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Segregation Analysis of Hemophilia A and B

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SUMMARY

We analyzed a sample of 1,485 families with hemophilia A and B and with unknown diagnosis. The frequency of sporadic cases was estimated to be .166 and .078 for the two types of hemophilia, respectively. The sex ratio of mutation rates did not differ significantly from unity.

The average age of maternal grandfathers of probands at birth of mothers with a single child, affected by hemophilia B, and of maternal grandfathers of probands at birth of mothers with more than one child affected by hemophilia B, was higher than the age in appropriate control groups.

INTRODUCTION

In 1968, we presented results of segregation analysis of data collected from families of patients with hemophilia A and B [1]. The analysis showed that (1) there was no significant segregation distortion in hemophilia A or B, (2) there was a significant fraction of sporadic cases in each disorder, and (3) the average age of maternal grandfathers of probands at birth of heterozygous mothers, in sibships with no previous history of hemophilia, was not higher than in comparable control groups. We were unable to show that (4) there was a difference in the rate of mutation to the hemophilia allele in eggs and sperm.

The analysis has been criticized [2, 3] on the basis of the methods of ascertainment and the methods of segregation analysis used. Our response to this

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criticism has been published in this journal [4]. The criticism to our initial investigation originates in the controversy about presence or absence of sex differences in mutation rates [2]. For X-linked recessive disorders, such as hemophilia, differing mutation rates in eggs and sperm can be investigated at equilibrium through the estimate of the fraction of sporadic cases (over all cases) and the estimate of the selection coefficient for affected males [5, 6]. The precision of these estimates and the assumption of equilibrium between selection and mutation are crucial in the assessment of sex differences in mutation rates.

Modern methods of segregation analysis [7] provide precision in the estimate of the fraction of sporadic cases; however, the assumption of equilibrium is weak, since the fitness of the hemizygotes has been changing in the present century.

After our initial investigation, we continued to receive additional family data on hemophilia. We now have data on 1,485 families: 949 families with hemophilia A, 209 with hemophilia B, and 327 with hemophilia of uncertain or unspecified diagnosis; the latter, although considered in previous work [1], were not taken into account for segregation analysis in the present study. The expanded data have been analyzed to reestimate the sporadic cases frequency by segregation analysis. We have also analyzed the expanded data for age effects among parents and grandparents of probands for each type of hemophilia; the analysis is also pertinent to the issue of sex differences in mutation rates.

We present our data in such a way that anyone having access to a computer program for segregation analysis [7] may analyze them.

MATERIALS AND METHODS

Families were classified into one of six categories: hemophilia A, hemophilia B, and hemophilia of unknown type (U), with or without a family history of the disease. A family history was considered positive if hemophilia occurred in previous generations or in sibships other than that of the proband. For each family, we had available the number of probands and the number of affected and normal brothers in the male sibship. The sibships composed of one affected individual only were referred to as "uniplex"; when there were both one affected and other healthy male sibs, the sibship was referred to as "simplex"; when two or more hemophiliac males were present, the sibship was referred to as "multiplex." Besides the age of onset of the disease in the proband, the country of residence of the family and the dates of birth of the proband, of his parents, and of his grandparents were known. The procedure of data collection, with its possible advantages and shortcomings, has been discussed [1, 4].

The general plan of segregation analysis for each type of hemophilia was the following: (1) The probability of ascertainment π was estimated using all data for that type of hemophilia, and heterogeneity among countries was tested. Significant heterogeneity was the criterion for separate estimation of π by country of origin of families, with and without family history. (2) The hypothesis $p = .5$, or, in other words, of complete penetrance, was tested at the appropriate value of the probability of ascertainment in multiplex families from different countries. When the hypothesis did not fit at the observed value of π, the group of families was excluded from the total sample. (3) The frequency of sporadic cases was estimated for all data for each type of hemophilia at the appropriate values of p and π.

Finally, the average age of parents at birth of the proband, and of grandparents of the proband at birth of his parents, was estimated in the 18 groups resulting from classification of families as uniplex, simplex, or multiplex; by type of hemophilia; and by presence or absence of history.

RESULTS

Probability of Ascertainment

The probability of ascertainment, π, was estimated from the distribution of a probands among r affected individuals in multiplex sibships, from the following equation [6]:

$$P(a|r) = \frac{\binom{r}{a} \pi^a (1 - \pi)^{r-a}}{1 - (1 - \pi)^r} .$$

In table 1, we present the distribution of the families by number of probands in a male sibship for families with and without a history of hemophilia by country of origin.

