Editorial

A PROBABLE SEX DIFFERENCE IN SOME MUTATION RATES

Haldane's Indirect Method for Estimating Human Mutation Rates

Since Haldane in 1935 [1] suggested an indirect method for estimating the mutation rate for hemophilia, many human mutation rates have been estimated [2]. It is assumed that a genetic equilibrium exists between selection and mutation and that mutation rate can be derived from measures of selection: $\mu = 1/3\ (1 - f)x$ (μ = mutation rate; f = fertility of hemophiliacs compared with the population average; x = incidence of hemophilia in the population). For X-linked and autosomal dominant mutations leading to a strong selective disadvantage of affected individuals, such an equilibrium can be shown to exist.

To estimate mutation rate with this indirect method, two parameters must be determined: x, the incidence of hemophiliacs in a certain, well-defined population and f, their effective fertility. In most studies, fertility of hemophiliacs was found to be roughly one-third of normal. Apart from new mutations, the loss of genes per generation due to this reduced fertility of male hemizygotes could also be compensated, at least theoretically, by an above-average fertility of heterozygous females or by shifting the segregation ratio. The first possibility is unlikely for physiological reasons, and the second was excluded [3–5]. There seems to be agreement that the incidence of hemophilia and other deleterious, X-linked diseases has been maintained in the past by an equilibrium between mutation and selection. For hemophilia, this equilibrium no longer exists, as treatment with factor VIII concentrates has vastly improved the survival of hemophiliacs.

Mutation Rates in Male and Female Germ Cells

In his basic paper, Haldane [1] showed that in equilibrium, of all affected males a fraction, $m = (1 - f)\mu/(2\mu + \nu)$, should be sons of homozygous and normal mothers (μ = mutation rate in female germ cells; ν = mutation rate in male germ cells). In the special case $f = 0$ (i.e., no male hemizygote has a chance to reproduce) and $\mu = \nu$ (i.e., identical mutation rates in both sexes), this reduces to $m = 1/3$. In X-linked diseases that are so severe that no patient reproduces, roughly one-third of all patients in 1 generation should be sons of homozygous normal women. This hypothesis will be considered in application to Duchenne muscular dystrophy and the Lesch-Nyhan syndrome.

In hemophilia, however, with f estimated as 1/3, one would expect 2/9 or 22% of affected males to be new mutants in each generation, if mutation rates in both sexes are equal. If the mutation rate is much lower in female germ cells ($\mu < \nu$), almost all

mothers of the observed cases in the population would be expected to be heterozygotes; most of the mutations necessary to maintain the genetic equilibrium should occur in male germ cells. These genes, due to their selective disadvantage, run a high risk of being eliminated a few generations after their appearance. Many new mutations may even have occurred in the germ cells of the maternal grandfathers of our present-day patients.

Our problem boils down to the question: How many of the mothers of our present-day patients are heterozygous, and how many are normal homozygotes? There are two ways to examine this question. The first uses information from segregation analysis and the second, from the physiology of heterozygotes.

Segregation analysis. Among sons of heterozygous women, we expect a 1:1 segregation ratio between affected and normal. New mutants will be the only affected in their sibships (and will have no other affected relatives). Hence, if (almost) no new mutations have occurred in the germ cells of women, the segregation ratio among the sons of the mothers of our present-day population of affected males will be 1:1. If many of our present-day patients are new mutants, the segregation ratio will deviate from the expected 1:1 ratio, and the number of sibships with just one affected male will be more than the expected.

Physiology of heterozygotes. If the methods for heterozygote detection are highly efficient, the problem becomes relatively easy: the mothers of patients can be tested. Even if there is some overlap in laboratory values between heterozygotes and normal homozygotes, a comparison between genetically confirmed and suspected heterozygotes may help. Mothers are considered genetically confirmed heterozygotes if they have more than one affected son or if further cases have occurred among their male relatives. Suspected heterozygotes are mothers of single, sporadic cases, with no other cases in the family. If both distributions are more or less identical, most of the suspected heterozygotes should be heterozygous. If an appreciable fraction of them are normal homozygotes, the distribution of suspected heterozygotes should deviate from that of the obligate heterozygotes.

