Pathogenic non-coding variants in inherited retinal disease from 100KGP

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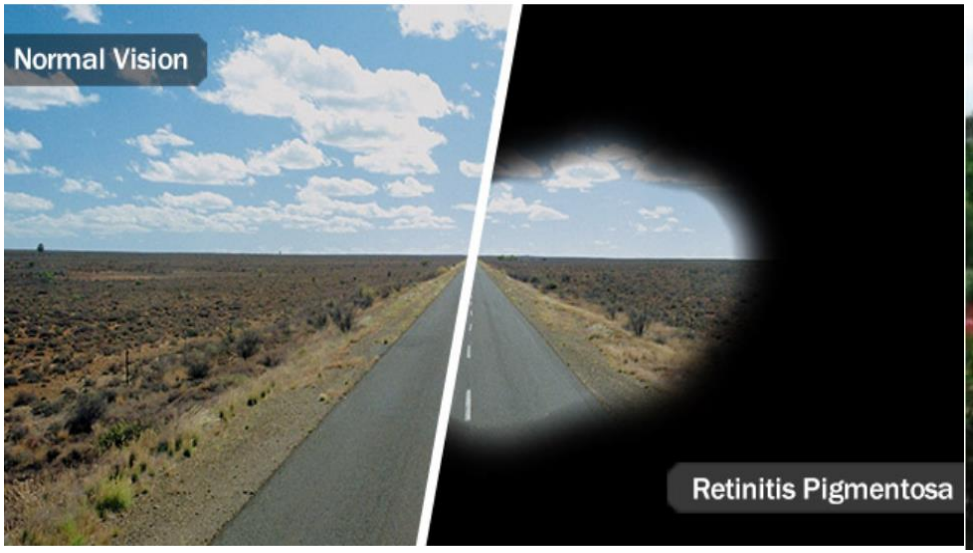
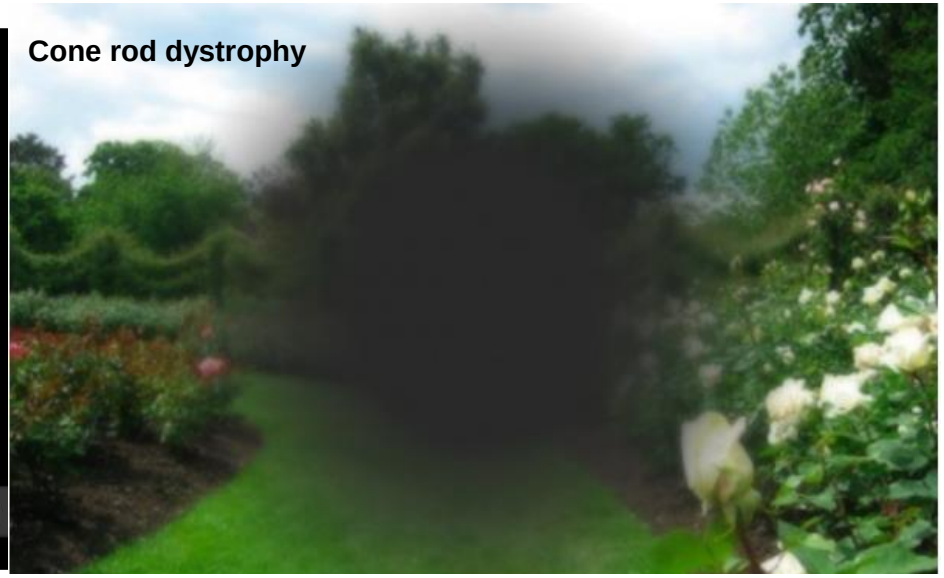
Inherited retinal disease (IRD)

- broad spectrum of disorders characterised by retinal cell dysfunction and/or cell death
- commonest cause of blindness in working age adults across the UK
- affects ~1 in 3000 individuals and over 2 million people worldwide