The probability of ascertainment was first estimated using all data for each type of hemophilia in multiplex families, and we obtained the following values for the chi-squares of heterogeneity among families:

Type of hemophilia	π	Chi-square	Degrees of freedom	P
A	.461	305.847	246	< .005
B	.571	80.575	59	< .05

Analysis of data for each type of hemophilia reveals significant heterogeneity of π among all groups (with or without family history and from all countries contributing data). For hemophilia A as well as for the B form, the heterogeneity is accounted for by variation of π among countries, while there is no significant difference in ascertainment of families with and without history of the disease (since some countries contributed no cases of hemophilia B, the degrees of freedom are diminished accordingly):

Type of hemophilia	All groups (country of origin and family history)		Country of origin		Family history	
	χ^2	df	χ^2	df	χ^2	df
A	109.275	23	98.475	11	10.800	12
B	42.966	16	38.437	9	4.529	7

On the basis of this analysis, we estimated π for each type of hemophilia in each country. Such estimates were based on the distributions of probands among affected males in multiplex sibships. Thus, in subsequent analyses, the

TABLE 1

DISTRIBUTION OF a PROBANDS AMONG r AFFECTED BY COUNTRY

	No history					History						
	$r\backslash a$	1	2	3	4	5	$r\backslash a$	1	2	3	4	5	6
						A. Hemophilia A							
Germany	1	45	–	–			1	16	–	–	–		
	2	5	2	–			2	6	2	–	–		
		...					3	–	–	1	–		
Argentina	1	48	–	–			1	32	–	–	–		
	2	8	6	–			2	5	4	–	–		
		...					4	1	–	–	–		
		...					5	1	–	–	–		
Yugoslavia	1	26	–	–			1	14	–	–	–		
	2	8	3	–			2	1	5	–	–		
	3	1	1	–			3	–	2	1	–		
Sweden	1	2	–	–			1	6	–	–	–		
	2	1	–	–			2	3	–	–	–		
	3	1	–	–			3	1	–	–	–		
Norway	1	7	–	–			1	10	–	–	–		
	3	2	–	–			2	4	–	–	–		
	4	1	–	–			3	2	–	–	–		
Italy	1	22	–	–			1	9	–	–	–		
	2	2	2	–			2	1	1	–	–		
	3	2	1	–					...				
	4	–	1	–					...				
Netherlands	1	4	–	–			1	11	–	–	–		
	2	1	3	–			2	1	5	–	–		
		...					3	–	–	1	–		
		...					4	–	–	–	1		
USA and Canada ..	1	172	–	–			1	134	–	–	–		
	2	26	11	–			2	26	9	–	–		
	3	7	1	1			3	11	1	4	–		
	6	1	–	–			4	1	–	1	–		
		...					5	1	–	–	–		

Table 1 continued on next page

TABLE 1 *(continued)*

A. Hemophilia A *(continued)*

Australia	1	14	–	–			1	8	–	–	–	
	2	1	1	–			2	4	–	–	–	
	3	3	–	–			3	1	–	–	–	
	5	1	–	–			4	2	–	–	–	
Turkey	1	4	–	–			1	1	–	–	–	
	2	1	–	–			2	1	–	–	–	
	6	–	1	–					...			
United Kingdom ...	1	71	–	–			1	2	–	–	–	
	2	8	–	–			2	3	–	–	–	
	3	2	–	–					...			
Denmark	1	19	–	–			1	25	–	–	–	
	2	–	5	–			2	–	9	–	–	
	3	–	–	1			3	–	–	2	–	

B. Hemophilia B

Germany	1	17	–	–	–	–	1	7	–	–	–	–
	2	–	1	–	–	–				...		
Argentina	1	7	–	–	–	–	1	4	–	–	–	–
	2	–	1	–	–	–	3	–	1	–	–	–
Yugoslavia	1	3	–	–	–	–	1	3	–	–	–	–
	2	–	1	–	–	–	2	1	–	–	–	–
			...				3	–	1	–	–	–
Sweden	2	1	–	–	–	–	2	1	–	–	–	–
			...				3	1	–	–	–	–
Norway	1	4	–	–	–	–	1	2	–	–	–	–
			...				2	6	–	–	–	–
Italy	1	4	–	–	–	–	1	2	–	–	–	–
			...				2	1	–	–	–	–
Netherlands	2	1	1	–	–	–	1	7	–	–	–	–
			...				2	2	–	–	–	–

USA and Canada ..	1	32	–	–	–	–	1	30	–	–	–	–	
	2	5	8	–	–	–	2	5	4	–	–	–	
	3	2	–	–	–	–	3	–	–	1	–	–	
	4	1	–	–	–	–	5	1	–	–	–	–	
			...				6	1	–	–	–	–	
Australia	1	4	–	–	–	–	1	9	–	–	–	–	
	2	1	–	–	–	–	3	3	–	–	–	–	
Turkey	1	1	–	–	–	–				...			
Denmark	1	4	–	–	–	–	1	9	–	–	–	–	
	3	–	–	1	–	–	2	–	3	–	–	–	
	5	–	–	–	–	1	3	–	–	2	–	–	
			...				5	–	–	–	–	1	

