Foveal photopigment kinetics – abnormality: an early sign in myotonic dystrophy?

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Abstract

Twelve subjects with minimal expression of the myotonic dystrophy (MyD) gene were investigated by retinal densitometry, a technique which has been used to study the properties of photopigments in the living eye and to detect photoreceptor abnormalities. Other investigations included slit-lamp examination, funduscopy, raleigh matches with the anomaloscope, tonometry, and neurological examination, including electroretinography (ERG) and pattern visual evoked potentials recording. Foveal densitometry demonstrated reduced values of the macular photopigment density difference with normal photopigment kinetics in early phases of the disease, even in asymptomatic individuals. The densitometric values correlated with decreased amplitudes of the photopic ERG a-wave. These findings may be explained by loss or dysfunction of the outer segments of foveal receptors. It is yet unknown whether or not these changes are secondary to other observed neuroretinal abnormalities in MyD. The most likely explanation might be an abnormality of the Na, Ca:K exchanger at the level of the outer segments of the photoreceptors whether or not in combination with a dysfunction of voltage generation systems, involving both photoreceptors and retinal pigment epithelium.

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Myotonic dystrophy (MyD) or Curschmann-Steinert disease is an autosomal dominant multi-system disorder of which the gene abnormality is localised on chromosome 19.^{1,2} DNA diagnosis of MyD is possible with both sensitivity and specificity higher than 96%.² It shows variable gene penetrance and expressivity, and the onset of MyD varies from early infancy to old age.³ An alteration in the intrinsic biochemical composition of the cell membrane, affecting the entire body, has been proposed as the pathogenesis of MyD.¹

Ocular abnormalities associated with MyD are cataract, hypotonia, and chorioretinal changes. Several authors reported the considerable electroretinal alterations in MyD patients, even before fundal changes and other expression of the abnormal gene.^{1,4–14} A common finding was the reduction of the b-wave amplitude in the electroretinogram (ERG) both in scotopic and photopic conditions.^{4–12} Visual evoked potential (VEP) recording in MyD repeatedly showed prolongation of latency or decrease of amplitude.¹² Recordings of the pattern ERG (PERG) and pattern VEP (pVEP) simultaneously demonstrated abnormalities that occurred independently of one another. The authors also found

subnormal electro-oculogram Arden indices. They suggested that the pathological process may affect both the retina and post-retinal intrinsic visual pathways.

Both clinical^{4,5} and histopathological^{15–21} evidence of photoreceptor and other neuroretinal abnormalities has been reported in progressive stages of MyD. The purpose of this study was to investigate the early expression of retinal abnormalities in MyD, including those of foveal photoreceptors. We have used retinal densitometry to examine the macular photoreceptors of eyes of a group of minimally affected MyD patients. To our knowledge this technique has never been utilised in MyD.

Material and methods

SUBJECTS

Twelve patients, eight females and four males, were examined. Informed consent was given by all patients. They all either had a positive family history for MyD and myotonia, or a clinical diagnosis of MyD, confirmed by DNA investigation (Laboratory of Clinical Genetics, Radboud Hospital, Nijmegen, the Netherlands). All patients were submitted to neurological and ophthalmic examination, including testing of visual acuity, funduscopy, slit-lamp examination, and tonometry (Goldmann applanation tonometer). Inclusion criteria for the study were a visual acuity of at least 0.8 after correction and no or minimal lens abnormalities (sporadic polychromatic crystals or white dots) and a Karnofsky index exceeding 80. An age matched group of 12 healthy subjects was used as control.

INVESTIGATIONS

Densitometry

The density of foveal cone pigments was measured with the Utrecht densitometer which has been described in detail by van Norren and van der Kraats.²²

Briefly, the light reflection at the fundus is determined at a range of wave lengths through the visual spectrum. The light needed for this measurement is so dim that influence on the visual pigments is minimal. The spot of light (30 W halogen lamp) at the fundus is restricted to 2.8 degrees in this measurement. Reflections of the fundal lights are collected by a photomultiplier in photon-counting mode and processed by a computer. Bleaching is possible with a separate light path which provides a retinal illumination of 5.6 log Td. The change in reflection from the retina of a healthy person before and after bleaching determines the 'two-way density'. The

