

GENETIC RISK ESTIMATES FOR DUCHENNE DYSTROPHY (DMD) IN THE ABSENCE OF DNA DELETIONS IN THE CENTRAL REGION OF THE DYSTROPHIN GENE

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ABSTRACT

In a screening of 164 unrelated Duchenne patients (DMD) done with cDNA probes cf23a and cf56a (which detects about 70% of DNA deletions), it was observed that the frequency of DNA deletions was significantly greater for sporadic (0.56) than inherited cases (0.34). Such a finding has important implications since it is shown that the absence of DNA deletions leads to a greater genetic risk for further pregnancies than if a molecular deletion is found in the proband. It is suggested also that in the absence of a molecular deletion in the propositus a muscle biopsy should be performed for dystrophin study to exclude the diagnosis of an autosomal recessive "Duchenne-like" muscular dystrophy.

INTRODUCTION

Duchenne muscular dystrophy (DMD) is a lethal X-linked disease caused by a defect in the muscle protein dystrophin (Hoffman *et al.*, 1987, 1988).

Several independent investigations performed with the use of cDNA have reported that 40 to 60% of affected patients have deletions within the DMD gene (Forrest *et al.*, 1987, 1988; Darras *et al.*, 1988; Read *et al.*, 1988; Liechti-Gallati *et al.*, 1989; Sugino *et al.*, 1989; Passos-Bueno *et al.*, 1990; Rapaport *et al.*, 1990). In 6% duplication mutations in the dystrophin gene were found (Hu *et al.*, 1988, 1990; Den Dunnen *et al.*, 1989). In the remaining patients the disorder is probably due to a point mutation or a small deletion (or rearrangement) not detected by current technology.

The identification of a DNA deletion or duplication in an affected proband allows direct prenatal diagnosis for further male pregnancies. However, when no molecular defect is detected, genetic counseling and recurrence risks estimates depend on polymorphic DNA markers located within, proximal or distal to the DMD gene.

In a preliminary screening of 34 DMD patients with cDNA probes cf23a and cf56a (which detects about 70% of DNA deletions, Forrest *et al.*, 1988) it was observed that the frequency of DNA deletions was significantly greater in sporadic than inherited cases (Passos-Bueno *et al.*, 1990). This study, extended to 164 unrelated DMD patients (Rapaport *et al.*, 1990) gave further support to our previous observation. Molecular deletions were found in 43% of the patients but the frequency among sporadic cases (56%) was significantly greater than among inherited ones (34%).

In addition, we had estimated that 3-5% of isolated DMD patients may have the autosomal recessive DMD-like dystrophy (AR-"DMD-like") and that this proportion may be around 10% (8-12%) among DMD patients with no detected DNA deletion in the central part of the gene (Zatz *et al.*, 1989; Vainzof *et al.*, 1990). Such patients may now be identified through dystrophin assessment of muscle biopsies which shows, in contrast to X-linked DMD a positive immunostaining pattern.

These observations have important implications for genetic counseling since, as shown below, in families in which no DNA deletion is detected in the proband with probes cf23a and cf56a, there is an increased recurrence risk for further pregnancies.

ESTIMATION OF HETEROZIGOSITY RISKS AND DISCUSSION

Based on the deletion detection rate found with the cDNA probes cf56a and cf23a, the probabilities of not finding a molecular deletion in a proband diagnosed as DMD are:

- a) for inherited cases: $(1 - 0.34) = 0.66$ or 66%;
- b) for sporadic cases: $(1 - 0.56) = 0.44$ or 44%.

Taking such data into account, it is estimated, through Bayesian calculation, that a mother of an isolated boy diagnosed as DMD in whom no DNA deletion is detected with these probes will have a probability of 75% of being a carrier of the DMD gene. If this mother has normal serum CK (taking into account that 2/3 of the DMD carriers and 1/20 of the normal female controls have elevated serum CK levels), her risk of being a carrier of the DMD gene will still be 51%. (See appendix).

In addition, in order to calculate the recurrence risk for further pregnancies, the probability (approximately 10%) that an isolated boy diagnosed as DMD is

affected by AR-"DMD-like" dystrophy also has to be taken into account. With this additional information, the final estimated recurrence risk for parents of an affected isolated DMD boy in which no DNA deletion was detected is: a) 19% if there is no information on serum CK and; b) 14% if the mother has normal CK, as shown in the appendix (situation 1).

On the other hand, if a DNA deletion is detected in the proband, the possibility of autosomal recessive inheritance is excluded. In this case (appendix, situation 2), the final estimated recurrence risk for another affected child will be approximately 15% if there is no information on serum CK and 7.5% if normal enzyme activity is found in the proband's mother, that is, approximately half of the risk estimated for situation 1b.

The above risk are still an underestimate since it has recently been reported that the presence of germinal mosaicism leads to an estimated recurrence risk of approximately 7% for mothers of apparently new DMD mutants (Bakker *et al.*, 1989; Passos-Bueno *et al.*, 1990).

