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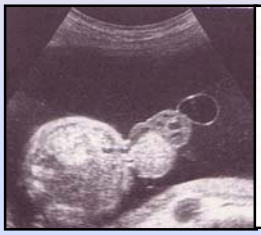
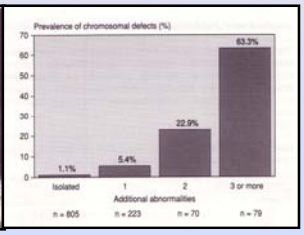
Birmingham Women's NHS NHS Foundation Trust

The use of new genomic technologies for the prenatal diagnosis of chromosome abnormalities when a fetus has a structural anomaly

Professor Mark. D Kilby, Centre for Women's & Children's Health, University of Birmingham & Fetal Medicine Centre, Birmingham Women's Foundation Trust. ***Jornadas sobre Medicina Fetal, 2015.***

Congenital structural anomaly



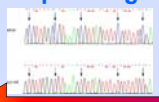


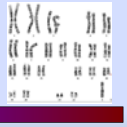
- **Different fetal anomalies are associated with different risks of chromosomal abnormalities.**
- **Increasing additional anomalies increases chromosomal difference.**

Increased size of imbalance

1bp | 6Kb | Resolution of 'array platform' | 5Mb | >10Mb

Sequencing **Chromosomal Microarrays** **Cytogenetics**



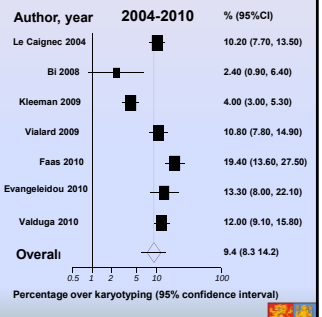


Increased resolution whole genome arrays

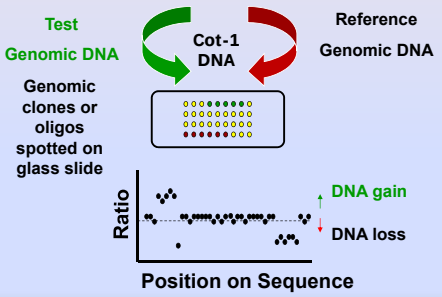
Chromosomal microarrays

Test used for children:

- Dysmorphic appearance.
- Unexplained neurodevelopmental delay.
- Malignancy risks.
- Psychological morbidity



Chromosomal microarray method



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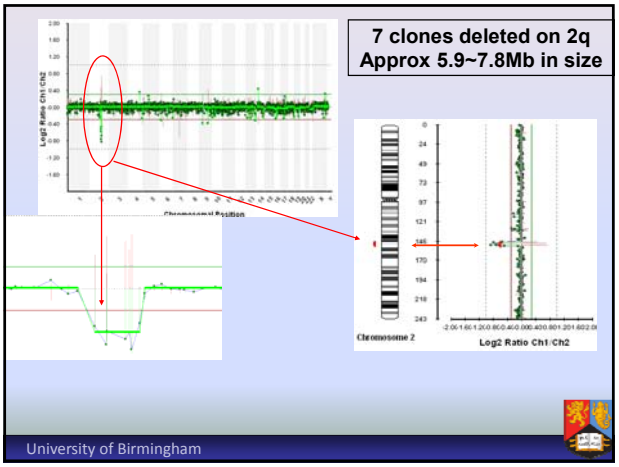

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Strengths of microarrays

- High resolution ⟶ increase detection rates.
- Detects microdeletions and duplications.
- Removes culturing that can introduce clonal selection.
- Small amount of DNA
- Fast turn around times.
- Protocols are/can be automated for higher throughput.

