DIAGNOSI PRENATALE INTEGRATA

CARIOTIPO + ARRAY-CGH

La tecnica di array-CGH (Comparative Genomic Hybridization) ha visto recentemente uno sviluppo massiccio, imponendosi prepotentemente come realtà diagnostica e strumento indispensabile nella genetica medica. L'evoluzione scientifica e l'ottimizzazione sperimentale ha infatti consentito di snellire e semplificare in maniera importante i protocolli analitici e di diminuire i costi, permettendo ad un numero sempre crescente di laboratori di avvicinarsi a questo tipo di tecnica. I risultati ottenuti si sono dimostrati incoraggianti soprattutto per quanto riguardo lo studio di pazienti con ritardo mentale idiopatico e dismorfismi, per i quali la probabilità di positività al test è stata stimata intorno al 10-12% (Sagoo et al, 2009, Miller et al, 2010). Lo studio delle regioni coinvolte e del contenuto genico e il confronto con i dati raccolti in banche dati ha permesso l'individuazione di regioni associate a sindromi note e, in molti casi, aiutato nella correlazione genotipo-fenotipo prestandosi come valido supporto alla consulenza genetica. I vantaggi addotti dall'utilizzo di questa nuova tecnologia hanno aperto nuovi scenari, fino al recente dibattito riguardo un suo possibile utilizzo in diagnosi prenatale dove il cariotipo è considerato ad oggi la tecnica di elezione. L'interrogativo principale risiede nel fatto che la citogenetica permette di individuare alterazioni numeriche e strutturali ad un livello di risoluzione più basso (10 Mb circa) e in tempi maggiori *rispetto* all'array-CGH.

Non esiste attualmente una regolamentazione che stabilisca in maniera precisa l'utilizzo dell'array CGH in diagnosi prenatale. L’array permette di evidenziare con un unico saggio tutte le sindromi da microdelezione/duplicazione note (diversamente da altri tests molecolari commerciali che si limitano all’analisi di un limitato gruppo di patologie) tuttavia esisten la possibilità di individuare risultati dal significato clinico incerto e senza alcun valore predittivo fetale. Ad oggi la percentuale di risultati dal significato clinico incerto può essere stimata intorno al 1-3% contro dati con rilevanza patologica in gravidanze senza indicazioni dello 0,1-0,3%.

Il recente trial federale US NIH condotto sotto la storica egida Wapner – Jackson e pubblicato in NEJM definisce che, nelle gravidanze a rischio le differenze significative fra analisi ArrayCGH e cariotipo sono:

1) **evidenze ecografiche: ArrayCGH trova anomalie 6% in più del cariotipo**
2) **Età avanzata e altro: ArrayCGH trova anomalie 1.7% in più del cariotipo.**
3) **Solo il cariotipo riscontra triploidie, anomalie bilanciate e mosaicismi <30**

Table 3. Frequency and Clinical Interpretation of Microdeletions and Duplications on Chromosomal Microarray in the 3822 Samples with a Normal Karyotype, According to Indication for Prenatal Testing.

Indication for Prenatal Diagnosis	Normal Karyotype	Common Benign	Pathogenic	Uncertain Clinical Significance (N=130)		Total Known Pathogenic and Potential for Clinical Significance*
				Likely to Be Benign	Potential for Clinical Significance	
	no.	*no. (%)*				*no. (%) [95% CI]†*
Any	3822	1234 (32.3)	35 (0.9)	69 (1.8)‡	61 (1.6)	96 (2.5) [2.1–3.1]
Advanced maternal age	1966	628 (31.9)	9 (0.5)	37 (1.9)	25 (1.3)	34 (1.7) [1.2–2.4]
Positive on Down's syndrome screening	729	247 (33.9)	3 (0.4)	13 (1.8)	9 (1.2)	12 (1.6) [0.9–2.9]
Anomaly on ultrasonography	755	247 (32.7)	21 (2.8)	16 (2.1)	24 (3.2)	45 (6.0) [4.5–7.9]
Other§	372	112 (30.1)	2 (0.5)	3 (0.8)	3 (0.8)	5 (1.3) [0.6–3.1]

* Total includes those predetermined as known to be pathogenic and those classified by the clinical advisory committee as clinically relevant.
† CI denotes confidence interval.
‡ Includes 36 samples determined likely to be benign by the study geneticist and 33 determined by the independent clinical advisory committee on the basis of size, gene content, inheritance, the literature, and ultrasonography findings.
§ Other indications include family history, previous pregnancy with chromosomal abnormalities, and elective decision.

