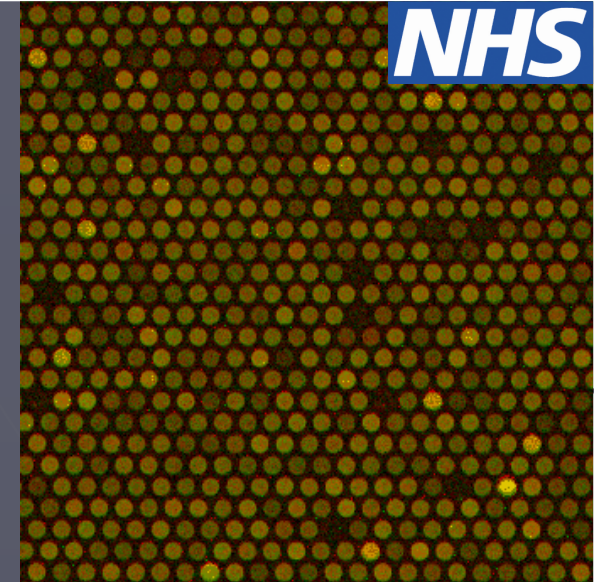
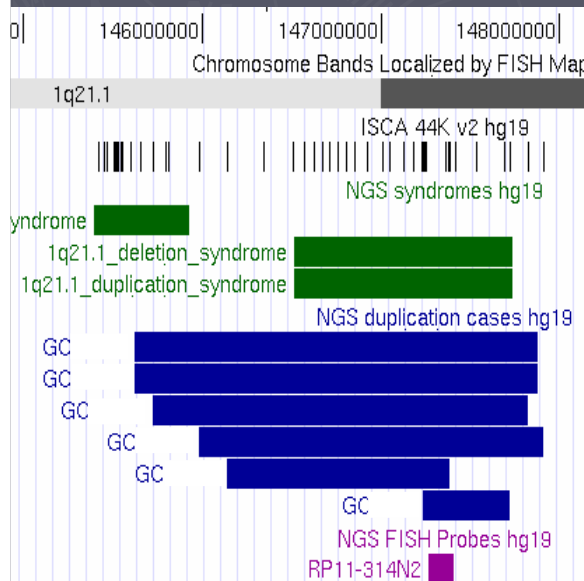




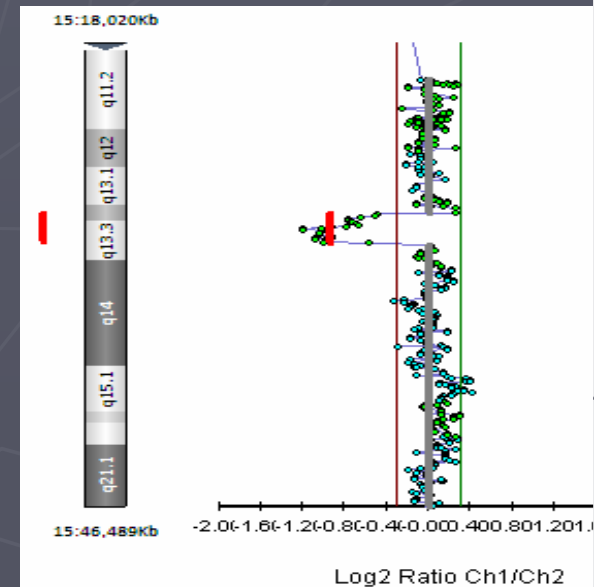
Simon Zwolinski  
Christopher Kettle  
Gareth Breese  
Stephen Hellens



# Oligo Array CGH in Newcastle: One Year On



Institute of  
Genetic Medicine  
Newcastle-upon-Tyne



From October 2010, the Northern Genetics Service made a commitment to head towards undertaking array CGH on developmentally delayed and dysmorphic children instead of G-banding as a first line investigation

