Gaps between the bedside and the bench: Perspectives from the bench

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THE MEGAN FOUNDATION
SUPPORT FOR USHER SYNDROME

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Sponsored by the Office of the Director National Institutes of Health,
the National Human Genome Research Institute,
the National Institute of Child Health & Development,
the National Institute on Deafness & Other Communication Disorders,
the National Eye Institute,
the Usher 1F Collaborative, and the Megan and Vision for a Cure Foundations

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Case study 1: Positive results validate candidate genes

Case study 2: Negative results reveal incorrect diagnoses

Mind the gaps

Undiagnosed Diseases Network

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Usher syndrome - the leading cause of deafblindness

- **Prevalence ≈ 1 per 6,000 births in the US**
  (more common than ALS or Huntington's Disease)

- **Congenital deafness (~4% of deaf have Usher)**
  Sensorineural hearing loss
  Vestibular dysfunction

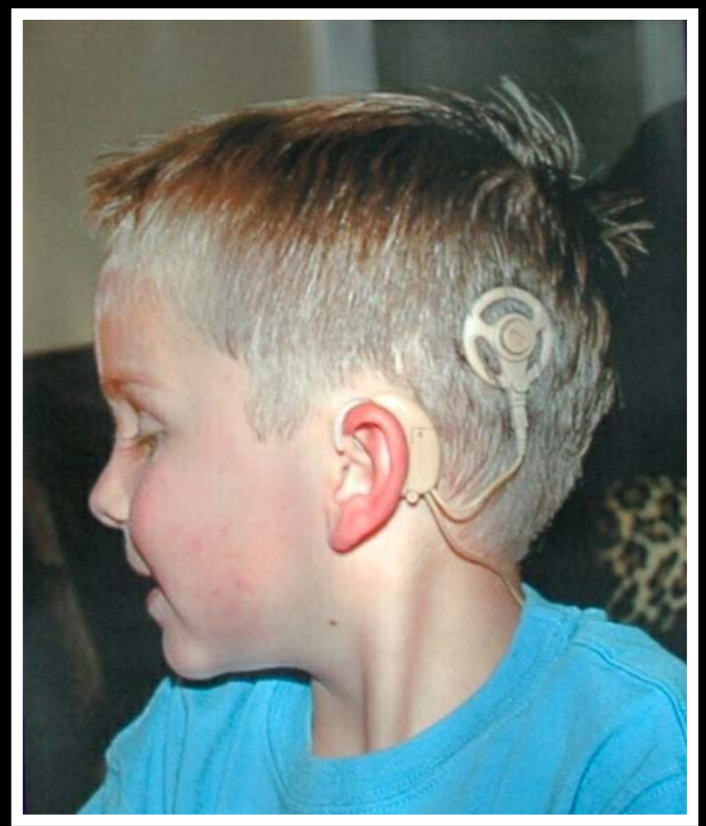
- **Retinitis pigmentosa**
  Loss of rod photoreceptors
  Progressive tunnel vision as cones die

Multiple Usher genes with multiple functions

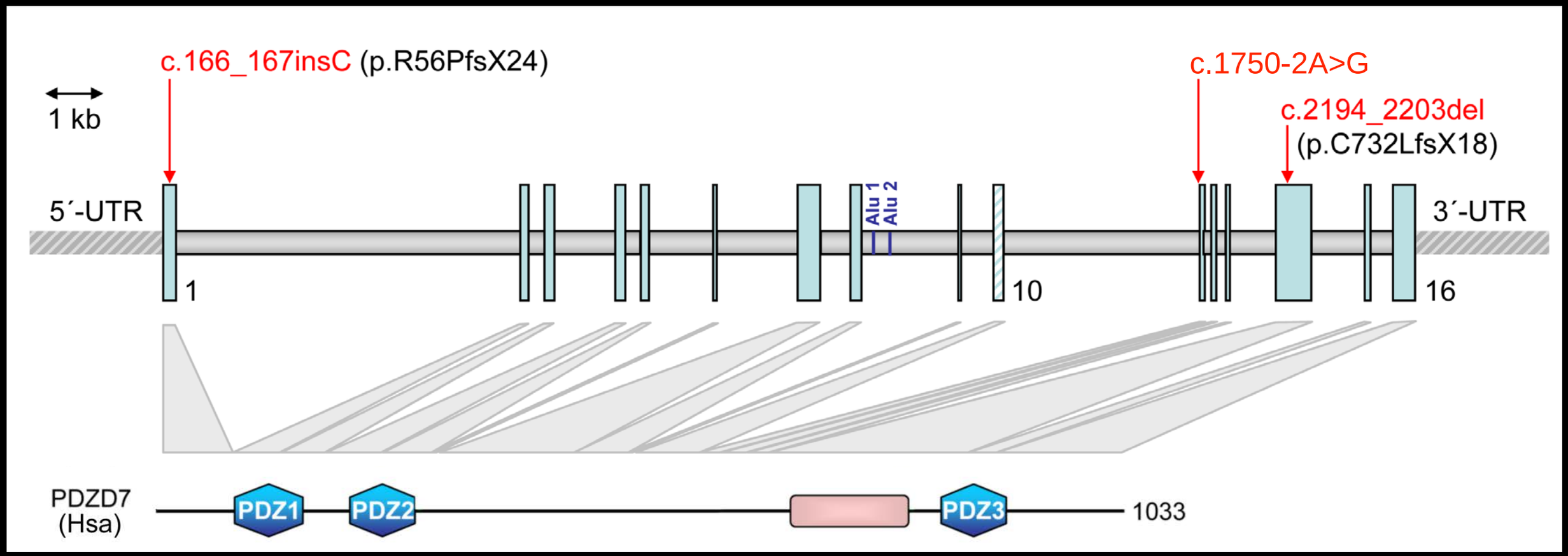
Type	Human	Protein: potential function
USH1B	*MYO7A*	MyosinV11A: motor activity
USH1C	*USH1C*	Harmonin: scaffold
USH1D	*CAD23*	Cadherin: calcium dependent adhesion
USH1E	-	Unknown
USH1F	*PCDH15*	Protocadherin15: adhesion, signaling
USH1G	*USH1G*	SANS: membrane associated scaffold
USH1H	-	Unknown
USH1J	*CIB2*	Calcium and integrin binding protein
USH1K	-	Unknown
USH2A	*USH2A*	Usherin: Laminin-like transmembrane protein
USH2C	*GPR98*	Vlgr1: G-protein coupled receptor, signaling
USH2D	*CIP98*	Whirlin: scaffold
USH3A	*CLRN1*	Clarin1: 4-pass transmembrane protein
USH3B	*HARS*	Histidyl-tRNA Synthetase

Genetic counseling is important for Usher patients

Gene discovery is important for Usher patients



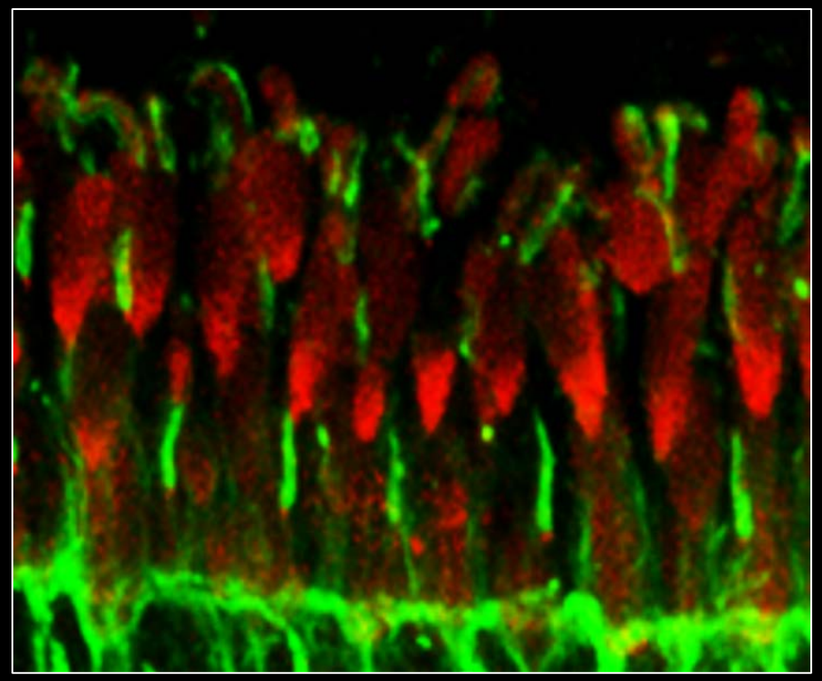
Exome sequencing of undiagnosed patients identifies mutations in PDZD7, a gene of unknown function



