Biallelic mutations in the autophagy regulator *DRAM2* cause retinal dystrophy with early macular involvement

Mohammed E. El-Asrag[1,2,10], Panagiotis I. Sergouniotis[3,4,10], Martin McKibbin[5], Vincent Plagnol[6], Eamonn Sheridan[7], Naushin Waseem[3], Zakia Abdelhamed[1], Declan McKeefry[8], James A. Poulter[1], UK Inherited Retinal Disease Consortium, Colin A. Johnson[1], Ian M. Carr[1], Chris F. Inglehearn[1], Andrew R. Webster[3,9], Carmel Toomes[1]**, Manir Ali[1]*

[1] Section of Ophthalmology and Neuroscience, Leeds Institute of Biomedical and Clinical Sciences, University of Leeds, Leeds, UK.
[2] Department of Zoology, Faculty of Science, Benha University, Benha, Egypt.
[3] UCL Institute of Ophthalmology, University College London, London, UK.
[4] Institute of Human Development, Faculty of Medical and Human Sciences, University of Manchester, Manchester, UK.
[5] The Eye Department, St. James's University Hospital, Leeds, UK.
[6] UCL Genetics Institute, University College London, London, UK.
[7] Yorkshire Regional Genetics Service, St. James's University Hospital, Leeds, UK.
[8] Bradford School of Optometry and Visual Science, University of Bradford, Bradford, UK.
[9] Moorfields Eye Hospital, London, UK.

[10] These authors contributed equally to this work.

Correspondence:
*medma@leeds.ac.uk
**c.toomes@leeds.ac.uk

Short title: *DRAM2* mutations cause retinal dystrophy.

Abstract

Retinal dystrophies are an overlapping group of genetically heterogeneous conditions resulting from mutations in over 250 genes. Here we describe four families affected by an adult onset retinal dystrophy with initial macular degeneration that is characterized by central visual loss in the third/fourth decade of life where the affected individuals were found to harbor disease-associated variants in *DRAM2 (DNA-damage regulated autophagy modulator protein 2).* Homozygosity mapping and exome sequencing in a large, consanguineous British family of Pakistani origin revealed a homozygous frameshift variant (c.140delG, p.Gly47Valfs*3) in nine affected family members. Then, Sanger sequencing of *DRAM2* in 322 retinal-dystrophy unrelated probands revealed one European subject with compound heterozygous *DRAM2* changes (c.494G>A, p.Trp165* and c.131G>A, p.Ser44Asn). Independently, a gene-based case-control association study was conducted using an exome sequencing dataset of 18 phenotypically similar cases and 1,917 controls. Using a recessive model and a binomial test for rare, presumed biallelic, variants, *DRAM2* was found to be the most statistically-enriched gene; one subject was a homozygote (c.362A>T, p.His121Leu) and another a compound heterozygote (c.79T>C, p.Tyr27His and c.217_225del, p.73_75del). *DRAM2* is a transmembrane protein that has previously been localized to lysosomes and has been implicated in inducing autophagy. Immunohistochemical analysis showed DRAM2 co-localisation with rhodopsin in the photoreceptor outer segment where lysosomes are absent. This surprising observation suggests a novel role for DRAM2 in the retina.

Retinal dystrophies are a clinically and genetically heterogeneous group of disorders characterized by progressive photoreceptor degeneration.[1] The pattern of visual loss and retinal appearance varies and is related to the degree to which cone and rod photoreceptors are affected. In subjects with retinitis pigmentosa (RP), for example, the rods are affected more severely and earlier than the cones, and the presenting symptoms are typically night blindness and/or visual field loss. Disorders in which the cones are more severely affected than the rods include macular dystrophies (MD; localized loss of central/macular cones as a primary or secondary event) and cone-rod dystrophies (CRD; central and peripheral cone involvement). MD and CRD show clinical overlap and loss of central vision is often the common presenting symptom. Frequently, subjects with CRD also report light sensitivity, a symptom which can suggest generalized cone system dysfunction. Assigning a disease category can be challenging and confounding factors include inter- and intra-familial phenotypic variability and the presence of age-dependent phenotypic transitions. RP, MD and CRD can be transmitted in a dominant, recessive or X-linked manner and, to date, variants in 73, 16 and 30 genes respectively have been shown to give rise to these conditions (RetNet, accessed October 2014).