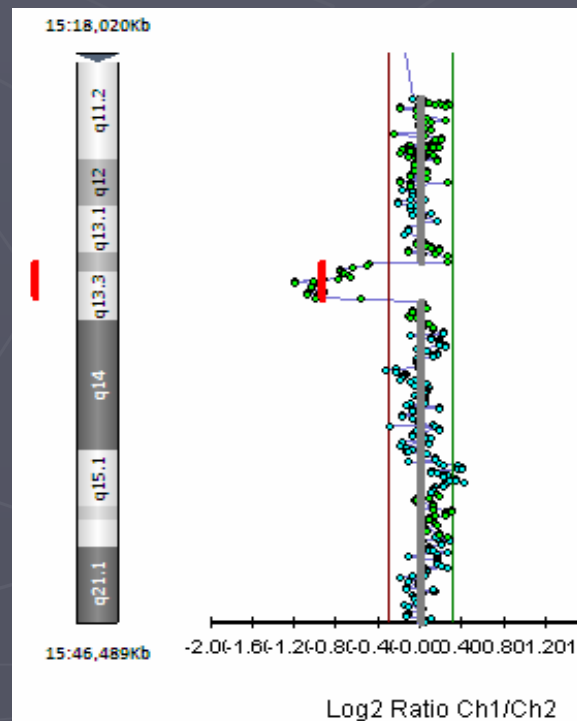
To date, the majority of our array CGH data has been generated using BAC arrays. Whilst recent BAC arrays have been limited in resolution to approximately 0.5Mb, in our experience the BAC platform is extremely sensitive and precise

Started validation of 4x44K and 8x60K Oligo array CGH in February 2010 by undertaking studies on 36 cases previously undertaken by BAC array at Newcastle

The 36 were a mixture of “abnormals” and “normals” and we were able to refine the accuracy of the breakpoints in every abnormal case and in one case we changed our clinical interpretation by showing that there were no OMIM genes in a deletion

We have now undertaken oligo array CGH on over 270 new clinical cases

Our number of reported copy number changes has increased from approximately 22% by BAC array to 24% by Oligo array



Started clinical cases with oligo arrays beginning of May and in the following eight months undertook 217 cases

Originally found we needed to repeat ~20% patients but since improving our DNA clean-up procedures, we now only need to repeat less than 3%

Total number of patients failing is only 13 (6%) including two DNA samples extracted from mouth washes. The current figures, although unavailable, are improving

Interestingly, there have been changes in the types of referral and we think this partly explains our apparent rise in “abnormality” rate

Original proportion with no G-banding = 7%

Current proportion with no G-banding = 24%

Original mean referral age (BACs) = 13.3 yrs

Current mean referral age (oligos) = 7.5 yrs

Known G-band abnormalities = 11

Patients reported with new abnormality = 52 (24%)

Reported new copy number changes = 82

Deletions = 36 (44%)

Duplications = 32 (39%)

Complex results = 14 (17%)



Location of changes important (by FISH) = 11 (13%)

Changes in known syndrome regions = 16 (20%)

