

Oligonucleotide array-CGH analysis of fetuses with selected congenital abnormalities

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Aims and Objectives

AIMS:

Develop and implement the use of array CGH on fetal samples in the Newcastle Cytogenetics Laboratory

Identification of causative copy number changes in previously karyotyped fetuses with selected congenital abnormalities

OBJECTIVES:

1. Validation of technique on archived cultured fetal cells
2. Testing of cohort of samples presenting with
 - i. Holoprosencephaly
 - ii. Anencephaly
 - iii. Congenital diaphragmatic hernia

HOLOPROSENCEPHALY (HPE)

- Incomplete or failed cleavage of the forebrain during fetal development
- 1:250 conceptuses, 1:10-16,000 live births
- Varying clinical spectrum
- Chromosome abnormalities in 20-45% (Bendavid *et al* 2010)
- 4 major genes
 - SHH (7q36)
 - sonic hedgehog - brain and CNS development
 - ZIC2 (13q32)
 - zinc finger transcription factor – neurulation
 - SIX3 (2p21)
 - homeobox containing gene – head midline and eye formation
 - TGIF (18p11)
 - homeodomain transcription factor – inhibits signalling through TGF β pathways

ANENCEPHALY

- Extreme form of a neural tube defect resulting from failure of rostral neuropore closure
- Brain has neural tissue exposed
- 0.5-2:1000 conceptuses
- Large no of studies undertaken to elucidate causative genes – very few consistently reported
 - MTHFR (1p36.3)
 - Two polymorphisms linked with 1.8 fold increased risk
- Chromosomal abnormalities in ~ 2% (Kennedy *et al* 1998)
 - +18, +13, triploidy
 - del(13q), r(13), dup(2p)

CONGENITAL DIAPHRAGMATIC HERNIA (CDH)

- Protrusion of the abdominal contents through a defect in the structural integrity of the diaphragm.
- 1:2500-4000 live births
- Isolated and non-isolated forms
- Chromosomal abnormalities in ~ 10% (Pober, 2007)
- A number of clinically relevant loci have been identified – likely to contain genes contributing to CDH
 - 15q26 (DIH1)
 - 8p23.1 (DIH2)
 - 8q23.1 (DIH3)

Materials and Methods

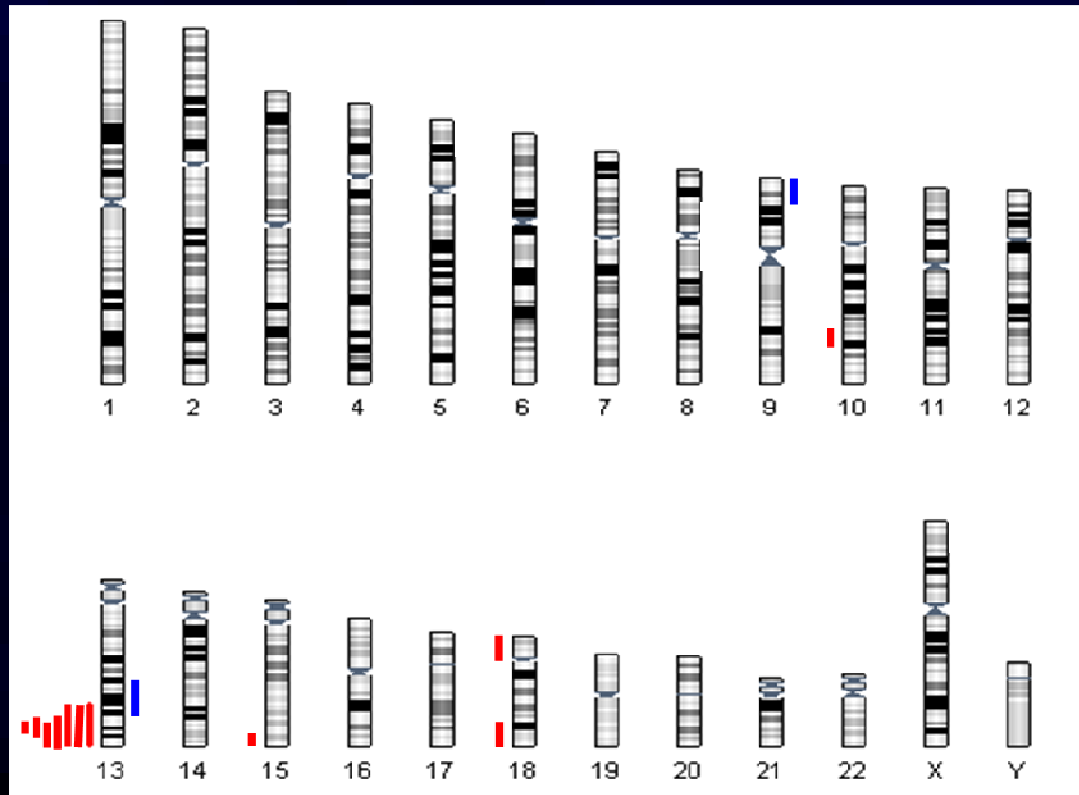
- Archived fetal cells (skin, muscle, membrane and villi) were retrieved from liquid N₂ where they had been stored following cell culture
- DNA was extracted from cultured, retrieved cells
- 72 samples
 - 36 holoprosencephaly (10 with abnormal karyotype)
 - 20 anencephaly
 - 16 congenital diaphragmatic hernia (1 abnormal karyotype)
- Whole genome CytoChip ISCA 4x44K (v2.0) oligonucleotide array (BlueGnome, Cambridge, UK)

