Correlation between electroretinogram findings and molecular analysis in the Duchenne muscular dystrophy phenotype

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Abstract

Fifteen consecutive patients with the Duchenne muscular dystrophy (DMD) phenotype were studied. Each patient was asked to undergo an ophthalmic examination, an electroretinogram (ERG), and to donate a blood sample for molecular diagnosis. All 15 patients had a normal ophthalmic examination. Electroretinography was successful in 14/15 patients. The ERG tracings were normal in seven patients, abnormal in seven, and unreliable in one. Blood for molecular analysis was obtained in 12/15 patients. In the seven patients with a normal ERG, five underwent molecular analysis, and in these five no deletion was detected in the dystrophin gene. In the seven patients with an abnormal ERG, six had molecular analysis available, and all six were found to have a deletion. These results suggest that patients with a classic DMD phenotype are genetically heterogeneous, and that this heterogeneity is reflected in the ERG. (*Br J Ophthalmol* 1994; 78: 719–722)

Duchenne muscular dystrophy (DMD) is one of the most common and severe neuromuscular disorders, with an incidence of one in 3500 newborn males,¹ and a prevalence of 2.48/100 000 in the general population.² The muscle atrophy progresses rapidly: patients are classically wheelchair bound by the age of 12 and die of respiratory insufficiency or cardiac failure in their early twenties.³

In DMD, the large causative gene located at Xp21 may undergo deletions, duplications, or point mutations. The defective protein product, dystrophin, is produced inadequately to maintain its normal functions.^{3–5} Dystrophin is a very high molecular weight protein essential to the cytoskeleton of striate muscle.^{6,7} Dystrophin deficient fibres have a mechanically weakened plasma membrane⁸ and may have abnormal calcium channels.⁹ Several isoforms of dystrophin exist.⁷ Some are found in neurons and glial cells of the central nervous system,^{10,11} where they seem to localise more specifically to dendrites and synaptic regions, thus suggesting a role in neurotransmission.¹² Other dystrophin isoforms are present in normal human retina at the level of the outer plexiform layer and it has been reported that patients with DMD have an electronegative scotopic ERG.^{13,14} It has been suggested that the ERG could become a non-invasive probe to measure the DMD gene function, or a diagnostic or prognostic tool for DMD.^{13,14}

Because approximately 35% of patients with

DMD have no identifiable deletion in the dystrophin gene,³ we chose to study 15 consecutive patients with DMD phenotype, rather than only those with a proved deletion. We report on the ophthalmic findings, the ERG, and the molecular analysis in these subjects.

Materials and methods

PATIENTS

Fifteen consecutive patients with a DMD phenotype were identified from the neuromuscular clinic. The diagnostic criteria are described in Table 1. Molecular diagnosis was not part of the diagnostic criteria. All 15 patients were asked to undergo an ocular examination and electroretinography, as well as to donate a blood sample for molecular analysis. One DMD patient followed at our institution has complex glycerol kinase deficiency (CGKD), a contiguous gene syndrome of the Xp21 region. This patient was included in a previous report,^{15,16} and he did not participate in the present study.

MOLECULAR ANALYSIS

Blood was collected in EDTA vacutainer tubes and the DNA extracted by standard procedures.¹⁷ The samples were examined for commonly deleted regions of the DMD gene by PCR amplification using the multiplex conditions of Chamberlain *et al.*¹⁸ and Beggs *et al.*¹⁹ These reactions will detect 98% of DMD deletions. In those patients in which deletions were not detected by multiplex PCR, Southern blots were performed using the probes cDMD 4–5a (ATCC), cDMD 5b–7 (ATCC), and J66 (van Ommen, Leiden) to examine regions of the DMD gene less commonly involved in deletions.

OPHTHALMIC EXAMINATION

Each patient was asked with insistence about the presence of nyctalopia or any other ocular complaint. Visual acuity was measured with a portable illuminated HOTV chart calibrated for use at 3 metres. Pupils were examined for afferent defect or paradoxical reaction. Nystagmus was looked for clinically. Dilated fundus examination was done with direct and indirect ophthalmoscopy, and attention was paid particularly to the aspect and coloration of the optic nerves, the retinal blood vessels, the maculae, the quality of the foveal reflex, and the overall chorioretinal background. A cycloplegic refraction was done if uncorrected visual acuity was less than 1.0 (6/6) in either eye.

