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Introducing array-CGH into routine prenatal diagnosis practice: a prospective study on 1900 consecutive clinical cases

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Array-CGH on prenatal samples

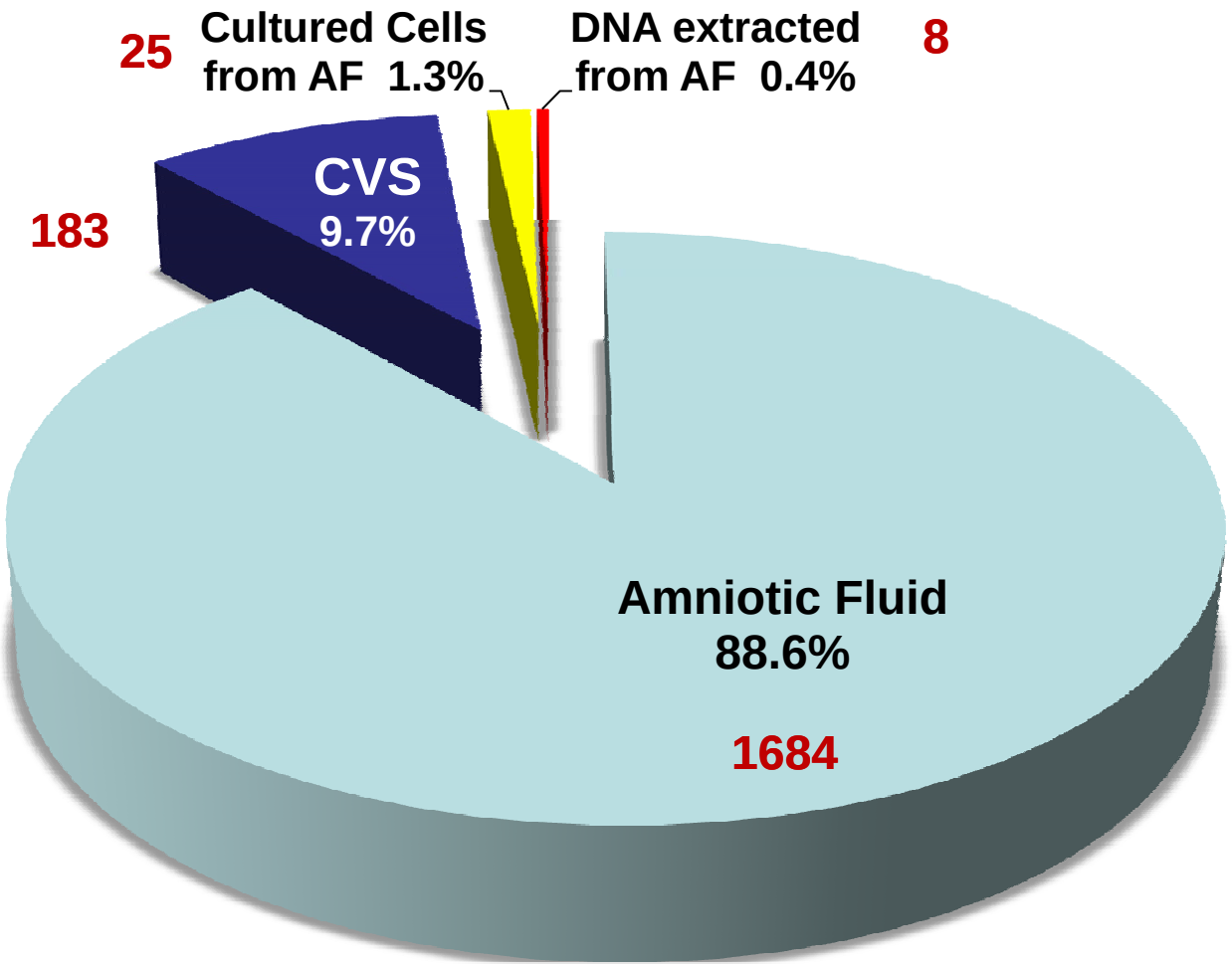
- aCGH is a useful assay for detection of common and submicroscopic chromosome abnormalities, widely used in the pediatric population as a **first-line test** in place of traditional karyotype analysis.
- While experience with aCGH in the pediatric patients is extensive, experience with its use for clinical **prenatal diagnosis** is still relatively limited.
- Published studies exploring aCGH usefulness **on prenatal samples**:
  - **retrospective** (Rickman *et al*., 2006; Le Caignec *et al*., 2005)
  - **prospective** (Sahoo et al., 2006; Shaffer et al., 2008; Kleeman et al. 2009; Coppinger et al., 2009; Van den Veyver *et al*., 2009; Maya et al., 2010)
- **reduced cohort of samples** processed (a total of 1112);
- Need of larger population-based **prospective trials** before aCGH can be recommended for routine clinical use in a prenatal diagnosis setting as a **first-line test** (ACOG Committee Opinion no. 446, 2009).

Aim of the study

- To perform a **prospective blind study**, comparing the results obtained using a BAC-based aCGH platform with those obtained from a standard G-banding karyotype.
- We aimed to assess the **feasibility** of offering aCGH in prenatal diagnosis on routine basis.
- Issues to address:

  1) aCGH **accuracy** in detection of common and submicroscopic chromosome abnormalities in prenatal samples;
  2) if the technique **improves** the detection rate of genetic aberrations or, on the contrary, whether aCGH **misses** potential pathogenic chromosomal abnormalities, compared with conventional karyotyping;
  3) if there is an increase in **results of unclear clinical relevance**;
  4) whether aCGH should be applied to all prenatal samples as **first-line test** or its use should be **limited to specific indications** (e.g., in cases of abnormal ultrasound findings but normal karyotype).

Prenatal samples analysed



1900 prenatal samples (referred from October 2010 to September 2011)

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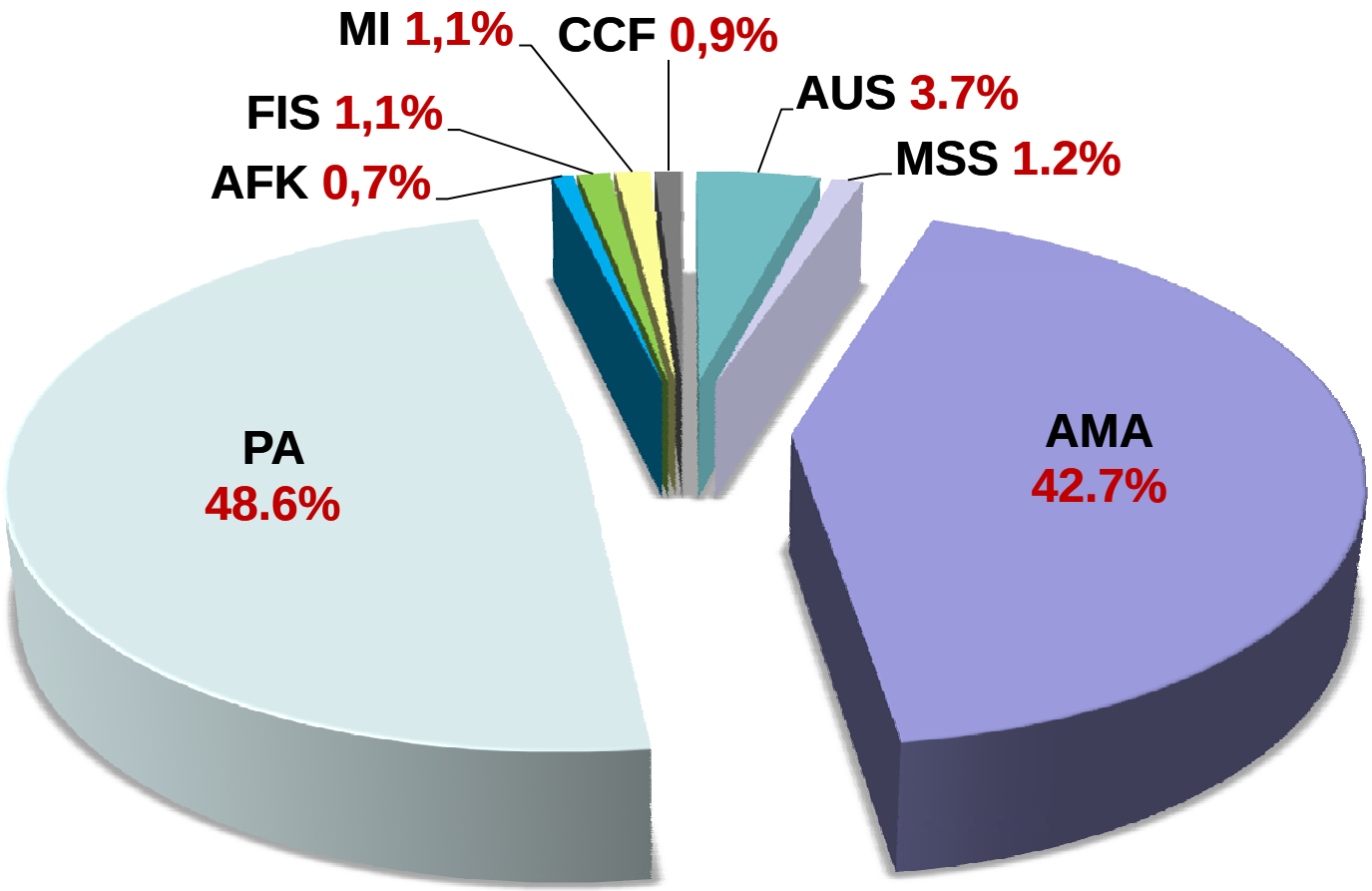
Indication for prenatal diagnosis



