SIGU Societa' Italiana di Genetica Umana

SIEOG SOCIETA' ITALIANA DI ECOGRAFIA OSTETRICA E GINECOLOGICA E METODOLOGIE BIOFISICHE

SEGRETERIA PERMANENTE E TESORERIA: Via dei Soldati, 25 ROMA – TEL/FAX 06 6808142 - Tel.06 6875119 - C/C postale N.20857009

SIGU – Italian Society of Human Genetics
SIEOG – Italian Society of Obstetric and Gynecologic Ultrasound and Biophysical Methods

Recommendations for the prenatal use of Chromosomal Microarray Analysis

Antonio Novelli[1+], Giuseppe Calì[2+], Francesca Forzano[3*], Anna Aprile[4], Mattia Gentile[5], Mario Lituania[6], Enrico Periti[7], Federico Prefumo[8], Georgios Rembouskos[9], Gioacchino Scarano[10], Andrea Sciarrone[11], Giuliana Simonazzi[12], Lorenzo Sinibaldi[13], Bruno Dallapiccola[14]

1 IRCCS Fondazione Casa Sollievo della Sofferenza, Laboratorio Mendel, San Giovanni Rotondo, Italy

2 ARNAS CIVICO, UOS Medicina Perinatale ed Assistenza alla Nascita Palermo

3 IRCCS Ospedali Galliera, SSD Genetica Medica Genova, Genova, Italy

4 Università degli studi di Padova, Dipartimento di Medicina Molecolare, Laboratorio di Bioetica Clinica

5 ASL di Bari, Ospedale di Venere, UOC Genetica Medica, Bari, Italia

6 IRCCS Ospedali Galliera, SSD Fisiopatologia Preconcezionale e Prenatale, Genova, Italy

7 Azienda Sanitaria di Firenze, SSD Diagnosi Prenatale, Dipartimento Materno Infantile, Firenze, Italy

8 Università di Brescia, Dipartimento di Ostetricia e Ginecologia, Italy

9 Ospedali Di Venere e Sarcone, Unità di Medicina Fetale, Bari, Italy

10 AORN "G. Rummo", UOC di Genetica Medica, Benevento, Italy

11 Azienda Ospedaliera Universitaria, Città della Salute e della Scienza, SSCVD di Ecografia e Diagnosi Prenatale, Torino

12 Policlinico Universitario Sant'Orsola-Malpighi, Università di Bologna, Dipartimento di Scienze Mediche e Chirurgiche, UO Ostetricia e Medicina dell'Età Prenatale

13 IRCCS Fondazione Casa Sollievo della Sofferenza, Laboratorio Mendel, San Giovanni Rotondo, Italy and UOSD Biologia Molecolare, Citogenetica, Citomorfologia E. e V., AUSL Viterbo, Complesso Ospedaliero di Belcolle, Italy

14 IRCCS Ospedale Pediatrico Bambino Gesù, Roma, Italy

[+] These authors equally contributed to this work.

[*] Corresponding author: francesca.forzano@galliera.it.

Introduction

Over the past few years, Chromosomal Microarray Analysis (CMA) [i.e. array-Comparative Genomic Hybridisation (CGH) and single nucleotide polymorphism (SNP)-array analyses] has been used along with karyotyping in postnatal diagnosis of chromosome anomalies, and at times has replaced standard karyotyping[1].

From several studies, dealing with the application of CMA in prenatal diagnosis, some issues related to the use of these techniques in this setting became evident. In particular, the lack of adequate clinical data [most of which are based on ultrasound (US) examinations], the limited time for reporting and the short time lasting from CMA results, and the parents' decision about the pregnancy showed to be extremely sensitive topics. The use of CMA in prenatal diagnosis is complex, especially when the results are of uncertain significance. Both technical aspects and psychological implications of prenatal CMA, have been widely discussed at international level, leading to controversial conclusions[2-9].

With the present document, the Italian Society of Human Genetics (SIGU) and the Italian Society of Obstetric and Gynaecologic Ultrasound and Biophysics Methodologies (SIEOG) are recommending a shared strategy for implementing the prenatal application of CMA.

