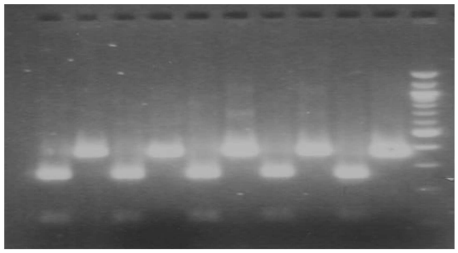
Summary of supplemental data: Figure1S; Figure Legend 1S; Figure 2S; Figure Legend 2S; Tables 1-3S; Summary of other AEs.

Figure 1S

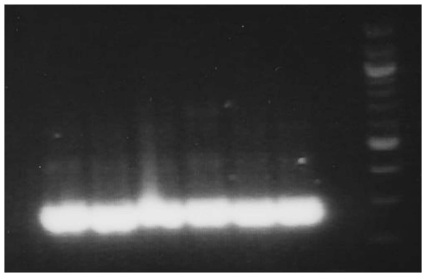
a



RE LE RE LE RE LE RE LE RE LE 100bp
mouse # 0104 0201 0203 0301 0302 Ladder

BanI digest of PCR product from cDNA of retinas of mice injected with D477G (RE) and WT (LE) RPE65 AAV2/9.

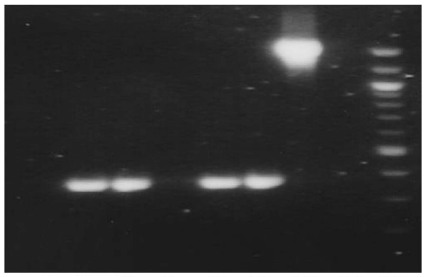
b



Liv RE LE Liv RE LE H_2O 100bp
mouse # 0104 mouse # 0301 Ladder

PCR of cDNA extracted from liver, RE (D477G AAV) and LE (WT AAV) from two animals using ßeta-globin primers.

c

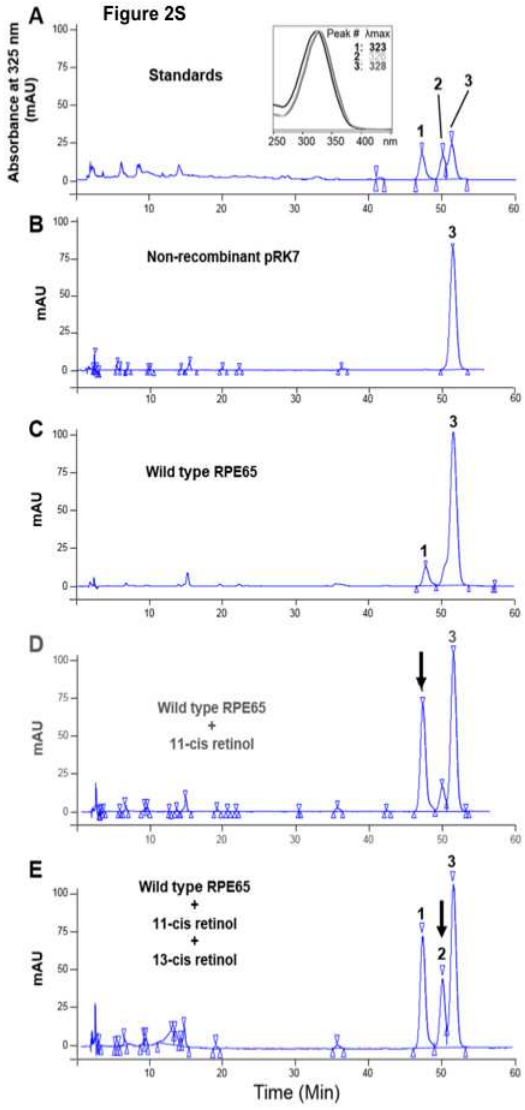


Liv RE LE Liv RE LE gDNA H_2O 100bp
mouse # 0104 mouse # 0301 ladder

PCR of amplified product from cDNA with primers spanning exons 11-14 of RPE65 gene. Product size for cDNA = 369bp and for genomic DNA = 1705bp.

Figure 1S Legend: Confirmation of AAV-mediated expression of RPE65 transcripts following sub- retinal inoculation of virus.

