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# IDENTIFICATION OF CLINICALLY SIGNIFICANT, SUBMICROSCOPIC CHROMOSOME ALTERATIONS AND UPD IN FOETUSES WITH ULTRASOUND ANOMALIES USING GENOME WIDE 250K SNP ARRAY ANALYSIS

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**IDENTIFICATION OF CLINICALLY SIGNIFICANT, SUBMICROSCOPIC  
CHROMOSOME ALTERATIONS AND UPD IN FOETUSES WITH ULTRASOUND  
ANOMALIES USING GENOME WIDE 250K SNP ARRAY ANALYSIS**

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## **ABSTRACT**

**Background:** The implementation of microarray analysis in prenatal diagnostics is a topic of discussion, as rare CNVs with unknown/uncertain clinical consequences are likely to be found. The application of targeted microarrays limits such findings, but the potential disadvantage is that relevant, so far unknown, aberrations might be overlooked. Therefore, we explore the possibilities for the prenatal application of the genome wide 250k SNP array platform.

**Methods:** Affymetrix 250k Nspl SNP array analysis was performed on DNA from 38 prenatally karyotyped fetuses with ultrasound anomalies. Analyses were performed after TOP, IUFD, or birth on DNA isolated from foetal or neonatal material.

**Results:** Aberrations were detected in 17/38 fetuses, six of whom with a previously identified chromosomal abnormality and eleven with previously normal or balanced karyotypes. Of the latter, the detected aberration occurred *de novo* and was considered of clinical relevance in five cases (16%), inherited from a healthy parent in four cases (12%), and *de novo* yet with unclear clinical relevance in two cases (6%). The clinically relevant abnormalities were either novel copy number variants (CNVs) (N=3) or concerned a uniparental disomy (UPD; N=2).

**Conclusion:** In at least 16% of fetuses with ultrasound anomalies and a normal or balanced karyotype, causal (submicroscopic) aberrations were detected, illustrating the importance of the (careful) implementation of microarray analysis in prenatal diagnosis. The fact that the identified, clinically relevant, aberrations would have gone undetected with most targeted approaches underscores the added value of a genome wide approach.

## INTRODUCTION

Chromosome abnormalities have been reported in 9 to 27% of pregnancies in which (major) ultrasound anomalies have been observed. [1, 2, 3] Although limited by its relatively low resolution, conventional cytogenetic analysis using G- or R-banded metaphase spreads still remains the gold standard in prenatal cytogenetics. As a consequence, aberrations smaller than 5–10 Mb may remain undetected. Microarray analysis, a widely accepted technique in postnatal diagnosis, allows for a genome wide scan of unbalanced genomic aberrations at a higher resolution without the need to culture cells. The majority of pregnancies with ultrasound anomalies show a normal karyotype and are therefore likely to benefit from high resolution analysis. However, as reviewed by Friedman [4] and Kuehn [5], the use of microarrays in prenatal diagnosis remains an ongoing debate, in particular due to the possible detection of Copy Number Variants (CNVs) for which the clinical consequences are uncertain or unknown. To avoid such findings, most papers so far report on the use of targeted or relatively low-resolution arrays, either during or after pregnancy. [6-13] The application of such targeted arrays has the advantage of being able to carefully select the genomic regions under study, with the ability to focus on regions with known clinical relevance. However, as Tyreman *et al.* [14] recently stated, the obvious disadvantage of using a targeted approach is that yet unknown clinical important aberrations might be missed. Moreover, to be kept up-to-date, targeted array platforms need to be redesigned every time a new relevant locus is discovered. For postnatal analysis genome wide screening with a high resolution array is now general practice and the application of the same platform in prenatal diagnosis would, from a laboratory point of view, be more practical.

In our laboratory, the Affymetrix 250k SNP array is routinely used for genome wide postnatal analysis. We studied the applicability of this array platform in prenatal diagnosis for pregnancies with referral reason “ultrasound anomalies, highly suspect for a chromosomal aberration”. Bearing in mind that its high resolution might give rise to results with unknown significance, the analyses were not performed during pregnancy, but only after Termination of Pregnancy (TOP), Intra Uterine Foetal Death (IUFD) or birth. Although in the setting of this study there was no limited time frame, in clinical prenatal practice this usually is the case. Therefore, DNA isolated from uncultured amniocytes was mostly used, to test the suitability of this DNA on our platform.

## **MATERIALS AND METHODS**

### **Samples**

SNP array analyses were performed on DNA from 38 fetuses or newborns who, during pregnancy, showed significant structural malformations with or without intrauterine growth restriction (defined as an estimated fetal weight below the 5th percentile for gestational age), mostly observed at routine ultrasound examination, offered to all pregnant women in The Netherlands around 20 weeks of gestation.

