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Choroideremia: Analysis of the retina from a Female Symptomatic Carrier

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Abstract

Purpose—To define the retinal pathology in a 91 year-old affected matriarch of a three-generation choroideremia family with multiple manifesting carriers.

Methods—Tissue from three different retinal areas was processed for immunohistochemistry. The macular area was processed for transmission electron microscopy. Cryosections were studied by indirect immunofluorescence, using well-characterized antibodies to cone cytoplasm, rhodopsin and cone opsins. The affected donor eyes were compared to a postmortem matched normal eye.

Results—The retina displayed areas of severe degeneration, with no photoreceptor outer segments, photoreceptor nuclear atrophy, and atrophy of the inner retina. Other retinal areas were near to normal. The RPE was severely degenerated, with thinning, pigment clumping and sub-epithelial debris deposition in all the areas examined. The choroid displayed depigmentation. Labeling with cone opsin antibodies revealed that cones were drastically affected: blue opsin was almost completely absent, while red/green opsins were distributed along the entire plasma membrane of the cell. Rhodopsin was also distributed along the entire rod plasma membrane. Ultrastructural analysis of the affected macula revealed the absence of RPE apical microvilli and basal infoldings. Instead, RPE's basal surface and choroid displayed the presence of banded fibers composed of clumps of wide-spacing collagen. Bruch's membrane was filled with vesicular structures, some smooth and others with bristle-like projections.

Conclusions—The histological data suggests that the clinical manifestation in this donor is related to degenerative changes in the retina, RPE and choroid.

Keywords

Choroideremia; carrier state; immunohistochemistry; cone opsins; rhodopsin

Introduction

Choroideremia is a rare (1:50,000 males) X-linked recessive degeneration characterized by progressive atrophy of the photoreceptors, RPE, and choroid in affected males. Vision loss in males is similar to that observed in X-linked retinitis pigmentosa, with night blindness, severe peripheral vision loss, and central visual acuity loss late in the course of the disease.

Female carriers of choroideremia often demonstrate characteristic fundus changes including patchy depigmentation of the RPE and coarse pigmentary granularity in the periphery. However, most carriers retain good visual function throughout life, with either no symptoms or mild to moderate night blindness. Significant visual impairment in female carriers of choroideremia is uncommon, and typically attributed to skewed X-inactivation leading to a high percentage of cells expressing the mutant X chromosome¹, although subretinal neovascularization or fibrosis has been reported in at least 2 cases^{2,3}. Full-field ERG recordings in carrier females are typically normal or very mildly reduced^{4,5}; multifocal ERGs may be more sensitive given the patchy nature of cellular loss in the carrier state⁶.

Pathologically, male patients with choroideremia show complete loss of the choroid and outer retina, displaying a thin line of atrophic inner retina lying against the sclera⁷⁻⁹. The complete atrophy of the retina and choroid in male patients precludes identification of the primary defect in these structures. However, the milder changes in female patients present an opportunity for understanding of the mechanisms involved in this pathological process.

Choroideremia is caused by mutations in the *CHM* gene, which encodes component A of Rab geranylgeranyl-transferase, referred to as Rab escort protein (REP-1)¹⁰⁻¹². However, it is still unclear how mutations in this protein lead to the degeneration of the choroid, RPE and retina. In the present study we analyzed the retina, RPE and choroid morphology and the distribution of photoreceptor markers in a donor eye from a female symptomatic carrier of X linked choroideremia.

Materials and Methods

Patient information

The clinical evaluation of the affected members was carried out at the Casey Eye Institute at Oregon Health & Science University in Portland, OR with the approval of the OHSU Institutional Review Board (IRB). The donor requested eye donation prior to her death; organ donation was coordinated through National Retinitis Pigmentosa Foundation Donor Program (donation number #788).

The patient and several other affected members in the family were clinically evaluated.

