



THE UNIVERSITY of EDINBURGH

Edinburgh Research Explorer

Late-onset autosomal dominant macular degeneration caused by deletion of the CRX gene

Citation for published version:

Yahya, S, Smith, CEL, Poulter, JA, McKibbin, M, Arno, G, Ellingford, J, Kämpjärvi, K, Khan, MI, Cremers, FPM, Hardcastle, AJ, Castle, B, Steel, DHW, Webster, AR, Black, GC, El-Asrag, ME, Ali, M, Toomes, C, Inglehearn, CF, Ingram, S, Taylor, R, Manson, F, Sergouniotis, P, Pontikos, N, Cheetham, M, Fiorentino, A, Downes, S, Yu, J, Halford, S, Broadgate, S, van Heyningen, V, Ambrose, JC, Arumugam, P, Bevers, R, Bleda, M, Boardman-Pretty, F, Boustred, CR, Brittain, H, Caulfield, MJ, Chan, GC, Elgar, G, Fowler, T, Giess, A, Hamblin, A, Henderson, S, Hubbard, TJP, Jackson, R, Jones, LJ, Kasperaviciute, D, Kayikci, M, Kousathanas, A, Lahnstein, L, Leigh, SEA, Leong, IUS, Lopez, JF, Maleady-Crowe, F, McEntagart, M, Minneci, F, Moutsianas, L, Mueller, M, Murugaesu, N, Need, AC, O'Donovan, P, Odhams, CA, Patch, C, Pereira, MB, Perez-Gil, D, Pullinger, J, Rahim, T, Rendon, A, Rogers, T, Savage, K, Sawant, K, Scott, RH, Siddiq, A, Sieghart, A, Smith, SC, Sosinsky, A, Stuckey, A, Tanguy, M, Taylor Tavares, AL, Thomas, ERA, Thompson, SR, Tucci, A, Welland, MJ, Williams, E, Witkowska, K & Wood, SM 2023, 'Late-onset autosomal dominant macular degeneration caused by deletion of the CRX gene', *Ophthalmology*, vol. 130, no. 1, pp. 68-76. <https://doi.org/10.1016/j.ophtha.2022.07.023>

Digital Object Identifier (DOI):

[10.1016/j.ophtha.2022.07.023](https://doi.org/10.1016/j.ophtha.2022.07.023)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

Ophthalmology

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.





Late-Onset Autosomal Dominant Macular Degeneration Caused by Deletion of the *CRX* Gene

Samar Yahya, MBBS, MSc,^{1,2,*} Claire E.L. Smith, PhD,^{1,*} James A. Poulter, PhD,¹ Martin McKibbin, FRCOphth,^{1,3} Gavin Arno, PhD,⁴ Jamie Ellingford, PhD,⁵ Kati Kämpjärvi, PhD,⁶ Muhammad I. Khan, MS, PhD,^{7,8} Frans P.M. Cremers, PhD,^{7,8} Alison J. Hardcastle, PhD,⁴ Bruce Castle, MD, MRCOG,⁹ David H.W. Steel, FRCOphth, MD,^{10,11,‡} Andrew R. Webster, FRCS, FRCOphth,⁴ Graeme C. Black, PhD,^{5,12} Mohammed E. El-Asrag, MRes, PhD,^{1,13,14} Manir Ali, PhD,¹ Carmel Toomes, PhD,¹ Chris F. Inglehearn, PhD,¹ for the UK Inherited Retinal Dystrophy Consortium[‡] and the Genomics England Research Consortium[§]

Purpose: To characterize the phenotype observed in a case series with macular disease and determine the cause.

Design: Multicenter case series.

Participants: Six families (7 patients) with sporadic or multiplex macular disease with onset at 20 to 78 years, and 1 patient with age-related macular degeneration.

Methods: Patients underwent ophthalmic examination; exome, genome, or targeted sequencing; and/or polymerase chain reaction (PCR) amplification of the breakpoint, followed by cloning and Sanger sequencing or direct Sanger sequencing.

Main Outcome Measures: Clinical phenotypes, genomic findings, and a hypothesis explaining the mechanism underlying disease in these patients.

Results: All 8 cases carried the same deletion encompassing the genes *TPRX1*, *CRX*, and *SULT2A1*, which was absent from 382 control individuals screened by breakpoint PCR and 13 096 Clinical Genetics patients with a range of other inherited conditions screened by array comparative genomic hybridization. Microsatellite genotypes showed that these 7 families are not closely related, but genotypes immediately adjacent to the deletion breakpoints suggest they may share a distant common ancestor.

Conclusions: Previous studies had found that carriers for a single defective *CRX* allele that was predicted to produce no functional *CRX* protein had a normal ocular phenotype. Here, we show that *CRX* whole-gene deletion in fact does cause a dominant late-onset macular disease. *Ophthalmology* 2023;130:68-76 © 2022 by the American Academy of Ophthalmology. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).



Supplemental material available at www.aaojournal.org.

Cone-rod homeobox (*CRX*; OMIM*602225) is a transcription factor highly expressed in rod and cone photoreceptors, which is essential for their development and survival.^{1,2} Mutations in the *CRX* gene were first implicated in dominant cone-rod dystrophy (CRD) in a single, large family.³ Subsequently, pathogenic *CRX* mutations were identified as the cause of dominant CRD,^{1,4} dominant retinitis pigmentosa,⁵ and both dominant and recessive forms of Leber congenital amaurosis (LCA).⁵ More recently, *CRX* variants have been shown to cause dominant adult-onset macular disease (MD) said to simulate benign concentric annular MD, with a bull's-eye macular lesion and relatively well-preserved visual acuity.⁶⁻¹⁰ In total, >100 disease-causing *CRX* variants have been reported (www.lovd.nl/CRX), including missense, stop

codon, and frameshift changes and multiple exon deletions, most of which cause dominant disease.¹¹

Hull et al⁶ reviewed 41 heterozygous and 2 homozygous pathogenic variants and noted that all but 2 consisted of either missense mutations in exon 3, which primarily encodes the homeodomain, or frameshift and stop mutations in the large terminal exon 4. Normally, stop codon or frameshift variants in other genes are expected to lead to nonsense mediated decay (NMD), making them effectively null alleles that produce no protein.¹² However, in *CRX*, almost all pathogenic frameshift and stop variants occur in the last exon, meaning that they are likely to escape NMD¹³ so that a truncated protein would be produced. It is therefore not clear whether any of the known disease-causing *CRX* variants are truly null or what

phenotype would be caused by a heterozygous *CRX* null mutation.

