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Sex ratio of the mutation frequencies in haemophilia A: estimation and meta-analysis

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Summary. A hereditary disease with excess mortality such as haemophilia is maintained in the population by the occurrence of new cases, i.e. mutations. In haemophilia, mutations may arise in female or male ancestors of a 'new' patient. The ratio of the mutation frequencies in males over females determines the prior risk of carriership of the mother of an isolated patient. An estimate of this prior risk is required for the application of Bayes' theorem to probability calculations in carriership testing. We have developed a method to estimate the sex ratio of the mutation frequencies; it does not depend on the assumption of genetic equilibrium, nor require an estimate of the reproductive fitness of haemophilia patients and carriers. Information from 462 patients with severe or moderately severe haemophilia A was gathered by postal questionnaires in a survey that included practically all Dutch haemophiliacs. Pedigree analysis was performed for the 189 patients of these 462, who were the first haemophiliacs in their family. By the maximum likelihood method, the ratio of the mutation frequencies in males and females was estimated at 2.1, with a 95% confidence interval of 0.7–6.7. In addition, we performed a meta-analysis of all published studies on the sex ratio of the mutation frequencies. When the results of six studies were pooled, it was estimated that mutations originated 3.1 times as often in males as in females. The 95% confidence interval was 1.9–4.9. This implies that 80% of mothers of an isolated patient are expected to be haemophilia carriers.

Introduction

Only by the occurrence of new cases via mutation is a disease such as haemophilia maintained in the population despite excess mortality. Mutations may occur either in females or in males and give rise to male patients or female carriers. Patients produce additional carriers when they procreate, whereas carriers may give birth to patients or may silently pass the gene on to their daughters. Therefore, an isolated patient, i.e. a patient without prior family history, may be the result of a mutation in his mother and may then be called a true sporadic case, or he may be the son of a carrier. In the latter case, the mutation occurred in the grandparental or a further removed generation.

The probability that an isolated patient is a truly sporadic case is the complement of his mother's risk of being a carrier, and depends on the relative mutation frequencies in both sexes. If mutations occur predominantly in males, it becomes likely that the mutated gene carried by an isolated patient originated in one of the male ancestors of the patient's mother, who then would be a carrier. On the other hand, if mutations occur predominantly in females, the mutated gene may well have originated in the mother of the isolated patient. Therefore, the sex ratio of the mutation frequencies determines the risk of carriership of the mother of an isolated patient.

The indirect method

In his classic article of 1935, Haldane presented the indirect method to estimate the mutation rate of a sex-linked disease under the assumption of a balance of selection and mutation. He showed that, in genetic equilibrium, the proportion of sporadic cases to all cases can be used to estimate the sex ratio of the mutation frequencies of an X-linked disease. When patients' reproductive fitness ((f_A) is nil, such as in Duchenne's muscular dystrophy, and the mutation frequencies in males (ν) and females (μ) are equal, this proportion x is one third of the total number of patients. According to Haldane's equation, $x = \mu/(2\mu + \nu)$, x will be lower than one third when mutations occur more frequently in males than in females. This has been called the 'deficit of sporadic cases'.

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When fitness (f_A) increases, the distribution of sporadic and hereditary cases will shift towards hereditary cases and x will decrease. This can be corrected for, i.e. $x_f = (1\text{-}f_A)x_0$. A method to adjust for a decreased fitness of carriers has also been proposed (Holloway and Smith 1973). The usual methods in segregation analysis use pedigree information to estimate the proportion of sporadic cases to all cases (Morton and Chung 1959). These methods, however, have serious drawbacks. First, they depend on the assumption of genetic equilibrium. Secondly, they require a good estimate of patients' fitness. Thirdly, they are sensitive to incomplete ascertainment and ascertainment bias.

Since the beginning of this century, the life expectancy of patients with severe haemophilia has increased steadily, spectacularly so since the 1960s, from a mere 11 years in 1900 to 65 years most recently (Ikkala et al. 1982; Larsson 1985; Rosendaal et al. 1989). The effective fitness that has led to the present distribution of sporadic and hereditary cases is thus difficult to assess, whereas the assumption of a steady state is completely unwarranted. Segregation analysis is usually performed under incomplete ascertainment (Morton 1959), which can only be corrected for by estimation of the ascertainment probability. Hereditary cases are often more likely to be included in a study than isolated cases; this may lead to biased results (ascertainment bias).

