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Do we really need a screening test for open spina bifida?

In a recent issue of the Journal were published a series of articles[1–4] and an Opinion[5] regarding possible approaches to early diagnosis of open spina bifida (SB) by way of a screening examination of the fetal brain posterior fossa, specifically the cerebral fourth ventricle, as imaged as an intracranial translucency (IT) between the brain stem and choroid plexus. Each of the studies applied a slightly different imaging approach and varying biometry to the target area, with the aim of flagging up suspected cases or ruling out SB during the late first trimester of pregnancy.

The WHO defines certain criteria for screening tests[6]. The disease must be of sufficient prevalence and severity and should have a fixed spectrum of symptoms, the screening method should be simple and acceptable, screening should be accurate, confirmation and follow-up should be available, the disease should be treatable and screening should show improved outcome and a positive cost/benefit ratio. First and foremost, therefore, a screening test is performed because the definitive diagnostic test is too invasive, too expensive, too difficult technically, or otherwise unacceptable or unavailable to the general population. Screening acts as a 'sieve' (hence its name): screen-positive cases are caught in the sieve and are referred for further examination, i.e. either a definitive diagnostic test or more invasive, expensive or technically challenging screening.

Clearly, prenatal diagnosis of SB as an entity fulfills the criteria of a target lesion for a screening test. SB prevalence is approximately 1 : 2000 and each case represents a significant cost to society in long-term care; it is treatable, i.e. avoidable, by pregnancy termination, or early treatment *in-utero* may ameliorate its damage. However, intrauterine repair of SB is performed only during the second half of the second trimester, at 23–26 weeks of gestation[7,8]. Thus, whether the lesion was diagnosed in the first or early second trimester is irrelevant. The advantage of early diagnosis is only in earlier provision of pregnancy termination, when this is the parents' wish.

Does screening for fetal IT, as an indicator for SB, fulfill the criteria of screening tests? Is it easier, more accurate or more acceptable to patients than is direct ultrasound scanning of the fetal spine at 11–13 weeks' gestation for diagnosis of SB? The authors of the various papers that appeared in the Journal did not show that their scanning method increased screening performance, was easier to perform or was more acceptable to patients compared with direct visualization of the fetal spine. What advantage, then, does the method confer? According to the authors, five or seven specified reference points must be imaged to visualize satisfactorily the IT, and all authors reported low success rates for satisfactory imaging of IT as well as low sensitivity of the test. No comparison was made with transvaginal imaging of the spine. It would seem that if image quality is sufficient to allow for IT imaging and measurement, direct imaging of the fetal spine would be possible, whether by transabdominal or transvaginal route. At these gestational ages, with the rapid changes of fetal position, or with simple bimanual manipulation, the whole spine can be visualized and SB or meningomyelocele can be diagnosed.

In our center we have been performing late first- and early second-trimester targeted fetal organ scanning by the transvaginal route for about 20 years. Examination of the fetal spine is of course an integral part of this examination. During this period we have scanned about 15 000 fetuses and diagnosed about 20 cases of open SB, while we have not missed any; since this anomaly is subject to national registration, and because of the medicolegal climate here, we would be informed promptly if an undiagnosed case were to be delivered, whether in our hospital or elsewhere.

In their recent Opinion[5] Profs Chaoui and Nicolaides claim that the transvaginal approach is not acceptable for screening of SB. However, in the pyramid of prenatal diagnosis put forward by Nicolaides[9], the first-trimester screening test includes transvaginal ultrasound to evaluate cervical length, and transvaginal ultrasound has also long been applied to uterine artery pulsatility index measurement. An overwhelming majority of women agree to undergo transvaginal ultrasound examination. We maintain that if women agree to transvaginal sonography for cervical length or uterine artery Doppler measurement, they will surely agree to it to exclude open SB.