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Weaknesses of microarrays

Can not detect:
- Balanced translocations
- Inversions

Low sensitivity:
- Triploidy
- Mosaicism (<10-20%)

Copy Number Variance (CNV), what is normal?
- Wellcome Trust Case Control Consortium

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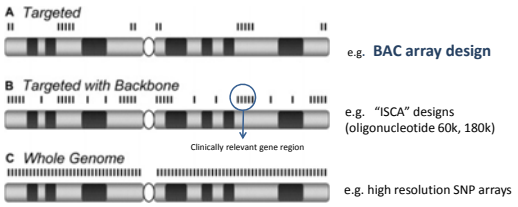
Evolution of Chromosomal Microarray platforms



Prospective Cohort Aims

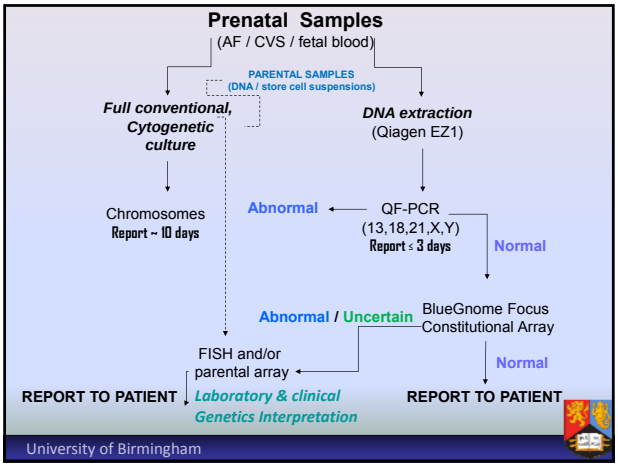
- Would prenatal CMA for fetuses with **abnormal scans** give clinically useful information over conventional karyotyping?
- Is it feasible within the pregnancy time-frame?
- Would the DNA quality from amnio/CVS make a difference?
- What would women and their partners think/feel about it?
- Is it economically viable in the NHS?

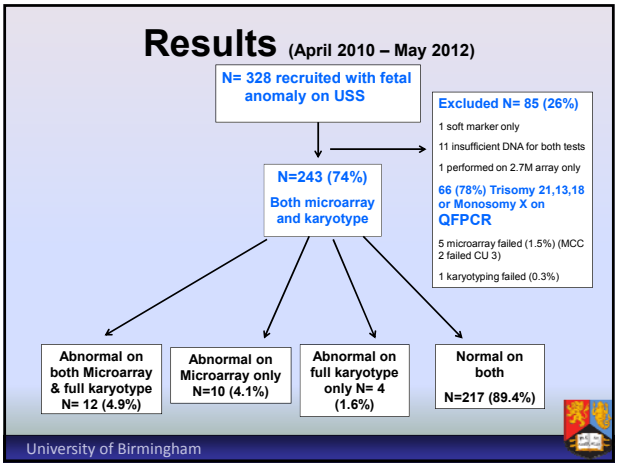
Methods

- Birmingham Women's Hospital Foundation Trust
- Prospective study.
- Collaborative Fetal Medicine/Clinical genetics/laboratory : University of Birmingham
- Ethics : Written consent & information.
- Parental blood taken (fetal 'trios').
- Results fed back to patients with Clinical Geneticists
- Semi structured interviews / qualitative research (in sample)

Focused array, Why?

- **1 Mb targeted-BAC microarray** (Conservative).
- Experience in the WMGL, good results
- Conservative to minimize results of unknown significance (same lab uses 60K array on paediatric samples)
- Works with prenatal sample DNA quality (poor) and quantity (less) than postnatal samples
- Give results in a timely fashion





Human Phenotype Ontology (HPO) classification of anomalies

Structural anomaly	N=	Percentage
Single	228	94%
Central nervous system	51	21%
Cardiovascular system	41	17%
Increased NT>3.5mm / Cystic hygroma	40	16.5%
Musculoskeletal system	25	10.5%
Genitourinary system	20	8.5%
Congenital diaphragmatic hernia	15	6%
Abdominal wall defect	11	4.5%
Head/face/neck	7	3%
Respiratory system	5	2%
Spina Bifida/ encephalocele	5	2%
Hydrops	4	1.5%
Gastrointestinal tract	3	1%
Tracheal/oesophageal fistulae	1	0.5%
Multiple systems	15	6%