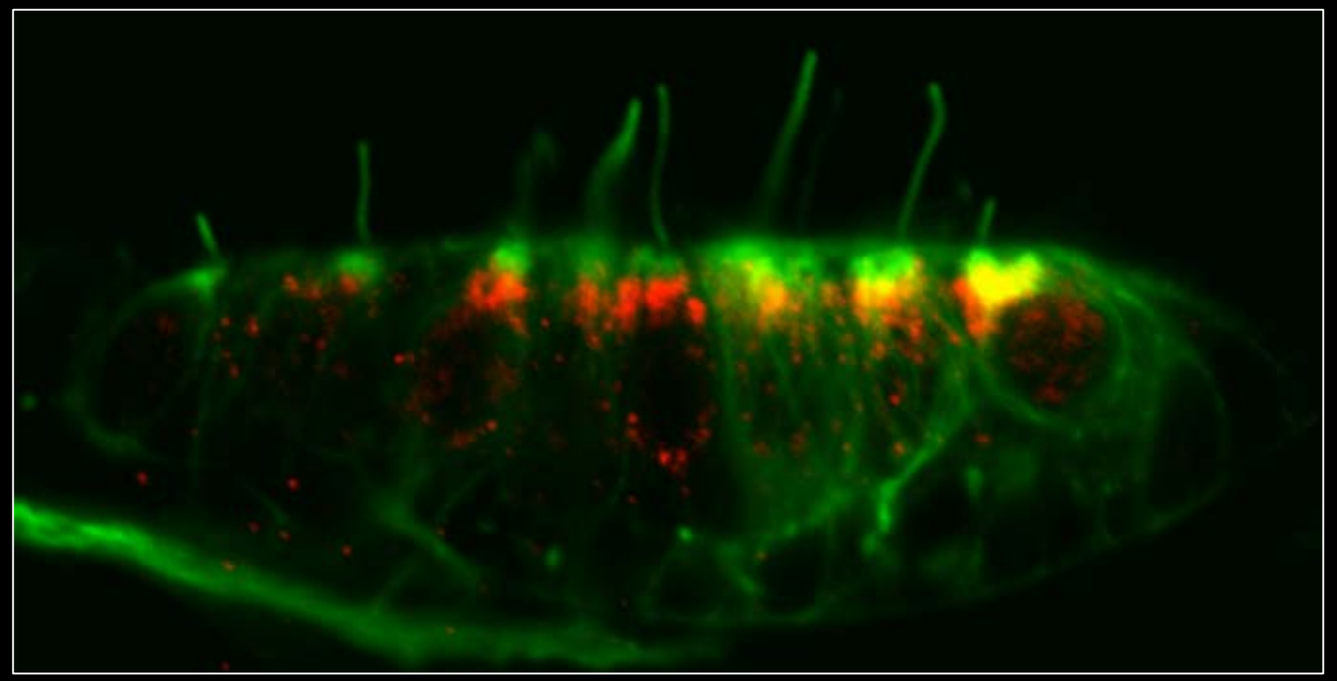
(Hanno Bolz & Inga Ebermann)

Zebrafish Pdzd7a is localized with other Usher proteins

Pdzd7 + ac-tubulin



Eye

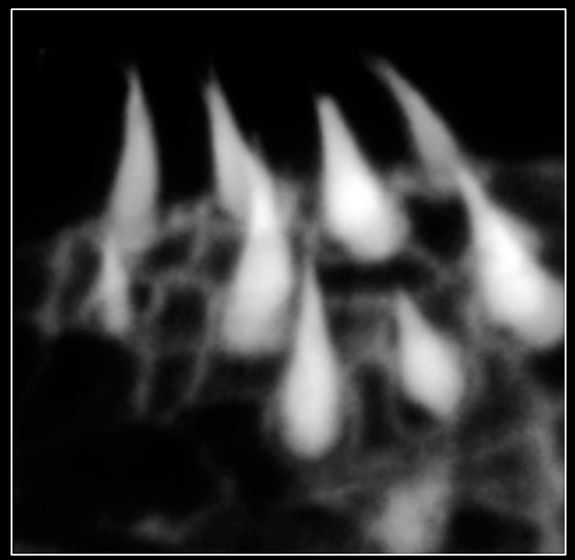


Ear

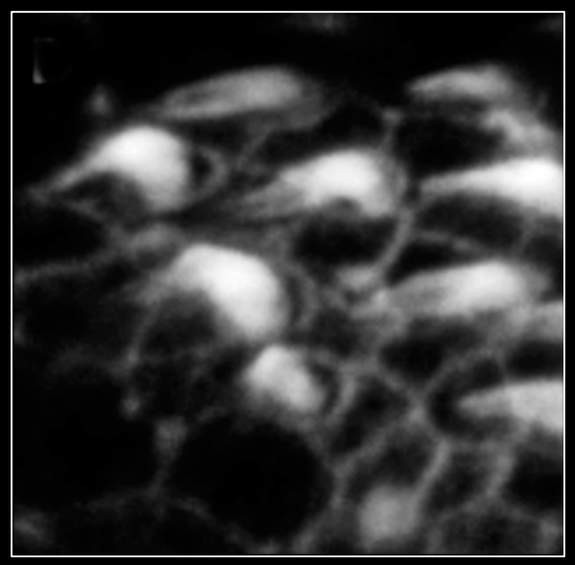
(Jennifer Phillips)