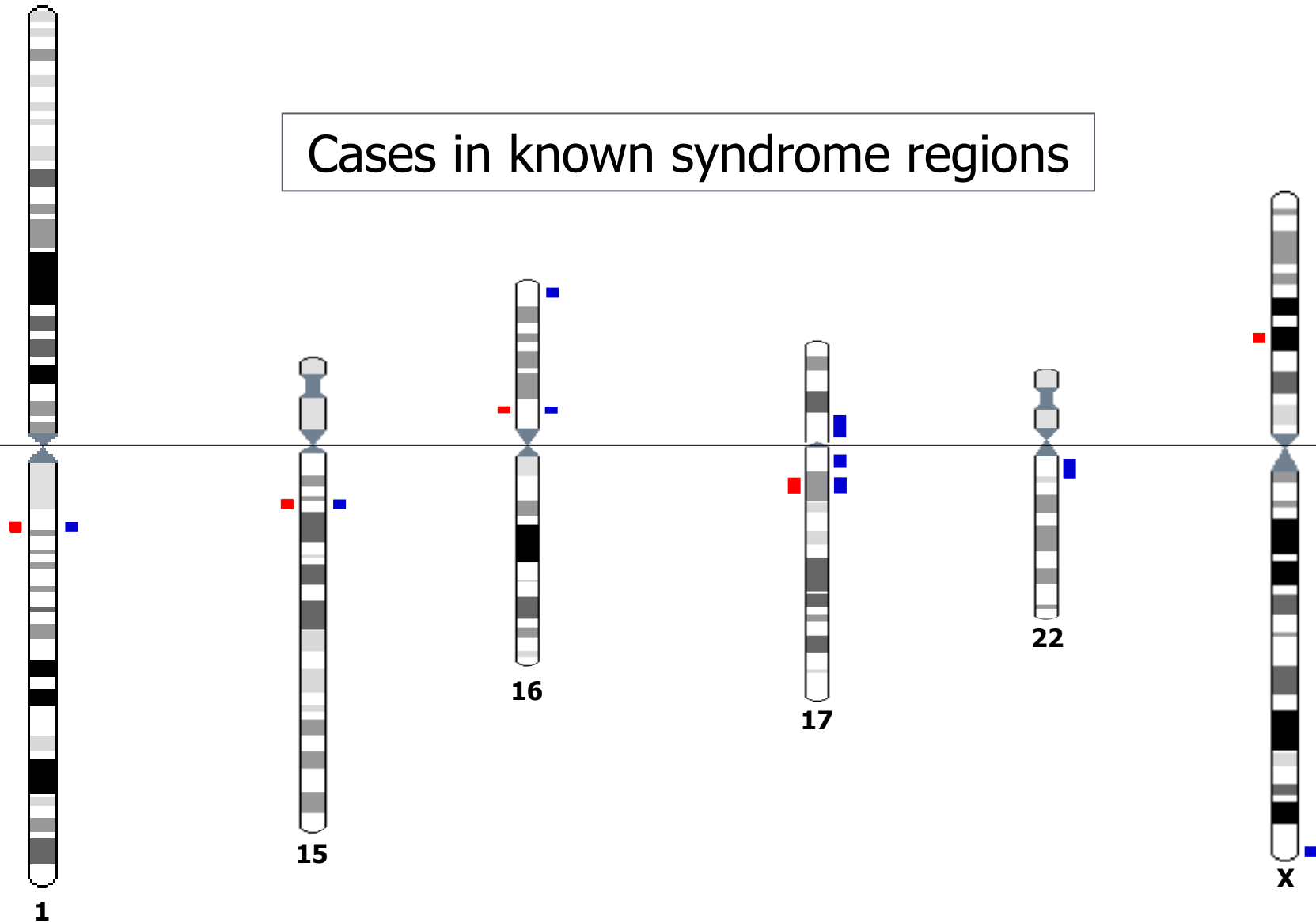
## Cases in known syndrome regions = 16 (20%)

Examples include:

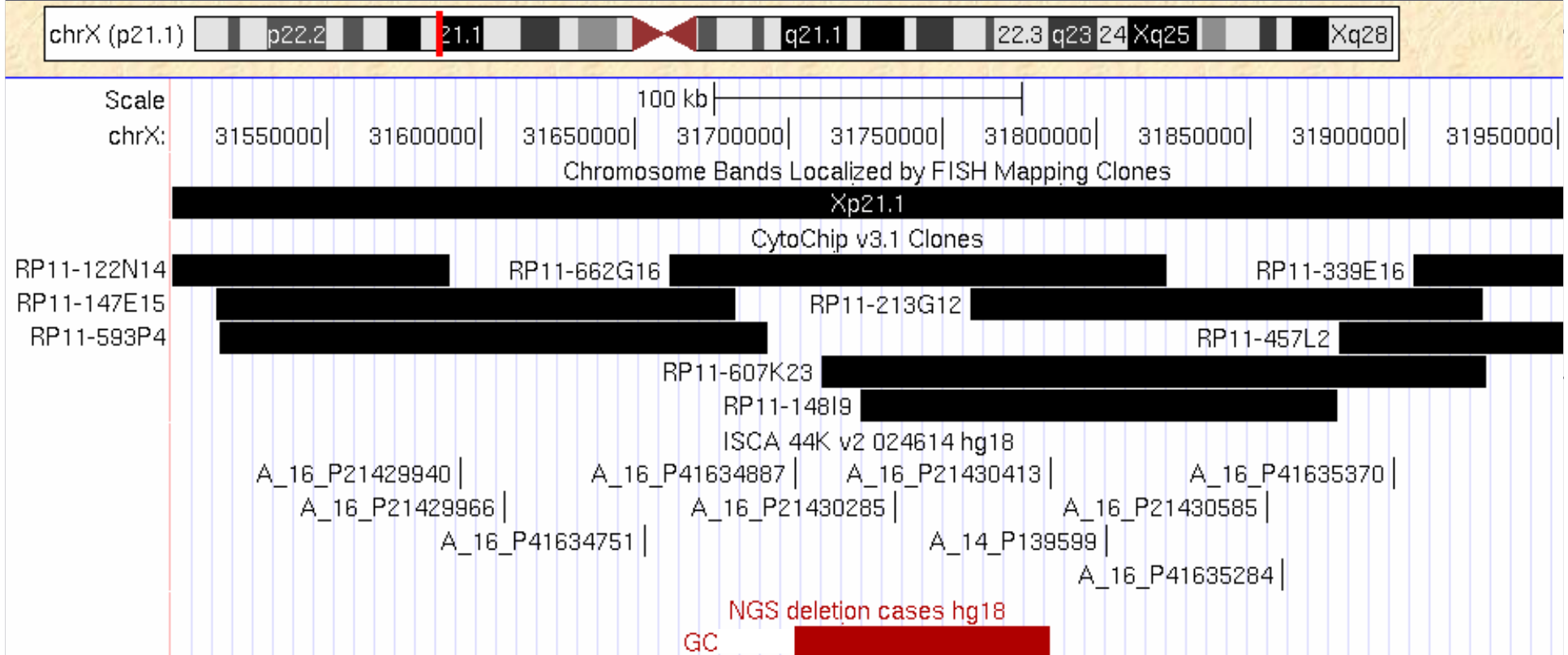
- 1q21.1 deletion / duplication
- 15q13.3 deletion /duplication
- 16p11.2 deletion / duplication
- CREBBP duplication (16p13.3)
- Potocki-Lupski (17p11.2)
- NF1 atypical deletion (17q11.2)
- RCAD deletion (17q12)
- 22q11.2 duplication
- DMD dystrophin gene 82kb deletion (Xp21.1)
- MECP2 duplication (Xq28)



Cases in known syndrome regions



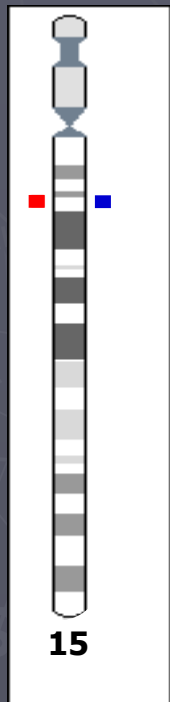
We have retrospectively examined our cases reported by oligo array CGH to determine how many would have been missed by BAC arrays



3 features on an oligo- but 4 clones on a BAC-array

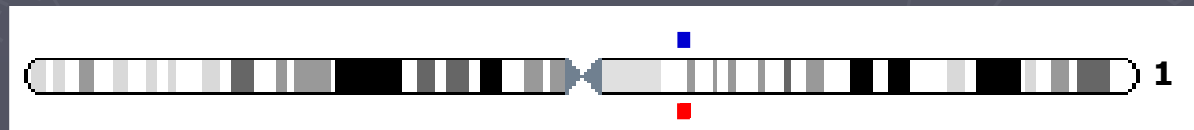
Between start of May 2010 and 15<sup>th</sup> March 2011, using UCSC genome tracks (hg19, GRCh37), for all the oligo arrays reported

- Only 13 copy number changes would have been missed by our previous BAC array platform (CytoChip v3.1)
  - Four cases whose significance is still unknown because we are still awaiting parental samples
  - Five cases that are not clinically significant, including two that were not reported because they were inherited from normal parents and had another syndrome abnormality
  - Three cases inherited from normal parents. No known OMIM genes in region identified
  - One case where the true complexity would have been missed but clinical significance unchanged



# Conclusion to the move from BAC to Oligo array CGH

1. We would have missed no known clinically significant copy number changes if we had continued with BAC arrays
2. Extrapolating, there is nothing to be gained by reprocessing the previous normal BAC array cases with oligo arrays
3. The higher resolution of the oligo arrays helps to make clinical decisions easier regarding copy number changes
4. Our experience so far is that the maximum estimated size of oligo arrays rarely makes a difference to the genetic content of an abnormality



In total, including all BAC and Oligo arrays,  
in over 5 years at Newcastle (713 cases)

