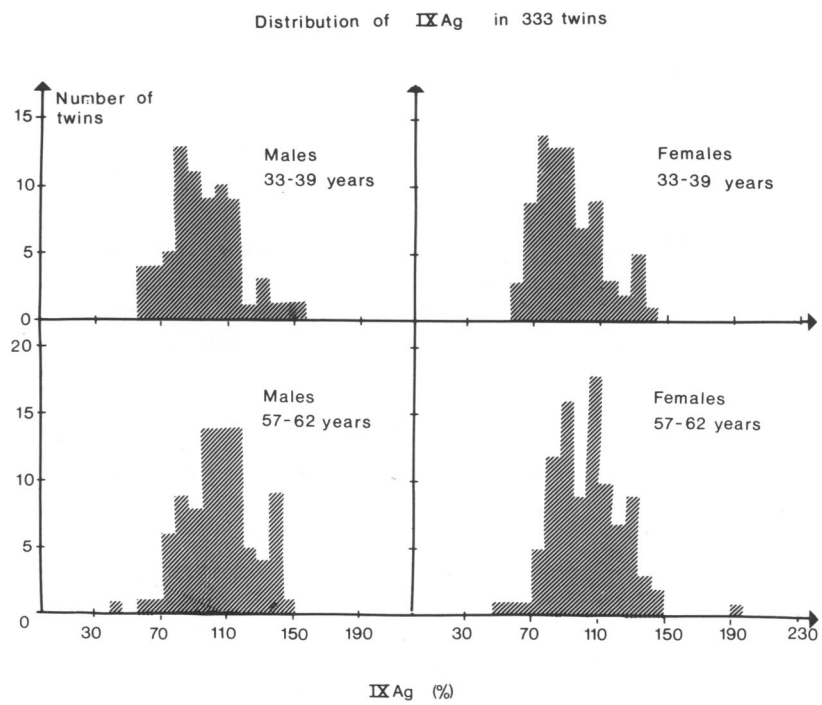

FIG. 3.—Distribution of plasma concentration of IXAg according to age and sex

TABLE 3

EFFECT OF AGE AND SEX ON PLASMA FACTOR VIII AND FACTOR IX CONCENTRATION

SEX	AGE (YRS)	VIIIRAg No.	VIIIRAg Mean (ln%)	VIIICAg No.	VIIICAg Mean (ln%)	IX No.	IX Mean (%)
Females	33–39	79	4.29 ± 0.05	80	4.66 ± 0.06	79	90 ± 2.26
Females	57–62	95	4.57 ± 0.05	84	4.92 ± 0.06	95	105 ± 2.29
Males	33–39	72	4.28 ± 0.05	66	4.71 ± 0.08	72	97 ± 2.45
Males	57–62	87	4.60 ± 0.05	85	5.04 ± 0.06	87	105 ± 2.29
Total		333	4.447 ± 0.03	315	4.841 ± 0.03	333	100 ± 1.21
F test			$P < .00001$		$P = .00005$		$P < .00001$

of VIIICAg, which is the X-linked protein, in female and male DZ twin pairs. As can be seen from table 9, no difference was found.

FIX

For factor IX, no sex effect was apparent and no difference between total variances for young and old twins was found. Although the distribution was moderately skewed, this was much less marked than for factor VIII (fig. 3). Therefore, the data were not transformed, and models were fitted to the whole set of data. Again, a model including V_{EW} only did not explain the variation. However, the model with V_{EW} and V_{EB} could not be discarded ($\chi^2 = 4.42$; $P > .5$) and the model with V_G and V_{EW} gave no better fit ($\chi^2 = 6.61$; $P > .25$). The full model of V_G, V_{EW}, and V_{EB} gave a chi-square of 3.14 (5 df; $P > .5$) (table 7). Since the estimate of genetic variance of .20 is not significantly different from zero, the heritability estimate of .20 permits no definite interpretation.

DISCUSSION

The present data show that there is a wide range in plasma concentration of VIIIRAg and VIIICAg in individuals without symptoms of a bleeding disorder. For VIIIRAg, the widest range was found in older twins, which was expected since several diseases are known to influence the level of VIIIRAg [16]. It was surprising, however, to find a large number of individuals with less than 40% VIIIRAg and no signs of a bleeding disorder. It is possible that further studies on these individuals might reveal mild cases of von Willebrand disease, which is characterized by a low concentration of VIIIRAg.