Molecular Genetics

Genomic DNA was prepared from peripheral blood lymphocytes using a commercially available kit Gentra DNA kit (Puregene; Gentra Systems, Minneapolis, MN). All fifteen exons of the *CHM* gene and their respective splice site junctions were PCR amplified and sequenced by bi-directional fluorescence sequencing using BigDye Terminator cycle sequencing version 3.1 and the model ABI 3130 sequencer according to the manufacturer's recommendations (Applied Biosystems, Foster City, CA). Primers sequences and PCR conditions are shown in Table 1.

Histopathology

The immunocytochemistry analysis was performed in the Cleveland Clinic Foundation and is exempt of IRB approval. The donor globes were fixed 8hrs postmortem in a mixture of

4% paraformaldehyde and 0.5% glutaraldehyde made in 0.1M phosphate buffer, pH 7.3. After 1 month in fixative, the globes were transferred and stored in 2% paraformaldehyde prepared in the same buffer. The eyes from a 68 year-old female and a 91 year-old male were used as controls, and were fixed 14.5hr and 7.5hr post-mortem in 2% paraformaldehyde made in the same buffer. Tissue from three different retinal areas were cut, dehydrated through a series of ethanol solutions and embedded in paraffin using an automated tissue processor (Leica Microsystems TP1020, Benneck Burn, IL). 7–8µm sections were cut on a Leica RM2125 microtome (Leica Microsystems) and sections were collected on Superfrost/Plus Slides (Fisher Scientific, Pittsburg, PA). Sections were stretched on the slides on water and adhered to the slides by room temperature incubation overnight followed by 2hs incubation in a HI1210 slide warmer at 60°C (Fisher Scientific). Prior to labeling, paraffin was removed through two consecutive xylene incubations for 10 min. The tissue was then gradually re-hydrated by sequential incubation of ethanol 100, 90, 70, 50 and 30% for 5 min. each and processed for immunofluorescence labeling as previously described¹³. Briefly, tissues were blocked in PBS supplemented with 1% BSA (PBS/BSA) for 30 min and incubated with the antibodies in PBS/BSA overnight at 4°C. Cryosections of both the matched control and affected donor tissues were labeled with the following antibodies: rabbit polyclonal antibody AB5407 to blue cone opsin (1: 1200, Chemicon International, Inc., Temecula, CA), rabbit polyclonal antibody AB5405 to red/green cone opsins (1: 1200, Chemicon International, Inc., Temecula, CA), monoclonal antibody B6-30N to rhodopsin (1:50, from Dr. P. Hargrave, University of Florida, Gainesville, FL, U.S.A.), and the monoclonal antibody 7G6 to cone cytoplasm (1:100, from Dr. P. MacLeish, Morehouse School of Medicine, Atlanta, GA). Cell nuclei were labeled with TO-PRO®-3 iodide (1mg/ml, Molecular Probes, Eugene, OR). Secondary antibodies (goat anti-mouse or anti-rabbit IgG; 1:1000) were labeled with Alexa Fluor 488 (green; Molecular Probes) and Alexa Fluor 594 (red; Molecular Probes). Sections were analyzed using a Leica laser scanning confocal microscope (TCS-SP2, Leica, Exton, PA). A series of 1 µm *xy* (*en face*) sections were collected. Each individual *xy* image of the retinas stained represents a three-dimensional projection of the entire cryosection (sum of all images in the stack). Microscopic panels were composed using AdobePhotoshop 5.5 (Adobe, San Jose, CA).

Ultrastructural analysis

A small area of the retina/RPE/choroid tissue from the macula of the affected choroideremia donor and matched-controls (88 and 91 year-old males with 7.5 and 12 hr post-mortem) were fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer, sequentially dehydrated in ethanol and embedded in Epon as previously reported (Bonilha et al., J. Cell Biol. 1999). Thin sections were prepared and electron micrographs were taken on a Tecnai 20 200 kV digital electron microscope (Philips, Hillsboro, OR) using a Gatan image filter and digital camera at 3600 diameters and are printed at identical magnifications.

