Estimate of severe autosomal recessive limb-girdle muscular dystrophy (LGMD2C, LGMD2D) among sporadic muscular dystrophy males: a study of 415 families

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Abstract
Ninety-five percent of cases of severe muscular dystrophy with early childhood onset result from mutations in the dystrophin region of the human X chromosome (DMD, McKusick 310200), whereas 5% are thought to result from mutations in autosomal genes. We examined a total of 415 families with at least one living patient whose clinical features suggested DMD. Based on formal genetics, haplotype analysis, and dystrophin determinations, we estimate that one in eight (11.8%) sporadic male patients carries autosomal rather than X chromosomal mutations.

Duchenne muscular dystrophy (DMD) is one of the most common monogenic disorders; it is caused by X linked recessive mutations at the dystrophin locus on band Xp21.1,5 The autosomal recessive forms of muscular dystrophy (Duchenne-like autosomal recessive muscular dystrophy, McKusick 253700,6 LGMD2 types A, B, C, and D (limb-girdle muscular dystrophy types 2 A, B, C, and D);2 McKusick 253600, 253601, 253700, 600506, 600119) are heterogeneous since gene assignments have been made to different loci: 15q (LGMD2A),8 2p13.3 (LGMD2B),9 13q12 (LGMD2C, previously SCARMD1),10 and 17q12-21.33 (LGMD2D, previously SCARMD2).11,12 The autosomal recessive childhood form of SCARMD (severe childhood autosomal recessive muscular dystrophy) has recently been classified as LGMD2C and LGMD2D7 and this nomenclature will be used throughout this paper.

LGMD2 is characterised by normal dystrophin,13 whereas the muscle biopsies of typical X linked DMD patients show virtually no dystrophin. In autosomal recessive LGMD, a deficiency of adhalin (50 kDa dystrophin associated glycoprotein (50 kDa DAG)) has been shown either as a primary gene defect in the adhalin gene on chromosome 17q1213 or as a secondary event to a separate gene on chromosome 13q.10

Case reports
Two pedigrees were chosen to illustrate our reasoning in favour of LGMD2C/LGMD2D in families presenting with single affected males. In family A (fig 1), III-1 was suspected of having DMD because of his clinical findings (pseudohypertrophic body, muscle weakness, spinal lordosis, calf pseudohypertrophy, waddling gait, discrete Gower’s sign) and high serum creatine kinase (CK) levels. No deletion in Xp21 was detectable. Indirect genetic diagnosis showed that he had inherited the grandmaternal (I-2) alleles from his mother (II-2). The two healthy uncles (II-3 and II-4) inherited different haplotypes from the patient’s grandmother. This leads to the conclusion that the grandmother cannot be a carrier for DMD. The proband (III-1) could either be the result of a new mutation in one of his mother’s germ cells or in one of his grandmother’s germ cells. In 1989 the mother of the proband became pregnant again. Prenatal diagnosis was carried out and the fetus showed a female karyotype. To confirm his diagnosis, the proband underwent muscle biopsy. Dystrophin analysis yielded dystrophin of normal size and abundance. Therefore the mother was counselled that the risk for her female fetus being affected was 25%; the mother decided to continue pregnancy. After the birth the newborn daughter had extremely high CK levels (2065 IU/l) and at the age of 1 year she showed clinical signs of muscular dystrophy like her brother. Thus, autosomal recessive muscular dystrophy is the most likely explanation in this family.

The first son (III-1) in family B (fig 2) was diagnosed as having DMD because of his clinical manifestations (muscle weakness, Gowers’ sign, calf pseudohypertrophy, spinal lordosis)
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Evaluation of families
Between 1982 and 1995, 415 families with at least one living patient with clinical signs of DMD were referred to our laboratory. All patients were screened for deletions either with the 14 kb cDNA of human dystrophin6 (until 1990) or with multiplex PCR using 18 (in part 19) exon specific primers15,16 (from 1990 onwards). These assays detect about 98% of all deletions within the Duchenne gene region. If we did not find a deletion in the latter cases but a completely negative dystrophin status, we performed further deletion screening with cDNA. In 256 families a deletion, duplication, or point mutation was detected by DNA analysis of the DMD gene. Thirty-one of the remaining 159 families showed typical X linked inheritance with one or more affected sons in at least two generations. The remaining 128 families consisted of sporadic/sib cases in which autosomal recessive inheritance had to be taken into consideration. Assuming a prior probability of 5% (21 of 415 families) for autosomal recessive inheritance among all families with a clinical diagnosis of DMD,17 21 of these 128

and raised CK levels. Deletion screening was negative. Indirect DNA analysis yielded the same haplotype in the region of Xp21 in the affected and unaffected brother (III-2). If the gene defect were X linked, the explanation would have to be a de novo mutation in the affected boy. His normal dystrophin status suggests autosomal recessive inheritance.

