The Effect of Parental Age on Rates of Mutation for Hemophilia and Evidence for Differing Mutation Rates for Hemophilia A and B

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INTRODUCTION

A fully penetrant, dominant gene, lethal before reproductive age, would offer optimal possibilities to test hypotheses about mutation rates. In man, there are several dominant genes which are lethal or sublethal, but their penetrance is incomplete or their expression is variable. The frequency of most such genes is very low, and a project aimed at estimating the relative contribution of various factors to mutation would be made prohibitively expensive by the cost of ascertainment.

Hemophilia is a sex-linked recessive trait, almost fully penetrant, highly deleterious if not lethal, and ubiquitous enough not to pose unusual ascertainment problems. There is no evidence of incomplete penetrance, nor of segregation distortion. The frequency of sporadic cases has been estimated as approximately 0.18 (Kosower *et al.*, 1962).

We have used the trait for testing the effect of parental age on mutation rate. In this investigation we are testing two models of mutation in man. According to the first model, mutation arises as a result of failure to copy the gene correctly in dividing cells. Were this hypothesis true, mutation rates should be related to paternal age but not to maternal age, because in the male, gamete formation, and so the copying of hereditary material, continues throughout most of life. In the second model, mutation is independent of cell division. It increases with age irrespective of sex. In this case, the age of mothers at birth of mutant children (sporadic cases) should be higher than at birth of normal children.

Variation in the secondary sex ratio has been studied as a function of parental and grandparental age. Novitski (1953), Novitski and Sandler (1956), and Novitski and Kimball (1958) found a significant effect of paternal age on sex ratio, independent of maternal age and birth order. Cavalli-Sforza (1961, 1962) found an effect of maternal

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grandfather's age on the secondary sex ratio of the offspring of Italian mothers and used this effect to estimate cumulative mutation rates of recessive lethals on the *X* chromosome. Krehbiel (1966, see also for review) confirmed this result for Caucasian mothers in the state of Minnesota. However, Cann and Cavalli-Sforza (1968) were unable to find the same effect on a larger sample of birth certificates. Penrose (1955, 1957) found a correlation between paternal age and frequency of achondroplasia.

Apart from the effect of paternal age on the sex ratio, which probably has another explanation (meiotic drive), these results support the model of mutation as a copying error. On the other hand, Lejeune and Turpin (1957) found an effect of maternal age on the secondary sex ratio, which was not confirmed by Novitski and Kimball (1958). This effect was not found by Morton and Chung (1959) for sporadic cases of muscular dystrophy, using intrafamilial controls.

To test for evidence of one or the other model, relating to frequency of mutations, we have collected data from a large sample of hemophiliacs. For analytical purposes we distinguish between families with and without a history of hemophilia. Sibships which have only one male, who is also a proband, are defined as uniplex. If the proband has at least one normal brother and no other affected brothers, the family is simplex; if there are at least two affected males, the family is multiplex (Morton, 1959).

Uniplex and simplex families without a family history of hemophilia may represent a mutation in the mother or in either of her parents, or gene segregation in an earlier female maternal ancestor. There is no way of discriminating between the first and the latter two possibilities except by testing mothers for plasma antihemophilic globulin (AHG) (Rapaport *et al.*, 1960) or plasma thromboplastin component (PTC) levels (Barrow *et al.*, 1960), and these tests do not provide complete discrimination.

Multiplex families without a family history of hemophilia can result from a mutation occurring in a gamete of a maternal grandparent which has been transmitted to a daughter, conferring carrier status upon her. Other multiplex families without a family history of hemophilia will terminate a number of generations of heterozygotes who produced no males, only normal males, or unreported affected children.

If it is possible to detect an effect of mothers' ages on the frequency of the hemophilia mutation, then the average age at birth of a proband will be higher in mothers of uniplex and simplex sibships with no previous history of hemophilia than in the general population. Such a finding will support the nonreplication model of mutation. If there is an effect of maternal grandfathers' ages, the age will be higher in multiplex families with no history than in the general population, supporting a replicating error model of mutation.

MATERIALS AND METHODS

Questionnaires were sent to hematologists in Argentina, Germany, Holland, Italy, Norway, and Sweden. They were asked to complete them with the data pertaining to their hemophiliac patients. The numbers of hematologists who were questioned and who replied are shown in Table 1. Questionnaires were also sent to the United States where the National Hemophilia Foundation collaborated in the data collection both

in the United States and Canada.* In these countries, the forms mostly were completed by the patients or their parents. The general questions were: (1) Identification of the patient, with birthplace and year of birth, and age of onset of hemorrhagic manifestations. (2) Type of hemophilia. (3) Number of affected and normal brothers. (4) Does the patient have any sister(s) who proved to be carriers? (5) Does the patient have any maternal uncle(s) who suffered from hemophilia? (6) Does the patient have any maternal aunt(s) who proved to be carriers? (7) Are there any other cases of hemophilia known in the family and, if yes, in which relatives?

The important questions pertained to the ages of the parents at birth of the patient and of his grandparents at birth of the parents. These were: (1) year of birth of the patient's father, (2) year of birth of the patient's mother, (3) year of birth of the patient's maternal grandfather, (4) year of birth of the patient's maternal grandmother, (5) year of birth of the patient's paternal grandfather, and (6) year of birth of the patient's paternal grandmother.

TABLE 1

RESPONSES BY HEMATOLOGISTS TO QUESTIONNAIRES CONCERNING HEMOPHILIA

Country	Questioned	Replied
Germany	4	2
Netherlands	3	1
Sweden	1	1
Norway	1	1
Argentina	1	1
Italy	1	1
Total	11	7

The ages of paternal grandparents were included as controls, because no effect of the age of paternal ascendants on *X*-linked mutation is expected. We assume that there is no difference between the average ages of parents at the birth of males and females.

RESULTS

We received 1,038 completed forms over a three-year period ending December 31, 1965. Four were rejected. The remainder included 621 forms for hemophilia A, 165 for hemophilia B, and 248 forms which did not specify the type of hemophilia. We considered a person for whom a questionnaire was completed a proband; we counted, at most, three probands per family.

The distribution of families by type of segregation and by history of hemophilia is shown in Table 2.

