REPORT

Clinical Findings in Members of a Czech Family with Retinitis Pigmentosa Caused by c.2426_2427delAG Mutation in RPGR

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SUMMARY

Purpose: To describe the phenotype of members of the first Czech retinitis pigmentosa family with an identified molecular genetic cause (c.2426_2427delAG in RPGR), followed for more than 13 years.
Methods: Medical records were reviewed and a detailed ophthalmic examination including spectral-domain optical coherence tomography and full-field and multifocal electroretinography (ERG) was performed in two affected males, three female carriers and one unaffected female.
Results: A 22-year-old male who denied suffering from nyctalopia had a best corrected visual acuity (BCVA) of 0.63 in both eyes. Moderate myopia and myopic astigmatism were present bilaterally. Color vision and contrast sensitivity were normal. There was an eccentric constriction of the visual fields that spared the central 20 degrees in both eyes. Fundus examination revealed bilateral pigmentary changes in the mid-periphery. Full-field ERG documented a 10% rod and 20% cone response. The phenotype of his cousin, also aged 22 years, was more severe. He complained of nyctalopia since 12 years of age. His BCVA was 0.3 in the right eye and 0.5 in the left eye. Myopia and astigmatism were present bilaterally. Contrast sensitivity and color vision were severely impaired. Full field ERG was extinct, but some activity on multifocal ERG was still detectable. The constriction of the visual fields reached 5 degrees in both eyes. Fundus examination showed the typical retinitis pigmentosa appearance. All carriers denied that they suffered from nyctalopia, but two of them had decreased BCVA in at least one eye. None exhibited typical bone spicules or a tapetal-like reflex. Significant refractive errors were present in all eyes of the carriers.
Conclusion: The finding of moderate or high myopia and astigmatism in males with retinitis pigmentosa as well as refractive errors in female relatives indicates possible X-linked inheritance, which may be especially important in pedigrees where the transmission pattern can not be clearly established. Our study highlights the inter-individual variability in phenotype observed in similar aged patients with identical ORF15 RPGR mutations.

Key words: retinitis pigmentosa, phenotype, multifocal ERG, mutation, RPGR

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INTRODUCTION

Retinitis pigmentosa (RP) is a genetically heterogeneous group of progressive degenerative retinal disorders, a common symptom of which is primary affliction of the rod cells. Nyctalopia is the first symptom, followed by loss of peripheral vision. In the later phases, withering of the cone cells also takes place, with an attendant reduction of central visual acuity. Concomitant loss of rod and cone cells may also occur, or only affliction of the cone cells, in the case of "cone-rod dystrophies", which are however detached as a separate clinical unit (20, 21). The disorder is characterised by a large variability of clinical findings, even amongst members of the same family. A typical symptom upon examination of the fundus is considered to be a finding of pigment clusters, reminiscent of bone cells (20, 19).
RP appears either as a separate disorder or as part of a range of syndromes. The prevalence of the most common non-syndrome RP is stated at 1/4000 (20).
With few exceptions, RP is a monogenic disorder, i.e. manifesting Mendelian inheritance. Autosomal recessive form of transmission is determined most frequently, in approximately 20-25% of cases. Autosomal dominant heredity is represented in 15-20% of cases. Less frequent is RP linked to the sex chromosome, which is determined in 10-15% of cases. Approximately 40-55% of all cases of RP are represented by sporadic patients, in whom there is no present family anamnesis, and in these persons heredity therefore cannot reliably be determined without knowledge of molecular genetic causes (8, 44).
To date at least 50 genes have been identified or evidence has been submitted for their existence, in which it is estimated that a further 40-50% of genes have not yet been discovered (6, 20) (http://www.sph.uth.tmc.edu/Retnet/).
At present two genes conditioning RP and bound to the sex chromosome are known. Most frequently, in up to 80% of cases of retinitis pigmentosa, mutation is determined in the gene for the GTPa-

se regulator (Retinitis Pigmentosa GTPase Regulator, RPGR, MIM *312610). Less often, in approximately 20% of cases, the disorder is conditioned by mutations in the gene retinitis pigmentosa 2 (RP2 MIM *300757). Evidence has also been submitted concerning the existence of at least three further loci, in which new causal genes for this type of RP are found, though their percentage representation is minimal (34).

RP bound to the sex chromosome is one of the most serious forms of human degenerative retinal disorders, both with regard to the age at which the first manifestations occur and to the speed of progression. Nyctalopia occurs in men frequently as early as in the first decade of life. In the second decade this is accompanied by narrowing of the visual field, followed by a reduction of central visual acuity in the third and fourth decades of life. The disorder is characterised by the presence of pigment deposits, which are reminiscent in appearance to bone cells, as well as narrowing of the retinal arterioles, atrophy of the disc of the optic nerve, posterior subcapsular opacity of the lens and undetected or reduced amplitude upon electroretinographic (ERG) examination (20, 40).

Female carriers may be entirely without clinical symptoms of the disorder, but mostly manifest slight changes on the fundus and a slight reduction of the function of the retina. Myopia and astigmatism is often detected, as well as a slight deterioration of visual acuity, a small quantity of pigment deposits in the periphery of the retina or the appearance of the fundus indicated as a tapetal (metallic) reflex, which is considered a characteristic finding (13, 14, 23).

