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A common missense mutation in the adhalin gene in three unrelated Brazilian families with a relatively mild form of autosomal recessive limb-girdle muscular dystrophy

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Autosomal recessive limb-girdle muscular dystrophies (AR LGMD) represent a heterogeneous group of diseases with a wide spectrum of clinical variability, classified phenotypically into two main groups, the most severe forms (Duchenne-like muscular dystrophy, DLMD, or severe childhood autosomal recessive muscular dystrophy, SCARMD) and the milder forms. Four genes causing AR LGMD have been mapped: the 15q (LGMD2a), the 2p (LGMD2b), the 13q locus (LGMD2c) and the adhalin gene on chromosome 17q (LGMD2d). In the present report we have performed linkage analysis with 17q markers in three mild AR LGMD and in four DLMD families with adhalin deficiency and unlinked to 2p, 15q or 13q genes. Linkage was observed only among the mild cases. Patients from these three 17q-linked families showed near or total deficiency of adhalin in muscle biopsies. An identical missense mutation was identified in all three 17q-linked unrelated families. These results indicate that AR LGMD with a mild phenotype is caused by mutations in the adhalin gene. In addition, they demonstrate that there is at least one other locus for DLMD associated with adhalin deficiency.

INTRODUCTION

Autosomal recessive limb-girdle muscular dystrophies (AR LGMD) represent a heterogeneous group of diseases, with a wide spectrum of clinical variability, involving muscles from the pelvic and scapulae girdles without affecting the facial musculature. The rate of progression of the disease is usually highly correlated with the age of onset which may vary from early to late childhood or even adulthood (1,2). The more severe forms, with onset before the age of 5 and loss of ambulation before 16 years old, have been named as 'Duchenne-like muscular dystrophy (DLMD) or severe childhood autosomal muscular dystrophy, SCARMD' (3,4).

Two loci associated with the milder forms of AR LGMD have been mapped, one on chromosome 15q (5) and the other on chromosome 2p (5). These results were confirmed in other populations, including Brazilian families (7–9). The 15q-linked LGMD was termed LGMD2a while the 2p-linked form was classified as LGMD2b (10). However, we have verified that some families are not linked to any of these loci, demonstrating the existence of at least one other locus for this disease (8,11).

Linkage analysis using 15q as well as 2p markers have been performed in families with the most severe forms of limb-girdle muscular dystrophies (DLMD). However, none of the Brazilian DLMD families were found to be linked to these loci (12,13 and unpublished data). Ben Othmane *et al.* (13) linked a more severe phenotype of AR LGMD to chromosome 13q in Tunisian families. This finding has been confirmed in other families from Africa (14,15) and this form has been classified as LGMD2c (10). Only one among the 16 studied Brazilian LGMD families was found to be apparently linked to the 13q gene; however, in contrast to the Tunisian families, the three affected patients from this genealogy have a relatively mild phenotype (11). On the other hand, no evidence of linkage was observed with 13q markers in four DLMD families from different ethnic groups, suggesting the existence of another locus for this severe form of muscular dystrophy (16).

Due to the fact that the AR LGMDs are clinically similar to the dystrophinopathies, that is, the Xp21 Duchenne and Becker muscular dystrophies, it has been hypothesized that the proteins and/or glycoproteins associated with dystrophin could be candidate genes for one or more forms of AR LGMD. Indeed, deficiency of one of these glycoproteins (50 DAG or adhalin) in muscles from patients with the DLMD phenotype has been reported by Matsumara *et al.* (17). This finding was confirmed by us in six other DLMD Brazilian patients (18). However, none of the families found to be deficient for this protein were linked to the 13q gene (16), suggesting that the gene for adhalin is not on 13q or that deficiency of this glycoprotein could be due to a primary or secondary effect (16,18).