Equilibrium independent method

We have developed an approach to estimate the sex ratio of the mutation frequencies; our method avoids these difficulties. In the absence of equilibrium, the increased fertility of patients that causes the imbalance will only lead to more hereditary cases. The mutation rates will still be constants. The number of initially isolated cases, i.e. sons of mothers without a prior family history of haemophilia, will therefore be dependent only on the mutation rates in males and females. A number of these will be sporadic patients, i.e. sons of non-carrier women; their number will be dependent only on the female mutation rate. Therefore, when the analysis is limited to initially isolated cases, the assumption of a steady state is not needed, nor is the fitness of patients relevant to the calculation. This restriction to initially isolated cases also limits the possibility of ascertainment bias, as has been suggested before (Winter 1980). In our calculation, the distribution of healthy and affected sibs of initially isolated cases is used to estimate the sex ratio of the mutation frequencies. We have set up a model in which the probability that a woman has more than one affected son, given that she has one affected son, is expressed as a function of the sex ratio of the mutation frequencies, which is then estimated by the maximum likelihood method. By this method, it is possible to estimate the sex ratio of the mutation rates of an X-linked disorder in the absence of genetic equilibrium.

Mosaicism

Recently, it has been shown that a mutation may cause a clone of cells with the affected gene to develop, thereby giving rise to individuals who bear a mosaic of cells with and without the mutated gene. These individuals may pass on the affected gene to more than one of their offspring. Mosaicism may exist in the gonads only (germ line mosaicism) or may be extended to all body tissues (somatic mosaicism) of males or females (Bakker et al. 1987; Higuchi et al. 1988; Maddalena et al. 1988; Hall 1988; Bröcker-Vriends et al. 1990). With respect to mutation rates, these two types of mosaicism are not different; both result from mutations during an individual's development from a fertilized zygote to the gamete-producing adult. The population mutation rate is defined as the proportion of X-chromosomes per generation changing from the unaffected state to the affected state, and not as the mutation rate per cell division (Haldane 1935; Murphy et al. 1974). Generally, the mutation rate can be viewed as the probability that an individual who did not inherit a haemophilia gene passes on such a gene to his or her offspring. The phenomenon of mosaicism does not alter this definition of the mutation rate or its sex ratio, both of which are the results of several possible events including mosaicism. Theoretically, it implies that if the sex ratio differs from unity, this may not only be caused by a higher rate of mutation per cell division in one of the sexes, or by a larger number of cell divisions in one of the sexes at an equal mutation rate per division, but also by mutations generally occurring at an earlier stage of development in one of the sexes. Moreover, it is no longer justified to discuss mutation rates in sperm and ova: this should be the mutation rates in males and females.

Meta-analysis

For the two most common X-linked recessive diseases, Duchenne muscular dystrophy and haemophilia, several studies using various methods have been carried out, with different results. In a recent review of 17 studies of Duchenne muscular dystrophy (Moser 1984), it was concluded that there was no evidence for a difference in the mutation rates in males and females. For the less common Lesch-Nyhan disease, only a few studies have been performed; here, the conclusion tends to be that the male mutation rate exceeds the female rate (Francke et al. 1976, 1977; Winter 1980).

In haemophilia, the results are not in agreement. As early as 1947, Haldane calculated a higher mutation frequency for haemophilia in males than in females. Based on a study of Andreassen of 63 pedigrees (Andreassen 1943), he suggested a ratio of ν to μ of more than 10:1. Several studies have been conducted since, some of which have confirmed this finding (Hermann 1966; Biggs and Rizza 1976; Winter et al. 1983), whereas others did not (Kosower et al. 1962; Barraï et al. 1985). An important reason for the seemingly conflicting results of these studies may be the lack of statistical power to detect a difference in the mutation rates (Karel et al. 1986). This power can be increased by pooling the results of all available studies in a so-called meta-analysis (Sacks et al. 1987). The aim of a meta-analysis is to obtain a more reliable and precise estimate than was possible in the indi-

vidual studies We therefore included the result of our analysis in a meta-analysis of all available studies on the sex ratio of the mutation frequencies in haemophilia A

Practical importance

Since the sex ratio of the mutation frequencies determines the risk of carriership of the mother and other female relatives of an isolated patient, knowledge of this ratio is of practical importance in clinical genetics.