https://www.webmd.com/eye-health/what-is-retinitis-pigmentosa#1

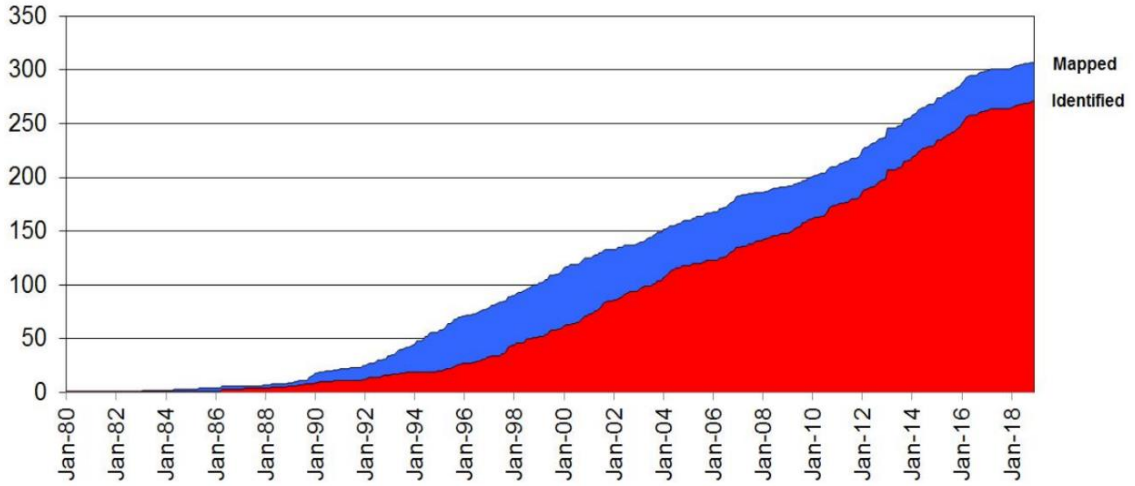


https://stargardtsdisease.weebly.com/what-its-like.html

Missing heritability of IRD

- Diagnostic rate is ~50-60%

- Main challenges
  - >250 genes
  - genetic, allelic and phenotypic heterogeneity
  - All inheritance patterns



https://sph.uth.edu/retnet/sum-dis.htm#D-graph

- **Unsolved cases?**
  - Non-coding variants
  - Structural variants
  - Novel genes
  - Mosaicism
  - Regulatory variants *in cis*
  - Hypomorphic alleles
  - Epigenetic
  - Non-mendelian inheritance patterns
  - Non-genetic phenocopies

