Data double take:
Three examples of atypical alterations detected in exome sequencing data

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Disclosures

I am a full-time paid employee of Ambry Genetics

Outline

Brief overview of exome sequencing at Ambry Genetics

Case 1 – 17y old male with pigmentary maculopathy

CRB1 – compound heterozygous microduplication and nonsense

Case 2 – 14y old male with high IgE and immunodeficiency

DOCK8 – compound heterozygous microtranslocation and small insertion

Case 3 – 5y old female with a neuromuscular disorder with reduced calpain3 staining

CAPN3 – Homozygous microdeletion

Overview of clinical exome sequencing at Ambry Genetics

Sample

Library Prep
Kapa
Biosystems

Exome
Capture
IDT

Alignment
NovoAlign

Haplotype
caller

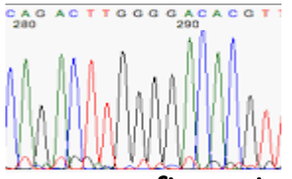
In-house
annotation

Filtering

Inheritance
models

Ambry Variant
Analysis
Software

Results
and gene
review



Sanger confirmation

Clinical
Report

Case -1 Overview

Patient Phenotype

- 17 year old Caucasian male
- Poor central, peripheral, and night vision
- Complete color blindness
- Exotropia, hyperopia

Retinal exam

- Posterior vitreous detachment
- Pigmentary maculopathy

Fundus autofluorescence

- Large nummular areas of hypofluorescence

Electroretinogram

- severely abnormal

Optical Coherence Tomography:

- Loss of normal retinal architecture
- Thin maculae with abnormal foveal contour
- Surface gliosis

Family History

- 2 distant maternal cousins blind in 20's
- Paternal and maternal families from a small greek island.

Exome sequencing

- Proband, unaffected mother, unaffected full brother

Case -1 *CRB1* nonsense alteration

Paternally inherited[#] nonsense alteration

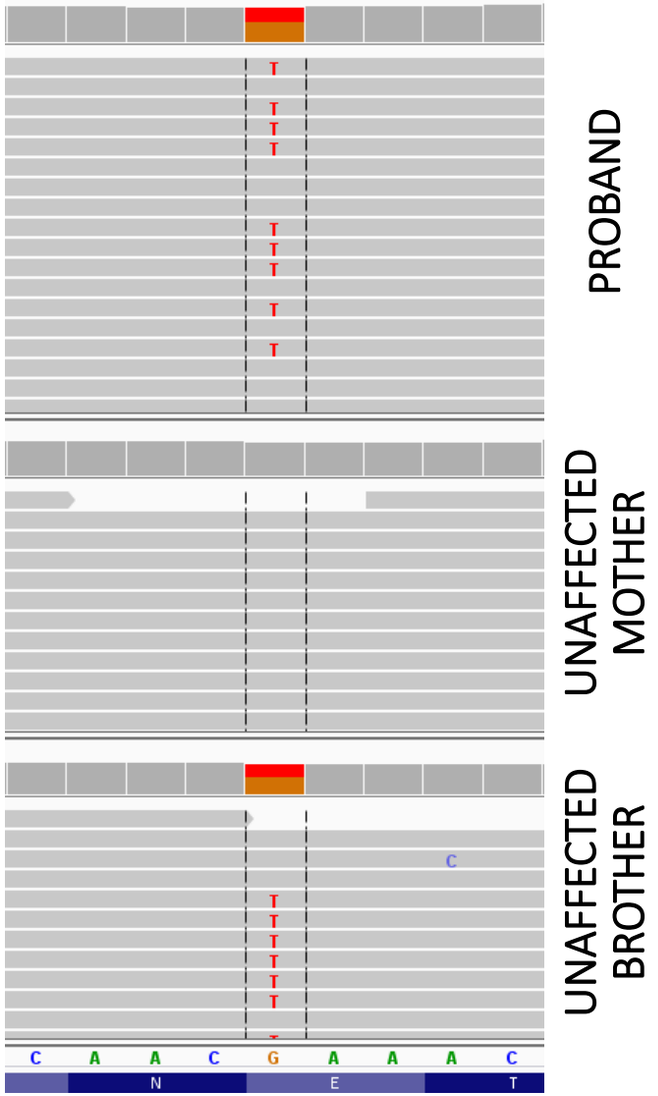
CRB1

g.1:197404165

c.3172G>T (NM_201253)

p.E1058*

Father not available for sequencing but unaffected brother carries this alteration.



