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1 **Fundus albipunctatus associated with compound heterozygous mutations in *RPE65***

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19 This article contains online-only material. The following should appear online-only: Figs 6, 7 and 8.

20 Running head: Fundus albipunctatus and *RPE65* mutations.

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24

1 **Abstract**

2 **Purpose:** To describe a family with an 18-year old woman with fundus albipunctatus and  
3 compound heterozygous mutations in *RPE65* whose unaffected parents and one female sibling  
4 harboured single heterozygous *RPE65* mutations.

5 **Design:** Observational study.

6 **Participants:** Four family members.

7 **Methods:** Clinical examinations included full-field ERG (ffERG) after standard (30 min) and  
8 prolonged (17 h) dark adaptation, multifocal ERG (mfERG), optical coherence tomography (OCT)  
9 and fundus autofluorescence (FAF). Molecular genetic testing included sequencing of *RDH5* and  
10 *RLBP1* and screening for known autosomal recessive retinitis pigmentosa mutations by a  
11 commercially available microarray technique. *RPE65* sequencing was performed following the  
12 identification of a known heterozygous splice-site mutation by array screening.

13 **Main Outcome Measures:** FfERG and mfERG amplitudes, OCT characteristics, FAF intensity  
14 index and outcomes of DNA sequencing regarding *RPE65* mutations.

15 **Results:** Uniform yellow-white dots typical of fundus albipunctatus were demonstrated in the  
16 proband. These dots corresponded to discrete hyperreflective formations extending from the  
17 Bruch's membrane and retinal pigment epithelium (RPE) into the level of the external limiting  
18 membrane (ELM), thus spanning along the entire RPE and photoreceptor outer and inner segments.  
19 A reduced thickness of the central retina and the RPE-outer segment complex was demonstrated.  
20 FAF intensity was severely reduced in the entire fundus. FfERG demonstrated a barely recordable  
21 rod response after standard dark adaptation and normalization (increase by >700%) of the response  
22 after prolonged dark adaptation. The cone 30 Hz flicker response was reduced after standard dark  
23 adaptation and increased by >50% after prolonged dark adaptation. MfERG demonstrated reduced  
24 central and peripheral responses. Molecular genetic analysis demonstrated compound heterozygous

1 mutations (IVS1+5G>A and c.344T>C) in *RPE65*. No mutations were found in *RDH5* or *RLBP1*.  
2 No significant abnormalities of retinal structure or function were detected in the parents and sister  
3 carrying single heterozygous mutations in *RPE65*.

4 **Conclusions:** This is the first reported association between compound heterozygous *RPE65*  
5 mutations and fundus albipunctatus, indicative of a mutation-specific phenotypic effect in this gene.  
6 This finding, together with the reduced fundus autofluorescence, supports that disruption of retinoid  
7 recycling in the RPE is essential for the development of fundus albipunctatus.

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1 The visual cycle refers to the regeneration of the chromophore 11-cis retinal after its isomerization  
2 and release from the opsin protein in response to light, a process that is fundamental for vision and  
3 is affected in various forms of retinal degeneration. As a side effect of the visual cycle, the bis-  
4 retinoid derivative A2E (*N*-retinylidene-*N*-retinyl-ethanolamine) is formed, a major constituent of  
5 lipofuscin which is incriminated in the pathogenesis of for example age related macular  
6 degeneration, Stargardt disease and Best Macular Dystrophy.<sup>1-2</sup> Therefore, recently, several  
7 attempts have been made to investigate the components of the visual cycle as potential therapeutic  
8 targets (for review, see Travis et al. 2007).<sup>2-6</sup> It is thus of great interest to characterize phenotypes  
9 associated with specific defects of the visual cycle.

10 11-cis retinol dehydrogenase (11-cis RDH) is an abundantly expressed transmembrane protein in  
11 the RPE cell somata.<sup>7,8</sup> The enzyme catalyzes the final step in the visual cycle, the biosynthesis of  
12 11-cis retinal before it binds to opsin in rods and to the cone opsin in cones, to form rhodopsin and  
13 the cone opsins. To date, at least 18 different mutations in the gene for 11-cis RDH (*RDH5*) have  
14 been associated with fundus albipunctatus, which is a rare autosomal recessive condition  
15 characterized by numerous discrete white dots scattered over the fundus.<sup>7-9</sup> In contrast to other  
16 disorders resulting from mutations in enzymes of the visual cycle, e.g., Leber congenital amaurosis,  
17 there is a variable recovery of dark adaptation and full-field ERG responses after prolonged dark  
18 adaptation, which implies remaining retinal function.<sup>10</sup> Furthermore, the phenotype features reduced  
19 formation of lipofuscin, implying that a pharmacologic interference at this specific step of the visual  
20 cycle may be a feasible treatment strategy for retinal disorders featuring excessive lipofuscin  
21 accumulation.

22

23 RPE65 is the isomerase of the visual cycle, catalyzing the reaction just upstream to the one  
24 catalyzed by 11-cis retinol dehydrogenase; the conversion of all trans retinol to 11-cis retinol.<sup>11</sup>

1 *RPE65* mutations are associated with Leber congenital amaurosis and early onset rod-cone  
2 dystrophy (EOSRD), and the gene has been in focus recently in the clinical trials on gene therapy  
3 for Leber congenital amaurosis.<sup>12-15</sup> Similar to fundus albipunctatus, phenotypes associated with  
4 homozygous or compound heterozygous mutations in *RPE65* show a lack of lipofuscin formation,  
5 however there is a severe loss of retinal function from birth with later deterioration to blindness.<sup>14,15</sup>  
6 It is not known why mutations that seemingly affect the function of two different enzymes  
7 catalyzing successive steps in the same pathway, lead to very different phenotypes in terms of  
8 retinal function.

9 In this paper, we present clinical and molecular genetic data in an 18-year old Danish female patient  
10 with fundus albipunctatus, with compound heterozygous mutations in *RPE65*. Considering a recent  
11 report of widespread drusenoid deposits in a single heterozygous subject with the IVS1+5g→a  
12 splice-site mutation on one *RPE65* allele, we also investigated family members extensively to  
13 examine possible phenotypic effects of each of the heterozygous mutations.<sup>16</sup>

14

#### 15 **Methods:**

16 The study included an 18-year old female with night blindness since childhood, her one sibling, a  
17 20-year old sister and their 2 parents (Fig. 1). All the subjects were healthy and did not take any  
18 medication. There was no parental consanguinity or family history of retinal disease. Informed  
19 consent was obtained. The study was performed in accordance with institutional guidelines and the  
20 Declaration of Helsinki. Ethics committee approval was obtained.