In Czech patients, a molecular genetic cause of RP has been determined to date in one family, in which causal mutation c.2426_2427delAG was detected in exon ORF15 of gene RPGR (30). Our aim is to describe in detail the clinical findings in the members of this family, including the results of an examination obtained using contemporary illustrative and electro-physical methods, and to demonstrate certain specific phenotype characteristics determined in connection with RP bound to the sex chromosome, conditioned by mutations in the RPGR gene.

METHODS

We examined 6 members of a family with RP bound to the sex chromosome (fig. 1), in which we had previously demonstrated causal mutation c.2426_2427delAG in exon ORF15 of the RPGR gene. Specifically this concerned two afflicted males, two obligatory female carriers, one female carrier with diagnosis confirmed by molecular genetic examination and one healthy woman in whom the possibility of her being a carrier was excluded (30). The family under study was one of a branch of an extensive genealogy, in which Klouček et al. attempted to demonstrate a linkage to the sex chromosome using the then available markers in 1979 (29). The variability of the finding in certain female carriers of this genealogy was then described by Otradovec et al. in 1979 (33).

In the case of all the afflicted males and the female carriers, we conducted an examination of visual acuity using ETDRS optotypes and examination of near vision using two types of Jaeger tables with a scope from the smallest to the largest text 0.4-1.1 and 1-12. Contrast sensitivity was determined on Pelli-Robinson tables (HS Clement Clarke International, Great Britain) (23) and colour sensitivity by a Lanthony desaturated panel D-15 test. The visual field was evaluated by a kinetic (Goldmann kugel perimeter, Carl Zeiss Jena, Germany) and a static automatic perimeter (M-700, Medmont International Pty Ltd., Australia). The finding on the fundus upon biomicroscopic examination in mydriasis was photographically documented (FF 450 plus IR Fundus Camera, Carl Zeiss Meditec AG, Germany). An examination was also conducted by optical coherence tomography with a spectral domain (SD-OCT, Spectral OCT/SLO, OTI Ophthalmic Technologies Inc., Canada), displaying the individual layers of the retina in the area of the macula and the retina nerve fibre layer (RNFL) within a circuit with a diameter of 3.4 mm around the disc of the optic nerve. An electrophysiological assessment of the function of the retina was conducted by classic flash and multifocal electroretinography (ERG; RETI-port plus mfERG system, Roland Consult GmbH, Germany), according to the international standards compiled by the International Society for Clinical Electrophysiology of Vision (31). We observed the rod cell response under scotopic conditions (night vision) using classic ERG, as well as the maximum response and oscillation potential, and under photopic conditions (day vision) we observed the cone cell response and the response to flashing stimuli with a frequency of 30 Hz. Multifocal ERG

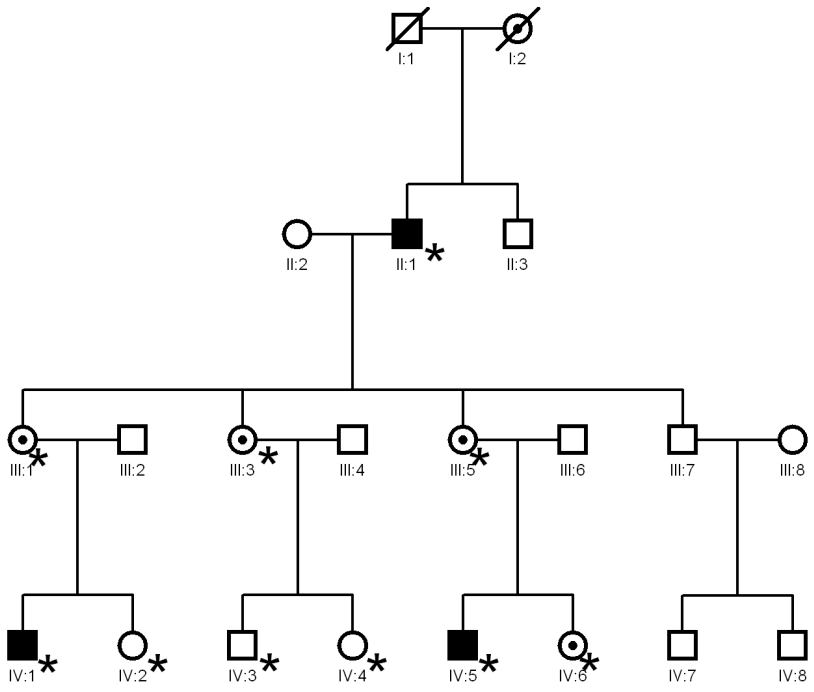

Fig. 1. Examined family with RP bound to sex chromosome with demonstrated pathogenic mutation c.2426_2427delAG in ORF15 gene RPGR. Individuals in whom molecular genetic testing was conducted are indicated with a star, black squares represent afflicted males, circles with a dot indicate female carriers, empty symbols are unaffected family members (30)

provided 61 local responses in the central 30° of the visual field.
We evaluated amplitudes and latencies of the positive peak (P1) via the response of the first alignment in five concentric circles around the fovea. The three-dimensional image of the density of the responses illustrated the central, foveolar response and declining activity in the direction towards the periphery. The amplitudes of 61 local responses of the given patient were compared with the normal values of a control group of corresponding age.