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pupils of all subjects were dilated with tropicamide. A bar made of dental impression compound, and two temple pads were used for immobilisation of the head. The fundus camera was adjusted to obtain the highest possible reflection as measured by the photomultiplier. Subjects fixated cross-hairs centred with the stimulus field. The fovea was bleached for 2 minutes. After switching off the bleaching light the regeneration of cone pigment was followed until the density trace had reached a stable level. Then the bleaching light was switched on again for 2 minutes. The density trace should then return to the fully bleached condition, permitting the reliability of the measurements to be checked. The changes in retinal transmittance between fully dark adapted and fully bleached states of the retina are related to the photopigment density of the test area.

Basically, two values can be extracted from the data: the density difference and the time constant of pigment regeneration. The density difference is the change in the logarithm of the measuring light reflectance to the reference light reflectance, from fully bleached to fully adapted states. The time constant of pigment regeneration can be estimated from the initial 180 seconds of adaptation following extinction of the bleaching light. In this study we only present the data obtained at 554 nm, being the peak of the absorption spectrum. Peripheral measurements, investigating the rods take considerably longer, since the adopses regenerates slow. In our subjects occasionally denture fitting problems and lack of concentration – common signs of MyD – interfered with the measurements.

VEP and ERG

Pattern visual evoked potentials and ERG measurements were made with a laboratory built system for clinical electrophysiology of vision.²³ The ERG was obtained in both scotopic and photopic conditions, using corneal electrodes and three different white light stimulus intensities. VEP recording was done under standard conditions, using 11', 46', and 92' settings in the black-and-white chequerboard reversal pattern.

Anomaloscope

Colour matching was investigated with a Nagel type I anomaloscope by a standard technique.²⁴

Results

The ages of the patients ranged from 12 to 39 years (mean 26 years) and the general disability rated by Karnofsky index²⁵ varied from 80 to 100 (mean 90). The duration of clinical disease varied from 0 to 408 months (mean 71 months).

A summary of the main ophthalmic results is displayed in Table I. Slit-lamp examination revealed minimal lens opacities in four patients (30%) and characteristic polychromatic crystals in three patients (25%), while in five patients (42%) no abnormalities were detected. Funduscopy did not show morphological changes of the retina in any of the patients. The visual acuity varied from 1.2 to 0.8 (mean 0.9). Anomaloscopy showed no protan or deutan defects.

Seven of nine patients had low intraocular pressures bilaterally, while two patients had low normal pressures (mean 12.7±2.3; normal values 15.5±2.5).²⁶

In four out of 23 eyes (17%) the scotopic ERG showed a significant decrease of the a-wave amplitude and in only 1/23 eyes (4%) a significant decrease of the b-wave. An increase in implicit time was noticed in 10/23 eyes (43%) for the scotopic a-wave and 3/23 (12%) for the scotopic b-wave. In photopic conditions 8/23 eyes (35%) showed a decrease of the a-wave amplitude and in 7/23 eyes (30%) a decrease of the b-wave amplitude. An increase in implicit time was recorded in 2/23 (8%) eyes for the photopic a-wave and in 4/23 (16%) for the photopic b-wave.

Pattern VEP recording with 11' pattern reversal blocks showed a significant prolongation of the P100-latency in four patients (30%). In five eyes (42%) a decrease of the VEP amplitude was noted. No significant hemispheric asymmetry was seen. In two eyes (16%) both VEP amplitude and latency were abnormal. Only five eyes (42%) had normal VEP data. There was no clear correlation between VEP and ERG data.