Stereocilia are defective after *pdzd7a* knockdown

Control

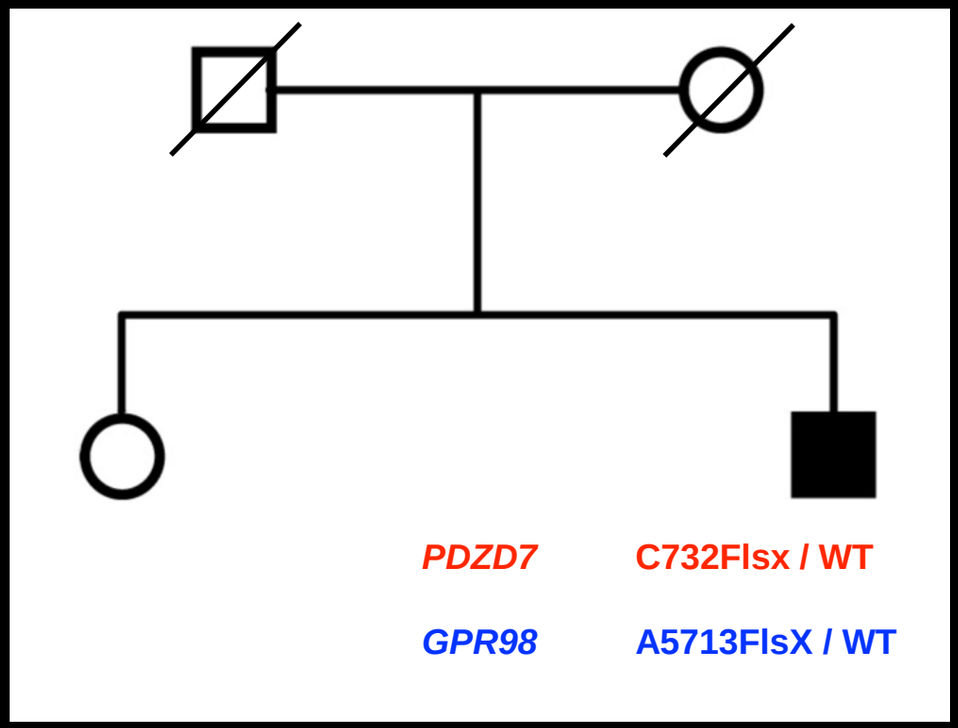


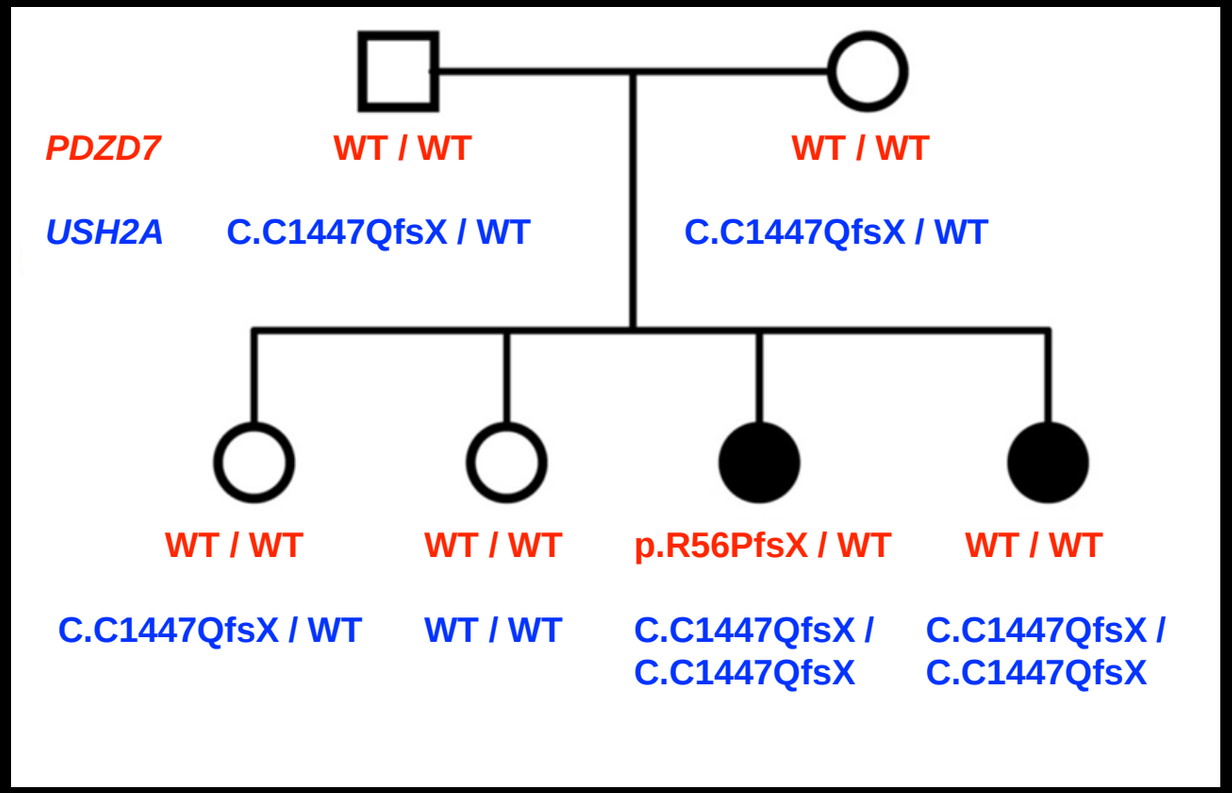
pdzd7a MO



(Bernardo Blanco)

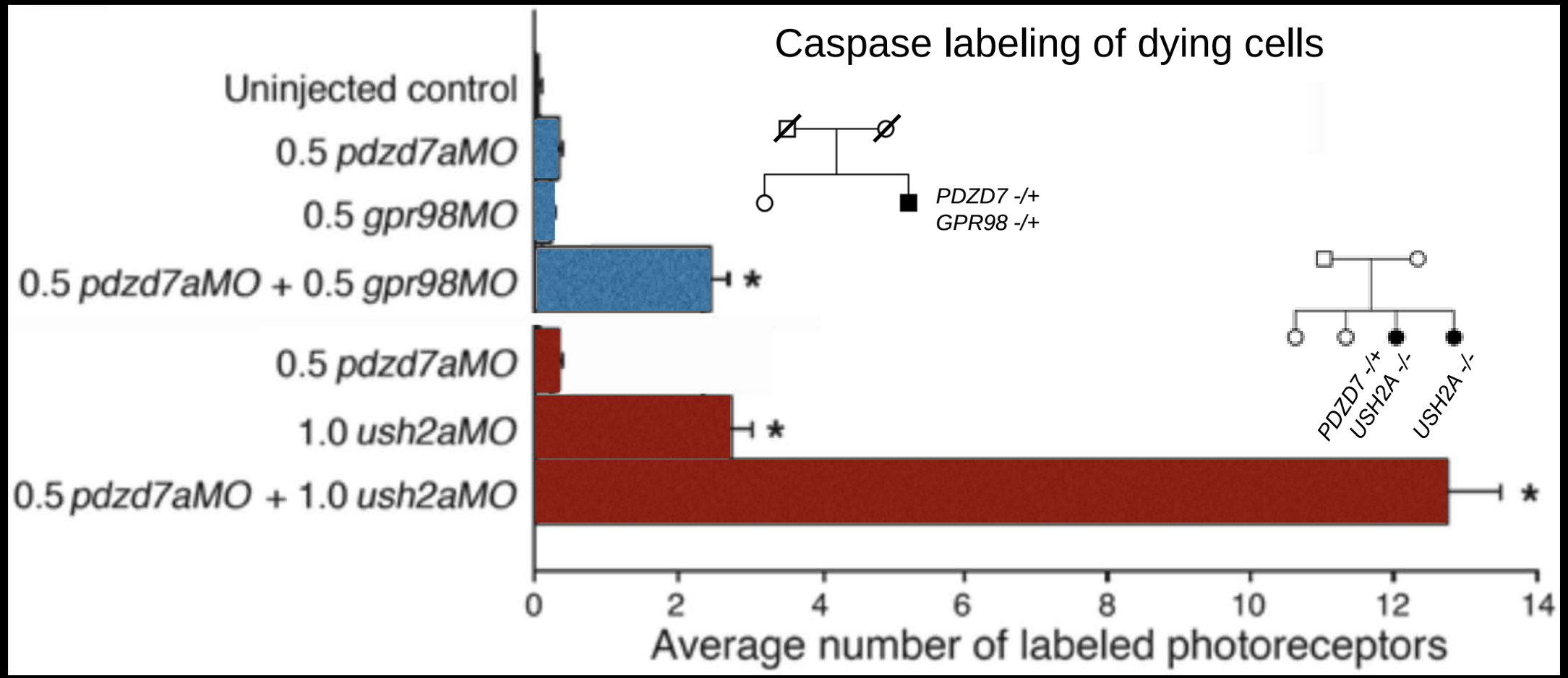
PDZD7 mutations are heterozygous in patients with known Usher gene mutations





(Hanno Bolz & Inga Ebermann)