A. Purpose and implementation of prenatal testing

Prenatal investigations have two main purposes:

- to screen for Down syndrome (DS), other chromosomal aneuploidies, and congenital developmental abnormalities;
- to diagnose specific disorders, such as chromosomal imbalances associated with fetal defects, US anomalies pinpointing to congenital anomalies, inheritable genetic disorders amenable to be prenatally investigated in case of positive family history.

The first group of indications includes standard tests offered to all pregnant woman, while the second group refers to targeted analyses performed only in pregnancies presenting with specific risk factors.

Several papers discussed the employ of prenatal CMA in a range of circumstances, including positive biochemical screening pointing to an increased risk of DS; women aged ≥ 35 years; maternal anxiety; need of a more accurate diagnosis following US and/or cytogenetic results not fully resolved by standard karyotyping. Comparison between these studies has intrinsic limitations, due to the differences among the platforms used, in terms of type (i.e. "*targeted*" platforms having a non-homogeneous resolution, higher in chromosome regions previously associated to specific disorders; "*whole genome*" platforms performing an uniform analysis of all chromosome regions) and/or resolution power[5]. In addition to these technical discrepancies, the non-unique interpretation of copy number variants (*CNVs*) pathogenicity in some genome discrete regions should be considered.

CNVs may be classified into five groups: benign; probably benign; of unknown significance (VOUS); possibly pathogenic; pathogenic. They may arise *de novo* or be maternally/paternally inherited.

Pathogenic CNVs can be associated to a substantial phenotypic variability and may be incompletely penetrant. Some CNVs are considered pathogenic or likely pathogenic for a clinical condition different from the tested indications (incidental findings). Therefore, when 'clinically significant' CNVs are mentioned in reports and papers, it is not always possible to discriminate and establish if they include also CNVs regarded as "possibly" pathogenic, although not consistent with the case under examination, which, in other contexts, could be interpreted in a different way[5]. In some cases it has been clearly stated that CNVs of "clinical relevance" also include "possibly pathogenic" CNVs[4].

Noteworthy clinical features are often incompletely expressed at prenatal evaluation, ascertained anomalies are often nonspecific or not fully defined, and, finally, their long term consequences cannot be always predicted. Taken together, these issues further complicate the CNVs classification into stringent categories.

With these major limitations in mind, a recent review[5] suggested that CMA could increase by approximately 1% the detection rate of genomic anomalies in samples analyzed for screening purposes (i.e. parental anxiety, maternal age ≥35, increased risk for DS based on screening), and up to 7% in pregnancies presenting with US anomalies[10-12]. The VOUS rate was variable ranging between 1% and 3%, mainly depending on the employed platform[9,13,14]. These results point to a disproportion between the potential diagnostic gain based on CMA and other drawbacks, such as the presence of VOUS, the complexity and cost of analysis, the problems related to data interpretation, and the need of an accurate post-test genetic counseling. So far, no Health Technology Assessment of prenatal CMA is available. As consequence, no cost-benefit ratio, nor wide scale economic sustainability of CMA for the National Health Service have been investigated.

B. Nuchal Translucency.

Separate considerations were drawn with regard to increased fetal nuchal translucency (NT). This US marker can be associated with chromosome aneuploidies, prompting NT evaluation in the combined/integrated screening test. On the other hand, NT increase often resolves spontaneously

and the pregnancy results in a chromosomally normal developed fetus[15]. However, NT increase can be associated with fetal anomalies, such as cardiac abnormalities, extracardiac malformations, some Mendelian disorders, and spontaneous fetal demise. For these reasons, a dedicated follow-up of these pregnancies is recommended. In the presence of US anomalies, the laboratory tests should be driven by the clinical suspicion.

Different studies proposed the use of CMA in the presence of an increased NT, even when NT is an isolated feature[13]. Available data, although potentially pointing to an increased detection rate of chromosome anomalies, do not offer a unique interpretation, because of the low number of investigated samples, and lack of discussion about associated fetal anomalies. Other studies did not clarified if isolated NT increase can be regarded as a fetal structural abnormality, or it just represents an inclusion criteria for CMA. Finally, the pregnancies outcome is unknown, with the exception of those lumped to the group of fetal anomalies[11,14,17].