In six patients (50%) foveal densitometric measurements showed a significant decrease of

Table 1 Ophthalmic findings in myotonic dystrophy

Patient	Duration (months)					Scot ERG				Phot ERG				
		Vision		Densito		Amplitude		Latitude		Amplitude		Latitude		
		Right	Left	Right	Left	A	B	A	B	A	B	A	B	
AB ^a	12	1.0	1.0	cp	–	0.21	–	N	–	N	L	L	N	H
AB	24	1.0	0.8	vac.	0.22	0.18	N	N	N	N	L	L	N	N
GF ^a	60	1.0	1.0	pcc	0.25	–	N	N	N	N	–	–	–	–
EG	144	0.8	1.0	dots	0.35	0.37	N	N	H	N	N	N	N	N
JM ^a	0	1.2	1.2	dot	0.21	–	–	H	–	N	L	N	N	H
JM	0	0.9	0.9	N	0.33	0.35	N	N	N	N	N	N	N	N
JS	408	1.0	0.6	dcp	0.37	0.40	N	N	H	N	N	N	N	N
JS	118	1.0	1.0	cp	–	0.23	N	N	N	H	N	N	H	N
JS	72	1.0	1.0	dcp	0.36	–	L/N	N	H	N	N	N	N	N
JV	19	1.0	–	dot	0.18	–	L/N	L/N	N	H/N	L/N	L/N	N	N
MV	0	1.0	1.0	dots	0.12	–	N	N	H	N	L/N	L	N	N
GY	0	0.9	0.9	N	0.39	0.43	L	N	H	N	N	N	N	N

N, normal; L, decreased; H, prolonged or increased; –, no result; pcc, polychromatic crystals; cp, crystals in lens cortex posterior; dots, dots in lens cortex posterior; dcp, dot(s) and crystals in lens cortex posterior; densito, foveal two-way densities – normal: age matched: 0.38 (SD 0.12).

^aValues of peripheral densitometry

patient 1: 0.20 (N)

patient 3: 0.13 (abnormal)

patient 5: 0.17 (N)

Figure 1 The amplitudes of a-wave of the photopic ERG are plotted against the values of foveal densitometry. The best fitting line has an r value of 0.57 (Spearman's rank correlation) and $p=0.02$ (Wilcoxon rank sum test) to reject the null option.

the two-way density. The mean density of all patients tested (0.29 (SD 0.09)) was significantly lower than the mean of the age-matched control group (0.38 (SD 0.12)). The time constants of pigment regeneration of the patients (mean 66 (SD 6) seconds) were in the normal range (mean 69 (SD 8) seconds).

Both peripheral retinal densitometry and ERG in patients 1 and 5 showed no abnormalities, but foveal densitometry was significantly abnormal in both cases. In patient 3 the peripheral densitometric measurement was low, 0.13 (normal 0.18 (SD 0.04)) (Table I).

The Arden index of the EOG was subnormal in 6/12 eyes (mean 181% (SD 15.7), range 161–193).

Myotonia was noted in seven patients (58%). Ptosis was absent in six patients (50%), minimal in one (8%), moderate in three patients (25%), and severe in the remaining two patients (16%). Weakness of the limb muscles was found in three patients (25%), varying from minimal to moderate.

Discussion

Foveal densitometry demonstrated subclinical abnormalities of foveal photoreceptors in 50% of our patients with minimal (early) expression of the MyD gene (Table I). Even in a 12-year-old asymptomatic DNA positive individual (patient 11) a strikingly abnormal value was noticed.

The reduced two-way density may be caused by loss or dysfunction of photoreceptors or by a reduced effective optical density of foveal cones due to shortened outer segments. Other causes include active or chronic serous leakage of subretinal fluid with photoreceptor disorientation and abnormally high stray light levels secondary to lenticular opacities. The latter is unlikely because lens opacities in our patients were negligible. Serous leakage of subretinal fluid can be excluded by normal funduscopy findings. The results of the normal pigment regeneration times excludes serous leakage because this phenomenon is usually associated with increased pigment regeneration times.²⁷ Normal findings with the Nagel anomaloscope, which would be most sensitive to cone pigment density changes occurring in individual cones, such as shortened outer

segments,²⁸ make such alterations less probable. Therefore, we argue in favour of loss or dysfunction of photoreceptors to explain the reduced densitometric values.

Additionally, the peripheral retina of three cases was examined using retinal densitometry. Whilst in all three patients the foveal densitometry findings were abnormal, in two out of three the peripheral two-way densities were normal.

Though the number of investigated eyes is limited, these findings may indicate predominant involvement of the macular area in early stages of MyD. The predictive power for densitometry for family members of patients with MyD is very high (odds ratio: 6, 95% CI, 0.728–277 CIA statistics) compared with the total of all performed ophthalmic tests (odds ratio: 10, 95% CI 1.42–442).

