Prenatal chromosomal microarray testing of fetuses with ultrasound structural anomalies

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Title: Prenatal chromosomal microarray testing of fetuses with ultrasound structural anomalies: A prospective cohort study of over consecutive 1000 cases

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What is already known about this topic?

Chromosome Microarray Analysis (CMA) provides an additional genetic diagnostic yield (sub-microscopic deletions/rearrangements) of 3-5% over and above standard testing strategies in fetuses with structural anomalies.

What does this study add?

This study assesses the clinical utility of CMA in the current day and demonstrates that with time and experience the diagnostic yield for fetal structural anomalies has doubled with significantly lower rates of variants of uncertain significance (0.6%)

Data availability: The full anonymised dataset is available from the corresponding author on request

ABSTRACT

OBJECTIVE Evaluate the diagnostic yield of prenatal submicroscopic chromosome anomalies using prenatal comparative genomic hybridisation (aCGH) microarray.

METHOD Prospective cohort study conducted between March 2013 – June 2017 including fetuses where an elevated nuchal translucency (NT) or structural anomaly was identified on ultrasound and common aneuploidy testing was negative. aCGH was performed using an 8-plex oligonucleotide platform with a genome wide backbone resolution of >200kb and interpretation in line with American College of Medical Genetics guidance.

RESULTS 1129 fetuses were included; 371 fetuses with an increased NT (32.9%) and 758 with a structural anomaly (67.1%). The rate of pathogenic CNVs and VUS was 5.9% (n=22) and 0.5% (n=2) in the elevated NT group and 7.3% (n=55) and 0.8% (n=6) in the mid-trimester anomaly group, respectively. No pathogenic CNVs were identified in fetuses with a NT <4.0mm. Multisystem and cardiac anomalies had the greatest yield of pathogenic CNV with a 22q11.2 microdeletion present in 40% [12/30].

CONCLUSION Prenatal array CGH is a useful diagnostic tool in the investigation of fetuses with a significantly elevated NT or structural anomaly. With time and experience, rates of pathogenic CNVs have increased and VUS have reduced, supporting the prenatal application of increasingly high resolution aCGH platforms.

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INTRODUCTION

Up to 3% of pregnancies are complicated by sonographically detected fetal structural anomalies (FSAs), ranging from those which are isolated anomalies to those affecting multiple systems.[1] FSAs are associated with a significantly increased risk of perinatal morbidity and mortality[2] and pose a substantial global health burden. A fetal prognosis is typically worsened if there is a co-existing chromosome abnormality.[3]

Standard prenatal chromosomal testing prior to the introduction of chromosome microarray (CMA) traditionally involved using quantitative fluorescent polymerase chain reaction (QF-PCR) to exclude common autosomal and sex chromosome aneuploidies followed by a G-banding karyotype.[4] CMA using array Comparative Genomic Hybridization (aCGH) technology facilitates a greater level of variant detection to which may for specific targeted regions involve CNVs as small as 1kb through identifying the presence of microdeletions, rearrangements and microduplications.[1] aCGH compares proband DNA to control samples and through hybridization and fluorescence, identifies copy number variation (CNV).[1]

Postnatally, in children with FSAs and neurodevelopmental disability, CMA has been proven to provide an additional diagnostic yield over conventional chromosomal analysis. Yields have been reported to be up to 27% compared to when performed as a prenatal test in fetuses with structural anomalies ~~standard strategies of up to 27% with lower rates in prenatal testing for FSAs of~~ (3-5%).[5-10] It has been demonstrated to be a cost-effective strategy in this instance and is now recommended in the investigation of FSAs where QF-PCR does not detect a common aneuploidy (and costs declining with more routine use).[11]

CMA interpretation has improved with time and it is important to reassess the clinical utility in the UK in the more modern clinical post-guideline era. Hence, the aim of this large

prospective study is to evaluate the incidence of pathogenic CNVs and variants of uncertain significance (VUS) using aCGH microarray in cases where FSAs are detected on prenatal ultrasound based upon contemporary clinical guidance.

