Ahn JW, Bint S, Irving MD, Kyle PM, Akolekar R, Mohammed SN, Mackie Ogilvie C. 2014. A new direction for prenatal chromosome microarray testing: software-targeting for detection of clinically significant chromosome imbalance without equivocal findings. PeerJ 2:e354 https://doi.org/10.7717/peerj.354

TITLE: A new direction for prenatal chromosome microarray testing: software-targeting for detection of clinically significant chromosome imbalance without equivocal findings.

ABSTRACT

Purpose. To design and validate a prenatal chromosome microarray testing strategy that moves away from size-based detection thresholds, towards a more clinically relevant analysis, providing higher resolution than G-banded chromosomes but avoiding the detection of imbalances of unclear prognosis that cause parental anxiety.

Methods. All prenatal samples fulfilling our criteria for karyotype analysis (n=342) were tested by chromosome microarray and only copy number variants of established deletion/duplication syndrome regions and any other imbalance >3Mb were detected and reported. A retrospective full-resolution analysis of 249 of these samples was carried out to ascertain the performance of this testing strategy.

Results. Using our prenatal analysis, 23/342 (6.7%) samples were found to be abnormal. Of the remaining samples, 249 were anonymized and reanalyzed at full-resolution; a further 46 regions of imbalance were detected in 44 of these traces (17.7%). None of these additional imbalances were of clear clinical significance.

Conclusion. This prenatal chromosome microarray strategy detected all CNVs of clear prognostic value and did not miss any imbalances of clear clinical significance. This strategy avoided both the problems associated with interpreting imbalances of uncertain prognosis and the parental anxiety that are a result of such findings.

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INTRODUCTION

Chromosome microarray analysis (CMA) detects genome-wide copy number variation (CNV) with a higher resolution than traditional G-banded chromosome analysis, and has therefore been implemented as a diagnostic service for paediatric referrals in most cytogenetics laboratories. The resolution will depend on the specific platform used; the oligonucleotide platform used at our centre has an 8x60K format, and a resolution across the genome of approximately 120kb; this results in detection of imbalance in 25% of our patients after exclusion of known benign CNV (Ahn et al. 2013). However, approximately 40% of these imbalances are small in size and of unknown clinical significance.

Some centers have already implemented this powerful tool for investigation of prenatal samples (usually following detection of a fetal anomaly by ultrasound) (Evangelidou et al. 2013; Fiorentino et al. 2011; Liao et al. 2013; Srebniak et al. 2011), whilst others await international guidelines before embarking on this diagnostic approach. The problems and challenges involved in the use of CMA for prenatal testing have been extensively discussed in the literature (Evangelidou et al. 2013; Ganesamoorthy et al. 2013; Vetro et al. 2012; Wapner et al. 2012), as well as at conference debates (Crolla et al. 2013). The advantages of increased resolution compared with G-banded karyotype analysis must be balanced against the potential distress and anxiety caused to a couple by reporting CNVs of unknown significance, and/or incidental findings of clinical significance but of no relevance to the fetal abnormalities. Different models have been proposed, most of which focus on testing only pregnancies with fetal abnormalities on ultrasound, although application to all those having invasive testing has been suggested (Brady et al. 2013). These models generally recommend setting a size cut-off for reporting, with all imbalances above this size being subject to detailed scrutiny and discussion with clinicians (Liao et al. 2013; Vetro et al. 2012) and/or

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committees (http://www.nets.nihr.ac.uk/projects/eme/106003) in order to arrive at a consensus as to which imbalances to report.

We have developed a novel strategy to detect all imbalances greater than 3Mb and at the same time detect imbalances of any other specific regions of known clinical significance, regardless of size (see Table 1). In this paper we present the results from 342 prenatal samples, representing 20 months of diagnostic testing. Anonymisation and re-examination of the array traces from 249 of these samples, using standard postnatal, full resolution analysis, was carried out. The results of this retrospective re-analysis have allowed us to gauge the validity of the software-targeted approach, and to assess its advantages in terms of clinical utility, throughput and turnaround times.

MATERIALS AND METHODS

Prenatal CMA testing strategy

The choice of 3Mb as a cut-off for "calling" imbalance outside the targeted regions was informed by our experience with results of postnatal CMA (>19,000 reported tests). Our data from postnatal samples showed that clinically benign imbalances were generally smaller in size and that they were also more likely to be inherited. Figure 1 shows the number of inherited imbalances when grouped by size. It indicates that using a 3Mb backbone resolution for prenatal samples would exclude ~97% of the inherited CNVs that would be detected if we were to use the full potential of our CMA platform (Table S1). This was desirable as we sought to minimize the uncertainty of prenatal CMA results.