Methods

Data collection

The data were obtained by postal questionnaires These were sent to 1162 haemophilia patients, who were identified by an extensive search covering most Dutch hospitals, the Dutch Haemophilia Society and the files of our earlier surveys of 1972 and 1978 Of the 81% (947 patients) returning the form, 935 were suitable for analysis The analysis was restricted to haemophilia A and to patients with severe or moderately severe haemophilia only, i e with less than 5% (5 IU/dl) coagulant activity factor level, assayed in the standard fashion (Veltkamp et al 1968) Of these 466 patients, a further 4 had to be excluded 2 because they were part of homozygous twins (one individual of each pair of twins was included), and another 2 since they had been adopted and all pedigree information was missing Consequently, the data presented here refer to 462 patients

The questionnaire was standardized and contained questions concerning the number of affected and unaffected siblings and whether the patient was the first haemophiliac in the family, it also included questions pertaining to demographic and general medical variables An extensive report of the findings has been published elsewhere (Smit et al 1989)

In order to estimate the ratio of male to female mutation frequencies and the probability of carriership of the mother of an isolated patient, only information about the progeny of those women who had no family history of haemophilia before the birth of their affected son was considered We used information about all other sons of these mothers in our calculations, and not only of the sons born after the haemophilic child, this resembles the subsequent sibs method (Lane et al 1983) Our approach obviously increases the statistical power, but would introduce an error if women tended to limit the size of their families after the birth of the haemophilic child This can be understood by looking at the extreme if no women at all had additional children after the birth of the affected child, we would never see sibships with more than one affected child, and we would grossly under-estimate the sex ratio of the mutation frequencies Therefore, we proceeded with the analysis only after it was established that family size restriction was absent in our pedigrees, by comparing observed and expected birth ranks (see Results)

Estimation method

Let ν be the mutation frequency in males, μ in females and let Ω be the probability of carriership of a woman without a family history of haemophilia Let r be the number of affected sons and s the total number of sons The status of the other sons of a woman without a prior family history, born before or after the haemophilic son (the initially isolated patient) determines the probability of carriership When we assume that the recurrence risk for non-carriers is negligible, all subsequent births of affected sons will increase the risk of carriership to unity, whereas the occurrence of unaffected sons will decrease it The probability of having more than one affected son, given one affected, is

$$P(r>1|r>0)=\frac{1-(s+1)(\tfrac{1}{2})^s}{1-(\tfrac{1}{2})^s+\Theta s} \quad \text{Eq I}$$

where $\Theta=\mu/\Omega$ (see Appendix for derivation of equations) Since a carrier woman without family history will either be the result of a mutation or the daughter of a carrier (without family history), $\Omega=\nu+\mu+\tfrac{1}{2}\Omega$, so that $\Omega=2\nu+2\mu$ Since $\Theta=\mu/\Omega$ it follows that the ratio of the mutation frequencies is

$$\nu/\mu=\frac{1-2\Theta}{2\Theta} \quad \text{Eq II}$$

The probability that the mother of an isolated patient is a carrier (C) is $\tfrac{1}{2}\Omega/(\tfrac{1}{2}\Omega+\mu)$, which is

$$P(C|\text{mother of isolated patient})=\frac{\nu+\mu}{\nu+2\mu}=\frac{1}{1+2\Theta} \quad \text{Eq III}$$

An estimate of Θ will yield the ratio of male to female mutation frequencies and the risk of carriership of the mother of the isolated patient

The distribution of sibships with one affected son and more than one affected son served to produce a maximum likelihood estimate of Θ and its standard error using Eq I (see Appendix for maximum likelihood method) The standard error for Θ was estimated via the second derivative of the log-likelihood function Confidence intervals for Eqs II and III were calculated after log transformation, i e via $\log(\Theta)$ for the probability of carriership of the mother of an isolated patient and via $\log(\nu/\mu)$ for the sex ratio

Meta-analysis

We searched for studies in which an estimate of the sex ratio of the mutation frequencies was present This also included studies in which analyses were performed on data collected by other workers The studies were gathered by cross referencing and an on-line search (database Medline, Cologne, FRG)