C. Hemophilia with unknown diagnosis

Germany	1	1	–	–	–	–				...			
Argentina	1	2	–	–	–	–				...			
Yugoslavia	2	1	–	–	–	–	1	2	–	–	–	–	–
	3	–	1	–	–	–	2	–	1	–	–	–	–
			...				4	1	–	–	–	–	–
Norway	1	2	–	–	–	–	1	2	–	–	–	–	–
	2	3	–	–	–	–	2	2	–	–	–	–	–
	3	1	–	–	–	–	3	1	–	–	–	–	–
Italy	1	1	–	–	–	–	1	1	–	–	–	–	–
USA and Canada ..	1	72	–	–	–	–	1	64	–	–	–	–	–
	2	13	5	–	–	–	2	18	13	–	–	–	–
	3	7	1	2	–	–	3	5	–	–	–	–	–
			...				4	1	–	–	–	–	–
			...				5	1	–	–	–	–	–
			...				6	1	1	–	–	–	–
Australia	1	2	–	–	–	–	1	3	–	–	–	–	–
	2	1	–	–	–	–	2	1	–	–	–	–	–
			...				3	1	–	–	–	–	–
Denmark	1	21	–	–	–	–	1	44	–	–	–	–	–
	2	–	3	–	–	–	2	–	14	–	–	–	–
	3	–	–	1	–	–	3	–	–	5	–	–	–
	4	–	–	1	–	–	5	–	–	–	–	1	–
	5	–	–	–	–	2	6	–	–	–	–	–	1

genetic parameters of interest were estimated by country of origin with the value of π appropriate for that country and for that form of hemophilia.

Segregation Frequency

In order to test that the segregation frequency, p, is equal to the expected Mendelian value .5, we evaluated the maximum likelihood scores and the information about p and π when there is more than one affected individual in a family [7]. The distribution of r affected in families of size s is

$$P(r|r > 1) = \frac{\binom{s}{r} p^r q^{s-r} [1 - (1 - \pi)^r]}{1 - (1 - p\pi)^s - sp\pi q^{s-1}} .$$

The distribution of affected by male sibship size and country is given in table 2. A summary of the segregation analysis of the multiplex families irrespective of family history is presented in table 3, where U is the score for a parameter, K is the amount of information for the parameter, and U^2/K is distributed as a chi-square with 1 degree of freedom (df). There is a good fit of the hypothesis that $p = .5$ for hemophilia B. The hypothesis did not fit for hemophilia A; the deviation from $p = .5$ is significant.

From table 3, we observe that the only score that deviates significantly, and which is responsible for the heterogeneity, is the score for the families from Argentina (chi-square = 19.40 with 1 df). When the families from Argentina are excluded, no significant deviation from $p = .5$ is observed in multiplex families (chi-square = .114, df = 1, nonsignificant) or any heterogeneity of p between countries (chi-square = 12.34, df = 10, nonsignificant). Therefore, we separated the families from Argentina from the set; segregation distortion in the data from this country, which might be due to any cause, would influence the estimate of the frequency of the sporadic cases, and presumably did in our study of 1968 [1]. The remaining families are then used for subsequent analysis.

Frequency of Sporadic Cases

The frequency of sporadic cases, x, is estimated by comparing the observed distribution of simplex families of sibship size s with that of the multiplex families having the same size, irrespective of presence or absence of family history. The distribution of simplex families is

$$P(r = 1|s, p, x, \pi) = \frac{sp\pi [x + (1 - x) q^{s-1}]}{sp\pi x + (1 - x) [1 - (1 - p\pi)^s]} ,$$

and the distribution of multiplex families is

$$P(r > 1|s, p, x, \pi) = \frac{(1 - x) [1 - (1 - p\pi)^s - sp\pi q^{s-1}]}{sp\pi x + (1 - x) [1 - (1 - p\pi)^s]} .$$

The results of the tests of the hypothesis that $p = .5$ and $x = 0$ at the values of π estimated for each country are presented in table 4 for hemophilia A and B, both for families with and without history of the disease.

In families with history, nonsignificant disturbance of the segregation fre-

TABLE 2

DISTRIBUTION OF r AFFECTED BY MALE SIBSHIP OF SIZE s BY COUNTRY

	No history						History					
	$s\backslash r$	1	2	3	4	5	6	$s\backslash r$	1	2	3	4	5
						A. Hemophilia A							
Germany	1	32	–	–	–	–	–	1	7	–	–	–	–
	2	8	3	–	–	–	–	2	6	7	–	–	–
	3	1	2	–	–	–	–	3	3	1	1	–	–
	4	3	2	–	–	–	–			...			
	5	1	–	–	–	–	–			...			
Argentina	1	19	–	–	–	–	–	1	10	–	–	–	–
	2	17	3	–	–	–	–	2	8	3	–	–	–
	3	3	4	–	–	–	–	3	3	3	–	–	–
	4	1	1	–	–	–	–	4	1	–	–	–	–
	5	3	2	–	–	–	–	5	6	1	–	–	–
	6	3	2	–	–	–	–	6	1	1	–	1	–
	7	1	1	–	–	–	–	7	2	1	–	–	–
	8	1	–	–	–	–	–	10	1	–	–	–	–
	11	–	1	–	–	–	–	14	–	–	–	–	1
Yugoslavia	1	21	–	–	–	–	–	1	10	–	–	–	–
	2	1	7	–	–	–	–	2	1	4	–	–	–
	3	3	3	1	–	–	–	3	2	1	1	–	–
	4	1	–	1	–	–	–	4	1	1	2	–	–
	5	–	1	–	–	–	–			...			
Sweden	1	1	–	–	–	–	–	1	4	–	–	–	–
	2	–	1	–	–	–	–	2	2	1	–	–	–
	3	1	–	1	–	–	–	3	–	1	–	–	–
				...				4	–	1	1	–	–
Norway	1	6	–	–	–	–	–	1	7	–	–	–	–
	2	1	–	–	–	–	–	2	3	4	–	–	–
	4	–	–	–	1	–	–	3	–	–	2	–	–
	6	–	–	1	–	–	–			...			
	8	–	–	1	–	–	–			...			

