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CNV and SNV analysis of 844 intellectual disability genes in singleton referrals

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EAST MIDLANDS AND EAST OF ENGLAND NHS GENOMIC LABORATORY HUB

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Background

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- Gemini analysis for large gene panels and exomes can be cumbersome as all data is loaded and managed in spread-sheets
- CNV and SNV analysis in the lab is streamlined but we still use multiple software packages
- Nexus Clinical (NxC), offers an alternative analysis platform to our Gemini and Array analysis pipelines for combined CNV/SNV detection
- NxC is currently used to analyse all data for the intellectual disability gene panel at CUH
  - 844 'green' PanelApp genes for ID
  - All variants VUS and above go to a weekly Clinical MDT to be reviewed

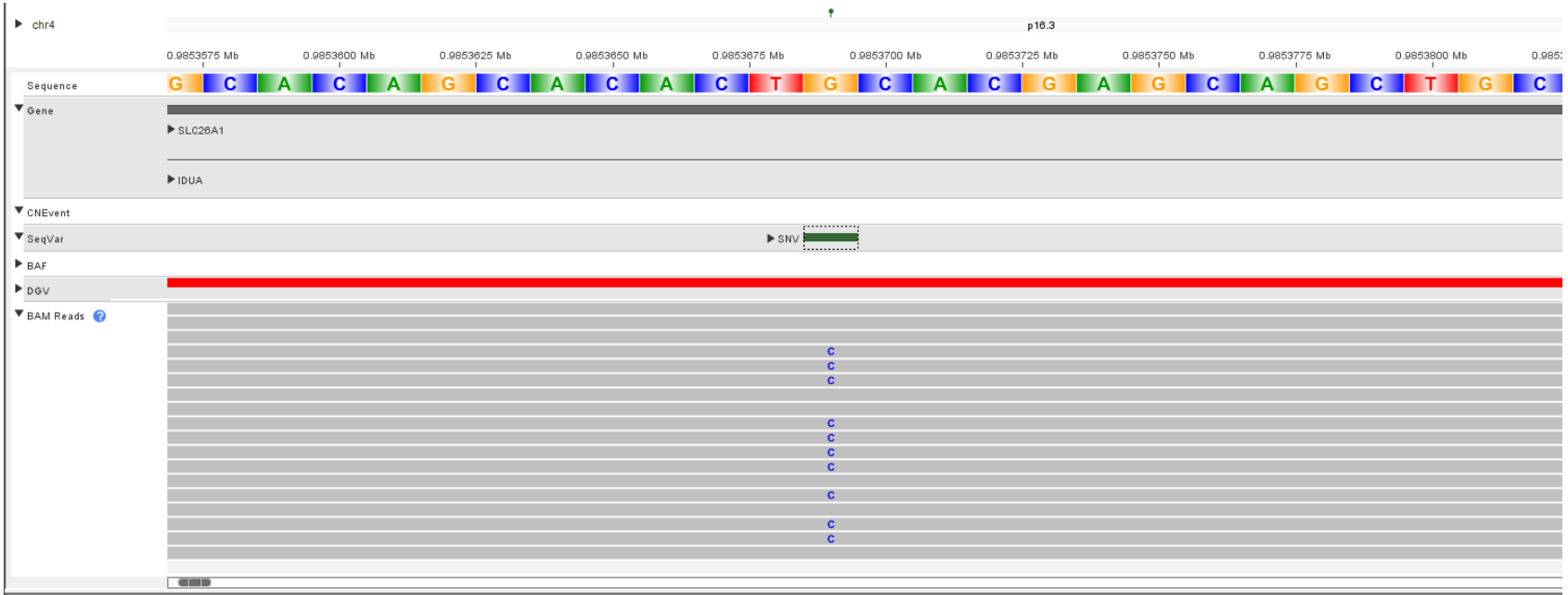

Table | Deleted Events | Whole Genome | Report

Similar Previou...	HGVSc	Phenotypes	Genes	Transcript Overview	Event	Genotype	Variant Rea...	Total Depth	Allele Depth	Pop. Allele Freq %	GMAF %	Quality	ClinVar	dbSNP	Chromosome Region	Length	Classification
0.01% (1/11271)	c.2453delT	Umbilical hernia, Om...	SPECC1L, ...	Frameshift variant	Deletion	Heterozygous	30.61	98	30			738.73	no records		chr22:24,730,432-24,7...	1	
0.01% (1/11271)	c.5788delA	Multicystic kidney dy...	KMT2A	Frameshift variant	Deletion	Heterozygous	37.63	279	105			3,191.73	no records		chr11:118,368,774-118...	1	
0.21% (24/11271)	c.-14-1G>T	Joint contracture of ...	ERCC6	Splice acceptor varia	SNV	Heterozygous	11.9	42	5			63.77	no records		chr10:50,741,025-50,7...	1	
0.18% (20/11271)	c.-14-2A>T	Joint contracture of ...	ERCC6	Splice acceptor varia	SNV	Heterozygous	11.9	42	5			63.77	no records		chr10:50,741,026-50,7...	1	