The excess of simplex and uniplex families and the deficit of multiplex families

* Unfortunately, questionnaires sent to other countries were received too late to be included in the present survey, which is based only on the forms received by December 31, 1965. Questionnaires received after that date will be processed in the future.

without a family history of hemophilia A is not significant. There is no heterogeneity among types of sibship for hemophilia B. However, among families in which the type of hemophilia was not specified, there is a departure from homogeneity among the distribution of uniplex, simplex, and multiplex sibships with and without a family history of the condition ($\chi^2_{[2]} = 7.986$).

To insure that the material was suitable for our purpose, it was tested for the following requirements: (1) the trait must show evidence of X-linked recessive inheritance; (2) the disease must be severe; and (3) the segregation frequency for either type of hemophilia must be 0.5.

If there is no segregation distortion, the penetrance must be close to unity. We can, however, test penetrance independent of segregation frequency by analyzing distribution of age at clinical onset of the disease.

TABLE 2

HISTORY AND TYPE OF HEMOPHILIA IN REPORTED FAMILIES

Type of Hemophilia	Family History	Uniplex	Simplex	Multiplex
A	No	124	110	65
	Yes	114	85	78
B	No	34	18	20
	Yes	34	17	24
Unspecified	No	26	49	32
	Yes	37	31	47

Sex Linkage

We received five forms which listed a female name for the proband. Of these individuals, four were affected with hemophilia B, three from Germany and one from the United States. The three cases from Gemany referred to uniplex families with no previous history. In view of inconsistencies in these forms, the cases were rejected. The case from the United States was a sister of a proband and the mother of another proband; her father was affected. This individual is probably a female homozygote, and for the purpose of this study she will be considered a proband resulting from segregation of her carrier-mother's X chromosomes. We excluded from the analysis the only case of hemophilia A in which the proband had a female name. Although her father was affected, we were unable to find evidence for the mother of this individual being a carrier.

The other questionnaires were all consistent with sex-linked inheritance of hemophilia, that is, affected individuals were males, and for those cases with a positive family history, the mutant allele seemed to be segregating in the family of the mother of the proband.

Severity of Hemophilia A

In Table 3 we give the distributions of year of birth of probands, in decennial classes (except for the present decade, for which a five-year class is used), and the

mean and standard error of age, in months, of onset of hemophilia in probands born in these decades.

Each of the age distributions has a mode between 5 and 15 years, with a sharp decline after 25 years, and then a gradual decay in the older groups. It may be assumed that the depletion of probands after 25 years of age is due to mortality. From Andreassen's data (1943), Haldane (1947) calculated a selection coefficient against hemophilia of 0.714. Considering that in Andreassen's data the distinction between hemophilia A and B was not possible, the estimate given by Haldane is presumably an inferior limit.

From the distributions of Table 3, we observe that the older the proband the later is the age of onset of the disease. We therefore tested the linear dependence of the

TABLE 3

MEAN AGE AT ONSET IN MONTHS AS A FUNCTION OF YEAR OF BIRTH OF PROBANDS FOR EACH TYPE OF HEMOPHILIA

Year of Birth	Type of Hemophilia					
	A		B		Unspecified	
	Mean Onset Age in Months	Number of Probands	Mean Onset Age in Months	Number of Probands	Mean Onset Age in Months	Number of Probands
1960–1965.....	6.28± 0.67	81	7.79± 2.70	14	5.41± 0.94	29
1950–1959.....	11.19± 1.05	214	14.83± 2.51	47	11.56± 2.30	77
1940–1949.....	19.78± 3.12	125	37.12± 8.23	34	16.48± 3.81	56
1930–1939.....	29.54± 6.50	52	58.09±17.88	22	18.71± 7.88	24
1920–1929.....	60.58±14.13	40	71.00±33.67	6	45.56±15.29	9
1910–1919.....	76.62±27.57	21	29.20±13.26	5	52.50±30.08	8
1897–1909.....	189.00±52.57	13	42.00±33.05	3	36.00±12.17	9
Total.....	24.54± 1.34	546	30.87± 1.67	131	16.85± 0.83	212

present age of probands on age of onset. The regression was weighted by the number of probands in each class. For the values of age of probands, class midpoints were used. The regression equation of proband's age on onset age for hemophilia A is

$$\text{age}_P = 8.55 + 0.39\ \text{age}_{ons}\ ,$$

where age_P represents present age of probands (computed from date of birth) and age_{ons} represents age at onset. The regression coefficient ($b = 0.3855 \pm 0.0095$; $t = 40.57$; $P < .01$) is highly significant and indicates that a delay in onset of one month increases by approximately five months the survival of probands affected with hemophilia A. Assuming that the age distribution represents a life table, the data suggest that early onset is correlated with severity. However, we note that the estimate of the regression coefficient will be biased because our data undoubtedly include individuals who were diagnosed at birth because of bleeding from circumcision. Such individuals will show an earlier mean age of onset than their uncircumcised cohorts.

In Table 3, the variances of ages at onset for the different groups increase approxi-

mately as the square of the means, suggesting that severity of the disease is not solely dependent on age at onset. If early onset alone were the only indicator of severity, the variances should decrease with age because early death would truncate the distribution of age at onset.

Significant regressions of proband age on mean age of onset were also obtained with data from probands with hemophilia B ($b = 0.5311 \pm 0.0394$; $t = 13.98$; $P < .01$) and from those for whom the type of hemophilia was not specified ($b = 1.1492 \pm 0.0121$; $t = 94.87$; $P < .01$).

Penetrance

The ages of onset of affected siblings of probands were not available to us. However, it is possible to estimate an upper limit for penetrance due to delayed onset using ages at onset of probands and their age distribution at death or last contact. This is given by

$$\nu = \int_0^\infty f(a)G(a)da \,,$$

where $f(a)$ is the age distribution of probands and $G(a)$ the cumulative distribution of onset ages (see Morton, 1959). In the present sample, the estimate for hemophilia A was

$$\nu = 0.971 \,,$$

suggesting almost complete penetrance at early ages.