CRB1 exon 9

Variant classification = PATHOGENIC

- Nonsense
- Rare (Not in 1Kg, ESP, ExAC, dbSNP, HGMD).
- In trans with another mutation

CRB1

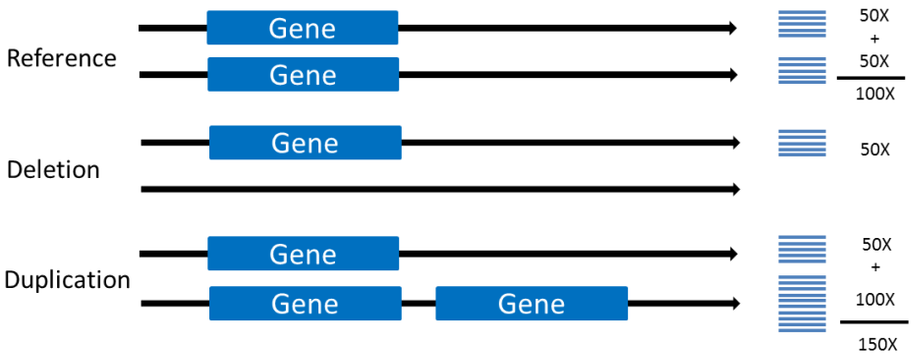
- autosomal recessive retinitis pigmentosa
- No second alteration identified by standard analysis

Ambry's bioinformatics team ran a fusion detection algorithm.

Bioinformatics pipeline for structural variation detection

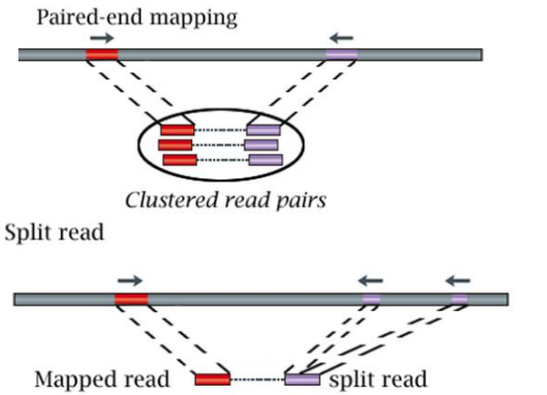
Analysis-ready BAM files

Read-depth component



Fromer, M. et al. (2012) Discovery and statistical genotyping of copy-number variation from whole-exome sequencing depth. American JourNot availablel of Human Genetics, 91:597-607

