

A novel mutation (Cys83Tyr) in the second zinc finger of *NR2E3* in enhanced S-cone syndrome

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Abstract

Background Enhanced S-cone syndrome (ESCS) is an autosomal recessive retinal disorder characterized by an increased number of S-cones over L/M cones and rods. Mutations in the *NR2E3* gene, encoding a photoreceptor-specific nuclear receptor, are identified in patients with ESCS. The purpose of this study is to report the ophthalmic features of a 25-year-old Portuguese male with a typical ESCS phenotype and a novel homozygous *NR2E3* mutation.

Methods The patient underwent a detailed ophthalmic examination including fundus photography, fluorescein angiography (FAF), fundus autofluorescence imaging (FAI), and spectral domain optical coherence tomography (SD-OCT). Full-field electroretinography (ERG), S-cone ERG, and multifocal ERG were performed. Mutation screening of the *NR2E3* gene was performed with polymerase chain reaction amplification and direct sequencing.

Results The patient had poor visual acuity but good color vision. Funduscopy showed degenerative changes from the vascular arcades to the midperipheral retina. The SD-OCT revealed macular schisis and cystoid changes that had no fluorescein leakage. The posterior pole showed diffusely increased autofluorescence compared with eccentric areas in both eyes. International-standard full-field ERG showed the typical pathognomonic changes associated with ESCS and the short-wavelength flash ERG was simplified, delayed, and similar to the standard photopic flash ERG. Multifocal ERG showed widespread delay and reduction. Genetic analysis revealed a novel homozygous mutation (p.C83Y), which resides in the second zinc finger of the DNA-binding domain.

Conclusions This homozygous mutation is likely to affect binding to target DNA sites, resulting in a non-functional behavior of NR2E3 protein. It is associated

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with a typical form of ESCS with a nondetectable rod response and reduced/delayed mfERG responses at all eccentricities.

Keywords Enhanced S-cone syndrome, NR2E3 · S-cone · Retina · ERG

Introduction

Most retinal dystrophies are the result of either a generalized rod dysfunction, a generalized cone dysfunction (involving all three cone subtypes), or a generalized dysfunction of both rods and cones either simultaneously or successively. In 1990, Marmor [1] described a series of eight patients with night blindness, cystoid maculopathy, degenerative changes in the region of the vascular arcades, and loss of visual field. These patients showed S-cone hypersensitivity in electrophysiological testing; this condition was designated enhanced S-cone syndrome (ESCS). The ESCS has an autosomal recessive inheritance pattern and is characterized by hypersensitivity to short wavelength flashes with a concomitant decreased rod and L and M cone response [1–3].

Over ten mutations of the *NR2E3* gene have been identified in European patients with ESCS [4]. This gene encodes a photoreceptor-specific nuclear receptor. It consists of eight exons mapped on chromosome 15q24 [5]. Functionally, *NR2E3* protein regulates the proper differentiation and maturation of rod and cone photoreceptors [6–10]. To date, more than 30 mutations in the *NR2E3* gene have been described not only in ESCS [4, 11–15] but also in Goldman–Favre syndrome (GFS) [11, 16], autosomal recessive retinitis pigmentosa [17, 18], autosomal dominant retinitis pigmentosa [17, 19], and clumped pigmentary retinal degeneration [11]. Because appropriate ERG analyses demonstrated a relatively enhanced S-cone function and the presence of *NR2E3* mutations in GFS, it was concluded that the two diseases are likely to be one clinical entity, being the GFS the severe phenotype [11, 16, 20].

Although characteristic electroretinographic responses are usually essential for diagnosis, there is a variable spectrum of disease severity in patients with ESCS. We describe the clinical findings of a Portuguese patient with ESCS with a novel homozygous mutation in the *NR2E3* gene and exhibited a typical phenotype.

Materials and methods

Clinical studies

Ophthalmic examination included best-corrected visual acuity (BCVA), slit-lamp and dilated fundus observation,

Goldmann kinetic perimetry, chromatic vision testing, fluorescein angiogram (FAF), fundus autofluorescence imaging (FAI), spectral domain optical coherence tomography (SD-OCT), contrast sensitivity, dark adaptometry, and electrophysiological testing. Color vision testing included color contrast sensitivity threshold and the Farnsworth 100-hue test. The color contrast sensitivity thresholds were measured as proposed by Arden et al. (1988). In brief, the system uses a calibrated 21-inch color monitor to present random letters as targets to be identified. The letters are of equal luminance to the background, and can only be recognized because their hue differs from the background. All stimuli are equiluminant with the background. This is ensured by a preliminary adjustment (in every patient) of the relative luminance of the red and green and green and blue phosphors. Then a modified binary search is carried out to determine the threshold color contrast along the protan, deuteran, and tritan color confusion lines [21]. All these color vision tests were performed monocularly. Cross-sectional retinal images were evaluated using SD-OCT (SPECTRALIS Spectral-domain OCT, Heidelberg Engineering, Heidelberg, Germany). The SD-OCT was taken horizontally through the fovea (transverse width of 20°). Dark adaptometry was performed based on the Goldman–Wecker adaptometer. Briefly, the patient was pre-adapted under photopic conditions (30 cd/m²) for 5 min. The dark adaptation characteristic was then assessed by measuring detection thresholds under scotopic conditions over a 30-min period.