Ideal Information to Apply in These Two Approaches

Complete ascertainment is necessary of all cases of a given X-linked anomaly born in a (predefined) number of years in a relatively well-defined population. For their families, reliable firsthand data on the occurrence of other cases with the same condition among brothers, and at least first-degree male maternal relatives, should be available. Since the clinical diagnosis of rare disease is often wrong, it should be confirmed, and the family study should be carried out by a team familiar with the disease. Personal examination of family members is also required. Moreover, all the mothers should be examined directly for heterozygosity using a test that permits diagnosis in every case. Given a sufficiently large sample size, such data would permit an unequivocal answer to our question.

So far, such ideal data have not been available for any X-linked condition in humans. The criticism of the paper of Francke et al. [6, 7] discussed in the letter to the editor of Morton and Lalouel [8] is largely centered around the question of which

conditions one could compromise, and how these compromises will influence the outcome.

Evidence for Hemophilia

Haldane [4], analyzing data on hemophilia families ascertained in a specific time period in Denmark [9], concluded that the mutation rate in female germ cells is lower (about one-tenth) than in male germ cells. A similar result was found from Swiss data [10, 5]. Because these conclusions were based on assertions about coagulation defects in female carriers that are now obsolete, the problem was reexamined using new information [11].

The statistical argument. The conditions of nearly exhaustive ascertainment of affected families in a population were met by the following four studies from Denmark [12], Finland [13], Switzerland [10, 14], and Germany [15]. A statistical analysis of the segregation ratio among hemophiliacs and their brothers (hemophilia A and B) was carried out for all families in which no other close relatives of the mothers were reported to suffer from hemophilia, because only in these families were new mutations in the mothers' germ cells expected. There was a perfect fit with the expected segregation ratio if all mothers of sporadic hemophiliacs were heterozygous. There was no increase in the number of sibships with just one affected male. Because the analysis of the data from the four series was fairly comprehensive, the alternative hypothesis that many mothers were, indeed, homozygous and normal and that the segregation ratio was shifted by chance in the opposite direction could be excluded.

In a disease like hemophilia, the additional problem of whether early death due to this disease leads to systematic underrepresentation of families with just one affected member arises. This, however, was not the case [11].

Examination of heterozygotes. Reliable comparisons between obligate and suspected heterozygotes of hemophilia A (in the above definition) were available for only two of the four series [13, 15], and for other case series that had not been collected on an epidemiological basis [16–18]. The two distributions coincided very well in all of these series.

Thus, segregation analysis and heterozygote testing were in perfect agreement with the hypothesis that all (or almost all) mothers of hemophiliacs are heterozygous and that the mutation rate in females is much lower than in males. With the improved techniques for heterozygote detection, especially the success in showing a cross reacting material (CRM) protein in hemophilia A patients and heterozygotes, new data on heterozygotes have recently become available [19, 20]. Again, confirmed and suspected heterozygotes show about the same distribution of anti-hemophilic factor (AHF) and CRM values, much to the surprise of the investigators.

In a recent American study, for example, 94% of 87 obligate carriers, and at least 85% of 37 mothers of sporadic cases were unequivocally identified as carriers [20]. In a British study, 39 of 41 mothers of sporadic cases were shown to be carriers [19].

Two statistical analyses were published that suggest an equal mutation rate in both sexes, accounting for the surplus of sporadic cases expected if the mutation rate were equal. The data collection in both studies, however, suffers from severe deficiencies.

In the first study [21], the authors criticized some of the outdated data which they utilized. More recent surveys [12–15] were not available to them.

Ascertainment was less controlled in a more recent study [22] which has recently been criticized in detail [2]. Statistical information was collected using questionnaires that were sent to hospitals and completed by the patients' families; no attempt was made at direct verification of diagnoses or family data. Population basis and mode of ascertainment were undefined. Segregation analysis to test for the surplus of sporadic cases was apparently performed in both studies irrespective of whether additional patients occurred in the mothers' families. Obviously, mothers might be homozygous if they have no other affected relatives apart from their sons. Therefore, to neglect this aspect would dilute the relevant information. Moreover, neither study utilized data from heterozygote testing. Because these last discussed studies [21, 22] suffer from such obvious deficiencies, the results are not convincing. A higher mutation rate of the gene for hemophilia A in male germ cells is very likely.