Table 2 continued on next page

TABLE 2 *(continued)*

	No history						History					
	*s**r*	1	2	3	4	5	6	*s**r*	1	2	3	4	5
						A. Hemophilia A *(continued)*							
Italy	1	13	–	–	–	–	–	1	6	–	–	–	–
	2	6	2	–	–	–	–	2	–	2	–	–	–
	3	1	–	1	–	–	–	3	1	–	–	–	–
	4	1	1	–	1	–	–	5	2	–	–	–	–
	5	–	1	–	–	–	–			...			
	6	–	–	1	–	–	–			...			
	7	1	–	–	–	–	–			...			
	12	–	–	1	–	–	–			...			
Netherlands	1	1	–	–	–	–	–	1	8	–	–	–	–
	2	3	2	–	–	–	–	2	2	3	–	–	–
	3	–	2	–	–	–	–	3	1	1	–	–	–
				...				4	–	–	–	1	–
				...				5	–	1	–	–	–
				...				6	–	1	–	–	–
				...				7	–	–	1	–	–
USA and Canada	1	90	–	–	–	–	–	1	78	–	–	–	–
	2	62	17	–	–	–	–	2	42	18	–	–	–
	3	15	16	6	–	–	–	3	12	11	7	–	–
	4	4	3	–	–	–	–	4	1	5	6	2	–
	5	–	1	2	–	–	–	5	1	1	2	–	–
	6	–	–	1	–	–	1	6	–	–	–	–	1
	7	1	–	–	–	–	–	7	–	–	1	–	–
Australia	1	8	–	–	–	–	–	1	2	–	–	–	–
	2	5	1	–	–	–	–	2	4	2	–	–	–
	3	1	1	2	–	–	–	3	1	2	–	–	–
	4	–	–	1	–	–	–	4	1	–	–	1	–
	7	–	–	–	–	1	–	5	–	–	1	–	–
				...				7	–	–	–	1	–

Turkey	1	1	–	–	–	–	–	2	1	1	–	–	–
	2	2	1	–	–	–	–			...			
	5	1	–	–	–	–	–			...			
	7	–	–	–	–	–	1			...			
United Kingdom	1	40	–	–	–	–	–	2	1	1	–	–	–
	2	25	3	–	–	–	–	4	1	1	–	–	–
	3	2	3	1	–	–	–	6	–	1	–	–	–
	4	3	2	1	–	–	–			...			
	6	1	–	–	–	–	–			...			
Denmark	1	12	–	–	–	–	–	1	10	–	–	–	–
	2	4	3	–	–	–	–	2	11	4	–	–	–
	3	1	2	–	–	–	–	3	3	4	2	–	–
	4	1	–	–	–	–	–	4	1	1	–	–	–
	5	1	–	1	–	–	–			...			

	No history						History						
	s\r	1	2	3	4	5	*s\r*	1	2	3	4	5	6
							B. Hemophilia B						
Germany	1	12	–	–	–	–	1	4	–	–	–	–	–
	2	5	–	–	–	–	2	1	–	–	–	–	–
	3	–	1	–	–	–	3	2	–	–	–	–	–
Argentina	1	5	–	–	–	–	1	3	–	–	–	–	–
	2	1	–	–	–	–	2	1	–	–	–	–	–
	4	1	1	–	–	–	4	–	–	1	–	–	–
Yugoslavia	1	2	–	–	–	–	1	2	–	–	–	–	–
	2	1	–	–	–	–	2	1	–	–	–	–	–
	3	–	1	–	–	–	3	–	1	–	–	–	–
			...				4	–	–	1	–	–	–
Sweden	6	–	1	–	–	–	3	–	1	–	–	–	–
			...				7	–	–	1	–	–	–
Norway	1	3	–	–	–	–	1	2	–	–	–	–	–
	2	1	–	–	–	–	2	–	4	–	–	–	–
			...				4	–	1	–	–	–	–
			...				7	–	1	–	–	–	–

