Building the skull – normal and abnormal development

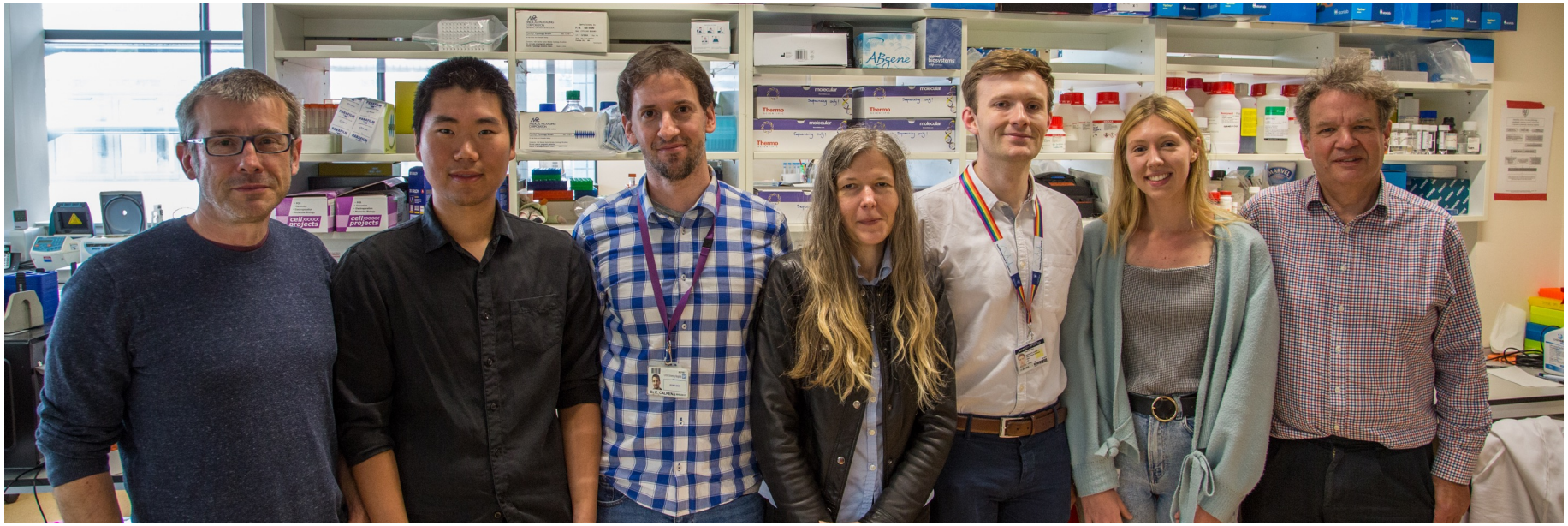
Steve Twigg, Becky Tooze, and Yang Pei

Clinical Genetics Group

Talk outline

- Craniosynostosis
- Becky – SNPs
- Yang - SVs

Becky Tooze

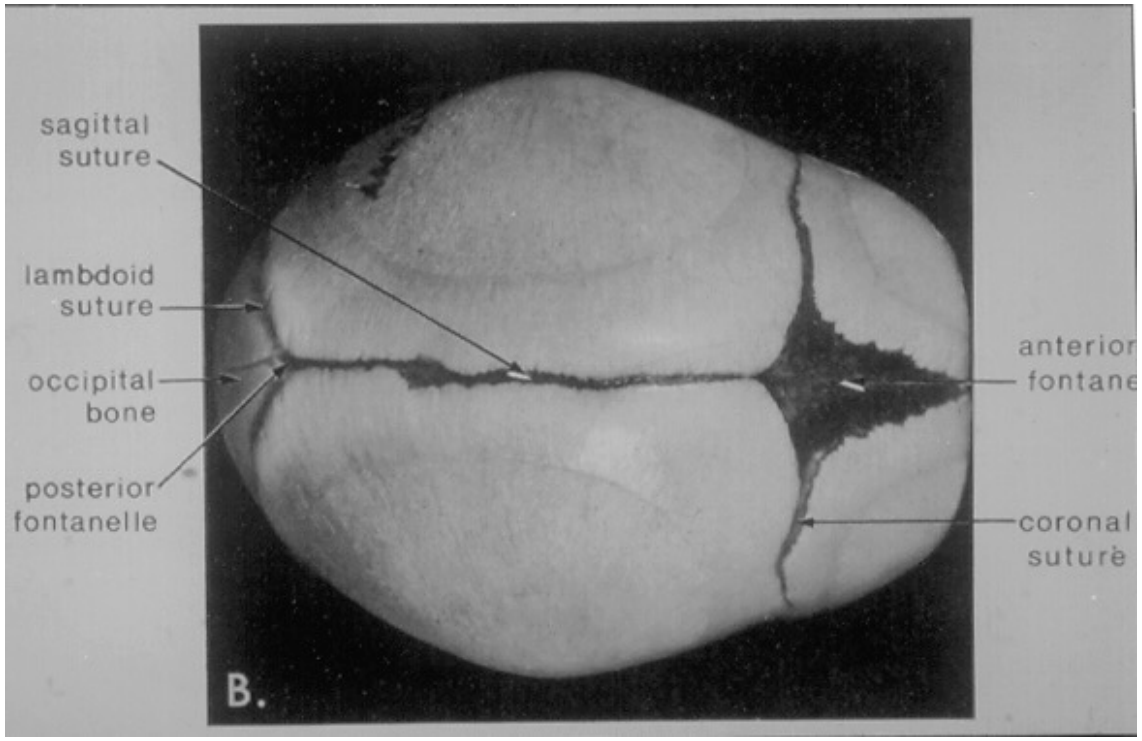


Yang Pei

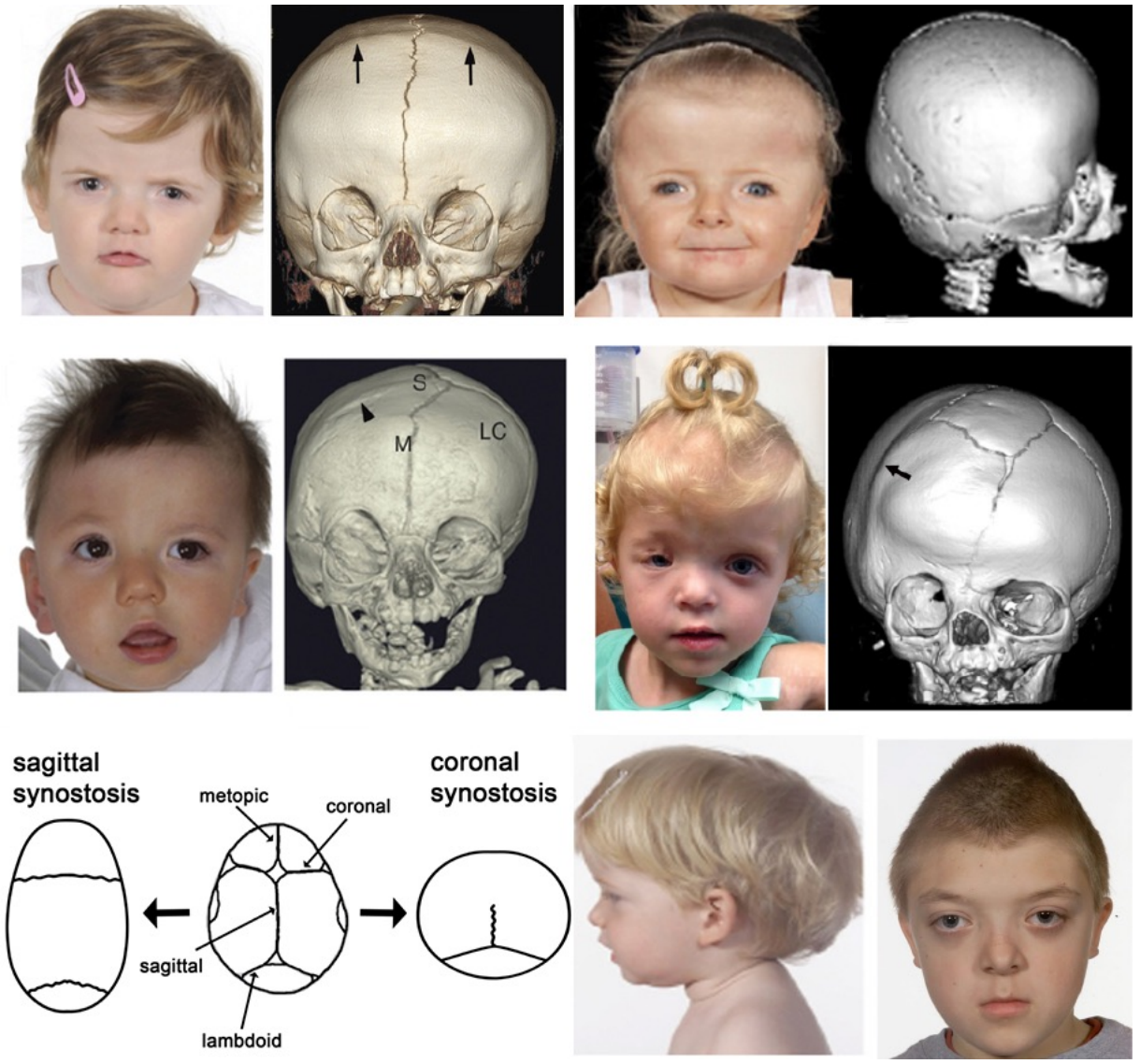
Andrew Wilkie

Craniosynostosis

Prevalence ~1 in 2,000

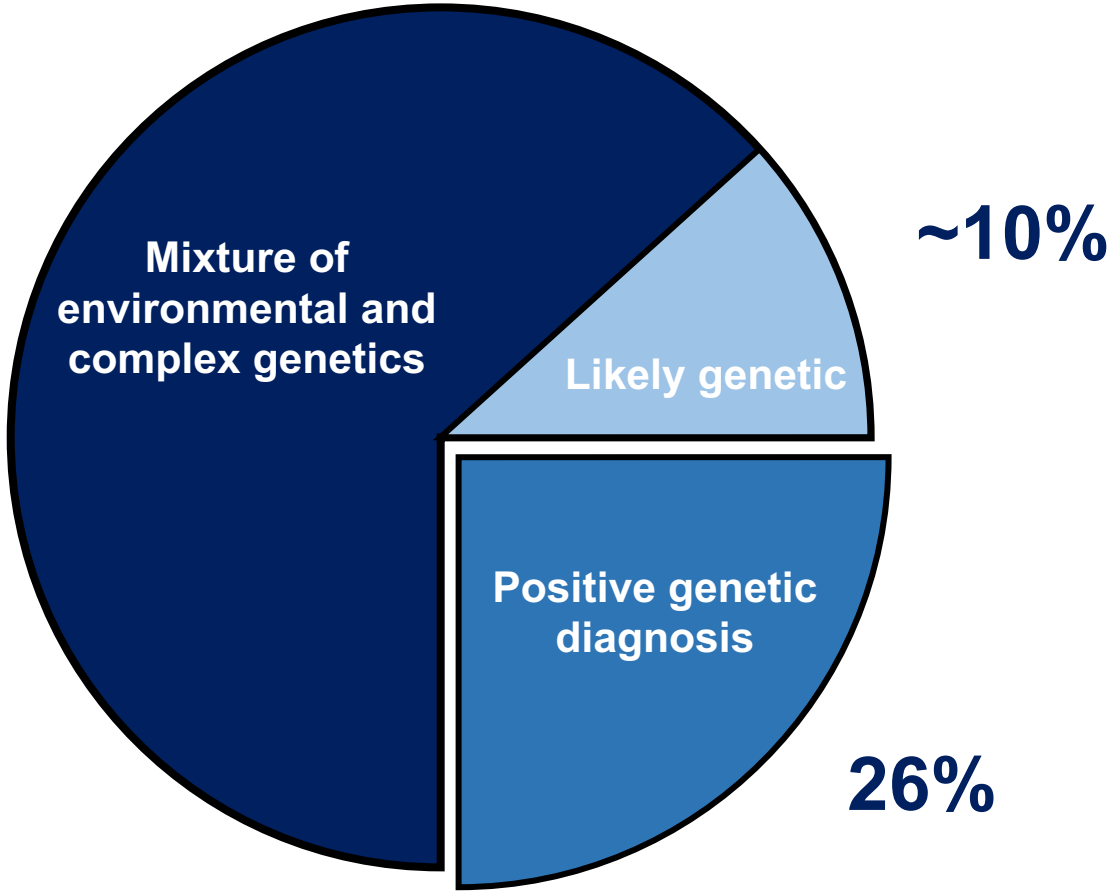


normal sutures



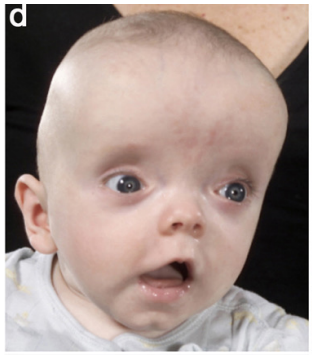
Craniosynostosis causes

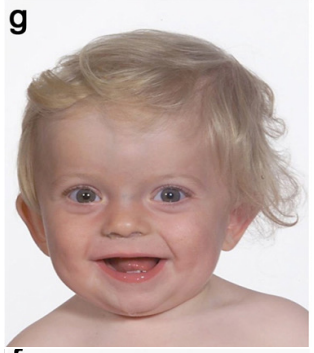
17 yr Oxford craniosynostosis cohort study n=932