La Società Italiana di Genetica Umana ha approvato e pubblicato una posizione ufficiale con le indicazioni per l'uso di array-CGH in diagnosi prenatale solo assieme al cariotipo.

Il test di array CGH può e si consiglia venga proposto alla facoltà di scelta delle pazienti che appartengono suesposte categorie a rischio (*standard care practice*) e venga considerato come un esame integrante di secondo livello, che affianchi il cariotipo. In particolare:

- *Caratterizzazione di riarrangiamenti cromosomici de novo apparentemente bilanciati*
- *Presenza di anomalie ecografiche*
- *Presenza di un microriarrangiamento strutturale bilanciato nei genitori*
- *La età materna e presenza di test biochimici a rischio*

I nostri laboratori, nell'intento di rispondere alle esigenze di medici e pazienti, sono oggi in grado di offrire la diagnosi prenatale con la combinazione del cariotipo e array-CGH.

CARIOTIPO+ARRAY-CGH

La informativa al consenso per la indagine citogenetica prenatale vede inserita la clausola relativa alla scelta della tecnica supplementare ArrayCGH offerta alla valutazione e sottoscrizione della paziente.

I colleghi medici e i pazienti interessati possono rivolgersi direttamente ai nostri laboratori per ulteriori informazioni e modalità degli esami.

Campioni necessari: campione liquido amniotico o villi coriali, campione di sangue periferico in EDTA dei genitori.

Original Article

Chromosomal Microarray versus Karyotyping for Prenatal Diagnosis

Ronald J. Wapner, M.D., Christa Lese Martin, Ph.D., Brynn Levy, M.Sc.(Med.), Ph.D., Blake C. Ballif, Ph.D., Christine M. Eng, M.D., Julia M. Zachary, Melissa Savage, M.S., Lawrence D. Platt, M.D., Daniel Saltzman, M.D., William A. Grobman, M.D., M.B.A., Susan Klugman, M.D., Thomas Scholl, Ph.D., Joe Leigh Simpson, M.D., Kimberly McCall, B.S., Vimla S. Aggarwal, M.B., B.S., Brian Bunke, B.S., Odelia Nahum, M.Sc., Ankita Patel, Ph.D., Allen N. Lamb, Ph.D., Elizabeth A. Thom, Ph.D., Arthur L. Beaudet, M.D., David H. Ledbetter, Ph.D., Lisa G. Shaffer, Ph.D., and Laird Jackson, M.D.

N Engl J Med
Volume 367(23):2175-2184
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The NEW ENGLAND JOURNAL of MEDICINE

Study Overview

- This large, systematic study of prenatal diagnosis shows that chromosomal microarray analysis provided additional, clinically significant cytogenetic information as compared with karyotyping but did not identify triploidies and balanced translocations.

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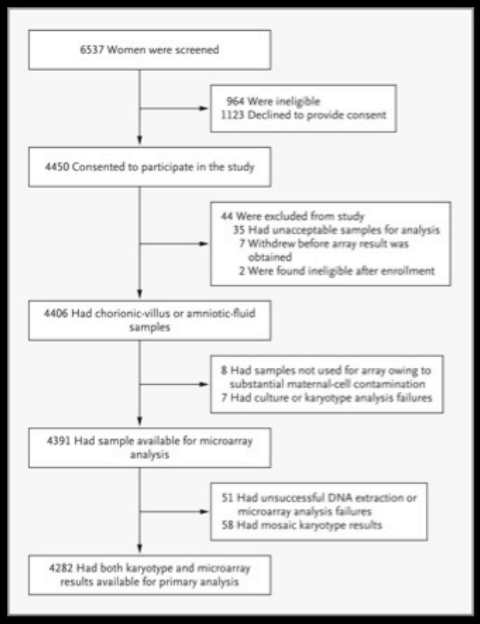
Screening and Enrollment of the Study Participants.