We then examined the imbalances that would be reported at various backbone resolutions, excluding syndromic regions. Our postnatal data suggested that ~1 in 5 imbalances reported

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with a 3Mb backbone resolution would be inherited (Figure S1). However, if we were to use a 2Mb resolution CMA platform, this would increase to ~1 in 3 non-syndromic imbalances being inherited. While our postnatal CMA experience informed us that the inheritance pattern of an imbalance is not a perfect predictor of clinical significance and clear prognosis, we felt that the inheritance pattern was a useful proxy for these attributes here.

In addition to the low resolution backbone, we selected 29 regions associated with genetic syndromes that would have a clear prognosis, should they be detected in a fetus (see Table 1). These regions were not restricted by size as we designed a custom software module to detect imbalances >3Mb and these deletion syndrome regions. Susceptibility loci such as 15q11.2 BP1-BP2, 1q21.1 (OMIM 612474 & 612475) and proximal 16p11.2 (OMIM 611913 & 614671) were excluded as they would be incidental to the ultrasound findings, would present interpretational difficulties and would be of little prognostic value.

As both the backbone resolution and the smaller, specifically targeted regions were configurable options in the software, this system was flexible and future-proof as we are free to change the resolution and add or remove targeted regions as our ability to interpret findings improved, or should we wish to refocus the test. While any such changes would require further validation, that process would be far simpler and more cost-effective than if we were using a fixed CMA platform.

Diagnostic analysis

All prenatal samples received at our center receive QF-PCR testing to exclude trisomy for chromosomes 13, 18 and 21 (Hills et al. 2010). Those samples referred for fetal ultrasound anomalies (excluding soft markers for Down syndrome), where trisomies have been

excluded, proceed to CMA by array CGH, using previously published protocols. Briefly, DNA was extracted from chorionic villi and amniotic fluid samples using the Puregene Tissue kit (Qiagen, UK), DNA was processed with the CGH Labeling Kit (Enzo Life Sciences, UK), unincorporated nucleotides were removed with the QIAquick Minelute kit (Qiagen, UK), and then hybridized to arrays following the manufacturer's recommendations (Agilent, UK). Samples were incorporated into our postnatal array CGH pipeline, which paired two diagnostic samples, differentially labeled, together on a single Agilent oligonucleotide 60K array (AMADID 028469) (Ahn et al. 2013), thereby decreasing costs and increasing throughput. Prenatal samples are sexed by QF-PCR and then paired with clinically normal, sex-matched parental samples for postnatal patients that were undergoing inheritance studies.

Following quantification of fluorescence signals (Feature Extraction software, Agilent, UK), a combination of the ADM2 algorithm (threshold 6, Genomic Workbench, Agilent, UK) and a custom software module was used to detect imbalances >3Mb and of specific deletion/duplication syndrome regions (see Table 1). These regions were chosen after consideration and discussion with clinical colleagues. If any imbalances were called by the software, the signal intensity across the CNV was assessed for the cyanine-3 and cyanine-5 labeled samples (relative to 5 other patient arrays on the same run showing no imbalance in the called region) in order to confirm which sample carried the imbalance as prenatal samples were hybridized with a second patient sample. This novel analysis method allowed confident identification of the abnormal sample on a paired array, as well as further cost and time savings.

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All imbalances were confirmed by either karyotyping or in situ hybridization studies, prior to reporting, in order to confirm sample identity; parental samples were requested where appropriate. No karyotype analysis was carried out for samples with no detected imbalance.

Average reporting time for this cohort was 7 days, with a cost estimated to be approximately three-fifths that of culture and karyotyping of prenatal samples.

Validation analysis

Array traces from 249 samples were anonymised, then re-analysed using a standard postnatal, 3-probe cut-off for imbalance calling (Ahn et al. 2010). The size and gene content of any region called by the software was scrutinized to assess the likely clinical significance of the imbalance.

RESULTS AND DISCUSSION

Diagnostic prenatal CMA testing results

Following the strategy detailed in this paper, we have tested 342 prenatal samples. Of these, 23 (6.7%) samples were found to be abnormal (see Table 2) and were reported following confirmation of sample identity. All other samples were reported as "No Abnormality Detected", with information on the size cut-off used and the deletion syndrome imbalances which had been excluded.

Our software targeted system provides flexibility to make changes "live", in response to information on specific cases. For example, in one of our cases (indicated by an asterix in Table 2), the fetus was reported to have ultrasound anomalies suggestive of thrombocytopenia-absent radius syndrome (OMIM 27400). An additional targeted region was

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therefore added to the analysis pipeline and the copy number of RBM8A could therefore be analyzed. The array showed deletion of this gene, consistent with the referral indication and this finding was reported. This type of adaptive testing has the potential to be hugely effective in a prenatal setting.