Unpublished, updated from Wilkie et al., 2017 CO Pediatrics

Why does a genetic diagnosis matter?





Medical

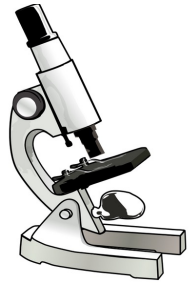
- There may be specific complications to screen for
- Management implications
- Treatment

Families:

- Ends the diagnostic odyssey
- Having a name opens doors for extra support
- Better counselling: recurrence risks
- Testing of wider family
- Preimplantation or prenatal genetic testing

Research:

- New information about skull and suture development
- Essential for future prevention and treatment strategies



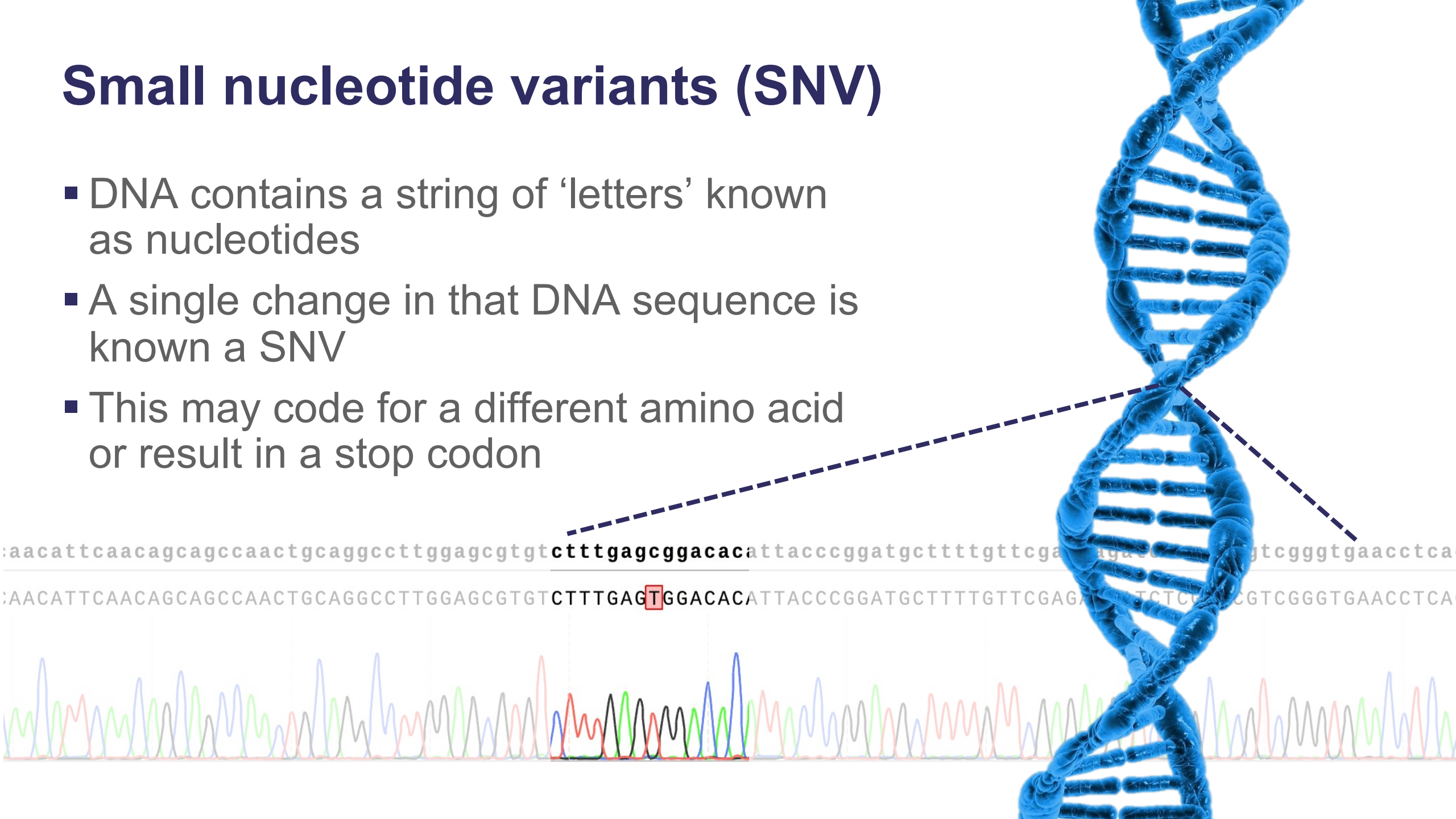
Identification of small nucleotide variants in patients with craniosynostosis

Rebecca Tooze

I have no commercial disclosure

Small nucleotide variants (SNV)

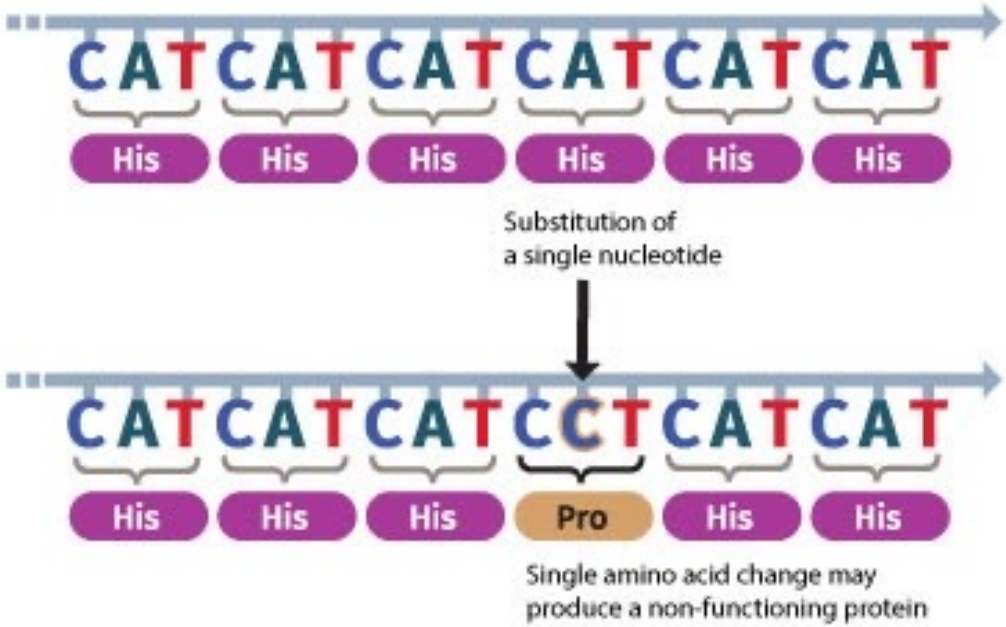
- DNA contains a string of ‘letters’ known as nucleotides
- A single change in that DNA sequence is known a SNV
- This may code for a different amino acid or result in a stop codon

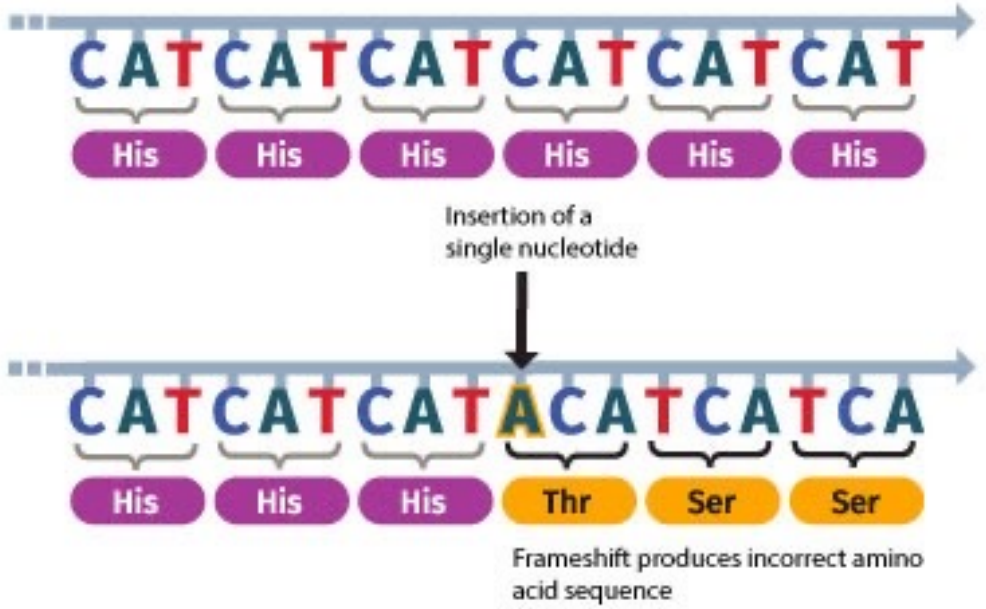


Consequence of the SNV…

- Combinations of three letters code for an amino acid

- Consequence:
  - Silent mutation (same amino acid produced)
  - Missense mutation (codes for a different amino acid)
  - Insertion or deletion (frameshift)
  - Stop codon (protein is truncated or lost)



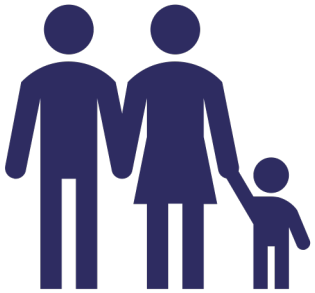


The 100kGP

- Patients were recruited to the UK 100,000 Genomes
- Whole genome sequencing
- Primary Aim – return to the patients' variants with sufficient evidence for diagnostic reporting related to their primary condition (i.e., craniosynostosis)

Genomic Medicine Centres (GMC)