0.13% (15/11271)	c.748-2A>T	Ichthyosis, Skeletal ...	PEX7	Splice acceptor varia	SNV	Heterozygous	26.92	26	7	1.234 (gnomAD FIN)		138.77	★likely pathogenic	rs7788...	chr6:137,193,334-137,...	1	
0.05% (6/11271)	c.2656C>G	Hyperactivity, Intell...	AFF2	Probably damaging	SNV	Homozygous	99.25	266	264	0.327 (gnomAD A...	0.053	8,054.77	★benign	rs1510...	chrX:148,039,954-148,...	1	
0.41% (46/11271)	c.123C>G	Macrocephaly, Umbil...	SLC26A1, I...	Probably damaging	SNV	Heterozygous	48.35	364	176	3.311 (gnomAD ASJ)	0.459	5,698.77	no records	rs1439...	chr4:985,369-985,369	1	
5.39% (607/11...	c.3152C>T	Cortical visual impair...	DIAPH1	Probably damaging	SNV	Heterozygous	25	28	7	1.685 (gnomAD FIN)		61.77	no records	rs8685...	chr5:140,907,261-140,...	1	
0.14% (16/11271)	c.1991T>A	Proptosis, Generaliz...	TAF1, BCY...	Probably damaging	SNV	Heterozygous	12.96	162	21	0.146 (gnomAD O...		181.77	no records		chrX:70,602,998-70,60...	1	
0.01% (1/11271)	c.568C>T	Cardiac rhabdomyo...	TSC1	Probably damaging	SNV	Heterozygous	14.08	142	20	0.029 (gnomAD FIN)		245.77	★likely benign	rs1182...	chr9:135,797,301-135,...	1	
0.19% (21/11271)	c.1987C>A	Proptosis, Generaliz...	TAF1, BCY...	Probably damaging	SNV	Heterozygous	13.48	178	24	0.145 (gnomAD O...		285.77	no records		chrX:70,602,994-70,60...	1	
0.29% (33/11271)	c.1989C>A	Proptosis, Generaliz...	TAF1, BCY...	Probably damaging	SNV	Heterozygous	13.94	165	23	0.145 (gnomAD O...		205.77	no records		chrX:70,602,996-70,60...	1	
5.26% (593/11...	c.1851_1853delTCC	Cortical visual impair...	DIAPH1	Inframe deletion	Deletion	Heterozygous	11.21	232	26			381.73	★★benign (★uncer	rs7671...	chr5:140,953,564-140,...	3	
9.57% (1079/1...	c.138_143delGCTGGC	Hepatomegaly, Ath...	SMPD1	Inframe deletion	Deletion	Heterozygous	99.64	555	252			18,511.77	★★benign (★likely	rs3838...	chr11:6,411,936-6,411...	6	

