No sex difference in mutation rates of Duchenne muscular dystrophy*

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SUMMARY Segregation analysis was performed to evaluate possible differences in mutation rates in man. It was based on 514 males with Duchenne type muscular dystrophy (DMD) from five of nineteen hospitals for muscular disease in Japan. The estimated proportion of sporadic cases (new mutations) was 0.291 ± 0.046 , which is in excellent agreement with the expected 0.333 if there is no sex difference in mutation rates. The rate of mutation was estimated to be 6.3×10^{-5} per generation. The incidence and prevalence rates among males were estimated to be 217×10^{-6} and 49.9×10^{-6} , respectively.

Duchenne muscular dystrophy (DMD) is a fatal hereditary disease resulting from a sex-linked gene with a high mutation rate.¹ Expression typically occurs only in boys, with onset usually in the first five years of life, but occasionally as late as the second decade. Female heterozygotes may occasionally have atypical dystrophic symptoms. Death occurs from inanition or respiratory infection usually in the second decade. This genetic trait is of particular interest because it offers an excellent opportunity not only to estimate the mutation rate but also to test the hypothesis of a possible sex difference in mutation rates in man.^{2,3}

In men, spermatogenesis begins about the age of 12, whereas in women primary oocytes are already formed at birth and have begun their first reduction division. This division is completed at puberty.⁴ These sex differences in gametogenesis lead to differences in the predictions of three putative models of mutation: (1) mutation rates depend on total chronological age of parent (so there is no sex difference); (2) mutation occurs predominantly during mitosis, (so mutation rates are higher in men than in women); and (3) mutation occurs predominantly after cessation of cell division (so

mutation rates are higher in women than in men). Hence, while the definitive resolution of the biological mechanism in mutation depends on cytological and biochemical studies in the laboratory, population surveys provide a statistical test of the hypothesis that there is no sex difference, as well as an estimate of the magnitude of mutation.

Methods

Taking u and v to be the mutation rates in eggs and sperms, respectively, the proportion of sporadic cases (x) as a result of new mutations among men with an X linked trait in the population is

$$x = \frac{mu}{2u+v} \quad (1)$$

where m is the selection coefficient ($m=1$ for a lethal disease such as DMD), or

$$\frac{v}{u} = \frac{m}{x} - 2.$$

If $u=v$, we have $x = m/3$ or $1/3$ in the case of DMD.

Incidence (I) is given by

$$I = \frac{u}{x} \quad (2)$$

so that the mutation rate in eggs is estimated by

$$u = xI.$$

Combining (1) and (2), we can estimate the male mutation rate as $v = (m-2x)I$, and the gene frequency in women as $(1-x)I$.⁵ The proportion of sporadic cases (x) and other relevant parameters have

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been estimated from segregation models,^{6,7} to which a method of maximum likelihood scoring has been applied.

Material

Two sets of data were studied by segregation analysis. The senior author reviewed medical records and found 234 male probands from 206 sibships (so ascertainment was multiple) with the aid of clinicians at the Shimoshizu hospital in Chiba prefecture and at the hospital affiliated to Tokyo University (data set 1). All probands were clinically diagnosed as having DMD. The Becker type was excluded. A questionnaire was sent to relatives (usually to the proband's mother) to confirm age, sex, inbreeding, and other relevant information about the proband and his sibs. This information was also checked with *Koseki*, an official household record of Japan.⁸ A proband was defined as an affected person who had been examined in hospital. Thus, it is unlikely that all probands would always be ascertained independently of one another. For instance, a younger brother may be admitted to hospital for evaluation if his elder brother had already been diagnosed as DMD. This inflates the estimate of the ascertainment probability in sib material.