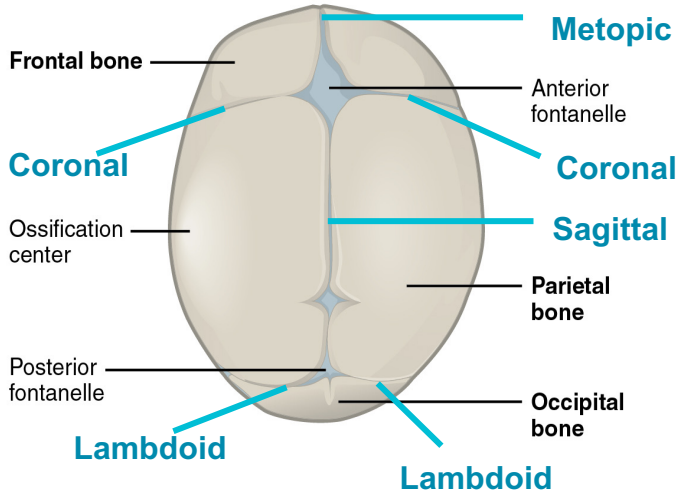
Analysis of variants in known genes associated with craniosynostosis



The 100kGP craniosynostosis cohort

Inclusion criteria:

1. Negative for mutations in known craniosynostosis genes
2. Negative for chromosomal abnormalities



Cases which are more likely to have a genetic cause:

1. Multi-suture
2. One suture +
   - additional clinical feature → syndromic
   - a relative with craniosynostosis → familial

The 100kGP craniosynostosis cohort

Total number of families: 114 families

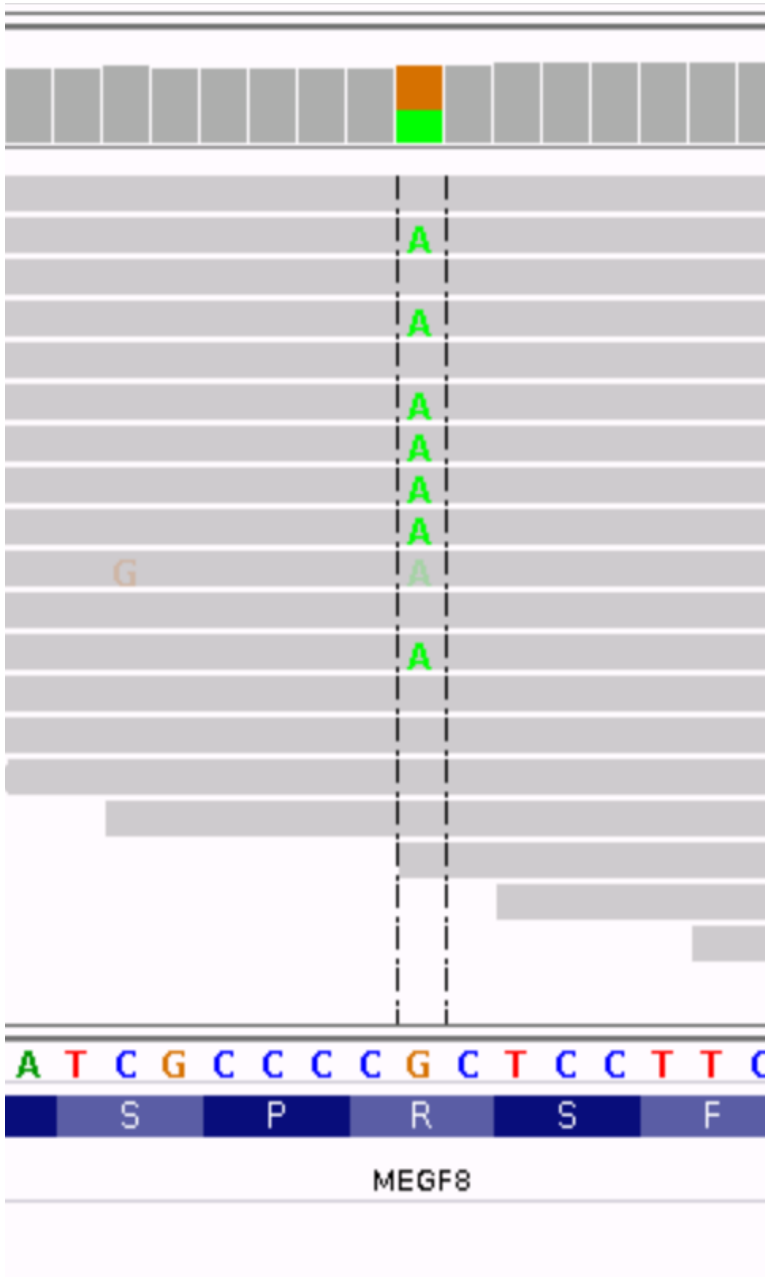
- With craniosynostosis as their primary phenotype
- Either syndromic or familial

Total diagnoses

- Genome sequencing identified a likely pathogenic variant in 29.8% of patients with previously undiagnosed craniosynostosis

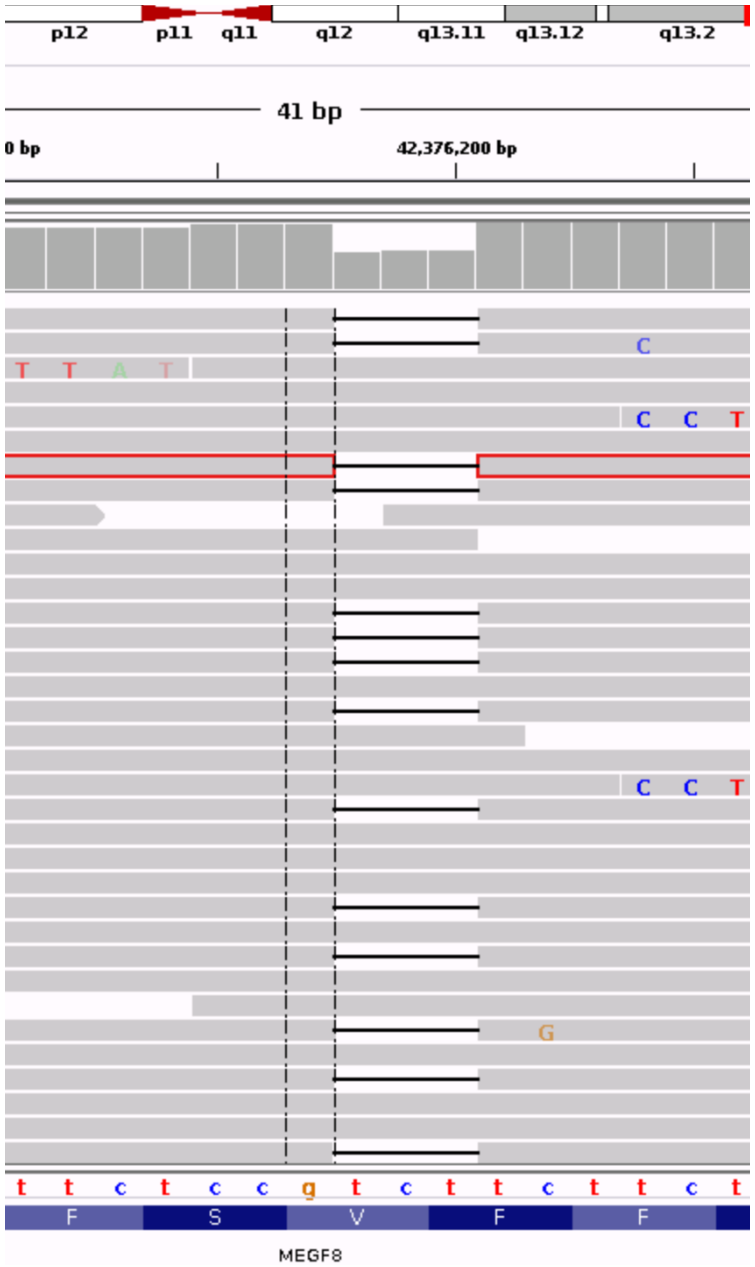
Example SNV: *MEGF8*

- Patient was diagnosed with Carpenter syndrome
- Carpenter syndrome is caused by recessive mutations in *RAB23* or *MEGF8*
- We identified a heterozygous missense variant in *MEGF8* (G>A)

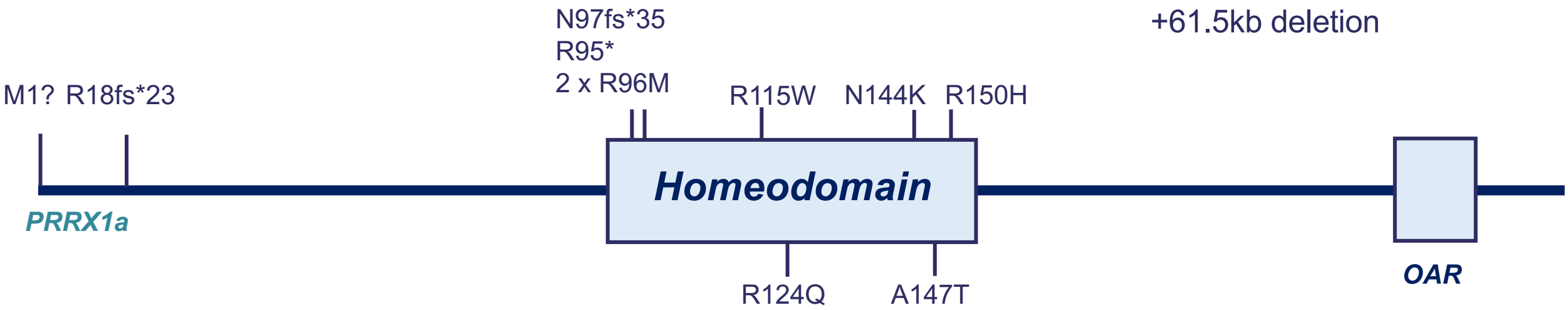


Example SNV: *MEGF8*

- Patient was diagnosed with Carpenter syndrome
- Carpenter syndrome is caused by recessive mutations in *RAB23* or *MEGF8*
- We identified a heterozygous missense variant in *MEGF8* (G>A)
- Further scrutiny identified a three nucleotide (one amino acid) deletion inherited from the other parent



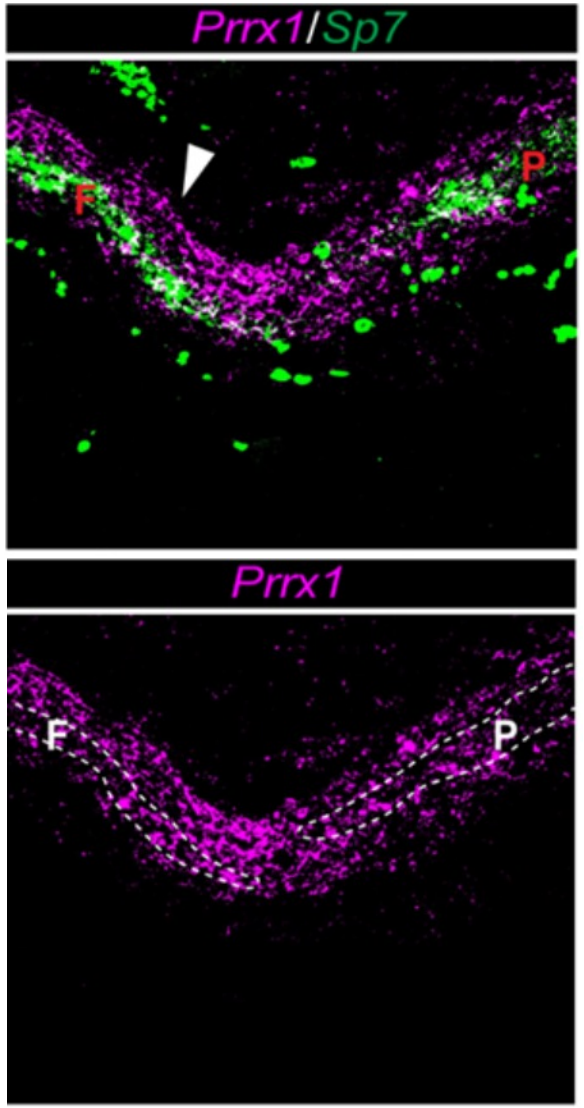
We've identified 12 families with mutations in *PRRX1*