Duchenne Muscular Dystrophy

Haldane [23], on the basis of data from Britain [24] and Northern Ireland [25, 26], reported a deficit of sporadic cases that seemed to indicate a lower mutation rate in female germ cells. However, a new analysis with inclusion of additional series gave evidence that two of the three series agreed remarkably well with the expectation $m = 1/3$ (i.e., about one-third of all sporadic cases are sons of homozygous and normal women). This would mean that the mutation rates in the two sexes are identical. One series showed a deficit of sporadic cases even if a complete lack of mutations in female germ cells could be assumed—obviously an ascertainment bias [27, 28].

The current evidence rather favors equal mutation rates in both sexes for this mutation. However, the evidence is not very strong, especially as no reliable biochemical data from heterozygotes were included. Since these analyses have been published, a number of other population studies have become available (for references cf. [29]). Clinical determination of true muscular dystrophies from superficially similar, nongenetic diseases has improved [30], and heterozygote tests have become available. Clearly, the problem deserves a second look.

Lesch-Nyhan Syndrome

The Lesch-Nyhan syndrome is especially well suited for analysis because the clinical symptoms are distinct, the male hemizygotes never reproduce, and reliable heterozygote tests are available. Francke et al. [6] asserted in their paper that (1) there are significantly less than the expected one-third new mutants among the patients observed; (2) there are significantly more heterozygotes among the daughters of confirmed heterozygotes than expected if the segregation ratio were 1:1; and (3) the age of the fathers of heterozygous mothers (i.e., the maternal grandfathers of probands) was higher in cases in which the maternal grandmothers were normal homozygotes. The data were interpreted tentatively as indicating a higher mutation rate in male germ cells and an increase of the mutation rate with age in males.

Morton and Lalouel [8] discount these conclusions assuming an ascertainment bias due to correlation of ascertainment probability with number of affected family

members and, more specifically, to underreporting of sibships with just one affected male. To correct this bias by using the number of probands per family, they estimated the ascertainment probability as only 7.5%. Francke et al. [6], on the other hand, overcorrected this bias by limiting the analysis to those families with just one affected boy; there was still a paucity of patients with +/+ mothers. This would strongly favor a higher mutation rate in male germ cells if it were not for the strange overrepresentation of heterozygous (+/−) as compared with +/+ daughters among the female offspring of +/− women. The new data of Francke et al. [7] seem to disprove the most obvious interpretation that some of the heterozygote tests misclassified some +/+ women as +/−. In a somewhat tedious argument, Francke et al. [6] explain this deviation as an ascertainment effect. Disturbance of the primary segregation ratio by meiotic drive would be an alternative explanation. There are a few proposed examples of this in humans, but the evidence is not convincing. However, critical examinations of segregation ratios with this problem in mind are rare.

On balance then, an ascertainment bias seems to be the most likely interpretation. However, this shows the general difficulties inherent in utilizing data collected by a poorly defined ascertainment process.

The paternal age effect suggested by Franke et al. was criticized by Morton and Lalouel [8] because controls from the general population rather than intrapedigree controls were used. For some problems, use of intrapedigree controls is, indeed, the only way to get around the difficulty of socioeconomic or ethnic stratification; consanguinity, for example, is dependent on such factors. A strong consanguinity effect on perinatal mortality in France that had been assumed on the basis of population controls [31] was disproved by intrapedigree controls [32]. However, there is no obvious reason to assume that the incidence of spontaneous mutations is influenced by socioeconomic factors. Therefore, the statistical figures from the basic American population as used by Francke et al. [6, 7] in their comparison are fully adequate and, considering the smaller standard deviation, even superior. Moreover, the increase reported by these authors is in excellent agreement not only with those found in dominant mutations showing a paternal age effect [2] but also in the only two reliably ascertained series of maternal grandfathers of hemophilia A patients [2, 33, 34] with 77 patients (not so very small a sample size). It should be noted that although in one of these series, intrapedigree controls were used, and in the other, controls from the general population, the results agreed remarkably well. Thus, the observations on the Lesch-Nyhan syndrome do indeed suggest that mutation rate increases with age in male germ cells.