The early diagnosis of open SB can be achieved confidently by transvaginal scanning of the fetal spine as part of the 11–13-week scan, after measurement of uterine artery Doppler flow and cervical length measurement. A separate screening test for a marker for open SB is not necessary.

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References

1. Chaoui R, Benoit B, Heling KS, Kagan KO, Pietzsch V, Sarut Lopez A, Tekesin I, Karl K. Prospective detection of open spina

bifida at 11–13 weeks by assessing intracranial translucency and posterior brain. *Ultrasound Obstet Gynecol* 2011; **38**: 722–726.
2. Fong KW, Toi A, Okun N, Al-Shami E, Menezes RJ. Retrospective review of diagnostic performance of intracranial translucency in detection of open spina bifida at the 11–13-week scan. *Ultrasound Obstet Gynecol* 2011; **38**: 630–634.
3. Mangione R, Lelong N, Fontanges M, Amat S, Rosenblatt J, Khoshnood B, Jouannic JM. Visualization of intracranial translucency at the 11–13-week scan is improved after specific training. *Ultrasound Obstet Gynecol* 2011; **38**: 635–639.
4. Scheier M, Lachmann R, Petros M, Nicolaides KH. Three-dimensional sonography of the posterior fossa in fetuses with open spina bifida at 11–13 weeks' gestation. *Ultrasound Obstet Gynecol* 2011; **38**: 625–629.
5. Chaoui R, Nicolaides KH. Detecting open spina bifida at the 11–13-week scan by assessing intracranial translucency and the posterior brain region: mid-sagittal or axial plane? *Ultrasound Obstet Gynecol* 2011; **38**: 609–612.
6. Wilson JMG, Jungner G. Principles and practice of screening for disease. *WHO Chronicle*. World Health Organization: Geneva, 1968; pp.26–27.
7. Bruner JP, Tulipan N, Reed G, Davis GH, Bennett K, Luker KS, Dabrowiak ME. Intrauterine repair of spina bifida: preoperative predictors of shunt-dependent hydrocephalus. *Am J Obstet Gynecol* 2004; **190**: 1305–1312.
8. Fichter MA, Dornseifer U, Henke J, Schneider KT, Kovacs L, Biemer E, Bruner J, Adzick NS, Harrison MR, Papadopulos NA. Fetal spina bifida repair–current trends and prospects of intrauterine neurosurgery. *Fetal Diagn Ther* 2008; **23**: 271–286.
9. Nicolaides KH. A model for a new pyramid of prenatal care based on the 11 to 13 weeks' assessment. *Prenat Diagn*; **31**: 3–6.

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

We read with interest the paper by Novelli *et al.*[1], concerning the use of chromosomal microarray analysis (CMA) in prenatal diagnosis. The authors recommended that it should be offered only after standard karyotyping, as a second-line test for selected groups of high-risk pregnancies.

Recently, numerous independent prospective studies[2–7], involving the use of various strategies and validated with several different array platforms, have demonstrated the effectiveness and usefulness of CMA in clinical prenatal diagnosis. In a large-scale prospective study headed by our center[6], the average improvement in detection rate using CMA compared with traditional karyotyping was 0.9%. Other similar large-cohort prospective studies have been published[4,5,7], all reporting results concordant with our and previous findings. The combined experience from the prospective analysis of a cohort of over 10 000 prenatal samples[2–7] (12 000 including the updated results from our study), with parallel processing for both CMA and conventional cytogenetic analysis, indicates that the use of CMA in prenatal diagnosis produces a substantial improvement, of ~1–3%, in the detection rate of pathogenic chromosomal abnormalities compared with conventional karyotyping. In our opinion, there is now no doubt that CMA markedly enhances the detection of fetal chromosomal aberrations, both when it is performed for any clinical indication and when it is performed because congenital malformations are noted on ultrasound investigation[2–7].