Results

Objective 1. Validation of Technique

11 samples (from cohort of 72) with unbalanced karyotypes

All abnormalities were identified by array CGH



Results

Objective 2. Cohort Testing

	Holoprosencephaly	Anencephaly	Diaphragmatic hernia	Total
Benign Variants	74	51	17	142
Discounted due to nature of abnormality	-	-	2	2
Uncertain Significance	11	8	11	30
Pathogenic	4	-	3	7

Total abnormality rate – 20% [37/181] (unconfirmed)

Results

Objective 2. Cohort Testing

Drawbacks of study

- Aberrations not confirmed by alternative methods
 - Expected that false positive rate is extremely low
- Inheritance information not available

Results

Objective 2. Cohort Testing

1. F94/0452

PM: Alobar holoprosencephaly

Cyclopia

Encephalocele

Cardiac and pulmonary abnormalities

G-banding: fetal skin and muscle fibroblasts – 46,XY,t(2;4)



Results

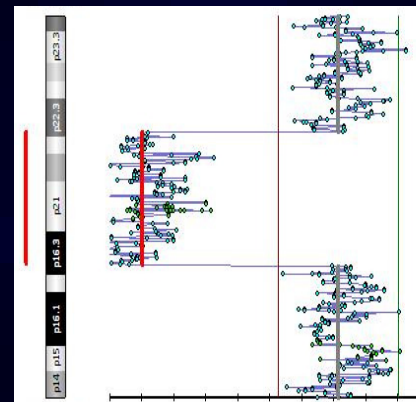
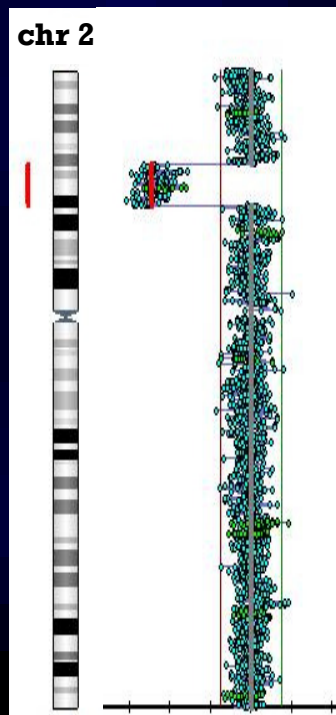
Objective 2. Cohort Testing

aCGH:

6 copy number changes

- 5 benign copy number variants

N...	Start C...	End Cyto	Type	Assessment	R...	Size
2	1p36.23	1p36.23	Gain	Unknown?	yes	309676.0
15	8p11.23	8p11.22	Gain	Benign?	yes	127205.0
5	2p22.3	2p16.3	Loss	Pathogenic?	yes	15673677.0
13	8p23.1	8p23.1	Loss	Unknown?	yes	861042.0
32	Xq28	Xq28	Loss	Unknown?	yes	3016.0
35	Yp11.2	Yp11.2	Loss	Unknown?	yes	75076.0



Copy number change of 227 features

DELETION of 2p16.3-22.3

15.6Mb

Incorporates 58 OMIM genes

- SIX3 (HPE2) (2p21)

Results

Objective 2. Cohort Testing

2. F00/466

Holoprosencephaly (no PM)

G-banding: Chorionic villi cultures – 46,XY

aCGH: 3 copy number changes

- 2 benign copy number variants

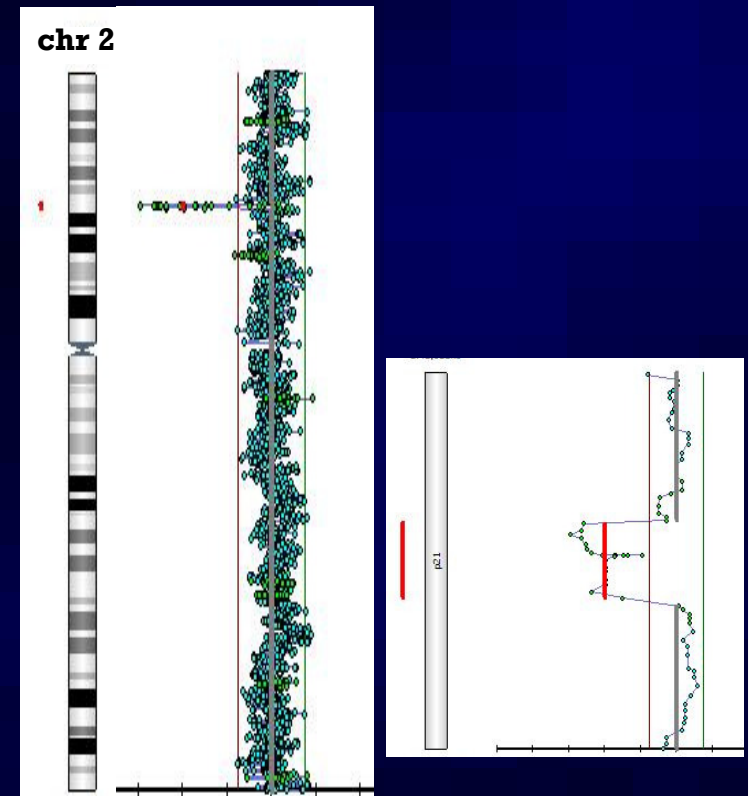
1 copy number change of 27 features

DELETION of 2p21

1.0Mb

Incorporates 3 OMIM genes

- SIX3 (HPE2)



Results

Objective 2. Cohort Testing

3. F94/0251

PM: Alobar holoprosencephaly

Secondary adrenal hypoplasia

G-banding: Fetal skin fibroblasts – 46,XY

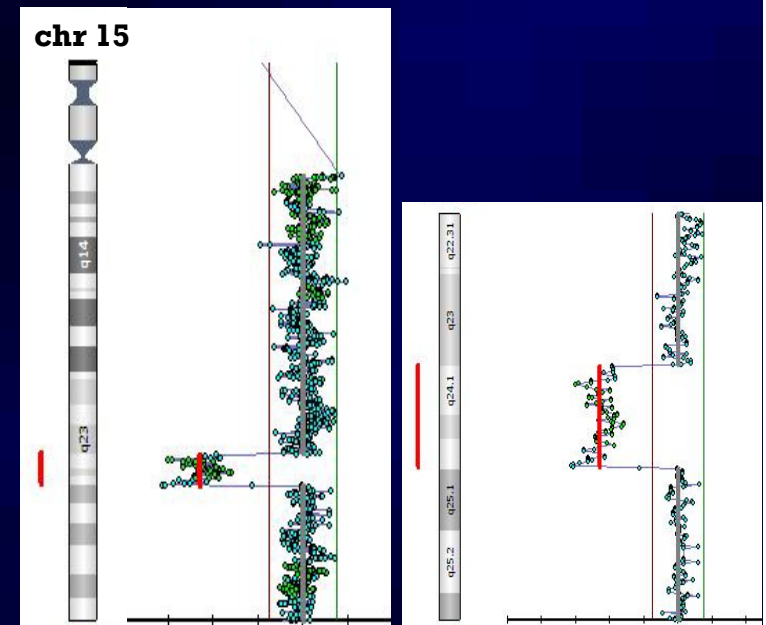
aCGH: 1 copy number change – 98 features