The initial aim of this study was to identify the genetic basis of an adult-onset retinal dystrophy with early macular involvement (Figure 1) in a consanguineous Pakistani family with multiple affected members living in the UK (family ES1; Figure 2). Affected individuals became symptomatic early in the third decade, describing increasing difficulty with close visual tasks. Neither light sensitivity nor night blindness were significant early symptoms. There was progressive loss of visual acuity in all symptomatic individuals but only three of eleven reported light sensitivity as the condition progressed; difficulty seeing in dim illumination was an inconsistent feature. Fundus examination revealed maculopathy in all symptomatic individuals tested, with peripheral retinal degeneration being a frequent finding in older subjects. Notably, optical coherence tomography (OCT) imaging in the pre-symptomatic second decade (subject IV.8, family ES1; Figure 2) suggested early para-central photoreceptor degeneration (loss of the photoreceptor layer and ellipsoid zone in the para-foveal area).

This study was approved by the Leeds East (Project number 03/362) and Moorfields Eye Hospital Research Ethics Committees and adhered to the tenets of the Declaration of Helsinki. Peripheral blood was collected from affected individuals, parents and unaffected relatives where these were available. Genomic DNA was extracted from blood leukocytes according to standard procedures.

Homozygosity mapping was performed using Affymetrix 250K single nucleotide polymorphism (SNP) arrays on genomic DNA from seven affected individuals from family ES1. Data were analysed with the AgileMultiIdeogram software. Two homozygous regions were shared among all seven affected individuals: a 10.1Mb interval on chromosome 1 (between rs6677953 and rs814987; containing 160 genes) and a 2.9Mb region on chromosome 7 (between rs17140297 and rs12706292; containing 5 genes) (Figure S1). Given the absence of genes previously reported to be associated with retinal dystrophy within these intervals, a whole exome sequencing (WES) strategy was utilized to identify the molecular pathology in the family. DNA from one affected family member (subject IV.6, family ES1; Figure 2) was analysed. For exon capture, the SureSelectXT Human All Exon V4 target enrichment reagent (Agilent) was used; paired-end sequencing was performed on a HiSeq2000 system (Illumina). The raw sequence data files were processed on the Galaxy platform[2] and aligned to the human reference genome sequence (hg19/GRCh37) using Bowtie2.[3] The alignment was processed in BAM format[4] with Picard and the Genome Analysis Toolkit[5] to correct alignments around insertions-deletions, and to identify and remove duplicates and sequencing reads with quality scores less than 20. The Unified Genotyper[6] reported variants in the VCF format which were annotated using ANNOVAR.[7] We excluded variants (i) with a read depth of less than 10, (ii) that are outside the exon and flanking two base-pair splice donor and acceptor sites, (iii) that are synonymous and (iv) with a minor allele frequency (MAF) >1% in the NHLBI Exome Sequencing Project Exome Variant Server (EVS; release version v.0.0.30). A list of the 33 homozygous variants remaining after filtration is shown in Table S1. Only one of these sequence alterations mapped within the shared regions of homozygosity identified in family ES1. This was a homozygous single-base deletion in *DRAM2* (*DNA-damage regulated autophagy modulator protein 2* [MIM 613360], NM_178454.4), that creates

a frameshift and is predicted to lead to premature truncation of the protein (c.140delG, p.Gly47Valfs*3). Segregation of this variant with the disease in the family (Figure 2) was confirmed by Sanger sequencing of *DRAM2* exon 4 (Figure 3A; primer pairs are shown in Table S2). This change was excluded from 159 ethnically matched control individuals and was not present in the dbSNP and EVS databases. It was found once in heterozygous state in WES data from 61,486 unrelated individuals sequenced as part of various disease-specific and population genetic studies (accessed via. the Exome Aggregation Consortium [ExAC] browser, version 0.2). Notably, no homozygous presumed loss-of-function variant in *DRAM2* was present in the ExAC dataset. A maximum two point LOD score of 2.4 was obtained between c.140delG and the disease in nine genotyped family members using Superlink.[8] For this analysis the c.140delG change was treated as a genetic marker with a MAF of 0.01%, and the disease was assumed to segregate in the family in a recessive fashion with full penetrance.