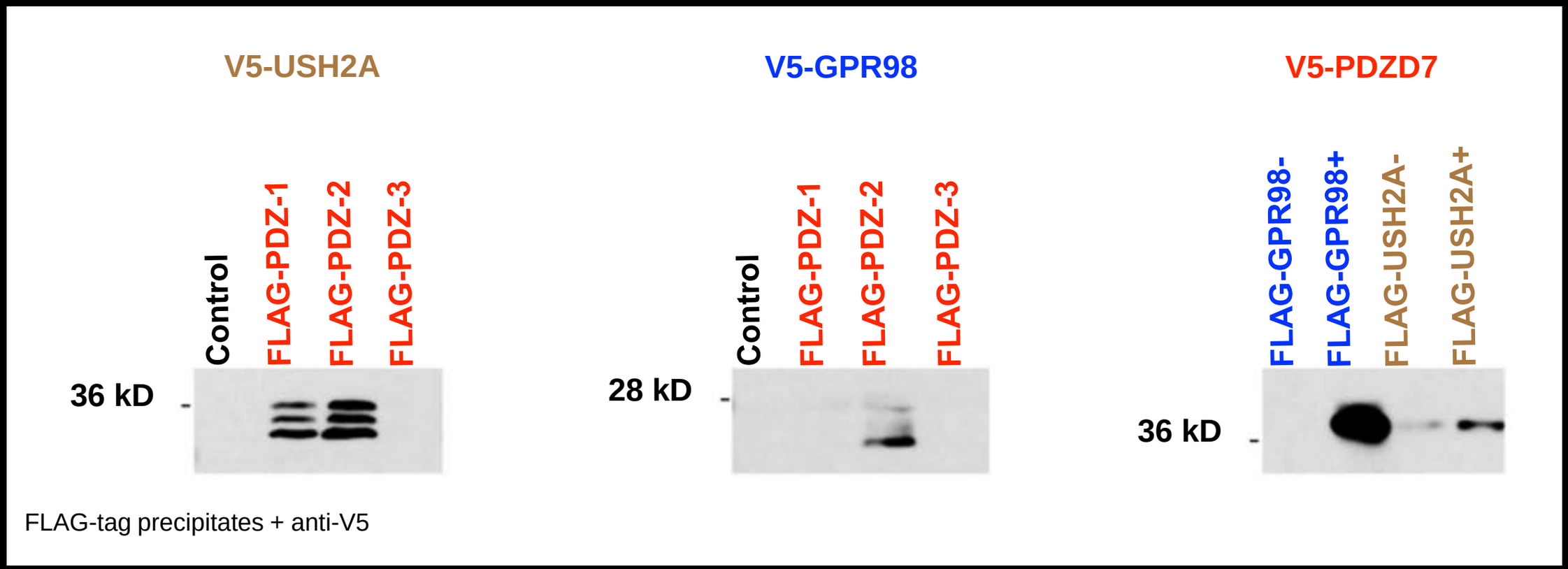
pdzd7a interacts with *ush2a* & *gpr98* in photoreceptor cell death



(Ebermann et al., 2010)

PDZD7 binds to USH2A & GRP98 proteins

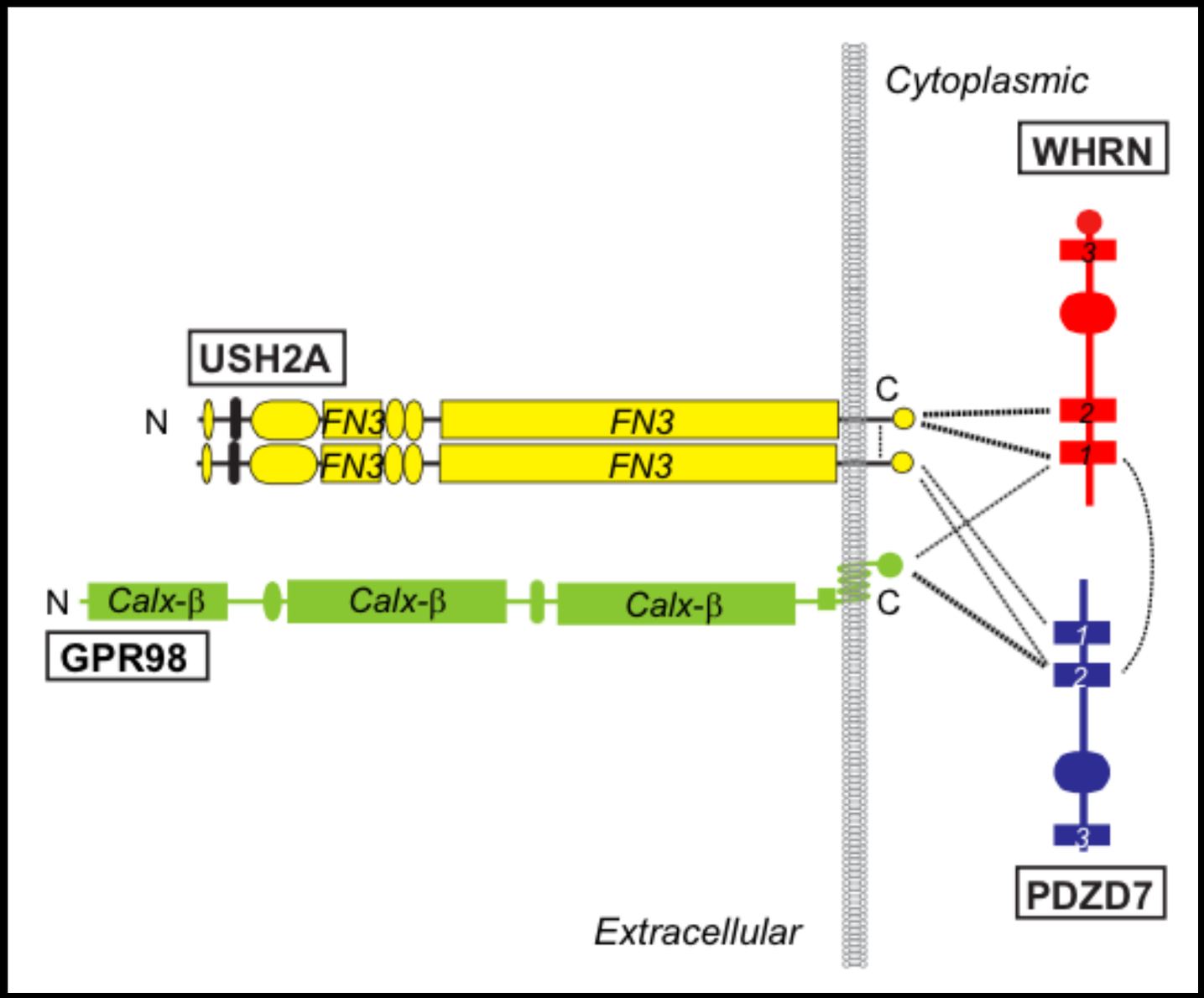
HEK293T cells



FLAG-tag precipitates + anti-V5

(Hanno Bolz & Inga Ebermann)

PDZD7 forms a quaternary complex of USH 2 proteins



(Chen et al., 2014)

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PDZD7 causes disease

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PDZD7 causes disease

Where are the missing homozygous and compound heterozygous patients?

- embryonic lethal? (model organism data suggest not)
- patient pool too small?
  - limited access to patient data?
  - lack of communication (or sharing) among clinicians?

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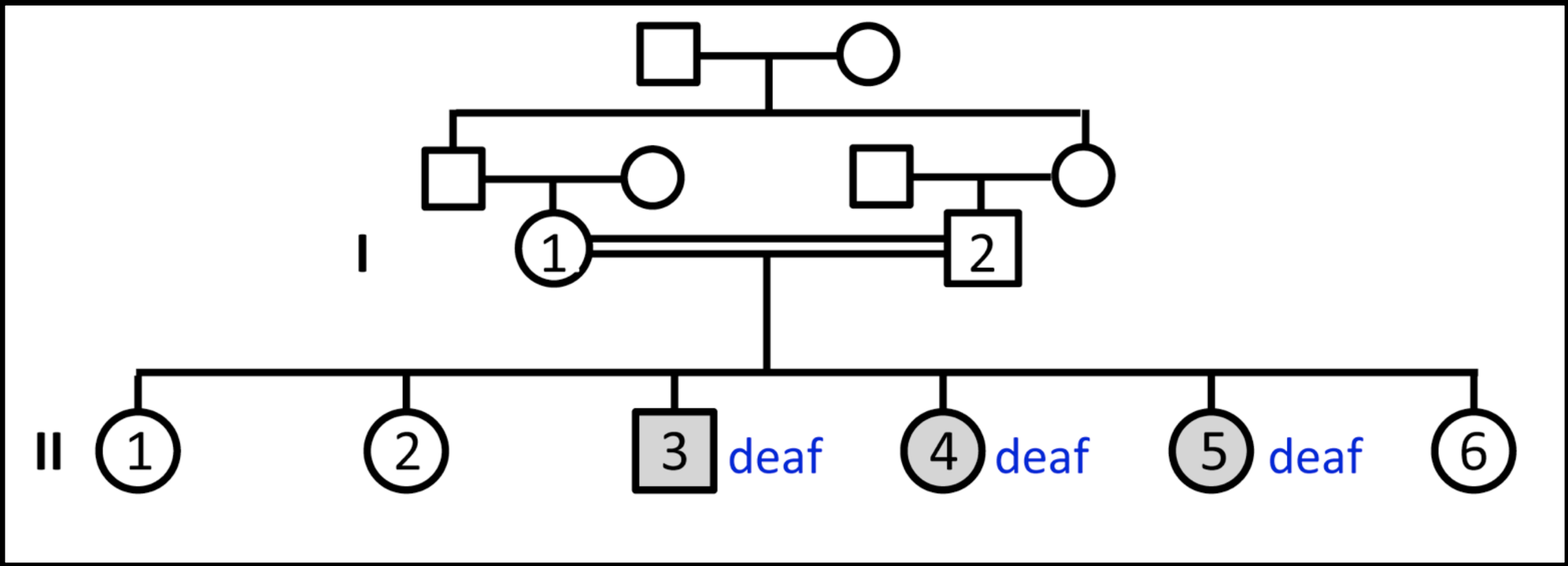
Mind the gaps