Electrophysiological evaluation included full-field electroretinograms (ERG) using a Ganzfeld dome, long duration ON-OFF ERG, S-cone ERG, Electro-oculogram (EOG), and multifocal ERG (mf ERG). The ERG testing was performed according to the protocol of the International Society for Clinical Electrophysiology of Vision [22]. Briefly, under dilation and after dark adaptation (30 min), a dim white flash of 0.01–0.05 cd·s/m² was used for the scotopic (rod) response and a single white bright-flash (3 cd·s/m²) for the combined response. After light adaptation (10 min; 25 cd/m²), a brief white flash (3 cd·s/m²) was superimposed for the photopic response. The 30-Hz ERG was obtained in the same conditions using a 30-Hz flickering stimulation. The S-cone ERG was performed, under photopic conditions, using a blue stimulus (440 nm; 10 ms; 65 cd/m²) on an orange (660 nm; 350 cd/m²) background [23, 24]. For the L/M cones ON-OFF ERG, the 200-ms orange (660 nm; 350 cd/m²) stimulus was used on a green (530 nm; 130 cd/m²) background [25]. The EOG was obtained after a room light pre-adaptation period of 10 min [26]. Acquisitions were made after 15 min of dark adaptation and repeated after 15 min of light adaptation. The mfERG was acquired using an array of 61 stimulus (200 cd/m²) according to the recommendations of ISCEV [27]. All the electrophysiological exams were performed using the Metrovision

vision monitor (Pérenchies, France). For the ON-OFF ERG and the S-cone ERG, an additional external led stimulator was used (CH electronics).

Molecular genetic studies

The protocol adhered to the Declaration of Helsinki, and informed consent was obtained from the participant. Genetic analysis was performed at the Department of Ophthalmology at The Jikei University as previously described [12, 15]. Briefly, genomic DNA was extracted from venous blood samples using a Puregene Blood DNA Isolation kit (Gentra Systems, Minneapolis, MN). For mutation screening, all exons (exon 1 to exon 8) and the promoter region of the *NR2E3* gene were amplified by polymerase chain reaction (PCR) using previously reported primers [12, 15]. The PCR products were purified with a QIAquick PCR Purification kit (Qiagen, Tokyo, Japan) and

used as the template for sequencing. Both strands were sequenced on an automated sequencer (3730xl DNA Analyzer, Applied Biosystems, Foster City, CA) using a BigDye Terminator kit V3.1 (Applied Biosystems). A nucleotide variation in exon 3 was analyzed in 100 normal controls without any retinal diseases.

Results

Clinical findings

A 25-year-old male patient was referred to our hospital with suspected X-linked juvenile retinoschisis. The patient was one of the three children from a consanguineous couple (second-degree cousins) and was the only family member with visual symptoms. His chief complaint was of decreased vision but, even after inquiry, he denied nyctalopia. His BCVA was 0.5 in

Fig. 1 Fundus photographs and fluorescein angiograms (FAF) images. Color fundus montages of the right eye (a) and left eye (b) show degenerative lesions in the midzonal retinal areas, near the arcades with nummular pigmentary changes at the RPE level. Absence of foveal reflex and the presence of schisis-like changes in both maculae are also observed. Midphase fluorescein angiograms of the right eye (c) and left eye (d) show no leakage in the maculas. The fundus autofluorescence images (e and f) show a diffusely increased auto fluorescence (AF) associated with the arcades with some sparing of central and inferior macular area. The SD-OCT images show the presence of macular schisis with cystoid changes in the right (g) and left maculas (h) in the inner and outer nuclear layers