Recently, Roberds *et al.* (19) mapped the gene encoding adhalin to chromosome 17q and identified point mutations in

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this gene in patients from one family with an apparently mild phenotype which was previously demonstrated to be adhalin negative (20).

The aims of the present study are to report linkage analysis with 17q markers in three mild AR LGMD and in four DLMD families with adhalin deficiency and unlinked to 2p, 15q or 13q genes. We observed that only the families with a mild phenotype are linked to this locus, which was confirmed by the identification of a missense mutation in the adhalin gene.

RESULTS

Two-point lod scores between D17S1319 and the disease locus for all the seven families are described in Table 1. The families were analysed separately in two groups according to the severity of the disease.

All the four families clinically classified as DLMD showed negative lod scores with the intragenic adhalin marker, suggesting that this is not the gene responsible for this muscular dystrophy in these genealogies, despite the fact that they are adhalin-negative, as previously reported (18).

All the three genealogies with mildly affected patients (pedigrees with haplotypes summarized in Figure 1) showed positive lod scores with the intragenic adhalin marker D17S1319 and/or the flanking markers (Family 7: $Z = 2.43$ and 1.55 at $\theta_{max} = 0$, respectively for D17S806 and D17S941; Family 16: $Z = 2.55$ and 1.02 at $\theta_{max} = 0$, respectively for D17S806 and D17S941; Family 30: $Z = 1.01$ at $\theta_{max} = 0$ for both flanking markers).

To identify possible causative mutations in these families we performed reverse transcription-PCR (RT-PCR) analysis on total RNA prepared from a muscle biopsy of affected member 6 of family 7. Two pairs of primers were used to amplify the entire adhalin mRNA, and both produced normal sized products. Direct sequence analysis of the RT-PCR products from this affected patient identified one missense mutation, a C→T transition at nucleotide 229 [according to the sequence from Roberds *et al.*(19)], which results in an Arg→Cys substitution (Fig. 2). The mutant base was detected as a homozygous substitution on the directly sequenced RT-PCR product using three separate sequencing primers.

This mutation abolishes a restriction site for the enzyme *Nla*IV in exon 3 of the adhalin gene. Amplification of exon 3 from genomic DNA of all 10 individuals of this family followed by restriction digestion with *Nla*IV demonstrated that the mutation at nucleotide 229 is present in only one of the alleles (the maternal allele) from the parents. Since RT-PCR products were homozygous for the mutation, this patient's paternal allele is either not transcribed, or leads to expression of a very unstable mRNA. No deletion was detected at the 5′ end of the gene through Southern blot analysis (data not shown).

The patients from the other two 17q-linked families were also tested at the genomic level for this mutation, and we observed that all the affecteds are homozygous for this same mutation (Fig. 3). To ensure that the observed base change was not a common polymorphism, we screened genomic DNA from 100 unrelated, healthy Brazilian individuals, as well as 74 unrelated parents from the CEPH reference families. This mutation was not observed in any of these 348 normal chromosomes.

Table 1. Two point lod scores between the intragenic adhalin gene and the disease locus

Family	Recombination fraction (θ)					
	0	0.01	0.05	0.1	0.2	0.3
Mild forms (LGMD)						
7	1.53	1.50	1.38	1.23	1.91	0.56
16	2.68	2.62	2.35	2.01	1.31	0.64
30	0.98	0.95	0.85	0.71	0.45	0.22
Total	5.19	5.07	4.58	3.95	2.67	1.42
Severe forms (DLMD)						
3	−∞	−1.89	−0.60	−0.14	0.15	0.15
26	−∞	−6.07	−2.88	−1.66	−0.67	−0.25
43	−∞	−2.64	−1.35	−0.82	−0.37	−0.16
55	−∞	−3.14	−1.70	−1.08	−0.48	−0.19
Total	−∞	−14.85	−6.99	−3.93	−1.43	−0.46

