

Sources of Variability in Antihemophilic Factor (Factor VIII) Procoagulant Titers and Precipitating Antigen Levels Among Obligate Carriers of Classic Hemophilia

By Paul K. Jones and Oscar D. Ratnoff

Chediak et al. have reported that the titer of procoagulant antihemophilic factor (AHF:C; factor VIII:C) was significantly lower in obligate carriers of classic hemophilia who were daughters of affected men (paternal carriers) than in those whose fathers were normal by history (maternal carriers). In contrast, among 113 obligate carriers of hemophilia, no significant difference in procoagulant AHF

titers was observed between paternal and maternal carriers. The concentration of AHF-like precipitating antigens, however, was significantly higher in maternal than in paternal carriers. This difference may have reflected in part the greater severity of disease in affected males in the families of maternal carriers.

CHEDIAK ET AL.¹ reported recently that the procoagulant titer of antihemophilic factor (AHF:C; factor VIII:C) was significantly lower in obligate carriers of classic hemophilia (hemophilia A) who were daughters of affected men than in those carriers whose fathers were normal by history. The concentrations of precipitating AHF-like antigens (AHF:Ag; factor VIII:Ag) detected by heterologous antiserum were similar in the two groups. They concluded that the lower procoagulant AHF titers in carriers whose fathers were hemophiliacs "may indicate dominance of the father's affected X chromosome during X-chromosome selection in embryogenesis."

of hemophilia in the affected males in families of maternal compared to paternal carriers.

MATERIALS AND METHODS

Carrier Groups

Obligate carriers of classic hemophilia were classified as paternal or maternal carriers. Paternal carriers ($n = 45$) were daughters of patients with classic hemophilia recognized by history or by test. Maternal carriers ($n = 68$) were women who had at least one hemophilic son and were daughters of known carriers ($n = 46$), or mothers of multiple hemophilic sons in families in which there was no earlier family history of the disease ($n = 22$). Results are reported separately for the two subgroups of maternal carriers as well as for the combined group. Carriers were excluded if pregnant or diabetic.

This interesting study has prompted a review of our own data obtained in 113 obligate carriers of classic hemophilia. Contrary to Chediak's experience, no significant difference in procoagulant AHF titer was observed between carriers who were daughters of hemophiliacs (called "paternal carriers" by Chediak et al.¹) and those whose fathers were said to be normal ("maternal carriers"). In contrast, the concentration of AHF-like antigens was significantly higher in maternal than in paternal carriers. This difference appeared to have reflected in part the greater severity

Blood Collection and Definition of Severity of Disease

Citrated plasma was prepared from the blood of volunteer normal individuals, patients with classic hemophilia, and obligate carriers, as reported earlier;² venipuncture was performed after obtaining informed consent. Procoagulant AHF activity was measured by a modified partial thromboplastin time, described earlier;^{3,4} the geometric standard deviation of replicate assays was $\pm 7.5\%$ of any given value.² Precipitating AHF-like antigens were measured by a modification^{5,6} of Laurell's⁷ semiquantitative immunoelectrophoretic technique. In early cases ($n = 19$ carriers), this assay was performed using an ethanol-insoluble fraction of plasma;⁵ in later cases ($n = 94$ carriers), the assay was carried out with whole plasma.⁶ The geometric standard deviation for replicate antigen assays was $\pm 12\%$ of any given value.² As the results of the two methods did not differ significantly, they have been pooled in the data presented.

From the Departments of Biometry and Medicine, Case Western Reserve University, School of Medicine, and University Hospitals of Cleveland, Cleveland, Ohio.

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Address reprint requests to Oscar D. Ratnoff, M.D., Department of Medicine, University Hospitals of Cleveland, Cleveland, Ohio 44106.

