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Non-invasive prenatal testing (NIPT) for trisomy 13, 18 and 21; challenging cases and reporting conundrums!

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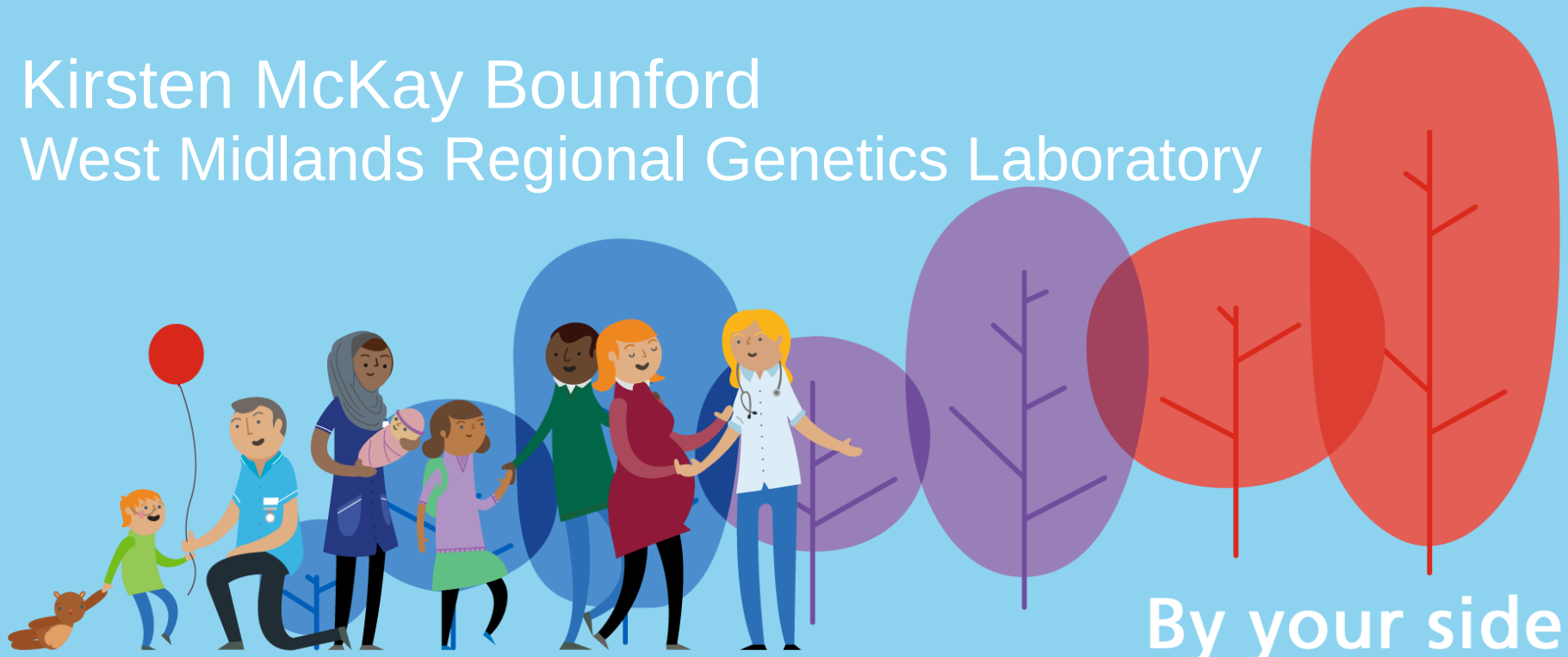

Implementation at WMRGL

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- Non-invasive prenatal testing already established
  - Fetal sexing
  - NIPSIGEN project (for single gene disorders)
- Procurement for technology transfer; Illumina Verifi®
- Method had published literature to show low failure rate and high detection rate
- Implemented September 2015
  - £275 private test
  - 14 calendar day target turnaround time
  - >300 patients tested
  - Some samples sent to Illumina, CA

Verifi® testing strategy

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- Blood collected in Streck tubes (min 10 weeks)
- Extract cell-free DNA from maternal plasma
- Library prep followed by genome-wide NGS