For both coagulation factors, a higher mean was found in the older- than in the younger-age group. This age effect is in agreement with a previous study [17] where an increase in the concentration of VIIIC of 0.8% per year was found. We found the effect of age to be mainly an effect on VIIIRAg concentration. VIIIRAg is synthesized in vascular endothelial cells, whereas VIIICAg, according to one recent report, is synthesized in hepatic sinusoidal endothelial cells [18]. A higher plasma concentration in older individuals might be due to damage to endothelial cells and a leakage of VIIIRAg into plasma [19]. A similar mechanism has been invoked to explain the increased concentration of VIIIRAg in individuals

TABLE 4

RELATIONSHIP BETWEEN FACTOR VIII Concentration and Age after Correction of VIIICAg FOR VIIIRAg EFFECT AND OF VIIIRAg FOR VIIICAg EFFECT

AGE (YRS)	VIIICAg		VIIICAg CORRECTED FOR VIIIRAg		VIIIRAg		VIIIRAg CORRECTED FOR VIIICAg	
	No.	Mean (ln%)	No.	Mean (ln%)	No.	Mean (ln%)	No.	Mean (ln%)
33–39	146	4.68 ± 0.05	136	4.90 ± 0.04	151	4.28 ± 0.04	136	4.34 ± 0.03
57–62	169	4.98 ± 0.04	156	4.81 ± 0.04	182	4.58 ± 0.04	156	4.52 ± 0.03
Total	315	4.84 ± 0.03	292	4.86 ± 0.03	333	4.45 ± 0.03	292	4.44 ± 0.02
F test		$P = .00001$		$P = .10$		$P < .00001$		$P = .00008$

TABLE 5

FACTOR VIII AND ABO BLOOD TYPE

ABO BLOOD TYPE	VIIIRAg		VIIIRAg CORRECTED FOR VIIICAg		VIIICAg		VIIICAg CORRECTED FOR VIIIRAg	
	No.*	Mean (ln%)	No.*	Mean (ln%)	No*	Mean (ln%)	No.*	Mean (ln%)
O	60	4.18 ± 0.06	55	4.27 ± 0.06	59	4.66 ± 0.07	55	4.85 ± 0.06
A_2	20	4.47 ± 0.11	17	4.41 ± 0.09	18	4.96 ± 0.17	17	4.97 ± 0.14
A_1	68	4.60 ± 0.05	55	4.55 ± 0.05	59	4.90 ± 0.07	55	4.83 ± 0.07
B	15	4.63 ± 0.15	14	4.64 ± 0.13	16	5.01 ± 0.12	14	4.80 ± 0.15
A_1B	4	4.78 ± 0.09	4	4.53 ± 0.17	4	5.39 ± 0.25	4	4.97 ± 0.14
Total	167	4.44 ± 0.04	145	4.44 ± 0.04	156	4.84 ± 0.05	145	4.86 ± 0.04
F-test		$P < .00001$		$P = .001$		$P = .01$		$P = .62$

* One twin randomly chosen from each twin pair.

TABLE 6

OBSERVED MEAN SQUARES (MS) AND DEGREES OF FREEDOM (df) FOR VIIIRAg, VIIICAg, AND IXAg

	Age	VIIIRAg	(df)	VIIICAg	(df)	IXAg	(df)
MS_WMZ	33–39	0.0529	(37)	0.1353	(36)	0.0187	(37)
MS_BMZ	33–39	0.3055	(36)	0.5087	(35)	0.0563	(36)
MS_WDZ	33–39	0.1510	(36)	0.1886	(33)	0.0227	(36)
MS_BDZ	33–39	0.1909	(35)	0.2957	(32)	0.0706	(35)
MS_WMZ	57–62	0.0786	(37)	0.1236	(34)	0.0242	(37)
MS_BMZ	57–62	0.3519	(36)	0.4003	(33)	0.0814	(36)
MS_WDZ	57–62	0.1582	(48)	0.2081	(44)	0.0290	(48)
MS_BDZ	57–62	0.3172	(47)	0.4475	(43)	0.0590	(47)

NOTE: MS_WMZ = mean square within MZ twin pairs; MS_BMZ = mean square between MZ twin pairs; MS_WDZ = mean square within DZ twin pairs; MS_BDZ = mean square between DZ twin pairs.

