Introducing array comparative genomic hybridization into routine prenatal diagnosis practice: a prospective study on over 1000 consecutive clinical cases

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INTRODUCTION

While experience with diagnostic aCGH in the pediatric population is extensive, experience with its use for clinical prenatal diagnosis is still relatively limited (Sahoo *et al.*, 2006; Shaffer *et al.*, 2008; Coppinger et al., 2009; Van den Veyver *et al.*, 2009; Maya et al., 2010).

Here we present the cytogenetic findings of a prospective study, performed on a cohort of 1037 consecutive prenatal samples, comparing the results obtained using a BAC-based aCGH platform with those obtained from standard G-banded karyotyping, to assess the feasibility of offering aCGH as a first-line test in the clinical prenatal diagnostic setting.

MATERIALS AND METHODS

Samples included in this dataset were received between 1 October 2010 and 30 April 2011 from healthcare providers in Italy. The indications for invasive prenatal testing are listed in Table 1.

Prenatal samples were processed in parallel using both aCGH and G-banding for standard karyotyping.

High molecular weight DNA was extracted from 5 ml of AF and 1 mg of CVS using the QIAamp DNA Blood Mini Kit (Qiagen).

Differently fluorescently labelled test and reference DNAs of the same gender were then competitively hybridized to whole-genome BAC microarrays CytoChip Focus Constitutional (BlueGnome).

Detected copy number gains or losses were compared to known CNVs in publicly available databases (e.g. Database of Genomic Variants – DGV; Decipher; etc.) and in our own database of results, in order to ascertain the clinical significance of the variation. Array results were confirmed by fluorescence in situ hybridisation (FISH) or by quantitative fluorescent PCR or STR markers genotyping.

Table 1: *Number and types of prenatal samples processed for study according to primary indication*

Indication	Amniotic Fluid (AF)			CVS	Total (%)
	Direct AF	Cultured amniocytes	DNA from uncultured amniocytes		
Advanced maternal age (AMA) (≥35 years at conception)	376	3	1	64	444 (42.8)
Abnormal ultrasound findings (AUS)	30	0	0	18	48 (4.6)
Known abnormal fetal karyotype (AFK)	4	3	1	0	8 (0.8)
Abnormal results of maternal serum screening tests (MSS)	11	0	0	2	13 (1.3)
Family history of a genetic condition or chromosome abnormality (FIS)	6	0	0	5	11 (1.1)
Parental anxiety (PA)	476	8	0	0	484 (46.7)
Cell culture failure (CCF)	1	1	2	0	4 (0.4)
Multiple indications (MI)	15	0	0	10	25 (2.4)
- AMA+AUS	9	0	0	8	17 (1.6)
- AMA+MSS	2	0	0	0	2 (0.2)
- AMA+FIS	3	0	0	2	5 (0.5)
- MSS+AUS	1	0	0	0	1 (0.1)
Total (%)	919 (88.6)	15 (1.5)	4 (0.4)	99 (9.5)	1037

RESULTS

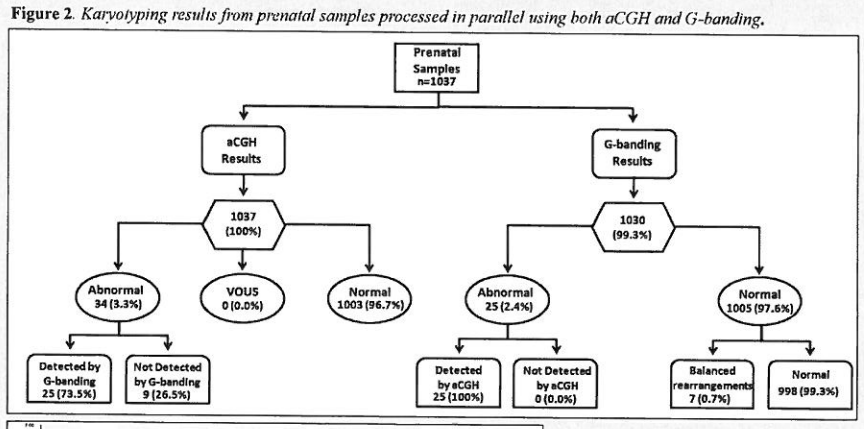
A total of 1037 prenatal samples were processed, 919 (88.6%) of which were AF, 99 (9.5%) CVS, 15 (1.5%) CA, 4 (0.4%) DEUA (Table 1).

The average amount of DNA obtained per mL of amniotic fluid was 99±98 ng (range 7-1694), and 2894 ±2420 ng (range 306-12807) on from CVS tissue. The average quantity of DNA used in the aCGH process was 264±109 ng (range 28-510). The average turnaround time for aCGH results was 2.6±0.6 (range 2-7) working days from sample's receipt.