- Normalised chromosome values (ncvs)
- Blood to report in 4 days

Oct 2016- March 2017 n=236

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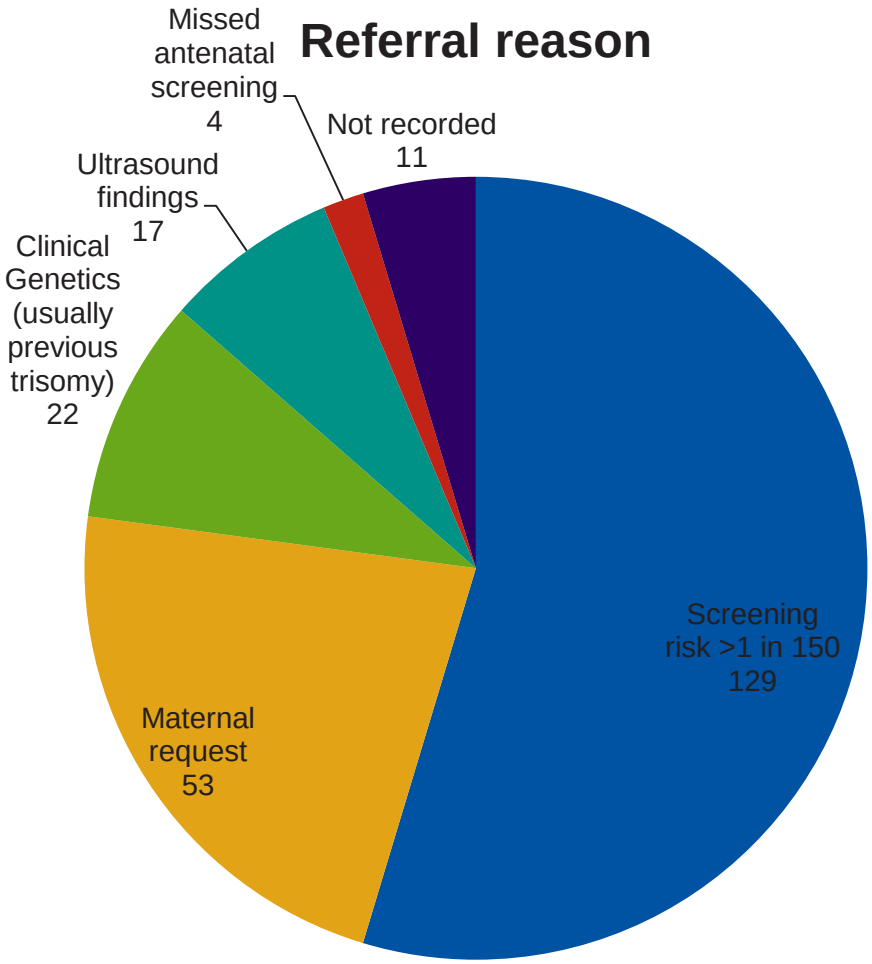
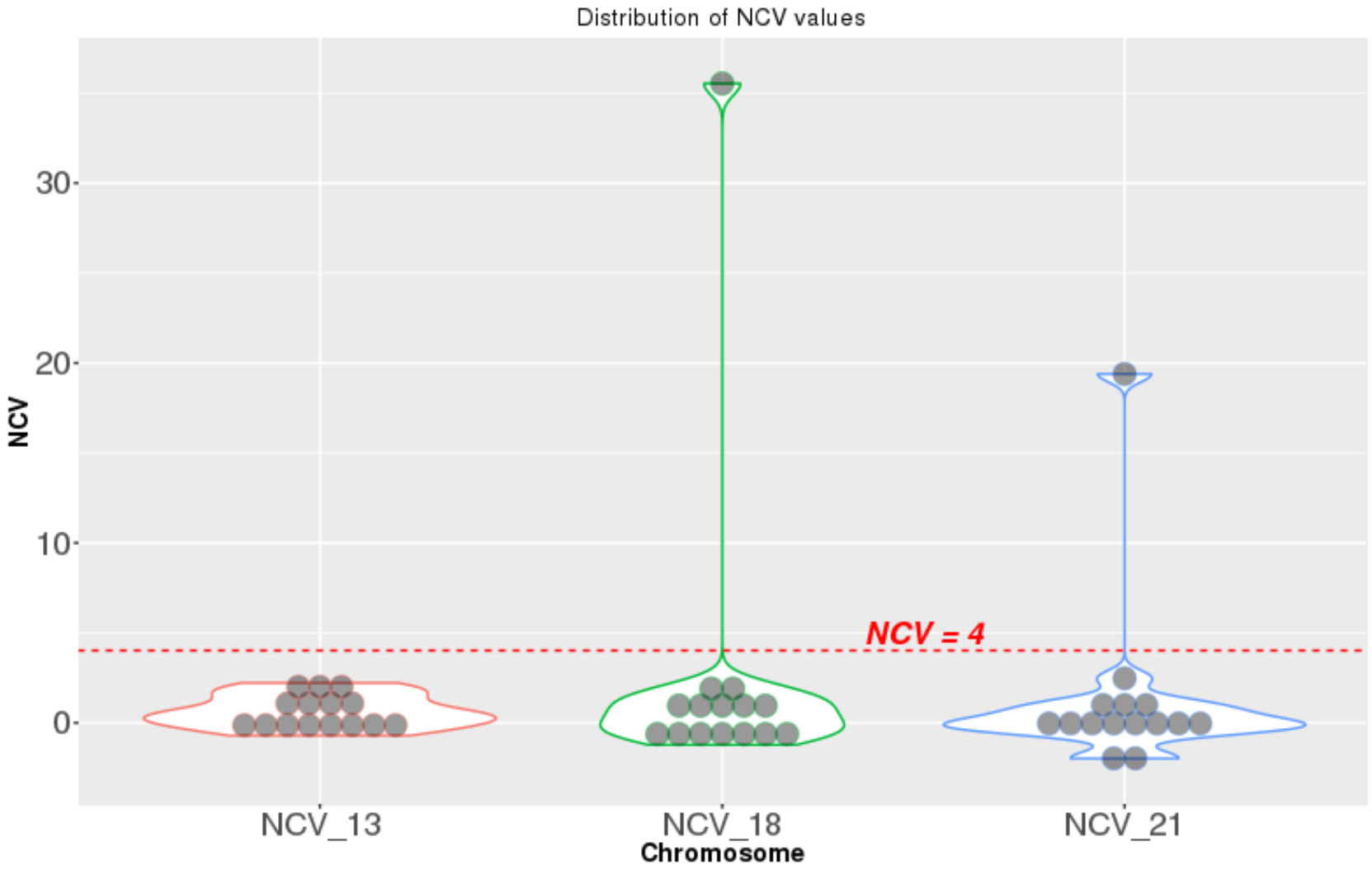