In summary, it is suggested that in the absence of a detected DNA deletion in the proband, a muscle biopsy should be performed to exclude the diagnosis of AR-DMD. If X-linked DMD is confirmed, the parents should be aware that the absence of a DNA deletion leads to a greater genetic risk for further pregnancies than if a deletion is found in the proband. Prenatal diagnosis and identification of female carriers among proband's sisters, in such families should be based on DNA polymorphic markers. On the other hand, in the case of AR-DMD the recurrence risks for the probands' sisters or for the patients' parents in the case of another marriage are negligible.

ACKNOWLEDGMENTS

The collaboration of the following persons is gratefully acknowledged: Drs. Mariz Vainzof, Rita Pavanello, Ivo Pavanello-Filho, Mrs. Marta Cánovas, Martha A.B.O. Lima, Sabine Eggers, Simone Campiotto and Marcia Neves, Mr. Reinaldo Issao Takata and Roberto Schreiber for their invaluable help; this work was supported with grants for FAPESP, CNPq, Secretaria de Ciência e Tecnologia, FINEP and ABDIM.

Publication supported by FAPESP.

RESUMO

Em um estudo de triagem de deleções em 164 pacientes afetados por distrofias de Duchenne (DMD) não aparentados, com as sondas de DNA *cf23a* e *cf56a* (que detectam cerca de 70% das deleções

de DNA), observou-se que a frequência de deleções era significativamente maior nos casos esporádicos (0.56) do que naqueles herdados (0.34). Estes achados têm implicações importantes para o Aconselhamento Genético, pois mostram que a ausência de deleções de DNA no probando levam a uma estimativa maior de risco genético de recorrência para futura prole, do que se este apresentar uma deleção molecular. Sugere-se também que seja estudada a distrofia muscular no afetado para exclusão de um possível diagnóstico de uma forma autossômica recessiva de Duchenne, sempre que não for encontrada deleção molecular no propósito.

APPENDIX

BAYESIAN CALCULATION

1. No DNA deletion is found in the proband

1.a) There is no information on serum CK for the proband's mother

Conditional Probabilities	Mother is a DMD-carrier	Mother is not a DMD-carrier
A priori	4μ	$1 - 4\mu \cong 1$
Genetic risk (affected son)	0.5	μ
No DNA deletion (probes cf23a, cf56a)	0.66	0.44
Final	$4\mu \times 0.5 \times 0.66 = 1.32\mu$	$1 \times \mu \times 0.44 = 0.44\mu$
A posteriori:	$\frac{1.32\mu}{1.32\mu + 0.44\mu} \cong 0.75$	$\frac{0.44\mu}{1.32 + 0.44\mu} \cong 0.25$

The final recurrence risk for situation 1.a) will be: 0.9 (probability for X-linked DMD) x 0.75 (probability that the mother is a carrier) x 0.25 (risk for affected child independently of sex) + 0.1 (probability for AR-DMD) x 0.25 (risk for further affected child for this couple) $\cong 0.19$ or approx. 19%.

1.b) The proband's mother has normal serum CK

Conditional Probabilities	Mother is a DMD-carrier	Mother is not a DMD-carrier
A priori	4μ	$1 - 4\mu \cong 1$
Affected son	0.5	μ
Normal CK	0.33	0.95
No DNA deletion	0.66	0.44
Total:	$4\mu \times 0.5 \times 0.33 \times 0.66 = 0.44\mu$	$1 \times \mu \times 0.95 \times 0.44 = 0.42\mu$
A posteriori	$\frac{0.44\mu}{0.44\mu + 0.42\mu} \cong 0.51$	$\frac{0.42\mu}{0.44\mu + 0.42\mu} \cong 0.49$

The final recurrence risk will be for situation 1.b): $0.9 \times 0.51 \times 0.25 + 0.1 \times 0.25 \cong 0.14$ or approx. 14%

2) *The proband has a DNA deletion*

2.a) There is no information on serum CK for the proband's mother

Conditional Probabilities	Mother is a DMD-carrier	Mother is not a DMD-carrier
A priori	4μ	$1 - 4\mu \cong 1$
Affected son	0.5	μ
Presence of DNA deletion	0.34	0.56
Total:	$4\mu \times 0.5 \times 0.34 = 0.8\mu$	$1 \times \mu \times 0.56 = 0.56\mu$
A posteriori:	$\frac{0.8\mu}{0.8\mu + 0.56\mu} \cong 0.59$	$\frac{0.56\mu}{0.8\mu + 0.56\mu} \cong 0.41$

The final recurrence risk will be for situation 2.a): $0.59 \times 0.25 \cong 0.15$ or approx. 15%.

2.b) The proband's mother has normal serum CK

Conditional Probabilities	Mother is a DMD-carrier	Mother is not a DMD-carrier
A priori	4μ	$1 - 4\mu \cong 1$
Genetic risk (affected son)	0.5	μ
Normal CK	0.33	0.95
Presence of DNA deletion	0.34	0.56
Final:	$4\mu \times 0.5 \times 0.33 \times 0.34 \cong 0.22\mu$	$1 \times \mu \times 0.95 \times 0.56 \cong 0.53\mu$
A posteriori:	$\frac{0.22\mu}{0.22\mu + 0.53\mu} \cong 0.30$	$\frac{0.53\mu}{0.22\mu + 0.53\mu} \cong 0.70$

The final recurrence risk will be for situation 2.b): $0.30 \times 0.25 \cong 0.075$ or approx. 7.5%.

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