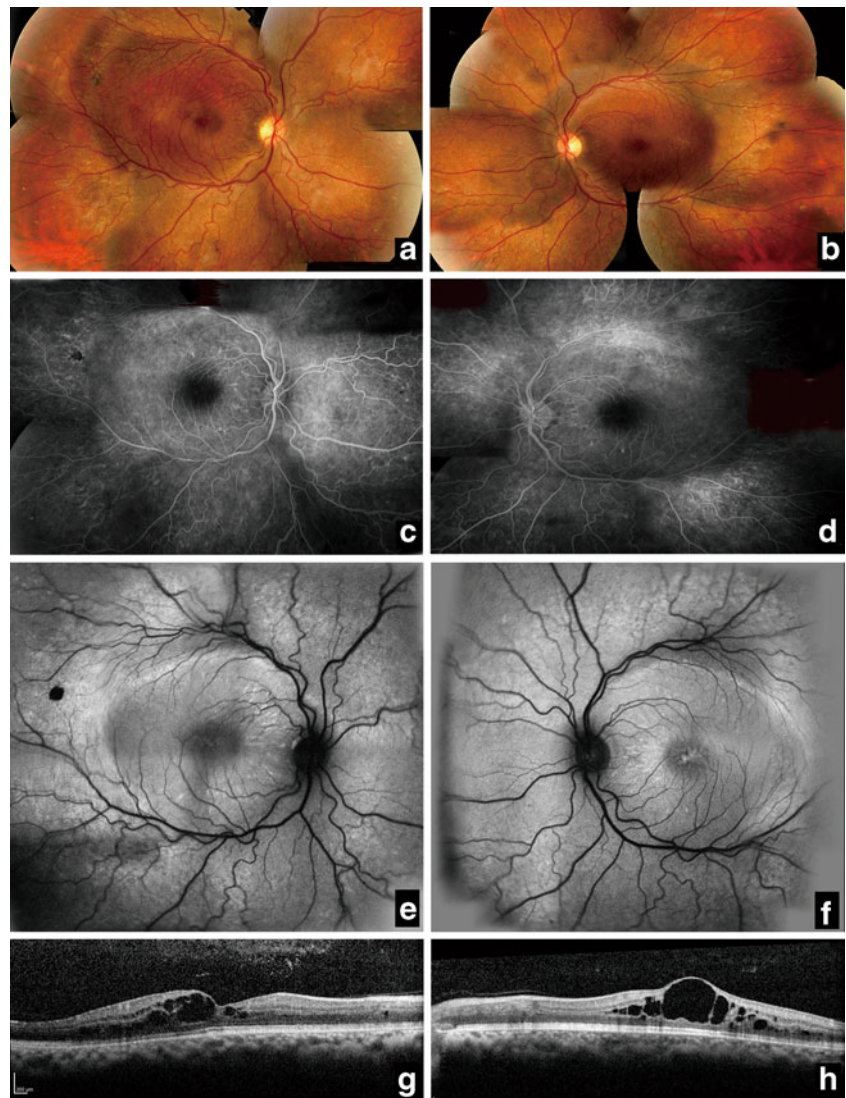
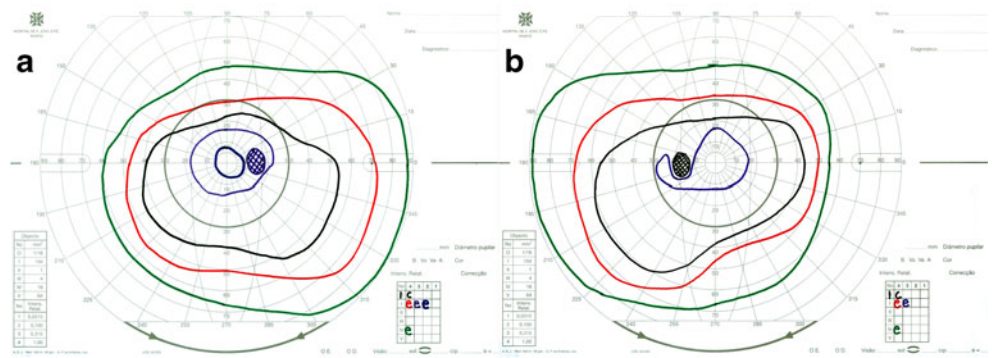


Fig. 2 Goldmann perimetry shows decreased central sensitivity with constriction of the I/2e and/or I/3e isopters (**a** I/2e: 10° of fixation in the right and **b** I/3e: 20° of fixation in the left), but normal peripheral visual fields



the right eye and 0.25 in the left eye. The anterior segment examination was unremarkable. Intraocular pressure was within the normal range. Fundoscopy showed bilateral foveal schisis-like changes (Fig. 1a, b). In addition, nummular pigmentary deposits were observed at the RPE level in the mid-periphery and along the vascular arcades, without vascular attenuation. FAF did not reveal any hyperfluorescence or leakage at the macular lesions (Fig. 1c, d). FAI showed diffusely increased AF over broad, crescent-like areas associated with the vascular arcades and optic disc in both eyes. More eccentric areas were hypofluorescent in comparison. Parafoveal hyperfluorescence was greater in the left eye compared to the right eye (Fig. 1e, f). The SD-OCT images confirmed the presence of macular schisis with cystoid changes in both maculae (Fig. 1g, h).

Functional evaluation

Color vision testing did not demonstrate a preferred axis of chromatic confusion, with 100 and 186 error scores in the right and left eye respectively on the Farnsworth 100-hue. The CCS thresholds were within normal limits. Goldmann perimetry revealed bilaterally decreased central sensitivity with constriction of the I/2e and/or I/3e isopters (I/3e: 20° of fixation in the left and I/2e: 10° of fixation in the right). However, there was no constriction of the visual fields (Fig. 2). The dark adaptometry curve showed a monophasic pattern with elevated cone threshold without the characteristic decrease that is attributed to rod function in normal individuals (Fig. 3).

Electrodiagnostic tests revealed characteristic patterns for ESCS. Scotopic dim flash rod ERG were undetectable. The waveforms of combined (rod-plus-cone) responses were similar to those of the photopic responses. Flicker ERGs of 30-Hz were smaller than the single flash cone a-waves (Fig. 4). The ERG results of specific chromatic stimulation showed that most of the ERG responses were arising from S-cone systems. S-cone ERG were delayed, simplified, and resemble the single flash cone ERG (Fig. 5). The ON-OFF responses with 200-ms orange stimulation, which are originated from the L/M cones [23], were markedly decreased in the patient, indicating minimal

responses to L/M cone stimulation (Fig. 5). The OFF L/M cones response was absent.

The EOG light rise was detected, with a reduction on the Arden index (1.56 and 1.65, for a normal value of 1.85).

Comparing the mf ERG to the mf ERG of normal subjects, reduced amplitudes and delayed implicit times, of both N1 and P1 components, were observed at all eccentricities and at all retinal locations (Fig. 6).