NIPT result	n
No trisomy	223 (95%)
Trisomy 21	9 (4%)
Borderline T21*	1
Trisomy 18	2 (<1%)
Not reportable*	1


Distribution of NCV values
NCV
30
20
10
0
NCV = 4
NCV_13
NCV_18
NCV_21
Chromosome

Negative result

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- Highly unlikely be affected with trisomy 13, 18 or 21.
- This is a screening test, and therefore false positive and false negative results can occur.
- This test is for these chromosomes only and will not detect other numerical or structural abnormalities, small segment imbalance or mosaicism.

Trisomy	Sensitivity	Specificity
21	>99.9% (95% CI 96-100%)	99.8% (95% CI 98.7-100%)
18	97.4% (95% CI 86.2-99.9%)	99.6% (95% CI 98.5-100%)
13	87.5% (95% CI 61.7-98.5%)	99.9% (95% CI 99.2-100%)

Positive – trisomy 21

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- Highly likely to be affected with Down syndrome
- Confirmation of the diagnosis should be offered by chorionic villus sampling or amniocentesis.
- There was no evidence of trisomy for chromosomes 13 or 18.
- Same caveats, exclusions and performance data

Case 1

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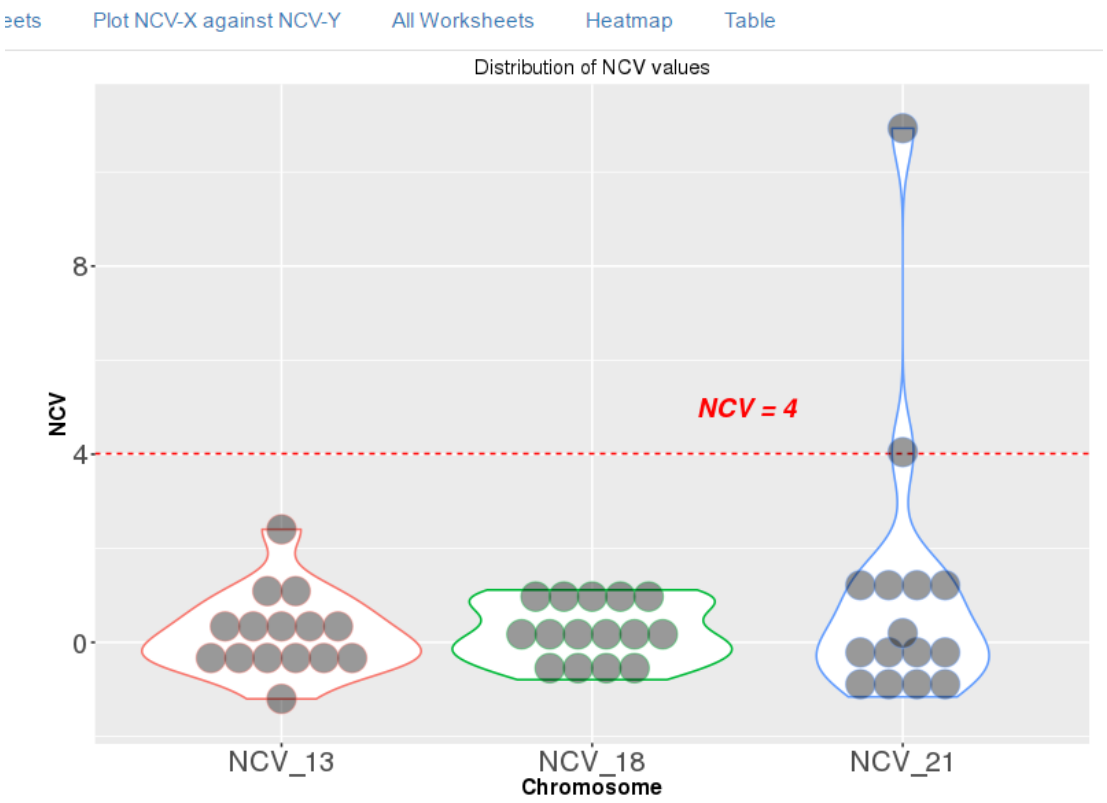
- Age: 37
- Reason: maternal request
- Gestation: 11+3 weeks

