Duchenne muscular dystrophy: negative electroretinograms and normal dark adaptation. Reappraisal of assignment of X linked incomplete congenital stationary night blindness

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Abstract

Åland Island eye disease (ÅIED) and X linked congenital stationary night blindness (CSNB) have been mapped to Xp11.3. Patients have been described with deletions of the Duchenne muscular dystrophy (DMD) gene who also had a negative electroretinogram (ERG) similar to that seen in patients with CSNB and ÅIED. This seems to confirm that some cases of ÅIED and CSNB map to Xp21.

We examined 16 boys with DMD/BMD (Becker muscular dystrophy) of whom 10 had negative ERGs, eight of them having deletions downstream from exon 44. Normal dark adaptation thresholds were observed in all patients and there were no anomalous visual functions. Hence, CSNB cannot be assigned to Xp21 and negative ERG in DMD/BMD is not associated with eye disease. Six boys with DMD/BMD had normal ERGs. We speculate that a retinal or glial dystrophin may be truncated or absent in the boys with negative ERGs.

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There are two types of X linked congenital stationary night blindness (CSNB), complete and incomplete. Linkage studies in families with incomplete CSNB,¹ complete CSNB,²⁻⁶ and Åland Island eye disease (ÅIED)^{7,8} have shown that they all map to Xp11.3. X linked incomplete CSNB and ÅIED presumably constitute the same genetic disorder.¹⁷⁻¹¹

In CSNB and ÅIED, the electroretinographic response to a bright light of the dark adapted eye shows a normal a wave and a low or absent b wave, so that the ratio of the amplitudes of the b to the a wave is below 1.0. This is called a negative electroretinogram (ERG). A negative ERG can also be found in a number of other ocular disorders (table).

In a series of papers, Weleber *et al*¹⁰ and Pillers *et al*¹⁵⁻¹⁷ described patients with complex deletions comprising glycerol kinase deficiency and DMD. The patients had a negative ERG. The observation led them to hypothesise that incomplete CSNB and ÅIED could also be induced by a deletion in the DMD gene at Xp21.

Five patients with DMD deletions described by Cibis *et al*¹⁸ and six patients with DMD gene deletions observed by De Becker *et al*¹⁹

had negative ERGs, similar to ÅIED and incomplete CSNB. This seemed to confirm that some cases of ÅIED and CSNB mapped to Xp21. Sigesmund *et al*²⁰ described 26 patients with DMD/BMD. Five patients, of whom three were brothers, had electroretinographic b/a amplitude ratios <1.0. Their deletions were exons 14-41, 48-49, and 45. Ten patients had no deletions and four of them had a b/a ratio <1.0. The rest had normal b/a amplitude ratios. No dark adaptation thresholds were routinely examined in the studies mentioned.

We have observed eight boys with DMD/BMD resulting from deletions downstream from exon 44. The ERGs had a b/a amplitude ratio <1.0, dark adaptation thresholds were normal, and there were no visual functional defects. We conclude that a negative ERG associated with deletion of the dystrophin gene is insufficient evidence for mapping CSNB and ÅIED to Xp21.

Methods

PATIENTS AND CLINICAL EXAMINATIONS

The Danish Patient Association of People with Muscular Disorders agreed to write to their members with DMD/BMD suggesting that they reported to us for a full ophthalmological examination, including ERG tracings. Letters were mailed to 48 members with DMD/BMD, 16 of whom, aged 9 to 26 years, accepted the invitation. Two patients were brothers.

The ophthalmological examination consisted of determination of visual acuity for near and distance vision, accommodation, refractive errors, motility, alignment, transillumination of the irides, colour vision, contrast thresholds, Goldmann visual fields, and dark adaptation measured in the right eye by the Goldmann-Weekers apparatus. The patients were tested for glycerol excretion in the urine.

Retinal disorders with negative ERG adapted from references 10, 12, 13, and 14.

X linked congenital stationary night blindness (CSNB)
Åland Island eye disease (ÅIED)
Autosomal recessive CSNB
Juvenile X linked retinoschisis
The enhanced S cone syndrome
Goldmann-Favre syndrome
Retinitis pigmentosa, special type
Carriers of some types of retinitis pigmentosa
Cone dystrophy
Optic neuropathy
Diabetic retinopathy
Other retinal vascular disorders

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Figure 1 Dark adaptation in patients with DMD/BMD. The patients were examined with the Goldmann-Weekers' apparatus. The dashed line shows mean results from normal subjects. The panel shows dark adaptation in boys with deletions in the middle part of the Duchenne gene. The bars present the range of clinical measurements. The abscissa shows time of adaptation in minutes, the ordinate shows the reduction of light in arbitrary units given on the instrument.

Figure 2 Deleted exons in 16 boys with Duchenne and Becker muscular dystrophy.

Figure 3 The top curve is an electroretinogram from a patient with a deletion of the Duchenne gene and a b/a wave amplitude ratio of <1.0 . The bottom curve shows a normal electroretinogram for comparison. The a and b waves are indicated.