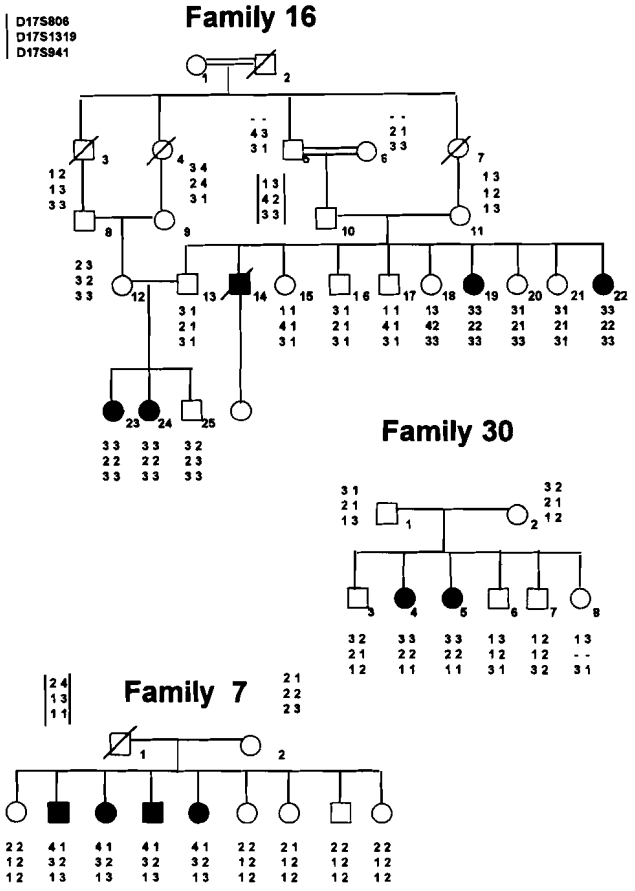

Figure 1. Genealogies and haplotypes in the three families with mild affected patients. The order of the markers in each haplotype is: -D17S806-D17S1319-D17S941.

Four patients from the three unrelated 17q-linked families were also tested for adhalin and dystrophin by immunocytochemistry: patient 6 from family 7, patients 19 and 22 from family 16 and patient 5 from family 30. The patients from families 16 and 30 presented a negative pattern for adhalin while in patient 6 from family 7, a partial although very weak

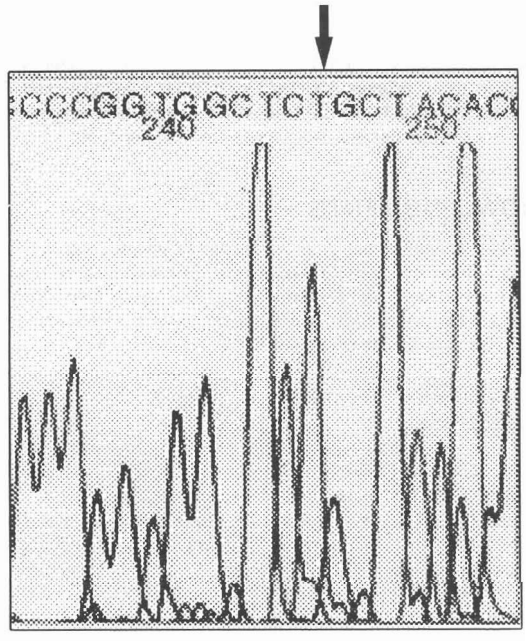

Figure 2. Partial sequence of adhalin cDNA fragment from affected 6, family 7. Circled nucleotide is the only base found to differ from the wild-type adhalin sequence (19).