21 Standard clinical examination included determination of best-corrected visual acuity (BCVA) and  
22 Goldmann perimetry. Achromatic, automated perimetry was performed with a Humphrey Visual  
23 Field Analyzer II (model 750, Carl Zeiss Meditec, Dublin, CA, USA) using the 30-2 program with

1 the SITA (Swedish Interactive Threshold Algorithm) fast protocol. Values of the mean deviation  
2 (MD) and pattern standard deviation (PSD) were analysed.

3 Full-field ERG (ffERG) was performed in the proband (patient 2:2) at age 10y according to ISCEV  
4 standard (ISCEV=International Society for Clinical Electrophysiology of Vision).<sup>17</sup> FfERG was  
5 repeated at age 18y in patient 2:2, when it was also performed in the healthy parents and sister  
6 according ISCEV standard with a few modifications as described previously.<sup>10</sup> The examination  
7 was performed after subjects were dark adapted for 30 minutes in their left eyes and for 17 hours in  
8 their right eyes, accomplished by overnight patching. Multifocal electroretinography (mfERG) were  
9 recorded using a visual evoked response imaging system (VERIS 4; EDI, San Mateo, CA), in the  
10 right eyes of all family members.<sup>10</sup>

11 Optical Coherence Tomography (OCT; OCT-3 and OCT-4; Zeiss Humphrey Instruments, Dublin,  
12 CA) was performed with 5 mm single line scans over the fovea. In addition, in the right eye of  
13 proband 2;2, OCT scans were also analyzed after multiple scan averaging.<sup>18,19</sup> Furthermore, to  
14 determine the retinal sublayer location of the albipunctate dots in the patient, spectral domain OCT  
15 (Spectralis HRA-OCT, Heidelberg Engineering, Germany), was performed in the paracentral retina,  
16 where the density of the dots was highest.

17 Fundus autofluorescence (FAF) was examined using a scanning laser fundus camera (Spectralis  
18 HRA-OCT, Heidelberg Engineering, Heidelberg, Germany) in both eyes of all four family  
19 members. To extract semiquantitative measures of fluorescence intensity we used the HRA  
20 software as described previously.<sup>10</sup> The ratio between the fluorescence intensity in the temporal  
21 macula to that of the optic disc (macula/optic disc) served as an index of fundus autofluorescence  
22 intensity. Six healthy eyes from age-matched subjects served as controls.

23 Molecular genetic *RDH5* sequencing using standard methods was carried out. A commercially  
24 available *RLBP1* sequence analysis was performed (Centogene, Rostock, Germany). Screening for

1 known mutations in autosomal recessive retinitis pigmentosa was performed with microarray  
2 technique (Asper Ophthalmics, Tartu, Estonia), including mutations in *CERKL*, *CNGA1*, *CNGB1*,  
3 *MERTK*, *PDE6A*, *PDE6B*, *NR2E3*, *RDH12*, *RGR*, *RLBP1*, *SAG*, *TULP1*, *CRB*, *RPE65*, *USH2A*,  
4 *USH3A*, and *LRAT* genes.

5 *RPE65* sequencing was performed following the identification of a heterozygous splice-site  
6 mutation by array screening. Mutation segregation with disease was verified by sequencing of  
7 *RPE65* in unaffected family members.

8

### 9 **Results:**

10 No subjective complaints were reported by single heterozygotes, whereas the patient with fundus  
11 albipunctatus (Patient 2:2) reported night blindness since childhood (Fig. 2). No significant  
12 abnormalities of retinal structure or function were recorded in the remaining family members  
13 (single heterozygotes), who all had normal fundi and normal Humphrey 30-2 perimetry. None of  
14 the family members had any significant media opacities. The following presentation refers to the  
15 findings in patient 2:2 with fundus albipunctatus.

16 Patient 2:2 reported night blindness since childhood. She was diagnosed with fundus albipunctatus  
17 at the National Eye Clinic at the age of eight. Best corrected visual acuity was reported as 0.9 at age  
18 8y and was reduced to 0.5 in both eyes at 18y. Goldmann perimetry did not reveal any significant  
19 abnormalities in the visual fields at age 12 and 14y. At age 18, Humphrey 30-2 perimetry  
20 demonstrated patchy reduction of sensitivity in the central visual fields in both eyes (mean deviation  
21 [MD] was -12.79 and -13.42 dB, and the mean pattern standard deviation [PSD] was 4.86 and  
22 7.5dB for the right and left eye, respectively). The scattered homogenous yellow-white dots  
23 throughout both fundi corresponded to discrete hyperreflective formations extending from the RPE  
24 to the external limiting membrane (ELM) on OCT (Figs. 2,3). At age 18y, reduction of the central



1 retinal thickness was documented, and an analysis of the retinal sublayers in patient 2:2 revealed  
2 reduced thickness of the RPE-OS complex (66  $\mu\text{m}$ , compared to median 76 [range 70-87] in 25  
3 normals).<sup>18,19</sup> The corresponding measure was 70 in a 19-year old patient with fundus albipunctatus  
4 due to homozygous mutations in *RDH5*.<sup>10</sup> (Figs. 4, 5 and Table 1). Fundus autofluorescence was  
5 homogenously reduced in both fundi, and did not change after prolonged (17 hours) dark adaptation  
6 (Figure 3).

7 The temporal macula/optic disk autofluorescence index was 1.4 (right eye) and 1.6 (left eye) in the  
8 proband (healthy subjects  $\geq 2.5$ ). The corresponding estimate was 1.3 in a 19-year old patient with  
9 fundus albipunctatus due to homozygous mutations in *RDH5*.<sup>10</sup> In the remaining family members  
10 the corresponding estimate was 1.9 or more.

11 There was a pronounced reduction of central and peripheral responses by mfERG (Table 1 and Fig.  
12 4).

13 ffERG performed at age 10y showed no recordable rod response after standard dark adaptation, but  
14 a normal combined response and a normal cone response. At age 18y, ffERG including prolonged  
15 dark adaptation, demonstrated a barely recordable rod response after standard dark adaptation and  
16 normalization (increase by  $>700\%$ ) of the response after prolonged dark adaptation. The cone 30 Hz  
17 flicker response was reduced after standard dark adaptation and increased by  $>50\%$  after prolonged  
18 dark adaptation (Table 1 and Fig. 6 [available at <http://aaojournal.org>]). There was a striking  
19 similarity in the response dynamics, compared to the response recorded by us previously in a 19-  
20 year old patient with fundus albipunctatus due to homozygous mutations in *RDH5* (Fig. 6 [available  
21 at <http://aaojournal.org>]).<sup>10</sup>

22 In the three remaining heterozygous family members, no significant changes in the ffERG  
23 responses were seen after prolonged dark adaptation compared to after standard dark adaptation  
24 (Fig. 7 [available at <http://aaojournal.org>]).