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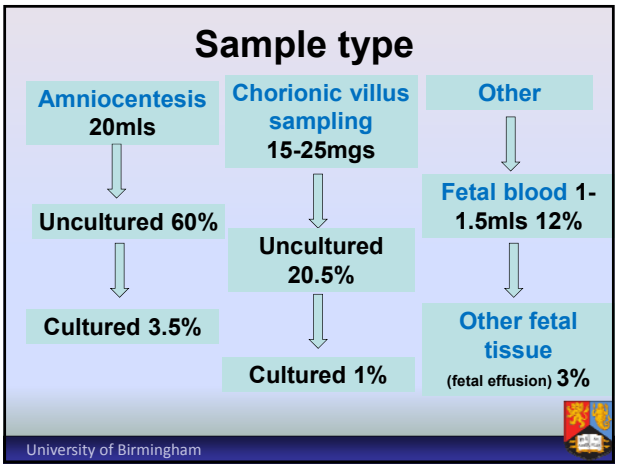

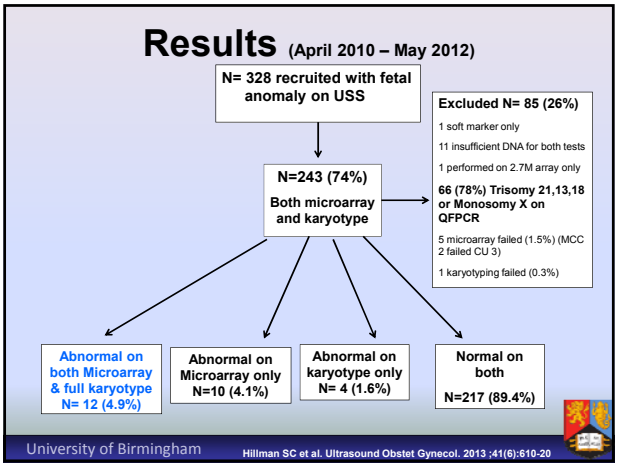

Abnormal on microarray and karyotype

- In 12 fetal samples (4.9%) 17 pathological CNVs
  - 4 aneuploidy: Two cases Trisomy 9 & two cases Trisomy X.
  - 7 large structural chromosome anomalies:
    - 46,XX,der(2)del(2)(p16.2p22.3)(q14.1q22.1)dn,t(5;6)(q22;p11)dn
    - 45,X,idic(Y)(q11.2)[27]/45,X[7]
    - 46,XX,del(6)(q11.1q16.1)dn
    - 47,XX,+r(8)(p10p21.2)[12]/46,XX[8]
    - 46,XX,del(7)(q3.2)dn
    - 46,XX,del(6)(q2.5.1)dn
    - 46,XX,der(8)del(8)(p23)invdup(8)(p23.1p11.2)dn
  - 1 at limit of cytogenetic resolution (Smith-Magenis Syndrome) : 46,XY,del(17)(p11.2p11.2)dn

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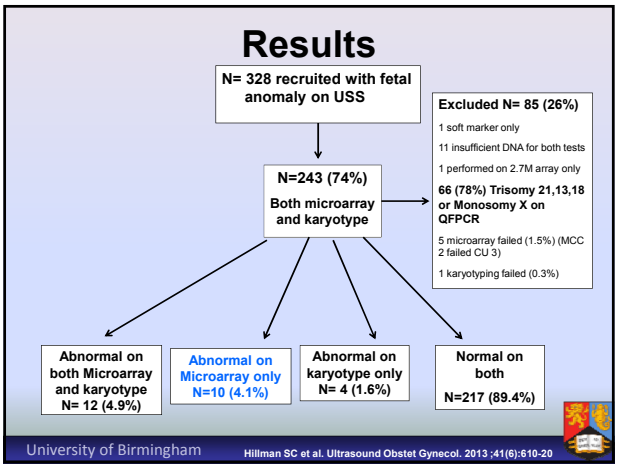

Detectable only by microarray

- 4.1% fetal samples / subjects.
  - 4 cases of 22q11.2 deletion (Di George syndrome)
  - 1 case of mosaic isochromosome 12p (Pallister Killian syndrome)
  - 1p36 microdeletion syndrome
  - Deletion 17p12 including PMP22 (HNPP)
  - 6-8Mb duplication 11q24.2 &11q25 (50 HGNC genes)
  - Submicroscopic (3.2 Mb) deletion 5q35.3