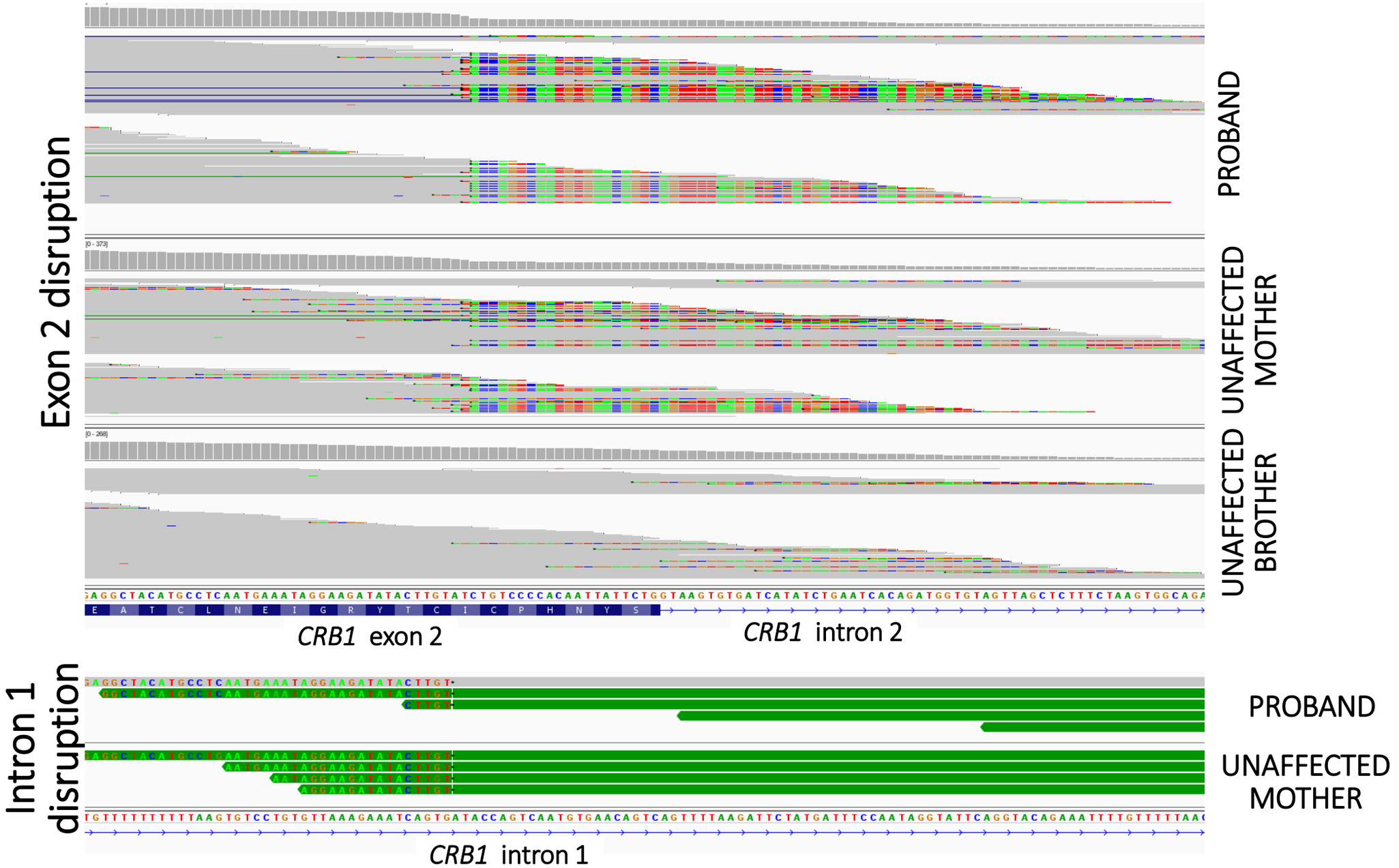
Split-read component



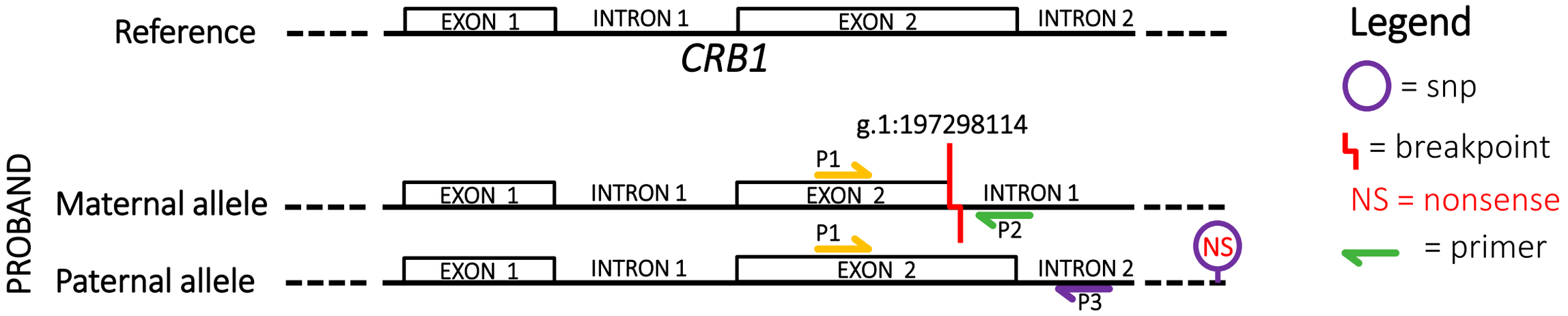
Zhao M. et al. (2013) Computational tools for copy number variation (CNV) detection using next-generation sequencing data: features and perspectives. Bioinformatics, 14.