RNA was extracted from wild type retinas of C57BL/6J mice injected with WT or D477G RPE65 AAV2/9 using Qiagen RNeasy kit (Cat No./ID:74104) and cDNA synthesis was carried out on 250 ng RNA using Applied Biosystems High Capacity cDNA Reverse Transcription Kit. Total RNA was also extracted from the livers of injected animals for assessment of possible systemic distribution of virus following sub-retinal inoculations. PCR was carried out using primers spanning exons 11/14 of the human RPE65 cDNA (F Primer 5'-CTGCAATTCTGTGCAGTGACG-3' and R primer 5'-GGGCAACTTCACTTAAGTCCT-3'), producing an amplification product of 369 bp. PCR reactions were carried out in a volume of 25 µl in the presence of 100 ng DNA, 50 pmol each of oligonucleotide primers, 200 µM each of dCTP, dGTP, dATP and dTTP, 50 mM KCl, 10 mM Tris (pH8.4), 15 mM $MgCl_2$ and 0.75 units of Taq polymerase. Reactions were carried out for 35 cycles using the following parameters: 94^{O}C, 1 min; 60^{O}C, 1 min; 72^{O}C 1 min x 35 cycles, 72^{o}C, 5 min; 4^{o}C hold. Overnight *Ban*I restriction endonuclease digestion produced bands of 255 bp and 114 bp in retinas that received D477G AAV2/9 and a single band of 369 bp in samples that received WT AAV2/9 and visualized on a 2% agarose gel (see figure S1a). Presence of normal and mutant transcripts extracted from retinas was also confirmed by direct sequencing. PCR was also carried out using the same conditions on cDNA generated from RNA extracted from liver and retinas of WT mice which had been injected with D477G and WT RPE65 AAV2/9 virus (right and left eye respectively) using mouse ß-actin primers (F 5'-GTCACCCACACTGCCCATCTA -3' and R 5'GTGATGACCTGGCCGTCAG-3') producing a product of 272 bp and primers spanning Exons 11-14 of the RPE65 gene using conditions as above (See Figure S1b and c). No PCR product was observed from cDNA generated from liver, confirming that the AAV expression was restricted to the eye. A fragment of 1,705bp was observed in a control human genomic sample.



Kenna PF, *et al. BMJ Open Ophth* 2020; 5:e000462. doi: 10.1136/bmjophth-2020-000462

Figure 2S Legend: HPLC separation profiles of 11-*cis*-retinol, 13-*cis*-retinol and all-*trans*-retinol. Retinol isomers were analyzed by reverse-phase C18 HPLC column and detected by UV absorbance at 325 nm. **A**, chromatogram of standard solutions: (1) 11-*cis*-retinol, (2) 13-*cis*-retinol, (3) all-*trans*-retinol. **B-E**, representative chromatograms of retinol in 293-L cells stably expressing LRAT, transiently transfected with mammalian expression vector pRK7 empty (**B**) and containing wild type human RPE65 (**C-E**), and incubated with all-trans retinol (2 μM) for 24h. Retinoid extracts were injected alone (**B, C**) or added of standard solutions (**D, E**) as mentioned. Peaks were identified based on their characteristic elution times and UV absorbance spectra (top inset).

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Table 1S Overview of Treatment Emergent Adverse Events

	All Adverse Events	**Adverse Drug Reactions[a]**
All Adverse Events	5(100%)	5 (100%)
Treatment Discontinuations due to AEs	0	0
Serious Adverse Events	0	0
Deaths	0	0
Treatment Discontinuation due to SAEs	0	0
Other SAEs [b]	0	0

Number (%) of Subjects (N = 5).

[a] Adverse drug reactions are those suspected to be related to treatment

[b] Other: Excluding serious adverse events that led to death or withdrawal

Legend: All 5 subjects enrolled in the study experienced at least one adverse event (AE) and at least one adverse drug reaction (ADR). There were no deaths, no treatment discontinuations due to an adverse event or adverse drug reaction and no serious adverse events or serious adverse drug reactions.

