

## Supplementary Information

### *EYS* mutations and implementation of minigene assay for variant classification in *EYS*-associated retinitis pigmentosa in northern Sweden

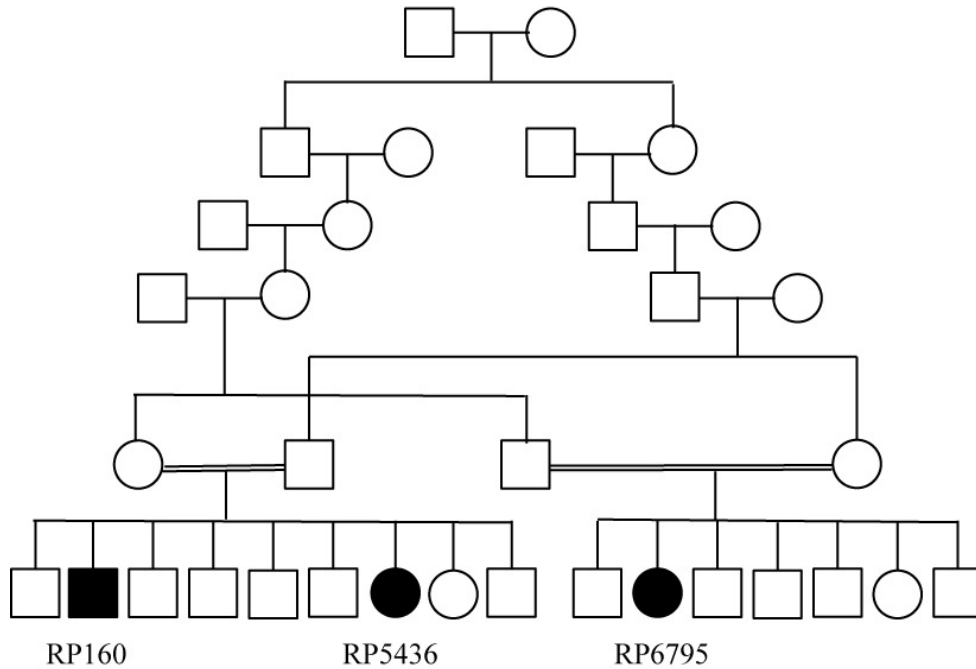
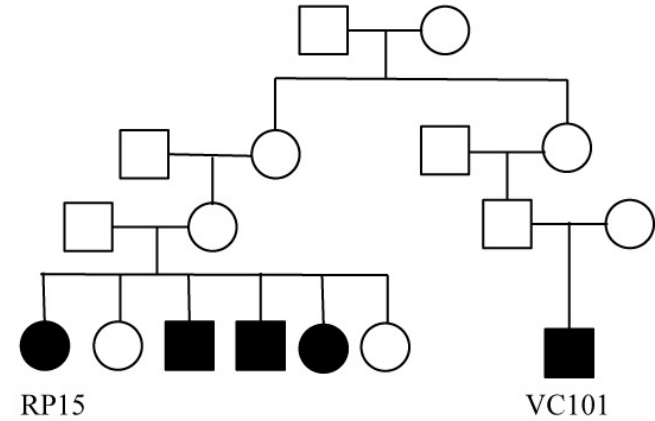