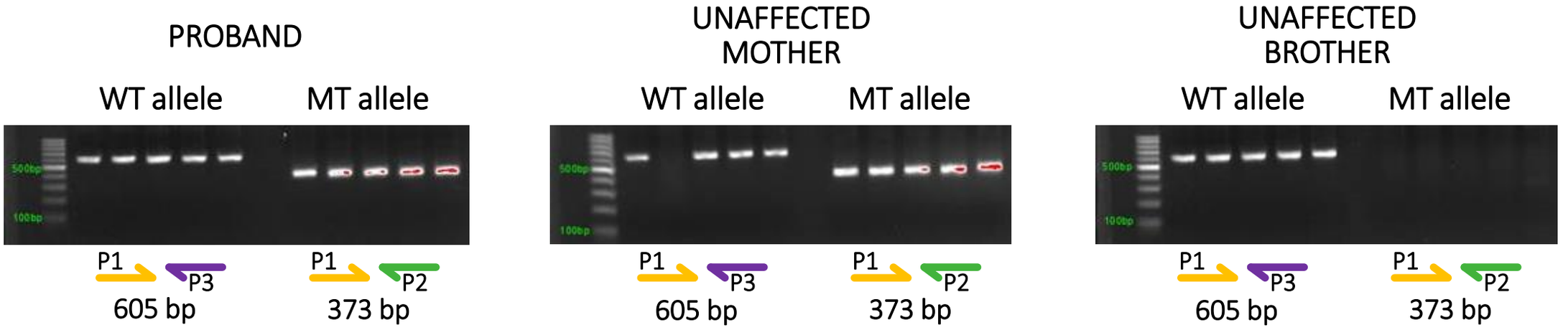
Confirm SVs by aCGH/MLPA/PCR/Gel

Case -1 Maternally inherited *CRB1* microduplication



Case -1 *CRB1* microduplication validation





PCR products were Sanger sequenced and confirmed the anticipated sequences

Case -1 Report

Results Summary

Gene Symbol	Gene Inheritance	Characterized/Novel Gene*	Protein Change	Nucleotide Change	Genotype	Alteration Type	Alteration Classification	Gene Overlap
CRB1	Autosomal recessive	Characterized	p.E1058*	c.3172G>T	Heterozygous	Nonsense	Pathogenic	Positive, Partial
			N/A	Exon 2 Rearrangement	Heterozygous	Complex	Uncertain	

Case -2 Overview

Patient Phenotype

- 14 year old Hispanic boy
- Primary immunodeficiency
- Frequent and recurrent infections
- progressive bronchiectasis
- Chronic eczema

Lab findings

- Longstanding isolated CD4 T-cell lymphopenia
- T-cell proliferation defect
- High IgE, Low IgM
- Normal CD8 and NK cell counts

