

Managing pregnancy losses: Arrays replacing karyotypes

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Introduction

- Traditional method of cell culture performed for cytogenetic analysis of placental tissue sample metaphases.
- Several limitations associated with this method including:
 - Long culture times (up to 4 weeks)
 - High failure rate
 - Labour intensive chromosome analysis of poor quality preparations
- Project to study an alternative diagnostic tool.
 - To overcome these limitations and get more accurate picture on causes behind pregnancy loss.

Aims

- Use 12x135K NimbleGen array-CGH to analyse ~100 anonymised DNA samples from placental tissues received by the laboratory in the course of 6 months.
 - 1) Prospectively compare the diagnostic performance of both G-banded chromosome analysis and CGH.
 - Assess success/failure rate and the abnormality detection rate.
 - 2) Investigate performance of triploid samples and formalin fixed samples.

Sample preparation

- ~100 consecutive placental tissue samples received for cytogenetic analysis.
- 6 months collection period (April – September 2010).
- Each sample had routine G-banding analysis where successful, and DNA extracted for the project.
- DNA was isolated using Qiagen DNeasy blood and tissue kit.

12x135K NimbleGen array-CGH

- 12plex
- 135,000 oligos
- Test and male reference DNA samples labelled with NimbleGen's Dual-Colour DNA labelling kit.
- Blind analysis with SignalMap software and checked against annotation files for any underlying genes and CNVs.
- Criteria
 - >5 consecutive probes (losses/gains)
 - Log_2 ratio $>\pm 0.3$
- Independent blind analysis with Nexus software.

aCGH results

Karyo-code	G-banding	SignalMap	Nexus
46,XX	37	39	39
46,XY	32	35	35
Monosomy X	2	2	2
Single Trisomy	8	10 (+1)	10
Double Trisomy	1	0 (-1)	1
? Unbalanced Translocation		1	1
Fail	9	1	1
Total	89	89	89

aCGH abnormalities

- The additional abnormalities identified on aCGH included:
 - Mosaic monosomy X and Trisomy 7
 - ? Unbalanced translocation
- The abnormalities that were missed included:
 - Trisomy 13
 - A double trisomy (missed by SignalMap software but detected by Nexus software)

Overall detection rate of aneuploidies

	G-banding	aCGH
Success Rate	80 (90%)	88 (99%)
Abnormality detection rate	11/89 (12%)	14/89 (16%)

Additional imbalances

- In addition, 12 small imbalances were detected.
- Two of the samples were :
 - Sample T024 del(7)(q22.1q22.1)
 - Sample T028 del(21)(q22.3)

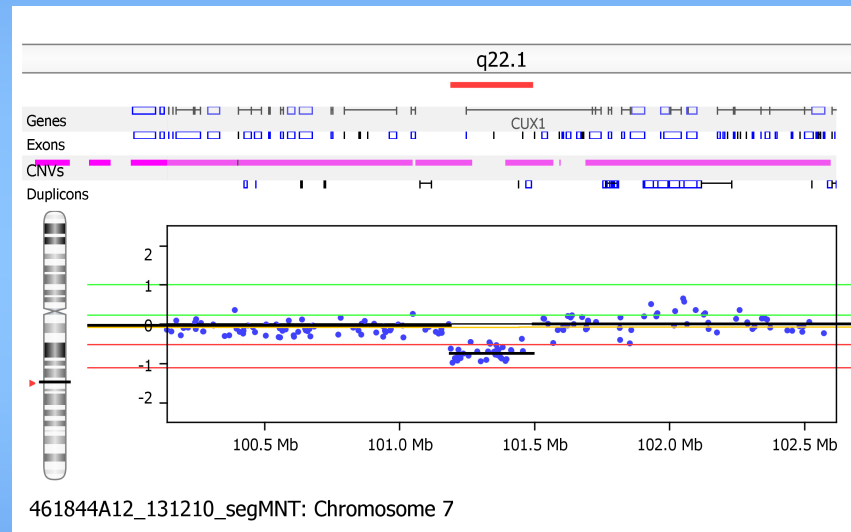
7q deletion

- Approximately 266kb interstitial deletion.
- Same deletion not seen in WTCCC2 controls.
- One gene in deletion:

- **CUX1**; Cut-like homeobox 1
 - Decipher: likely haploinsufficient
 - Integral golgi membrane protein
 - May regulate gene expression, morphogenesis, differentiation, and cell cycle progression.
 - broad role in mammalian development as a repressor of developmentally regulated gene expression.

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- In routine practise, this imbalance would be reported and followed up with parental bloods samples.