1 Microarray testing of proband DNA for autosomal recessive retinitis pigmentosa identified a  
2 heterozygous *RPE65* splice-site mutation, IVS1+5G>A (Table 1), previously described in  
3 association with early-onset severe retinal dystrophy (EOSRD) in homozygous and compound  
4 heterozygous patients.<sup>14-16</sup> This prompted sequencing of *RPE65*, which led to identification of a  
5 previously unreported mutation [p.I115T (c.344T>C)] predicted by mutation analysis software  
6 (SIFT and Polyphen [<http://blocks.fhcrc.org/sift/SIFT.html>, <http://genetics.bwh.harvard.edu/pph/>.  
7 2010 May]) to be pathogenic. Alignment of sequences of several species from mammal to diptera  
8 revealed only one species presenting an amino acid other than the highly conserved isoleucine at  
9 position 115, namely methionine in the sea squirt *ciona intestinalis* (Fig. 8 [available at  
10 <http://aaojournal.org>]). The mutation was not identified in any sample among >500 patients and  
11 controls we sequenced in the search for mutations in *RPE65*. This mutation was subsequently  
12 verified in the clinically unaffected mother and sister through direct sequencing of *RPE65*. The  
13 father was found to harbour the IVS1+5G>A mutation in *RPE65* (Table 1). No mutations were  
14 found in *RDH5* or in *RLBP1*.

15

## 16 **Discussion:**

17 This is, to the best of our knowledge, the first report of fundus albipunctatus associated with  
18 compound heterozygous mutations in *RPE65*. The proband presented the characteristic whitish  
19 punctate lesions that were localized by OCT in the outer retina at a unique depth, namely in the  
20 photoreceptor inner and outer segment and RPE layers where the lesions were interspersed between  
21 normal-appearing stretches of outer retina. This has previously been reported by us in patients with  
22 fundus albipunctatus associated with *RDH5* mutations,<sup>10</sup> and also in a patient with cone dystrophy  
23 and *RDH5* mutations.<sup>20</sup> Furthermore, our compound heterozygous *RPE65* proband also  
24 demonstrated reduction of autofluorescence of the fundus and shortening of the RPE-OS complex

1 by OCT, which we also found in *RDH5* fundus albipunctatus.<sup>10</sup> Finally, retinal function recovered  
2 substantially after prolonged dark adaptation in a similar manner to that found in fundus  
3 albipunctatus associated with mutations in *RDH5*.<sup>10</sup> We therefore argue that our patient indeed  
4 represents a case of fundus albipunctatus, with all its typical phenotypic features.

5

6 Fundus autofluorescence in the retinal pigment epithelium is mainly attributable to slow  
7 accumulation of the retinoid dimer A2E (N-retinylidene-N-retinyl-ethanolamine), a major  
8 component of lipofuscin. We have now demonstrated subnormal fundus autofluorescence in two  
9 types of interruption of the classical visual cycle involving the rod photoreceptors and the retinal  
10 pigment epithelium. These observations support that accumulation of A2E in the RPE requires a  
11 normal flux of retinoids through the classical visual cycle. Additional support is found in fundus  
12 autofluorescence being absent in Leber congenital amaurosis and EOSRD caused by *RPE65*  
13 mutations.<sup>14</sup>

14

15 There is evidence that the *RPE65*, *RDH5* and *RLBP1* (cellular retinaldehyde-binding protein)  
16 interact physically in a retinoid processing complex, where the latter, being expressed throughout  
17 the RPE cell, mediates diffusion of retinoids from the apical processes of the retinal pigment  
18 epithelium into its cell somata, in which *RDH5* and *RPE65* localize.<sup>21-22</sup> *RLBP1* mutations are  
19 associated with retinitis punctata albescens (RPA), which shares phenotypic similarities with fundus  
20 albipunctatus, including delayed dark adaptation.<sup>23</sup> *RPE65* mutations on the other hand are  
21 associated with Leber Congenital Amaurosis and EOSRD,<sup>14-16</sup> and have hitherto not been  
22 associated with either of the aforementioned conditions fundus albipunctatus or RPA.

23 The presumed accumulation of cis-retinol and cis-retinyl esters in the RPE because of 11-cis-retinol  
24 dehydrogenase deficiency may be responsible for the formation of white dots in *RDH5* mutation

1 associated fundus albipunctatus.<sup>24</sup> It is not clear, however, why such accumulation should result in  
2 highly focal rather than diffuse accumulation of material. Furthermore, the nature and content of  
3 these dots remain uncertain. Theoretical possibilities include retinyl ester storage pools in the RPE  
4 in intracellular organelles termed retinosomes,<sup>25,26</sup> and/or uninhibited phagosome release and  
5 subsequent accumulation of debris, which may be ingested by subretinal microglia.<sup>27,28</sup>

6

7 The mechanism whereby slow dark adaptation can exist in fundus albipunctatus is not clear. Recent  
8 studies support that the RPE has an alternative pathway for the production of 11-cis-retinal,  
9 involving RDH10 for the production of 11-cis-retinal, which may account for the delayed dark  
10 adaptation in patients with fundus albipunctatus and *RHD5* or *RPE65* mutations.<sup>29</sup> This subnormal  
11 production of photopigment may be important for the long-term integrity of the retina and may  
12 explain the increase of the ffERG response after prolonged dark adaptation in fundus albipunctatus.

13

14 In our patient with compound heterozygous mutations in *RPE65*, the cone 30 Hz flicker response  
15 was reduced after standard dark adaptation and increased by >50% after prolonged dark adaptation  
16 (Table 1 and Figs. 6,7 [available at <http://aaajournal.org>]). The implicit time of the cone 30-Hz  
17 flicker, a sensitive marker for progressive retinal degenerations affecting the cones, however, was  
18 normal. This predominant affection of the rod system and relative sparing of the cone response may  
19 be due to the intraretinal cone-specific visual cycle, by-passing the RPE.<sup>30</sup> However, the enzyme  
20 responsible for converting 11-cis retinol to 11-cis retinal in cones remains unknown.