In an attempt to identify further families with *DRAM2*-associated retinopathy, the seven coding *DRAM2* exons and flanking splice sites, were PCR amplified and Sanger sequenced in 74 individuals diagnosed with RP, 154 with CRD or MD and 94 with infantile-onset retinal dystrophy (Leber Congenital Amaurosis) (primer pairs are shown in Table S2). This screen identified an isolated female case (subject 1325) of European ancestry in the CRD/MD panel that was compound heterozygous for a nonsense variant in exon 6 (c.494G>A, p.Trp165*) and a missense change in exon 3 (c.131G>A, p.Ser44Asn). The latter affects a serine residue that is highly conserved from human to nematodes (Figures 3 and S2). This missense change was predicted to be pathogenic by a number of bioinformatics prediction tools (Table S3) and was not present in dbSNP, EVS or ExAC databases. The p.Trp165* change is an annotated variant in dbSNP (rs201422368) with a MAF of 0.008% (1/13,003) in EVS and 0.003% (3/118,572) in ExAC; it is only reported in heterozygous state in these databases. Subject 1325 experienced blurred vision at age 29 and was soon after found to have maculopathy on fundus examination. At age 35, she also complained of night vision problems and sensitivity to light; fundus examination revealed mild peripheral retinal degeneration in addition to the maculopathy. At the age of 47, she had acuity of 1.0 logMAR in each eye and electrophysiology revealed severely

attenuated or absent dark- and light-adapted electroretinograms (ERGs) and pattern ERGs (Figure S3).

Independently, in a study designed to identify novel genes causative of recessive macular dystrophy, 28 families from the inherited retinal disease clinics at Moorfields Eye Hospital, London were ascertained. Details on this cohort have been previously reported.[9] Briefly, the main inclusion criteria were a CRD or MD phenotype, an absence of retinal imaging findings suggestive of *ABCA4*-associated retinopathy and a negative screen for *ABCA4* mutations and other known retinal disease genes using AsperBiotech APEX technology and Sanger sequencing of other candidate genes. DNA samples from the 28 probands were extracted and analyzed by WES (exon capture by SureSelectXT Human All Exon V5, Agilent; sequencing by HiSeq2000, Illumina). Downstream analysis and variant filtering were performed as previously described. The molecular diagnosis was identified in 10 of 28 families (methods and results have been reported before).[9] On the 18 unsolved cases, a gene based case-control association analysis was performed using exome sequencing data from a consortium of UK based researchers ("UCL-exomes"). Aiming to minimize bias,[10] UCL-exomes controls were initially split between two sets. The first set of 500 randomly selected samples was used in conjunction with EVS to determine variant frequency for inclusion in case control tests. In that context, "rare" variants are variants with MAF<0.5% in EVS and no more than 2 occurrences in this first set of 500 UCL-exomes control samples. The second set of 1,917 unrelated UCL-exomes controls was used to directly compute gene based association p-values, using a recessive disease mode, *i.e*. samples were labeled as potential carriers only if they carried at least two rare (using the definition stated above) and potentially functional (presumed loss-of-function, non-synonymous or splice site altering) variants. A binomial test was used for excess of such potential bi-allelic variants in the 18 cases compared to the 1,917 controls (Table S4).

The most significant gene-based p-value was obtained for *DRAM2* (Table S4). Two of the 18 cases were found to harbor likely disease-associated variants in this gene. A 37-year-old female proband (family gc17004, Figure 2) of European ancestry was a compound heterozygote for a missense variant (c.79T>C, p.Tyr27His) and an in-

frame deletion (c.217_225del, p.73_75del). Furthermore, a 47-year-old male proband of South Asian origin (family gc4728, Figure 2; parents not knowingly related) was homozygous for a missense change (c.362A>T, p.His121Leu). None of these three changes was present in dbSNP, EVS or ExAC and both missense variants (p.Tyr27His and p.His121Leu) were evolutionarily conserved from human to nematodes (Figure S2) and reported to be pathogenic by a number of prediction tools (Table S3). All changes were confirmed by Sanger sequencing (Figure 3) and segregated with the disease phenotype in the family as expected for a recessive condition (Figure 2).

