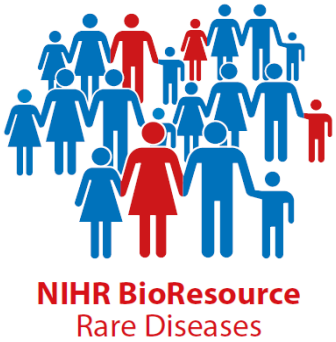
Comprehensive Rare Variant Analysis via Whole-Genome Sequencing to Determine the Molecular Pathology of Inherited Retinal Disease

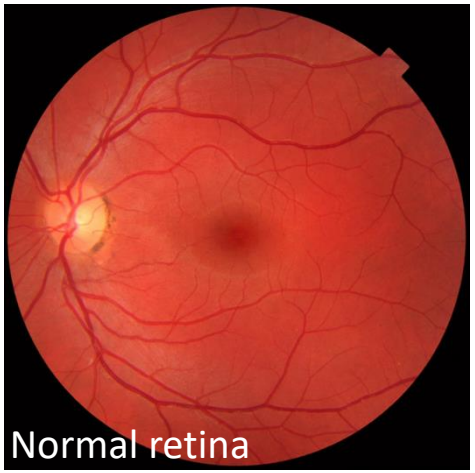
Keren Carss



Tuesday 27th June 2017

Inherited retinal disease (IRD)

- Phenotypically heterogeneous
    - Progressive or stationary
    - Rods or cones
    - Non-syndromic or syndromic
- Genetically and allelically heterogeneous
- Good candidates for WGS
- No large WGS studies of IRD published


Normal retina

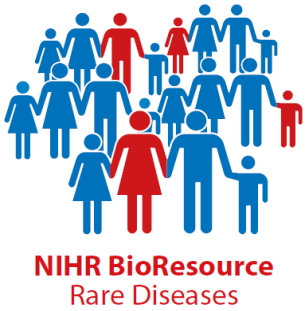

Inherited retinal disease

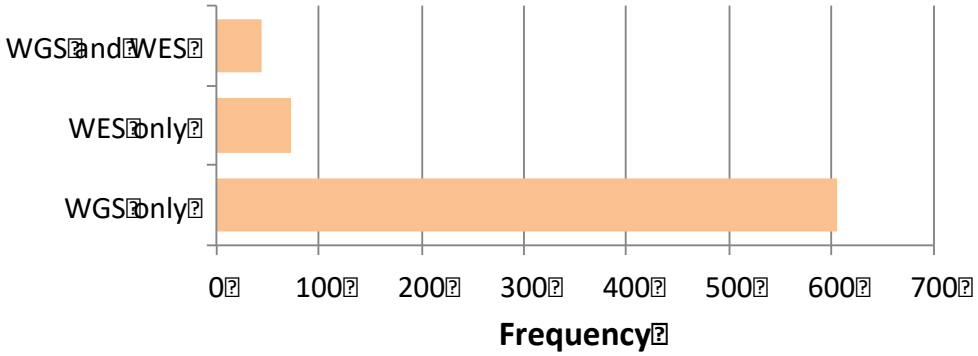
Aims

- Identify pathogenic variants including intractable cases
- Explore advantages and disadvantages of WGS
- Identify novel IRD-associated genes and provide new insights into phenotypes and the genetic architecture of IRD

Cohort

- Part of NIHR BioResource-Rare Diseases study
- High-throughput sequencing on 722 inherited retinal disease (IRD) patients





- Phenotypes include RP, cone-rod dystrophy, Stargardt disease, Usher syndrome
- Most recruited at Moorfields Eye Hospital (London)
- Most had some previous negative genetic test

Methods

WGS Sequencing pipeline: Illumina

PCR-free library preparation

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Paired-end WGS (Illumina HiSeq 2500 or HiSeq X Ten)