Molecular genetic findings

The genetic analysis identified a novel homozygous mutation in exon 3. This mutation leads to the substitution of an adenine for a guanine base (c.248G>A). At the proteic level, this causes the substitution of a tyrosine for a cysteine at position 83 on the highly conserved DNA-binding

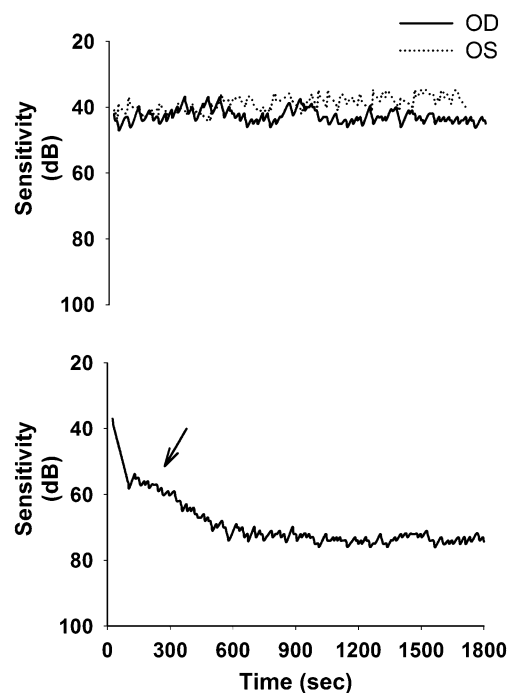


Fig. 3 Dark adaptometry curve of the patient (*upper panel*) shows a monophasic pattern without the characteristic decline attributed to rod function (*arrow*) that is seen in normal individuals (*lower panel*)

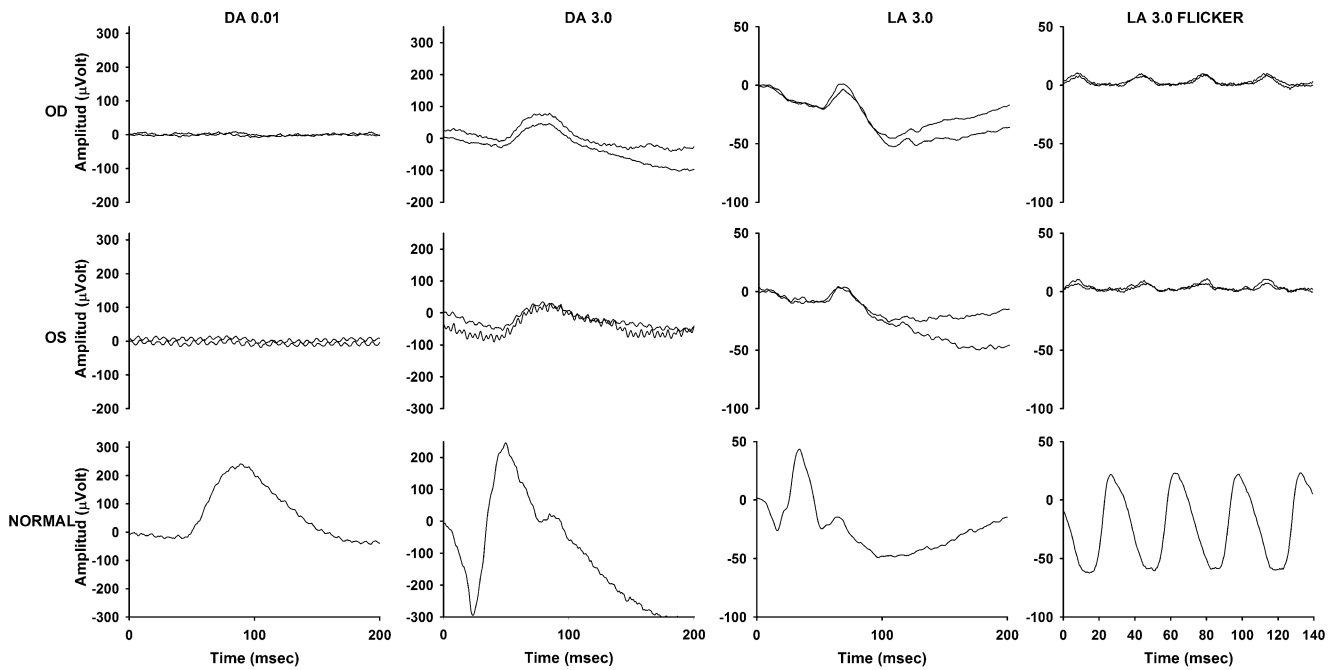


Fig. 4 Full-field electroretinograms (ERGs) of the patient and a normal subject. In the patient, there is no scotopic response (DA 0.01); the waveforms of the combined (DA 3.0) and photopic (LA 3.0) ERGs are very similar. The photopic ERG a- and b-waves are delayed

almost 20 ms when compared to the normal subject. Delayed 30-Hz flicker ERG (LA 3.0 flicker), with lower amplitude than the single-flash photopic a-wave are also detected

domain (DBD), which consists of 84 amino acids (cysteine 47 to valine 130) (Fig. 7). No other nucleotide substitution was detected in the patient. The cysteine residue at position 83 (Cys83) within the second zinc finger motif of the DBD (arrow) is conserved among orthologs of other vertebrate species (Fig. 7), predicting a functionally important amino acid residue.