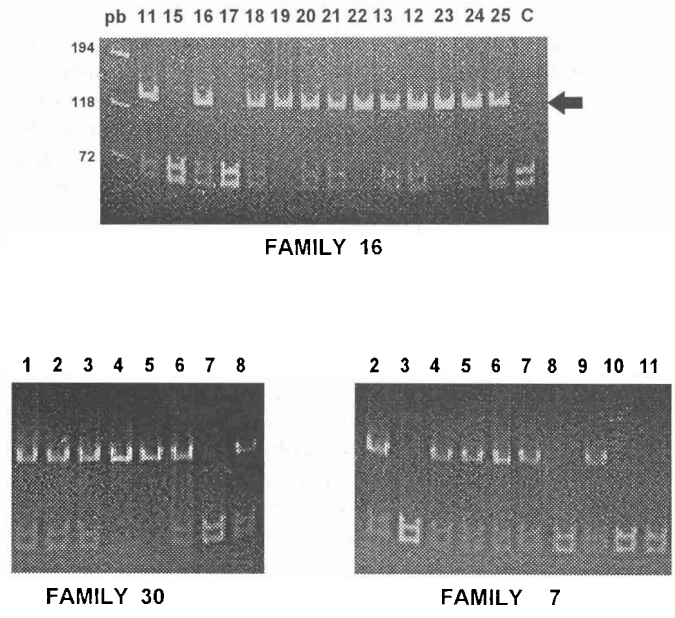

Figure 3. *Nla*IV digested PCR products from adhalin exon 3, amplified from genomic DNA from each member of the three 17q-linked families (as described in Materials and Methods); the individuals are numbered as in Figure 1.

labelling was observed in some fibers (Fig. 4). A positive dystrophin pattern was observed in all these cases (Fig. 4).

DISCUSSION

The present results confirm that families linked to the adhalin gene may have a mild form of limb-girdle muscular dystrophy, as previously suggested (19).

Adhalin is a muscle-specific glycoprotein having a single transmembrane domain, in which the N-terminal 290 amino acids are extracellular (21). The mutation reported here, a substitution of Arg→Cys at residue 77 (R77C) may therefore change the secondary and/or tertiary structural conformation of adhalin, interfering with the adhalin extracellular functions. In addition, an important finding is that dystrophin is apparently normal in these 17q-linked patients, suggesting that this glycoprotein is not critical for the maintainance of the dystrophin-associated complex as originally reported (21) .

Interestingly, the affected patients from the three unrelated 17q-linked families have different haplotypes, suggesting different origins for this mutation. These results were unexpected, since point mutations among unrelated affecteds from the same population in other autosome recessive diseases, such as cystic fibrosis (22,23) and phenylketonuria (24), typically share the same haplotype.

The clinical progression of the disease in the 17q-linked patients is relatively mild, although there is a great variability within and between families, even among those carrying the same genotype. Patients homozygous for mutation R77C from families 16 and 30 showed a negative adhalin pattern through immunocytochemistry; however, the patients from family 16 have a more severe clinical course. In family 16, one patient

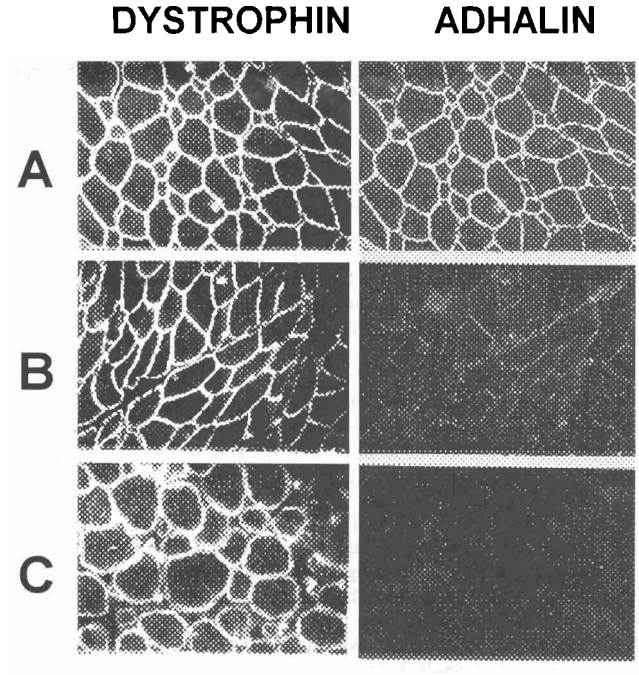

Figure 4. Dystrophin (I) and adhalin (II) immunocytochemistry in muscle from a non-adhalin-linked patient (A and B) and two adhalin-linked affecteds (C and D: 6, family 7; E and F: 22, family 16).