In contrast with previous authors (see Introduction), who reported reduced amplitudes of the scotopic b-wave as the most frequent ERG abnormality, our study showed a significant predominance of photopic ERG a and b-wave abnormalities. The latter is not due to a sample error. Compared with the study of Pinto *et al*¹² a difference of proportions is significantly different (difference of confidence interval: 0.560, 99% CI 0.151–2.58, CIA statistics).

Furthermore, the photopic ERG a-wave amplitude showed a significant (Wilcoxon test, $p=0.02$) correlation with the foveal densitometric values. The decrease of amplitude of 11' pVEP shows a trend for correlation with foveal densitometric findings (Wilcoxon test, $0.05 < p < 0.10$). These findings also point at subclinical (macular) cone changes in early onset of MyD.

Only in a minority of this selected patient group, a decrease of the b-wave of the scotopic ERG could be detected (Table I). Presumably, the scotopic b-wave abnormality seems a late manifestation.²¹

Several authors described a decrease of the scotopic b-wave,^{29,30} which may be associated with a disturbance in retinal Mueller cells. Another report¹² suggested involvement of ganglion cells. Retinal pigment epithelial (RPE) changes have been clinically^{4,5,31} and histologically observed.^{15–18} Whether the photoreceptor and other neuroretinal (Mueller and ganglion cells) alterations are primary or secondary to changes in the RPE, remains a matter for discussion. Sarks *et al*²¹ argue in favour of the principal pathological changes to occur at the level of the RPE, based on the minimal fallout of photoreceptors, despite relatively good vision and slightly affected electrophysiological tests; while, in those cases the RPE was grossly irregular and hypertrophic, with distorted villi, containing – in particular under the fovea – large amounts of (melano) lipofuscin.²¹ If the EOG is abnormal, a much larger area than the foveal (receptors) must be affected (see above). It is intriguing to speculate that in MyD some as yet unknown metabolic disturbance of the RPE cells may result in dysfunction and subsequent variable atrophy of photoreceptors.

Where does this leave us with the retinal pathophysiology? The difficulty could lie in the voltage generating mechanisms of the slow

oscillations at the basal membrane, the concentration of the 'light peak substance (lps)' at the level of the photoreceptors or the transmittance of the message across the apical membrane through the RPE cytoplasm to the basal membrane. Less likely hypotheses we cannot exclude are a delay or a block in the messenger system or a defective voltage generation system of RPE and photoreceptors, similar to the one demonstrated in the muscle and erythrocyte membranes. Nevertheless, the 'lps' concentration change abnormality at the level of the photoreceptors³² might explain both ERG, EOG, and densitometry findings. Therefore, we postulate an abnormality in a Na, Ca:K dependent exchanger in the outer segments to be the primary defect, taking into account knowledge of membrane pathophysiology. The mRNA of the MyD gene encodes a protein kinase polypeptide, which is known to modulate the activity of excitable cells by phosphorylation of ion channels.^{33,34} The (muscle) membrane has a decreased resting potential, with a value close to the threshold for activation of Na⁺ channels and the presence of specific (apamin sensitive) Ca²⁺ activated K⁺ channels.^{35,36} Besides, the Na⁺-K⁺-ATPase activity is decreased and the voltage dependent Ca²⁺ channels are active under conditions in which they are normally inactive.³⁷ Therefore, instead of 3 moles of Na⁺ ion exchange for 2 moles of K⁺ ion in control cells, a 2 Na⁺ for 2 K⁺ ion exchange occurred in MyD cells.³⁸ Altered inward sodium transport and extracellular leaking of potassium is involved in the generation of action potentials or regulation of resting potentials.³⁹⁻⁴¹ The defective regulation of ion transport could initiate or contribute to abnormal cellular functions in MyD.⁴¹ Remarkably, in more advanced disease the Mueller glial cell becomes affected. In particular this cell, with a high resting potential, appears to be most sensitive to potassium changes at physiological levels compared with neurons.⁴² We hope that future studies may obtain additional information on the locality and mechanisms of the retinal dysfunction in MyD.

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