Table 2 continued on next page

TABLE 2 *(continued)*

	No history						History						
	s\r	1	2	3	4	5	*s\r*	1	2	3	4	5	6
							B. Hemophilia B *(continued)*						
Italy	1	1	–	–	–	–	1	2	–	–	–	–	–
	2	3	–	–	–	–	2	–	1	–	–	–	–
			...				6	1	–	–	–	–	–
Netherlands	2	–	1	–	–	–	1	5	–	–	–	–	–
	3	–	1	–	–	–	2	1	2	–	–	–	–
			...				3	1	–	–	–	–	–
USA and Canada	1	19	–	–	–	–	1	18	–	–	–	–	–
	2	6	6	–	–	–	2	6	6	–	–	–	–
	3	6	6	–	–	–	3	5	2	–	–	–	–
	4	–	1	–	–	–	4	1	1	1	–	–	–
	5	1	–	–	–	–	8	–	–	–	–	1	–
	6	–	–	1	–	–	9	–	–	–	–	–	1
	7	–	–	1	–	–				...			
	8	–	–	–	1	–				...			
Australia	1	1	–	–	–	–	1	4	–	–	–	–	–
	2	3	1	–	–	–	2	3	–	–	–	–	–
			...				3	2	–	3	–	–	–
Turkey	4	1	–	–	–	–				...			
Denmark	1	4	–	–	–	–	1	5	–	–	–	–	–
	3	–	–	1	–	–	2	3	2	–	–	–	–
	6	–	–	–	–	1	4	1	–	–	–	–	–
			...				5	–	1	2	–	1	–
							C. Hemophilia with unknown diagnosis						
Germany	1	1	–	–	–	–				...			
Argentina	1	1	–	–	–	–				...			
	4	1	–	–	–	–				...			

Norway	1	1	–	–	–	–	1	1	–	–	–	–	–
	2	1	3	–	–	–	2	–	1	–	–	–	–
	4	–	–	1	–	–	3	–	1	1	–	–	–
	...						4	1	–	–	–	–	–
Yugoslavia	2	–	1	–	–	–	1	1	–	–	–	–	–
	3	–	–	1	–	–	2	–	1	–	–	–	–
	...						3	1	–	–	–	–	–
	...						4	–	–	–	1	–	–
Italy	1	1	–	–	–	–	1	1	–	–	–	–	–
USA and Canada	1	24	–	–	–	–	1	34	–	–	–	–	–
	2	29	14	–	–	–	2	17	19	–	–	–	–
	3	10	3	6	–	–	3	7	5	3	–	–	–
	4	6	1	2	–	–	4	3	5	–	–	–	–
	5	2	–	1	–	–	5	2	2	1	–	–	–
	6	1	–	1	–	–	6	1	–	1	–	–	1
	...						8	–	–	–	–	1	–
	...						9	–	–	–	1	–	1
Australia	1	1	–	–	–	–	1	3	–	–	–	–	–
	2	1	1	–	–	–	3	–	1	1	–	–	–
Denmark	1	9	–	–	–	–	1	20	–	–	–	–	–
	2	4	2	–	–	–	2	15	5	–	–	–	–
	3	5	–	1	–	–	3	7	4	–	–	–	–
	4	1	1	–	–	–	4	1	–	1	–	–	–
	5	1	–	–	–	–	5	1	3	1	–	1	–
	6	–	–	–	–	1	6	–	1	1	–	–	1
	7	1	–	–	1	–	7	–	–	2	–	–	–
	8	–	–	–	–	1	9	–	1	–	–	–	–

TABLE 3

TEST OF THE HYPOTHESIS $p = .5$ IN MULTIPLEX FAMILIES

	π	U_p	U_π	K_{pp}	$K_{\pi\pi}$	L
			A. Hemophilia A			
Germany	.519	−5.233	.582	27.192	.337	−3.620
Argentina	.487	−72.304	6.378	269.387	1.610	−21.810
Yugoslavia	.638	.639	−.214	58.578	.509	−8.452
Sweden	.001	−.378	.047	22.781	.356	−3.199
Norway	.001	2.051	.257	58.922	.920	−7.074
Italy	.481	−11.579	.512	91.318	.523	−9.361
Netherlands	.921	−8.518	.200	65.840	.029	−6.792
USA/Canada	.386	9.567	−.906	392.133	5.130	−44.499
Australia	.079	11.583	−1.451	89.217	1.349	−9.816
Turkey	.230	8.400	−.842	21.685	.254	−2.564
England	.001	−15.077	1.855	62.875	.982	−7.527
Denmark	.999	−1.720	0.000	42.355	0.000	−6.060
		−82.569		1202.283		−130.774
			B. Hemophilia B			
Germany	.999	−1.000	.000	3.000	.000	−.288
Argentina	.779	−.466	−.003	13.880	.058	−1.616
Yugoslavia	.650	−.434	.025	13.294	.122	−1.588
Sweden	.001	−11.841	1.481	42.925	.670	−3.664
Norway	.001	−11.044	1.381	29.893	.467	−3.197
Netherlands	.400	−1.160	.144	3.294	.051	−.342
USA/Canada	.494	−8.985	1.089	170.026	1.201	−11.644
Australia	.001	8.001	−.999	10.665	.167	−3.297
Denmark	.999	15.794	−.003	65.493	.000	−9.762
		−10.775		352.470		−35.398

NOTE: Scores (U), information (K), and log likelihood (L).