Case 1

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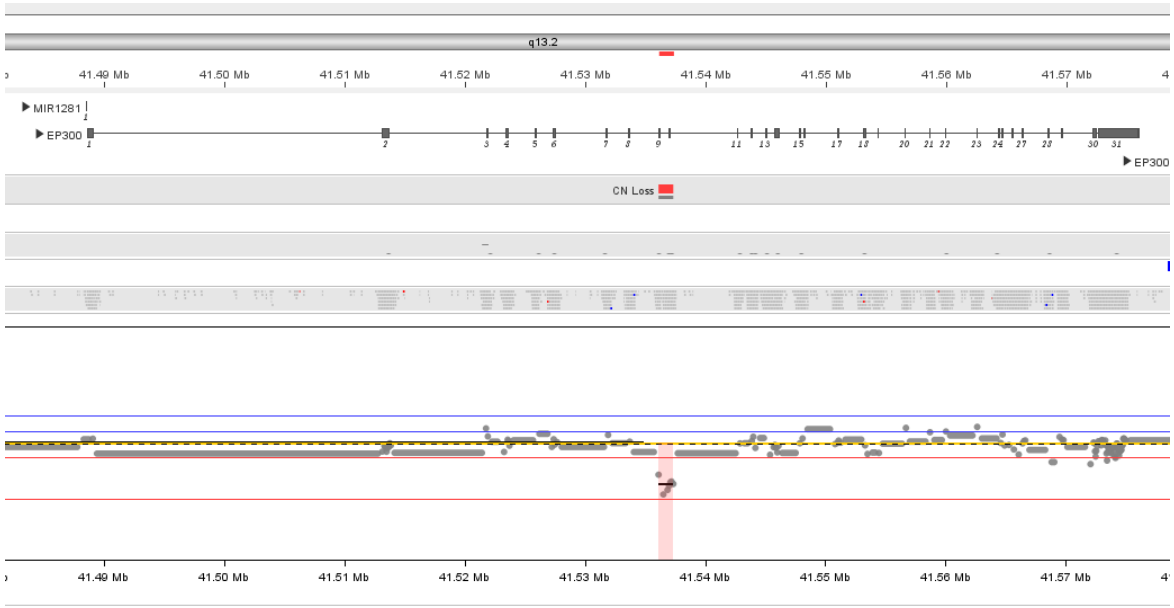
- 4 year old girl with global developmental delay, microcephaly, IUGR and dysmorphic features

- Previous microarray analysis using the 750K Affymetrix SNP array was negative

- The ID panel was requested to identify the cause of the clinical phenotype
  - No clinically relevant SNVs were detected on the panel
  - One small CNV was identified



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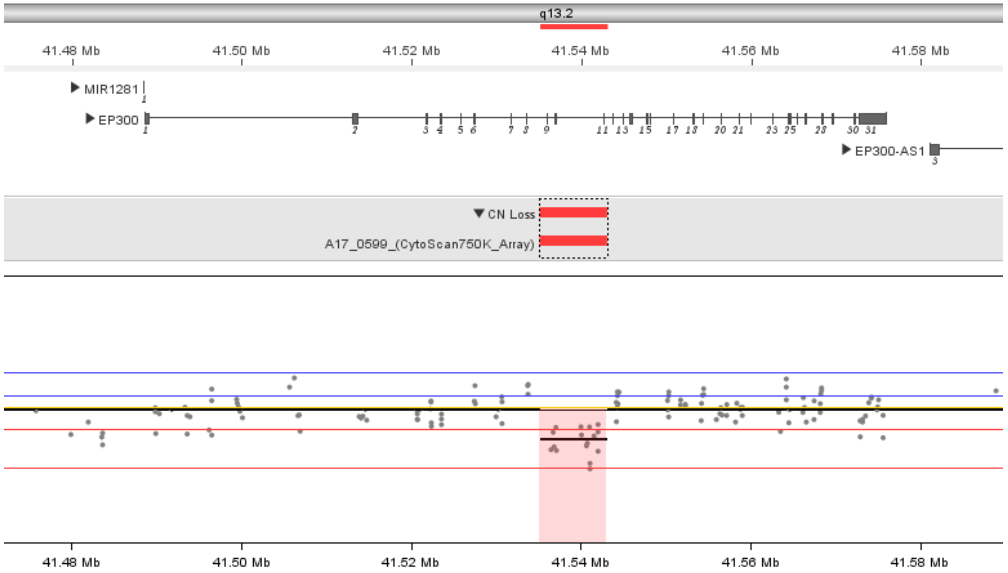
- 8kb deletion of 22q13.2
- Removes exons 9 and 10 of the EP300 gene
- HET deletions of EP300 associated with Rubinstein-Taybi syndrome