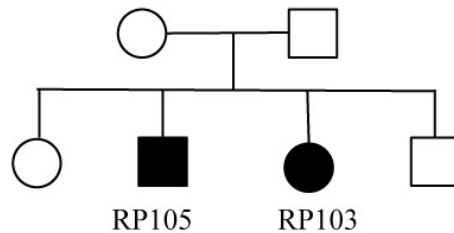
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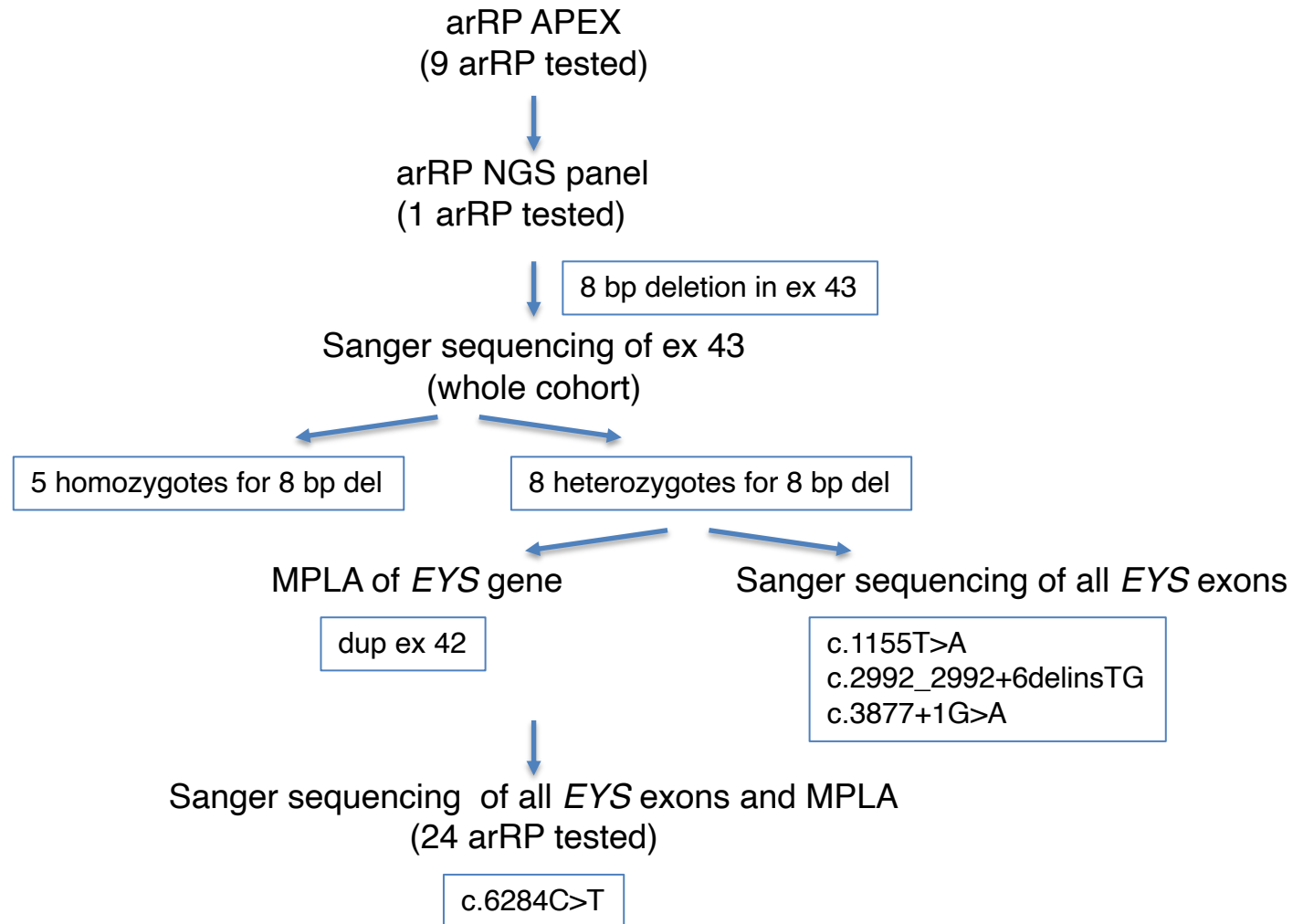
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## Pedigrees of the families in this study