Segregation Analysis

The distribution of hemophiliac males in our sample, classified by male sibship size and type of hemophilia, is given in Table 4. The distribution of probands by type of hemophilia and number of affected males is presented in Table 5. We were able to estimate the probability of ascertainment, π, from the distribution of a probands among r affected in a sibship using Morton's problem 9 (Morton, 1959):

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

This distribution is based on the assumption that ascertainments are independent and that π is constant for every proband. The distribution is independent of both the segregation frequency and the frequency of sporadic cases. Pooling data from all families, the estimate of π was

$$\pi = 0.384 \pm 0.029 \,.$$

We tested the heterogeneity of this estimate of π between families with and without a history of hemophilia, among types of hemophilia, and among reporting countries. The χ^2 for heterogeneity was computed using the formula

$$\chi^2_{[m-1]} = \Sigma(U_\theta^2/K_{\theta\theta}) - (\Sigma U_\theta)^2/\Sigma K_{\theta\theta} \,,$$

where m is the number of classes, U_θ is the maximum likelihood score for a parameter θ, and $K_{\theta\theta}$ the variance. The results of the tests are given in Tables 6, 7, and 8. The probability of ascertainment is homogeneous between families with and without a history of hemophilia ($\chi^2_{[1]} = 0.418$) and among types of hemophilia ($\chi^2_{[2]} = 1.023$). The heterogeneity among reporting countries was tested for each type of hemophilia. It is significant for hemophilia A ($\chi^2_{[7]} = 37.016$). The nine informative families re-

TABLE 4

DISTRIBUTION OF HEMOPHILIAC MALES PER SIBSHIPS SIZE

	Types of Hemophilia																			
	A							B							Unspecified						
	1	2	3	4	5	6	Total	1	2	3	4	5	6	Total	1	2	3	4	5	6	Total
Sibship size:																					
1	238						238	68						68	63						63
2	137	55					192	19	18					37	47	37					84
3	35	33	13				81	14	11	0				25	17	10	11				38
4	10	12	6	3			31	1	4	2	0			7	11	6	4	1			22
5	7	5	4	0	0		16	1	0	0	1	0		2	4	2	2	0	0		8
6	2	4	1	0	1	1	9	0	1	1	0	0	0	2	1	0	2	0	0	1	4
7	3	1	2				6	0	1	2	0	0	0	3							0
8			1				1	0	0	0	1	1	0	2					1		1
9							0						1	1				1		1	2
10	1						1							0							0
14					1		1							0							0
Total	433	110	27	3	2	1	576	103	35	5	2	1	1	147	143	55	19	2	1	2	222

TABLE 5

DISTRIBUTION OF PROBANDS PER NUMBER OF AFFECTED AND TYPE OF HEMOPHILIA

	Type of Hemophilia											
	A				B				Unspecified			
	1	2	3	Total	1	2	3	Total	1	2	3	Total
Affected males:												
1	238*			238	68*			68	63*			63
1	195			195	35			35	80			80
2	78	32		110	20	15		35	37	18		55
3	20	3	4	27	3	1	1	5	15	1	3	19
4	2		1	3	2			2	2			2
5	2			2	1			1	1			1
6	1			1	1			1	1	1		2
Total	536	35	5	576	130	16	1	147	199	20	3	222

* Sibship size equals one.

TABLE 6

HETEROGENEITY OF π BETWEEN FAMILIES WITH AND WITHOUT HISTORY OF HEMOPHILIA ($\pi = 0.384$)

Families	n	Score (U)	Variance (K)
With history	149	−11.228	722.11
Without history	117	11.228	518.12
Total	266	0	1,239.23

$\chi^2_{[1]} = 0.418$. The χ^2 is calculated according to the formula:

$$\chi^2_{[m-1]} = \sum_n \frac{(U_\pi)^2}{K_{\pi\pi}}$$

TABLE 7

HETEROGENEITY OF π BETWEEN TYPES OF HEMOPHILIA ($\pi = 0.384$)

Type of Hemophilia	n	U	K
A	143	−3.372	635.89
B	44	12.775	209.01
Unspecified	79	−9.403	394.33
Total	266	0	1,239.23

$\chi^2_{[1]} = 1.023$

TABLE 8

HETEROGENEITY OF π BETWEEN COUNTRIES FOR EACH TYPE OF HEMOPHILIA

Country	Type of Hemophilia		
	A	B	Unspecified
	$U^2_\pi/K_{\pi\pi}$		
Argentina	0.029	2.734	.....
Germany	0.214	3.200	.....
Italy	3.200	.....	.....
Netherlands	25.530	0.151	.....
Norway	5.091	2.854	2.834
Sweden	2.523	1.261	.....
United States	0.441	1.940	0.052
Other	0.006	.....	1.295
$\Sigma(U^2_\pi/K_{\pi\pi})$	37.034	12.140	4.181
$(\Sigma U_\pi)^2/\Sigma K_{\pi\pi}$	0.018	0.781	0.224
df	7	5	2
χ^2	37.016	11.359	3.957

ported from the Netherlands account for the heterogeneity. In this group, there is an excess of probands, which accounts for the discrepancy. Excluding the nine Dutch families, the heterogeneity becomes nonsignificant ($\chi^2_{[6]} = 9.885$). However, we have decided not to reject these families from the analysis.

For hemophilia B, the significance of the heterogeneity among countries is marginal ($\chi^2_{[5]} = 11.359$). Heterogeneity in the estimate of π was nonsignificant among countries from which probands with hemophilia of unspecified type were reported ($\chi^2_{[2]} = 3.957$). We conclude that the estimate of π is not biased by gross heterogeneity, and we have used it for further tests.

In multiplex and simplex families arising from carrier mothers, the a priori probability that a child be affected, p (the segregation frequency; $q = 1 - p$), is considered constant. The distribution of sibships of size s males with one affected is:

$$P(r = 1 \mid r > 0) = \frac{sp\pi[x + (1 - x)q^{s-1}]}{xsp\pi + (1 - x)[1 - (1 - p\pi)^s]} \quad \text{(Morton, 1959),}$$

and with r affected is

$$P(r \mid r > 1) = \frac{\binom{s}{r} p^r q^{s-r}(1 - \pi)^r}{1 - (1 - p\pi)^s - sp\pi q^{s-1}} \quad \text{(Morton, 1959).}$$

Morton refers to these distributions as problem 2 and problem 3, respectively; we shall follow his nomenclature.

Pooling data from all families, we estimated the frequency of sporadic cases, x, using $p = .5$ and the estimate of $\pi = 0.384$ for the three types of hemophilia. The details of the analysis are given in Table 9. The estimates, which are maximum likelihood solutions, are, for the three types of hemophilia, shown in Table 10. At these values of x, the hypothesis fits the observed data for the three types of hemophilia. The variation between problems for p in hemophilia A is not significant, and there is no evidence of segregation disturbance for hemophilia A, B, nor for the group of families for which the type of hemophilia was not specified.