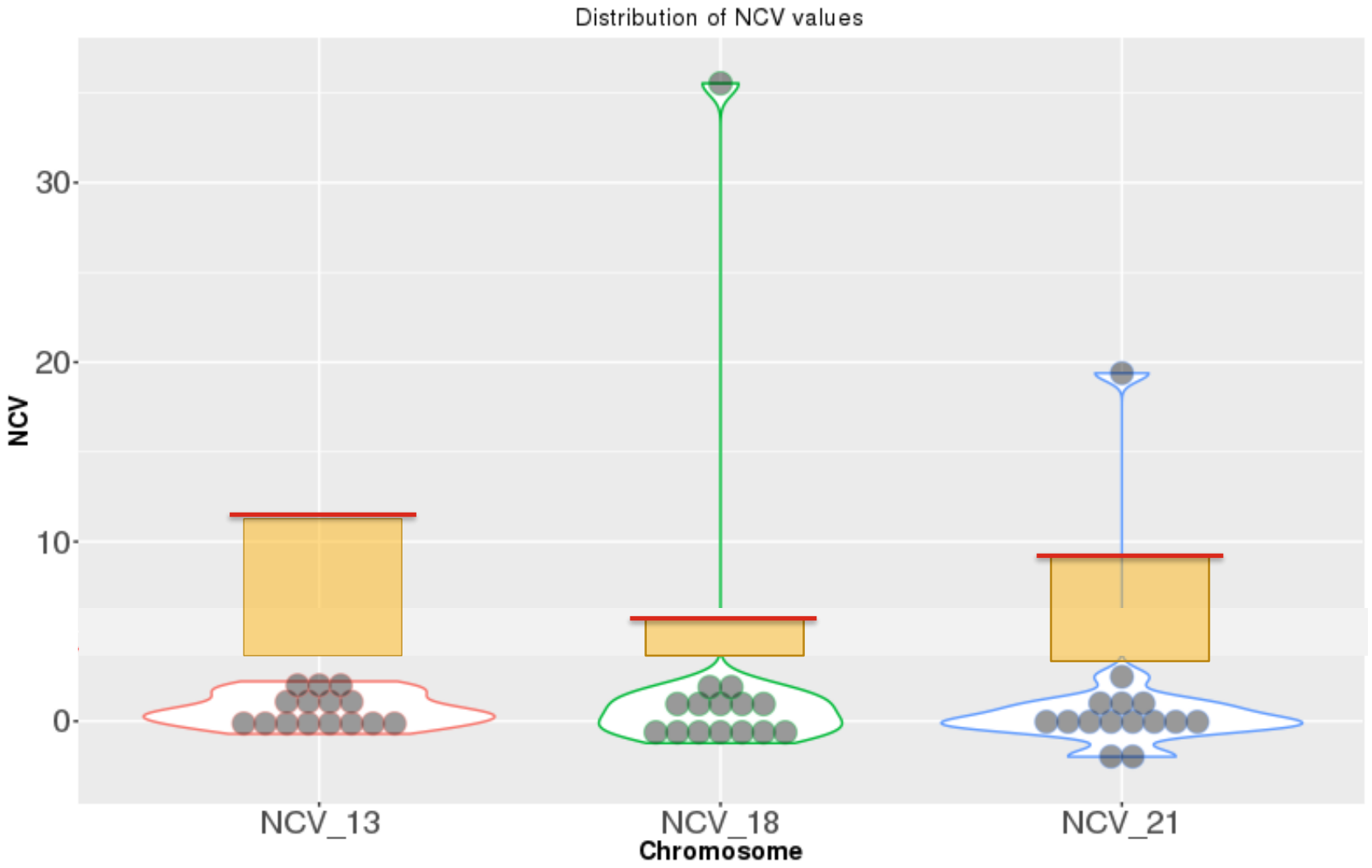


Ch21 NCV 4.044

- Possible explanations?
  - Abnormality at limits of detection; mosaicism, partial trisomy, CNV
  - Repeat NIPT sample not indicated
- Audit of ncv values for all samples tested where positive or negative results were known.
  - Positive and negative distinct clusters
  - Borderline ranges established for each chromosome

Chr	Lower threshold	Upper threshold
13	3.5	11.68
18	3.5	5.65
21	3.0	9.56

- NCVs falling into these ranges are discussed with wider team
- Data reviewed periodically to refine ranges


Distribution of NCV values
NCV
30
20
10
0
NCV_13
NCV_18
NCV_21
Chromosome

Intermediate report

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- Increased risk of trisomy 21
- The results of non-invasive prenatal testing (NIPT) showed an increased risk of trisomy for chromosome 21. The current pregnancy is therefore at an increased risk of Down syndrome. For definitive diagnosis, chorionic villus sampling or amniocentesis should be offered.