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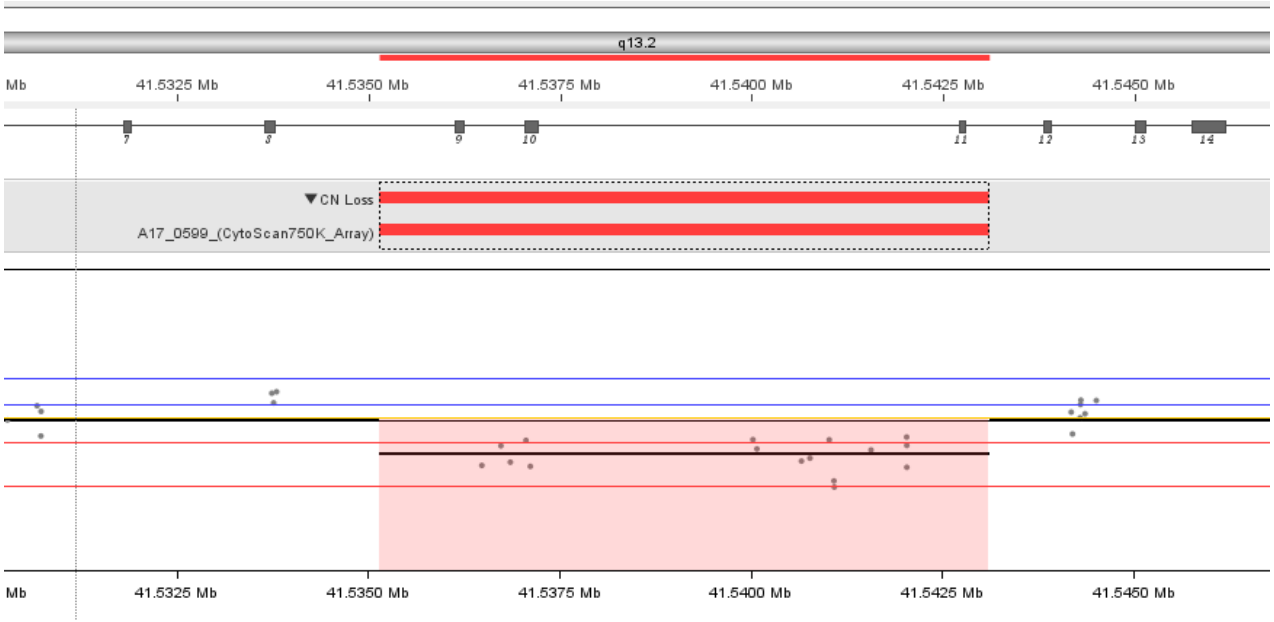
- The 750K Affymetrix SNP array (using ChAS software) has a resolution of 200kb
- When the previous array data is loaded into NxC, the deletion is called



- Parental testing showed the deletion to be de novo
- Outcome – deletion reported as likely pathogenic
- Increased resolution for detecting small CNVs using NGS data and NxC

