Expanding the genetic spectrum of choroideremia in an Australian cohort: report of five novel CHM variants

Terri L. McLaren1,2, John N. De Roach1,2, Jennifer A. Thompson1, Fred K. Chen1,2,3,4,5, David A. Mackey1,2,3, Ling Hoffmann1, Isabella R. Urwin1 and Tina M. Lamey1,2

Abstract
Choroideremia is an X-linked chorioretinal dystrophy caused by mutations in the CHM gene. Several CHM gene replacement clinical trials are in advanced stages. In this study, we report the molecular confirmation of choroideremia in 14 Australian families sourced from the Australian Inherited Retinal Disease Registry and DNA Bank. Sixteen males (14 symptomatic) and 18 females (4 symptomatic; 14 obligate carriers) were identified for analysis. Participants’ DNA was analyzed for disease-causing CHM variants by Sanger sequencing, TaqMan qPCR and targeted NGS. We report phenotypic and genotypic data for the 14 symptomatic males and four females manifesting disease symptoms. A pathogenic or likely pathogenic CHM variant was detected in all families. Eight variants were previously reported, and five were novel. Two de novo variants were identified. We previously reported the molecular confirmation of choroideremia in 11 Australian families. This study expands the CHM genetically confirmed Australian cohort to 32 males and four affected carrier females.

Introduction
Choroideremia (CHM, OMIM: 303100) is a chorioretinal dystrophy inherited in an X-linked recessive manner with an incidence between 1:50,0007 and 1:100,0007. It is characterized by progressive degeneration of the retinal pigment epithelium (RPE), photoreceptors, and choroid3. Individuals with choroideremia usually present with a distinctive fundus appearance, featuring a scalloped choroid due to atrophy of the choroidal vessels4.

Choroideremia is caused by mutations in the CHM gene (OMIM: 300390), which is located at Xq21.2 and comprises 15 exons5 encoding Rab escort protein 1 (REP-1). Currently, 293 disease-causing variants in the CHM gene are listed in the Human Genome Mutation Database6.

Due to the monogenic nature and distinctive phenotype of this disease, direct sequencing of the CHM gene, with follow-up deletion/duplication analysis where required, has been highly effective for genetic confirmation of clinically diagnosed individuals7. However, next-generation sequencing (NGS) has sometimes unexpectedly identified CHM mutations in male and female individuals with an alternative clinical diagnosis, such as retinitis pigmentosa (RP)8–10. Thus, choroideremia may have a variable phenotype, leading to underreporting11.

Clinical trials for therapeutic gene replacement of the CHM gene are at an advanced stage. Following the first of these trials (NCT0146121312), further phase 1/2 trials are complete or underway. A phase 3 trial (NCT03496012) is underway for 140 participants across clinical sites in the United States, Canada, Europe, and the United Kingdom13. No CHM gene therapy trials are underway in Australia.

The Australian Inherited Retinal Disease Registry and DNA Bank (AIRDR) previously genetically confirmed

Correspondence: Terri L. McLaren (terri.mclaren@health.wa.gov.au)

1Australian Inherited Retinal Disease Registry and DNA Bank, Department of Medical Technology and Physics, Sir Charles Gairdner Hospital, Hospital Avenue, Nedlands, Western Australia, Australia
2Centre for Ophthalmology and Visual Science, The University of Western Australia, 35 Stirling Highway, Crawley, Perth, Western Australia, Australia
Full list of author information is available at the end of the article

© The Author(s) 2020

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

Official journal of the Japan Society of Human Genetics
choroideremia in individuals from 11 Australian families. The primary aim of the present study was to genetically characterize recently recruited pedigrees to update the spectrum and prevalence of CHM mutations in Australian families and to further consolidate potential candidates for future gene-specific clinical trials or treatments. Here, we identified 16 additional males with genetically confirmed choroideremia, two of whom are currently asymptomatic, and 14 asymptomatic carrier females sourced from 14 families. We also identified four female carriers with a vision-threatening phenotype. Such female patients are also relevant to gene-specific clinical trials or treatments but tend to be overlooked.

In the cohort described in our previous study, we genetically confirmed 16 affected males and 12 asymptomatic carrier females sourced from 11 families. Here, we present the combined mutation spectrum, which includes five novel CHM mutations, for all 32 genetically confirmed males from 25 Australian families.

Methods

Research participants

Participants were identified from the AIRDR. Interrogation of the registry identified nine pedigrees not previously reported with at least one individual clinically diagnosed with choroideremia. Three additional pedigrees, each containing one participant clinically diagnosed with RP, were included in this study where previous analyses revealed potentially disease-causing CHM variants. Two families were added after testing negative for the X-linked RP genes RP2 and RPGR, resulting in a suspected diagnosis of choroideremia. In all, 34 participants from 14 families were included in this present study. They comprised 14 symptomatic males, two asymptomatic males, four affected females of varying clinical severity and 14 unaffected, suspected carrier females. For 10 out of 14 families, the proband was a male with clinical features consistent with choroideremia. For two families, there were no consenting affected male participants in the registry. These families are identified here by the numbers 12–25 to distinguish them from families 1 to 11 in our previously published study.

