Multi-omics to advance genetic diagnostics of rare eye diseases

Elfride De Baere

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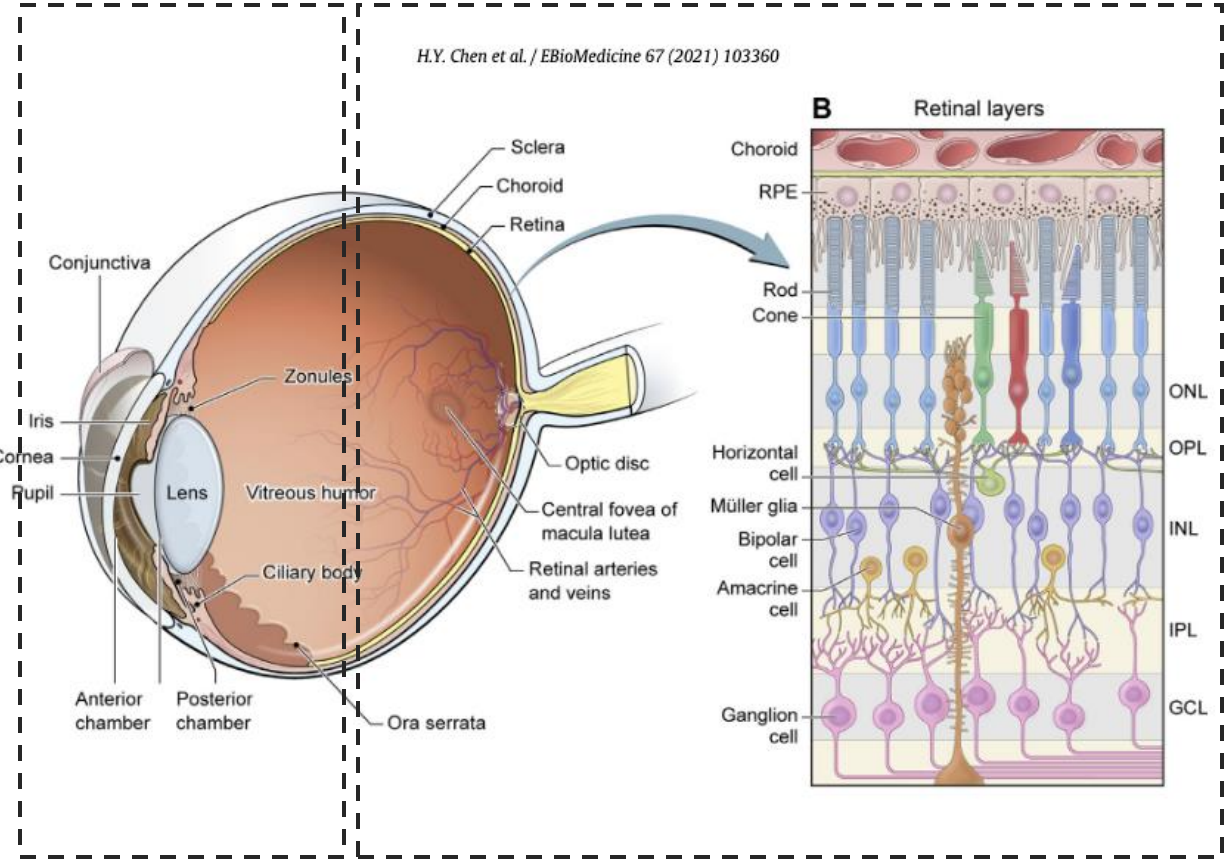
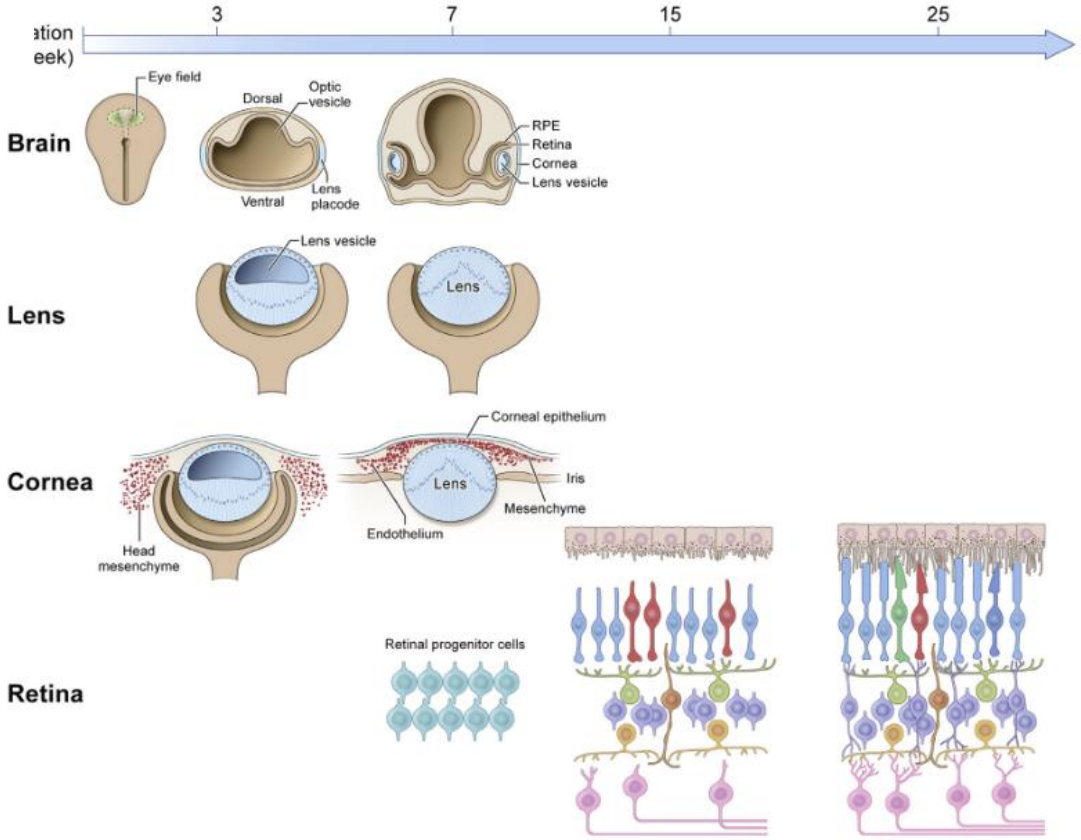
Center for Medical Genetics

Rare eye diseases (RED): a major cause of vision loss and target for treatment



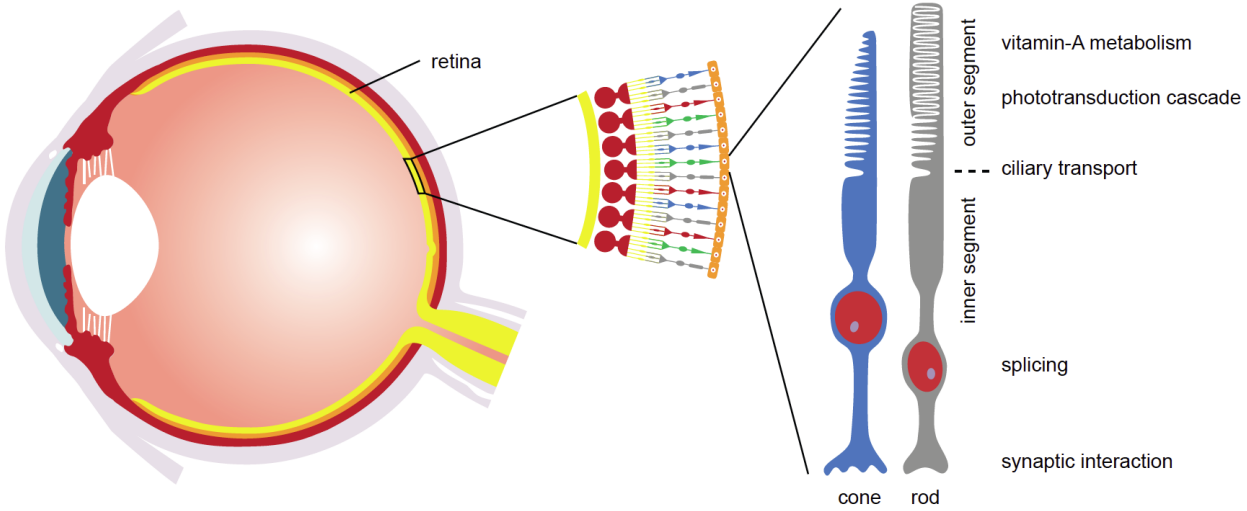
Diverse spectrum: from anterior to posterior segment

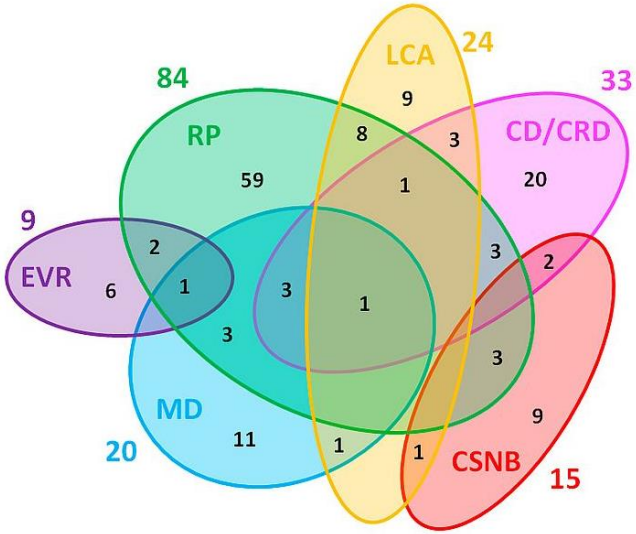


Development is tightly regulated

Chena, Lehmann & Swaroop, EBioMedicine 2021

Inherited retinal diseases (IRD): clinical and genetic heterogeneity





2+ million patients worldwide, 1/3,000

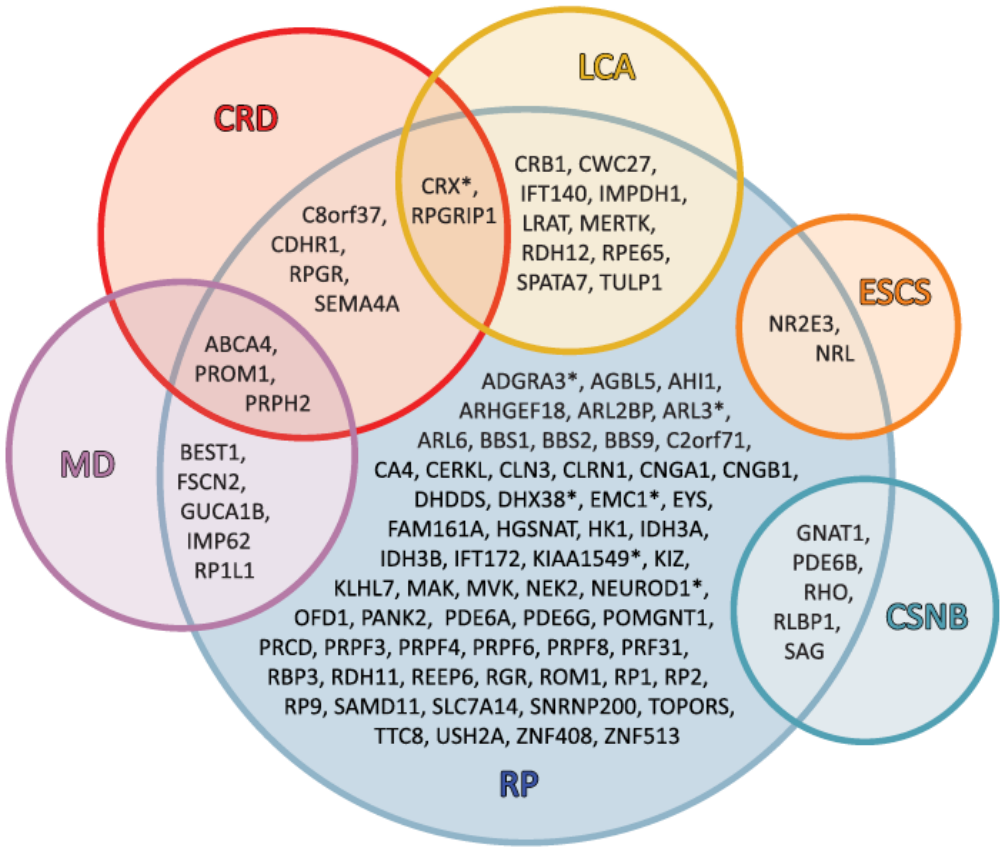
Genetic heterogeneity, 300+ genes

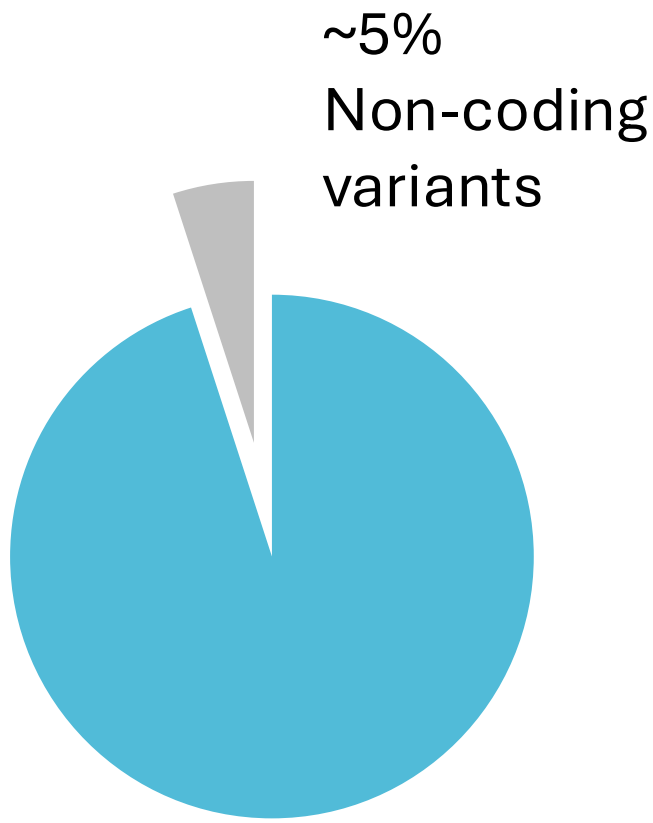
Rod involvement

Clinical heterogeneity

Cone involvement

Genetic basis of IRD



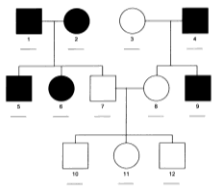


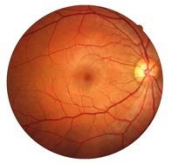
>300 IRD genes, RetNet

~60% molecular diagnosis

Genetic testing of RED: standard of care

Deep phenotyping





Patient with unexplained IRD
Family recruitment
Sample collection
Consent

Exome sequencing (ES)
Targeted gene panels
Single gene sequencing


Reverse phenotyping:
Re-evaluating clinical findings in light of (likely) pathogenic variant(s)

Multidisciplinary interpretation of variants based on inheritance, clinical features, predictions, literature

Reanalysis based on clinical information, family information, time elapsed, pipeline changes

ES-based panels

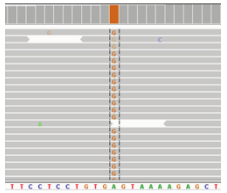
RetNet v7 (324 genes) (**56%** 'solved' rate)
Cataract v3 (77 genes)
Corneal dystrophy v3 (37 genes)
Glaucoma v2 (31 genes)
MAC-ASD v3 (102 genes)
OCA-OA-nystagmus v2 (30 genes)
Optic atrophy v2 (35 genes)

Targeted gene panels

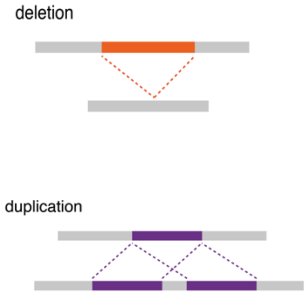
Customized
XLRP panel including *RPGR*

Single-gene testing

ABCA4 including deep intronic variants



SNVs, indels



CNVs

Limitations of genetic testing in IRD:
missing heritability

Limitations of genetic testing in IRD:
missing heritability

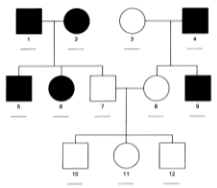
After WES, 44% of patients are molecularly unresolved

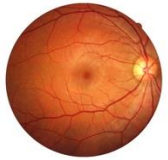
Limitations of exome-oriented genetic testing in IRD: missing heritability

Causes of missing heritability
Non-coding variants
‘Gaps’ in exome or genome
Complex structural variants (SVs)
Complex alleles
Hypomorphic alleles
Mosaicism
Uniparental disomy
Non-coding RNAs
Digenic/oligogenic inheritance

From exome- to genome-based testing

Deep phenotyping





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Genome sequencing (GS)
SR-WGS, LR-WGS, OGM

SNVs, indels

SVs

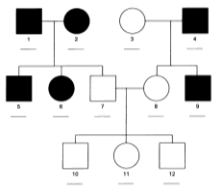
Reverse phenotyping:
Re-evaluating clinical findings in light of (likely) pathogenic variant(s)

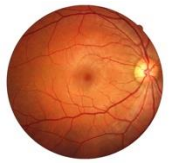
Multidisciplinary interpretation of variants based on inheritance, clinical featurres, predictions, literature

Enrollment based on prior genetic result (e.g. monoallelic), clinical information, family information

Future: from single genomics to multi-omics

Deep phenotyping





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Customized functional assays

Transcriptomics

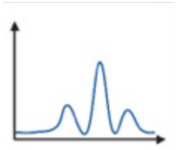
Epigenomics

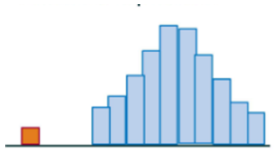
Minigene & luciferase assays

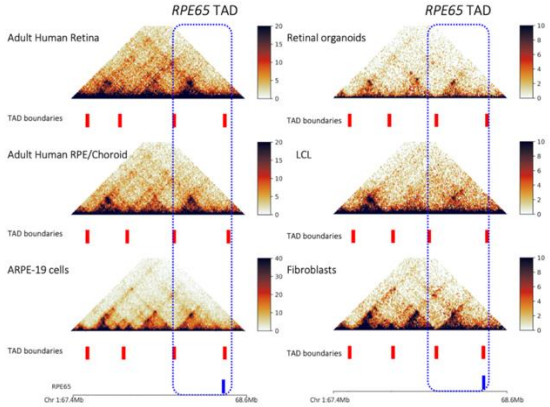
Biochemical assays

Aberrant expression

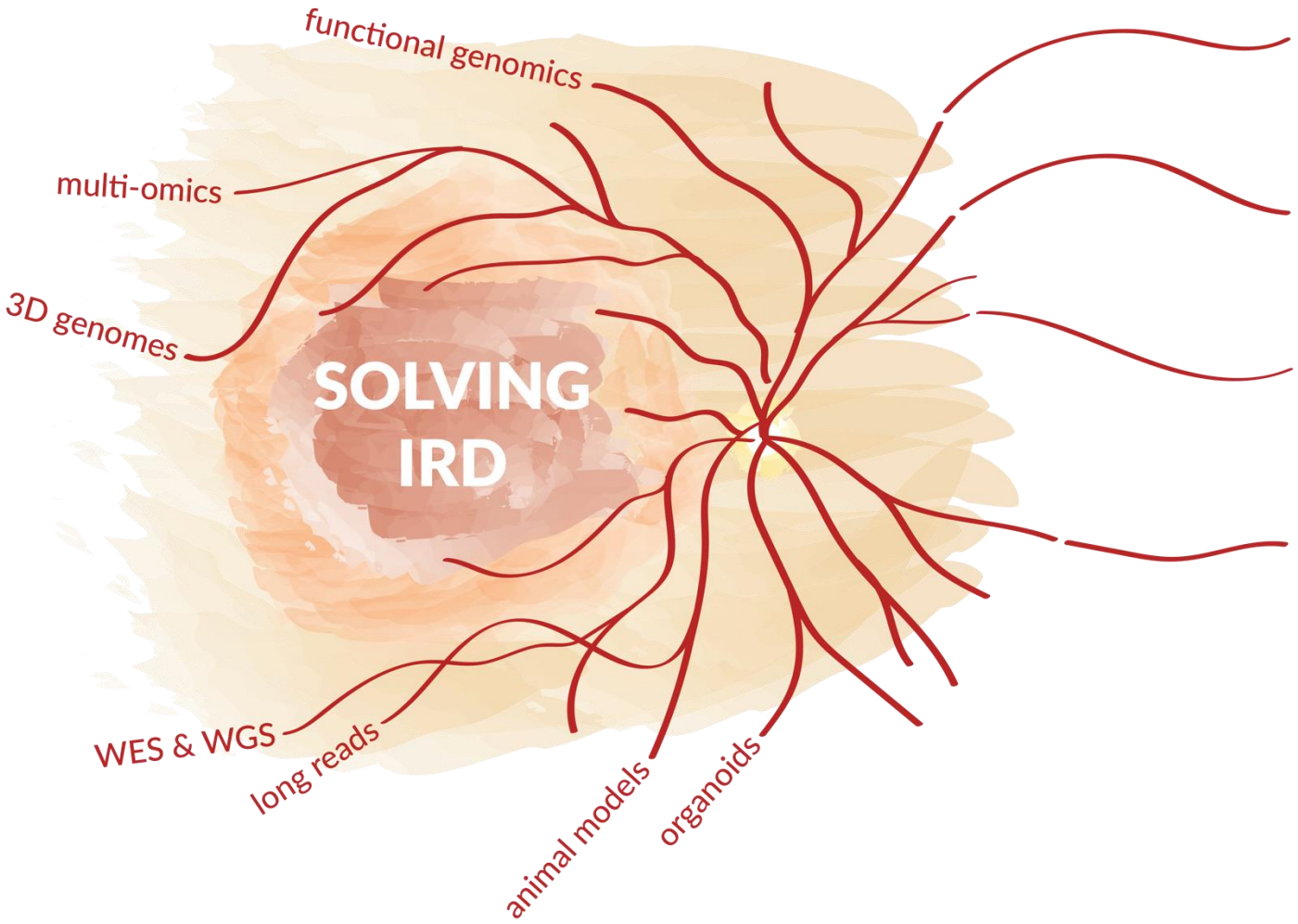
Aberrant splicing







Outline multi-omics



Courtesy of Eva D'haene

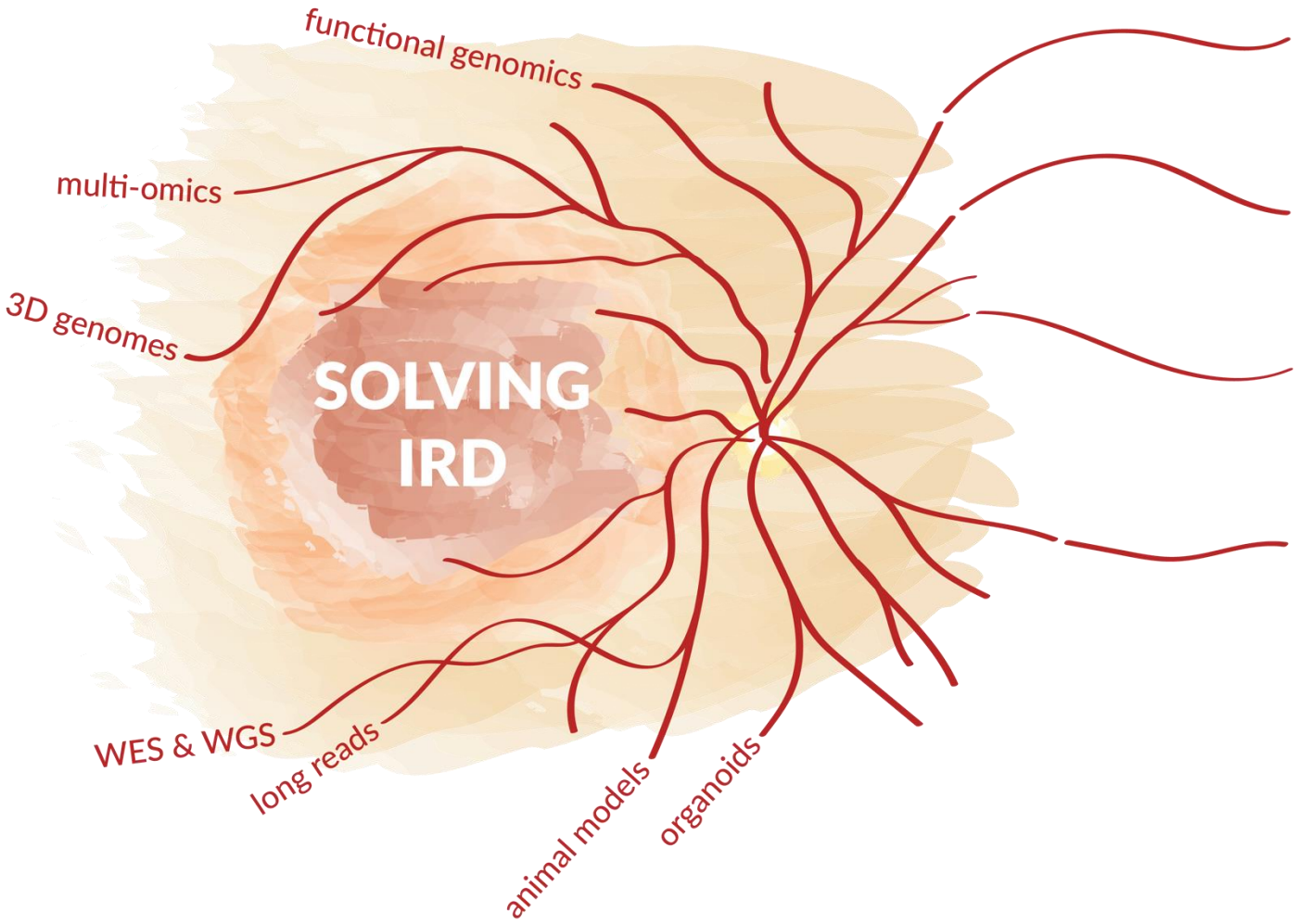
Integrated multi-omics approach in IRD

Coding SNVs in known IRD genes

Non-coding variants increase the diagnostic yield in IRD

Coding variants in novel candidate genes

Outline multi-omics



Integrated multi-omics approach in IRD

Coding SNVs in known IRD genes

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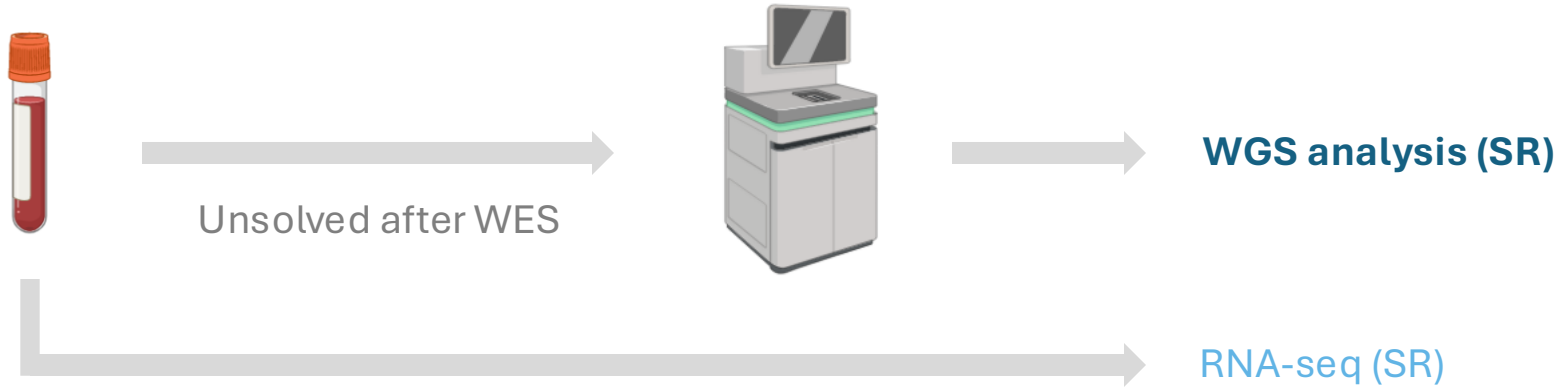
Coding variants in novel candidate genes