Similarly the junior author found 433 probands, including 16 women from five of 19 hospitals for muscular disease, one each in Hokkaido and Kyushu islands and three (including Shimoshizu Hospital) in Honshu or the mainland of Japan (data set 2). In those 433 families the ascertainment was assumed to be single. Since 137 families of 417 male probands in data set 2 were duplicated in data set 1, we used the duplicated portion for reconfirmation of data set 1. This left 280 men and 16 women as probands in data set 2. Although the ascertainment in data set 2 was assumed to be single, the duplicated portion (data set 1) showed multiple ascertainment after careful, independent examination by the senior author. Since the material was gathered from federal hospitals, we believe there to be an equal probability of ascertainment in the two sets of data. Medical records of the patients admitted to hospital in data set 2 were reviewed to identify DMD. When the type of dystrophy appeared questionable, the case was accepted as a proband only after personal re-examination. Age at the proband's death, sex, and muscle wasting disease were studied for sibs of the probands and maternal sibs as well. Muscle wasting disease in a relative was presumed to be DMD unless there was evidence to the contrary. Questionnaires were sent to relatives to confirm the pertinent items. A check with *Koseki* was also made on demographic items as in data set 1.

SEGREGATION ANALYSIS

Three parameters are involved in valid segregation analysis: p , the segregation frequency of the disease among sibs; x , the proportion of sporadic cases; and π , the probability of ascertainment. The present material provides an estimate of π from the distribution of probands among affected sibs (table 1). We have 0.686 ± 0.059 for data set 1 and 0 for data set 2.

The segregation frequency of fully penetrant sex-linked genes in men is expected to be $p=1/2$. However, it is necessary to correct for bias in estimates of p because of differential selection (such as death of DMD hemizygotes in utero) and because of delayed onset in sibs. The penetrance y may be obtained from $\Sigma f(t)G(t)$, where $f(t)$ is the frequency of age t at death or at last examination among normal and affected sibs or this frequency with the index cases excluded, and $G(t)$ is the cumulative frequency of onset at age t among affected cases.⁹ After such correction we have $p=y/2=0.460$ for the ages of the men, with an average penetrance (y) of 0.911 for affected men who are not probands, 0.903 for all sibs, and 0.948 for affected men.

Taking $p=0.460$ and $\pi=0.686$ for data set 1, the hypothesis of no sex difference was tested by assuming $x=1/3$, as expected from equation 1 if $m=1$ and $u=v$. The estimated proportion of sporadic cases in data set 1 was $x=0.201 \pm 0.070$ and this did not differ significantly from $x=0.333$ ($\chi^2=(0.333-0.201)^2/0.070^2=3.56$, $df=1$) (table 2). In contrast, taking $p=0.460$ and $\pi=0$ in data set 2, the estimate of x , which differed significantly from $1/3$, was $x=0.530 \pm 0.054$ ($\chi^2=14.13$, $df=1$). Suspecting that $\pi=0$ was an underestimate, the proportion of sporadic cases was re-estimated for data set 2 assuming $\pi=0.686$ as in data set 1 (table 3). In this case, $x=0.359 \pm 0.061$ and does not differ significantly from $1/3$. Combining the two sets of data, the hypothesis that $p=0.460$, $x=0.333$, and $\pi=0.686$ is compatible with the present material, giving an estimate $x=0.291 \pm 0.046$. No significant heterogeneity between the two x s was

TABLE 1 Distribution of probands among affected sibs (data set 1: Chiba and Tokyo)

No of affected sibs	No of probands	No observed	No expected	χ^2
1	1	157	157.00	—
2	1	22	21.50	0.012
2	2	23	23.50	0.011
3	1	1	0.84	0.032
3	2	1	1.83	0.376
3	3	2	1.33	0.333
Total		206	206.00	0.764

$$\hat{\pi} = 0.686 \pm 0.059$$

TABLE 2 *Distribution of DMD boys among sibships (data set 1)*

Sibship size	No of affected	No observed	No expected(1)	χ^2	No expected(2)	χ^2
1	1	74	—	—	—	—
2	1	53	62.00	1.31	57.92	0.42
2	2	27	18.00	4.49	22.08	1.10
3	1	21	22.76	0.14	19.63	0.10
3	2	12	9.38	0.73	11.78	0.00
3	3	2	2.86	0.26	3.59	0.71
4	1	7	8.23	0.19	6.59	0.03
4	2	5	3.31	0.87	4.25	0.13
4	3	2	2.02	0.00	2.59	0.14
4	4	0	7.44	0.44	0.56	0.56
5	2	1	0.18	3.64	0.24	2.40
5	others	0	0.82	0.82	0.76	0.76
6	1	2	1.12	0.69	0.82	1.69
6	others	0	0.88	0.88	1.18	1.18
Total		206		14.46		9.22