Exome sequencing

- Proband, unaffected mother, unaffected father

Case -2 *DOCK8* small deletion

Paternally inherited 7bp frameshift insertion

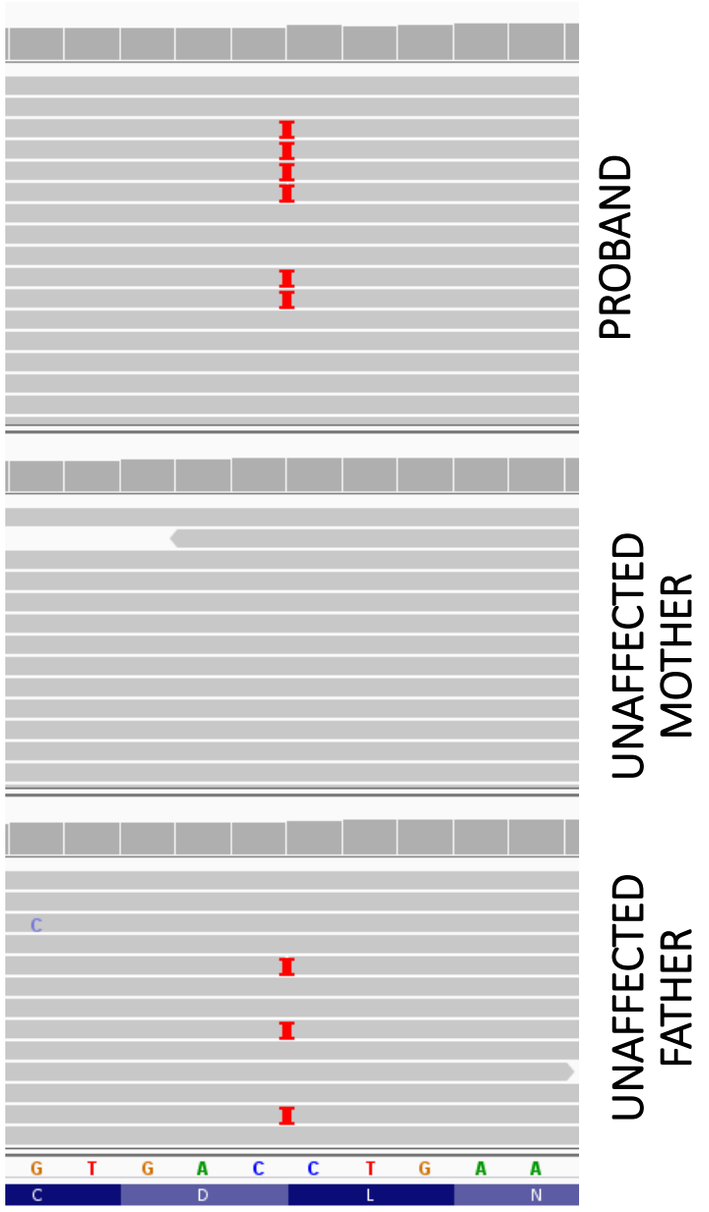
DOCK8

g.9:328045

c.918_919INSTTGAACT

NM_203447

Exon 9



DOCK8 exon 9

Variant classification = PATHOGENIC

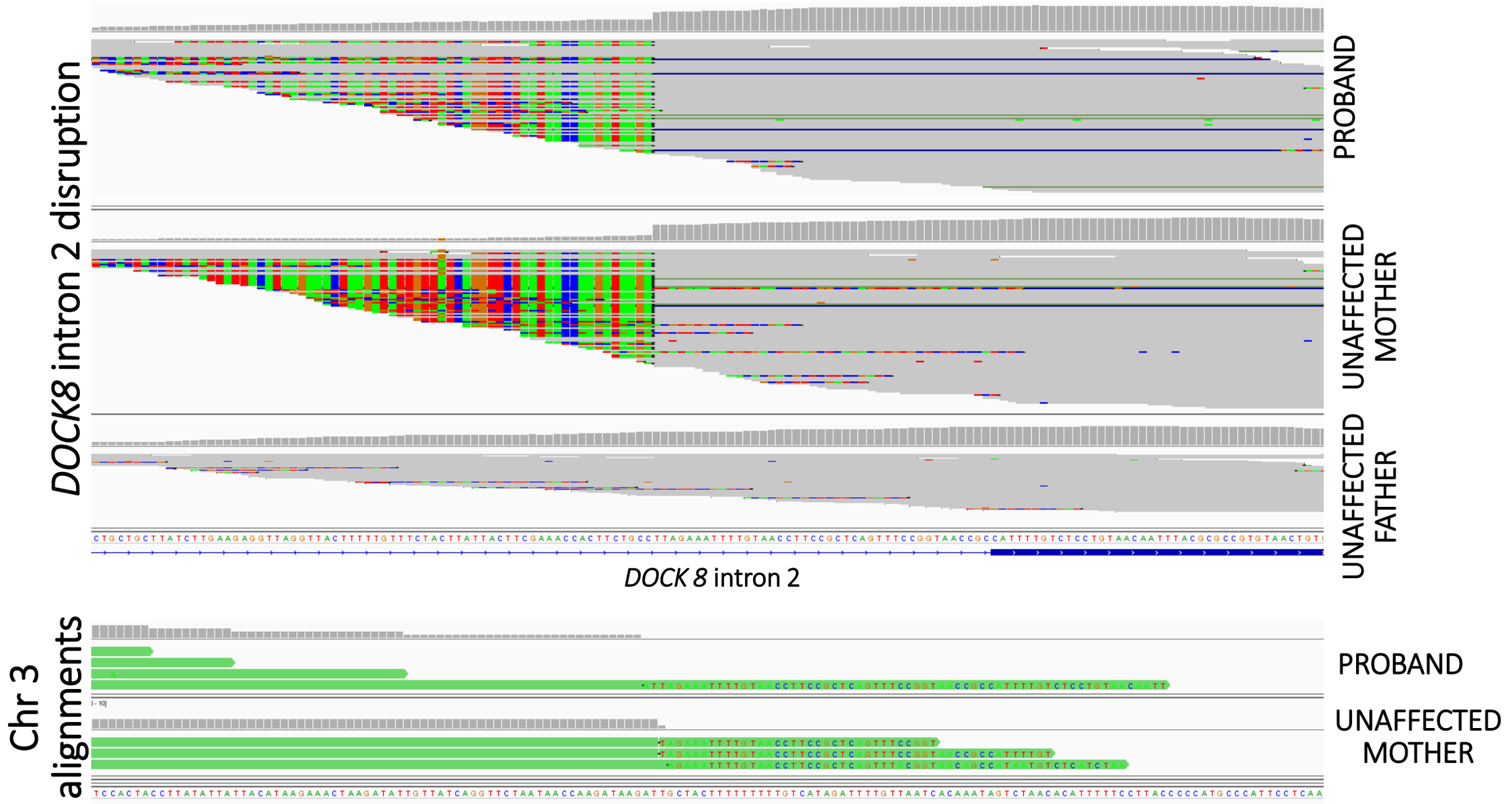
- FRAMESHIFT -The majority of variants listed in HGMD are loss of function alterations
- Rare – not present in (ExAC, ESP, 1KG, dbSNP, HGMD).