In 36 cases, routine prenatal chromosome analysis was performed following standard procedures as part of the routine diagnostic work-up. Subsequently, after birth, TOP or IUFD, array analysis was diagnostically carried out since, despite having normal or balanced karyotypes, the foetus / child remained suspect for a chromosomal aberration (N = 31: fetuses 1-27, 29-32), or to further characterize a previously identified chromosomal aberration (N = 5: fetuses A-D and F). In two

cases (cases E and 28), culturing of the foetal material could not be carried out and therefore only array analysis was performed.

Table I gives an overview of the clinical aspects of the foetuses / newborns.

## **DNA**

The starting foetal / newborn material that was used for DNA isolation is mentioned in Table I. All foetal DNA isolations were performed using the QIAamp DNA Mini Kit (QIAGEN, Westburg bv, The Netherlands), following the instructions of the manufacturer. For DNA isolation from uncultured amniocytes, 3-4 ml of amniotic fluid was used and the isolated DNA was subsequently eluted in 50  $\mu$ l of elution buffer.

DNA from uncultured blood cells from parental blood was isolated following standard procedures (Chemagic Magnetic Separation Module 1 from Chemagen).

## **Affymetrix Nspl SNP array hybridisation and analysis**

All analyses were carried out on the Affymetrix GeneChip 250k (Nspl) SNP array platform (Affymetrix, Inc., Santa Clara, CA), which contains 25-mer oligonucleotides representing a total of 262,264 SNPs. Hybridisations were performed according to the manufacturer's protocols, with quality criteria of SNP call >85% (based on the dynamic model algorithm GTYPE) and a standard deviation <0.20. Copy numbers were determined using CNAG version 2.0 (Copy Number Analyzer for Affymetrix GeneChip mapping) software package [15], by comparing SNP intensities from patient DNA with those of a sex-matched pooled reference DNA sample (DNA from either ten healthy male or ten healthy female individuals). The normalized  $^2\log$  ratios were subsequently analyzed for genomic imbalances by a standard Hidden Markov

Model (HMM) [17]. Losses were considered significant if at least five consecutive SNP probes showed a value of  $\leq -0.38$  and gains if at least seven consecutive SNPs showed a value of  $\geq 0.3$  [16]. CNVs called by the HMM were compared with publicly available and in-house databases (essentially as described in Koolen *et al.* [18]) and excluded from further analysis if the observed gain or loss was reported three times or more in the Database of Genomic Variants (DGV: <http://projects.tcag.ca/variation/>) and/or twice or more in our own database of more than 800 healthy individuals [19; Vrijenhoek *et al.*, Homozygous deletions in the human genome; what don't we need?, *Submitted*]. Gains or losses of <200 kb or <150kb, respectively (patients), or <600 kb and <450 kb, respectively (parental samples), were only further investigated if genes / gene-rich regions were involved, based on the 95% power analysis described by Hehir-Kwa *et al.* [16] The breakpoint positions of each aberrant region were remapped to UCSC hg18 (UCSC Genome Browser, release March 2006) using hgLiftOver (<http://genome.ucsc.edu/cgi-bin/hgLiftOver>).

In addition to copy number analysis, SNP array data analysis also enables genotyping of the array data and the detection of homozygous stretches.

## RESULTS

All array experiments resulted in data that met our quality criteria. The SNP calls from arrays with DNA from uncultured amniocytes versus cultured cells were respectively: 10 vs 12% for SNP calls between 85 and 90%, 52 vs 41% for calls between 90 and 95% and 38 vs 47% SNP calls > 95%. In 15 samples CNVs were detected. Furthermore, in three fetuses large stretches of homozygosity were identified, in two due to uniparental disomy, in the third (case 26) due to consanguinity of the parents. (For overview of all results: see Table I).

## Copy number variants

In 15 cases, one or more potentially clinically significant CNV was detected (see Table I; in each of the cases A, F and 27 two CNVs were detected).

These CNVs can be categorized into one of the groups of fetuses and CNVs listed below:

1. CNVs detected in fetuses with previously identified chromosomal aberrations (N=5: fetuses A-D, F)

The analyses of DNA from fetuses in this category were performed to further characterize previously identified pathogenic chromosomal aberrations.

In fetus A, intrauterine growth retardation and pericardial effusion were noticed upon ultrasound examination at 27.4 weeks of gestation. Chromosome analysis on amniocytes revealed a recombinant chromosome 4, resulting from a paternal  $inv(4)(p16.3q27)$ . The pregnancy was terminated upon this finding. Further mapping of the breakpoints resulted in  $46,XX,rec(4)dup(4q)inv(4)(p16.3q27)pat.arr\ snp\ 4p16.3(211,623-2,693,667)x1\ dn, 4q27q35.2(121,562,709-191,167,888)x3\ dn$ , with a terminal 2.5 Mb loss of 4p16.3 (including the Wolf-Hirschhorn critical region) and a terminal 69 Mb gain of 4q27q35.2.

Fetus B was prenatally suspect of a 22q11.2 deletion due to a cardiac defect.