Retrospective reanalysis results

Retrospectively, 249 of the prenatal samples were anonymised and reanalysed at full resolution, using a 3-probe cut-off as for our postnatal samples. A further 46 regions of imbalance were detected in 44 of these samples (17.7%). These imbalances ranged in size from 3kb to 2Mb for deletions (see Table 3) and 0.5kb to 2.5Mb for increased copy number (see Table 4). There were only two CNVs between 2 and 3 Mb in size. The size and gene content of all 46 imbalances indicated that none of them was likely to be of clear clinical significance. A few imbalances included genes/loci that had some association with a clinical phenotype (e.g. PAX3, SLC9A9, and susceptibility loci such as 15q11.2 BP1-BP2), but none of these had a clear prognosis and thus would have presented the clinicians with difficult counseling issues and couples with difficult decisions, a situation which our prenatal CMA testing strategy was designed to avoid.

Figure 2 shows the effect of increasing backbone resolution in terms of the increased number of CNVs that would have been detected for our reanalyzed prenatal cohort. Our reanalysis has shown that lowering the threshold would greatly increase the time and associated costs, with no added clinical utility in this cohort. Furthermore, these additional findings would have potentially caused anxiety whilst inheritance studies were in progress, and further into the pregnancy and early years of the resulting progeny; the possibility of an unnecessary pregnancy termination should also be considered.

CONCLUSION

It is generally agreed that CMA should be used for prenatal detection of chromosome imbalance, as this test has a higher resolution than traditional G-banded karyotype analysis and can thus identify clinically important imbalances not previously detectable. However, pregnant women are vulnerable and anxious, and therefore an ideal prenatal test should provide results in the shortest possible time-frame, and these results should be clear-cut and should provide straightforward choices for the couples. Findings of uncertain significance and "toxic knowledge" associated with incidental findings of clinical significance but of no relevance to the fetal anomalies, cause distress and anxiety not only throughout the pregnancy, but beyond (Bernhardt et al. 2013); ambiguous results and incidental findings are therefore considered by some to present ethical dilemmas (Mikhaelian et al. 2013).CMA platforms have therefore been designed and implemented that target regions associated with deletion/duplication syndromes and that have a low resolution "backbone" with widely spaced probes (Park et al. 2010); these platforms detect syndrome-associated deletions and duplications, and provide information on large imbalances across the rest of the genome. However, they are inflexible, as new platforms must be developed each time a new region of clinical significance emerges. Therefore more recently, centers have begun using higher density arrays, and assigning CNVs to various categories, with different reporting frameworks for each category (Brady et al. 2013; Ganesamoorthy et al. 2013; Vetro et al. 2012). The importance of discussion between laboratory and clinician before assigning a CNV to a specific category is emphasized (Liao et al. 2013; Vetro et al. 2012), with one study suggesting a pan-centre committee for scrutinizing imbalances and making recommendations (http://www.nets.nihr.ac.uk/projects/eme/106003). However, these approaches involve delays in reporting until scrutiny and discussion is complete, and the complexity of the decision-

making process increases the cost of the test. In addition, the possession of information that will not be conveyed to the couple could be considered an infringement of their right to autonomy.

The software-targeting approach described in this paper has three key advantages over previously described prenatal CMA strategies. Firstly, it allows far greater test adaptability than hardware-restricted platforms, and thus permits the incorporation of new clinically significant regions (e.g. adding emergent syndromes), or changes in backbone resolution. Second, it provides the ability to personalize the prenatal CMA test to an individual clinical presentation, e.g. as demonstrated in the example above of a fetus suspected to have TAR syndrome; it is for situations such as this that some laboratories have adopted high resolution CMA. Finally, and most importantly, the problems associated with incidental findings and CNVs of uncertain significance are minimized, as is the anxiety for the parents that would receive these results.

The results of the anonymization of array traces from our cohort showed that no imbalances of serious clinical significance were unreported (see Tables 3 and 4). There were only two CNVs between 2Mb and 3Mb. One was an ~2.5Mb duplication of material from the long arm of chromosome 10 (64,902,961-67,399,362bp), that contained no genes associated with any clinical phenotype. The other imbalance was an ~2Mb deletion in the long arm of chromosome 2 (indicated by an asterix in Table 3); this region includes the PAX3 gene, deletion of which is causative of Waardenburg syndrome, Type 1 (OMIM 193500). The main features of this syndrome are a white forelock, deafness, and ocular anomalies, including dystopia canthorum, although the penetrance of these features is variable. Interestingly, while this manuscript has been in preparation, a neonate has been referred to our center with

features of Waardenburg syndrome. The prenatal test, carried out using our strategy, had reported "no abnormality detected"; the referring clinician was now requesting a full CMA result. Unmasking of the prenatal trace showed that this was indeed the case with the PAX3 deletion described above.

We feel that these two cases provide support for using a 3Mb threshold, rather than reducing the size threshold to 2Mb, as one was not clinically significant and prenatal reporting of the other imbalance would have caused serious counseling difficulties, due not only to the nature of the clinical features, but also to uncertainty as to the severity of the phenotype; any such reporting would be likely to cause distress and uncertainty for the pregnant woman and her partner, and would have presented them with extremely difficult decisions on the future of the pregnancy.

