Educational cases and exercises

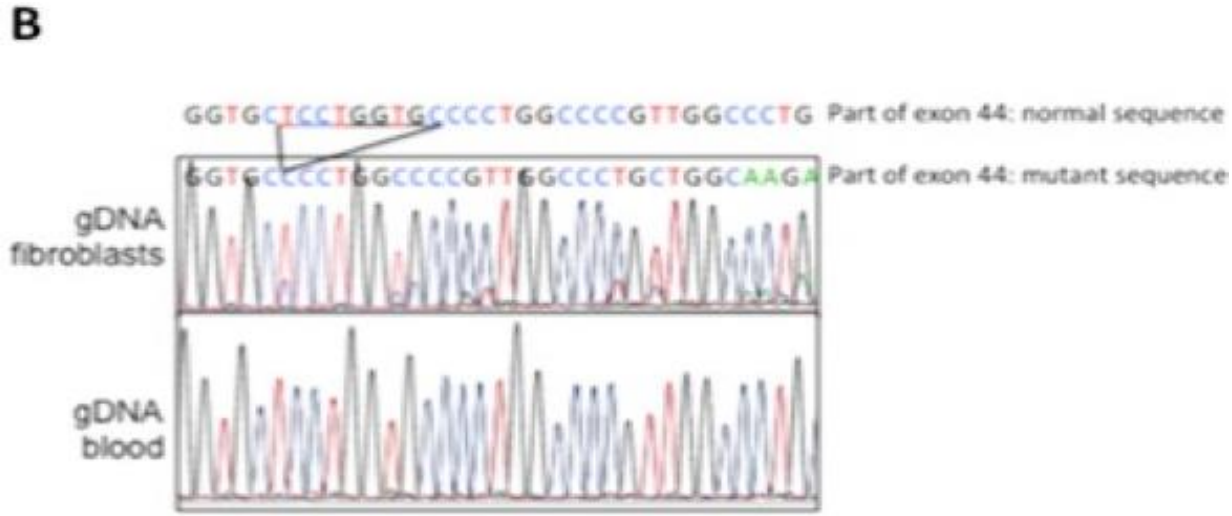
ANNELIES DHEEDENE

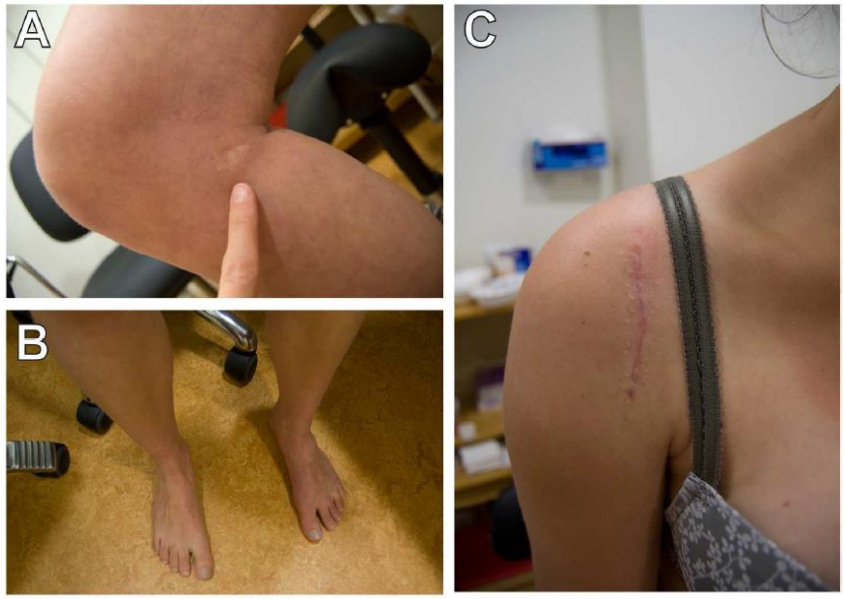
SOFIE SYMOENS

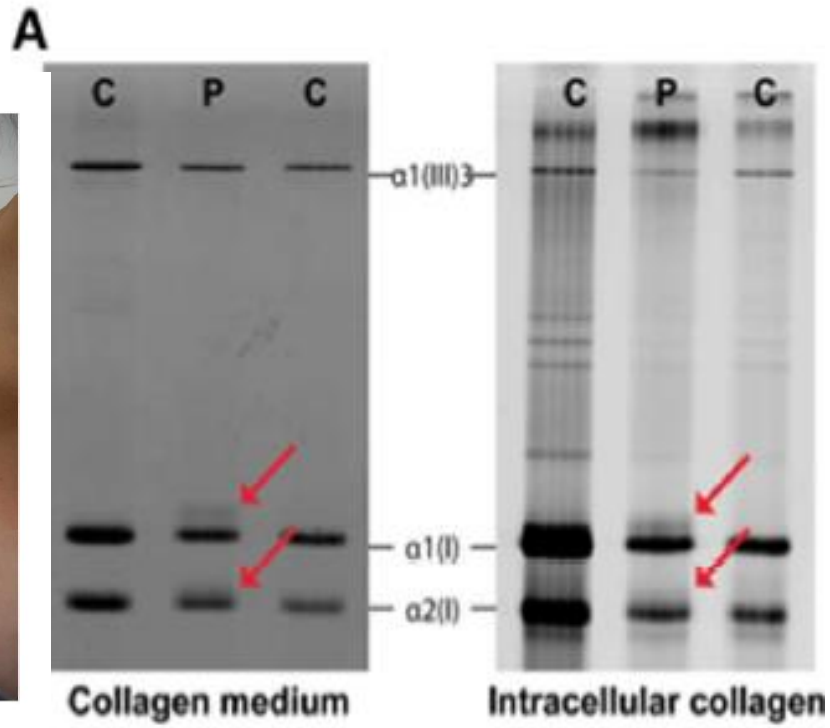
Illustrative case

IMPORTANCE OF RNA AND FUNCTIONAL STUDIES

MAIN MESSAGE: IF YOU ARE CLINICALLY CONVINCED, GO FURTHER!







Exercise

Variant classification SMARCB1

Clinical information: woman 33 years with intellectual disability

--> ID&epilepsy gene panel sequencing (WES based)

SMARCB1 gene: (NM_003073.4) c.1101C>G & p.(Asp367Glu).

Heterozygous, de novo

Class?

Tip: REVEL damaging effect

- PM2: not in population database
- PP2: Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease. [Z = 3.71 (>3,09)]
- PP3: Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.).
- PM5: Novel missense change at amino acid residue where a different missense change determined to be pathogenic has been seen before
    - (p.Asp367Gly in Decipher patient 301245)
- PM1: Located in a mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation.
- PS2 - supporting: De novo (both maternity and paternity confirmed) in a patient with the disease and no family history.

Class 4

Educational case

gnomAD is not always right!

Clinical information: girl with epilepsie and cerebral palsy

--> ID&epilepsy gene panel sequencing (WES based)

Heterozygous missense variant in exon 4/4 of the **TUBB2A** gene (NM_001069.3): g.3154458G>A (GRCh38/hg38), c.743C>T, p.(Ala248Val), rs2808001

De novo

OMIM #615763: complex cortical dysplasia with other brain malformations-5 (CDCBM5) – autosomal dominant

Clinvar: conflicting interpretation - **Benign(1);Likely benign(1);Likely pathogenic(1);Pathogenic(2)**

Phenotype fits!