Integrated multi-omics approach
to unravel missing heritability in IRD

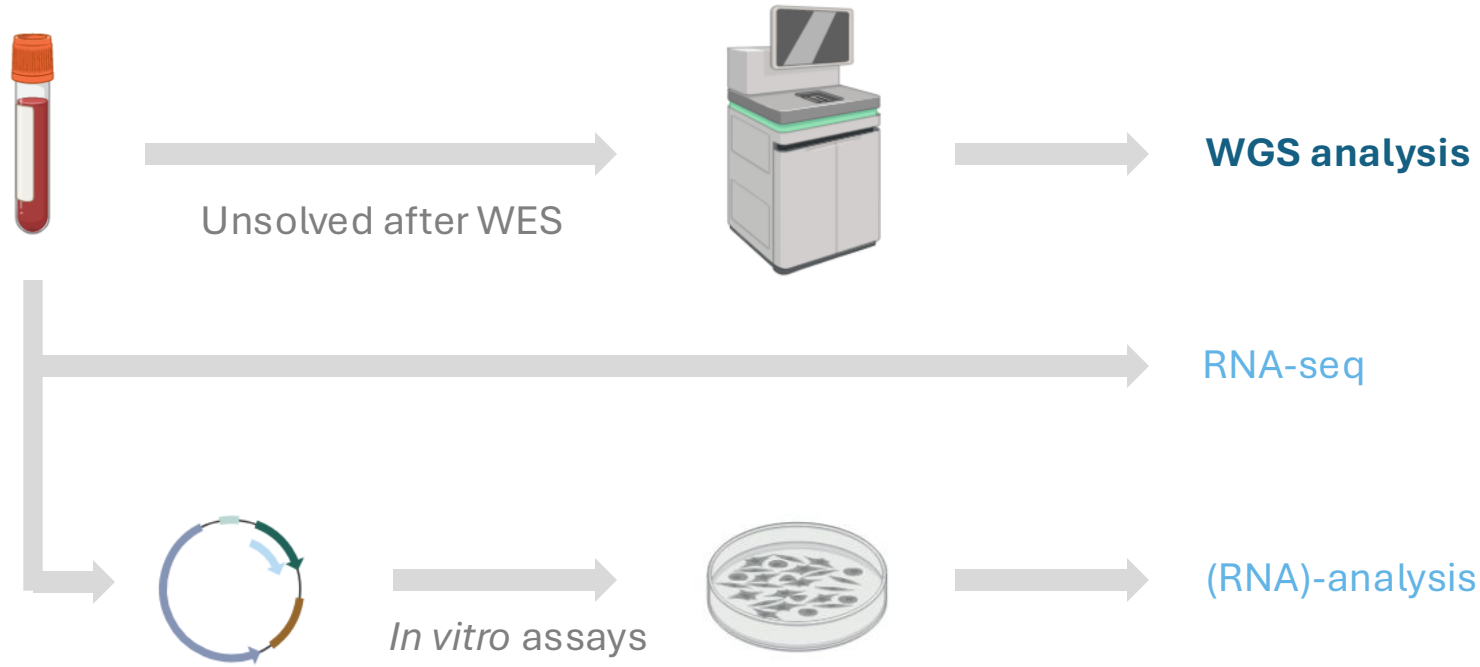
Unsolved after WES

WGS analysis (SR)

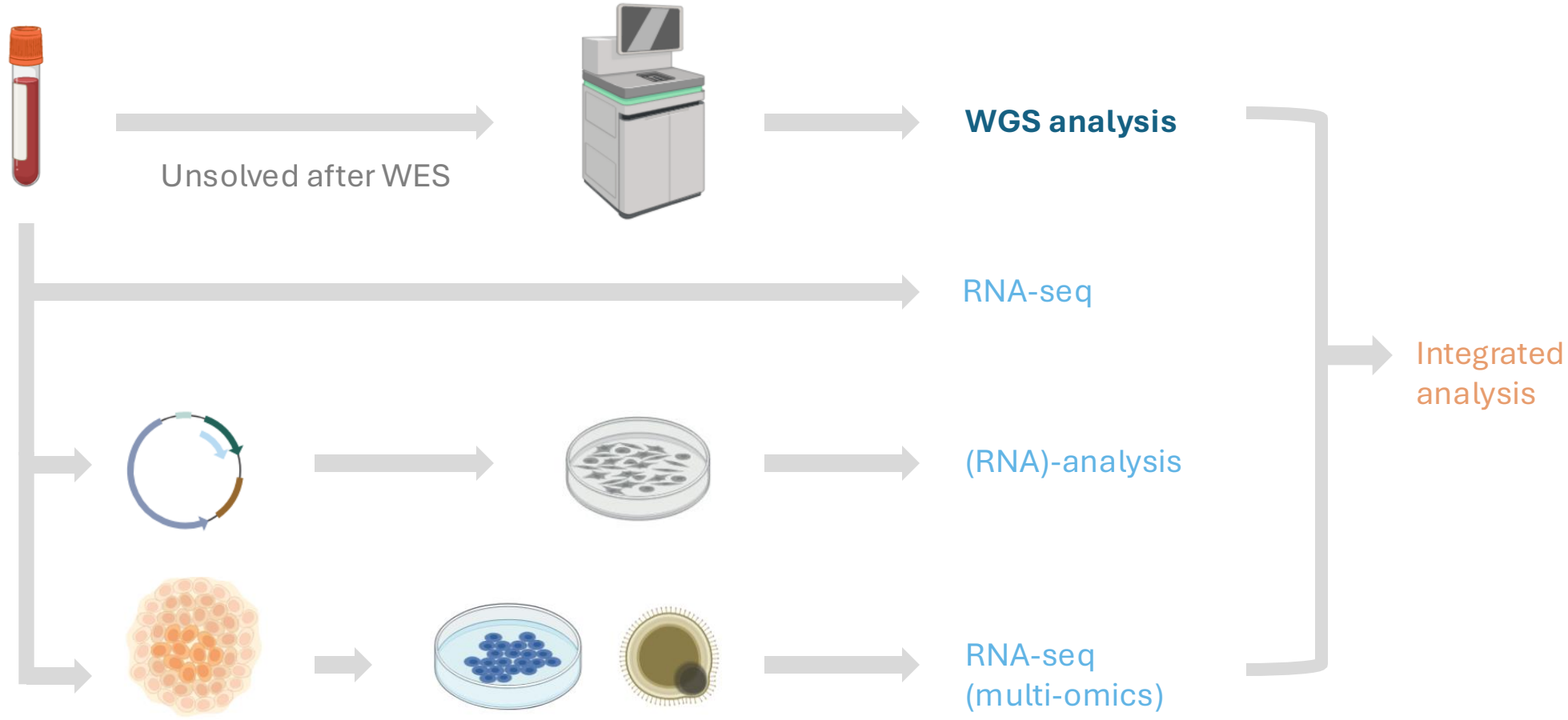
Integrated multi-omics approach
to unravel missing heritability in IRD



Integrated multi-omics approach
to unravel missing heritability in IRD

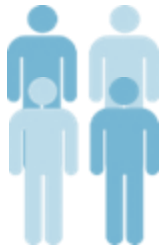


Integrated multi-omics approach
to unravel missing heritability in IRD



Courtesy of Miriam Bauwens

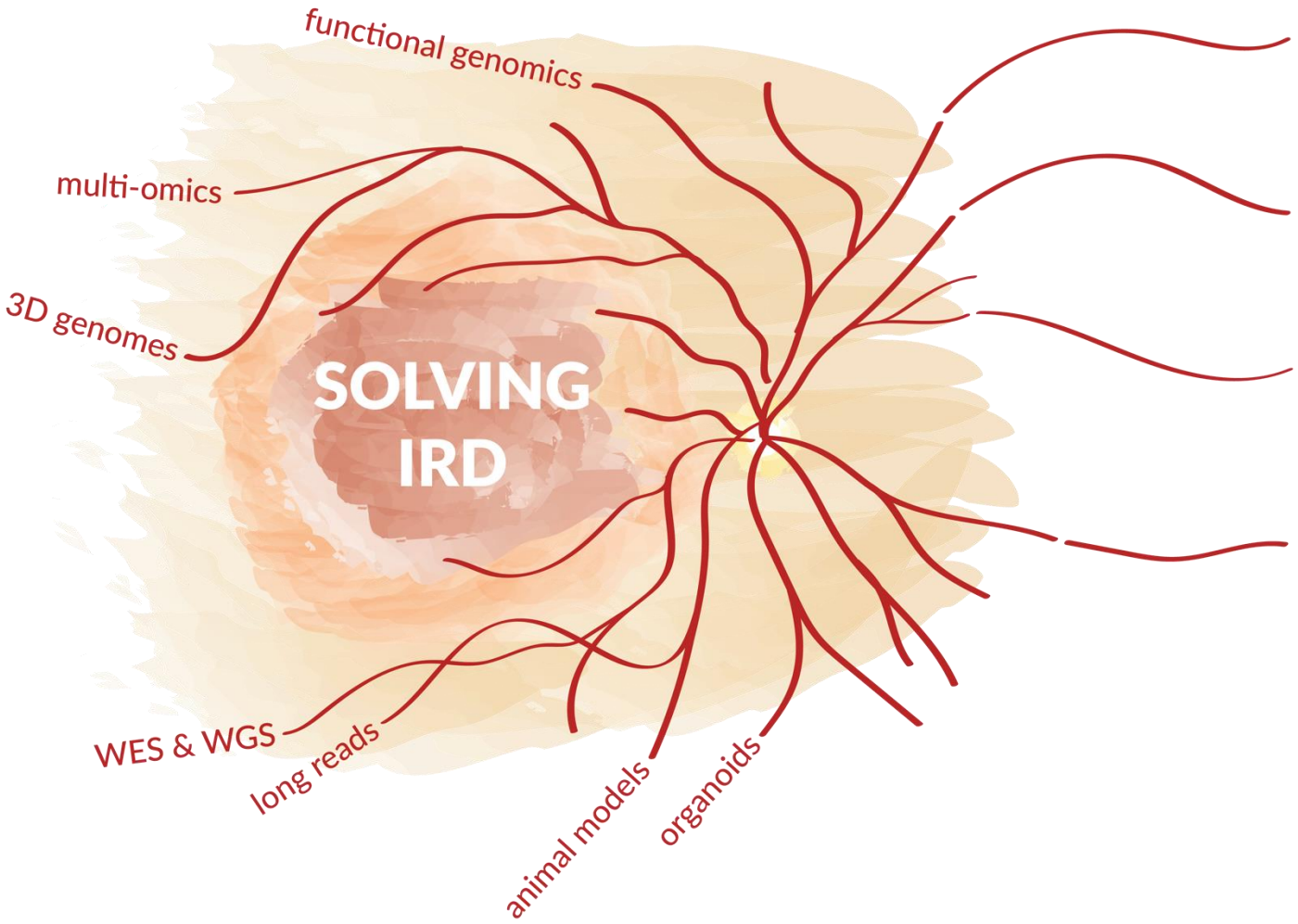
WGS analysis makes use of a multi-level filtering approach



N=185

- CODING SNVs IRD GENES
- NON-CODING SNVs IRD GENES
- SVs IRD GENES
- CANDIDATE GENES

Outline multi-omics



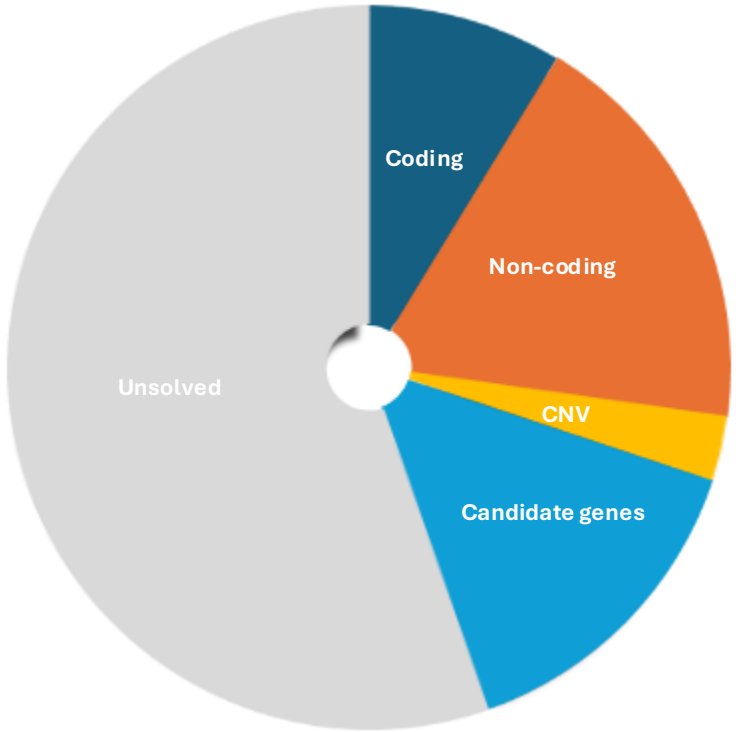
Integrated multi-omics approach in IRD

Coding SNVs in known IRD genes

Non-coding variants increase the diagnostic yield in IRD

Coding variants in novel candidate genes

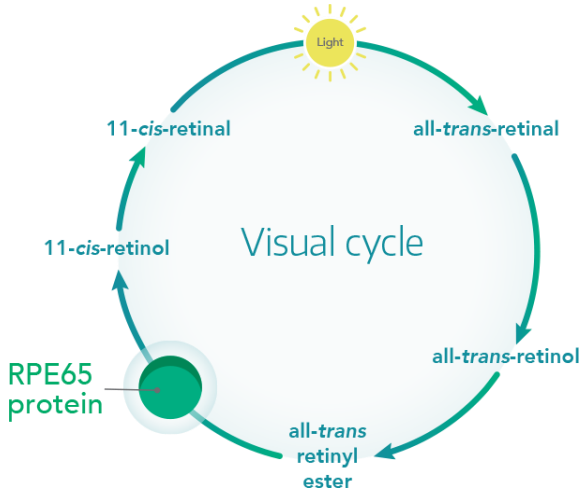
Coding SNVs in known IRD genes were identified in 8.5%

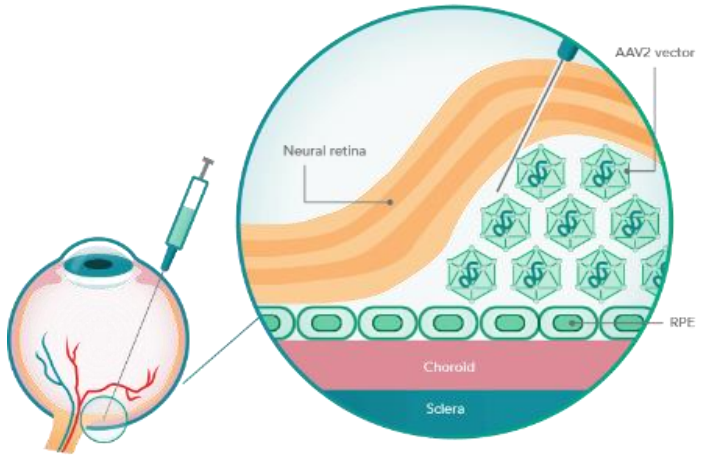


Coding SNVs in known IRD genes (8.5%)

Coding variants were identified in 8.5%

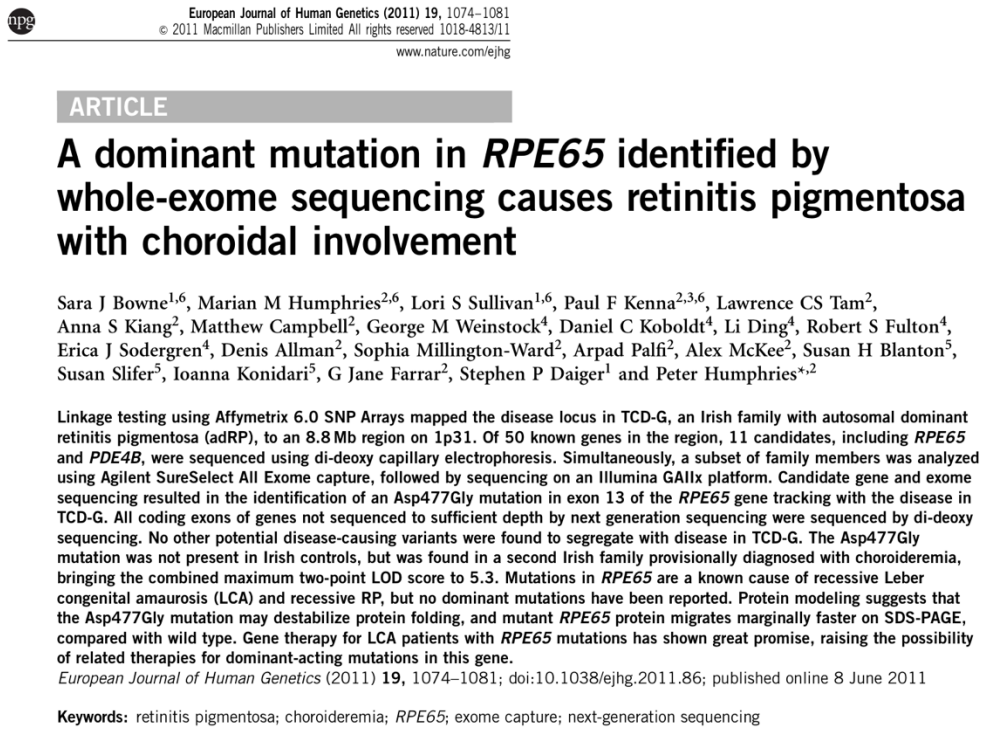
- Retinal pigment epithelium-specific 65 kDa
- Isomerohydrolase in visual cycle
- Associated with severe autosomal recessive IRD
  - Over 200 *RPE65* variants
  - Luxturna gene therapy





RPE65 and autosomal dominant retinopathy

- One dominant *RPE65*-IRD
- c.1430A>G, D477G
  - Irish origin
  - Mild late-onset disease with non-penetrance
  - Splicing effect
  - Mechanism?



European Journal of Human Genetics (2011) 19, 1074–1081

www.nature.com/ejhg

ARTICLE

A dominant mutation in *RPE65* identified by whole-exome sequencing causes retinitis pigmentosa with choroidal involvement

Sara J Bowne[1,6], Marian M Humphries[2,6], Lori S Sullivan[1,6], Paul F Kenna[2,3,6], Lawrence CS Tam[2], Anna S Kiang[2], Matthew Campbell[2], George M Weinstock[4], Daniel C Koboldt[4], Li Ding[4], Robert S Fulton[4], Erica J Sodergren[4], Denis Allman[2], Sophia Millington-Ward[2], Arpad Palfi[2], Alex McKee[2], Susan H Blanton[5], Susan Slifer[5], Ioanna Konidari[5], G Jane Farrar[2], Stephen P Daiger[1] and Peter Humphries*[,2]

Linkage testing using Affymetrix 6.0 SNP Arrays mapped the disease locus in TCD-G, an Irish family with autosomal dominant retinitis pigmentosa (adRP), to an 8.8 Mb region on 1p31. Of 50 known genes in the region, 11 candidates, including *RPE65* and *PDE4B*, were sequenced using di-deoxy capillary electrophoresis. Simultaneously, a subset of family members was analyzed using Agilent SureSelect All Exome capture, followed by sequencing on an Illumina GAIIx platform. Candidate gene and exome sequencing resulted in the identification of an Asp477Gly mutation in exon 13 of the *RPE65* gene tracking with the disease in TCD-G. All coding exons of genes not sequenced to sufficient depth by next generation sequencing were sequenced by di-deoxy sequencing. No other potential disease-causing variants were found to segregate with disease in TCD-G. The Asp477Gly mutation was not present in Irish controls, but was found in a second Irish family provisionally diagnosed with choroideremia, bringing the combined maximum two-point LOD score to 5.3. Mutations in *RPE65* are a known cause of recessive Leber congenital amaurosis (LCA) and recessive RP, but no dominant mutations have been reported. Protein modeling suggests that the Asp477Gly mutation may destabilize protein folding, and mutant *RPE65* protein migrates marginally faster on SDS-PAGE, compared with wild type. Gene therapy for LCA patients with *RPE65* mutations has shown great promise, raising the possibility of related therapies for dominant-acting mutations in this gene.
European Journal of Human Genetics (2011) **19,** 1074–1081; doi:10.1038/ejhg.2011.86; published online 8 June 2011

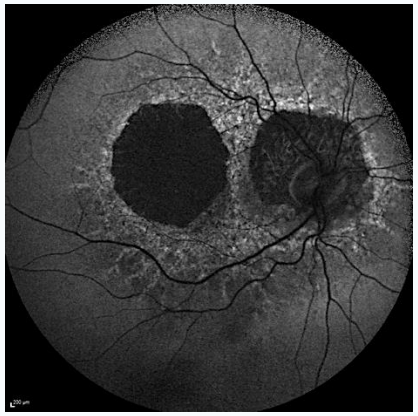
Keywords: retinitis pigmentosa; choroideremia; *RPE65*; exome capture; next-generation sequencing