DOCK8

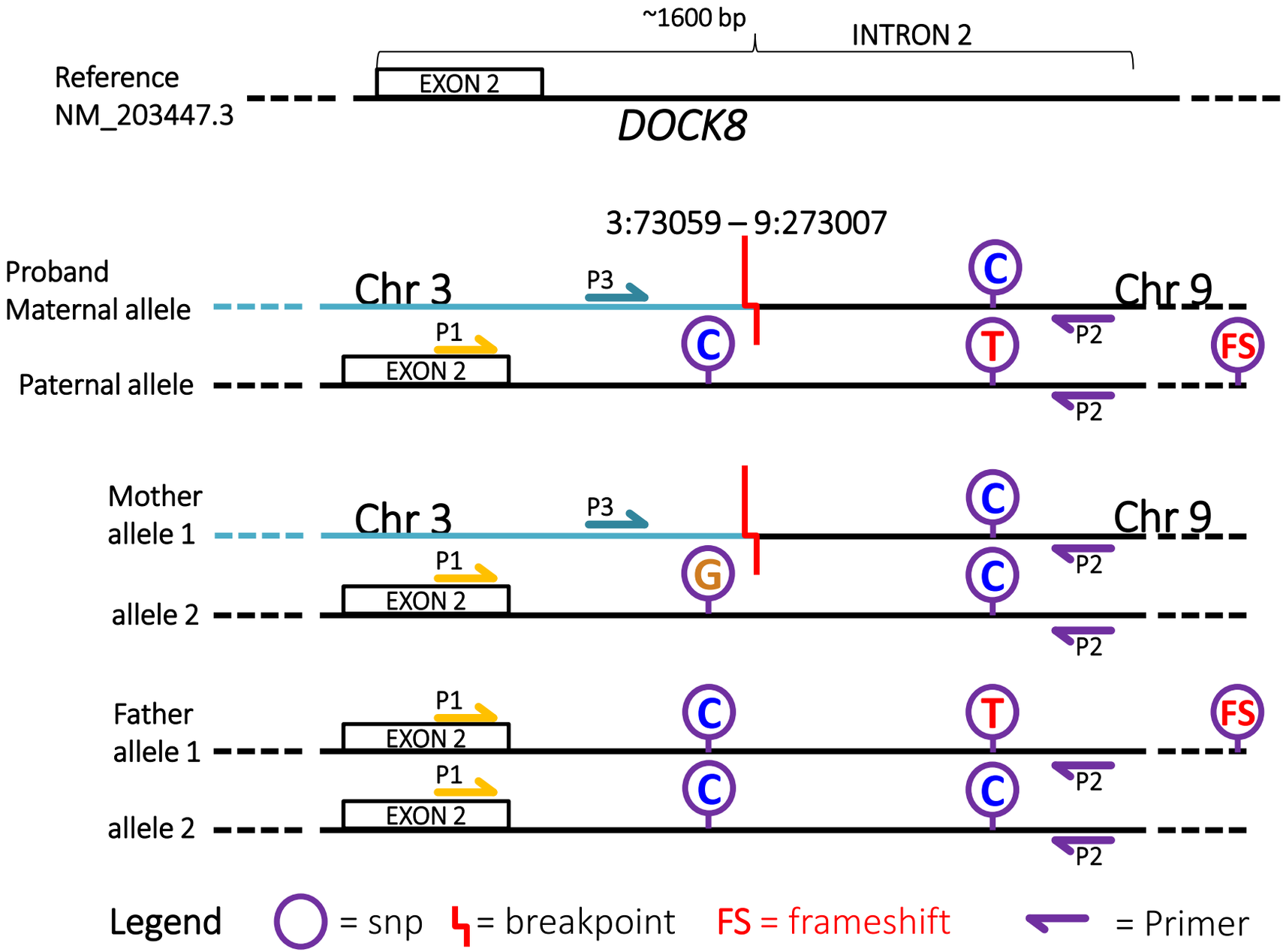
- autosomal recessive Hyper-IgE recurrent infection syndrome
- No second alteration identified by standard analysis

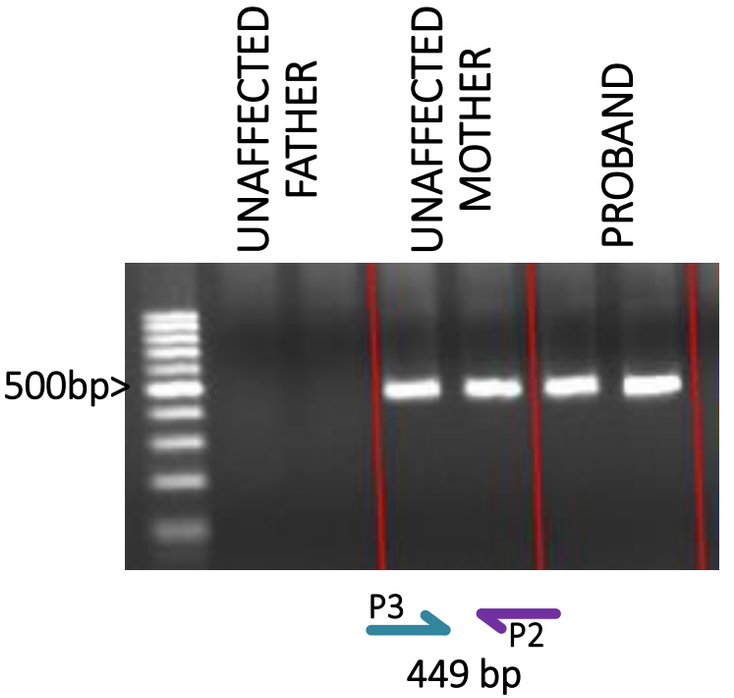
Ambry's bioinformatics team ran a fusion detection pipeline.