Novelli *et al.*, in their review of the recent literature, omitted to cite three important large-scale prospective studies[4–6] that were listed in PubMed and other similar databases at the time of submission of their manuscript. A recent prospective study[7] also provides a considerable amount of additional clinical data. The conclusions reached by Novelli *et al.* should therefore be considered flawed. These missing data, totaling over 9000 prenatal samples, represent nine times the amount of combined data included in their paper. Had the authors also considered the above papers, they would have found an answer to most of their questions and concerns, especially regarding the diagnostic yield of CMA in different categories of indications.

Novelli *et al.* stated that their position was based on review of the recent literature and the knowledge and experiences of the members of the SIGU Committee[1]. Considering the number of papers which provide substantial evidence for the feasibility of introducing CMA into routine prenatal practice as a first-line diagnostic test[5–11], we would be interested to learn more about the Committee's knowledge and experience particularly concerning large-scale prospective clinical trials which might contradict the findings of the recent literature. It is also curious that one of the authors of the position statement[1] has recently expressed herself as being strongly in favor of substituting traditional karyotyping with CMA[11].

Our view in this discussion is firmly that CMA should be used as a first-line test, since offering CMA only as a second-line test in high-risk pregnancies may substantially limit the diagnostic potential of this assay, missing pathological copy number variations (CNV). The most relevant point in relation to this argument relates to the nine (0.9%) fetal conditions detected in the preliminary results of our still ongoing large-scale prospective trial[6] that would have remained undiagnosed if only conventional karyotyping had been performed. More importantly, four (0.4%) of these findings would have been overlooked following the diagnostic strategy of using CMA as a second-line test only after standard karyotyping, as suggested by Novelli *et al.*[1]. The latest data from our trial continue to show a similar outcome, with 22/2500 (0.9%) submicroscopic chromosomal abnormalities that would have been missed, 15 (0.6%) of which would still have been missed using the above proposed strategy. Similar results have also been reported by other independent large-scale prospective studies[4,5,7].

In our opinion, this technology should also be available to all pregnant women undergoing invasive prenatal testing, regardless of risk factors. The updated results of our study speak clearly in favor of such a testing paradigm, showing that the use of aCGH gives an

increased detection rate regardless of the indication for analysis. This became especially evident when examining the data from high-risk groups, in which the detection rate was elevated to 6.5% (7/107), but also in groups with *a priori* low risk for detection of submicroscopic chromosomal abnormalities, such as advanced maternal age (7/958, 0.7%) and parental anxiety (8/1355, 0.6%) groups (unpubl. data). Lee *et al.*[7] found the average improvement in detection rate in advanced maternal age and parental anxiety groups to be 0.5% (11/1911) and 0.7% (7/973), respectively. Armengol *et al.*[5] recently reported similar results.

Novelli *et al.*[1] also raised concerns about the potential detection by CMA of mild or unpredictable phenotypes and variations of unknown clinical significance (VOUS). We agree with them that the current challenge in the application of CMA in routine prenatal diagnostic practice is minimizing the potential to detect VOUS whilst maximizing the detection of pathogenic CNVs. However, it is well known that the differences in proportions of VOUS detected is related mainly to the array platform used and its resolution. In our study[6], we carefully selected a platform specifically developed for prenatal application, with a balance between increased resolution in locations of known constitutional disorders and less coverage in polymorphic regions. This platform allowed us to detect a single VOUS occurrence out of 1037 samples. In a recent systematic review[2], VOUS were reported to occur in $\sim$1% of prenatal samples. Combining the above data, the average probability of detecting such findings in prenatal samples can be estimated at around 0.3%, similar to the value (5/3171, 0.2%) reported by Lee *et al.*[7] in their large-scale prospective study. It is also well known that the frequency of detection of VOUS increases if parental samples are not available. This was the case in several papers included in the manuscript of Novelli *et al.* for calculation of the incidence of VOUS.

