Neurosensoriy Hearing Loss in Secondary Adhalinopathy

By K. Oexle, R. Herrmann, Catherine Dode, F. Leturcq, Ch. Hübner, J.-C. Kaplan, Y. Mizuno, E. Ozawa, K. P. Campbell and T. Voit

1Abteilung Neuropädiatrie, Charité, Humboldt-Universität, Berlin, Germany; 2Zentrum für Kinderheilkunde des Universitätsklinikums, Essen, Germany; 3Institut National de la Santé et de la Recherche Médicale Unité 129 and Laboratoire de Biochimie Génétique, Centre Hospitalier Universitaire Cochin, Université René Descartes, Paris, France; 4Department of Cell Biology, National Institute of Neuroscience, NCNP, Tokyo, Japan 187, and 5Department of Physiology and Biophysics, The University of Iowa College of Medicine, Iowa City; Iowa 52242, USA

Abstract

We report mild-to-moderate neurosensoriy hearing loss and severe childhood autosomal recessive muscular dystrophy with adhalin-deficiency in two siblings from a Bulgarian sibship of Turkish origin. Microsatellite analysis excluded linkage to the adhalin gene, mutations of which cause limb girdle muscular dystrophy (LGMD) 2D, but was compatible with linkage to the gene locus of LGMD 2C on chromosome 13q12. Compound heterozygosity of the affected siblings was detected in this chromosomal region. A severe autosomal recessive form of neurosensoriy deafness has been linked to the same region (locus NSRD1) which is now contained in a 7 Mb YAC contig. Using polymorphic markers and STS PCR primers mapping in this contig, we did not find evidence for major rearrangements in the suspected region. These preliminary findings are not in favor of, but do not completely exclude a contiguous gene syndrome in these cases. Therefore, we consider a potential role of the putative 13q12 gene product and/or adhalin in neurosensoriy hearing.

Key words

Limb-girdle muscular dystrophy (LGMD) - Non-syndromic recessive deafness (NSRD) - Adhalin - Dystrophin - Chromosome 13q12

Abbreviations

DAG: dystrophin-associated glycoprotein
DMD: Duchenne muscular dystrophy
LGMD: limb-girdle muscular dystrophy
NSRD: non-syndromic recessive deafness
STS: sequence-tagged site

Introduction

X-chromosomal recessive Duchenne muscular dystrophy (DMD) is caused by deficiency of dystrophin, a submembraneous cytoskeletal protein (17, 19). Dystrophin is associated with an oligomeric complex of sarcolemmal glycoproteins linking the intracellular actin skeleton with the extracellular matrix (8, 11, 12). Dystrophin deficiency leads to reduced expression of these dystrophin-associated glycoproteins (DAGs) (11, 23). Autosomal recessive limb girdle muscular dystrophy of variable severity is related to the deficiency of adhalin, a 30 kD-component of the DAG complex, while dystrophin expression is preserved (22). In some patients LGMD, subsequently termed LGMD 2D, is associated with mutations of the adhalin gene on chromosome 17q (1, 7, 27, 30). However, in some North African patients adhalin-deficient LGMD, subsequently termed LGMD 2C, has been linked to chromosome 13q12 (4, 5, 7); adhalin-deficiency is supposed to be secondary in these cases.

Genetic markers within the dystrophin gene have been linked to an X-chromosomal, non-syndromic form of neurosensoriy hearing loss (20) although auditory dysfunction has not been implicated with DMD (2, 33). Recessive (13) and dominant (9) non-syndromic forms of deafness (NSRD1 locus) were mapped to the 13q11 region in close proximity with the LGMD 2C locus. In these cases of hearing loss, evidence of muscular dystrophy has not been reported. Here, we describe the combination of neurosensoriy hearing loss and severe muscular dystrophy due to secondary adhalinopathy in a Bulgarian sibship of Turkish origin. The suspected region was explored with markers and sequence-tagged sites (STSs) included in a recently obtained YAC contig covering the NSRD1 and the LGMD 2C loci (14).

Case report

Family history

A neuromuscular disorder or auditory dysfunction did not occur before in this Bulgarian family of Turkish origin. The parents of the affected sibship were not consanguinous. Two brothers were healthy.
were placed on Poly-L-Lysine (Sigma, St. Louis, US) coated and A3b was detectable only in traces (Fig. 2).