(1) Hypothesis: $p = 0.46$, $x = 1/3$, $\pi = 0.686$.(2) Hypothesis: $p = 0.46$, $\hat{x} = 0.201$, $\pi = 0.686$.

	p	x	π	p	x	π
U	21.015	-27.227	-7.879	-3.283	0.000	-0.009
$\chi^2 = U^2/K$	1.50	4.09*	3.37	0.03	0.00	0.00
K	295.06	181.34	18.42	422.26	204.09	20.34

*Significant at 5% level ($df = 1$).

U, the first derivative of the log-likelihood of observation.

K, reciprocal of variance. For details, see Morton.⁶TABLE 3 *Distribution of DMD boys among sibships (data set 2)*

Sibship size	No of affected	No observed	No expected(1)	χ^2	No expected(2)	χ^2
1	1	93	—	—	—	—
2	1	83	72.91	1.40	81.37	0.03
2	2	22	32.09	3.17	23.63	0.11
3	1	33	29.11	0.52	35.76	0.21
3	2	15	18.18	0.56	14.74	0.01
3	3	7	7.70	0.07	4.50	1.39
4	1	15	8.37	5.26	11.17	1.31
4	2	3	5.10	0.86	4.49	0.50
4	3	1	4.32	2.55	2.74	1.11
4	4	0	1.22	1.22	0.60	0.60
5	1	2	1.96	0.00	2.82	0.24
5	2	1	0.97	0.00	0.92	0.01
5	3	2	1.23	0.48	0.84	1.61
5	others	0	0.84	0.84	0.43	0.43
6	1	1	0.73	0.10	1.12	0.01
6	2	1	0.26	2.09	0.26	2.05
6	others	0	1.01	1.01	0.62	0.62
7	1	1	0.35	1.19	0.57	0.32
7	others	0	0.65	0.65	0.43	0.43
Total		280		21.97		10.99

(1) Hypothesis: $p = 0.46$, $x = 1/3$, $\pi = 0$.(2) Hypothesis: $p = 0.46$, $x = 1/3$, $\pi = 0.686$.

	p	x	π	p	x	π
U	-73.459	59.591	16.077	-6.667	6.965	1.975
$\chi^2 = U^2/K$	8.98**	11.85**	12.53**	0.10	0.18	0.14
K	602.38	299.55	20.62	435.60	271.73	27.08

**Significant at 1% level ($df = 1$).

U, the first derivative of the log-likelihood of observation.

K, reciprocal of variance.

detected. Therefore, we conclude that the Japanese material is consistent with the hypothesis that mutation rates are equal in both sexes.

Corroboration of the sib analysis can be sought in the distribution of affected maternal uncles, with ascertainment through nephews. Information on affected maternal uncles was obtained through interviews with the mother, or relatives, or both, of the proband so that it is possible that not all the affected uncles were reported. The analysis was performed on the distribution of affected uncles strictly identified through medical records. This provides an estimate of the lower limit of x since affected uncles will be overrepresented among those with medical records and healthy uncles will be less likely to have medical records. The expected probability that the brother of a carrier woman would not be at risk is $x' = 1/2$, since the probability that a carrier woman acquired the gene through an affected father is $I(1-m)$, and by mutation is $u+v$; in both these cases she will have no affected brothers. But the gene frequency in women is $I(1-m) + u + v$ and since the approximate frequency of carrier women of rare traits like DMD is twice the gene frequency in women, we would expect $x' = 1/2$.¹⁰

Table 4 shows the result of segregation analysis on maternal uncles if there is more than one affected nephew. Test of the null hypothesis that $p = 0.460$ and $x' = 0.5$ showed a lower morbidity risk and a higher sporadic risk than expected, indicating a tendency to underrate affected uncles by the mother (presumably because of forgetfulness or other reasons). For maternal uncles ascertained through sibs with only one affected nephew (table 5), the