The point to debate is whether we are able or willing to deal with such a level of VOUS detection. We believe this $\sim$0.3% rate of finding a VOUS does not differ dramatically from that observed with cytogenetic karyotype analysis, with which findings of unclear significance or with unclear clinical consequences are occasionally encountered. Genetic counselors are also familiar with the problem related to CNVs of incomplete penetrance and variable expressivity, and preliminary guidelines are already available[12]. VOUS identified by prenatal CMA might be approached in a similar manner and managed by providing patients with thorough pre- and post-test counseling[13], keeping the family fully informed and remaining respectful of patients' autonomy.

Although detection of VOUS cannot be excluded completely with CMA, the growing clinical experience with genome-wide arrays and the increasing availability, sophistication and size of CNV databases for both healthy and affected individuals, together with parental analysis, allow classification and interpretation of most alterations, thus minimizing the number of VOUS detected and making CMA increasingly applicable to clinical prenatal diagnostic practice.

We now move on to other aspects mentioned in the position statement of Novelli *et al.*[1], in which a strategy is recommended for prenatal diagnosis involving conventional karyotyping with CMA as a second-line test in high-risk pregnancies. It is evident that by using such a strategy patients would obtain their results more than 3 weeks after initial testing. The rapid confirmation of prenatal findings is essential for purposes of best-practice clinical management. The logic of this approach is therefore questionable since, if CMA is performed and reported to patients only after results from conventional karyotyping are available, instead of simply offering CMA as first-line test, patient anxiety is prolonged.

We should also bear in mind the further disadvantages of conventional karyotyping in routine prenatal diagnosis: the resolution limits of conventional cytogenetic analysis, the requirement for cell culture and the possibility of culture failure or contamination; the variable quality of chromosomal preparations; the need for subjective interpretation which requires skilled and experienced cytogeneticists to carry out the analysis; the labor-intensive nature of karyotyping which limits the possibility for high-throughput automated analysis.

Novelli *et al.*[1] also stated that CMA is unable to detect mosaicism with rates of lower than 30% (low-level mosaicism, LLM). On the contrary, several studies performed in postnatal samples have demonstrated that CMA may detect mosaicism with rates as low as 8–10%[14–16]. In our study[6], we were able to detect LLM at rates of 10% in prenatal samples.

Novelli *et al.*[1] also focused on the risk of phenotypic consequence related to the impossibility of CMA detecting *de novo* balanced chromosomal rearrangements. Although we agree with this, in a recent paper this risk has been estimated to be very low (0.0001%)[17].

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References

1. 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 on behalf of the Italian Society of Human Genetics (SIGU), November 2011. *Ultrasound Obstet Gynecol* 2012; **39**: 384–388.
2. Hillman SC, Pretlove S, Coomarasamy A, McMullan DJ, Davison EV, Maher ER, Kilby MD. Additional information from array comparative genomic hybridization technology over conventional karyotyping in prenatal diagnosis: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 2011; **37**: 6–14.