A recent review of 18 studies[12] reported a CNVs incidence of 3.1% (95% C.I. 0.4-5.7) in fetuses with NT >3.5 mm. Since this figure is the lowest among the fetuses with structural anomalies, it is assumed that this US marker is not sufficiently specific. Huang *et al.*[17] recently confirmed the limited benefit of the use of CMA in fetuses with isolated NT. CMA did not identify any pathogenic CNV of clinical significance out of 215 chromosomally normal fetuses with an increased NT, in respect to a VOUS rate of 1.4%.

In conclusion, present data are not adequate nor a shared opinion does exist about the use of CMA in pregnancies with an isolated NT increase. Unfortunately, the quoted studies did not properly classified the different rates of NT increases, also including cases of cystic hygroma.

SIEOG and SIGU recommend that dedicated prenatal diagnostic trials or pilot screening programs are devised to ascertain clinical efficacy of CMA applied to pregnancies presenting with fetuses with isolated NT increase (stratified into distinct measurement intervals), and cystic hygroma.

Otherwise CMA should be reasonably offered in pregnancies presenting with NT significantly increased (i.e. >5.5 mm), which are at very high risk of abnormal outcome (≥ 70%) and may be associated with abnormalities detectable at a later follow-up[18,19].

The SIGU 2013 Guidelines for cytogenetic diagnosis recommended to store fetal DNA samples in all pregnancies presenting with an increased NT, undergoing invasive investigations, in order to make possible CMA analysis or other genetic testing at follow-up, if additional fetal abnormalities become manifest.

C. Ultrasound anomalies.

The detection of fetal anomalies on US shifts the pregnancy category from low (i.e. physiologic) risk to moderate or high risk, or even recognizes distinct pathological conditions in pregnancies originally classified at increased risk. Fetal anomalies can be isolated or multiple, can be the markers of a syndromic disorder, can be associated with significant early-onset growth restriction in the absence of major alterations of utero-placental Doppler velocimetry. The so-called soft markers are not regarded as significant anomalies[21, 22].

In the presence of fetal US anomalies, laboratory testing has diagnostic rather than screening purposes. Whenever a specific diagnosis is suspected, one or more targeted tests can be indicated. When a precise diagnosis is not possible, CMA may increase the ability to detect chromosome

imbalances up to 7% when compared to standard karyotyping[12,14,16,23]. This figure is even higher in the presence of multiple abnormalities (9.1%)[12].
Despite the availability of large case series[10], conclusive data on the rate of imbalances detectable using CMA are not available for the different types of fetal malformations. Therefore, at present a CMA application procedure linked to distinct anomalies detected by US does not yet exist.

D. Critical issues and management aspects.

Prenatal CMA presents various critical issues at the technical, clinical and psychosocial level. As reported before, the interpretation of the results can be difficult, in particular in the presence of VOUS, and because of unavailability of a shared definition of clinically significant CNVs. Indeed, almost all published results and databases refer to postnatal CMA series, driven by specific clinical indications. Accordingly, these are often selected cases, usually without complete clinical phenotyping[24,25]. Similarly, databases collecting healthy controls do not include clinical data of the enrolled subjects, making the results not completely reliable[26]. All these points represent limiting factors for the proper interpretation of prenatal findings.
Furthermore, many CNVs are associated to disorders displaying variable expression and incomplete penetrance, or late-onset diseases, raising prognostic doubts when diagnosed during fetal life. The identification of a variant inherited from an apparently healthy parent can destabilize health expectations and family balances, and prompts further investigations[9]. The impact of this unexpected information on parents is often strong and negative, as shown by a qualitative study where this experience was reported as "toxic"[27].

E. Analytic stage.

The strength of the diagnostic power, which is the major benefit of CMA, needs to be balanced with the critical issues described above, according to the principles of opportunity and proportionality.
Prenatal CMA currently lies in a grey area between research and diagnostic[28]. Available results and rapid technological developments do not recommend to favor any specific platform for prenatal testing. Major critical findings include VOUS, susceptibility variants, and variants associated with late-onset disorders, which need to be elucidated, and carefully handled[28]. For this reason, it is mandatory to select a filter for reading and interpreting CMA findings. There are different procedures, essentially including two main approaches:

- To reduce/restrict the platform resolution power (technical filter). In this case, an analytic system, allowing a high resolution examination of regions already known to be associated with distinct disorders, in which the resolution is lower for the rest of the genome ("*backbone*") is used[8,13,16]. This approach is referred to as "*targeted*" and can vary according to the resolution used by the laboratory. The major benefit of the "*targeted*" approach is the exclusion of many potentially identifiable VOUS.
- To enroll a panel of experts (*expert review panel*), not directly involved in the clinical management of the case, to evaluate the CMA results and select the variants that should be indicated in the medical report ("interpretative filter"). The panel can be central (single

laboratory)[4] or national, as in Belgium[29] or UK with the EACH project. The expert review panel approach has apparently a larger flexibility; however, it can be biased by individual interpretations, variable according to the experts panel composition. In addition, the difficulties in managing such an organization must be also considered. These two approaches can be used in combination[4].

The choice of the platform and its resolution is a critical point. The strategy should address the clinical question and the proportionality among utility, uncertainty and complexity of the results.

If CMA is used for characterizing a *de novo* rearrangement identified through karyotyping (i.e. a supernumerary chromosome marker, a reciprocal translocation), the resolution level should be selected case by case by the laboratory geneticist.

Based on the available data, in other instances the use of targeted platforms with a backbone resolution not exceeding 500 kb seems appropriate. This threshold represents an adequate compromise for identifying the most clinically relevant CNVs and reduce the number of VOUS[11,30,31].

F. Other technical and operational aspects.

The laboratory providing prenatal CMA should guarantee the completion of the diagnostic process within the analytical stage.

At present, it is debated which is the most suitable sample (if any) for prenatal CMA between trophoblasts and amniocytes, and what might be the best DNA extraction approach (directly from tissue samples vs. cultured cells). Potential limitations include sample quality and possible placental mosaicisms. A collaborative effort aiming at collecting this information is highly recommended.

CMA results always need to be confirmed by means of other molecular techniques. Parental blood samples should be available, since they are often necessary for interpreting fetal results[31].

Since parental analysis can detect additional CNVs to those present in the fetal specimen, informed consent must be obtained independently from each parent, to define which results they want to know (i.e. only information about fetal CNVs, information about all the relevant variants, etc.), and separate reports must be prepared for each tested subject.

At the time of writing, a European system for external quality control of array-CGH in postnatal analysis is available (CEQAS: Cytogenetic External Quality Assessment Service), while a similar system for the prenatal diagnosis does not exist yet. We propose that each laboratory running prenatal CMA participate to the quality control for the use of array-CGH in postnatal analysis.

We recommend also implementing national studies for investigating the diagnostic use of CMA, with particular reference to clinical utility, cost-benefit analysis, population impact, including the psychological impact and the weight of decision-making for parents.

Recommendations

1. Prenatal CMA should not be offered as a first-tier test, replacing standard karyotyping. Maternal anxiety, advanced maternal age, increased risk of chromosomal aneuploidies based on combined or biochemical screening, and presence of US soft markers, are not indications to perform CMA.
2. Present evidence does not support the clinical utility of CMA in chromosomally normal fetuses with an isolated NT increase. In these cases, CMA can be proposed only within trials approved by an Ethical Committee, aiming to assess the clinical utility of this indication. At present, CMA should be considered in pregnancies presenting with significantly increased NT.
3. CMA analysis could be indicated as a second-tier test in fetuses presenting with an ultrasound anomaly (not including the so-called soft markers), after a multidisciplinary evaluation by a clinical geneticist and an obstetrician with extensive experience in prenatal medicine. This analysis should be carried out by an experienced laboratory. The testing indication should be based on the evaluation of different aspects, including clinical history, available scientific data, risk related to the sampling procedure, gestational age, utility of analysis, parental decision following information on CMA characteristics.
4. The complexity of prenatal CMA management, result interpretation and communication, recommends its careful and thoughtful use. This analysis should always be considered "possible" and not "necessary". Its integration into the prenatal diagnostic plan should be managed only by a centre with adequate experience, where a multidisciplinary team (obstetrician, clinical geneticist, laboratory geneticist) is operating and undertakes the full management of both pregnancy and parents. Psychological support to the couple shall be available within the same team.
5. At present, with only a few notable exceptions, a national plan for implementing an "expert review panel" approach, is not achievable. Therefore, the use of a "technical" filter by means of a targeted analysis, with a backbone resolution lower than that used in postnatal diagnosis, appears as a better choice. The backbone resolution has to be less than 500 kb, with the exception of analyses aiming at characterizing *de novo* chromosome rearrangements detected by standard karyotyping. In these instances, the resolution should be chosen conveniently and findings evaluated by a multidisciplinary team. The decision of reporting non clearly pathogenic variants, not related to the relevant anomaly, should be taken in agreement with the parents, who have previously given their informed consent.
6. Laboratories providing prenatal CMA should be part of an integrated pathway of care, within the framework of a multidisciplinary team, including an obstetrician, a clinical geneticist with expertise in fetal medicine, and a laboratory geneticist expert in CMA. These laboratories must fulfill the following requisites: to complete the diagnostic procedure in the analytic phase; to confirm the results by using other molecular techniques; when indicated, to compare fetal and parental DNAs. Laboratories should also participate in the currently available quality control systems and in those hopefully implemented for prenatal analysis.