We used the natural logarithm of the sex ratio ν/μ from each study and the standard error of $\log(\nu/\mu)$ Initially, the estimates (as log ratio) were pooled in the classical precision weighted manner, with the inverse squared standard errors as weights [$w=1/(s\,e\,)^2$], the standard error of the weighted average was approximated as the inverse of the square root of the sum of the weights ($s\,e\,=1/\sqrt{(\Sigma w)}$) This method depends on a stringent assumption of homogeneity among the studies (Greenland 1987)

We investigated whether the studies were homogeneous, i e whether the differences in the reported outcomes could be attributed solely to chance variation around the 'true' parameter Homogeneity was tested by calculating $G=\Sigma\, w_i(k_i-k_e)^2$ in which w_i is the weight given to each study that was used to obtain the weighted average, k_i the estimate of $\log(\nu/\mu)$ in each study, and k_e the pooled estimate G follows a χ^2-distribution with n-1 degrees of freedom for n studies

Since the studies proved heterogeneous, we applied two other methods of obtaining a summary estimate First, the 'odd man out' method, a graphical approach recently presented by Walker et al (1988) With this method, heterogeneity will, according to Walker et al , lead to intervals remaining wide, or to the emergence of two intervals Secondly, we pooled the study results by treating the point estimates as single observations, i e without variation estimates The summary estimate is simply the unweighted average (of the log ratio) of the point estimates, whereas the standard error is calculated in the standard fashion for n (number of studies) observations The conservative method allows for heterogeneity

Results

Estimation of the sex ratio

Two hundred and seventy-three (59%) of the patients reported that they had relatives with haemophilia at the time of their birth, and may be considered hereditary cases One hundred and eighty-nine patients (41%) re-

Table 1. Affected male sibs by male sibshipsize for initially isolated patients ($n = 189$)

No affected (r)	Sibshipsize (index patient included) (s)							
	1	2	3	4	5	6	7	8
$r = 1$	74	56	18	5	1	1	2	0
$r > 1$	–	14	6	3	2	4	2	1
$r = 2$	–	14	6	2	1	1	0	0
$r = 3$	–	–	0	0	1	2	2	1
$r = 4$	–	–	–	1	0	1	0	0

ported that they had been the first haemophiliac in the family (initially isolated)

To determine whether women had limited family size after the birth of the haemophilic child, we compared observed and expected birth ranks of the initially isolated patients The observed birth rank of the 189 patients, $(\Sigma s)/n$ for n sibships, was 2 15 The expected birth rank, calculated as $\{\Sigma(s+1)/2\}/n$, was 2 16 If a tendency to limit family size had been present, the observed birth rank would have exceeded the expected birth rank Since this appeared not to be the case, information on all sibs of the initially isolated patient, including those born before him, could be used in the analysis

The distribution of healthy and affected male sibs of the initially isolated patients is shown in Table 1 The 74 patients who had no brothers were not included in the maximum likelihood estimate, since they contributed no information to the genetic status of their mother The remaining 115 patients had a total of 210 brothers, 42 affected and 168 unaffected We used Eq I to obtain a maximum likelihood estimate for Θ of 0 16046 ($s\ e\ = 0\ 06407$)

The ratio of male to female mutation frequencies can now be calculated by substitution from Eq II, $\nu/\mu = (1 - 2\Theta)/2\Theta = 2\ 12$ This implies that mutations originate more than twice as often in males as in females The 95% confidence interval, which was made log-symmetrical around ν/μ, was 0 7–6 7

Since the probability of carriership for the mother of an isolated patient is $(\nu + \mu)/(\nu + 2\mu) = 1/(1 + 2\Theta)$, this probability has a theoretical range from 50%–100% From Eq III, we find that 76% of mothers of an isolated patient will be carriers, with a 95% confidence interval, via $\log(\Theta)$, of 59%–87%[1]

Meta-analysis

We found six studies, in addition to ours, in which an estimate of the sex ratio of the mutation frequencies was reported (Haldane 1947, Kosower et al 1962, Biggs and Rizza 1976, Ananthakrishnan and D'Souza 1979, Winter et al 1983, Barrai et al 1985) In two studies, the analysis had been performed on data collected previously by other workers (Andreassen 1943 in Haldane 1947, Birch 1937, Hoogvliet 1942, Andreassen 1943, Fonio 1954 in Kosower et al 1962) In four studies, the results of carrier detection tests in the mother of isolated patients had been used to determine the fraction of sporadic patients (Haldane 1947, Biggs and Rizza 1976, Ananthakrishnan and D'Souza 1979, Winter et al 1983), whereas in the other studies, only pedigree information had been used (Kosower et al 1962, Barrai et al 1985) The number of patients ranged from 21 isolated cases to 669 sibships The reported estimates of the sex ratio ν/μ varied from 1 2–29 3