- Total: 12 families and 15 individuals with craniosynostosis

PRRX1

- *PRRX1* is a transcription factor which is highly expressed during limb bud formation and craniofacial development
- Skeletal stem cells expressing *PRRX1* reside exclusively in the sutural mesenchyme
- Involved in regulating several key developmental pathways, including Wnt/β-Catenin and Notch signalling



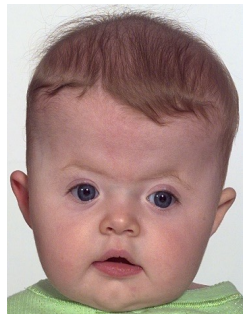
Farmer et al. (2021). *Nat. Commun.* 4797

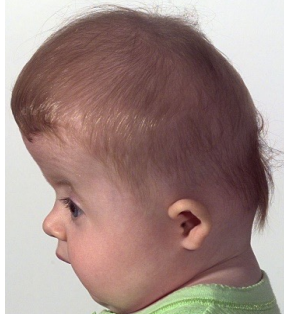
The sagittal and coronal sutures are most commonly fused

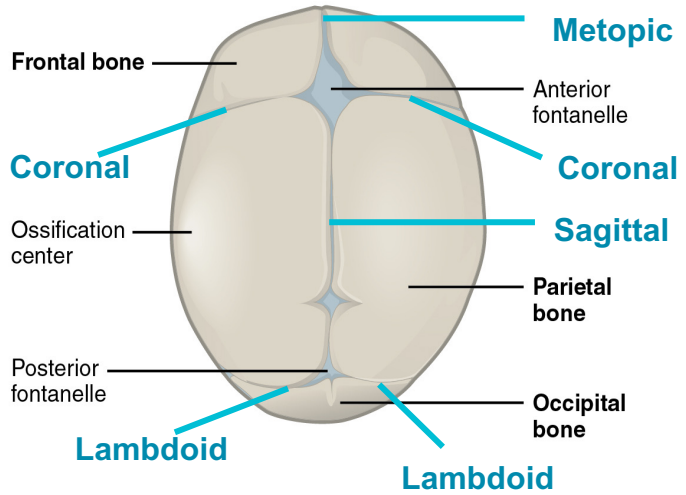
NS Uni-lambdoid (R)

NS Sagittal

NS Bi-coronal







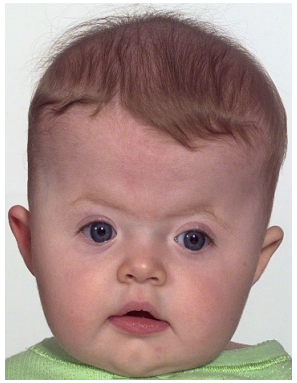
Suture	M1?	R18fs *23	R95*	R96M	R96M	N97fs *35	R115W	R124Q	N144K	A147T	R150H	61.5kb del	Total
Metopic													0
Left Coronal		X				X			X	(X)	X		4
Right Coronal		X	X	X		X			X	(X)	X		6
Sagittal	X		X		X		X			X			5
Left Lambdoid													0
Right Lambdoid	X							X					2
Multi-suture												X	1

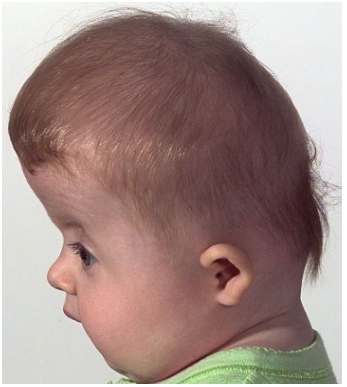
The sagittal and coronal sutures are most commonly fused

NS Uni-lambdoid (R)

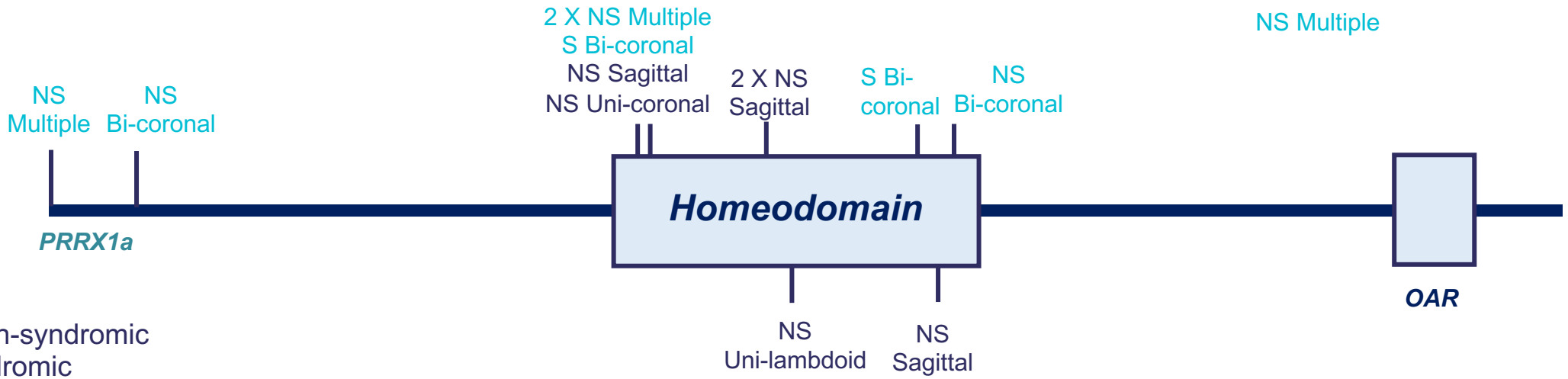
NS Sagittal

NS Bi-coronal



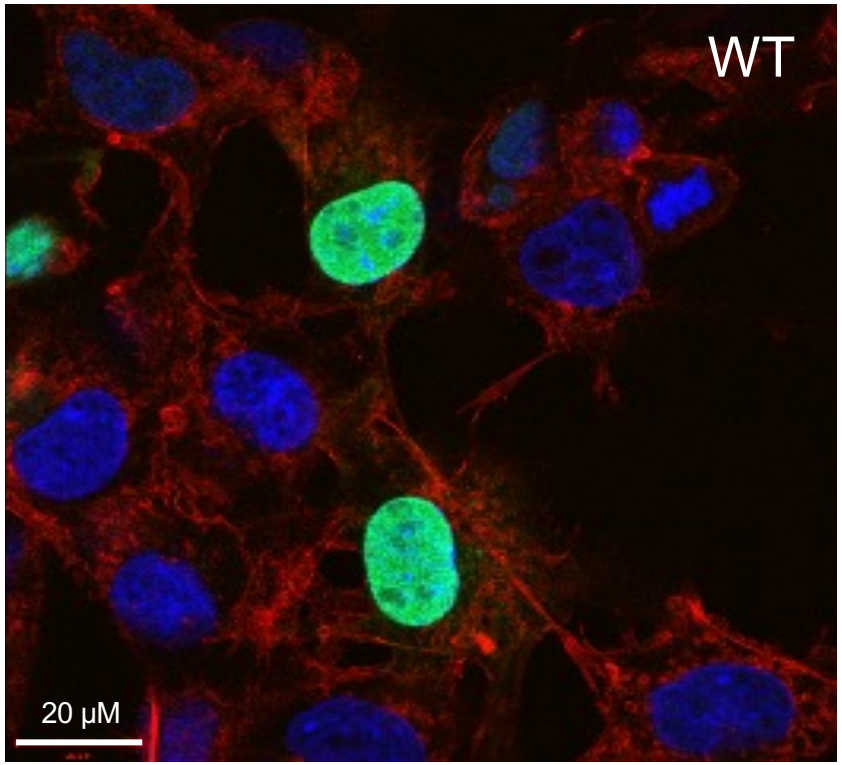


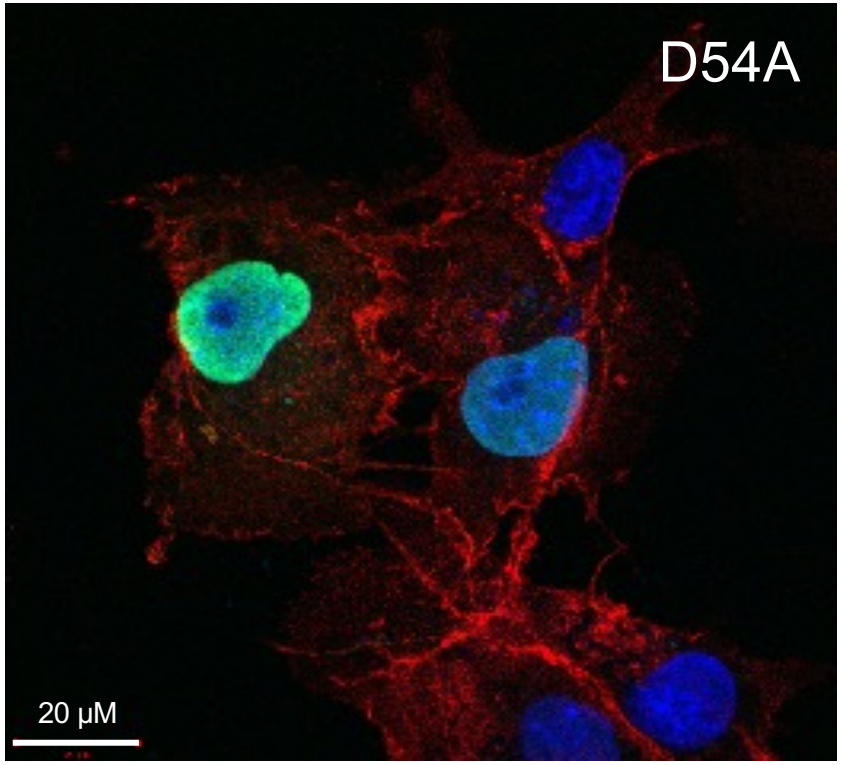
- More than one suture fused in 58% of the families
- No recurrent syndromic features