DELETION of 15q23-q24.3

5.6Mb

Incorporates 51 OMIM genes

15q24 microdeletion syndrome region

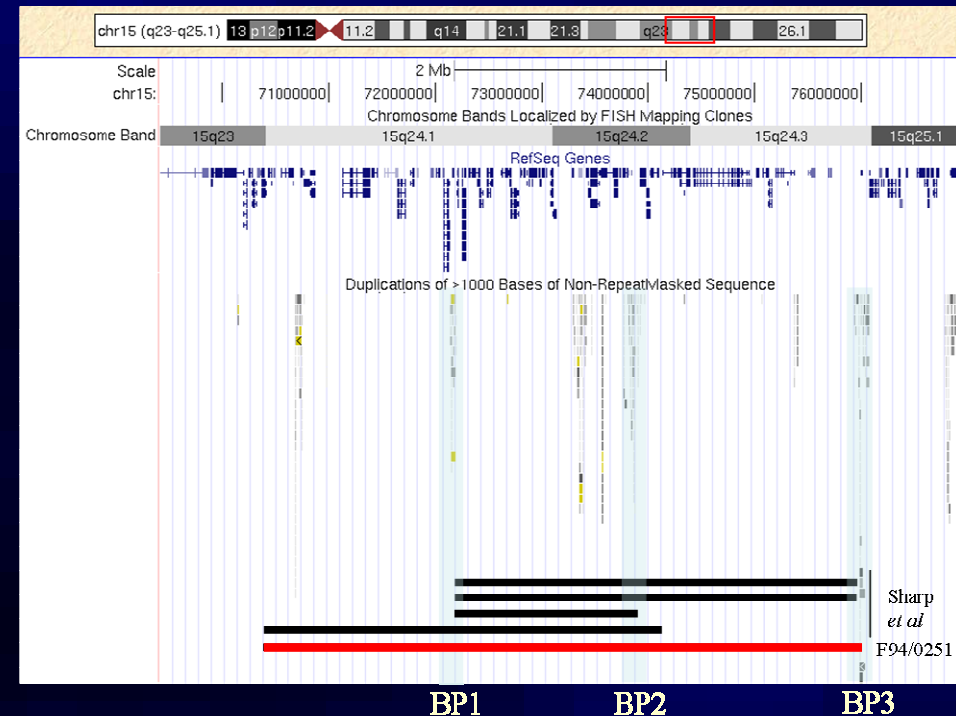


Results

Objective 2. Cohort Testing

Postnatal Phenotypes:

- developmental delay
- unusual facial features
- growth retardation
- microcephaly



- Postnatal phenotypes much less severe
- Poorly understood how microdeletions/duplications manifest prenatally
 - may be the cause of more severe phenotypes
- Clinical spectrum may be larger than initially thought