Bowne et al., EJHG 2011

Miriam
Bauwens

We found a novel monoallelic *RPE65* variant in WGS data

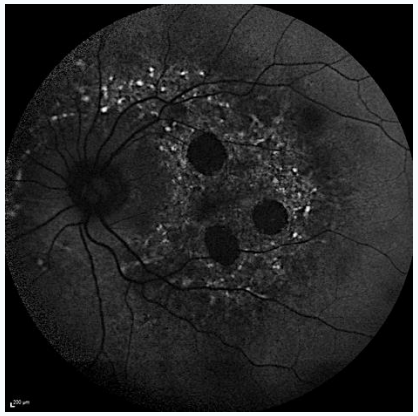
Patient 1



Age of onset: 60

Indications
Macular dystrophy
MIDD-like

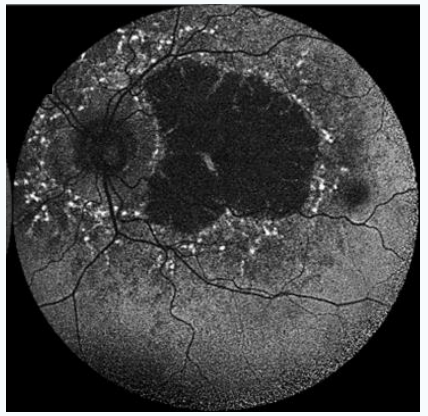
Patient 2



Age of onset: 41

Indications
Macular dystrophy
MIDD-like

Patient 3

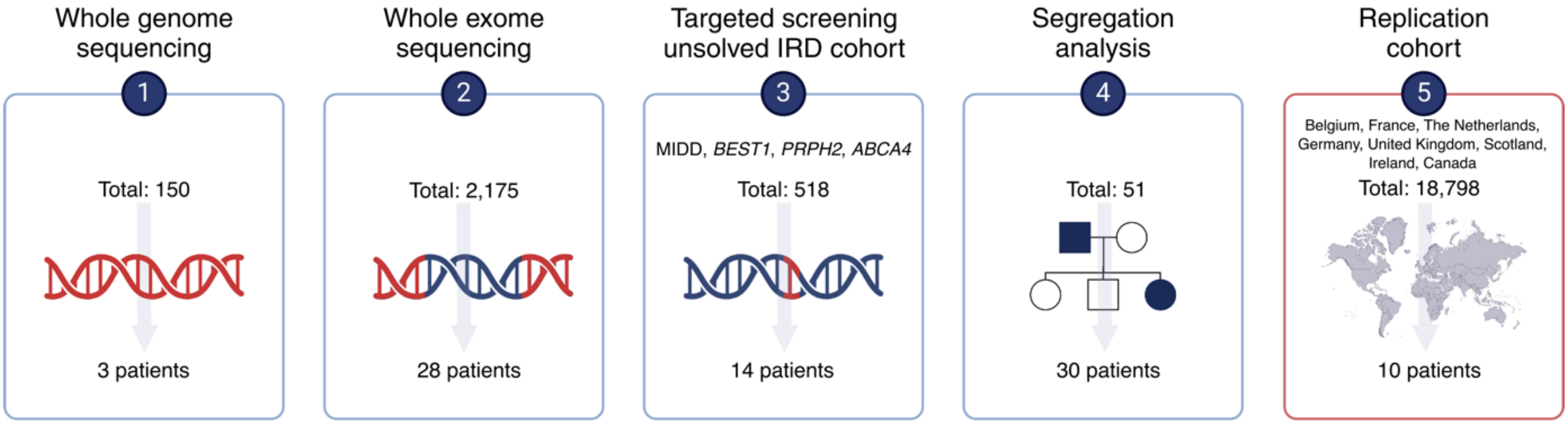


Age of onset: 45

Indications
Macular dystrophy
MIDD-like

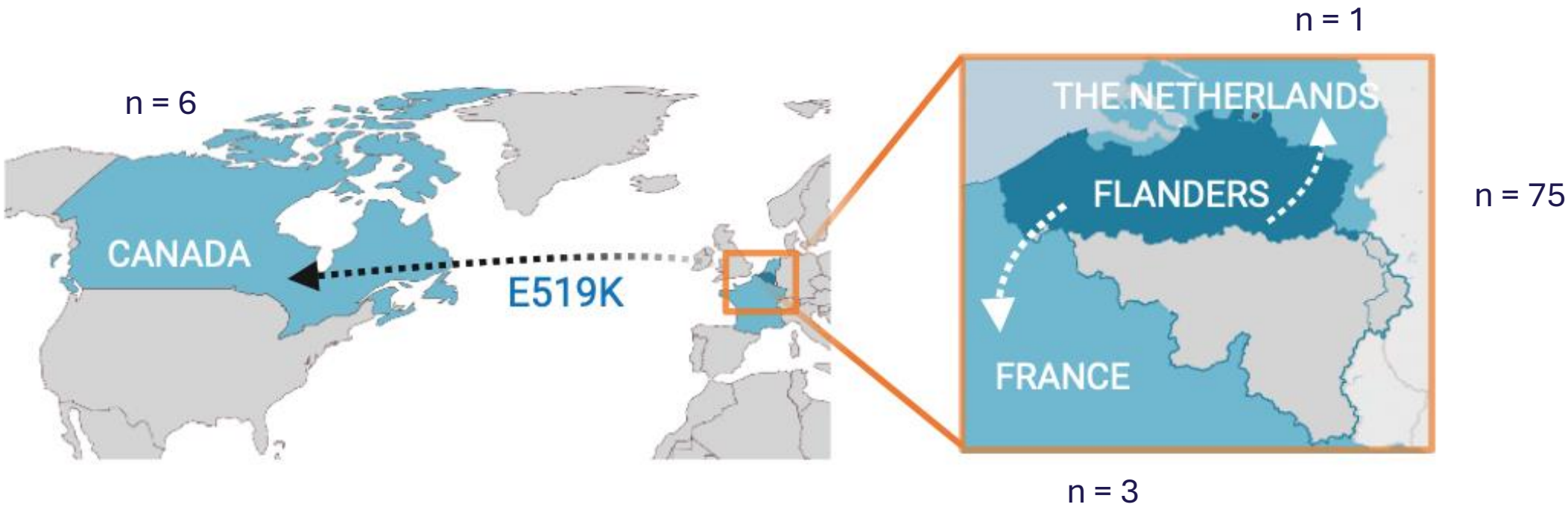
c.1555G>A, p.(E519K) in *RPE65*

Overall we identified 85 IRD patients with novel *RPE65* variant E519K



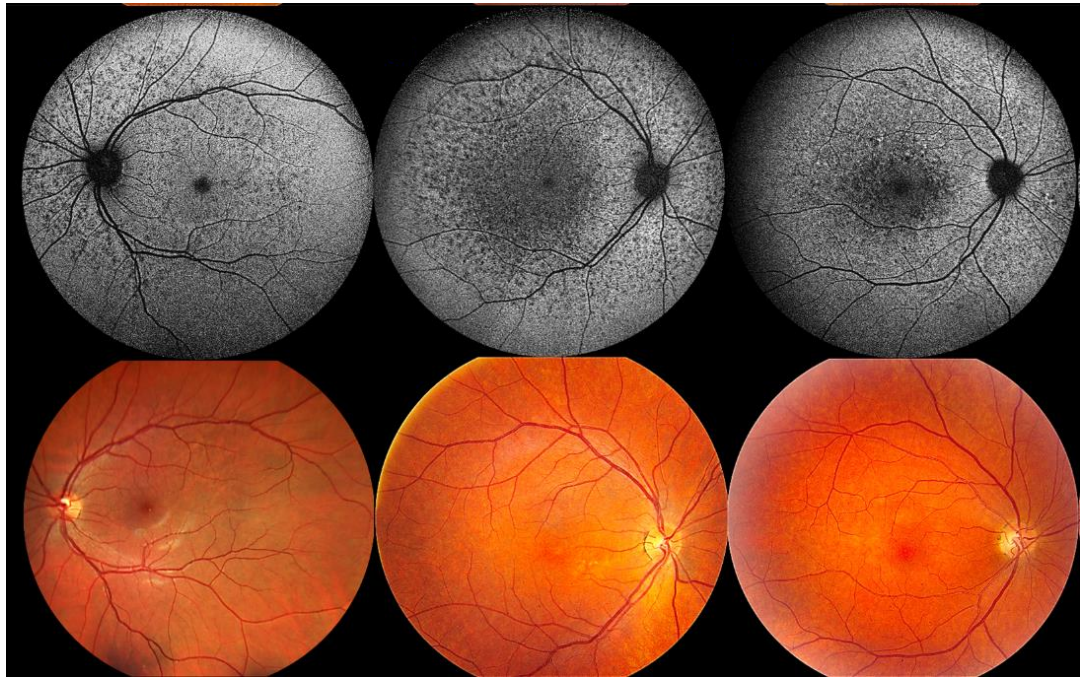
Van Vooren *et al.*, under review

E519K found in a replication cohort, with new patients from the Netherlands, France and Canada

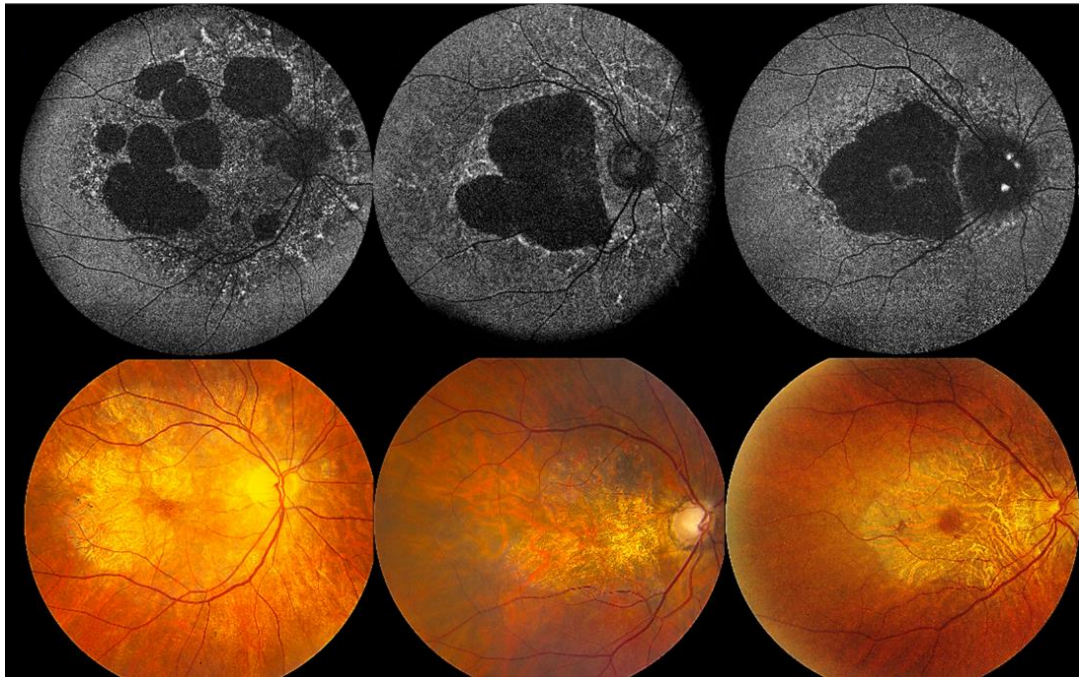


Two distinct and recognizable late-onset E519K-IRD phenotypes are observed

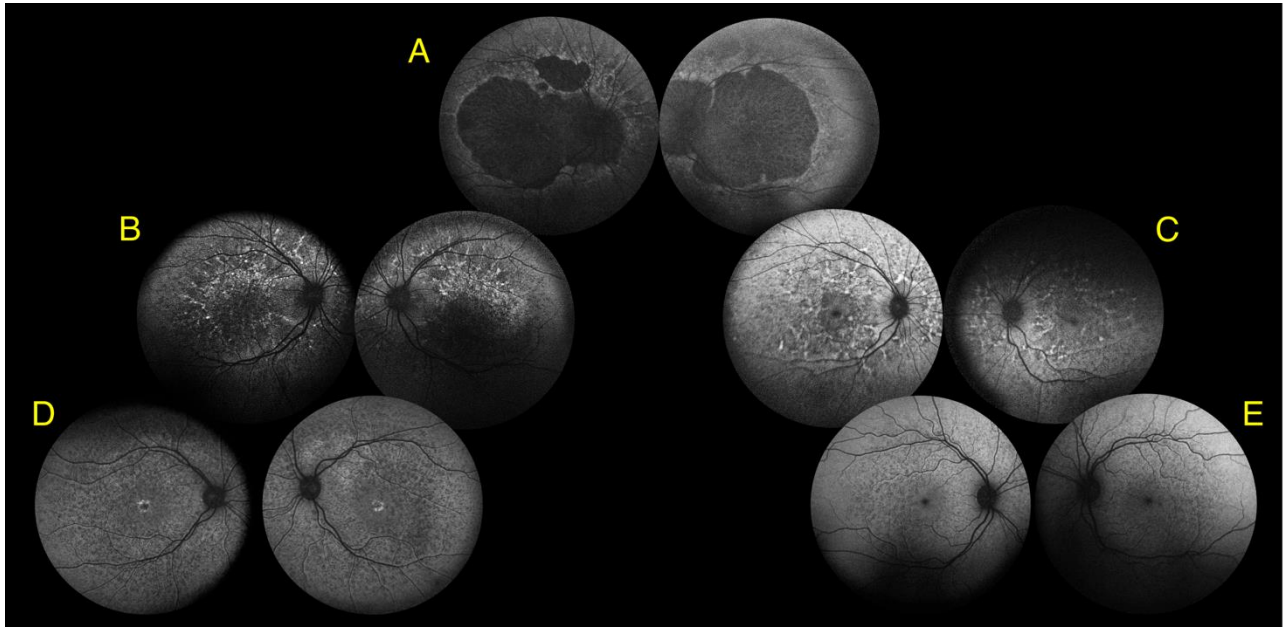
Mottled

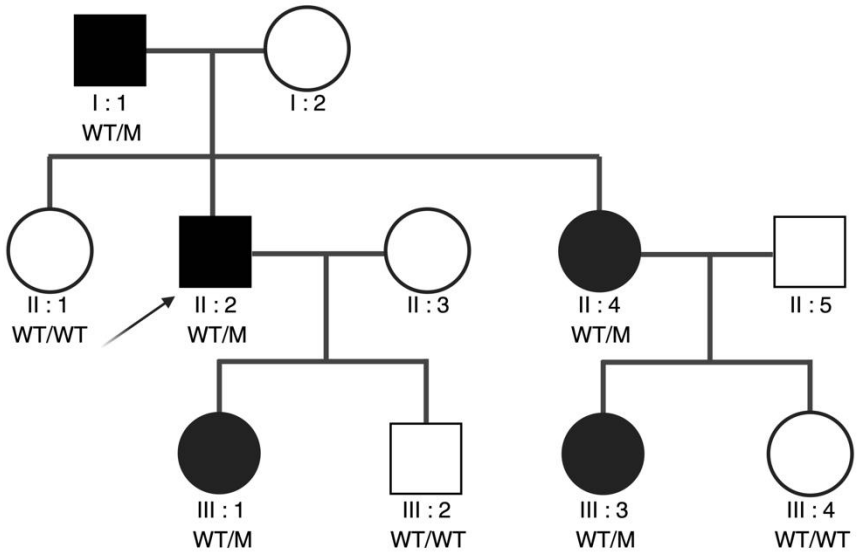


Pattern dystrophy



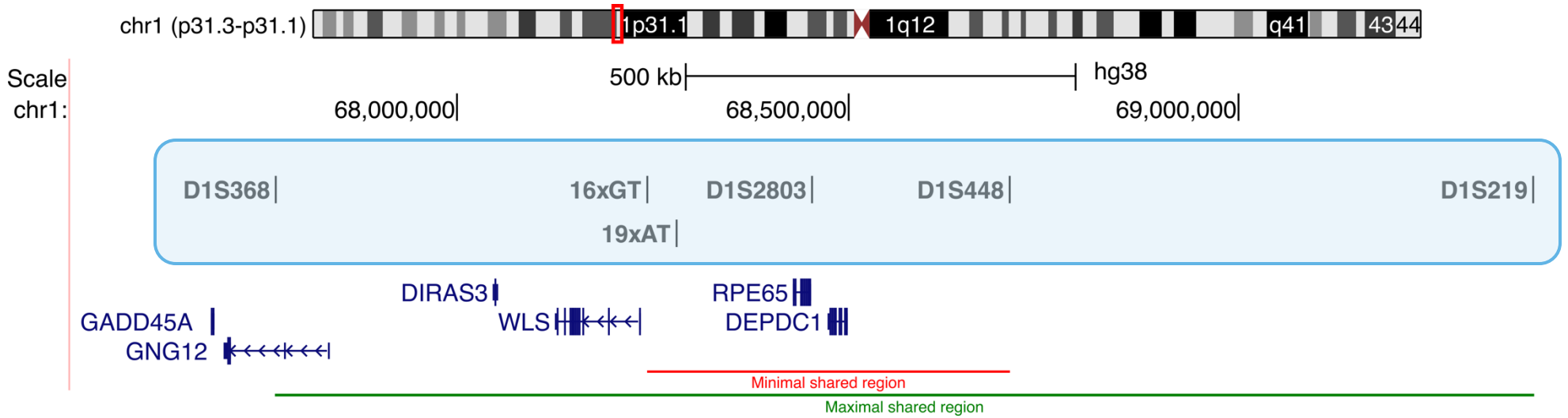
We observed inter- and intrafamilial variability and a dominant inheritance pattern





Van Vooren *et al.*, under review

Common haplotype of 464 kb supports a Flemish founder



	F1I:1		F1II:1		F2I:1		F2II:1		F3II:2		F3III:4		F4I:1		F4II:1		F5III:2		F5IIII:3		F6I:1		F6II:1		F7II:1		F7I:1		F8II:3		F8II:4		F9I:1		F9II:1		F10II:1		F10III:2		F11II:1	
D1S368	5	9	1	9	7	7	7	7	1	9	1	9	9	9	3	8	1	8	9	9	1	4	4	7	4	8	9	9	7	7	NA	NA	9	9	1	8	1	8	1	8	8	8
16xGT	1	6	2	6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1	3	NA	NA	1	1	1	1	1	1
19xAT	5	1	5	1	5	7	5	7	5	7	5	7	5	7	5	2	5	7	5	7	5	7	5	7	5	7	5	7	5	7	5	7	5	7	NA	NA	NA	NA	5	7	5	7
RPE65	**A**	G	**A**	G	**A**	G	**A**	G	**A**	G	**A**	G	**A**	G	**A**	G	**A**	G	**A**	G	**A**	G	**A**	G	**A**	G	**A**	G	**A**	G	**A**	G	**A**	G	**A**	G	**A**	G	**A**	G	**A**	G
D1S2803	4	4	4	3	4	6	4	3	4	3	4	2	4	3	4	3	4	2	4	3	4	2	4	2	4	4	4	4	4	5	4	4	4	7	4	3	4	2	4	2	4	2
D1S448	3	4	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3	1	3	3	3	1	3	3	3	3	3	3	3	3	3	1	3	3	3	3	3	3	3	3	3	1
D1S219	2	3	2	2	2	2	2	4	5	6	4	6	2	2	2	6	2	2	2	6	2	2	2	5	2	7	2	4	2	5	2	7	2	6	2	2	2	6	2	6	2	6

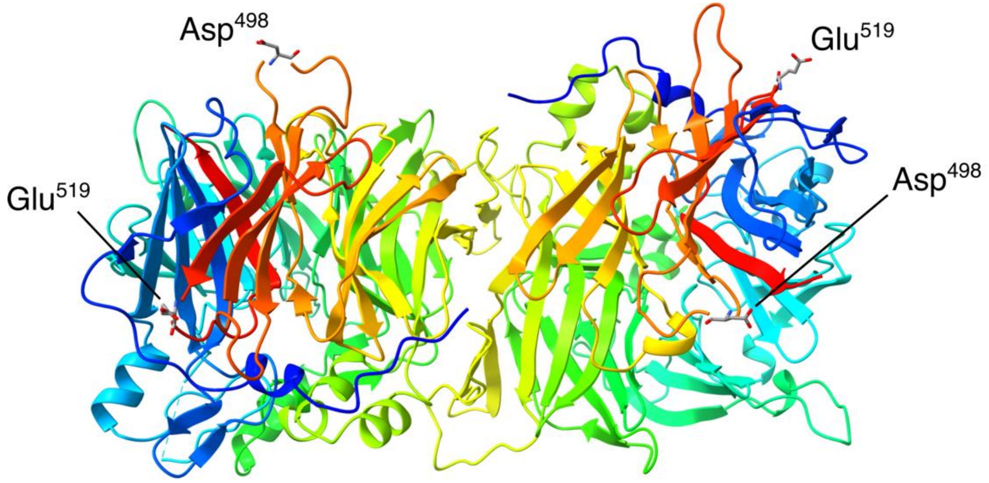
Van Vooren et al., under review

Protein modeling suggests a destabilizing effect of E519K

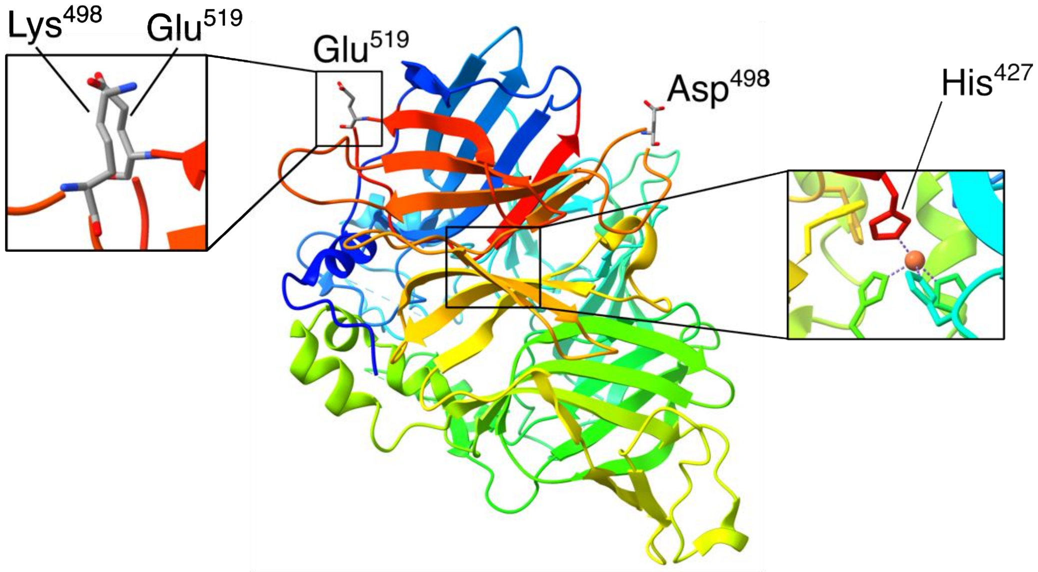
D477 E519

```
Homo sapiens                SEPIFVSHPDALEEDDGVVLSVVVSPGAGQKPAYLLILNAKDLSEVARAEV-EINIPVT--FHGLFK-KS--
Macaca mulatta              SEPIFVSHPDALEEDDGVVLSVVVSPGAGQKPAYLLILNAKDLSEVARAEV-EINIPVT--FHGLFK-KS--
Bos taurus                  SEPIFVSHPDALEEDDGVVLSVVVSPGAGQKPAYLLILNAKDLSEVARAEV-EINIPVT--FHGLFK-KS--
Gallus gallus               SEPIFVSHPDALEEDDGVVLSIVISPGSGPKPAYLLILNAKDMSEVARAEV-EVNIPVT--FHGLFK-RA--
Mus musculus                SEPIFVSQPDALEEDDGVVLSVVVSPGAGQKPAYLLVLNAKDLSEIARAEV-ETNIPVT--FHGLFK-RS--
Danio rerio (rpe65a)        SEPLFVQTPDGVDEDDGILMTIVVSPGA-QRPTYCLILNAKDLSEIARAEV-EILTPVT--FHGMYK-P---
Drosophila melanogaster     SEPIFVPSPDPKSEDDGVILASMVLGGLNDRYVGLIVLCAKTMTELGRCDF-HTNGPVPKCLHGWFA-PNAI
Caenorhabditis elegans      GEPIFVPNPEGVREDDGILIVPVMTISDGQRP-FVLILEAKNLTEIARYTIPEARIPLG--FHAFYQGRT--
```