REFERENCES

1. Miller DT, Adam MP, Aradhya S, Biesecker LG, Brothman AR, Carter NP, Church DM, Crolla JA, Eichler EE, Epstein CJ, Faucett WA, Feuk L, Friedman JM, Hamosh A, Jackson L, Kaminsky EB, Kok K, Krantz ID, Kuhn RM, Lee C, Ostell JM, Rosenberg C, Scherer SW, Spinner NB, Stavropoulos DJ, Tepperberg JH, Thorland EC, Vermeesch JR, Waggoner DJ, Watson MS, Martin CL, Ledbetter DH. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *Am J Hum Genet* 2010; **86**: 749-764.

2. Novelli A, Grati FR, Ballarati L, Bernardini L, Bizzoco D, Camurri L, Casalone R, Cardarelli L, Cavalli P, Ciccone R, Clementi M, Dalprà L, Gentile M, Gelli G, Grammatico P, Malacarne M, Nardone AM, Pecile V, Simoni G, Zuffardi O, Giardino D. Microarray application in prenatal diagnosis: a position statement from the cytogenetics working group of the Italian Society of Human Genetics (SIGU), November 2011. *Ultrasound Obstet Gynecol* 2012; **39**: 384-388.

3. Vetro A, Bouman K, Hastings R, McMullan DJ, Vermeesch JR, Miller K, Sikkema-Raddatz B, Ledbetter DH, Zuffardi O, van Ravenswaaij-Arts CM. The introduction of arrays in prenatal diagnosis: a special challenge. *Hum Mutat* 2012; **33**: 923-929.

4. Wapner RJ, Martin CL, Levy B, Ballif BC, Eng CM, Zachary JM, Savage M, Platt LD, Saltzman D, Grobman WA, Klugman S, Scholl T, Simpson JL, McCall K, Aggarwal VS, Bunke B, Nahum O, Patel A, Lamb AN, Thom EA, Beaudet AL, Ledbetter DH, Shaffer LG, Jackson L. Chromosomal microarray versus karyotyping for prenatal diagnosis. *N Engl J Med* 2012; **367**: 2175-2184.

5. Callaway JL1, Shaffer LG, Chitty LS, Rosenfeld JA, Crolla JA. The clinical utility of microarray technologies applied to prenatal cytogenetics in the presence of a normal conventional karyotype: a review of the literature. *Prenat Diagn* 2013; **33**: 1119-1123.

6. Evangelidou P, Alexandrou A, Moutafi M, Ioannides M, Antoniou P, Koumbaris G, Kallikas I, Velissariou V, Sismani C, Patsalis PC. Implementation of high resolution whole genome array CGH in the prenatal clinical setting: advantages, challenges, and review of the literature. *Biomed Res Int* 2013; 2013: e346762.

7. Ganesamoorthy D, Bruno DL, McGillivray G, Norris F, White SM, Adroub S, Amor DJ, Yeung A, Oertel R, Pertile MD, Ngo C, Arvaj AR, Walker S, Charan P, Palma-Dias R, Woodrow N, Slater HR. Meeting the challenge of interpreting high-resolution single nucleotide polymorphism array data in prenatal diagnosis: does increased diagnostic power outweigh the dilemma of rare variants? *BJOG* 2013; **120**: 594-606.

8. Crolla JA, Wapner R, Van Lith JM. Controversies in prenatal diagnosis 3: should everyone undergoing invasive testing have a microarray? *Prenat Diagn* 2014; **34**: 18-22.