(14) lost ambulation at age 16 and deceased at age 27 (according to his parents' information). His two sisters, currently aged 26 and 19 (patients 19 and 22, respectively), present severe weakness both in the upper and lower limbs, walking only

with support. In family 30, individual 5, currently aged 25, had disease onset at age 16 and currently has severe weakness both in the upper and lower limbs. In contrast, her sister (4), currently 26 years old, had disease onset only at the age of 23 and is very mildly affected, being able to perform all her functional abilities (walking and climbing stairs) without support. Patients from family 7 also have a very mild clinical course, comparable to patient 4 from family 30, even though some traces of adhalin were observed in one of the tested patients from this family. Although more individuals should be studied, these preliminary results suggest that there is not a direct correlation between levels of adhalin and severity of the disease.

The negative lod scores for the 17q markers in the Brazilian DLMD families indicate that there is at least one other locus for this form of muscular dystrophy. The adhalin negative pattern in the affected patients from these families have been previously described (18). These findings support our previous suggestion that deficiency of adhalin in patients with the severe phenotype may be a secondary effect and indicate that other important proteins associated with adhalin and/or dystrophin might be altered in these forms of muscular dystrophy.

MATERIALS AND METHODS

Families

Seven families with AR LGMD patients (ascertained at the Centro de Miopatias, Universidade de São Paulo) have been included in the present analysis. Linkage analysis for 17q markers in two of these families (7 and 16) have been previously reported (11). All of them have been excluded for the 2p, 13q and 15q genes (11 and unpublished data). Diagnosis was based on clinical examination of at least one affected person from each genealogy, clinical course of the disease, family history, elevated serum creatine-kinase levels and assessment of dystrophin (through IF and Western blot) in muscle biopsies. Patients were classified as: (i) mild LGMD when the onset of the disease occurred during or near the end of the first decade of life and loss of ambulation at or after 16 years old; (ii) severe DLMD or SCARMD when loss of ambulation occurred before age 16 in at least one patient in each genealogy (3,4,11).

Three (families 7, 16 and 30) out of the seven families have a relatively mild phenotype and the other four have the more severe clinical course and have been classified as DLMD (families 3, 26, 43 and 55). Consanguinity was observed in four families (16, 26, 30 and 43).

Methods

DNA was extracted from whole blood, according to the method of Miller *et al.* (25). Linkage analysis was done using the intragenic adhalin microsatellite marker (D17S1319); in cases in which this marker was not informative, the closest available markers for this region were then used (D17S579, D17S806 and/or D17S941). PCR conditions for these markers were denaturation at 94°C for 1 min, 55°C for 1 min and 72°C for 1 min for 30 cycles. Two point linkage analysis between the disease gene and the marker was performed using the computer program Linkage (26). The gene frequency for the AR LGMD was considered as 0.001. The recombination rate was assumed to be equal in males and females.

Adhalin immunocytochemistry was studied by double labeling with the mouse monoclonal antibody IVD31 (27,28) and the rabbit polyclonal anti-dystrophin 303–7 antibody (29), as already described (30). Adhalin immunocytochemistry of the DLMD patients included in the present study were previously reported (18).