However, prenatal FISH analysis with the region-specific LSI HIRA probe (Abbott Laboratories, Illinois, USA) revealed normal signals on both chromosomes 22.

Postnatally, the child remained suspect for a 22q11.2 deletion and MLPA analysis using the SALSA MLPA kit P250 was carried out according to instructions of the manufacturer (MRC Holland, Amsterdam, the Netherlands). With this kit, a 22q11.21

deletion was detected that did not encompass the *HIRA* gene and overlapped with the distal part of the commonly deleted 3 Mb region in DiGeorge syndrome.[20] Array analysis resulted in a 46,XX,arr snp 22q11.21q11.22(19,390,359-20,690,650)x1 dn karyotype (1.3 Mb loss), with the deleted region being comparable to the region previously reported by Ogilvie *et al.* [21] in a patient with cardiac defects.

Foetus C prenatally showed a cardiac defect. Despite a normal prenatal karyotype, chromosome analysis was repeated postnatally as the child remained suspect of a chromosomal aberration. This revealed a 22qter deletion, that was not noticed upon prenatal analysis. Obviously, the GTG banding level in prenatal karyotyping was inferior to the postnatal metaphase spreads. Accurate determination of the breakpoints revealed a 46,XX,del(22)(q13qter).arr snp 22q13.31q13.33(43,444,530-49,576,671)x1 dn karyotype (6.1 Mb loss).

In foetus D, with prenatal karyotype 46,XX,del(5)(q35) dn, ultrasound examination at 24.5 weeks of gestation showed several anomalies (see Table I). The child was born at 36.1 weeks of gestation. Axial hypotonia was present from birth. Because of central apnoea and respiratory insufficiency, she was admitted to the neonatal intensive care unit and array analysis was requested for accurate determination of the deleted region. This resulted in a 46,XX,del(5)(q35) dn.arr snp 5q35.2q35.3(175,503,757-180,629,495)x1 karyotype.

In foetus F severe ultrasound anomalies such as cardiac defect and renal anomalies were noticed upon ultrasound examination. Karyotype analysis revealed a 46,XX,del(8)(p23) karyotype and the parents decided to terminate the pregnancy. Subsequent array analysis not only showed the expected loss of 8p, but also a gain of 9p (arr snp 8p23.2p22(180,568-18,676,556)x1,9p24.3p23(30,910-9,903,373)x3).

Karyotype and FISH analysis (subtelomeric regions of 8p and 9p) of both parents showed this unbalanced translocation to be *de novo*.

2. CNVs detected in foetuses in which no routine cytogenetic analysis could be performed (N=2: foetuses E and 28)

The parents of foetus E, in whom severe ultrasound malformations were observed, were each carrier of a balanced translocation. As the couple had opted for assisted reproduction using a donor egg, the unbalanced karyotype of the father was expected. However, with karyotype arr snp 4p16.3q12(19,099-56,358,811)x3 (56.5 Mb gain), the foetus appeared to have inherited the unbalanced karyotype from the mother, who is a t(4;22)(q12;q11.1) carrier, hinting to an unexpected spontaneous pregnancy. No aberrations on chromosome 22 or elsewhere in the foetal genome were observed.

Only a limited amount of amniotic fluid was submitted for foetus 28 because of IUID with unknown cause. Array analysis showed a male profile with an 800 kb maternally inherited loss in Xp21.3 (see Figure 1D), a region encompassing three RefSeq genes: *MAGEB6*, *MAGEB18*, and *VENTXP1*.

3. CNVs detected in foetuses with a normal or balanced prenatal karyotype but suspect of a chromosomal aberration (N=8: foetuses 19, 20, 22, 23, 24, 26, 27 and 30)

a. *Rare, de novo and pathogenic CNVs (foetuses 22, 23, and 24)*

Foetus 22 showed several ultrasound anomalies (see Table 1) based on which the pregnancy was terminated. Array analysis showed a loss in 11q14.1q14.2 that was originally only detected by visual inspection of the array plots and not by significantly

lowered HMM values (see Figure 1A: mean <sup>2</sup>log ratio -0.19). As this profile was suspect for a mosaic deletion 11q14, metaphase FISH analysis was performed with probes located within the aberrant region of 11q (BAC probes RP11-118L16 and RP11-157B22). This indeed revealed an 11q14.1q14.2 deletion in ~50% of the cells, the other cells showing a normal pattern. As the starting material was DNA isolated from cultured chorionic villi and the foetus was a female, QF-PCR (Aneufast, Genomed, UK) was performed (according to the instructions of the manufacturer) to rule out maternal contamination. No maternal contamination was detected. Parental analysis showed normal profiles and the final karyotype was 46,XX, arr snp 11q14.1q14.2(82,843,560-86,402,152)x1~2 dn (3.6 Mb loss (mosaic)).