Case 2

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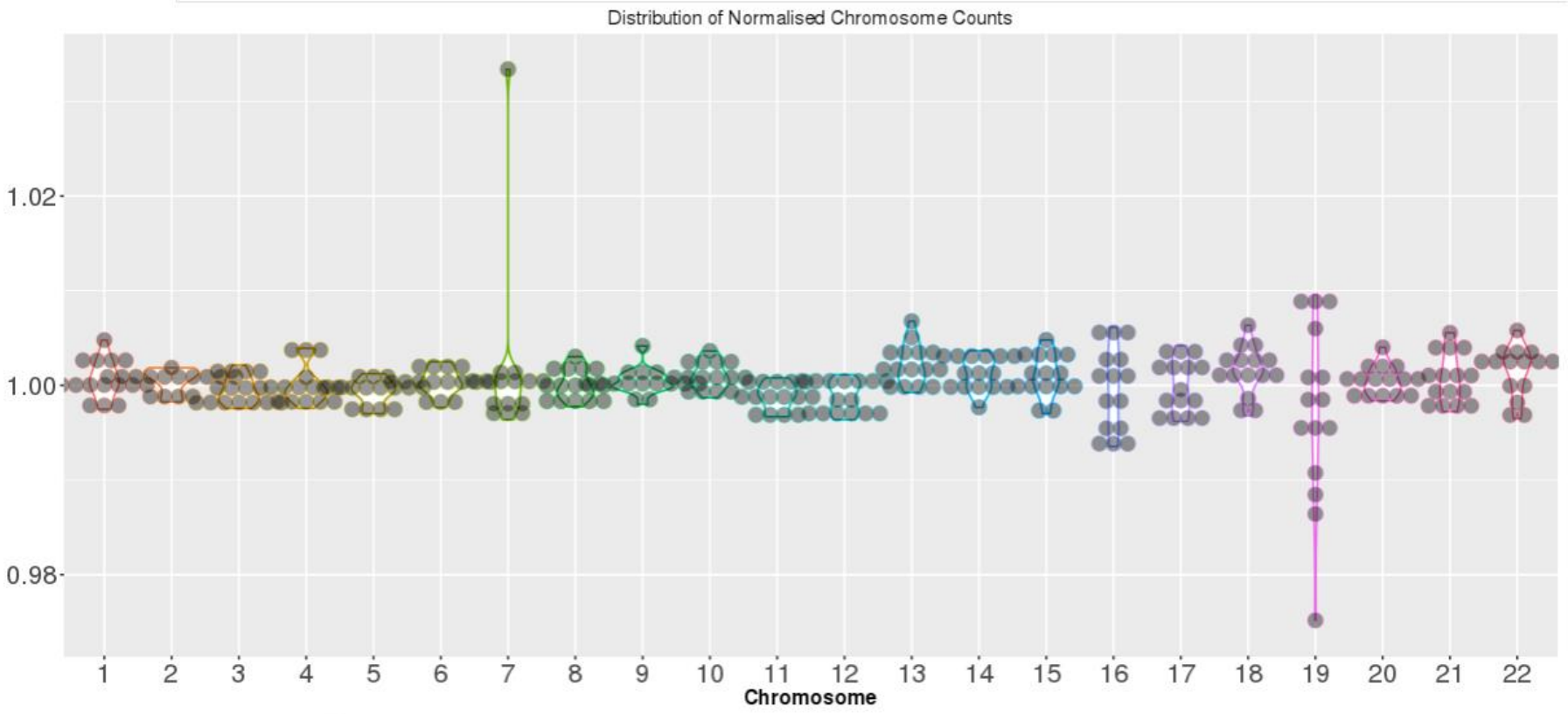
- Age: 41
- Reason: first trimester screening risk 1 in 125
- Gestation: 13+5 weeks
- NIPT result: No NCVs generated



Case 2

- Possible explanations?
  - Mosaicism/confined placental mosaicism for trisomy 7, vanishing twin
- Significance?
  - May be a risk for placental insufficiency
  - Repeat NIPT sample not indicated
- Assay not validated to detect other trisomies

- NIPT has failed to give a reportable result.

- Implicated an imbalance elsewhere in the genome.
- Indicated this may represent a benign change in the placenta, or may have implications for the pregnancy.
- Recommended follow-up by ultrasound scan, and patient should be offered invasive testing by amniocentesis.

Outcome: Male infant born at 37 weeks, SGA, otherwise healthy

Case 3

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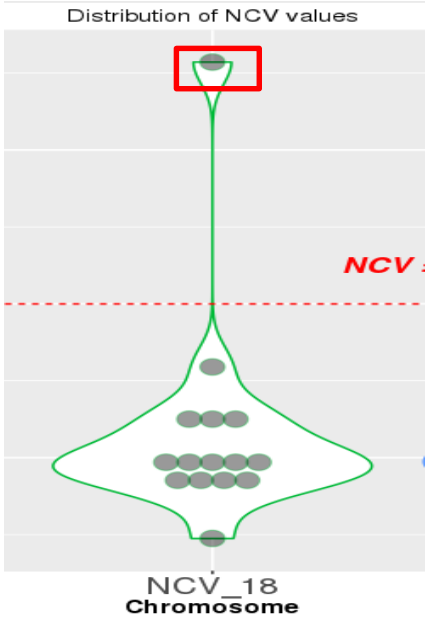
- Age: 36
- Reason: first trimester screening risk 1 in 7
- Gestation: 13 weeks
- NIPT sample sent to Illumina
  - Highly likely to be affected with Edwards syndrome

- Amniotic fluid was taken 15+3 weeks
- QF-PCR – no evidence of trisomy 13, 18, 21 or sex chromosome aneuploidy
- Results confirmed on second AF aliquot