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Baseline Characteristics and Primary Indication for Prenatal Testing and Characteristics of the 4406 Study Participants with Adequate Samples for Analysis.

Table 1. Baseline Characteristics and Primary Indication for Prenatal Testing and Characteristics of the 4406 Study Participants with Adequate Samples for Analysis.*

Characteristic	Indication for Invasive Sampling				
	Anomaly on Ultrasonography (N=1109)	Maternal Advanced Age (N=2054)	Positive Result on Down's Syndrome Screening (N=827)	Other (N=416)	All (N=4406)
Maternal age — yr	32.2±5.8	38.5±2.5	34.0±5.2	33.1±4.5	35.6±5.1
Gestational age at procedure — wk					
Chorionic-villus sampling	12.5±1.6	11.8±0.8	12.8±0.8	11.9±0.8	12.1±1.1
Amniocentesis	21.1±4.0	17.4±1.3	18.3±1.9	17.8±2.1	18.8±3.1
Race or ethnic group — no. (%)†					
Black	114 (10.3)	80 (3.9)	65 (7.9)	27 (6.5)	286 (6.5)
Hispanic	164 (14.8)	163 (7.9)	110 (13.3)	46 (11.1)	483 (11.0)
Other	831 (74.9)	1811 (88.2)	652 (78.8)	343 (82.5)	3637 (82.5)

* Plus–minus values are means ±SD. Pregnancies in which the fetus had a nuchal translucency of 3.5 mm or greater or a septated cystic hygroma are included as anomalies on ultrasonography. Nuchal translucencies of less than 3.5 mm were considered a component of Down's syndrome screening. Other indications for prenatal testing include family history, previous pregnancy with chromosome abnormality, and elective decision. Of the 4406 study participants, 2275 had chorionic-villus sampling and 2131 had amniocentesis.
† Race or ethnic group was self-reported.

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Results of Karyotype and Microarray Analysis in 4282 Samples with a Nonmosaic Karyotype, According to Cytogenetic Abnormality.

Table 2. Results of Karyotype and Microarray Analysis in 4282 Samples with a Nonmosaic Karyotype, According to Cytogenetic Abnormality.

Abnormality	Detected on Karyotyping	Detected on Microarray*		
		Total	Full Complement	Mosaic Complement
	no. (%)	*no. (%)*	*no.*	*no.*
Any autosomal or sex-chromosome abnormality	374 (8.7)	374 (100)	366	8
Any common autosomal trisomy	317 (7.4)	317 (100)	312	5
Trisomy 21	188	188 (100)	185	3
Trisomy 18	93	93 (100)	91	2
Trisomy 13	36	36 (100)	36	0
Other autosomal trisomy	4 (0.1)	4 (100)	4	0
Any sex-chromosome aneuploidy	57 (1.3)	57 (100)	54	3
45,X	39	39 (100)	36	3
47,XXX; 47,XXY; 47,XYY	18	18 (100)	18	0
Structural rearrangement	65 (1.5)			
Balanced	40	0	0	0
Unbalanced	22	22 (100)	21	1
Marker	3	2 (66.7)	2†	0
Triploidy	17 (0.4)	0‡	0	0

* All results are reported from uncultured samples where available, and otherwise from cultured samples.
† No euchromatin was identified on fluorescence in situ hybridization in the marker with a normal result on chromosomal microarray.
‡ A total of 15 of the 17 triploidy cases (88.2%) were identified in maternal-cell contamination studies. One other was recorded as mosaic 47,XXY on microarray.

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Frequency and Clinical Interpretation of Microdeletions and Duplications on Chromosomal Microarray in the 3822 Samples with a Normal Karyotype, According to Indication for Prenatal Testing.

Table 3. Frequency and Clinical Interpretation of Microdeletions and Duplications on Chromosomal Microarray in the 3822 Samples with a Normal Karyotype, According to Indication for Prenatal Testing.

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Conclusions

- In the context of prenatal diagnostic testing, chromosomal microarray analysis identified additional, clinically significant cytogenetic information as compared with karyotyping and was equally efficacious in identifying aneuploidies and unbalanced rearrangements but did not identify balanced translocations and triploidies.