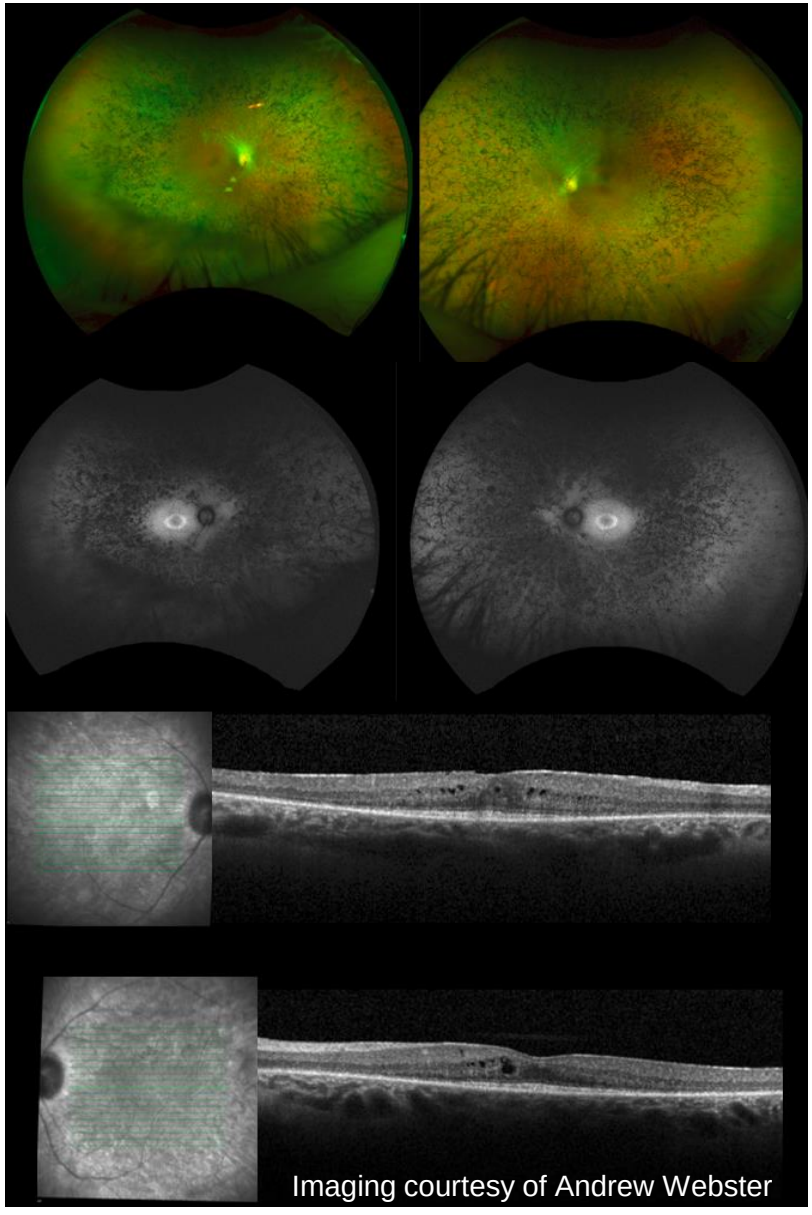
100KGP ophthalmology analysis

- 1459 patients recruited to 100KGP by MEH
- Passed through the diagnostic pipeline
  - WGS by Illumina
  - Automated bioinformatics analysis of **313 virtual gene panel** for posterior segment abnormalities
  - Screening/ scoring of selected variants by Clinical Scientists in North Thames GMC
  - Discussion at MDT
- Moorfield's diagnostic rate → 50-55%
- Unsolved cases who were heterozygous for a coding *USH2A* variant with corresponding phenotype and family history.

USH2A

- *USH2A* is the commonest cause of recessive RP
- Also causes type 2 Usher's syndrome (USH2)- sensorineural HL and RP

- 47YO Female
- Diagnosis of USH2
- Non consanguineous parents
- No FHx of hearing impairment or retinal disease



Imaging courtesy of Andrew Webster

100K analysis

- Recruited as trio with unaffected mother and unaffected brother
- Clinical pipeline identified *USH2A* c. 1036A>C, p.Asn346His (heterozygous)- previously identified
  - sibling- carrier of p.Asn346His
  - mother- not a carrier

→ Asn346His paternally inherited

Missing maternal allele

USH2A non-coding variant analysis

Filters applied:

- Heterozygous variants in *USH2A* shared between proband and mother
- Rarer than 1% in control datasets (GEL, EXAC, 1KGP)

→ 9 variants. 3 excluded as they were in low complexity regions of highly polymorphic repeats

→ 6 variants analysed using human splice finder

→ 1 variant was absent from gnomAD and predicted to strongly alter splicing **c.4885+375A>G**

In silico prediction

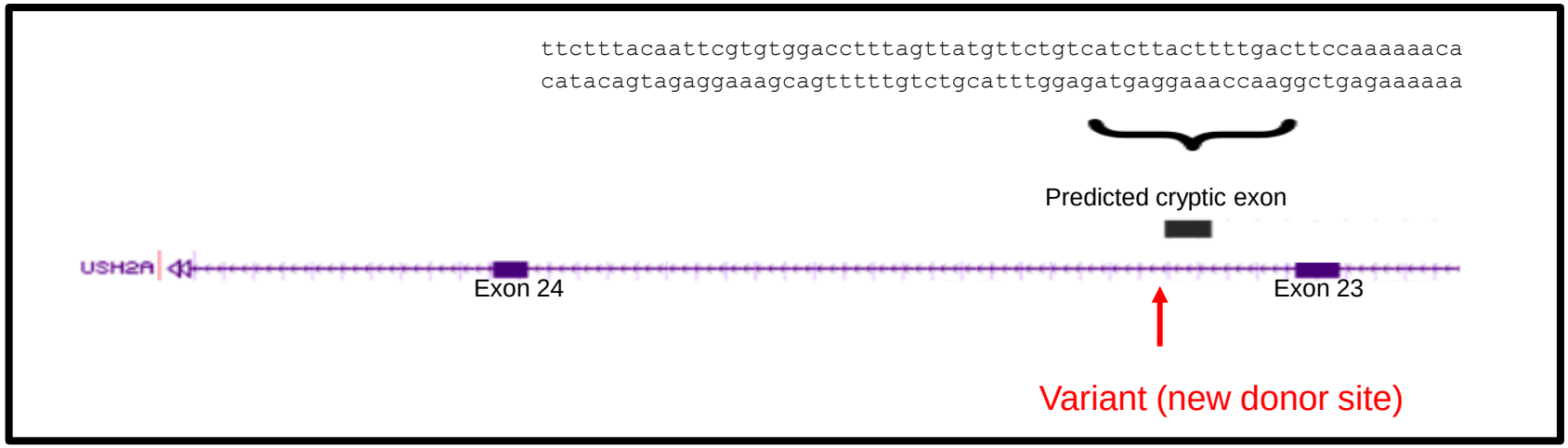
Splice site type	Motif	New splice site	Wild Type	Mutant	Variation (%)
Donor	aaagtaaat	AAAgtaagt	76.3	88.47	+15.95