Case 2

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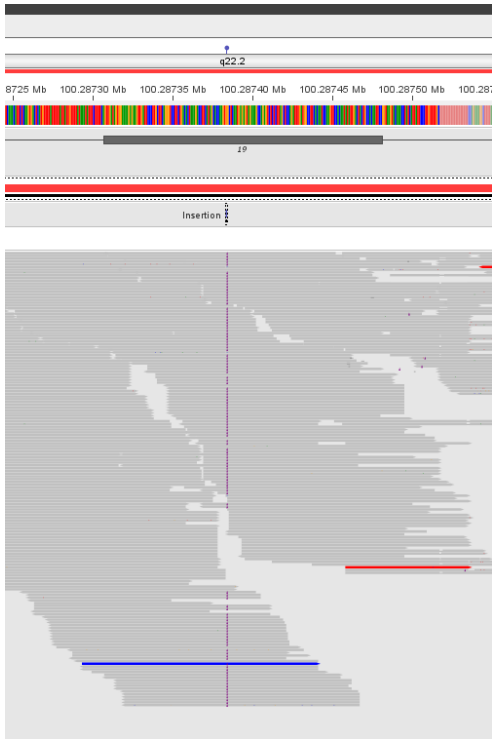
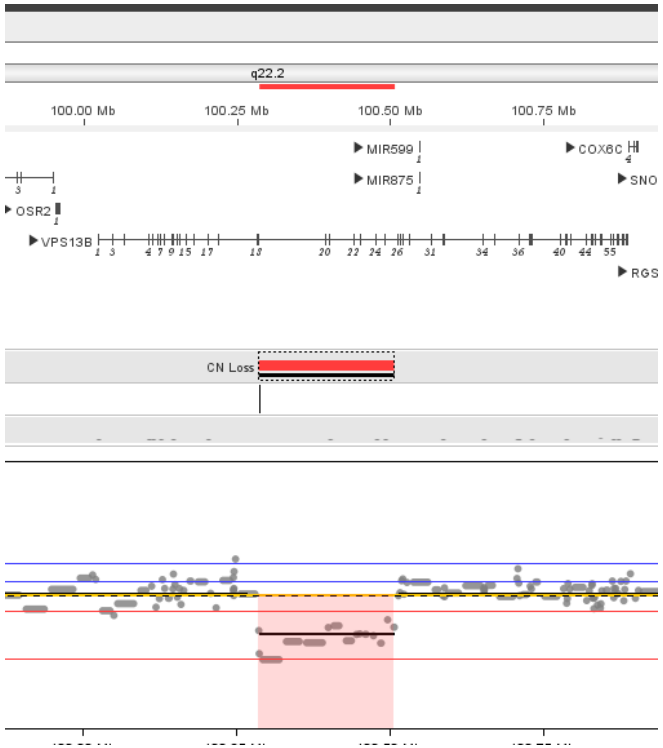
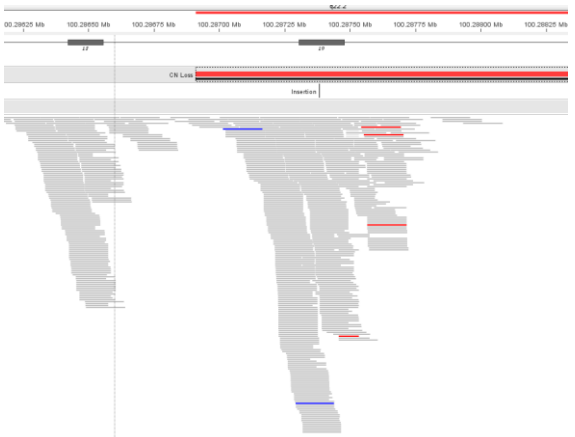
- 15 year old boy with microcephaly, short stature, severe learning difficulties and hypotonia
- Previous PWS/AS testing was negative
- No microarray testing has previously been carried out
- The ID panel was requested to identify the cause of the clinical phenotype
  - A SNV and CNV were both detected in the VPS13B gene