TABLE 4

HEMOPHILIA A: TEST OF THE HYPOTHESIS ($x = 0$, $p = .5$) AT THE VALUE OF π OBSERVED IN EACH COUNTRY

	U_p	U_x	K_{pp}	K_{xx}	χ^2 ($p = .5$)	χ^2 ($x = 0$)	L
Hemophilia A, families with history	−58.373	53.013	1025.064	1281.824	3.324	2.193	−141.127
Hemophilia A, families without history	−245.411	322.380	1230.401	7689.624	48.949	13.516	−192.930
Hemophilia B, families with history	−25.554	34.538	303.364	1341.288	2.153	.889	−42.939
Hemophilia B, families without history	−19.445	15.464	251.446	715.613	1.504	.334	−34.566

NOTE: Scores (U), information (K), log likelihood (L).

quency is observed (chi-square = 3.324, df = 1, $P < .10$) and there is no indication of presence of sporadic case (chi-square = 2.193, df = 1, nonsignificant), as expected in families in which previous hemophilia cases have been recorded. In this analysis, simplex families are, of course, included.

In families where there is no history of the disease, no fit of the hypothesis is observed; the score for p is negative, indicating a significant depression of the segregation frequency, and the score for x is positive, indicating the presence of sporadic cases among simplex families. A slight, nonsignificant disturbance of segregation is apparent also in hemophilia B.

The frequency of sporadic cases is defined as the fraction of cases that are sporadic among all cases; therefore, we proceed to estimate x from pooled data, irrespective of family history. The resulting estimate is $x = .158$ for hemophilia A and $x = .078$ for hemophilia B.

The scores and the information on parameters are given in table 5 at the maximum likelihood value of x. However, it is observed that for hemophilia A there is heterogeneity of x among countries, and three sets of families, coming from Britain, the Netherlands, and Yugoslavia, contribute the heterogeneity. Since we want to estimate x with the greatest possible precision, we exclude the families from these three countries, and use only the homogenous set of families. We obtain, as given in table 5, a new and final estimate of the frequency of sporadic cases: $x = .164 \pm .034$, with no residual heterogeneity among countries. This estimate is based on 392 families with hemophilia A out of the 562 available for segregation analysis.

We observe that this estimate is lower than the estimate of x in our 1968 study, when we did not dissect the probability of ascertainment by country; it is surprisingly close to the estimate of $x = .179$ obtained by Kosower et al. in 1961 for hemophilia A [8].

For hemophilia B, the analysis is also given in table 5. We note that the heterogeneity in π does not greatly affect the estimates. Using the average value of π for all B families ($\pi = .571$), the value of x, the scores and variances of p and x are practically the same as those obtained under the more accurate analysis, in which data from each country are analyzed at the proper value. The frequency of sporadic cases for hemophilia B has a large standard error ($x = .078 \pm .044$), and significance of the estimate is borderline; from the likelihood ratio ($x = 0$ vs. $x = .78$), the probability of such difference is equal to 8%, close to marginal significance.

Sex Ratio of the Mutation Rates

The average year of birth of the probands with hemophilia A in this study was 1946, with an average age at diagnosis of 2 years, 1 month; the median year of birth was 1950. Therefore, we cannot use for the estimation of the sex ratio of mutation rates the selection coefficient (m) calculated by Haldane in 1947 ($m = .714$, [5]). A smaller selection coefficient, consistent with the progress of medical therapy for hemophilia, should be used. The selection coefficient $m = .62$ estimated in 1955 [9] should be appropriate for this set of data. Some of our probands were born as late as 1965, and we would predict that for these the

TABLE 5

TEST OF THE HYPOTHESES ($p = .5$, $x = .158$), AND ($p = .5$, $x = .166$) FOR HEMOPHILIA A AND ($p = .5$, $x = .078$) FOR HEMOPHILIA B, AT THE VALUE OF π OBSERVED IN EACH COUNTRY

	x	U_p	U_x	K_{pp}	K_{xx}	$\chi^2(p)$	$\chi^2(x)$	L
Hemophilia A	.158	−25.397	−.142	1231.161	1078.785	.524	.000	−315.136
Hemophilia A*	.164	−23.044	1.912	977.179	846.215	.543	.000	−247.198
Hemophilia B	.078	−13.444	.321	368.270	452.741	.491	.000	−74.651

NOTE: Scores (U), information (K), and log likelihood (L).
* Data from Yugoslavia, Netherlands, and United Kingdom excluded.

selection coefficient is even smaller. The value $m = .62$ seems therefore a *conservative* estimate for the calculation of the sex ratio of the mutation rates.