One previous report noted homozygosity for a putative null variant, c.25insG (p.Pro9fs9*), causing LCA in a family.¹⁴ The authors noted that heterozygous carriers were unaffected, suggesting that carriers with a mutation causing loss of function of 1 copy of *CRX* are asymptomatic. Two more recent reports also described putative null variants that seem to be tolerated. The first described a family with dominant CRD carrying a heterozygous deletion of *CRX* exons 3 and 4, predicted to lead to NMD.¹⁵ The second reported a consanguineous family in which a homozygous 56 kb deletion of *CRX* and 2 flanking genes caused LCA.¹⁶ The heterozygous carrier parents were examined, and 2 of the 4 were found to have subclinical macular abnormalities but not clinical disease.

We identified 7 individuals (6 families) with late-onset MD and 1 with age-related macular degeneration (AMD), all apparently unrelated, and showed that they carry a heterozygous ~126 kb deletion encompassing *CRX* and neighboring genes *TRPX1* and *SULT2A1*. Here, we characterize the deletion and the phenotypes in those carrying it and carry out genetic analyses, the results of which suggest these families share a common ancestral founder.

Methods

Patients

Eight affected individuals from 7 unrelated families were recruited, and written informed consent was obtained from all patients. Research was conducted in accordance with the principles of the Declaration of Helsinki. Patients were of White British or White US origin. Ethical approval was obtained from the Leeds East Research Ethics Committee (REC reference 17/YH/0032) or the North West Regional Ethics Committee (REC reference 15/YH/0365). The 100,000 Genomes Project is covered by REC reference approval 14/EE/1112.

Clinical Assessment and DNA Sampling

Medical history was obtained, and family members underwent ophthalmic assessment, including fundus examination, OCT, visual acuity, Goldmann visual field testing, and electroretinographic testing. Genomic DNA was extracted from blood by standard protocols.

Genome and Exome Sequencing

Genome and exome sequencing were carried out as described previously.¹⁷ All genomic coordinates given refer to the GRCh37/hg19 human genome assembly. Genome sequencing was performed by Edinburgh Genomics, United Kingdom. Exome sequencing was performed by the University of Leeds' in-house Sequencing Facility. Fastq files were processed using the Maximal Exact Match option of the Burrows-Wheeler Aligner (BWA-MEM) to align sequences to the human reference genome. Genome Analysis Toolkit¹⁸ was used for indel realignment and base recalibration. Haplotype caller was used to create a genomic Variant Call File (gVCF). Variants were annotated using Ensembl's Variant Effect Predictor¹⁹ and filtered based on frequency in publicly available databases using vcfhacks perl scripts written by Dr David Parry (<https://github.com/david-a-parry/vcfhacks>). ExomeDepth was used for copy number variant detection according to the developer's guidelines.²⁰

Microsatellite Genotyping

Microsatellite markers were polymerase chain reaction (PCR) amplified with HotShot Diamond PCR Mastermix (Cient Life Science), and 1 µl of product was mixed with 0.5 µl ROX-500 size standard (Applied Biosystems) and 8.5 µl Hi-Di formamide (Applied Biosystems). PCR fragments were size fractionated on an ABI3130XL capillary sequencer, and data were analyzed using Gene Mapper, version 4, software (Applied Biosystems).

Gel Extraction and TA Cloning

Polymerase chain reaction was performed using a HotShot Diamond Mastermix, and products were excised from gels and purified using a MinElute gel extraction kit (Qiagen). The TOPO TA cloning kit for subcloning (ThermoFisher) was used to clone the purified products into pCR2.1-TOPO vector using the manufacturer's instructions. Plasmid DNA was extracted using the QIAprep Spin Miniprep kit (Qiagen) and sequenced.

Sanger Sequencing

Polymerase chain reaction products were treated with ExoSAP-IT (ThermoFisher); then plasmids and PCR products were sequenced using the primers detailed in Table S1 (available at www.aaojournal.org), or with M13 Forward (-20) or M13 Reverse, using the BigDye Terminator, version 3.1, kit (ThermoFisher). Products were run on an ABI3130xI sequencer (ThermoFisher). Sequences were analyzed on SeqScape, version 2.5 (ThermoFisher), and assembled using DNA baser software (Heracle BioSoft SRL).

Results

Variant Identification

In a female patient who received a diagnosis of CRD with central macular atrophy in her 50s (patient 4776, Table 1, Fig 1), exome sequencing and variant filtering identified no candidate causative variants. However, read depth analysis identified a minimum heterozygous deletion of chr19:48,305,033-48,389,514 (Fig 2A), which included the entire coding regions of *TRPX1*, *CRX*, and *SULT2A1*. In a second female patient who received a diagnosis of bull's-eye maculopathy at age 78 (patient 3888, Table 1, Fig 1), read depth analysis in data generated by sequencing 108 inherited retinal disease-associated genes²¹ identified a heterozygous deletion minimally spanning chr19:48,337,702-48,343,224 (Fig 2B), which covered all *CRX* exons. No significant second *CRX* variant was identified in either case. A third individual, a male patient given a diagnosis of late-onset MD at age 20 (patient GC16591, Table 1, Fig 1), was found to have a heterozygous deletion of the *TRPX1*, *CRX*, and *SULT2A1* genes in genome sequence generated by the UK 100,000 Genomes Project (Fig 2C). The centromeric breakpoint was located at approximately chr19:48,296,800. The telomeric breakpoint was difficult to place accurately owing to the presence of a block of 10 tandem ~5.3 kb repeats containing small NF-90 associated RNAs, spanning approximately chr19:48,407,000-48,465,000. Functional Analysis Through Hidden Markov Models (version 2.3)²² was used to predict the consequences of all variants in the region chr19:47,010,944-49,499,828, but again no significant second *CRX* variant was identified.

