

Cone and rod dysfunction in the NARP syndrome

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Abstract

Aims—Description of the ophthalmic manifestations of the NARP (neuropathy, ataxia, retinitis pigmentosa) syndrome that is associated with a point mutation in position 8993 of the mitochondrial DNA (mtDNA).

Methods—A mother and her two children, all carrying the 8993 mtDNA mutation, were examined. Two had manifestations of the NARP syndrome. A complete ocular and systemic examination was performed on all three patients.

Results—The clinical examination, electroretinogram, and visual fields revealed a typical cone-rod dystrophy in the son, and a typical cone dystrophy in the daughter. The mother had no ocular manifestations of the disease.

Conclusions—NARP is a recently described, maternally inherited mitochondrial syndrome in which a retinal dystrophy, among other abnormalities, is related to a mutation of the mtDNA at nucleotide 8993. This study demonstrates the great variability of the ocular manifestations in the NARP syndrome. It also indicates that the retinal dystrophy in at least some NARP patients affects primarily the cones.

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Mitochondrial diseases associated with mutations in the mitochondrial DNA (mtDNA) frequently show ocular manifestations.¹⁻¹⁰ Pigmentary retinopathy is found in Kearns-Sayre syndrome,⁴⁻⁶ in chronic progressive external ophthalmoplegia (CPEO), in mitochondrial myopathies without external ophthalmoplegia,^{7,8} in mitochondrial encephalomyopathy overlap syndrome,⁹ and in the NARP syndrome.¹⁰ Salt and pepper retinopathy is the common fundus manifestation in these diseases, but a typical retinitis pigmentosa (RP)-like retinopathy and other types of retinal pigment epithelium (RPE) changes and choriocapillaris atrophy were also described.^{7-9,11} Macular abnormalities are frequently associated with the pigmentary retino-

pathy, usually as hyperpigmentation and/or hypopigmentation.¹²

In 1990 Holt *et al*¹⁰ first described the NARP syndrome (neuropathy, ataxia, retinitis pigmentosa), caused by a point mutation in nucleotide 8993 of the mtDNA. Since then, several families with members affected by NARP have been described with a spectrum of neurological findings such as migraine, ataxia, neuropathy, and mental retardation.^{11,13-16} The ocular manifestations of the NARP syndrome were described as RP-like disease in most of these reports.

We report here on three members of a family that carried the mtDNA 8993 mutation; one of them showed the clinical and electrophysiological characteristics of cone-rod dystrophy (CRD), the second, of cone dystrophy (CD), and the third was normal. Such findings in association with the NARP syndrome have not been described previously.

Patients and methods

PATIENTS

We examined a mother, her son, and her daughter. The family history showed three generations with an ocular disease that was described as "progressive reduction in visual acuity" (Fig 1). The patients available for our examination underwent ophthalmological and neurological examinations, ERG, and fluorescein angiography. One patient underwent Goldmann visual fields.

ELECTRORETINOGRAM (ERG)

ERG was done as described in detail elsewhere.¹⁷ In short, full field ERG was recorded using corneal electrodes (Henke's type), Cyberscan 4000 computerised system (Microshev, Efrat, Israel), and a Grass PS22 Photostimulator (Quincy, MA, USA). The forehead served as a reference and the ear as a ground. In the dark adapted state, two responses were acquired: a rod response to a dim blue flash using a Wratten 47b filter (Kodak, Rochester, NY, USA), and a mixed cone-rod response to a white flash (2.75 lumen/s/ft²). In the light adapted state, a background light of 20 ft candelas was used to suppress rods and the cone response to 1 Hz white light (11 lumen/s/ft²), to 30 Hz flicker were acquired. All ERG responses were signal averaged (n = 4). With this method the minimal normal values in our laboratory for adults are 100 µV and 400 µV for the scotopic white a and b wave respectively, 200 µV for the scotopic blue b wave, 20 µV and 90 µV for the photopic white a and b wave respectively, and 80 µV to the 30 Hz flicker. The lower limit of sensitivity using this method is 10 µV.