From Haldane's formula, one obtains for such sex ratio in hemophilia A: $v/u = m/x - 2 = 1.78$, where v is the mutation rate in sperm, and u, the mutation rate in eggs. The 95% fiducial interval is $.69 < v/u < 4.37$, and the mutation rate could be four times greater in sperm than in eggs, or 1.5 times greater in eggs than in sperm. We still have no evidence that the sex ratio of mutation rates is significantly different from unity. However, since Haldane's formula is derived on the assumption of equilibrium due to balance between selection and mutation, the changing equilibria for hemophilias introduce further uncertainty.

Our estimate of the frequency of sporadic cases for hemophilia B is close to marginal significance, and we do not evaluate the sex ratio of mutation rates at that locus. If we assume that mutation rates are equal in the two classes of gametes, we obtain $m = .23$ for hemophilia B.

From the analysis of segregation we conclude that: (1) there is significant fraction of sporadic cases in hemophilia A; (2) the estimate of the frequency of sporadic cases for hemophilia B is of borderline significance; and (3) at the level of the resolution power of these data, we are still unable to find a difference in mutation rates in sperm and eggs for hemophilia A.

Investigation of Age Effects

Our method of investigation of age effects is based (1) on the comparison between the average age of mothers at birth of probands in families with history and no history of hemophilia and (2) on the comparison between average ages of maternal grandfathers of probands at the birth of mothers, with the average age of paternal grandfathers of probands at the birth of fathers. Theoretical considerations about these comparisons for X-linked recessive disorders have been presented by various authors [1–3].

The first comparison, in which the underlying hypothesis is that mutations might accumulate in eggs, that is, with maternal age (MA), has a control group that may not be satisfactory; mothers having a family history of the disease and possibly knowing the risk of being carriers, or knowing they are, might reproduce later than women in the general population. However, the observed average ages of mothers with and without family history are not significantly different, and in each of the three groups of families (uniplex, simplex, and multiplex), they are all in the range of 26.6 to 28.5 years.

The second comparison (table 6), which tests the hypothesis that older men have a higher chance of producing sperm carrying mutations, is made between average age of paternal grandfathers (PGFA, the controls) and maternal grandfathers (MGFA), namely, fathers of obligate heterozygous women (mothers of multiplex sibships and mothers of uniplex and simplex sibships with family history) and fathers of potentially heterozygous women (mothers of uniplex and simplex sibships with no family history). Given the strict correlation existing between ages at marriage in spouses, it seems that the control group is adequate.

The data relative to all age effects are available on request. Paternal ages and

TABLE 6

PAIRED *t*-TESTS FOR THE DIFFERENCE BETWEEN THE AGE OF MATERNAL GRANDFATHERS AT BIRTH OF THE MOTHER AND PATERNAL GRANDFATHERS AT BIRTH OF THE FATHER OF PROBANDS (NO. AGE PAIRS IN PARENTHESES)

TYPE OF HEMOPHILIA	HISTORY	TYPE OF FAMILY		
		Uniplex	Simplex	Multiplex
A	No	.65 (196)	.45 (148)	.60 (94)
	Yes	.72 (99)	.18 (83)	.58 (72)
B	No	3.40 (*x*) (35)	.61 (23)	.16 (19)
	Yes	.07 (27)	.35 (18)	4.15 (*xxx*) (17)
Unknown	No	.27 (26)	.59 (46)	1.83 (24)
	Yes	.16 (32)	1.00 (20)	1.11 (32)

NOTE: (*x*) significant at the .01 level, (*xxx*) significant at the .001 level.

paternal (PGMA) and maternal (MGMA) grandmothers' ages have been included to test consistency of any possible effect found. From these comparisons, we have no indication of a maternal-age effect in hemophilia of any type. For maternal grandfathers, we find significant differences from the ages of paternal grandfathers only in hemophilia B, for uniplex families without history and multiplex families with history.

In the case of uniplex families, the paired *t*-test is 3.40 for 35 comparisons, highly significant. The average age of maternal grandfathers at birth of mothers of a child with hemophilia B, with no history of the disease in the family, is 33.8 ± 1.7 years; the age of paternal grandfathers at the birth of the husbands of such women is 27.5 ± .9 years.

For multiplex families with history of hemophilia B, only 17 pairs of grandfathers were available; the paired *t*-test was 4.15, again highly significant. The average age of the 17 maternal grandfathers at birth of a carrier daughter was 33.1 ± 1.4 years, and the average age of 17 paternal grandfathers at the birth of the husbands of such women was 28.6 ± 1.6 years. These outcomes are not easy to explain since the possible effect of paternal age should affect all kinds of families without history of the disease.

DISCUSSION

The results of segregation analysis of our expanded data sample from 13 countries indicate no evidence of segregation distortion for hemophilia A and hemophilia B. To reach this conclusion, we had to test the segregation frequency for both disorders from family data of each participating country because of heterogeneity in the probability of ascertainment among countries. We also used this analytic strategy to estimate *x*, the frequency of sporadic cases.