**Family 501****Family 012****Family 335**

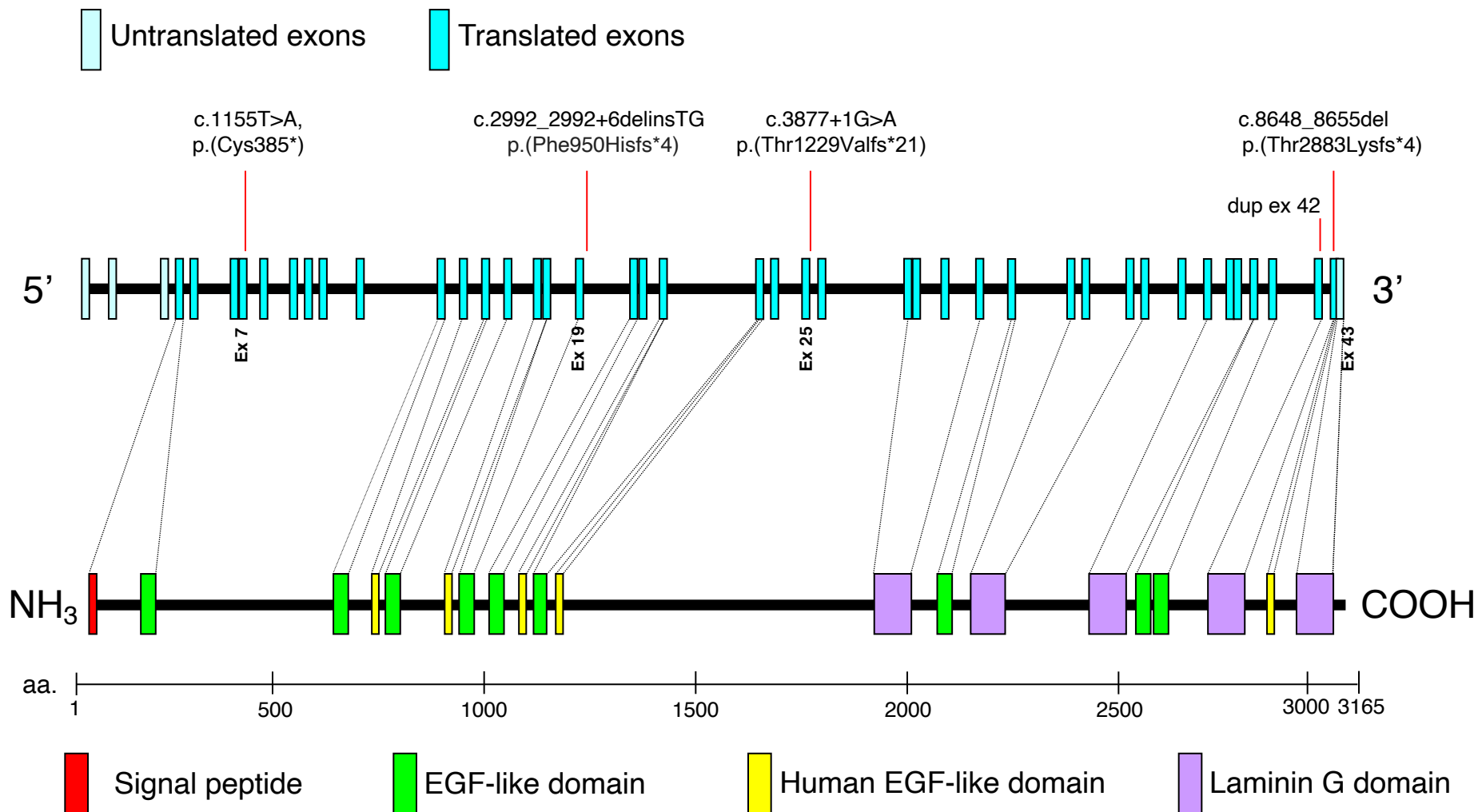
Circles and squares represent females and males, black and white symbols represent affected and unaffected, respectively; double lines - consanguineous marriage