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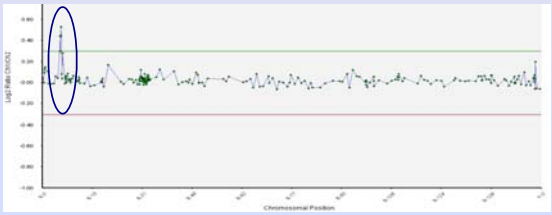
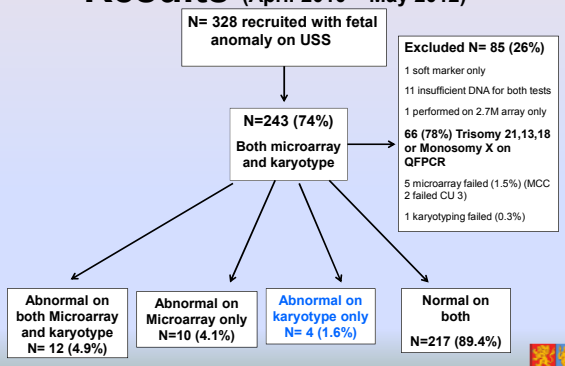
VOUS : Only **one** in our series (0.4%).

- **0.5-1Mb duplication between Xp22.32 and Xp22.31 in a male fetus.**
- **Prenatal diagnosis of complex CHD : Truncus Arteriosus.**



- Partial duplication/possible disruption of the gene *NLGN4*
- *N*o known cardiac link but is potentially (but not certain) associated with neuro-developmental delay/autism
- Shown to be maternally inherited - unknown significance (VOUS)

Results (April 2010 – May 2012)



Hillman SC et al. Ultrasound Obstet Gynecol. 2013 ;41(6):610-20

Did microarray miss anything?

4 cases (1.6%)

- **3 balanced inversions**
  - Very unlikely to have phenotypic effect
  - Microarray would have been reassuring.
- **1 sample mosiac :** 47,XXX and Monosomy X (47XXX[45]/45X[15]) likely to have a phenotypic effect (cystic hygroma/hydrops and fatal at 23 weeks)

Conclusion of cohort

- BAC array added information over conventional karyotyping in **4.1%**.
- Only one **VOUS (0.4%)** much lower then contemporary studies (1.2 - 4.7%)
- Robust, accurate and valuable method
- Turn around under <10 days
- Conservative focused but relatively low resolution, 1 Mb targeted BAC microarray

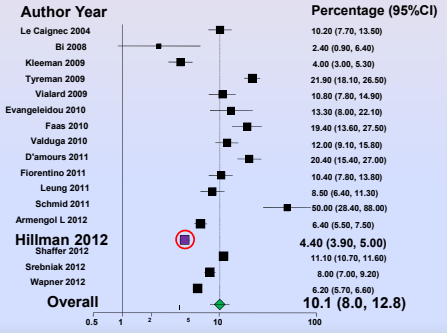
Hillman SC et al. Ultrasound Obstet Gynecol. 2013 ;41(6):610-20

Prenatal comparison of BAC and 60k array platforms

- 62 fetal cases with fetal anomaly (1 Mb BAC array) then at TOP (60 k oligonucleotide array).
- BAC array noted a detection rate of pathogenic CNVs in 4.1% (95% CI 2.1-7.6) of cases and VOUS rate of 0.4% (95% CI 0.07-2.2) over G-band karyotyping.
- The 60 K array had an additional pathogenic detection rate of 4.8% (95% CI 1.6-13.3) over the BAC array but also detected an additional 8% (95% CI 1.3-14.8) VOUS.

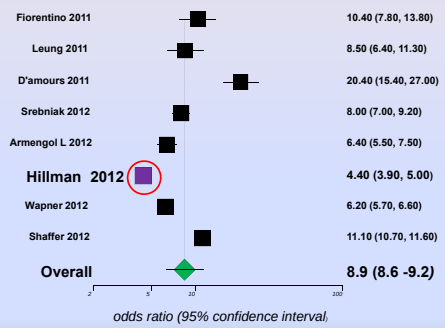
(Hillman SC et al. *J Mat Fetal Neonatol Med.* 2014 In Press).