Table 1. Demographics, Phenotype, Screening Method Used to Detect the Deletion, and Method of Confirmation of Breakpoint for Each Patient

Lab ID/Sex	Age at Onset	Diagnosis	Clinical Notes	Deletion Detected by	Breakpoint Confirmed in
4776/F	50s	Bull's-eye maculopathy	Nyctalopia second decade, reduced central visual acuity (6/60 OD, 6/24 OS) sixth decade. Posterior pole staphyloma. Sister affected, same diagnosis.	Exome sequencing	Sequenced PCR product
3888/F GC16591/M	78 20	Bull's-eye maculopathy Bull's-eye maculopathy	Mild photoaversion, normal peripheral fields, no family history. Acuities 6/60 bilaterally, symmetrical area of degeneration at posterior pole. Electrophysiology has shown minimal progression in 10 years, generalized retinal dysfunction affecting rods and cones.	MIPs screening Genome sequencing	Cloned amplicon Sequenced PCR product
5587/F	36	Macular dystrophy	Photophobic, poor accommodation, normal color vision. MfERG - poor central responses. pERG extinguished R/L. Snellen visual acuity 6/12 OD, 6/18 OS.	Targeted sequencing	ND
5588/F	50	Macular dystrophy	Vision never good, sat at front of class at school. Noticed distortion at 50. Pendular nystagmus, not photophobic, no nyctalopia. LogMAR visual acuity 0.46 R, 0.64 L. Vitreomacular traction on OCT. MfERG - low amplitude bilaterally, pERG nonrecordable. Mother of 5587.	Targeted sequencing	Sequenced PCR product
5581/F	Late 40s	Macular dystrophy	Father developed rapidly progressive macular degeneration at 52, died at 73 severely sight-impaired. No other family members symptomatic.	Targeted sequencing	PCR
5598/M	53	Cone-rod dystrophy	Visual acuity 20/300 both eyes, vision loss began at 53 yrs. AF with few hyperfluorescent lesions (not pisciform). Low photopic flicker on FFERG, MfERG with low foveal waveforms. OCT with foveal atrophy.	Exome sequencing	Sequenced PCR product
3258/M	Late 60s	Wet AMD	Presented with poor vision, scar in left eye. Asymptomatic in right eye despite early wet AMD. No previous visual problems. Visual acuity 6/18 OD, 3/60 OS.	PCR	Cloned amplicon

AF = autofluorescence imaging; AMD = age-related macular degeneration; FFERG = full-field electroretinography; LogMAR = logarithm of the minimum angle of resolution; MfERG = multifocal electroretinography; MIP = molecular inversion probe; ND = not done; PCR = polymerase chain reaction; pERG = pattern electroretinography.

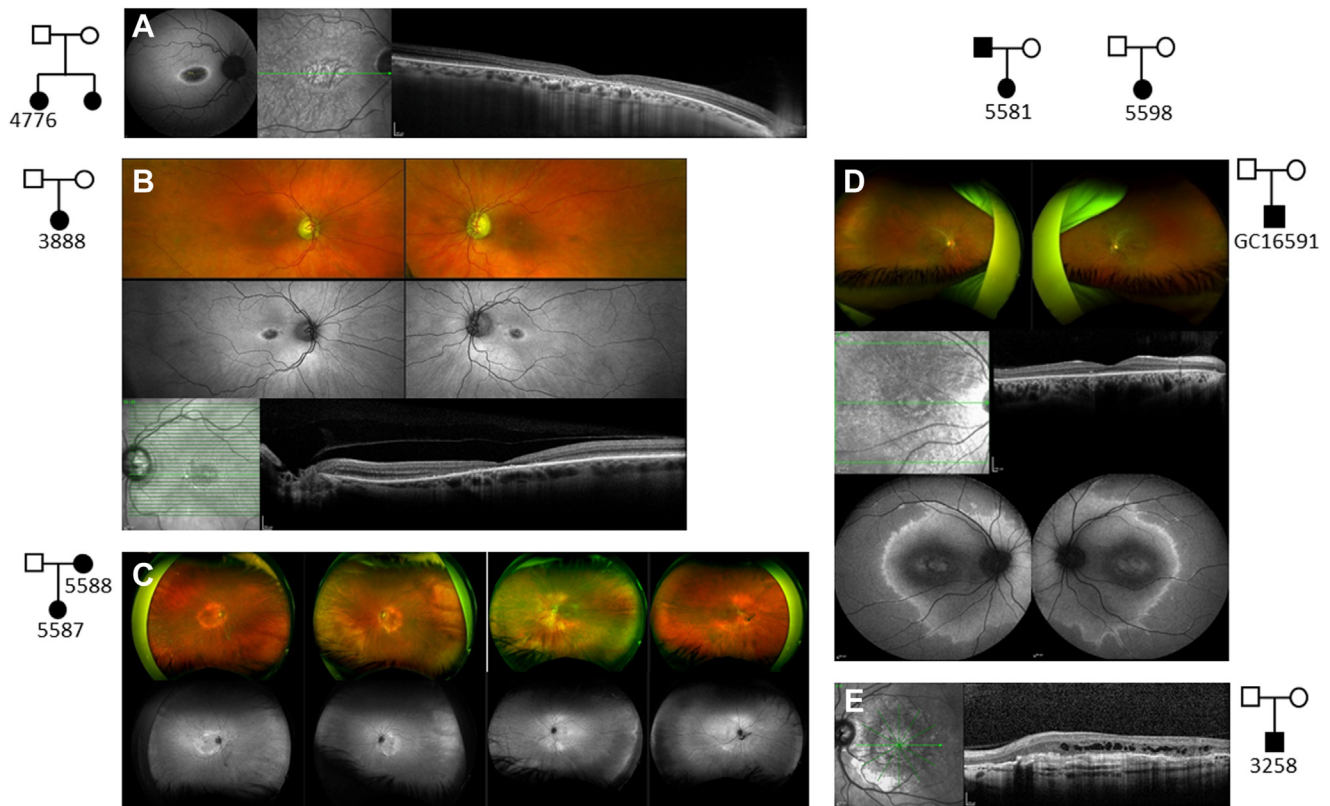


Figure 1. Clinical and family information in individuals with *CRX* deletion. **A**, Family tree, fundus autofluorescence image, and OCT for individual 4776. Fundus shows a central area of reduced autofluorescence with a surrounding ring of increased autofluorescence, consistent with the bull’s-eye maculopathy seen on clinical examination. On the OCT image, there is central loss of the outer retinal layers with choroidal hypertransmission. **B**, Family tree, pseudo-color fundus photographs, and autofluorescence images and OCT for individual 3888. There is reduced fundus autofluorescence centrally in both eyes, with a surrounding ring of increased autofluorescence, consistent with a diagnosis of bull’s-eye maculopathy. The structural OCT image shows loss of outer retinal lamination centrally. **C**, Family tree and pseudo-color and autofluorescence fundus images for individuals 5588 (mother, left images) and 5587 (daughter, right images). A ring of increased autofluorescence is visible in the mother, whereas in the daughter patches of increased autofluorescence are also visible in the macular region. **D**, Pseudo-color fundus, OCT, and autofluorescence images and family tree for individual GC16591. Bull’s-eye maculopathy is evident, but there are also changes nasal to the optic disc and a ring of increased autofluorescence around the central retina and posterior pole. **E**, Structural OCT images and family tree for individual 3258. Subretinal hyperreflective material and intraretinal cysts are evident on the OCT image, indicating a diagnosis of wet age-related macular disease.

