Theoretical considerations on germline mosaicism in Duchenne muscular dystrophy

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Abstract

A newly formulated mutation selection equilibrium for lethal X linked recessive traits such as Duchenne muscular dystrophy is presented, which allows for both male and female germline mosaicism. Estimates of the additional parameters used are given, thus allowing the incorporation of germline mosaicism into the calculation of genetic risks.

Germline mosaicism, that is, the occurrence of a mutation of a certain gene during the development of germ cells, has been observed or suggested in a variety of diseases. These include ectrodactyly,^{1 2} aniridia,³ achondroplasia,⁴⁻⁸ haemoglobin Köln,⁹ Apert's syndrome,¹⁰ Rett's syndrome,^{11 12} tuberous sclerosis,^{13 14} Duchenne muscular dystrophy,¹⁵⁻²² ornithine transcarbamylase deficiency,²³ pseudo-chondroplasia,²⁴ haemophilia A,²⁵ osteogenesis imperfecta type II,²⁶ Crouzon's syndrome,²⁷ and chromosome abnormalities.²⁸

The aim of this paper is to investigate how the existence of germline mosaicism may be incorporated into the estimation of risks in DMD. DMD is a good model for the investigation of germline mosaicism owing to the high rate of new mutations, the availability of direct gene and carrier tests in a high proportion of families, and the high incidence of the disease.

Methods

In order to include germline mosaicism in risk estimations in DMD, it is necessary to redefine the mutation selection equilibrium formulated by Haldane²⁹ in 1935 (table 1).

After the model of Haldane, the mutation has to occur in the first or second meiotic division. A

Table 1 Mutation selection equilibrium (μ =mutation rate).

	Frequency of	
	Heterozygotes	Affected males
Parent generation	4 μ	3 μ
Mutations inherited Mutations occurring de novo	2 μ	2 μ
	2 μ	μ
Generation of children	4 μ	3 μ

mutation occurring after the primordial germ cell will lead to more than one oocyte or sperm carrying the mutation. This will be called germline mosaicism in the following. Mutation in the meiotic divisions may be considered an extreme case of germline mosaicism with only one oocyte or sperm instead of many carrying the mutation.

Mutations occurring after fertilisation and before differentiation of the primordial germ cell will present as somatic mosaics. Only those cells descending from the cell which first carried the mutation will be affected. If the primordial germ cell is among those descendent cells, about 50% of oocytes and spermatocytes will carry the mutation.

Therefore in order to define a new mutation selection equilibrium, two additional parameters have to be included. They are:

g-proportion of mutations leading to germline mosaicism.

f-mean proportion of half of oocytes or spermatocytes with an X chromosome in the population carrying a mutation resulting from germline mosaicism.

Equal transmission probabilities and equal mutation rates in males and females are assumed.³⁰ There was no evidence for different mutation rates in males and females in our own data (211 DMD families) (Grimm *et al*, in preparation). Mutation rate as used in this paper is defined as the rate of mutations per gene (in this case the gene coding for dystrophin) per generation.³¹ The reformulated mutation selection equilibrium is shown in table 2. In this model, which has been formulated under the above assumptions, a carrier has inherited her DMD allele from her

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Table 2 Mutation selection equilibrium under the assumption of germline mosaicism (μ =mutation rate, g =proportion of mutations leading to germinal mosaicism, f =mean proportion of half of oocytes or spermatocytes with an X chromosome in the population carrying a mutation owing to germline mosaicism).

