On the origin of deletions and point mutations in Duchenne muscular dystrophy: most deletions arise in oogenesis and most point mutations result from events in spermatogenesis

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Abstract

We present the results of a study of the rate and origin of mutations in Duchenne muscular dystrophy (DMD). Depending on the type of mutation (deletion/duplication or point mutation) present in the patient, there are widely varying ratios of male to female mutation rates. In deletions, the male mutation rate is only 30% of the female one. In non-deletional/non-duplicational mutations (presumably containing a high proportion of point mutations) the male mutation rate is at least 2·2 as high as the female one and probably much higher. Allowing for the presence of autosomal recessive phenocopies we find that k in non-deletional/non-duplicational mutations is 40·3.

These findings mean that the vast majority of deletions arise in oogenesis, while most point mutations stem from spermatogenesis. Previous investigations have shown that in other diseases and genes, most notably haemophilia B and A, but also the ZFY and ZFX genes, the male mutation rate for point mutations tends to be higher than the female one. Our results can be seen as a confirmation of this for the special case of DMD.

The influence on risk figures is considerable. As an example, the risk of the mother of an isolated case of DMD without an apparent structural anomaly of the gene of being a carrier increases from 67% to at least 76%. Given the estimate of 40·3 for k, allowing for the presence of autosomal recessive phenocopies mentioned above, it increases even further to 98%. However, as confidence intervals are still large, more data are needed to improve the estimates. Germinal mosaicism in this context is discussed.

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The estimation of the ratio of male and female mutation rates in Duchenne muscular dystrophy (DMD), an X linked recessive, genetically lethal disorder, has been a matter of some interest, both for scientific reasons and for genetic counselling, as this ratio, which will be called k in the following, has, among others, implications for risk calculation. It should also give some insight into the origin of DMD mutations. We present the results of a retrospective study, analysed by the indirect method of Müller and Grimm,[1] in order to estimate k for different types of mutations separately. A large collaborative study[2] using the same methodology has shown that the overall mutation rates for mutations causing DMD are about equal in males and females. The results of the present analysis not only give indications of the ratio of mutation rates, but also the origin of mutations.

Material and methods

FAMILIES

Data were collected at the genetic service of the Department of Human Genetics, University of Würzburg. To be included in the study, the following criteria had to be met by the families studied. (1) There had to be a definite diagnosis of DMD in the index patient. (2) Families had to be referred for carrier/prenatal diagnosis. (3) A blood sample from the index patient had to be available for deletion screening. There were 280 families fulfilling these three criteria.

Intragenic recombinations in families were excluded and a correction for the possible bias thus introduced was performed as described by Müller *et al.*[2] Blood samples from both the mother and the two maternal grandparents had to be available, too, or, if this was not the case, families were used only if the missing haplotypes could be reconstructed without ambiguities from the genotypes of the patient, his mother, and one of the grandparents. No information from other family members was used to avoid any bias for familial cases.

In a total of 117 families the grandparental origin of the mutated haplotype could be determined. These families were thus informative for the study. A duplication was found in six of these families. Detailed analysis of duplications was not pursued further because of the small numbers.

The distribution of mutation types in these families is given in table 1. It should be noted that what is called a "point mutation" in the text probably consists of a mixture of different mutation types, including point mutations, small deletions, and small duplications. They do, however, share the common feature of not being detectable on deletion screening. We did not find significantly different prevalences of proximal and distal deletions in familial versus

Table 1 Distribution of families

	Deletion	Duplication	Point mutation	Total
Sporadics cases	114	8	76	198
?	72	4	38	114
GMH	26	2	13	41
GPH	16	2	24	42
Affected sibs	22	2	14	38
?	17	0	11	28
GMH	4	1	1	6
GPH	1	1	2	4
Extended family history	21	0	23	44
?	10	0	10	20
GMH	11	0	13	24
GPH	0	0	0	0
All family configurations	157	10	113	280
?	99	4	59	162
GMH	41	3	27	71
GPH	17	3	26	46

? Grandparental haplotype could not be determined.
GMH = grandmaternal haplotype.
GPH = grandpaternal haplotype.

Table 2 Distribution of grandparental haplotypes

	Deletion	Point mutation
(A)		
GMH	41	27
GPH	17	26
(B)		
GMH	42·44	26·04
GPH	15·56	26·96
(C)		
GMH	42·44	23·74
GPH	15·56	22·64

GMH = grandmaternal haplotype.
GPH = grandpaternal haplotype.

sporadic cases, contrary to the findings of Passos-Bueno *et al*,[3] so we did not consider them separately (data not shown).