Although we have described a set of regions to target and a backbone resolution, other laboratories would be free to determine their own threshold and targeting if using the CMA approach described here. Perhaps more appropriately, best practice guidelines could be produced; these could be reviewed periodically and updated to incorporate advances in the field.

For severe fetal phenotypes detected on ultrasound scan, a decision to terminate may be based on the ultrasound findings alone; in these cases, the CMA result will have value in determining the etiology and recurrence risk for any causative imbalances detected. For milder phenotypes, such as a heart defect or isolated raised nuchal thickness, a normal CMA result will provide reassurance that the ultrasound finding is not due to a deletion syndrome with associated neurodisability. Imbalance in the deletion syndrome regions currently

targeted by our software, shown in Table 1, is of known pathogenic effect, although in some cases there may be variation in severity. For instance, although the phenotype associated with the "common" 22q11.2 deletion is generally severe, the features associated with the reciprocal duplication are very variable and relatively mild. Unfortunately, even with the targeted approach described here, it was not possible to avoid detecting these duplications, as this region is targeted. However, we are now developing the software further to differentiate between reduced and increased copy number, and so will be able to increase further the selectivity of this test.

Many deletion syndromes would not be expected to produce abnormal features detectable by ultrasound; for this reason, the possibility of using array CGH to test all prenatal samples has been raised (Brady et al. 2013). The objections to this are generally based on the concomitant detection of CNVs and incidental findings, with the associated problems discussed above. A software-targeted approach as described here would circumvent these concerns, and could provide exclusion of deletion syndromes for all pregnancies undergoing prenatal sampling.

Sequencing approaches for the prenatal detection of fetal genomic imbalance using free fetal DNA in maternal blood samples have recently become available for detection of the common trisomies (Chiu et al. 2011; Jiang et al. 2012; Palomaki et al. 2011); these approaches are currently expensive, and may take around two weeks to report. Until this technology becomes cheaper, results available more rapidly, and imbalances accurately detected across the genome, CMA will continue to be an important tool in obstetric practice, and should become the standard of care at all centers. The approach described in this paper, backed by international and national guidelines on size cut-offs and regions to be targeted, should allow the rapid introduction of this test for the benefit of all women having prenatal diagnosis.

ACKNOWLEDGEMENTS

We thank our colleagues in the Cytogenetics Department for their contributions to this work; in particular, Michael Coldwell, for his technical assistance, and Celia Donaghue for her expertise in DNA extraction and development of protocols for prenatal samples.

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FIGURES

Figure 1. Size distribution of inherited CNVs detected by postnatal CMA.