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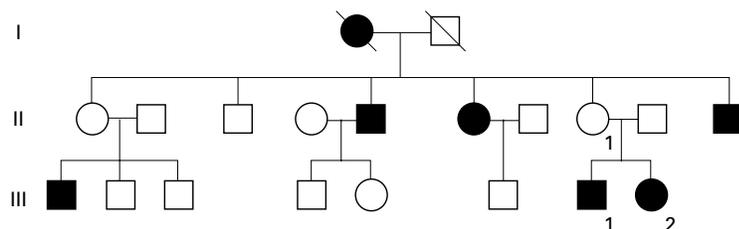


Figure 1 Pedigree of the family. Patients II-1, III-1, and III-2 were available for our examination (filled square/circle = progressive reduction in visual acuity).

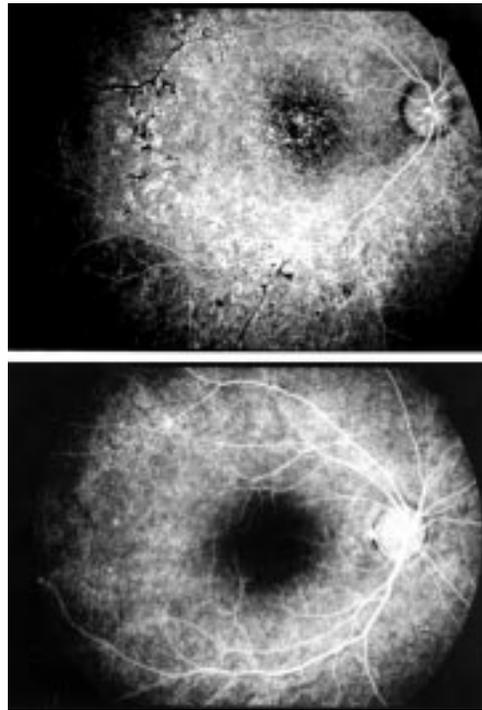


Figure 2 Fluorescein angiography of patients with NARP syndrome. Patient III-1 in arteriovenous phase (upper) shows multiple spots of hyperfluorescence in the macular and perimacular areas in addition to dark spots of pigment and arteriolar narrowing. Patient III-2 in arteriovenous phase (lower) shows the sign of the silent choroid in the centre with a perimacular wide ring of hyperfluorescence.

MITOCHONDRIAL DNA ANALYSIS

Total DNA was extracted from blood lymphocyte of the three patients by proteinase K using the phenol chloroform method. A 2379 bp mtDNA fragment encompassing nucleotide 8993 was amplified by the polymerase chain reaction (PCR) technique with a pair of primers (L8161-8180 and H10540-10521) under the following conditions: 36 cycles of denatura-

tion at 94°C for 1 minute, annealing at 57°C for 1 minute, and extension at 70°C for 2 minutes. Seven μ l of the PCR product were digested with 10 units of MSPI at 37°C for 16 hours. The digested DNA was then electrophoresed on 1% agarose gel at 100 V for 1 hour and the band intensities were determined using scanning densitometry. In a healthy control, the 2379 bp fragment was cleaved to 1123 bp and 1248 bp fragments; in the presence of the 8993 T→G mutation the 1132 fragment was cleaved into two bands of 831 bp and 301 bp. The proportion of the mutant mtDNA was calculated as the ratio between the intensity of the 831 bp plus 301 bp and the 1132 bp bands.

Results

Patient II-1, a 44 year old female, is the mother of patients III-1 and III-2. She had no ocular symptoms and was generally healthy. Her late mother, two brothers, sister, and nephew were known to suffer from progressive reduction in visual acuity but unfortunately no further reliable data concerning these patients could be acquired. Her corrected visual acuity was 6/6 in each eye. Anterior and posterior segments were normal, and the electroretinogram showed normal cone and rod response

Patient III-1, a 16 year old male, the son of patient II-1, was generally healthy and had no ocular problems, until the age of 15 years, when he noticed reduced night vision and was referred to an ophthalmologist. His best corrected visual acuity was 6/21 in the right eye and 6/9 in the left eye. The anterior segments and intraocular pressures were normal. Ophthalmoscopy and fluorescein angiography revealed a similar picture in both eyes, consisting of bull's eye maculopathy, blots of pigment in the posterior pole and in the mid-periphery, many small retinal scars, and arteriolar narrowing. The optic discs were normal (Fig 2). Goldmann visual perimetry showed central and paracentral scotomas in the visual field of the right eye, and mild peripheral constriction in the left eye. The ERG showed markedly decreased amplitudes and prolonged implicit time of the photopic b wave and the flicker response, with a significant, but relatively smaller, decrease in amplitude and prolonged implicit time of the scotopic b wave (Fig 3).