TUBB2A

gnomAD

Single nucleotide variant: 6-3154458-G-A (GRCh38)

	Genomes
Filter	AS_VQSR
Allele Count	389
Allele Number	104640
Allele Frequency	0.003718
Popmax Filtering AF (95% confidence)	0.01260
Number of homozygotes	0

References

- dbSNP (rs2808001)
- UCSC
- ClinVar (127101)

Report

- Report this variant
- Request additional information

Annotations

This variant falls on 1 transcript in 1 gene.

missense

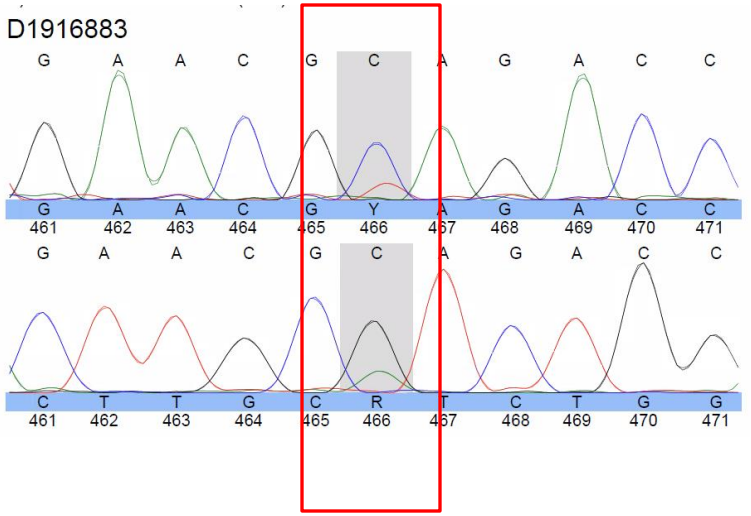
- TUBB2A
  - ENST00000333628.3
    Different version of MANE Select transcript for TUBB2A
    HGVSp: p.Ala248Val
    Polyphen: benign
    SIFT: deleterious_low_confidence

Population Frequencies

Population	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
African	342	24780	0	0.01380
East Asian	14	2172	0	0.006446
Other	5	1526	0	0.003277
South Asian	4	1688	0	0.002370
Latino	13	10256	0	0.001268
European (Finnish)	8	6468	0	0.001237
European (non-Finnish)	3	54062	0	0.00005549
Amish	0	750	0	0.000
Ashkenazi Jewish	0	2938	0	0.000
Female	211	55642	0	0.003792
Male	178	48998	0	0.003633
Total	**389**	**104640**	**0**	**0.003718**

TUBB2A

Sanger confirmation



exome data

proband

mother

father

PMID: 20191564

*"Interestingly, we noticed that human **TUBB2A and TUBB2B genes are located in tandem in chromosome 6p** and encode **proteins** that **only differ in two amino acids**."*

TUBB2A: chr6:3154599

TGGAAAACACAGATGAAACCTACTGCATTGACAACGAGGCCCTGTATGACATCTGCTTCC

TUBB2B: chr6:3225486

TGGAAAACACAGATGAAACCTACTCCATTGATAACGAGGCCCTGTATGACATCTGCTTCC

TUBB2A

Variant: c.743C>T & p.Ala248Val

AA201 different from TUBB2B

Variant + AA TUBB2A
in same read

- mapping error due to overlap in sequence between two genes (TUBB2A and TUBB2B)
- TUBB2A de novo missense variant explains phenotype in this patient
- Class 4
- Long-read sequencing?

Educational case

Choose your transcript(s) wisely!

Clinical information: boy, 2 yrs old, severe OI with multiple fractures and bowing

--> OI targeted gene panel sequencing

Genes: ACAN, ALPL, B3GALT6, **BMP1**, COL1A1, COL1A2, CREB3L1, CRTAP, FAM46A, FKBP10, IFITM5, LEPRE1, LRP5, LRP6, MBTPS2, NBAS, P4HB, PLOD2, PLS3, PPIB, SEC24D, SERPINF1, SERPINH1, SP7, SPARC, TAPT1, WNT1, TMEM38B, LIFR

--> MANE transcript: NM_006129.4 --> this is also the RefSeq sequence used in published studies and LOVD database.

BUT: BMP1 has an alternative exon --> two different biologically active isoforms of the protein --> do not only include the MANE transcript, but look for ALL biologically active transcripts!

--> Likely pathogenic variant in the case: c.2191T>C; p.(Ter731ArgextTer81) homozygous in NM_001199.4

In NM_006129.4: c.2108-602T>C

Educational case

Get to know your gene panel

D1915563

Analysed gene panel: Skin (337 genes)

--> filtersetting

--> variant impact

--> population frequency

--> **interaction lab and clinical geneticst (referring physician) yielded the identification of the FECH variant**

Exercise

Variant classification: BRCA2

c.516+1G>A

Female, 56 yrs old, bilateral breast cancer. She has a sister, 53 yrs old, with breast cancer. They also have a paternal aunt and paternal niece (deceased at young age) with breast cancer.

Molecular analysis reveals the following variant in the BRCA2 gene: c.516+1G>A

--> Class?

Exercise BRCA2 – c.516+1G>A

CLASSIFICATIE CLASS 5 (Pathogenic)

Criteria: 1 very strong pathogenic argument (PVS1) and >= 2 supporting pathogenic arguments (PP3,PP5)

- PM2: de variant is afwezig in Exome Sequencing Project, 1000 Genomes Project, Exome AggregationConsortium

- PP5: variant is pathogeen volgens betrouwbare bronnen. ClinVar ID: RCV000258397.1 (Pathogenic - Breast-ovarian cancer, familial 2), RCV000563773.2 (Pathogenic -Hereditary cancer-predisposing syndrome), RCV000478432.1 (Pathogenic), RCV000586303.1(Pathogenic - Hereditary breast and ovarian cancer syndrome) --> also see Enigma Database

https://www.ncbi.nlm.nih.gov/clinvar/variation/51786/

- PP3: meerderheid (3/5) van de predictieprogramma's voorspellen dat de variant schadelijk is. Human Splicing Finder: 0.00% (var: - wt:). Genesplicer: 0.00% (var: - wt:). NNSPLICE: 0.00% (var: - wt:). MaxEntScan: 0.00% (var: 0.702 - wt: 0.702). Splicesite finder like: 0.00% (var: - wt:)*

- PVS1: splice site verandering (positie +/-1 of +/-2) in een gen waar 'verlies van functie' een gekend ziektemechanisme is (m.a.w. indien eerder in dit gen voor deze aandoening dit type varianten als pathogene varianten gedefinieerd werden).

Exercise

PGT-M

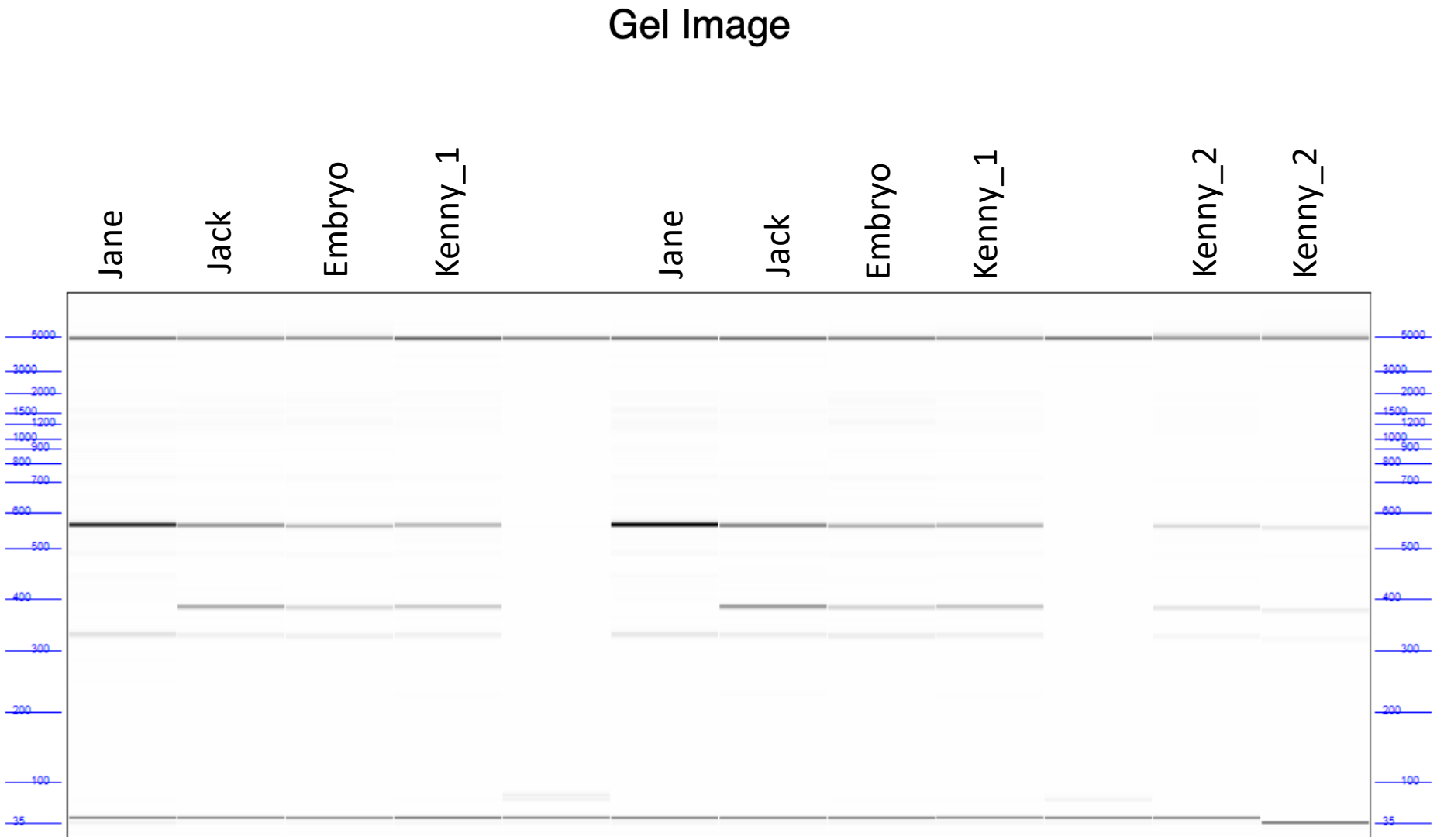
Mr Jack Doe and Mrs Jane Doe come to consult the clinical geneticist with their son Kenny. Jane is affected with NF1 and the couple has started the PGT-M procedure. After transfer of a healthy embryo, Kenny is born. However, Kenny is affected with NF1.