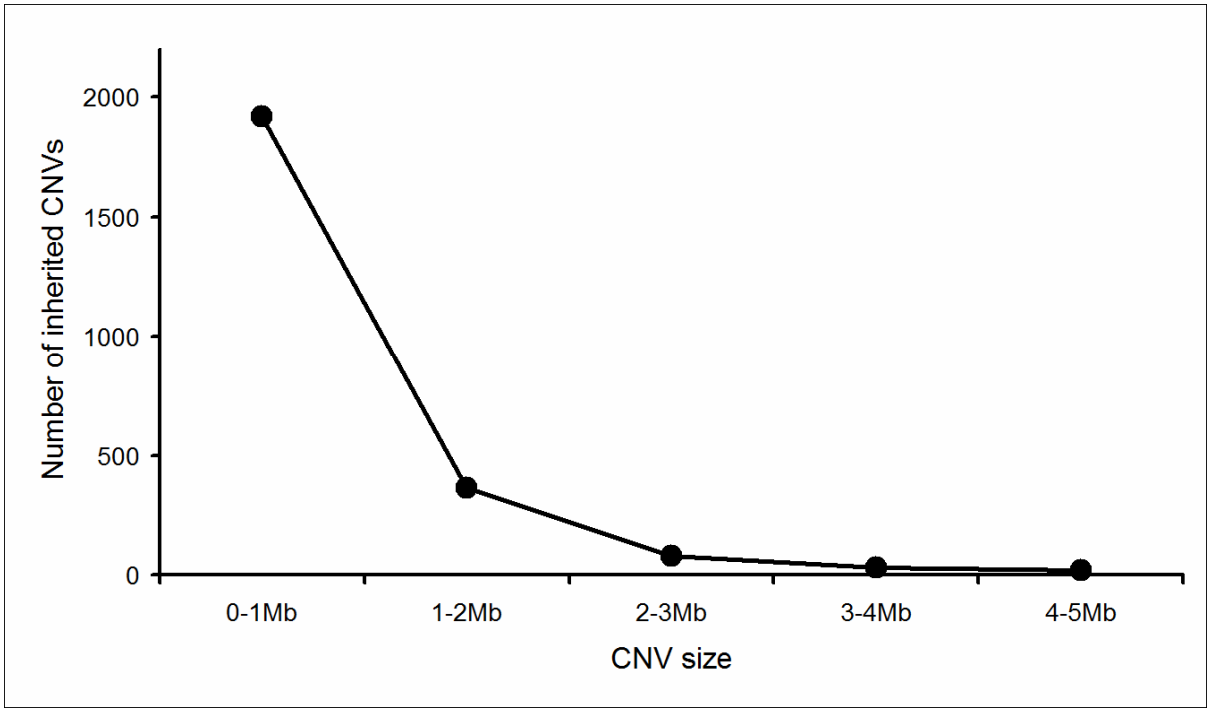
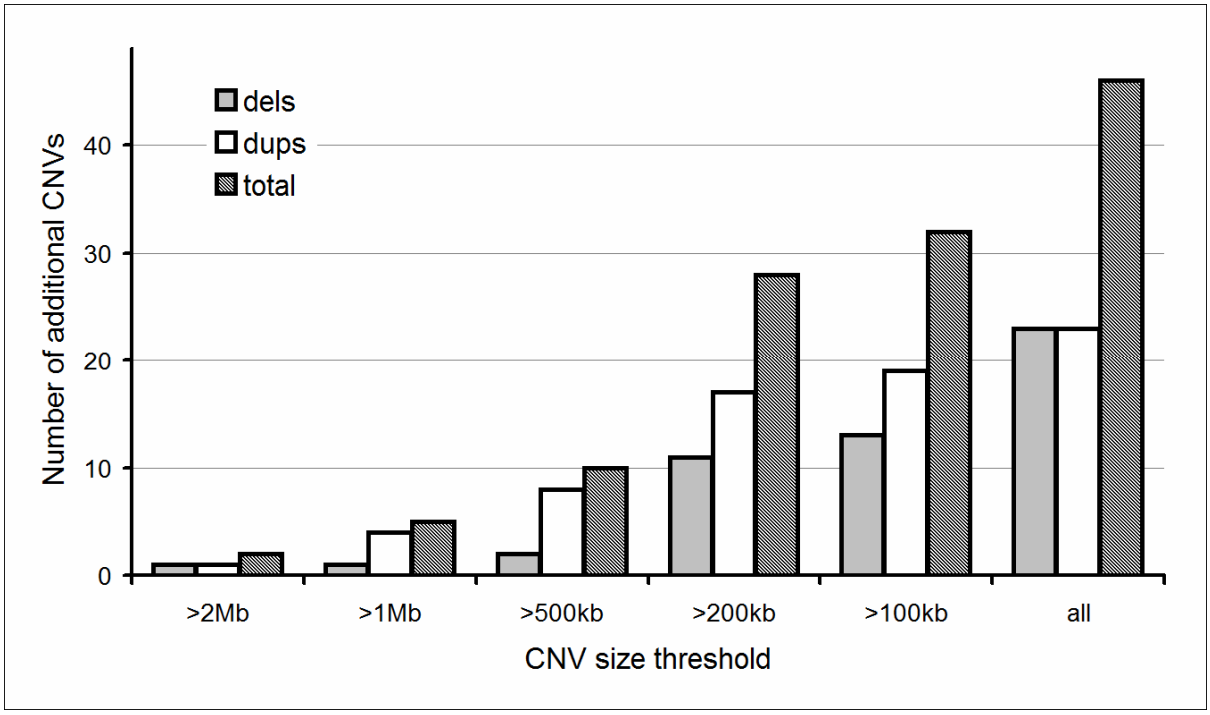


Figure 2. Additional CNVs detected if backbone resolution of prenatal CMA is increased.



TABLES

Table 1. Regions targeted for prenatal CMA.

Syndrome (OMIM ID)	Chromosome Band
1p36 deletion (607872)	1p36
2q37 deletion (600430)	2q37
3q29 deletion (609425) / duplication (611936)	3q29
Wolf-Hirschhorn (194190)	4p16.3
Cri du Chat (123450)	5p15.2
Sotos (117550)	5q35.2q35.3
Williams-Beuren (194050)	7q11.23
8p23.1 deletion	8p23.1
Kleefstra (610253)	9q34.3
WAGR 11p13 deletion (194072)	11p13
Potocki-Shaffer (601224)	11p11.2
Angelman (105830) / Prader-Willi (176270)	15q11.2
15q24 deletion (613406) / duplication (613406)	15q24
16p13.3 deletion (610543) / duplication (613458)	16p13.3
Miller-Dieker (247200) / 17p13.3 duplication (613215)	17p13.3
Hereditary Liability to Pressure Palsies (162500) / Charcot-Marie-Tooth type 1A (118220)	17p12
Potocki-Lupski (duplication)	

Smith-Magenis (182290) / Potocki-Lupski (610883)	17p11.2
17q11.2 deletion (613675)	17q11.2
Koolen-De Vries (610443)	17q21.31
Cat-Eye (115470)	22q11
DiGeorge (188400) / Velocardiofacial (192430) / 22q11.2 duplication (608363)	22q11.2
Phelan-Mcdermid (606232)	22q13.33
Pelizaeus-Merzbacher (312080)	Xq22.2
Rett (312750) / Lubs X-Linked Mental Retardation (300260)	Xq28

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Table 2. Reported prenatal CMA results.