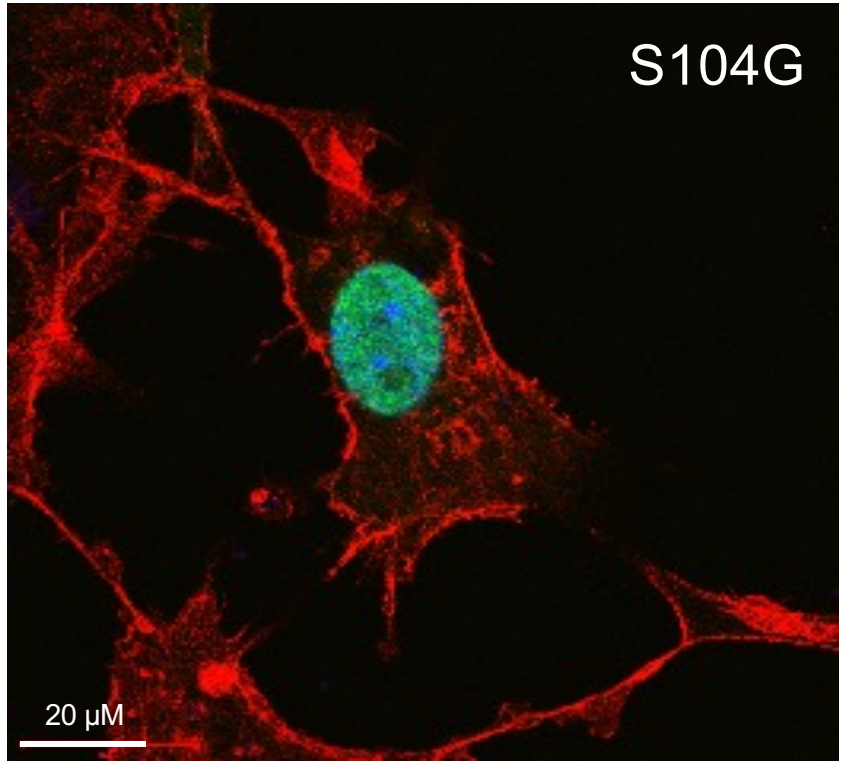


NS = Non-syndromic
S = Syndromic

Nuclear localisation



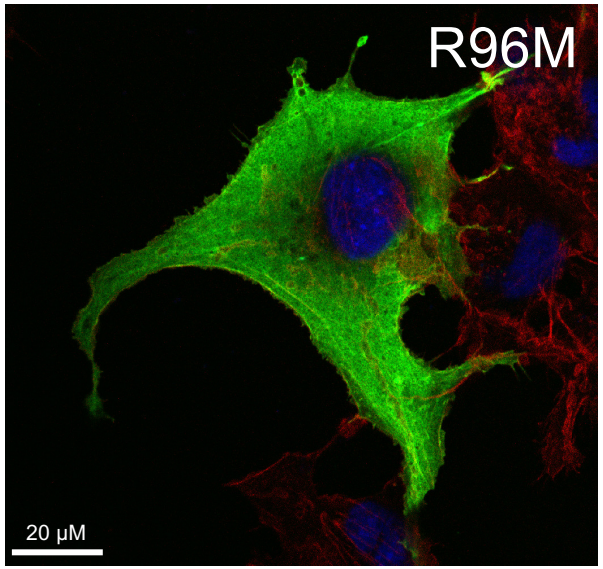


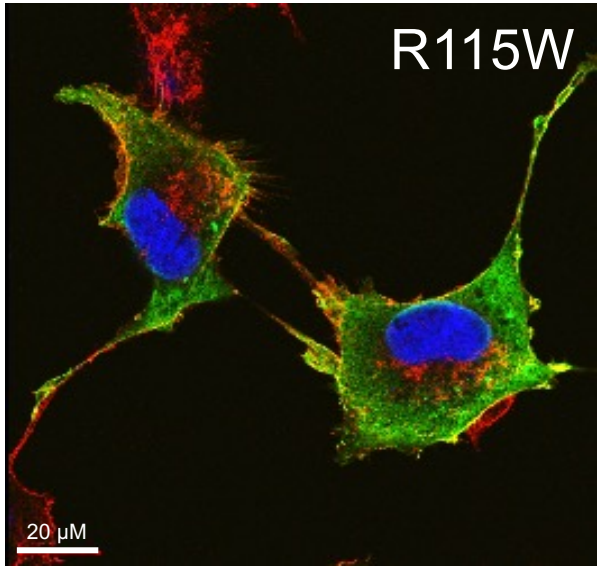


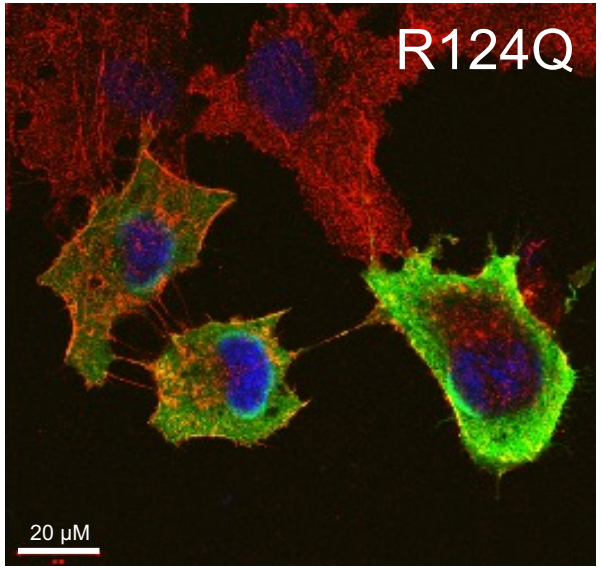
- Wildtype PRRX1 localises to the nucleus
- D54A – variant outside of the homeodomain
- S104G – polymorphism within the homeodomain (gnomAD AF: 0.0011)

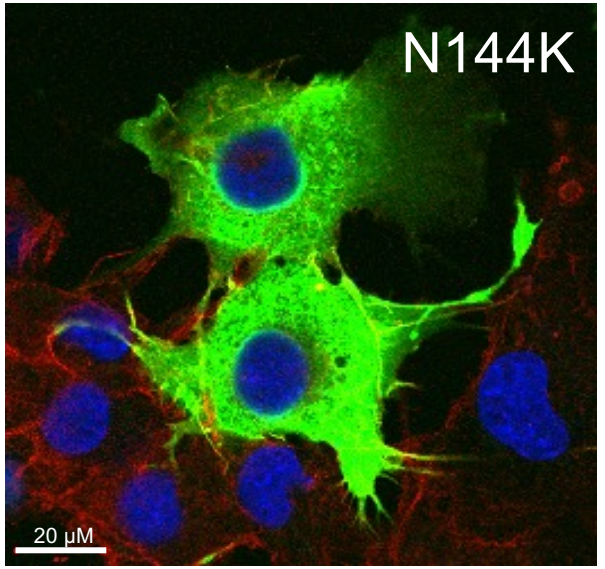
PRRX1
Nucleus
Actin

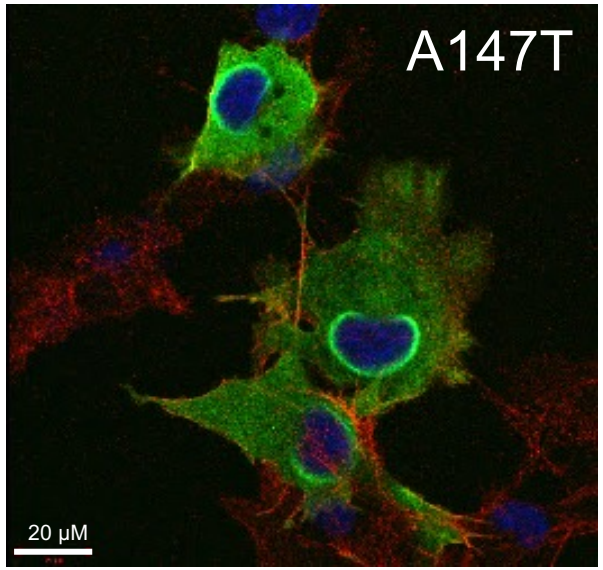
Variants within the homeodomain cause abnormal nuclear localisation

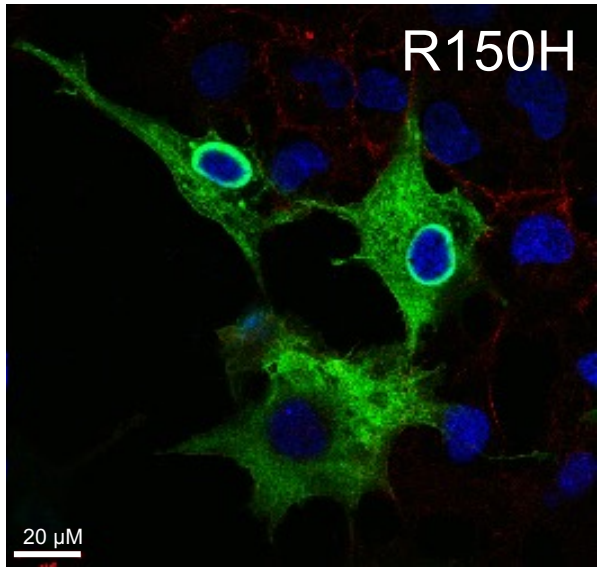






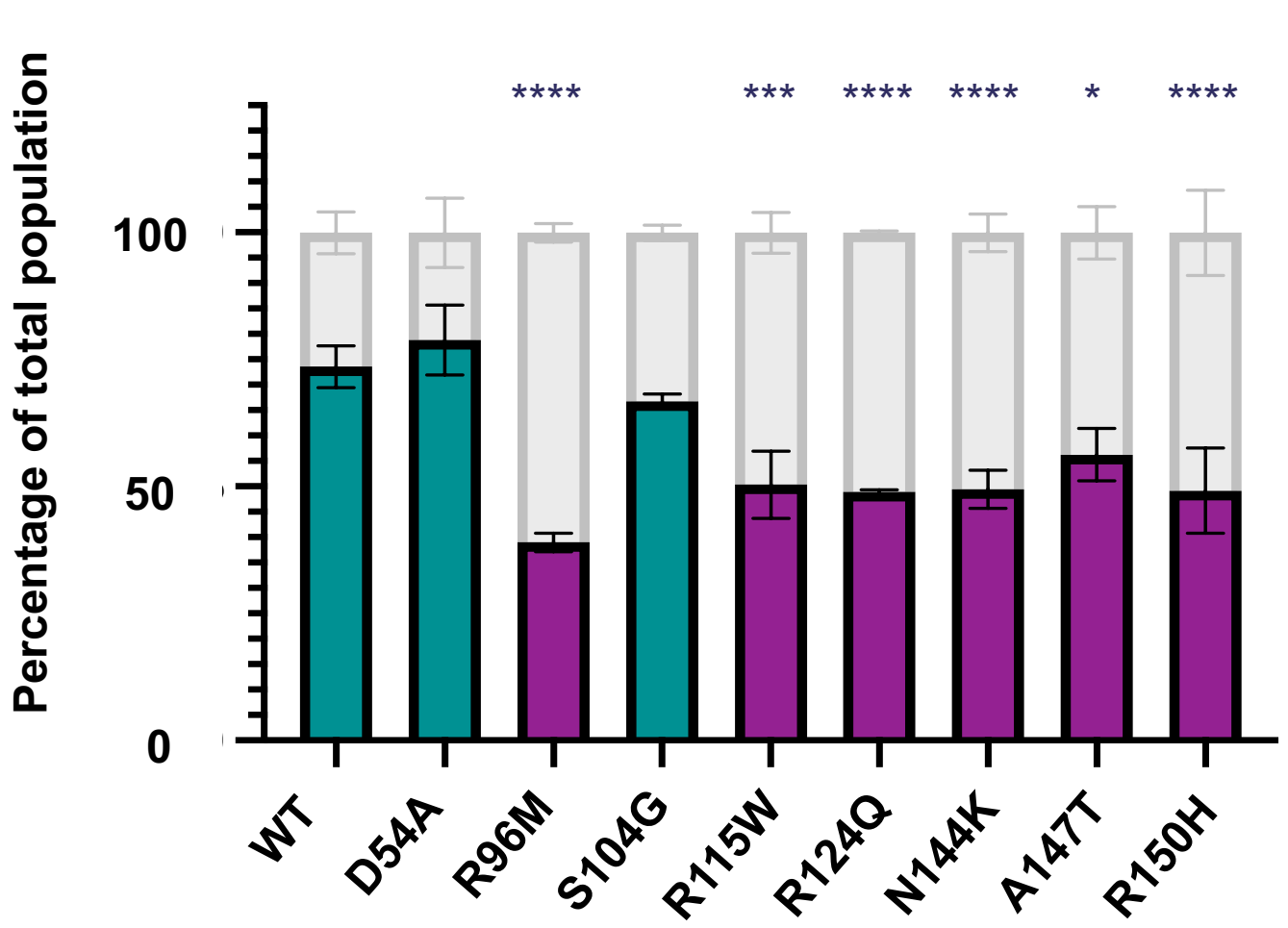






PRRX1
Nucleus
Actin

Mutations within the homeodomain cause abnormal nuclear localisation



Nuclear

Cytoplasmic

****<0.0001

**<0.001

*<0.01

Data is representative of three repeats, counting a total of 700-1200 cells per variant