ELECTRORETINOGRAPHY

ERGs were performed on the right eye on all patients and their mothers following the international guidelines.²¹ The stimuli were elicited with a Nicolet Ganzfield dome and the patients were dark adapted for 30 minutes. The response of the dark adapted eye to a bright white flash was assessed with maximal light intensity.

DELETION ANALYSES

DNA was isolated by routine methods. Deletion analysis was performed by PCR including the 9-plex PCR kit, designed by Chamberlain *et al*²² and Beggs *et al*²³ respectively. Furthermore, *Hind*III digested DNA was subjected to Southern blot analysis using cDNA probes²⁴ in order to determine the extension of the deletions.

Results

The 16 boys complied bravely with all examinations. The patients had normal visual acuities, contrast threshold, colour vision, visual fields, and dark adaptation (fig 1). The irides were not transparent and glycerol excretion was not increased in any patient.

The deletions observed are shown in fig 2. Eight boys had deletions in the middle part of the gene, and two in the 5' end of the dystrophin gene; in six patients no deletion was detected.

Ten boys had negative ERGs (fig 3), eight of whom had deletions of exons downstream from exon 44, while two had no discernible deletion. ERGs showed b/a amplitude ratios >1.0 in two boys with deletions of exons 3-4 and 8-11, respectively, and in four patients without deletions, two of whom were brothers. ERGs were normal in all mothers.

Discussion

DIFFERENTIAL DIAGNOSIS

ERG is negative in CSNB; in complete CSNB there is no trace of rod dark adaptation, while in incomplete CSNB dark adaptation is present, but the thresholds are raised. Even in the oldest of our patients with negative ERGs we found no sign of defective dark adaptation; hence complete and incomplete X linked congenital stationary night blindness could be excluded.

Dark adaptation in patients with the AIED showed defective rod dark adaptation, similar to curves from patients with incomplete CSNB.^{11 25} Typical findings in AIED are negative ERGs, impaired visual acuity, nystagmus, myopia, astigmatism, and colour vision defect.²⁶ Apart from the negative ERGs, none of these signs was observed in our patients.

A negative ERG is found in a number of retinal disorders (table), but none of them was present in our patients with DMD/BMD. Our patients had normal fundi, no abnormalities of their visual psychophysics responses, and normal visual fields. There were no signs of cone dystrophy.

DYSTROPHIN DELETIONS AND ERG RESPONSE

We found ERGs with a b/a amplitude ratio <1.0 in all patients with deletions of exons downstream from exon 44 (fig 2). ERGs with a b/a amplitude ratio >1.0 were present in boys with deletions of exons 3–4 and 8–11, and in four boys with no detectable deletion. Others^{17–20} have also found negative ERGs in patients with DMD/BMD who had deletions including exons 41–48, but De Becker *et al*¹⁹ also had a patient with a deletion of exon 3 who had a negative ERG.

Our patients were a random series of young boys who presented themselves in response to a letter from their patient organisation. Deletions were found in 63%, a percentage in accordance with other unselected series of DMD patients.²⁷

Dystrophin can be shown in the retina by immunohistochemical methods. Transcription of the dystrophin gene involves at least five distinct promoters, each of them driving cell specific dystrophins.²⁸ Different dystrophins are present in the brain, muscle, Purkinje cells, Schwann cells, and glial cells,²⁸ and are transcribed by alternative splicing.

Dystrophin is normally present in the photoreceptor-bipolar synaptic complex^{29–31} of the retina in humans and mice. Signals derived from these synapses and the Müller cells drive the b wave of the ERG and Fitzgerald *et al*³² found evidence of dysfunction of the photoreceptor-bipolar pathway in patients with DMD.

Brain dystrophin, muscle dystrophin, and some of the C-terminal splice variants are present in the outer nuclear layer of the retina. A specific retinal dystrophin with its own promoter has not been reported, and the function of the dystrophins in the retina is unknown.

THE POSITIONAL RELATION OF THE GENES FOR DMD/BMD AND X LINKED CSNB

Complete and incomplete CSNB and ÅIED are mapped to Xp11.3^{1–8} and incomplete CSNB and ÅIED presumably constitute the same nosological entity^{7–11} or allelic disorders.¹

Patients with DMD/BMD but without complex deletions also had negative ERGs similar to those observed in incomplete CSNB.^{17–20} It was therefore surmised that incomplete CSNB and ÅIED might also be assigned to Xp21.¹⁰

Our results show that although patients with deletions downstream from exon 44 do in fact have negative ERGs they have no deficiency of their dark adaptation thresholds or other clinical visual functions. Thus the patients have an asymptomatic ERG anomaly, and the assignment of CSNB or ÅIED to Xp21 cannot be upheld.

Conclusion

In 16 unselected patients with DMD/BMD, 10 had negative ERGs, similar to the responses in ÅIED and CSNB, but they had normal clinical ophthalmological functions and no measurable night blindness and therefore no CSNB. This shows that CSNB cannot be as-

signed to Xp21 and that negative ERG in DMD/BMD is not associated with eye disease.

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