Reported Result	Referral Indication
1p36.32p36.12(4,481,264-22,855,001)x1	Nuchal 2.9 mm, cleft lip and palate, adjusted risk for trisomy 21 of 1/361, adjusted risk for trisomies 13 & 18 of 1/53.
1q21.1(145,413,387-145,747,269)x1*	Suspected TAR syndrome, absent or shortened radius & ulna bilaterally, bilateral radial aplasia with shortened phalanges and di-phalyngeal thumbs, both hands acutely abducted, both ulnae are approx 2/3 the normal length, humeri on the 5th centile, long bones of the lower limbs are on the 50th centile.
3p21.31(45,266,030-48,311,229)x3	Known familial insertion of chr3 material into another chromosome.
3p24.1p22.3(30,485,387-34,962,363)x1	Bilateral borderline ventriculomegaly, absent cavum septum pellucidum.
4p16.3p14(72,446-35,935,983)x1	Nuchal 3.1mm.
4p16.3p16.1(514,449-8,667,610)x1	Bilateral talipes, cleft lip, single umbilical artery.
4p15.33p15.32(13,625,716-17,418,852)x1	Interuterine growth retardation, low PAPPA and combined tests.
4p15.31p15.1(20,541,127-28,451,250)x1	Nuchal 2.0mm, exomphalos, absent nasal bone, reverse ductus.

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6p25.3p22.2(259,527-25,416,824)amp	Mild ventriculomegaly, potential brain abnormality.
6q23.2q24.2(135,056,331-143,515,719)x1	Oligohydramnios, echogenic bowel.
9p24.3(214,366-2,197,859)x1, 9p24.2p13.1(2,418,074-40,508,819)x3	Bilateral genu recurvatum, moderate ventriculomegaly.
9p24.3p13.1(214,366-40,508,819)x4	Nuchal 8.3mm.
9q33.3q34.3(126,795,265-141,008,915)x3	Muscular ventral-septal defect, tricuspid regurgitation, abnormally shaped aortic arch.
10q11.22q11.23(46,951,236-52,004,151)x1	Bilateral ventriculomegaly.
11q23.3q25(116,693,628-134,446,160)x3, 22q11.1q11.21(16,053,472-20,311,763)x3	Possible micrognathia with prominent upper lip, brain cyst, dilated 3rd ventricle, stomach not clearly insulated, complete or partial agenisis of corpus callosum, deficient cerebellar vermis.
13q31.3q34(94,493,888-115,059,020)x1	High risk for trisomy 13, holoprosencephaly, diaphragmatic hernia, cleft palate
15q21.2q25.1(51,740,270-79,762,418)x3	Shortened femur, bilateral hydronephrosis, interuterine growth retardation, 2x vessel cord, dysmorphic, downslanting palpebral fissures, low set ears.
17p11.2(16,532,735-20,221,695)x3	Long bones around or below 3rd centile (including femur), dilation of the intra-abdominal portion of the umbilical vein (varix).

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22q11.1q13.33(17,096,854-51,178,264)x3	Nuchal 4.4mm, trisomy 21 risk of 1:61.
22q11.21(18,896,971-21,440,514)x1	Nuchal 4.2mm, trisomy 21 risk of 1:70.
22q11.21(18,896,971-21,801,661)x3	Ventriculomegaly, hydronephrosis, shortening of the long bones.
22q13.31q13.33(45,576,756-51,178,264)x1	Echogenic bowel, urinary tract/renal anomaly.
Xp11.3p11.21(44,307,282-58,051,765)x1~2	Intrauterine growth retardation, talipes, stomach not visible on ultrasound.

*This fetus was referred for suspected TAR syndrome and therefore an additional targeted region was added for RBM8A.

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Table 3. Further deletion CNVs uncovered by retrospective reanalysis of prenatal CMA data at full resolution.