3. Maya I, Davidov B, Gershovitz L, Zalzstein Y, Taub E, Coppinger J, Shaffer LG, Shohat M. Diagnostic utility of array-based comparative genomic hybridization (CMA) in a prenatal setting. *Prenat Diagn* 2010; **30**: 1131–1137.
4. Park SJ, Jung EH, Ryu RS, Kang HW, Ko JM, Kim HJ, Cheon CK, Hwang SH, Kang HY. Clinical implementation of whole-genome array CGH as a first-tier test in 5080 pre and postnatal cases. *Mol Cytogenet* 2011; **9**: 4–12.
5. Armengol L, Nevado J, Serra-Juhé C, Plaja A, Mediano C, García-Santiago FA, García-Aragonés M, Villa O, Mansilla E, Preciado C, Fernández L, Mori MA, García-Pérez L, Lapunzina PD, Pérez-Jurado LA. Clinical utility of chromosomal microarray analysis in invasive prenatal diagnosis. *Hum Genet* 2012; **131**: 513–523.
6. Fiorentino F, Caiazzo F, Napolitano S, Spizzichino L, Bono S, Sessa M, Nuccitelli A, Biricik A, Gordon A, Rizzo G, Baldi M. Introducing array comparative genomic hybridization into routine prenatal diagnosis practice: a prospective study on over 1000 consecutive clinical cases. *Prenat Diagn* 2011; **31**: 1270–1282.
7. Lee CN, Lin SY, Lin CH, Shih JC, Lin TH, Su YN. Clinical utility of array comparative genomic hybridization for prenatal diagnosis: a cohort study of 3171 pregnancies. *BJOG* 2012; DOI: 10.1111/j.1471-0528.2012.03279.x. [Epub ahead of print].
8. Shaffer LG, Coppinger J, Alliman S, Torchia BA, Theisen A, Ballif BC, Bejjani BA. Comparison of microarray-based detection rates for cytogenetic abnormalities in prenatal and neonatal specimens. *Prenat Diagn* 2008; **28**: 789–795.
9. Ogilvie CM, Yaron Y, Beaudet AL. Current controversies in prenatal diagnosis 3: For prenatal diagnosis, should we offer less or more than metaphase karyotyping? *Prenat Diagn* 2009; **29**: 11–14.
10. Faas BH, van der Burgt I, Kooper AJ, Pfundt R, Hehir-Kwa JY, Smits AP, de Leeuw N. Identification of clinically significant, submicroscopic chromosome alterations and UPD in fetuses with ultrasound anomalies using genome-wide 250k SNP array analysis. *J Med Genet* 2010; **47**: 586–594.
11. Bui TH, Vetro A, Zuffardi O, Shaffer LG. Current controversies in prenatal diagnosis 3: is conventional chromosome analysis necessary in the post-array CGH era? *Prenat Diagn* 2011; **31**: 235–243.
12. Berg JS, Potocki L, Bacino CA. Common recurrent microduplication syndromes: diagnosis and management in clinical practice. *Am J Med Genet A* 2010; **152A**: 1066–1078.
13. Darilek S, Ward P, Pursley A, Plunkett K, Furman P, Magoulas P, Patel A, Cheung SW, Eng CM. Pre- and postnatal genetic testing by array-comparative genomic hybridization: genetic counseling perspectives. *Genet Med* 2008; **10**: 13–18.
14. Menten B, Maas N, Thienpont B, Buysse K, Vandesompele J, Melotte C, de Ravel T, Van Vooren S, Balikova I, Backx L, Janssens S, De Paepe A, De Moor B, Moreau Y, Marynen P, Fryns JP, Mortier G, Devriendt K, Speleman F, Vermeesch JR. Emerging patterns of cryptic chromosomal imbalance in patients with idiopathic mental retardation and multiple congenital anomalies: a new series of 140 patients and review of published reports. *J Med Genet* 2006; **43**: 625–633.
15. Scott SA, Cohen N, Brandt T, Toruner G, Desnick RJ, Edelmann L. Detection of low-level mosaicism and placental mosaicism by oligonucleotide array comparative genomic hybridization. *Genet Med* 2010; **12**: 85–92.
16. Valli R, Marletta C, Pressato B, Montalbano G, Lo Curto F, Pasquali F, Maserati E. Comparative genomic hybridization on microarray (a-CGH) in constitutional and acquired mosaicism may detect as low as 8% abnormal cells. *Mol Cytogenet* 2011; **9**: 4–13.
17. Ahn JW, Mann K, Walsh S, Shehab M, Hoang S, Docherty Z, Mohammed S, Mackie Ogilvie C. Validation and implementation of array comparative genomic hybridisation as a first line test in place of postnatal karyotyping for genome imbalance. *Mol Cytogenet* 2010; **3**: 9.