RESULTS

A 22 year old male (IV:1, fig. 1) denied suffering from any subjective complaints, including nyctalopia, despite the fact that symptoms of incipient RP with isolated peripheral pigment shifts in the shape of clusters had been documented in him at the age of 9 years, and visible pigment changes of the character of bone cells at the age of 15 years. At the age of 10 the following corrections were used in order to attain BCVA – 2.5 D = -2.0 Dcyl ax 25° right and -0.5 D = -2.0 ax 15° left, at age 15 -3.0 D =
-2.0 Dcyl ax 30° right and -2.5 D = -2.0 Dcyl ax 180° left. Upon the last examination at age 22, visual acuity was bilaterally 0.63 with -3.75 D = -2.0 Dcyl ax 15° right and with -3.5D = -2.0 Dcyl ax 165° left. Near vision was bilaterally normal.
The biomicroscopic examination of the fundus in mydriasis revealed a bilaterally preserved macula including a layer of retinal pigment epithelium (RPE). Typical chorioretinal atrophy with characteristic pigment shifts in the shape of bone cells, circulary localised, was determined in the periphery of the retina. There was also present attenuation of the retinal capiallries, especially the arteries (fig. 2a, b). SD-OCT examination did not reveal pathology in the macula in either eye, but in the area around the macula there was present reduction of the height of the retina and the choroid (fig. 2c). The disc of the optic nerve bilaterally appeared pink, with the aid of RNFL the physiological thickness of the layer of nerve fibres was confirmed. No defect of colour sensitivity or contrast sensitivity was demonstrated.
An examination of the scope of the visual field by means of computer perimetry was conducted every year from the age of 10 years. The first assessable changes in the sense of isolated relative scotomas were recorded at the age of 15 years. Absolute hemianopsias and characteristic presence of annular scotoma between 10-40° was documented at the age of 20 and 22 years (fig. 2d). An examination by kinetic perimetry at the age of 22 years confirmed narrowing of the visual field from above to 20°, in other direction the scope was evaluated as normal.
An ERG examination determined residual scotopic (approx. 10% of norm) and photopic activity (10% of norm in right eye and 25% of norm in left eye) with pronounced extension of latencies (table 1, fig. 2e). Multifocal ERG demonstrated reproducible but markedly reduced foveolar and parafovoelar activity of the retina (approx. 20% of norm) with extended latencies. Activity externally from 14° eccentricity was only residual (fig. 2f, g).
22 year old male (IV:5, fig. 1) in whom nyctalopia is observed from the age of 12 years. First pigment shifts reminiscent of bone cells in documentation substantiated at the age of 15 years. Central visual acuity and myopic correction did not change over the course of the years, and upon our last examination BCVA was 0.3 with correction -7.0 D = -1.25 Dcyl ax 10° in right eye and 0.5 with correction -6.0 D = -1.0 Dcyl ax 180° in left eye. Near vision was reduced bilaterally to reading text with Jaeger number 1-2. The anterior segment in the left eye was without a pathological finding, in the right eye we determined an incipient cataract in the posterior cortex of the lens. There were certain characteristic symptoms of RP on the fundus of both eyes: lighter disc of the optic nerve, slight granulation in the maculas, circulary in central periphery pigment shifts in shape of bone cells and very narrow capillaries up to the periphery, also predominant pronounced chorioretinal atrophy and visible large choroidal capillaries (fig. 3a-d).