164 (23%) patients where we have requested parental  
samples

204 copy number changes in 164 patients

- Average of 1.2 changes per case
- 59 (29%) known to be de novo
- 55 (27%) copy number changes have been inherited [including those with syndromes known to lack 100% expressivity / penetrance]
- 90 (44%) still awaiting parental samples, including 25 in known syndrome regions

In over 5 years at Newcastle (713 cases)

204 copy number changes in 164 patients

60 (29%) copy number changes  
are in previously known  
clinically significant regions  
(eg, syndrome and subtelomere regions)

Where we have both parents, 57% of the  
reported changes are de novo

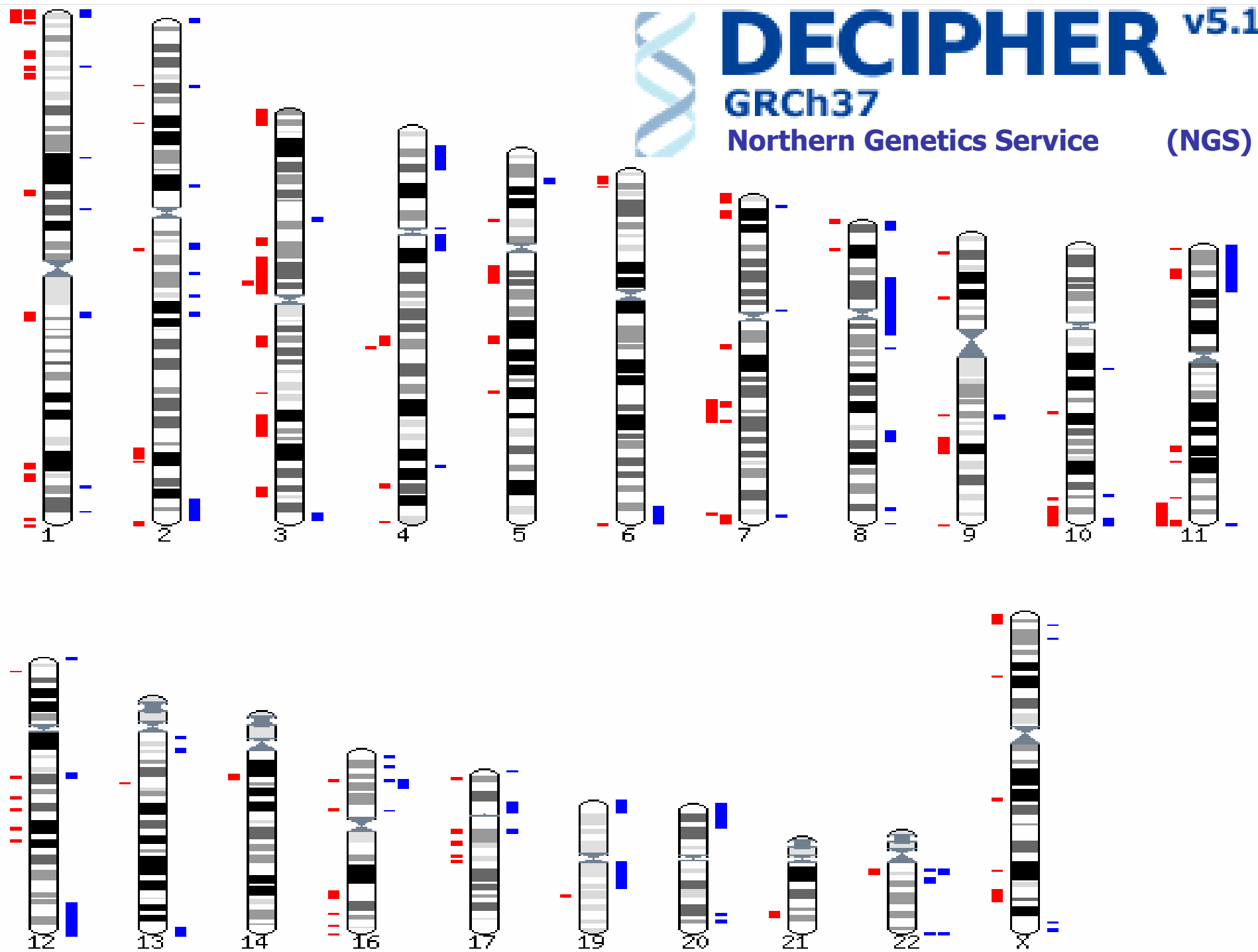


# DECIPHER v5.1

GRCh37

Northern Genetics Service

(NGS)