Ascertainment

As described above, a request for carrier or prenatal diagnosis or both was one of the selection criteria. While avoiding a possible bias in favour of cases diagnosed through deletion screening, this could possibly lead to bias for a certain family configuration. In a recent Dutch study with near complete ascertainment,[4] sporadic cases accounted for 62% of the families studied, affected sibs for 16%, and cases with an extended family history for 22%. In our sample these proportions were significantly different (χ^2 9·95, 2 df, $p<0·01$) with most of the difference resulting from an excess of sporadic cases (71%). This may have been caused by our selection criteria demanding the index patient to be available for testing. As this finding would bias our estimate of k we corrected the respective proportions of sporadic cases, cases with affected sibs, and cases with an extended family history in our sample to conform with the Dutch results.

DELETION SCREENING

Deletion screening was done by standard methods.[5]

HAPLOTYPE ANALYSIS

Haplotype analysis was done using a total of 15 RFLPs partly within or close to either end of the gene. Recombinant haplotypes were excluded from the analysis and corrected for, as mentioned above.

ANALYSIS

First, we analysed the proportions of grandmaternal and grandpaternal haplotypes in patients with deletions and presumed point mutations by means of a two by two contingency table (tables 2A and B) after correcting for ascertainment bias as described above.

In a second step we estimated the ratio k of male to female mutation rates both for deletions and point mutations. This approach was based on the indirect method of Müller and Grimm[1] with the correction introduced and described in detail by Müller *et al*.[2]

The method used may be briefly outlined as follows. The mutation present in a DMD patient may have arisen de novo in either the patient himself (with a probability of the female mutation rate μ on the grandpaternal X chromosome and with the same probability on the grandmaternal X chromosome) or the mother of the patient (on either the paternal X chromosome with probability v, the male mutation rate, or the maternal X chromosome with probability μ). The third possibility is that the grandmother of the patient is already a carrier, which according to Haldane's equilibrium[6] has a probability of 2 $(\mu+v)$.

Then the distribution of grandmaternal and grandpaternal haplotypes is determined only by the ratio k of the male and female mutation rates. The proportion of grandpaternal haplotypes among all DMD index patients is given by

$$\frac{GPH}{(GMH+GPH)}=\frac{v+\mu}{(4\mu+2v)},$$ as can be derived from the above.

Then k, which is v/μ, becomes

$$k=\frac{3\,GPH-GMH}{GMH-GPH}.$$

A factor r has to be introduced to allow for a possible bias owing to the omission of cases with intragenic recombinations:

$$k=\frac{(3-r)GPH-(1-r)\,GMH}{GMH-GPH} \qquad (1)$$

with GPH and GMH being the absolute numbers of grandpaternal and grandmaternal haplotypes in our sample and r the probability of intragenic recombination, which we set to 0·1.

We also calculated the proportion of deletions stemming from oogenesis and spermatogenesis, respectively, as well as the corresponding proportions for point mutations.

The proportion of mutations arising in spermatogenesis is, in general, given by

$$\frac{2k}{4+2k},$$

which simplifies to

$$\frac{k}{2+k} \quad (2).$$

In analogy the proportion of mutations arising in oogenesis is given by

$$\frac{2}{2+k} \quad (3).$$

Applying the above formulae using the ratio k found in deletions or point mutations, respectively, we find the proportions pertaining to deletions and point mutations.

Germinal mosaicism does not alter our findings very much. Indeed, it can be shown that, following our model of germinal mosaicism published in this journal,[7] the extra factors needed to introduce germinal mosaicism into the mutation-selection equilibrium for DMD are deeply confounded with the ratio of mutation rates. Incidentally and very nicely, it turns out that the estimate of k obtained neglecting the possibility of germinal mosaicism is equal to the estimate of k'd,

$$\hat{k} = \widehat{k'd} \quad (4)$$

where k' is again the ratio of male and female mutation rates ($k' = v/\mu$), and d is a function of the male and female factors needed to introduce germinal mosaicism.

$$d = \frac{(1 - g_f + g_f f_f)}{(1 - g_m + g_m f_m)} \quad (5)$$

where g_f and g_m are the proportions of mutations leading to germinal mosaicism in males and females, respectively. The parameters f_f and f_m are the proportions of affected germ cells in males and females, respectively. While at first it appears that the confoundment of k' and d may be considered somewhat annoying, it turns out that some major facts in counselling remain untouched, that is, the product k'd is equivalent to k for the calculation of some risks also.