Results

Clinical Findings

Figure 1 shows the pedigree of the family affected with an X-linked choroideremia. The donor (III-4 of Fig 1) first presented to the Ophthalmic Genetics Clinic at the Casey Eye Institute at the age of 77 with a visual acuity of 20/25 OD, 20/30 OS and no subjective limitation of night vision or peripheral vision. Fundus examination disclosed a striking reticular pigmentary dispersion with fine clumping, but no frank bone spicule pigmentation, in the mid and far periphery of each eye. A large pigmented retinal scar was observed temporal to the right macula OD. Full field electroretinography was normal. Over the

ensuing 14 years, her night vision and central vision gradually decreased; by 91, her family reports that she was significantly visually impaired from loss of central vision.

The donor's family history is unique in that several of the female members appear to be affected to an equal, if not greater degree than the men. The donor's daughter (IV-2 of Fig. 1) was initially diagnosed with retinitis pigmentosa in her early 20's. When she presented to ophthalmic genetics at the age of 41, she suffered from reduced night vision and blurred central vision (20/40-2, 20/25-2). Full-field ERG responses were mildly subnormal. By the age of 54, acuity worsened to 20/300, 20/60+1, and she was unable to drive. ERG responses decreased to approximately one-third of those in the earlier study. Her son and daughter (V-1 and V-2 of Fig 1), who both carry the familial CHM mutation, remain asymptomatic at the age of 35 and 34 years, respectively. The donor, her daughter, and her granddaughter and grandson were molecularly confirmed to carry a splice site mutation on the 5' splice site (donor site) of intron 11 in the *CHM* gene c1413+1G>. This mutation is expected either to cause the retention of intron 11 or skipping of exon 11 which in either case will produce an unstable mRNA leading to a nonsense-mediated mRNA decay (NMD) and the absence of the **REP-1**.

Morphological Pathology

Semi-thin sections of epon-embedded tissue were analyzed and compared to matched controls [Fig. 2 A–B]. In female patients, the severity of disease expression is related to the proportion of cells expressing the mutant X chromosome. As a consequence, we focused our observations in three different regions in the periphery of the donor. The schematic drawing in the upper corner of this figure depicts the regions harvested and processed for observation in both the morphological and immunohistological assays [periphery regions 1, 2 and 3 and macular region 4]. The retina of the affected donor displayed different degrees of photoreceptor degeneration in each of the regions observed when compared to the morphology of the control retina in the periphery [Fig. 2A] and macular [Fig. 2B] region of the eye. Extensive degeneration could be observed in all retinal layers in all analyzed regions [Fig. 2C1, D2, E3]. Some pigmented cells are seen invading the degenerating retina [Fig. 2C1, arrowheads]. The RPE of the affected donor displayed typical thinning, pigment clumping and basal laminar deposits [Fig. 2F1, G2, H3] when compared to the matched control [Fig. 2A and B]. Extensive accumulation of amorphous material underneath the RPE layer was observed [Fig. 2 G2, *] while in another area the presence of inflammatory cells [Fig. 2H3, small arrows] was observed in the choroid. In the macula, the RPE was thinned with pigment clumping, soft and hard drusen deposits [Fig. 2I4, J4, K4, large arrows], widespread photoreceptor degeneration, and giant lipophilic drops in the choroid when compared to the matched control [Fig. 2B].

Histological Pathology

To further understand some of the molecular changes associated with this X-linked choroideremia phenotype in this family, tissue from the same regions described above [regions 1–3 in Fig. 2] were also processed for paraffin embedding and immunofluorescence. Initially, the distribution of the cone cytoplasmic marker 7G6 was analyzed in both the symptomatic carrier and a matched-control eye [Fig. 3A–D]. No significant differences were observed between the control sample [Fig. 3A] and all the three other areas observed in the affected donor [Fig. 3B–D] with cones displaying normal morphologic characteristics of cone photoreceptors. Moreover, cell nuclei, labeled with TO-PRO-3, did not display any significant difference to the matched control. However, a striking abnormal distribution of the red/green opsins throughout the entire cone cell body was observed in all the affected donor regions [Fig. 4B–D] when compared to the matched

control eye [Fig. 4A]. This abnormal distribution was observed from the outer segments to the cone pedicles in the inner nuclear region of the retina of the affected donor.