AMA: advanced maternal age
AUS: abnormal ultrasound findings
PA: parental anxiety
AFK: a known abnormal fetal karyotype

MSS: Abnormal maternal serum screening test
FIS: Family history of a genetic condition or chr. abn.
CCF: Cell culture failure
MI: Multiple indications

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DNA recovery from prenatal samples

- Potential limitations on the use of the aCGH assay on prenatal samples:
  - inability to isolate **sufficient quantities of fetal DNA**, especially from AF specimens;
  - **suboptimal quality of DNA** isolated from prenatal samples, due to the presence of dead cells, small degraded DNA fragments, and other unknown inhibiting factors.
- All prenatal samples that were processed in this study:
  - yielded **sufficient DNA** for successful aCGH analysis (**99 ng/ml** AF);
  - provided **high-quality profiles** with as little as **28 ng**.

DNA recovery from prenatal samples

	Amniotic Fluid (AF)				CVS[#]	All samples
	Direct AF*		Cultured amniocytes	DNA from uncultured amniocytes		
	ng/ml	Total				
Average DNA quantity (+SD) in aCGH		264 (±109)	291 (±121)	188 (±65)	397 (±28)	276 (±111)
- Min		28	92	94	222	28
- Max		510	399	244	498	510
Average quantity (+SD) of extracted DNA	99 (±98)	496 (±492)	705 (±643)	255 (±89)	2894 (±2420)	712 (±1100)
-Min	7	36	120	123	306	36
- Max	1694	8482	1947	318	12807	12807

* 5 ml of Amniotic Fluid
[#] 2 mg CVS

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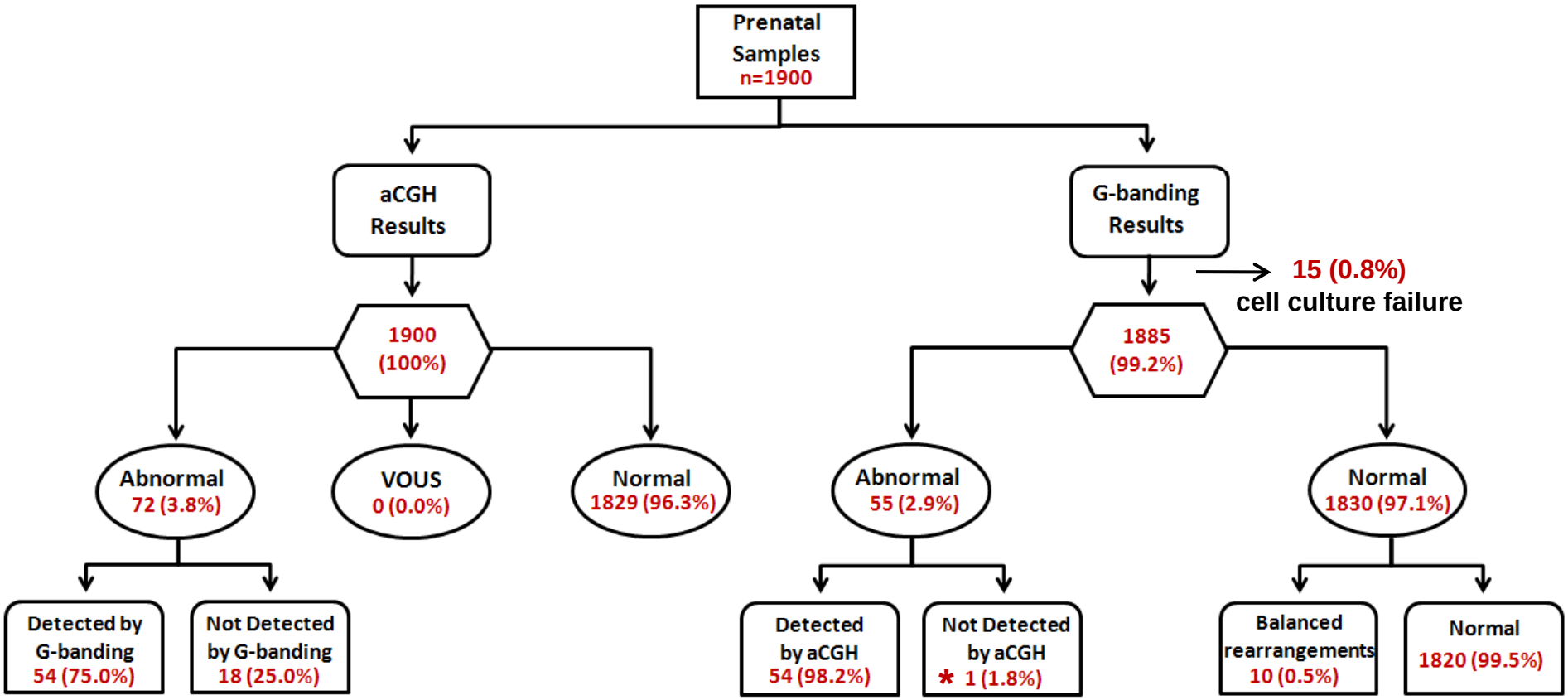
aCGH results turnaround time

- aCGH using direct DNA extraction from prenatal samples also led to **rapid turnaround time** (**2.5** working days), an important issue for prenatal diagnosis.

Chromosome abnormality type	Average turnaround time* (SD)	Min	Max
Normal	**2.4 (±0.5)**	**2**	**3**
Abnormal results with microscopic aberrations	**2.2 (±0.4)**	**2**	**3**
Abnormal results with submicroscopic aberrations	**6.3 (±1.0)**	**5**	**7**
Total	**2.5 (±0.6)**	**2**	**7**

* Working days

Results



* *In vitro* artefact

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Array-CGH results according to the indication

Indication	No. Samples analysed	No. Samples with chr. abnormalities	No. Samples with chr. Abnormalities not detectable by conventional karyotyping	aCGH detection rate	
				% whole samples	% abnormal results
Abnormal ultrasound findings	70	22 (31.4%)	5	7.1%	22.7%
Abnormal results of maternal serum screening tests	23	3 (13.0%)	0	0%	0%
Advanced maternal age	811	28 (3.5%)	6	0.7%	21.4%
Parental anxiety	**924**	**18 (1.9%)**	**7**	**0.8%**	**38.9%**
Known abnormal fetal karyotype	14	1 (7.1%)	0	0%	0%
FIS +CCF+MI	58	0 (0%)	0	0%	0%
Totale	**1900**	**72 (3.8%)**	**18**	**0.9%**	**25.0%**

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Array-CGH results according to the indication

Indication	No. Samples analysed	No. Samples with chr. abnormalities	No. Samples with chr. Abnormalities not detectable by conventional karyotyping	aCGH detection rate	
				% whole samples	% abnormal results
Abnormal ultrasound findings	**70**	**22 (31.4%)**	**5**	**7.1%**	**22.7%**
AMA + MSS + PA + others	**1830**	**50 (2.7%)**	**13**	**0.7%**	**26.0%**
Totale	**1900**	**72 (3.8%)**	**18**	**0.9%**	**25.0%**

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Results comparison between G-banding and array-CGH