Both probands presented with central visual loss (at age 29 for the proband of family gc17004 and at age 39 for the proband of family gc4728). At presentation, there was no light sensitivity or night blindness. Fundus examination and retinal imaging revealed macular photoreceptor loss with an apparently normal peripheral retina. These observations were consistent with electrophysiological findings which in both showed macular dysfunction (attenuated pattern ERG) with no evidence of generalised retinal involvement (dark- and light-adapted ERGs were within normal limits). Notably, 8 years after presentation, the central areas of atrophy have expanded and peripheral changes were observed. Electrodiagnostic testing was repeated and revealed more widespread retinal dysfunction in both cases. The phenotype was notably similar to the affected members of family ES1 and subject 1325 described above.

Given that affected members of family ES1 are homozygous for a *DRAM2* variant that is likely to lead to either nonsense mediated decay of the encoded mRNA, or to a truncated protein of only 47 amino acids, the molecular pathology of the disease is likely to be loss of DRAM2 function. This speculation is further supported by the biallelic state and predicted severity of the additional five likely disease-causing variants identified as well as by the similar phenotype in all four families.

DRAM2, also known as TMEM77 (transmembrane protein 77), encodes a 266 amino acid protein containing six putative transmembrane domains (Figure 3B). Previous overexpression studies in HEK293 cells localised it to lysosomal membranes[11,12]

where it initiates the conversion of endogenous LC3-I (microtubule-associated protein light chain 3) to the general autophagosome marker protein, LC3-II (LC3-1/phosphatidylethanolamine conjugate). This suggests that DRAM2 induces the autophagy process.[11] Autophagy is a natural cell survival mechanism triggered in response to stress stimuli such as nutrient starvation or the accumulation of damaged organelles. It is responsible for degrading and recycling cytoplasmic proteins and lipids as well as organelles within the cell.[13] This usually begins with isolation of the macromolecules and organelles within the cytoplasm into single membrane vesicles, which fuse together to produce an autophagosome. These autophagosomes subsequently fuse with lysosomes containing acid hydrolases and form a double-membrane autolysosome.[14,15] Although the aim of autophagy is to relieve cellular stress, its excessive induction can in some cases lead to apoptosis rather than protection from cell death.[16]

There is also some evidence to suggest that DRAM2 may have tumour suppressor capability. DRAM2 transcript and protein expression are reduced in ovarian tumours compared to normal matched tissues.[11] Also, siRNA knockdown of endogenous DRAM2 results in reduced conversion to LC3-II in cells subject to starvation-induced autophagy[17] and increased survival in deoxyrubicin treated cells that would normally undergo p53-mediated apoptosis.[11] We note that examination of medical histories in the reported subjects with *DRAM2*-associated retinal dystrophy provided no evidence of increased susceptibility to cancer.

Although *DRAM2* is transcribed ubiquitously (Figure S4),[12] in light of the finding that human *DRAM2* variants cause retinal dystrophy, we investigated the precise distribution of the normal protein in the mouse retina. Serial sections were taken from mouse eyes at postnatal day 30 and were stained with a goat polyclonal antiserum against DRAM2 (Figure 4). Confocal immunofluorescence microscopy showed that DRAM2 co-localized with rhodopsin to the outer segment of the photoreceptor layer. This coincides with the primary pathology observed on pre-symptomatic OCT analysis in which photoreceptor outer segments appeared specifically affected (Figure 1). However, this result is unexpected as the photoreceptor outer segments are thought to be devoid of lysosomes. Autophagy takes place in the cytoplasm, so a

role in photoreceptor autophagy would predict localization in the photoreceptor inner segments. Given the disc shedding process and requirement to recycle phototransduction components, a high level of autophagy occurs in the RPE. Given that outer segments consist almost entirely of densely packed membrane discs filled with phototransduction cascade proteins, these data therefore suggest that DRAM2, a transmembrane protein, localizes to disc membranes where its precise role is unknown.

To summarize, we have shown that biallelic missense, nonsense and frameshift variants in *DRAM2* cause retinal dystrophy with early macular cone photoreceptor involvement. The clinical features and course of retinal degeneration were highly similar among affected individuals from the four reported families. Our findings suggest that DRAM2 is essential for photoreceptor survival and further studies are expected to provide important insights into its role in the photoreceptor outer segments.