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

We have read the recommendations proposed by the Italian Society of Human Genetics (SIGU) Committee[1] for the use of chromosomal array (CMA) in prenatal testing. The SIGU Committee recommends that CMA in prenatal testing should never be used as a substitute for conventional karyotyping. Its application is strictly for specific diagnostic purposes in selected pregnancies, not for general prenatal screening. The reason for this is mainly concerns regarding: (1) additional parental anxiety resulting from the detection of variations of uncertain significance (VOUS); (2) the low rate of detection of known, disability-causing pathogenic copy number variations (CNV) by CMA in all pregnant women (0.16–0.3% as quoted in the SIGU recommendation); (3) the impossibility to detect balanced rearrangements with CMA; and (4) the inability to detect polyploidy and low-rate mosaicism.

Since 2011, more and more studies with large prenatal cohorts have been published supporting prenatal screening by CMA. However, these studies were not reviewed in the SIGU article[2–4]. These new published data may provide us with answers to the four major concerns proposed by Novelli *et al.*[1]. We would like to clarify the newly reported evidence in favor of substituting traditional karyotyping with CMA. First of all, the rate of detection of VOUS was 5/3171 (0.3%) in our study[4] and 1/1037 (0.1%) in that of Fiorentino *et al.*[2]. Secondly, the reported detection rates of pathologic microdeletions or microduplications in all pregnant women were 34/3171 (1.1%)[4], 9/1037 (0.9%)[2] and 15/4073 (0.4%)[3]. These differences between the detection rates of VOUS and pathogenic CNVs further justify the use of CMA technology in the prenatal setting without a specific clinical indication. Thirdly, CMA is limited in the detection of balanced translocation; however, the risk of *de-novo* balanced translocation is very low and translocations in phenotypically abnormal patients are more likely to be associated with genomic imbalances at the breakpoints or elsewhere and with chromosomal complexity[5]. In our prospective survey, array comparative genomic hybridization (CGH) identified genomic losses in two of 17 (11.8%) fetuses carrying *de-novo* balanced translocations, while the remaining 15 newborns had normal phenotype. In clinical scenarios, when *de-novo* balanced translocation is identified by karyotyping and CMA shows no genetic dosage gain or loss, further ultrasonographic follow-up is suggested, rather than surveying for disruption or modulation of the expression of genes located at the breakpoint, inactivation (position effect) of genes or imprinting syndromes. As for the concern of triploidy, array CGH would only miss cases with 69,XXX mixed with female reference DNA (46,XX). Taking this into consideration, we recommend using a

male reference to avoid misdiagnosis. Last but not least, whilst we agree that CMA is hampered by low rates of mosaicism, this applies to rates lower than 10%, not 30% as Novelli *et al*. stated.

Nevertheless, we are not stating that CMA should be used as the only prenatal diagnostic technology. The primary disadvantage of karyotyping is its requirement of cell culture, because of possible culture failure or contamination. Additionally, we have studied two cases with inconsistent results from uncultured and cultured amniotic fluid; one was a case of trisomy 2 and the other trisomy 12p[6,7]. Conventional karyotyping requires culturing of amniocytes, but abnormal cells may disappear after long-term culturing. Array CGH analysis of DNA from uncultured cells thus offers an advantage over karyotyping in this situation. Besides CMA, regular prenatal examinations and ultrasonographic survey for fetal structural anomalies are still necessary. Novelli *et al*. recommended a strategy for prenatal diagnosis involving conventional karyotyping and CMA as a second-line test in high-risk pregnancies. This strategy may lead to a prolonged wait, need for repeat amniocentesis and involves the drawbacks of cell culture. Due to its high diagnostic rate and short turnaround time, the role of CMA in prenatal diagnosis should be far more than as an adjunct tool preserved for specific purposes. However, it is still worthwhile considering karyotyping as back-up technology to rule out low-rate mosaicism or balanced translocations.