METHODS

Population

The cohort was comprised of all fetuses where an FSA was identified or confirmed by a fetal medicine sub-specialist on ultrasound and 'fetal karyotyping' using prenatal aCGH testing had been performed between March 2013 and June 2017. aCGH was performed at the West Midlands Regional Genetics Laboratory, Birmingham Women's and Children's NHS Foundation Trust, which serves an area in the UK of approximately 5.5 million patients. Fetal DNA was obtained via prenatal invasive testing (amniocentesis, chorionic villous or fetal blood sampling), and both singleton and multiple pregnancies were included. For multiple gestations the data from the fetus with FSA was analysed (for this study). In line with laboratory policy, QF-PCR using markers for chromosomes 13,18,21 and the sex chromosomes was performed initially, followed by aCGH where QF-PCR testing was negative. Parental samples were tested when required to aid interpretation. Clinical data were obtained from computerised hospital and maternal handheld records for maternal demographics, type of invasive test and ultrasound phenotype. FSAs were categorised based upon groups of anomalies outlined in the Fetal Anomaly Screening programme[12] and disagreements in classification were resolved through an expert, multi-disciplinary group consensus. Categorical statistical analysis was performed using GraphPad Prism 5.0 (La Jolla, California, USA). In relation to measurements of the NT, the first measurement obtained between 11-13+6 weeks by an accredited sonographer within or outside a specialist fetal medicine unit was utilised. Testing may have occurred not at the time of elevated NT detection (if the measurement <3.5mm or first trimester combined screening

risk was prospectively <1 in 150), but at a later gestation based upon the presence of additional detected anomalies.

Pregnancies were categorised into two phenotypic groups for the purposes of analysis; Group 1 comprised of fetuses with an increased NT (>3.5mm). Fetuses with an NT ≤3.5mm were only included if septations were visible, suggestive of a cystic hygroma. Group 1 fetuses were subdivided into two groups for analyses: Group 1A - fetuses with an isolated increased NT and; Group 1B - fetuses with an increased NT that were subsequently found to have an FSA. Group 2 comprised of fetuses with a defined FSA on detailed mid-trimester ultrasound examination. Fetuses previously identified in Group 1B were not included in this group.

Chromosomal microarray

aCGH was performed using a dosage sensitivity consortium-based array design. This consisted of an 8-plex platform of 60,000 60-mer oligonucleotides [BlueGnome v.2 ISCA or Oxford Gene Technology (OGT) v3.0 Constitutional]. Data was analysed using genome build GRCh37 (hg19) and the analysis was capable of detecting intragenic CNVs in key clinically relevant genes and with a genome wide backbone resolution of >200kb. Detected gains or losses were compared with known CNVs in publicly available databases,[13-15] were interpreted following American College of Medical Genetics guidelines[16] and subsequently reported following current UK guidelines.[11] In instances where CNVs were categorised prior to issued guidance, these were retrospectively re-classified (in conjunction with clinical genetics specialists). Any results of uncertain significance with respect to the presenting ultrasound finding were discussed by the local expert panel and only reported in specific

circumstances. Incidental findings were also discussed and reported where there was potential relevance to future pregnancies or other family members.

RESULTS

A total of 1525 consecutive fetuses with FSA underwent karyotypic assessment during the study interval. 396 fetuses were excluded from further analyses including; (i) n=387 (25.4%) fetuses with positive findings by QF-PCR (or conventional karyotyping where QF-PCR failed) [monosomy X (n=45), triploidy (n=9) and trisomy 13 (n=33), 18 (n=116) or 21 (n=184)]; (ii) n=7 (0.5%) fetuses that were investigated for fetal hydrops and subsequently found to have human parvovirus B19 infection or complications of monochorionic twining and; (iii) n=2 (0.1%) fetuses with incomplete ultrasound descriptors which prohibited classification. The final study population included 1129 fetuses with detectable FSAs on ultrasound examination. Demographics of the study population at the time of invasive prenatal testing are demonstrated in Table 1.