Acknowledgements

We would like to thank the patients and their families who participated in this study. We thank our colleagues at University College London, UK for kindly contributing to the UCL-exomes control panel. Additional members of the UK Inherited Retinal Disease Consortium include Graeme Black (Manchester Academic Health Science Centre, University of Manchester), Alison Hardcastle (UCL Institute of Ophthalmology and Moorfields Eye Hospital) and Andrea Nemeth (Division of Clinical Neurology, University of Oxford). This work was supported by grants from NERC (Yorkshire branch), Macular Society UK (www.maculardisease.org), the UK National Institute for Health Research (Biomedical Research Centre, Moorfields Eye Hospital and Institute of Ophthalmology), RP Fighting Blindness and Fight For Sight (RP Genome Project GR586). CAJ and CFI were supported by a grant from the Jules Thorn Charitable Trust and MEE is funded by an Egyptian Government Scholarship.

Web Resources

The URLs for data presented herein are as follows:

AgileMultiIdeogram, http://dna.leeds.ac.uk/agile/AgileMultiIdeogram/
ANNOVAR, http://www.openbioinformatics.org/annovar/
CADD, http://cadd.gs.washington.edu/
ClustalW, http://www.ebi.ac.uk/clustalw2/
dbSNP, http://www.ncbi.nlm.nih.gov/SNP/
ExAC browser, http://exac.broadinstitute.org/
Galaxy, https://usegalaxy.org
GATK, http://www.broadinstitute.org/gsa/wiki/index.php/The_Genome_Analysis_Toolkit
IGV (Interactive Genomics Viewer), http://www.broadinstitute.org/software/igv/
Mutation Taster, http://www.mutationtaster.org/
MutPred, http://mutpred.mutdb.org/
NHLBI Exome Sequencing Project Exome Variant Server (EVS), http://evs.gs.washington.edu/
Online Mendelian Inheritance in Man (OMIM), http://www.omim.org/
Picard, http://picard.sourceforge.net/
PolyPhen2, http://genetics.bwh.harvard.edu/pph2/
Primer3, http://frodo.wi.mit.edu/cgi-bin/primer3/
PROVEAN, http://provean.jcvi.org/
RetNet, https://sph.uth.edu/retnet/home.htm
SIFT, http://sift.jcvi.org/
Superlink, http://cbl-hap.cs.technion.ac.il/superlink-snp/

References

1. Wright, A.F., Chakarova, C.F., Abd El-Aziz, M.M., Bhattacharya, S.S. (2010). Photoreceptor degeneration: genetic and mechanistic dissection of a complex trait. Nat. Rev. Genet. 11, 273–284.

2. Blankenberg, D., Gordon, A., Von Kuster, G., Coraor, N., Taylor, J., Nekrutenko, A. (2010). Manipulation of FASTQ data with Galaxy. Bioinform. 26, 1783-1785.

3. Langmead, B. and Salzberg, S.L. (2012). Fast gapped-read alignment with Bowtie 2. Nat. Methods 9, 357-359.

4. Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., and Durbin, R. 1000 Genome Project Data Processing Subgroup. (2009). The Sequence Alignment/Map format and SAMtools. Bioinform. 25, 2078–2079.

5. McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M., et al. (2010). The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 20, 1297–1303.

6. DePristo, M.A., Banks, E., Poplin, R., Garimella, K.V., Maguire, J.R., Hartl, C., Philippakis, A.A., del Angel, G., Rivas, M.A., Hanna, M., et al. (2011). A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat. Genet. 43, 491–498.

7. Wang, K., Li, M., Hakonarson, H. (2010). ANNOVAR: Functional annotation of genetic variants from next-generation sequencing data. Nuc. Acids Res. 38, e164.

8. Silberstein, M., Tzemach, A., Dovgolevsky, N., Fishelson, M., Schuster, A., Geiger, D. (2006). Online system for faster multipoint linkage analysis via parallel execution on thousands of personal computers. Am. J. Hum. Genet. 78, 922-935.