Take as an example the risk for the mother of an affected boy without a previous family history of being a carrier. Neglecting germinal mosaicism, this risk is given as $\frac{1+k}{2+k}$. Allowing for this, the risk is $\frac{1+k'd}{2+k'd}$, the value of which is identical to $\frac{1+k}{2+k}$, given the relationship (4).

Another problem is the possible presence of autosomal recessive phenocopies (for example, Duchenne-like autosomal recessive muscular dystrophy, MIM 253 700) in our sample. This recessive disease has been estimated to be at most a twentieth as frequent as DMD.[8] Moreover these autosomal recessive cases may be assumed to be present in our sample exclusively in cases where no mutation in the DMD gene is present in the patient and in the absence of a family history typical of X linkage. If we assume that overall about 5% of cases with apparent DMD are in fact such phenocopies, then in the subset of sporadics or sib cases with apparent point mutations in our sample, which constitutes 90 of the total 280 patients in table 1 (32%), we can expect the proportion of phenocopies to be around 16%. The presence of autosomal recessive phenocopies will tend to overestimate k. The possible presence of autosomal recessive phenocopies should be corrected for. It should be noted again that the presence of autosomal recessive phenocopies plays a role only in cases without a detectable structural anomaly of the DMD gene.

Results

The distribution of grandparental haplotypes conditional on the presence of either a deletion or a point mutation in the index patient is given in table 2A (before the correction mentioned in ascertainment), table 2B (after the correction), and in table 2C (after the correction for the presence of 16% autosomal recessive phenocopies in sporadic or sib cases with supposed point mutations). Table 2B, which is the relevant one for the analysis neglecting the presence of autosomal recessive phenocopies, yields a χ^2 of 6·77, 1 df, $p < 0{\cdot}01$ (table 2A gives a χ^2 of 4·55, $p < 0{\cdot}05$). Table 2C gives a χ^2 of 5·36, 1 df, $p < 0{\cdot}03$.

Our estimate of k in deletions is 0·3 (95% confidence interval 0–2·9). In point mutations, assuming absence of autosomal recessive phenocopies, no valid point estimate is reached as, because of small sample variation, GPH is larger than GMH, which yields a negative and thus meaningless estimate of k. However, the lower limit of the confidence interval for k in point mutations is 2·2, so we may assume that, neglecting the presence of autosomal recessive phenocopies, the value of 2·2 is a reasonable minimum estimate for k in point mutations. However, k is probably considerably higher. The difference between k in deletions and k in point mutations appears significant, as suggested by the χ^2 in table 2B (χ^2 of 6·77, 1 df, $p < 0{\cdot}01$). The difference between the estimate for k in deletions and the lower limit for k in point mutations misses significance applying a likelihood ratio test of the hypothesis of equal k values in both deletions and point mutations against the hypothesis of different values in these types of mutations (likelihood ratio of 5·0, χ^2 3·22, 1 df, $0{\cdot}10 < p < 0{\cdot}05$). It should be noted, however, that this test is probably overly conservative, as we tested the lower limit for the estimate of k in point mutations owing to the absence of a valid estimate. The overall estimate of k in all 117 families is 2·4 (95% confidence interval 0·5–18·9), which is in agreement with Müller *et al*[2] ($k = 1{\cdot}14$). This is not very surprising, however, because of the large overlap of data sets in both studies.

Allowing for the presence of autosomal recessive phenocopies, which is the more realistic assumption, we find an estimate of k in point mutations of 40·3 (95% confidence interval $2{\cdot}0 - \infty$). The overall estimate of k in both deletions and point mutations becomes 1·83. The likelihood ratio test as described above is significant (likelihood ratio of 14·6, χ^2 5·36, 1 df, $p < < 0{\cdot}03$).

Given the above estimates for k (0·3 in deletions and 40·3 in point mutations) we find

that only about 13% of deletions arise in spermatogenesis and 87% occur in oogenesis. We also estimate that around 95% of point mutations arise in spermatogenesis while only about 5% result from an event in oogenesis. Disregarding the presence of autosomal recessive phenocopies and assuming the lower limit of 2·2 for k in point mutations, we would estimate that at least 52% of point mutations arise in spermatogenesis while at most 48% result from an event in oogenesis.