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All carrier assays were compared to assays performed on standard pools of 24 or 25 normal male plasmas;⁵ the pools were arbitrarily said to contain 1.00 U/ml of procoagulant AHF and of AHF-like antigens. Obligate carriers tend to have about half the procoagulant AHF levels of normals and may have significantly elevated AHF-like antigens.² Hemophilic patients were described as severely affected if the procoagulant titer was <0.01 U/ml; moderately affected if the titer was 0.01–0.04 U/ml; and mildly affected if the titer was 0.05 U/ml or higher. These definitions vary slightly from those used by Chediak et al.,¹ who defined severe hemophiliacs as those patients with procoagulant AHF titers of <0.01 or <0.02

Table 1. Age, AHF Procoagulant Activity, and AHF Precipitating Antigen in Obligate Carriers of Classic Hemophilia

Group	Number	Age (yr) Mean \pm SD	AHF Procoagulant Activity (U/ml)		AHF-like Antigen (U/ml)	
			Geometric Mean \pm SE	Range	Geometric Mean \pm SD	Range
Paternal carriers	45	29* \pm 15	0.51 \pm 6%	0.24–1.22	1.00* \pm 5%	0.43–1.96
Maternal carriers	68	45* \pm 14	0.58 \pm 6%	0.14–1.80	1.33* \pm 6%	0.48–3.50
Daughters of carriers	46	44 \pm 14	0.57 \pm 7%	0.14–1.55	1.38 \pm 7%	0.48–3.50
Mothers of multiple patients —no family history known	22	48 \pm 16	0.58 \pm 12%	0.18–1.80	1.23 \pm 10%	0.73–2.90
Total	113	39 \pm 17	0.55 \pm 4%	0.14–1.80	1.19 \pm 4%	0.43–3.50

* $p < 0.01$ for t test difference in arithmetic or geometric means between paternal and maternal carriers.

U/ml, and mild hemophiliacs as those with titers of >0.03 or >0.04 U/ml. Affected individuals who were not examined but who had histories of repeated hemarthroses were arbitrarily assigned to the severe group.

Statistical Methods

Logarithmic transformation of procoagulant AHF and AHF-like antigen values was performed to reduce skewedness.^{2,8} The geometric mean was defined as the antilogarithm of the mean of the transformed values. The geometric standard error (expressed in percent form) was obtained as follows. First, the standard deviation of the logarithmic AHF values was divided by the square root of the sample size (number of carriers). Second, the antilogarithm of this quotient was obtained. Third, unity was subtracted and the resultant difference was multiplied by 100 to convert from decimal to percent form.

Comparison of geometric mean procoagulant AHF titers and AHF-like antigens in paternal and maternal carriers was performed in two ways. In unadjusted comparisons (i.e., ignoring the carrier's age or severity of disease in affected male patients), ordinary two-sample t tests or analyses of variance were employed. Here, each obligate carrier was classified according to the type of family history (paternal or maternal). In adjusted comparisons, each subject was cross-classified by type of family history and by the severity of hemophilia (i.e., severe, moderate, or mild) in affected males. Two-way analysis of variance⁸ was employed in order to examine variation in geometric mean procoagulant AHF or AHF-like antigen according to the type of family history within each category of severity. Two-way analysis of covariance⁸ was also used

to determine whether any existing differences in procoagulant AHF or AHF-like antigen might reflect age-related increases in titers. The covariate used was the age of the carrier at the time of testing.

RESULTS

On the average, paternal carriers of classic hemophilia were 16 yr younger than maternal carriers (29 versus 45 yr, $p < 0.01$) (Table 1). No difference was observed in geometric mean AHF procoagulant activity (0.51 U/ml versus 0.58 U/ml), but paternal carriers had lower geometric mean AHF-like antigen concentrations (1.00 versus 1.33 U/ml, $p < 0.01$). No significant differences were observed in the mean age or geometric mean procoagulant activity or AHF-like antigen between those maternal carriers who were daughters of carriers and those carriers who were mothers of multiple patients who had no other known affected relatives.