In contrast, the blue cone opsin of the affected donor eye [Fig. 5 B–D] was almost completely abolished when compared to the matched-control eye [Fig. 5A]. However, region 2 displayed a few cones that still expressed blue opsin distributed throughout the inner and outer segments of this cone type [Fig. 5B, arrow].

The rod outer segments were visualized through labeling with rhodopsin antibodies. Similar to the red/green opsin, a striking unrestricted distribution of rhodopsin was observed throughout the entire rod cell body of the affected eye [Fig. 6 B–D] when compared to the control-matched eye [Fig. 6A].

Ultrastructural pathology

The ultrastructure of RPE and Bruch's membrane in the macular area was analyzed by TEM. Observation at low magnification showed photoreceptor outer segments (POS) lying on top of a collapsed RPE apical surface. No RPE microvilli are visible on the RPE apical surface. Moreover, the RPE cytoplasm is filled with small vacuoles and pigments (P) [Fig. 7A]. Examination of the RPE's basal surface revealed complete absence of basal infoldings and the presence of banded fibers composed of clumps of banded material with 1000Å periodicity consistent with long spaced collagen [Fig. 7B–D, arrows, B, C, D]. The choroid, Bruch's membrane, and the space between the RPE basal membrane was filled with vesicular structures, some smooth and others like bristle-coated vesicles [Fig. 7B and C]. Higher magnification of this area is shown in Fig. 7C. Beneath the RPE, the banded fibers composed of clumps of wide-spacing collagen are continuous with a drusen characterized by the presence of residual bodies, vesicular material and filaments [Fig. 7D]. The basement membrane is not visible in this area.

Discussion

This study demonstrated substantial redistribution in the photoreceptor proteins, specifically red/green cone opsin and rhodopsin, as compared to normal control tissues. These changes included loss of opsin restriction to the cone and rod outer segment resulting in its distribution throughout the entire cell body. Moreover, the blue cone opsin was significantly decreased in the affected retina. With the exception of one of the three observed areas, no blue opsin was detected in the affected retina. Rhodopsin was also found to be distributed along the entire cell body.

Opsins are the protein component of the photopigments, which absorb photons of light. This reaction leads to a change in molecular shape and the subsequent release of energy, the first step in the visual transduction cascade. Two families of opsins are generally recognized due to different spatial expression and evolutionary histories. Rhodopsin, which is used in night vision, is a high-sensitivity, low-acuity opsin found in the rod photoreceptor cells. Cone opsins, employed in color vision, are low-sensitivity, high-acuity opsins located in the cone photoreceptor cells. Cone opsins are further subdivided according to their absorption maxima ($\lambda_{\max}$), the wavelength at which the highest light absorption is observed. Evolutionary relationships, deduced using the amino acid sequence of the opsins, are also frequently used to categorize cone opsins into their respective group. Both methods predict four general cone opsin groups in addition to rhodopsin¹⁴. Our immunofluorescence studies were carried out with antibodies specific to both red/green and the blue cone opsins. Our labeling of the red/green opsins detected a large population of these cone types throughout the entire retina when compared to those with blue cone opsin. This result is in agreement

with several reports demonstrating that the human retina is composed of roughly 90% red/green and 10% blue cones^{15–18}.

In the present case, a striking cone abnormality was the presence of red/green opsins throughout the entire cone cell body instead of the usual restriction to the outer segment. A previous study of enhanced S-cone syndrome donor eyes reported the presence of abnormal distribution of both S- (blue) and L/M- (red/green) cone opsins along the entire plasma membranes of the cell, including their inner segments, cell bodies, axon and pedicles¹⁹. Moreover, a patient with X-linked cone degeneration (a known 6.5-kilobase deletion in the red cone pigment gene) showed a significant diminution of the red and green cone population when compared with an age-matched control eye. However, some remaining red and green cones without outer segments did stain weakly with the antibody to red and green opsin in the inner segments²⁰. More recently, an affected member of a clinically well characterized family with an autosomal dominant form of cone dystrophy was also shown to exhibit distribution of the red/green cone opsin from the tip of the outer segment to the synaptic base of this cone type¹⁵. A recent retinitis pigmentosa GTPase regulator (RPGR)-deficient murine model also was shown to have initial ectopic or unrestricted distribution of both red/green and blue opsins and a reduced level of rhodopsin followed by the subsequent degeneration of cones and rods²¹. This report suggested that mislocalization of cone opsins may precede cone cell death. This conclusion was strengthened by data from another report showing that both rod- and cone-mediated function declined with age as did the central retinal thickness in a group of clinically defined and genotyped patients studied²².