In only two studies, including ours, had the analysis been limited to more severe forms of haemophilia (Winter et al 1983) in order to reduce the possibility of ascertainment bias In all studies except ours, a method was used that was dependent on genetic equilibrium The estimates of patients' fitness used in these studies varied from 0 28–0 70

We excluded Haldane's paper (1947) from the final analysis, since the coagulation time, used by Andreassen, is not generally prolonged in haemophilia carriers (Merskey and MacFarlane 1951) Moreover, Andreassen's pedigrees were also included in the analysis of Kosower et al (1962) Although the data of Kosower et al (1962) also comprised haemophilia B patients, we decided to include their results, since these patients are only a small fraction of all haemophilia patients (Larsson et al 1982, Smit et al 1989)

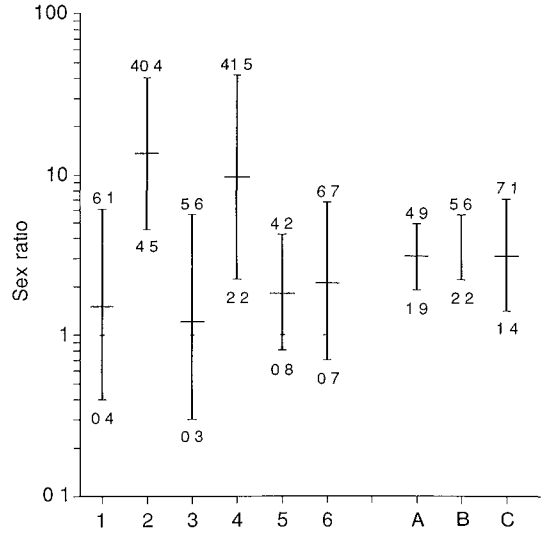

Fig. 1. Meta-analysis of six studies The point estimates are shown of six studies of the sex ratio of the mutation frequencies in males and females, with the 95% confidence interval Both the summary results by the precision weighted method *(A)*, and the 'odd man out procedure *(B)* are shown, together with the estimate calculated by treating the study results as single observations *(C)* Studies *1* Kosower et al (1962), *2* Biggs and Rizza (1976), *3* Ananthakrishnan and D'Souza (1979), *4* Winter et al (1983), *5* Barrai et al (1985), *6* Rosendaal et al (this study)

[1] List of symbols

- ν mutation rate in males
- μ mutation rate in females
- f_A fitness of patients
- x proportion sporadic patients on all patients
- i number of affected males in a sibship
- s total number of males in a sibship
- Ω probability of carriership of a woman without a family history of haemophilia
- Θ μ/Ω

We modified the results reported by Biggs and Rizza (1976). Among 41 mothers of isolated patients, from a total of 86 mothers, they found 37 carriers by factor VIII measurements. Subsequently, in the families of the four women considered homozygous, carriership testing was performed in other female relatives; this led to two more women being considered carriers. We ignored this biased search, which reduced the estimate from 29.3 to 13.5. The standard errors reported by Kosower et al. (1962) and Barrai et al. (1985) were modified to obtain log-symmetrical confidence intervals. When only a point estimate was reported (Biggs and Rizza 1976; Ananthakrishnan and D'Souza 1979), we calculated the standard error for the proportion of mothers reported to be carriers.

The summary estimate of the sex ratio as calculated in the precision-weighted method was 3.1, with a 95% confidence interval of 1.9–4.9. The confidence interval obtained by the 'odd man out' method was almost identical: 2.2–5.6 (Fig. 1). Since the studies were heterogeneous ($G = 13.3$, $P < 0.025$), we calculated a straight average and a standard error between the six 'observations'; this resulted in a summary estimate of 3.1 (95% confidence interval 1.4–7.1).