21

22 Patient 2:2 was heterozygous for a splice mutation in *RPE65* (IVS1+5G>A), known for its  
23 pathogenic capacity because of loss of function due to inactivation of a splice site leading to  
24 aberrant splicing.<sup>14-16</sup> This mutation has been considered a null mutation. In previous reports,

1 compound heterozygous patients with this mutation (ins144T/IVS1+5G>A and  
2 (1114delA+T457N/IVS1+5G>A), had no recordable fERG responses by 5-10 years of age. It thus  
3 seems that the remaining retinal function in our patient was likely attributable to some function  
4 remaining in the transcript from the other *RPE65* allele, which carried the p.I115T mutation, located  
5 close to a palmitoylated cysteine residue (C112) involved in membrane binding.<sup>31</sup> PSIC and SIFT  
6 indicate a probably disease causing mutation with impact on protein structure which results from  
7 the charge introduced by threonine on substitution of the uncharged isoleucine. Position I115 is  
8 included in a somatostatin (amino acid 106 – 123) and a  $\beta$ -tubulin (amino acid 100 – 152) motif.  
9 Therefore the deficit may not be a loss of isomerohydrolase function but impaired interaction with  
10 11-cis retinol dehydrogenase, which is necessary to release the isomerised chromophore from  
11 RPE65, thus establishing a situation like in RDH5 deficiency when the RLBP1 pool is overloaded  
12 with 11-cis retinal. Alternatively, there may be other enzymes in the RPE with a residual  
13 isomerohydrolase activity, similar to the residual 11-cis-Retinol Dehydrogenase activity  
14 demonstrated for RDH10.<sup>29</sup>

15

16 In summary, we have identified RPE65 dysfunction as a likely cause of fundus albipunctatus.  
17 Indirectly, a reduced autofluorescence of the fundus in a patient with a relatively well preserved  
18 retinal function may indicate that pharmacotherapy interfering at specific points in the visual cycle  
19 may be a feasible treatment strategy for disorders featuring excess lipofuscin accumulation.

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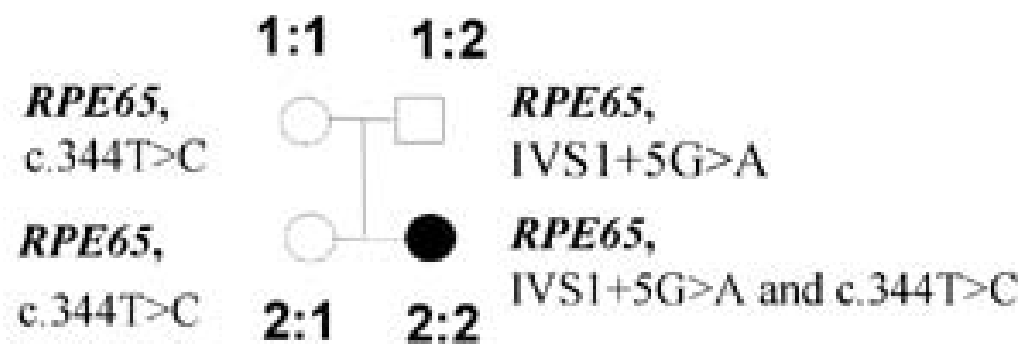
Fig. 1. Pedigree with proband 2:2 with fundus albipunctatus.

Fig. 2. Fundus photographs of subject 2:2 with scattered yellow-white fundus elements typical of fundus albipunctatus.

Fig. 3. Fundus autofluorescence in all family members and optical coherence tomography (OCT) from a region of high density of albipunctate dots in patient 2:2. Reduced fundus autofluorescence as demonstrated in patient 2:2, necessitates an upregulation the laser light of the Heidelberg instrument to obtain a signal. This leads to increased noise, so that the optic disc and vessels appear less dark, and the autofluorescence from the retina appears less regular. Arrows in the Spectralis OCT scan denote Bruch's membrane and the external limiting membrane, the borders between which the hyperreflective lesions corresponding to the albipunctate dots span, comprising the entire retinal pigment epithelium and the photoreceptor outer and inner segments.

Fig. 4. Multifocal electroretinography (mfERG) demonstrates reduced central retinal function and foveal optical coherence tomography demonstrates reduced retinal thickness in patient 2:2.

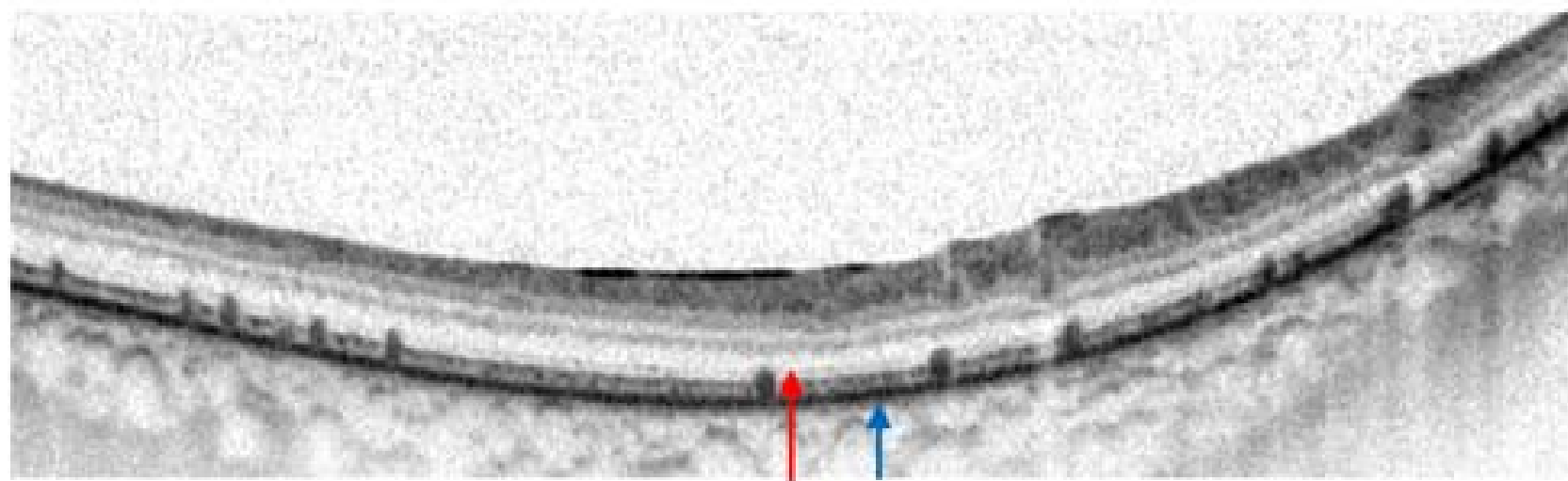
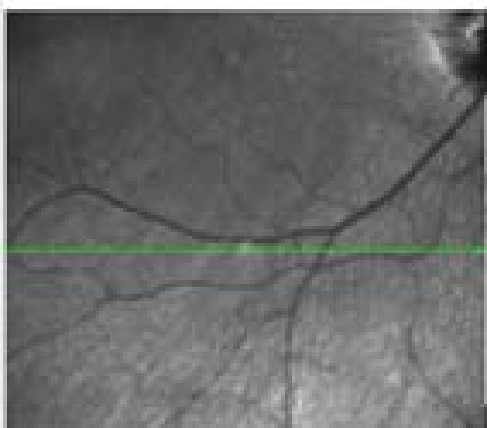
Fig. 5. Optical coherence tomography (OCT) with multiple scan averaging demonstrates reduced length of the photoreceptor outer segments in patient 2:2. Arrows in the OCT from the normal subject delineate the length of normal outer segments.