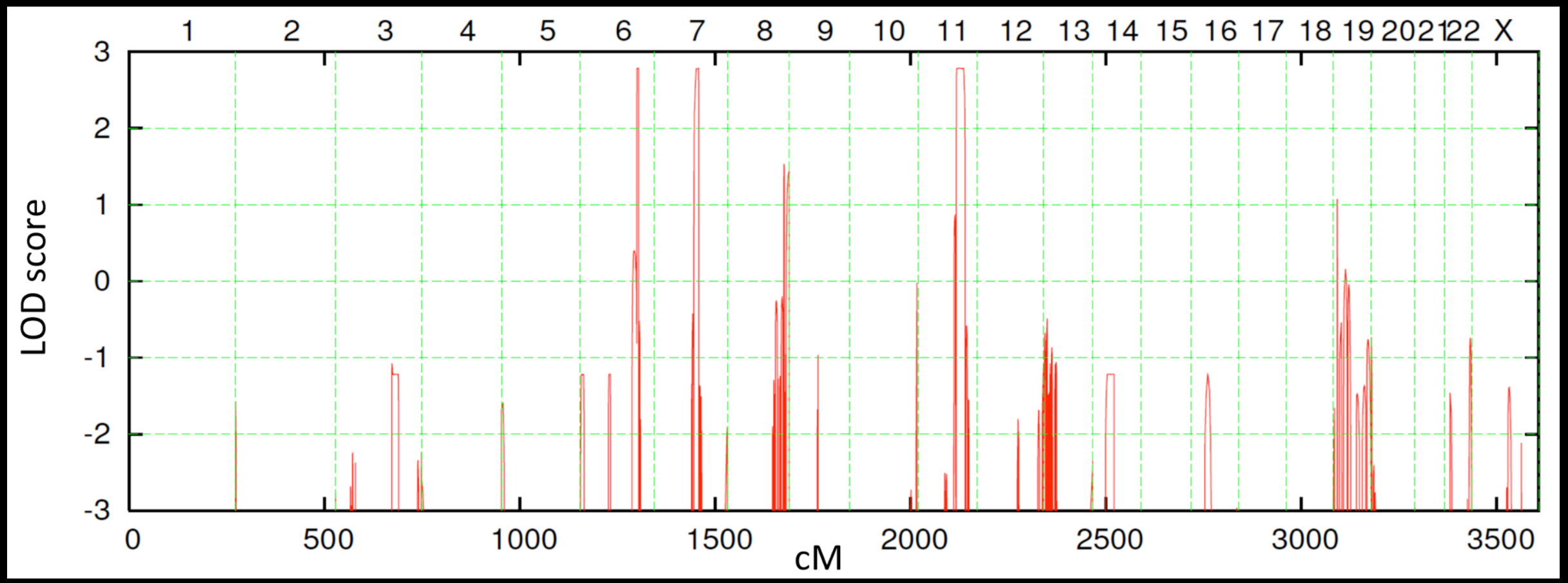
Undiagnosed Diseases Network

Consanguineous family with deafness



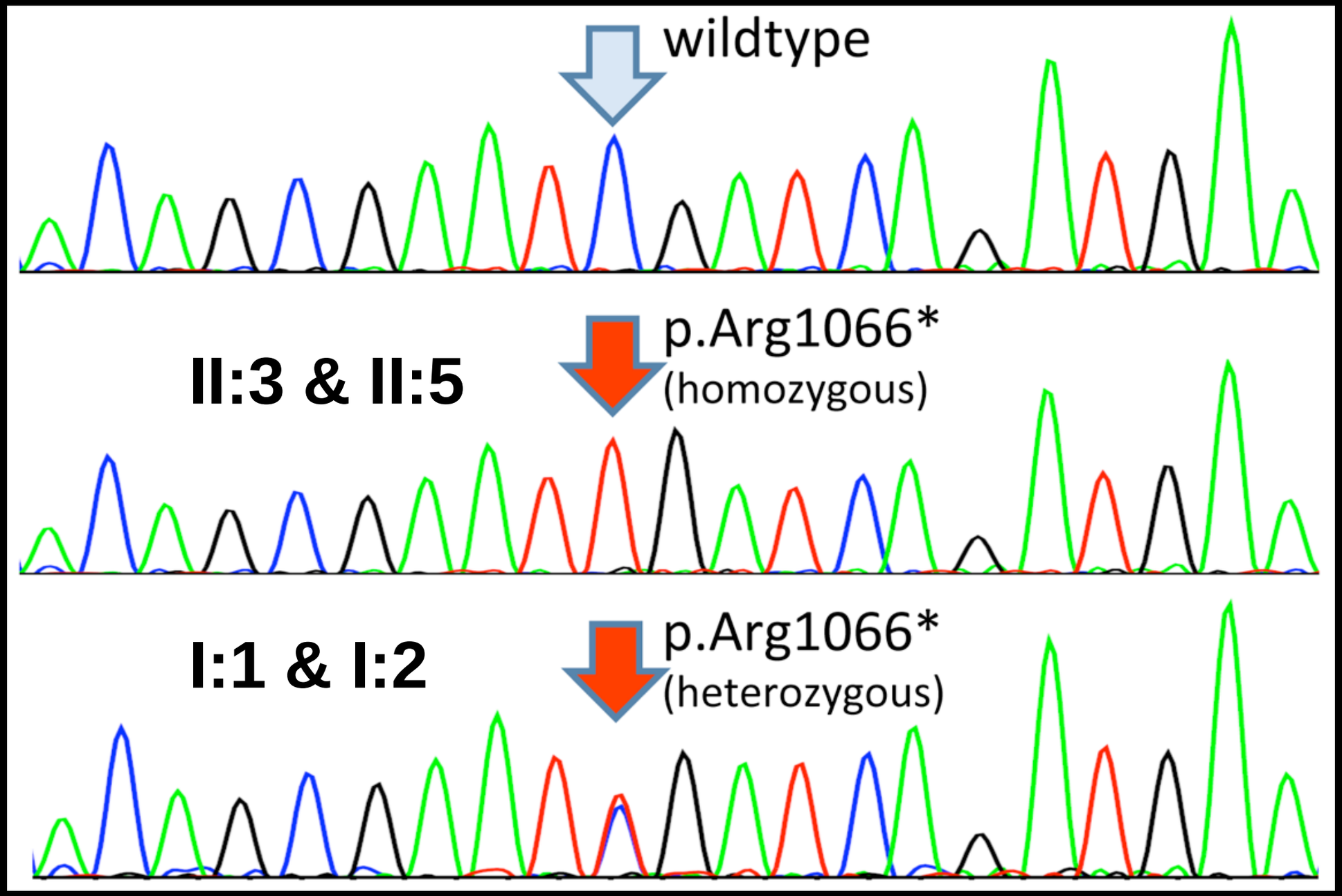
(Solaf Elsayed & Hanno Bolz)