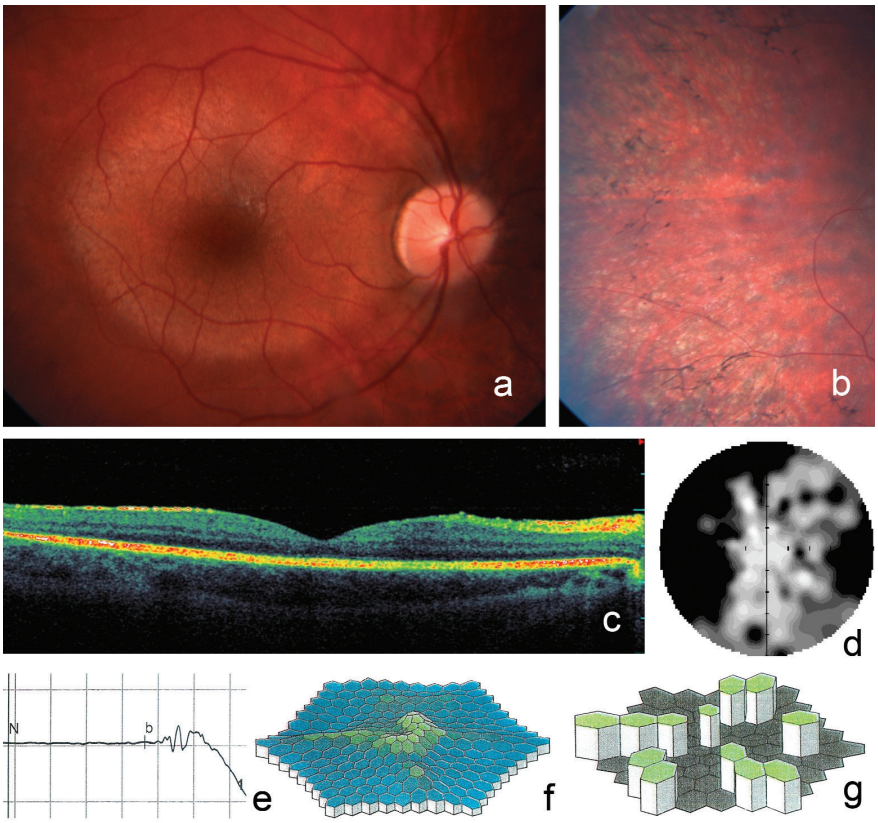

Fig. 2 Clinical findings determined in right eye of 22 year old man (IV:1) with retinitis pigmentosa conditioned by mutation in ORF15 of gene RPGR. The photograph of the fundus documents a normal appearance of the papilla, relatively preserved macular with circular clarification contrasts with chorioretinal atrophy in periphery a). Periphery with pigment clusters in the shape of bone cells b), horizontal scan SD-OCT displays normal macula and reduction of height of retina and choroid around macula c), visual field with annular scotoma d), residual rod-cell response e), reduction of density of responses to three-dimensional image upon multifocal ERG f), residual areas of electrical activity of retina represented by green hexagons g)

Table 1. Values recorded upon electro-physical examination of retina. Results determined in man IV:5 with already extinct electrical activity of retina are not stated. Values of amplitudes are stated in μV, values of latencies in ms, pathological values are highlighted in bold

	Scotopic conditions																		Photopic conditions											
	Rod response				Maximum response								Oscillation potential						Cone response								30-Hz filter			
Individual	Amplitude of wave b		Peak latency of wave a		Amplitude of wave a		Amplitude of wave b		Peak latency of wave a		Peak latency of wave b		Amplitude of OS2		Peak latency of N2		Peak latency of P2		Amplitude of wave a		Amplitude of wave b		Peak latency of wave a		Peak latency of wave b		Amplitude of N1-P1		Peak latency of P1	
	OP	OL	OP	OL	OP	OL	OP	OL	OP	OL	OP	OL	OP	OL	OP	OL	OP	OL	OP	OL	OP	OL	OP	OL	OP	OL	OP	OL	OP	OL
Male IV:1	2,8	4,82	77	73	15,6	6,6	15,2	18,9	23	24	54	50	10,4	1,46	19	18	22	23	6,77	1,95	10,1	8,18	18	18	40	40	14,2	13,4	70	72
Female carrier III:1	198	395	92	110	85,8	79,8	244	225	18	20	48	50	25,1	23,4	23	24	27	28	33,6	6,92	46,6	112	17	15	32	33	52,1	49,6	63	65
Female carrier III:5	46,1	58	80	82	53,5	85,5	146	169	21	20	50	50	16,7	20,1	24	24	28	28	7,45	17,3	33,8	51,6	18	16	34	34	24,9	45,1	63	63
Female carrier III:6	219	107	97	100	144	71,9	393	214	17	17	47	46	68,5	31	23	23	26	26	21,6	20	119	64,9	16	15	31	31	97,4	59,1	61	62
Norm	95-305		67-91		155-356		290-654		14-22		33-46		21-64		-		22-24		26-62		103-250		13-16		29-33		57-223		58-64	

Progressive narrowing of visual field occurred at the age of 16 years, with rapid progression between the ages of 19 and 21. At the age of 16 years practically circular hemianopsias were determined, leading to the periphery and infringing in both eyes to 20-30°. At the age of 22 years only the central remainder of the visual field was recorded, with a scope of less than 5° (fig. 3d). Serious dyschromatopsia and defect of contrast sensitivity (right 0.90, left 1.35) were also determined. The finding on SD-OCT revealed a reduction of the height of the retina and choroid with atrophy of the layer of photoreceptors (fig. 3c). Only residual electrical activity of the retina, which was difficult to distinguish from the background noise, was determined using ERG (fig. 3e-g).

Female, 44 years old (III:1, fig. 1), subjectively did not state any complaints. We determined best corrected visual acuity (BCVA) of 1.0 right and 0.4 left with correction +2.0 D = -2.25 Dcyl ax 15° right and +1.0 D = -3.25 Dcyl ax 175° left. Near vision was reduced in left eye (Jaeger's no. 1.1). The anterior segment of both eyes was entirely without pathological finding. On the fundus we observed slight pigment shifts without similarity to bone cells (fig. 4a, b). Examination of the visual field bilaterally did not show any abnormal hemianopsia, but in the left eye was hampered by a high percentage of loss of fixation. All the layers of the retina appeared as normal on the SD-OCT image of the right eye, but in the left eye in comparison with the right eye a lower reflexivity of the complex of exterior segments of the photoreceptors was recorded, as well as RPE with a more pronounced illustration of the structure of the choroid. Contrast sensitivity was within the norm in the right eye (1.65), slightly reduced in the left (1.35). The result of the examination of colour sensitivity revealed defects in the blue and red OL axis. Upon classic flash ERG examination a slight alteration of the electrical activity of the retina was determined. Scotopic and photopic activity of the retina was within the limits of the norm, with the exception of reduced maximum response at 90% of norm bilaterally and low cone response in right eye (50% of norm) with extended latency (table 1). Multifocal ERG detected bilaterally reduced foveolar (85% of norm), parafoveolar (50% of norm) and peripheral (80% of norm) activity. Several localised areas of reduced amplitudes were determined bilaterally (fig. 4c, d).

