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WORKING GROUP ITALIAN SOCIETY OF HUMAN GENETICS (SIGU) POSITION STATEMENT. Microarray application in prenatal diagnosis: a position statement from the working group on behalf of the Italian Society of Human Genetics (SIGU), *November 2011*

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ABSTRACT

Objectives: At present, a precise guideline establishing chromosome microarray analysis (CMA) applications and platforms in the prenatal setting does not exist. The actual controversial question is whether CMA technologies can or should shortly replace the standard karyotype in prenatal diagnosis practice.

Methods: Based on review of the recent literature and actual knowledge and experiences of all participants, the SIGU Committee proposes recommendations for the use of CMA in prenatal testing.

Results: Dataset collections reported in the medical literature clearly show a significant incidence of pathogenic CNVs at 6.4% in the group of pregnancies with ultrasound fetal abnormalities and normal karyotype and the detected CNVs are more likely to have a relevant role in terms of nosology for the fetus and for the assessment of reproductive risks for the couples. The estimation of the frequencies of variations of unclear significance (VOUS) varies depending on the different CMA platforms used spanning from targeted arrays, for which a 0-4% frequency of VOUS has been reported, to high resolution whole genome SNP arrays for which the estimated incidence of VOUS was of 9-12%.

Conclusions: Presently CMA analysis can be considered a second-tier diagnostic test to be used after a standard karyotype in selected group of pregnancies, such as those with single (apparently isolated) or multiple US fetal abnormalities, with *de novo* chromosomal rearrangements, even if apparently balanced, and those with supernumerary marker chromosomes.

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BACKGROUND

In the last few years chromosomal microarray (CMA) technologies (array Comparative Genomic Hybridization, aCGH; single nucleotide polymorphism array, SNP array) have acquired more and more relevance becoming a fundamental diagnostic tool in Medical Genetics. In fact, the technological evolution and the experimental optimization determined a notable simplification of the analytic protocols, leading to the decreasing of the costs and

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enabling the progressive spreading of this technology in many laboratories all over the world. Extremely encouraging results, in terms of detection rate, were obtained on patients affected by unexplained developmental delay/intellectual disability (DD/ID), autism spectrum disorders (ASD), or multiple congenital anomalies (MCA), in which the diagnostic yield over karyotyping was estimated to be ~10-20%[1-3]. The accurate evaluation of the gene content of the imbalanced genomic regions together with the comparison with data collections present in publicly available repository databases (DGV http://projects.tcag.ca/variation/, DECIPHER http://decipher.sanger.ac.uk/, OMIM http://www.ncbi.nlm.nih.gov/omim) enabled the detection of the critical regions related to known syndromes allowing in several cases genotype-phenotype correlations. For such

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reasons, the SIGU Committee proposed in 2010 a national document in which, based on the literature and on the experiences of all participant Institutions, recommended CMA as the first-tier diagnostic test for patients with DD/ID, ASD, or MCA in the postnatal setting (www.sigu.net).

The incredible advantages offered by CMA technology opened new scenarios regarding its possible application in prenatal diagnosis (PD), where the traditional karyotype is still considered the gold standard method for all categories of indications for invasive sampling. Compared to conventional karyotyping, CMA can detect imbalances with an extremely higher resolution (up to few Kb) in a shorter time with standardized protocols[4].

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LITERATURE REVIEW

At present, a precise guideline establishing CMA applications and platforms in the prenatal setting does not exist and this situation has led to the explosion of debates and controversies[5-11] concerning whether CMA technologies can or should shortly replace the standard karyotype in prenatal diagnosis practice.