The mother of foetus 23 underwent amniocentesis at 16.2 weeks of gestation because of advanced maternal age, and a 46,XX,t(3;18)(q26.2;q21.3)dn karyotype was found. At 20 weeks gestational age, the foetus developed a hydrocephalus and the pregnancy was terminated. Array analysis on cultured amniotic fluid cells revealed a 5 Mb loss in the long arm of chromosome 3 at the breakpoint of the translocation: 46,XX,t(3;18)(q26.2;q21.3)dn, arr snp 3q26.33q27.2(181,214,043-186,173,910)x1 (see Figure 1B).

Foetus 24 showed a polyhydramnios and a possible oesophagus atresia upon ultrasound examination and died unexpectedly intrauterine. Array analysis showed a 2.9 Mb loss in 17p13.2p13.1 (46,XY, arr snp 17p13.2p13.1(4,421,993-6,972,362)x1 dn) (see Figure 1C).

*b. Rare, de novo and suspect CNVs (foetus 30)*

In foetus 30 (see Table I) a novel and *de novo* 640 kb gain in 7p21.2 was found, encompassing (part of) the *DGKB* gene (karyotype 46,XY, arr snp

7p21.2(14,352,157-14,991,857)x3 dn: see Figure 1F). The *DGKB* gene product belongs to the family of diacylglycerol kinases (DGKs), regulators of the intracellular concentration of the second messenger diacylglycerol (DAG), which play a key role in cellular processes.

*c. Rare, inherited and most likely without clinical relevance CNVs (foetuses 19, 20, 26, and 27)*

Foetus 19 showed cerebral abnormalities upon ultrasound examination. SNP array analysis revealed a 50 kb loss in 7p21.3, that involved an intronic region of the *NXPH1* gene. Parental array analysis showed the mother of the foetus to be carrier of the same loss, the final karyotype being 46,XX.arr snp 7p21.3(8,476,409-8,526,074)x1 mat.

Foetus 20 showed exencephalus upon ultrasound examination. SNP array analysis showed a 340 kb paternally inherited gain in 17q12 (foetal karyotype: 46,XY.arr snp 17q12(31,159,050-31,496,575)x3 pat).

In foetus 26 (for ultrasound anomalies: see Table I), array analysis showed a 150 kb gain in 16q23.2 (79,755,648-79,904,890), encompassing two RefSeq genes, *PKD1L2* and *BCMO1*. Moreover, a high percentage of homozygosity (~79 Mb), due to consanguinity of the parents, was observed genome wide in the array profile of this foetus, especially at chromosome 17 (~31 Mb). Parental analysis not only showed the father of the foetus to be carrier of the same gain in 16q23.2, but also revealed an unexpected finding in the (healthy) mother, as she carried a 610 kb loss in 3p25.1p24.3 (arr snp 3p25.1p24.3(15,953,709-16,538,531)x1). The loss comprises four RefSeq genes that have not previously been described to be involved in any

pathogenesis. As the mother is a healthy woman, this loss is therefore probably without clinical significance.

Because of a Dandy Walker malformation, the parents of foetus 27 decided to terminate their pregnancy. Upon array analysis, two CNVs were found, an 890 kb paternally inherited gain in 1q42.3q43 and a 400 kb maternally inherited gain in 9p24.3 (foetal karyotype: 46,XY.arr snp 1q42.3q43(233,888,438-234,778,604)x3 pat,9p24.3(619,468-1,022,153)x3 mat).

### **Uniparental disomy**

In two cases, SNP array analysis revealed a single, large homozygous stretch. In foetus 21 (see Table 1) was prematurely born at 35.6 weeks gestational age by caesarean section. Postnatally, the child remained suspect for a chromosomal anomaly (see Table 1). As can be seen in Figure 1F, SNP array analysis showed a 9.5 Mb homozygous region at the q-terminal part of chromosome 16. Subsequent array analysis of parental samples enabled patient-parent trio analysis and revealed that the child received both chromosomes 16 from his mother, the 9.5 Mb isodisomic region being generated by recombination during meiosis I.

Foetus 29 prenatally showed renal anomalies and polyhydramnios. Postnatally, the boy showed several anomalies (see Table 1) and array analysis revealed homozygosity of chromosome 4 (see Figure 1G). Patient-parent trio analysis revealed a paternal (iso)uniparental disomy.

## DISCUSSION

In the present study, we have evaluated the application of the Affymetrix whole genome 250k SNP array, which is routinely used in our laboratory for postnatal diagnostic analyses in patients with mental retardation and / or congenital anomalies.

In our series of 38 samples, six cases were subjected to array analysis for further characterization of a previously identified aberration or, because both parents were translocation carrier and karyotyping was not possible (cases A-F). In all these cases no interpretational dilemmas were experienced and all detected CNVs were classified as clinically significant.

Thirty-two other foetuses (cases 1-32) were analysed because ultrasound anomalies were detected and yet a normal or balanced foetal karyotype was found (N=31), or because insufficient vital cells were available for routine cytogenetic analysis (N=1). In this group, 11 foetal DNA samples revealed CNVs that could not be detected by routine chromosome analysis (11/32 = 34%). Five of these could be classified as clinically relevant (5/32 = 16%), two as with unclear clinical significance (2/32 = 6%) and four (4/32 = 12%) were inherited.