Discussion

We have estimated the sex ratio of mutation frequencies using an equilibrium independent method, and have subsequently performed a meta-analysis of all available studies on this subject. We conclude that mutations that cause haemophilia A occur more often in males than in females, at a ratio of about 3:1. This implies that about 80% of mothers of an isolated patient carry the gene for haemophilia. Therefore, carrier testing by factor VIII discriminant analysis and DNA analysis remains important in these women.

Estimation of the sex ratio

The results of a segregation analysis may be flawed because of ascertainment bias, which occurs when the probability of being a study subject is dependent upon genetic status. Usually, the bias results from an overrepresentation of hereditary cases, because these are more readily identified as patients, since they are registered as families in hospitals or genetic centres. When it is the aim of a study to estimate the proportion of sporadic cases to all patients, it is obvious that this might lead to erroneous conclusions. The problem of ascertainment bias was limited in the method presented in this paper, because only initially isolated patients and their male siblings were included. Furthermore, patients with mild haemophilia were excluded, since their disease may remain undetected for many years, whereas detection of these patients will be much more likely for familial cases. We carried out an extensive search that included most Dutch hospitals, with the help of the patients' and haemophilia physicians' organization. We are convinced that we reached virtually all Dutch haemophiliacs, since it is inconceivable that patients with severe or moderately severe haemophilia would not have been diagnosed. Indeed, we noticed that many patients were known in several hospitals. The non-response was 19%, but is unlikely to be related to genetic status. We conclude therefore that ascertainment of patients was unbiased.

Our method to estimate the sex ratio of mutation frequencies is independent of patients' fitness f_A, and is insensitive to distortion of equilibrium. It does rely on the patients' or their mothers' recollection that no cases occurred previously in a family. Haldane has shown that the mean life of a haemophilia gene in the population is $3/(1-f) - 3\mu/(2\mu + \nu)$, which is, for $f_A = 0.25$–0.40, three to four generations (Haldane 1935). Since a fair recollection up to three generations may be expected, and since a gene will be passed on silently from female to female over several generations only rarely, we feel that this does not introduce an error of importance.

Meta-analysis

The studies that we used to calculate a summary estimate were not homogeneous, i.e. the results showed more variation around the summary estimate than could be explained by chance alone. Nevertheless, we conclude that the mutation frequency for haemophilia A in males exceeds that in females, since even the most conservatively calculated summary confidence interval did not include one, whereas in none of the six studies was an estimate of the sex ratio of less than one found.

This conclusion is in accordance with studies in which, instead of the calculation of an estimate of the sex ratio, a hypothesis was tested. Bitter (1964) could not reject the hypothesis that all mothers of haemophilia patients were carriers in a survey in the Hamburg area. Vo-

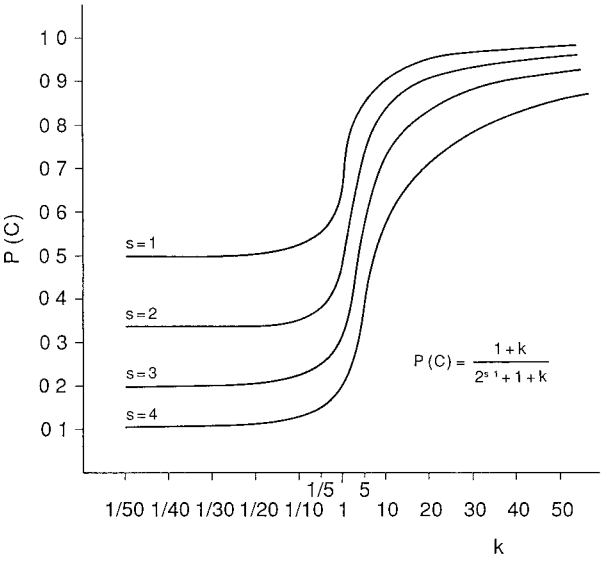

Fig. 2. The risk of carriership of the mother of an isolated patient with haemophilia A is shown as a function of s (total number of sons, of whom one is the haemophilia patient) and k, the ratio of the mutation frequencies in men and women ($k = \nu/\mu$). The function can be derived by calculating the odds of carriership, with a prior probability of carriership of $2\nu + 2\mu$

gel (1965), who used data from Sjölin (that included Andreassen's pedigrees), Fonio, Ikkala and Bitter obtained the same result. He also reported that the data were very clearly in disagreement with a hypothesis of equal mutation rates in males and females ($\mu = \nu$).