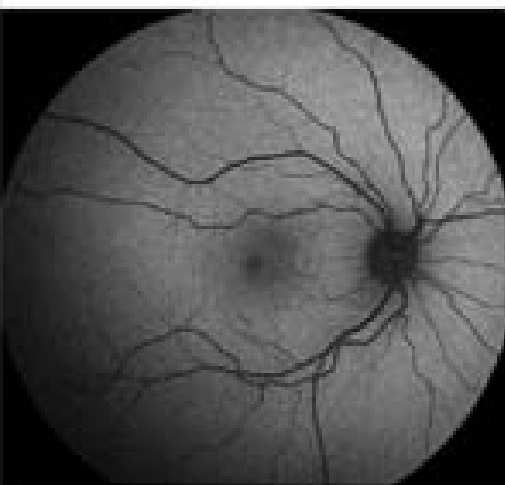
**2:2**

*RPE65*, IVS1+5G>A/c.344T>C



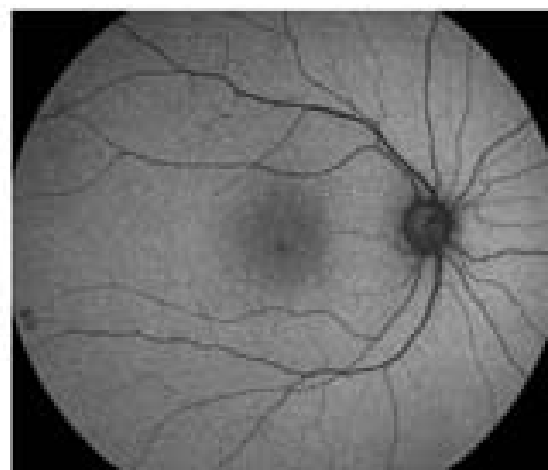
**2:2**

*RPE65*, IVS1+5G>A/c.344T>C



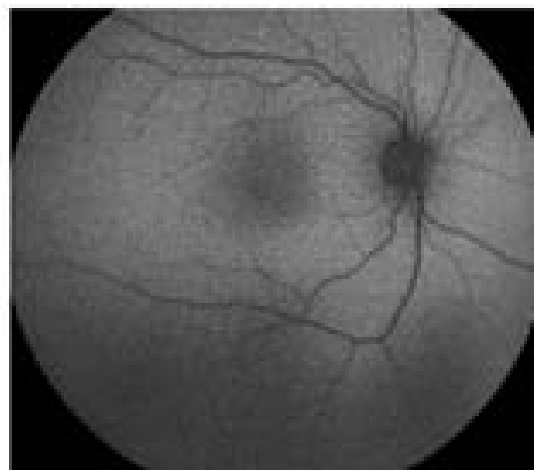
**1:1**

*RPE65*, c.344T>C



**1:2**

*RPE65*, IVS1+5G>A



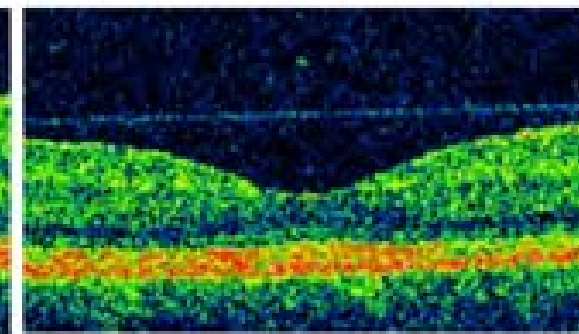
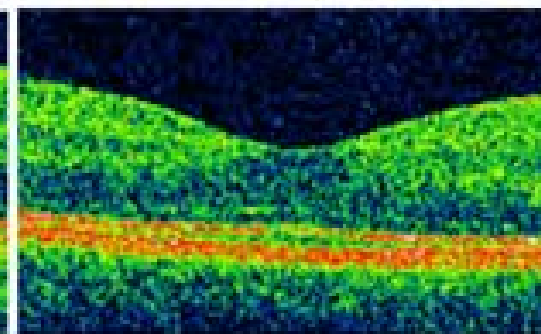
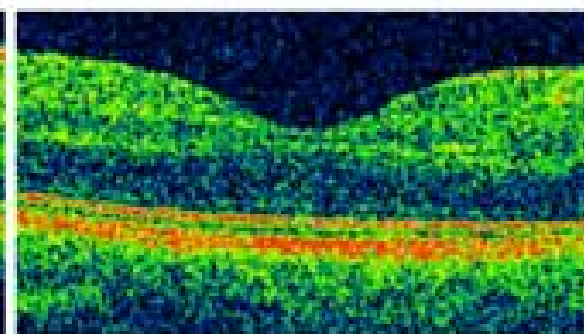
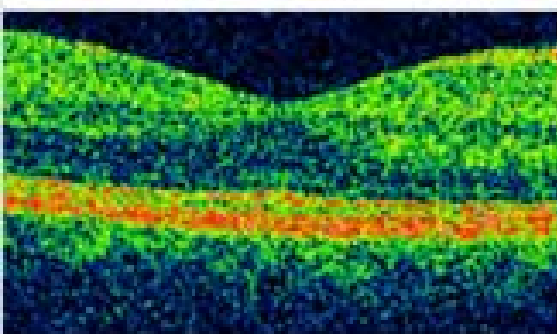
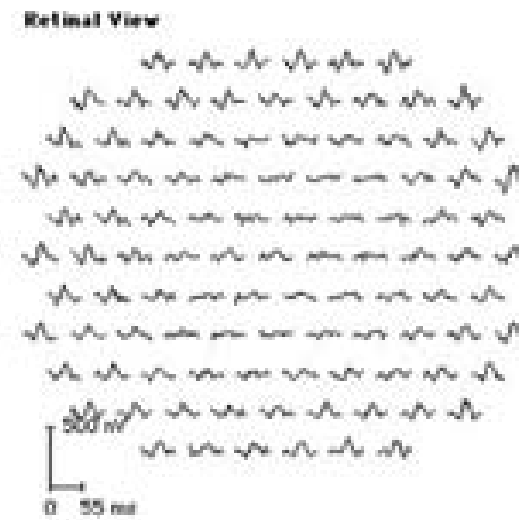
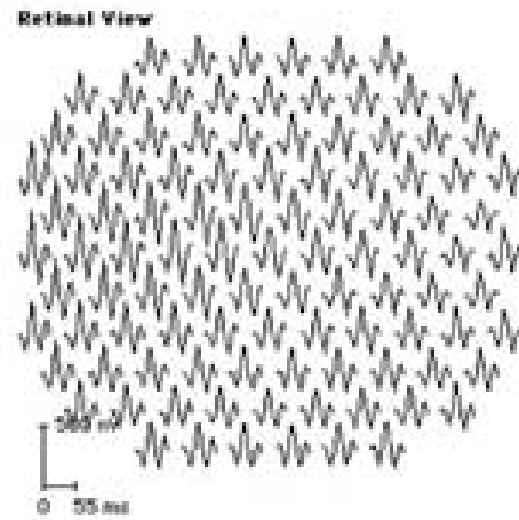
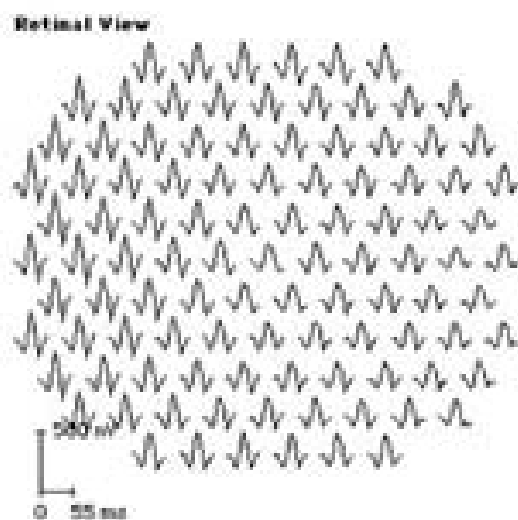
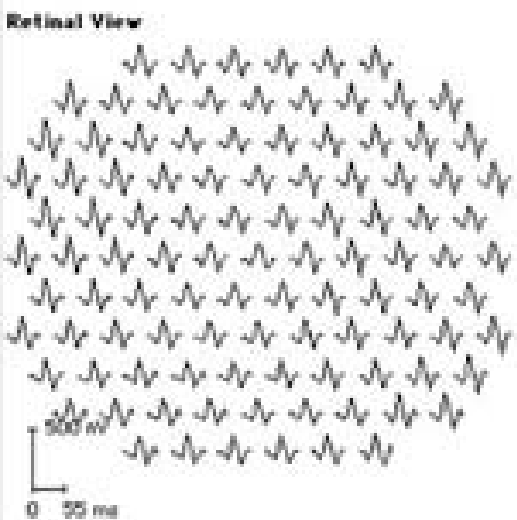
**2:1**