Patient III-2, a 12 year old female, is the sister of patient III-1. She presented in early childhood with episodic ataxia and mild mental retardation. A metabolic evaluation including blood lactate, ammonia, amino acids and carnitine, and urine organic acids was normal. She had no ocular complaints and was examined by an ophthalmologist because of the family history. Her best corrected visual acuity was 6/9 in each eye, and the anterior segments and intraocular pressures were normal. The fundus manifested a typical bull's eye maculopathy with temporal optic disc pallor in both eyes, but without any pigmentation or scars. The electroretinogram showed reduction in amplitude and prolonged implicit time of the photopic b wave, while the scotopic a and b wave amplitudes and implicit time were

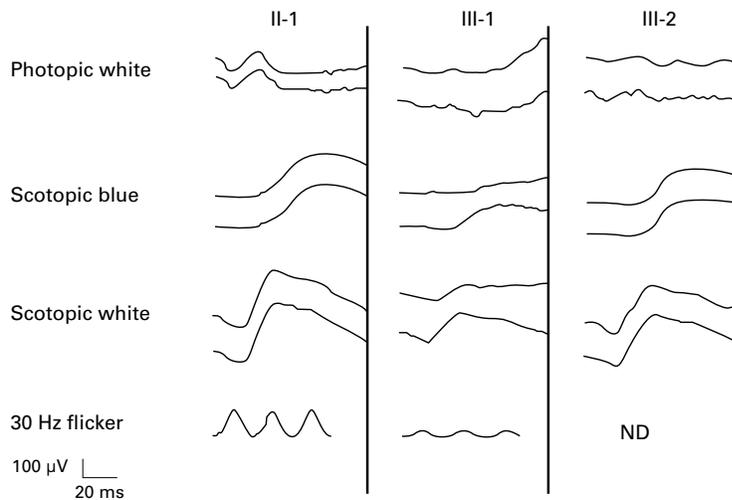


Figure 3 Electrorretinogram results of patients II-1, III-1, and III-2. After photopic white stimulus patient II-1 showed normal amplitude and implicit time of the a and b wave, while patients III-1 and III-2 showed decreased amplitude and prolonged implicit time. After dark adaptation, bright white light and blue light evoked normal responses from patients II-1 and III-2, but in patient III-1 the a and b wave had reduced amplitude and prolonged implicit time, more in the right eye. The 30 Hz flicker test evoked normal response in patient II-1; however, patient III-1 had reduced amplitudes and prolonged implicit time. Patient III-2 could not be examined by the flicker test due to poor patient cooperation (ND = not done).

normal (Fig 3). Goldmann perimetry and the flicker response could not be obtained because of poor patient cooperation.

Lymphocytes from blood samples of the three patients were tested for the mtDNA 8993 point mutation. In patients II-1, III-1, and III-2, 17%, 80%, and 85%, respectively, of the lymphocyte mtDNA showed the 8993 point mutation.