Several studies have been conducted to determine the number of carriers among mothers of isolated patients, with very different results. Ratnoff and Jones (1977) classified 85% of 39 mothers of isolated patients as carriers by factor VIII level discriminant analysis, whereas Ekert (1977), using the same method, found only 47% (of 32 women). These results, however, should not be compared and give no information on the sex ratio of the mutation frequencies. The risk of carriership of the mother of an isolated patient is dependent on the sex ratio of the mutation frequencies (the prior probability) and on the number of (unaffected) sons, i.e. in a population with a high average number of children, mothers of isolated patients will rarely be carriers. Figure 2 shows the risk of mothers of isolated patients as a function of the number of children and the sex ratio of the mutation frequencies.

Recently, the results of carrier testing by restriction fragment length polymorphisms (RFLP) in 17 families with an isolated patient were reported (Bernardi et al. 1987). In 8 families, carriership of the maternal grandmother could be excluded. In all of these families, the mothers were carriers: 6 had inherited the X-chromosome carrying the mutant gene from their father, 2 from their mother. This study, although it may be questioned since mutations in the grandpaternal X-chromosome are more readily identified than in the grandmaternal, is compatible with a sex ratio of 3.

Mosaicism

In 1935, Haldane hinted at the possibility of somatic mosaicism for haemophilia when he wrote that the mutation in the mother of two patients with haemophilia might have occurred 'during her early embryonic life'. In his paper of 1947, he offered as a general possibility for the occurrence of new haemophilia genes: 'the gene for haemophilia may have arisen by mutation in part of her body, including the ovaries in whole or part' (Haldane 1947).

Although the possibility of mosaicism for haemophilia was considered long before it was actually demonstrated, it has become customary in the practice of clinical genetics to discount it. This is also reflected in the guidelines for recognition of obligatory carriers, as all mothers of two or more haemophilic sons are included (Akhmeteli et al. 1977).

Theoretically, female mosaicism would affect our method of estimation, since in our model, we assume mothers of two or more haemophilic sons to be carriers. This would lead to an under-estimate of the female mutation rate, i.e. an over-estimate of the sex ratio. It has been shown that when a constant mutation rate per cell division is assumed, mosaicism has very little effect on the posterior probabilities (Murphy et al. 1974). This can intuitively be understood as follows: when mutations can occur at each cell division, this will only rarely take place at the very few first postzygotic divisions. Therefore, in mosaic women, only a small fraction of cells will eventually bear the affected gene. Although the recurrence risk will be much higher than the mutation rate in these cases, it will still be negligibly small. A sizeable effect of mosaicism will only be present when the mutation occurs in the earliest phase of embryonic development or gonadal development. This seems generally unlikely, unless the human organism is more sensitive to mutations in the early developmental phase than in the later stages of development. Moreover, as pointed out by Hall (1988), the frequency of somatic mosaicism might be different for different mutations. Since mosaicism affects the reliability of carrier testing in clinical genetics, an estimate of its importance for different disorders is needed. A possible approach would be to investigate the distribution of affected and unaffected sons in sibships with two or more affected children, to observe a possible departure from a segregation frequency of 0.50.

Conclusion

One mechanism that has been offered as an explanation for a possible difference in mutation frequencies in males and females is that female germ cells are produced by a limited number of mitotic cell divisions occurring before birth and only two divisions (the meiotic divisions) in the adult woman. In the male, however, numerous mitotic divisions occur throughout life, and so the number of divisions is dependent on age (Thompson and Thompson 1966; Vogel 1977).

In one study (Hermann 1966), it was reported that the maternal grandparents of haemophiliacs were older than expected, whereas mothers of isolated patients had a higher birth-rank than expected. This result, obtained by a completely different approach, gives further evidence that the mutation rate is higher in male gametes possibly because more cell divisions occur in the development of male germ cells than of female gametes.