We tested for dependence of the number of affected in the sibship (r) on mother's age at birth of a proband (x_1) and on sibship size (s), in multiplex families segregating for hemophilia A. The regression equations are as follows:

1. families with a history of hemophilia A,
   $r = 1.24 + 0.0105x_1 + 0.2328s$;
2. families without a history of hemophilia A,
   $r = 2.07 + 0.0243x_1 + 0.0906s$.

As expected, there is a significant regression on sibship size ($t = 20.389$ in families with a history, and $t = 1.996$ in families without a history). There is no effect of mother's age. This may mean that there is no segregation distortion with increasing age of the mother, namely that the probability of generating a hemophiliac male is constant throughout the reproductive history of a carrier mother. Similar results were obtained for the other types of hemophilia.

Our estimate of the frequency of sporadic cases for hemophilia A, $x = 0.275$, is

TABLE 9

SEGREGATION ANALYSIS

HEMOPHILIA A

Problem	p	x	π	U_p	U_x	U_π	K_{pp}	K_{px}	$K_{p\pi}$	K_{xx}	$K_{x\pi}$	$K_{\pi\pi}$
2	.5	0.275	0.384	10.91	0.15	−7.61	598.31	−553.87	−140.48	769.08	189.34	47.27
3	.5	.....	0.384	−60.18	....	6.19	673.99	.....	− 69.68	.....	.....	234.05
9	....	.....	0.384	.....	....	−3.37	.....	.....	.....	.....	.....	635.89
Total	....	.....	.....	−49.27	0.15	4.79	1,272.30	−553.87	−210.16	769.08	189.34	917.21

	df	χ^2		df	χ^2
$p=\frac{1}{2}$, total	1	1.908	$\pi=0.384$, total	1	0.003
$p=\frac{1}{2}$, between problems	1	3.664	$\pi=0.384$, between problems	2	1.404

HEMOPHILIA B

Problem	p	x	π	U_p	U_x	U_π	K_{pp}	K_{px}	$K_{p\pi}$	K_{xx}	$K_{x\pi}$	$K_{\pi\pi}$
2	.5	0.093	0.384	− 3.62	−0.24	−0.74	237.76	−201.51	−45.15	326.36	56.47	10.76
3	.5	.....	0.384	−29.69	.....	3.35	274.20	.....	−26.46	.....	.....	75.42
9	....	.....	0.384	.....	.....	12.78	.....	.....	.....	.....	.....	209.01
Total	....	.....	0.384	−33.31	−0.24	15.39	511.96	−201.51	−71.61	326.36	56.47	295.19

	df	χ^2		df	χ^2
$p=\frac{1}{2}$, total	1	2.167	$\pi=0.384$, total	1	0.802
$p=\frac{1}{2}$, between problems	1	1.103	$\pi=0.384$, between problems	2	0.179

HEMOPHILIA OF UNSPECIFIED TYPE

Problem	p	x	π	U_p	U_x	U_π	K_{pp}	K_{px}	$K_{p\pi}$	K_{xx}	$K_{x\pi}$	$K_{\pi\pi}$
2	.5	0.209	0.384	22.39	−0.09	− 4.72	333.70	−303.18	− 75.25	407.98	94.34	22.25
3	.5	.....	0.384	20.63	.....	− 2.29	336.27	.....	− 34.64	.....	.....	112.84
9	....	.....	0.384	.....	.....	− 9.40	.....	.....	.....	.....	.....	394.33
Total	....	.....	.....	43.02	−0.09	−16.41	669.97	−303.18	−109.89	407.98	94.34	529.42

	df	χ^2		df	χ^2
$p=\frac{1}{2}$, total	1	2.762	$\pi=0.384$, total	1	0.509
$p=\frac{1}{2}$, between problems	1	0.005	$\pi=0.384$, between problems	2	0.763

TABLE 10

Type of Hemophilia	x
A	0.275 ± 0.037
B	0.093 ± 0.056
Unspecified	0.209 ± 0.051

higher than the estimate of $x = 0.176$ obtained by Kosower *et al.* (1962). However, they could not discriminate between hemophilia A and B (Aggeler *et al.*, 1952; Biggs *et al.*, 1952) since their analysis was performed on data collected before hemophilia B was recognized as a genetic and clinical entity. Thus, our group of cases of hemophilia for which the type was not specified, presumably a mixture of individuals with hemophilia A and hemophilia B, is comparable to their data. Indeed, their estimate of $x = 0.176$ does not differ significantly from our estimate of $x = 0.209$.

If we accept Haldane's estimate for the selection coefficient against hemophilia, $m = 0.714$, as an inferior limit for hemophilia A, we can estimate the 5% fiducial interval of the sex ratio of the mutation rates. If ν is the mutation rate in sperm, and μ the mutation rate in eggs, at equilibrium

$$\nu/\mu = (m/x) - 2 \qquad \text{(Haldane, 1947).}$$

The 5% confidence limits for the estimate of x for hemophilia A are

$$0.202 < x < 0.347$$

so that the corresponding interval for ν/μ is

$$1.53 < \nu/\mu < 0.06 .$$

The mutation rate could be $1\frac{1}{2}$ times greater in sperm than in eggs, or 17 times higher in eggs than in sperm. Using the maximum likelihood estimate of x, one finds $\nu/\mu = 0.60$, too close to unity to be indicative of a differential in mutation rates.

We have poor information on the selective disadvantage of hemophilia B and of hemophilia of unspecified type. Assuming (in the absence of contrary evidence) equality of mutation rates in the two sexes, we may estimate the value of m from the frequency of sporadic cases. Thus, for hemophilia B, $m = 0.279$, and for hemophilia of unspecified diagnosis, $m = 0.627$.

The sample of hemophiliacs of unspecified type should consist of an admixture of individuals with hemophilia A and hemophilia B. If the two types of hemophiliacs are present in the proportions found in our over-all sample, then the selection coefficient for the group with the unspecified type of hemophilia can be computed from the weighted mean of the selective coefficients for hemophilia A and B:

$$m = [(762 \times 0.714) + (207 \times 0.279)]/969 = 0.621 .$$

This value is in good agreement with the value obtained independently from the frequency of sporadic cases assuming equality of the sex ratio of mutation rates ($m = 0.627$).