Considering the present, limited knowledge in this field, the SIGU Committee has focused on disadvantages related to this technology and currently advises against its unlimited and unselected application in routine prenatal diagnosis. Without strict guidelines for the use of CMA in PD, this approach could actually be more harmful than useful when applied during prenatal life because of the unclear results. In fact, the current knowledge has gaps regarding the clinical interpretation of copy number changes. This is because of the detection of a not previously described imbalance, the lack of knowledge about the function of many genes, a relatively poor understanding of the gene-gene and gene-environment interactions, and the role of epigenetic modifications in modulating the penetrance and expressivity of copy number changes[12-14]. There are additional questions related to the detection of variations of uncertain significance (VOUS) without a real and concrete predictive value about fetal and future health during the prenatal diagnostic period that could lead to useless information and cause increasing parental anxiety[7,15]. In addition, the diagnostic yield of CMA in prenatal setting has not been clearly established in all categories of indications because the majority of the published papers included selected cases with fetal abnormalities detected by ultrasound (US) with an apparently normal karyotype. In this group of pregnancies the CMA detection rate is in average 6.4% (range: 0-15.6%) (Table 1). In fact, dataset collections reported in the medical literature clearly show a significant incidence of pathogenic CNVs in this group of pregnancies and the detected CNVs are more likely to have a relevant role in terms of nosology for the fetus

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2.5 and for the assessment of reproductive risks for the couples[16-32]. In cases with US fetal abnormalities the sum of the detection rates of conventional cytogenetic analyses (28% in

2.10 chorionic villi and 12% in amniotic fluids; in average ~20%)[33] and CMA (6.4%) provide an overall yield of detection of ~27% combining the first-tier karyotype with the second-tier CMA.

The estimation of the frequencies of VOUS seems to be difficult to assess due to different CMA platforms used in the studies spanning from targeted arrays (reported VOUS frequency ranging from 0 to 4%), to high resolution whole genome SNP arrays (VOUS estimated incidence of ~9-12%) (Table 1)[16-32]. In contrast, the rate of detection of a known,

2.6 disability-causing pathogenic copy number variation (CNV) by CMA in all pregnant women has been estimated to be between 0.16% and 0.3% [6]. The comparison between the percentages of ambiguous findings and of pathogenic CNVs shows that the use CMA technology in prenatal setting without a specific clinical indication is currently not justified. Another important limitation related to the application of CMA as a first-tier test is represented by the impossibility to detect balanced rearrangements without genetic losses or gains. This would lead to the under-estimation of the risks of phenotypic consequences related to i) disruption or modulation of the expression of gene(s) located at the breakpoint(s), ii) inactivation (position effect) of gene(s) at the breakpoint region(s) and iii) missing the opportunity to investigate and detect uniparental disomy conditions related to imprinting syndromes in cases involving imprinted chromosomes[34-36]. SNP array has the

2.7 advantage of being able to detect long continuous stretches of homozygosity (LCSH), representing whole chromosome or segmental uniparental isodisomies (a duplicate of one chromosome from a parent and no chromosome from the other parent). It cannot however detect heterodisomies (the most common form of uniparental disomy where both chromosomes in a pair are inherited from one parent and nothing from the other parent)

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without testing parents in conjunction with the fetal specimen. In addition, SNP array provides consanguinity information (occurrence of incest) that raises important ethical issues, therefore its use in terms of LCSH may be limited[37]. Finally, polyploidies and mosaicisms lower than 30%, that are relatively common findings in chorionic villi and amniotic fluid samples[33], cannot currently be detected by aCGH[38,39].

On the other side, CMA is useful to clarify abnormal karyotype results. In cases with supernumerary marker chromosomes different studies demonstrated the practical usefulness of CMA for their classification and characterization improving the diagnostic accuracy and allowing to offer a specific genetic counselling[40-42]. The role of CMA in prenatal cases with de novo apparently balanced chromosomal rearrangements has not extensively studied; however in postnatal dataset collections of patients with *de novo* apparently balanced chromosomal rearrangements and an abnormal phenotype CMA detect cryptic imbalances in 35-40% of samples with reciprocal translocations and in 72-75% of samples with complex rearrangements[43-45].

