

# Sonogenetics: A Breakthrough in Prenatal Diagnosis

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## ABSTRACT

G-band and rapid FISH/QF-PCR are regarded as the gold standards for prenatal chromosomal diagnosis. Numerous microdeletion/microduplication syndromes, however, are not detectable by conventional karyotyping. So far, we had a dilemma between fetal developmental/structural abnormalities with strong suspicion of chromosomal abnormalities and normal karyotype results. Fetal DNA chip includes more than 6,450 genetic loci and covers more than 100 common genetic diseases with numeric, structural chromosomal anomalies.

In April 2009, we launched prenatal diagnosis by fetal DNA chip of amniotic fluid samples or chorionic villi samples in the selected fetuses with sonographic abnormalities and suspicion of familial genetic disorders. We had seven cases with both abnormal ultrasound findings and pathologic copy number variations by DNA chip. In all cases, normal karyotype was confirmed by G-banding analysis.

Fetal DNA chip (array CGH) may become a strong modality to solve some part of this dilemma. Although we have to be prudent to select the patients, deal with DNA chip results and parental counseling, "sonogenetics" is one of the breakthroughs in prenatal diagnosis, and the further accumulation of case studies will be required in this new field.

**Keywords:** Prenatal diagnosis, Sonogenetics, Microarray, Fetal DNA chip, Ultrasound.

## INTRODUCTION

Recent advances of fetal imaging technology have been remarkable, and prenatal detection and diagnoses have been shifted from the second and third trimesters to the first trimester. However, we still have dilemma in fetal diagnoses of normal karyotype cases with strong suspicion due to sonographic abnormalities.

G-band and rapid FISH/QF-PCR are regarded as the gold standards for prenatal chromosomal diagnosis. Numerous microdeletion/microduplication syndromes, however, are not detectable by conventional karyotyping. The latest development in microarrays enables the detection of submicroscopic deletions/duplications. Relevance of genetic alteration in developmental and behavioral etiology remains to be determined. A number of genetic and environmental factors are taken into account as responsible for congenital structural abnormalities, intrauterine growth restriction and organ developmental delay.

Array-comparative genomic hybridization (aCGH) was developed as a high-resolution analysis of DNA copy number variations, initially in cancer studies, and subsequently extended to postnatal evaluation of mental retardation and multiple congenital anomalies. Array-CGH offers a rapid analysis of the DNA copy number variations with results of a comprehensive genome-wide picture available within 3 days' time, much shorter than the G-banded analysis, which takes

at least 2 weeks. In addition, its superior resolution allows detection of submicroscopic microdeletions or microduplications, and a more precise delineation of chromosomal aberrations leading to improved genotype-phenotype correlation. However, aCGH cannot detect truly balanced chromosomal rearrangements or polypoidy, and may even generate data with unknown significance. Knowing its limitations and with proper counseling of the advantages and shortcomings, aCGH will become the first-line diagnostic test for management of pregnancy with fetal sonographic anomalies.

Submicroscopic microdeletions and microduplications have been reported to be associated with developmental and behavioral abnormalities.<sup>1</sup> Thus, aCGH increased the ability to detect segmental genomic copy number variations in patients with global developmental delay, mental retardation, autism, multiple congenital anomalies and dysmorphism, and is becoming a powerful tool in disease gene discovery and prenatal diagnostics.<sup>2</sup> Clinical investigation using aCGH in the field of prenatal diagnosis has recently introduced, and several reports on relations between fetal abnormalities and abnormal aCGH results have been published.<sup>3,4</sup> Fetal DNA chip is a specially designed diagnostic chip to interrogate over 100 recognized genetic syndromes plus every region known to be involved in cytogenetic abnormalities with 50-fold-higher-resolution than conventional karyotyping. Exam duration of fetal DNA chip is

quite fast (2-4 days, at longest 7 days from sampling). Fetal DNA chip v1.0.