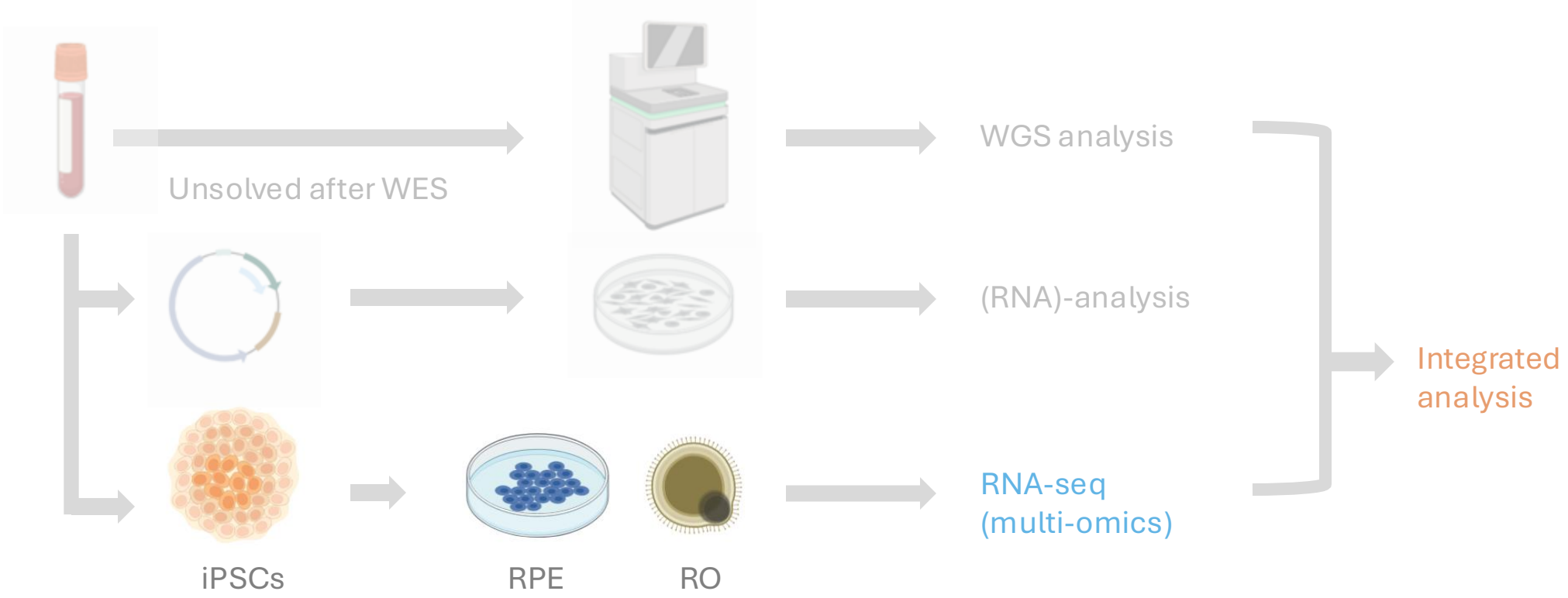
Dimer crystal structure

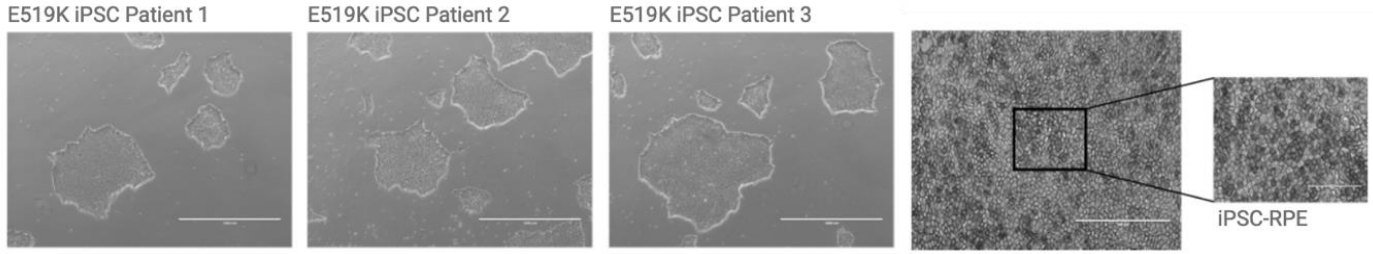


Monomer crystal structure

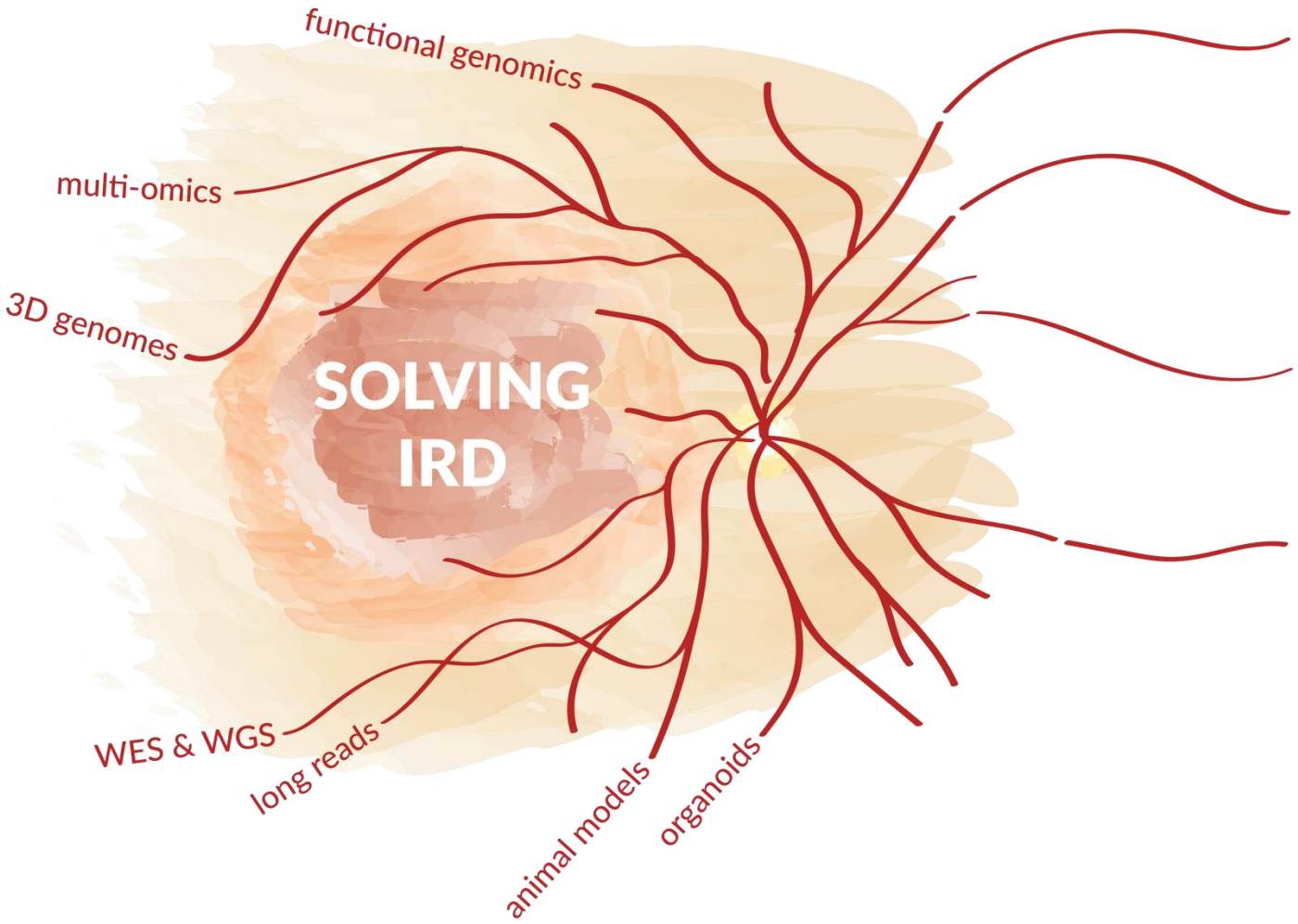


Novel variant E519K in known IRD gene *RPE65* explains missing heritability in dominant IRD





Outline multi-omics



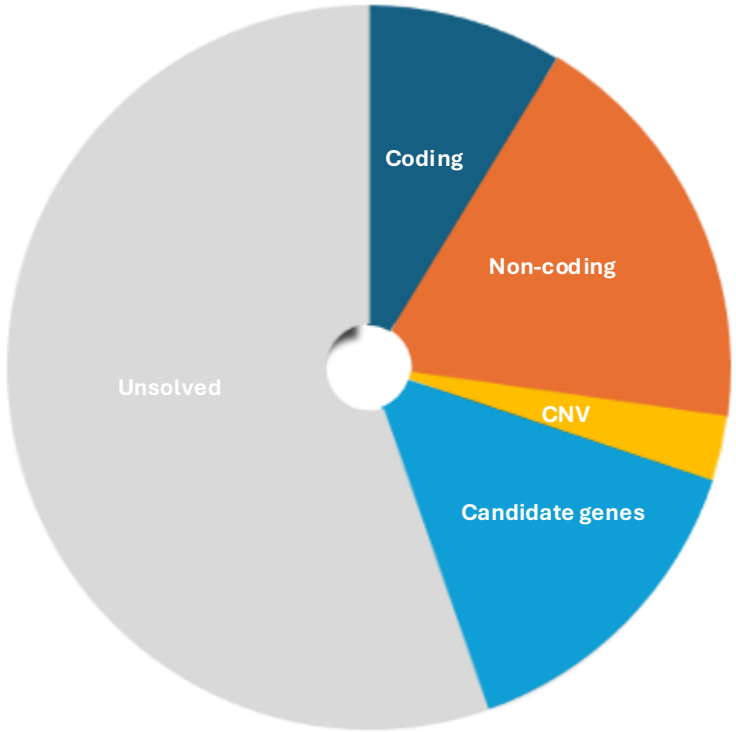
Integrated multi-omics approach in IRD

Coding SNVs in known IRD genes:
A novel dominant *RPE65*-related retinopathy

Non-coding variants increasing the diagnostic yield in IRD

Coding variants in novel candidate genes

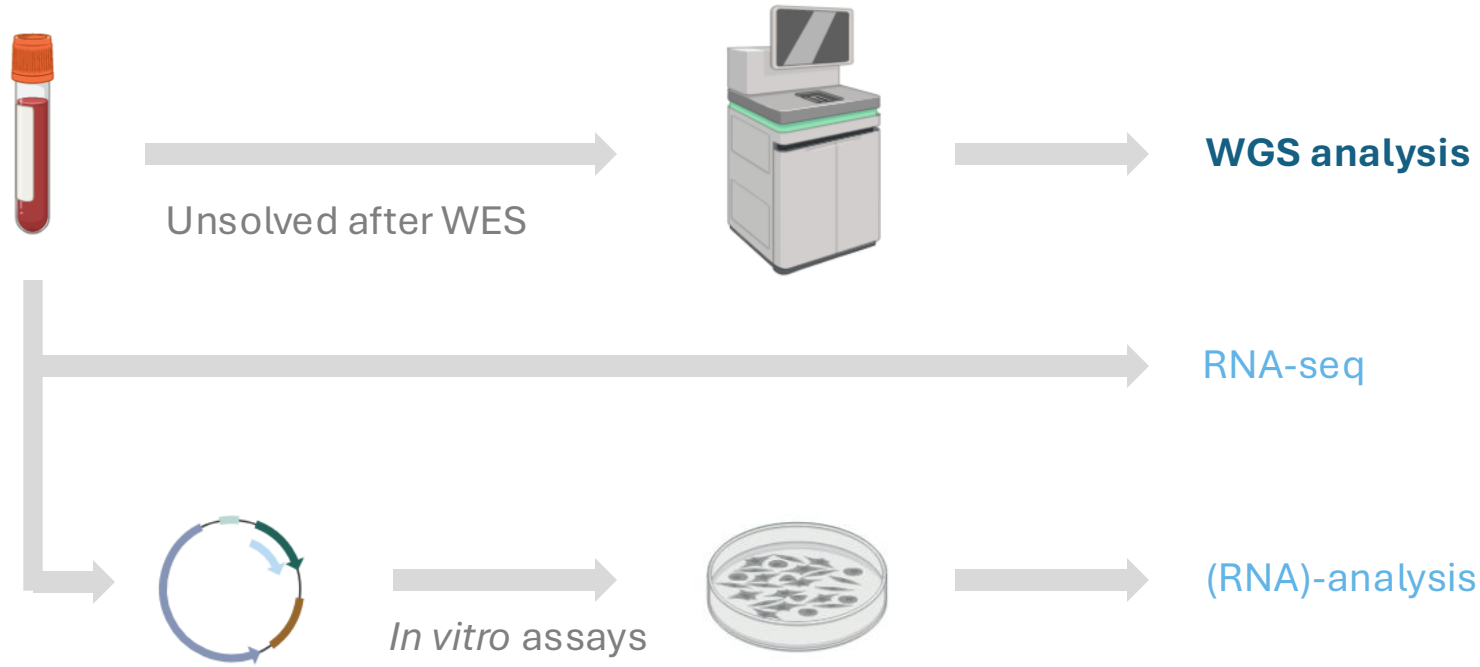
Putative pathogenic non-coding variants were identified in 18.5%



Coding SNVs in known IRD genes (8.5%)

Non-coding splice and regulatory SNVs in known IRD genes (18.5%)

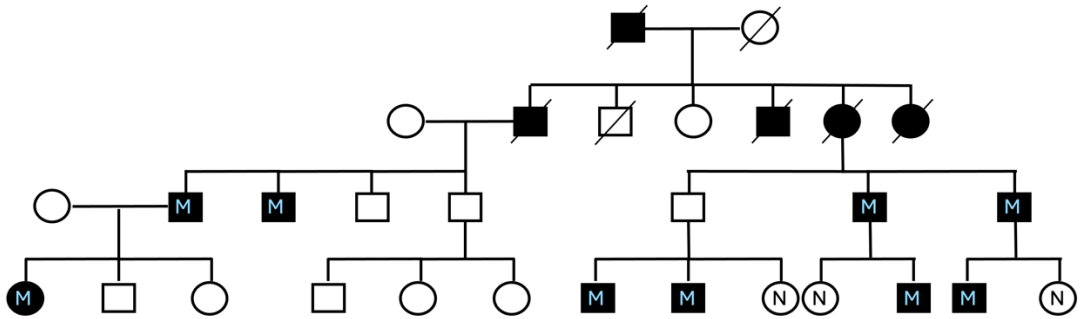
RNA-seq and *in vitro* minigene assays detect aberrant splicing



WGS identifies a deep-intronic *OPA1* variant in a family with 14 affected individuals

Large family with AD optic atrophy

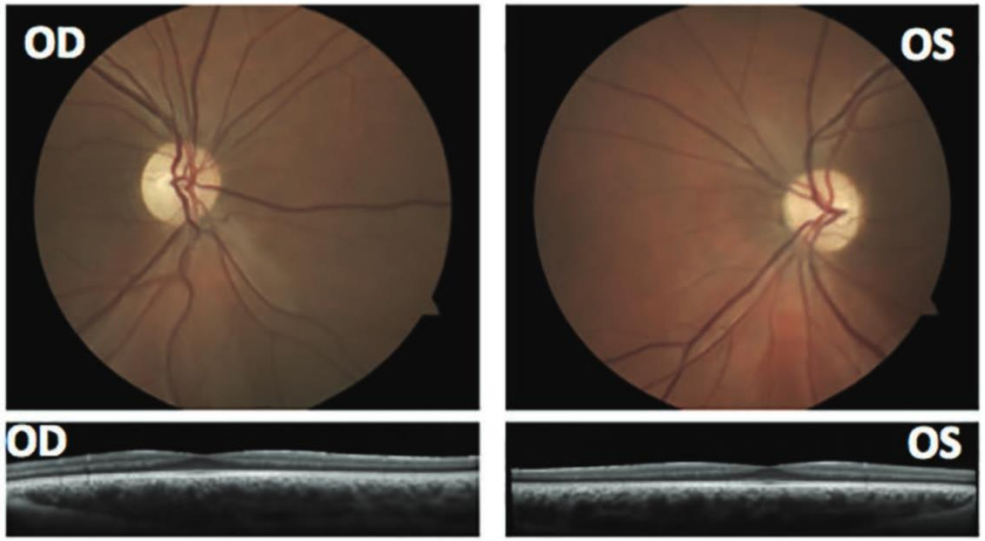
- WGS: c. 1608+622 A>G, segregates with disease
- 11 family members available: 9 aff. and 3 unaff.



WGS: *OPA1* c.1608+622 A>G

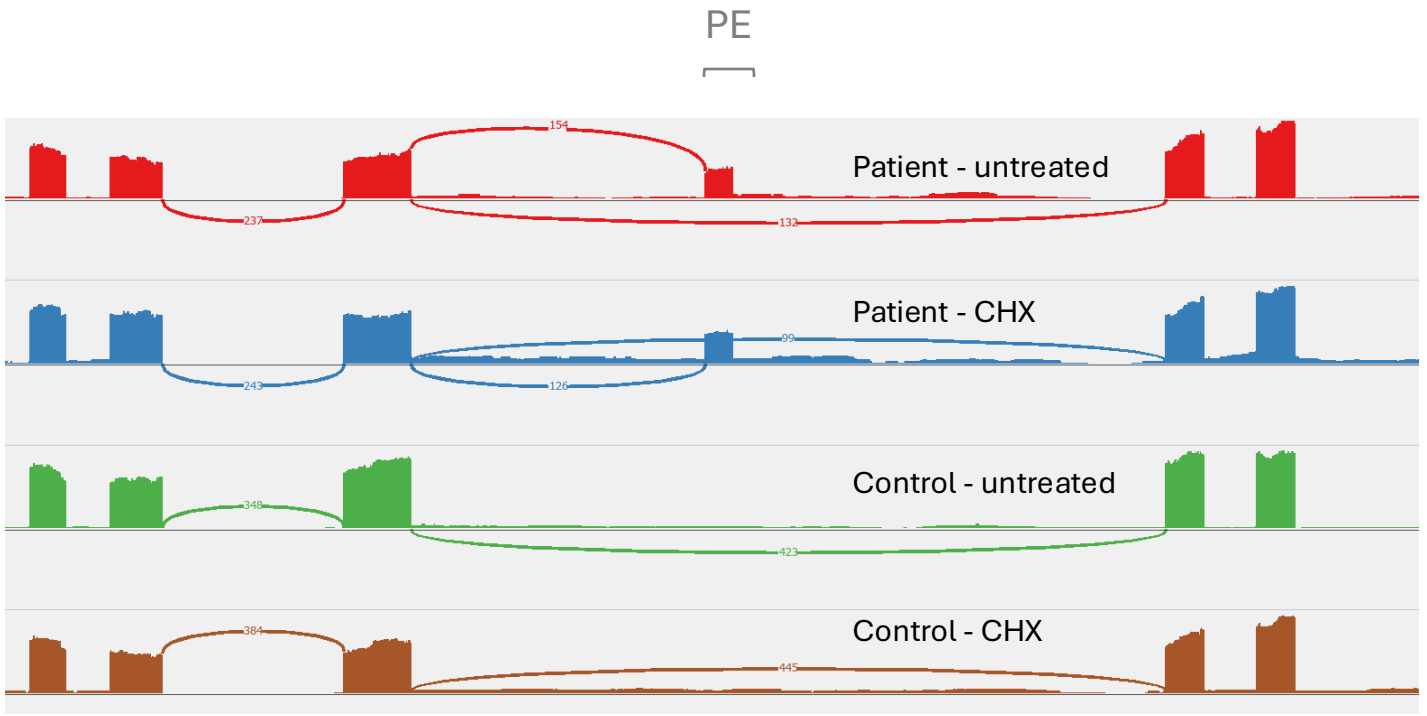
- Previously found once in a 17 year old male
- Optic atrophy type 1, no family history
- MG reveals PE inclusion

SPLICE AI			
DG	AG	DL	AL
0.52	0.76	0.13	0.00



Qian *et al*. 2021

RNA-seq (whole blood) confirms an in-frame pseudo-exon inclusion



-CHX:
54% of junction reads with PE

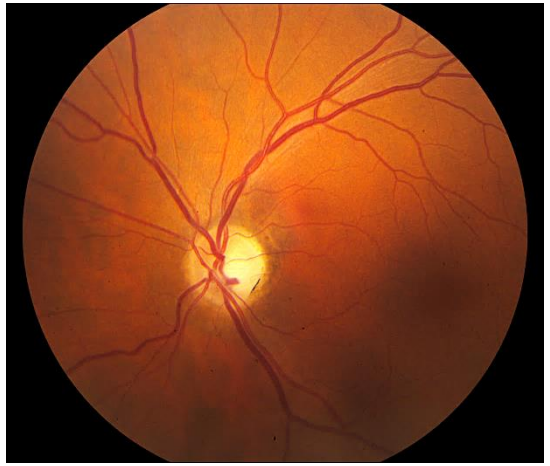
+CHX:
56% of junction reads with PE

In-frame insertion (54 bp)

WGS identifies a novel deep-intronic *OPA1* variant

Single patient with optic atrophy

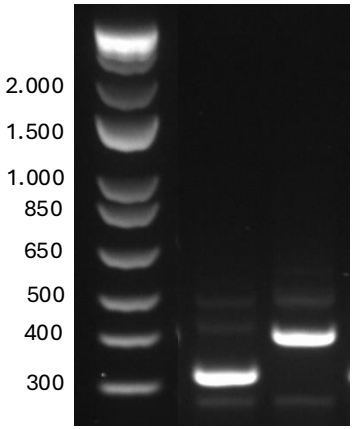
- No familial history
- No coding *OPA1* variant

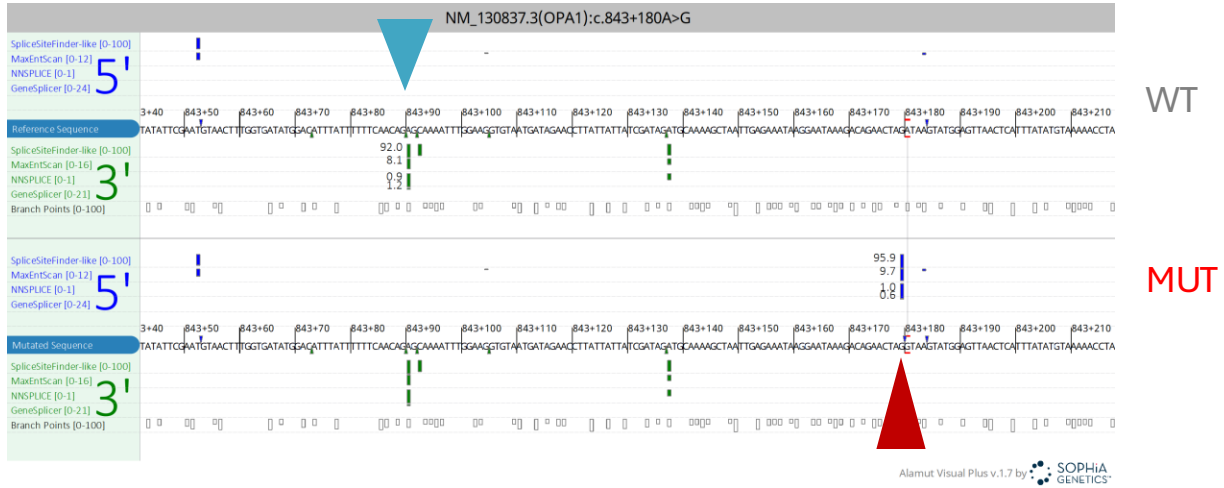


WGS: *OPA1* c.843+180 A>G

- Not in public databases
- SpliceAI: suggestive for PEI (90 bp)

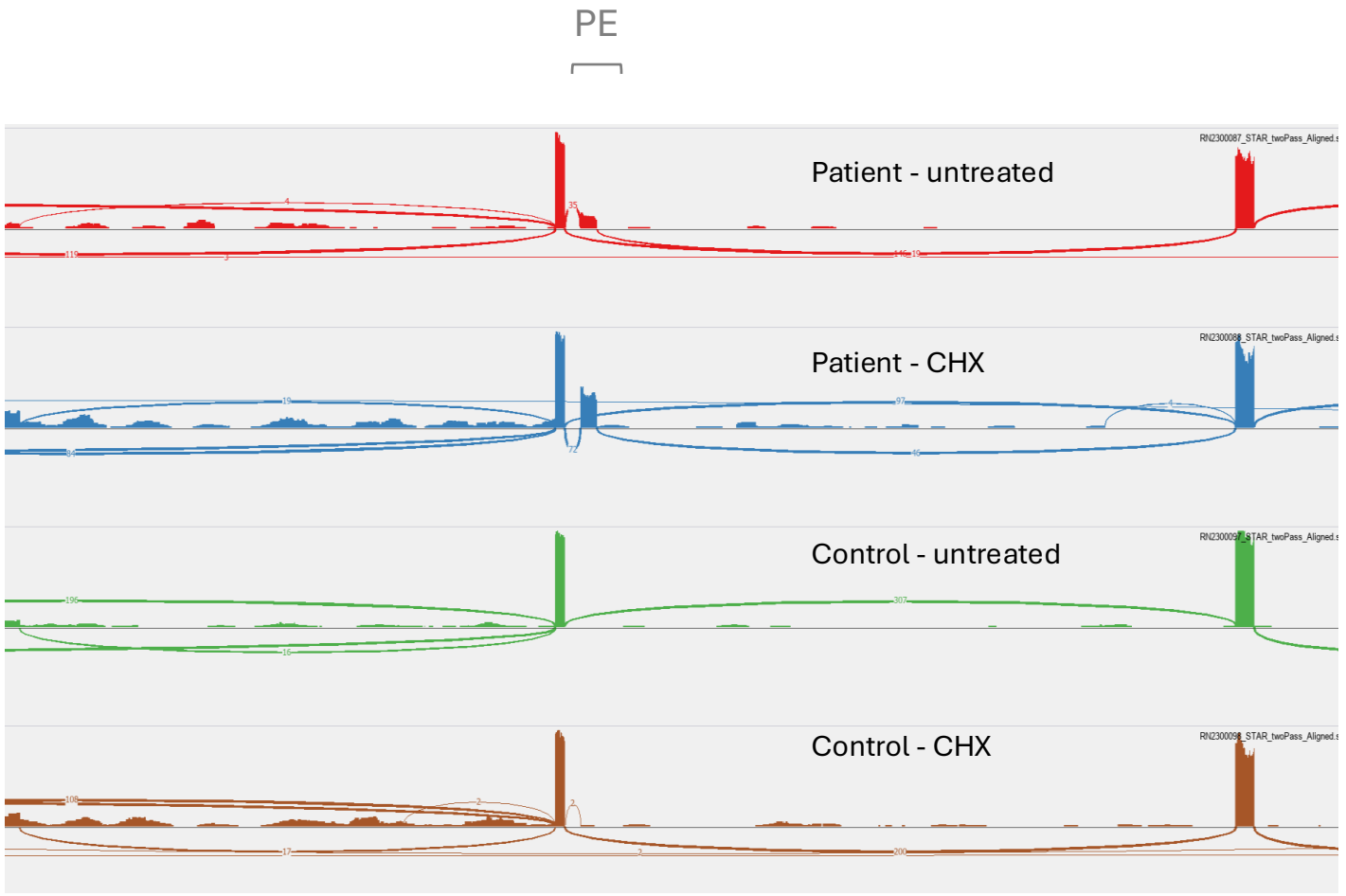
SPLICE AI			
DG	AG	DL	AL
0.94	0.67	0.02	0.00





RNA-seq (blood) confirms a splice effect & NMD

2 bp difference compared to SpliceAI predictions & minigene results



-CHX:
junction reads with PE: 19%

+CHX:
Junction reads with PE: 43%

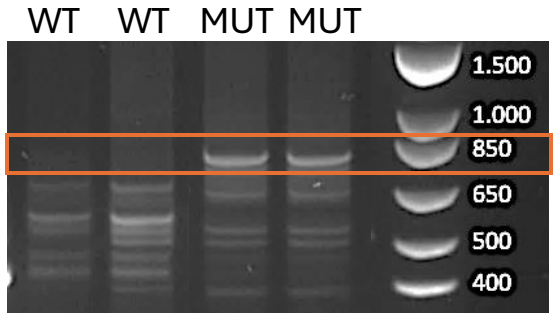
Out-of-frame insertion (88 bp)