The AHF-like antigen levels in carriers is higher in those in whom the disorder in the affected male family members is severe.² We therefore classified both paternal and maternal carriers according to the severity of disease (Table 2). Among paternal carriers, 18 of 45 (40%) were mildly affected versus 8 of 68 (12%) among maternal carriers ($p < 0.01$ by chi square test

Table 2. Obligate Carriers and the Severity of Hemophilia in Affected Male Family Members

Group	Number (%)	Severity of Hemophilia*			Unknown
		Severe <0.01 (U/ml)	Moderate 0.01–0.04 (U/ml)	Mild 0.05 + (U/ml)	
Paternal carriers	45 (100%)	14 (31%)	13 (29%)	18 (40%)	0 (0%)
Maternal carriers	68 (100%)	47 (69%)	11 (16%)	8 (12%)	2 (3%)
Daughters of carriers	46 (100%)	30 (65%)	9 (20%)	5 (11%)	2 (4%)
Mothers of multiple patients— no family history known	22 (100%)	17 (77%)	2 (9%)	3 (14%)	0 (0%)
Total	113 (100%)	61 (54%)	24 (21%)	26 (23%)	2 (2%)

*Severity of hemophilia is determined by the average AHF procoagulant activity titer (U/ml) in affected family members. Each row sums to 100%.

Table 3. Age, AHF Procoagulant Activity, and AHF Precipitating Antigen in Obligate Carriers by Severity of Disease in Affected Male Patients

Severity of Hemophilia	Number	Age (yr) Mean \pm SD	AHF Procoagulant Activity (U/ml)		AHF-Like Antigen (U/ml)	
			Geometric Mean \pm SE	Range	Geometric Mean \pm SE	Range
Severe (<0.01 U/ml)	61	42* \pm 16	0.58 \pm 7%	0.18–1.80	1.36† \pm 6%	0.61–3.50
Moderate (0.01–0.04)	24	32* \pm 20	0.53 \pm 10%	0.14–1.16	1.12† \pm 8%	0.54–2.56
Mild (0.05 +)	26	35* \pm 12	0.49 \pm 6%	0.26–0.90	0.90† \pm 8%	0.43–1.96
Unknown	2	48 \pm 11	0.50 \pm 8%	0.46–0.54	1.48 \pm 35% –26%	1.10–2.00
Total	113	39 \pm 17	0.55 \pm 4 %	0.14–1.80	1.20 \pm 4%	0.43–3.50

*Mean age of obligate carriers differs significantly according to severity of disease in affected male patients ($p < 0.01$ for severe versus moderate, $p < 0.05$ for severe versus mild).

†Geometric mean antigen titer differs significantly according to disease in affected male patients ($p < 0.05$ for severe versus moderate, $p < 0.01$ for severe versus mild, $p < 0.05$ for moderate versus mild).

of association). Conversely, only 14 of 45 (31%) of the paternal carriers were severely affected, versus 47 of 68 (69%) of the maternal carriers. Daughters of carriers and mothers of multiple patients who had no other family history did not differ significantly in the distribution of the degree of severity within the family.

The mean age of carriers and the geometric mean procoagulant activity and AHF-like antigen for 113 obligate carriers were grouped according to the degree of severity of disease in the affected individuals (Table 3). Obligate carriers whose male relatives were severely affected were, on the average, about 10 yr older than obligate carriers with moderately affected relatives ($p < 0.01$ by two-sample *t* test), and were, on the average, about 7 yr older than obligate carriers with mildly affected relatives ($p < 0.05$). With both AHF procoagulant activity and AHF-like antigen, there was an apparent trend such that obligate carriers in families with mildly affected hemophiliacs had

lower geometric mean titers. The trend was significant, however, only for AHF-like antigens ($p < 0.05$ for severe versus moderate, $p < 0.01$ for severe versus mild, and $p < 0.05$ for moderate versus mild).

The combined influence of severity of illness and age on procoagulant AHF and AHF-like antigens was examined for paternal and maternal carriers (Table 4). In the "unadjusted" column, the ordinary geometric means were recorded. In the "age-adjusted" column, the calculated geometric mean was adjusted upward or downward by covariance adjustment to reflect the procoagulant AHF mean that would be observed were the mean age in each cross-classified group 39 yr, that is, the mean age of the total sample.⁸ This adjustment reflects the fact that, within the subgroups examined, procoagulant AHF increased at about 1.7% of any given value per decade (95% confidence interval was -4.5% – 8.3% /decade). Weighted means for paternal and maternal carriers were obtained by multiplying the logarithmically trans-

Table 4. AHF Procoagulant Activity and Precipitating Antigen Geometric Means (U/ml) in Obligate Carriers by Type of Inheritance and Severity of Disease