Although the molecular mechanism of visual excitation in the cone is not as well understood as in the rod, it is generally thought to involve a cone-specific G protein (cone transducin) that couples the cone visual pigment to a cGMP phosphodiesterase. The functional significance of the red/green opsin delocalization is not known but it could result in a major loss of sensitivity, which, in turn, could lead to sufficient cone degeneration to cause the visual symptoms experienced by the donor. Mura et al⁵ performed light- and dark-adapted thresholds in an obligate CHM carrier, and found that while most of the 500-nm dark-adapted thresholds were within reference limits, 650-nm fully dark-adapted thresholds were significantly elevated at all loci tested. The authors suggest that impaired cone function in the dark-adapted state is the most likely explanation for reduced night vision in symptomatic carriers of CHM. In this context, our present observations might contribute to the night vision and central vision loss observed in this donor.

The blue cone opsin reactivity was significantly diminished in the affected donor. To our knowledge this is the first report of such pathology in the retina of a choroideremia patient. One of the possible explanations for this observation is that the antigenic epitopes might be masked in this donor since there was no alteration of the labeling with the cone cytoplasmic marker. Another possibility is loss of blue cones in this donor.

This case was also characterized by the striking abnormal distribution of rhodopsin throughout the entire rod cell body. A previous study carried out in a female carrier of choroideremia also detected patchy ectopic distribution of rhodopsin²³.

It has been shown that choroideremia is caused by mutations in the REP-1 gene that encodes component A of Rab geranylgeranyl-transferase or REP-1, a protein involved in intracellular vesicular transport^{10–12, 24}. However, little is known about how mutations cause disease and degeneration of the choroid, RPE and retina. Rab geranylgeranyl-transferase A-B holoenzyme attaches (3)H-geranylgeranyl groups to Rab GTPases, a reaction which regulates Rab intracellular trafficking since the mutation in Rep1 prevents the modification

of Rab proteins, thereby disrupting Rab-mediated intracellular trafficking²⁵. Recently, REP-1 was localized to rod inner segments, cell bodies and synapses suggesting that rods are the primary site of degeneration in this disease²³. Our observations of cone degeneration suggest an important role of cones in the choroideremia pathology. Also recently, the retinal pathology of zebrafish rep1 mutants carrying a nonsense mutation in the homologous REP1 gene was described²⁶. However, the author did not see any abnormal distribution of rhodopsin, red/green and blue opsins in the photoreceptor cells of the mutant zebrafish.

The ultrastructural observation of the affected macula revealed absence of RPE apical microvilli and basal infoldings. Moreover, the RPE's donor basal surface was dominated by the presence of banded fibers composed of clumps of wide-spacing collagen. Bruch's membrane was filled with vesicular structures, some smooth and others like bristle-coated vesicles. Similar findings were previously reported when the functions of the RPE begin to fail in the senile maculopathies²⁷. The reported RPE ultrastructure is consistent with RPE cells that are not able to carry out several of their nurturing functions.

In conclusion, we report here the clinical findings and abnormal distribution of the red/green cone opsins and rhodopsin throughout the entire cone and rod cell bodies from a member of a family with an X-linked recessive retinal degeneration. The blue cones were also affected since they exhibited very low reactivity to the blue cone opsin antibody. These results are important to our understanding of one of likely several molecular mechanisms underlying choroideremia in the context of high clinical variability. It also suggests that vision loss in choroideremia may result not only from abnormal REP-1 but also from secondary neuronal death and corrupted retinal circuitry due to the irreversible effects of retinal remodeling.