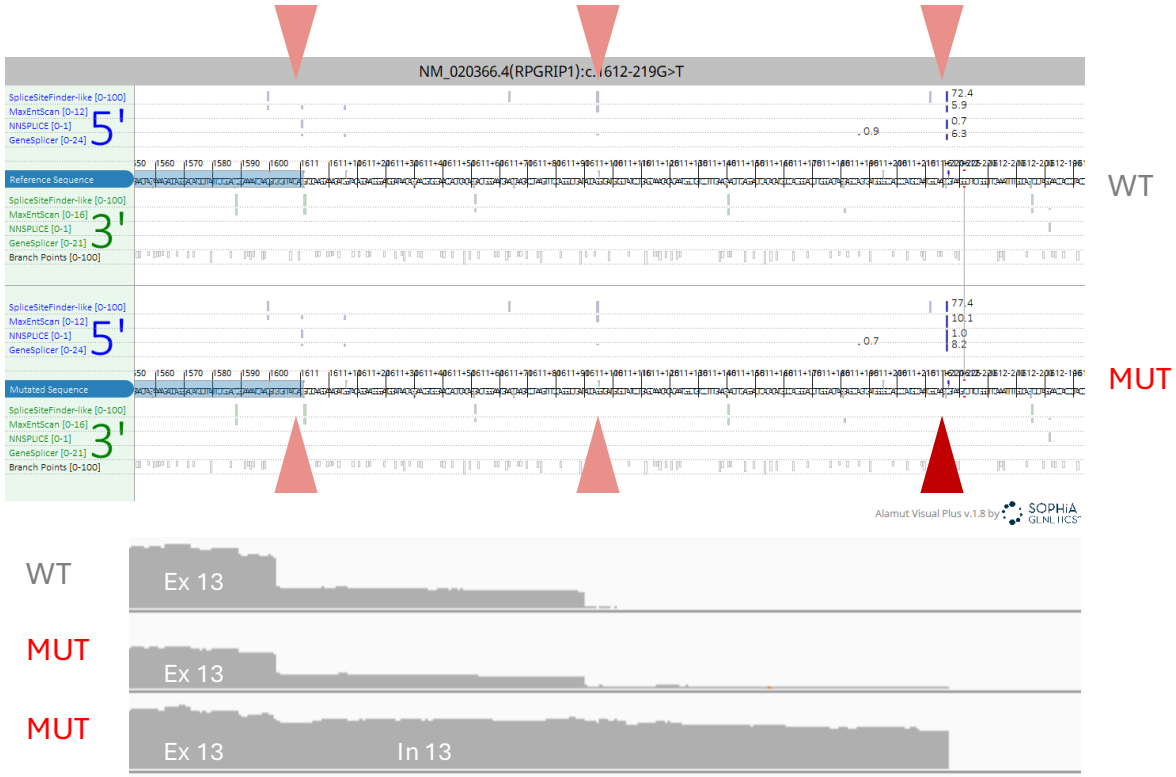
WGS identifies two non-coding *RPGRIP1* variants

Patient with LCA

- WGS: *RPGRIP1*: Non-coding splice variant c.2367+23delG *(Jamshidi et al. 2019)*
- WGS: *RPGRIP1*: Novel deep-intronic variant c.1612-219 G>T: OOF extension of exon 13 (227 bp) *(in trans)*

SPLICE AI			
DG	AG	DL	AL
0.34	0.04	0.18	0.03

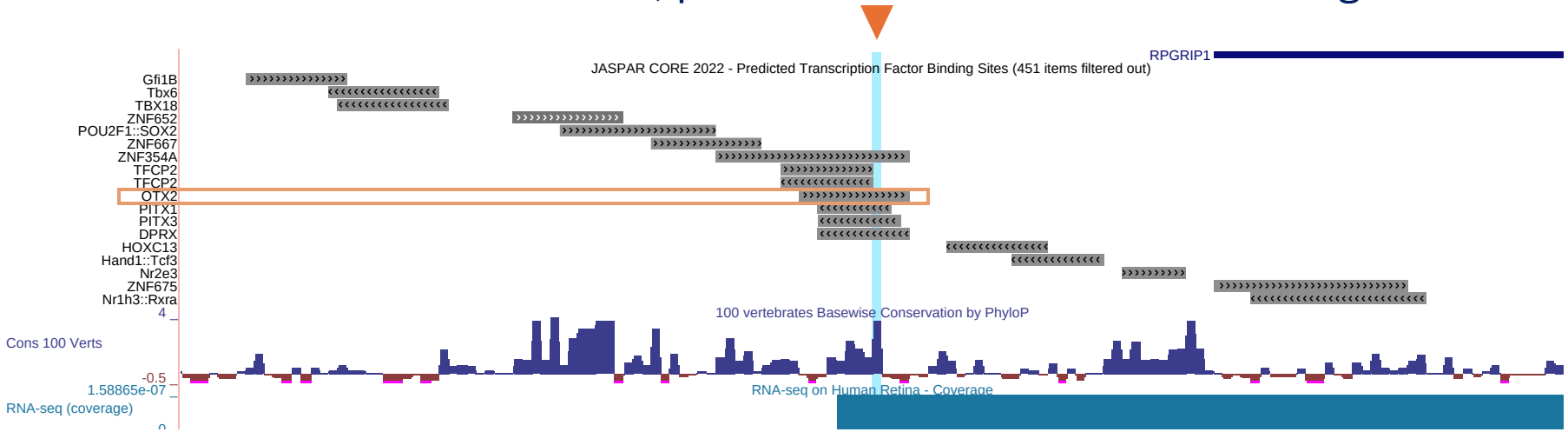




WGS identifies a novel *RPGRIP1* promoter variant

IRD patient

- WES: *RPGRIP1* variant c.1930C>T; p.Gln644*
- WGS: *RPGRIP1* c.-152A>C, promoter variant in a OTX2 binding site



Retina-specific database RegRet

- ChIP-seq of histon modifications and retinal TFs in adult retina

  by T. Cherry

- ATAC-seq of adult retina

  by J.L. Gómez-Skarmeta

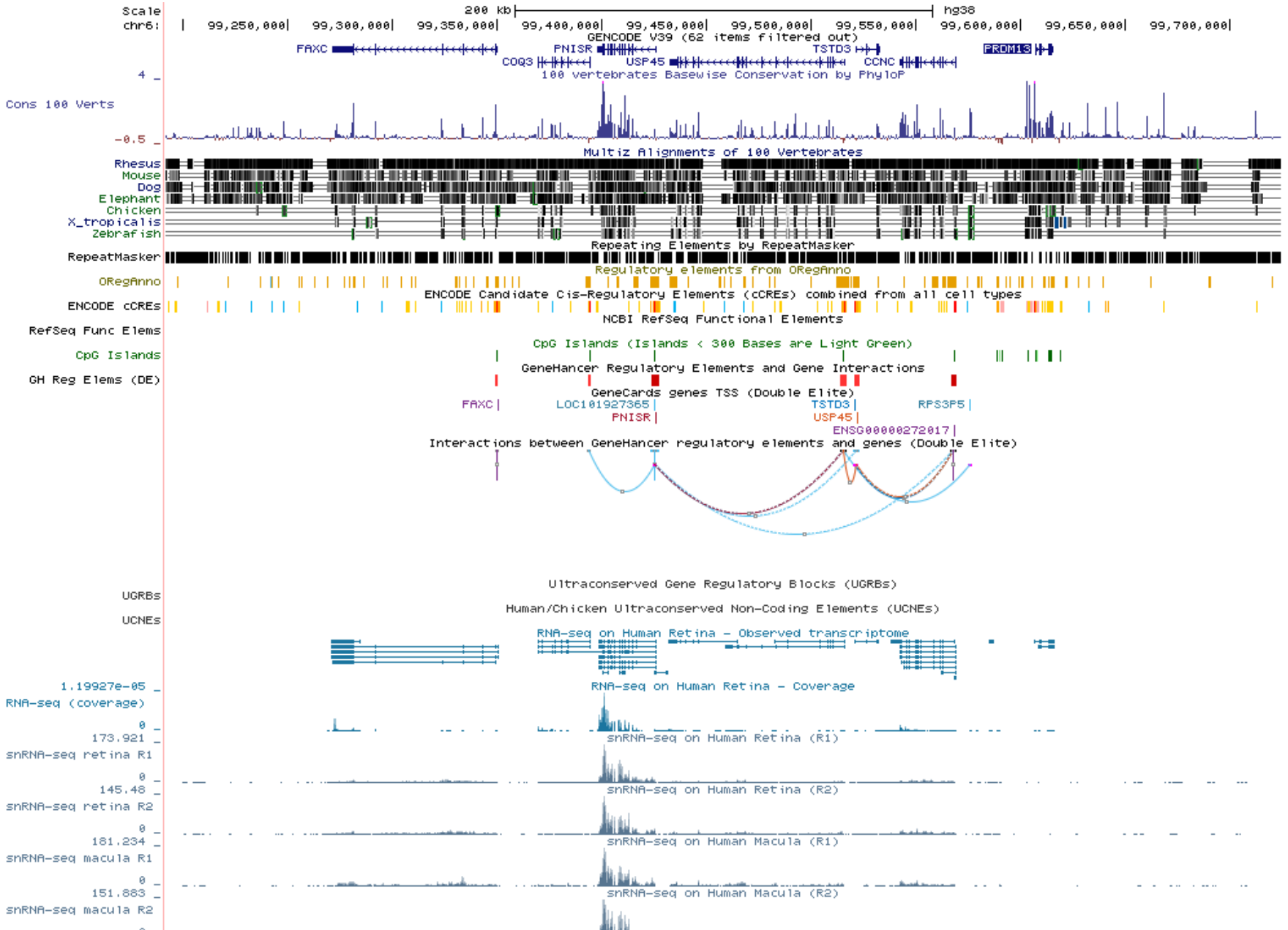
- RNA-seq of adult retina

  by S. Banfi

- DNase-seq of embryonic retina (5 stages)

  by J. Stamatoyannopoulos

Van de Sompele *et al.*
AJHG 2022



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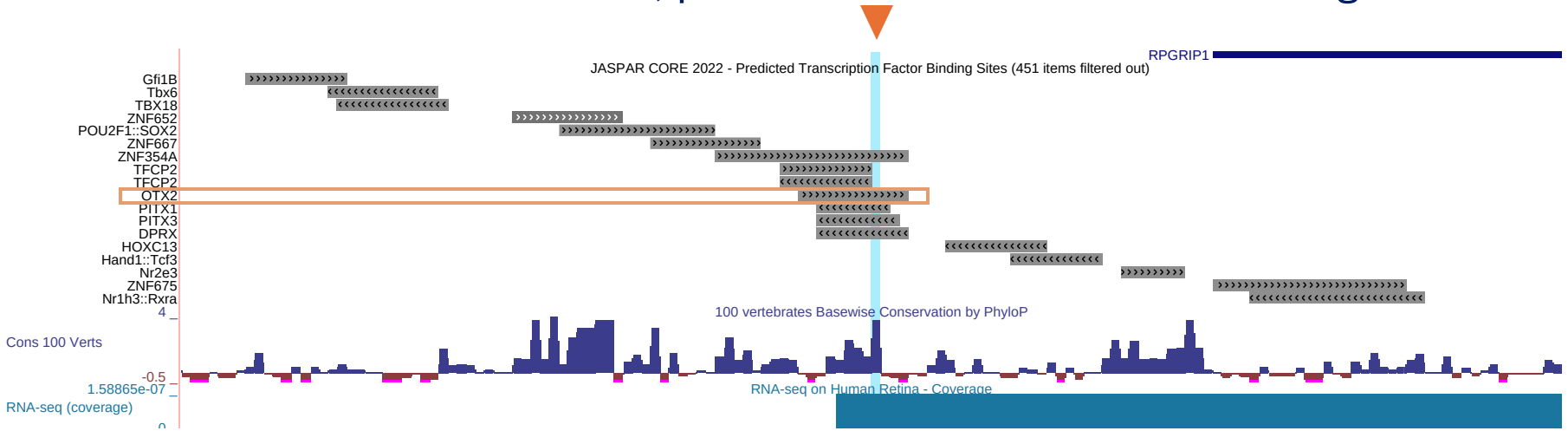
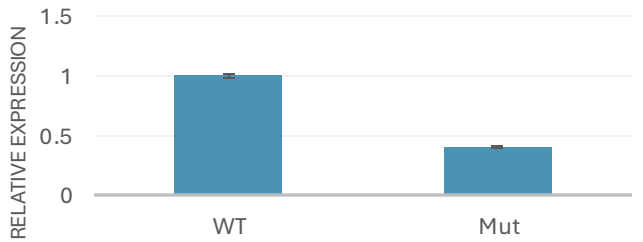


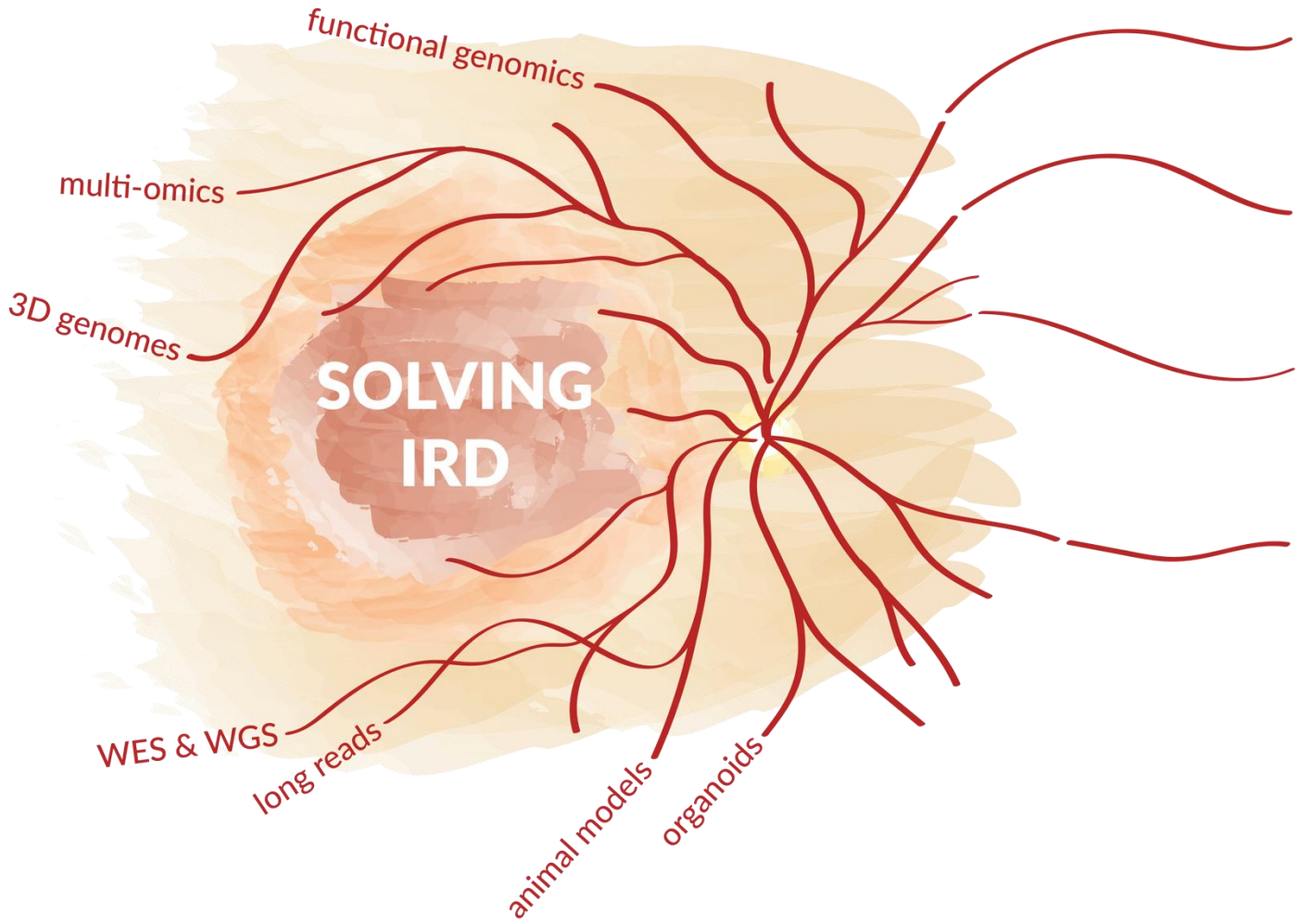
Predicted loss of the OTX2 binding site

wild	mutant	diff	z_score	p_value	binding_status	TF_gene
GGATT**A**GCTCC	GGATT**C**GCTCC	-1.19979	-35.1431	**1.482E-270**	bound>**unbound**	OTX1,**OTX2**

Luciferase assay + OTX2



Outline multi-omics



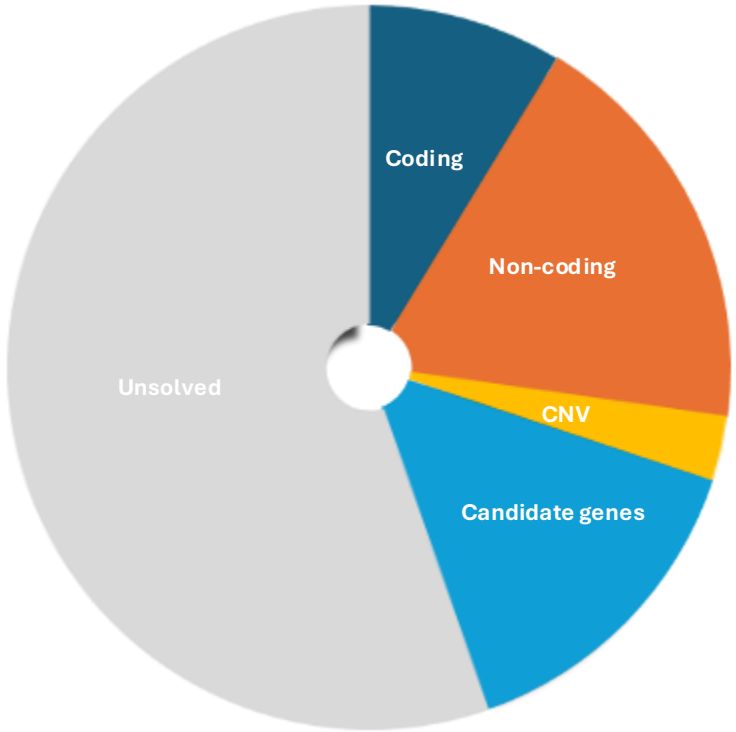
Integrated multi-omics approach in IRD

Coding SNVs in known IRD genes:
A novel dominant *RPE65*-related retinopathy

Non-coding variants increase the diagnostic yield ‘beyond the exome’ in IRD

Coding variants in novel candidate genes

Variants in novel candidate genes were identified in 14.5%



Coding SNVs in known IRD genes (8.5%)

Non-coding splice and regulatory SNVs in known IRD genes (18.5%)

SVs in known IRD genes (3%)

Variants in novel and candidate IRD genes (14.5%)

snRNAs as novel major cause of NDD

HOT TOPIC

Article | Open access | Published: 11 July 2024

De novo variants in the *RNU4-2* snRNA cause a frequent neurodevelopmental syndrome

Yuyang Chen, Ruebena Dawes, Hyung Chul Kim, Alicia Ljungdahl, Sarah L. Stenton, Susan Walker, Jenny Lord, Gabrielle Lemire, Alexandra C. Martin-Geary, Vijay S. Ganesh, Jialan Ma, Jamie M. Ellingford, Erwan Delage, Elston N. D'Souza, Shan Dong, David R. Adams, Kirsten Allan, Madhura Bakshi, Erin E. Baldwin, Seth I. Berger, Jonathan A. Bernstein, Ishita Bhatnagar, Ed Blair, Natasha J. Brown, ... Nicola Whiffin + Show authors

Nature **632**, 832–840 (2024) | Cite this article

Case Reports > Clin Genet. 2024 Oct;106(4):512-517. doi: 10.1111/cge.14574. Epub 2024 Jun 11.

Re-analysis of whole genome sequencing ends a diagnostic odyssey: Case report of an RNU4-2 related neurodevelopmental disorder

Rachel Schot [1 2], Federico Ferraro [1], Geert Geeven [1], Karin E M Diderich [1], Tahsin Stefan Barakat [1 2]

Affiliations + expand

PMID: 38859706 DOI: 10.1111/cge.14574

> Genet Med. 2024 Oct 2;26(12):101288. doi: 10.1016/j.gim.2024.101288. Online ahead of print.

Deep phenotyping of 11 individuals with pathogenic variants in RNU4-2 reveals a clinically recognizable syndrome

Irene Valenzuela [1], Marta Codina-Solà [2], Elida Vazquez [3], Anna Cueto-González [2], Jordi Leno-Colorado [2], Amaia Lasa-Aranzasti [2], Laura Trujillano [2], Bárbara Masotto [2], Miriam Masas [2], Mar Escobar [2], Elena García-Arumí [2], Eduardo F Tizzano [2]

> Nat Med. 2024 Aug;30(8):2165-2169. doi: 10.1038/s41591-024-03085-5. Epub 2024 May 31.

Mutations in the U4 snRNA gene RNU4-2 cause one of the most prevalent monogenic neurodevelopmental disorders

Daniel Greene [1 2], Chantal Thys [3], Ian R Berry [4 5], Joanna Jarvis [6], Els Ortibus [7 8], Andrew D Mumford [5 9], Kathleen Freson [3], Ernest Turro [10 11 12 13]

Affiliations + expand

PMID: 38821540 PMCID: PMC11333284 DOI: 10.1038/s41591-024-03085-5

> medRxiv [Preprint]. 2024 Sep 4:2024.09.03.24312863. doi: 10.1101/2024.09.03.24312863.

Mutations in the U2 snRNA gene *RNU2-2P* cause a severe neurodevelopmental disorder with prominent epilepsy

Daniel Greene, Koenraad De Wispelaere, Jon Lees, Andrea Katrinecz, Sonia Pascoal, Emma Hales, Marta Codina-Solà, Irene Valenzuela, Eduardo F Tizzano, Giles Atton, Deirdre Donnelly, Nicola Foulds, Joanna Jarvis, Shane McKee, Michael O'Donoghue, Mohnish Suri, Pradeep Vasudevan, Kathy Stirrups, Natasha P Morgan, Kathleen Freson, Andrew D Mumford, Ernest Turro

PMID: 39281759 PMCID: PMC11398430 DOI: 10.1101/2024.09.03.24312863

nature genetics

Letter https://doi.org/10.1038/s41588-025-02159-5

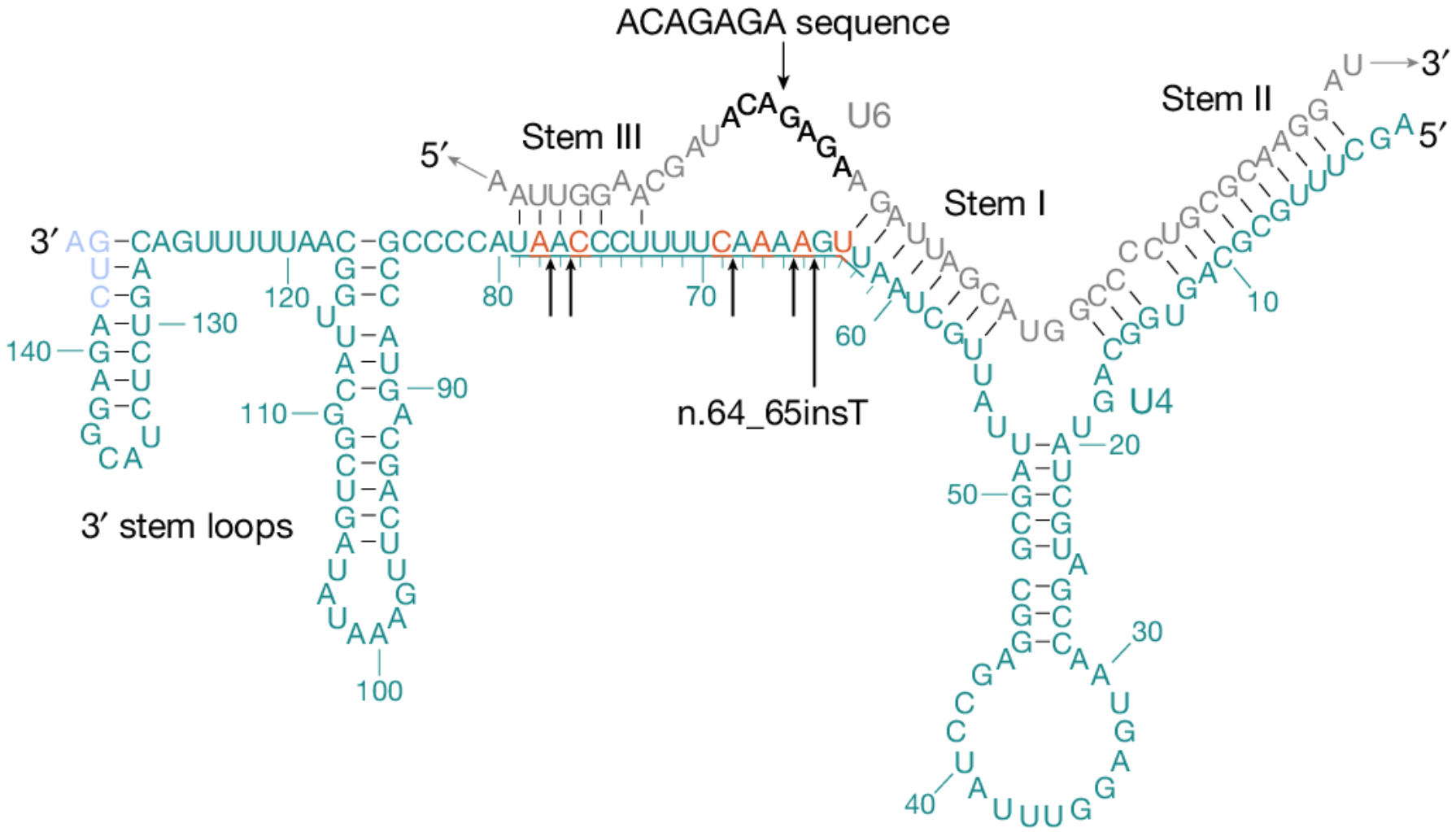
Mutations in the small nuclear RNA gene *RNU2-2* cause a severe neurodevelopmental disorder with prominent epilepsy