9. Klugman S, Suskin B, Spencer BL, Dar P, Bajaj K, Powers J, Reichling J, Wasserman D, Dolan SM, Merkatz IR. Clinical utility of chromosomal microarray analysis in prenatal diagnosis: report of first 6 months in clinical practice. *J Matern Fetal Neonatal Med* 2014; **27**: 1333-1338.

10. Shaffer LG, Dabell MP, Fisher AJ, Coppinger J, Bandholz AM, Ellison JW, Ravnan JB, Torchia BS, Ballif BC, Rosenfeld JA. Experience with microarray-based comparative genomic hybridization for prenatal diagnosis in over 5000 pregnancies. *Prenat Diagn* 2012; **32**: 976-985.

11. Shaffer LG, Dabell MP, Rosenfeld JA, Neill NJ, Ballif BC, Coppinger J, Diwan NR, Chong K, Shohat M, Chitayat D. Referral patterns for microarray testing in prenatal diagnosis. *Prenat Diagn* 2012; **32**: 344-350.

12. de Wit MC, Srebniak MI, Govaerts LC, Van Opstal D, Galjaard RJ, Go AT. Additional value of prenatal genomic array testing in fetuses with isolated structural ultrasound abnormalities and a normal karyotype: a systematic review of the literature. *Ultrasound Obstet Gynecol* 2014; **43**: 139-146.

13. Hillman SC, McMullan DJ, Hall G, Togneri FS, James N, Maher EJ, Meller CH, Williams D, Wapner RJ, Maher ER, Kilby MD. Use of prenatal chromosomal microarray: prospective cohort study and systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 2013; **41**: 610-620.

14. Brady PD, Delle Chiaie B, Christenhusz G, Dierickx K, Van Den Bogaert K, Menten B, Janssens S, Defoort P, Roets E, Sleurs E, Keymolen K, De Catte L, Deprest J, de Ravel T, Van Esch H, Fryns JP, Devriendt K, Vermeesch JR. A prospective study of the clinical utility of prenatal chromosomal microarray analysis in fetuses with ultrasound abnormalities and an exploration of a framework for reporting unclassified variants and risk factors. *Genet Med* 2014; **16**: 469-476.

15. Bilardo CM, Timmerman E, Pajkrt E, van Maarle M. Increased nuchal translucency in euploid fetuses - what should we be telling the parents? *Prenat Diagn* 2010; **30**: 93-102.

16. Ahn JW, Bint S, Irving MD, Kyle PM, Akolekar R, Mohammed SN, Mackie Ogilvie M. 2014 A new direction for prenatal chromosome microarray testing: software-targeting for detection of clinically significant chromosome imbalance without equivocal findings. *Peer J* 2014; **22**: 2.e354.

17. Huang J, Poon LC, Akolekar R, Choy KW, Leung TY, Nicolaides KH. Is high fetal nuchal translucency associated with submicroscopic chromosomal abnormalities on array CGH? *Ultrasound Obstet Gynecol* 2014; **43**: 620-624.

18. Souka AP, von Kaisenberg CS, Hyett JA, Sonek JD, Nicolaides KH. Increased nuchal translucency with normal karyotype. *Am J Obstet Gynecol* 2005; **192**: 1005–1021.

19. Bilardo CM, Müller MA, Pajkrt E, Clur SA, van Zalen MM, Bijlsma EK. Increased nuchal translucency thickness and normal karyotype: time for parental reassurance. Ultrasound Obstet Gynecol 2007; **30**: 11-18.

20. SIGU.NET: SIGU (Italian Society of Human Genetics) Diagnostic Cytogenetics Guidelines. Constitutional Cytogenetics Procedural notes, 2013. Available from www.sigu.net/index.php?option=com_docman&task=cat_view&gid=57&Itemid=85.

21. FNCO.IT: SIEOG (Italian Society of Obstetric and Gynecologic Ultrasound and Biophysical Methods) Guidelines, 2010. Available from www.fnco.it/news/linee-guida-sieog-societa-italiana-di-ecografia-ostetrico-g.htm.