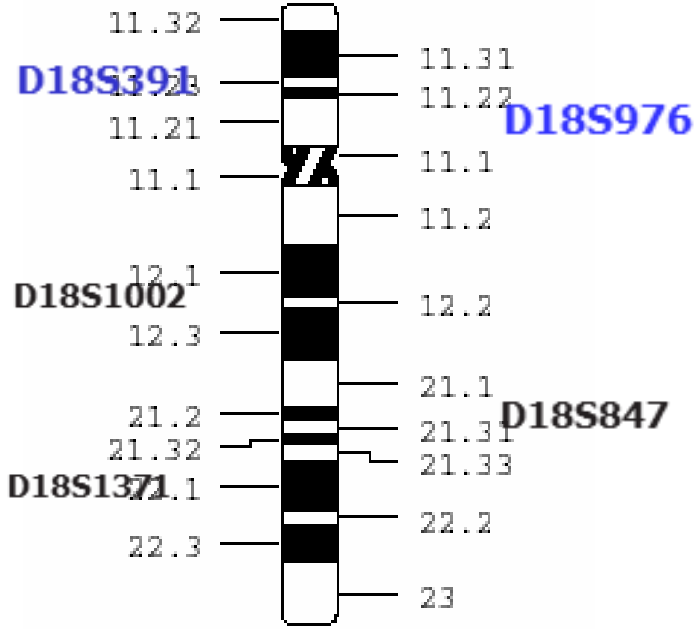
- Microarray – No significant copy number imbalance detected
- Karyotype – 46, XX



- Second NIPT sample received
  - Tested in-house NCV 18 10.28

- Possible explanations?
  - Confined placental mosaicism, vanishing twin
  - Maternal mosaicism for T18, benign CNV; excluded by microarray

- Apparently healthy baby girl born
  - Cord blood and 5 pieces of placenta
- Cord blood
  - QF-PCR showed no trisomy
  - Targeted microarray – no evidence of trisomy 18
  - Karyotype – 46, XX
- Placenta
  - QF-PCR showed trisomy for markers on 18p
  - Microarray - profile consistent with trisomy 18p
  - Cultures failed



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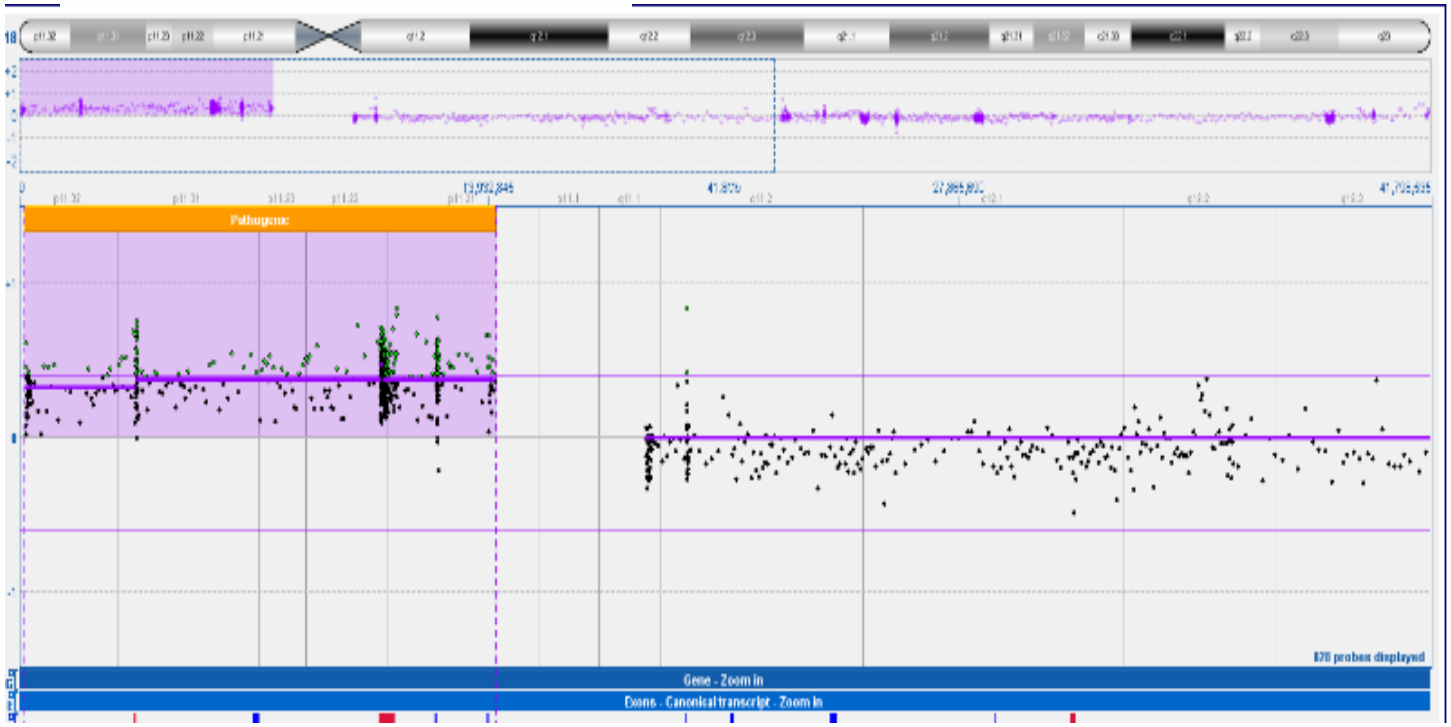
Parental samples?