	Frequency of			
	Heterozygotes	Germline mosaicism in females	Germline mosaicism in males	Affected males
Parent generation	4 $(1-g+gf)\mu$	2 $g\mu$	$g\mu$	3 $(1-g+gf)\mu$
Mutations inherited	2 $(1-g+gf)\mu + fg\mu + fg\mu$	$1/2f$	f	2 $(1-g+gf)\mu + fg\mu$
Mutations occurring de novo	2 $(1-g)\mu$	2 $g\mu$	$g\mu$	$(1-g)\mu$
Generation of children	4 $(1-g+gf)\mu$	2 $g\mu$	$g\mu$	3 $(1-g+gf)\mu$

mother, who is a carrier herself $[2(1-g+gf)\mu]$ or is a germline mosaic $[g\mu]$, or from a mosaic father $[g\mu]$. As can be seen, the probabilities for a carrier woman to inherit her DMD allele from either a germline mosaic father or mother are equal $[g\mu]$. Finally, she can also be a new mutation $[2(1-g)\mu]$. A male can be affected because his mother is a carrier $[2(1-g+gf)\mu]$, a germline mosaic $[g\mu]$, or because he is a new mutation $[(1-g)\mu]$.

Under this model an average of 2/3 of mothers of DMD patients are carriers, a proportion of $gf/[3(1-g+gf)]$ are germline mosaics, and in $(1-g)/[3(1-g+gf)]$ the patient represents a new mutation.

Results

In order to include the mutation selection equilibrium formulated in table 2 in risk calculations, it is helpful to estimate the parameters g and f . Both g and f have to be within the interval $[0;1]$. Although attempts have been made to estimate the proportion of germ cells carrying a mutation based on sibship structure,^{32,33} we will restrict ourselves to estimating f , which is a population value. This approach is independent of sibship size and greatly facilitates computations.

Germline mosaicism can be shown directly only in families with at least two gene carriers in one sibship (affected males or heterozygous females) with a recognisable structural anomaly in the DMD gene. In the case of germline mosaicism, this anomaly will be demonstrable in neither the mother nor the father of these gene carriers. For the estimation of g and f only families with two affected sons with no previous family history will be considered.

A family of this type may be found owing to:

- (1) mother being a carrier
owing to a mutation $=2(1-g)\mu$
having two affected sons $=1/4$

- (2) mother being a carrier
owing to germline mosaicism
in her father $=g f \mu$
having two affected sons $=1/4$
- (3) mother being a carrier
owing to germline mosaicism
in her mother $=g f \mu$
having two affected sons $=1/4$
- (4) both mother and grand-
mother being carriers $=2(1-g+gf)\mu$
having two affected sons $=1/4$
having no family history $=x$
- (5) germline mosaicism in the
mother $=2 g \mu$
having two affected sons $=1/4 f^2$

Thus the probability (p) of germline mosaicism in this subgroup of mothers is:

$$p = \frac{2/4 g \mu f^2}{1/2(1-g)\mu + 1/4 g f \mu + 1/4 g f \mu + 1/2 x(1-g+gf)\mu + 2/4 g \mu f^2} \quad (1)$$

or

$$p = \frac{g f^2}{(1+x)(1-g+gf)+g f^2} \quad (2)$$

Solving (2) for g yields (3):

$$g = \frac{1}{1-f+f^2(1-p)/p(1+x)} \quad (3)$$

Darras *et al*¹⁸ estimated p to be about 0.1.

Bakker *et al*²⁰ reported an increased recurrence risk for 'new' Duchenne muscular dystrophy mutations. They pointed out that in the case of an apparent 'new mutation' in DMD there was a recurrence risk of at least 7%. This figure was then given as the risk to be used in counselling mothers and sisters of apparent new mutations.

After the new mutation selection equilibrium (table 2), using a Bayesian approach the recurrence risk (B) for the mother of an apparent new mutation may be expressed as

Table 3 Risk calculation under the condition of germline mosaicism.