Polymerase Chain Reaction Assay

Given the repetitive nature of the sequence adjacent to the deleted region, the common ethnic origin of the subjects, and the fact that panel and exome sequencing do not define breakpoints, we hypothesized that the deletion might be the same or similar in all 3 cases. Multiple primers were designed using whole-genome sequencing data. It was impossible to design unique PCR primers owing to the repetitive nature of the region. However, primers CCTCCATCTCAGCCTCCTA and CAAGGAGGAATGTGCAGTGG amplify a unique product of approximately 1150 bp only when the deletion is present (Fig S1, available at www.aojournal.org). In each of the above cases, these primers amplified the same-sized band, implying the deletion breakpoints were similar in each case. Figure 3 shows a graphic representation of the genomic locus and deletion.

Additional Screening

We used these primers to screen suspected *CRX* deletion cases identified in other cohorts for deletions with the same or similar breakpoints. Patients 5588 and 5587 are a mother and daughter

who received an MD diagnosis aged 50 and 36, respectively. Patient 5581 is a female who received an MD diagnosis in her 40s, whose father developed rapidly progressive MD at 52. These 3 were identified through screening for mutations in 180 inherited retinal disease-associated genes.²³ Read depth analysis identified a heterozygous deletion of chr19:48337702-48389514 encompassing all *CRX* exons in each of these cases. Patient 5598, a man in the United States who received a diagnosis of CRD at age 53, was identified as having a deletion covering the *CRX* gene in a whole-exome sequencing-based panel assay for patients with inherited eye diseases.²⁴ These 4 individuals were tested by PCR amplification with the above primers, and in each case, a band of approximately 1150 bp was amplified, confirming the presence of a deletion with similar breakpoints to those described above. Clinical results and screening data for these patients are detailed in Figure 1 and Table 1.

To test the hypothesis that the late-onset macular condition in these patients could overlap with AMD, we used primers CCTCCATCTCAGCCTCCTA and CAAGGAGGAATGTGCAGTGG to test for the presence of the same deletion in a cohort of 405 White British patients with exudative (wet) AMD. Amplification in 1 of these, patient 3258, who was diagnosed with wet

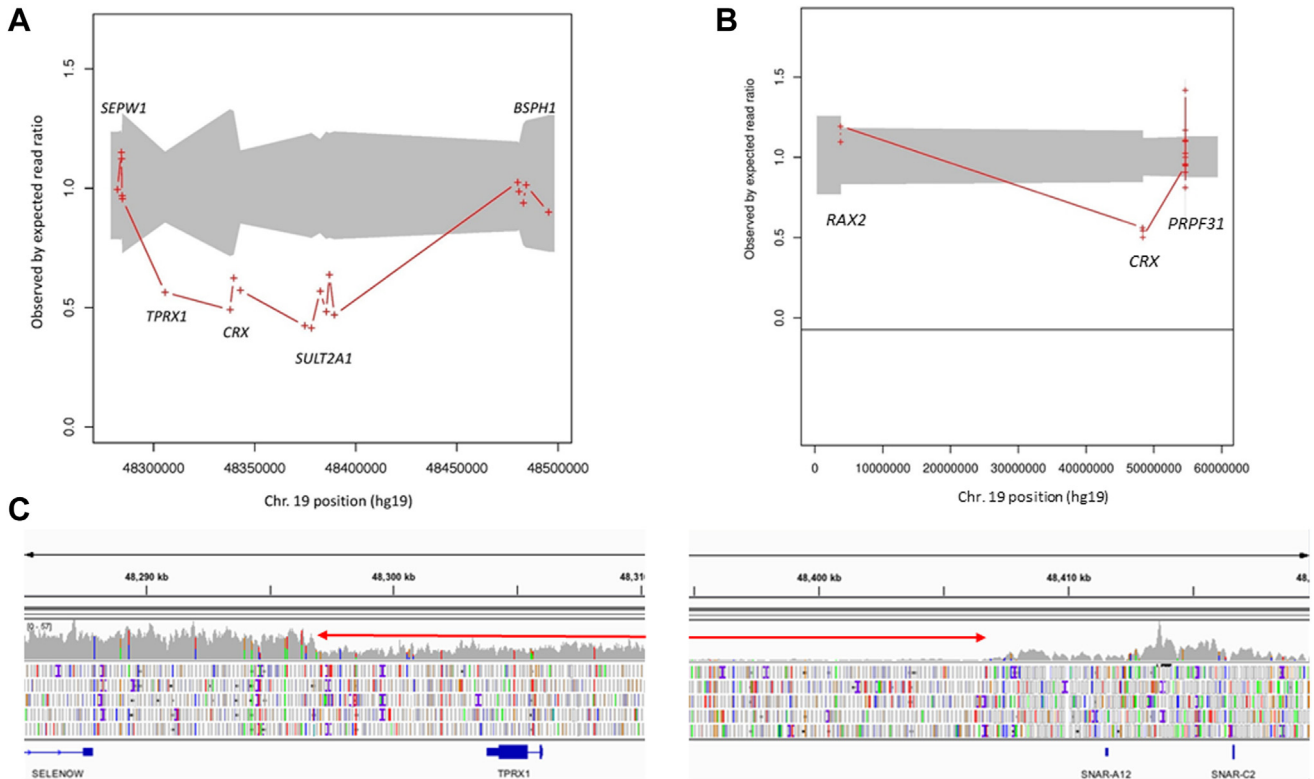


Figure 2. Sequence analysis in individuals with CRX deletion. **A**, ExomeDepth plot of whole-exome sequencing data across the deleted region in individual 4776, showing the drop in read depth over *TPRX1*, *CRX*, and *SULT2A1*. **B**, ExomeDepth plot of molecular inversion probes—generated sequence data from individual 3888, showing a drop in read depth for all exons of the *CRX* gene. **C**, Integrative Genomics Viewer image of genomic sequence at each end of the deletion in individual GC16591. The left-hand image shows a clear drop in read depth across the deleted region at the centromeric end of the deletion. However, the 5.3 kb tandem repeat cluster at the telomeric end means sequences align multiple times, giving the false impression of a sudden increase in read depth at the point where these repeats start, masking the precise end point of the deletion in the genomic sequence. An increase in read depth at this point is also visible in normal genomic sequence (not shown).

AMD in his late 60s, yielded a band of approximately 1150 bp, confirming the presence of a deletion with similar breakpoints. The remaining cases did not amplify any product with these primers.