Human Splicing Finder

Acceptor site predictions

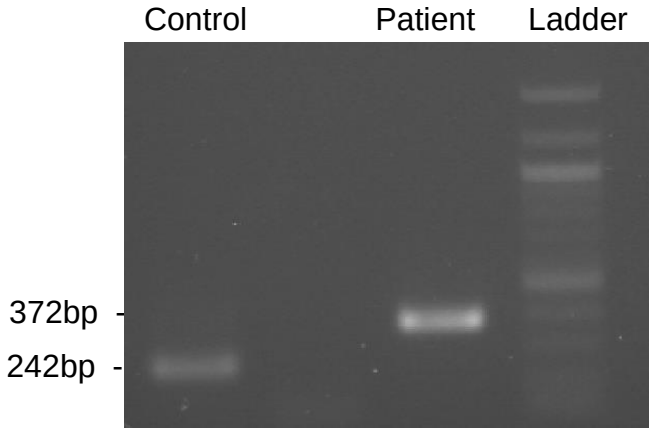
Score	Intron	Exon
0.47	accagtttttcatccatgaag	gtctttggtaaagaaaaaaa
0.86	agtcacctatgtcttttacag	ttctttacaattcgtgtgga

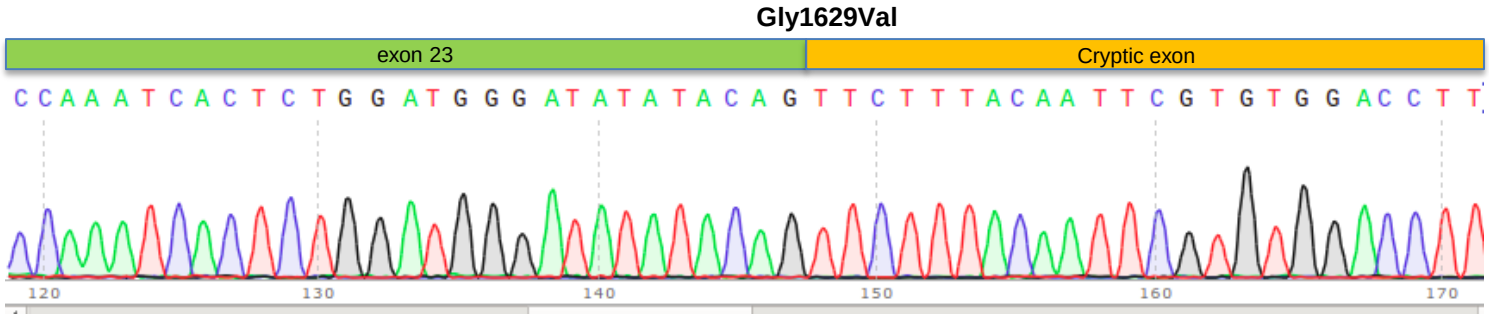
BDGP



RT-PCR transcript analysis

- *USH2A* transcripts isolated from nasal epithelial cells
- Nasal cell RNA extraction
- Reverse transcription-PCR
- Nested PCR Ex 23-24
- Pseudoexon- 130bp
- p.Gly1629ValfsTer52





Summary

- Non-coding variants are emerging as one of the causes unsolved IRD cases
- Unable to analyse these on a genome-wide scale, but they can be identified through careful case selection
- Simplex RP accounts for the highest proportion of unsolved IRDs
- *USH2A* is the commonest cause of recessive RP/ USH2
- *USH2A* is a useful model for the non-coding mutation hypothesis
- Could inform development of new bioinformatics pipelines

Acknowledgements

Gavin Arno

Monica Armengol

Rola Ba-Abbad

Michael E Cheetham

Omar Mahroo

Samantha Malka

Michel Michaelides

Anthony T Moore

Mariya Moosajee

Elena Schiff

Andrew R Webster

Genevieve Wright

Andrew Buckton

Lyn Chitty

Emma Clement

Savita Nutan

Genomics england

Great Ormond Street Hospital for Children NHS Foundation Trust

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