The aim of fetal DNA chip is to diagnose both common and less common, but clinically significant, cytogenetic aneusomies, including microdeletion/microduplication. Its average genome wide resolution is 100 kb. Fetal DNA chip includes more than 6,450 genetic loci and covers more than 100 common genetic diseases with numeric, structural chromosomal anomalies. In April 2009, we launched prenatal diagnosis by fetal DNA chip of amniotic fluid samples or chorionic villi samples in the selected fetuses with sonographic abnormalities and suspicion of familial genetic disorders.

### Sonographic Abnormalities with Pathologic Copy Number Variations

**Patients and methods:** Seven cases with sonographic abnormalities suspected as an abnormal karyotyping, but not common aneuploidy, were selected. Immediately after confirming negative QF-PCR result of chromosomes 13, 18, 21, X and Y on the next day of sampling, fetal DNA chip (Fig. 1) was done. Sonographic abnormalities of case 1, 2, 4, 6 and 7 are shown in Figures 2, 3, 4, 6 and 7 respectively.

**Results:** Seven cases with both abnormal ultrasound findings and pathologic copy number variations by DNA chip are shown in Table 1. The DNA chip result of case 4 showed three different microdeletions with copy number losses at the genomic regions

spanning 13q32.3, 13q34 and Xp21.3 involving ZIC2, SOX1 and ARX genes (Figs 5A to C). In all cases, normal karyotype was confirmed by G-banding analysis.

### DISCUSSION

The advantages of fetal DNA chip comparing to conventional chromosomal analysis of karyotyping are as below.

- Higher resolution
- No need to culture
- Direct mapping of aberration
- Short reporting time (2-7 days).

Fetal development and structural abnormality may be strongly related to chromosomal aberration. So far, we had a dilemma between fetal developmental and structural abnormalities with strong suspicion of chromosomal abnormalities and normal karyotype results. Fetal DNA chip (aCGH) may become a strong modality to solve some part of this dilemma. The introduction of microarray analysis has provided considerable benefit to clinical practice, compared to traditional approaches. Most importantly, it has enabled specific diagnoses to be made in a number of patients with subsequent benefits to the family in terms of the provision of accurate prognostic information and recurrence risk counseling.

Cytogenetic studies have demonstrated that duplications or deletions of entire chromosomes or microscopically visible aberrations are associated with specific congenital disorders.