Summary

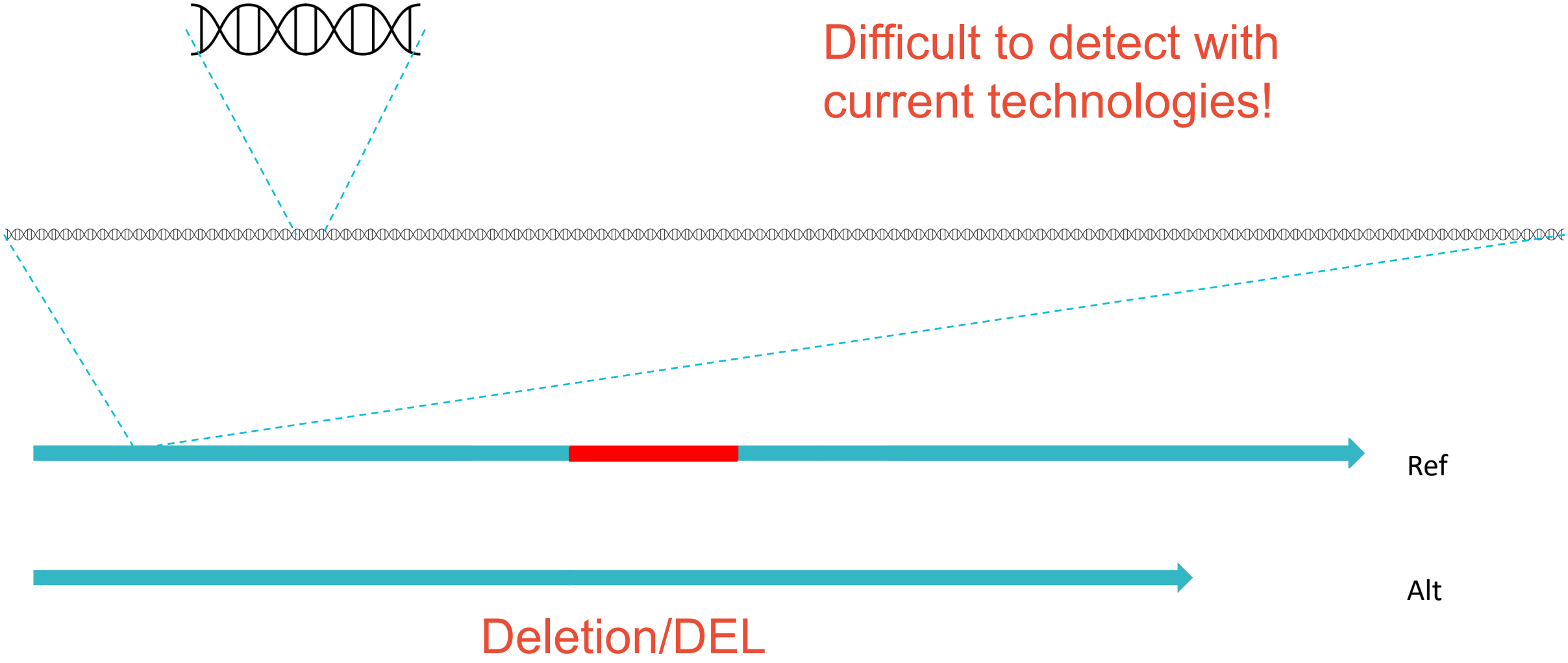
- Why are we doing this?
  - Positive genetic diagnosis for the individual and the family
  - Family planning
  - Clinical monitoring
  - Advance knowledge on craniosynostosis

Characterising pathogenic **structural variants** in patients with craniosynostosis

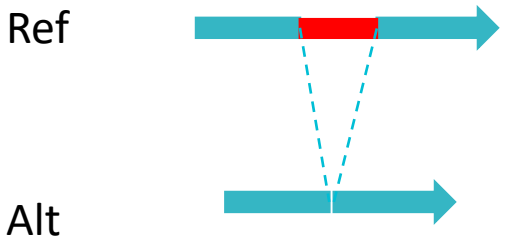
Yang Pei

I have no commercial disclosure

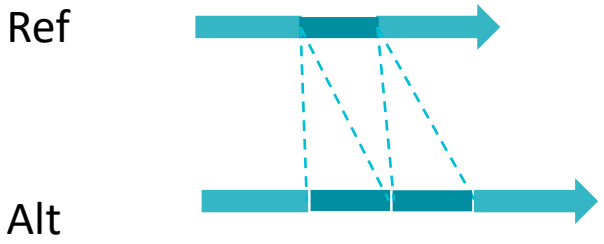
What are structural variants?



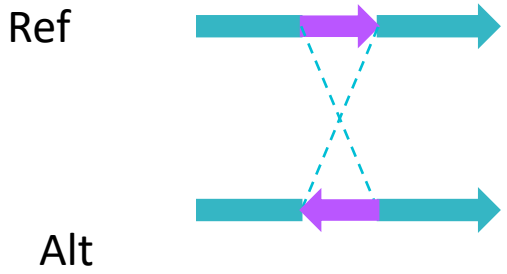
Structural variants (SVs)



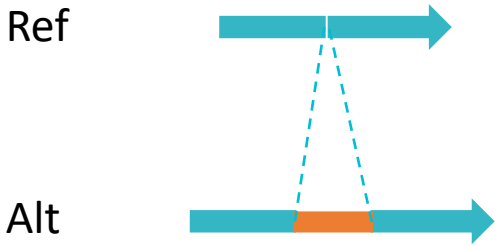
Deletion/DEL



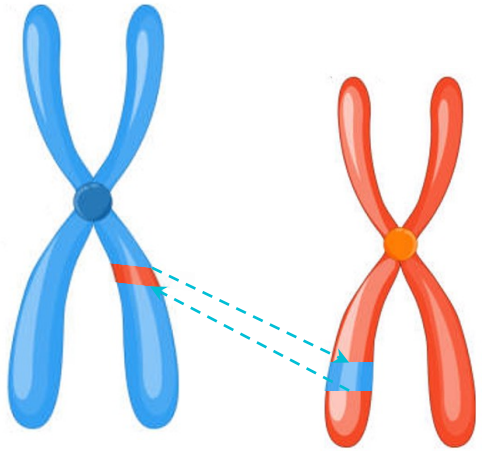
Duplication/DUP



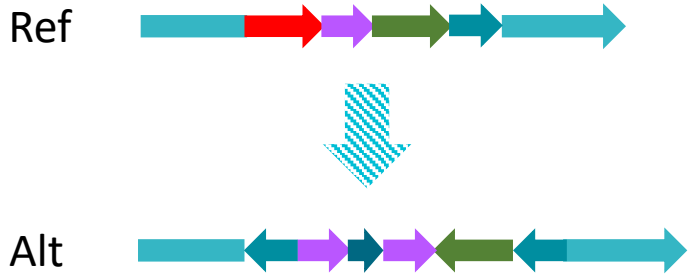
Inversion/INV



Insertion/INS



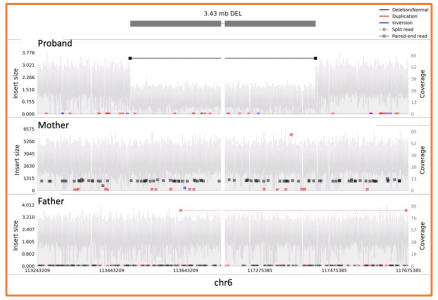
Translocation

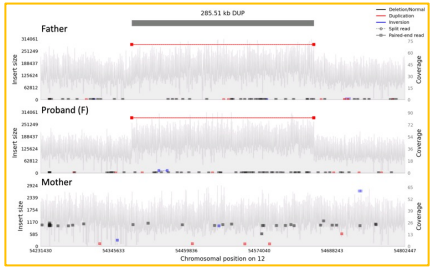


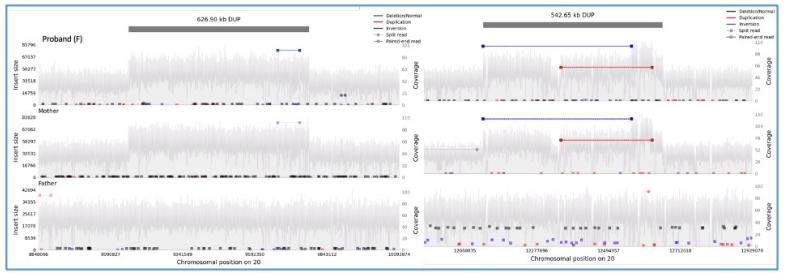
Complex

3 SVs of interest

chr6	DEL	*HDAC2*, *COL10A1*, *etc.*
chr12	DUP	*HOXC* cluster
chr20	CPX	*PLCB4 (?), etc.*



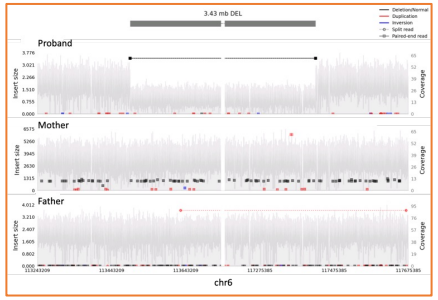




3 SVs of interest

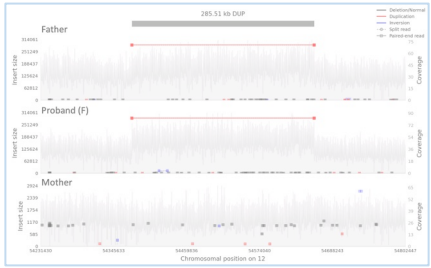
chr6 **DEL**

HDAC2, COL10A1, etc.