Percentage of positive CMA findings over Karyotyping when indication *is abnormal USS*



Percentage positive findings by CMA over karyotype

Percentage of positive CMA findings over conventional karyotyping when indication *is abnormal USS* 2010-2012



Systematic Review : VOUS

- 2.1% (95%CI 1.3-3.3) for *abnormal ultrasound scan*
- Our own cohort was 0.4%
- Conventional karyotyping detected an extra 0.6% (95%CI 0.2-1.6%) when microarrays were normal.
  - *Mosaicism*
  - *Balanced inversion/translocation*

VOUS : 'This is "toxic" information'

Qualitative study in Birmingham.

"*Like I thought today, we would be walking out of here guaranteed to know what we need to know (sic: about the babies outcome). But we are in the same place. I hadn't prepared myself , you never think a doctors going to go say "phew, don't know what it is*"!

Hillman SC, Skelton J, Quinlan-Jones E, Wilson A, Kilby MD.
"If it helps..." the use of microarray technology in prenatal testing: patient and partners reflections.
Am J Med Genet A. 2013 ;161 A(7):1619-27

Qualitative element of NICHD study Bernhardt et al.

- Increased anxiety
- Concern continued after pregnancy
- 50% had lingering worries about their child's development

Bernhardt,B.A., Soucier,D., Hanson,K., Savage,M.S., Jackson,L., and Wapner,R.J. 2012. Women's experiences receiving abnormal prenatal chromosomal microarray testing results. Genet.Med, 2013 .

ORIGINAL ARTICLE

Secondary findings from non-invasive prenatal testing for common fetal aneuploidies by whole genome sequencing as a clinical service

Tze Kin Lau[1], Fu Man Jiang[2], Robert J. Stevenson[3], Tsz Kin Lo[4], Lin Wai Chan[5], Mei Ki Chan[2], Pui Shan Salome Lo[1], Wei Wang[2], Hong-Yun Zhang[2], Fang Chen[2] and Kwong Wai Choy[6,7]*

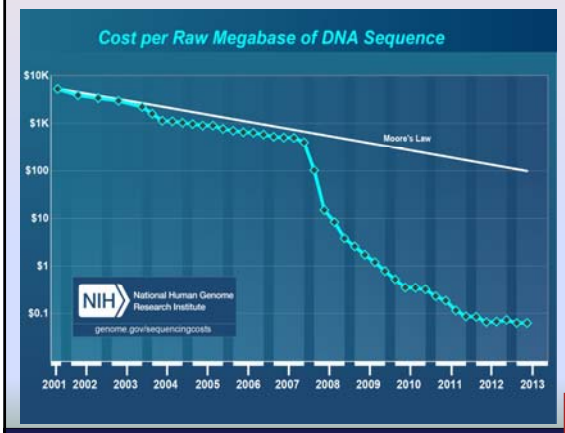
[1]Fetal Medicine Centre, Paramount Clinic, Hong Kong, China
[2]BGI-Shenzhen, Shenzhen, China
[3]Hong Kong Adventist Hospital, Hong Kong, China
[4]Department of Obstetrics and Gynaecology, Kwong Wah Hospital, Hong Kong, China
[5]Department of Obstetrics and Gynaecology, United Christian Hospital, Hong Kong, China
[6]Department of Obstetrics and Gynaecology, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong, China
[7]CUHK-Shenzhen Research Institute, Chinese University of Hong Kong, Shenzhen, China

In Case 1, NIPT revealed a large duplication in chromosome 18p, with final karyotype showing mosaic tetrasomy 18p. In Case 2, a deletion in the proximal long arm of chromosome 18 of maternal origin. In Case 3, NIPT a deletion in the proximal long arm of chromosome 3 was found. In Case 4, NIPT correctly predicted confined placental mosaicism with triple trisomy involving chromosomes X, 7 and 21. In Case 5, NIPT correctly detected a 45X.