Prevalence and Incidence

Prevalence is defined as the number of cases of a trait existing in a given area at a given time. From the estimate of prevalence and from the population size, it is possible to determine the frequency at birth of individuals who will develop the trait, namely, its incidence, I (Barrai *et al.*, 1965).

The prevalence, n, is obtained from the equation:

$$\pi = A/n$$

where π is the probability of ascertainment and A the number of probands. Clearly, $n = A/\pi$ so that the error of n is

$$\sigma_n = A\sigma_\pi/\pi^2 ,$$

where π and σ_π are estimated from segregation analysis. We will estimate prevalence and incidence only for the United States and Canada, using data from families reported by the National Hemophilia Foundation. There were 416 families with 482 probands: 244 probands with hemophilia A, 66 with hemophilia B, and 172 for whom the type of hemophilia was not specified. Using the values $\pi = 0.384$ and $\sigma_\pi = 0.029$, we obtain the estimates in Table 11.

TABLE 11

Type of Hemophilia	Prevalence
A	635.4 ± 48.0
B	171.9 ± 13.0
Unspecified diagnosis	447.9 ± 33.8

If we assume that cases of hemophilia A and B are proportionally distributed in the group with the unspecified type as they are in the total sample, we may compute the expected number of probands having hemophilia A or B among those with unspecified diagnoses. We find that 135.4 probands may be added to the 244 with hemophilia A and 36.6 to the 66 with hemophilia B. The revised estimates of prevalence for hemophilia A, n_A, and for hemophilia B, n_B, become:

$$n_A = 988.0 \pm 74.6 , \quad n_B = 267.2 \pm 20.2 .$$

Now, if we assume that Haldane's estimate of the selection coefficient for hemophilia pertains to hemophilia A, although some cases of hemophilia B may have been included in the sample he studied, we may compute the incidence of hemophilia A (I_A):

$$I_A = \frac{n_A}{(1 - m)N} \pm \frac{\sigma_{n_A}}{(1 - m)N} ,$$

where N is the total male population of the areas covered by the chapters of the National Hemophilia Foundation participating in this study, and is approximately 62 million males (Canada Dominion Bureau of Statistics, 1962; U.S. Bureau of Census, 1963). Then,

$$I_A = (5.51 \pm 0.41) \times 10^{-5}$$

and for hemophilia B, assuming $m = 0.279$, we obtain

$$I_B = (5.91 \pm 0.45) \times 10^{-6} .$$

The incidence of hemophilia A is approximately ten times as much as the incidence of hemophilia B. Differential mortality, with elimination of relatively more individuals with hemophilia A, probably accounts for the smaller ratio of the prevalences, n_A/n_B.

Given the incidence, it is possible to compute the mean mutation rate per generation. According to Haldane, at equilibrium,

$$2\mu + \nu = mI ,$$

so that when the mutation rates are equal in sperm and eggs the average mutation rate is:

$$\bar{\mu} = \tfrac{1}{3}mI \pm \tfrac{1}{3}m\sigma_I .$$

In the present case,

$$\bar{\mu}_A = (1.31 \pm 0.10) \times 10^{-5}$$

for hemophilia A, and

$$\bar{\mu}_B = (0.55 \pm 0.04) \times 10^{-6}$$

for hemophilia B. The mutation rate for hemophilia B is about one-twentieth the mutation rate for hemophilia A.

Effect of Age on Mutation Rate

We now turn to testing the hypotheses implicit in the models about parental and grandparental age and mutation frequency. The object is to seek a difference between parental age at birth of mutant progeny—in this case hemophiliacs or carriers—and parental age at birth of nonmutant-bearing individuals.

Assuming that (1) mutation rates will increase linearly with age and (2) the ages of parents at birth of nonmutant individuals are normally distributed, and given the following definitions:

μ_0 = the mutation rate before the increase with age;
b = the rate of increase in mutation rate per year;
$\bar{x}$ = the mean of the distribution of ages of parents (in the general population) at birth of nonmutant progeny, with variance σ^2;
$\bar{x}_s$ = the mean of the distribution of ages of parents at birth of mutant progeny, with standard error $\sigma_{\bar{x}_s}$,

it is possible to obtain an explicit formulation for the yearly variation of mutation rate as a function of μ_0.

The mutation rate at age x, μ_x, is

$$\mu_x = \mu_0 + bx .$$

Let $f(x)$ represent the probability distribution function of ages of parents at birth of their children. Then, the distribution of parental ages at birth of a mutant child is

$$\phi(x) = \frac{\mu_x f(x)}{\Sigma \mu_x f(x)} ,$$

and the mean age at birth of a mutant child is

$$\bar{x}_s = \sum \frac{x\mu_x f(x)}{\Sigma \mu_x f(x)} = \sum \frac{x(\mu_0 + bx)f(x)}{\Sigma(\mu_0 + bx)f(x)} = \frac{\mu_0 \bar{x} + b(\sigma^2 + \bar{x}^2)}{\mu_0 + b\bar{x}} . \qquad (1)$$

When $\mu_0 = 0$, one obtains

$$\bar{x}_s = \bar{x} + \frac{\sigma^2}{\bar{x}} ,$$

as given by Penrose (1955).

By rearranging terms in (1), and dividing by μ_0, we can express the yearly change in mutation rate as a fraction of μ_0:

$$\frac{b}{\mu_0} = \frac{\bar{x}_s - \bar{x}}{\sigma^2 - \bar{x}_s\bar{x} + \bar{x}^2} . \tag{2}$$

An approximate standard error of this ratio can be calculated assuming that the mean ($\bar{x}$) and variance (σ^2) of the general population are free of sampling error. The standard error of the ratio becomes a function of the error of $\bar{x}_s$:

$$\sigma(b/\mu_0) = \sigma_{\bar{x}_s} \cdot \frac{\sigma^2}{(\sigma^2 - \bar{x}\bar{x}_s + \bar{x}^2)^2} .$$

Thus, we can estimate the expected yearly increase in mutation frequency, relative to the initial mutation rate, using the mean and variance of the distribution of age at birth in the general population for $\bar{x}$ and σ^2 and the sample values for $\bar{x}_s$ and $\sigma_{\bar{x}_s}$.