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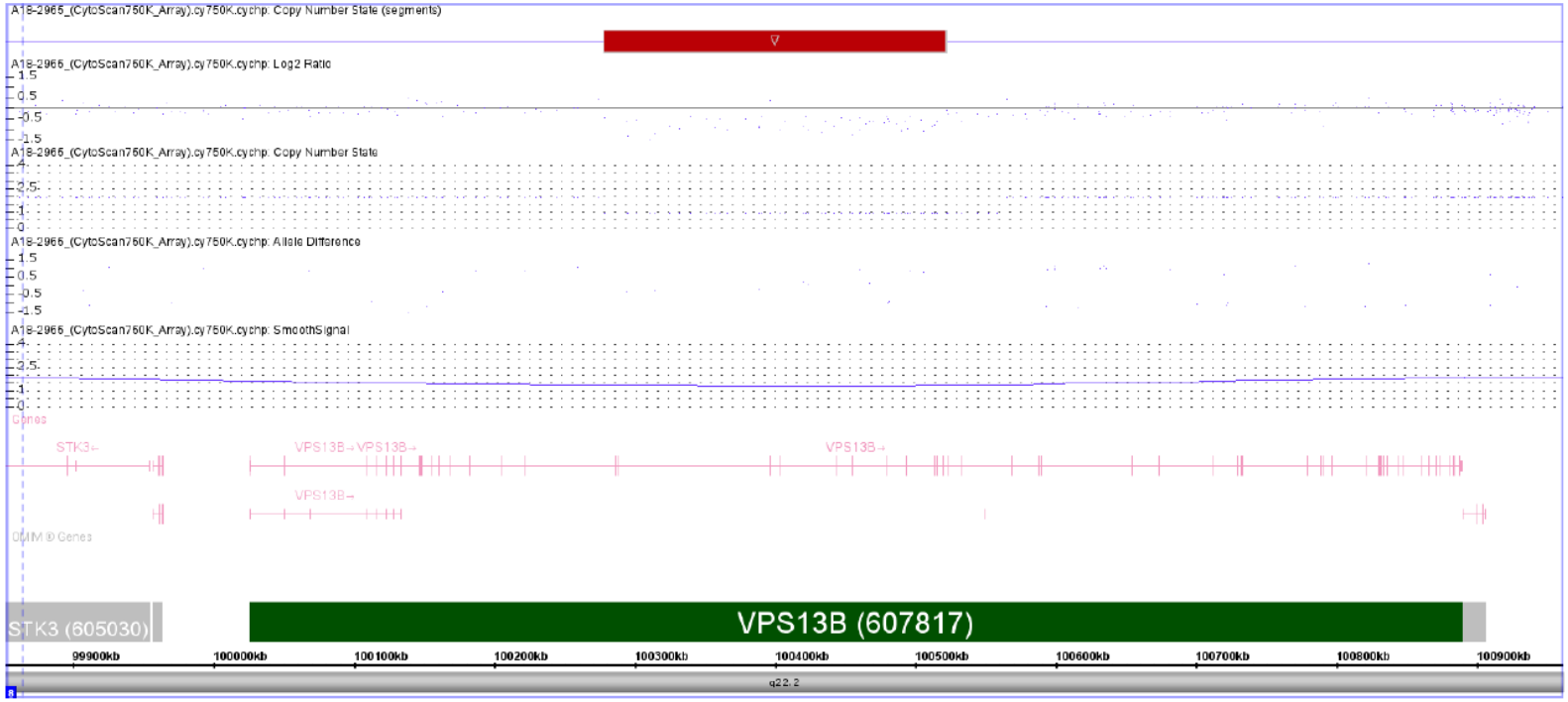
- VPS13B frameshift variant in exon 19 – c.2727_2730dupGCTC p.(Asn911AlafsTer3)
- 228kb intragenic heterozygous loss within the VPS13B gene spanning exons 18 to 25
- VPS13B associated with Cohen syndrome
  - AR multisystem disorder – facial dysmorphism, microcephaly, ID, intermittent neutropenia
  - c.2727_2730dupGCTC variant has previously been reported in a HOM state in two siblings with Cohen syndrome
  - Compound HET for SNV and deletion also previously described as pathogenic