Sample type	No. of samples	Indication	Chromosomal findings		Concordance	Final diagnosis
			G-banding results	aCGH result		
AF-CVS	27	AMA, MSS, AUS, PA	47,XX,+21 or 47,XY,+21	47,XX,+21 or 47,XY,+21	Y	Trisomy 21
AF-CVS	8	AMA, MSS, AUS, PA	47,XX,+18 or 47,XY,+18	47,XX,+18 or 47,XY,+18	Y	Trisomy 18
AF-CVS	2	AMA - AUS	47,XX,+13	47,XX,+13	Y	Trisomy 13
AF	2	AMA	47,XYY	47,XYY	Y	47,XYY
AF	1	AMA	47,XXX	47,XXX	Y	Trisomy X
AF	1	PA	45,X	45,X	Y	Monosomy X
CVS	1	AUS	46, XY,18p-	46, XY,18p-	Y	18p Deletion
AF	1	AUS	46,XY,del(8)(p22p21.1)	46,XY,del(8)(p22p21.1)	Y	Del. p22-p21.1
AF	1	PA	46,XX,dup(15)(q21.2q25.2)	46,XX,dup(15)(q21.2q25.2)	Y	Dup 15q21.2-q25.2
CVS	1	AMA	46,XX (80%) /47,XX+7(20%)	47,XX+7 mosaic	Y	Trisomy 7 mosaic*
AF	2	AMA	46,XX (80%) /45,X(15%) 46,XX (90%) /45,X(10%)	45,X mosaic	Y	Monosomy X mosaic
AF	1	AUS	46,XY (65%) /47,XXY(35%)	47,XXY mosaic	Y	XXY Mosaic
CVS	1	AMA	46,XX (80%) /47,XX+5p(20%)	47,XX+5p mosaic	Y	Trisomy 5p mosaic*
CVS	1	AUS	46,XY (80%) /47,XY+19(20%)	47,XY+19 mosaic	Y	
AF	1	AUS	46,XX (94%) /47,XX+6p(6%)	47,XX+6p mosaic	Y	Trisomy 6p mosaic
CVS	1	MSS	46,XY(80%) /47,XY+18(20%)	47,XY+18 mosaic	Y	Trisomy 18 mosaic
AF	1	AUS	46,XX(65%)/47,XX+22(35%)	47,XY+22 mosaic	Y	Trisomy 21 mosaic
AF	1	PA	46,XX (16%) /47,XX+20(84%)	46, XX	N	46,XX§
CA	1	AMA, AK	Suspected duplication 5q	46,XY,dup(15)(q24.2q26.3)	N	Dup.15q24.2-qter

* Normal after AF karyotyping

§ *in vitro* artefact

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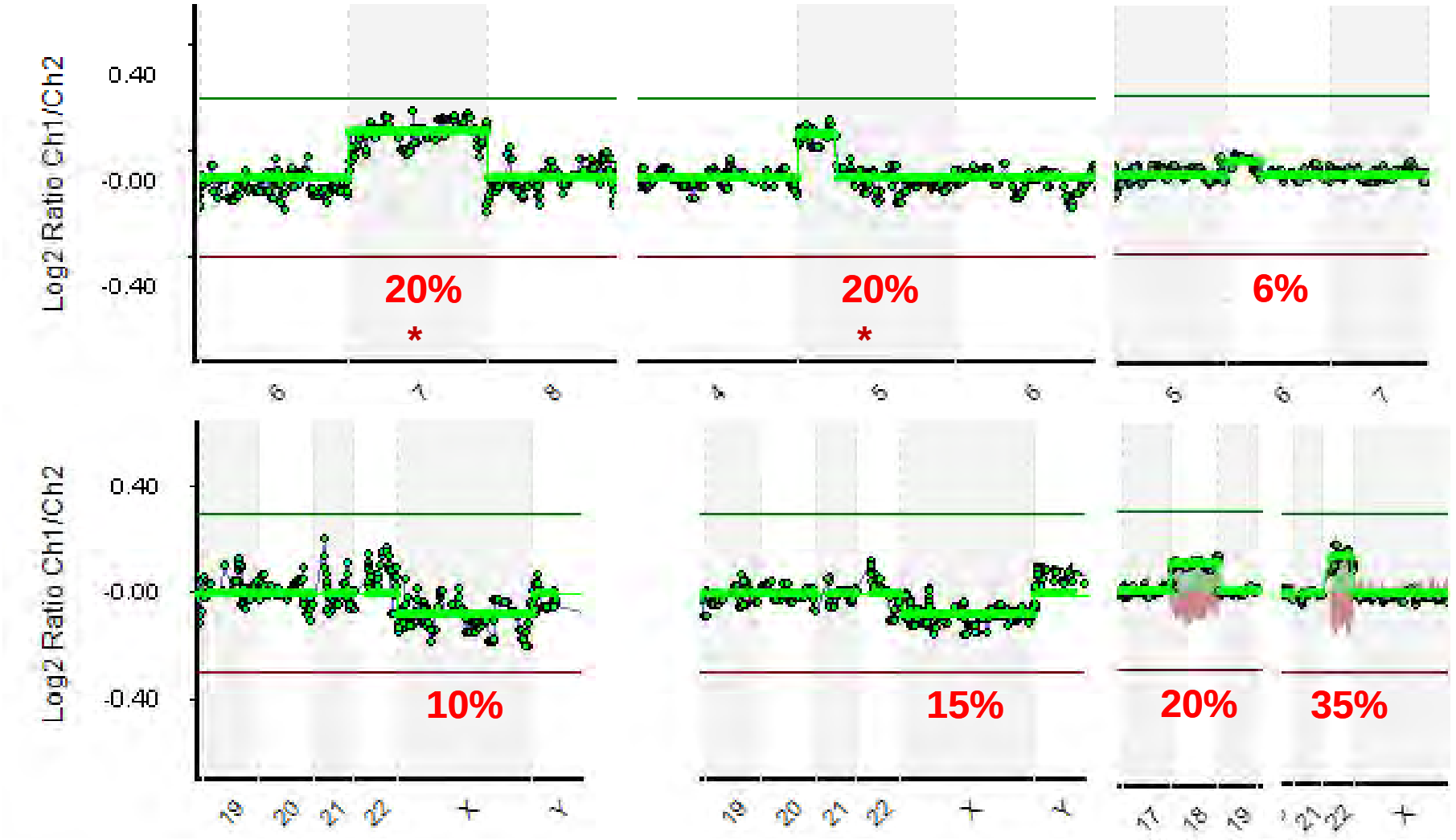
Examples of chromosomal mosaicism in prenatal samples



* Normal after AF karyotyping

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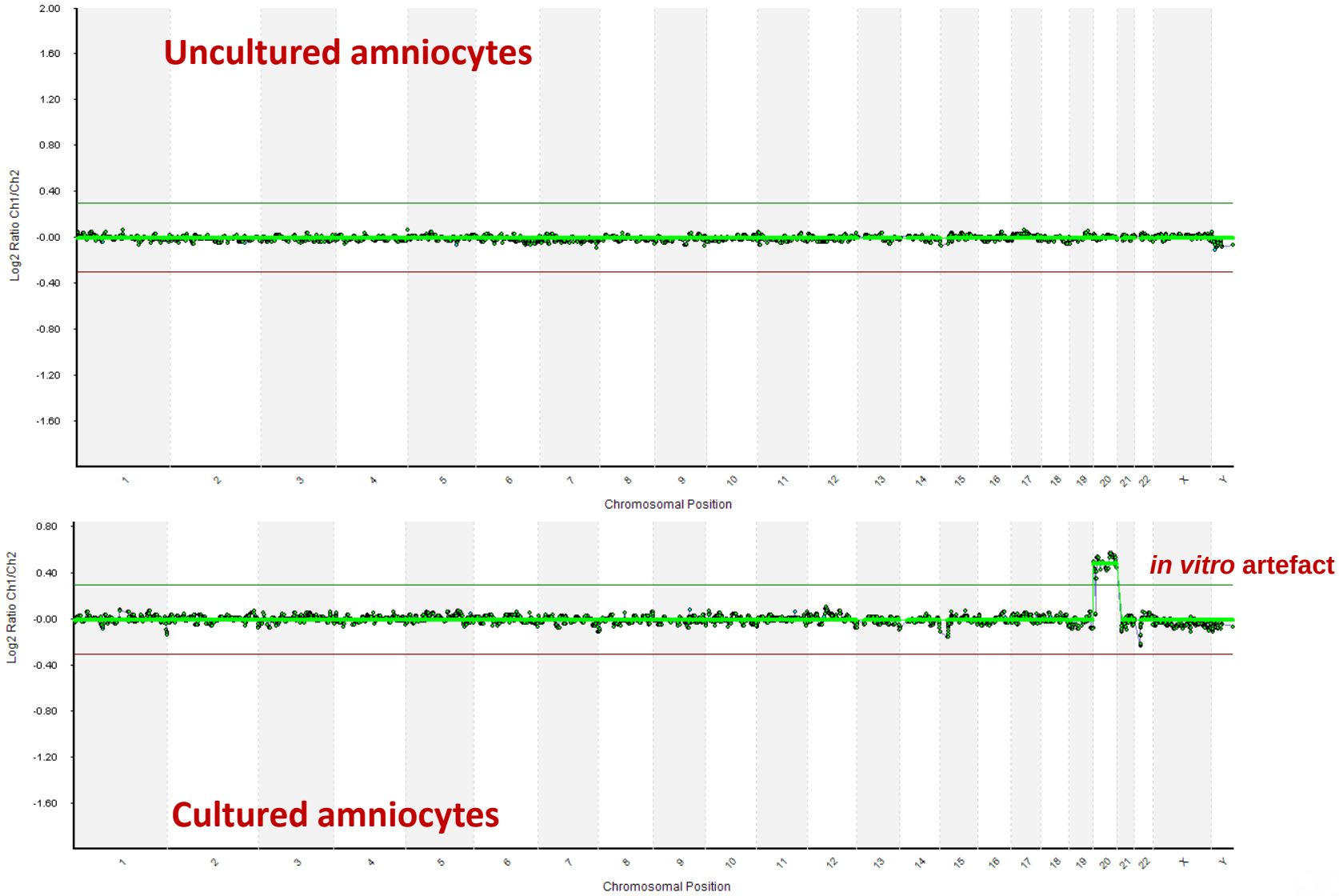
In vitro artefact in cultured amniocytes