TABLE 4 *Distribution of maternal uncles whose number of affected nephews is greater than one.*

s	a	No observed	No expected(1)	χ^2
1	—	12	10.01	0.40
1	+	1	2.99	1.32
2	—	19	13.56	2.18
2	+	2	7.44	3.98
3	—	20	15.05	1.63
3	+	6	10.95	2.24
4	—	9	8.14	0.09
4	+	6	6.86	0.11
5	—	9	6.80	0.71
5	+	4	6.20	0.78
6	—	1	1.02	0.01
6	+	1	0.98	0.01
7	—	1	0.51	0.48
7	+	0	0.49	0.49
Total		91		14.43

s, sibship size of maternal uncles; a, affection status, — none affected; + at least one affected.

(1) Hypothesis: $p = 0.46$, $x' = 0.5$. $U_{x1} = 53$ and $Kx'x' = 247$ so that $\chi^2 = U^2/K = 11.20$ ($df = 1$) significant at 1% level.

TABLE 5 Distribution of maternal uncles whose number of affected nephews is one

n	s	0	1	2	3	4	5	6	7	8	9
1	—	20 (20.00)	28 (27.09)	29 (23.68)	28 (22.29)	19 (15.99)	14 (10.91)	9 (6.75)	4 (3.36)	1 (0.67)	1 (0.67)
1	+	0 (0.00)	4 (4.91)	2 (7.32)	3 (8.71)	4 (7.01)	2 (5.09)	1 (3.25)	1 (1.64)	0 (0.33)	0 (0.33)
2	—	16 (16.00)	27 (24.66)	21 (18.77)	19 (17.19)	23 (18.30)	12 (9.03)	6 (5.23)	2 (1.49)	1 (0.74)	—
2	+	0 (0.00)	1 (3.34)	2 (4.23)	3 (4.81)	1 (5.70)	0 (2.97)	1 (1.77)	0 (0.51)	0 (0.26)	—
3	—	6 (6.00)	7 (6.41)	16 (13.91)	11 (10.98)	6 (5.82)	3 (2.47)	3 (2.46)	1 (0.82)	—	—
3	+	0 (0.00)	0 (0.59)	0 (2.09)	2 (2.02)	1 (1.18)	0 (0.53)	0 (0.54)	0 (0.18)	—	—
4	—	2 (2.00)	5 (4.72)	4 (4.58)	2 (1.80)	3 (2.67)	2 (1.77)	1 (0.88)	1 (0.88)	—	—
4	+	0 (0.00)	0 (0.28)	1 (0.42)	0 (0.20)	0 (0.33)	0 (0.23)	0 (0.12)	0 (0.12)	—	—
5	—	1 (1.00)	1 (0.97)	—	1 (0.94)	—	—	—	—	—	—
5	+	0 (0.00)	0 (0.03)	—	0 (0.06)	—	—	—	—	—	—
6	—	—	—	—	—	1 (0.96)	1 (0.96)	1 (0.96)	—	—	—
6	+	—	—	—	—	0 (0.04)	0 (0.04)	0 (0.04)	—	—	—
7	—	—	—	—	—	—	—	0 (0.98)	—	—	—
7	+	—	—	—	—	—	—	1 (0.02)	—	—	—

n, sibship size of nephews; s, sibship size of maternal uncles; — none of maternal uncles affected; + at least one maternal uncle affected. The expected number is given in parentheses after the observed number. The χ^2 for good fit is 82.95, df = 35. The expected rate is obtained from the hypothesis that $p = 0.46$, $x = 1/3$, $\pi = 1/2$.

$U_x = 101.17$, $K_{xx} = 353.84$, $\chi^2 = U^2/K = 28.93^{**}$. $U_{x'} = 109.66$, $K_{x'x'} = 375.08$, $\chi^2 = U'^2/K' = 32.06^{**}$.

**Significant at 1% level.

probability x' was overestimated as 0.793 ± 0.036 . Again we think there is a tendency to underrate affected uncles in the present material.