Total RNA was isolated from 30 mg of skeletal muscle from patient 6, family 7, using RNAzol™ (Gibco) according to the directions of the manufacturer. Fragments of adhalin cDNA were amplified using two pairs of oligonucleotide primers (product 1: 5′TCTGTCACTCACCGGGCGG3′ and 5′TCGGGGG-ATGCCACCATC3′; product 2: 5′GCCGAAAAGAAGGGGTATAC3′ and 5′TGGGATGTGCCACTCGTG3′); the products were sequenced directly on both strands with the use of additional internal oligonucleotide primers, using an Applied Biosystems model 373a automated DNA sequencer. PCR conditions for these markers were denaturation at 94°C for 1 min, 58°C for 1 min and 72°C for 2 min for 35 cycles.

To detect the C→T substitution in the exon 3 at the genomic level, DNA was amplified using primers 5′CCACCCGCTGTCCACAT3′ and 5′AATG-ACCTGGAGCCCACG3′ and digested with the restriction enzyme *Nla*IV; the restriction site for this enzyme is abolished by the mutation.

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REFERENCES

1. Shields, R.W., Jr. (1986) Limb girdle syndromes. In Engel, A.G. and Banker BQ (eds), *Myology*, vol. II. McGraw-Hill Inc., USA
2. Walton, J.N. and Gardner-Medwin, D. (1988) The muscular dystrophies. In John Walton (ed.), *Disorders of voluntary muscle*, 5th edition. Churchill Livingstone, UK
3. Ben Hamida, M., Fardeau, M. and Attia, N. (1983) Severe childhood muscular dystrophy affecting both sexes is frequent in Tunisia. *Muscle Nerve* **6**, 469–480.
4. Zatz, M., Passos-Bueno, M.R., Rapaport, D. (1989) Estimate of the proportion of the Duchenne muscular dystrophy with autosomal recessive inheritance. *Am. J. Med. Genet.* **32**, 407–410.
5. Beckmann, J.S., Richard, I., Hillaire, D., Broux, O., Antignac, C., Bois, E., Cann, H., Cottingham Jr, R.W., Feingold, J., Kalil, J., Lathrop, G.M., Marcadet, A., Masset, M., Mignard, C., Passos-Bueno, M.R., Pellerain, N., Zatz, M., Dausset, J., Fardeau, M. and Cohen, D. (1991) A gene for limb-girdle muscular dystrophy maps to chromosome 15 by linkage analysis. *C. R. Acad. Sci. Paris* **312**, 141–148.
6. Bashir, R., Strachan, T., Keers, S., Stepheson, A., Mahjneh, I., Marconi, G., Nashef, L., Bushby, K.M.D. (1994) A gene for autosomal recessive limb-girdle muscular dystrophy maps to chromosome 2p. *Hum. Mol. Genet.* **3**, 455–457
7. Young, K., Williams, P., Foroud, C.E. *et al.* (1991) Confirmation of linkage of limb-girdle muscular dystrophy to chromosome 15. *Cytogenet. Cell Genet.* (HGM11): Abstract 26966.
8. Passos-Bueno, M.R., Richard, I., Vainzof, M., Fougerousse, F., Weissenbach, J., Broux, O., Cohen, D., Akiyama, J., Marie, S.K.N., Carvalho, A.A., Guilherme, L., Kalil, J., Tsanaclis, A.M., Zatz, M. and Beckmann, J.S. (1993) Evidence of genetic heterogeneity for the autosomal recessive adult forms of limb-girdle muscular dystrophy following linkage analysis with 15q probes in Brazilian families. *J. Med. Genet.* **30**, 385–387.
9. Passos-Bueno, M.R., Bashir, R., Moreira, E.S., Vasquez, L., Marie, S.K., Vainzof, M., Iughetti, P., Bakker, E., Keers, S., Stephenson, A., Strachan, T., Bushby, K. and Zatz, M. (1994) Confirmation of the 2p locus for mild autosomal recessive limb-girdle muscular dystrophy (AR LGMD) in two Brazilian and one Palestinian families and refinement of this candidate region. *Genomics* (in press).
10. Bushby, K. and Beckmann, J.S. (1995). Report of the 30th and 31st ENMC International workshop—the limb-girdle muscular dystrophies and proposal for a new nomenclature. *Neuromusc. Disorders* (in press).
11. Passsos-Bueno, M.R., Moreira, E.S., Vasquez, L., Marie, S.K., Vainzof, M., Iughetti, P., Oliveira, J.R., Bakker, E., Strachan, T., Bashir, R., Bushby, K. and Zatz M. (1994) Confirmation of a third locus, at 2p, for autosomal recessive limb-girdle muscular dystrophy indiates that at least 4 genes are responsible for this condition. *Am. J. Hum. Genet.* **55**, A199 (Abstract).
12. Passos-Bueno, M.R., Bakker, E., Marie, S.K.N., Pavanello, R.C., Vainzof, M., Carvalho, A.A., Cohen, D., Beckmann, J. and Zatz, M. (1992). Exclusion of the 15q locus as a candidate gene for severe childhood autosomal recessive Duchenne-like muscular dystrophy in Brazilian families. *Hum. Mol. Genet.* **2**, 201–202.
13. Ben Othmane, K., Ben Hamida, M., Pericak-Vance, MA., Ben Hamida, C., Blel, S., Carter, S.C., Bowcock, A.M., Petrukhin, K., Gilliam, T.C., Roses, A.D., Hentati, F. and Vance, J.M.. (1992) Linkage of Tunisian autosomal recessive Duchenne-like muscular dystrophy to the pericentromeric region of chromosome 13q. *Nature Genet.* **2**, 316–317.