Discussion

A T to G point mutation in nucleotide 8993 of the mtDNA causes a substitution of a highly conserved leucine by an arginine residue in the ATPase 6 protein.¹⁰ This is a heteroplasmic mutation, meaning that both normal and mutant mtDNA are found in the same subject, but the proportion of the mutant mtDNA is different among patients. Usually, a high proportion of mutant mtDNA is associated with severe clinical manifestations.^{2 14 16} Two clinical syndromes were described in association with the 8993 mtDNA mutation. The first, subacute necrotising encephalomyopathy or Leigh syndrome, is a severe disease, with patients showing developmental delay, ataxia, psychomotor regression, seizures, peripheral neuropathy, and optic atrophy. Onset is usually during infancy with progression to death within months to years. The 8993 mutation was found to be a common cause of Leigh syndrome and most of the patients had more than 90% of mutant mtDNA.^{2 3} The second entity, NARP syndrome, was first reported by Holt *et al* in 1990¹⁰ and is usually milder than Leigh syndrome. Patients show a variety of clinical manifestations such as migraine, sensory neuropathy, proximal muscle weakness, ataxia, seizures, dementia, and pigmentary retinopathy.¹⁰⁻¹⁶ Patients with the NARP syndrome usually carry around 80% of mutant mtDNA. At levels below 75% carriers may either show pigmentary retinopathy, suffer from migraines, or be totally asymptomatic.^{14 16}

The ocular abnormalities in the NARP syndrome were reported in most cases as typical RP or as pigmentary retinopathy.^{10 13 14 16} However, in the ophthalmic literature we found only two reports. In the first, Puddu *et al* reported three patients with the NARP syndrome, two of whom had ocular manifestations. The fundus examination showed bone spicule pigmentation in the mid-periphery and in spite of being described as RP, they had a normal ERG except for subnormal photopic responses.¹⁵ In the second report, Ortiz *et al* described eight patients from two families with the 8993 mutation,¹¹ the ocular manifestations ranged from a mild salt and pepper retinopathy to severe RP-like changes with maculopathy. Four of these patients underwent ERG examination. One was normal and three revealed a rod-cone type of dysfunction. Perimetry was performed in five patients; four of them had normal visual fields and one had a paracentral scotoma.

We describe three members of a family who carry the mtDNA 8993 mutation and show a great variability in the clinical manifestations of the disease. Two of the three had ocular abnor-

malities that are different from classic RP. The clinical findings and the results of visual fields and ERG in patient III-1 are typical of cone-rod dystrophy.¹⁸ Patient III-2 shows findings typical of progressive cone dystrophy.¹⁹ Cone-rod dystrophy and cone dystrophy have not been described before in NARP or in Leigh syndromes. However, one previous report indicated that cone function is a more sensitive factor than rod function for assessing early retinal changes in patients with mitochondrial myopathy.⁷ Bull's eye maculopathy was reported previously in one patient with NARP and a rod-cone type of retinal dystrophy.¹¹ We found bull's eye maculopathy in two of our patients, both with a retinal dystrophy that affects primarily the cones. Bull's eye maculopathy is a typical manifestation of cone dystrophy with or without rod dystrophy.¹⁹

How can the great variability in the ocular and neurological manifestations found in patients with the mtDNA 8993 mutation be explained? A high proportion of mutant mtDNA in blood lymphocytes is associated with severe clinical manifestations. However, this is only a rough guide. Patient III-1 had a lower percentage of mutant mtDNA in the blood lymphocytes, a more severe ocular disease, but no neurological involvement in contrast with patient III-2. Similar findings were also reported in previous studies. The proportion of mutant mtDNA in the retina can differ from that in the blood or other tissues, especially the central nervous system, but such differences have not yet been demonstrated.¹⁴ It is possible that the ocular findings reflect different stages in the development of the same disease that may progress to typical retinitis pigmentosa. However, Ortiz *et al*¹¹ reported two patients, aged 12 and 14 years, who suffered from NARP syndrome and had a rod-cone type of ERG dysfunction. The fundus findings of these patients were much less advanced than in the patients presented here, who are of a similar age but with a predominant cone dysfunction. These differences show that patients with the NARP syndrome may manifest either rod or cone dominant retinal dystrophy at a similar age. It is also possible that other factors, not yet understood, modulate the phenotypic expression of the 8993 mtDNA mutation. This is conceivable since in two of the three reported NARP pedigrees that were studied by an ERG, all patients had a predominantly cone dysfunction, while in the third pedigree all had predominantly rod dysfunction. This finding cannot be explained by the differences in age, stages of the disease, or percentage of mutant mtDNA among families.

This study emphasises the great variability in the clinical expression of the NARP syndrome. It also indicates that, for reasons that are not yet understood, the dominant ocular manifestation of the NARP syndrome may be either a rod or a cone dysfunction in different families.

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