Received: 13 September 2024

Accepted: 10 March 2025

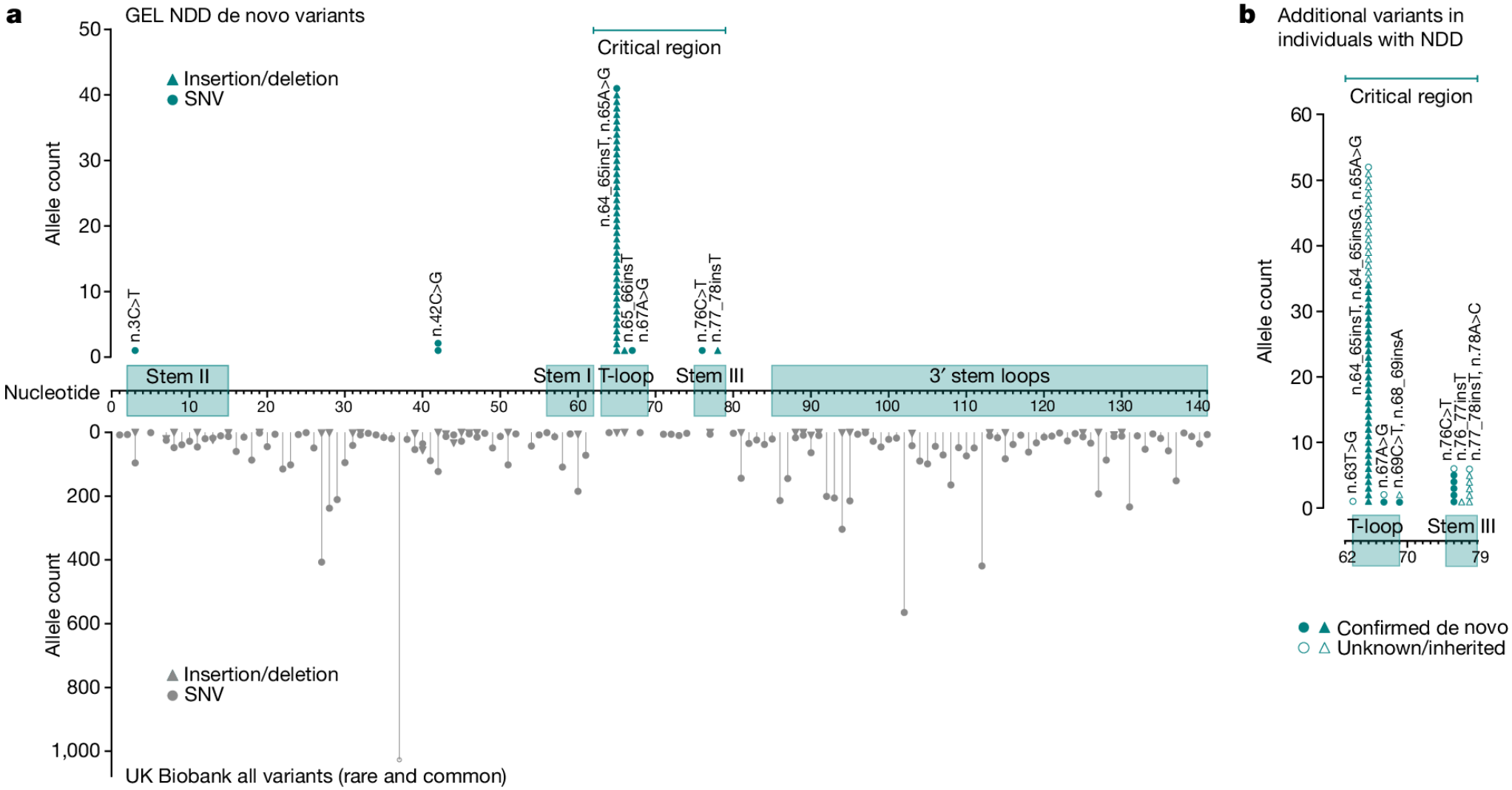
Published online: 10 April 2025

U4:U6 duplex



Chen, et al. Nature 2024

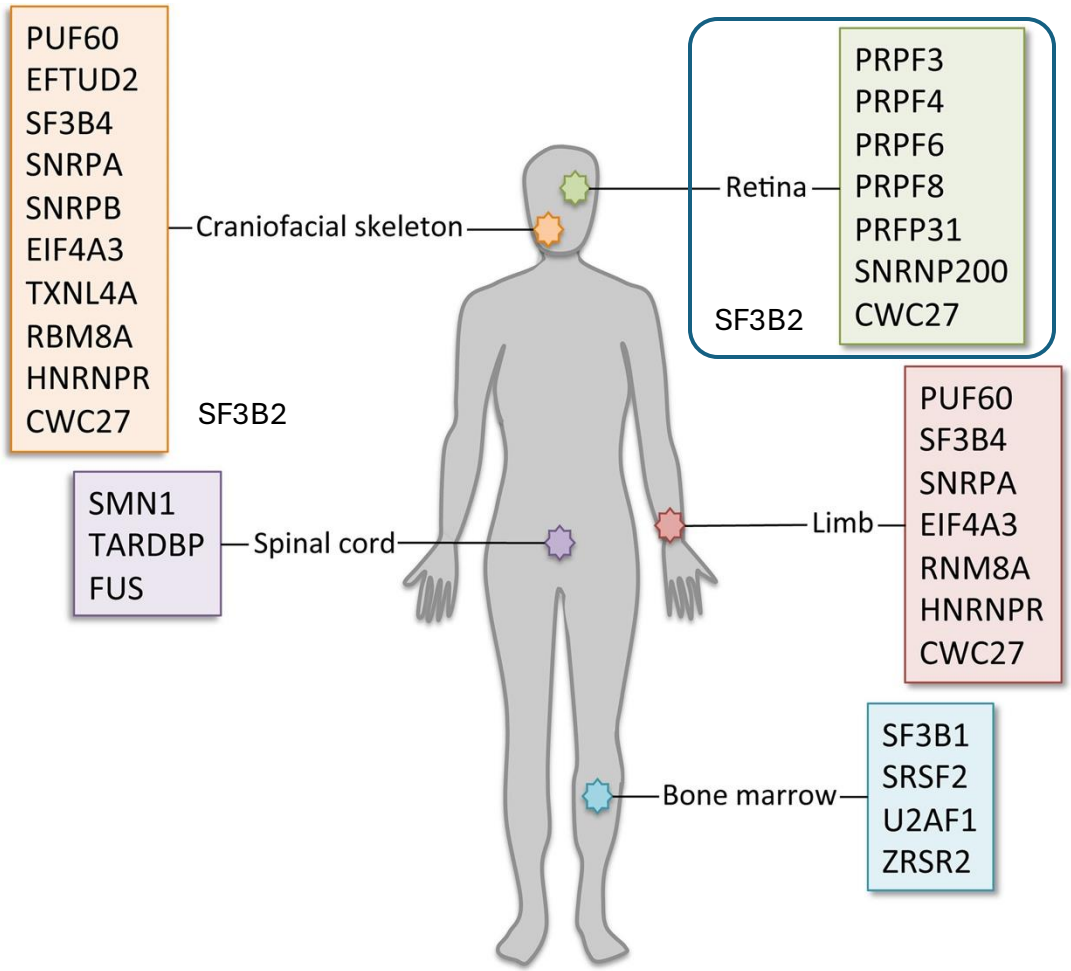
RNU4-2 gene

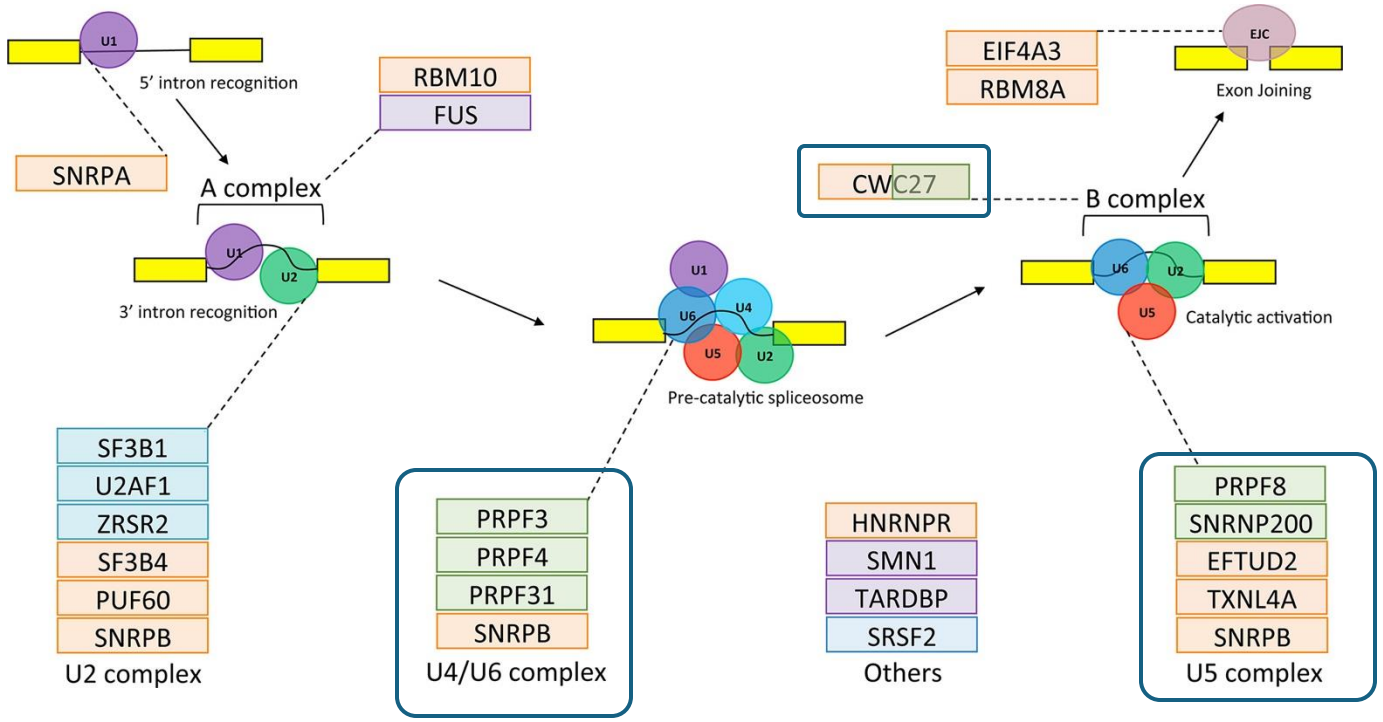


Chen, et al. Nature 2024

Spliceosomopathies

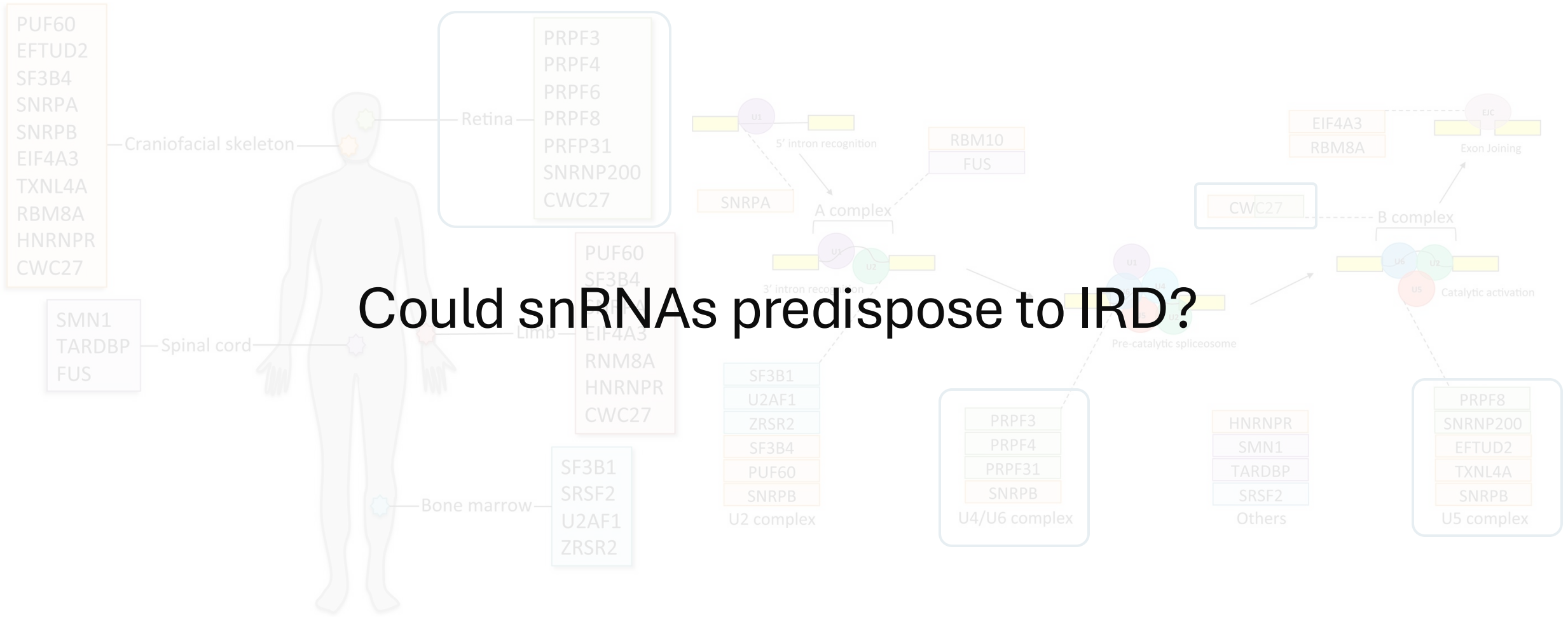
Also known to be implicated in inherited retinal diseases





Griffin C, Saint-Jeannet J-P. Spliceosomopathies: Diseases and mechanisms. *Developmental Dynamics*. 2020

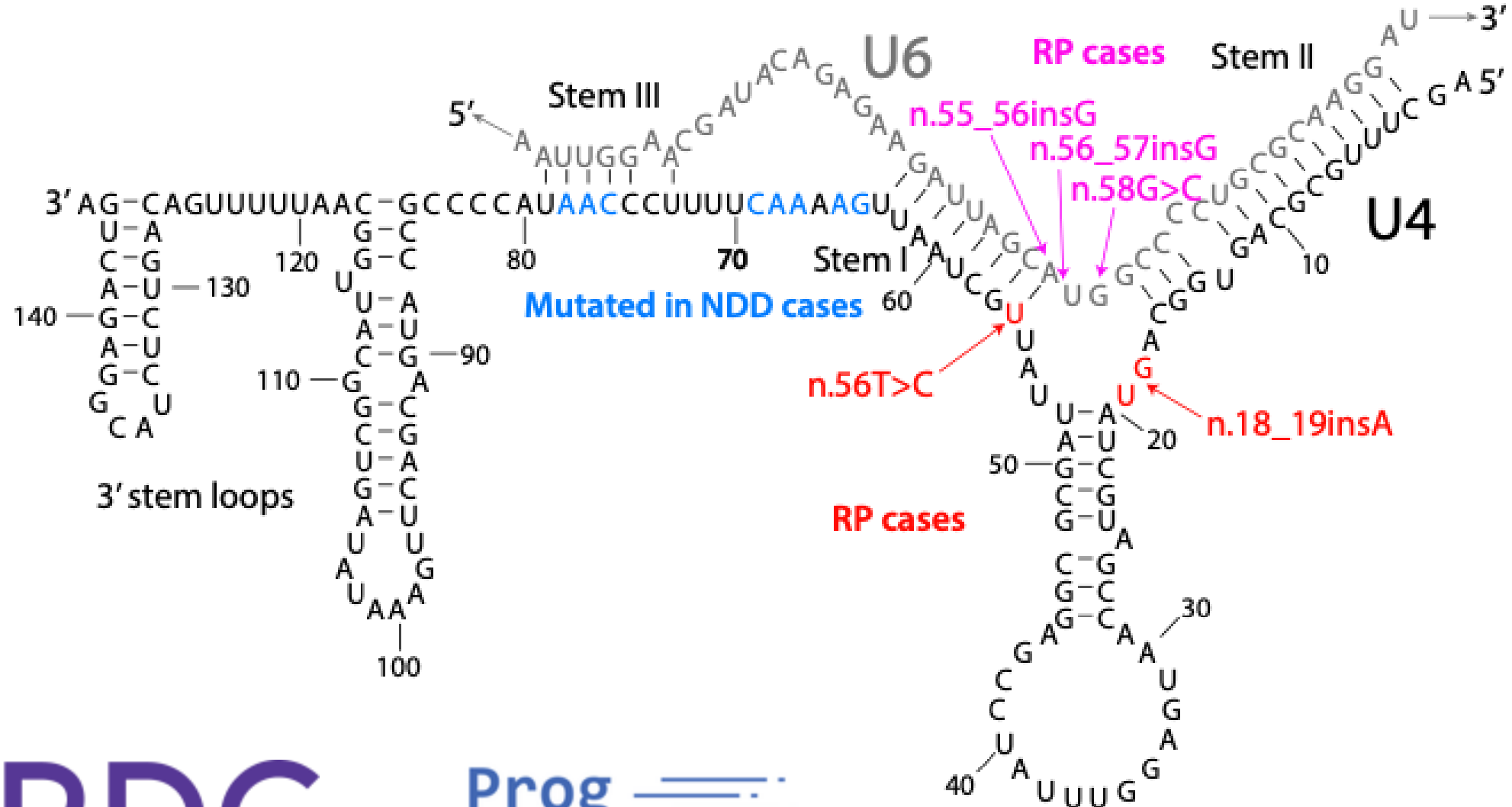
Spliceosomopathies



Could snRNAs predispose to IRD?

Images from: Griffin C, Saint-Jeannet J-P. Spliceosomopathies: Diseases and mechanisms. *Developmental Dynamics*. 2020; 249: 1038–1046. https://doi.org/10.1002/dvdy.214

RNU gene variants cause dominant RP



ERDC
European Retinal Disease Consortium

Prog RET

Image from Mathieu Quinodoz (IOB), ERDC Oct 2024

RNU gene variants cause dominant RP

De novo and inherited dominant variants in U4 and U6 snRNAs cause retinitis pigmentosa

Mathieu Quinodoz, Kim Rodenburg, Zuzana Cvackova, Karolina Kaminska, Suzanne E de Bruijn, Ana Belén Iglesias-Romero, Erica G M Boonen, Mukhtar Ullah, Nick Zomer, Marc Folcher, Jacques Bijon, Lara K Holtes, Stephen H Tsang, Zelia Corradi, K Bailey Freund, Stefanida Shliaga, Daan M Panneman, Rebekkah J Hitti-Malin, Manir Ali, Ala'a AlTalbishi, Sten Andréasson, Georg Ansari, Gavin Arno, Galuh D N Astuti, Carmen Ayuso, Radha Ayyagari, Sandro Banfi, Eyal Banin, Mirella T S Barboni, Miriam Bauwens, Tamar Ben-Yosef, David G Birch, Pooja Biswas, Fiona Blanco-Kelly, Beatrice Bocquet, Camiel J F Boon, Kari Branham, Alexis Ceecee Britten-Jones, Kinga M Bujakowska, Elizabeth L Cadena, Giacomo Calzetti, Francesca Cancellieri, Luca Cattaneo, Peter Charbel Issa, Naomi Chadderton, Luísa Coutinho-Santos, Stephen P Daiger, Elfride De Baere, Berta de la Cerda, John N De Roach, Julie De Zaeytijd, Ronny Derks, Claire-Marie Dhaenens, Lubica Dudakova, Jacque L Duncan, G Jane Farrar, Nicolas Feltgen, Lidia Fernández-Caballero, Juliana M Ferraz Sallum, Simone Gana, Alejandro Garanto, Jessica C Gardner, Christian Gilissen, Kensuke Goto, Roser Gonzàlez-Duarte, Sam Griffiths-Jones, Tobias B Haack, Lonneke Haer-Wigman, Alison J Hardcastle, Takaaki Hayashi, Elise Héon, Alexander Hoischen, Josephine P Holtan, Carel B Hoyng, Manuel Benjamin B Ibanez IV, Chris F Inglehearn, Takeshi Iwata, Kaylie Jones, Vasiliki Kalatzis, Smaragda Kamakari, Marianthi Karali, Ulrich Kellner, Krisztina Knézy, Caroline C W Klaver, Robert K Koenekoop, Susanne Kohl, Taro Kominami, Laura Kühlewein, Tina M Lamey, Bart P Leroy, María Pilar Martín-Gutiérrez, Nelson Martins, Laura Mauring, Rina Leibu, Siying Lin, Petra Liskova, Irma Lopez, Victor R de J López-Rodríguez, Omar A Mahroo, Gaël Manes, Martin McKibbin, Terri L McLaren, Isabelle Meunier, Michel Michaelides, José M Millán, Kei Mizobuchi, Rajarshi Mukherjee, Zoltán Zsolt Nagy, Kornelia Neveling, Monika Ołdak, Michiel Oorsprong, Yang Pan, Anastasia Papachristou, Antonio Percesepe, Maximilian Pfau, Eric A Pierce, Emily Place, Raj Ramesar, Florence Andrée Rasquin, Gillian I Rice, Lisa Roberts, María Rodríguez-Hidalgo, Javier Ruiz-Eddera, Ataf H Sabir, Ai Fujita Sajiki, Ana Isabel Sánchez-Barbero, Asodu Sandeep Sarma, Riccardo Sangermano, Cristina M Santos, Margherita Scarpato, Hendrik P N Scholl, Dror Sharon, Sabrina Giovanna Signorini, Francesca Simonelli, Ana Berta Sousa, Maria Stefaniotou, Katarina Stingl, Akiko Suga, Lori S Sullivan, Viktória Szabó, Jacek P Szaflik, Gita Taurina, Carmel Toomes, Viet H Tran, Miltiadis K Tsilimbaris, Pavlina Tsoka, Veronika Vaclavik, Marie Vajter, Sandra Valeina, Enza Maria Valente, Casey Valentine, Rebeca Valero, Joseph van Aerschot, L. Ingeborgh van den Born, Andrew R Webster, Laura Whelan, Bernd Wissinger, Georgia G Yioti, Kazutoshi Yoshitake, Juan C Zenteno, Roberta Zeuli, Theresia Zuleger, Chaim Landau, Allan I Jacob, Frans P M Cremers, Winston Lee, Jamie M Ellingford, David Stanek, Carlo Rivolta, Susanne Roosing

ERDC
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Prog RET

medRxiv
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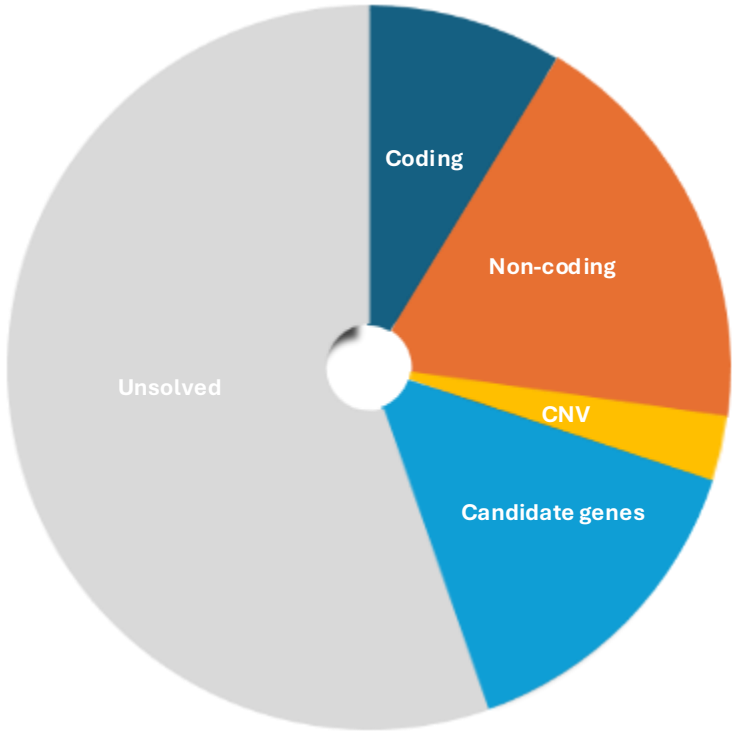
doi: https://doi.org/10.1101/2025.01.06.24317169

A multi-omics approach in clinically accessibe tissues reveals a genetic diagnosis in 44.5% of a prescreened IRD cohort



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Non-coding splice and regulatory SNVs in known IRD genes (18.5%)

SVs in known IRD genes (3%)

Variants in novel and candidate IRD genes (14.5%)

A multi-omics approach in clinically accessibe tissues reveals a genetic diagnosis in 44.5% of a prescreened IRD cohort

1. WGS improves the diagnostic yield

2. A multi-omics approach is essential for variant interpretation

3. Non-coding splice variants and novel disease genes are key contributors in our pre-screened IRD cohort

4. Novel targets for therapy uncovered

How to treat genetic eye diseases?

Elfride De Baere

Ghent University & GU Hospital
Center for Medical Genetics

Why is the eye an ideal target for gene therapy?