## The abnormal de novo cases (not seen by G-banding)

	number of cases	mean size Mb	range in size Mb
deletions	29	3.1	0.1-6.2
duplications	10	2.4	0.3-5.9
total	39	2.9	0.1-6.2

# Power of oligo arrays

female child now 5yrs old

- Dysmorphic
- Small mouth
- Heart murmur
- Odd digits on hands and feet
- Learning difficulties
- Father has similar features

Clinical photo removed

	5q23.1 loss				16p13.3 gain			
	number of features	size range Mb	number OMIM genes	clinically significant?	number of features	size range Mb	number OMIM genes	clinically significant?
BAC	2	0.44 - 2.13	0 to 5	???	6	0.74 - 1.18	1	YES
Oligo	10	0.63 - 0.64	0	NO	32	0.93 - 0.93	1	YES



## Duplications of the critical Rubinstein-Taybi deletion region on chromosome 16p13.3 cause a novel recognizable syndrome

Bernard Thienpont, Frédérique Béna, Jeroen Breckpot, et al.

*J Med Genet* published online October 14, 2009

doi: 10.1136/jmg.2009.070573

Clinical photo removed



Clinical photo removed



## Quotes from our Clinical Team

“Array CGH has dramatically increased our diagnosis rate. Being able to give a family a diagnosis can be a healing process in itself.”

“In some families, having a diagnosis of a de novo abnormality has allowed couples to have more children without worrying.”

“Array CGH is the gold standard..... so if we have a family with a normal array result, we can discharge the family saying there is nothing more we can do..... that might change of course with the DDD study.”

## More quotes from our Clinical Team

“Every time you give an abnormal array result to a member of our team they get excited.”

“For known abnormalities, arrays allow a long term follow up by finding out the exact genes involved, which leads to happier families who can understand the ‘why’ things are happening or being done.”

“You can’t run a modern genetics service without array CGH.”

# Work with Clinical Geneticists

- Work together adding cases to DECIPHER
- Include DECIPHER type report with the array CGH report
- Good communications / regular meetings
- Discussing individual cases with unclear significance
- Run genome browser workshops / array CGH interpretation
- Created genome browser tracks for cases and FISH probes
- Created a "Clinician" Genome Browser log-in with array tracks and cases curated / managed by Molecular Cytogeneticists with different "Sessions" for the different genome builds

# Use of 'Blue Elephant Definition' files (BED files/genome browser tracks)




position/search  [gene](#)    size 5,852,616 bp.







chr1 (q21.1-q21.2) 




Scale 2 Mb  
chr1: | 145000000 | 145500000 | 146000000 | 146500000 | 147000000 | 147500000 | 148000000 | 148500000 | 149000000 | 149500000 |


Chromosome Band 1q21.1 1q21.2

ISCA 44K v2 hg19 

1q21.1\_TAR\_syndrome   
1q21.1\_deletion\_syndrome   
1q21.1\_duplication\_syndrome 