Hearing loss had not been detected before our examination. Hearing loss was slightly more pronounced at higher frequencies (Fig. 1). There was no history of damage due to excessive noise, of sudden hearing loss after an infection, or of potentially harmful drug intake.

Affected sibship

Pre- and immediate postnatal development was uneventful. Early motor milestones were reached in time. However, the parents reported that first signs of muscular weakness appeared during the following year in both cases, and that progressive deterioration occurred at the age of 9 in the girl (Patient II-2) and at the age of 7 in the boy (Patient II-3). Aged 12 yrs (Patient II-2) and 10 yrs (Patient II-3) at the time of our examination both had a waddling gait. They were not able to stand up from a sitting position. The degree of muscular weakness was similar in both patients. Marginal calf enlargement possibly was non-specific and due to generalized adiposity. There were no joint contractures. Both patients suffered from planovalgus deformity of the feet. There was no evidence of cardiomyopathy by echocardiography. Intelligence was normal. Activity of serum creatin kinase was 7840 U/l in the boy and 11557 U/l in the girl.

Both siblings suffered from hearing loss which had not been detected before our examination. Hearing loss was slightly more pronounced at higher frequencies (Fig. 1). Similar results of air- and bone-conduction tests indicated a neurosensory disturbance. The boy (max. loss of 55 dB at 6 kHz) was more severely affected than his sister (max. loss of 40 dB at 6 kHz). There was no history of damage due to excessive noise, of sudden hearing loss after an infection, or of potentially harmful drug intake.

Methods

Immunofluorescence

Five μm cryosections of the muscle specimens were placed on Poly-L-Lysine (Sigma, St. Louis, US) coated slides and incubated with the primary antibody in PBS, containing 1% BSA, for 1.5 h at 37 °C, followed by the corresponding secondary antibodies for 1/2 h at 37 °C, visualized (except for A3b) with streptavidin-Texas Red (Amersham, Braunschweig, FRG) 1:200 for 3/4 h, and photographed on a Zeiss III RS photomicroscope equipped for epifluorescence. The following antibodies were used: mAb against adhalin (22) 1:2, followed by goat anti-mouse IgG1 (Amersham) 1:200; mAb against 35 DAG (36) 1:8, and mAbs against β-dystroglycan, spectrin and dys-2 (all from Novocastra, Newcastle upon Tyne, UK) 1:100, followed by sheep anti-mouse Ig (Amersham) 1:200; mAb anti-laminin α2 1:1000 (Gibko, Eggstein, FRG), followed by sheep anti-mouse Ig 1:200 (Amersham); mAb anti-laminin β1 (Gibko) 1:1000, followed by goat anti-mouse IgG1 (Amersham); mAb anti-laminin γ1 (Gibko) 1:200, followed by goat anti-mouse IgG2a (Amersham) 1:200; pAb against A3b [37] 1:100, followed by dichlorotriazinyl amino-fluorescein-labelled goat F(ab')2 anti-guinea-pig IgG (Chemicon, Temecula, CA, US) 1:200.

DNA analysis

Linkage analysis was performed using seven microsatellites on chromosome 13 covering a distance of about 6 cm in the 13q11-q12.1 region: D13S292 (AFM351x9), D13S283 (AFM290v9b), D13S200 (AFM323v1) (15); D13S141, D13S143 (26); D13S115 (18); D13S221 (AFM248ve1) (35). For the chromosome 17q21 region we used one microsatellite located within the intron 6 of the adhalin gene (D17S1319) (1) and the extragenic microsatellites D17S791 (AFM155xd12) and D17S941 (AFM269x1) (15).

Deletions in the 13q11-q12 region were searched by PCR using six STSs (D13S1115, D13S1122, D13S1123, D13S1124, D13S1125, D13S1128) comprised in a 7 Mb YAC contig established between D13S143 and D13S292 (14).

Audiometry

Unspecific disturbances of the middle ear were excluded by local inspection. Subjective hearing capacity was tested by pure tone threshold audiometry. Both air and bone conduction of sound were used for testing.