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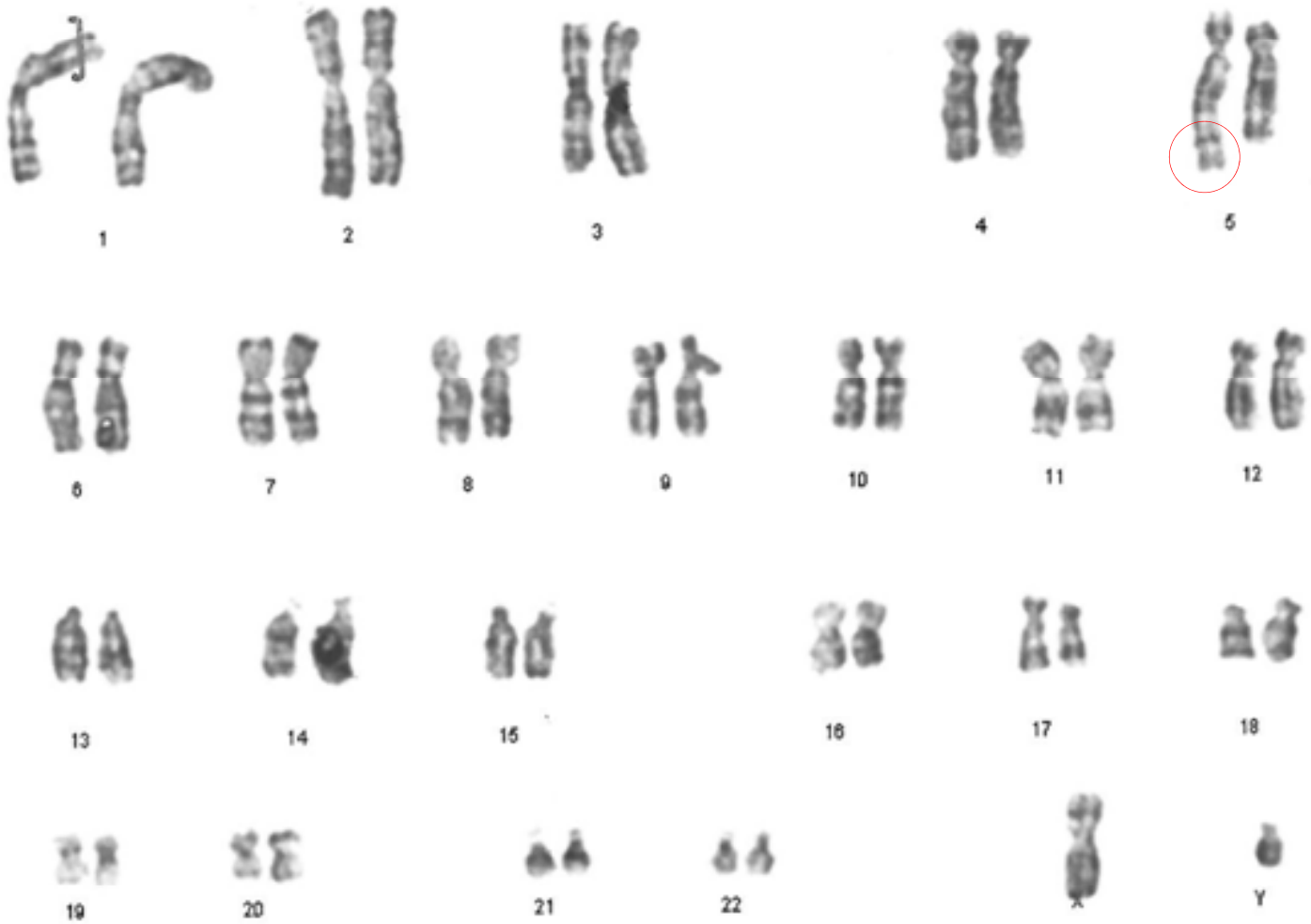
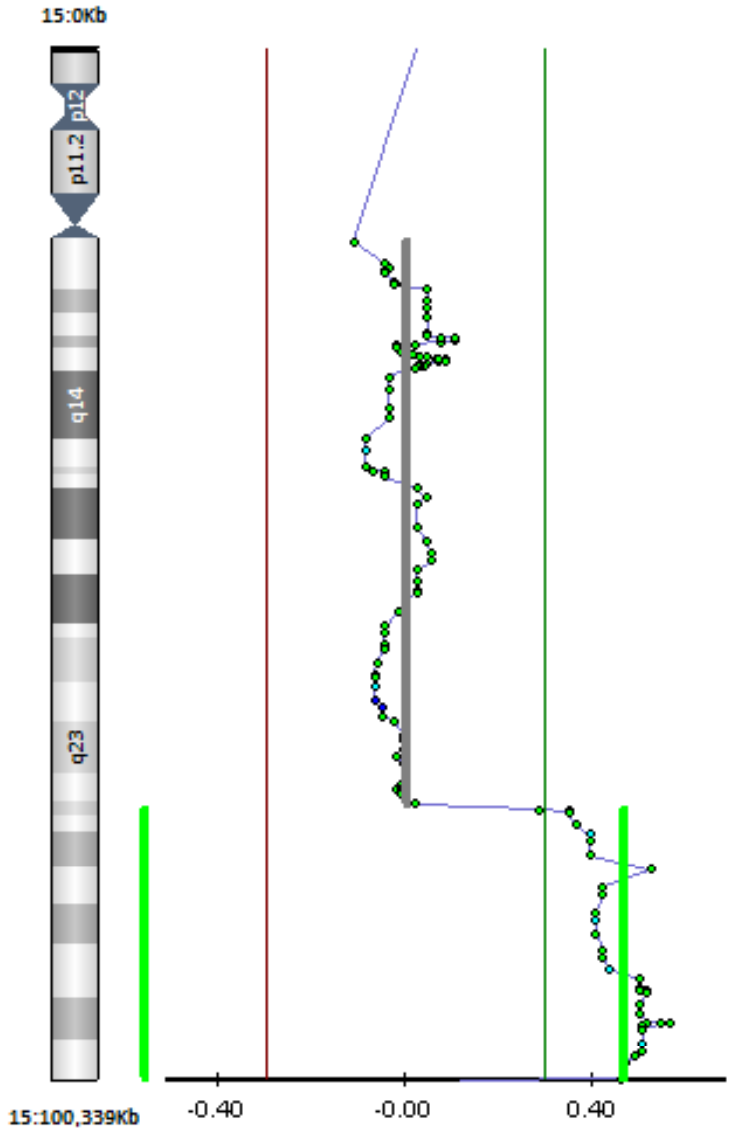
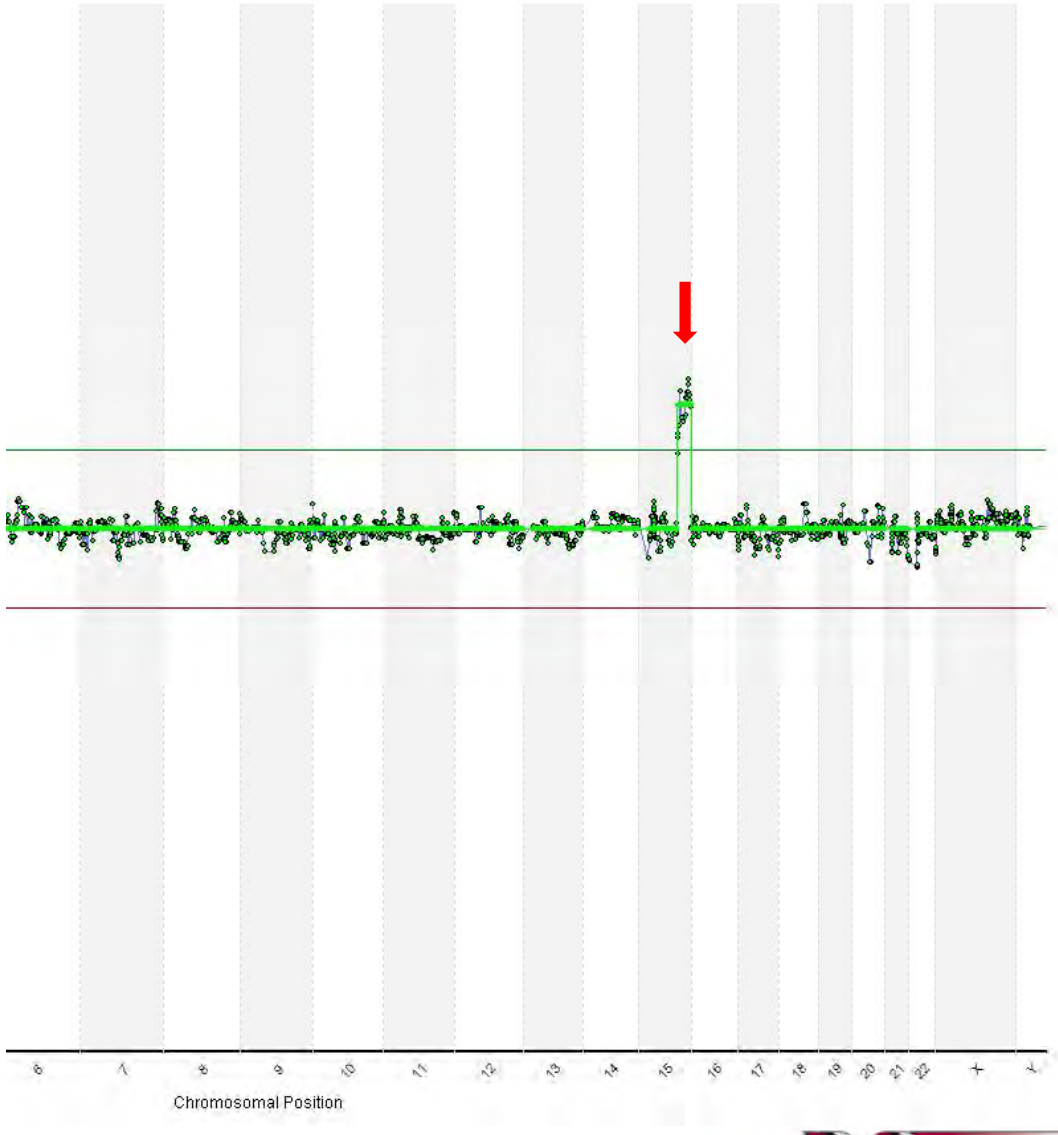
Karyotype from a fetus with a suspected partial dupl chr 5q