*RPE65*, c.344T>C



**2:2**

*RPE65*, IVS1+5G>A/c.344T>C



**1:1**

*RPE65*, c.344T>C

**1:2**

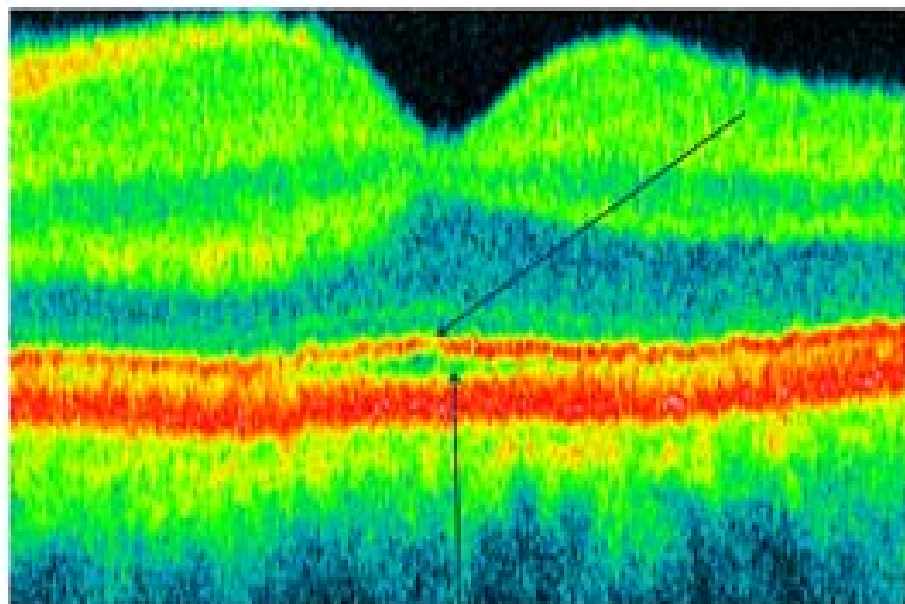
*RPE65*, IVS1+5G>A

**2:1**

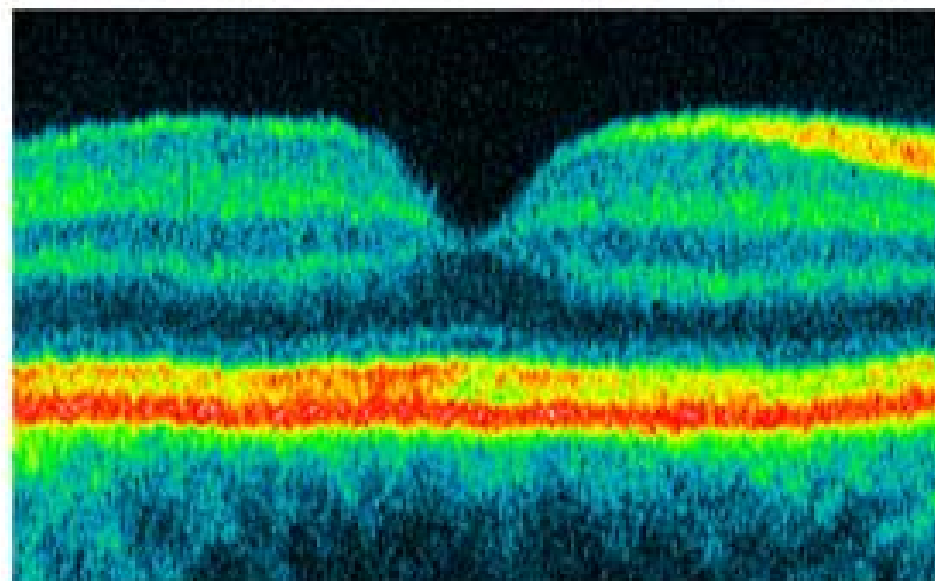
*RPE65*, c.344T>C

**2:2**

*RPE65*, IVS1+5G>A/c.344T>C



**Normal**



**2:2**

*RPE65, IVS1+5G>A/c.344T>C*

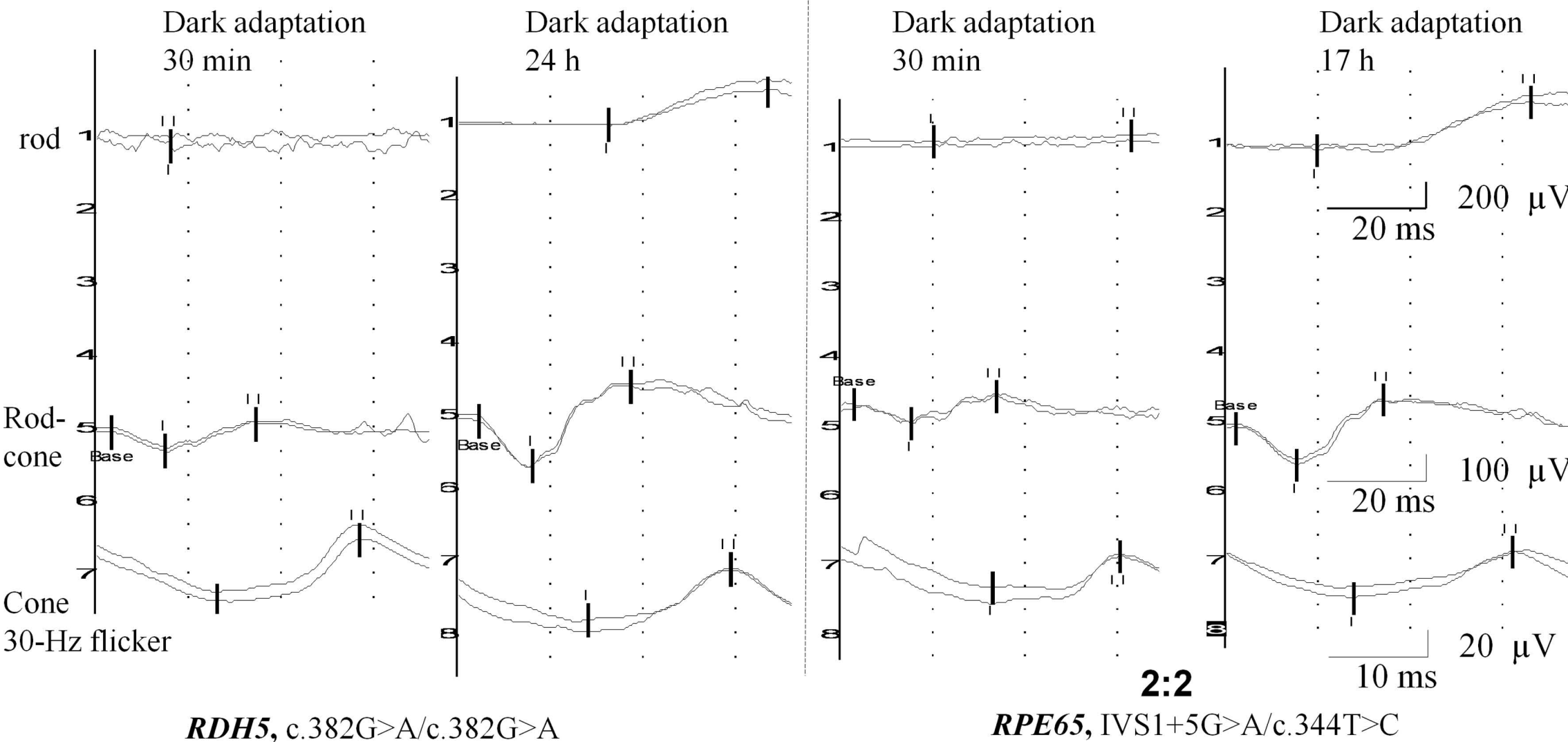


Fig. 6. Left panel: ffERG responses recorded by us previously in a 19-year old patient with fundus albipunctatus due to homozygous mutations in *RDH5*.

Right panel: Full-field electroretinography (ffERG) in patient 2:2 demonstrates a characteristic response featuring a barely recordable rod response after standard dark adaptation and normalization, increase by >700%, of the response after prolonged dark adaptation. The cone 30 Hz flicker response is reduced after standard dark adaptation and increased by >50% after prolonged dark adaptation. There is a striking similarity in the response dynamics in the two patients.