9. Sergouniotis, P.I., Chakarova, C., Murphy C., Becker M., Lenassi E., Arno G., Lek M., MacArthur D.G., UCL-Exomes Consortium, Bhattacharya S.S., et al. (2014). Biallelic variants in TTLL5, encoding a tubulin glutamylase, cause retinal dystrophy. Am. J. Hum. Genet. 94, 5, 760-769.

10. Pearson, R.D. (2011). Bias due to selection of rare variants using frequency in controls. Nat. Genet. 43, 392–393, author reply 394–395.

11. Park, S.M., Kim, K., Lee, E.J., Kim, B.K., Lee, T.J. Seo, T., Jang, I.S., Lee, S.H., Kim, S., Lee, J.H., et al. (2009). Reduced expression of DRAM2/TMEM77 in tumor cells interferes with cell death. Biochem. Biophys. Res. Commun. 4, 1340-1344.

12. O'Prey, J., Skommer, J., Wilkinson, S., Ryan, K.M. (2009). Analysis of DRAM-related proteins reveals evolutionarily conserved and divergent roles in the control of autophagy. Cell Cycle 8, 2260– 2265.

13. Levine, B., Klionsky, D.J. (2004). Development by self-digestion: molecular mechanisms and biological functions of autophagy. Dev. Cell 6, 463-477.

14. Yoshimori, T. (2004). Autophagy: a regulated bulk degradation process inside cells. Biochem. Biophys. Res. Commun. 313, 453-458.

15. Kroemer, G., Jäättelä, M. (2005). Lysosomes and autophagy in cell death control. Nat. Rev. Cancer 5, 886-897.

16. Yuan, J., Lipinski, M., Degterev, A. (2003). Diversity in the mechanisms of neuronal cell death. Neuron 40, 401-413.

17. Yoon, J.H., Her, S., Kim, M., Jang, I.S., Park, J. (2012). The expression of damage-regulated autophagy modulator 2 (DRAM2) contributes to autophagy induction. Mol. Biol. Rep. 39, 1087-1093.

Figure 1. Clinical features of individuals from family ES1 with retinal dystrophy and early maculopathy caused by recessive *DRAM2* mutations.

Colour fundus photograph (A), fundus autofluorescence (C), infra-red reflectance (E) and OCT (F) images from the right eye of case IV.8 at 25 years. A colour fundus photograph (B), fundus autofluorescence (D) infra-red reflectance (G) and OCT image (H) from a normal control individual are provided for comparison. There is a dull foveal reflex on fundus photography. On autofluorescence imaging, there is a central area of reduced autofluorescence with a ring of increased autofluorescence. On OCT imaging, there is loss of the outer nuclear, external limiting membrane and ellipsoid layers in the central macula. The composite colour photograph from the left eye of case III.1, at the age of 48, shows macular atrophy with white dots in the temporal macula and intra-retinal pigment migration, extending towards the equator (I). On the infra-red reflectance images, the horizontal green lines show the position and direction of the corresponding OCT scan. The scale bars represent 200μm.

Figure 2. Pedigrees of families/cases reported in this study and *DRAM2* mutation segregation data.

Affected individuals are shaded black. The maternal grandmother of individual gc17004 has age-related macular degeneration in her 90s (shaded grey). The genotypes for all tested family members are shown below each individual, with M representing the mutant allele and + representing the wild-type allele.

Figure 3. Variants in *DRAM2* cause retinal dystrophy.

(A) Schematic representation of the *DRAM2* genomic structure and major transcript (NM_178454.4) showing the location and sequence traces of the six disease-causing variants identified in this study. (B) Schematic diagram of the DRAM2 protein showing the location of the affected amino-acids within the protein domains.

Figure 4. Localization of Dram2 in photoreceptor outer segments.

Radial 6μm cryosections of mature mouse retina (P30) were labeled with anti-Dram2 (M-12, Santa Cruz Biotechnology) and anti-Rhodopsin (Sigma-Aldrich) followed by the secondary antibody Alexa Fluor 488-conjugated donkey anti-goat immunoglobulin (green) (Molecular Probes Incorporation) and Alexa Fluor 568-

conjugated goat anti-rabbit immunoglobulin (red) (Invitrogen) respectively, and the nuclei counterstained with DAPI (Vector Laboratories). Immunofluorescence was analyzed with an Eclipse TE2000-E inverted confocal microscope (Nikon Instruments) and shows co-localisation of dram2 with rhodopsin to the outer segment of the photoreceptor layer (POS). The other layers are the retinal pigment epithelial layer (RPE), outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL) and the ganglion cell layer (GCL). Scale bars represents 50 μm.