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Accepted for publication
5 May 1994

Table 1 Diagnostic criteria for DMD phenotype, molecular analysis, and ERG findings

Case	Age	Diagnostic criteria						DNA deletion (exons)	ERG
		Muscle weakness	Muscle biopsy	Elevated CPK	EMG	FH	WCB		
3	20	+	+	+	+	—	9	47	Electronegative
4	24	+	+	+	ND	—	10	45	Electronegative
5	15	+	+	+	ND	—	10	49–52	Electronegative
9	16	+	+	+	ND	—	8	45–48	Electronegative
1	22	+	+	+	+	—	10	ND	Electronegative
8	5	+	+	+	ND	—	NY	3	Electronegative
15	4	+	+	+	ND	—	NY	48–51	Electronegative
2	15	+	+	+	ND	+	9	ND	Normal
10	24	+	+	+	+	—	12	No deletion	Normal
6	11	+	+	+	ND	—	NY	ND	Normal
13	22	+	+	+	+	—	13	No deletion	Normal
7	17	+	+	+	+	—	12	No deletion	Normal
12	10	+	+	+	+	—	NY	No deletion	Normal
14	19	+	+	+	+	+	12	No deletion	Normal
11	7	+	+	+	ND	—	NY	No deletion	Unreliable

Elevated CPK = creatine phosphokinase >140 $\mu\text{m}/\text{ml}$; muscle biopsy = dystrophy changes observed; EMG = electromyography; FH = family history; WCB = age at which the patient is wheelchair bound; ERG = electroretinogram in response to bright flash stimulation; ND = not done; NY = not yet wheelchair bound.

ELECTRORETINOGRAPHY

All ERGs were done without sedation. The electroretinographic procedure used in this study has been described in full detail elsewhere.²⁰ Briefly, this included an initial period of 30 minutes of dark adaptation under controlled dim red illumination during which the procedures were explained to the patient. Cutaneous gold electrodes were fixed to the forehead (reference) and right earlobe (ground) after skin preparation. After pupil dilatation and topical corneal anaesthesia, a contact lens (Lovac type, Medical Workshop, the Netherlands) was set on the cornea. In one young uncooperative patient, a carbon fibre electrode (Instruments for Medicine, Salt Lake City, USA) was placed in the inferior conjunctival fornix. A Ganzfeld stimulator (Nicolet GS2000, Madison, USA) produced a series of short (Kodak Wratten No 47, a, b, Rochester, USA) and long (Kodak Wratten No 26) wavelength stimulation in scotopic conditions, in order to characterise the dark adapted rod and cone components. Bright white flash stimulations ($0.8 \text{ cd}\cdot\text{m}^{-2}\cdot\text{s}$) were also used to estimate the total retinal output. Single white flash stimulation ($10.1 \text{ cd}\cdot\text{m}^{-2}\cdot\text{s}$) under photopic conditions ($34 \text{ cd}\cdot\text{m}^{-2}$) and 33.3 Hz flicker stimulation were delivered after a minimum of 3 minutes of light adaptation to provide

an estimate of the cone activity. The amplifier's bandpass was set at 1 Hz to 1 kHz in a first channel to record the standard ERG and in a second channel to 100 Hz to 1 kHz to record the oscillatory potentials (OPs). An artefact rejection device eliminated data contamination from eye movements and an averager (Nicolet PathFinder II) cumulated and stored the raw data. This procedure fulfils the minimum requirements as stated by the International Society for Clinical Electrophysiology of Vision's Standardisation Committee.²¹

STATISTICAL ANALYSIS

For the ERG results, a *t* test (two tails, 0.05 level) was used to compare the group average of each of the parameters measured to a normative group, which contained 24 normal patients age-matched with the DMD group (aged 5–24 years; mean 16 years).

Results

Fifteen consecutive males with a DMD phenotype, age 5 to 24 years, were examined. The 10 oldest patients were wheelchair bound. Visual acuity was 1.0 (6/6) or better in all eyes examined, except for one, that had suffered a

Figure 1 Representative electroretinograms in DMD patients with (recordings 1–3) and without (recordings 4–6) electronegative ERG to scotopic bright flash stimulation. The top line illustrates the cone related responses after adaptation to a photopic background. The second line shows recordings of the rod related component, after 30 minutes of dark adaptation. The characteristic response to a bright flash is in the bottom line. Examples of normal recordings are provided in the right hand column. Arrowhead = stimulus onset. Horizontal calibration mark = 20 ms (traces 1, 4, 7); 40 ms (traces 2, 3, 5, 6, 8, and 9). Vertical calibration mark = 100 μV .