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Microarray application in prenatal diagnosis: a position statement from the cytogenetics working group of the Italian Society of Human Genetics (SIGU), November 2011

A. NOVELLI[1], F. R. GRATI[2], L. BALLARATI[3], L. BERNARDINI[1], D. BIZZOCO[4], L. CAMURRI[5], R. CASALONE[6], L. CARDARELLI[7], P. CAVALLI[8], R. CICCONE[9], M. CLEMENTI[10], L. DALPRÀ[11], M. GENTILE[12], G. GELLI[13], P. GRAMMATICO[14], M. MALACARNE[15], A. M. NARDONE[16], V. PECILE[17], G. SIMONI[2], O. ZUFFARDI[9] and D. GIARDINO[3]

[1]*Mendel Laboratory, Casa Sollievo della Sofferenza Hospital, IRCCS, San Giovanni Rotondo, Italy;* [2]*TOMA Advanced Biomedical Assays S.p.A., Busto Arsizio (VA), Italy;* [3]*Laboratory of Medical Cytogenetics and Molecular Genetics, IRCCS Istituto Auxologico Italiano, Milan, Italy;* [4]*Artemisia Fetal-Maternal Medical Centre, Rome, Italy;* [5]*Genetics Department, RDI Rete Diagnostica Italiana, Padova, Italy;* [6]*SS Dipartimentale Genetica Azienda Osp. Universitaria Ospedale di Circolo e Fondazione Macchi, Varese, Italy;* [7]*Laboratorio Citotest, Consorzio GENiMED, Sarmeola di Rubano, Padova, Italy;* [8]*Servizio di Genetica, AO Istituti Ospitalieri, Cremona, Italy;* [9]*Biologia Generale e Genetica Medica, Universita' degli Studi di Pavia, Pavia, Italy;* [10]*U.O. Complessa di Genetica Clinica, Dipartimento di Pediatria, Università di Padova, Padova, Italy;* [11]*Dipartimento di Neuroscienze e Biotecnologie Mediche, Università di Milano-Bicocca, Monza, Italy;* [12]*Dipartimento di Genetica Medica, Ospedale Di Venere, ASL Bari, Italy;* [13]*UOSD di Genetica Medica, Centro per la Salute della Donna S.Anna, ASL Rome, Italy;* [14]*Medical Genetics, Department of Molecular Medicine, Sapienza University, San Camillo-Forlanini Hospital, Rome, Italy;* [15]*Laboratorio di Genetica, Ospedale Galliera, Genova, Italy;* [16]*Laboratory of Medical Genetics, Fondazione PTV-Policlinico Tor Vergata, Rome, Italy;* [17]*S.C. Laboratorio di Genetica Medica, IRCCS Burlo Garofalo, Trieste, Italy*

KEYWORDS: microarray; position statement; prenatal diagnosis; SIGU; ultrasound fetal abnormalities

ABSTRACT

A precise guideline establishing chromosomal microarray analysis (CMA) applications and platforms in the prenatal setting does not exist. The controversial question is whether CMA technologies can or should soon replace standard karyotyping in prenatal diagnostic practice. A review of the recent literature and survey of the knowledge and experience of all members of the Italian Society of Human Genetics (SIGU) Committee were carried out in order to propose recommendations for the use of CMA in prenatal testing. The analysis of datasets reported in the medical literature showed a considerable 6.4% incidence of pathogenic copy number variations (CNVs) in the group of pregnancies with sonographically detected fetal abnormalities and normal karyotype. The reported CNVs are likely to have a relevant role in terms of nosology for the fetus and in the assessment of reproductive risk for the couple. Estimation of the frequency of copy number variations of uncertain significance (VOUS) varied depending on the different CMA platforms used, ranging from 0–4%, obtained using targeted arrays, to 9–12%, obtained using high-resolution whole genome single nucleotide polymorphism (SNP) arrays. CMA analysis can be considered a second-tier diagnostic test to be used after standard karyotyping in selected groups of pregnancies, namely those with single (apparently isolated) or multiple ultrasound fetal abnormalities, those with de novo *chromosomal rearrangements, even if apparently balanced, and those with supernumerary marker chromosomes. Copyright © 2012 ISUOG. Published by John Wiley & Sons, Ltd.*