Case -2 *DOCK 8* Microtranslocation (3:73059-9:273007)(3p26.3;9p24.3)



Case -2 *DOCK8* translocation validation





All PCR products were Sanger sequenced. SNPs confirmed paternal and maternal alleles as well as the absence of exon2 in the translocation allele.

Case -2 *DOCK8* translocation validation: Proband Sanger traces

Case -2 Report

Results Summary

Gene Symbol	Gene Inheritance	Characterized/ Novel Gene*	Protein Change	Nucleotide Change	Genotype	Alteration Type	Alteration Classification	Gene Overlap
DOCK8	Autosomal recessive	Characterized	p.K307Lfs*3	c.918_919insTTGAACT	Heterozygous, paternal	Frameshift	Pathogenic	Positive
			N/A	Disruption of coding exons 1-2	Heterozygous, maternal	Translocation	Pathogenic	

Engelhardt et al. 2015 and Mizesko et al. 2013

- immunodeficiency cases with deletion of exon 1 -2of *DOCK8*

Case -3 Overview

Patient Phenotype

- 5 y. old girl – Hispanic / Honduran descent
- History of muscle fatigue and pain
- Slightly hypertrophy of calves
- Negative Gower's response
- Patient can run, jump, climb stairs
- Endurance and motor skills have remained stable