Figure 1

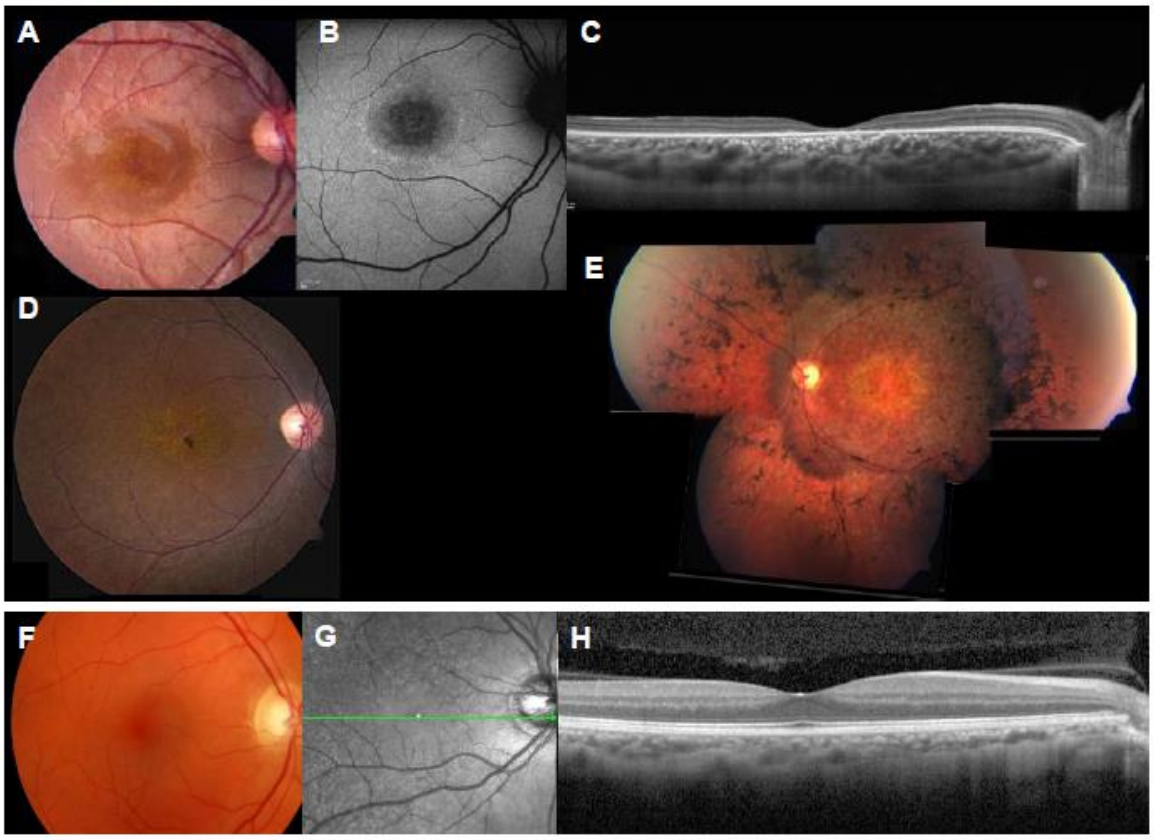

Figure 2