Mapping homozygosity by descent identifies no good candidates



(Solaf Elsayed, Raoul Heller & Hanno Bolz)

Whole exome sequencing for homozygous SNPs identifies mutation in *AHI1*, *a* gene responsible for Joubert syndrome

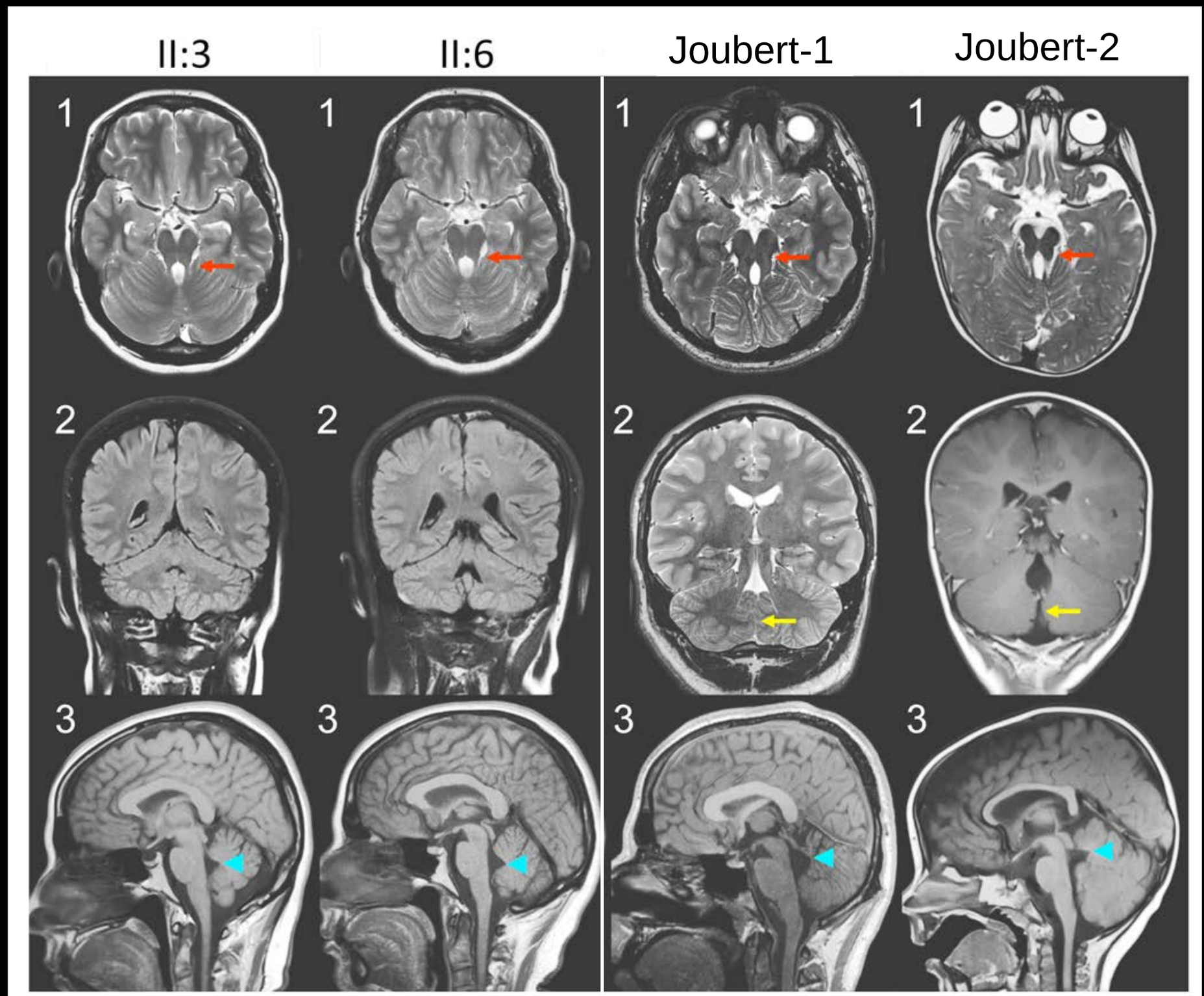


(Solaf Elsayed, Raoul Heller & Hanno Bolz)

Joubert syndrome - a severe ciliopathy disease

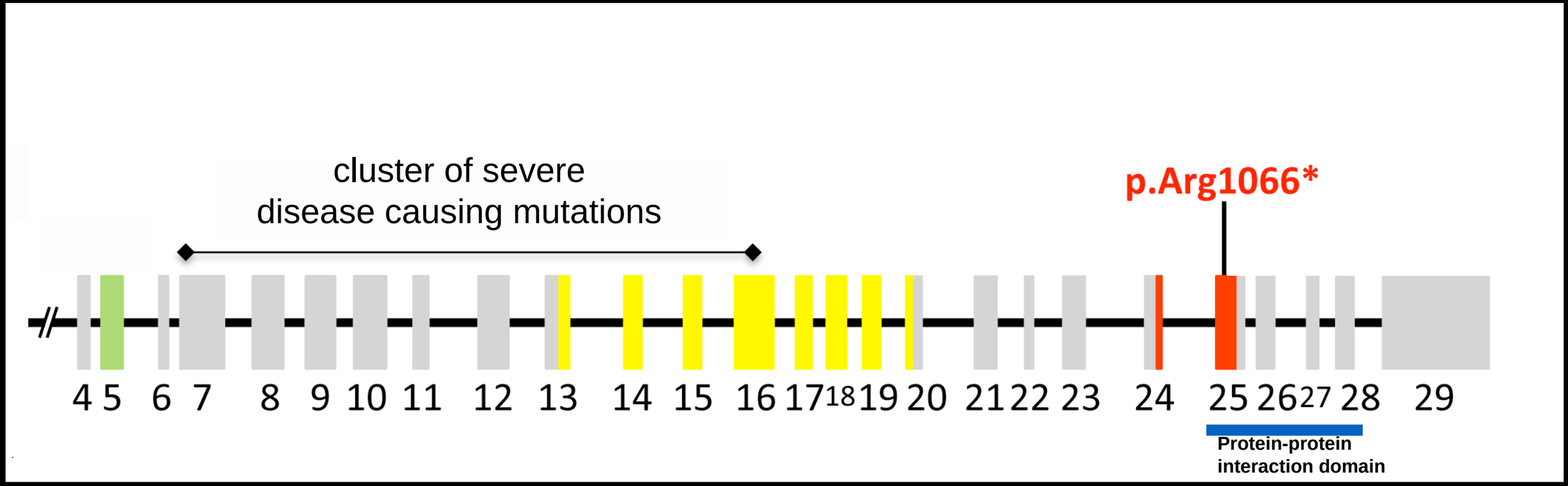
- Underdevelopment of the cerebellum and brainstem*
- Impaired intellectual development, seizures
- Retinitis pigmentosa
- Developmental abnormalities
- Kidney and liver abnormalities

Homozygous patients have normal CNS MRIs



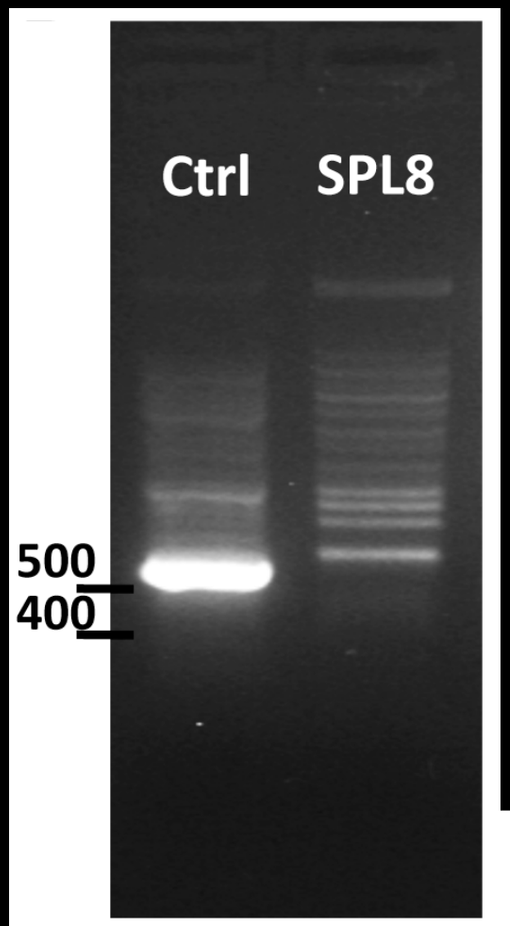
(Raoul Heller & Hanno Bolz)