To study the effect of age on mutation rate in the maternal gametic pool, the values for $\bar{x}_s$ and $\sigma_{\bar{x}_s}$ should be derived from the distribution of ages of mothers at birth of probands of uniplex and simplex families with no family history of the particular type of hemophilia under scrutiny. As we have stated above, new mutants (the mutation occurring in the mother) will be found only in these families. Segregating sibships will also be found among these families, and we are unable to discriminate between the two groups. Inclusion of maternal age data from segregating sibships imparts a bias to the estimates of $\bar{x}_s$ and $\sigma_{\bar{x}_s}$ which, if indeed there is an effect of maternal age on mutation rate, tends to obscure the effect. The distribution of ages of heterozygous mothers at birth of probands will be the same as that for general population or, perhaps, will differ from the general population for reasons other than mutation.

Mutation occurring in the X chromosomes of males are transmitted to female offspring, each of whom has the potential of producing multiplex sibships. Such sibships will have no family history of hemophilia. The relevant parameter for the examination of the effect of age on mutation rate in the males, then, is the age of maternal grandfather at birth of the mother of a proband for a multiplex sibship without previous family history of the condition. Some of these sibships will arise from heterozygous mothers who are mutant individuals—the ages of maternal grandfathers of these sibships provide the relevant data for computing $\bar{x}_s$ and $\sigma_{\bar{x}_s}$, and we cannot discriminate them from sibships whose mothers are heterozygous because of gene segregation in maternal grandmothers. The estimates of $\bar{x}_s$ and $\sigma_{\bar{x}_s}$ will be biased by inclusion of data about ages of maternal grandfathers of the latter sibships.

We will present our analysis of only the hemophilia A data. The data for hemophilia B are too few for a meaningful analysis.

Effect of Age on Mutation Rate in Females

A preliminary examination of maternal ages at birth of probands with hemophilia A was undertaken. Analysis of variance of the age of mothers for uniplex, simplex, and multiplex families, with and without a previous history of hemophilia, showed that there is a maternal age difference among types of sibships ($F = 3.48$; $P < .05$). Mothers of uniplex families are significantly younger than mothers of simplex and

multiplex families ($t = 2.64$), irrespective of history. The presence or absence of a family history of hemophilia does not affect the age of mother at birth of a proband within each type of sibship and for all the data. In the following tables, we give the mean maternal ages at birth of probands and the analysis of variance (see Tables 12 and 13).

For the examination of the model describing the relationship between age and mutation rate, birth information from the United States for the period 1957–63 (U.S. Department of Health, Education, and Welfare, 1965) was used to compute $\bar{x}$ and σ^2. It is appropriate to use the U.S. data because (1) the United States contributed more cases than any of the other countries participating in this study, and (2) the data are derived from so large a sample that we can ignore sampling errors in the estimation procedures. The mean of the distribution of ages of mothers in the general population at birth of a child is $\bar{x} = 26.43$ years, and the variance of the age distribution is $\sigma^2 = 37.01$ years. We assume that the distribution of ages of mothers at birth of males is the same as that for birth of females.

In the group of families with no history of hemophilia, there are 116 uniplex sibships and 106 simplex sibships for which the year of birth of the mother is known. Table 14

TABLE 12

Type of Sibship	Family History of Hemophilia A			
	Present		Absent	
	Mean Age of Mother at Birth of Child (Years)	Number of Sibships	Mean Age of Mother at Birth of Child (Years)	Number of Sibships
Uniplex	27.08	106	26.52	116
Simplex	28.04	81	28.25	106
Multiplex	27.90	71	28.24	59

TABLE 13

ANALYSIS OF VARIANCE

Sources of Variation in Age of Mother at Birth of Child	SS	df	MS	F	P
Between family history and type of sibship (discrepancy)	254.94	5	.....	.....	.....
Type of sibship	231.28	2	115.64	3.48	$<.05$
Family history of hemophilia	0.75	1	0.75	0.02	n.s.*
Interaction between family history and type of sibship	22.91	2	11.46	0.34	n.s.*
Residual	17,729.20	533	33.26	.....	.....
Total	17,984.14	538	.....	.....	.....

*$P > .05$.

TABLE 14

MEANS AND STANDARD ERRORS OF AGES OF ASCENDANTS OF PROBANDS

	Type of Family											
	Uniplex				Simplex				Multiplex			
	History of Hemophilia				History of Hemophilia				History of Hemophilia			
	−		+		−		+		−		+	
	Ages at Birth	Number Sibships	Ages at Birth	Number Sibships	Ages at Birth	Number Sibships	Ages at Birth	Number Sibships	Ages at Birth	Number Sibships	Ages at Birth	Number Sibships
(1) Hemophilia A:												
Mother	26.52±0.49	116	27.08±0.58	106	28.25±0.53	106	28.04±0.65	81	28.24±0.78	59	27.90±0.73	71
Father	29.73±0.57	120	30.31±0.65	107	32.03±0.64	105	31.49±0.81	81	31.33±0.97	58	31.06±0.78	69
Maternal grandfather	32.25±0.69	109	32.59±0.52	92	30.45±0.74	93	30.88±0.79	73	32.96±1.13	53	32.95±1.09	58
Maternal grandmother	28.12±0.57	110	27.89±0.75	91	27.67±0.68	95	27.45±0.74	74	28.02±0.80	53	28.62±0.88	58
Paternal grandfather	31.27±0.75	109	32.21±1.02	84	31.75±0.94	92	31.83±0.90	64	32.87±1.21	54	33.54±1.03	50
Paternal grandmother	27.61±0.62	110	28.54±0.79	84	28.91±0.78	94	28.45±0.83	64	29.51±0.88	53	29.38±1.11	50
(2) Hemophilia B:												
Mother	27.04±0.90	28	27.59±0.94	32	28.52±1.38	17	26.71±1.29	17	26.53±1.42	19	29.06±1.52	18
Father	29.90±0.80	30	31.21±1.08	33	29.94±1.54	17	30.06±1.83	16	31.00±1.51	19	29.47±1.27	19
Maternal grandfather	32.50±1.72	25	28.96±1.41	28	32.47±2.17	13	33.67±2.08	15	31.53±1.68	17	33.38±1.63	15
Maternal grandmother	29.58±1.40	25	24.93±0.88	29	29.53±2.46	13	29.75±2.18	16	26.62±1.87	16	29.06±1.59	17
Paternal grandfather	27.58±0.99	26	29.27±1.20	22	31.40±2.35	15	34.14±2.50	14	32.67±2.44	13	27.57±1.43	14
Paternal grandmother	26.81±0.86	26	25.77±1.22	22	28.40±1.70	15	29.73±1.59	15	27.50±2.19	14	23.79±1.61	14
(3) Unspecified hemophilia:												
Mother	24.92±1.18	25	28.40±1.14	35	29.36±0.79	48	28.68±1.20	29	28.07±1.29	29	30.91±1.03	42
Father	27.96±1.36	25	32.48±1.26	35	32.43±0.79	46	32.45±1.37	29	30.70±1.53	30	27.81±1.01	44
Maternal grandfather	29.33±1.00	24	33.96±1.37	32	33.38±1.26	44	29.91±1.15	22	28.84±1.31	25	32.44±1.23	36
Maternal grandmother	25.87±1.17	23	28.45±1.29	33	29.07±0.90	44	28.34±1.24	23	26.08±1.20	25	28.13±0.95	37
Paternal grandfather	30.04±1.56	23	34.61±1.37	31	31.80±1.05	42	31.10±1.64	20	32.73±1.58	22	30.60±1.34	35
Paternal grandmother	24.70±1.06	24	30.77±1.38	31	28.21±0.88	42	29.00±1.35	19	28.09±1.33	23	27.78±1.29	36