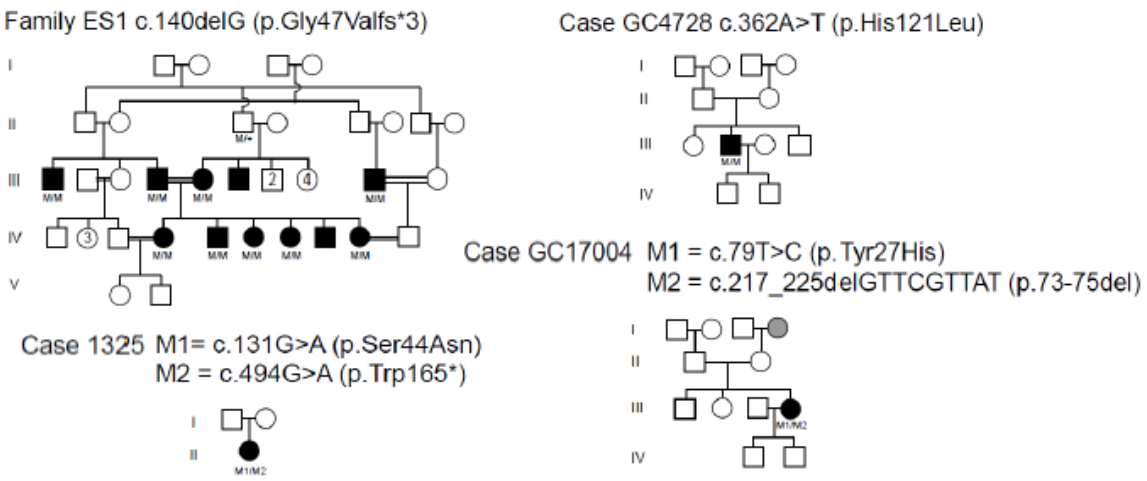

Figure 3

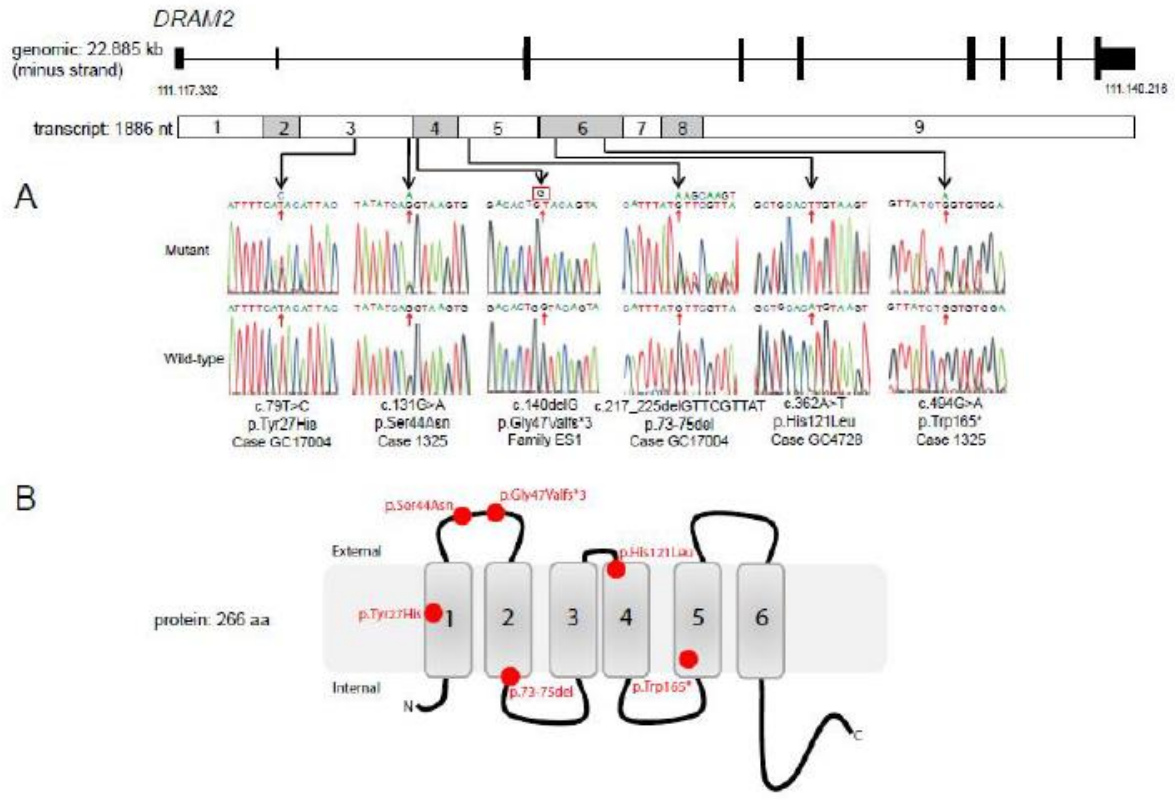

Figure 4