39 year old sister (III:5, fig. 1) in whom reduction of visual acuity is observed from the age of 10 years despite correction by eyeglasses, in recent years states deteriorated vision in darkness. In childhood she wore an eye patch and always had worse vision in the left eye, to which the determined mic-

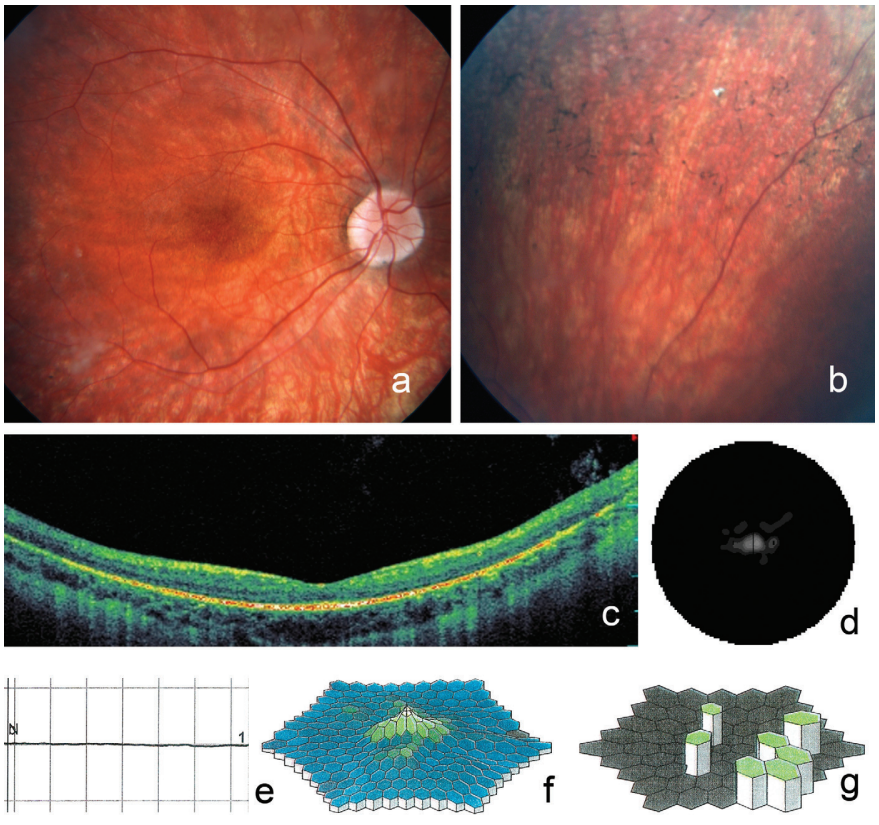

Fig. 3. Clinical findings determined in right eye of 22 year old male (IV:5) with retinitis pigmentosa conditioned by mutation in ORF15 of gene RPGR. Thinning of the retinal pigment epithelium is evident on the photograph of the fundus, as well as narrowing of the artery, pale disc of optic nerve a) and a larger number of pigment clusters in the shape of bone cells b), vertical SD-OCT of the macula documents reduction of the thickness of the neuroretina and thin choroid with accentuation of its structures upon diminution of RPE c). Concentric narrowing of visual field d), extinct rod response e), reduction of density of responses on three-dimensional image upon multifocal ERG f), residual areas of electrical activity of retina represented by green hexagons g)