RECOMMENDATIONS FOR MICROARRAY APPLICATION IN PRENATAL DIAGNOSIS

The SIGU Committee members belong to both public and private institutions. Based on review of the recent literature and actual knowledge and experiences of all participants on the committee, the SIGU Committee recommends the use of CMA in prenatal testing:

[1] never as a substitute of the conventional karyotype;

[2] for specific diagnostic purposes in selected pregnancies and not for general screening in all pregnancies;

[3] only in prenatal cases with specific indications, such as:

i) single (apparently isolated) or multiple US fetal abnormalities;

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ii) de novo chromosomal rearrangements, even if apparently balanced, detected by standard karyotype to investigate the possible presence of cryptic imbalance(s) related to the structural chromosome abnormality

iii) supernumerary marker chromosomes to characterize their origin and genetic content.

2.1 In these groups of pregnancies the application of a genome-wide, and not a targeted, platform enriched in probes containing dosage-sensitive and disease-causing genes is recommended with an average spatial resolution of at least 250Kb with calls in the

2.12 backbone of at least 500Kb. When an uncommon CNV is found parental testing is needed to help in the interpretation of genotype-phenotype correlations.

Further data are needed about the application of CMA in other groups of pregnancies such as those with:

- abnormal maternal serum screening with an increased risk for Down syndrome and normal karyotype;
- 1 or more soft markers (i.e. choroid plexus cysts, intestinal hyperechogenicity, renal pielectasy, single umbilical artery, hyperecogenic cardiac foci...);
- IUGR and/or amniotic fluid volume alteration without major structural abnormalities (i.e. cardiac malformations, diaphragmatic hernia, central nervous system abnormalities).

Robust genotype-phenotype correlations collected from large scale research studies are necessary before future introduction of this technique in *all* pregnancies as a screening tool and in substitution of the standard karyotype.

CONCLUSIONS

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Presently, CMA analysis can be considered a second-tier diagnostic test which can be integrated with, but not used as a replacement of a standard karyotype, in selected group of pregnancies.

QUALITY ASSURANCE

Laboratories providing microarray-based analysis are encouraged to participate in an external assessment quality program and in proficiency testing among laboratories to monitor their performance.

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Table 1: Incidence of pathogenic variations and unclear results from a dataset collection of published studies

Study	N° investigated cases with US abnormalities and normal karyotype	N° Abnormal CMA results	Percentage of pCNVs -%	N° VOUS/total prenatal population analysed	Percentage of CNVs of VOUS - %
Le Caignec et al. [16]	49	4	8.2	1/49	2.0
Vialard et al. [18]	37	4	10.8	NR	NR
Van den Veyver et al. [20]	84	5	6	3/300	1
Shaffer et al. [21]	110	2	1.9	1/151	0.7
Coppinger et al. [22]	155	6	3.9	1/213	0.5
Kleeman et al. [23]	50	0	0	1/50	2
Tyreman et al. [24]	106	10	9.4	13/106	12.2
Valduga et al. [25]	50	5	10	NR	NR
Faas et al. [26]	32	3	9.4	3/38	7.9
Maya et al. [27]	102	2	2	0/269	0
Evangelidou et al. [34]	15	1	6.6	1/25	4
Gruchy et al. [35]	38	3	7.9	0/38	0
D'Amours et al. [36]	49	4	8.2	6/49	12.2
Zuffardi et al, ISPD 2010 *(oral communication)*	63	5	9.5	1/63	1.6
De Toffol et al. [37]	32	5	15.6	1/32	3.1
Leung et al. [38]	48	6	12.5	NR	NR
Overall studies	1020	65	**6.4**	29/1383	**2.1**
Estimated incidence of pCNVs in all pregnant women [Ogilvie et al. [6]]			0.16-0.3 (1:600-1:300)		

pCNVs= pathogenic copy number variation; VOUS= variation of unknown clinical significance

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