NGS duplication cases hg19  
GC   
GC   
GC   
GC   
GC   
GC 

NGS deletion cases hg19  
GC   
GC   
GC 

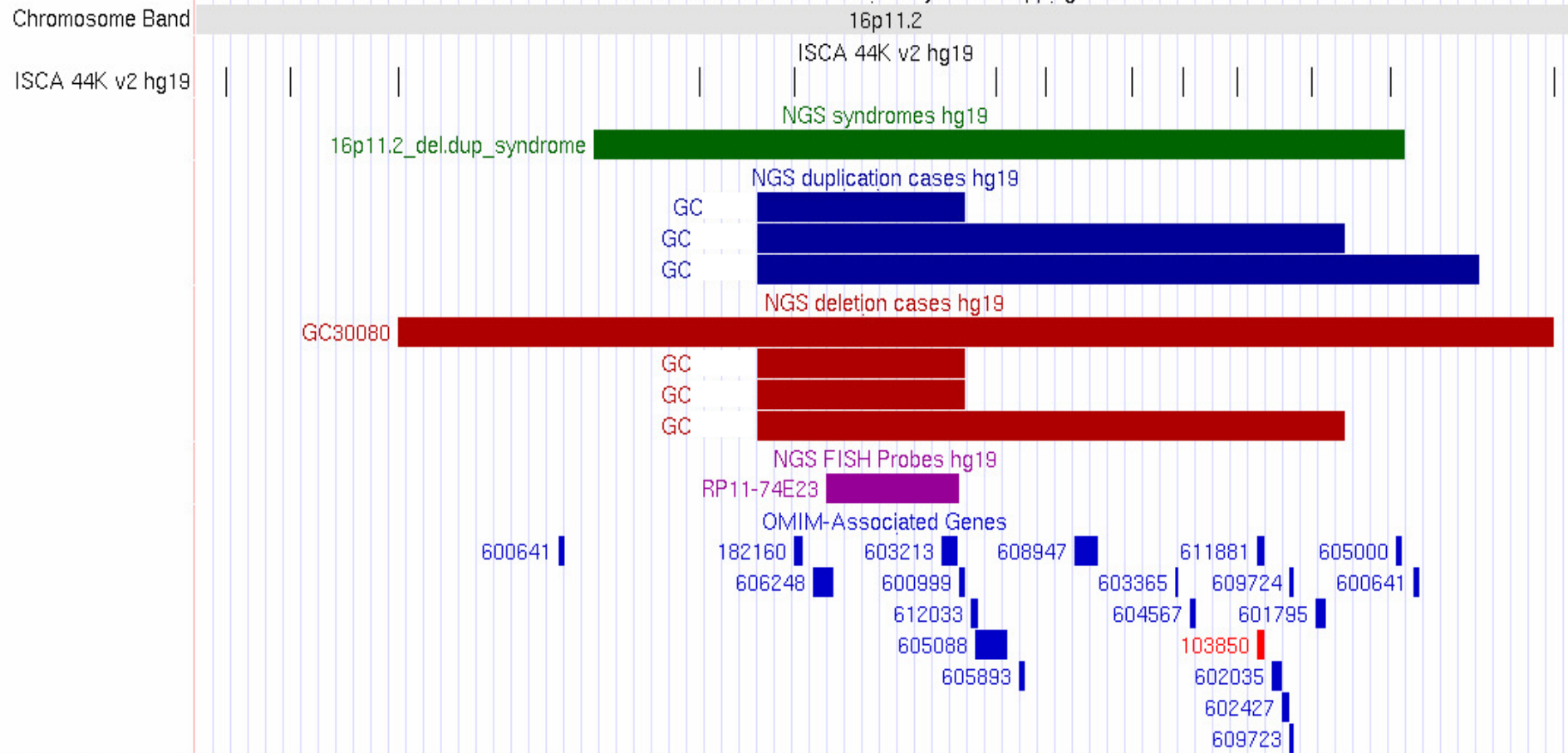
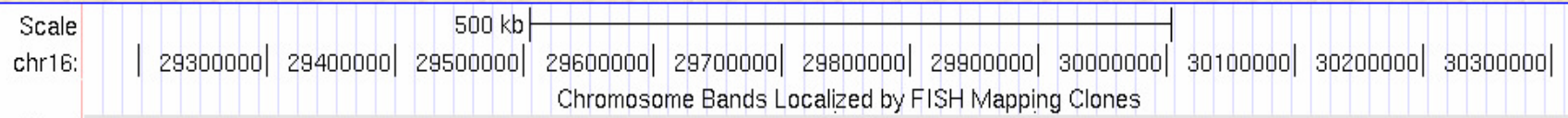
NGS FISH Probes hg19  
RP11-314N2 

Genetic Association Studies of Complex Diseases and Disorders

NRG2 | HFE2 | GJA5 | FCGR1A |  
TXNIP | GJA8 |

# Allows visualisation of patient clusters and available "in-house" FISH probes

position/search  [gene](#)    size 1,192,298 bp.



# Thank You

- Every one in all the Newcastle Genetics Laboratories  
for G-banding / FISH / MLPA / DNA extraction
- Our Clinical Geneticists  
for their clinical input, feedback and particularly  
for their encouragement
- Special thanks to Kate Rennie  
for creating the BED files / genome browser tracks