Genetic analyses

DNA samples were collected, processed, and stored, as previously described. Proband DNA was analyzed using various methods (Table 1).

Proband DNA was analyzed by Sanger sequencing of all 15 exons and flanking intronic regions of the CHM gene (Molecular Vision Laboratory (MVL), Oregon, or Australian Genome Research Facility (AGRF), Perth). Where a candidate disease variant was not detected, the possibility of a large deletion/duplication was investigated by TaqMan quantitative PCR (qPCR) (MVL or Casey Eye Institute (CEI), Oregon). Targeted Sanger sequencing was used to verify detected variants, where required, and for testing familial variants in family members.

RefSeq Accession NM_000390.2/3 was used in genetic analyses. Nucleotide 1 corresponds to the A of the ATG translation initiation codon. Sequence variant nomenclature is reported in accordance with the recommendations of the Human Genome Variation Society.

Classification of variant pathogenicity

The pathogenicity of detected CHM variants was ascertained by interrogation of the scientific literature and disease- and locus-specific databases and by in silico analysis, as detailed previously. Variant pathogenicity was classified in accordance with recommendations of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG). Splice site variants at consensus dinucleotides (± 1/2) were automatically assigned a pathogenic status.

Results

Phenotypic data

As the AIRDR is a national registry containing information from participants throughout Australia, phenotypic data such as imaging or electrophysiology results contained in the registry are often incomplete or self-reported. Nevertheless, we report here those phenotypic data that were available.

Male participants

At the time of this study, the age range of the 14 affected males was 8–60 years. Their reported ages of onset ranged from 3 to 28 years. All symptomatic males reported night blindness as a presenting feature, with six reporting constricted fields at this time. One male also reported photophobia as a presenting symptom (Table 2).

Self-reported presenting symptoms were similar among the four families containing more than one symptomatic male (Families 13, 14, 15, and 20), as was age of onset within three families. One exception was Family 14, where self-reported ages of onset differed by nine years (Table 2). This disparity may relate to the generational gap between the proband and his maternal uncle, with the uncle’s existing diagnosis possibly alerting the family to the possibility of disease in the proband.

For each of the two families, family testing revealed the presence of the familial CHM mutation in an asymptomatic male with retinal features consistent with choroideremia.