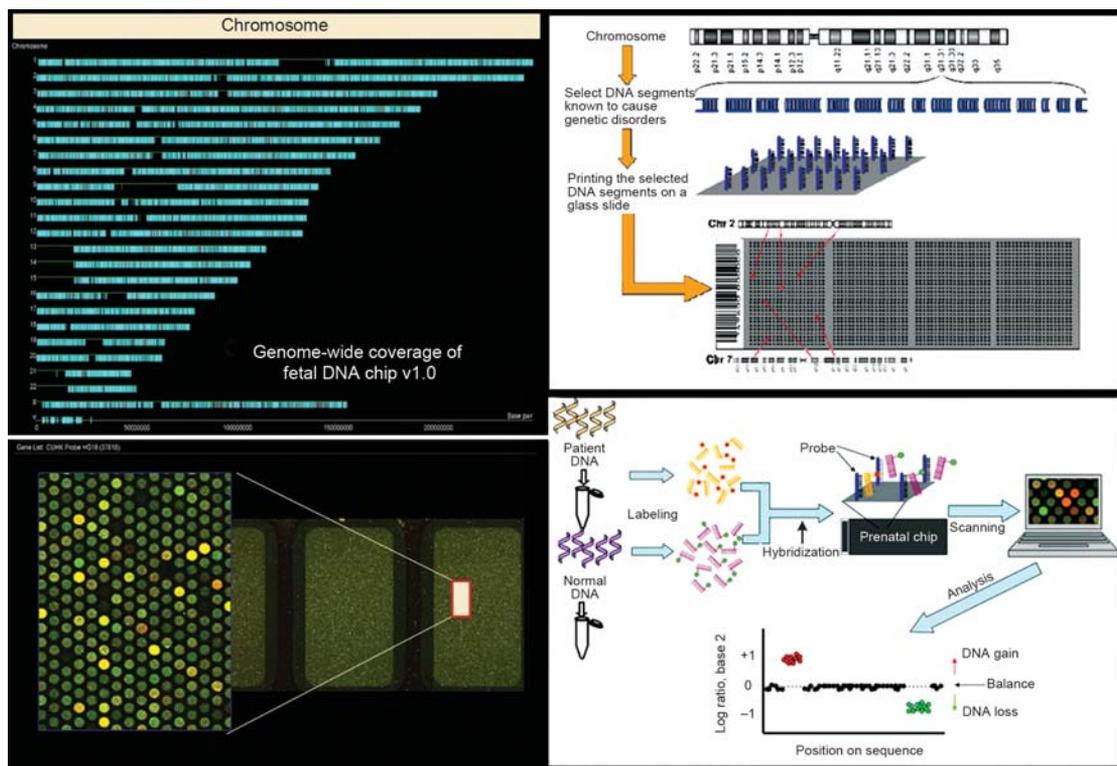
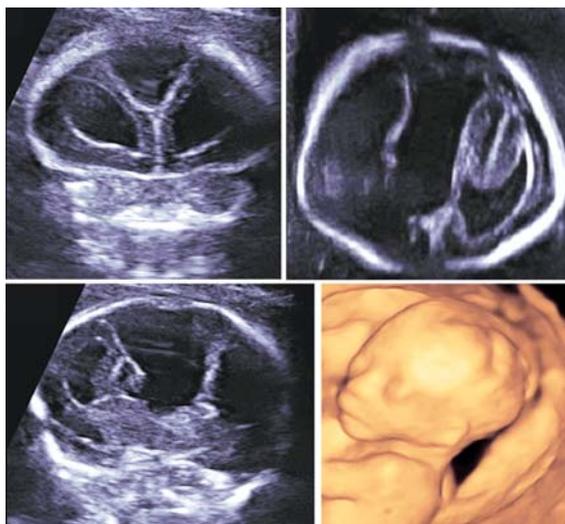