14. Azibi, K., Bachner, L., Beckmann, J.S., Matsumara, K., Hamouda, E., Chaouch, M., Chaouch, A., Ait-Ouarab, R., Vignal, A., Weissenbach, J., Vinet, M.-C., Leturcq, F., Collin, H., Tomé, F.M.S., Reghis, A., Fardeau, M., Campbell, K.P. and Kaplan, J.-C. (1993) Severe childhood autosomal recessive muscular dystrophy with the deficiency of the 50 kDa dystrophin-associated glycoprotein mapes to chromosome 13q12. *Hum. Mol. Genet.* **2**, 1423–1428.
15. El Kerch, F., Sefiani, A., Azibi, K., Boutaleb, N., Yahyaoui, M., Bentahila, A., Vinet, M.-C., Leturcq, F., Bachner, L., Beckmann, J.S., Campbell, K.P., Tomé, F.M.S., Fardeau, M. and Kaplan, J.-C. (1994) Linkage analysis of families with severe childhood autosomal recessive muscular dystrophy (SCARMD) in Morocco indicates genetic homogeneity of the disease in North Africa. *J. Med. Genet.* **31**, 342–343.
16. Passos-Bueno, M.R., Oliveira, J.R., Bakker, E., Anderson, R.D., Marie, S.K., Vainzof, M., Roberds, S., Campbell, K.P. and Zatz, M. (1993) Genetic heterogeneity for Duchenne-like muscular dystrophy (DLMD) based on linkage and 50 DAG analysis. *Hum. Mol. Genet.* **2**, 1945–1947.
17. Matsumara, K., Tomé, F.M.S., Collin, H., Azibi, K., Chaouch, M., Kaplan, J.-C, Fardeau, M. and Campbell, K.P. (1992) Deficiency of the 50 K dystrophin-associated glycoprotein in severe childhood autosomal recessive muscular dystrophy. *Nature* **359**, 320–322.
18. Zatz, M., Matsumara, K., Vainzof, M., Passos-Bueno, M.R., Pavanello, R.C.M., Marie, S.K. and Campbell, K.P. (1994) Assessment of the 50-kDa dystrophin-associated glycoprotein in Brazilian patients with severe childhood autosomal recessive muscular dystrophy. *J. Neurol. Sci.* **123**, 122–128.
19. Roberds, S.L., Leturcq, F., Allamand, V., Piccolo, F., Jeanpierre, M., Anderson, R.D., Lim, L.E., Lee, J.C., Tomé, F.M.S., Romero, N.B., Fardeau, M., Beckmann, J.S., Campbell, K.P., Kaplan, J.-C. and Campbell, K.P. (1994) Missense mutations in the adhalin gene linked to autosomal recessive muscular dystrophy. *Cell* **78,** 625–633.
20. Romero, N.B., Tomé, F.M.S., Leturcq, F., El Kerch, F., Azibi, K.F., Bachner, L., Anderson, R.D., Roberds, S.L., Campbell, K.P., Fardeau, M. and Kaplan, J.-C. (1994) Genetic heterogeneity of severe childhood autosomal recessive muscular dystrophy with adhalin (50-kDa dystrophin-associated glycoprotein) deficiency. *C. R. Acad. Sci. Paris Ser. III, Sci. Vie* **317**, 70–76.