Table 2S Summary of All Treatment-Emergent Adverse Events

SYSTEM ORGAN CLASS: Event	# and % of Subjects	# of Events
ANY EVENT	5 (100%)	40
EYE DISORDERS:		
Any event	1 (20%)	1
Photopsia	1 (20%)	1
GASTROINTESTINAL DISORDERS:		
Any event	3 (60%)	5
Abdominal discomfort	1 (20%)	1
Diarrhoea	1 (20%)	1
Gingival disorder	1 (20%)	1
Nausea	1 (20%)	1
Toothache	1 (20%)	1
GENERAL DISORDERS & ADMINISTRATION SITE CONDITIONS:		
Any event	2 (40%)	2
Fatigue	2 (40%)	2
INFECTIONS & INFESTATIONS:		
Any event	2 (40%)	4
Nasopharyngitis	1 (20%)	1
Respiratory tract infection	1 (20%)	1
Upper respiratory tract infection	2 (40%)	2
INJURY, POISONING & PROCEDURAL COMPLICATIONS:		
Any event	1 (20%)	3
Excoriation	1 (20%)	1
Fall	1 (20%)	1
Wound	1 (20%)	1
INVESTIGATIONS:		
Any event	3 (60%)	4
Blood Cholesterol increased	1 (20%)	1
Intraocular pressure increased	1 (20%)	1
Low density lipoprotein increased	2 (40%)	2
MUSCULOSKELETAL & CONNECTIVE TISSUE DISORDERS:		
Any event	4 (80%)	7
Back pain	2 (40%)	2
Muscle Strain	1 (20%)	1
Musculoskeletal pain	1 (20%)	1
Myalgia	1 (20%)	1
Neck pain	1 (20%)	1
Pain in extremity	1 (20%)	1
NERVOUS SYSTEM DISORDERS:		
Any event	5 (100%)	8
Dizziness	1 (20%)	2
Headache	5 (100%)	6
RENAL & URINARY DISORDERS:		
Any event	1 (20%)	1
Pollakiuria	1 (20%)	1
REPRODUCTIVE SYSTEM & BREAST DISORDERS:		
Any event	1 (20%)	1
Dysmenorrhoea	1 (20%)	1
SKIN & SUBCUTANEOUS TISSUE DISORDERS:		
Any event	2 (40%)	3
Dermatitis allergic	1 (20%)	1
Erythema	1 (20%)	1
Skin reactions	1 (20%)	1
VASCULAR DISORDERS:		
Any event	1 (20%)	1
Flushing	1 (20%)	1

Table 3S Summary of All Treatment-Emergent Adverse Drug Events

SYSTEM ORGAN CLASS: Event	# and % of Subjects	# of Events
ANY ADVERSE DRUG REACTION	5 (100%)	20
EYE DISORDERS: Any event Photopsia	 1 (20%) 1 (20%)	 1 1
GASTROINTESTINAL DISORDERS: Any event Diarrhoea Gingival disorder Nausea	 2 (40%) 1 (20%) 1 (20%) 1 (20%)	 3 1 1 1
GENERAL DISORDERS & ADMINISTRATION SITE CONDITIONS: Any event Fatigue	 2 (40%) 2 (40%)	 2 2
INVESTIGATIONS: Any event Blood cholesterol increased Low density lipoprotein increased	 2 (40%) 1 (20%) 2 (40%)	 3 1 2
MUSCULOSKELETAL & CONNECTIVE TISSUE DISORDERS: Any event Back pain Myalgia Pain in extremity	 2 (40%) 1 (20%) 1 (20%) 1 (20%)	 3 1 1 1
NERVOUS SYSTEM DISORDERS: Any event Headache	 5 (100%) 5 (100%)	 5 5
SKIN & SUBCUTANEOUS TISSUE DISORDERS: Any event Erythema Skin reaction	 2 (40%) 1 (20%) 1 (20%)	 2 1 1
VASCULAR DISORDERS: Any event Flushing	 1 (20%) 1 (20%)	 1 1

Summary of other AEs occurring in more than one subject (see also supplemental Tables 1-3S)

These included fatigue (2 subjects), upper respiratory tract infection (2 subjects) and increase in low-density lipoprotein (LDL) levels (2 subjects). Of those AEs, all events of fatigue and LDL increase were considered ADRs. Other ADRs occurring in one subject each included photopsia, diarrhea, gingival disorder, nausea, increase in blood cholesterol, back pain, myalgia, pain in extremity, erythema, skin reaction and flushing. The majority of AEs occurring in the study were mild in intensity. The six ADRs that were moderate in intensity included myalgia, pain in extremity, and four events of headache. There were no AEs or ADRs that were severe in intensity. Laboratory parameters that shifted from normal pre-dose to high or low within 14 days post-treatment included serum retinol (shifting to low in three subjects), cholesterol (shifting to high in two subjects), bicarbonate (shifting to low in two subjects), LDL (shifting to high in two subjects), and triglycerides (shifting to high in two subjects). There were no findings from vital signs, ECG, dilated fundus examination, biomicroscopy or intraocular pressure measurement that indicated a safety concern. In summary, treatment with 40 mg/m2 QLT091001 Oral Solution once daily for 7 days in RP subjects aged 40-68 years with an autosomal dominant RPE65 mutation was safe and well tolerated. The effect of retinoid supplementation lasted at least six months, providing promise for enhanced visual function (in visual acuity and/or visual field) in individuals with this late-onset dominant IRD.