The patient's parents were heterozygotes for the mutation (p.C83Y). This mutation was not found in the 100 normal controls or in database searches of PubMed, The Human Gene Mutation Database (URL: <http://www.hgmd.cf.ac.uk/>), and of the Leiden Open Variation Database (<http://www.lovd.nl/>) [20].

Discussion

In this study, we describe the clinical, electrophysiological, and psychophysical findings of a 25-year-old male patient who was diagnosed with ESCS. Genetic analysis revealed a novel homozygous *NR2E3* mutation (p.C83Y). This is the first report of an ESCS patient with any *NR2E3* mutation in the Portuguese population.

The DBD of *NR2E3* is composed of highly conserved two zinc finger motifs that facilitate binding to target DNA sites [19]. Four cysteine residues are coordinated with one zinc atom in each zinc finger. Cys83 is the first cysteine residue of the second zinc finger (Cys83 to Cys103) [19].

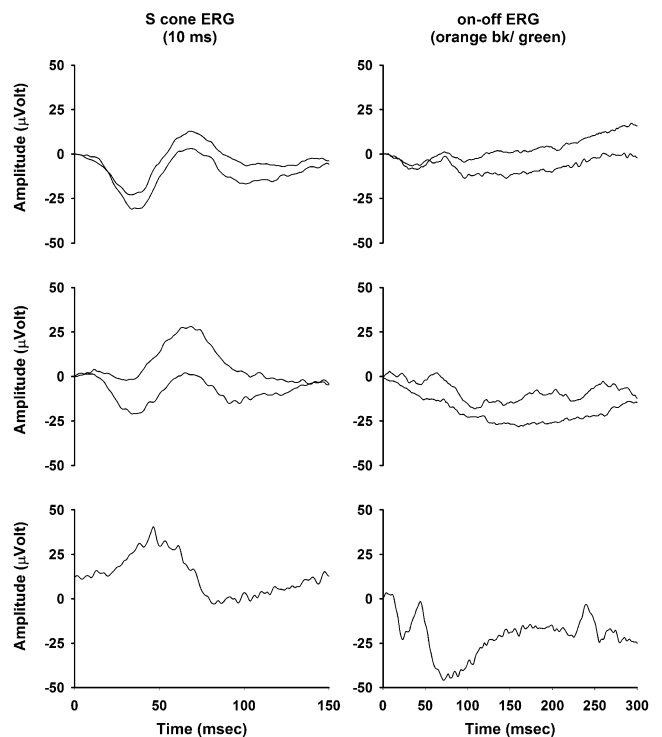


Fig. 5 Electroretinograms of specific chromatic stimulation. S-cone ERGs were delayed, simplified, and resemble the single flash cone ERGs. The ON-OFF responses with 200-ms orange stimulation are markedly decreased. The S-cone ERGs waveforms were not superimposable due to limited patient compliance

Fig. 6 Multifocal ERG. The analysis of the central response and four concentric rings are shown in **a**. Amplitudes and implicit time are compared with a database of normal subjects in **b**. There is an increase in the latency and a decrease in the amplitude of the N1 and P1 components in all the analyzed responses, even in the most central (Z1)

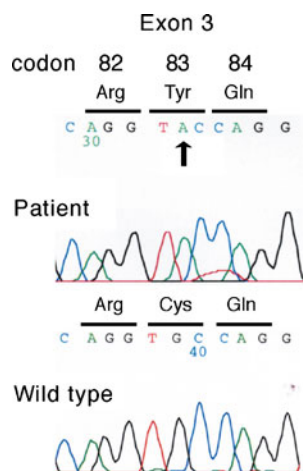
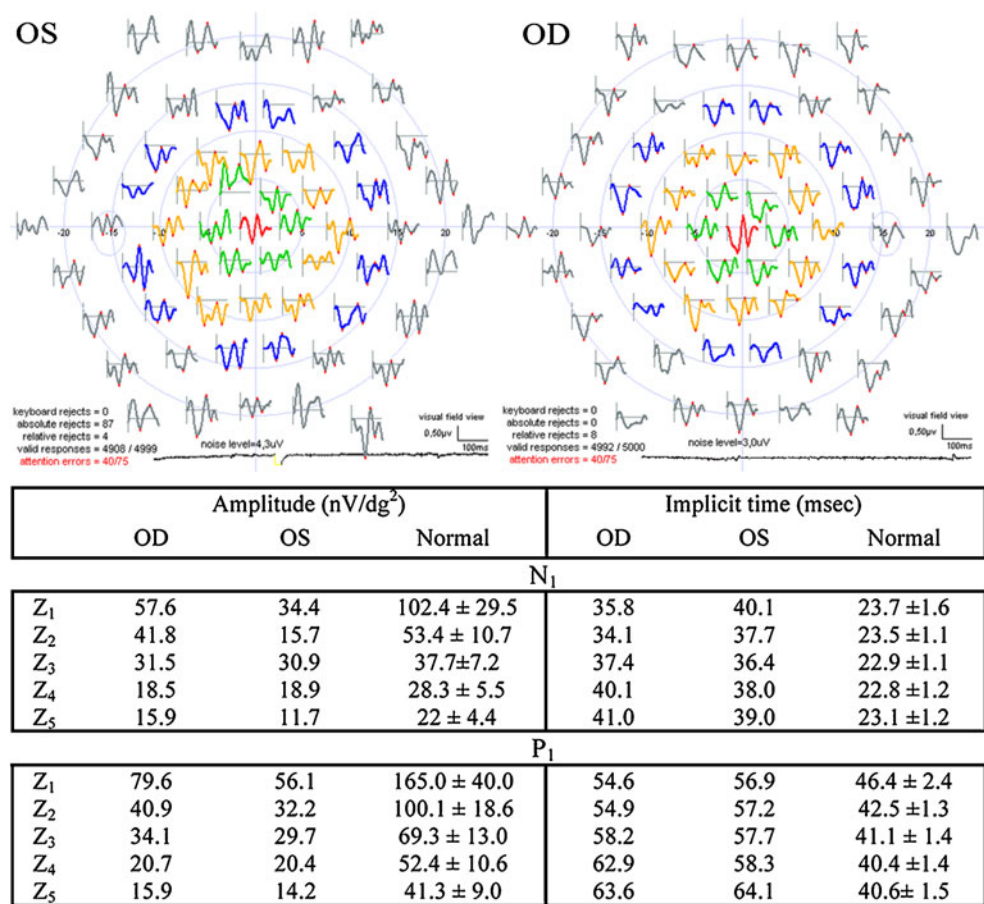