In 3/5 cases with clinically relevant CNVs (cases 22, 23 and 24), the *de novo* losses were several Mb in length, encompassing tens of genes (~10-50). In all these cases none of the genes were known to be directly linked to known diseases that could account for the ultrasound anomalies. In the two other foetuses uniparental disomies of chromosome 16 (maternal) and 4 (paternal) were observed. As maternal UPD(16) has been reported in literature [22] and ECARUCA ([www.ecaruca.net](http://www.ecaruca.net)) to cause growth retardation, renal agenesis and heart defects, this finding is considered as most likely causative for the clinical features of the boy. Pathological consequences of paternal UPD(4) have not been described so far. [22] However, we

consider it highly likely that this finding in foetus 29 is clinically relevant since, as a result of the UPD, a recessive gene mutation may be unmasked. The possible presence of a low percentage of mosaicism in the foetus can also not be excluded, although this is not very likely as only a rescue from a monosomy 4 can explain the complete iso-uniparental profile. The results of the array and amniotic fluid analysis do not suggest a mosaic profile in these tissues.

In two other foetuses, aberrations were detected of which, even after parental analysis, the clinical relevance remains unclear. In case 28, the clinical relevance of the inherited X-chromosomal loss encompassing three genes, that have not previously been reported to be involved in any pathogenesis, is difficult to determine.

Although copy variants in the *MAGE* and *VENXP1* genes are not likely to cause pathogenesis, a clinical relevance cannot be ruled out with certainty without further family investigation.

In the mother, the loss might be compensated by the remaining X chromosomal allele, possibly in combination with skewed X-inactivation, compensatory processes that are obviously absent in the male foetus. The 7p21.2 gain in foetus 30 has not previously been reported and as the *DGKB* gene is not known to be involved in pathogenic processes, the clinical relevance of this gain, although suspect, remains unclear.

Our results illustrate the additional value and diagnostic yield of the use of genome wide SNP array analysis in prenatal diagnosis in pregnancies with ultrasound anomalies and a normal or balanced karyotype. Since most of the clinically significant aberrations had not previously been recognized as pathogenic CNVs, they would have gone undetected with most of the targeted array approaches

described by others.[6-13] Nonetheless, whole genome screening can, even with the inclusion of parental analysis, also lead to the detection of CNVs with unknown or uncertain clinical relevance, as is illustrated by the CNVs in cases 28 and 30. Whole genome parental analysis can also lead to the detection of new, unclear CNVs in healthy parents, as illustrated for case 26. We, however, argue that these disadvantages do not outweigh the great diagnostic power of whole genome SNP array analysis, as clearly illustrated by the high diagnostic yield in this study (16% clinical significant aberrations vs 6% with unknown clinical relevance).

Our results also illustrate the importance of the availability of parental DNA, in order to be able to distinguish between a rare, inherited variant and a *de novo*, (most likely) pathogenic imbalance, as in 4/11 fetuses with novel CNVs these could be classified as inherited from a healthy parent. Although rare inherited CNVs cannot always with certainty be classified as without clinical relevance, they are, dependent on the size and type of CNV, less likely to directly lead to a clinical phenotype: small CNVs (<0.1 Mb) and gains are less likely to be pathogenic than large CNVs (>1 Mb) and losses, respectively. [23,24] We agree with Friedman [4] and Coppinger [25] that in a prenatal setting, it is not only highly recommended to be able to include parental analysis upon the finding of a suspicious CNV, but also to be able to obtain the parental samples simultaneously with the foetal sample. This because of the limited time frame prenatal diagnosis is dealing with but obviously also to avoid as much as possible that parents will receive results with unknown clinical significance.

Previously, Tyreman *et al.* [14] studied the possible prenatal use of the high resolution Affymetrix GeneChip 6.0 SNP array and retrospectively studied 106 anonymized cultured samples of fetuses that were prenatally karyotyped because of

major ultrasound anomalies or multiple soft markers. In 9% of the fetuses they detected likely pathogenic CNVs, in 11% likely benign CNVs and in 12% CNVs of unknown clinical significance. But, as the study was performed with anonymized samples, parental analysis to obtain more information on the nature of the CNVs could not be carried out. As Tyreman *et al.* used detection criteria comparable to ours, the difference between the results (9 vs 16% likely pathogenic) can probably be explained by the difference in numbers of samples that were included and the inclusion of parental samples in our study.