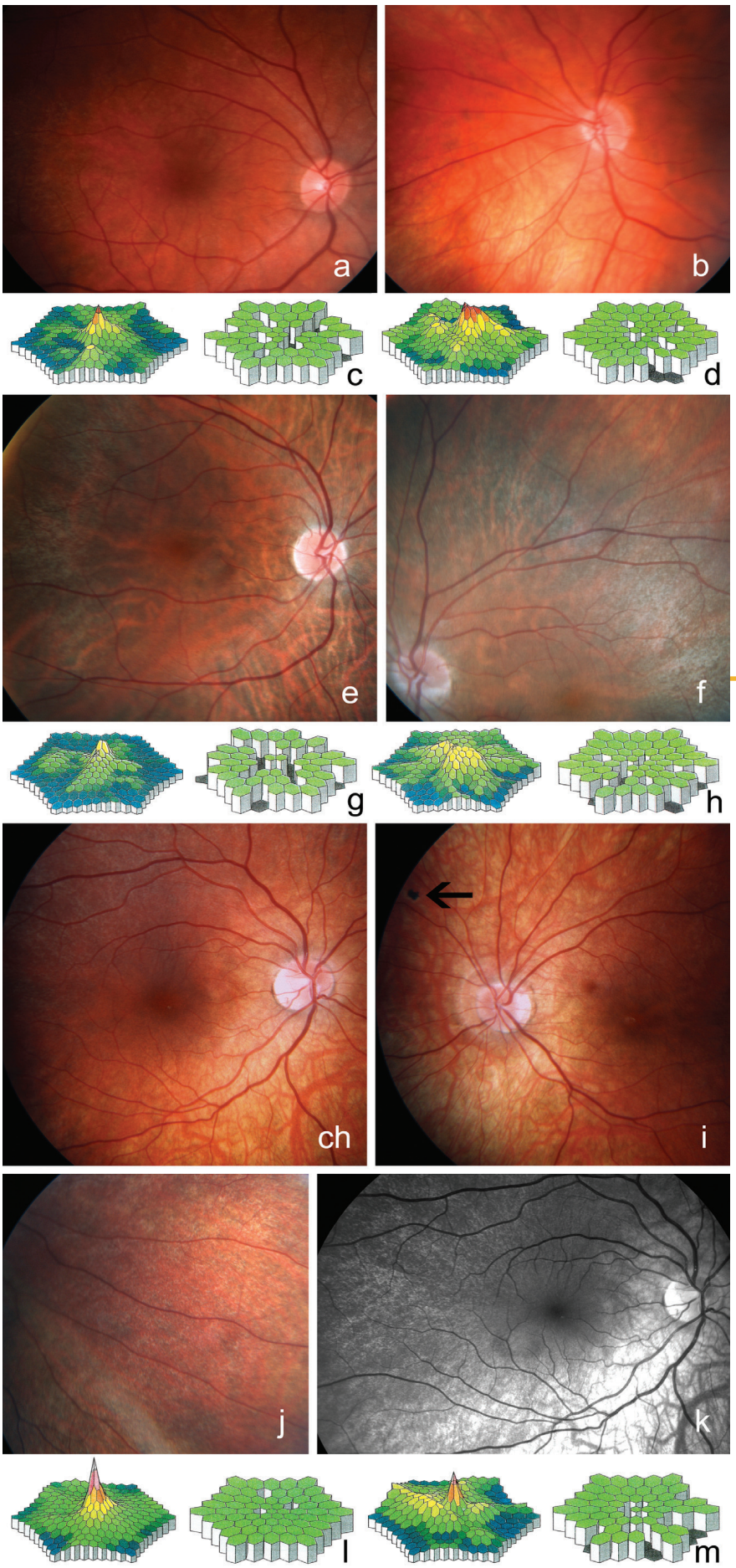

Fig. 4. Clinical findings of three female carriers of retinitis pigmentosa. Photograph of fundus showing thinning of retinal pigment epithelium and narrowing of retinal arteries in right eye a), left eye of female III:1 b), overall foveolar alteration of signal and localised reduction of function of retina in right eye c) and left eye d) determined using multifocal ERG in same patient. Thinning of retinal pigment epithelium, narrowing of retinal arteries and grainy appearance of retina in central periphery in right e) and left eye in female III:5 f); changes upon multifocal ERG right g) and left in same patient h). Thinning of retinal pigment epithelium and narrowing of retinal arteries in right eye in female IV:6 ch), similar finding in left eye and peripapillary atrophy within the framework of severe myopia and in nasal upper quadrant pigment cluster in shape of lump (indicated by arrow) i), grainy appearance of retina in lower temporal quadrant in right eye j); black and white image of right eye k) and virtually normal finding upon multifocal ERG in right eye l) and in left eye in same female m)

roestropia in the left eye corresponds. BCVA was 0.8 right with correction -1.0 D = +3.0 Dcyl ax 90° and 0.5 left with correction -1.0 D = +3.0 Dcyl ax 90°. The patient read a text indicated with Jaeger's no. 1 in the right eye and no. 4 in the left eye with correction. In this obligatory female carrier we determined symmetrically slight chorioretinal atrophy in both eyes, as well as slight narrowing of the retinal capiallaries and in the central periphery typical change residing in irregular distribution of pigment. Particularly in this area the retina had a grey tint and increased visibility of choroidal capiallaries (fig. 4e, f). Upon SD-OCT examination, lower reflexivity of the RPE boundary and neuroretina was recorded, with more pronounced illustration of the structure of the choroid. Neither automatic perimetric examination nor examination using kinetic perimetry with indication IIIe4 showed an abnormal finding. Examination of contrast sensitivity and colour sensitivity was within the norm. ERG examination demonstrated a reduction of amplitudes of scotopic and photopic responses at 50% of the norm with extension of latencies. Multifocal ERG detected bilaterally reduced amplitudes of average responses at 50% of the norm with mosaic distribution of dysfunctional areas (fig. 4g, h).