BACKGROUND

In the last few years chromosomal microarray analysis (CMA) technology (array comparative genomic hybridization, aCGH; single nucleotide polymorphism array, SNP array) has acquired increasing relevance, becoming a fundamental diagnostic tool in medical genetics. In fact, technological evolution and experimental optimization have resulted in a notable simplification of analytic protocols, leading to a decrease in costs and enabling the progressive spread of this technology in many laboratories all over the world. Encouraging results, in terms of detection rate, were obtained in patients affected by unexplained developmental delay/intellectual disability (DD/ID), autism spectrum disorders (ASD) or multiple congenital anomalies (MCA), in whom

Correspondence to: Dr A. Novelli, Istituto CSS Mendel, Viale Regina Margherita, 261, 00198 Roma, Italy (e-mail: a.novelli@css-mendel.it)

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the diagnostic yield was improved over that obtained by karyotyping by an estimated 10–20%[1–3]. Accurate evaluation of the gene content of the imbalanced genomic regions, together with 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 detection of critical regions related to known syndromes, allowing genotype–phenotype correlations in several cases. For such reasons, in 2010 the Italian Society of Human Genetics (SIGU) Committee proposed a national document in which, based on the literature and on the experience of all participating institutions, CMA was recommended as the first-tier diagnostic test in the postnatal setting for patients with DD/ID, ASD or MCA (http://www.sigu.net).

The advantages offered by CMA technology have opened up new avenues regarding its possible application in prenatal diagnosis, where traditional karyotyping is still considered the gold standard method for all indications for invasive testing. Compared with conventional karyotyping, CMA can rapidly detect imbalances with a resolution of up to a few Kb using standardized protocols[4].

LITERATURE REVIEW

A precise guideline establishing CMA applications and platforms in the prenatal setting does not exist and this situation has led to debates and controversies[5–11] concerning whether CMA technology can or should replace standard karyotyping in prenatal diagnostic practice. Considering the 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 prenatal diagnosis, it could potentially be more harmful than it is useful when applied during prenatal life, because of the unclear results it can provide. Current knowledge has gaps regarding the clinical interpretation of copy number variations (CNVs). This is because of the possibility of detecting an imbalance not previously described, the lack of knowledge about the function of many genes, our relatively poor understanding of gene–gene and gene–environment interactions, and the role of epigenetic modifications in modulating the penetrance and expressivity of CNVs[12–14]. There are additional questions related to the detection during the prenatal diagnostic period of variations of uncertain significance (VOUS), which have no known predictive value with regards to fetal and future health, and can thus cause increased parental anxiety[7,15]. In addition, the diagnostic yield of CMA in the prenatal setting has not been established clearly in all categories of indications because the majority of published papers included selected cases with fetal abnormalities detected by ultrasound and an apparently normal karyotype. In this group of pregnancies the CMA detection rate is, on average, 6.4% (range, 0–15.6%) (Table 1). Datasets reported in the medical literature clearly show a significant incidence of pathogenic CNVs in this group of pregnancies and these detected CNVs are likely to have a relevant role in terms of nosology for the fetus and for the assessment of reproductive risks for the couple[16–32]. In cases with sonographic fetal abnormalities, the sum of the detection rates of conventional cytogenetic analyses (28% for chorionic villi and 12% for amniotic fluid: ~20% on average)[33] and CMA (6.4%), i.e. combining the first-tier karyotype with the second-tier CMA, provide an overall detection of ~27%.

Frequencies of VOUS seem to be difficult to assess due to the different CMA platforms used in the various studies, and range from 0–4% when assessed by targeted arrays to ~9–12% when assessed by high resolution whole genome SNP arrays (Table 1)[16–32]. In contrast, the rate of detection of known, disability-causing pathogenic CNVs by CMA in all pregnant women has been estimated to be between 0.16% and 0.3%[6]. Analysis of the proportion of ambiguous findings compared to pathogenic CNVs shows that using CMA technology in the prenatal setting without a specific clinical indication is not justified at present.