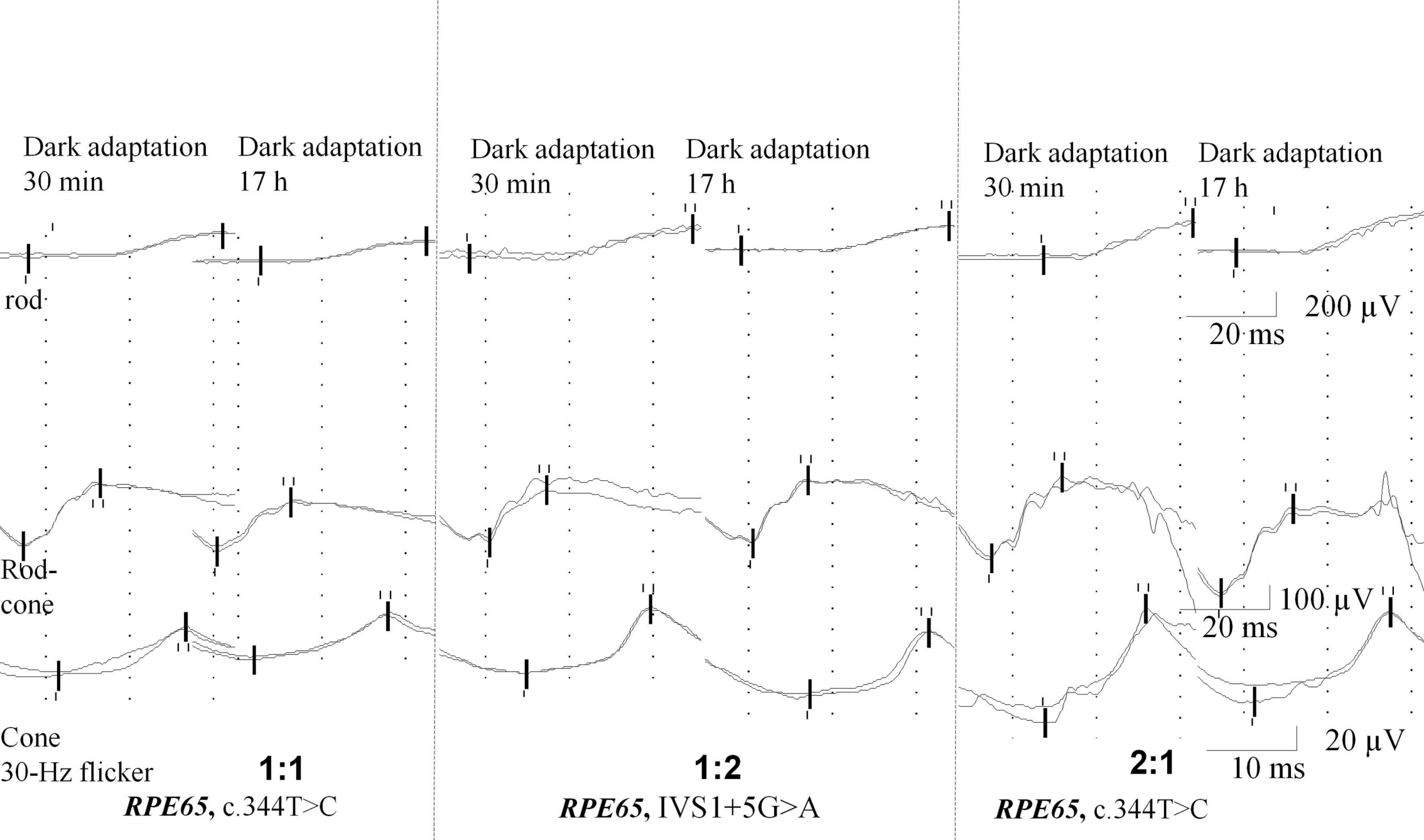


Fig. 7. Full-field electroretinography (ffERG) responses in the remaining family members. No significant change was seen after prolonged dark adaptation compared to after standard dark adaptation.

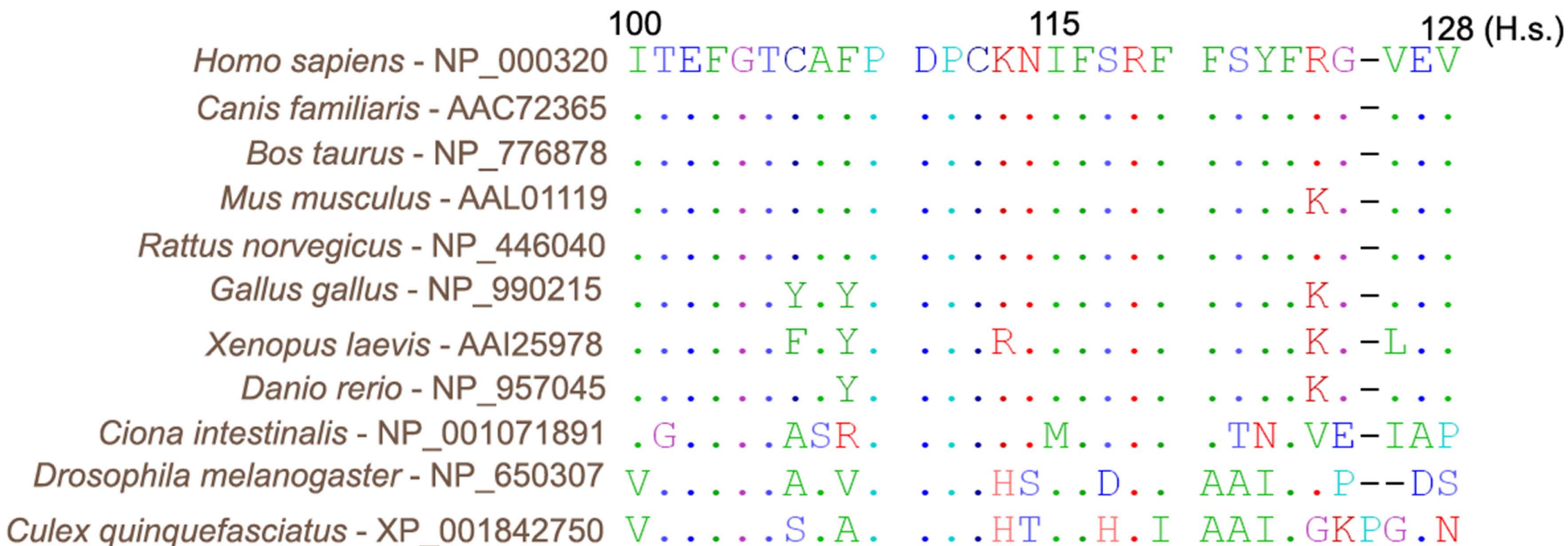


Fig. 8. Clustal W alignment of protein sequences of 11 species from man to diptera. Amino acid numbering refers to the human sequence, H.s. Isoleucine is highly conserved with the exception that methionine is found in *Ciona intestinalis*, a tunicate.

**Table 1.** Clinical, electrophysiological and molecular genetic data in a family with compound heterozygous mutations in *RPE65*.

Patient		1;1	1;2	2;1	2;2	Normal median (range)	
Age, sex		49, F	47, M	20, F	18, F		
<i>RPE65</i> mutation		c.344T>C	IVS1+5G>A	c.344T>C	IVS1+5G>A/ c.344T>C		
Effect		p.I115T	splice	p.I115T	splice/p.I115T		
OCT foveal thickness OD/OS (µm)		206/204	208/202	216/215	132/134	182 (157-207)	
Visual acuity OD/OS		0.9/0.9	0.9/0.9	0.9/0.9	0.5/0.5		