Female, 20 years (IV:6, fig. 1), denied

nyctalopia. Since childhood has blunt-sighted left eye, and since the age of 15 has observed reduction of BCVA, which in our examination was 1.0 with correction -4.0 D = -1.5 Dcyl ax 180° right and 0.16 with correction -10.0 = -2.5 Dcyl ax 160° left. Upon assessment of the mutual position of the eyes, microexotropia of the left eye with eccentric fixation was determined. The finding on the fundus corresponded to myopic correction, no pigmentations in the shape of bone cells were found, only in the nasal upper quadrant of the left eye one pigment cluster was present. Temporally from the macula we then observed bilaterally slight grainy greying, sometimes also described as "salt and pepper fundus" (fig. 4ch-k). On the SD-OCT scans there was an evident myopic character of the retinogram with lower reflexivity of the RPE boundary and neuroretinas with more pronounced illustration of the structure of the choroid. Examination of the visual fiend, contrast sensitivity, colour sensitivity did not show any abnormalities. The ERG examination showed normal amplitudes of scoptopic and photopic responses in the right eye and in the left eye abnormal maximum (80% of norm) and cone (65% of norm) response upon normal to borderline latencies bilaterally (table 1). Responses of the right eye upon multifocal ERG evaluated as normal, in the left eye reduction of foveolar responses to approx. 30% of norm was determined, parafoveolar activity, as with responses externally from macula at 80% of norm (fig. 4l, m).

Female, 23 years old (IV:2, fig. 1), did not state any complaints. Determined visual acuity was bilaterally 1.0 in right eye without correction, in left with correction -1.0 D. Examiantion of the fundus did not show any pathological finding in accordance with the results of the molecular genetic testing.

None of the examined individuals underwent a intraocular surgical procedure. Intraocular pressure was within the physiological range in all individuals.

DISCUSSION

The aim of our work is to describe the clinical findings in members of a Czech family with RP bound to the sex chromosome, which was the first Czech family in which a molecular genetic cause was published (30). In our previous work we demonstrated that the disorder in the studied genealogy is conditioned by pathogenic mutation c.2426_2427delAG, located in exon ORF15 of the RPGR gene, which leads to a shift of the open sensory framework from the amino-acid residue to position 809 (p.Glu809fs) (30) and thus to a premature termination of protein synthesis (1). Pathogenic changes in ORF15 are not only the most frequent cause of RP bound to sex chromosome, but appear in an estimated up to 15% of all cases with this disorder, i.e. with the highest frequency of all loci for RP (3, 22). With regard to the prevalence in other populations (7, 35), we estimate that in the Czech Republic there may be a total of approximately 200-300 individuals of male sex with RP bound to the sex chromosome.

The product of the RPGR gene is a protein which has been localised to the transition of the exterior and interior segment of the rod and cone cells and shares in intraflagellar transport, but its detailed function is not yet known (25). In accordance with the expression in both types of photoreceptor elements, mutations in the RPGR cause not only a clinical picture of typical RP, but also dystrophies of cone cells, mixed cone-rod cell dystrophies or atrophic macular degeneration (2, 9, 10, 46).

The clinical findings for the members of the family we observed were described by Otradovec et al. in 1979 (33). By studying the literature we came to the conclusion that we had re-examined two of the obligatory female characters of the original genealogy (indicated in fig. 1 by symbols III:1 and III:5), at that time aged 2-9 years (33). Otradovec et al. (1979) documented four afflicted men, in all of whom the first subjective complaints originated at the beginning of their second decade of life, and at around the age of 40 they all manifested a visual defect to the degree of practical blindness (visual acuity below 3/60 or binocular visual field lower than 10° (33). We also observed this course ourselves in male IV:5, who at the age of 22 no longer showed signs of ERG activity and whose clinical finding thus contrasted with the results of the examination for his male cousin of the same age IV:1, who still had relatively preserved visual functions as well as hitherto functional electrophysiological activity of the retina. ERG examination in the case of this male demonstrated mixed damage to rod and cone cells, so far without reduction of near visual acuity, without changes to colour sensitivity or central hemianopsias.

The differences between both of the individuals we examined are an example of the frequently observed variability of the clinical finding, which hampers consultancy for patients and their family members upon estimating the prognosis for the development of loss of visual functions. The cause of this phenomenon has not been unequivocally clarified to date. It is believed that the basis could be modifying genetic and epigenetic factors (11, 35).

A further specific characteristic of the clinical finding in individuals with RP conditioned by mutations in the genes RPGR and RP2 localised in the sex chromosome is the occurrence of refractive defects with a mechanism of origin for which to date there is no satisfactory explanation. Myopia is frequently determined in combination with myopic astigmatism or mixed astigmatism (16, 28, 34, 35). We have also observed these refractive defects in our own patients. During long-term observation of individual family members conducted over the course of more than 13 years, in one of the males we demonstrated a progressive increase of myopia and astigmatism. Otradovec et al. also determined medium to high myopia from -6.0 to -15.0 D (33) in three out of four of the afflicted examined men.

The clinical findings for all three of the female carriers we examined were relatively similar. BCVA was reduced in at least one eye, and we also observed more serious refractive defects with anisometropia and connected amblyopia. All the female carriers had changes on the fundus in the sense of slight thinning of the RPE, narrower capillaries in the periphery and isolated shifts to clusters of pigment without the typical shape of bone cells, which is a frequent finding in younger and middle age (35). In carrier IV:6, with a high level of myopia, it was however impossible to differentiate the extent to which this defect shares in the determined changes on the fundus. We did not determine a tapetal reflex, which is considered to originate probably upon the background of increased reflexivity of the external segments of the photoreceptors, in any of the three female carriers (5). In the literature it is stated that this finding is present in patients with mutations in RPGR in approximately one half of cases (15).