Breakpoint Characterization

To determine the precise breakpoints in each case, the breakpoint PCR product was cloned and Sanger sequenced for patients 3888 and 3258 and directly Sanger sequenced in 4 other deletion carriers (Table 1). Breakpoint locations were determined using the BLAT function of the University of California Santa Cruz (UCSC) Genome Browser. The deletion was identical in each case, but the exact site of the breakpoint is ambiguous owing to local sequence homology. It was therefore assigned as the most 3' base, according to Human Genome Variation Society guidelines. The centromeric breakpoint was assigned as chr19:48,296,898. The telomeric breakpoint gave 100% alignment within the fourth 5.3 kb small NF-90 associated RNA-containing repeat, giving a location of chr19:48,422,892, making this a deletion of 125 995 bp (g.48,296,898_48,422,892del). However, owing to the presence of the 5.3 kb repeats, the telomeric sequence also aligned at 10 other sites between chr19:48,406,792 and chr19:48,460,162, with between 99.5% and 96.5% sequence identity.

Analysis of the sequence adjacent to the breakpoint using Emboss Matcher (EMBL-EBI) revealed 294 bp of sequences with 78% homology immediately adjacent to the deletion breakpoints at

each end. The repeat masker function of the UCSC Genome Browser revealed that these are AluSx1 sequences (Fig 3).

To determine whether this deletion is common in the normal population, we tested primers CCTCCCATCTCAGCCTCCTA and CAAGGAGGAATGTGCAGTGG in 382 White British control DNA samples, all of which were negative. We also used these primers to confirm this deletion is not the same as that reported by Ibrahim et al¹⁶ (data not shown), which deleted these same 3 genes and was reported as causing subclinical foveal abnormalities in 2 of the 4 carriers examined. The heterozygous deletion carriers described by Ibrahim et al were younger than the patients described here (Rob Koenkoop, personal communication, June 29, 2018) and may therefore be at risk of developing MD in later life.

Haplotype Analysis

Genotyping was carried out with microsatellite markers spanning the ~3 Mb (chr19:47,010,944-49,858,428) or 6.5 cM²⁵ of chromosome 19 containing *TPRX1*, *CRX*, and *SULT2A1*. Marker D19S606 genotypes show the deletion segregating on at least 2 different haplotype backgrounds, whereas marker D19S879 genotypes imply segregation with at least 3 different haplotype backgrounds (Table S2, available at www.aaojournal.org). In contrast, comparison of the breakpoint PCR product sequences to the hg19 reference genome identified 2 non-reference variants relatively rare in European populations (Fig S1). The presence of these variants, with allele frequencies of 17.4% and 2.5%, within the 500 bp immediately

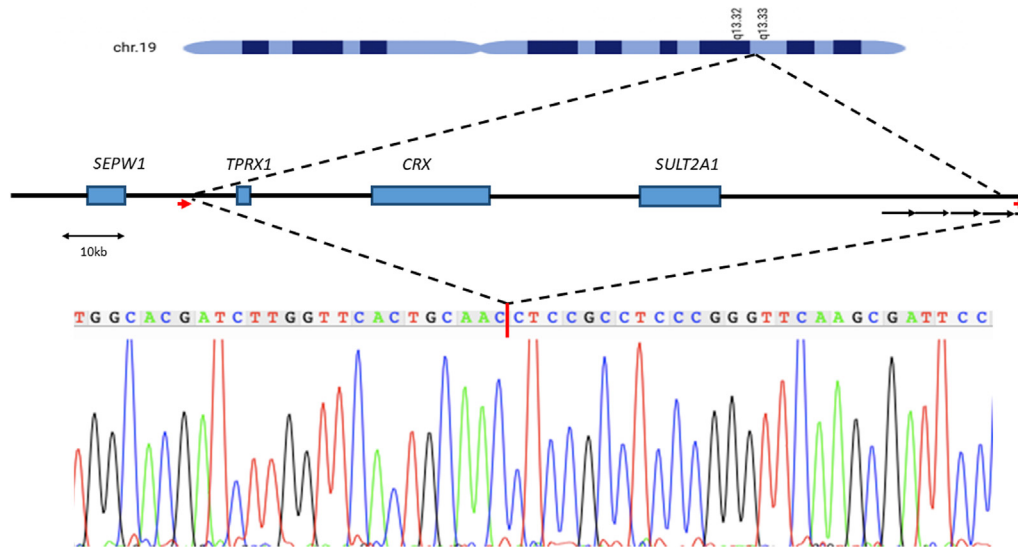


Figure 3. Graphical representation of the CRX deletion. 19q13.33 genomic region spanning *TPRX1*, *CRX*, *SULT2A1*, and adjacent genes, with cut-away showing the deleted region and below that a sequence trace of the breakpoint. Red arrows indicate the AluSx1 short, interspersed repeats within which the breakpoints occur, and the black arrows at the distal end of the locus denote small NF-90 associated RNA-containing tandem 5.3 kb repeats.

adjacent to the breakpoint in all deletion carriers tested, suggests this deletion has arisen only once, with these alleles representing a founder haplotype.

Discussion

Here, we show that a deletion encompassing the *TPRX1*, *CRX*, and *SULT2A1* genes causes late-onset dominant MD in 6 apparently unrelated families. It seems highly unlikely that loss of *SULT2A1* or *TPRX1* contributes to the phenotype. *TPRX1* (MIM*61166) is a homeodomain protein like *CRX*, but variants in it are not associated with any condition or trait in any species; it shows little sequence conservation (<https://genome.ucsc.edu>) and is expressed at very low levels in all tissues except testis (Protein Atlas, version 19.3, <https://www.proteinatlas.org/ENSG00000178928-TPRX1/tissue>). *SULT2A1* (MIM*125263) is an enzyme that catalyzes the sulfonation of hydroxysteroids, xenobiotics, and bile acids; it is highly expressed in the liver, and variants in it contribute to prostate cancer risk.²⁶ In contrast, MD has been well documented in patients with variants throughout the *CRX* gene.^{6–10}

However, many *CRX* variants may not be true null alleles because premature stop and frameshift variants in or near the large fourth exon will escape NMD, so a truncated protein may be produced. Previous reports suggested that carriers of true heterozygous *CRX* null alleles were unaffected, implying that loss of function of 1 copy of *CRX* is tolerated.^{14–16} The variant we report deletes *CRX* and is an unambiguous *CRX* null allele that will almost certainly result in insufficient *CRX* protein. Our findings therefore prove that lack of *CRX* protein owing to complete loss of function of 1 allele of the gene, or haploinsufficiency, is not asymptomatic but rather causes a late-onset macular phenotype, with variation in age at onset (20–78 years) and fundus appearance. Cases 4776 (Fig 1A) and

3888 (Fig 1B) have a classic bull’s-eye maculopathy with an incomplete ring of central macular atrophy. Cases 5588 and 5587 (Fig 1C) have a much larger ring of atrophy located closer to the temporal arcades. Case GC16591, like 4476 and 3888, has a bull’s-eye maculopathy, but there are also changes nasal to the optic disc and a ring of increased autofluorescence around the posterior pole. For cases 5581 and 5598, no clinical images were available, but diagnoses of CRD and MD and information available from notes are consistent with the range of phenotypes described above.