22. FETALANOMALY.SCREENING.NHS.UK: NHS Fetal Anomaly Screening Programme, 4.3 Normal variant screening (formerly known as soft markers), 2010. Available from http://fetalanomaly.screening.nhs.uk/fetalanomalyresource/images/stories/Downloads/2.4.3_Normal_variants/normal_variant_statement_%20final_2010.pdf.

23. Shaffer LG, Rosenfeld JA, Dabell MP, Coppinger J, Bandholz AM, Ellison JW, Ravnan JB, Torchia BS, Ballif BC, Fisher AJ. Detection rates of clinically significant genomic alterations by microarray analysis for specific anomalies detected by ultrasound. *Prenat Diagn* 2012; **32**: 986-995.

24. Riggs ER, Jackson L, Miller DT, Van Vooren S. Phenotypic information in genomic variant databases enhances clinical care and research: the International Standards for Cytogenomic Arrays Consortium experience. *Hum Mutat* 2012; **33**: 787-796.

25. Riggs ER, Wain KE, Riethmaier D, Savage M, Smith-Packard B, Kaminsky EB, Rehm HL, Martin CL, Ledbetter DH, Faucett WA. Towards a Universal Clinical Genomics Database: the 2012 International Standards for Cytogenomic Arrays Consortium Meeting. *Hum Mutat* 2013; **34**: 915-919.

26. Duclos A, Charbonnier F, Chambon P, Latouche JB, Blavier A, Redon R, Frébourg T, Flaman JM. Pitfalls in the use of DGV for CNV interpretation. *Am J Med Genet* 2011; **155A**: 2593-2596.

27. Bernhardt BA, Soucier D, Hanson K, Savage MS, Jackson L, Wapner RJ. Women's experiences receiving abnormal prenatal chromosomal microarray testing results. *Genet Med* 2013; **15:** 139-145.

28. de Jong A, Dondorp WJ, Macville MV, de Die-Smulders CE, van Lith JM, de Wert GM. Microarrays as a diagnostic tool in prenatal screening strategies: ethical reflection. *Hum Genet* 2014; **133**: 163-172.

29. Vanakker O, Vilain C, Janssens K, Van der Aa N, Smits G, Bandelier C, Blaumeiser B, Bulk S, Caberg JH, De Leener A, De Rademaeker M, de Ravel T, Desir J, Destree A, Dheedene A,

Gaillez S, Grisart B, Hellin AC, Janssens S, Keymolen K, Menten B, Pichon B, Ravoet M, Revencu N, Rombout S, Staessens C, Van Den Bogaert A, Van Den Bogaert K, Vermeesch JR, Kooy F, Sznajer Y, Devriendt K. Implementation of genomic arrays in prenatal diagnosis: The Belgian approach to meet the challenges. *Eur J Med Genet* 2014; **57**: 151-156.

30. Cooper GM, Coe BP, Girirajan S, Rosenfeld JA, Vu TH, Baker C, Williams C, Stalker H, Hamid R, Hannig V, Abdel-Hamid H, Bader P, McCracken E, Niyazov D, Leppig K, Thiese H, Hummel M, Alexander N, Gorski J, Kussmann J, Shashi V, Johnson K, Rehder C, Ballif BC, Shaffer LG, Eichler EE. A copy number variation morbidity map of developmental delay. *Nat Genet* 2011; **43**: 838-846.

31. Rooryck C, Toutain J, Cailley D, Bouron J, Horovitz J, Lacombe D, Arveiler B, Saura R Prenatal diagnosis using array-CGH: A French experience. *Eur J Med Genet* 2013; **56**: 341-345.

APPENDIX

INFORMATION SHEET FOR PRENATAL CHROMOSOMAL MICROARRAYS ANALYSIS

What is a chromosomal microarrays analysis? What can be detected by this test?

Chromosomal Microarrays Analysis (CMA) technique allows a deep and simultaneous investigation of all chromosomes. In this way very small chromosomal alterations, overlooked by conventional karyotype, can be detected. Since this technique presents some limitations, its prenatal application should be carefully evaluated.

What are the indications to CMA?

The most appropriate and frequent indications to prenatal CMA include the following:

1. deeper characterization of some fetal chromosomal anomalies;
2. the detection of fetal structural anomalies on ultrasound;
3. early-onset fetal growth restriction of unclear origin.

What are the limits of this diagnostic tool?