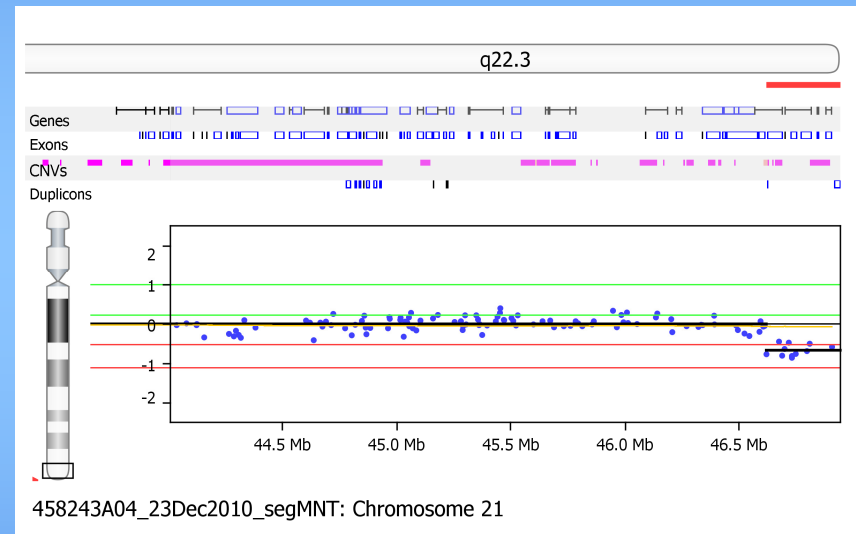


21qter deletion

- Approximately 286kb terminal deletion.
- Same deletion not seen in WTCCC2 controls.

- Underlying genes include:
 - **PCNT**; Pericentrin, recessive mutations in PCNT cause microcephalic primordial dwarfism and absence results in disorganised mitotic spindles and missegregation of chromosomes. Decipher: unlikely haploinsufficient
 - **RPL18AP2**: Ribosomal protein L18a pseudogene 2
 - **DIPZA**; disco-interacting protein 2 homolog A
 - **S100B**; S100 calcium binding protein B

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Triploid samples

- Most pregnancy losses due to chromosome abnormalities are numerical, and triploid pregnancies account for 15% (Gardner and Sutherland, 2004).
- aCGH is unable to detect triploid samples unless it is combined with genotyping capabilities.
- Three known triploid samples (1 female, 2 males) were investigated on the NimbleGen array.
- X and Y chromosome \log_2 ratios were compared to normal female and male samples respectively.
 - XXX Triploid, very subtle difference.
 - XXY Triploid, less subtle than female triploid but sex chromosome mosaicism would be suspected instead.
- Therefore, these samples would require confirmation studies with e.g. FISH, qPCR to determine polyploidy status.

Formalin-fixed samples

- Objective: Formalin-fixed samples cannot be cultured therefore G-banding is not possible.
- Disadvantage for these patients is that they do not get a result for their fixed tissue samples.
- 5 placental biopsies were fixed in formalin for varying lengths of time.

aCGH results of formalin-fixed samples

Sample ID	Duration in formalin	Protocol	Results
T107F	7 days	Failed to form pellet after labelling	None
T108F	6 days	Yielded <20ug labelled product	Yes
T109F	2 days	Successful labelling and hybridisation	Yes
T110F	28 hours	Successful labelling and hybridisation	Yes
T111F	21 hours	Successful labelling and hybridisation	Yes

- **Conclusions:**
 - Can successfully run DNA from formalin fixed samples.
 - Less time in formalin, better the outcome.

Comparison of methods

Tissue culture	Total duration	NimbleGen CGH	Total duration
Cells established and grown in flasks	3-4 weeks	DNA extraction	1 day
Harvesting, slide making (average 6 per batch)	2 hours	Labelling (batched), hybridisation (72hrs) Washing, scanning and Data alignment	5 days
Karyotype Analysis (x1)	1 - 2 hours	Analysis (SignalMap) (12plex)	~1/2 - 1 day
Independent check	1/2 hour	Independent check (Nexus)	1/2 hour
Total duration per sample	> 28 days	Total duration per 12	7 days
Reporting times per sample	>28 days	Reporting times per batch	<14days

Comparison of costs

Tissue Section	Approx cost	NimbleGen CGH	Approx cost
Full time MTO Section Scientist Second Analyst	£65,000	3 days MTO 1-2 days Scientist	£25,000
Consumables	£10,500	Consumables	£35,000
Total	£75,500	Total	£60,000

Conclusions

- A variety of cytogenetic imbalances; large and small, were identified using aCGH.
- This project has demonstrated that it is a valuable tool for the replacement of tissue culture:
 - Increased success rate
 - Increased abnormality detection rate
 - Decreased costs
 - Decreased reporting times
 - Successful analysis of formalin-fixed samples.
- The main drawback of aCGH is the inability to detect triploidy. Solution: arrays combined with SNPs.

Acknowledgements

- Amanda Clarkson
- Richard Nash
- Frankie Shaw
- Heather Kitson
- Patrick Bose
- Ingrid Simonic
- The patients and their families

- This study was funded from the grant awarded by:
ACT (Addenbrooke's Charitable Trust) Funding KDD/9793
- The study protocol was approved by Cambridgeshire 2 Research Ethics Committee, Project reference # 11/H0308/1