We found the same deletion in case 3258, with wet AMD, which could imply progression to or increased susceptibility to AMD in carriers of this deletion. However, this patient may have other AMD susceptibility alleles. A study applying whole-exome sequencing to a cohort of AMD patients found Mendelian retinal disease mimicking AMD due to a *PRPH2* gene variant, as well as enrichment of heterozygous *ABCA4* variants in AMD,²⁷ so the finding of apparently Mendelian alleles in AMD patients is not unprecedented.

Mechanisms giving rise to chromosomal rearrangements such as the one described here include recombination-based processes such as nonallelic homologous recombination²⁸ and replication-based processes such as microhomology-mediated break-induced replication or fork stalling and template switching.^{29,30} The finding of Alu sequences at each breakpoint suggests these sequences have driven nonallelic homologous recombination to create this deletion. Microsatellite genotyping showed that the deletion was segregating on multiple haplotype backgrounds, but analysis of single-nucleotide polymorphisms immediately adjacent to the breakpoint suggests it has arisen only once on a single founder allele. The contrast between the single haplotype detected by single-nucleotide polymorphisms within 500 bp of the breakpoints and the multiple haplotypes detected by markers 300 kb proximal (D19S606) and 1 Mb distal (D19S879) suggests that the deletion arose only once but has

existed in the population for many generations. This is plausible because this adult-onset condition would not be subject to significant negative selection. The late age at onset may also explain the apparent lack of a family history in most of these individuals because in most cases parents were deceased.

Early next-generation sequencing screens did not check for deletions, and more recent screens may miss heterozygous deletions when coverage is low. The finding of this variant in 7 apparently unrelated families with inherited retinal disease, the suggestion that it may have arisen many generations previously, and the existence of reports suggesting such an allele might be asymptomatic together imply that this could prove to be a relatively common cause of MD that has been overlooked in existing screening. The PCR test described here will facilitate quick, easy, and inexpensive screening of large patient cohorts and may lead to the identification of further cases.

Screening population controls did not reveal any further deletion carriers. In 13 096 Clinical Genetics patients with various inherited nonretinal conditions screened by array CGH (Leeds Teaching Hospitals, Leeds, UK), only 1 case was identified with a deletion encompassing *CRX*. This deletion spanned 3.4 Mb, but the individual also carried an inversion of chromosome 19 and was noted at birth to have exomphalos, urogenital sinus, congenital heart defect, annular pancreas, ductal aneurysm, and bowel atresia. The UCSC Genome Browser seems to show 14 copy number variants deleting the *CRX* gene. Of these, 2 encompassing a similar region, nsv1066782 and nsv3573812, were identified in a study of 29 084 patients with developmental

disorders,³¹ but precise breakpoints are unclear. The remaining 12 were identified by array Comparative Genomic Hybridisation (CGH) in a comparative screen of 30 normal humans and 30 chimpanzees,³² but the same study notes that copy number variant boundaries are likely to be overestimated with the platform used, and these findings remain unverified.

In conclusion, we have identified a heterozygous deletion of *CRX* as the cause of retinal disease in 6 apparently unrelated families with MD and an individual with wet AMD, confirming that complete loss of function in 1 copy of the *CRX* gene is pathogenic. The deletion seems to be a relatively old founder mutation caused by Alu sequences at each breakpoint. This mutation may have been overlooked in previous screens and may therefore be a relatively common cause of late-onset retinal disease. We describe a PCR assay that will facilitate the rapid screening of large patient cohorts.

Acknowledgment

The authors thank the families involved in this research. This research was made possible through access to the data and findings generated by the 100 000 Genomes Project. The 100 000 Genomes Project is managed by Genomics England Limited (a wholly owned company of the Department of Health and Social Care). The 100 000 Genomes Project is funded by the National Institute for Health Research and NHS England. The Wellcome Trust, Cancer Research UK, and the Medical Research Council have also funded research infrastructure. The 100 000 Genomes Project uses data provided by patients and collected by the National Health Service as part of their care and support.

Footnotes and Disclosures

Originally received: July 1, 2022.

Final revision: July 18, 2022.

Accepted: July 19, 2022.

Available online: August 5, 2022. Manuscript no. OPHTHA-D-22-01189R1.

¹ Leeds Institute of Medical Research, University of Leeds, St James's University Hospital, Leeds, United Kingdom.

² Department of Medical Genetics, School of Medicine, King Abdulaziz University, Rabigh, Saudi Arabia.

³ Department of Ophthalmology, St. James's University Hospital, Leeds, United Kingdom.

⁴ NIHR Biomedical Research Centre, Moorfields Eye Hospital NHS Foundation Trust & UCL Institute of Ophthalmology, London, United Kingdom.

⁵ Manchester Academic Health Science Centre, School of Biological Sciences, University of Manchester, Manchester, United Kingdom.

⁶ Blueprint Genetics Oy, Espoo, Finland.

⁷ Department of Human Genetics, Radboud University Medical Center, Nijmegen, the Netherlands.

⁸ Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, the Netherlands.

⁹ Peninsula Genetics Service, Royal Devon and Exeter Hospitals NHS Trust, Exeter, United Kingdom.

¹⁰ Sunderland Eye Infirmary, Sunderland, United Kingdom.

¹¹ The Bioscience Institute, Newcastle University, Newcastle upon Tyne, United Kingdom.

¹² Manchester Centre for Genomic Medicine, St. Mary's Hospital, Manchester University NHS Foundation Trust, Manchester, United Kingdom.

¹³ Department of Zoology, Faculty of Science, Benha University, Benha, Egypt.

¹⁴ Institute of Cancer and Genomic Science, University of Birmingham, Birmingham, United Kingdom.

*C.E.L.S. and S.Y. contributed equally to this work.

‡**UK Inherited Retinal Dystrophy Consortium:** Stuart Ingram, Rachel Taylor, Forbes Manson, Panagiotis Sergouniotis, Nikolas Pontikos, Michael Cheetham, Alessia Fiorentino, Susan Downes, Jing Yu, Stephanie Halford, Suzanne Broadgate, Veronica van Heyningen.