Immune-privileged: blood-retinal barrier

Retinal cells: differentiated and non-dividing

Bilateral disease: treated vs control eye

Non-invasive methods to monitor visual function

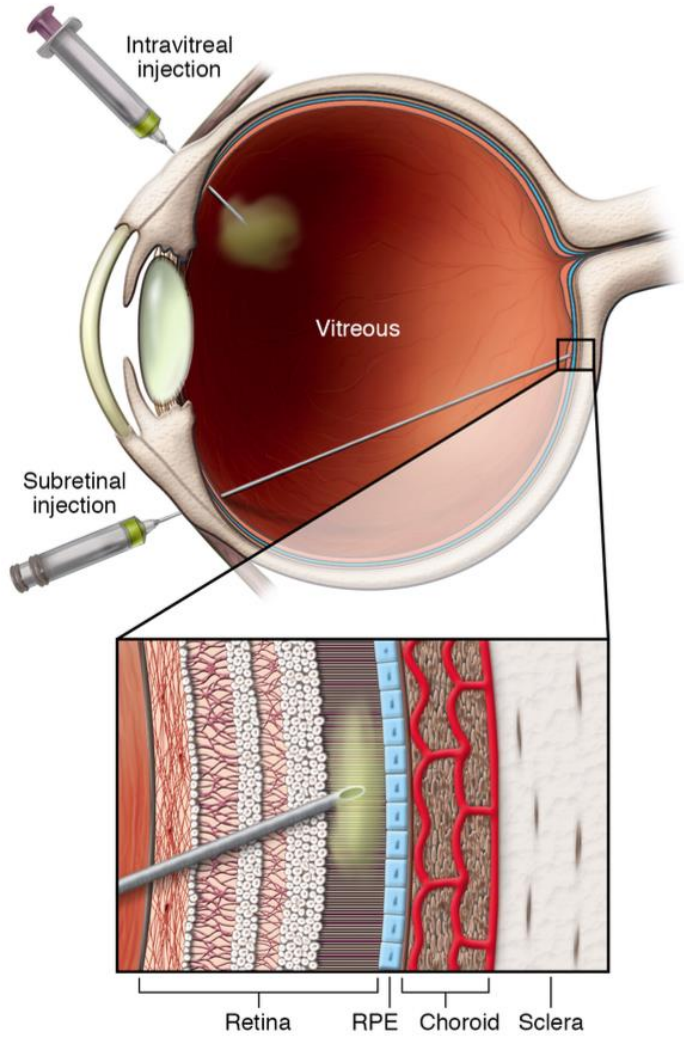
Small size, easily accessible by surgery

Subretinal injection

- Technically challenging, transient retinal detachment
- Higher concentration at the target tissue

Intravitreal injection

- Less invasive, fewer risks
- Widespread distribution but less concentrated delivery

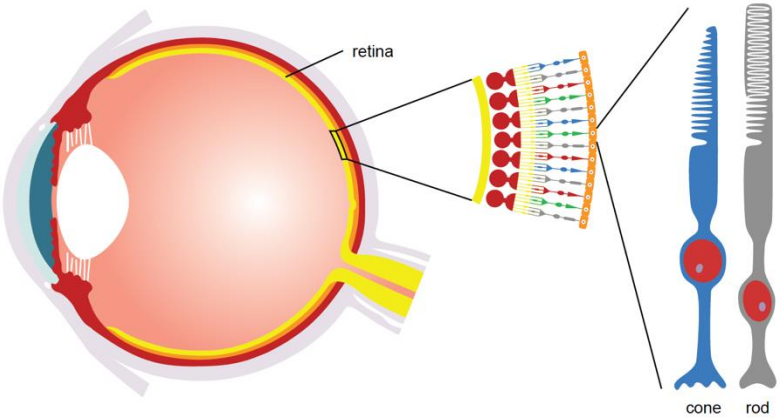


PMID 29856367

The ideal target: inherited retinal diseases IRD

Broad spectrum, overall prevalence 1/3.000

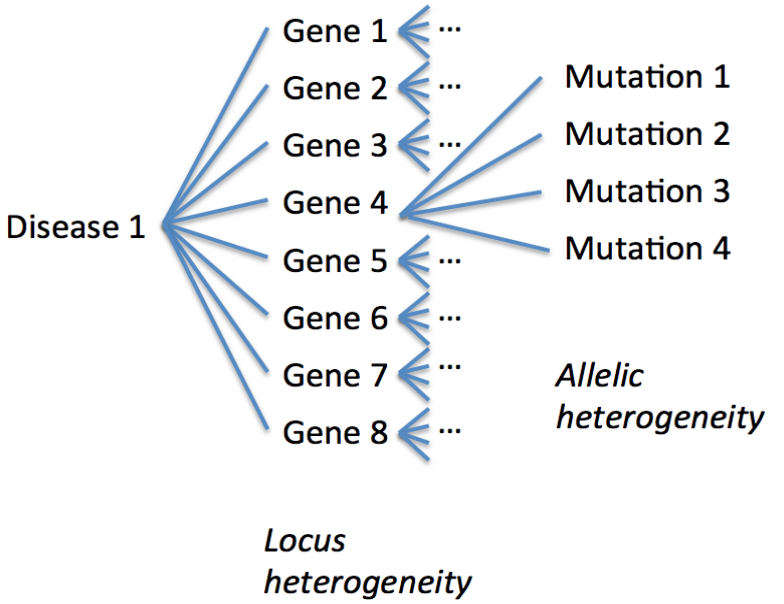
Degeneration of the photoreceptors (neuroretina) or the retinal pigment epithelium (RPE)



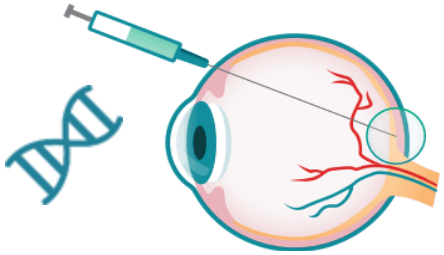
Monogenic

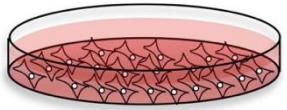
Genetically heterogeneous

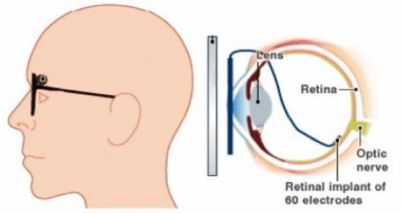
- Locus heterogeneity (> 300 genes)
- Allelic heterogeneity



Why is an early genetic diagnosis important?





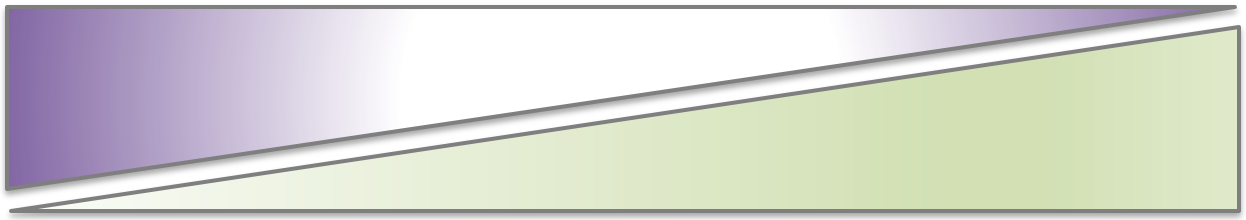


Gene therapy

Cell therapy

Prosthesis

Genetic diagnosis →



→ Progression of disease

DNA

RNA

Others

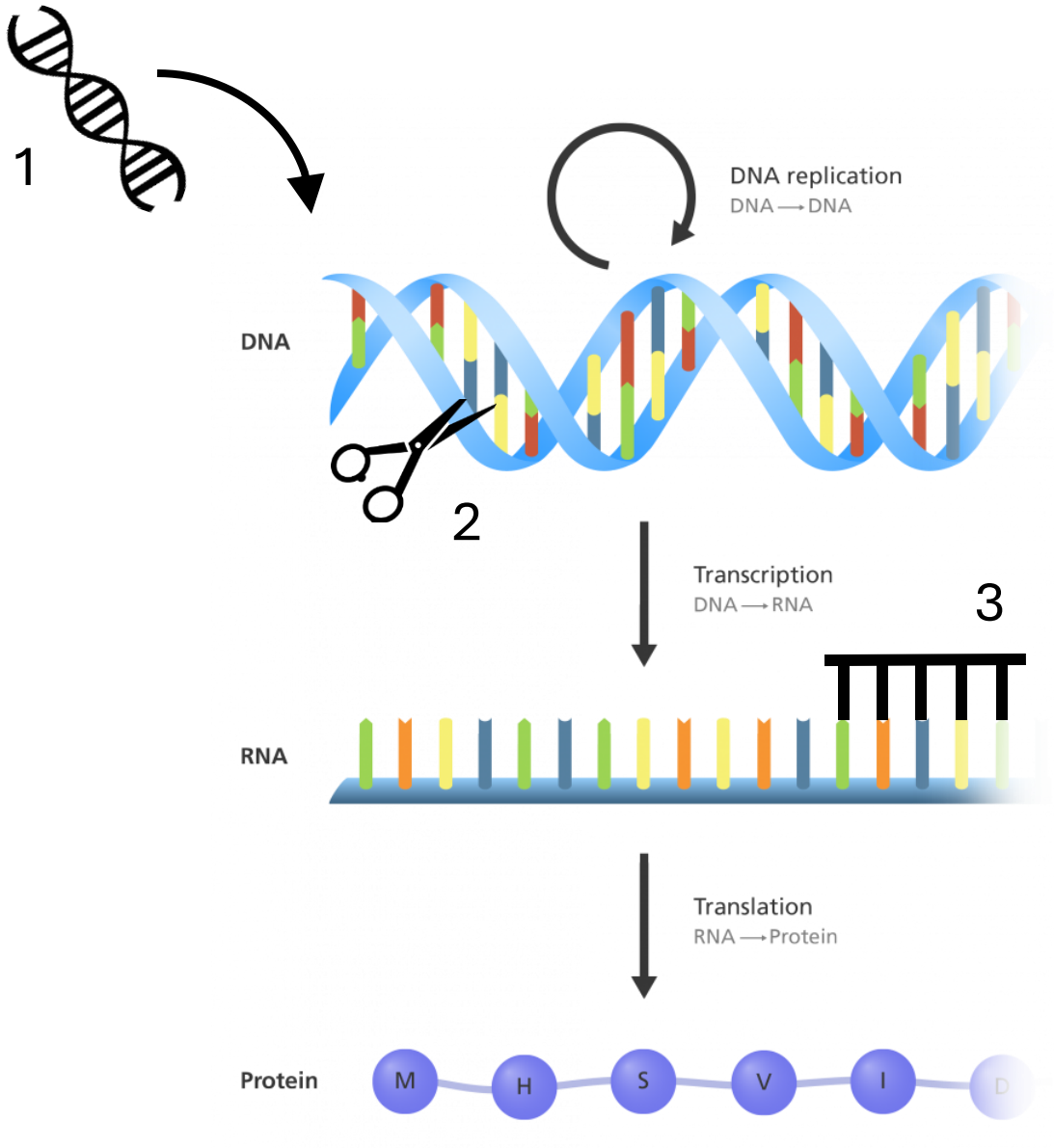
Cells

Optogenetics

Implant

Adapted from: Vázquez-Domínguez, Garanto & Collin. Genes 2019; PMID: 31466352

Gene therapy strategies



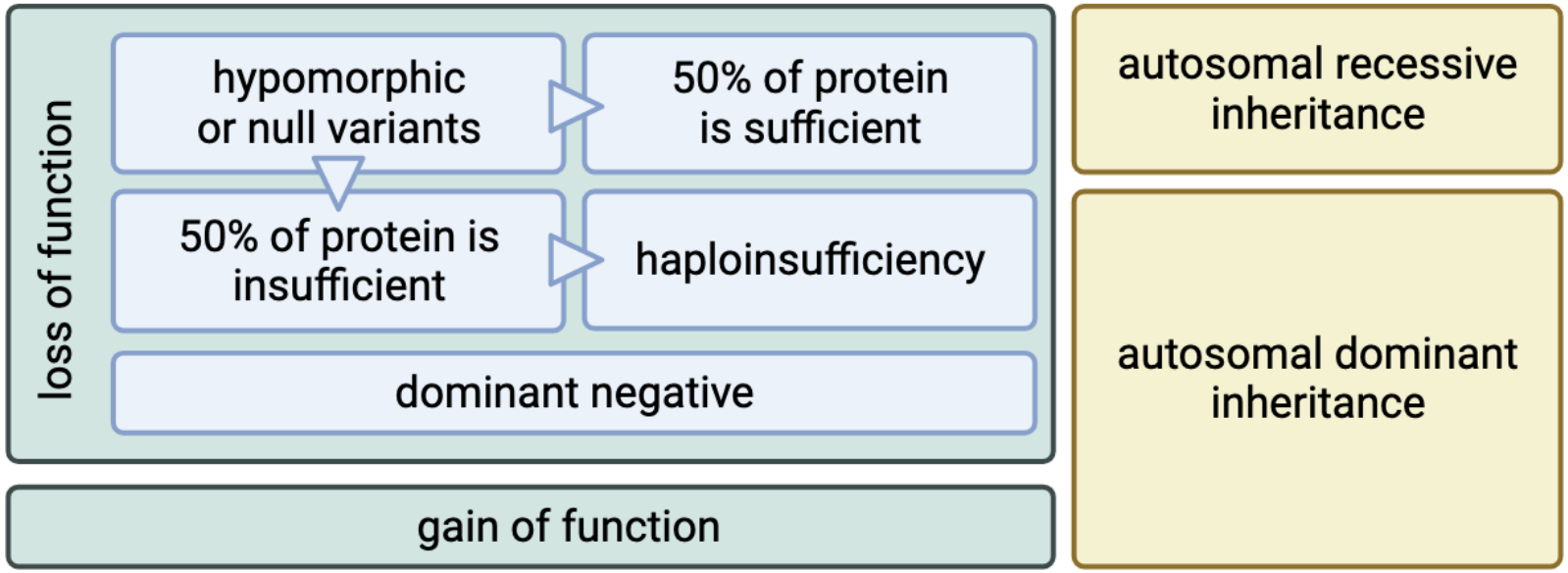
1. Add correct DNA to the cells of the patient
   = gene augmentation/supplementation

2. Modify the patient's DNA
   = gene editing (e.g. CRISPR/Cas9, base editing prime editing)

3. Interfere with the patient's RNA
   e.g. antisense oligonucleotides (ASOs/AONs)

Courtesy of
Frauke Coppieters

When to use which strategy?



1. Knowledge of the disease and the effect you want to achieve ➔ need for natural history studies

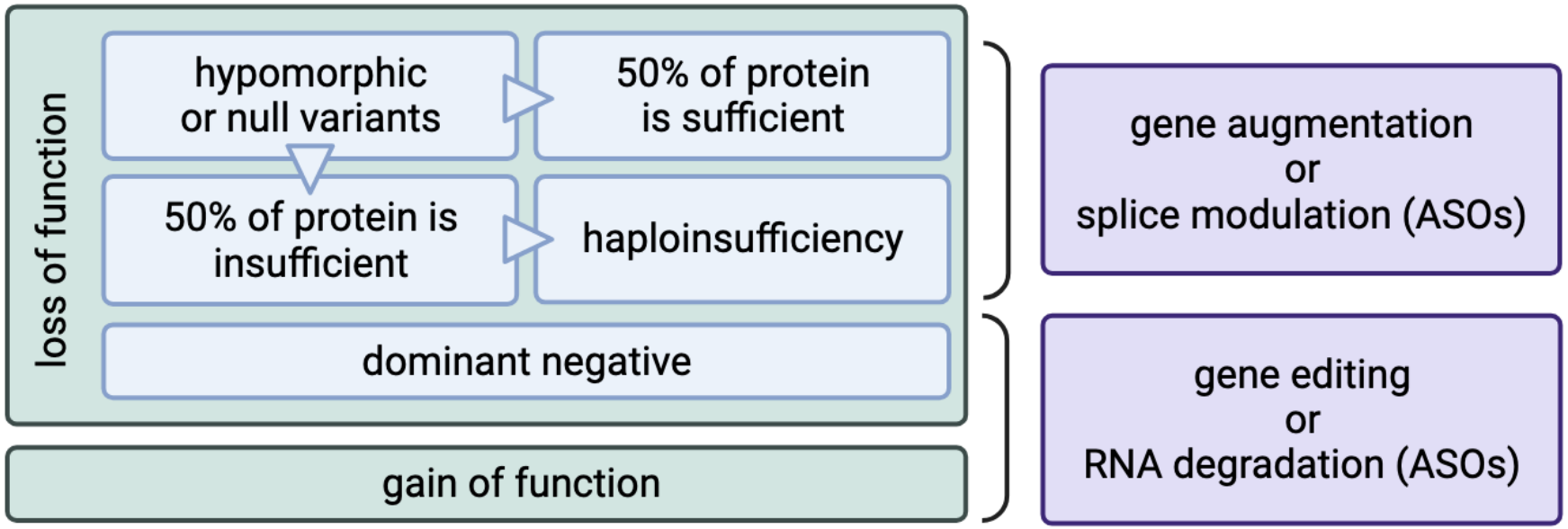
2. Underlying disease gene and molecular mechanism

- Loss-of-function (inactivating)
  - Hypomorphic allele: reduced expression
  - Null allele: complete loss of function
  - Haploinsufficiency: reduced dosage
  - Dominant negative effect

- Gain-of-function (activating)
- Hypermorphic allele: increased expression
- Neomorphic allele: new activity or product

Courtesy of Frauke Coppieters

When to use which strategy?



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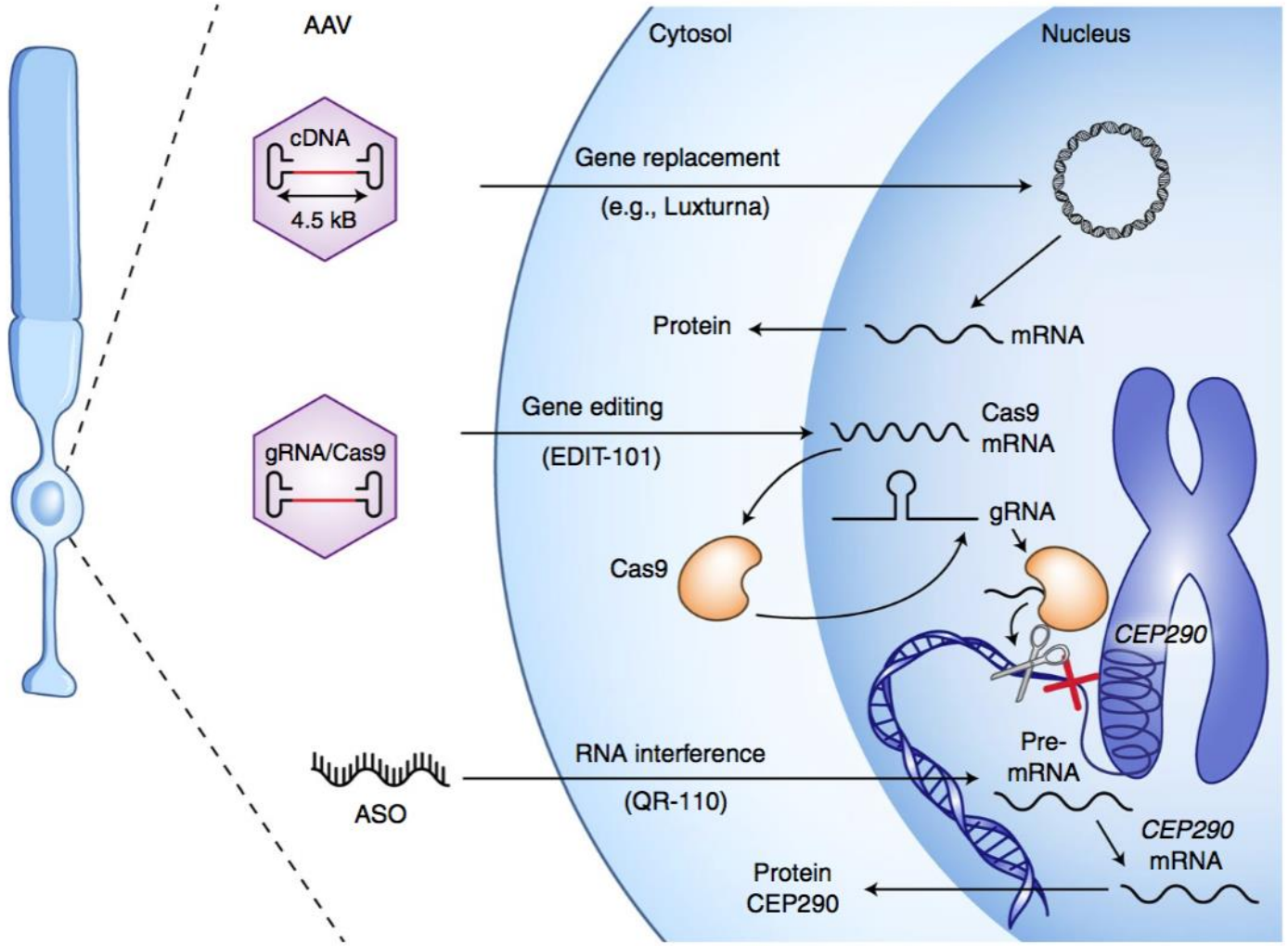
Courtesy of Frauke Coppieters

Overview of gene therapy for IRD

1. Gene augmentation
   - Luxturna for *RPE65* = first FDA/EMA approved gene therapy

2. Gene editing (e.g. CRISPR-Cas9) clinical trial for *CEP290* (EDIT-101)

3. Antisense oligonucleotides (ASOs) clinical trials for *CEP290* (QR-110), *USH2A* (QR-421a) and *RHO* (QR-1123)



PMID 30718907

Gene augmentation

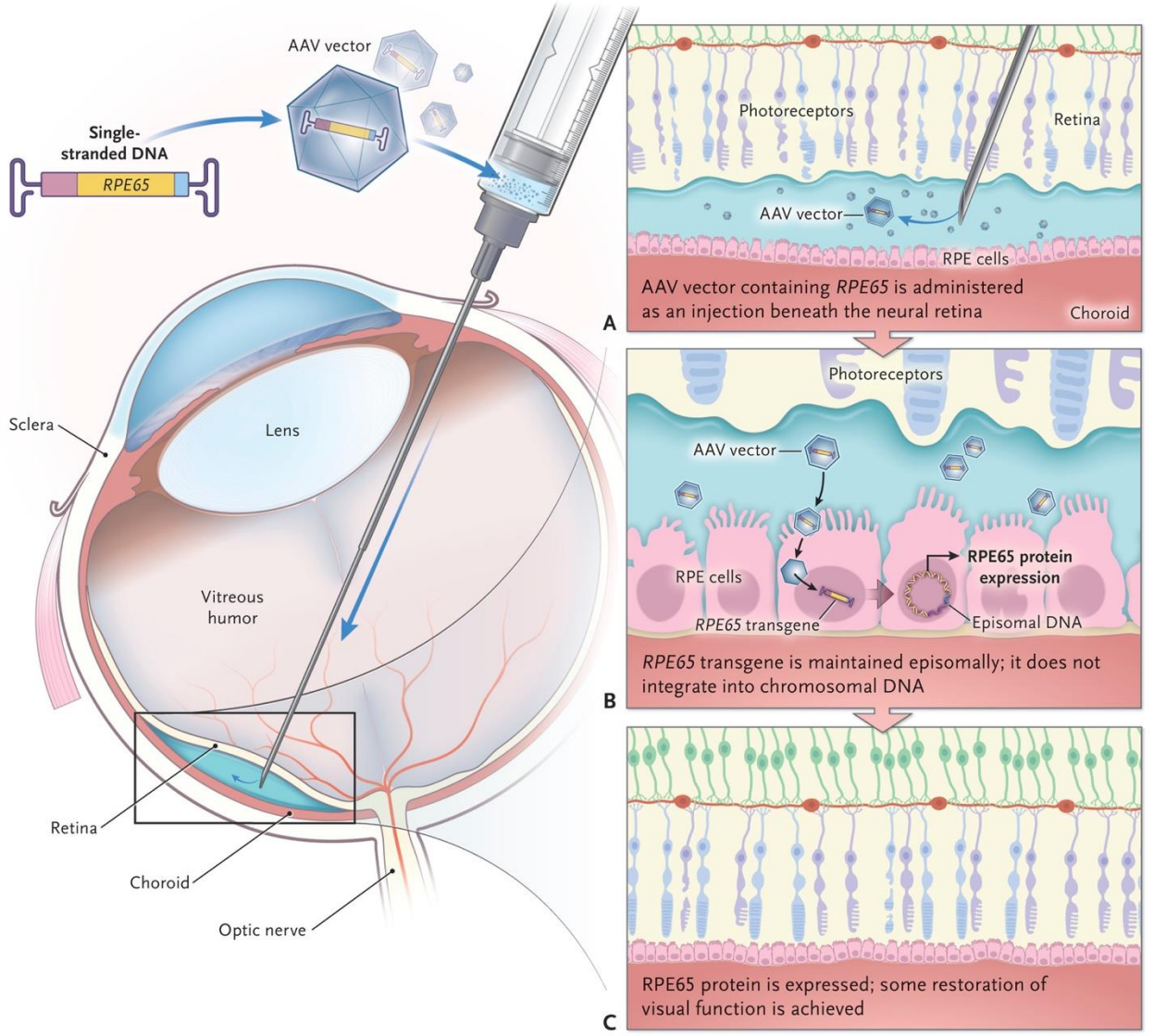
Method

- Pack wild-type cDNA in a vector
- Subretinal injection (A)
- Local transcription and translation of wild-type cDNA (B, C)

\+ One-time therapy

\+ Mutation-independent

\- Limited capacity of AAV vectors



PMID 31365802

Gene editing

What do you need?