Another important limitation related to the application of CMA as a first-tier test is represented by the impossibility of detecting balanced rearrangements i.e. those without genetic losses or gains. This would lead to underestimation 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 advantage of being able to detect long continuous stretches of homozygosity (LCSH), representing whole chromosomal 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, in which both chromosomes in a pair are inherited from one parent) 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 hand, CMA is useful to clarify abnormal karyotype results. In cases with supernumerary marker chromosomes, CMA can aid in their classification and characterization, improving the diagnostic accuracy and allowing specific genetic counseling to be offered to the couple[40–42]. The role of CMA prenatally in cases with *de novo* apparently balanced chromosomal rearrangements has not been studied extensively; however, in postnatal datasets of patients with *de novo* apparently balanced chromosomal rearrangements and an abnormal phenotype, CMA detects cryptic imbalances in 35–40% of samples with reciprocal translocations and in 72–75% of samples with complex rearrangements[43–45].

Table 1 Incidence of pathogenic variations and unclear results from published studies regarding use of chromosomal microarray analysis (CMA) in prenatal diagnosis

	Total prenatal population analyzed		*Cases with US abnormalities and normal karyotype*	
Study	n	*VOUS (n (%))*	n	*Pathogenic CNVs* (n (%))*
Le Caignec *et al.*[16]	49	1 (2.0)	49	4 (8.2)
Vialard *et al.*[18]	39	NR	37	4 (10.8)
Van den Veyver *et al.*[20]	300	3 (1)	84	5 (6)
Shaffer *et al.*[21]	151	1 (0.7)	110	2 (1.9)
Coppinger *et al.*[22]	213	1 (0.5)	155	6 (3.9)
Kleeman *et al.*[23]	50	1 (2)	50	0 (0)
Tyreman *et al.*[24]	106	13 (12.3)	106	10 (9.4)
Valduga *et al.*[25]	50	NR	50	5 (10)
Faas *et al.*[26]	38	3 (7.9)	32	3 (9.4)
Maya *et al.*[27]	269	0 (0)	102	2 (2)
Evangelidou *et al.*[28]	25	1 (4)	15	1 (6.6)
Gruchy *et al.*[29]	38	0 (0)	38	3 (7.9)
D'Amours *et al.*[30]	49	6 (12.2)	49	4 (8.2)
Zuffardi *et al.* (ISPD 2010)†	63	1 (1.6)	63	5 (9.5)
De Toffol *et al.*[31]	32	1 (3.1)	32	5 (15.6)
Leung *et al.*[32]	48	NR	48	6 (12.5)
Overall	1520	32 (2.3)	1020	65 (6.4)

*Pathogenic copy number variations (CNVs) detected by abnormal CMA results. †Oral communication. ISPD, International Society for Prenatal Diagnosis. NR, not recorded; US, ultrasound; VOUS, variation of unknown clinical significance.

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 the knowledge and experience of all members of the committee, we recommend the use of CMA in prenatal testing:

1) never as a substitute for conventional karyotyping;
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 sonographic fetal abnormalities;
   ii) *de novo* chromosomal rearrangements, even if apparently balanced, detected by standard karyotyping, to investigate the possible presence of cryptic imbalance(s) related to the structural chromosome abnormality;
   iii) supernumerary marker chromosomes in order to characterize their origin and genetic content.

In these groups of pregnancies we recommend the application of a genome-wide, and not a targeted, platform enriched with probes containing dosage-sensitive and disease-causing genes with an average spatial resolution of at least 250 Kb with calls in the backbone (the regions between known disease-causing regions) of at least 500 Kb. When an uncommon CNV is found, parental testing is needed to help in the interpretation of genotype–phenotype correlations.

Further data are needed on 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;
- one or more soft markers (e.g. choroid plexus cysts, intestinal hyperechogenicity, renal pyelectasis, single umbilical artery, echogenic cardiac foci);
- intrauterine growth restriction and/or amniotic fluid volume alteration without major structural abnormalities (e.g. 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 place of standard karyotyping.

CONCLUSIONS

Presently, CMA analysis can be considered a second-tier diagnostic test which can complement, but not replace, standard karyotyping in a selected group of pregnancies.

QUALITY ASSURANCE

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

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