21. Roberds, S.L., Anderson, R.D., Ibraghimov-Beskrovnaya, O. and Campbell, K.P. (1993) Primary structure and muscle-specific expression of the 50-kDa dystrophin-associated glycoprotein (adhalin). *J. Biol. Chem.* **268**, 23739–23742.
22. Kerem, E., Corey, M., Kerem, B., Rommens, J., Markiewicz, D., Levison, H., Tsui, L.-C. and Durie, P. (1990) The relation between genotype and phenotype in cystic fibrosis—analysis of the most common mutation (delta F508). *N. Engl. J. Med.* **323**,1517–1522
23. Sereth, H., Shoshani, T., Bashan, N. and Kerem, B.-S. (1993) Extended haplotype analysis of cystic fibrosis mutations and its implications for the selective advantage hypothesis. *Hum. Genet.,* **92,** 289–295.
24. Konecki, D.S., Schlotter, M., Trefz, F.K. and Lichter-Konecki, U. (1991) The phenylketonuria locus: current knowledge about alleles and mutations of the phenylalanine hydrozylae gene in various populations. *Hum. Genet.* **87**, 377–388.
25. Miller, S.A., Dykes, D.D. and Polesky, H.F. (1988) A simple salting out procedure for extrating DNA from human nucleated cells. *Nucleic Acids Res.* **16**, 1215.
26. Lathrop, C.M., Lalouel, J.M., Julier, C. and Ott, J. (1984) Strategies for multilocus linkage analysis in humans. *Proc. Natl Acad. Sci., USA* **81**, 3443–3446.
27. Ervasti, J.M., Ohlendieck, K., Kahl, S.D., Gaver, M.G. and Campbell, K.P. (1990) Deficiency of a glycoprotein component of the dystrophin complex in dystrophic muscle. *Nature* **345**, 315–319.
28. Ohlendieck, K., Ervasti, J.M., Snook, J.B. and Campbell, K.P. (1991). Dystrophin–glycoprotein complex is highly enriched in isolated skeletal muscle sarcolemma. *J. Cell. Biol.* **112**, 135–148.
29. Cox, G.A., Cole, N.M., Matsumara, K., Phelps, S.F., Hauschka, S.D., Campbell, K.P., Faulkner, J.A. and Chamberlain, J.S. (1993) Overexpression of dystrophin in transgenic *mdx* mice eliminated dystrophic symptoms without toxicity. *Nature* **364**, 725–729.
30. Vainzof, M., Zubrzycka-Gaarn, E.E., Rapaport, D., Passos-Bueno, M.R., Pavanello, R.C.M., Pavanello, I. and Zatz, M. (1991) Immunofluorescence dystrophin study in Duchenne dystrophy through the concomitant use of two antibodies directed against the carboxy-terminal and the amino-terminal region of the protein. *J. Neurol. Sci.* **94,** 137–146.