presents the mean and standard error of the distribution of ages of mothers at birth of a proband for these sibships. Pooling the data from these families, we obtained the following estimates of the mean maternal age at birth of a mutant child: $\bar{x}_s = 27.35$ years, with standard error $\sigma_{\bar{x}_s} = 0.37$ years. The estimate of the yearly increase of mutation rate in females, relative to the initial mutation rate, the ratio b/μ_0 given by equation (2), is

$$b/\mu_0 = 0.0729 \pm 0.0845 .$$

When setting 95% confidence limits, the estimate of b is not significantly different from zero:

$$-0.0927\ \mu_0 \leq b \leq 0.2385\ \mu_0 .$$

These results suggest that, under the conditions imposed for estimating changes in mutation rate relative to the initial rate, there is no effect of age on mutation rate in females, at least for the hemophilia A mutation. However, an annual increase in mutation rate of less than $0.24\ \mu_0$ could not be detected with the present data.

The means and their standard errors for the distributions of ages of mothers at birth of probands of uniplex, simplex, and multiplex sibships for hemophilia B and for families in which the type of hemophilia was not specified are also presented in Table 14.

Effect of Age on Mutation Rate in Males—Data from Multiplex Sibships

Our use of data from multiplex families is predicated upon the assumption that the probability of multiple sporadic cases occurring in the same sibship is so low that the occurrence can be safely ignored. Thus mothers of all multiplex sibships are heterozygotes, irrespective of the presence or absence of a family history of hemophilia.

If mutation rate increases with age in male gametes, carrier mothers of multiplex sibships with no family history of hemophilia should have been conceived, on the average, at a paternal age higher than that for fathers of mothers of multiplex sibships with a positive family history. This is the basis for comparing the grandparental ages at birth of the mothers of sibships with and without a history of the condition.

In examining the model relating change in mutation rate to the age of males, the assumptions concerning the normal distribution of ages of maternal grandfathers at birth of mothers of probands and the linear increase in mutation rate are necessary. The actively dividing spermatogonia, starting at puberty and continuing for perhaps 45 or 50 years, permit us to test, in males, the hypothesis that mutation is an error of DNA replication. Since the onset of puberty signals intensive DNA replication in male germ cells, the hypothesis specifies that mutation rate increases from the initial rate present at onset of puberty (μ_0). The onset of puberty, then, is age 0, and ages of maternal grandfathers at birth of mothers of probands of sibships must be referred to puberty. We have chosen 15 years as the onset of gamete formation in the male, so that 15 years will be subtracted from all age data.

The values of $\bar{x}$ and σ^2 were derived from data about maternal grandfathers of multiplex sibships in families with a history of hemophilia A, and $\bar{x}_s$ and $\sigma_{\bar{x}_s}$ were estimated from the distribution of age of maternal grandfathers at birth of mothers of probands in multiplex sibships in families reportedly free of the condition (Table

14). Age data from maternal grandfathers of simplex and uniplex sibships without a family history of hemophilia were not included in the estimation procedure because of the likelihood that some of the probands in these sibships represented sporadic cases.

The estimate of the ratio, b/μ_0, as obtained from data in Table 14, with its 5% fiducial interval is:

$$b/\mu_0 = 0.0002 \pm 0.0321 .$$

Clearly, we are far from statistical significance. Mutation rate does not increase in males with age, the increase is too small to be detected by our model with the present data, or the assumptions under which we are operating are invalid.

To pursue the analysis further, we studied families classified according to presence or absence of a family history of hemophilia A, and by type of sibship, for maternal grandfathers younger than 30 years and grandfathers being 30 or more years of age at birth of mothers of probands. The data are shown in Table 15. The χ^2 analysis of these

TABLE 15

	Maternal Grandfather 30 Years or Older			Maternal Grandfather under 30 Years	
	Positive History	Negative History		Positive History	Negative History
Uniplex and simplex sibships...	117	89	Uniplex and simplex sibships..	89	76
Multiplex sibships.....	33	34	Multiplex sibships.....	21	24

data showed no significant association between a family history of hemophilia A and age of maternal grandfather (younger than 30 years vs. 30 years and older) at birth of the mother of a proband for multiplex sibships ($\chi^2_{[1]}=0.072$) or for uniplex and simplex sibships ($\chi^2_{[1]}=0.301$).

Table 14 also presents grandparental age data for cases of hemophilia B and of hemophilia of unspecified type. The means and their standard errors for the following distributions are presented for uniplex, simplex, and multiplex sibships: (1) ages of maternal grandmothers at birth of mothers of probands, (2) ages of maternal grandfathers at birth of mothers of probands, (3) ages of paternal grandmothers at birth of fathers of probands, and (4) ages of paternal grandfathers at birth of fathers of probands.

Other Findings

Analysis of parental and grandparental age data revealed certain significant differences which do not pertain to the hypotheses being tested but which, nevertheless, we present here for their possible interest. These results pertain only to the hemophilia A group.

Fathers in uniplex families were found to be significantly younger at birth of

probands than fathers in simplex families ($F = 7.44$; $P < .01$). This can be explained by the correlation between parental age and sibship size, in this instance the maternal age being more closely correlated with sibship size and the paternal age being correlated with maternal age.