Female participants

Fourteen of 18 females in this study were asymptomatic. Four females had reported symptoms of varying severity.
| Family member | Status          | Sex | Relationship to proband | Service provider | Analysis method                        | Comment                                                                 |
|---------------|-----------------|-----|--------------------------|------------------|----------------------------------------|-------------------------------------------------------------------------|
| 12-1          | Affected        | F   | Proband                  | AGRF             | Targeted sequencing of familial CHM    | variant first identified via targeted NGS (performed based on externally provided results) |
| 13-1          | Affected        | M   | Proband                  | CEI              | Targeted NGS of ocular genes           | based on externally provided familial genetic data                     |
| 13-2          | Affected        | M   | Brother                  | AGRF             | Sanger sequencing of CHM               |                                                                         |
| 13-3          | Obligate carrier| F   | Mother                   | CEI              | TaqMan qPCR analysis                   |                                                                         |
| 14-1          | Affected        | M   | Proband                  | CEI              | Sanger sequencing of CHM               |                                                                         |
| 14-2          | Affected        | M   | Maternal uncle           | CEI              | Targeted sequencing of familial CHM    |                                                                         |
| 14-3          | Obligate carrier| F   | Mother                   | CEI              | Targeted sequencing of familial CHM    |                                                                         |
| 14-4          | Obligate carrier| F   | Maternal aunt            | AGRF             | Targeted sequencing of familial CHM    |                                                                         |
| 14-5          | Obligate carrier| F   | Maternal aunt            | AGRF             | Targeted sequencing of familial CHM    |                                                                         |
| 15-1          | Affected        | M   | Proband                  | CEI              | Targeted NGS of ocular genes           | based on externally provided results                                    |
| 15-2          | Affected        | M   | Brother                  | AGRF             | Targeted sequencing of familial CHM    |                                                                         |
| 15-3          | Obligate carrier| F   | Daughter                 | MVL              | Targeted sequencing of familial CHM    |                                                                         |
| 16-1          | Affected        | F   | Proband                  | CEI              | Sanger sequencing of CHM               |                                                                         |
| 17-1          | Affected        | M   | Proband                  | AGRF             | Sanger sequencing of CHM               |                                                                         |
| 17-2          | Obligate carrier| F   | Mother                   | AGRF             | Targeted sequencing of familial CHM    |                                                                         |
| 18-1          | Affected        | M   | Proband                  | AGRF             | Targeted sequencing for confirmation    |                                                                         |
| 18-2          | Obligate carrier| F   | Mother                   | AGRF             | Targeted sequencing of familial CHM    |                                                                         |
| 18-3          | Obligate carrier| F   | Daughter                 | AGRF             | Targeted sequencing of familial CHM    |                                                                         |
| 19-1          | Obligate carrier| F   | Proband                  | AGRF             | Targeted sequencing of familial CHM    |                                                                         |
| Family member | Status     | Sex | Relationship to proband | Service provider | Analysis method                  | Comment |
|---------------|------------|-----|--------------------------|------------------|----------------------------------|---------|
| 19-2          | Affected   | M   | Son                      | AGRF             | Targeted sequencing of familial CHM variant | Based on externally provided results; asymptomatic |
| 20-1          | Affected   | M   | Proband                  | AGRF             | Targeted sequencing of familial CHM variant | Based on externally provided results |
| 20-2          | Affected   | M   | Brother                  | AGRF             | Targeted sequencing of familial CHM variant |                     |
| 20-3          | Obligate carrier | F | Mother                  | AGRF             | Targeted sequencing of familial CHM variant |                     |
| 20-4          | Obligate carrier | F | Maternal aunt           | AGRF             | Targeted sequencing of familial CHM variant | Confirmation of externally provided results |
| 21-1          | Affected   | M   | Proband                  | MVL              | Sanger sequencing of CHM BrmM TaqMan qPCR analysis |                     |
| 21-2          | Obligate carrier | F | Mother                  | MVL              | Targeted sequencing of familial CHM variant |                     |
| 22-1          | Affected   | M   | Proband                  | AGRF             | Sanger sequencing of CHM MVL |                     |
| 22-2          | Obligate carrier | F | Mother                  | MVL              | Targeted sequencing of familial CHM variant |                     |
| 23-1          | Affected   | M   | Proband                  | MVL              | Sanger sequencing of CHM MVL |                     |
| 23-2          | Obligate carrier | F | Mother                  | MVL              | TaqMan qPCR analysis |                     |
| 24-1          | Affected   | F   | Proband                  | MVL              | Targeted NGS of ocular genes (Vision Panel v1; 537 genes) CHM variant first identified via targeted NGS (performed given the participant's initial diagnosis of retinitis pigmentosa) |                     |
| 24-2          | Affected   | M   | Son                      | MVL              | Targeted sequencing of familial CHM variant | Asymptomatic |
| 25-1          | Affected   | M   | Proband                  | MVL              | Sanger sequencing of CHM MVL | Analyzed first for variants in XLRP genes, RP2 and RPGR |
| 25-2          | Obligate carrier | M | Mother                  | MVL              | Targeted sequencing of the familial CHM variant |                     |

AGRF Australian Genome Research Facility.
CEI Casey Eye Institute.
MVL Molecular Vision Laboratory.
XLRP X-linked retinitis pigmentosa.
Gene Reference sequence utilized NM_000390.2; NM_000390.3 (GRC37).
| Family ID | Year recruited | Gender | Current age | Age of onset | Years affected | Age at diagnosis | Onset symptoms | Disease progression | Other comments |
|-----------|----------------|--------|-------------|--------------|----------------|-----------------|----------------|--------------------|-----------------|
| 12-1      | 2010           | F      | 90          | 55           | 35             | ND              | Yes            | ND                 | 85: retinae resemble lacework (CN); vision problems increasing; sees flashes of light; blind in one eye; decreased PV in the other |
| 13-1      | 2010           | M      | 33          | 16           | 17             | 17              | Yes            | ND                 | 17: legally blind |
| 13-2      | 2011           | M      | 35          | 16           | 19             | 20              | Yes            | ND                 | 20: stopped driving at night |
| 14-1      | 2014           | M      | 12          | 4            | 8              | 7               | Yes            | ND                 | 8: pigmentation of fundus (CN) |
| 14-2      | 2015           | M      | 23          | 13           | 10             | 20              | Yes            | ND                 | 51: reduced PV |
| 14-4      | 2015           | F      | 59          | 51           | 8              | 55              | Yes            | reduced PV        | 6: legally blind |
| 15-1      | 2012           | M      | 60          | 23           | 37             | 23              | Yes            | ND                 | 56: CV and photophobia very bad; no PV |
| 15-2      | 2012           | M      | 53          | 28           | 25             | 34              | Yes            | reduced PV        | 59: struggles to see dinner on his plate |