Group 1 (increased NT)

Group 1 included 371 fetuses [Table 2];

Group 1 included 371 fetuses [Table 2]; NT measurements were missing for 23 fetuses (6.2%). 99 (26.7%) fetuses had an NT >5mm and n=59 (15.9%) >6mm. Those with an NT < 5mm had an incidence of pathogenic CNV at ~~3.6~~4.4% (~~9~~11/249) rising to ~~9~~11.1% (11~~9~~/99) for an NT threshold >5.0mm. For those with missing data, 2 fetuses were found to have pathogenic CNVs.

~~Of those in this group n=105 (28.3%) fetuses had an NT >5mm and n=67 (18.1%) >6mm. Those with an NT ≤ 5mm had an incidence of pathogenic CNV at 3.6% (9/249) rising to 9% (9/99) for an NT threshold >5.0mm.~~ The corresponding VUS rate was 1.2% (3/249) and 1% (1/99) respectively. It was not possible to confirm that the particular CNV detected was causative of the increased NT in the absence of additional features. The presence of septation did not increase the likelihood of detection of a pathogenic CNV (p=0.24). Relevant data for Group 1 is demonstrated in Tables 1 and 2 and S1/2. Sub-analysis of group 1B (increased NT with subsequent detection of an FSA) revealed that 13.7% (n=51/371) of fetuses with an increased NT were subsequently found to have a further FSA which was most commonly cardiac in origin (n=27, 52.9%). Although there was no difference in the pathogenic CNV yield between Groups 1A and 1B [p=0.36 (5.3% vs 9.8%)], all of the pathogenic CNVs reported in group 1B were sub-classified as being fully causative of phenotype.

Group 2 (FSAs detected at mid-trimester ultrasound: 18-22 weeks)

758 fetuses (68.1%) were investigated for FSAs identified at the mid-trimester ultrasound. Relevant data is demonstrated in Table 2 and S3. Figures 1/2 demonstrate the breakdown per system of CNVs detected. Cardiac anomalies were the most common isolated structural anomaly 20.5% (114/557), followed by skeletal 16.2% (90/557) and brain anomalies 15.3% (85/557) [Supplementary Table 4]. Cardiac anomalies also represented the most common multisystem anomaly 43.8% (88/201), followed by skeletal 35.8% (74/201) and GI tract anomalies 25.4% (51/201) [Table S5]. Anorectal malformations were the least likely to be part of a multisystem phenotype 1.5%, (3/201).

The incidence of pathogenic CNVs was higher in fetuses with multiple compared to isolated FSAs ($p<0.0002$). The most common pathogenic CNV was a 22q11.2 microdeletion, identified in 40% (12/30) of cardiac cases. Mosaicism for a pathogenic CNV was detected in 0.7% (5/758) fetuses and was considered either fully or partially causative of the FSA in all cases. Details of the pathogenic CNVs are described in Table S5. There were 17 instances of inherited CNVs: 12 maternal (two involving the X chromosome in male fetuses); four paternally inherited CNVs, and one CNV inherited from both parents. Of the 17 inherited CNVs, n=10 were considered to be unrelated or of uncertain significance with respect to ultrasound findings.

The overall yields for group 1 and 2 were (27/371, 7.3%) and (62/758, 8.2%) respectively (Fisher's exact test: p=0.64).

DISCUSSION

This large prospective, consecutive cohort study has demonstrated that prenatal CMA, performed in the setting of a fetal structural anomaly and a normal screening QFPCR, provides a diagnostic yield of 6.8% which is superior to standard analysis by conventional cytogenetic analyses.

~~This large prospective, consecutive cohort study has demonstrated that prenatal CMA, performed in the setting of a fetal structural anomaly and a normal screening QFPCR, provides a diagnostic yield which is superior to standard analysis (6.8%).~~ This diagnostic yield was most optimal for multi-system anomalies, notably those of a cardiac nature and lower in cases of an isolated FSA. Overall rates of variants of uncertain significance (0.7%) and incidental findings (0.3%) were low.

This is one the largest prospective UK cohort series of CMA analysis in the presence of FSAs.[8] aCGH testing was performed using a standardised reproducible approach. Variant interpretation was performed using an expert panel when required and for the purposes of this study was re-checked for concordance by four genetic scientists (SH, FST, SA, DJM). The cohort was comprised of a well characterised group in which the ultrasound phenotypes were confirmed by a fetal medicine sub-specialist. Study limitations included the use of a lower resolution aCGH than is currently utilised, meaning that some smaller CNVs may not have been detected.[18] Also the NT group only captured subjects who requested screening and/or did not have a miscarriage prior to testing, introducing an element of bias.