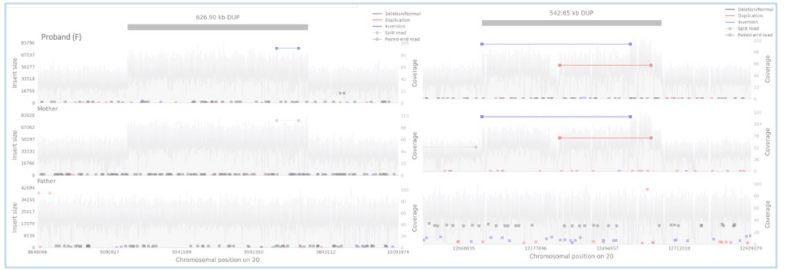
chr12 DUP

HOXC cluster

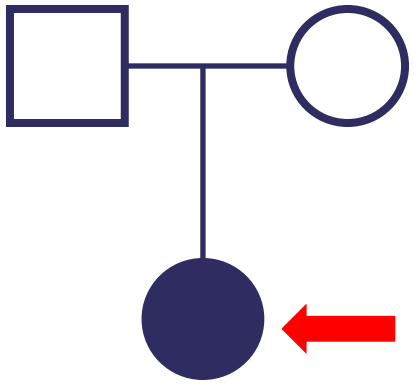


chr20 CPX

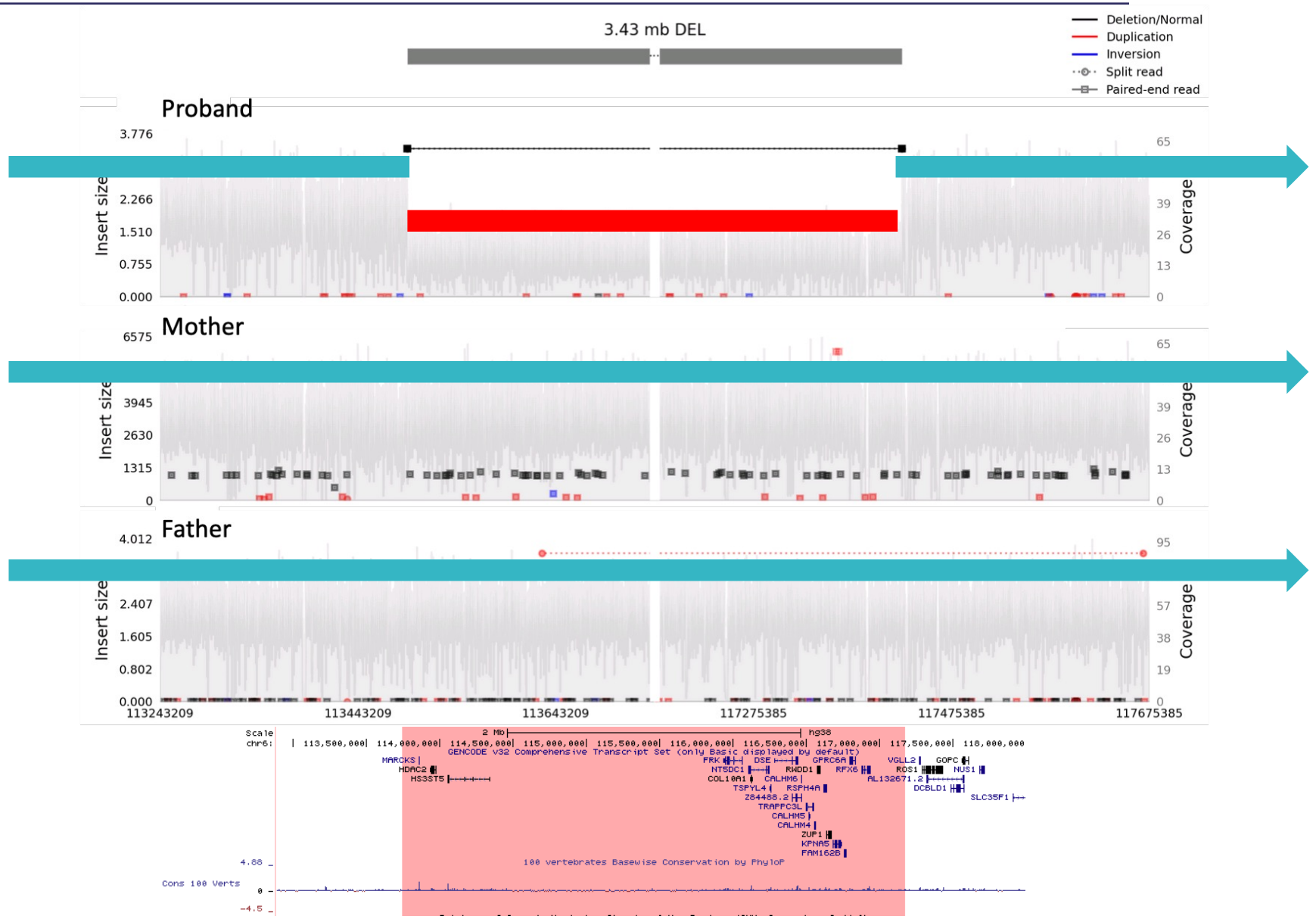
PLCB4 (?), etc.



Candidate genes	Location	Size	Type	Inheritance	Pathogenicity
HDAC2 & COL10A1	chr6	3.43 Mb	DEL	*De novo*	Pathogenic



Syndromic craniosynostosis



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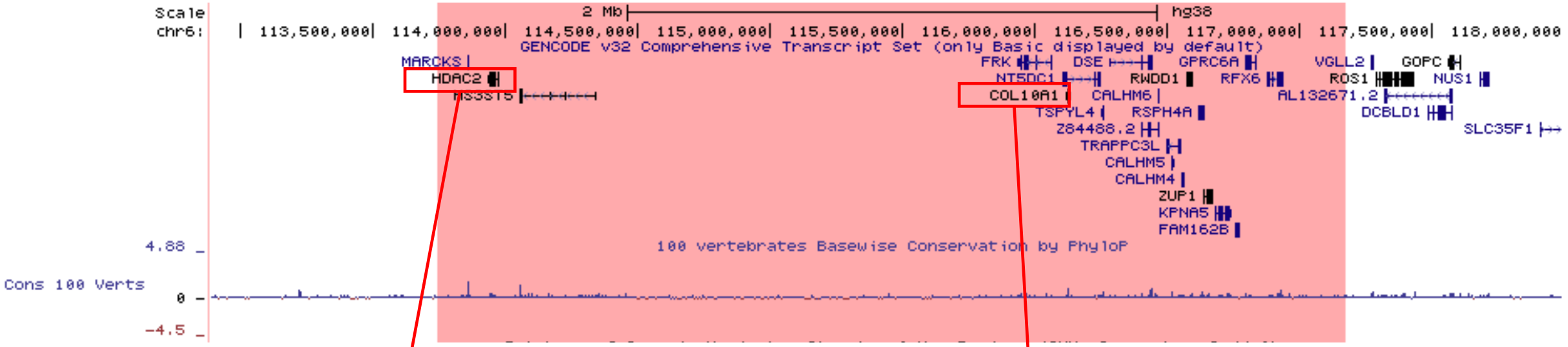


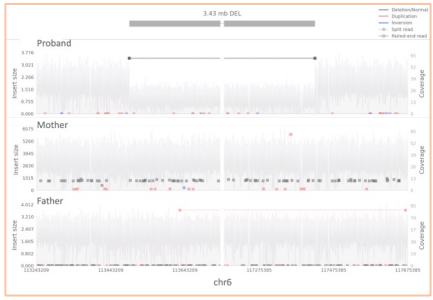
Interstitial 6q21q22.1 Deletion Syndrome

Metaphyseal chondrodysplasia, AD

3 SVs of interest

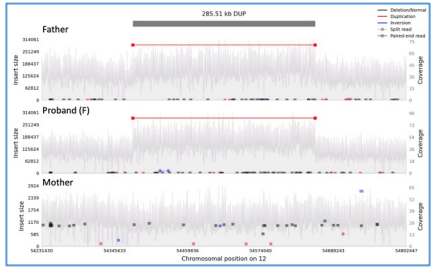
chr6 DEL

HDAC2, COL10A1, etc.



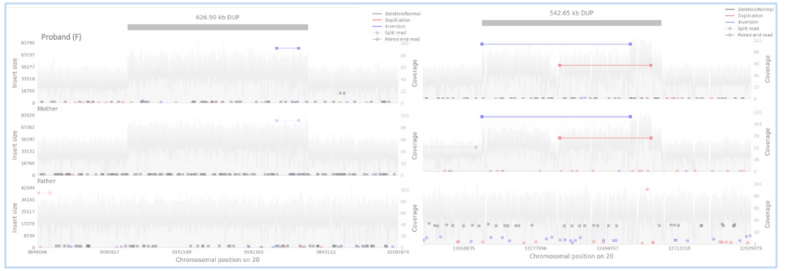
chr12 DUP

***HOXC* cluster**



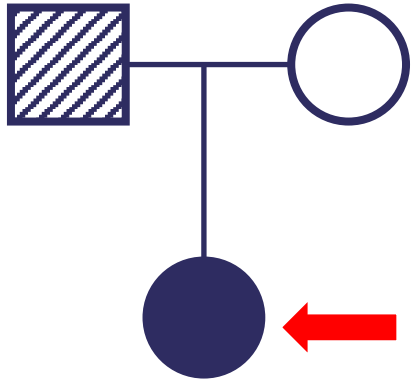
chr20 CPX

PLCB4 (?), etc.



Candidate gene(s)	Location	Size	Type	Inheritance	Pathogenicity
HOXC cluster	chr12	286 kb	DUP	Pat	Likely pathogenic

Fusion of middle ear ossicles
Severe conductive hearing impairment



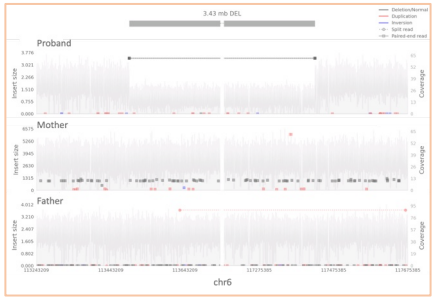
Pierre Robin sequence
Oral cleft
Hypertelorism
Downslanted palpebral fissures
Increased intracranial pressure
Microtia
Hearing impairment
Low-set ears
Abnormality of the auditory canal
Moderate conductive hearing impairment
Bicoronal synostosis

3 SVs of interest

chr6

DEL

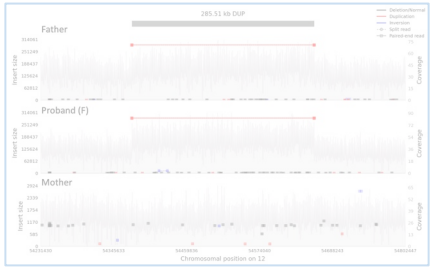
HDAC2, COL10A1, etc.



chr12

DUP

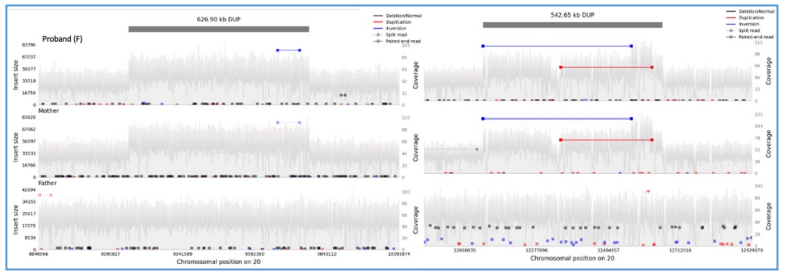
HOXC cluster



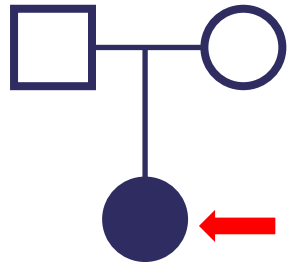
chr20

CPX

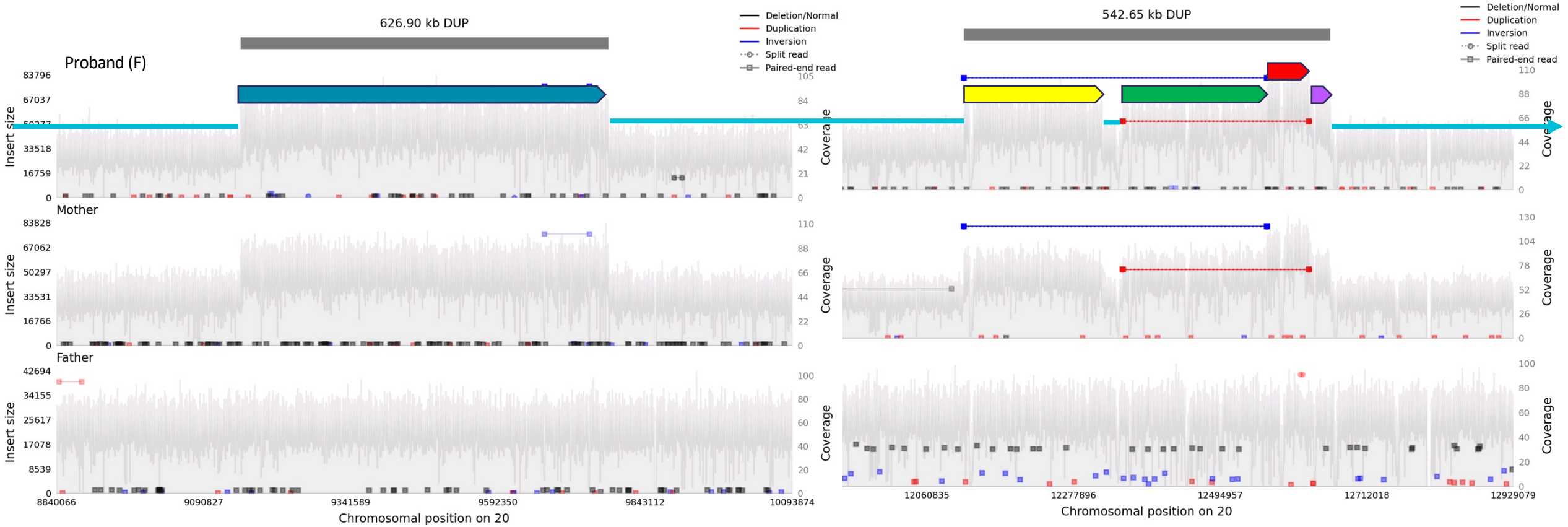
PLCB4 (?), etc.