Discussion

We present evidence that the ratio of male and female mutation rates in Duchenne muscular dystrophy, although overall they appear to be about equal, differ depending on the type of mutation present. In presumed point mutations, errors in spermatogenesis are the most likely cause of the mutations while the opposite appears true in deletions.

For a variety of other genes it has been shown that for point mutations the male mutation rate is higher than the female one. Shimmin *et al*[9] found a higher rate of nucleotide substitutions in males and estimated k (for substitutions in the ZFX and XFY genes in humans) to be around 6. Recently it has been shown for haemophilia B by Ketterling *et al*[10] that the ratio of mutation rates varies with the type of mutation. They found a higher mutation rate in males for single base substitutions (estimate of $k = 3{\cdot}5$). In deletions they found a sex ratio of the mutation rates of 1. In haemophilia A, a study published in this journal in 1991[11] also reported a higher mutation rate in spermatogenesis than in oogenesis. Depending on the method used they estimated k to be 12·1 or 5·2. Our findings in DMD point mutations show a similar trend to the above results.

Our data concerning deletions are in agreement with a large European study[12] in which a preferentially maternal origin of deletional mutations was found, although that finding was not significant, possibly because of smaller sample sizes and also the exclusion of certain family types which possibly biased their findings towards a more conservative estimate of k.

As far as genetic counselling is concerned, risk calculation depends very much on the ratio k. Take as an example the risk of the mother of a sporadic case of being a carrier. If k were equal to 1, her risk would be 2/3, which is the figure usually given. Now assume that this patient has a point mutation. His mother's risk of being a carrier is, as a function of k, equal to $\frac{1+k}{2+k}$. Thus if k is assumed to be 2·2 her risk increases from 67% to 76%. It is probably much higher. Allowing for the presence of autosomal recessive phenocopies and using the obtained estimate of 40·3 for k, that risk is calculated to be 98%, which is very high. Of course, such figures depend heavily on the value of k. The confidence intervals still being large and overlapping, more data are urgently needed to improve our estimates.

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1 Müller CR, Grimm T. Estimation of the male to female ratio of mutation rates from the segregation of X chromosomal DNA haplotypes in Duchenne muscular dystrophy families. *Hum Genet* 1986;**74**:181–3.
2 Müller B, Dechant C, Meng G, *et al*. Estimation of the male and female mutation rates in Duchenne muscular dystrophy (DMD). *Hum Genet* 1992;**89**:204–6.
3 Passos-Bueno MR, Bakker E, Kneppers ALJ, *et al*. Different mosaicism frequencies for proximal and distal Duchenne muscular dystrophy (DMD) mutations indicate difference in etiology and recurrence risk. *Am J Hum Genet* 1992;**51**:1150–5.
4 van Essen AJ, Busch HFM, te Meerman GJ, ten Kate LP. Birth and population prevalence of Duchenne muscular dystrophy in the Netherlands. *Hum Genet* 1992;**88**:258–66.
5 Koenig M, Hoffman EP, Bertelson CJ, Monaco AP, Feener C, Kunkel LM. Complete cloning of the Duchenne muscular dystrophy (DMD) cDNA and preliminary genomic organization of the DMD gene in normal and affected individuals. *Cell* 1987;**50**:509–17.
6 Haldane JBS. Mutation in the sex-linked recessive type of muscular dystrophy. A possible sex difference. *Ann Hum Genet* 1956;**20**:344–7.
7 Grimm T, Müller B, Müller CR, Janka M. Theoretical considerations on germinal mosaicism in Duchenne muscular dystrophy. *J Med Genet* 1990;**27**:683–7.
8 Emery AEH. Population frequencies of inherited neuromuscular diseases – a world survey. *Neuromusc Dis* 1991;**1**:19–29.
9 Shimmin LC, Chang BHJ, Li WH. Male-driven evolution of DNA sequences. *Nature* 1993;**362**:745–7.
10 Ketterling RP, Vielhaber E, Bottema CDK, *et al*. Germline origins of mutation in families with hemophilia B: the sex ratio varies with the type of mutation. *Am J Hum Genet* 1993;**52**:152–66.
11 Bröcker-Vriends AHJT, Rosendaal FR, van Houwelingen JC, *et al*. Sex ratio of the mutation frequencies in haemophilia A: coagulation assays and RFLP analysis. *J Med Genet* 1991;**28**:672–80.
12 van Essen AJ, Abbs S, Baiget M, *et al*. Parental origin and germline mosaicism of deletions and duplications of the dystrophin gene: a European study. *Hum Genet* 1992;**88**:249–57.