The findings determined by Otradovec et al., who examined 10 obligatory female carriers and 7 women whom they believed to be also carriers of a pathogenic mutation from the studied genealogy, were highly variable: from a fully developed image of typical RP, through sector retinitis punctata albescens, sector hemianopsias, presence of tapteal reflex in the area of the posterior pole up

to mere changes on the fundus, which were evaluated as myopic fundus. As in our group, all the female carriers had reduced visual acuity in at least one eye. In two of them, convergent strabismus and slight amblyopia of the strabismal eye was determined in childhood age, one of these was probably female III:5 who we examined. Of the refractive defects, hypermetropic astigmatism, medium and high myopia were present (33).

The occurrence of refractive defects in female carriers of RP is well documented in the literature (15, 27, 39), in 1986 Fishman et al. noted the fact that in these cases astigmatism greater than 1.5 Dcyl is frequently determined (14).

Classic flash ERG on the examined males showed affliction of both rod and cone cells, in which activity was extinct in patient IV:5. The female carriers manifested various findings, from slight alteration of electrical activity with amplitudes of scoptopic and photopic responses still within the normal range upon normal to borderline latencies, after reduction of amplitudes of varying degrees with extension of latencies. High myopia above -6.0 D may itself share in the reduction of amplitudes upon classic ERG (45).

Of interest were the results of multifocal ERG, which were published for the first time in connection with RP in Czech patients. Localised alteration was determined in the right eye of female IV:6, despite the fact that the overall electrophysiological activity of the retina was evaluated as normal. This mosaic distribution of retinal dysfunction upon examination by multifocal ERG is a characteristic finding in female carriers (43). Multifocal ERG demonstrated several preserved segments of activities primarily in the foveolar areas in both males, clinically corresponding to so far relatively good BCVA. Upon multifocal ERG it is frequently possible to demonstrate photopic responses in the area of the macula also in males with advanced loss of visual functions with a no longer detectable classic flash ERG recording, the main component of which is responses from the periphery of the retina. We also observed this ourselves in one of the afflicted patients (IV:5), similarly to other authors (17, 24, 37).

The variability of the clinical findings in the female carriers was previously explained by the uneven inactivity of chromosome X, but this theory has been excluded by experiments. At present it is believed that as with afflicted males, the main influence on phenotype is expression is due to the presence of modifying genetic factors, either in the causal gene itself or in other genes (32, 34). With regard to the fact that Otradovec et al. (33) determined entirely normal findings, including activity upon classic flash ERG examination, in a number of female carriers from the studied family (38, 43), demonstration or exclusion of pathogenic mutation is particularly beneficial in the case of the daughters of the obligatory female carriers, who have a 50% probability of becoming carriers of this sequential variant. This is also significant upon genetic consultation and stipulation of the risk of transmission of the disorder to further generations. In our genealogy (fig. 1) it concerned females IV:2 and IV:6.

Although it is mostly not possible to locate a specific gene which could condition the disorder in RP on the basis of the clinical finding, in patients with mutations in RPGR there was a determined more rapid progression of the disorder than in individuals with autosomal dominant RP caused by changes in the rhodopsin gene (RHO). The average age of occurrence of severe purblindness in patients with mutations in RPGR is 45 years, which is 32 years earlier than in the group of patients with mutations in the gene RHO (36).

It is necessary to consider the possibility of the presence of heredity with linkage to the sex chromosome also in males with a negative family anamnesis or in male siblings with frequent symptoms of the disorder. Causal mutations in genes localised to the X chromosome occur in this group in up to 30% (34). An important guide for choosing targeted genetic screening may also be the above-mentioned presence of refractive defects.

Knowledge of the molecular genetic cause of RP improves clinical consultancy for patients and their families. It also enables patients to undergo pre-implantation diagnosis, which is used upon in vitro fertilisation, in which an examination for the possible presence of a strain for hereditary disease is conducted before implantation of the embryo into the womb. In the case of eye disorders, this method has been documented in the literature e.g. for Stargardt's disease and retinoblastoma (12, 18, 41).

To date there is no effective known treatment for RP disorder, but on the basis of the promising results from the clinical trials in patients with retinal dystrophies conditioned by mutations in RPE65, a great deal of hope is being invested in gene therapy (26, 42). Very recently a study was published documenting the effectiveness of this treatment in a canine model for RP conditioned by mutations precisely in RPGR (4). With regard to the fact that pathogenic changes in this gene are one of the most frequent causes of this retinal dystrophy, it is expected that research shall lead to clinical tests, and for this reason also, knowledge of the molecular genetic causes of patients' disorders it may be important.

CONCLUSION

We conducted a comparison of genotype with phenotype in the first Czech family with described molecular genetic cause of RP. In the case of the presence of medium and high myopia and astigmatism in males with this disorder, it is necessary to consider heredity with linkage to the sex chromosome, which can then be verified by targeted screening of the causal genes.

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