- No evidence of a predisposing balanced chromosomal rearrangement



Case 3

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- Possible explanations?
  - Contribution of trisomy 18p led to increased NCV and positive result
  - CPM, mosaicism but low level in baby

Reports

- Baby – no clinically significant copy number imbalance detected
- Placenta – trisomy 18p most likely CPM
- Low recurrence risk

Case 4

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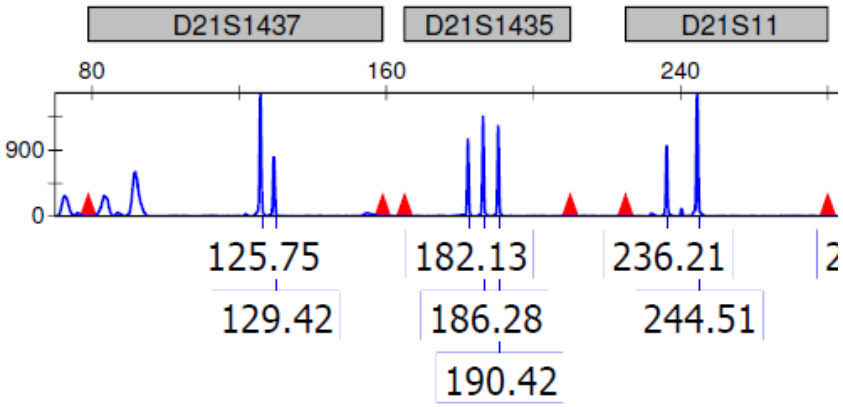
- Age: 35
- Reason: NT 3.8mm
- Gestation: 13+1 weeks
- NIPT sample sent to Illumina
  - Highly unlikely to be affected with trisomy 13, 18 or 21
- Blood received from neonate with same surname and address
  - Reduced tone, epicanthal folds, single palmar crease, ?T21
- Karyotype showed abnormal mosaic result
  - 25 cells with 47, XX, +21
  - 5 cells with 46, XX

- QF-PCR showed evidence of trisomy 21 with 3 informative markers



- Possible explanations
  - Mosaicism in placenta at level below limits of test
  - True false negative

- Informed Illumina lab, CA
  - NCV not reported but within 'normal' range
  - NIPT test repeated and same result obtained
- Letter to clinician acknowledging this result with possible explanations
- NT 3.8mm, therefore this patient should have been offered invasive testing

Conclusions

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- Mostly NIPT is extremely straightforward
- Occasionally not, but these situations are mostly predictable
  - NIPT is Screening, so false results will occur
  - Mosaicism/CPM/partial trisomy may not be

Recommended practice for laboratory reporting of non-invasive prenatal testing of trisomies 13, 18 and 21: a consensus opinion

Zandra C. Deans[1]*, Stephanie Allen[2], Lucy Jenkins[3], Farrah Khawaja[1], Ros J. Hastings[4], Kathy Mann[5], Simon J. Patton[6], Erik A. Sistermans[7] and Lyn S. Chitty[8]

- EQA and Best Practice important!

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