The estimate of x for hemophilia A is lower than that in our previous study [1]: $x = .164$ vs. $x = .271$, respectively. This difference is probably due to heterogeneity introduced in the 1968 study by data from Argentina, the only country showing a significantly low segregation frequency for hemophilia A; the segregation frequency affects the estimation of x. The origin of such a segregation distortion in a single country is not known.

For hemophilia B, fewer families contributed about one-quarter of the information available for hemophilia A. The resulting large standard error of x makes precise evaluation of the frequency of sporadic cases difficult for the B form.

Using a value of x more precise than in our previous study [1], and a reasonably conservative value of the selection coefficient, compatible with the chronology of our data sample, we still are unable to demonstrate that there is a significant difference between the rates of mutation to the hemophilia A allele in sperm and eggs. Although some investigators provide evidence for such a difference for X-linked recessive disorders [2, 3], others have not. For instance, Yasuda and Kondo found no evidence of differing mutation rates between eggs and sperm for Duchenne muscular dystrophy gene [10]. Indeed, this disease is a better model for the analysis of mutation rates, since the assumption of equilibrium of the deleterious allele frequency (due to balance between selection and mutation) is more reasonable than for hemophilia. However, a recent study in which data on Duchenne muscular dystrophy coming from different samples were analyzed together suggests that there might be a slight but significant excess of mutations in male gametes [11]. The sex ratio of mutation rates for Duchenne dystrophy is estimated to be equal to 1.62, a value that is very close to the estimate found in the present study for hemophilia A: 1.74. We believe that the only serious objection to our conclusion of very slight differences, if any, in mutation rates in sperm and eggs for hemophilia A is that of the assumption of equilibrium, which is necessary to evaluate such differences. Progress in medical therapy of this disease is clearly contributing to increased fitness of the patients. This progress has occurred in 1–2 generations, a time period too short for a new equilibrium to be achieved.

As for the analysis of age effects, we found two significant differences, both for hemophilia B. The maternal grandfathers of uniplex sibships with no family history of the disease are older than controls ($p = .0007$), and maternal grandfathers in multiplex sibships with a history of the disease are older than their controls too ($P = .0005$). Both levels of significance withstand correction for the number of comparisons. The first observation is compatible with an age effect on mutation rates in hemophilia B, but is not repeated in the same comparison for simplex sibships. It is difficult to explain the second significant difference, for multiplex sibships, in terms of mutations accumulating in male germ cells. Interpretation of the hemophilia B age-effects analysis is made even more complex because no effect whatsoever was found, with more data, for hemophilia A. Also, for Duchenne muscular dystrophy, no effect of parental age on the rate of mutation has been demonstrated [12].

On the basis of the results of segregation analysis and maternal- and grandpa-

rental-age comparisons presented here, we conclude that evidence does not support copy error as a major factor in the production of hemophilia A mutations.

REFERENCES

1. Barrai I, Cann HM, Cavalli-Sforza LL, De Nicola P: The effect of parental age on rates of mutation for hemophilia and evidence for differing mutation rates for hemophilia A and B. *Am J Hum Genet* 20:175–196, 1968
2. Vogel F: A probable sex difference in some mutations rates in man. *Am J Hum Genet* 29:312–319, 1977
3. Vogel F, Rathenberg R: Spontaneous mutation in man, in *Advances in Human Genetics,* edited by Harris H, Hirschhorn K, New York, Plenum Press, 1975, pp 223–318
4. Barrai I, Cann HM, Cavalli-Sforza LL: Segregation analysis of hemophilia A and B (Letter to the Editor). *Am J Hum Genet* 31:226–227, 1979
5. Haldane JBS: The rate of spontaneous mutation of a human gene. *J Genet* 31:317–326, 1935
6. Haldane JBS: The mutation rate of the gene for hemophilia and its segregation ratios in males and females. *Ann Eugen (Lond)* 13:262–271, 1947
7. Morton NE: Genetic tests under incomplete ascertainment. *Am J Hum Genet* 11:1–16, 1959
8. Kosower N, Christiansen R, Morton NE: Sporadic cases of hemophilia and the question of a possible sex difference in mutation rates. *Am J Hum Genet* 14:159–169, 1962
9. Vogel VF: Vergleichende Betrachtungen ueber die Mutationsrate der geschlechtsgebunden rezessive haemophilienformen in der Schweiz und in Danemark. *Blut* 1:91–109, 1956
10. Yasuda N, Kondo K: No sex difference in mutation rates of Duchenne muscular dystrophy. *J Med Genet* 17:106–111, 1980
11. Danieli GA, Barbujani G: Duchenne muscular dystrophy. Frequency of sporadic cases. *Hum Genet* 67:252–256, 1984
12. Yasuda N, Kondo K: The effect of parental age on rate of mutation for Duchenne muscular dystrophy. *Am J Med Genet* 13:91–99, 1982