	Carrier		Germline mosaicism		Non-carrier	
Mother	$4 \frac{(1-g+gf)\mu}{1/2}$		$2 \frac{g\mu}{1/2f}$		$1 \frac{(1-g)\mu}{(1-g)\mu}$	
Affected son	Affected	Unaffected	Affected	Unaffected	Affected	Unaffected
Fetus	$\frac{1/2}{(1-g+gf)\mu}$	$\frac{1/2}{(1-g+gf)\mu}$	$\frac{1/2f}{1/2gf^2\mu}$	$\frac{1/2+1/2(1-f)}{gf\mu-1/2gf^2\mu}$	$\frac{(1-g)\mu}{(1-g)^2\mu^2}$	$\frac{1}{(1-g)\mu}$
Fetus (affected)	$\approx \frac{(1-g+gf)+1/2gf^2}{3(1-g+gf)}$		Mother (germline mosaic) $\approx \frac{gf}{3(1-g+gf)}$			

$B = \frac{1/2 g f^2}{1-g+g f}$
 and solving (4) for g yields (5):
 $B = \frac{B+1/2 f^2-B f}{2 B-2 B p-p}$
 With the help of (3) and (5) x can be estimated:
 $x = \frac{p}{p}$
 If $B=0.07$ and $p=0.1$ then $x=0.26$. In our own DMD families we estimated x to be about 0.25.

DMD pedigree. The aunt of the affected boy is the consultand. DNA haplotyping shows that the affected boy has a grandpaternal haplotype (A=grandpaternal, B and C=grandmaternal haplotype).

Barbujani *et al*³⁴ estimated the proportion (D) of mothers of DMD patients being a germline mosaic to be at least 10%. This estimate was derived from segregation analysis in 1885 DMD families.

After the new mutation selection equilibrium, D will be:

$$D = \frac{g f}{3(1-g+gf)} = \frac{2B}{3f} \quad (7)$$

solving (7) for g yields (8):

$$g = \frac{1-f+f/(3 D)}{1-f+f/(3 D)} \quad (8)$$

$$\frac{B+1/2 f^2-B f}{2 B-2 B p-p} = \frac{1-f+f/(3 D)}{1-f+f/(3 D)} \quad (9)$$

(9) may be solved for f:

$$f = \frac{2 B}{3 D} \quad (10)$$

If $B=0.07$ and $D \geq 0.1$ then $f \leq 0.47$ and $g \geq 0.48$. The minimum for f will be about 0.14, because then $g=1$. With B fixed at 0.07 it follows from the constraints on f and g that D has to be equal or more than 0.05 (at $f=1.00$ and $g=0.14$) and equal or less than 0.33 (at $f=0.14$ and $g=1.00$).

The proportion of mothers being a germline mosaic among all female gene carriers (M) is:

Table 4 Risk calculation under the condition of germline mosaicism for the consultand in the figure.

	Germline mosaicism			Normal	
Grandfather	$\frac{g\mu}{\text{Carrier}}$			$1 \frac{\text{Germline mosaicism}}$	Not carrier
Mother	f		Carrier (new mutation)	$2 \frac{(1-g)\mu}{1/2 f}$	$1 \frac{(1-g)\mu}{(1-g)\mu}$
Son affected	$1/2$		$1/2$	$1/2$	$1/2$
Mutation on the grandpaternal haplotype	Carrier	Not carrier	Carrier	Not carrier	Not carrier
Aunt	$\frac{f}{1/2gf^2\mu}$	$\frac{1-f}{1/2gf(1-f)\mu}$	$\frac{1}{1/2(1-g)\mu}$	$\frac{1}{1/2gf\mu}$	$\frac{1}{1/2(1-g)\mu}$
Aunt (carrier)	$\approx \frac{gf^2}{2(gf+1-g)}$			Grandfather (germline mosaic) $\approx \frac{gf}{2(gf+1-g)}$	

$$M = \frac{g}{g+2(1-g+g f)} \quad (11)$$

Under the constraints put on f and g above:
 $0.24 \leq M \leq 0.78$.

EXAMPLE CALCULATIONS

The following calculations will show an application of the new mutation selection equilibrium to risk calculations in DMD.