Results

Muscle immunohistology

of both affected siblings showed preserved or marginally reduced expression of dystrophin (Fig. 2), β-dystroglycan, laminins α2, β1, γ1, and spectrin (not shown). Due to the marked dystrophic changes of the specimens, fibre surface staining was irregular in some places. In contrast, adhalin (50 DAG or α-sarcoglycan) was clearly reduced, with a few traces of immunoreactive material detectable on some fibres (Fig. 2). Moreover, a 35 kD dystrophin-associated protein detected with MA4-2 antibody in normal muscle (36) was lacking completely, and A3b was detectable only in traces (Fig. 2).
Genetic linkage analysis

Microsatellite markers of chromosome 17q21 including an intragenic marker of the adhalin gene (D17S1319) (1) indicated that the affected siblings share only one common allele of the adhalin gene. Therefore, linkage of recessive hearing loss or recessive muscular dystrophy to a mutation of this gene is most unlikely. Allelic distribution of 13q12-micro-
satellites was identical in both siblings and different in three unaffected family members. Hence, microsatellite data were compatible with secondary adhalin-deficiency related to chromosome 13q12 (Fig. 3). The six STSs scattered on the YAC contig gave a normal pattern after PCR amplification, suggesting the absence of large-scale deletion or rearrangement (not shown).
Discussion

The relation of muscular dystrophy in Patients II-2 and II-3 to adhalinopathy was indicated by markedly reduced expression of adhalin (50 DAG) and complete deficiency of a 35 kDa dystrophin-associated protein as opposed to the preserved expression of dystrophin, β-dystroglycan (43 DAG) and laminin (22, 24). Moreover, A3b labeling was vastly decreased revealing a generally reduced expression of the sacroglycan complex. The sacroglycan complex consists of dystrophin-associated and membrane-integrated proteins that do not seem to bind directly to dystrophin or laminin (24). Hence, the effects of adhalin-deficiency on sarcolemmal integrity and/or bridging between intra- and extracellular matrices deserve further evaluation.

Adhalinopathy of our patients was shown to be secondary by exclusion of a linkage with the adhalin gene locus (1, 30). Secondary adhalin deficiency in LGMD 2C has been linked to chromosome 13q12 (4, 5). Indeed, allelic distribution of 13q12-microsatellites was identical in the affected siblings but different in other family members, consistent with a linkage of LGMD and hearing loss in our patients to this chromosome region. The existence of a third gene locus of adhalinopathy cannot be ruled out, however. Microsatellite markers also indicated that our patients are compound heterozygotes, suggesting different mutations on each chromosome. In addition, deletions or large-scale rearrangements of the 13q12 gene locus have not been found. We conclude that the combination of hearing loss and muscular dystrophy in our patients is not likely due to a contiguous gene syndrome.

Neurosensory hearing loss is associated with distinct myopathies like MELAS-mitochondriopathy (16), myotonic dystrophy (32) and facioscapulohumeral dystrophy (6, 33). However, hearing impairment has not been implicated with DMD and DMD-like muscular dystrophies (2, 33). Nonetheless, a non-syndromic X-chromosomal form of sensorineural hearing loss maps to markers within the dystrophin gene (20). Furthermore, a dominant form of deafness (21) maps to the same region of chromosome 5 as a dominant form of limb-girdle muscular dystrophy (LGMD 1A) (7). Likewise, the 13q11-q12 region, where LGMD 2C maps, also contains a locus for recessive (13) and dominant (9) forms of deafness. However, neither has muscular dystrophy been reported in patients with these forms of deafness nor has hearing loss been described in LGMD 1A or 2C (4, 5, 7, 13, 23, 38). We show that LGMD with secondary adhalin deficiency may be associated with mild-to-moderate neurosensory hearing loss but we cannot completely exclude a coincidental combination of these disorders. In view of clinically silent electroretinographic abnormalities in DMD-patients (28) Lalwani et al (20) recommended elaborate audiological testing of muscular dystrophy patients. Our findings support this recommendation. Vice versa, we emphasize the equal necessity of appropriate (laboratory) analysis and report of muscle status including CK assessment in hereditary forms of hearing loss.

Defects of proteins that interact with the cytoskeleton may cause hearing impairment as has been shown in Usher syndrome type 1 (34). Hearing loss in our LGMD patients is compatible with a function of the putative 13q12 gene product and/or adhalin in the inner ear. However, to our knowledge expression of dystrophin and DAGs in the inner ear has not been investigated as yet (3, 10, 25, 29, 31, 39).

Addendum

Recently, dystrophin was detected in the hair cells of the cochlea (Dodson et al, J. Neurocytol. 24 [1995] 625-632).

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References


Dr. med. K. Oexle

Neuropädiatrie, Kinderklinik der Charité
Humboldt-Universität
Schumannstr. 20/21
D-10099 Berlin
Germany