Shaffer *et al.* [8] and Kleeman *et al.* [13] both studied the use of targeted aCGH using Signature microarrays and in their cohorts of patients only 1.3% and 2%, respectively, showed a chromosome abnormality that was considered causal for the phenotype. Van den Veyver *et al.* [9] tested DNA isolated from 84 fetuses with ultrasound anomalies during pregnancy, on targeted BAC arrays and in three of these fetuses (4%) CNVs of uncertain clinical significance that would have remained undetected with karyotyping were found. These relatively low percentages of abnormal findings can probably be explained by the use of a targeted array instead of high resolution whole genome arrays, as most of the clinically relevant abnormalities detected with our platform would not have been detected with the platforms used by these groups. **Indeed, in a recently published comparative study using aCGH, Coppinger *et al.* [25] also concluded that whole-genome array analysis is preferred over targeted array analysis.**

The group of Bi [10] also used targeted arrays for analysis of foetal material during pregnancy. In their study they showed the feasibility of prenatal genetic diagnosis using oligonucleotide arrayCGH analysis for direct analysis of amniocytes

without culturing cells. Since short handling times are an extremely important issue in prenatal diagnosis, especially as ultrasound anomalies are often only detected around 20 weeks of gestation, when structural ultrasound scans are routinely being carried out, we also tested the use of DNA isolated from uncultured amniotic fluid cells on the 250K SNP array platform. In all cases where this type of DNA was available (see Table I), array results that met our quality criteria could be obtained.

In conclusion, genome wide SNP array analysis offers a highly reliable way to accurately detect the highest number of clinically relevant imbalances and homozygous findings compared to any other (set of) test(s) in human clinical genetics. Therefore, despite potential results with unknown or uncertain clinical relevance, this technique is ready for implementation in daily practice of prenatal diagnosis for pregnancies highly suspect for chromosomal aberrations. Of course results with uncertain or unknown clinical significance are from a counselling and patient point of view undesired, but they are inherent to testing, regardless of the technique being used. The maximum effort possible should be undertaken to avoid unclear results and for genome wide array analysis in a prenatal setting, this could be achieved by adjusting both counselling and analytical and reporting approaches, as compared to the postnatal setting. [17] Criteria for losses and gains (such as size and number of probes) might be chosen differently from those used in a postnatal setting. One might agree to only report results with known clinical consequences in case of very small aberrations and screen genome wide for larger imbalances, based on constantly increasing knowledge in publicly available and in-house databases. If this is carefully surveyed by extensive pre- and posttest counselling by skilled clinical

geneticists and array analysis can be performed within a limited time frame, we are convinced that the time is right for implementation.

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## **COMPETING INTEREST**

None declared.

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Table I: Overview of the clinical aspects of the fetuses / newborns included in the study, the pregnancy history, the foetal / newborn material that was used for DNA isolation, array results and its clinical relevance.

Case number	Indication for array analysis (cases A-F) Ultrasound anomalies (cases 1-32)	Gestational age at invasive procedure (weeks)	Pregnancy follow-up*	Material used for DNA isolation**	Array result	Clinical relevance
A	Accurate determination of breakpoints.  Ultrasound anomalies: intrauterine growth retardation, pericardial effusion. Prenatal karyotyping: 46,XX,rec(4)dup(4q)inv(4)(p16.3q27)pat Postmortem examination: growth retardation. Facial features: microcephaly, prominent glabella, micrognathia, high forehead, small ears, hypertelorism, high nasal bridge, beaked nose, downturned corners of mouth, and a short upper lip. Lung and renal hypoplasia.	27.4	TOP	UC AF	46,XX,rec(4)dup(4q)inv(4)(p16.3q27).arr snp 4p16.3(211,623-2,693,667)x1 dn, 4q27q35.2(121,562,709-191,167,888)x3 dn  (terminal 2.5 Mb loss of 4p16.3, terminal 69 Mb gain of 4q27q35.2)	Clinically relevant
B	Characterization of deletion.  Ultrasound anomalies:cardiac defect. Prenatal FISH analysis (HIRA) for del(22)(q11.2q11.2): no deletion. Postnatal MLPA analysis (P250): small deletion outside the region of the FISH probe.	22.5	Liveborn child	UC AF	46,XX.arr snp 22q11.21q11.22(19,390,359-20,690,650)x1 dn  (1.3 Mb loss)	Clinically relevant
C	Accurate determination of breakpoint.  Ultrasound anomalies: hypoplastic right heart and soft markers for Down syndrome. Prenatal karyotyping: 46,XX. At postnatal analysis: 22qter deletion.	21.3	Liveborn child	UC AF	46,XX.ish del(22)(qter)(HIRA+,ARSA-).arr snp 22q13.31q13.33(43,444,530-49,576,671)x1 dn  (6.1 Mb loss)	Clinically relevant
D	Accurate determination of breakpoint.  Ultrasound anomalies: partial aplasia of the cerebellar vermis, VSD and rhizomelic limb shortening. Normal growth parameters. Prenatal karyotyping: 46,XX,del(5)(q35) dn. Birth: 36.1 wks. Axial hypotonia, central apnea, respiratory insufficiency. Echocardiography: ventricular septal defect (VSD) and open ductus arteriosus Botalli. Echography of the	21	Live born child	UC AF	46,XX,del(5)(q35) dn.arr snp 5q35.2q35.3(175,503,757-180,629,495)x1  (5.1 Mb loss)	Clinically relevant