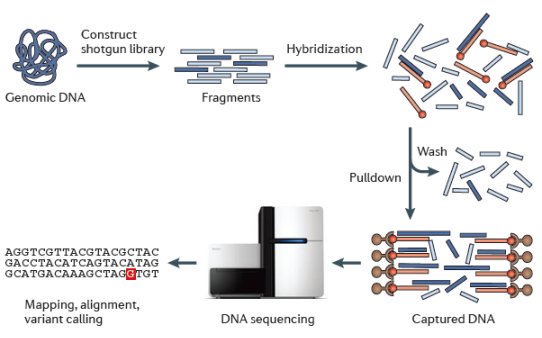
Microarray conclusions:

- aCGH is useful in prenatal diagnosis and identifies 'pathogenic' CNVs in approximately 5-10% of cases (as compared to conventional karyotyping).
- The choice of platform (depth and resolution) influences pathogenic and potentially unwanted VOUS detection.
- VOUS are considered by parents as being undesirable and 'toxic' information.

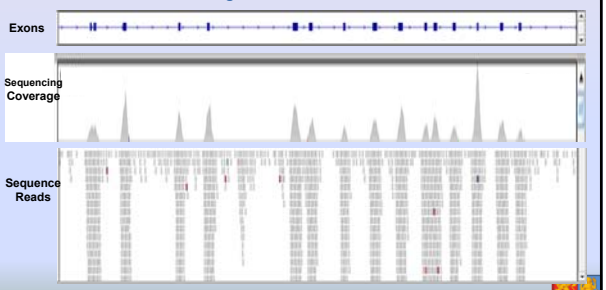
New Generation Sequencing : Exome sequencing



Targeted ('Exome') Sequencing



NGS can be used to identify SNVs and indels throughout the genome, Exome sequencing is often favoured over whole genome sequencing, as it targets only coding regions, which represent 1-2% of the entire genome, but contain around 85% of the mutations that cause known genetic disorders



Drs. Keren Carss & Matt Hurles at the Sanger Institute, Cambridge, UK

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Clinical Whole-Exome Sequencing for the Diagnosis of Mendelian Disorders

Yaping Yang, Ph.D., Donna M. Muzny, M.Sc., Jeffrey G. Reid, Ph.D., Matthew N. Bainbridge, Ph.D., Alecia Willis, Ph.D., Patricia A. Ward, M.S., Alicia Braxton, M.S., Joke Beuten, Ph.D., Fan Xia, Ph.D., Zhiyv Niu, Ph.D., Matthew Hardison, Ph.D., Richard Person, Ph.D., Mir Reza Bekheirnia, M.D., Magalie S. Leduc, Ph.D., Amelia Kirby, M.D., Peter Pham, M.Sc., Jennifer Scull, Ph.D., Min Wang, Ph.D., Yan Ding, M.D., Sharon E. Plon, M.D., Ph.D., James R. Lupski, M.D., Ph.D., Arthur L. Beaudet, M.D., Richard A. Gibbs, Ph.D., and Christine M. Eng, M.D.

CONCLUSIONS

Whole-exome sequencing identified the underlying genetic defect in 25% of consecutive patients referred for evaluation of a possible genetic condition. (Funded by the National Human Genome Research Institute.)

NEJM. 2013. 396; 1501-1511.

Clinical Description of Patients for Whom Whole-Exome Sequencing Was Ordered.

Table 1. Clinical Description of Patients for Whom Whole-Exome Sequencing Was Ordered.

Primary Phenotype Category	Age Group at Testing				
	Fetus	<5 Yr	5–18 Yr	>18 Yr	Total
Neurologic disorder*	0	31	27	2	60
Neurologic disorder and other organ-system disorder	1	74	54	11	140
Specific neurologic disorder†	0	5	5	3	13
Non-neurologic disorder	3	14	8	12	37
Total	4	124	94	28	250

* Neurologic disorders included developmental delay, speech delay, autism spectrum disorder, and intellectual disability.
† Patients in this category had a specific neurologic problem such as ataxia or seizure.

We identified 86 mutated alleles that were highly likely to be causative in 62 of the 250 patients, achieving a **25% molecular diagnostic rate** (95% CI, 20 to 31).

Among the 62 patients, **33 (53%) had autosomal dominant disease, 6 (9.6%) had autosomal recessive disease, and 9 (14.5%) had X-linked disease.**

A total of 4 probands received two non-overlapping molecular diagnoses, which potentially challenged the clinical diagnosis that had been made on the basis of history and physical examination.