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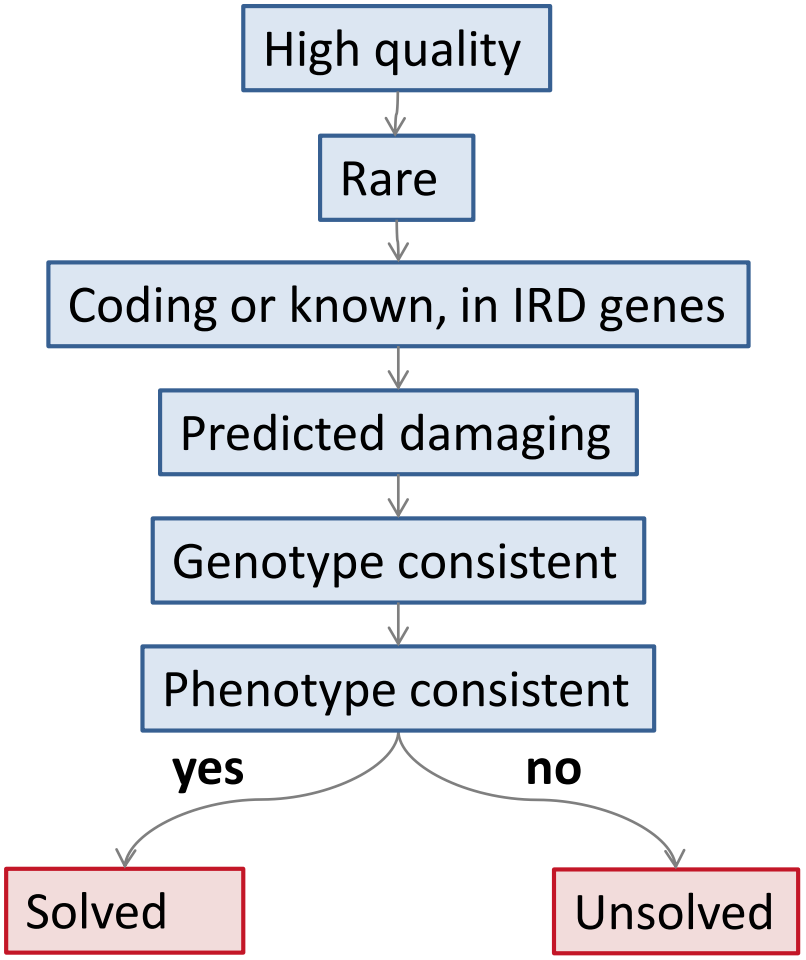
Reads aligned

↓

SNVs, indels and SVs identified

Average coverage 37X

Analysis pipeline



Pathogenic Variant Detection Rate

Sequencing Method	Total Cases	Cases Solved	Cases Partially Solved	Cases Unsolved
WGS	605	331 (55%)	31 (5%)	243 (40%)
WES	72	59 (82%)	3 (4%)	10 (14%)
WES and WGS	45	14 (31%)	2 (4%)	29 (64%)
TOTAL	722	404 (56%)	36 (5%)	282 (39%)

- WES initially solved 59/117 (50%) cases. Subsequently 45/58 unsolved cases also underwent WGS.
- 96/152 (63%) with no prescreening solved

14 cases solved by WES then WGS

- Variant not covered by WES baits (n=3)
- Large deletion or indel (n=3)
- Called by WES but variant did not pass QC (n=3)
- Called but WGS performed to exclude other possibilities (n=5)

The Effect of Ethnicity

Likely Ethnicity	Total Cases	Cases Solved	Cases Partially Solved	Cases Unsolved
EUR	467	259 (55%)	23 (5%)	185 (40%)
SAS	123	70 (57%)	4 (3%)	49 (40%)
AFR	43	13 (30%)	4 (9%)	26 (60%)
EAS	13	1 (8%)	1 (8%)	11 (85%)
AMR	4	2 (50%)	1 (25%)	1 (25%)
TOTAL	650	345 (53%)	33 (5%)	272 (42%)