Fig. 7 **a** Partial nucleotide sequences of exon 3 in a wild-type and in the patient. A homozygous variation c.248G>A (exon 3) is shown in the patient (arrow), resulting in a new homozygous missense mutation (Cys83Tyr) in the DNA-binding domain of NR2E3 protein. **b** Amino acid alignment of the DNA binding domain of the human NR2E3 protein. The cysteine residue at position 83 within the second zinc finger motif (arrow) is highly conserved among orthologs of human NR2E3. hs: *Homo sapiens* (human); mm: *Mus musculus* (mouse); gg: *Gallus gallus* (chicken); xt: *Xenopus tropicalis* (xenopus); dr: *Danio rerio* (zebrafish)

The D-box, a short loop with six amino acid residues between the first cysteine (Cys83) and second cysteine (Cys90) of the second zinc finger [5], is important for homodimerization of NR2E3 to be functionally active, suggesting that Cys83 is an important amino acid. In vitro experiments using electrophoretic mobility shift assay showed that varied NR2E3 proteins with mutations in the zinc finger motifs exhibited reduced binding to the target DNA sites [28, 29]. Those NR2E3 mutant proteins, which were localized at least partially in cell nuclei, exhibited reduced transcriptional activity of the target gene [28, 29] and impaired dimerization [29] using cultured cells. The p. C83Y mutated NR2E3 protein is not able to dimerize to become active. This results in disruption of the target DNA binding sites leading to a null function of the DBD. It is expected that the homozygous p.C83Y mutation causes severe non-functional behavior of NR2E3 protein.

ESCS shares several clinical features with GFS that is characterized by night blindness, pigmentary degeneration, macular and peripheral retinoschisis, posterior subcapsular cataract, markedly abnormal ERG and degenerative vitreous changes [30, 31]. Jacobson et al. demonstrated enhanced S-cone responses in patients with GFS [3], while NR2E3 mutations have been also found in GFS patients. Based on these findings, it was concluded that these two diseases are

likely to be one clinical entity [11], with two identifiable phenotypes in a wide-range spectrum of clinical expression of the same retinal degeneration [16, 20]. However, the absence of peripheral retinoschisis, posterior subcapsular cataract, and degenerative vitreous changes differentiates ESCS patients from patients with GFS phenotype. Our patient did not exhibit peripheral retinoschisis, cataract, or degenerative vitreous changes. The SD-OCT images revealed macular schisis with cystoid changes (Fig. 1), which are frequently seen in both ESCS and GFS [11, 16, 24] but are different from cystoid macular edema secondary to other conditions such as diabetic retinopathy and retinal vein occlusion that are characterized by the presence of leakage on fluorescein angiography.

The main ERG characteristics in our patient included the pathognomonic changes previously described in [24]. This included an absent rod ERG consistent with absence of rods. There was a simplified and delayed waveform to a standard flash under photopic and scotopic conditions, presumably both dominated by short-wavelength sensitive cones. The 30-Hz flicker ERG was severely abnormal and delayed and was smaller than the single flash cone ERG a-wave; these distinctive findings may be explained by considering the low temporal resolution of the S-cone system.

These results are in accordance with the histopathological studies that have demonstrated the absence of rods and the predominance of S-cone opsin over L/M cones in postmortem retinas of ESCS patients [32, 33]. Mild phenotypes of ESCS associated with compound heterozygous mutations have been found to have a residual rod response or a morphologically normal waveform of the combined ERG, either alone or together [15, 34]. Regarding the mfERG findings, Marmor et al. [35] previously described a normal waveform in the most central ring and a marked deterioration in the two paracentral rings in a patient with ESCS. Subsequently, similar mfERG findings have been described in five patients with ESCS, at least two of which had macular cysts [24]. Thus most of the patients had nearly normal central response [24, 35], suggesting preserved function of the most central macula retina in ESCS. However, our patient showed reduced amplitudes with delayed implicit times in both N1 and P1 components even in the most central ring (Z1) of both eyes (Fig. 6). The disorganization of laminar structure, namely macular schisis with cystoid changes (Fig. 1) can explain the reduced amplitudes of all the ring of the mfERG (Fig. 6). The condition of our patient with the homozygous p.C83Y mutation, causing expression of the putative non-functional NR2E3 protein, may be associated with a severer phenotype of ESCS that has reduced central mfERG responses compared with that of ESCS with preserved central responses.