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Appendix

Derivation of equations I, II and III

Let s be the total number of sons in a sibship, r the number of affected sons, Ω the probability of carriership of a woman without a family history of haemophilia and μ the female mutation rate In the general form, the probability of r affected sons can be written as

$$P(r) = \binom{s}{r}\left[\Omega(\tfrac{1}{2})^s + (1-\Omega)\,\mu^r(1-\mu)^{s-r}\right]$$

When we assume that μ and Ω are of the same small order of magnitude, terms involving $\mu\Omega$, $\mu^{>2}$, as denoted by , will be negligibly small It follows that

$$P(r=0) = (\tfrac{1}{2})^s\Omega + (1-\Omega)(1-\mu)^s$$
$$= 1 - \Omega\left[1-(\tfrac{1}{2})^s\right] - s\mu +$$

and

$$P(r=1) = \Omega s(\tfrac{1}{2})^s + s\mu +$$

These can be combined into:

$$P(r>1\,|\,r\geq 1)=\frac{P(r>1)}{P(r\geq 1)}=\frac{1-P(r=0)-P(r=1)}{1-P(r=0)}$$
$$=\frac{\Omega[1-(s+1)(\tfrac{1}{2})^s]+\ldots.}{\Omega[1-(\tfrac{1}{2})^s]+\mu s+\ldots.}$$

By substituting Θ for μ/Ω, dividing numerator and denominator by Ω, and leaving out the negligibly small terms, we obtain

$$P(r>1|r\geq 1)=\frac{1-(s+1)(\tfrac{1}{2})^s}{1-(\tfrac{1}{2})^s+\Theta s} \qquad \text{Eq. I.}$$

Ω can be approximated into an expression of the male mutation rate ν and the female mutation rate μ; this is most easily derived from its complement $1-\Omega$, by conditioning on the status of the maternal grandmother:

$1-\Omega = P(\text{non-carrier})$
$= \Omega\tfrac{1}{2}(1-\nu)(1-\mu)+(1-\Omega)(1-\nu)(1-\mu)$
$= (1-\tfrac{1}{2}\Omega)(1-\nu)(1-\mu)$, and therefore:

$$\Omega=\frac{1-(1-\nu)(1-\mu)}{1-\tfrac{1}{2}(1-\nu)(1-\mu)}$$
$$=\frac{\nu+\mu+\ldots.}{\tfrac{1}{2}+\tfrac{1}{2}(\nu+\mu)+\ldots.}=\frac{\nu+\mu}{\tfrac{1}{2}}=2\nu+2\mu$$

Equations II and III can now be derived by substitution:

$$\nu/\mu=\frac{2\nu}{2\mu}+1-1=\frac{2\nu+2\mu}{2\mu}-1=\frac{\Omega}{2\mu}-1=$$
$$=\frac{1}{2\Theta}-1=\frac{1-2\Theta}{2\Theta} \qquad \text{Eq. II.}$$

The probability of the mother of an isolated patient being a carrier (C) is:

$$P(C|\text{mother of isolated patient})=\frac{\tfrac{1}{2}\Omega}{\tfrac{1}{2}\Omega(1-\mu)+(1-\Omega)\mu}$$
$$=\frac{\nu+\mu}{\nu+2\mu+\ldots.}=\frac{1}{1+2\Theta} \qquad \text{Eq. III.}$$

Maximum likelihood estimate (MLE)

$P(r>1\,|r\geq 1)$ is a function of s and Θ: $p(s,\Theta)$.

MLE Θ of $\hat{\Theta}$ maximizes: $L(\Theta)=\prod_{s>1} p(s,\Theta)^{X_s}[1-p(s,\Theta)]^{Y_s}$

in which X_s is the number of families with $r>1$ and Y_s the number of families with $r=1$.

The standard error of $\hat{\Theta}$ is obtained in the usual way via the second derivative of the log-likelihood function, i.e.

$$s.e.^2=\frac{-1}{\dfrac{d^2}{d\Theta^2}\log[L(\Theta)]}\text{, at the MLE }(\hat{\Theta}).$$

Approximately, $\dfrac{\hat{\Theta}-\Theta}{s.e.\ (\hat{\Theta})}$ has the standard normal distribution. This does not imply that mean $(\hat{\Theta})=\Theta$, nor that var $(\hat{\Theta})=s.e.^2(\Theta)$, but it does imply that

$$P\left(\frac{\hat{\Theta}-\Theta}{s.e.\ (\hat{\Theta})}\leq z\right)\approx\int_{-\infty}^{z}\frac{1}{\sqrt{(2\pi)}}\ e^{-1/2x^2}\,dx$$

The normal approximation can be used to construct (approximate) confidence intervals for Θ.