Table 2 Quantitative data and statistical analysis of electroretinographic findings in patients with Duchenne muscular dystrophy

ERG measurements	Duchenne abnormal	p	Duchenne normal	p	Normal age matched
Amplitude (μ V) (SD)					
Photopic:					
a-Wave	82 (20)	0.68	88 (15)	0.18	79 (17)
b-Wave	159 (36)	0.19	178 (20)	0.96	178 (45)
Scotopic:					
Rod b-wave	140 (37)	<0.001	418 (60)	0.37	413 (81)
Bright-flash, a-wave	229 (43)	0.09	256 (120)	0.29	262 (47)
Bright-flash, b-wave	128 (55)	<0.001	469 (85)	0.92	516 (100)
b/a Ratio	0.58 (0.23)	<0.001	2.07 (0.23)	0.96	2.00 (0.33)
Implicit time (ms) (SD)					
Photopic:					
a-Wave	14.3 (1.5)	0.006	13.5 (0.8)	0.13	13.2 (0.4)
b-Wave	36.4 (2.3)	0.02	33.9 (1.0)	0.39	34.6 (1.7)
Scotopic:					
Rod b-wave	53.3 (3.4)	0.15	57.8 (2.7)	0.06	55.5 (29)
Bright-flash, a-wave	22.7 (0.9)	0.68	23.8 (1.9)	0.03	22.8 (0.6)
Bright-flash, b-wave	37.1 (4.1)	<0.001	45.3 (2.7)	0.56	45.1 (1.8)

p=p Value, t test, two tailed.

penetrating injury in early childhood. One patient was myopic (-6.00 dioptres) in both eyes. The fundi were within normal limits in all patients. In particular, there were no abnormalities of the optic nerve such as temporal hypoplasia, tilted disc, or temporal palor. None had paradoxical pupils, clinically detectable nystagmus, or nyctalopia.

Blood was obtained in 12/15 patients and all 12 samples underwent molecular analysis (Table 1). Six patients had a detectable deletion, and six had no detectable deletion. Blood was not obtained in 3/15 patients: two did not consent to venepuncture, and one had such marked pseudohypertrophy of the muscles that venepuncture was unsuccessful. We did not proceed to venous dissection.

Electroretinography was successful in 14/15 patients. Representative ERG tracings are shown in Figure 1. Table 2 details the quantitative data and statistical analysis as based on the 13 patients who underwent an ERG with the contact lens electrode. Seven subjects had normal ERGs (Fig 1, recordings 4–6). Seven had abnormal tracings (Fig 1, recordings 1–3). Their bright flash scotopic ERG had a normal a-wave amplitude, but a severely attenuated b-wave amplitude, resulting in an overall electronegative configuration (Fig 1: recording 3). In photopic conditions, amplitudes of the a and b waves were within normal limits (Fig 1: recording 1), and the wave morphology was normal.

Discussion

The ERG findings in this study divide the subjects with a DMD phenotype into two distinct groups: one group (7/14) with normal ERGs and another group (7/14) with an electronegative bright flash scotopic ERG. There were no obvious ophthalmic clinical characteristics predictive of the ERG results obtained.

An electronegative ERG has been linked clearly with DMD. Pillers *et al*¹³ report 11 patients with DMD/Becker muscular dystrophy (BMD) and an abnormal ERG. These authors also describe a group of 35 patients (five with CGKD, 23 with DMD, and seven with BMD), of which 31 had an abnormal ERG.²² Cibis *et al*¹⁴ report five DMD patients with an abnormal ERG.

In the present study only 50% of the patients with DMD have an electronegative ERG, while the other 50% have a normal ERG. This is possibly because we studied 15 consecutive patients with a classic DMD phenotype, rather than patients with a proved DMD genotype. It could also be that our DMD population differs from that studied in other geographical areas. In our series, patients with no detectable deletion in the dystrophin gene also had a normal ERG. It is possible that these patients have a smaller undetected mutation in the dystrophin gene or a duplication, not affecting normal transcription of the retinal isoforms. They may also have a mutation involving the promoter region for the striate muscle isoforms and not the brain and/or retinal isoforms. It is known that brain dystrophin and striate muscle dystrophin use different promoters.^{7,11} It is possible, but less likely, that a protein other than dystrophin is involved, such as a 'dystrophin associated protein' (DAP),²³ and 'dystrophin related protein', which maps to chromosome 6, is an autosomal homologue of dystrophin,^{24,25} causing a classic DMD phenotype, but no ERG abnormalities.

In conclusion, these results indicate there is genotypic and ERG heterogeneity within our group of patients with a classic DMD phenotype. There seems to be a correlation between the genotype and the ERG findings: our patients with a deletion have perturbed ERG while those with no detectable deletion have a normal ERG. This suggests that patients with DMD phenotype and a normal ERG may have an as yet undetected mutation in the dystrophin gene that does not affect the retinal isoforms. They could also have an intact dystrophin gene but an altered promoter. Lastly, it is possible that both the dystrophin gene and the promoter are intact and that the anomaly lies in a dystrophin related protein.

We wish to thank all the families who kindly gave their time to participate in this study. Many thanks also to Kevin Gordon, MD, for referring some patients, and to Judy MacSween, Brenda Walmsley, and Claudia MacPhee for the difficult task of coordinating the patients' appointments.

Presented in part at the Association for Research in Vision and Ophthalmology, Sarasota, 4 May 1993.

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