## Methods and outcome of cascade-targeted mutation analysis of *EYS* gene in arRP cohort from northern Sweden

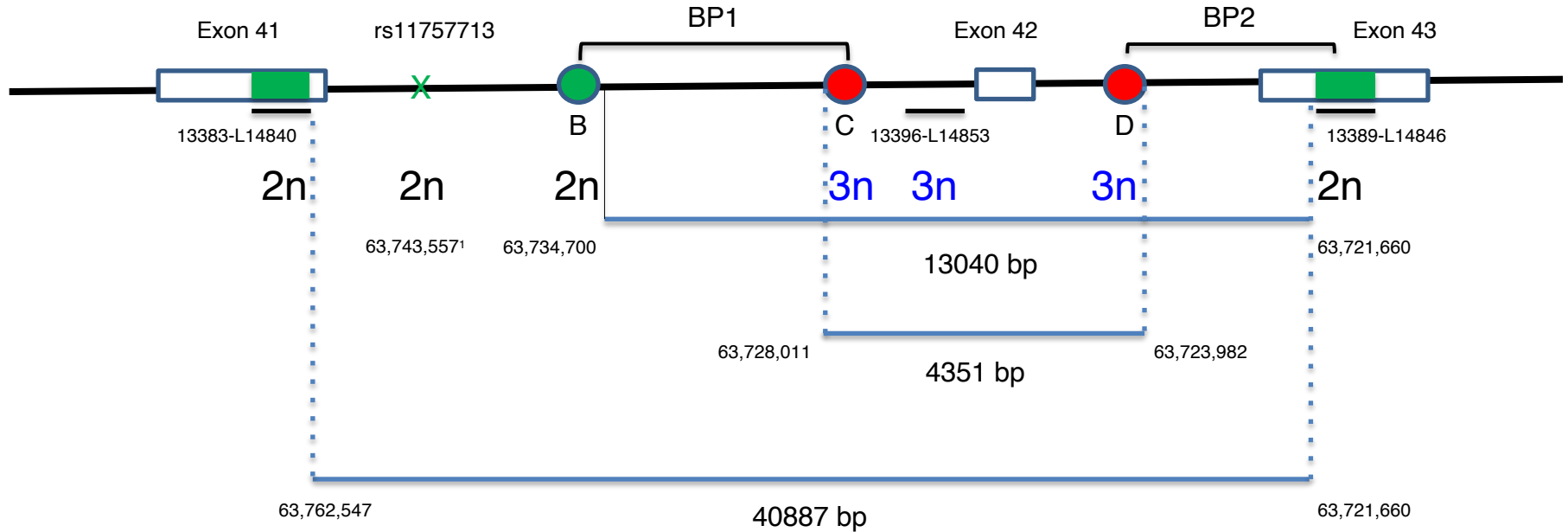


Our cohort (n=81) consisted of 65 arRP (3 probands, 4 family members and 58 simplex cases), 7 cases with either arRP or adRP and 9 adRP cases. Mutations detected at each step are shown framed.

# *EYS* mutations in the cohort of patients of northern Sweden



For protein source the following database was used. The Pfam protein families database: towards a more sustainable future by R.D. Finn, P. Coghill, R.Y. Eberhardt, S.R. Eddy, J. Mistry, A.L. Mitchell, S.C. Potter, M. Punta, M. Qureshi, A. Sangrador-Vegas, G.A. Salazar, J. Tate, A. Bateman. **Nucleic Acids Research** (2016) Database Issue 44:D279-D285

MLPA analysis of *EYS* duplication

*EYS* exons are shown as open boxes. Cross is an informative SNP, rs 11757713. BP – breakpoints' regions of the duplication. MLPA probes from SALSA Kit P328-A2-0217 (MRC Holland, Amsterdam, Netherlands) are shown as black lines. B, C and D – MLPA probes designed in this study, their sequences are shown in the Table below. <sup>1</sup> – genomic positions in *EYS* sequence (NM\_001142800.1) according to genome reference GRChr38/hg38. Two copies of MLPA probe is shown in green and denoted as 2n while duplication with three copies of MLPA probe is shown in red and described as 3n.