Figure 2. Pedigree of family B.

Figure 3. Estimation of the proportion of severe autosomal recessive limb-girdle muscular dystrophy (LGMD2C/ LGMD2D) among 415 muscular dystrophy families. *The estimate of 83.6% X linked of these sporadic/sib cases is an arithmetical derivative from the starting 5%, after having removed the familial cases and those with detectable Xp21 mutations.
families (16-4%) could be the result of autosomal recessive inheritance (fig 3). For 51 cases of the 128 families, dystrophin analysis of muscle biopsy was available in order to determine the presence or absence of dystrophin (western blot/imunohistochemical analysis). Forty-five patients lacked dystrophin, but six patients showed dystrophin of normal size and abundance. This yields a cumulative risk of 11-8% that sporadic/sib cases are affected by LGMD2C/LGMD2D. In 1991, a study by Vainzof et al estimated that about 8 to 12% of males with a clinical diagnosis of DMD, in whom X linked inheritance had been excluded, are thought to be affected by autosomal recessive muscular dystrophy.

The flow chart shown in fig 3 summarises the course and the results of our investigation.

Discussion

For both DMD and the milder form, BMD (Becker muscular dystrophy), an autosomal recessively inherited phenocopy is known. Two important aspects should be taken into account in genetic counselling of families with a sporadic/sib DMD case without a detectable anomaly in the dystrophin gene.

1) If one assumes that about 5% of all childhood MD cases are caused by autosomal recessive mutations,16 16-4% of all sporadic/sib cases without a detectable mutation are expected to show the autosomal recessive mode of inheritance. Our own dystrophin data showed normal dystrophin in six of 51 (11-8%) patients. A realistic estimate of the proportion of autosomal recessive muscular dystrophy therefore might lie between 11-8% and 16-4%. Sibs of both sexes of these affected males would have a probability of 0-25 of being affected.

2) With regard to sisters of affected males it is important to know whether the defect follows autosomal or X linked inheritance, since the risk for affected sons of these sisters differs greatly. If the sister is a carrier of X linked DMD, 50% of her sons would be affected. If the disease is autosomal recessive, the risk for her children would be minimal. For the determination of genetic risk and planning a preventive strategy in DMD families, it is essential to distinguish between X linked DMD and severe LGMD2C/LGMD2D. If there is no detectable mutation in the region of Xp21 in sporadic/sib cases we strongly recommend taking a muscle biopsy for dystrophin analysis.

A quite frequent situation in clinical practice is the lack of dystrophin determination in sporadic cases without evidence for a detectable structural anomaly in the dystrophin gene. In such cases, the following considerations apply (fig 4).

In the situation illustrated in fig 4, female offspring would carry a risk of 25% (a priori probability for autosomal recessive inheritance) multiplied by 11-8% (posterior probability for autosomal recessive inheritance), that is, 2-9%.

Male offspring would carry a risk of 45% (X linked inheritance, assuming that the mother has a probability of 90% of being heterozygous) multiplied by 88-2% (who were the 45 dystrophin negative patients in our investigation, meaning X linked inheritance) plus 2-9% (the mother and the father are heterozygous for autosomal recessive muscular dystrophy), that is, 42-6%.

In the case that there are two affected male sibs (fig 5), the risk for an affected son would amount to around 46%, while the risk for an affected girl, assuming autosomal recessive inheritance, does not decrease significantly.

So far there are no reliable published data about the distribution of CK values in heterozygotes in autosomal recessive LGMD. Therefore it would seem to be important to determine CK levels of all mothers and fathers of a sporadic/sib DMD case, in whom no detectable anomaly in Xp21 or dystrophin in muscle biopsy is found.


11 The mode of inheritance is autosomal recessive (fig 3). The risk for the mother 2/1 in the model pedigree being heterozygous for DMD is 66-7%, if the mutation in the affected son is unknown. If the patient has an undetectable mutation (point mutation) her risk of being heterozygous increases to about 90%. (fig 3) The risk for the mother 2/1 in the model pedigree being heterozygous for DMD increases to 99-9% because of her two affected sons; see also the legend to fig 4.
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