Deletion CNV	Referral Indication
1q21.1(145,413,387-145,747,269)x1	Nuchal 4.5mm.
2p21(44,507,914-44,531,188)x1	Brain ventricle/hemisphere >97th centile, trisomy 21 risk of 1:13.
2p16.3(50,881,995-50,947,729)x1	Complete transposition of the great arteries.
2p12(75,347,691-75,729,632)x1	Tricuspid valve dysplasia, fetal hydrops.
2q35(220,096,681-220,116,241)x1	Nuchal 1.6mm, absent ductus venous, trisomy 21 risk of 1:19121, trisomy 13/18 risk of 1:35067.
2q36.1(222,834,667-224,926,273)x1*	Nuchal 4.1mm, trisomy 21 risk of 1:112.
3p24.3(20,021,595-20,052,991)x1	Nuchal 1.5mm, echogenic bowel, liver anomaly.
4q24(107,063,807-107,248,637)x1	Isolated aberrant right subclavian artery.
6p22.2(26,440,746-26,463,502)x1	Tetralogy of Fallot, small for gestational age (<10th centile).
8q23.3(113,630,231-113,960,067)x1	Nuchal 4.1mm.
10q26.3(135,352,371-135,372,492)x1	Nuchal 2.5mm, trisomy 21 risk of 1:2005, stomach on right side, suspected arterioventricular defect.
11q22.1(97,762,150-98,228,688)x1	Trisomy 21 risk of 1:8.
14q24.3(76,352,571-76,522,811)x1	Nuchal 5mm.

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15q11.2(22,318,596-23,085,096)x1	Severe growth restriction, oligohydramnios.
15q11.2(22,765,627-23,085,096)x1	Aberrant right subclavian artery.
15q11.2(22,765,627-23,085,096)x1	Left-sided diaphragmatic hernia.
16q23.2(81,293,283-81,367,334)x1	Nuchal 5.7mm, Trisomy 21 risk of 1:204, Trisomy 13/18 risk of 1:129.
19p13.2(7,070,409-7,168,093)x1[$]	Short long bones, know early pregnancy haematoma (sub chorionic bleeding).
19q13.2(42,263,338-42,289,030)x1	Nuchal 3.2mm.
22q11.23(23,627,338-24,040,236)x1[$]	Short long bones, know early pregnancy haematoma (sub chorionic bleeding).
Xp22.33(1,378,590-1,689,610)x1	Borderline ventriculomegaly.
Xp22.33(2,066,580-2,343,577)x1	Nuchal 4.4mm.
Xp22.11(23,018,416-23,021,667)x1	Nuchal 3.1mm, abdominal cyst, crown-rump length small for gestational age, trisomy 21 risk of 1:37.

* PAX3 is deleted, which is indicative of Waardenburg syndrome, Type I.

[$] These two CNVs were carried by a single fetus.

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Table 4. Further amplification CNVs uncovered by retrospective reanalysis of prenatal CMA data at full resolution.

Amplification CNV	Referral Indication
2p16.3(50,625,488-51,057,883)x3	Echogenic bowel.
2q11.2(98,019,585-98,274,335)x3	Nuchal 1.0mm, omphalocele.
3q22.2(134,204,455-134,204,970)x4	Atrioventricular septal defect, coarctation of aorta.
3q24(142,840,204-143,579,847)x3	Nuchal >4mm.
4q24(102,735,053-102,897,983)x3	Polyhydramnios, ventriculomegaly, bilateral talipes, suspected Charcot-Marie-Tooth syndrome.
6p12.3(50,153,611-50,519,464)x3	Nuchal 5.2mm.
6q21(105,548,868-107,397,152)x3	Polyhydramnios, pleural effusion, hydronephrosis.
6q21(111,067,339-111,478,900)x3	Hypoplastic left heart syndrome, complex congenital heart disease, interstinal malrotation, suspected 22q11.2 deletion syndrome.
8p12(33,210,383-33,455,764)x3	Fetal cardiac abnormality.
10q21.3(64,902,960-67,399,362)x3	Coarctation of aorta, large ventricular septal defect, suspected 22q11.2 deletion syndrome.

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12p12.1(21,615,645-21,689,158)x3	Nuchal 2.3mm, double outlet right ventricle, spontaneous rupture of membranes.
14q11.2(22,323,878-22,964,922)x3	Nuchal 4.3mm, trisomy 21 risk of 1:10.
14q11.2(22,669,442-22,964,922)x3	Intrauterine growth retardation.
19p13.2 - p13.13(13,865,337-13,933,080)x3	Nuchal 3.8mm.
Xp22.33(658,210-1,259,140)x3	Bilateral talipes.
Xp22.33(919,416-1,259,140)x3[$$]	Nuchal 1.9mm, cardiac abnormality.
Xp22.33(970,702-2,017,358)x3	Polyhydramnios, trisomy 21 risk of 1:400.
Xp22.33(1,217,016-1,378,646)x3	Aberrant right subclavian artery, suspected 22q11.2 deletion syndrome.
Xp22.33(1,314,735-1,347,344)x3	Cardiac anomaly, ventriculomegaly.
Xp22.33(1,755,741-2,017,358)x3	Hydrops.
Xp22.31(6,551,154-8,032,120)x3	Bilateral talipes.
Xp22.2(16,147,216-16,809,305)x2	Nuchal 4.7mm, trisomy 13 risk of 1:82, trisomy 18 risk of 1:59.
Xq28(154,133,237-154,560,375)x2[$$]	Nuchal 1.9mm, cardiac abnormality.