Mutation Rate in Male as Compared with Female Germ Cells

It is known from molecular biology that in bacteria and bacteriophages, the only organisms for which critical data are available, most spontaneous gene mutations occur in connection with DNA replication. Until fairly recently [35], the existence of replication-independent mutations had not been proven. They are now known to occur but at a much lower rate. The relevant difference in human germ cell development between males and females is that all female germ cells can be formed by as few as 23 cell divisions occurring before birth. In the male, however, depending on the age at

reproduction, several hundred cell divisions are needed [2]. If many of the gene mutations are replication-dependent, we can predict not only a higher absolute mutation rate in male germ cells, but also an increase with paternal age [36]. This is exactly what the data on hemophilia A and, with qualification [6, 7], what the data on the Lesch-Nyhan syndrome show.

Therefore, the sex difference and the increase with paternal age are plausible. In addition, the limited data that are available from mice also point to a higher mutation rate in male germ cells [37]. This, however, should not seduce us to jump to premature conclusions.

Could There Be Differences in the Sex Ratio between Mutations Leading to Different Phenotypes?

The data presently available on the Duchenne muscular dystrophy favor the view that there is no sex difference in the mutation rate, whereas hemophilia A and the Lesch-Nyhan syndrome seem to show a difference. The available data on the paternal age effect of dominant mutations also suggest heterogeneity between mutations leading to different phenotypes [2]. The spectrum of mutants identified at the phenotypic level as one hereditary disease, if examined at the molecular level, reveals itself as a mixture of different molecular changes, some of which may be replication-dependent. Extrapolating from our experiences with genotype-phenotype relationships produced by mutations affecting the hemoglobin molecule, so far the most thoroughly analyzed example in humans [38], one would expect that different hereditary diseases for which only the phenotypes are known will represent quite different mixtures of primary molecular changes. This must not necessarily lead to differences in sex ratio or paternal age effect, but would plausibly explain such differences. At least, this assumption is not nearly so farfetched as Morton and Lalouel [8] seem to assume.

In conclusion, the data of Francke et al. [6, 7] suggest that a higher mutation rate in male germ cells, which is age dependent, is found in the Lesch-Nyhan syndrome. Data on additional families are needed before definite conclusions can be drawn.

Morton and Lalouel [8] correctly suggest that these family studies should come from a carefully planned epidemiological survey. This survey should comprise a sufficiently large population (e.g., that of the United States) over a suitable time period. On the basis of approximate incidence figures, the survey should be planned in a way that a sufficient sample size can be expected. (An incidence estimate derived from the suggestion [8] that at present, only 7.5% of all cases have been diagnosed would, in my opinion, be much too optimistic!) In such a survey, very incomplete ascertainment cannot be corrected adequately by statistical sophistication. There is only one feasible alternative for getting around the nuisance parameter of ascertainment: a determined attempt to ascertain *all* cases. For the Lesch-Nyhan syndrome, this should be much easier than for most other diseases.

Certainly, such an epidemiological study is a major effort. For the Lesch-Nyhan syndrome, however, it would be worthwhile not only for examining sex and grandpaternal age effects on mutation. The HPRT defect is the only human deficiency mutation for which data on mutations in single fibroblasts are available from in vitro studies. Would it not be worthwhile to compare the in vitro spectrum of events that are

supposedly due to mutation with a spectrum from naturally occurring germ cell mutations ascertained in an epidemiological survey?

Other, more practical issues could also be included in such a survey. For example, it could be a mechanism for offering genetic counseling to all families of affected cases, for studying the efficiency of such counsel, for looking at possible clinical effects, including behavioral characteristics, in heterozygotes, and for testing therapeutic approaches on a sufficiently large scale. Last but not least, cooperation in such a study could help to bridge the gap between human geneticists interested in population genetics and epidemiology and human biochemical geneticists primarily interested in enzyme defects and molecular mechanisms. Both groups need constructive criticism from the other side.

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