In summary, we reported the first Portuguese ESCS patient with a novel homozygous mutation (p.C83Y) in the *NR2E3* gene. The mutation, which resides within the

second zinc finger of the DBD, may cause a typical form of ESCS with nondetectable rod responses and reduced mfERG amplitudes in all eccentricities.

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References

- Marmor MF, Jacobson SG, Foerster MH, Kellner U, Weleber RG (1990) Diagnostic clinical findings of a new syndrome with night blindness, maculopathy, and enhanced S-cone sensitivity. *Am J Ophthalmol* 110:124–134
- Jacobson SG, Marmor MF, Kemp CM, Knighton RW (1990) SWS (blue) cone hypersensitivity in a newly identified retinal degeneration. *Invest Ophthalmol Vis Sci* 31:827–838
- Jacobson SG, Roman AJ, Roman MI, Gass JD, Parker JA (1991) Relatively enhanced S-cone function in the Goldmann-Favre syndrome. *Am J Ophthalmol* 111:446–453
- Haider NB, Jacobson SG, Cideciyan AV, Swiderski R, Streb LM, Searby C, Beck G, Hockey R, Hanna DB, Gorman S, Duhl D, Carmi R, Bennett J, Weleber RG, Fishman GA, Wright AF, Stone EM, Sheffield VC (2000) Mutation of a nuclear receptor gene, *NR2E3*, causes enhanced S-cone syndrome, a disorder of retinal cell fate. *Nat Genet* 24:127–131
- Kobayashi M, Takezawa S, Hara K, Yu RT, Umesono Y, Agata K, Taniwaki M, Yasuda K, Umesono K (1999) Identification of a photoreceptor cell-specific nuclear receptor. *Proc Natl Acad Sci USA* 96:4814–4819
- Haider NB, Naggert JK, Nishina PM (2001) Excess cone cell proliferation due to lack of a functional *NR2E3* causes retinal dysplasia and degeneration in *rd7/rd7* mice. *Hum Mol Genet* 10:1619–1626
- Cheng H, Khanna H, Oh EC, Hicks D, Mitton KP, Swaroop A (2004) Photoreceptor-specific nuclear receptor *NR2E3* functions as a transcriptional activator in rod photoreceptors. *Hum Mol Genet* 13:1563–1575. doi:10.1093/hmg/ddh173
- Peng GH, Ahmad O, Ahmad F, Liu J, Chen S (2005) The photoreceptor-specific nuclear receptor *Nr2e3* interacts with *Crx* and exerts opposing effects on the transcription of rod versus cone genes. *Hum Mol Genet* 14:747–764
- Cheng H, Aleman TS, Cideciyan AV, Khanna R, Jacobson SG, Swaroop A (2006) In vivo function of the orphan nuclear receptor *NR2E3* in establishing photoreceptor identity during mammalian retinal development. *Hum Mol Genet* 15:2588–2602. doi:10.1093/hmg/ddl185
- Haider NB, Demarco P, Nystuen AM, Huang X, Smith RS, McCall MA, Naggert JK, Nishina PM (2006) The transcription factor *Nr2e3* functions in retinal progenitors to suppress cone cell generation. *Vis Neurosci* 23:917–929
- Sharon D, Sandberg MA, Caruso RC, Berson EL, Dryja TP (2003) Shared mutations in *NR2E3* in enhanced S-cone syndrome, Goldmann-Favre syndrome, and many cases of clumped pigmentary retinal degeneration. *Arch Ophthalmol* 121:1316–1323
- Nakamura Y, Hayashi T, Kozaki K, Kubo A, Omoto S, Watanabe A, Toda K, Takeuchi T, Gekka T, Kitahara K (2004) Enhanced S-cone syndrome in a Japanese family with a nonsense *NR2E3* mutation (Q350X). *Acta Ophthalmol Scand* 82:616–622
- Nakamura M, Hotta Y, Piao CH, Kondo M, Terasaki H, Miyake Y (2002) Enhanced S-cone syndrome with subfoveal neovascularization. *Am J Ophthalmol* 133:575–577