The two largest prospective series reported to date, include the UK Evaluation of Array Comparative genomic Hybridisation in prenatal diagnosis of fetal anomalies [EACH] study (from the UK) which, based upon 1128 (n=494 elevated NT and n=629 structural anomaly) probands found a pathogenic CNV rate of 2.6% in the elevated NT group and 4.6% in the structural anomaly group where karyotype had been normal.[8] VUS rates were 3.4% and 3.3% respectively. Rates of pathogenic CNVs only became significant in the elevated NT group where it exceeded 5mm and similar to our own study, rates of pathogenic CNVs were greater in multi-system (11%) and cardiac anomalies (14.5%). The second study from Columbia University (New York, USA) as part of a US collaboration, assessed 3822 invasive tests which had been performed for a range of indications, not purely identified FSA. However, in the case of FSAs, where conventional karyotyping (by G-banding) had been normal, CMA achieved an additional rate of pathogenic CNVs of 2.85% with VUS rate of 5.3%.[6] Comparing our study to these, rates of pathogenic CNVs were greater in both groups yet there were much lower rates of VUS.

Interestingly, the EACH study also noted a relatively modest yield using CMA in cases of isolated elevated NT, until the NT measurement exceeded 5mm (5% pathogenic CNV and 1.8% VUS). This has important implications for current practice suggesting that CMA may only be diagnostically efficient in significantly elevated NTs.[8] Heterogeneity in relation to the incidence of CNVs may be explained by differences in the CMA platform used and its coverage, differing study populations, the timing of analysis and variations in microarray platforms and reporting policies.[18-20] It is likely that this difference in VUS rates between our study and the EACH study may be explained by increasing experience in variant

interpretation using internationally agreed classifications, more sophisticated bioinformatic pathways and further development of in silico tools and larger cohorts of samples in internal and public population and patient databases such as ClinVar, DECIPHER and the Database of Genomic Variants.[13-15] This suggestion supports the concept that the maximum resolution platform should be used in current practice as the rate of pathogenic CNVs to VUS has become much less of an issue than it once was.[21] Finally, similar to EACH, our study noted a particularly high rate of pathogenic CNVs in congenital cardiac anomalies, primarily due to the presence of 22q11.2 microdeletions, which were evident in 40% of suspected cardiac anomalies. This finding high-lights the importance of using targeted fluorescence in situ hybridization testing if CMA is not available in the presence of congenital cardiac anomalies. [22-23]

CONCLUSION

Prenatal CMA used following negative QF-PCR testing is an invaluable tool in the assessment of suspected FSAs from ultrasound assessment or NT>5mm. The EACH study supported the cost-effectiveness of this approach, and as time has progressed the turn-around time is now feasible in routine clinical practice within a 10-day working time period, supporting not only the efficacy but feasibility of this test outside a research setting. The multi-disciplinary team within a fetal medicine setting is an important component of this routine service to allow interpretation and appropriate reporting of VUS. Moving on from CMA there is potential for whole genome sequencing to replace both CMA and exome sequencing to become an 'all-in-one' test for the assessment of aneuploidy, copy number variation and single gene disorders, however as this still remains some way off from coming into routine practice,

requiring health economic evaluation and until this time, CMA remains an invaluable tool in the assessment of fetal structural anomalies.[24]

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TABLE AND FIGURE LEGENDS:

Table 1: Patient characteristics of included cases. [IQR = Interquartile range; CVS = chorionic villous sampling; FBS = fetal blood sampling]

Table 2: The diagnostic yield of copy number variants (CNVs) per group [VUS; variant of uncertain significance)

Figure 1: Incidence and breakdown of copy number variant (CNV) classifications (%). [GU = Genitourinary, GI = Gastrointestinal]. ,

Figure 2: Incidence of copy number variants (CNVs) according to organ system (%). Percentages are described according to categorical classification of either an isolated or multisystem finding. Numbers in each category denoted in brackets