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Fig. 1.

Pedigree of a family with an X-linked choroideremia showing the symptomatic carrier member (III.4) on whom the postmortem analysis was performed. * Molecularly confirmed to carry 1413+1 G>A in *CHM*; II-2: By report, totally blind by early 50's; III-2: By report, legally blind in adulthood; III-4: Propositus. Night blindness & central vision loss late in life (>75yo); IV-2: Night blindness & significant central vision loss in 50's. Abnormal rod & cone responses on ERG; V-1: Salt and pepper retinopathy; asymptomatic at 34 years old; V-2: Mottling of inferior RPE; asymptomatic at 33 years old.

The symbol x indicates patients examined personally; hatched symbols, patients who are affected by history but not personally examined; arrow, proband; single oblique line, deceased.

Fig. 2.

Degeneration in the retina of a female symptomatic carrier of choroideremia. Human 1 μ m plastic sections of both a matched control and an affected choroideremia stained with toluidine blue. **Top panel corner depicts a fundus image of the studied eye with a schematic drawing of the regions cut and processed for cryosectioning (A).** **Morphology of control retina in the perimacular region of the affected eye (B).** (C1, D2, E3) The retina of the affected donor displayed different degrees of photoreceptor degeneration in each of the regions observed. In C1 some pigmented cells are seen invading the degenerating retina (arrowheads). (F1, G2, H3). RPE of the affected donor displayed typical thinning, pigment clumping, basal laminar deposits. In G2 extensive accumulation of amorphous material underneath the RPE layer is observed (*) while in H3 the presence of inflammatory cells (small arrows) is observed in the choroid. (I4, J4, K4) Different areas of the affected donor displaying deposition of drusen under the RPE (large arrows), RPE thinning and pigment clumping, photoreceptor degeneration and the presence of giant lipophilic drops in the choroid (**). **RPE= retinal pigment epithelium; Ph=photoreceptors; ONL= outer nuclear layer; OPL= outer plexiform layer; INL= inner nuclear layer; IPL= inner plexiform layer; GCL= ganglion cell layer.** Bar= 200 μ m.

Fig. 3.

Cone cytoplasmic marker distribution is normal in a female symptomatic carrier of choroideremia. Human paraffin sections of both a matched control (A) and affected choroideremia donor (B, C, D) were labeled with an antibody specific to cone cytoplasm (green) while cell nuclei were labeled with TO-PRO-3 (blue). Sections were analyzed using a Leica laser scanning confocal microscope. Comparison of the samples showed that there was no significant difference in the overall morphology of the control and affected donor cones. Bar = 40 μ m.

Fig. 4.

Red/Green cone opsins are distributed along the entire plasma membrane of this cone type in a female symptomatic carrier of choroideremia. Human paraffin sections of both a matched control (A) and affected choroideremia donor (B, C, D) were labeled with antibodies specific to red/green cone opsins (green) while cell nuclei were labeled with TO-PRO-3 (blue). Sections were analyzed using a Leica laser scanning confocal. Comparison of the samples showed that red/green cone opsins are distributed along the entire plasma membrane of this cone type, from the tip of the outer segment to the synaptic base in all the observed regions of the affected eye. Bar = 40 μ m.

Fig. 5.

Blue cone opsins are significantly decreased in this cone type in a female symptomatic carrier of choroideremia. Human paraffin sections of both a matched control (A) and affected choroideremia donor (B, C, D) were labeled with antibodies specific to blue cone opsins (green) while cell nuclei were labeled with TO-PRO-3 (blue). Sections were analyzed using a Leica laser scanning confocal microscope. Comparison of the samples showed that blue cone opsins are significantly decreased in all the observed regions of the affected eye. Region 2 displays a few cones that still express blue opsin but it is distributed throughout the inner and outer segments of the cones (arrow). Bar = 40 μ m.

Fig. 6.