Yang Y et al. N Engl J Med 2013;369:1502-1511.

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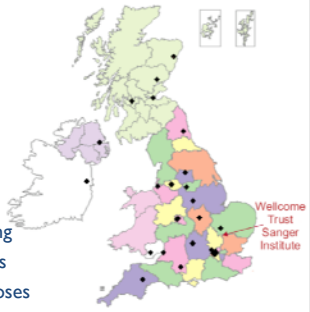
Deciphering Developmental Disorders

- Objectives:
  - Understand genetic basis
  - Catalyse improved diagnosis
- Collaboration:
  - Families + NHS + WTSI
- Strategy:
  - Recruit 12,000 families
  - Systematic clinical phenotyping
  - Genome-wide genetic analysis
  - Feedback likely genetic diagnoses

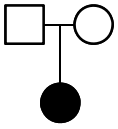


8,068 families recruited, >95% of all UK consultants

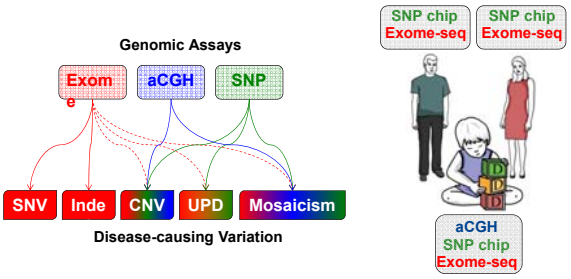
DDD families

- Record:
  - clinical presentation (Human Phenotype Ontology)
  - family history (75% singleton affected)
  - pregnancy/neonatal complications
  - growth metrics and developmental milestones
  - previous genetic tests
- Diversity representative of the UK clinical population in need of diagnosis
  - ~20% had an abnormal prenatal ultrasound
  - ~29% abnormal cranial MRI
  - ~11% heart defects
  - ~85% have neurodevelopmental disabilities
  - ~25% seizures
  - Diverse ancestry, 5% consanguinity
  - ~75% have had clinical microarray

90% trios



Genomic assays and analyses



Exome sequencing improves genetic diagnosis of structural fetal abnormalities revealed by ultrasound

Keren J. Carss, Sarah C. Hillman, Vijaya Parthiban, Dominic J. McMullan, Eamonn R. Maher, Mark D. Kilby, Matthew E. Hurles.

We performed exome sequencing on a cohort of **30 non-aneuploid fetuses (fetal trios)** with **diverse structural abnormalities identified by prenatal U/S**. We identified candidate pathogenic variants with a range of inheritance models, & evaluated these in the context of detailed phenotypic information.

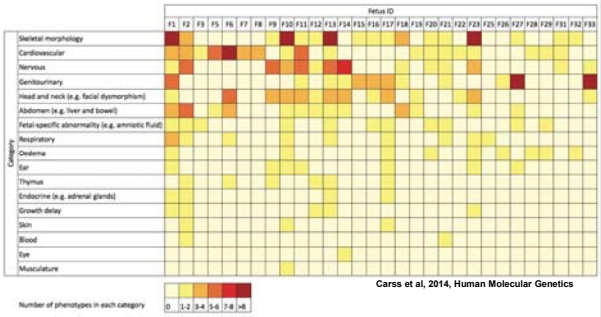
We identified 35 *de novo* single nucleotide variants (SNVs), small indels, deletions or duplications, of which **three** (**10% of the cohort**) are highly likely to **be causative**.

In **five** further cases (**17%**) we identified *de novo* or inherited recessive or X-linked variants in plausible candidate genes, which require additional validation to determine pathogenicity.

Our diagnostic yield of **10%** is comparable to, and supplementary to, the diagnostic yield of existing microarray testing for large chromosomal rearrangements and targeted CNV detection. The *de novo* nature of these events could enable couples to be counselled as to their low recurrence risk.

Human Molecular Genetics

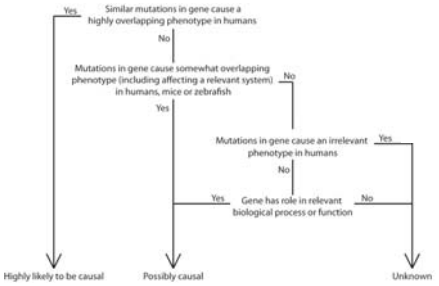
Phenotypes in pilot study of 30 fetuses



Ultrasound, post-mortem, or post-natal follow-up. Based on HPO terms.