The couple demands to the clinical geneticist how this is possible?

What do you tell Jack and Jane?

Which molecular tests do you request?

AMELF PCR = sexing PCR



	Jane		Jack		Embryo		Kenny_1		Kenny_2	
	Affected, proband		Healthy partner							
STR marker/path variant										
D17S1794	204	196	204	204	196	204	204	204	204	204
D17S1824	146	135	135	137	135	135	146	137	146	137
NF1 c.7127-12T>A	**A**	T	T	T	T	T	**A**	T	**A**	T
D17S1166	213*	222	213	222	222	213	213	222	213	222
D17S1800	294	285	283	292	285	283	294	292	294	292
D17S1880	199	191	209	199	191	209	199	199	199	199

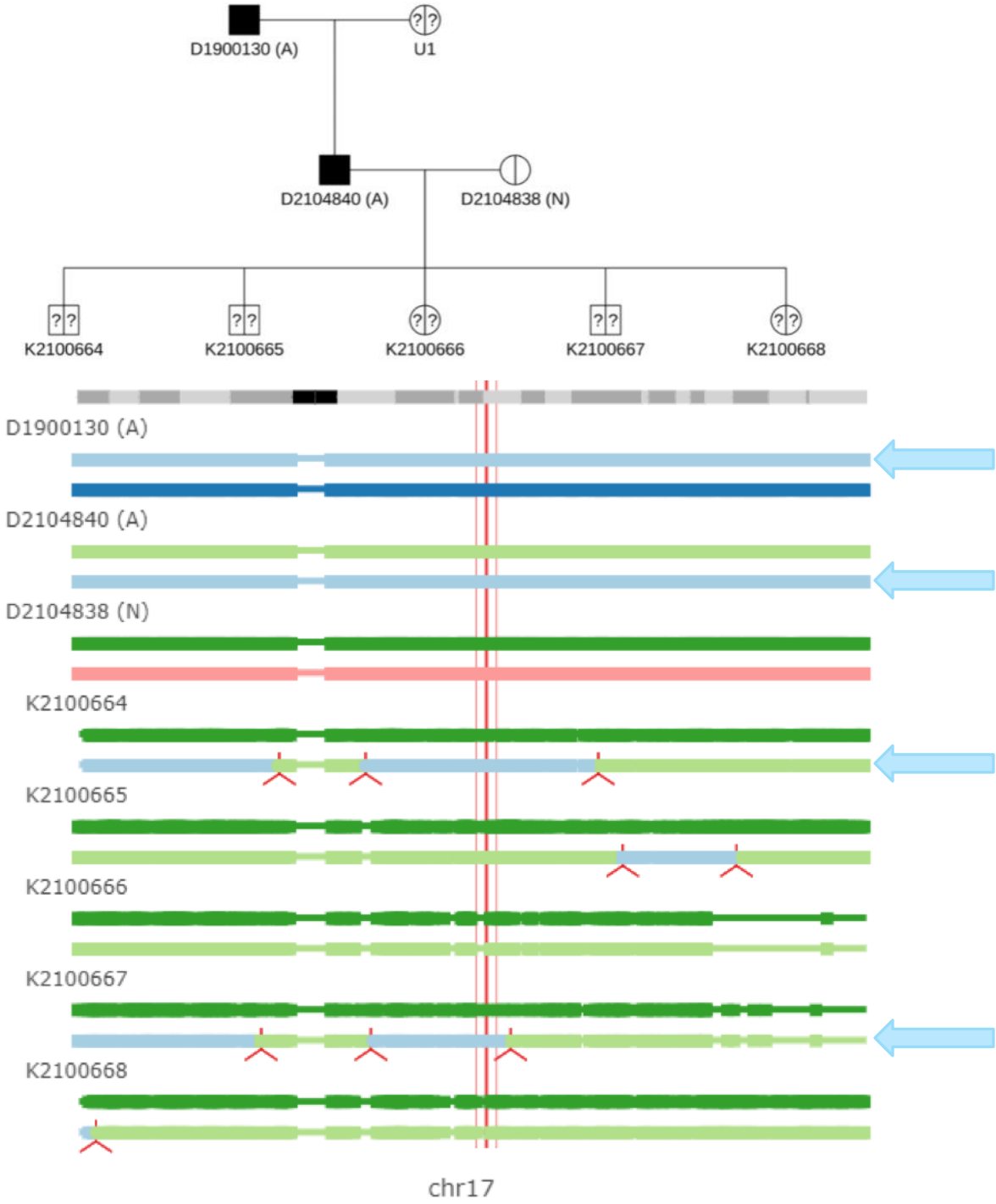
Identity testing (pp16) confirmed different identity of the transferred embryo and Kenny.
Conclusion: Kenny is the product of a spontaneous pregnancy.