Mferg	Amplitude (nV/deg <sup>2</sup> )	ring 1-2	42.7	56.2	83.3	10.6	29.3 (22.8-35.2)
	Latency (ms)		26.6	29.9	26.6	27.5	26.3 (25.8-29.5)
	Amplitude (nV/deg <sup>2</sup> )	ring 3-6	20.6	24.9	33.8	9.1	12.7 (10.6-20.2)
	Latency (ms)		25.0	27.4	24.9	27.5	26.3 (25.0-28.3)
Full field ERG	Amplitude (µV)	DA 30 min	122	143	174	14	137 (64-221)
		DA 17h	95	120	168	109	
		DA 30 min	292	255	387	63	305 (158-546)
		DA 17h	241	302	410	285	
		DA 30 min	47	62	109	26	53 (20-117)
		DA 17h	43	60	87	41	
	Implicit time (ms)	DA 30 min	26.6	29.6	25.8	30.2	29.1 (25.2-33.2)
		DA 17h	27.7	31.3	27.3	31.0	

OCT=optical coherence tomography, mfERG=multifocal electroretinography, DA=dark

adaptation, min=minute, h= hour, ms=milliseconds, nV/deg<sup>2</sup>=nanovolt per degree.

The control group for mfERG was based on examination of 8 individuals, mean age 43 years; range 14–58 years.

The control group for the full-field ERG was based on the examination of 23 individuals, mean age, 50 years; range 37–72 years.

The control group for the OCT was based on the examination of 20 eyes of 11 subjects.