Results

Objective 2. Cohort Testing

4. F97/350

PM: Diaphragmatic hernia

Hypoplastic left kidney

G-banding: Fetal skin fibroblasts – 46,XX

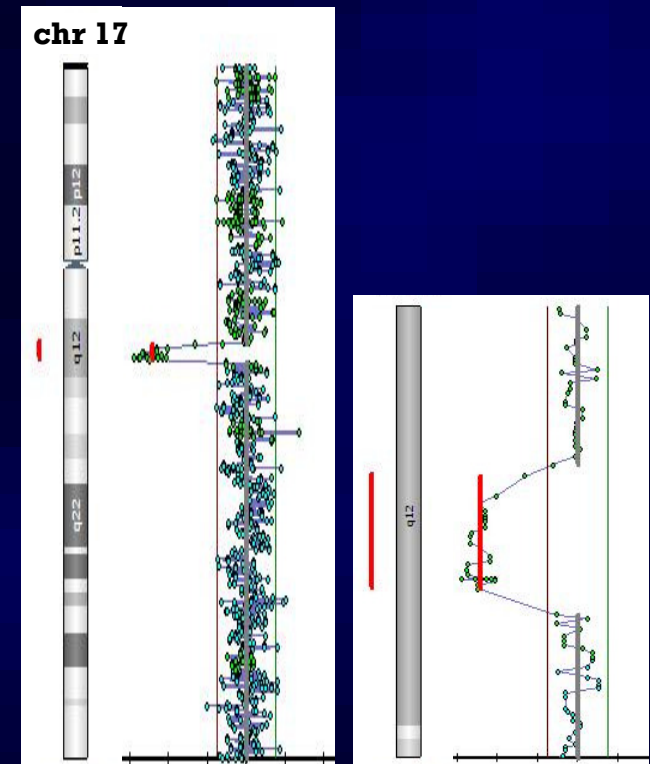
aCGH: 2 copy number changes

➤ 1 benign copy number variants

1 copy number change – 30 features

DELETION of 17q12

1.7Mb



Results

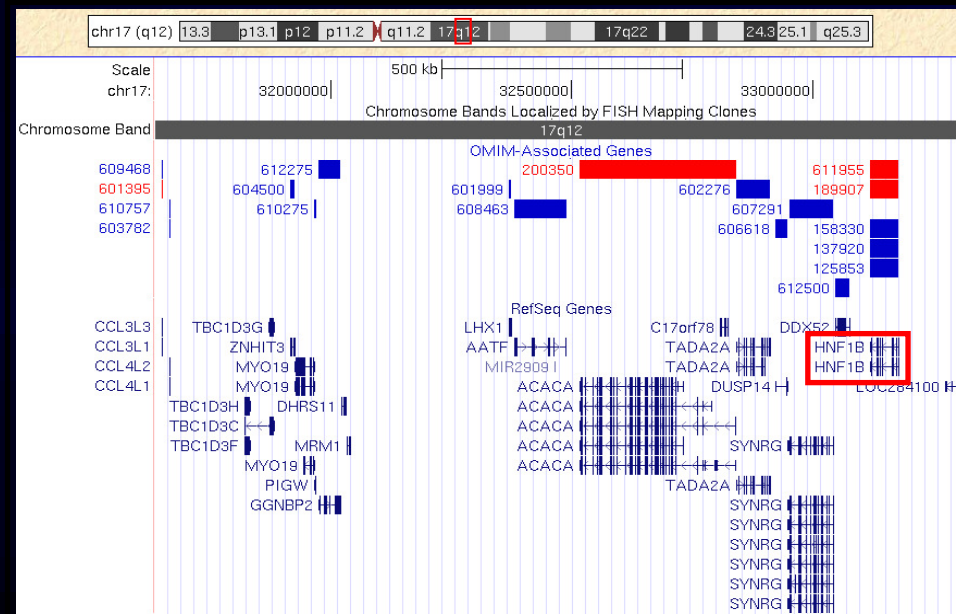
Objective 2. Cohort Testing

17q12 deletions: autistic spectrum disorders

schizophrenia

wide range of phenotypes

➤ HNF1B gene – cause of renal cysts and diabetes syndrome (RCAD)



Conclusions

	Holoprosencephaly	Anencephaly	Diaphragmatic hernia	Total
Uncertain Significance	11	8	11	30
Pathogenic	4	-	3	7
Total	15	8	14	37

- Total unconfirmed abnormality rate of 20%
 - Aberrations not confirmed by alternative methods
 - Inheritance information not available
- Array CGH successful using DNA from archived fetal cultured cells
- This technique is reliable in detecting abnormalities
- Previously unidentified abnormalities were detected
- Genotype-phenotype studies required prenatally

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