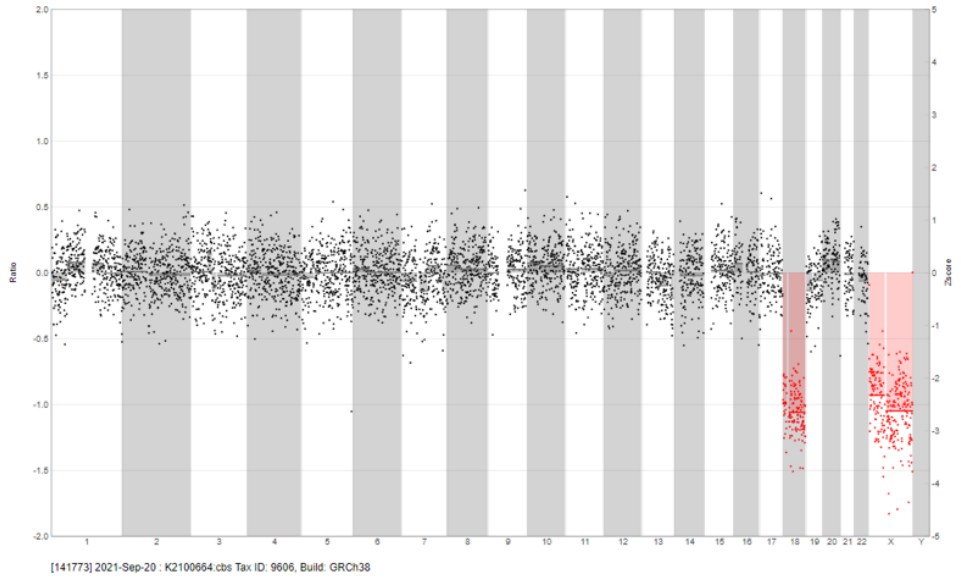
Educational case

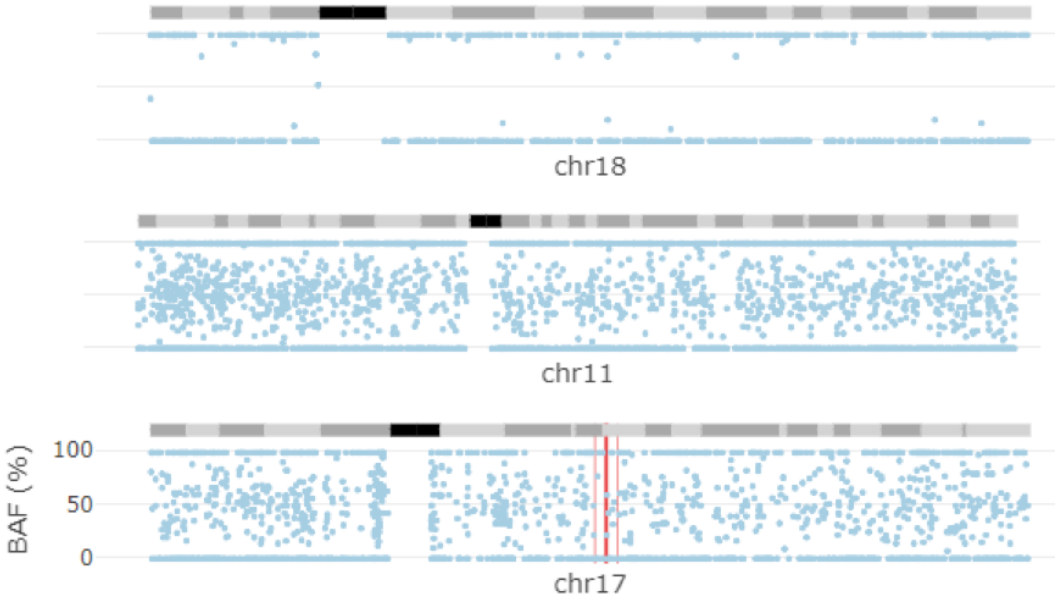
coPGT

BRCA1 variant and
46,XY,t(11;17)(q23.3;q25.1)

GENType - De Witte et al., 2021, submitted







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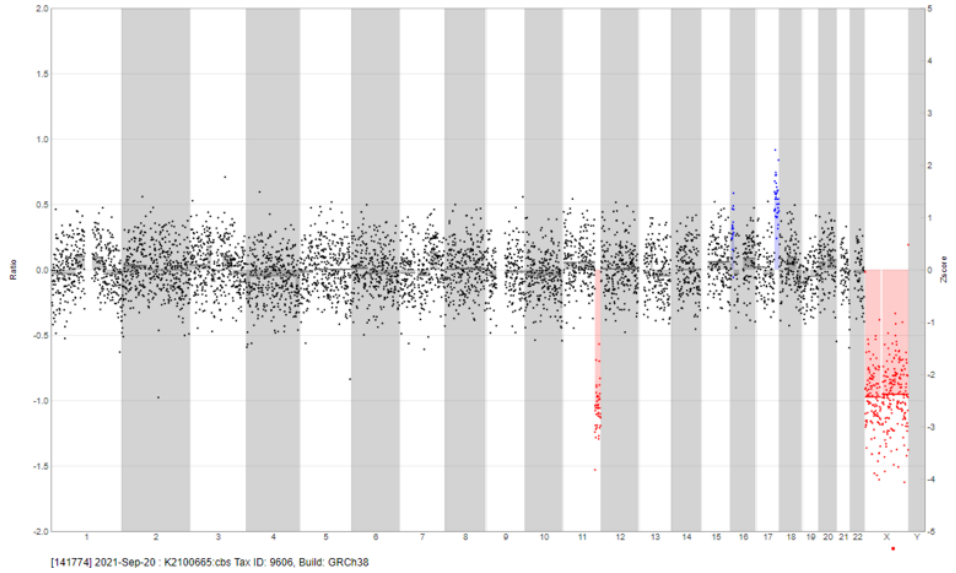

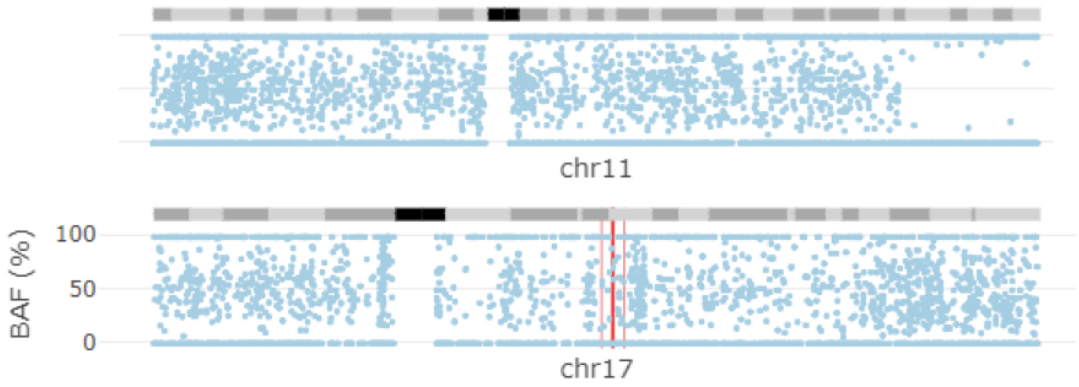

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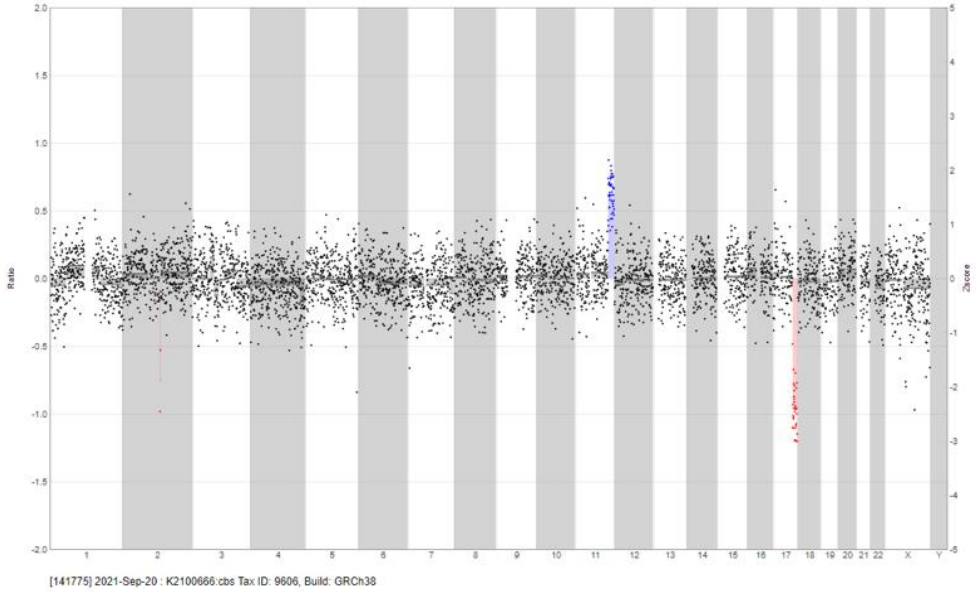