TABLE 7

ESTIMATE OF HERITABILITY (h^2) FOR VIIIRAg, VIIICAg, AND IXAg

	V_G	V_{EW}	V_{EB}	$h^2 = \frac{V_G}{V_G + V_{EW} + V_{EB}}$
VIIIRAg	0.6155 ± 0.0919	0.3172 ± 0.0492	...	0.66
VIIICAg	0.5056 ± 0.0913	0.3767 ± 0.0588	...	0.57
IXAg	0.088 ± 0.098	0.209 ± 0.034	0.1138 ± 0.084	0.20

NOTE: V_G = genetic variance, V_{EW} = within pair environmental variance, V_{EB} = between pair environmental variance.

TABLE 8

ESTIMATE OF GENETIC VARIANCE IN FACTOR VIII CONCENTRATION DUE TO ABO BLOOD TYPE

	VIIIRAg	VIIIRAg corrected for VIIICAg	VIIICAg	VIIICAg corrected for VIIIRAg
Variance within ABO blood type V_{ABO}	0.8133	0.5877	0.9401	0.7014
Total variance V	0.9975	0.6668	1.0018	0.7121
Genetic variance V_G	0.6155	0.4254	0.5056	0.2691
$\frac{V - V_{ABO}}{V_G}$	0.30	0.19	0.12	0.04

with diabetes [20]. The plasma concentration of VIIIRAg may, thus, be an indicator of endothelial damage [19].

The association between plasma factor VIII concentration and ABO blood type was found to be mainly an effect of ABO genes on VIIIRAg (table 5). The plasma concentration of factor VIII seems to be inversely related to the amount of H antigen (table 5), as the descending order of strength of H is O, A_2, B, A_1 [21]. Recently, Sodetz et al. [22] demonstrated that purified factor VIII possesses covalently bound oligosaccharides with A, B, and H blood group activities. It remains to be explained, however, how this finding can be related to the higher VIIIRAg concentration in individuals with blood type A. VIIIRAg is essential for platelet adhesion to vascular subendothelium. A lower risk of myocardial infarction [23] and atherosclerosis [24] has been found in persons with blood type O compared to blood type A. The possibility therefore existed that VIIIRAg is a risk factor for the development of coronary heart disease. However, the Northwick Park Heart Study, a prospective study of cardiovascular death, showed that VIIIC but not VIIIRAg was a risk factor [25]. It therefore does not seem probable that the lower risk for myocardial infarction in blood type O individuals could be explained by the associated low VIIIRAg concentration.

Twin studies are suitable to estimate the strength of the genetic influence but cannot give information on the mode of inheritance. Current information on the genetic control of the factor VIII complex was recently reviewed by Graham [5]. At least four genetic variants that affect factor VIII are known: classical X-linked hemophilia A, a dominant hemophilia A [26], dominant von Willebrand disease, and the combined factor V/factor VIII deficiency [27]. The genetic influence on the plasma concentration of VIIIRAg could be due to the existence of several

TABLE 9

INTRACLASS CORRELATION FOR VIIICAg IN DZ TWIN PAIRS

Age (yrs)	Females	Males
33–39	.213	.283
57–62	.396	.354

alleles at each of these loci, in addition to the effect of the *ABO* locus and other unknown loci. This is in agreement with previous family studies [1]. We found no support in our material for a large effect of X-linked isoalleles on the normal variation of VIIICAg (table 9) as was found by Filippi et al. [3] for VIIIC. However, our study was designed to estimate the heritability of factor VIII, and a significant effect of X-linked alleles on VIIICAg cannot be excluded. Furthermore, it is possible that a different result would be obtained if VIIIC instead of VIIICAg had been determined. The normal variation in factor VIII level may well be due to the effect of many autosomal genes in addition to X-linked isoalleles.

The relationship between IXAg and IXC seems to be a simple one, and the coefficient of correlation between the plasma concentration of IXC and IXAg is high [28]. Our present study gave no indication of a significant genetic influence on the plasma concentration of IXAg. Thus far, our knowledge of the loci affecting the factor IX molecule is limited to the X-linked locus for hemophilia B. However, Lester et al. [29] found a possible bimodality in the distribution of IXC in 206 young men, with only 2% of individuals belonging to the distribution with the highest activity. The number of male twin pairs in each age group in our present study was too small to uncover such a bimodality.

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