Maternal grandfathers of probands in simplex families were found to be significantly younger at birth of the mothers of probands than were maternal grandfathers at birth of mothers of uniplex and multiplex families, irrespective of presence or absence of a family history of hemophilia ($F = 6.37$; $P < .01$). We have no explanation for this finding.

DISCUSSION

In man, only mutation rates of dominant genes have been estimated directly. Estimates of mutation rates of recessive genes depend upon estimates of fitness and assumptions about mutation-selection equilibrium. Sex linkage provides opportunities for investigating recessive mutations because the hemizygous state in the male permits detection of such mutations occurring in the mother or in one of her parents. The problem is to distinguish between the isolated male mutant and the isolated male segregant individual in families which give no other evidence for a familial occurrence of the phenotype in question. Although sex linkage does not permit direct estimates of mutation rates of *X*-linked recessive genes, the genetics of the model do allow exploration of certain questions about recessive mutations which is impossible in the autosomal situation.

Analysis of variation in the secondary sex ratio provides information about the total mutation rate for recessive lethals on the *X* chromosome, but has disadvantages which could be eliminated in part by studying a single genetic entity. Thus, we studied a relatively well-defined recessive mutation located on the *X* chromosome. It is worth noting, incidentally, that in 1955 Penrose suggested studying "the effect of parental ages in sex-linked diseases such as hemophilia and the Duchenne type of muscular dystrophy" (Penrose, 1955).

Examination of the data indicated that they fit the genetic criteria for hemophilia. Although no diagnostic criteria were imposed for uniformity in this study, collecting data only from hematologists or from the National Hemophilia Foundation should insure that most cases were clinically hemophilia A or B. Attempts to insure clinical and genetic uniformity in the data are not sufficient for eliminating serious biases caused by inability to discriminate the mutant from the segregant among isolated cases. The design of this study does not allow this, and, of course, a complete discrimination is not yet possible. Still, assaying for AHG plasma levels offers the future possibility of distinguishing female carriers of the gene for hemophilia A from noncarriers (Rapaport *et al.*, 1960), and it is precisely this type of information which would help to approach the problem of separating mutant from segregant.

We must conclude that our data show no evidence of an age effect upon the hemophilia mutations. This is not to say that there is no effect of age upon frequency of recessive mutation—even more specifically, the hemophilia mutations—in man. We must improve our ascertainment of mutants and use the most appropriate analytical model to test the data before a final conclusion can be reached.

The difference between the frequencies of sporadic cases suggests a higher mutation rate for hemophilia A than for hemophilia B. In addition to new mutants, sporadic cases can represent instances of nondisjunction and expression of the abnormal phenotype in the individual heterozygous for a recessive gene, errors in diagnosis, and phenocopies; and their frequency among all affected individuals can be estimated from the excess in the observed over the expected frequency of simplex sibships (Morton, 1962). It is unlikely that chromosomal nondisjunction contributes appreciably to the frequency of sporadic cases of hemophilia A and B in our data because *XO* individuals are generally phenotypic females; almost all the probands were males. Similarly, we would not expect heterozygotes to account for the frequencies of sporadic cases found because, except for the infrequent occurrence of heterozygous Klinefelter males (*XXY*), only females can be heterozygous for *X*-linked alleles. Disorders of blood coagulation other than hemophilia A and B could account for sporadic cases in our data. Vascular hemophilia, an inherited disease associated with a prolonged bleeding time and decreased plasma AHG levels (Graham *et al.*, 1964), could be mistaken for classical hemophilia. We believe that ascertainment via hematologists tends to minimize erroneous diagnosis. We cannot present evidence indicating that phenocopies for hemophilia A and B do not occur, but it is unlikely that many sporadic cases in our sample are phenocopies. There is no a priori reason to expect different frequencies of phenocopies for the two conditions. Thus, it is a reasonable conclusion that the difference in sporadic case frequencies is due to a higher frequency of hemophilia A mutants. We must point out that the frequency of sporadic cases with hemophilia B is significantly less than the frequency of sporadic cases with hemophilia A; the difference is a real one.

The estimate of the mutation rate depends on the assumption of equilibrium between mutation and selection, certainly an approximation for hemophilia A and B with newer methods of treatment improving survival of patients with these conditions. In addition, for each disease, the estimate depends on the incidence of the condition. Biased estimates of the incidence will therefore be reflected in the mutation rates. It is important to use the appropriate population base in estimating the incidence, and this is why only data from the United States and Canada were used. The cases reported by each participating chapter of the National Hemophilia Foundation can be related to a population segment of a relatively well-defined size. An incorrect population base will bias the estimate of the mutation rate, but should not affect our ability to detect a difference between mutation rates for hemophilia A and B. In each case, the mutation rate will depend upon the same population base, so that the relationship between the two mutation rates will remain constant irrespective of the population size.

His analysis of Andreassen's data prompted Haldane (1947) to suggest that mutation rates for hemophilia are higher in sperm than in eggs. Kosower *et al.* (1962) also considered this question and, in their analysis of data on hemophilia from a number of large studies, they were unable to demonstrate differences between mutation rates in both sexes. Using an analysis similar to that of Kosower and co-workers but with different data, we too must conclude that there is no sex differential in the rate of

mutation to hemophilia A. To date, there is no compelling evidence for a sex differential of mutation rates in man.

With the discovery of hemophilia B, it is now possible to recognize two *X*-linked loci involved in the control of coagulation of the blood. Each is marked by a mutation of pathological significance in man: AHG deficiency and PTC deficiency. In this study we have presented evidence for differing mutation rates at these loci.

SUMMARY

The effect of parental age on mutation rates at the loci of hemophilia A and B was studied with data collected from various hematologists in Europe and Argentina and from the National Hemophilia Foundation in North America. No effect of maternal age at birth of the proband or maternal grandfather's age at birth of the mother of the proband on the rate of mutation to the hemophilia A gene could be detected.

Segregation analysis revealed a higher frequency of sporadic cases with hemophilia A than with hemophilia B. The difference in sporadic case frequencies was interpreted as reflecting a higher mutation rate for hemophilia A than for hemophilia B. The estimates of mutation rates for hemophilia A and hemophilia B for the North American data are $(1.31 \pm 0.10) \times 10^{-5}$ and $(0.55 \pm 0.04) \times 10^{-6}$, respectively.

We could not detect any evidence that the rate of mutation to the hemophilia A gene was different in sperm and eggs.

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