- The 750K Affymetrix SNP array was run for confirmation
- Outcome - The SNV and CNV were reported as pathogenic and consistent with the patient's clinical phenotype
- Parental testing is currently ongoing to determine the inheritance of both variants



Case 3

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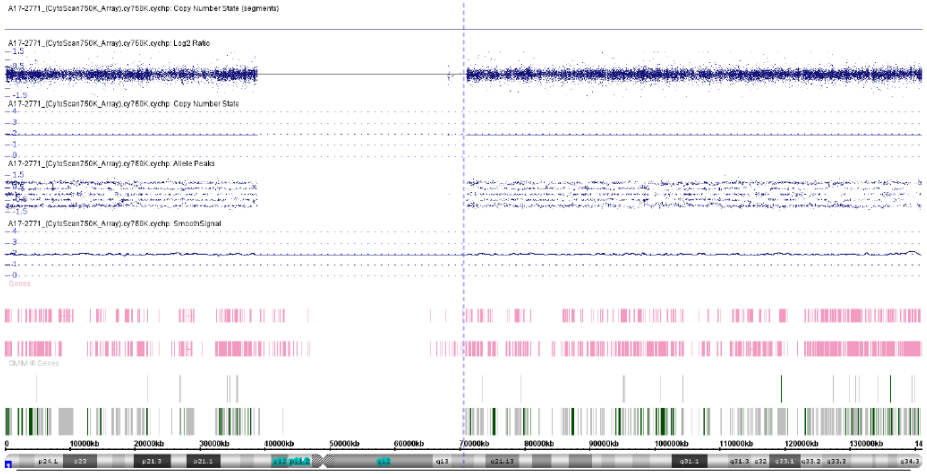
- 3 year old boy with learning difficulties, speech delay and minor ASD traits
- Previous microarray and QFPCR testing identified an unusual clustering of SNPs across all chromosomes, consistent with the blood DNA sample being tetragametic in origin
  - Two cell populations were present
  - One of which was identical to this patient's twin brother
- Chimerisim could be confined to one or more tissues
- Finding may obscure further genetic testing