	kidneys: bilateral - vesicoureteral reflux. Electroencephalography: no epileptic activity. MRI of cerebrum: delayed myelinisation, frontal polymicrogyria, partial holoprosencephaly, abnormal septum pallucidum, abnormal fossa posterior.					
E	No karyotyping possible.  Multiple ultrasound anomalies and both parents carrier of a balanced translocation.	30.1	IUFD	UC AF	arr snp 4p16.3q12(19,099-56,358,811)x3 mat  (56.5 Mb gain)  (maternal translocation: t(4;22)(q12;q11.1))	Clinically relevant
F	Accurate determination of abnormality.  Ultrasound anomalies: cardiac defect, renal abnormalities. Prenatal karyotyping: 46,XX,del(8)(p23)dn	18.5	TOP	C AF	46,XX,del(8)(p23)dn.arr snp 8p23.2p22(180,568-18,676,556)x1,9p24.3p23(30,910-9,903,373)x3  (terminal 18.5 Mb loss of 8p23, terminal 9.9 Mb gain of 9p24.3p23)	Clinically relevant
1	Cardiac defect (previous child with cardiac defect too, but a different one)	15.4	TOP	UC AF	normal	
2	Hydrops foetalis	28.5	IUFD	UC AF	normal	
3	Neural tube defect, omphalocele	10.4	TOP	C CHOR	normal	
4	Cardiac defect	21.1	TOP	UC AF	normal	
5	Prenatal suspect of trisomy 18 (postnatal suspect of Feingold syndrome)	20.1	Live born child (died postpartum)	Blood cells	normal (Several homozygous regions detected upon array analysis, among which the region in which the MYCN gene is located, haploinsufficiency of which can be causative for Feingold syndrome)	
6	Hygroma colli, spina bifida	16.5	IUFD	UC AF	normal	
7	Hydrops foetalis and abnormal position of feet (both sides)	22.2	TOP	C AF	normal	
8	Cardiac defect and multiple congenital anomalies	21.3	TOP	UC AF	normal	

9	Abnormal skull, corpus callosum agenesis, single umbilical artery	20.4	TOP	UC AF	normal	
10	Cardiac defect, multicystic kidney, single umbilical artery	21.3	TOP	UC AF	normal	
11	Microcephaly, hydrocephalus, partial vermis hypoplasia, micro-retrognathia	21.2	TOP	UC AF	normal	
12	Multiple congenital anomalies. Fraser syndrome?	20.5	TOP	C FIBRO	normal	
13	Hydrocephalus	29.4	IUFD	C AF	normal	
14	Diaphragmatic hernia (previous child with diaphragmatic hernia too)	20.3	TOP	UC AF	normal	
15	Cerebral anomalies (previous child with congenital anomalies and mental retardation)	19.6	TOP	UC AF	normal	
16	13 weeks of gestation: chorionic villus biopsy because of nuchal translucency (46,XX) 19 weeks of gestation: amniocentesis because of holoprosencephaly (only array analysis on this sample)	13 / 19	TOP	UC AF	normal	
17	IUGR, cardiac defect?, dysmorphic features (4p-syndrome?).	35.4	Live born child	UC AF	normal	
18	Megacysts, obstruction of the lower urinary tract	13.2	TOP	C CHOR	normal	
19	Vermis agenesis, corpus callosum agenesis.	20.3	Liveborn child	Blood cells	46,XX.arr snp 7p21.3(8,476,409-8,526,074)x1 mat  (50 kb loss; 1 RefSeq gene)	Unlikely clinically relevant
20	Exencephalus	13	TOP	C CHOR	46,XY.arr snp 17q12(31,159,050-31,496,575)x3 pat  (340 kb gain; 17 RefSeq genes)	Unlikely clinically relevant
21	Single umbilical artery, single kidney, oesophagus atresia?, abnormal genital  (Postnatal: prematurely born (35.6 wks), growth retardation (P3), hypospadias, absent right kidney, open ductus Botalli, fatty acid oxidation deregulation).	35.4	Live born child	Blood cells	46,XY,upd(16)mat.arr snp 16q23.2q24.3(77,669,950-87,193,742)x2 hmz	Clinically relevant
22	Dandy Walker malformation, cardiac defect, abnormal position of hands	22.5	TOP	C AF	46,XX.arr snp 11q14.1q14.2(82,843,560-86,402,152)x1~2 dn	Clinically relevant