DNA (Amniotic fluid) from a fetus with a suspected partial dupl chr 5q, diagnosed as dup15(q24.1->qter) by array-CGH





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Clinically significant array-CGH findings in prenatal samples not detected by conventional karyotyping

Sample type	No. of samples	Indication	aCGH result			Parental analysis	Interpretation
			Location	Gain / Loss	Size (Mb)		
AF	1	AMA + AUS (single umbilical artery)	17p12	Loss	3.4	Inherited	Hereditary neuropathy (HNPP)
AF	3	AMA - PA	17p12	Gain	0.35-1.1	Inherited	Charcot-Marie-Tooth 1A (CMT1A)
AF	1	AMA + AUS (tetralogy of Fallot)	22q11.21	Loss	0.67	*De novo*	22q11.2 microdeletion (DIGEORGE)
AF	2	AMA	22q11.21	Gain	0.67	Inherited	22q11.2 microduplication syndrome
AF	1	AMA	15q13.1-q13.3	Loss	2.9	*De novo*	15q13.3 microdeletion syndrome
CVS	1	AMA + AUS (abnormal NT)	5q35.2-q35.3	Loss	1.7	*De novo*	SOTOS Syndrome
AF	1	PA	7q11.22-q11.23	Loss	1.2	*De novo*	WILLIAMS-BEUREN syndrome
AF	1	PA	15q11.2-q13.1	Loss	4.6	Inherited	15q11-q13 duplication syndrome
CVS	1	PA	6q14.3q15	Loss	5.2	*De novo*	Clinically significant CNV
AF	1	AMA	Xp11.3-p11.23	Loss	1.9	*De novo*	Clinically significant CNV
AF	1	PA	2p24.3-p24.2	Loss	2.5	*De novo*	Clinically significant CNV
CVS	1	PA	19q13.41q13.43	Gain	7.5	*De novo*	Clinically significant CNV
AF	1	PA	Xp21.2-p21.1	Gain	0.60	*De novo*	Duplication including exons 56-77 of the DMD gene
CVS	1	AMA + AUS (Cystic Hygroma)	10q26.12-10q26.3 16q23.1-q24.3	Loss Gain	13.6 14.6	*De novo*	Clinically significant CNV
CVS	1	AUS (abnormal NT)	8p23.3-p23.1 8p22-p21.1	Loss Gain	6.5 14.6	*De novo*	Inv dup del(8p)

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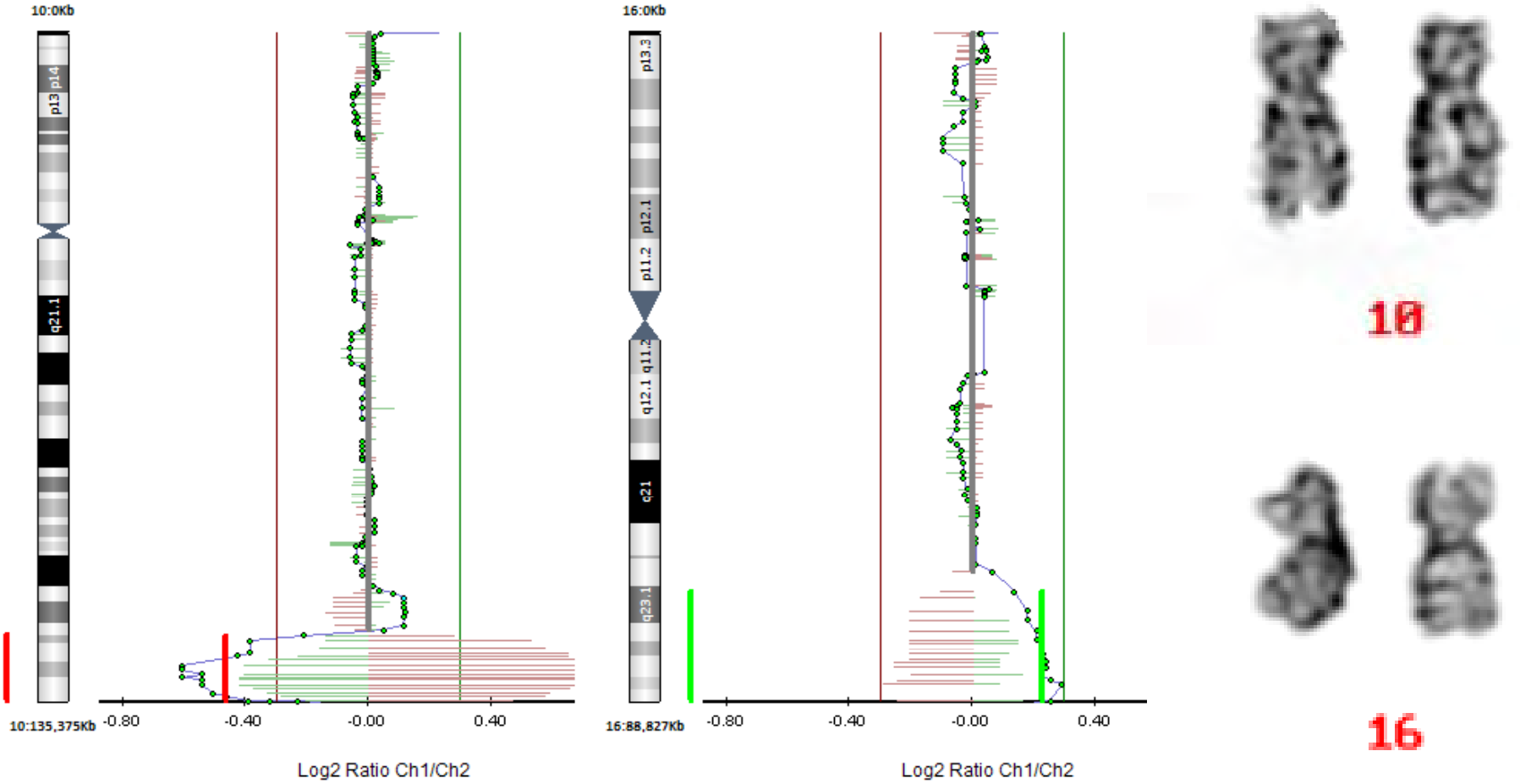
CVS with a de novo unbalanced translocation resulting in 13.6 Mb deletion 10q26.12-q26.3 and a 14.6 Mb duplication 16q23.1-q24.3 (ultrasound evidence: Cystic Hygroma)