Note: PV = photophobia; LE = left eye; RE = right eye; NB = no progression; arRP = atypical retinitis pigmentosa; RP = retinitis pigmentosa.
| Family ID | Year recruited | Gender | Current age | Age of onset | Years affected | Age at diagnosis | Onset symptoms | Disease progression | Other comments |
|-----------|----------------|--------|-------------|--------------|----------------|-----------------|---------------|-------------------|---------------|
| 16-1      | 2015           | F      | 72          | 58           | 14             | ND              | Yes photophobia | slow progression; PV not too bad; stopped driving in early morning (glare) and at night; depth perception problems; dry eyes | Isolated case |
| 17-1      | 2015           | M      | 22          | 16           | 6              | 18              | Yes ND         | no vision problems; slow progression thereafter | Isolated case |
| 18-1      | 2016           | M      | 44          | 10           | 34             | 41              | Yes photophobia | VA 6/6 BE; large areas of atrophy in peripheral and perimacular region and defects in RPE suggestive of choroideremia; ERG consistent with choroideremia (CN); NB in last 2-3 years | Isolated case; astigmatism; ERG: residual cone function, but extinguished rod response |
| 19-1      | 2015           | M      | 14          | N/A          | N/A            | N/A             | N/A           | Asymptomatic male |               |
| 20-1      | 2017           | M      | 27          | 3            | 24             | 6               | Yes ND         | very slow progression; CV fine; gradual loss in PV; gradual increase in NB; drives in daylight; sees flashes of light & floating spots; light to dark adaptation problems | Color blind |
| 20-2      | 2017           | M      | 25          | 6            | 19             | 17              | Yes reduced PV | very slow progression; CV a little blunted; gradual loss of PV; still day/night driving; sees flashes of light & floating spots; light to dark adaptation problems | Myopic |
| 21-1      | 2016           | M      | 8           | 4            | 4              | 4               | Yes ND         | no progression as yet; VA 6/9 BE; PV OK; peripheral pigmented changes; peripheral retinal mottling consistent with choroideremia (CN) | Normal color vision |
| Family ID | Year recruited | Gender | Current age | Age of onset | Years affected | Age at diagnosis | Onset symptoms | Disease progression | Other comments |
|-----------|----------------|--------|-------------|--------------|----------------|-----------------|----------------|--------------------|-----------------|
| 22-1      | 2014           | M      | 29          | 8            | 21             | 13              | Yes reduced PV | 21: CV OK; stopped driving; LE color perception problems | Isolated case |
|           |                |        |             |              |                |                 |                | 25: decreased CV and PV. NB worsened in past few years; LE worse than RE; can still read easily | |
|           |                |        |             |              |                |                 |                | 29: photophobia a recent development | |
| 23-1      | 2010           | M      | 53          | 21           | 32             | 21              | Yes reduced PV | 52: very gradual progression; drives in daylight | Initial diagnosis: RP |
| 24-1      | 2016           | F      | 61          | 28           | 33             | 58              | Yes reduced PV; photophobia | 59: VA 3/60 (RE); 6/18 (LE); NB; PV < 3°; photophobic; contrast sensitivity and color test grossly abnormal; flat or grossly reduced ERGs; slow progression (CN) | Initial diagnosis: RCD |
| 24-2      | 2016           | M      | 26          | N/A          | N/A            | N/A             | N/A            | N/A                | Asymptomatic male |
| 25-1      | 2009           | M      | 17          | 5            | 12             | 6               | Yes reduced PV | 10: VA RE 6/24; LE 6/30; (CN); no full field ERG responses; PV < 10° (CN) | Initial diagnosis: XLRP |

Choroideremia (CHM, OMIM: 303100).
Self-reported information unless indicated otherwise: CN, clinical notes; BE, both eyes; CV, central vision; ERG, electoretinogram; LE, left eye; N/A, not applicable; NB, night blindness; ND, no data; LP, light perception; PV peripheral vision; RCD, rod-cone dystrophy; RE, right eye; RP, retinitis pigmentosa; RPE, retinal pigment epithelium; VA, visual acuity; x, X-linked.
Initial diagnosis refers to the clinical diagnosis (if not choroideremia) at the start of this study. All ages are presented in years.
Gross deletions were identified in 11 families. Breakpoints were not identified for the exon 15 deletions, for the purpose of this paper, we classified them as a single variant. Thus, 13 different causative variants were identified among the 14 families included in this present study.

A genetic diagnosis of choroideremia was therefore confirmed for all nine families with a clinical diagnosis of choroideremia, as well as for the five families with a clinical diagnosis of RP at recruitment, for which no other candidate variants had been detected by previous genetic testing. A clinical re-evaluation of the diagnosis has been made for three of these RP families and is being sought for the other two.