Rhodopsin is distributed along the entire plasma membrane of the rods in a female symptomatic carrier of choroideremia. Human paraffin sections of both a matched control (A) and affected choroideremia donor (B, C, D) were labeled with antibodies specific to rhodopsin (Alexa488, green) while cell nuclei were labeled with TO-PRO-3 (blue). Sections were analyzed using a Leica laser scanning confocal microscope. Comparison of the samples showed that rhodopsin is distributed along the entire plasma membrane of rods, from the tip of the outer segment to the synaptic base in all the observed regions of the affected eye. Bar = 40 μ m.

Fig. 7.

Ultrastructural evidence of RPE degeneration in a female symptomatic carrier of choroideremia. The ultrastructure of RPE and Bruch's membrane was analyzed by TEM. Observation at low magnification showed photoreceptor outer segments (POS) lying on top of a collapsed RPE apical surface **while a control-matched eye displayed several apical microvilli supporting the POS (A)**. The matched control displayed extensive basal infoldings (BI) in their basal surface on top of a well-structured aged Bruch's membrane (BM). The choroideremia donor cytoplasm was filled with small vacuoles and pigments (P) (C). Observation of RPE's basal surface in the choroideremia donor revealed absence of basal infoldings and the presence of banded fibers composed of

clumps of wide-spacing collagen (arrows, D, E, F). The choroid (Ch), Bruch's membrane (BM) and the space between the RPE basal membrane was filled with vesicular structures, some smooth and others like bristle-coated vesicles (D, E). Higher magnification of this area is shown in E. Another area of the choroideremia donor eye displayed drusen (Dr) and beneath it Bruch's membrane contained residual bodies, vesicular material and filaments (F). Bars: C, D, F = 2 μ m and A, B, E = 1 μ m.

Table 1

Primers and conditions used for mutation detection in the CHM gene

Exon	Primer	Sequence: 5'>3'	Amplicon size	Tm
1	EX1FW	GACCTTCCACCCAAGAACTAC	218	55
	EX1RV	ACAGTCTTCCTAAACTTTGTCC		
2	EX2FW	AGGATGGGTCTCTTTGGTGGTT	497	55
	EX2RV	GGGGCAGGCATGAGGTTTC		
3	EX3FW	CTTTTAGCCTACCACTTCCACTTA	468	55
	EX3RV	CGCACCCGAGCTCTATTTAT		
4	EX4FW	TTGCATGTTTCACACTGCCCAC	222	55
	EX4RV	AGTCATTAATTTAGTTTACCTGCAG		
5	EX5AFW	CCCTGTCTGTCTTAAATTCTCAAAA	1096	60
	EX5BRV	TATCCCTTCCCTAACCCCAACC		
6	EX6FW	CCAATTTTCTACTATTTCAAC	214	55
	EX6RV	AACTTAAGCTGATGCCAGT		
7	EX7FW	GCCTGGCTAGAGTGGAACGAT	473	55
	EX7RV	ATGGGAGCCCTTGAAATACAGC		
8	EX8FW	AGTTTAGTTCTGATTTTAAGTG	309	55
	EX8RV	CACTTTTAGAAGGACAAGAA		
9	EX9bFW	TTTCGGGGGATAATTGGCTCTTG	387	53
	EX9RV	TATATATGAAGGTTACTTATATC		
10	EX10FW	AAGCCCTCAAAATAGCAACAAGAA	567	55
	EX10RV	TCACCTCCCAAAACCAAGTCAAGT		
11	EX11FW	CGAAACTTATCCATGGAATC	204	55
	EX11RV	GTGTAGTGATTAGTTCACCA		
12	EX12FW	ATGTCCTAATCTGAATATCTAAAATC	580	55
	EX12RV	ATCTCTACAAATACAACCTTCACAACT		
13	EX13FW	GCTCAGCTCTCTATTATCCAT	254	55
	EX13RV	GAAGATTATGATGGTTACAT		
14	EX14FW	TAGGCTACACAGTGTAGTAA	317	55
	EX14RV	GACTTCTCTCCTCCCAGAGG		
15	EX15FW	TGAACACTTGATAATGAACCTGATG	588	55
	EX15RV	CCCTTTTGGATTCTATTACCTATT		