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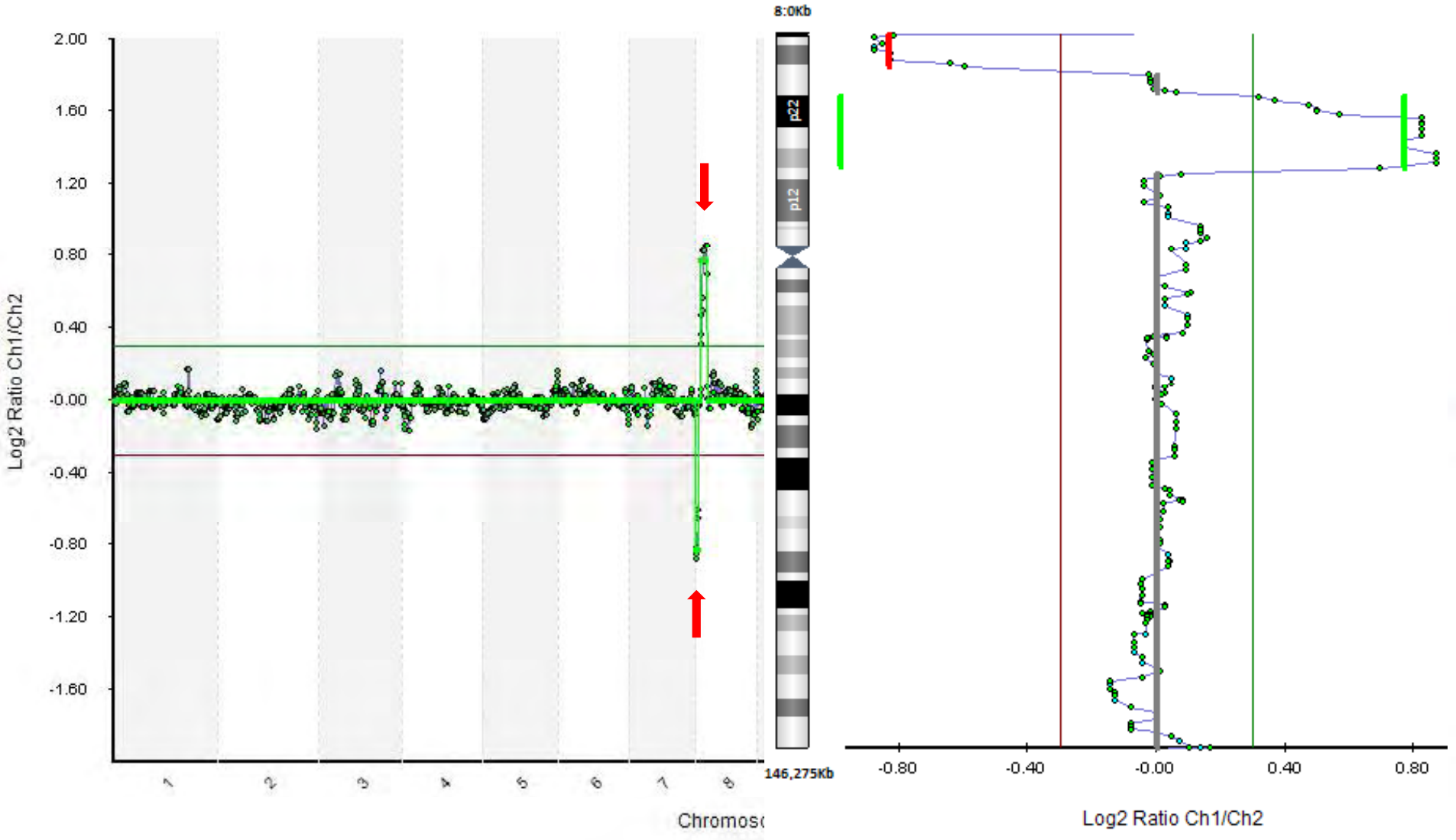
CVS with a *de novo* Inv dup del(8p) not detected by conventional Kariotype because of a cell culture failure (abnormal nuchal translucency)



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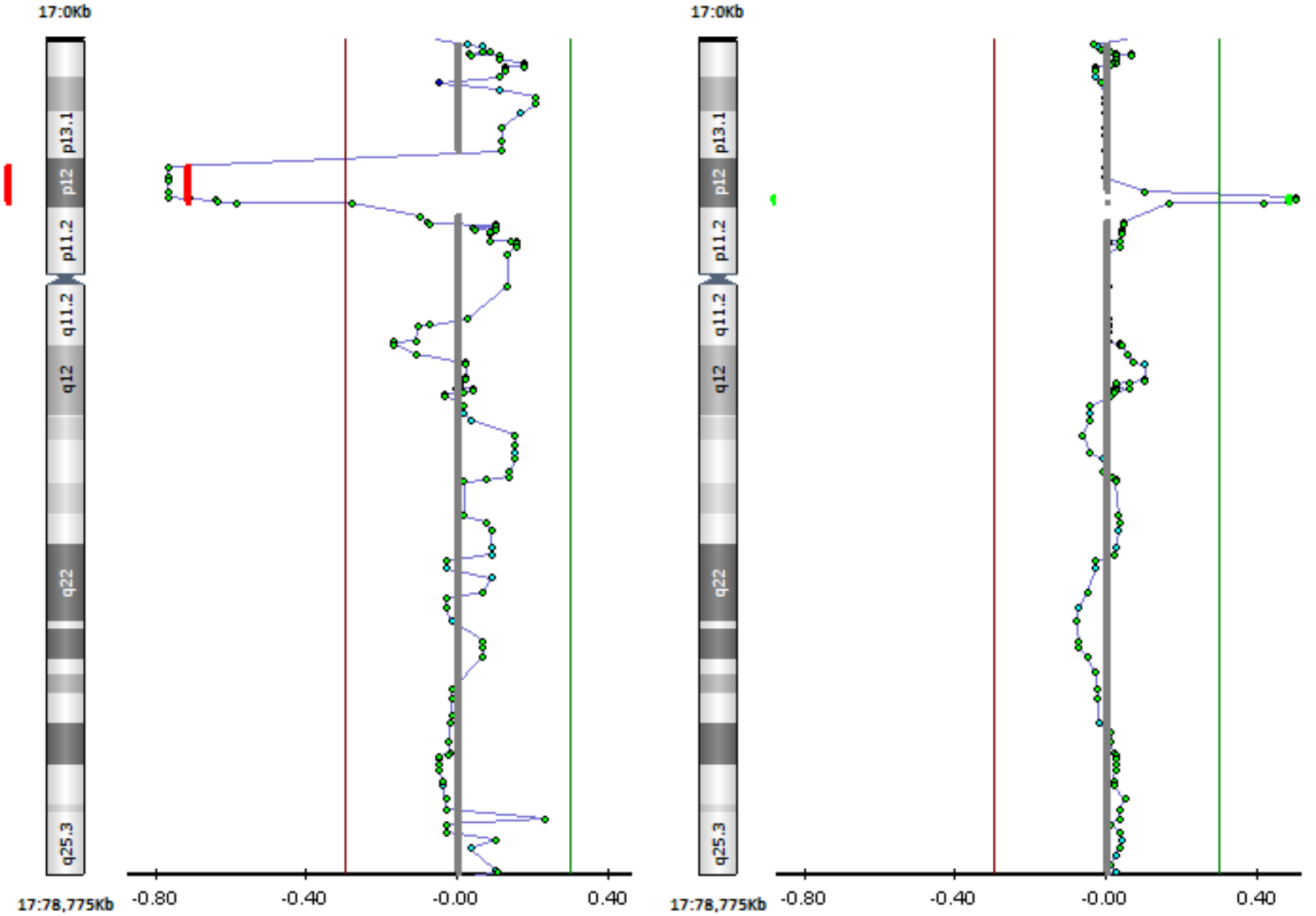
Hereditary neuropathy with liability to pressure palsies (HNPP) disease and Charcot-Marie-Tooth neuropathy type 1 A (CMT1A)