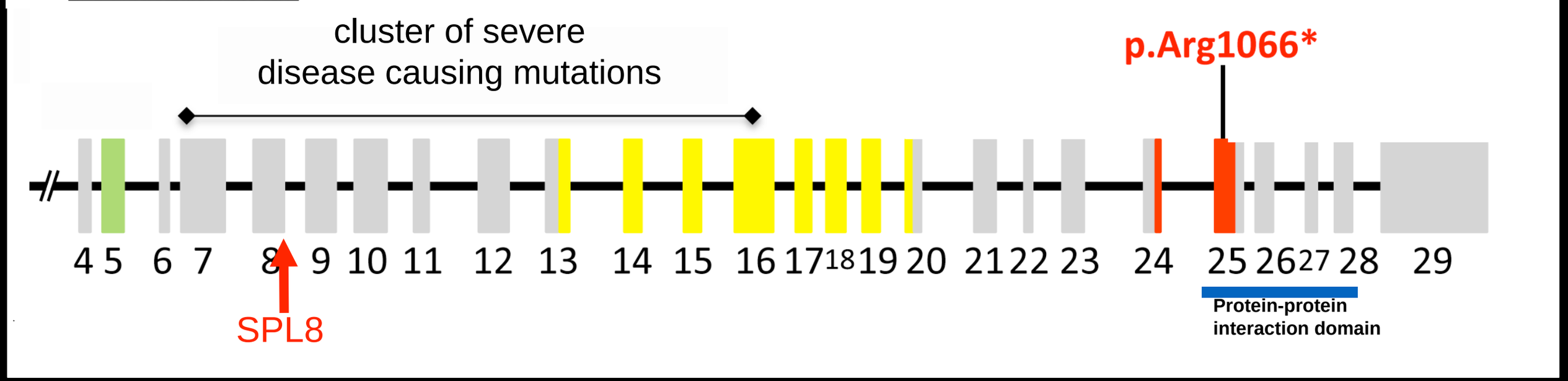
Nonsense mutation truncates the protein-protein interaction domain of *AHI1*



(Solaf Elsayed, Raoul Heller & Hanno Bolz)

Targeting upstream in zebrafish gene blocks expression



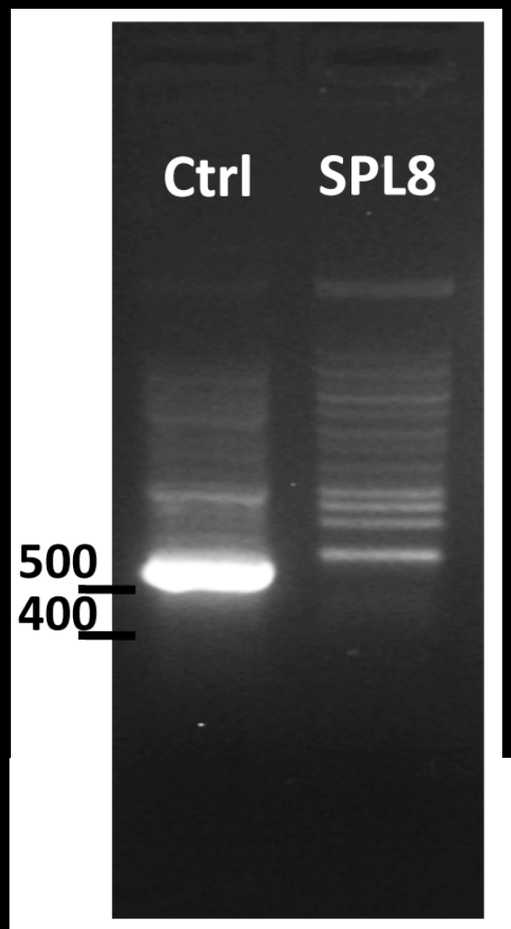


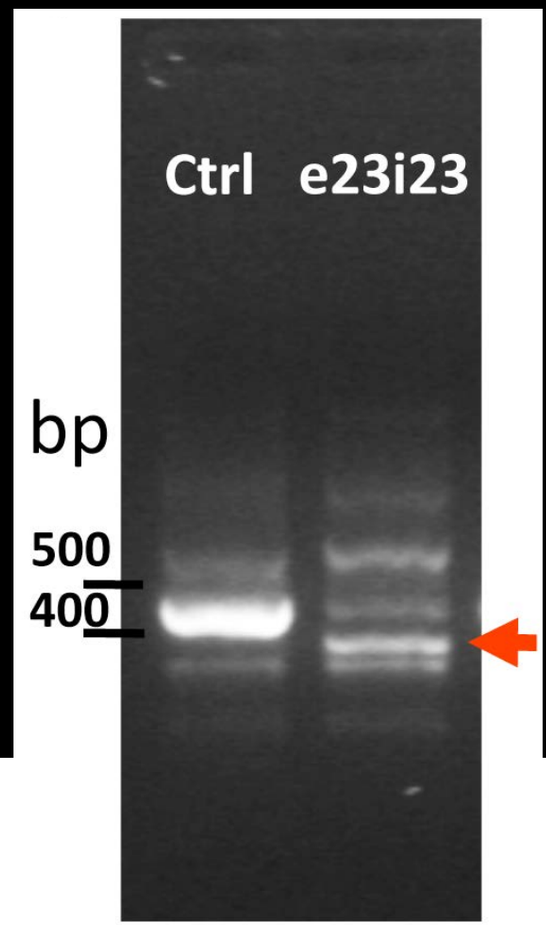
(Jennifer Phillips)

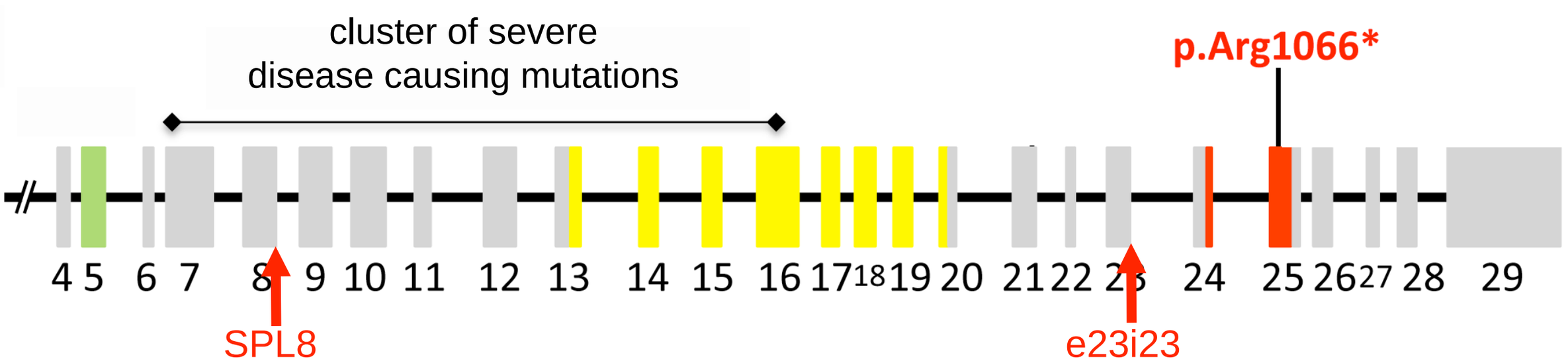
Upstream targeting produces strong ciliopathy phenotype

(Jennifer Phillips)