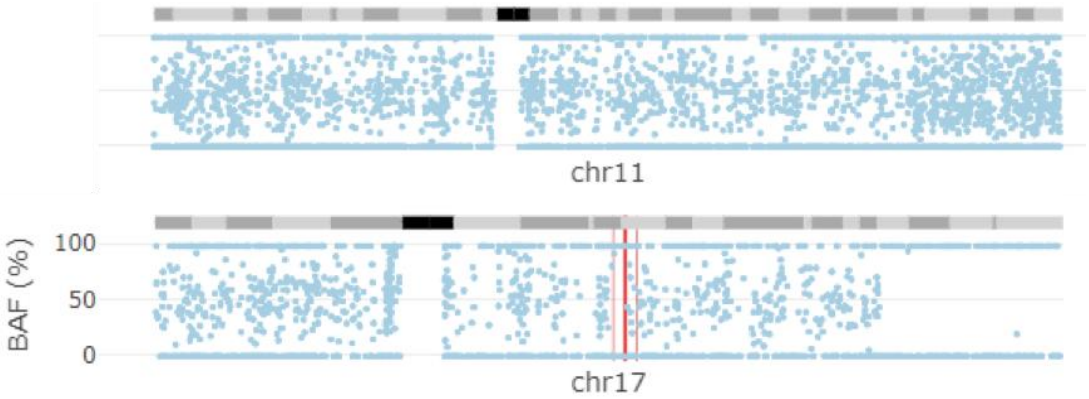

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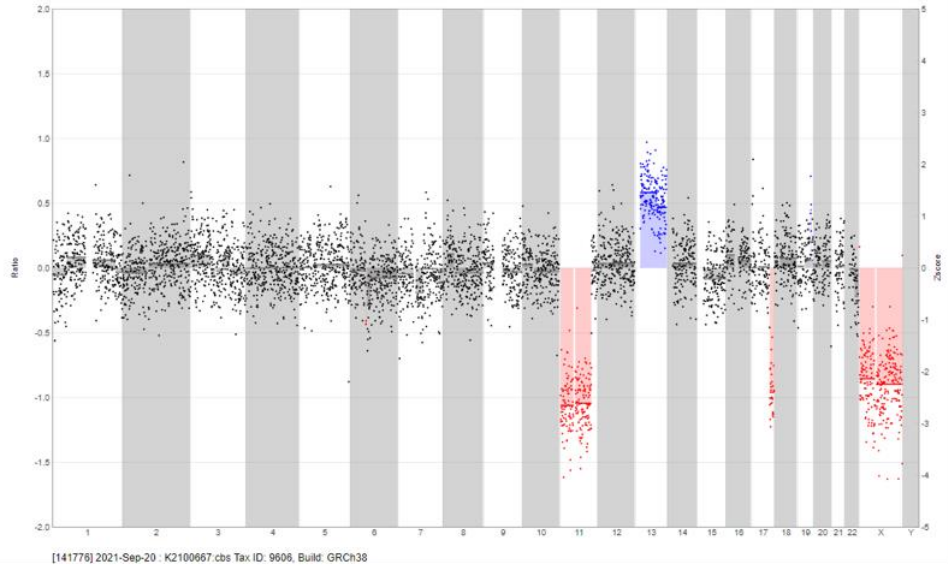

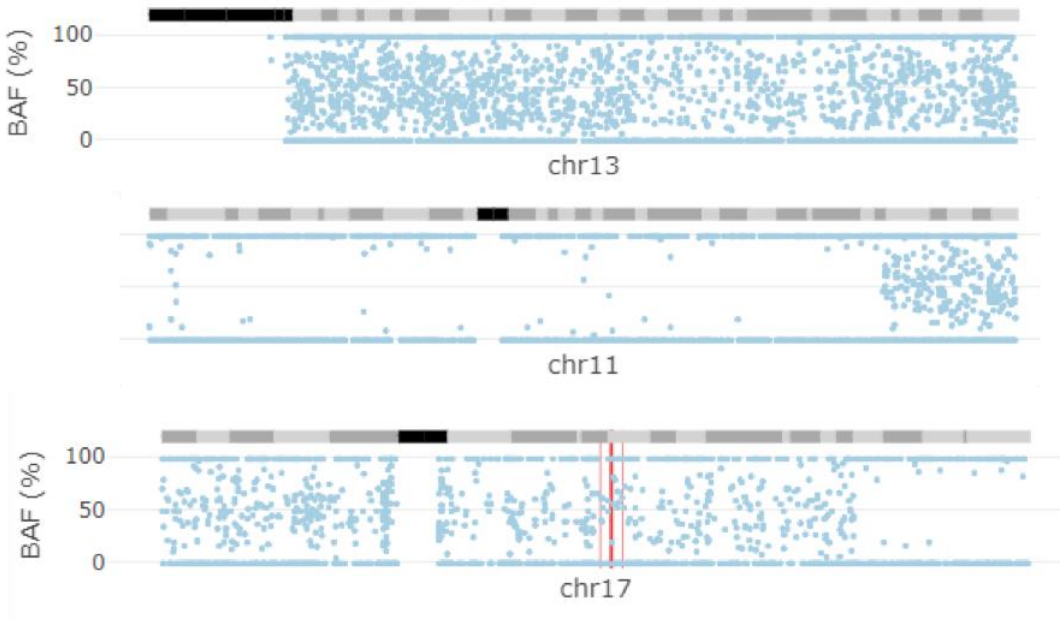

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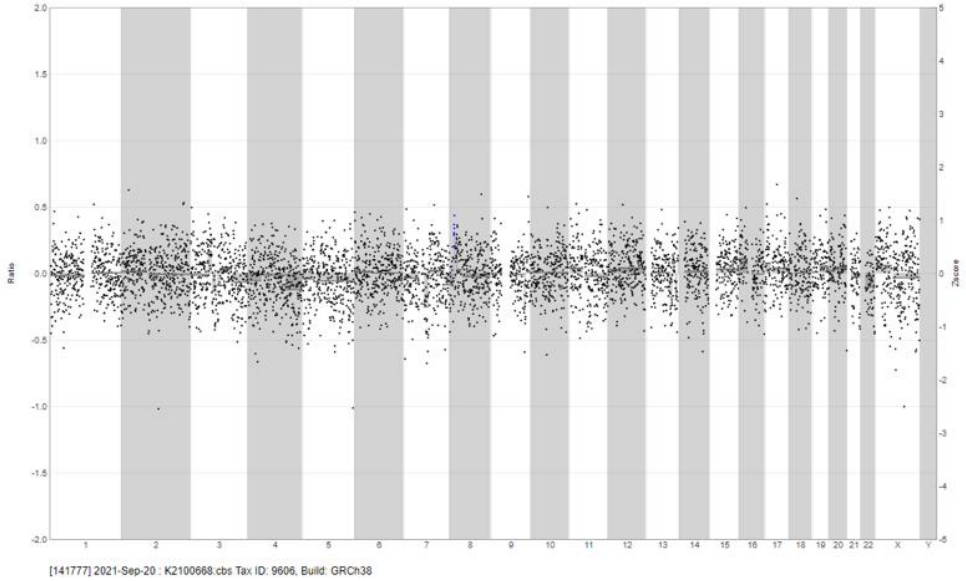
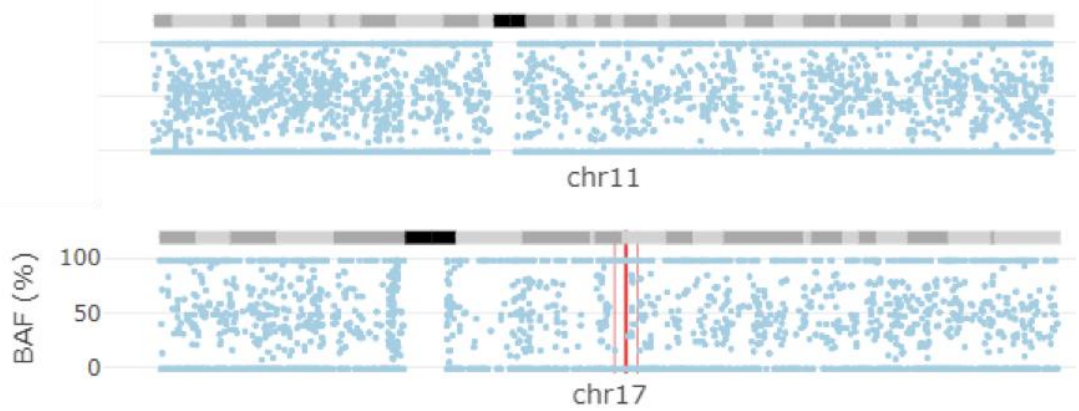