Sixteen female probands diagnosed as DMD provide a test of possible autosomal recessive inheritance. Two isolated cases were excluded and the remaining 15 sibs from normal parents with at least one affected girl were studied by segregation analysis (table 6) to test the hypothesis of autosomal recessive inheritance with no sporadic cases,

TABLE 6 Distribution of sibships with at least one affected girl from normal parents

Sibship size	No of affected	No observed	No expected(1)	χ^2	No expected(2)	χ^2
1	1	2	—	—	—	—
2	1	4	4.52	0.06	5.13	0.25
2	2	2	1.48	0.18	0.87	1.47
3	1	2	1.14	0.64	1.50	0.17
3	others	0	0.86	0.86	0.50	0.50
4	1	1	1.31	0.08	2.02	0.52
4	2	1	1.29	0.07	0.75	0.09
4	3	1	0.36	1.13	0.21	3.01
4	4	0	0.03	0.03	0.02	0.02
5	3	1	0.18	3.59	0.11	7.56
5	others	0	0.82	0.82	0.89	0.89
6	2	1	0.43	0.78	0.24	2.39
6	others	0	0.57	0.57	0.76	0.76
7	2	1	0.40	0.92	0.22	2.75
7	others	0	0.60	0.60	0.78	0.78
8	1	1	0.16	4.54	0.54	0.40
8	others	0	0.84	0.84	0.46	0.46
Total		17		15.71		22.02

(1) Hypothesis: $p = 1/4$, $x = 0$, $\pi = 0.686$.

(2) Hypothesis: $p = 1/4$, $x = 0.413$, $\pi = 0.686$.

	p	x	π	p	x	π
U	-2.830	3.118	0.110	11.386	-7.544	-2.559
$\chi^2 = U^2/K$	0.04	0.38	0.01	1.60	2.97	2.98
K	199.46	25.82	2.38	81.03	19.19	2.19

U, the first derivative of log-likelihood of observation.

K, reciprocal of variance.

assuming $p = 1/4$, $x = 0$, and $\pi = 0.686$. No statistical significance was detected. There were doubts about the diagnosis of DMD in these women: three female probands were recorded as having either limb-girdle muscular dystrophy (LG) or DMD. Morton and Chung¹⁰ reported 41.3% of LG, which occasionally resembles the symptoms of DMD, to be sporadic. The hypothesis that $p = 1/4$, $x = 0.413$, and $\pi = 0.686$ is compatible with the present material. Maximum likelihood estimate of the proportion of sporadic cases was $x = 0.081 \pm 0.214$, provided that $p = 0.25$ and $\pi = 0.686$. We therefore concluded that sufficient data were not yet available to show autosomal recessive inheritance definitely in DMD. Although some cases of DMD may be the result of an autosomal recessive locus, the effect of such heterogeneity on mutation rates seems minor.

PREVALENCE AND INCIDENCE

Let us suppose that P is the prevalence, defined as the frequency of a phenotype in a given population at a given time. Morton and Chung¹⁰ have shown that $P = A/N\pi$, in which A is the total number of probands and N is the size of population. To obtain the incidence I , the frequency at birth of patients who will develop DMD during their life time, N should be replaced approximately by $N' = \sum_t n(t)G(t)[1 - D(t)]$,

where $n(t)$ is the number in the general population at age t , $G(t)$ is the cumulative frequency of onset at age t , and $D(t)$ is the cumulative frequency of death at age t among affected subjects. $G(t)$ and $D(t)$ were obtained from the present material and $n(t)$ has been taken from a demographic year book.¹¹ The ratios of P/I were 0.250 in 1960 and 0.212 in 1970. We took an average of 0.230. The prevalence A/π is then 750 ± 51 , since $A = 514$ and $\pi = 0.686 \pm 0.059$.

The population size N should be determined by the male population of the area served by the six hospitals studied. Since no such information is available at present, we first assumed it to be the total number of men who reside in the prefectures from where at least one proband was reported. The size of the male population was 35 400 279 in 1965 from 29 prefectures.¹¹ However, only one proband was reported from two prefectures, Nara-ken and Ohsaka-fu, respectively. Ignoring these, we have $N=31\,223\,136$ so that $P=(24\pm2) \times 10^{-6}$ and $I=(104.3 \pm 8.7) \times 10^{-6}$. This is a half of the incidence currently observed.¹² Owing to uncertainty about the size of the population served by all six hospitals, it seems unwise to conclude that the mutation rate of the DMD gene is lower in Japanese women than in Caucasian women.