A small family with only one child, a boy affected with DMD, is assumed. A negative history for DMD is also assumed. In the absence of CK and DNA information a risk of $1/3$ or roughly 33.3% would be given for a male fetus to be affected.

Allowing for germline mosaicism, the Bayesian approach shown in table 3 applies and the risk of the fetus being affected (R_F) may, discounting a second, independent, new mutation, be given as:

$$R_F = \frac{1-g+g f + 1/2 g f^2}{3(1-g+g f)} = 1/3 + B/3 \quad (12)$$

Under the condition that $B=0.07$, the resulting risk figure is 0.36 instead of 0.33 without germline mosaicism.

In a second step, let the index patient have a grandpaternal haplotype in the dystrophin gene region; the possibility of undetectable crossover be discounted, and a detectable deletion not be present. The aunt of the affected boy is requesting her carrier risk (figure).

In the above situation the aunt is at risk only in the case of germline mosaicism in the grandfather or in the case of a second, independent, new mutation, the latter being discounted because of its low probability. The Bayesian approach for this situation is shown in table 4. The risk for the aunt (R_H) is:

$$R_H = \frac{g f^2}{2(1-g+g f)} = B \quad (13)$$

The resulting carrier risk (R_H) is 0.07 (if $B=0.07$). The probability that the grandfather is a germline mosaic (G) is:

$$G = \frac{g f}{2(1-g+g f)} = \frac{B}{f} \quad (14)$$

G is between 0.07 ($B=0.07$, $f=1.00$) and 0.5 ($B=0.07$, $f=0.14$). At $D=0.1$ G is 0.15 .

Discussion

Problems in risk estimation similar to those posed by germline mosaicism are caused by half chromatid mutations.^{35 36} However, these are not discussed in this paper.

Under the germline mosaicism hypothesis, risk figures are dependent on g and f . Hartl³² and Murphy

*et al*³³ followed approaches different from ours by developing methods to estimate the proportion of mutated germ cells for an individual subject. Our approach aims at the estimation of the parameters g and f , which we defined above as describing a population, not an individual subject. This has the great advantage of resulting in considerably easier algebra. By redefining the mutation selection equilibrium it may readily be used in Bayesian risk calculations and incorporating it into existing and widely used computer programs such as LINKAGE³⁷ seems desirable.

The published empirical data^{18 20 34} and our theoretical data show that the influence of germline mosaicism on risk figures in DMD is substantial.

As it is important to have reliable data on the frequency of germline mosaicism it is vital to define first what is meant by the frequency of germline mosaicism. Two definitions appear of practical value. The first defines the frequency of germline mosaicism as the proportion of germline mosaics among all female gene carriers, which is equivalent to formula (11). The frequency, then, would be M . As stated above, M should be between 0.24 ($B=0.07$, $D=0.10$) and 0.78 ($B=0.07$, $D=0.33$). A second definition of the frequency of germline mosaicism could be the proportion of patients whose mothers are germline mosaics, which is the one used by Barbujani *et al.*³⁴ This corresponds to the D in formula (7). We could show that D has to be equal to or more than 0.05 (at $f=1.00$ and $g=0.14$) and equal to or less than 0.33 (at $f=0.14$ and $g=1.00$).

It follows from this model that the mutation rate for the DMD locus should be notably higher than previously indirectly estimated. Disregarding germline mosaicism, the mutation rate is one third of the incidence (I).²⁹ Incorporating germline mosaicism the mutation rate is:

$$\mu = \frac{I}{3(1-g+g f)} \quad (15)$$

If $g=0.48$, $f=0.47$, and $I \approx 3 \times 10^{-4}$, then $\mu \approx 1.3 \times 10^{-4}$.

As a practical consequence of our considerations it would appear imperative to consider prenatal diagnosis in the mother of a seemingly 'new mutation', as she could very well be a germline mosaic for DMD.

Further investigation is needed to strengthen the estimates of g and f .

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