**Genetic results**

A pathogenic or likely pathogenic CHM variant was identified in all 14 families analyzed (Table 3). All males (14 affected and two asymptomatic) and 16 out of 18 females were hemizygous and heterozygous, respectively, for the detected familial CHM variant.

The mothers of two affected males did not possess the familial CHM variant, suggesting de novo events. The apparent de novo variants identified in unrelated, isolated males (17-1 and 22-1) are expected to result in premature termination codons (PTCs): the nonsense variant c.715 C > T, p.(Arg239*) has frequently been described in the literature, while the frameshifting duplication c.589dup, p.(Ser197Lysfs*2) is one of 5 novel variants identified in this study.

The frameshift variants c.589dup, p.(Ser197Lysfs*2), c.767_768del, p.(Glu256Valfs*2), c.999_1000insT, p. (Gln334Serfs*84) and c.1010_1015delinsCA, p.(Val337Alafs*6) are predicted to result in protein truncation and nonsense-mediated decay (NMD), with abolition of the protein. Accordingly, these variants have been classified as pathogenic.

The novel splice variant c.820-1 G > A is expected to be pathogenic because it occurs within a canonical splice site. This variant was heterozygous in an affected female proband (16-1) with no known family history of choroideremia. Other pathogenic nucleotide substitutions at this splice acceptor site have been described20. Notably, a similar CHM mutation (c.820-1 G > C) was reported in a female carrier who displayed a highly abnormal RPE without atrophy, with severe loss of visual acuity secondary to a presumed neovascular membrane20. In view of the absence of an affected male in Family 16 and this variant not previously described in the literature, we conservatively assessed c.820-1 G > C as likely pathogenic.

Overall, four nonsense, five frameshift and two canonical splice site mutations were detected in 11 families. Gross deletions were identified in the remaining three families, including an entire gene deletion in one family and deletion of exon 15 in two families. Although breakpoints were not identified for the exon 15 deletions, for the purpose of this paper, we classified them as a single variant. Thus, 13 different causative variants were identified among the 14 families included in this present study.

**Discussion**

Present study

In this study, 13 different CHM variants classified as pathogenic or likely pathogenic were identified in 14 Australian families. Five variants were novel, and two were de novo, including one novel variant.

As in other studies21,23, we identified a predominance of causative point mutations. Two gross deletions and one entire gene deletion were detected. Once considered rare, gross deletions now reportedly comprise approximately 20% of disease-causing CHM variants6. With the identification of an entire gene deletion or deletions of exon 15 among three families, gross deletions now comprise 12% of our combined Australian cohort. Although an exon 15 deletion has been previously reported24, the two cases presented in this study are the first reported in an Australian cohort. It is not known whether these families carry the same or distinct nucleotide deletions, as breakpoints were not determined. Similarly, owing to the absence of breakpoint data, it is not known whether the entire CHM gene deletion identified in this study is the same as those reported previously.

Entire gene deletions have been reported involving the CHM gene alone21,25,26 or in various combinations with other genes, which can result in complex syndromic choroideremia phenotypes11,24,27. In the absence of breakpoint data for these gross deletions, we cannot establish if they encompass only the CHM region or regions and/or regulatory elements of other genes. As associated medical conditions were self-reported as absent in all cases, it is likely that these deletions do not affect the function of other genes.

The clinical features and reported symptoms of carrier females in this study are consistent with the view that females are typically unaffected. Nevertheless, four female participants did show symptoms of varying severity. An underrepresentation of the contribution of CHM mutations to disease in affected carrier females may contribute to a diagnosis of RP with autosomal dominant transmission. It is important that severely affected female carriers of X-linked disease be included in considerations regarding inclusion in gene-specific clinical trials or treatments.