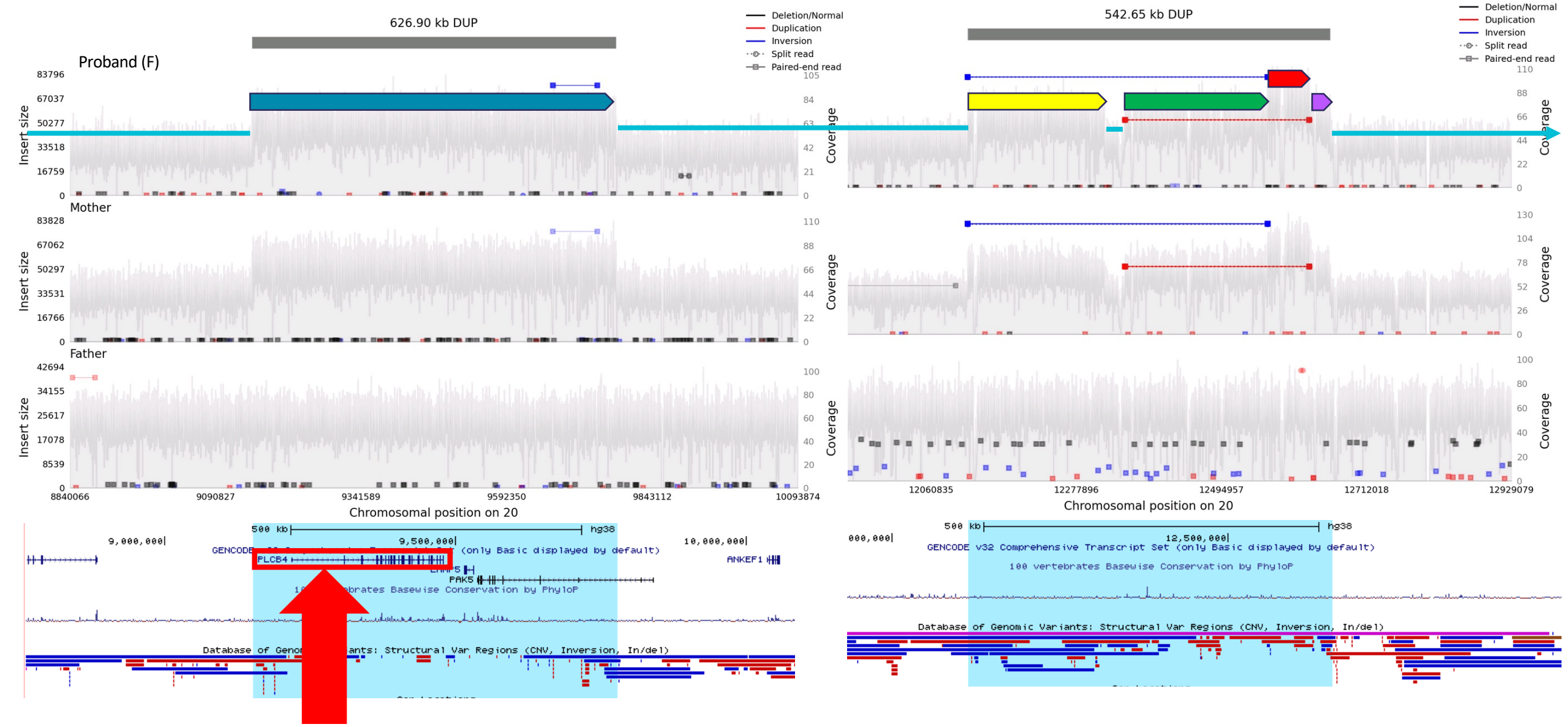


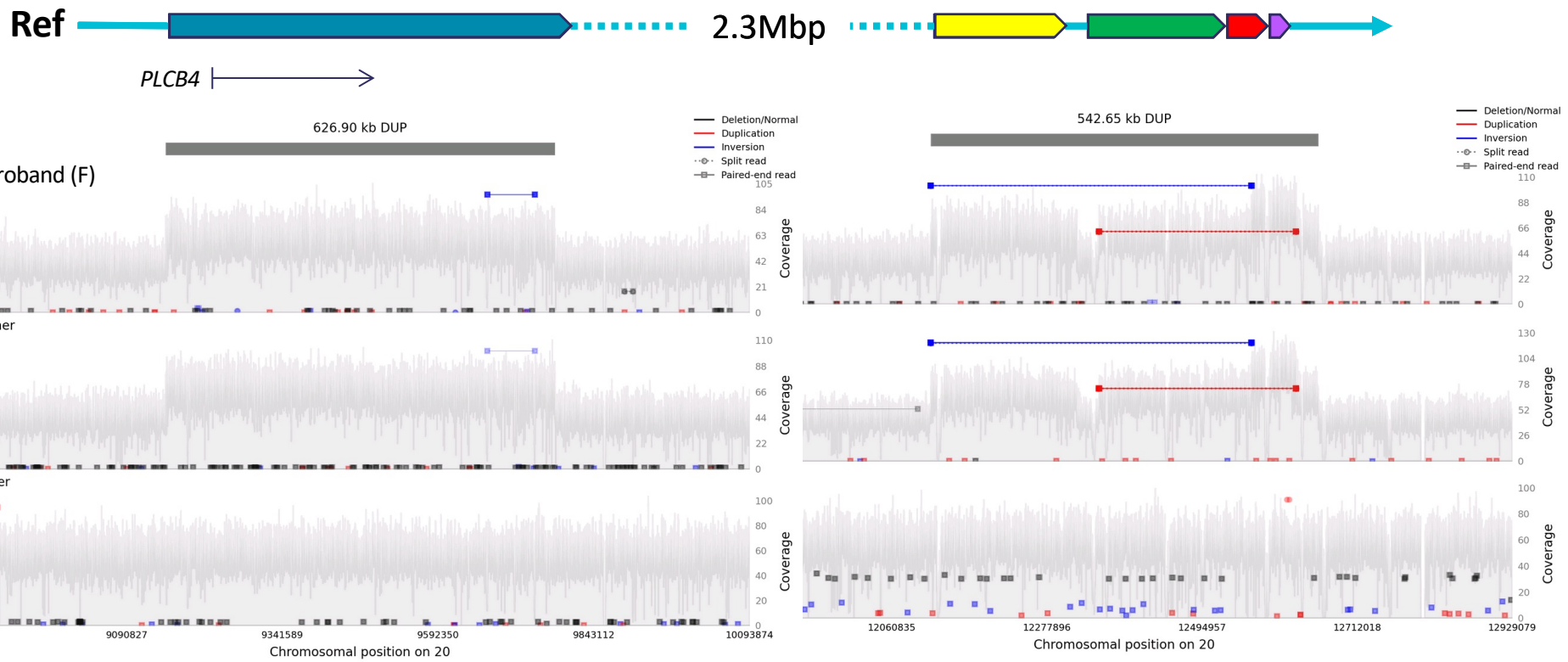
Candidate genes	Location	Size	Type	Inheritance	Pathogenicity
PLCB4	chr20	1.2 Mbp	INS	Mat	Unknown significance



Syndromic craniosynostosis
Multiple suture craniosynostosis




626.90 kb DUP
542.65 kb DUP
Deletion/Normal
Duplication
Inversion
Split read
Paired-end read
Proband (F)
Mother
Father
Insert size
Coverage
8840066
9090827
9341589
9592350
9843112
10093874
12060835
12277896
12494957
12712018
12929079
Chromosomal position on 20
500 kb
hg38
GENCODE v32 Comprehensive Transcript Set (only Basic displayed by default)
PLCB4
ANKEF1
PAK5
100 vertebrates Basewise Conservation by PhyloP
Database of Genomic Variants: Structural Var Regions (CNV, Inversion, In/del)

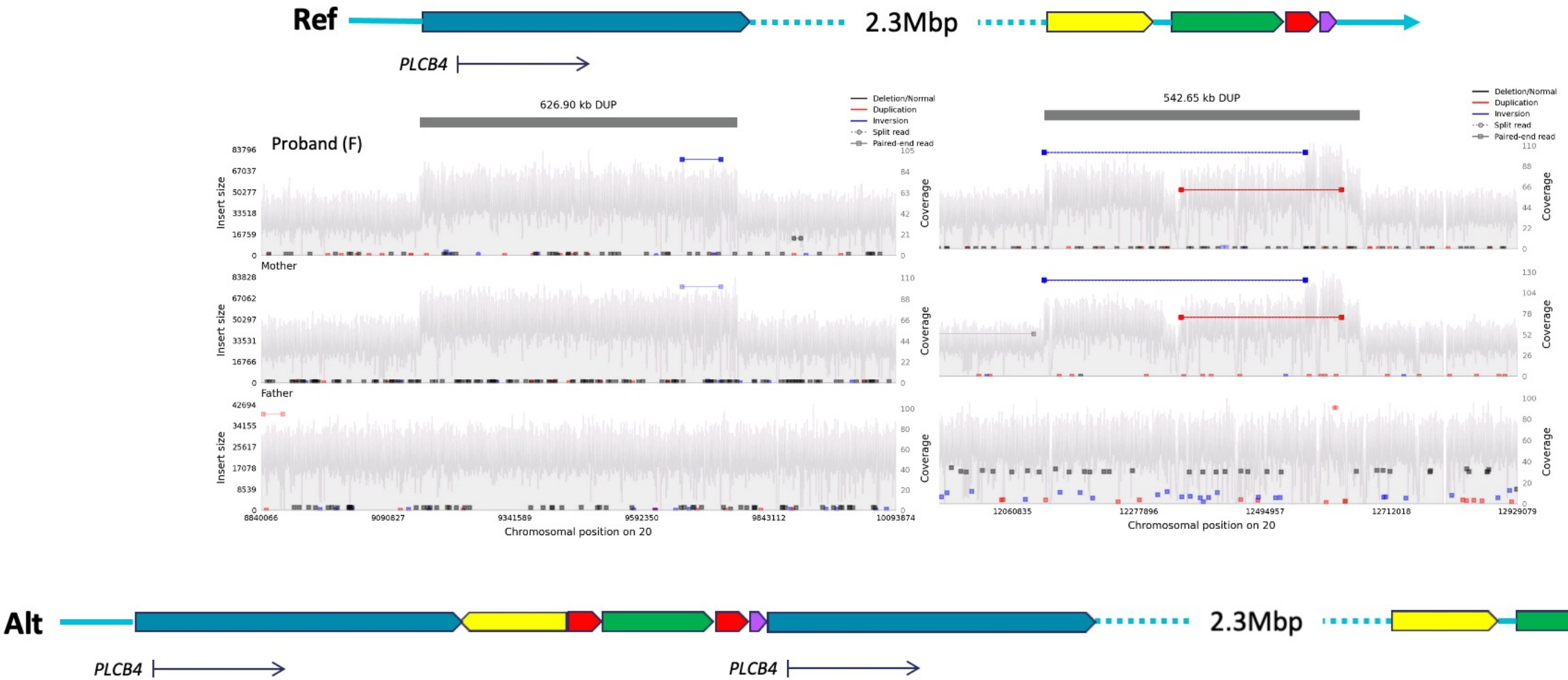


Alt

PLCB4 ⟶

PLCB4 ⟶

Many other hypotheses!



Many other hypotheses!

Summary

SVs == large genomic changes

Difficult to detect with current technologies

Highly complex -> hard to interpret

Essential diagnosis!

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Kerry Miller
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PRRX1 collaborators

Patients and their families

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@MRC_WIMM