Muscle biopsy

- necrotizing myopathy
- decreased staining for calpain 3

EMG – suggested myopathic changes

Labs

- Highly elevated CK levels
- Elevated liver enzymes

Research western blot

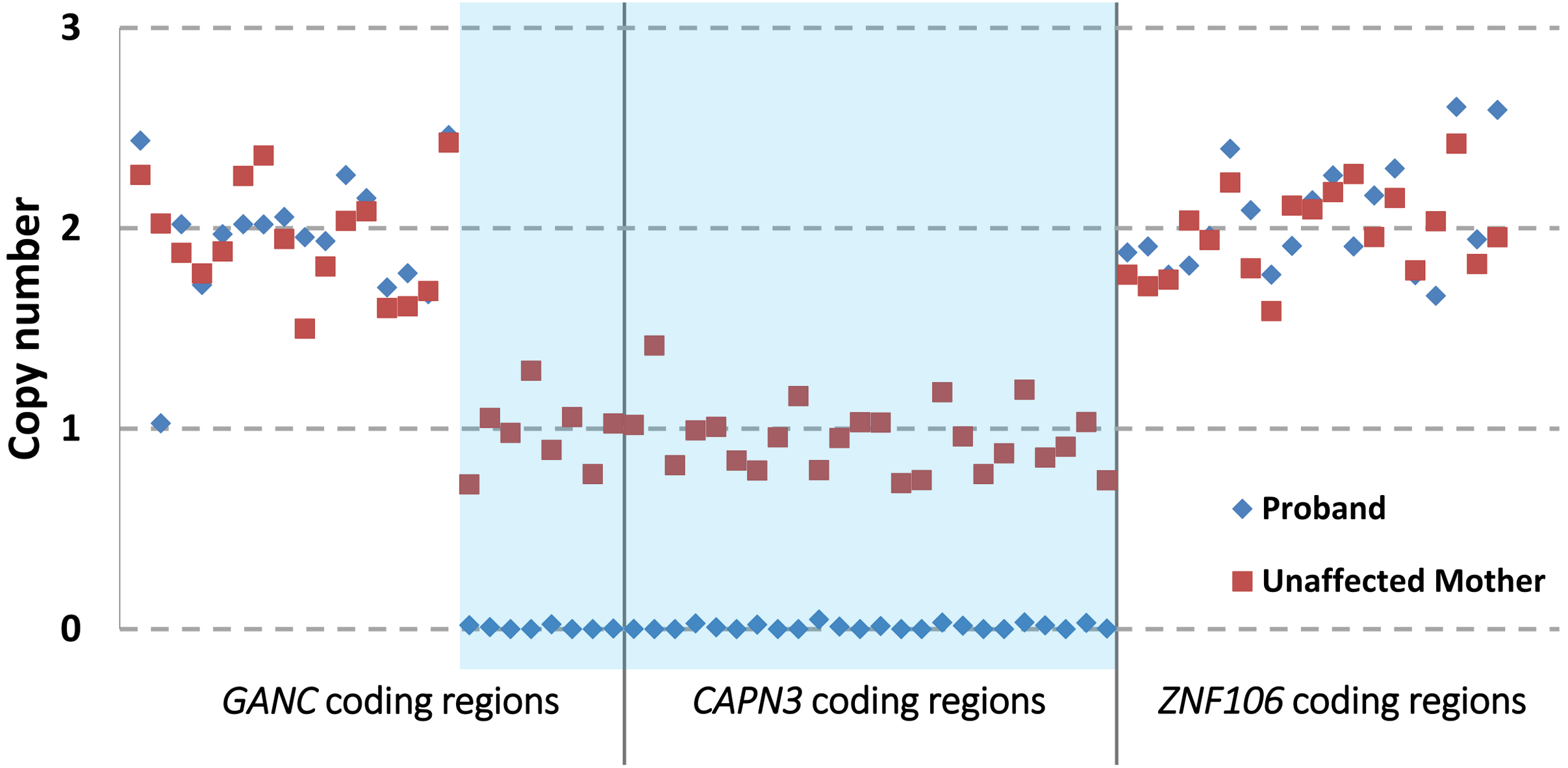
- Calpain 3 completely absent

Exome sequencing

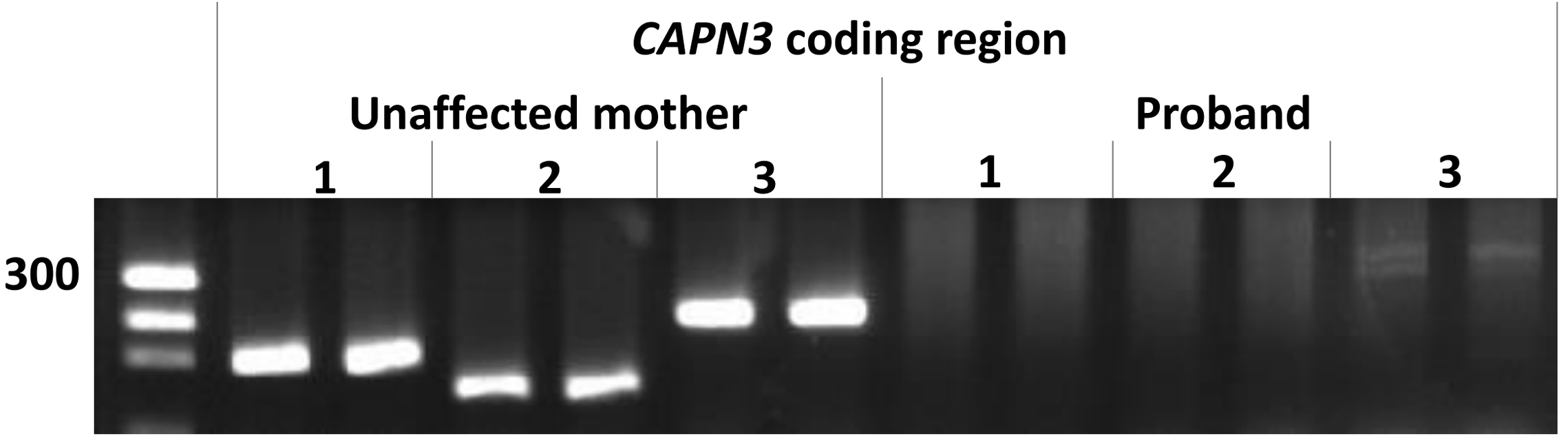
- Proband, unaffected mother

- No significant alterations identified with our standard pipeline.

Case -3 *CAPN3* deletion; exome coverage



Case -3 *CAPN3* deletion validation



All *CAPN3* assays failed in the proband
A control gene assay was normal

Case -3 Report

Results Summary

Gene Symbol	Gene Inheritance	Characterized/Novel Gene*	Nucleotide Change	Genotype	Alteration Type	Alteration Classification	Gene Overlap
CAPN3	Autosomal recessive	Characterized	Deletion of entire gene & part of *GANC*	Homozygous	Gross deletion	Deleterious	Positive

Conclusions

- We identified atypical pathogenic alterations in 3 different genes and unrelated disorders from exome sequencing data

- Exome data can sometimes contain sufficient information to identify alterations beyond the typical SNVs and small indels.

- Deeper analysis of exome data can improve clinical diagnositic yield and makes a difference for patients.

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Let’s find the answer.