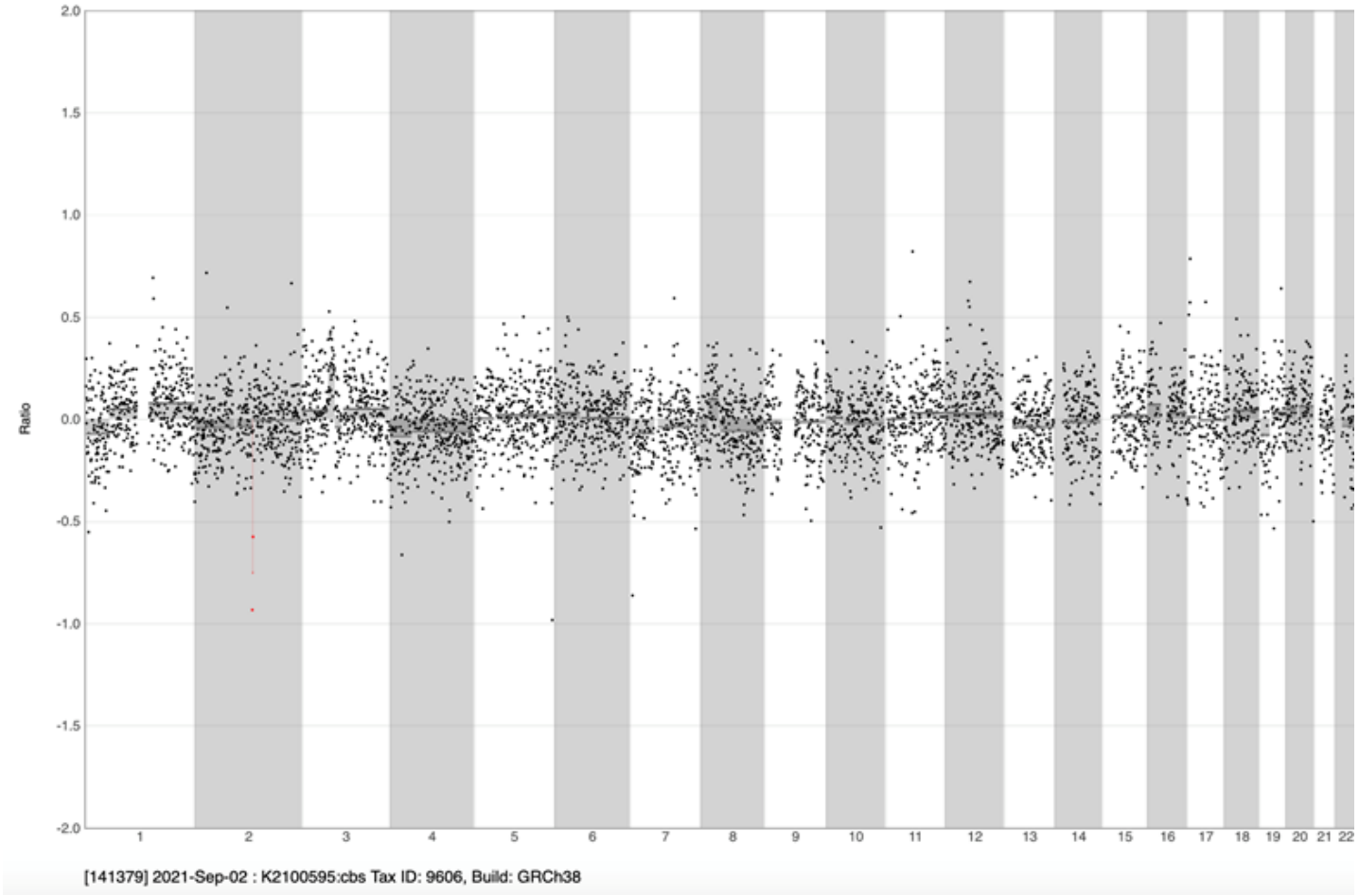


--> transferable!

1/5 embryo's suitable for transfer!

Educational case

coPGT



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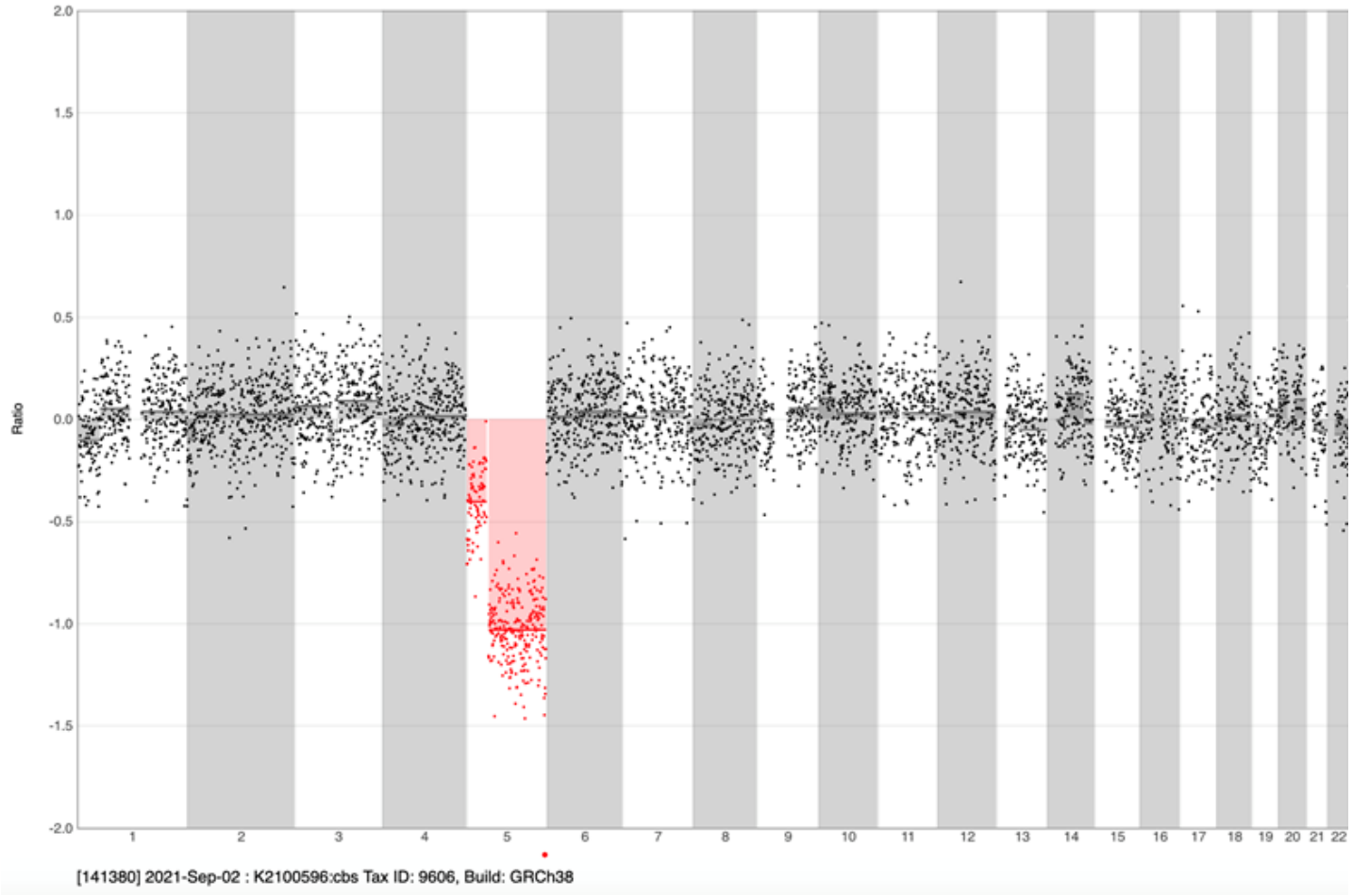
Educational case