The majority of prenatal samples (1003/1037; 96.7%) had normal results. Benign CNVs were identified in 135 samples (13.0%). Clinically significant chromosome alterations were identified in 34/1037 (3.3%) samples. Twenty-five (73.5%) clinically significant results were also identified by conventional karyotyping performed concurrent to aCGH (Table 2). aCGH was also able to detect chromosomal mosaicism in 4 samples, with the lower chromosome representation being at 10% level.

In 9 samples (26.5% of the chromosomal abnormalities detected and 0.9% of the samples included in the study), aCGH provided a diagnosis of clinically significant chromosomal abnormality, not detected by conventional karyotyping, that would have otherwise remained unascertained if only G-banded karyotype had been performed (Table 3). Seven of the above CNVs were concerning well-established syndromes described in OMIM database, 2 of which (Case 2 and 3) were classified as pathogenic CNVs (Figure 3). Following parental studies, no findings of unclear significance remained. These results are summarized in Table 3.

Figure 2. *Karyotyping results from prenatal samples processed in parallel using both aCGH and G-banding.*



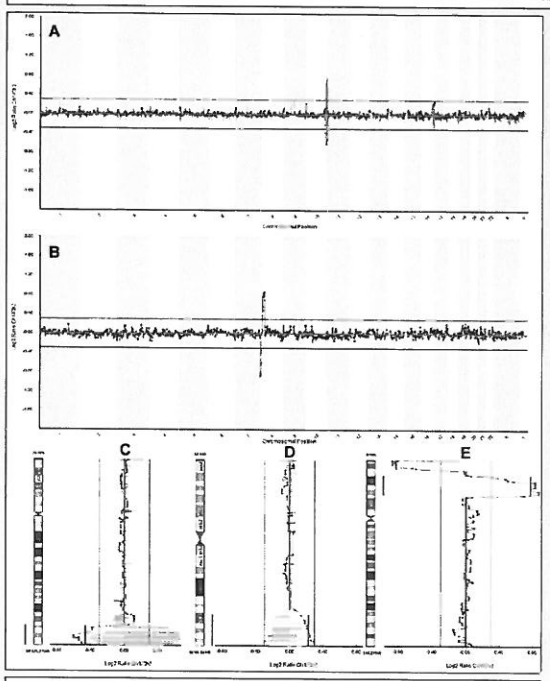


Figure 3. CNVs of unknown clinical significance identified in prenatal samples, classified as pathogenic following the decision criteria reported by Miller et al. (2010). A) Microarray plot for a de novo unbalanced translocation t(10;16)(q26.12;q23), identified in a CVS sample referred for AMA and cystic hygroma indications (Case 2), resulting in a 13.6 Mb deletion of 10q26.12-10q26.3 and a 14.6 Mb gain of 16q23.1-q24.3, detected as a shift of the BAC clones located in the above regions towards the red line (loss) and the green line (gain), respectively. B) Microarray plot for a de novo double segmental imbalances involving chromosome 8, identified in a CVS sample referred due to abnormal nuchal translucency (Case 3), characterized by a 6.5 Mb deletion of 8p23.3-p23.1 and a 14.6 Mb gain of 8p22-p21.1. (C) and (D) Chromosomal details for segmental imbalances from (A), and (E) segmental imbalances from (B).