					(3.6 Mb loss (mosaic); 14 RefSeq genes)  Mosaicism confirmed by FISH analysis (~50%). Maternal cell contamination excluded by QF-PCR.	
23	Foetal karyotyping because of advanced maternal age: 46,XX,t(3;18)(q26.2;q21.3)dn. At 22 weeks of pregnancy: hydrocephalus -> deletion at breakpoint?	16.2	TOP	C AF	46,XX,t(3;18)(q26.2;q21.3)dn.arr snp 3q26.33q27.2(181,214,043-186,173,910)x1 (5 Mb loss; ~40 RefSeq genes)	Clinically relevant
24	Oesophageal atresia with fistula, polyhydramnion and hydrocephalus	22.6	IUFD	C AF	46,XY.arr snp 17p13.2p13.1(4,421,993-6,972,362)x1 dn  (2.9 Mb loss; > 50 RefSeq genes)	Clinically relevant
25	Cardiac defect (AVSD)	21.6	Live born child (child died several months postpartum after cardiac surgery)	Blood cells	Normal  (Postnatal DNA analysis revealed a pathogenic c.922A>G in <i>PTPN11</i> , consistent with Noonan syndrome)	
26	Abnormal lumbal vertebrae (duplication?), intracranial anomalies, abnormal position of feet	18.4	TOP	UC AF	46,XX.arr snp 16q23.2(79,755,648-79,904,890)x3 pat  (150 kb gain; 2 RefSeq genes)  High percentage LOH across the genome, especially #17.  Maternal profile: arr snp 3p25.1p24.3(15,953,709-16,538,531)x1 (610 kb loss; 4 RefSeq genes)	Unlikely clinically relevant
27	Dandy Walker malformation	18	TOP	C AF	46,XY.arr snp 1q42.3q43(233,888,438-234,778,604)x3 pat,9p24.3(619,468-1,022,153)x3 mat  (890 kb gain in 1q42.3q43; 6 RefSeq genes; 400 kb gain in 9p24.3, 3 RefSeq genes)	Unlikely clinically relevant
28	IUFD e.c.i. (Foetus conform 16 weeks)	18	IUFD	UC AF	arr snp Xp21.3(25,724,049-26,543,804)x0 mat  (800 kb loss; 3 RefSeq genes)	Uncertain
29	Polyhydramnios, renal anomalies Postnatal: renal anomalies (ectopic kidney left-sided)	21	Live born child	Blood cells	46,XY.upd(4) pat.arr snp 4p16.2q35.2(19,099-	Clinically relevant

	and nephrectomy because of afunctional kidney right-sided), high forehead, small palpebral fissures, "rough" hands and feet with prominent distal phalanges, broad knees, normal psychomotor development.				191,167,888)hmz	
30	Cardiac defect	19.3	TOP	FIBRO	46,XY.arr snp 7p21.2(14,352,157-14,991,857)x3  (640 kb gain; one RefSeq gene)	Uncertain dn
31	Micro/retrognathia, corpus callosum agenesis, additional finger, abnormal foot, low implanted ears	21.5	TOP	UC AF	normal	
32	Cardiac defect (22.2 wks): double outlet right ventricle, arterial transposition). (FISH 22q11: normal).	22.1	TOP	UC AF	normal	

\*TOP = Termination of Pregnancy; IUFD = Intrauterine Foetal Death

\*\* UC = Uncultured; U = Cultured; AF = Amniotic Fluid; CHOR = Chorionic villi; FIBRO = fibroblasts

Legend to Figure 1:

Chromosome copy number variation and homozygous regions identified by high resolution SNP array analysis in patients 21 (A), 22 (B), 23 (C), 24 (D), 28 (E), 29 (F) and 30 (G). The  $\log_2$  T/R (test-over-reference) ratio values are plotted on the Y-axis versus the genomic position on the respective chromosome represented by the ideogram on the X-axis in the lower part of the figure. The red dots in the upper panel represent individual SNP values. The thin blue line in this panel represents the effective HMM outcome with a normal (N=2) T/R ratio of 0. A significant imbalance is indicated by a rise or fall of this line by 0.3 identifying a single copy number gain and -0.38 identifying a single copy number loss. In the lower panel (blue) each dot represents the averaged value of 10 neighbouring SNPs. An ideogram of the chromosome involved is shown on the bottom. A blue line below the ideogram reflects regions of homozygosity, not being caused by a loss.

The regions of interest are denoted by black circles.

A: Case 22. A 3.6 Mb loss in 11q14.1q14.2 is visible. Note the small decrease in that region, suggesting a mosaic loss.

B: Case 23. A 5 Mb loss in 3q26.2q21.3.

C: Case 24. A 2.9 Mb loss in 17p13.2p13.1.

D: Case 28. A 800 kb loss in Xp21.3. The blue line at the bottom of the figure indicates homozygosity of the X-chromosome, consistent with a male profile.

E: Case 30. A 640 kb gain in 7p21.2.

F: Case 21. The thin blue line at the bottom of the figure shows a 9.5 Mb homozygous region on chromosome 16.

G: Case 29: The blue line at the bottom of the figure shows homozygosity of chromosome 4.

Figure 1: SNP array profiles of aberrant cases