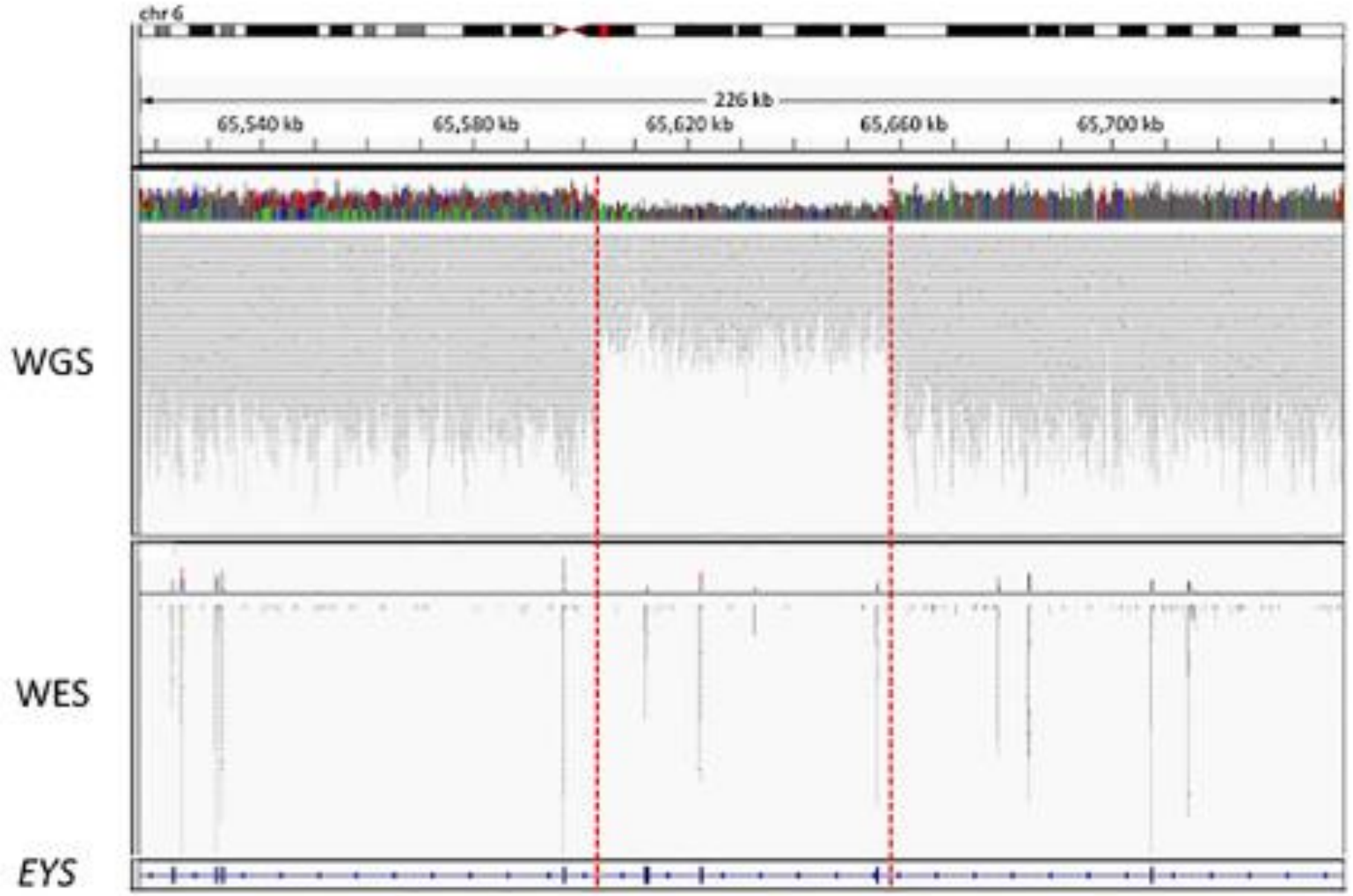
Likely ethnicity estimated from WGS data using principal component analysis. Table includes individuals who had WGS only. Abbreviations: EUR, European; SAS, South Asian; AFR, African; EAS, East Asian; AMR, Ad Mixed American.