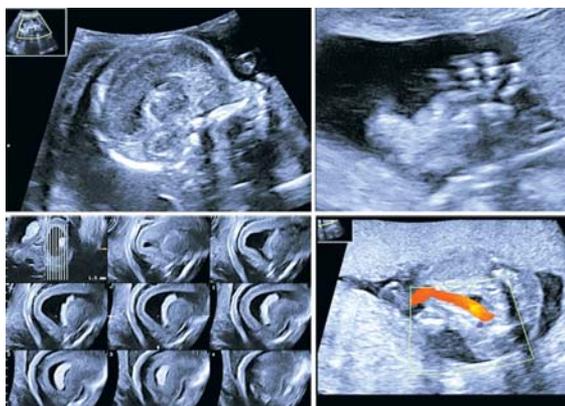
Fig. 1: Fetal DNA chip



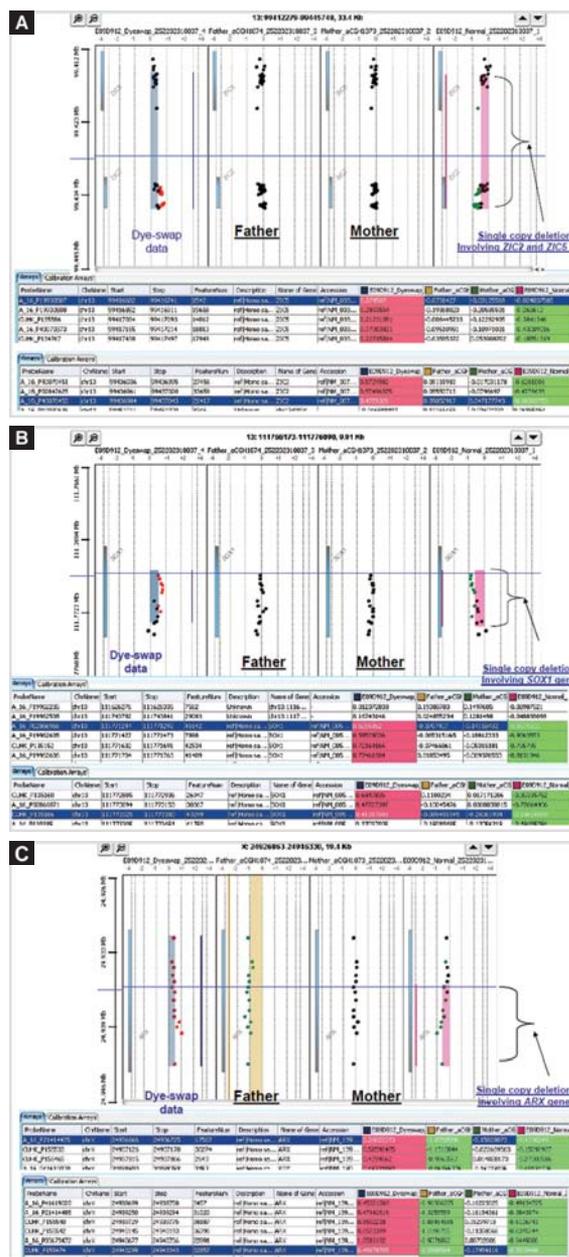
**Fig. 2:** Sonographic findings of case 1 at 16 weeks. Interhemispheric cyst, ventriculomegaly, asymmetrical migration disorder, micrognathia are demonstrated



**Fig. 3:** Sonographic findings of case 2. Upper figure shows the fetal face at 12 weeks. NT was within normal and no particular findings were seen. Lower figures were taken at 19 weeks. Hypogenesis of the corpus callosum (left, red circle) and ventriculomegaly (right) are demonstrated

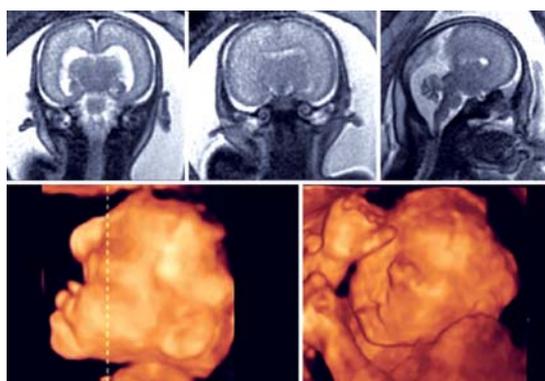


**Fig. 4:** Sonographic findings of case 4 at 18 weeks. Left upper figure shows hypoplastic corpus callosum, nasal bone defect and micrognathia. Right upper figure shows the abnormal curving 5th finger. Left lower figure shows ventriculomegaly. Right lower figure shows single umbilical artery



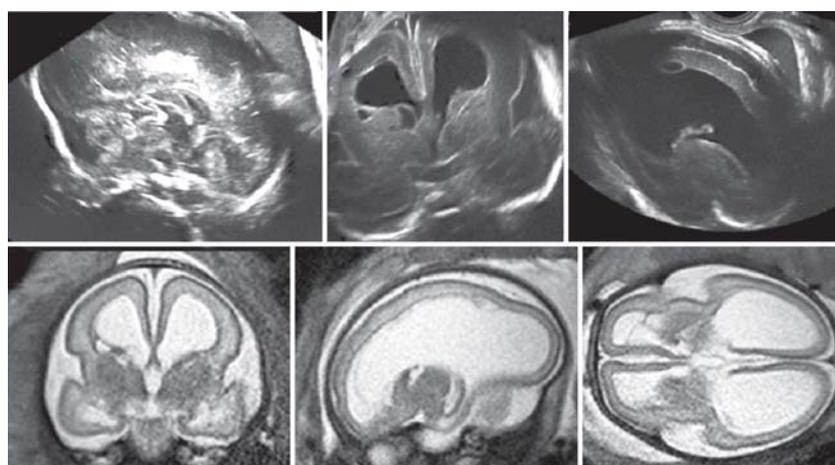
**Figs 5A to C:** The DNA chip result of case 4. Three different microdeletions with copy number losses at the genomic regions spanning 13q32.3 (A), 13q34 (B) and Xp21.3 (C) involving ZIC2, SOX1 and ARX genes respectively