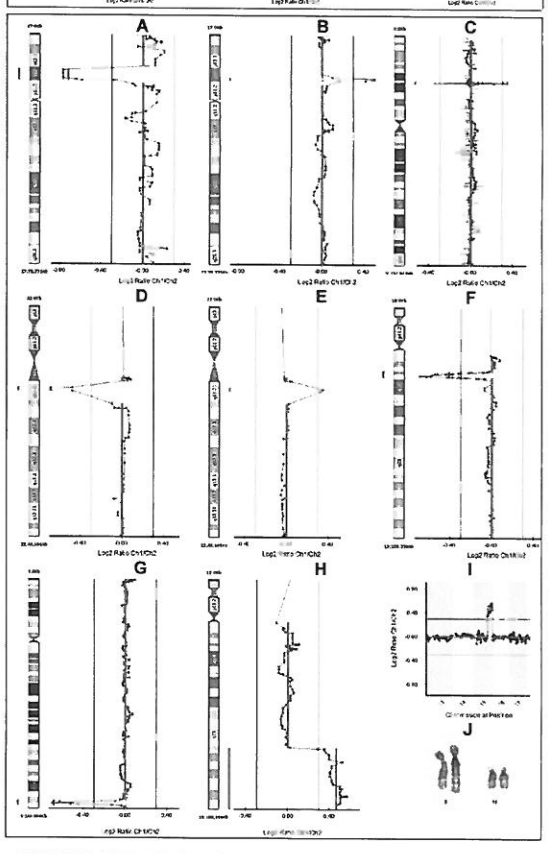


Figure 4. A) An inherited 3.4 Mb deletion of 17p12, associated with Hereditary neuropathy with liability to pressure palsies (HNPP) disease (Case 1). B) An inherited 1.1 Mb duplication at 17p12, consistent with Charcot-Marie-Tooth neuropathy type 1 A (CMT1A) (Case 4). C) A male fetus with a *de novo* clinically significant 600 Kb duplication at Xp21.2-p21.1, encompassing exons 52-79 of the Dystrophin gene, consistent with a diagnosis of male affected by Duchenne Muscular Dystrophy (DMD)(Case 5). D) A *de novo* clinically significant 670 Kb deletion at 22q11.21, consistent with 22q11.2 deletion syndrome (Case 7). E) An inherited 670 Kb duplication at 22q11.21, consistent with 22q11.2 microduplication syndrome (Case 9). F) A *de novo* clinically significant 2.9 Mb deletion at 15q13.1-q13.3, consistent with 15q13.3 microdeletion syndrome (Case 6). G) A *de novo* clinically significant 1.7 Mb deletion at 5q35.2-q35.3, consistent with Sotos Syndrome (Case 8). H) Chromosomal details for a sample of cultured amniocytes referred because of suspected duplication 5q, that after aCGH testing resulted a duplication 15q24.1-qter . I) Microarray plot from (H). J) G-banded karyotype from (H).

CONCLUSION

This study demonstrates that aCGH represents an improved diagnostic tool for prenatal detection of chromosomal abnormalities, allowing identification of submicroscopic clinically significant imbalances that are not detectable by conventional karyotyping, with no false positive or false negative findings, suggesting that the technique has the potential to replace the traditional cytogenetic analysis without missing significant results. Although larger studies are needed, our findings provide a further evidence on the feasibility of introducing aCGH into routine prenatal diagnosis practice as first-line diagnostic test to detect chromosomal abnormalities in prenatal samples.

Table 3: *Clinically significant array CGH findings in prenatal samples, not detected by conventional karyotyping*

Case No.	Sample type	Patient's age	Indication	Chromosomal findings				Parental analysis	Interpretation	Final diagnosis	Pregnancy outcome
				G-banding results*	aCGH result						
					Location	Gain / Loss	Estimated size (Mb)				
1	AF	35	AMA + AUS (single umbilical artery)	46,XX	17p12	Loss	3.4	Inherited (maternal)	Hereditary neuropathy with liability to pressure palsies (HNPP)(OMIM 162500)	Abnormal	Continued and delivered
2	CVS	39	AMA + AUS (Cystic Hygroma)	46,XY	10q26.12-10q26.3	Loss	13.6	*De novo*	Clinically significant CNV characterized by a *de novo* complex rearrangement involving relatively large chromosomal regions and containing clinically relevant genes	Abnormal	TOP
					16q23.1-q24.3	Gain	14.6				
3	CVS	33	AUS (abnormal nuchal translucency)	Cell culture failure	8p23.3-p23.1	Loss	6.5	*De novo*	Clinically significant CNV characterized by a de novo complex rearrangement involving relatively large chromosomal regions and containing clinically relevant genes	Abnormal	TOP
					8p22-p21.1	Gain	14.6				
4	AF	43	AMA	46,XY	17p12	Gain	1.1	Inherited (maternal)	Charcot-Marie-Tooth neuropathy type 1 A (CMT1A)(OMIM disease 118220)	Abnormal	Continued
5	AF	34	PA	46,XY	Xp21.2-p21.1	Gain	0.60	*De novo*	Duplication of the chromosomal region including exons 52-79 of the Dystrophin gene (OMIM: 300377) consistent with Duchenne Muscular Dystrophy (OMIM 310200)	Abnormal	TOP
6	AF	37	AMA	46,XX	15q13.1-q13.3	Loss	2.9	*De novo*	15q13.3 microdeletion syndrome (OMIM disease 612001)	Abnormal	TOP
7	AF	41	AMA + AUS (tetralogy of Fallot)	46,XX	22q11.21	Loss	0.67	*De novo*	22q11.2 deletion syndrome (OMIM disease 188400)	Abnormal	TOP
8	CVS	38	AMA + AUS (abnormal nuchal translucency)	46,XY	5q35.2-q35.3	Loss	1.7	*De novo*	Sotos Syndrome (OMIM disease 117550)	Abnormal	TOP
9	AF	35	AMA	46,XX	22q11.21	Gain	0.67	Inherited (maternal)	22q11.2 microduplication syndrome (OMIM disease 608363)	Abnormal	TOP