Normal SNP profile

Abnormal SNP profile



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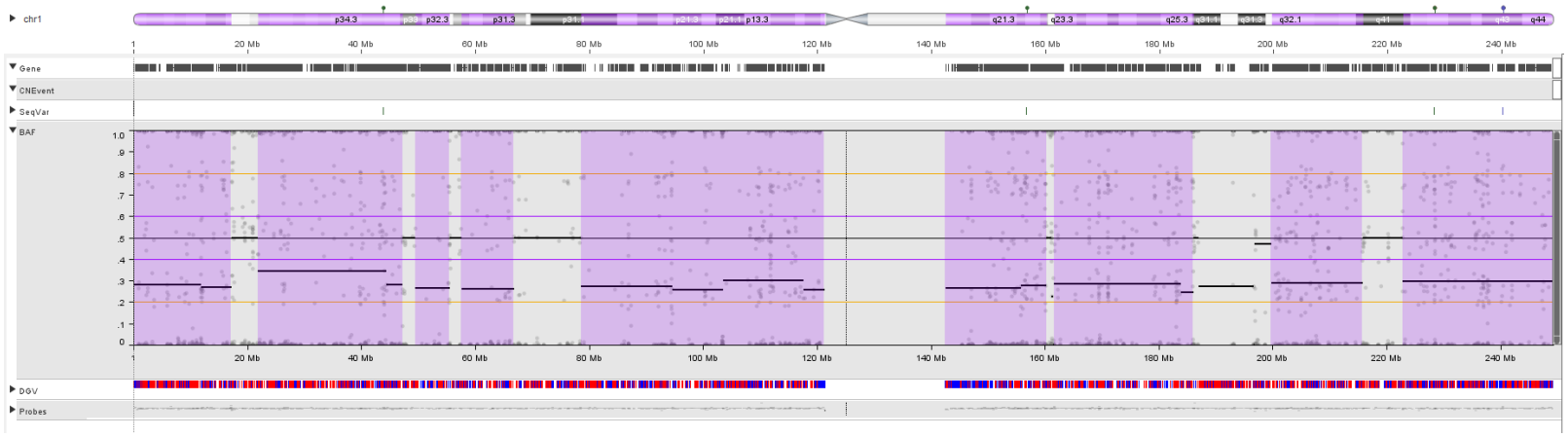
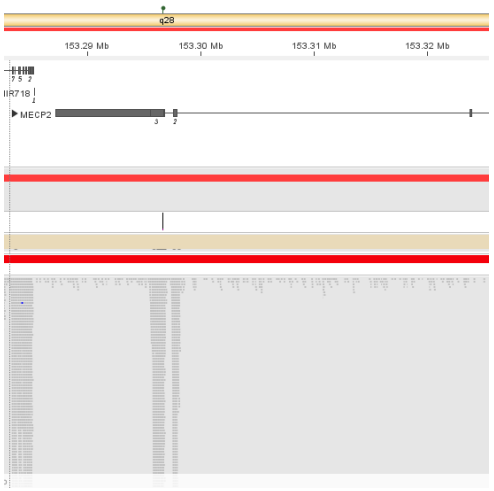
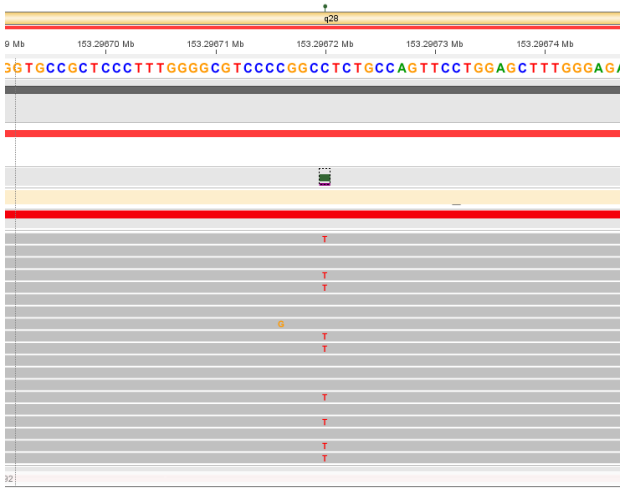
Chr 1



Chr X





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0.01% (1/11271)	c.595G>A	Macrocephaly, Drool...	MECP2	Probably damaging	SNV	Heterozygous	47.75	178	85			2,334.77	no records		chrX:153,296,720-153,...

- MECP2 variant identified with a read fraction of 0.47%
  - If pathogenic, must be present on the X chromosome not shared with twin brother
- Somatic mosaicism for a MECP2 variant has been previously reported associated with a classic Rett phenotype
- Currently awaiting further tissue samples from this patient and family to perform segregation analysis
- Analysis has shown that chimeras can be examined using NGS data, however caution must be taken when interpreting read fractions

Conclusions

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- By modifying the established in-house NGS pipeline, we have been able to detect SNVs, CNVs and LOH in a single test which has:
  - Helped to identify compound heterozygotes with a CNV and SNV
  - Provided more information for completeness
  - Increased accuracy and efficiency in interpretation
  - Reduced the cost and time associated with multiple tests
- Overall, NxC has allowed the analysis of very large gene panels without a significant burden of variants of uncertain significance requiring clinical assessment

Acknowledgements

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