3’ targeting truncates the protein

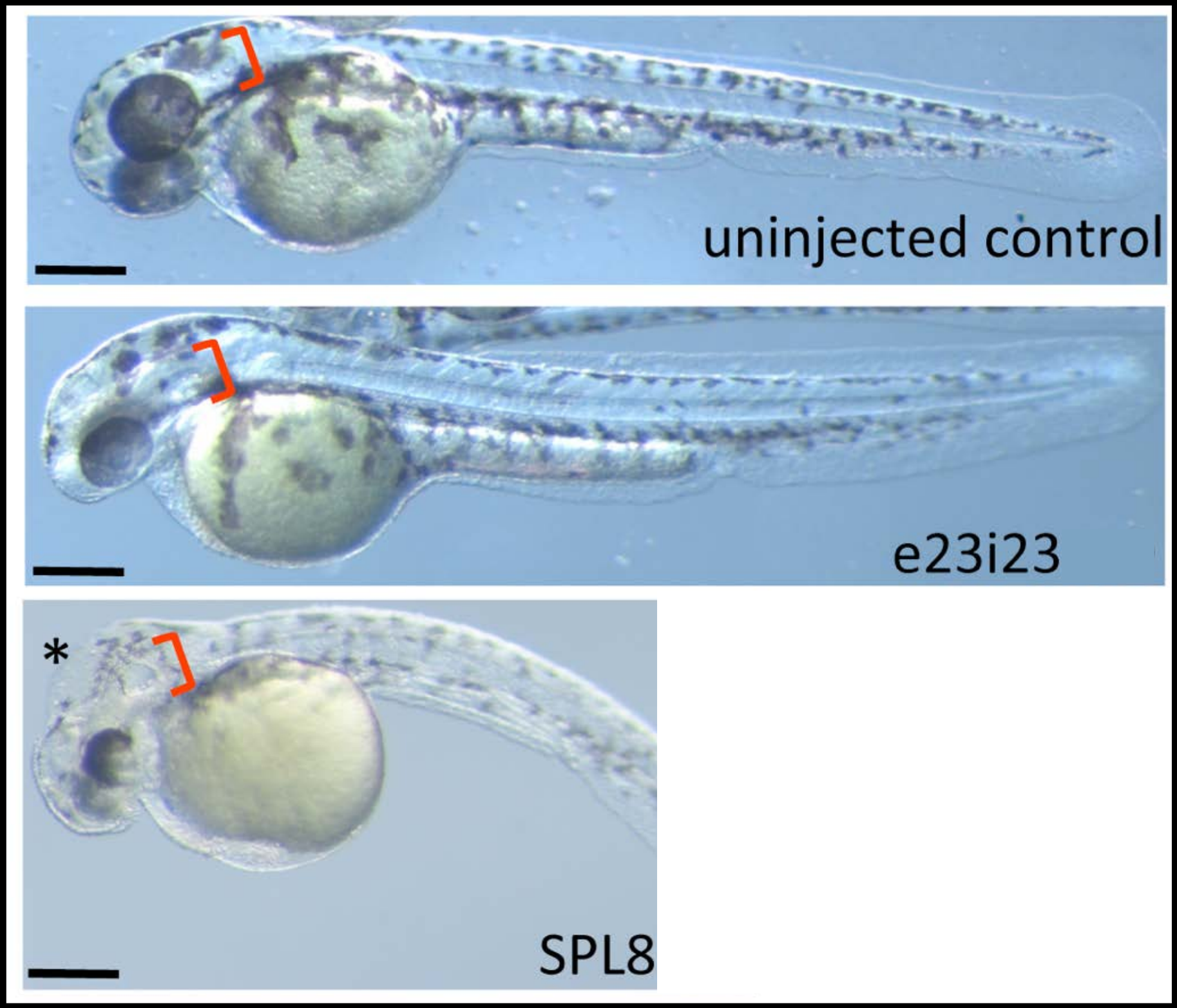






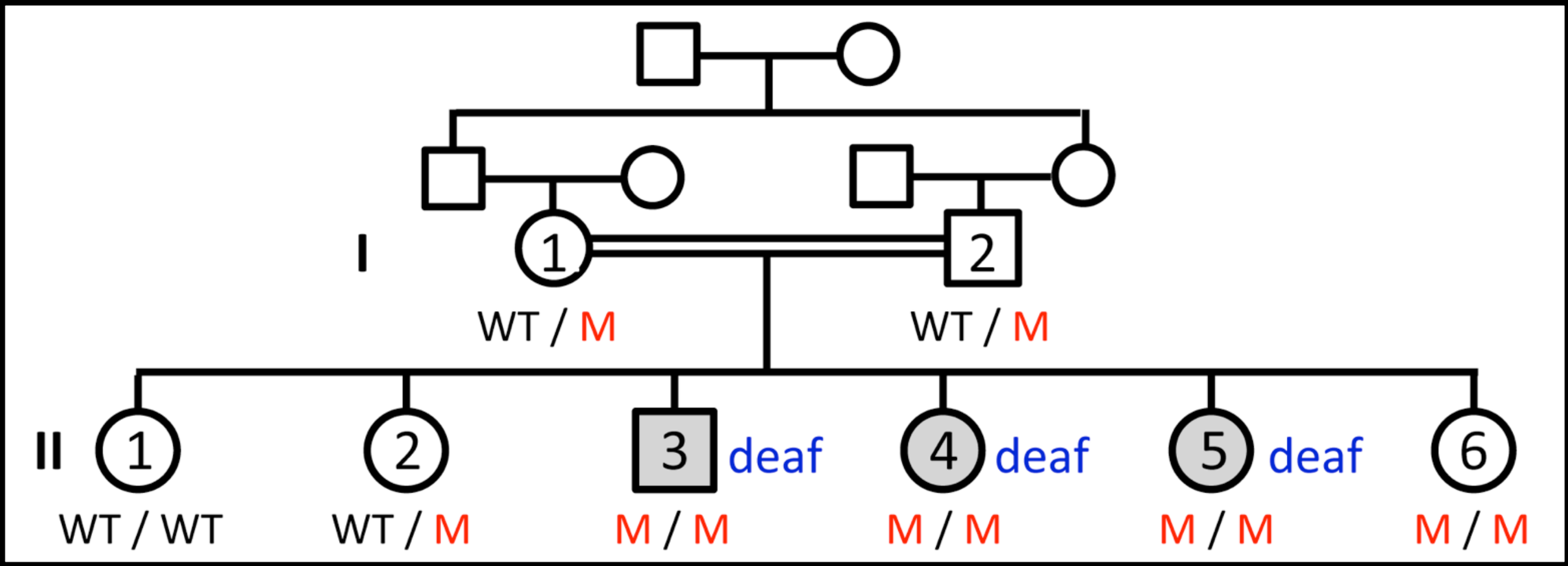
(Jennifer Phillips)

Truncated protein has no apparent phenotype



(Jennifer Phillips)

Nonsense *AHI1* mutation is not linked to deafness



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- Barriers to accessing patient data
  - Sociological: clinical vs basic research attitudes
  - Limited access to clinical records: de-identified vs IRB
- Limited patient data: horde vs share variant & phenotypic data

Undiagnosed Diseases Network

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Seven clinical sites, a coordinating center, two DNA sequencing cores, a metabolomics core, a model organisms screening center, and a central biorepository

University of Oregon

Battelle Pacific Northwest Laboratories
w/ Oregon Health & Science University

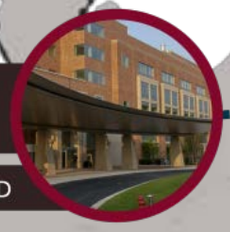
Richland, WA

Stanford Medicine

Stanford, CA

UCLA

Los Angeles, CA



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Boston Children's Hospital, Brigham and Women's Hospital, Massachusetts General Hospital

Boston, MA

Harvard Medical School

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Duke University
w/ Columbia University

Durham, NC

Vanderbilt University Medical Center

Nashville, TN

HudsonAlpha Institute for Biotechnology
w/ Illumina

Huntsville, AL

Baylor College of Medicine
w/ Univesity of Oregon

Houston, TX

- **Clinical site**
- **Coordinating center**
- **DNA sequencing core**
- **Central Biorepository**
- **Model Organisms Screening Center**
- **Metabolomics Core**

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