[$$] These two CNVs were carried by a single fetus.

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SUPPLEMENTARY INFORMATION

Table S1. Size distribution of *de novo* and inherited CNVs detected by postnatal CMA.

CNV size	Number of *de novo* CNVs	Number of inherited CNVs
0 - 1Mb	230	1918
1 - 2Mb	115	362
2 - 3Mb	101	79
3 - 4Mb	37	31
4 - 5Mb	28	18
5Mb +	163	17

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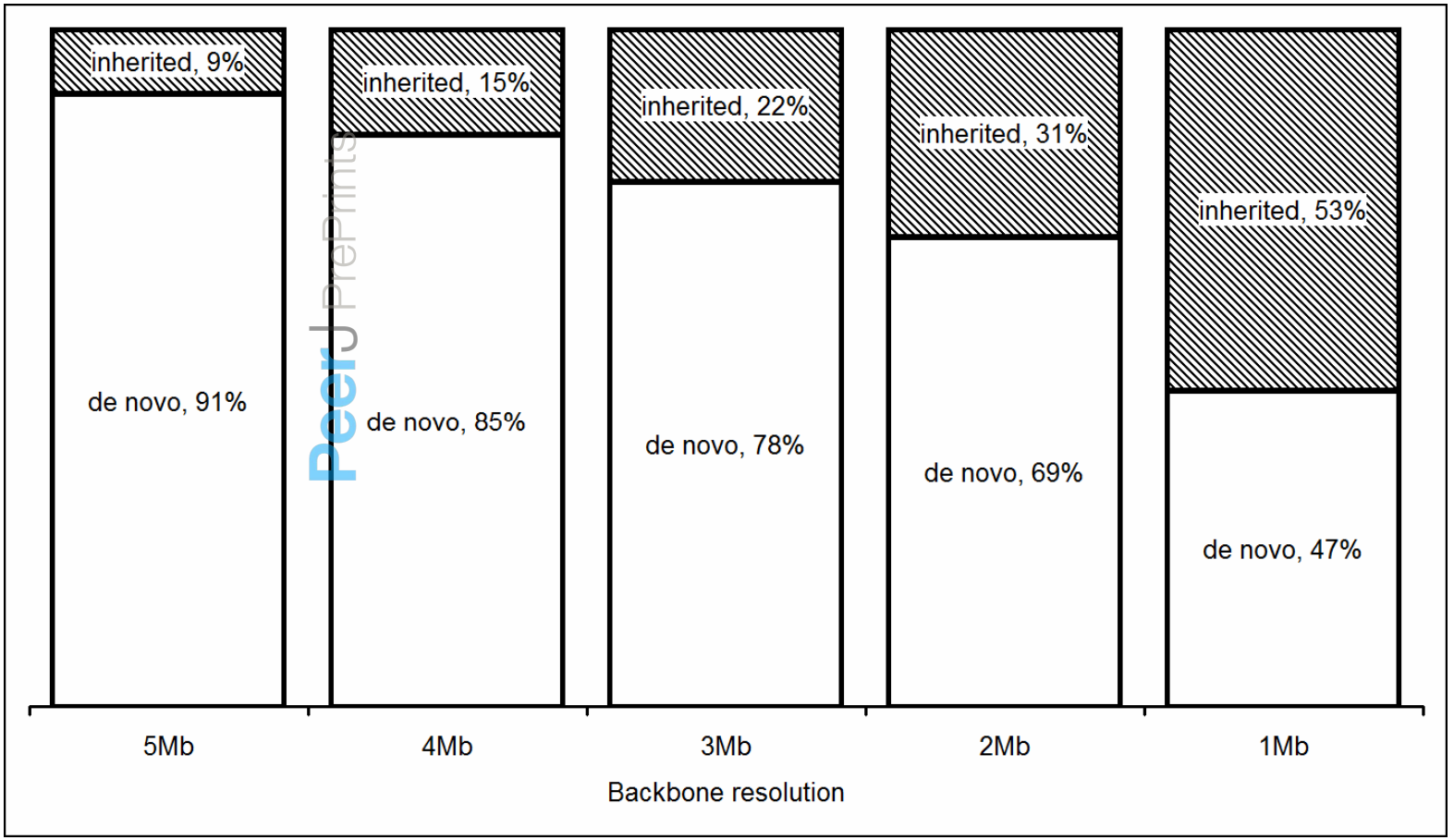
Figure S1. Inheritance pattern of CNVs detected by postnatal CMA if various backbone resolutions are applied.



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