CMA, similarly to other diagnostic techniques, has limitations. In particular, a number of anomalies cannot be resolved:

1. balanced chromosomal rearrangements (i.e. reciprocal translocations, inversions);
2. low-grade chromosome mosaicisms (<30%);
3. chromosomal variants/anomalies not detected by the microarray platform used by the laboratory;
4. inherited disorders not caused by chromosome duplication/deletions.

Maternal contamination (co-existence of maternal and fetal cells in the sample) can invalidate the CMA results. In some cases it is necessary to exclude this contamination, by means of molecular testing of fetal and maternal DNAs.

In the presence of chromosomal imbalances, additional analyses can be necessary for characterizing the rearrangement(s); analysis of parental DNA can be utilized for interpreting the results. For this reason, parental samples must be always collected together with fetal samples, and investigated only when the comparison between fetal and parental genetic profiles is required. In these cases, a longer time is usually needed to achieve a conclusive diagnosis.

In rare instances, these investigations reveal that fetal DNA does not match with parental DNA (i.e. in the case of heterologous insemination or non-paternity). Wrong information provided to clinicians about the biological parents could prevent a correct test interpretation.

Is the interpretation of CMA findings always clear?

At times, the evaluation of the results can be difficult, in particular in the presence of variants (so-called Copy Number Variants, CNVs) which not always allow a rapid and simple interpretation. These variants include:

- ✓ rare/uncommon CNVs, often referred to as VOUS (Variations Of Uncertain Significance). Current knowledge is inadequate to conclude if they are benign or potentially associated with distinct disorders.
- ✓ CNVs of pathogenic significance, whose relationship with the condition under investigation is uncertain;
- ✓ CNVs associated to disorders with variable expression and/or incomplete penetrance (i.e. the disorder associated to the variation could not become manifest during the lifetime or have a variable and unpredictable severity), or conferring susceptibility to severe disorders;
- ✓ CNVs with clinical implications different from those that have prompted the analysis (i.e. late-onset disorders, susceptibility to cancer, being carrier of a recessive disorder, etc.), which at times can be inherited.

What are the technical characteristics of this test?

In order to reduce the possibility of identifying variants of uncertain significance, the test will be performed by using some filters, allowing to detect mainly the unbalanced regions responsible for microdeletion/microduplication disorders, and/or including pathogenic genes. The resolution in these critical regions is at about 100-200 Kb, while other regions are analyzed with a 500 Kb filter.

When will the results be available?

Usually, the results will be available 10 days after fetal DNA extraction (cell cultures, chorionic villous sampling, amniocytes) and will be made available to parents during a genetic counseling session. In specific cases, a longer time may be needed.

CONSENT FORM
for Chromosomal Microarray Analysis on fetal samples

The undersigned Mr./Ms._____,
place and date of birth_____,
and the undersigned Mr./Ms._____,
place and date of birth_____,
acting as_____

DECLARE

- to have discussed during a genetic consultation with Dr._____ about the characteristics, potentialities and limits of the prenatal test performed with microarray technique;
- to have had the opportunity to ask questions;
- to have received clear and complete answers;
-to consider all the received information as adequate and complete (in accordance with the INFORMATION SHEET FOR PRENATAL CHROMOSOMAL MICROARRAY ANALYSIS attached to this form)

Hence, on the base of the information received, I/we

□ *Consent* □ *Do not consent*

To perform the test and to take a sample of my/our peripheral blood (for eventual additional analyses).

I/we declare that we desire to be informed about:
□ all detected variants-CNV
□ only the variants-CNV with clear pathogenic significance linked to the indications for the test
□ all the variants-CNV with clear pathogenic significance regardless of the indications for the test
□ _____ (Please indicate specific desires)

In addition, I/we:

□ give □ do not give permission to use sampled biological material for research in anonymized form

□ give □ do not give permission to inform Dr._____ about test results

I/we declare that the details provided above are true and I/we guarantee to promptly communicate any possible decision change on this matter.

Signature _____ Signature _____

I/we □ *Consent* □ *Do not consent*
the use of personal, sensitive and genetic data according to the Italian current Law 196/2003, and the conservation of the residual biological material for one calendar year from the sampling date.

Signature _____ Signature _____

Signature and stamp of the consultant
who completed this consent form _____

Date_____