Notably, over one-third of the choroideremia-affected pedigrees in the present study were not initially clinically diagnosed with choroideremia. This finding supports the view that choroideremia is underdiagnosed and...
| Family ID | Nucleotide change | Exon/Intron(i) | Predicted protein | Predicted effect^ | Novel or Reported | Variant classification (ACMG) |
|-----------|-------------------|----------------|------------------|------------------|------------------|-------------------------------|
| 12        | c.1358_1359delinsG | 11             | p.(Ser453*)      | Premature truncation of mRNA | Reported          | Pathogenic                   |
| 13        | c.(?_-1)_(*1_?)del| 1-15           | NIL              | Entire gene deletion# | Reported          | Pathogenic                   |
| 14        | c.1584_1587del    | 13             | p.(Val529Hisfs*7)| Premature truncation of mRNA | Reported          | Pathogenic                   |
| 15        | c.799 C > T       | 6              | p.(Arg267*)      | Premature truncation of mRNA | Reported          | Pathogenic                   |
| 16        | c.820-1 G > A     | i6             | p.?             | Abnormal splicing  | Novel            | Likely pathogenic           |
| 17        | c.589dup          | 5              | p.(Ser197Lysfs*2)| Premature truncation of mRNA | Novel            | Pathogenic                   |
| 18        | c.49 + 1 G > T    | i1             | p.?             | Abnormal splicing  | Reported          | Pathogenic                   |
| 19        | c.1010_1015delinsCA | 8        | p.(Val337Alafs*6)| Premature truncation of mRNA | Novel            | Pathogenic                   |
| 20        | c.1286_1287del    | 10             | p.(Ser429*)      | Premature truncation of mRNA | Reported          | Pathogenic                   |
| 21        | c.(1770 + 1_1771-1)_(*1962_?)del | 15 | p.? | Exon 15 deletion# | Reported          | Pathogenic                   |
| 22        | c.715 C > T       | 6              | p.(Arg239*)      | Premature truncation of mRNA | Reported          | Pathogenic                   |
| 23        | c.(1770 + 1_1771-1)_(*1962_?)del | 15 | p.? | Exon 15 deletion# | Reported          | Pathogenic                   |
| 24        | c.767_768del      | 6              | p.(Glu259Alafs*2)| Premature truncation of mRNA | Novel            | Pathogenic                   |
| 25        | c.999_1000insT    | 8              | p.(Gln334Serfs*84)| Premature truncation of mRNA | Novel            | Pathogenic                   |

**Bolded text** de novo variants are denoted in bold.

# deletion breakpoints not identified.

^ where premature truncation of mRNA is predicted, nonsense-mediated decay was considered likely.
sometimes misdiagnosed as RP owing to the overlapping clinical features and presenting symptoms of these related conditions and, in some cases, also owing to atypical fundus features or severe phenotype in a female\textsuperscript{29}. This highlights the value of nonhypothesis genetic diagnostic testing for suspected RP-affected individuals\textsuperscript{11,29,30}.

Combined studies

The age distribution of the participants in the combined studies, classified by gender and affection status, is shown in Table \textsuperscript{4}.

Self-reported phenotypic data for individuals with disease-causing CHM variants in the combined studies for males and symptomatic females are shown in Supplementary Table \textsuperscript{1}. One affected male (7-2) was added to our previous study.

The sequence variants established in this study combined with our previous study are detailed in Supplementary Table \textsuperscript{2}.

In this combined study, we identified a predominance of causative point mutations, including frameshift, nonsense and canonical splice site mutations, with an absence of missense mutations, which is well documented for this gene\textsuperscript{21–23,28,31}. Gross deletions were also reported in this study. The preponderance of such mutations suggests that most of the familial variants identified within this updated Australian cohort are likely to be null mutations, as found in other studies\textsuperscript{22,28,31}.

Only one mutation, c.1584_1587del (p. Val529Hisfs\textsuperscript{*7}), was detected in both studies. This frameshift variant is thought to occur at a mutation hotspot frequently reported in apparently unrelated pedigrees\textsuperscript{29,22,26,28,32–34}.

Of interest, all six nonsense mutations detected across the combined choroideremia cohort of 25 pedigrees are C > T transitions (24\% of pedigrees). Five of these are recurrent disease-causing CHM variants located at CpG dinucleotides, known mutational hotspots\textsuperscript{35,36}. Two were detected in the present study (\textit{de novo} c.715 C > T; c.799 C > T), and three were detected in our previous study (c.757 C > T; c.808 C > T; c.877 C > T). These results reflect the hypermutability for C > T transitions at CpG dinucleotides that occur at these five arginine residues (CGA), resulting in their conversion to a stop codon (TGA), as reported by others\textsuperscript{11,22,28,37,38}.

These results indicate that Sanger sequencing of the CHM gene in probands with a clinical diagnosis of choroideremia remains an efficient tool in the molecular diagnostic pipeline. In the three families in which a mutation was not detected by Sanger sequencing, follow-up qPCR analysis identified gross deletions in CHM. In addition to these reported Australian families, other undiagnosed, untested or unborn male family members may prove to be candidates for future gene therapy.

This Australian cohort now consists of 25 genetically confirmed choroideremia-affected families, with a total of 23 different CHM mutations identified.

Acknowledgments

The Australian Inherited Retinal Disease Registry and DNA Bank is financially supported by Retina Australia. The authors gratefully acknowledge the support of the Department of Medical Technology and Physics, Sir Charles Gairdner Hospital and the assistance of the Western Australian DNA Bank. We kindly thank our participants and our collaborating ophthalmologists and clinical geneticists. We acknowledge financial support provided by the National Health & Medical Research Council (NHMRC) of Australia (FKC: APP1116360, APP1142962 APP1054712), the McCusker Foundation (FKC) and the Miocevich family (FKC).