66% of pathogenic variants in SAS individuals were homozygous, compared to 18% in EUR individuals

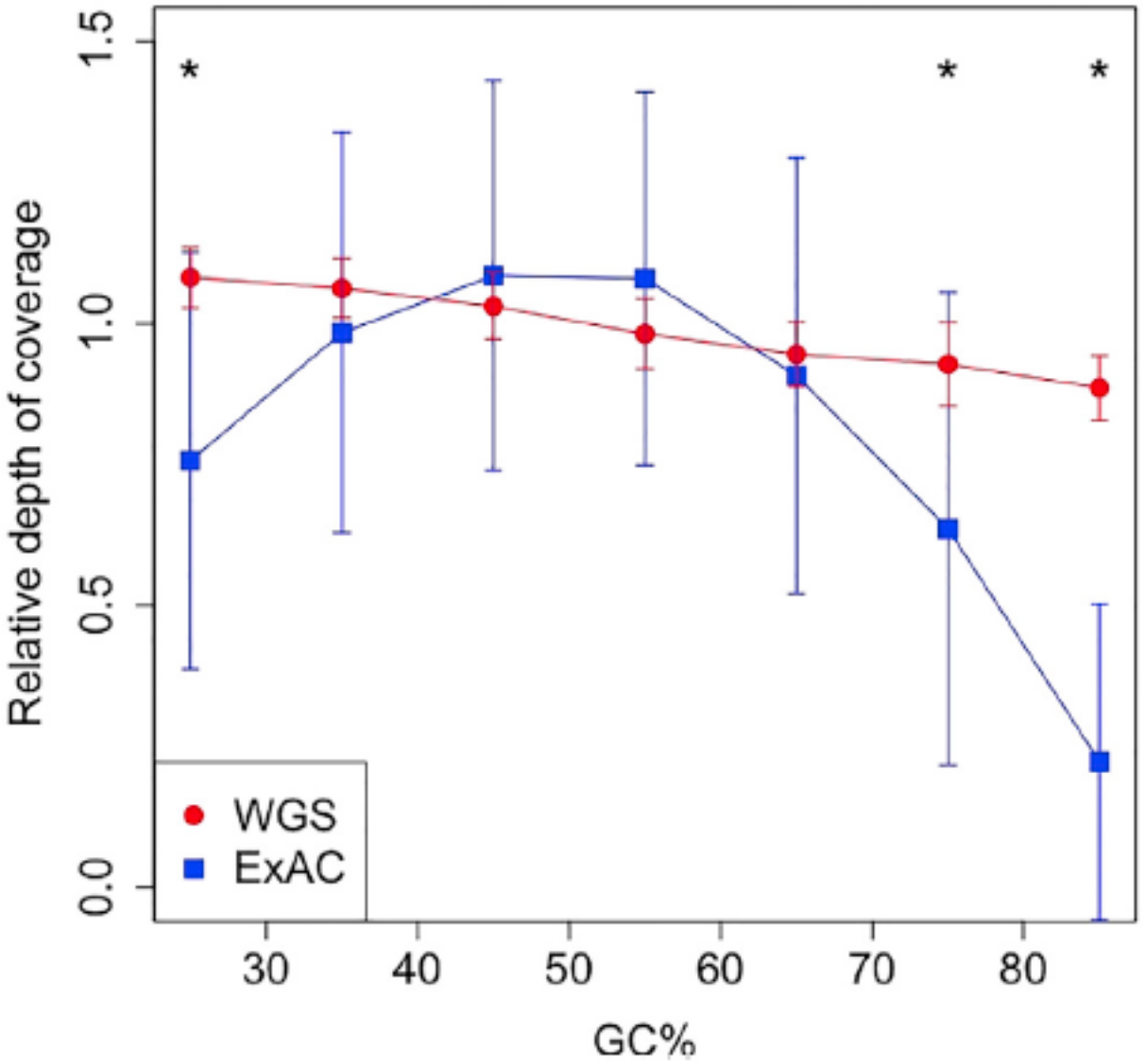
WGS: higher power to detect variants

- Structural variants
- Variants in GC-rich regions
- Variants in non-coding regions