Pathogenic CNVs are associated not only with birth defects and cancers but also with neurodevelopmental disorders at birth or neurodegenerative diseases in adulthood. Unfortunately, the limited knowledge of the phenotypic effects of most CNVs has led to the classification of many CNVs as genomic imbalances of unknown clinical significance. This has caused many clinicians to resist the introduction of microarray technologies in detecting CNVs in a genome-wide manner for prenatal applications.<sup>6</sup> Where the microarray result is normal, exclusion



**Fig. 6:** MRI and Sonographic findings of case 6 at 23 weeks. (Upper) MR images of fetal brain. Holoprosencephaly is clearly seen. (Lower) 3D reconstructed images of abnormal fetal face with exophthalmos, nasal hypoplasia and cleft lip

of a chromosomal etiology allows the clinician to shift the diagnostic focus onto other etiologies, such as Mendelian disorders and environmental insults.<sup>5</sup> The subsequent development and application of microarray-based assays have established the importance of copy number variants (CNVs) as a substantial source of genetic diversity in the human genome.<sup>6</sup> However, array-CGH is not an accomplished modality. Furthermore, interpretation of microarray data is complicated by the presence of both novel and recurrent copy number variants (CNVs) of unknown significance. Many of copy number variations exist, which are uncertain to be associated with abnormal phenotypes or to be considered as normal variations, and there should be more normal variations not reported yet. Evidence-based classification of pathogenic or benign status of a CNV in clinical genetics<sup>7</sup> is required.



**Fig. 7:** Sonographic and MRI findings of case 7 at 28 weeks. (Upper) Sonographic images. Midsagittal, anterior coronal and parasagittal sections from the left. Hypoplastic brain, hypogenesis of the corpus callosum, marked ventriculomegaly with irregular ventricular wall, and abnormal subependymal cystic formation are demonstrated. Sylvian fissure is very premature, compatible to 23 weeks of gestation. (Lower) MR images. Coronal, parasagittal and axial sections from the left

**Table 1:** Seven cases with abnormal ultrasound findings as well as DNA chip aberrations

Case	US weeks	Sonographic abnormalities	DNA chip procedure	Pathologic copy number variations	G-band
1	16	Interhemispheric cyst, ventriculomegaly, asymmetrical migration disorder, micrognathia	Amino	Single copy number gain (22q)	Normal
2	19	Mild ventriculomegaly, mildly slow brain development, CHD	Amino	Single copy number gain (15q), loss (11q)	Normal
3	13	Increased NT, CH, SUA, left renal cyst	CVS	Single copy number gain (17 q), loss (16 p)	Normal
4	18	IUGR, Ventriculomegaly, VSD, NB defect, micrognathia, SUA	Amino	Single copy number loss (13q32.3, 23q34, Xp21.3)	Normal
5	27	IUGR, TR	Amino	Single copy number gain (11p)	Normal
6	23	Holoprosencephaly, CHD (single ventricle and single atrium), facial anomaly	Amino	Single copy number loss (13q32.3)	Normal
7	28	Ventriculomegaly, brain developmental delay, migration disorder	Cord blood	Single copy number loss 17p12	Normal

Although we have to be prudent to select the patients, deal with DNA chip results and parental counseling, 'sonogenetics' is one of breakthroughs in prenatal diagnosis and the further accumulation of case studies will be required in this new field.

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