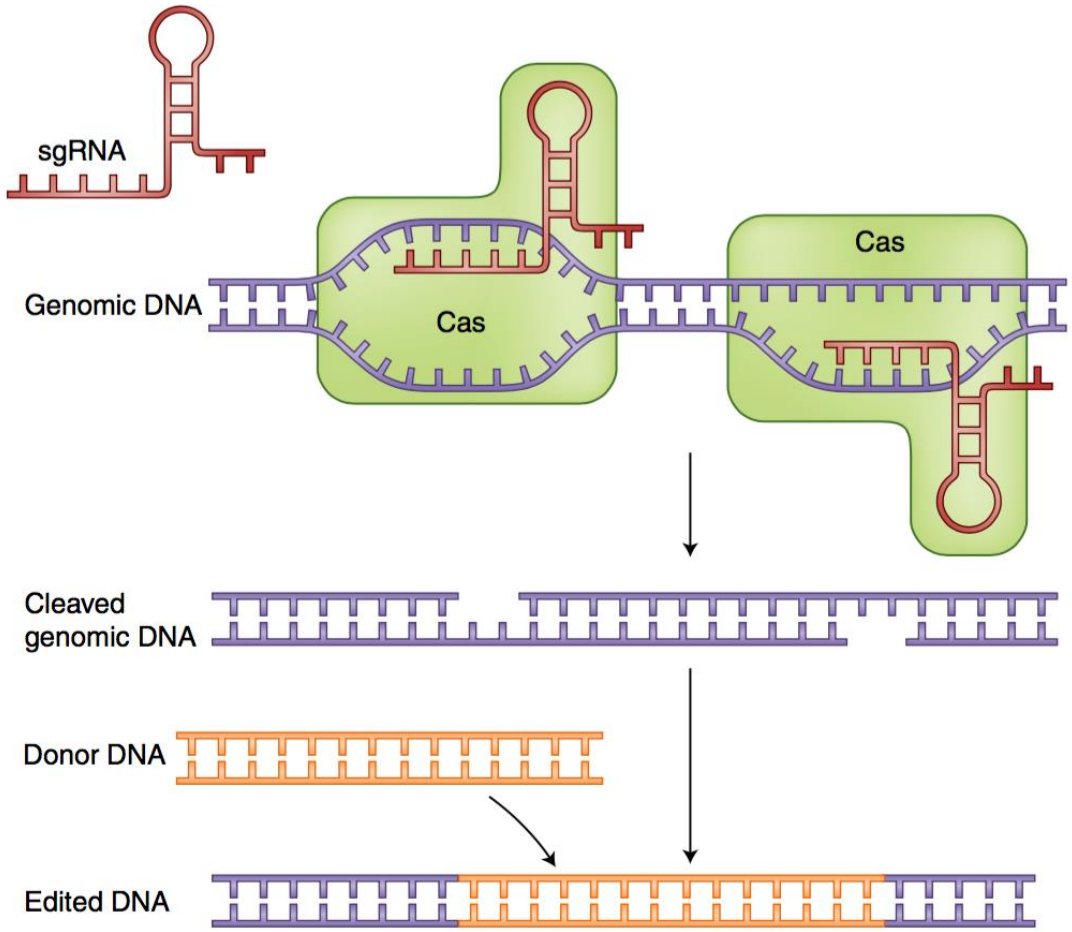
- sgRNA: single-guide RNA
- Cas9: DNA endonuclease enzyme
- (DNA repair template/donor DNA)

How does it work?

1. sgRNA guides Cas9 to region of interest
2. Cas9 enzyme creates DNA breaks
3. Trigger of endogenous repair mechanisms:
    - Non-homologous end joining (NHEJ)

      = used for **allele inactivation**
        - Error-prone, formation of indels
    - Homology-directed repair (HDR)

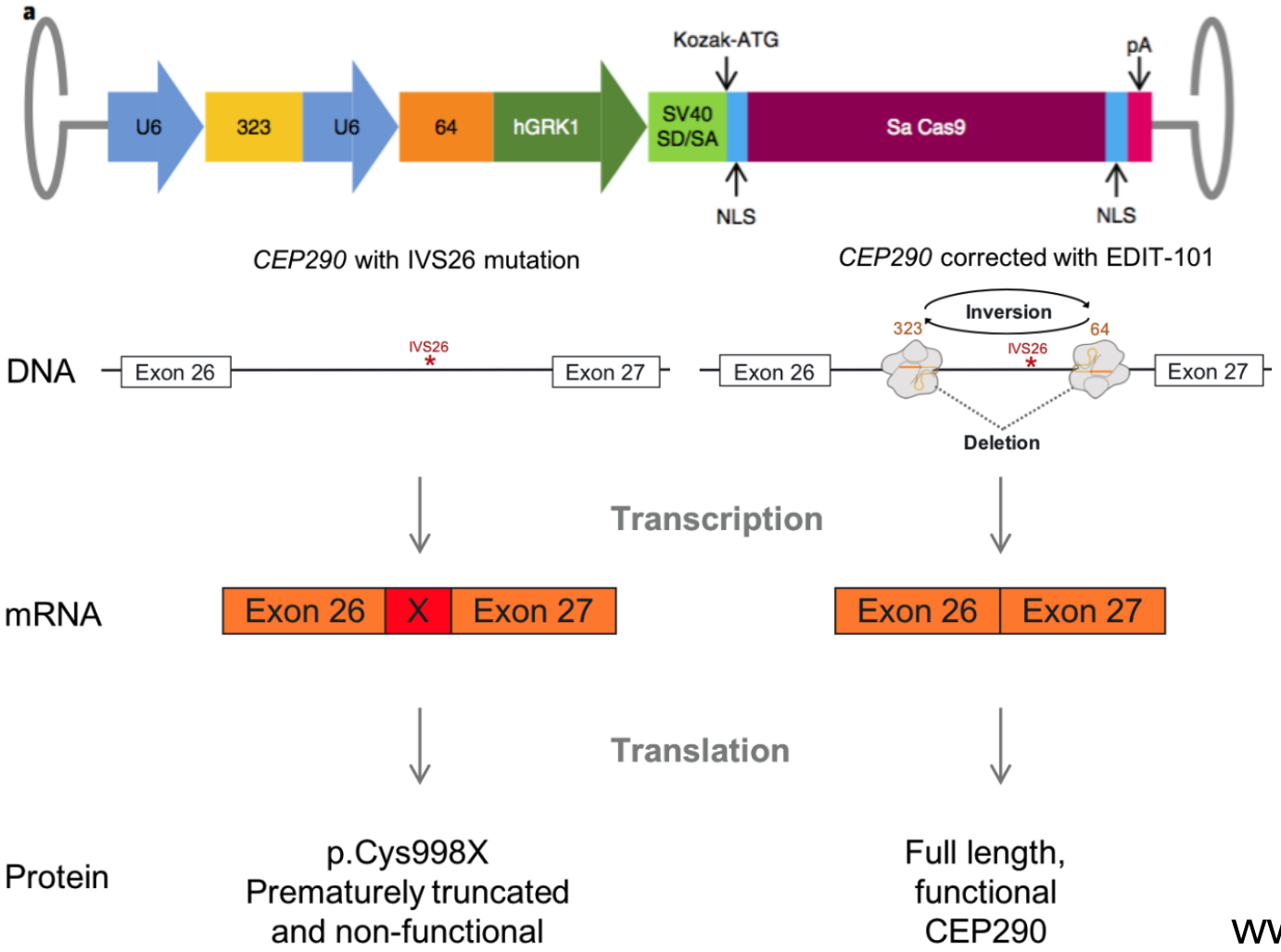
      = used for **variant correction**
        - Activated in the presence of a DNA repair template
        - Alters a DNA sequence at a specific locus



PMID 29662218

CRISPR/Cas9 for congenital blindness (LCA)

- Leber Congenital Amaurosis (LCA) caused by deep-intronic mutation ('IVS26') in *CEP290*
- EDIT-101 (Editas Medicine): CRISPR/Cas9 to remove an intronic sequence containing IVS26



www.editasmedicine.com

CRISPR TREATMENT INSERTED DIRECTLY INTO BODY FOR THE FIRST TIME

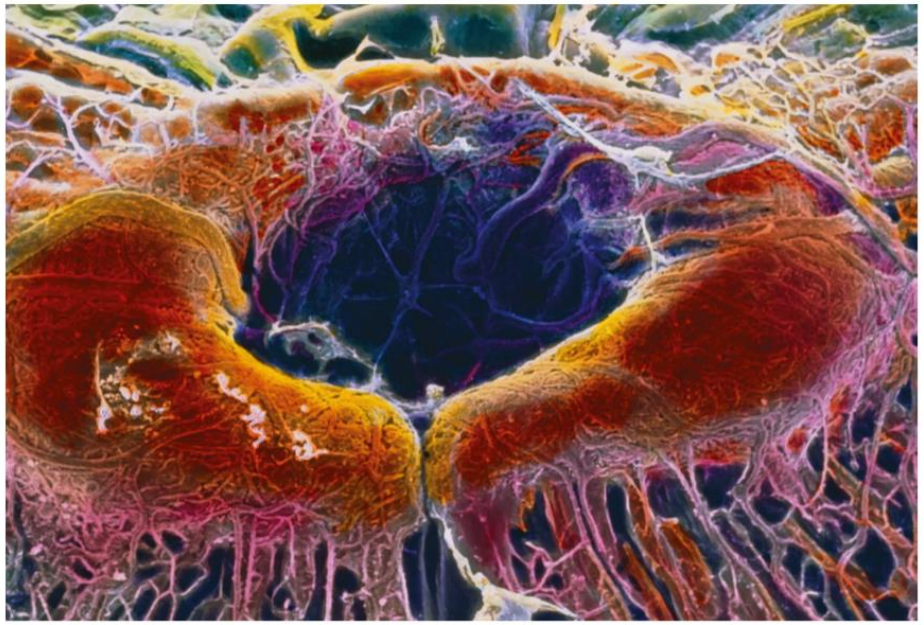
Experiment tests a gene-editing therapy for a hereditary blindness disorder.

By Heidi Ledford

A person with a genetic condition that causes blindness has become the first to receive a CRISPR–Cas9 gene therapy administered directly into their body.

The treatment is part of a landmark clinical trial to test the ability of CRISPR–Cas9 gene-editing techniques to remove mutations that cause a rare condition called Leber's congenital amaurosis 10 (LCA10). No treatment is currently available for the disease, which is a leading cause of blindness in childhood.

For the latest trial, the components of the gene-editing system – encoded in the genome of a virus – are injected directly into the eye, near photoreceptor cells. By contrast, previous CRISPR–Cas9 clinical trials have used



The human retina. A CRISPR therapy has been inserted directly into a person's eye.

Ledford, Nature 2020

First CRISPR/Cas9 clinical trial

- EDIT-101 Phase 1/2 BRILLIANCE trial
  - Subretinal injection
  - 12 adult and 2 pediatric patients
  - No ocular serious adverse events or dose-limiting toxicities
  - 3/14 patients met the responder threshold
  - 2/3 responders: homozygous for IVS26
    - ➢ Trial enrolment is paused

\+ One-time modification of the genome

\- (AAV) vector needed

\- Concerns for off-target effects

nature medicine

Article

https://doi.org/10.1038/s41591-024-03422-8

High-efficiency base editing in the retina in primates and human tissues

Alissa Muller[1,2], Jack Sullivan[3], Wibke Schwarzer[1,2], Mantian Wang[1,2], Cindy Park-Windhol[3], Pascal W. Hasler[2], Lucas Janeschitz-Kriegl[1,2], Mert Duman[1,2], Beryll Klingler[1,2], Jane Matsell[1,2], Simon Manuel Hostettler[1,2], Patricia Galliker[1,2], Yanyan Hou[1,2], Pierre Balmer[1,2], Tamás Virág[3], Luis Alberto Barrera[3], Lauren Young[3], Quan Xu[1,2], Dániel Péter Magda[4], Ferenc Kilin[4], Arogya Khadka[3], Pierre-Henri Moreau[5], Lyne Fellmann[5], Thierry Azoulay[6], Mathieu Quinodoz[1,2,7], Duygu Karademir[1,2], Juna Leppert[1,2], Alex Fratzl[1,2], Georg Kosche[1,2], Ruchi Sharma[8], Jair Montford[8], Marco Cattaneo[1,2,9], Mikaël Croyal[10,11], Therese Cronin[12], Simone Picelli[1,2], Alice Grison[1,2], Cameron S. Cowan[1,2], Ákos Kusnyerik[1,2], Philipp Anders[1,2], Magdalena Renner[1,2], Zoltán Zsolt Nagy[13], Arnold Szabó[4], Kapil Bharti[8], Carlo Rivolta[1,2,7], Hendrik P. N. Scholl[1,2,14,15], David Bryson[3], Giuseppe Ciaramella[3], Botond Roska[1,2,4] ✉ & Bence György[1,2] ✉

https://doi.org/10.1038/s41591-024-03422-8

c

STGD-gRNA wt-gRNA ms-gRNA

Model system	Target	Delivery	Targeted sites
HEK293T cells (lenti-*ABCA4*^{1961E})	A7 A8	Plasmid	Endogenous ·····GGA·····AGG ·····GGA·····AGG Lenti-integrated ·····GAA·····AGG
Human iPS cell-RPE cells (*ABCA4*$^{1961G/G}$)	A8	AAV	·····GGA·····AGG ·····GGA·····AGG
Human retinal organoid (*ABCA4*$^{1961E/E}$)	A7 A8	AAV	·····GAA·····AGG ·····GAA·····AGG
Human retinal explant (*ABCA4*$^{1961G/G}$)	A8	AAV	·····GGA·····AGG ·····GGA·····AGG
Human RPE/choroid explant (*ABCA4*$^{1961G/G}$)	A8	AAV	·····GGA·····AGG ·····GGA·····AGG
Mouse eye (*ABCA4*$^{hu1961E/ms1961G(KO)}$)	A7 A8	AAV	·····GAA·····AGG ····tGGA·····a·····AGG
Mouse eye (*ABCA4*$^{ms1961G/G}$)	A8	AAV	····tGGA·····a·····AGG ····tGGA·····a·····AGG
Macaque eye (*ABCA4*$^{1961G/G}$)	A8	AAV	·····GGA·····AGG ·····GGA·····AGG

Antisense oligonucleotides (ASO/AON)

- Short, chemically modified oligonucleotides that modulate gene expression

+ High specificity

+ Easy synthesis

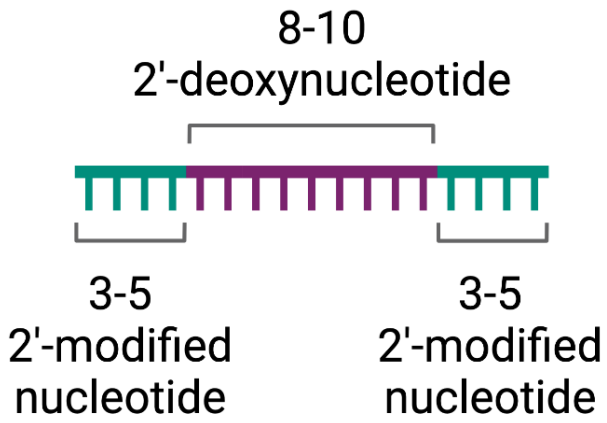
+ No vector needed for delivery (= suitable for large genes)

- Non-permanent: recurrent (intravitreal) injections needed

- 2 modes of action depending on ASO composition:

Gapmer ASOs

DNA-RNA hybrid, induces mRNA degradation

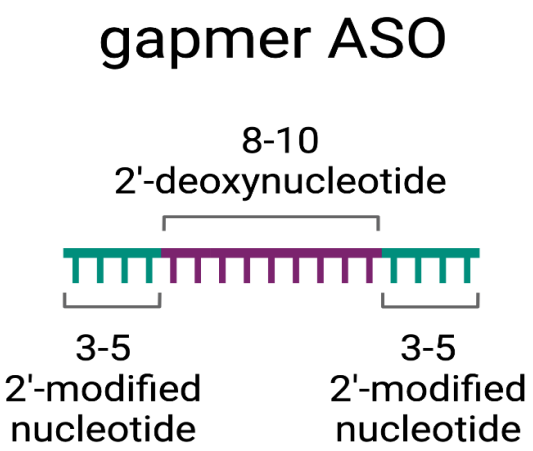


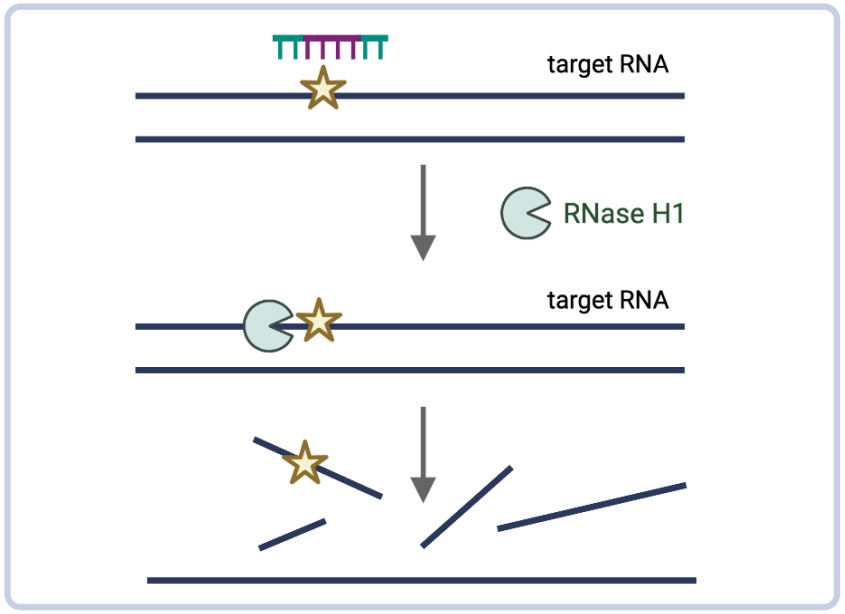
Steric-blocking ASOs

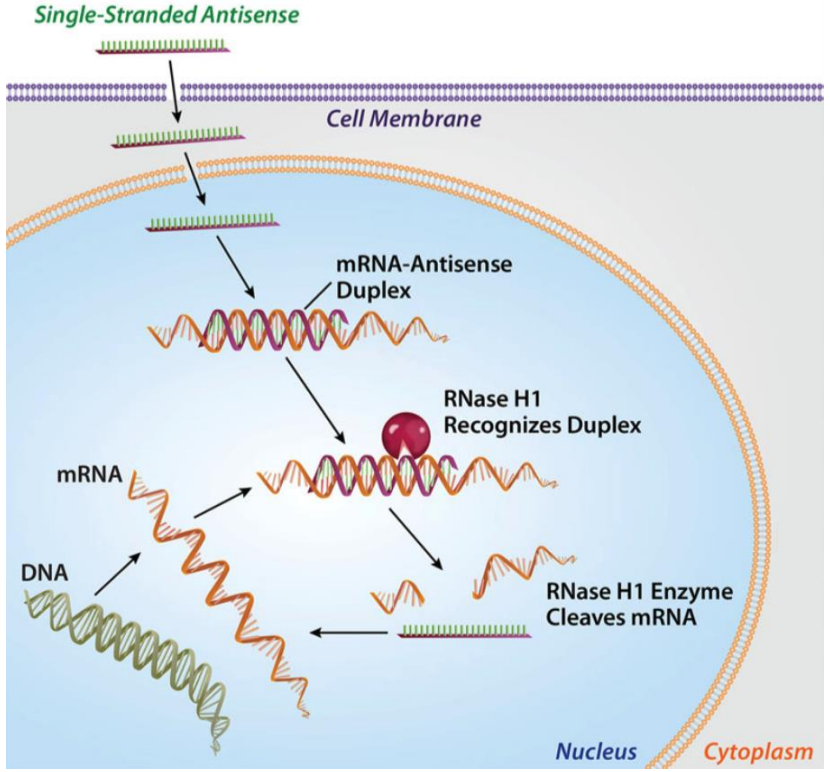
uniformly modified nucleotides, provide steric hindrance to for instance protein binding

Gapmer ASO (RNA degradation)

- Gapmer ASO ➔ degradation of patient mRNA through RNase H1 mediated cleavage
- Excellent approach for targeting gain-of-function or dominant-negative mutations
- Mutation-specific: limited patient population





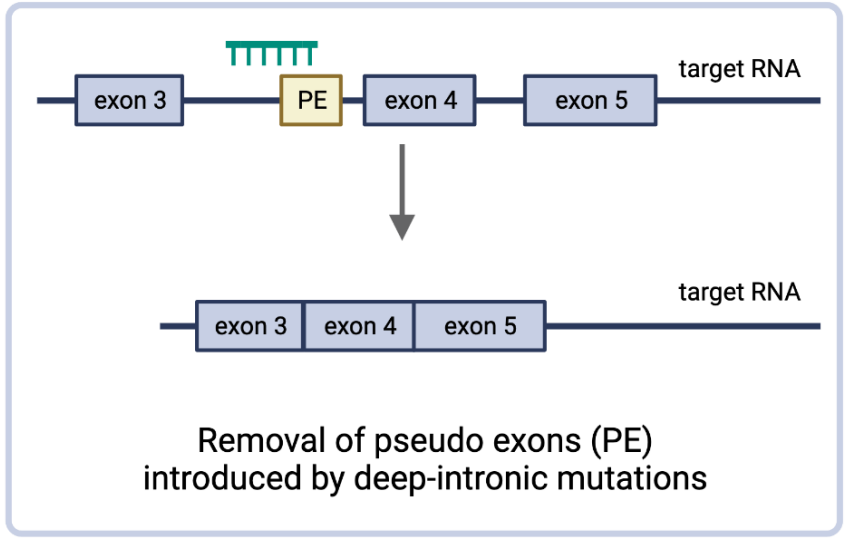


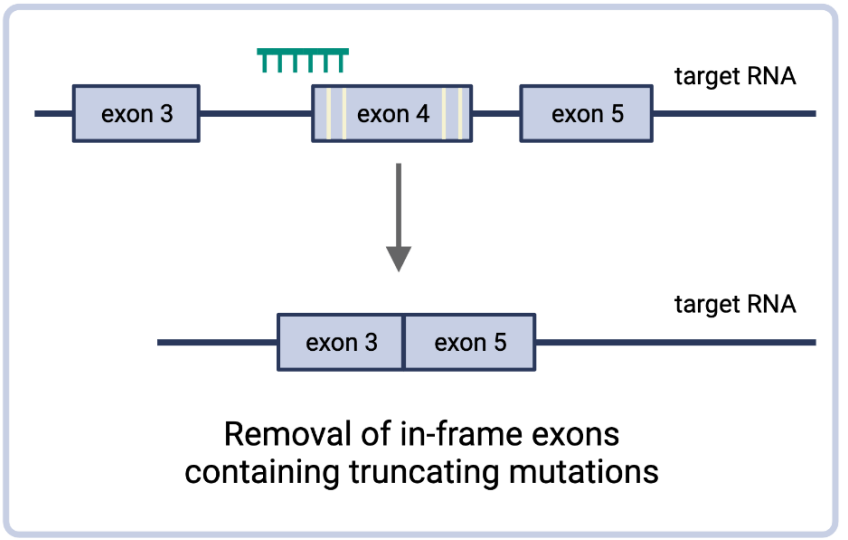
- Example: QR-1123 for allele-specific degradation of the frequent *RHO* P23H mutation (ProQR/Ionis Pharmaceuticals)

PMID 28921648

Splice modulating ASO

- Splice-modulation through steric hindrance for the splicing machinery
- Elegant strategy for large genes with loss-of-function mutations that do not fit AAV vectors
- Two main applications





- Mutation-specific
- E.g. Sepofarsen for IVS26 in *CEP290* (7.8 kb)
- > improved visual acuity and retinal sensitivity

- Exon-specific: removal of a non-redundant exon
- E.g. Ultevursen for truncating mutations in exon 13 of *USH2A* (15.6 kb)

Courtesy of Frauke Coppieters

What is the role of the clinical geneticists?

The evolving role of medical geneticists in the era of gene therapy: An urgency to prepare

Jerry Vockley[1,*], Nicola Brunetti-Pierri[2,3], Wendy K. Chung[4], Angus J. Clarke[5], Nina Gold[6], Robert C. Green[7], Stephen Kagan[8], Tara Moroz[8], Christian P. Schaaf[9], Martin Schulz[8], Elfride De Baere[10]

Genetics in Medicine (2023) 25, 100022

Timeframe	Action Needed
Immediate	• Better define educational needs for medical geneticists • Better define educational needs for other members of the multidisciplinary team (other specialists, referring physicians, pharmacists, nurses, other health care provider staff, and managerial staff) who are directly involved in the care of patients receiving gene therapy or decision-making for these patients
Intermediate term (1-3 y)	• Develop models of care that apply to the growing range of gene therapies that are anticipated to become available over the next decade • Develop educational programs tailored to the needs of different stakeholders in the pathway of Gene Therapists—medical geneticists who plan to focus on gene therapy specifically o Medical geneticists involved in initial decision-making, assessments, and long-term follow-up o Multidisciplinary team members directly involved in gene therapy administration o Specialists in the broader health care community o Primary care providers • Partner with disease-state specific societies to deliver educational programs that enhance awareness and understanding of basic genetics concepts and gene therapy • Train genetic counselors to deliver basic education on gene therapy and assist in counseling patients • Incorporate opportunities for genomic screening of newborns
Long term (≥3 y)	• Incorporate basic gene therapy training into medical and health care professional school curricula • Increase the number of medical geneticists to meet evolving needs • Increase the number of medical geneticists in regions where limited numbers exist • Consider adding gene therapy to training and certification requirements for medical geneticists

Take home messages

- Genetic therapies = exciting and emerging field with inherited blindness as a model
- Different strategies, the choice of which depends on the disease status, the underlying disease gene and the molecular mechanism

	Gene augmentation	Gene editing	ASOs
Injection site	mostly subretinal	subretinal	mostly intravitreal
Carrier required	yes	yes	no
Gene size restrictions	yes	no	no
Mutation-independent	yes	no	no/partially
Immunotoxicity	++	++	+
Dosing	one-time	one-time	multiple
Status	approved (Luxturna)/ in clinical trials	clinical trial	clinical trials

Courtesy of Frauke Coppieters

Eye and Developmental Genetics Lab

DE BAERE LAB
EYE & DEVELOPMENTAL GENETICS

De Baere lab

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