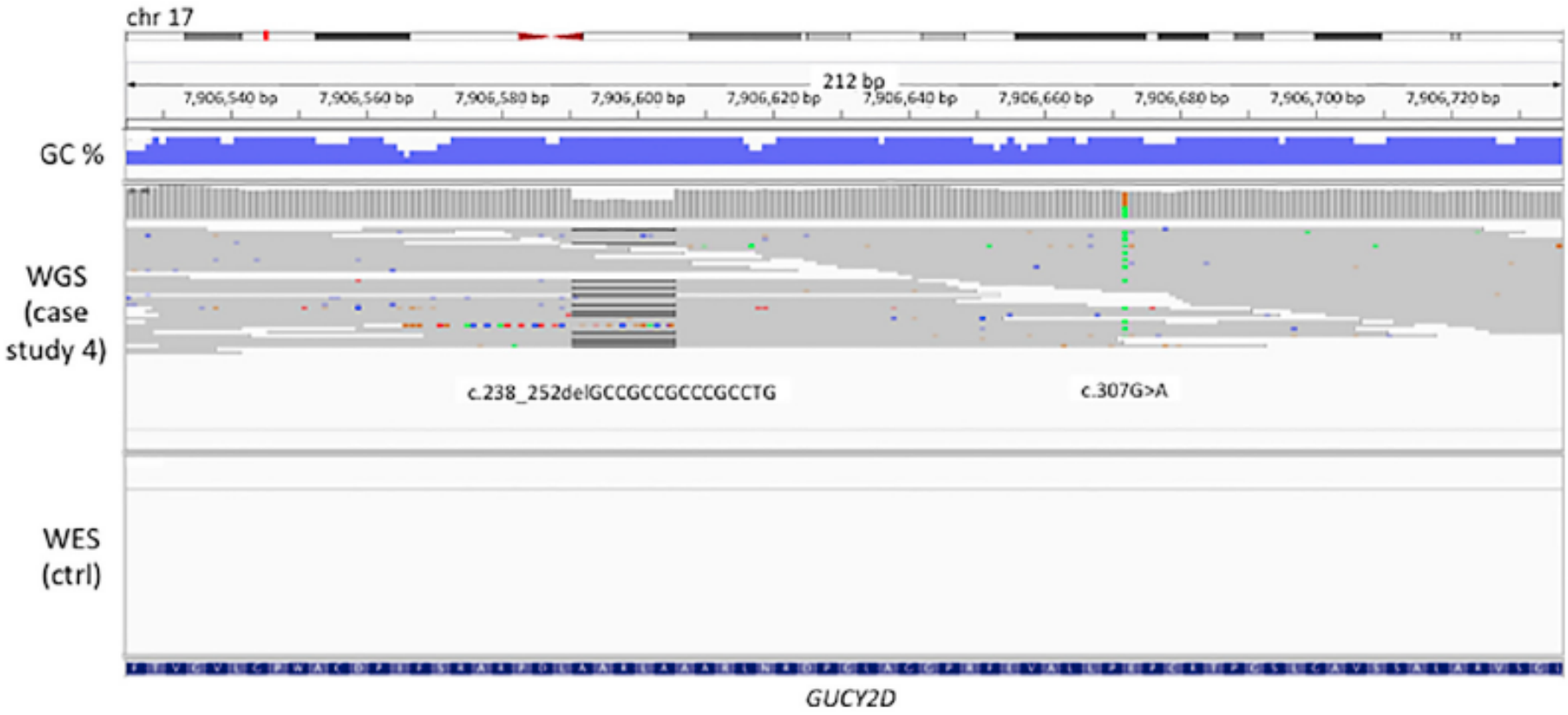
WGS Increases Power to Detect SVs



Sequencing Regions of Extreme GC Bias

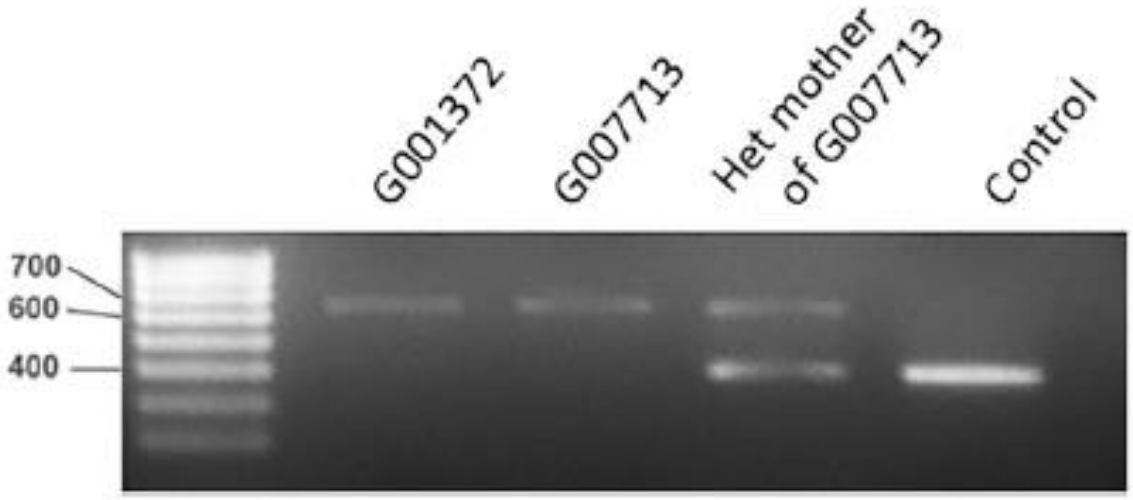


Pathogenic Variants in GC-Rich Regions



A Novel Pathogenic Intronic Variant

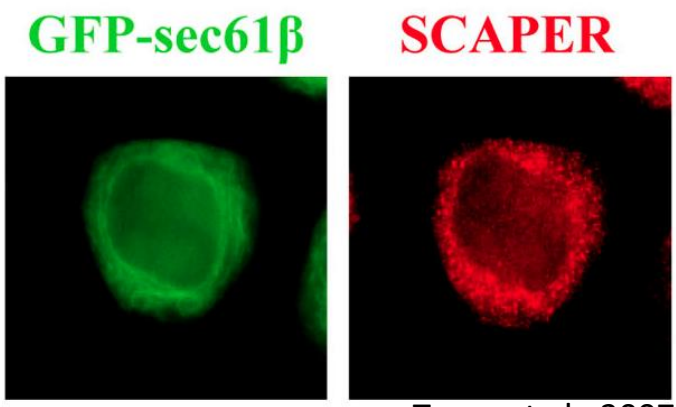
- 2 unrelated males with choroideremia
- Previously unreported deep intronic *CHM* Variant c.315-1536A>G
- Splice prediction analysis predicted introduction of cryptic splice acceptor site
- Inclusion of cryptic exon confirmed by RT-PCR



SCAPER

Case 1	Case 2	Case 3
WGS	WES	WES
UK	UK	USA
No family history	No family history	No family history
RP, DD, autism, and ADD	RP, possible mild DD	RP, obesity and DD
NM_020843: c.1116delT, p.Val373SerfsTer21	NM_020843.2: c.1495+1G>A	NM_020843.2: c.829C>T, p.Arg277Ter
NM_020843: c.2179C>T, p.Arg727Ter	NM_020843.2: c.3224delC, p.Pro1075GlnfsTer11	NM_020843.2: c.3707_3708delCT p.Ser1236TyrfsTer28

- *SCAPER* encodes S Phase Cyclin A-Associated Protein In The ER
- Widely expressed
- Involved in cell cycle progression



Tsang et al., 2007

Closing Comments

- Largest study of IRD using WGS to date
- Heterogeneous cohort
- Overall 56% solved
- Advantages of WGS
- Unsolved cases
- Limitations of WGS

Carss et al., The American Journal of Human Genetics *100*, 75-90, Jan 5, 2017

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