Candidate Gene Prioritization

- **OMIM, DDG2P, BioGPS, NHGRI GWAS catalog, IKMC, ZFIN and Pubmed**

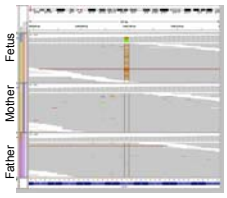


- **Found highly likely to be causal genes for 3/30 (10%)**
- **Found possibly causal genes for 5/30 (17%)**

Figure modified from Carss et al, 2014, Human Molecular Genetics

Diagnostic variants

De novo missense mutation (p.Y373C) found in *FGFR3*

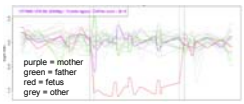


Male (F23)
Ultrasound found short limbs, large head, very narrow chest. Pregnancy terminated, post-mortem findings consistent with thanatophoric dysplasia

De novo missense mutation (p.G1164C) found in *COL2A1*

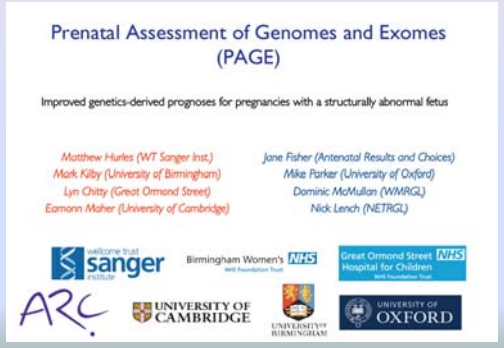
Male (F20)
Ultrasound found increased NT, tricuspid regurgitation, and very abnormal legs and feet with an extended posture and bilateral talipes equinovarus anomaly.. Miscarried , no post-mortem

De novo 21Kb deletion encompassing *OFD1*



Female (F14)
Ultrasound found ventriculomegaly and agenesis of the corpus callosum. Pregnancy terminated, post-mortem confirmed brain anomalies.

The PAGE Study : Wellcome HICF funding (£3.6M)



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Prenatal Assessment of Genomes and Exomes

The three primary objectives of the PAGE project are to:

1. **Elucidate the relative contribution of different forms of genetic variation to prenatal structural anomalies**
2. **Design cost-effective genome sequencing assays for improved prenatal diagnosis of structural anomalies**
3. **Catalyse the adoption by the NHS of prenatal diagnostic sequencing through translation of acquired know-how, rigorous health economic assessment and establishment of an ethical and social science framework for clinical implementation**

Work-packages

1. **Recruitment of families**
   - **1,000 parent-fetus trios over 2 years**
2. **Genetic investigation**
   - **1,000 trio exomes, 20 trio genomes**
3. **Assessing diagnostic yield and outcomes**
   - **Clinical review, analytical validation, neonatal and post-mortem outcomes**
4. **Health economics**
5. **Novel assay design**
   - **Cost-effective, targeted assays, explore the potential for the non-invasive approach**
6. **Ethics and social science**
   - **Develop principles of good practice**

Recruitment criteria

Inclusion criteria:

1. Women undergoing invasive testing because of nuchal translucency (NT) >4mm, or one or more structural anomalies identified by ultrasound any time in pregnancy after 11 weeks gestation (those with isolated NT>4mm will be capped at 10% of total recruitment).
2. Partners for women consenting to the PAGE study
3. QFPCR or conventional G-banding or non-invasive prenatal testing (NIPT) for aneuploidy is normal

Exclusion criteria:

1. Nuchal translucency ≤4mm, no obvious structural abnormalities present, only minor sonographic markers detected
2. QFPCR or conventional G-banding or NIPT for aneuploidy is abnormal
3. One or both parent is under the age of 16 years
4. One or both parent is unable to understand the study information
5. One or both parents decline to take part in the study

The presence of a large deletion or duplication shown by prior aCGH will NOT be an exclusion criterion

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