Author details

\textsuperscript{1}Australian Inherited Retinal Disease Registry and DNA Bank, Department of Medical Technology and Physics, Sir Charles Gairdner Hospital, Hospital Avenue, Nedlands, Western Australia, Australia.\textsuperscript{2}Centre for Ophthalmology and Visual Science, The University of Western Australia, 35 Stirling Highway, Crawley, Perth, Western Australia, Australia.\textsuperscript{3}Lions Eye Institute, 2 Verdon Street, Nedlands, Western Australia, Australia.\textsuperscript{4}Department of Ophthalmology, Royal Perth Hospital, Victoria Square, Perth, Western Australia, Australia.

Table \textsuperscript{4} Demographic information for CHM mutation carriers in the combined studies.

|                     | Symptomatic males | Asymptomatic males | Symptomatic females | Asymptomatic females |
|---------------------|-------------------|--------------------|---------------------|---------------------|
| Number              | 30                | 2                  | 4                   | 26                  |
| Average current age | 37                | 20                 | 71                  | 55                  |
| Age range           | 8–82              | 14–26              | 60–91               | 12–91               |
| 0–10                | 1                 | 0                  | 0                   | 0                   |
| 11–20               | 4                 | 1                  | 0                   | 1                   |
| 21–30               | 9                 | 1                  | 0                   | 3                   |
| 31–40               | 3                 | 0                  | 0                   | 1                   |
| 41–50               | 6                 | 0                  | 0                   | 1                   |
| 51–60               | 3                 | 0                  | 0                   | 1                   |
| 61–70               | 1                 | 0                  | 2                   | 3                   |
| 71–80               | 1                 | 0                  | 1                   | 4                   |
| 81–90               | 1                 | 0                  | 0                   | 1                   |
| 91–100              | 0                 | 0                  | 1                   | 1                   |
| Deceased            | 1                 | 0                  | 0                   | 0                   |
Conflict of interest

The authors declare that they have no conflict of interest.

Publisher’s note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Supplementary information is available for this paper at https://doi.org/10.1038/s41439-020-00122-w.

Received: 9 June 2020 Revised: 4 August 2020 Accepted: 11 September 2020.