Group	Severity of Hemophilia	Patient's Procoagulant AHF	Number of Obligate Carriers	AHF Procoagulant Activity		AHF-Like Antigen	
				Unadjusted	Age-Adjusted	Unadjusted	Age-Adjusted
Paternal	Severe	$<.01$	14	0.58	0.58	1.17	1.20
	Moderate	.01–.04	13	0.49	0.50	0.94†	1.02
	Mild	.05 +	18	0.48	0.48	0.93	0.96
	Weighted mean‡		(45)	0.53	0.53	1.06	1.10
Maternal	Severe	$<.01$	47	0.58	0.58	1.42	1.39
	Moderate	.01–.04	11	0.58	0.57	1.38†	1.33
	Mild	.05 +	8	0.54	0.54	0.82	0.81
	Weighted mean‡		(66)*	0.57	0.57	1.24	1.21

*Excludes two obligate carriers in which the severity of disease in affected family members was unknown.

† $p < 0.05$ for *t* test difference in geometric mean procoagulant AHF or AHF-like antigen levels between maternal and paternal carriers.

‡Correction for severity.

formed geometric means in each severity category by the corresponding proportion in the combined paternal and maternal carriers (Table 2), summing the products, and taking the antilogarithms. The procoagulant AHF weighted means were 0.53 and 0.57 U/ml for paternal and maternal carriers, respectively (Table 4). With adjustment for both age and severity of disease, the weighted procoagulant AHF means were also 0.53 and 0.57 U/ml, respectively. Neither unadjusted nor age-adjusted weighted mean procoagulant AHF values differed significantly for paternal and maternal carriers.

AHF-like antigen weighted means did not significantly differ between paternal and maternal carriers when adjustment was made for the severity of disease (1.06 U/ml versus 1.24 U/ml, not significant). Within the subgroups, AHF-like antigen geometric mean titers increased at a rate of about 4.4% of any given value per decade (95% confidence interval -1.1%–10.2%/decade). Using two-way analysis of covariance (adjusted for both severity of disease and age of the carrier at time of testing), paternal carriers did not differ from maternal carriers in weighted mean AHF-like antigen (1.10 U/ml versus 1.21 U/ml, not significant).

Although the weighted mean difference between paternal and maternal carriers was not significant for procoagulant AHF or AHF-like antigens, this was not true for a subset of carriers whose male relatives had procoagulant titers of 0.01–0.04 U/ml (i.e., moderately affected hemophiliacs). In this group the non-age-adjusted procoagulant AHF titer was 0.49 U/ml in 13 paternal carriers and 0.58 U/ml in 9 maternal carriers (not significant); the antigen titer in these carriers was 0.94 and 1.38 U/ml ($p < 0.05$). The significant difference in antigen titer was attributable to the inclusion of two maternal carriers who had procoagulant AHF titers of 0.86 and 1.16 U/ml, respectively, and antigen titers of 2.00 and 2.56 U/ml, respectively. We had no information to explain these high titers. These carriers' respective ages were 41 and 48 yr; after adjustment for age, no significant difference was observed in either procoagulant AHF or AHF-like antigen.

DISCUSSION

Chediak et al.¹ compared the procoagulant titer of AHF in the plasmas of 23 obligate carriers of classic hemophilia who were daughters of hemophiliacs ("paternal carriers") and 39 carriers whose fathers were normal by history ("maternal carriers"). Maternal carriers had a mean titer of AHF procoagulant activity that was 54% higher than that of paternal carriers

($p < 0.001$). These authors excluded from consideration those carriers who were taking oral contraceptives or who had acute or chronic illnesses, including thyroid disease.

In Chediak's¹ series, paternal carriers were, on the average, 28 yr younger than maternal carriers. This difference in age at the time of testing was predominantly due to the fact that maternal carriers can be identified only after they have had one or more hemophilic children, whereas paternal carriers are identified as such at birth. In their experience, the age at the time of testing correlated 0.197 with AHF procoagulant activity among paternal carriers, and 0.211 among maternal carriers, neither reaching significance.