§**Genomics England Research Consortium:** John C. Ambrose, Prabhu Arumugam, Roel Bevers, Marta Bleda, Freya Boardman-Pretty, Christopher R. Boustred, Helen Brittain, Mark J. Caulfield, Georgia C. Chan, Greg Elgar, Tom Fowler, Adam Giess, Angela Hamblin, Shirley Henderson, Tim J. P. Hubbard, Rob Jackson, Louise J. Jones, Dalia Kasperaviciute, Melis Kayikci, Athanasios Kousathanas, Lea Lahnstein, Sarah E. A. Leigh, Ivonne U. S. Leong, Javier F. Lopez, Fiona Maleady-Crowe, Meriel McEntagart, Federico Minnici, Loukas Moutsianas, Michael Mueller, Nirupa Murugaesu, Anna C. Need, Peter O'Donovan, Chris A. Odhams, Christine Patch, Mariana Buongiorno Pereira, Daniel Perez-Gil, John Pullinger, Tahrima Rahim, Augusto Rendon, Tim Rogers, Kevin Savage, Kushmita Sawant, Richard H. Scott, Afshan Siddiq, Alexander Sieghart, Samuel C. Smith, Alona Sosinsky, Alexander Stuckey, Mélanie Tanguy, Ana Lisa Taylor Tavares, Ellen R. A. Thomas, Simon R. Thompson, Arianna Tucci, Matthew J. Welland, Eleanor Williams, Katarzyna Witkowska, Suzanne M. Wood.

A list of members of the [‡]UK Inherited Retinal Dystrophy Consortium and the [§]Genomics England Research Consortium appear online at www.aaojournal.org.

Disclosure(s):

All authors have completed and submitted the ICMJE disclosures form.

The authors have made the following disclosures: K.K.: Employee – Blueprint Genetics, a Quest Diagnostics company.

Financial support: This work was funded by Retinitis Pigmentosa Fighting Blindness and Fight for Sight UK (RP Genome Project GR586) and by a scholarship from the Saudi Arabian Cultural Bureau and King Abdulaziz University (to S.Y.). Funding organizations had no role in the design and conduct of this research.

HUMAN SUBJECTS: Human Subjects were included in this study. Ethical approval was obtained from the Leeds East Research Ethics Committee (REC reference 17/YH/0032) or the North West Regional Ethics Committee (REC reference 15/YH/0365). Research was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all patients.

No animal subjects were used in this study.

Author Contributions:

Conception and design: Yahya, Smith, Poulter, Ali, Toomes, Inglehearn

Data collection: Yahya, Smith, Poulter, McKibbin, Arno, Ellingford, Kämpjärvi, Khan, Ali, Toomes, Inglehearn

Analysis and interpretation: Yahya, Smith, Poulter, McKibbin, Arno, Cremers, Hardcastle, Webster, Black, El-Asrag, Ali, Toomes, Inglehearn

Obtained funding: N/A

Overall responsibility: Yahya, Smith, Poulter, McKibbin, Arno, Ellingford, Kämpjärvi, Khan, Cremers, Hardcastle, Castle, Steel, Webster, Black, El-Asrag, Ali, Toomes, Inglehearn

Abbreviations and Acronyms:

AMD = age-related macular degeneration; **CRD** = cone-rod dystrophy; **CRX** = cone-rod homeobox; **LCA** = Leber congenital amaurosis; **MD** = macular disease; **NMD** = nonsense mediated decay; **PCR** = polymerase chain reaction.

Keywords:

AMD, CRX, Macular disease, Retinal disease, *TPRX1*, *SULT2A1*.

Correspondence:

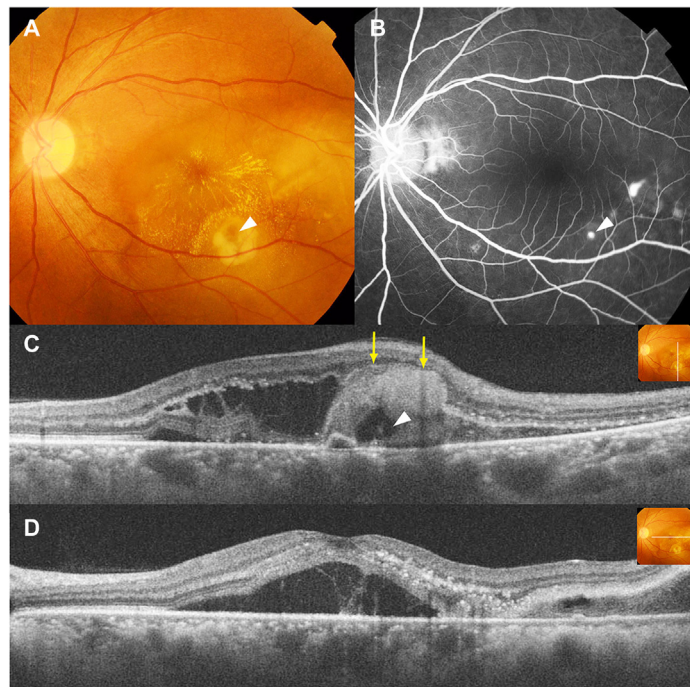
Chris F. Inglehearn, PhD, Leeds Institute of Medical Research, School of Medicine, University of Leeds, Wellcome Trust Brenner Building, St James's University Hospital, Beckett Street, Leeds, LS9 7TF, UK. E-mail: c.inglehearn@leeds.ac.uk.