Published online: 23 October 2020

References

1. MacDonald, I. M., Smoum, N. & Seabra, M. C. Choroideremia. In Choroideremia (eds Pagon R.A., Adam M.P., Ardinger H.H.) 1993–2014 (Seattle WA: University of Washington, Seattle, 2003) [Updated 2010].
2. van den Hurk, J. A. et al. Molecular basis of choroideremia (CHM): mutations involving the Rab escort protein-1 (REP-1) gene. Hum. Mutat. 9, 110–117 (1997).
3. Coussa, R. G. & Traboulsi, E. I. Choroideremia: a review of general pathogenesis. Ophthalmic Genet. 33, 57–65 (2012).
4. Roberts, M. F. et al. Retrospective, longitudinal, and cross sectional study of visual acuity impairment in choroideremia. Br. J. Ophthalmol. 86, 658–662 (2002).
5. van Bokhoven, H. et al. Cloning and characterization of the human choroideremia gene. Hum. Mol. Genet. 3, 1041–1046 (1994).
6. Stenson, P. D. et al. The human gene mutation database: towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next-generation sequencing studies. Hum. Genet. 136, 665–677 (2017).
7. McLaren, T. L. et al. Genetic analysis of choroideremia families in the Australian population. Clin. Exp. Ophthalmol. 43, 727–734 (2015).
8. Brea-Fernandez, A. J. et al. Novel splice donor site mutation in MERTK gene associated with retinitis pigmentos. Br. J. Ophthalmol. 92, 1419–1423 (2008).
9. Li, S. et al. Exome sequencing reveals CHM mutations in six families with atypical choroideremia initially diagnosed as retinitis pigmentosa. Int. J. Mol. Med. 34, 573–577 (2014).
10. Caragian, M. et al. Panel-based population next-generation sequencing for inherited retinal degenerations. Sci. Rep. 6, 33248 (2016).
11. Sanchez-Alcudia, R. et al. A comprehensive analysis of choroideremia: from genetic characterization to clinical practice. PLoS ONE 11, e0151943 (2016).
12. MacLaren, R. E. et al. Retinal gene therapy in patients with choroideremia: initial findings from a phase 1/2 clinical trial. Lancet 383, 1129–1137 (2014).
13. Nightstar Therapeutics: Nightstar Therapeutics Announces Initiation of STAR Phase 3 Registralional Trial for NSR-REP1 in Choroideremia. In Nightstar Therapeutics Announces Initiation of STAR Phase 3 Registralional Trial for NSR-REP1 in Choroideremia, Vol. 2018 GlobeNewswire, Inc., 2018.
14. De Roch, J. N. et al. Establishment and evolution of the Australian inherited retinal disease register and DNA bank. Clin. Exp. Ophthalmol. 41, 476–483 (2013).
15. Thompson, J. A. et al. The genetic profile of Leber congenital amaurosis in an Australian cohort. Mol. Genet. Genom. Med. 5, 652–667 (2017).
16. den Dunnen, J. T. et al. HGVS recommendations for the description of sequence variants: 2016 update. Hum. Mutat. 37, 569–569 (2016).
17. Richards, S. et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet. Med. 17, 405–424 (2015).
18. Hellen, B. Splice Site Tools: A comparative Report (National Genetics Reference Laboratory, Manchester, 2009).
19. Wallis, Y. P. et al. Practice Guidelines for the Evaluation of Pathogenicity and the Reporting of Sequence Variants in Clinical Molecular Genetics: Association for Clinical Genetic Science and Dutch Society of Clinical Genetic Laboratory Specialists, 2013.
20. Potter, M. J., Wong, E., Szabo, S. M. & MacTaggart, K. E. Clinical findings in a carrier of a new mutation in the choroideremia gene. Ophthalmology 111, 1905–1909 (2004).
21. Ramsden, S. C. et al. A clinical molecular genetic service for United Kingdom families with choroideremia. Eur. J. Med. Genet. 56, 432–438 (2013).
22. Freund, P. R., Sergeev, Y. V. & MacLaren, T. L. Analysis of a large choroideremia dataset does not suggest a preference for inclusion of certain genotypes in future trials of gene therapy. Mol. Genom. Genom. Med. 4, 344–358 (2016).
23. Esposto, G. et al. Comprehensive mutation analysis (20 families) of the choroideremia gene reveals a missense variant that prevents the binding of REP1 with Rab geranylgeranylation. Hum. Mutat. 32, 1460–1469 (2011).
24. Muro, V. et al. Retinal dystrophy and subretinal drusenoid deposits in female choroideremia carriers. Graefes Arch. Clin. Exp. Ophthalmol. 255, 2099–2111 (2017).
25. Pujicic, V. et al. Phenoype variants within a choroideremia family lacking the entire CHM gene. Ophthalmic Genet. 16, 143–150 (1995).
26. van Bokhoven, H. et al. Mutation spectrum in the CHM gene of Danish and Swedish choroideremia patients. Hum. Mol. Genet. 3, 1047–1051 (1994).
27. Lee, S. Y., Yu, W. K. & Lin, P. K. Large gene deletion and changes in corneal endothelial cells in a family with choroideremia. Invest. Ophthalmol. Vis. Sci. 56, 1887–1893 (2015).
28. Simunovic, M. P. et al. The spectrum of CHM gene mutations in choroideremia and their relationship to clinical phenotype. Invest. Ophthalmol. Vis. Sci. 57, 6033–6039 (2016).
29. Guo, H., Li, J., Gao, F., Li, J., Wu, X. & Liu, Q.Whole-exome sequencing reveals a novel CHM gene mutation in a family with choroideremia initially diagnosed as retinitis pigmentosa. BMC Ophthalmol. 15, 85 (2015).
30. Lin, Y. et al. Molecular analysis of the choroideremia gene related clinical findings in two families with choroideremia. Mol. Vis. 17, 2564–2569 (2011).
31. Skorczyk-Werner, A., Wawroda, A., Kochalak, N. & Krawczyński, M. R. Novel CHM mutations in Polish patients with choroideremia - an orphan disease with close perspective of treatment. Orphanet J. Rare Dis. 13, 221 (2018).
32. van den Hurk, J. A. et al. Detection and characterization of point mutations in the choroideremia candidate gene by PCR-SSCP analysis and direct DNA sequencing. Ann. J. Hum. Genet. 50, 1195–1202 (1992).
33. Cai, X. B., Huang, X. F., Tong, Y., Lu, Q. K. & Jin, Z. B. Novel CHM mutations identified in Chinese families with choroideremia. Sci. Rep. 6, 35360 (2016).
34. Schwartz, M., Rosenberg, T., van den Hurk, J. A., van de Pol, D. J. & Cremers, F. P. Identification of mutations in Danish choroideremia families. Hum. Mutat. 2, 43–47 (1993).
35. Pfeifer, G. P. Mutagenesis at methylated CpG sequences. Curr. Top. Microbiol. Immunol. 301, 259–281 (2006).
36. Kong, A. et al. Rate of de novo mutations and the importance of father’s age to disease risk. Nature 488, 471–475 (2012).
37. MacTaggart, K. E. et al. Mutational analysis of patients with the diagnosis of choroideremia. Hum. Mutat. 20, 189–196 (2002).
38. Chan, S. C. et al. Choroideremia research: report and perspectives on the second international scientific symposium for choroideremia. Ophthalmic Genet. 37, 267–275 (2016)