The small sample size in Chediak's series provides a lack of certainty regarding the magnitude of correlation coefficients that might be observed in larger samples. Were an association to exist between the age of the carriers at the time of testing and the AHF procoagulant titer observed, this effect would distort the difference between the titers of paternal and maternal carriers. For example, in Chediak's 62 obligate carriers, the correlations of procoagulant AHF with age correspond to an absolute increase of 2.5 U/decade in the paternal carriers and 3.9 U/decade in the maternal carriers—an average increase of about 3.4 U/decade in the combined sample. Thus, the difference in procoagulant arithmetic means for paternal versus maternal carriers (41.5 U versus 64.1 U) may partially reflect age-related increases in procoagulant AHF levels. Covariate adjustment indicated that perhaps four-tenths of the apparent difference might be attributed to age; the small sample sizes and the nonoverlapping age distributions precluded a more definitive answer. As noted above, the present series of 113 obligate carriers shows a slightly positive (but nonsignificant) association of age with both procoagulant AHF and AHF-like antigen.

A second possible confounding characteristic considered by Chediak et al.¹ was the severity of hemophilia in affected family members. Seventy-four percent of the paternal carriers were daughters of severe hemophiliacs, while 72% of the maternal carriers had severely affected sons. Severe hemophiliacs were said to have <0.01 or <0.02 U AHF procoagulant activity. When these authors examined only those carriers related to severe hemophiliacs, the difference in AHF procoagulant activity was more evident than in the complete series.

Contrary to the findings of Chediak et al.,¹ we found striking differences in the severity of disease in affected family members of paternal versus maternal

carriers. Only 31% of the paternal carriers versus 69% of the maternal carriers were severe by our criteria. This distribution might be anticipated, as until recently mildly affected hemophiliacs were more likely to have children than those more severely affected. As we reported earlier,² severe hemophilia in affected family members is associated with a relatively higher concentration of AHF-like antigens in obligate carriers. In the present series, neither age nor procoagulant activity nor AHF-like antigens systematically varied with paternal versus maternal carrier status when severity of disease in family members was taken into account.

As noted above, Chediak et al.¹ classified patients as either severe (<0.01 or <0.02 U/ml) or mild (>0.03 or >0.04 U/ml). Using their criteria, we would have 21 obligate paternal carriers and 50 obligate maternal carriers whose male relatives were severely affected. The procoagulant AHF geometric means did not differ significantly for paternal versus maternal carriers (0.58 U/ml versus 0.57 U/ml), but the AHF antigen titer geometric mean did (1.12 U/ml versus 1.40 U/ml, $p < 0.05$). After adjustment for age, however, neither procoagulant AHF nor AHF antigen titers differed significantly between paternal and maternal carriers classified in the "severe" group by Chediak's criteria. The geometric mean procoagulant AHF was half that of the normal control mean of 1.00 U/ml (within limits of sampling variability) for both paternal and maternal carriers.

We have confirmed the observations made by

Chediak et al.¹ that, within the group of maternal carriers, no systematic differences in age or AHF titers exist between daughters of carriers and mothers of multiple hemophilic sons with no family history of the disease. In our series, daughters of carriers had a mean age of 44 (SD = 14) while mothers of multiple patients had a mean age of 48 (SD = 16) (Table 1). The AHF procoagulant activities of these two groups were 0.57 and 0.58 U/ml, respectively, whereas the geometric means of AHF-like antigens were 1.38 and 1.23, respectively. Approximately the same percentage had severely affected family members (65% and 77%).

In summary, we have examined the difference in geometric mean procoagulant AHF and AHF-like antigen between paternal and maternal carriers. Two possible confounding variables have also been studied: age of carrier at time of testing and severity of disease in affected male family members. In the series of 62 obligate carriers presented by Chediak et al.,¹ the former variable appeared to account for four-tenths of the apparent difference in procoagulant AHF between paternal and maternal carriers. In the current series of 113 obligate carriers, the apparently significant difference in geometric mean AHF-like antigen between paternal and maternal carriers did not persist when comparison was made within degree of severity of disease within families. Thus, we conclude that there is no need to postulate dominance of the father's affected X chromosome during X-chromosome selection in embryogenesis.

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