References

- Fruend CL, Gregory-Evans CY, Furukawa T, et al. Cone-rod dystrophy due to mutations in a novel photoreceptor-specific homeobox gene (CRX) essential for maintenance of the photoreceptor. *Cell*. 1997;91:543–553.
- Furukawa T, Morrow EM, Li T, et al. Retinopathy and attenuated circadian entrainment in Crx-deficient mice. *Nat Genet*. 1999;23:466–470.
- Evans K, Fryer A, Inglehearn CF, et al. Genetic linkage of cone-rod retinal dystrophy to chromosome 19q and evidence for segregation distortion. *Nat Genet*. 1994;6:210–213.
- Swain PK, Chen S, Wang Q, et al. Mutations in the cone-rod homeobox gene are associated with the cone-rod dystrophy photoreceptor degeneration. *Neuron*. 1997;19:1329–1336.
- Sohocki MM, Sullivan LS, Mintz-Hittner HA, et al. A range of clinical phenotypes associated with mutations in CRX, a photoreceptor transcription-factor gene. *Am J Hum Genet*. 1998;63:1307–1315.
- Hull S, Arno G, Plagnol V, et al. The phenotypic variability of retinal dystrophies associated with mutations in CRX, with report of a novel macular dystrophy phenotype. *Invest Ophthalmol Vis Sci*. 2014;55:6934–6944.
- Griffith JF, DeBenedictis MJ, Traboulsi EI. A novel dominant CRX mutation causes adult-onset macular dystrophy. *Ophthalmic Genet*. 2018;39:120–124.
- Nasser F, Kurtenbach A, Kohl S, et al. Retinal dystrophies with bull's-eye maculopathy along with negative ERGs. *Doc Ophthalmol*. 2019;139:45–57.
- Khan AO, Neri P, Al Teneji AM. A recurrent arcuate retinopathy in familial cone-rod dystrophy secondary to heterozygous CRX deletion. *Ophthalmic Genet*. 2019;40:493–499.
- Ng CC, Carrera WM, McDonald HR, Agarwal A. Heterozygous CRX R90W mutation-associated adult-onset macular dystrophy with phenotype analogous to benign concentric annular macular dystrophy. *Ophthalmic Genet*. 2020;41:485–490.
- Yi Z, Xiao X, Li S, et al. Pathogenicity discrimination and genetic test reference for CRX variants based on genotype-phenotype analysis. *Exp Eye Res*. 2019;189:107846.
- Chang Y, Imam JS, Wilkinson MF. The nonsense-mediated decay RNA surveillance pathway. *Ann Rev Biochem*. 2007;76:51–74.
- Kurosaki T, Maquat LE. Nonsense-mediated mRNA decay in humans at a glance. *J Cell Sci*. 2016;129:461–467.
- Silva E, Yang J, Li Y, et al. A CRX null mutation is associated with both Leber congenital amaurosis and a normal ocular phenotype. *Invest Ophthalmol Vis Sci*. 2000;41:2076–2079.
- Jones KD, Wheaton DK, Bowne SJ, et al. Next-generation sequencing to solve complex inherited retinal dystrophy: a case series of multiple genes contributing to disease in extended families. *Mol Vis*. 2017;23:470–481.
- Ibrahim M, Alarcon-Martinez T, Lopez I, et al. A complete, homozygous CRX deletion causing nullizygosity is a new genetic mechanism for Leber congenital amaurosis. *Sci Rep*. 2018;8:1–6.
- Mahmood T, El-Asrag ME, Poulter JA, et al. A recessively inherited risk locus on chromosome 13q22-31 conferring susceptibility to schizophrenia. *Schizophr Bull*. 2021;47:796–802.
- Van der Auwera GA, O'Connor BD. *Genomics in the Cloud: Using Docker, GATK, and WDL in Terra*. 1st ed. O'Reilly Media; 2020.
- McLaren W, Gil L, Hunt SE, et al. The Ensembl Variant Effect Predictor. *Genome Biol*. 2016;17:122.
- Plagnol V, Curtic J, Epstein M, et al. A robust model for read count data in exome sequencing experiments and implications for copy number variant calling. *Bioinformatics*. 2012;28:2747–2754.
- Weisschuh N, Feldhaus B, Khan MI, et al. Molecular and clinical analysis of 27 German patients with Leber congenital amaurosis. *PLoS One*. 2018;13:e0205380.
- Shihab HA, Gough J, Cooper DN, et al. Predicting the functional, molecular, and phenotypic consequences of amino acid substitutions using hidden Markov models. *Hum Mutat*. 2013;34:57–65.
- Ellingford JM, Barton S, Bhaskar S, et al. Molecular findings from 537 individuals with inherited retinal disease. *J Med Genet*. 2016;53:761–767.

24. Guidugli L, Mehine M, Tuupanen S, et al. Characterization of copy number variants (CNVs) identified by genetic testing of inherited retinal disorders. *Invest Ophthalmol Vis Sci.* 2019;60:2944.
25. Kong A, Gudbjartsson DF, Sainz J, et al. A high-resolution recombination map of the human genome. *Nat Genet.* 2002;31:241–247.
26. Kwon EM, Holt SK, Fu R, et al. Androgen metabolism and JAK/STAT pathway genes and prostate cancer risk. *Cancer Epidemiol.* 2012;36:347–353.
27. Kersten E, Geerlings MJ, Pauper M, et al. Genetic screening for macular dystrophies in patients clinically diagnosed with dry age-related macular degeneration. *Clin Genet.* 2018;94:569–574.
28. Lupski JR. Genomic disorders: structural features of the genome can lead to DNA rearrangements and human disease traits. *Trends Genet.* 1998;14:417–422.
29. Lee JA, Carvalho CM, Lupski JR. A DNA replication mechanism for generating nonrecurrent rearrangements associated with genomic disorders. *Cell.* 2007;131:1235–1247.
30. Zhang F, Khajavi M, Connolly AM, et al. The DNA replication FoSTeS/MMBIR mechanism can generate genomic, genic and exonic complex rearrangements in humans. *Nat Genet.* 2009;41:849–853.
31. Coe BP, Witherspoon K, Rosenfeld JA, et al. Refining analyses of copy number variation identifies specific genes associated with developmental delay. *Nat Genet.* 2014;46:1063–1071.
32. Perry GH, Yang F, Marques-Bonet T, et al. Copy number variation and evolution in humans and chimpanzees. *Genome Res.* 2008;18:1698–1710.

Pictures & Perspectives



Macular Star Associated with Fibrinous Central Serous Chorioretinopathy

A 44-year-old woman with systemic lupus erythematosus, receiving prednisolone, presented with a macular star and an oval spot surrounded by a ring-shaped subretinal fibrin (Fig A, arrowhead). The oval spot corresponded with the site of leak on fluorescein angiography (Fig B). OCT showed subretinal fluid, intraretinal hyperreflective foci, and a hyporeflective vacuole associated with the leakage site (Figs C, arrowhead, D). The patient was diagnosed with central serous chorioretinopathy. Severe fibrinous exudate not only accumulated in the subretinal space but also invaded the outer plexiform layer of the retina (yellow arrows), resulting in macular star formation (Magnified version of Fig A–D is available online at www.aaojournal.org).

CHIKAKO HARA, MD, PhD¹

TAKU WAKABAYASHI, MD, PhD^{1,2}

KOHI NISHIDA, MD, PhD¹

¹Department of Ophthalmology, Osaka University Graduate School of Medicine, Osaka, Japan; ²Wills Eye Hospital, Mid Atlantic Retina, Thomas Jefferson University, Philadelphia, Pennsylvania