Therefore, further evaluation was concentrated on the Chiba prefecture where the ascertainment was performed more carefully. Having $A=46$, $\pi=0.686$, and $N=1\,343\,167$ in 1965 for Chiba prefecture, we obtain $P=(49.9\pm3.4) \times 10^{-6}$ and $I=(217.0\pm14.8) \times 10^{-6}$. These figures would be underestimates, since the ascertainment probability is likely to be overestimated, presumably because of higher ascertainment among proband brothers. If we take these figures as representative of Japan, then the effective size of the male population served by the six hospitals is $N=15\,011\,055$ or 48.1% of the total male population. Since the total number of men in the whole of Japan in 1965 was 48 692 138, the number of affected men in Japan is estimated to be $48\,692\,138 \times (49.9\pm3.4) \times 10^{-6} = 2430\pm17$, and the estimated mutation rate of the DMD gene in women is $0.291 \times 217 \times 10^{-6} = 6.3 \times 10^{-5}$ per generation. The corresponding figure in men is $(1-2 \times 0.291) \times 217.0 \times 10^{-6} = 9.1 \times 10^{-5}$ as a face value.

Discussion

The estimated rate of incidence $I = 217.0 \times 10^{-6}$ is comparable with the figure of Morton and Chung,¹⁰ although the latter give the figure 279×10^{-6} for DMD including benign forms of X linked muscular dystrophy. Some pertinent figures by other workers can be found in Brooks and Emery,¹² who obtained $I = 265 \times 10^{-6}$ in south-east Scotland and $I = 212 \times 10^{-6}$ as the crude mean of ten studies, some with clear evidence of incomplete ascertainment. All studies were of the same magnitude. Vogel and Rathenberg³ consider the figures 'surprisingly high'. The Japanese material also supports statistically the hypothesis that there is no sex difference in mutation rates. We found $x = 0.291 \pm 0.046$ to be the proportion of sporadic cases. Davie and Emery¹³

reanalysed some Caucasian material and found an average of x of 0.335 ± 0.098 . Morton and Chung¹⁰ gave a comparable estimate to the present one as 0.355 ± 0.050 . Most studies, except for Morton and Chung,¹⁰ did not state the proportion of sporadic cases with proper allowance for incomplete ascertainment. A deficiency of isolated cases might be observed on the incorrect assumption of truncated selection or the probability of ascertainment being $\pi=1$.¹⁴ In contrast, the assumption of single selection ($\pi=0$), as in the case of a part of the present data, results in an excess of sporadic cases. Therefore, proper correction of ascertainment bias is crucial before more complicated explanations of differences from theoretical expectation are sought. In segregation analysis, the segregation frequency p is rather robust against variation in the ascertainment probability π , whereas estimates of the proportion of sporadic cases x are quite sensitive to variation in the estimate of π .

The two locus model for DMD proposed by Tyler and Skolnick¹⁵ and Skolnick *et al*¹⁶ should be re-examined carefully with attention to ascertainment correction. The model predicts behaviour similar to a single locus disorder with $x = 1/2$, which does not agree with the present data nor with current estimates mentioned above. They have attempted to explain the two locus model by the results of Roses *et al*,¹⁷ who reported abnormalities indicative of carrier status in nearly all mothers of isolated cases. Carrier tests such as CK level¹⁸ and systemic membrane defect of leucocytes¹⁹ have rarely been applied to systematic sampling, thereby giving underestimates of the proportion of sporadic cases. Some isolated cases come to attention through an affected relative, without whom the diagnosis would not have been made.

Duchenne muscular dystrophy is unusually favourable for determining sex differences in mutation rates because the fertility of affected persons is zero. Therefore, mutation-selection equilibrium and the value of selection coefficients are not in question. Genetic drift, reproductive compensation,²⁰ ending reproduction after the birth of an affected boy, or selective abortion of sons of carriers may disturb this equilibrium, but at present Duchenne muscular dystrophy confirms the view that there is no sex difference in the mutation rate in man.

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