coPGT



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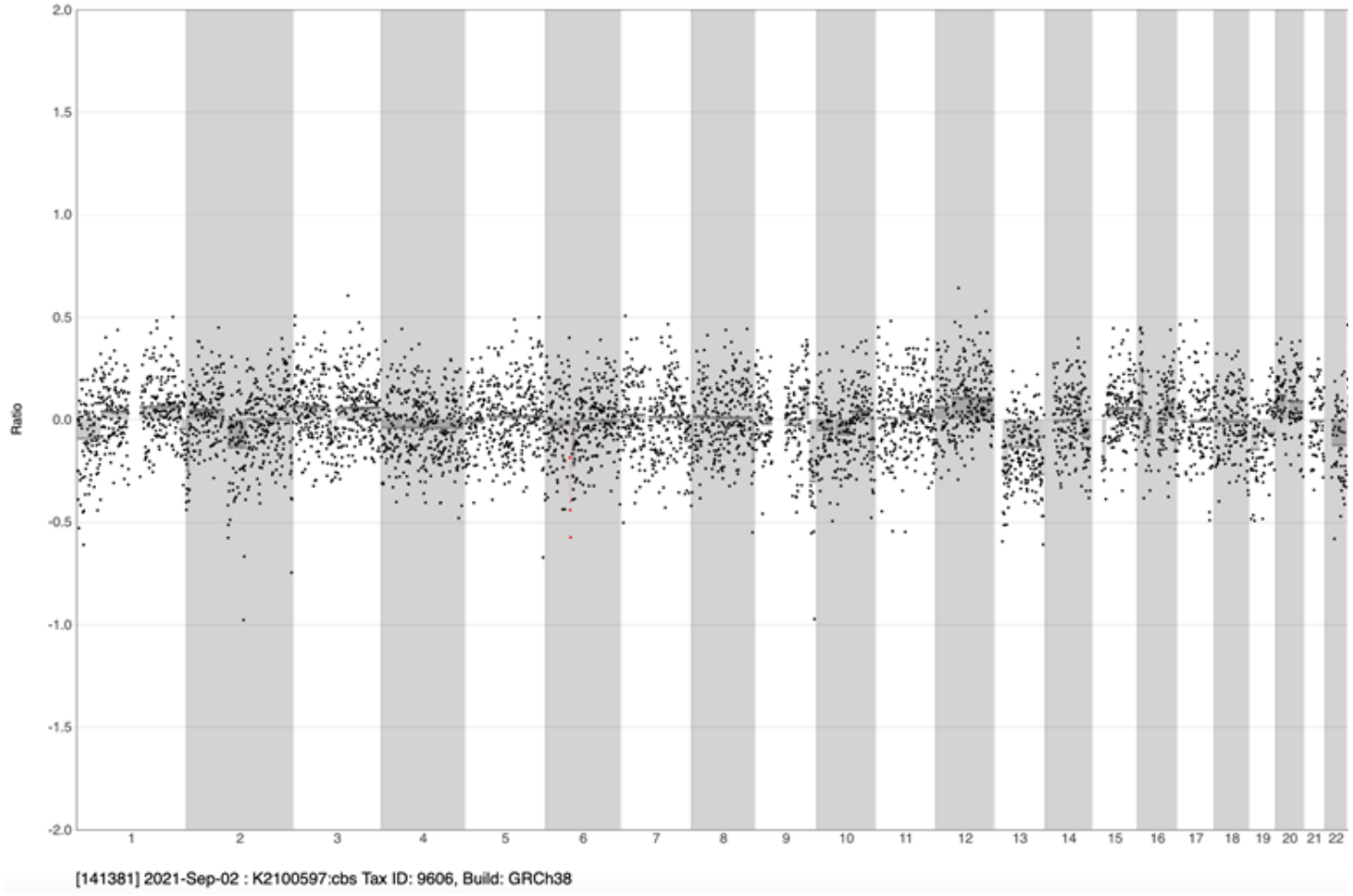
Educational case

coPGT



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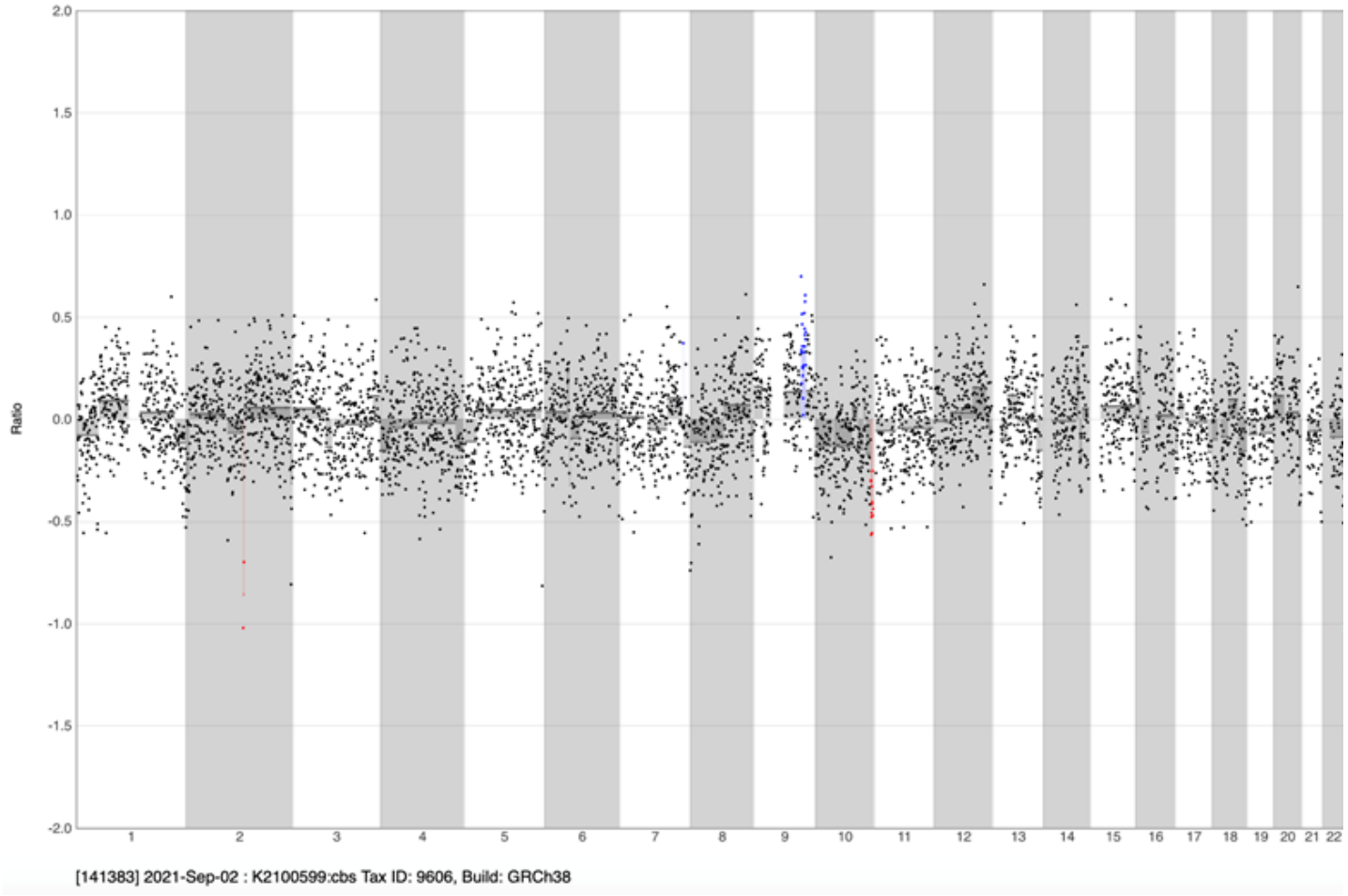
Educational case