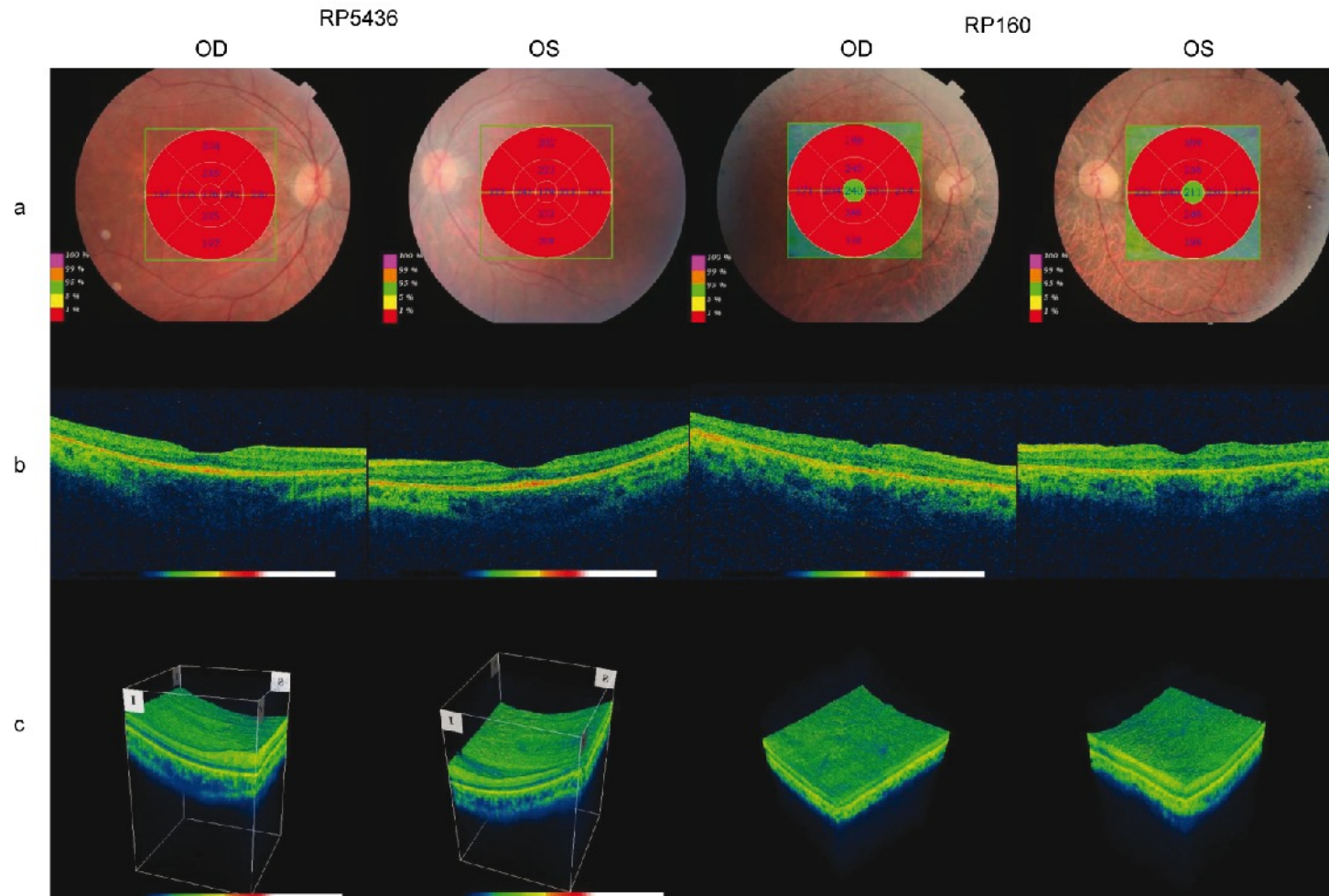
| Probe                      | Sequence                                                                                                                           |
|----------------------------|------------------------------------------------------------------------------------------------------------------------------------|
| Probe B-LHS<br>Probe B-RHS | 5'-GGGTTCCCTAAGGGTTGGAGTCCTACCTGAGCTCCACATCTTCTCCAAAATACC<br>5'-TGCTTCTCCCACAGTCTTCCCCATTTCAGTTAATGATCTAGATTGGATCTTGCTGGCAC        |
| Probe C-LHS<br>Probe C-RHS | 5'-GGGTTCCCTAAGGGTTGGACAAGGAACCCCTATGTCAGAAACACCAGGAGGGTTTA<br>5'-TTAAAAATGGGGATGCTTTCTGAATCCCATCCCAGCATTCTAGATTGGATCTTGCTGGCAC    |
| Probe D-LHS<br>Probe D-RHS | 5'-GGGTTCCCTAAGGGTTGGAGGCCGAGGTGGGCGGATCACCTGAAGTCAGGAGGTCAAG<br>5'-ACCAGCCTGGCCAACATGGTAAACCTCGCTCTACCCAAATCTAGATTGGATCTTGCTGGCAC |