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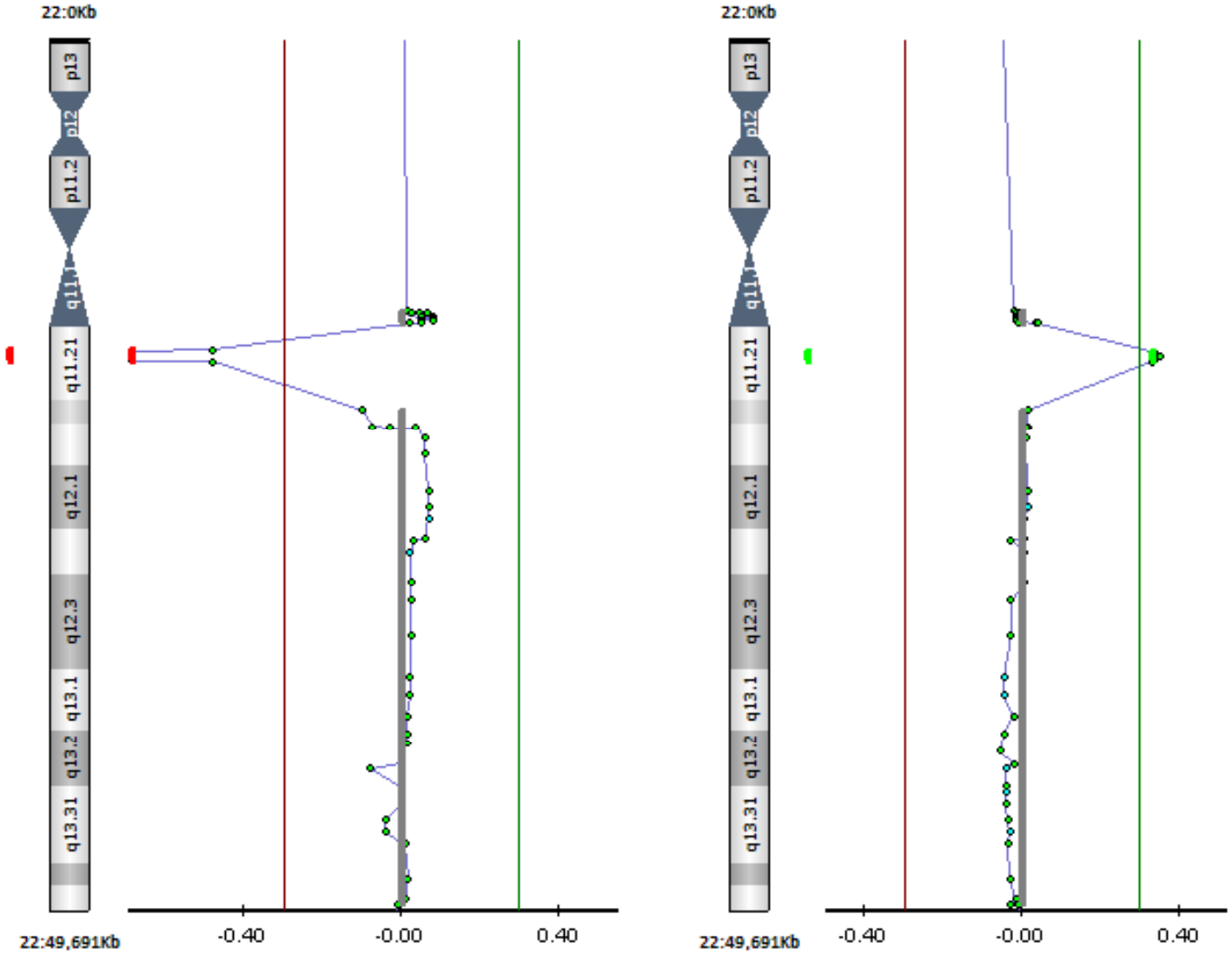
22q11.2 microdeletion syndrome (DIGEORGE) and 22q11.2 microduplication syndrome



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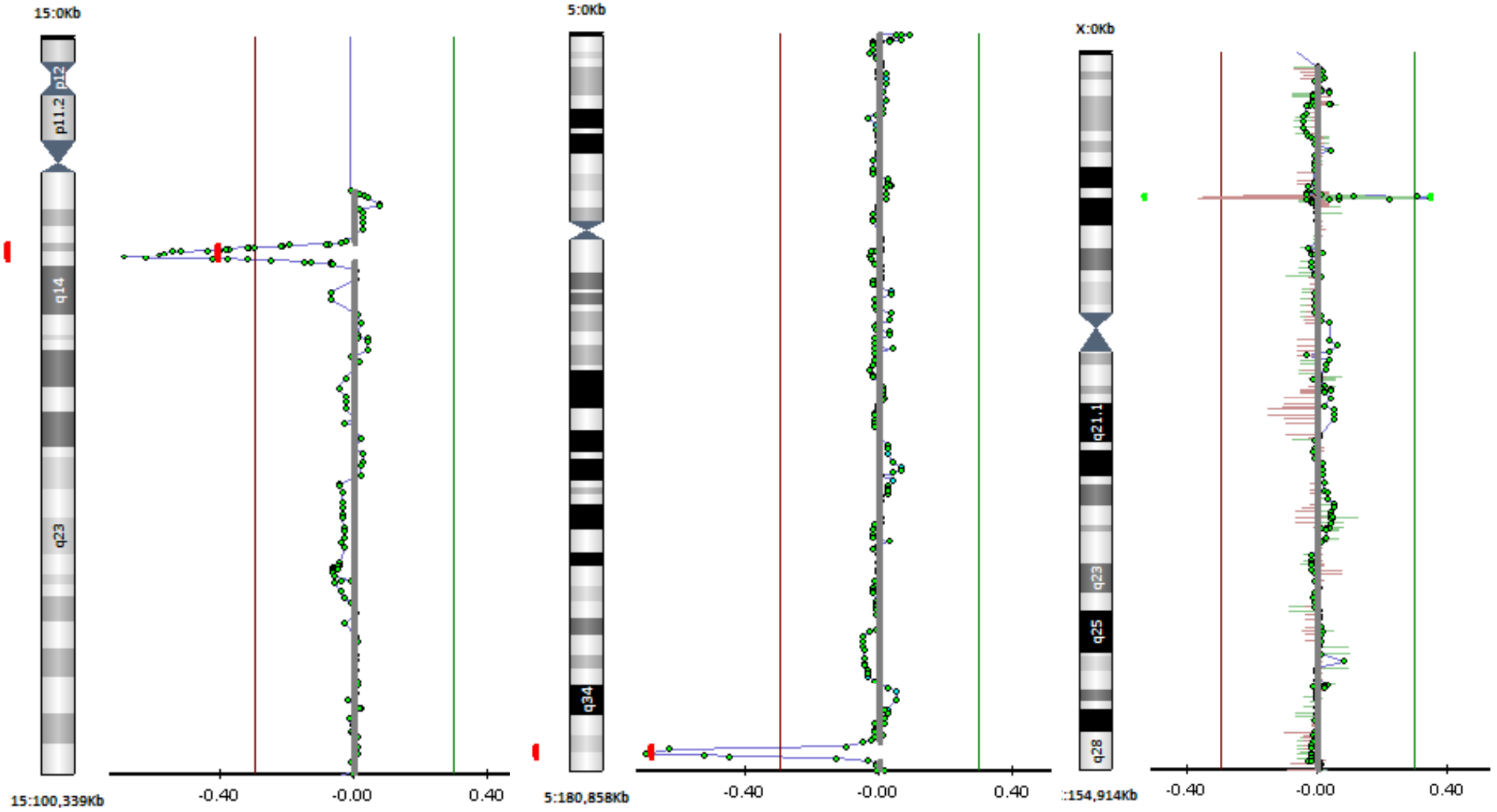
15q13.3 microdeletion syndrome
Sotos Syndrome - Duchenne Muscular Dystrophy (DMD)



5.2 Mb deletion (15q13.3 microdeletion syndrome)

1.7 Mb deletion at 5q35.2-q35.3 (Sotos Syndrome)

0.6 Mb dup. DMD gene (ex. 56-77)

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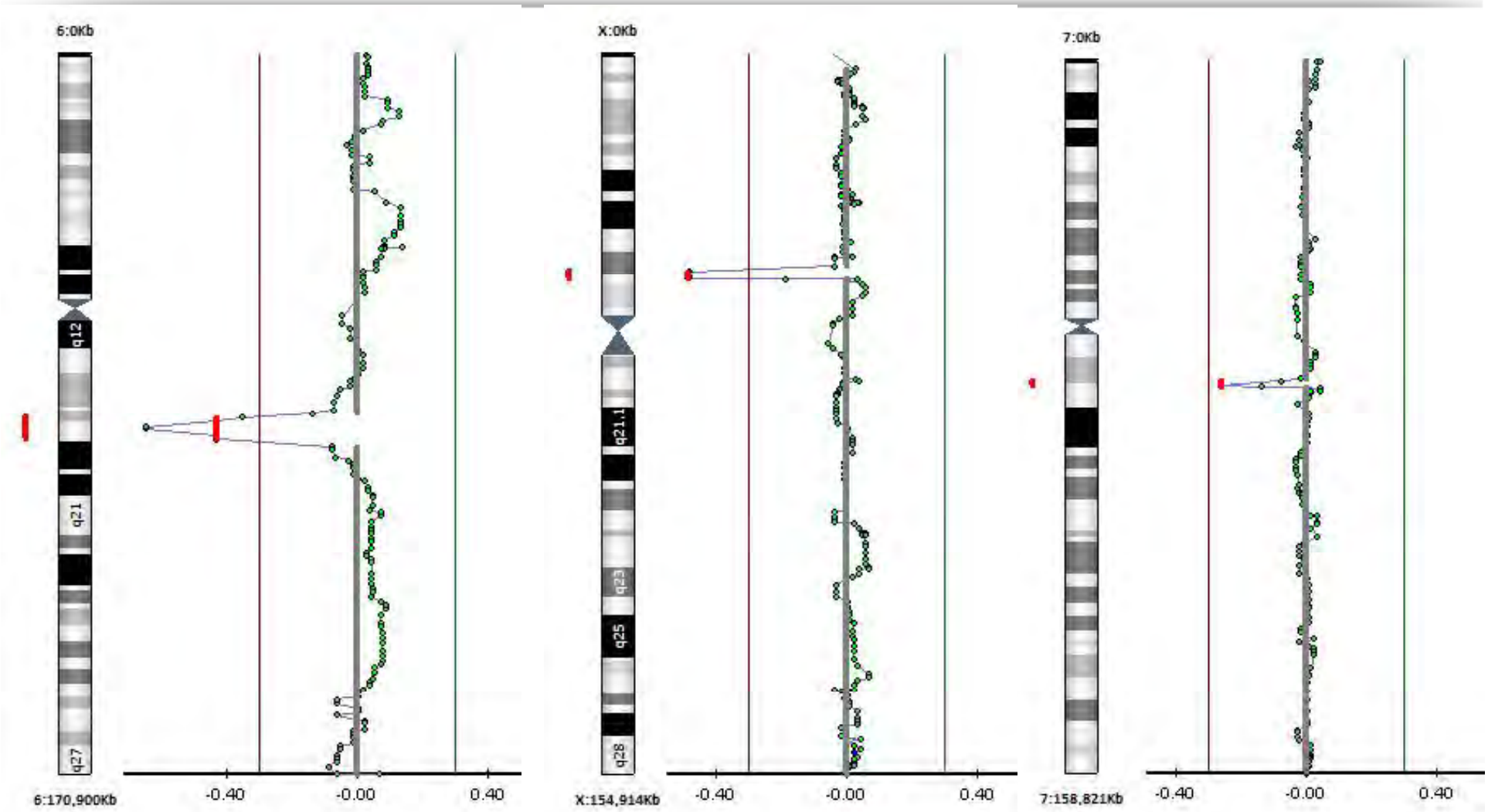
Other clinical significant CNVs