14. Wright AF, Reddick AC, Schwartz SB, Ferguson JS, Aleman TS, Kellner U, Jurklics B, Schuster A, Zrenner E, Wissinger B, Lennon A, Shu X, Cideciyan AV, Stone EM, Jacobson SG, Swaroop A (2004) Mutation analysis of NR2E3 and NRL genes in enhanced S-cone syndrome. *Hum Mutat* 24:439
15. Hayashi T, Gekka T, Goto-Omoto S, Takeuchi T, Kubo A, Kitahara K (2005) Novel NR2E3 mutations (R104Q, R334G) associated with a mild form of enhanced S-cone syndrome demonstrate compound heterozygosity. *Ophthalmology* 112:2115
16. Pachydaki SI, Klaver CC, Barbazetto IA, Roy MS, Gouras P, Allikmets R, Yannuzzi LA (2009) Phenotypic features of patients with NR2E3 mutations. *Arch Ophthalmol* 127:71–75. doi:10.1001/archophthalmol.2008.534
17. Escher P, Gouras P, Roduit R, Tiab L, Bolay S, Delarive T, Chen S, Tsai CC, Hayashi M, Zernant J, Merriam JE, Mermod N, Allikmets R, Munier FL, Schorderet DF (2009) Mutations in NR2E3 can cause dominant or recessive retinal degenerations in the same family. *Hum Mutat* 30:342–351. doi:10.1002/humu.20858
18. Gerber S, Rozet JM, Takezawa SI, dos Santos LC, Lopes L, Gribouval O, Penet C, Perrault I, Ducrocq D, Souied E, Jeanpierre M, Romana S, Frezal J, Ferraz F, Yu-Umesono R, Munnich A, Kaplan J (2000) The photoreceptor cell-specific nuclear receptor gene (PNR) accounts for retinitis pigmentosa in the Crypto-Jews from Portugal (Marranos), survivors from the Spanish Inquisition. *Hum Genet* 107:276–284
19. Coppieters F, Leroy BP, Beysen D, Hellemans J, De Bosscher K, Haegeman G, Robberecht K, Wuyts W, Coucke PJ, De Baere E (2007) Recurrent mutation in the first zinc finger of the orphan nuclear receptor NR2E3 causes autosomal dominant retinitis pigmentosa. *Am J Hum Genet* 81:147–157. doi:10.1086/518426
20. Schorderet DF, Escher P (2009) NR2E3 mutations in enhanced S-cone sensitivity syndrome (ESCS), Goldmann–Favre syndrome (GFS), clumped pigmentary retinal degeneration (CPRD), and retinitis pigmentosa (RP). *Hum Mutat* 30:1475–1485. doi:10.1002/humu.21096
21. Arden G, Gunduz K, Perry S (1988) Color vision testing with a computer graphics system: preliminary results. *Doc Ophthalmol* 69:167–174
22. Marmor MF, Fulton AB, Holder GE, Miyake Y, Brigell M, Bach M (2009) ISCEV Standard for full-field clinical electroretinography (2008 update). *Doc Ophthalmol* 118:69–77. doi:10.1007/s10633-008-9155-4
23. Arden G, Wolf J, Berninger T, Hogg CR, Tzekov R, Holder GE (1999) S-cone ERGs elicited by a simple technique in normals and in tritanopes. *Vision Res* 39:641–650
24. Audo I, Michaelides M, Robson AG, Hawlina M, Vaclavik V, Sandbach JM, Neveu MM, Hogg CR, Hunt DM, Moore AT, Bird AC, Webster AR, Holder GE (2008) Phenotypic variation in enhanced S-cone syndrome. *Invest Ophthalmol Vis Sci* 49:2082–2093. doi:10.1167/iovs.05-1629
25. Khan NW, Jamison JA, Kemp JA, Sieving PA (2001) Analysis of photoreceptor function and inner retinal activity in juvenile X-linked retinoschisis. *Vision Res* 41:3931–3942
26. Brown M, Marmor M, Vaegan ZE, Brigell M, Bach M (2006) ISCEV Standard for Clinical Electro-oculography (EOG) 2006. *Doc Ophthalmol* 113:205–212. doi:10.1007/s10633-006-9030-0
27. Hood DC, Bach M, Brigell M, Keating D, Kondo M, Lyons JS, Palmowski-Wolfe AM (2008) ISCEV guidelines for clinical multifocal electroretinography (2007 edition). *Doc Ophthalmol* 116:1–11. doi:10.1007/s10633-007-9089-2
28. Kanda A, Swaroop A (2009) A comprehensive analysis of sequence variants and putative disease-causing mutations in photoreceptor-specific nuclear receptor NR2E3. *Mol Vis* 15:2174–2184
29. Roduit R, Escher P, Schorderet DF (2009) Mutations in the DNA-binding domain of NR2E3 affect in vivo dimerization and interaction with CRX. *PLoS One* 4:e7379. doi:10.1371/journal.pone.0007379
30. Peyman GA, Fishman GA, Sanders DR, Vlcek J (1977) Histopathology of Goldmann–Favre syndrome obtained by full-thickness eye-wall biopsy. *Ann Ophthalmol* 9:479–484
31. Nasr YG, Cherfan GM, Michels RG, Wilkinson CP (1990) Goldmann–Favre maculopathy. *Retina* 10:178–180
32. Ben-Arie-Weintrob Y, Berson EL, Dryja TP (2005) Histopathologic-genotypic correlations in retinitis pigmentosa and allied diseases. *Ophthalmic Genet* 26:91–100
33. Milam AH, Rose L, Cideciyan AV, Barakat MR, Tang WX, Gupta N, Aleman TS, Wright AF, Stone EM, Sheffield VC, Jacobson SG (2002) The nuclear receptor NR2E3 plays a role in human retinal photoreceptor differentiation and degeneration. *Proc Natl Acad Sci USA* 99:473–478
34. Lam BL, Goldberg JL, Hartley KL, Stone EM, Liu M (2007) Atypical mild enhanced S-cone syndrome with novel compound heterozygosity of the NR2E3 gene. *Am J Ophthalmol* 144:157–159. doi:10.1016/j.ajo.2007.03.012
35. Marmor MF, Tan F, Sutter EE, Bearn MA Jr (1999) Topography of cone electrophysiology in the enhanced S-cone syndrome. *Invest Ophthalmol Vis Sci* 40:1866–1873