## Primer sequences for PCR amplification of *EYS* exons for minigene constructs

| <i>EYS</i> variant    | Forward primer                           | Reverse primer                          | Expected size of splice products |
|-----------------------|------------------------------------------|-----------------------------------------|----------------------------------|
| c.3877+1G>A           | 5'- <sup>1</sup> AAATTTGGGCATGCAACTGT    | 5'- <sup>2</sup> TGAGGTCCAGTTGTCTATTCGA | 453 bp                           |
| c.2992_2992+6delinsTG | 5'- <sup>1</sup> CACACATAAAGAACATTTGAGCA | 5'- <sup>2</sup> GCTCCCTGATGACTTTTGCC   | 406 bp                           |
| pSPL3                 | 5'-TCTGAGTCACCTGGACAACC                  | 5'-ATCTCAGTGGTATTTGTGAGC                | 260 bp (no insert)               |

<sup>1</sup>Forward primers have an EcoRI recognition sequence at the 5' end; <sup>2</sup>Reverse primers have a NotI recognition sequence at the 5' end

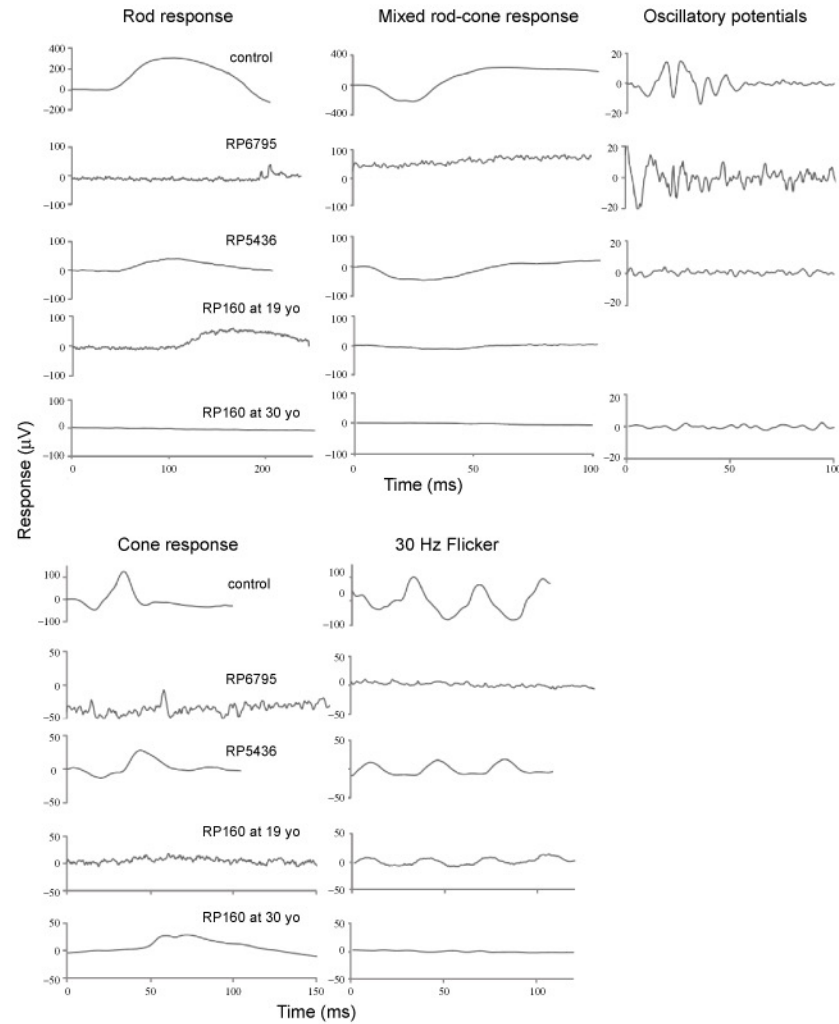
# Optical coherence tomography (OCT)



Optical coherence tomography (OCT) of two affected patients RP5436 examined at age of 18 yo and RP160 examined at age of 30 yo shows retinal thickness map (a), axial colour B-scan (b) and 3D scan (c).

I = inferior of the retina and S = superior of the retina

# Full-field ERG of the three affected family members



Case RP160 was examined twice at 19 and 30 years of age.  
F = female; M = male. Details are described in the Results.

Full-length gels of in vitro minigene splice assay – Fig.1b, Fig.1c, Fig 2b and Fig.2c