2.9 Mb deletion (6q14.3-q15)

1.9 Mb deletion (Xp11.3-p11.23)

1.9 Mb del. 7q11.22-q11.23 Williams-Beuren syndrome

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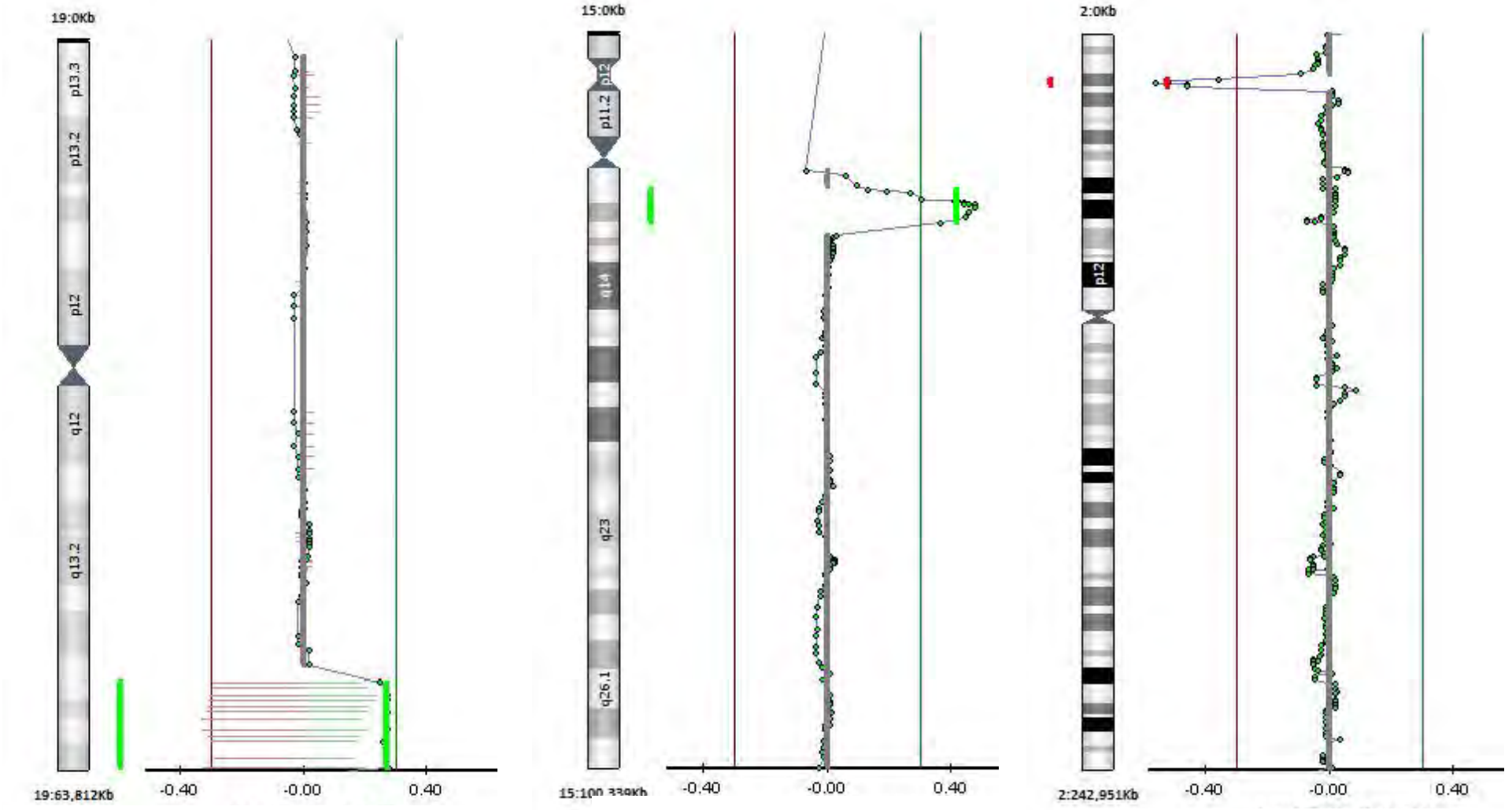
Other clinical significant CNVs



7.5 Mb duplication (19q13.41q13.43)

4.6 Mb 15q11-q13 duplication syndrome

2.5 Mb deletion (2p24.3-p24.2)

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Results comparison with previous prospective studies

Chromosome abnormality type	Sahoo *et al.* (2006) *n* = 98 (%)§	Shaffer *et al.* (2008) *n* = 151 (%)§	Kleeman *et al.* (2009) *n* = 24*+26§ (%)	Coppinger *et al.* (2009)		Van de Veyver *et al.* (2009) *n* = 190* +110§ (%)	Maya *et al.* (2010) n = 269 (%)*	**Fiorentino et al. (2011) *n* = 1900 (%)**	Combined *n* = 3012 (%)
				n = 182 (%)*	*n* = 62 (%)§				
No alteration	**51 (52.0)**	**136 (90.1)**	**46 (92.0)**	**158 (86.8)**	**57 (91.9)**	**242 (80.7)**	**229 (85.1)**	**1581(83.3)**	**2500 (83.0)**
Microscopic aberrations of clinical significance	**5 (5.1)**	**0 (0.0)**	**0 (0.0)**	**2 (1.1)**	**0 (0.0)**	**13 (4.3)**	**4 (1.5)**	**54 (2.8)**	**78 (2.6)**
Clinically significant submicroscopic aberrations	**0 (0.0)**	**2 (1.3)**	**1 (2.0)**	**5 (2.7)**	**0 (0.0)**	**2 (0.7)**	**3 (1.1)**	**18 (0.9)**	**31 (1.0)**
CNVs of Unclear significance	**2 (2.0)**	**1 (0.7)**	**0 (0.0)**	**1 (0.5)**	**0 (0.0)**	**3 (1.0)**	**0 (0.0)**	**0 (0.0)**	**7 (0.2)**
Benign CNVs	**40 (40.8)**	**12 (7.9)**	**3 (6.0)**	**16 (8.8)**	**5 (8.1)**	**40 (13.3)**	**33 (12.0)**	**247 (13.0)**	**396 (13.1)**

* Whole-genome arrays; § Targeted arrays

Fiorentino et al., Prenatal Diagnosis, *in press* (Updated)

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Conclusions

- aCGH has revealed **accurate** in detection of common and submicroscopic chromosome abnormalities in prenatal samples;
  - Detection of low level mosaicism (6%)
  - Correct scoring of abnormal cytogenetic findings
  - No *in vitro* artefact
- The technique increased the **sensitivity** and **accuracy** of the prenatal analysis, allowing identification of submicroscopic clinically significant imbalances that are not detectable by conventional karyotyping (**increased detection rate**)(~**1%**);
- No pathogenic chromosomal abnormalities were missed, compared with conventional karyotyping;
- No appreciable increase in results of unclear clinical significance
- Our findings provide a further evidence on the feasibility of introducing aCGH into routine prenatal diagnosis practice as **first-line diagnostic test**.