coPGT



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Educational case

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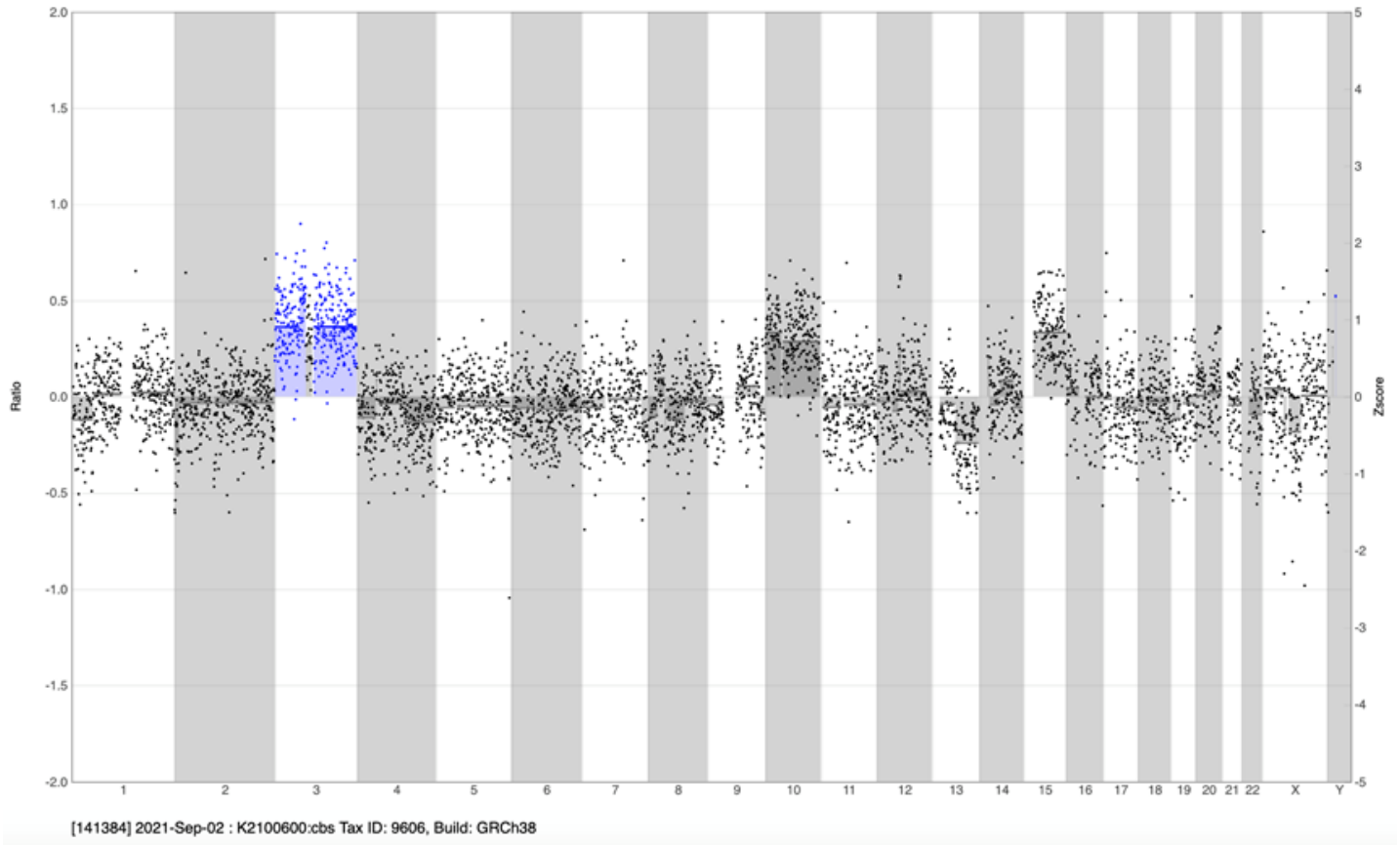

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Educational case

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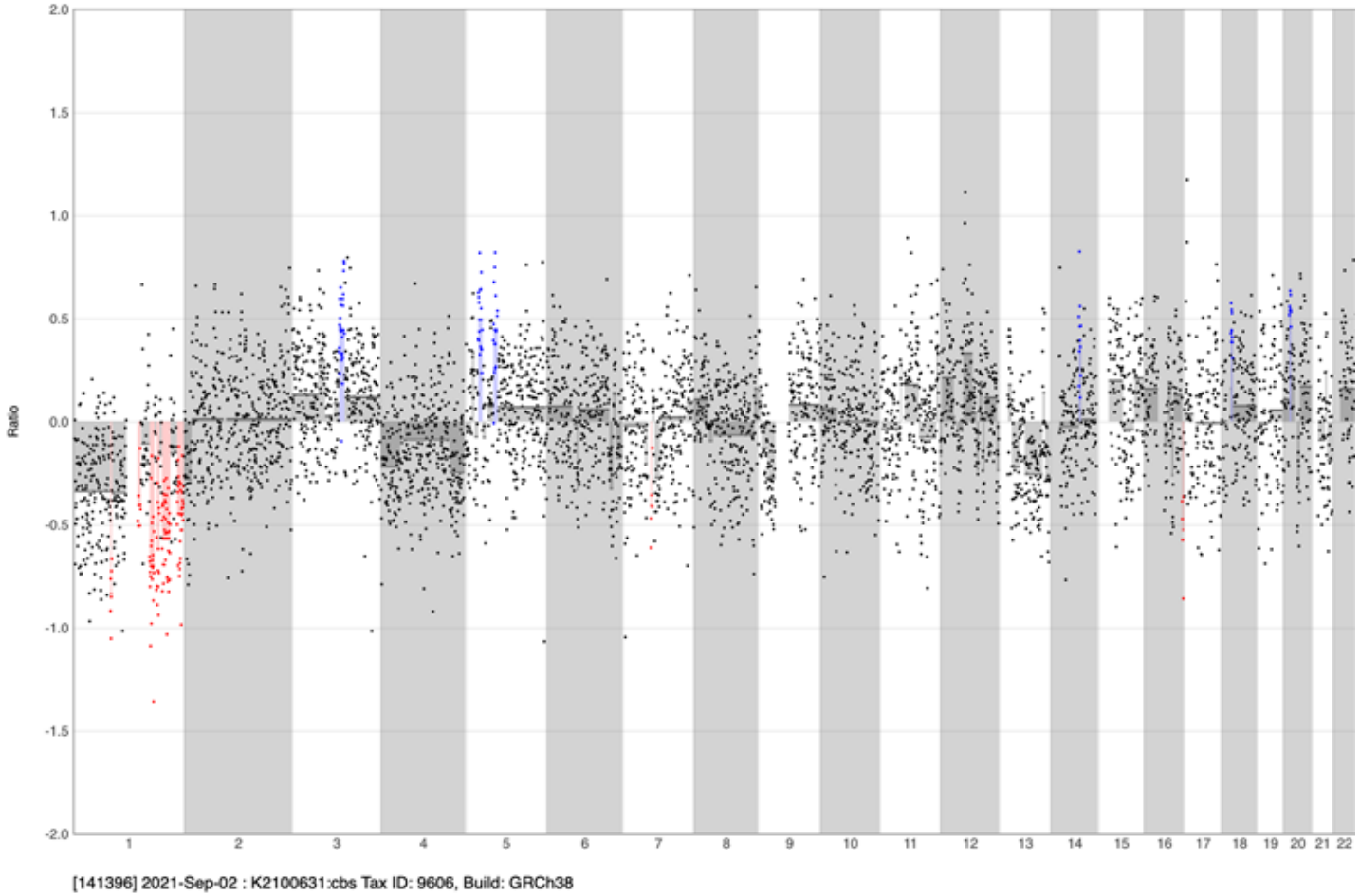

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Educational case

coPGT - Mosaicism

CLASS A: TRANSFER	CLASS B: TRANSFER WITH RANKING and/or AFTER COUNSELING	CLASS C: NO TRANSFER
Euploid embryos	chr X and Y, except for Turner (SCA'S) no transfer unless counseling chr 6, 7, 11, 14 and 15 (UPD chr) chr 2 and 22 chr 1, 3, 4, 5, 10, 12, 17, 19 and 20	chr 8 and 9 chr 13, 18 and 21 chr 16 Turner (monosomy X)

Educational case

Exceptions do occur!

Clinical information: boy 6 months old, microcephaly, bilateral deafness, cheiloschisis, dysmorphy (prominent eyebrows, long eyelashes and large ears), failure to thrive. Brother with Cornelia de Lange syndrome (clinical diagnosis)

→ ID & epilepsy gene panel (WES based)

Heterozygote missense variant in exon 13/25 van het **SMC1A** gen (NM_006306.3): g.53405077G>C (GRCh38/hg38), c.2131C>G, p.(Arg711Gly).

Cornelia de Lange syndrome type 2 (OMIM #300590), X-linked dominant

De novo

→ Class 5

Brother: boy 3 years old, FTT, psychomotor retardation, microcephaly, dysmorphic featurs (synophris, long eyelashes, triangular face)

Clinical question: check presence of SMC1A variant?

Sanger sequencing showed presence of pathogenic variant in brother

Implications: de facto (germline) mosaicism in mother (X-linked)

Excercise

CNV classification

arr[GRCh37] 1q44(247815979_248609997)x1

→ Class 1

- present in multiple individuals in Database of Genomic Variants (DGV).
- Several olfactory receptor genes (including pseudogenes) are located in the deletion, which are not associated with human disease.

arr[GRCh37] 15q13.2q13.3(31073668_32444261)x1

→ Class 5

- 15q13.3 microdeletion syndrome (OMIM # 612001)
- highly variable phenotype
- Individuals with the deletion may have mild to moderate mental retardation or learning difficulties, or may have no cognitive deficits.