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References

1. Novelli A, Grati FR, Ballarati L, Bernardini L, Bizzoco D, Camurri L, Casalone R, Cardarelli L, Cavalli P, Ciccone R, Clementi M, Dalpra 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.
2. Fiorentino F, Caiazzo F, Napolitano S, Spizzichino L, Bono S, Sessa M, Nuccitelli A, Biricik A, Gordon A, Rizzo G, Baldi M. Introducing array comparative genomic hybridization into routine prenatal diagnosis practice: a prospective study on over 1000 consecutive clinical cases. *Prenat Diagn* 2011; **31**: 1270–1282.
3. Park SJ, Jung EH, Ryu RS, Kang HW, Ko JM, Kim HJ, Cheon CK, Hwang SH, Kang HY. Clinical implementation of whole-genome array CGH as a first-tier test in 5080 pre and postnatal cases. *Mol Cytogenet* 2011; **4**: 12.
4. Lee CN, Lin SY, Lin CH, Shih JC, Lin TH, Su YN. Clinical utility of array comparative genomic hybridization for prenatal diagnosis: a cohort study of 3171 pregnancies. *BJOG* 2012; DOI: 10.1111/j.1471-0528.2012.03279.x. [Epub ahead of print].
5. Baptista J, Mercer C, Prigmore E, Gribble SM, Carter NP, Maloney V, Thomas NS, Jacobs PA, Crolla JA. Breakpoint mapping and array CGH in translocations: comparison of a phenotypically normal and an abnormal cohort. *Am J Hum Genet* 2008; **82**: 927–936.
6. Chen CP, Su YN, Lin SY, Chern SR, Chen YT, Lee MS, Wang W. Prenatal diagnosis of mosaic trisomy 2: discrepancy between molecular cytogenetic analyses of uncultured amniocytes and karyotyping of cultured amniocytes in a pregnancy with severe fetal intrauterine growth restriction. *Taiwan J Obstet Gynecol* 2011; **50**: 390–393.
7. Hung CC, Lin CH, Lin SY, Shin JC, Lee CN, Su YN. Prenatal diagnosis of a fetus with a de novo trisomy 12p by array-comparative genomic hybridization (array-CGH). *Gene* 2012; **495**: 178–182.

Reply

We read with interest the comments on our position statement from Drs Fiorentino and Baldi and from Dr Lee and colleagues. Their letters focus on several controversial issues regarding the use of chromosomal microarray analysis (CMA) in prenatal diagnosis.

First of all we would like to highlight that our report does not represent the opinion of any one individual but is a collaborative statement of the cytogenetic working group of the Italian Society of Human Genetics (SIGU). The recommendations were framed after several months of debate among SIGU members, including clinical and laboratory geneticists, and the literature quoted in the position statement was up-to-date at the time of official approval of the document by the working group. In order to keep this as a scientific debate rather than reduce it to a personal one, we will briefly discuss three main points regarding CMA in prenatal diagnosis, in response to the comments made by Fiorentino and Baldi and by Lee and colleagues and referring to the papers they cited to support their opinions.

Firstly, regarding the detection rate of CMA in low-risk prenatal populations (such as advanced maternal age and parental anxiety groups), Fiorentino and Baldi stated that the use of CMA in prenatal diagnosis would allow a substantial improvement of 1–3% in the detection rate of pathogenic chromosomal abnormalities. They then claimed that 0.4% (4/1000) of these findings would have been overlooked following our recommendations regarding the use of CMA. Park *et al.*[1] found 14 cases out of 4073 prenatal samples (0.3%) that would have been missed using conventional cytogenetic diagnosis. Using CMA, Lee *et al.*[2] identified 15 fetuses with an apparently normal karyotype and cryptic pathogenic

imbalances, among 2900 (0.4%) prenatal samples with an indication for advanced maternal age or parental concern. Finally, the report by Armengol *et al.*[3], comparing the currently available assays for detection of chromosomal abnormalities in women referred for advanced maternal age or anxiety, found 12 aberrations in 333 analyzed cases (their Table 1) of which four were not detectable by cytogenetic analysis (their Table 3). We would like to emphasize that in all these cases the pregnancies were continued, delivering healthy babies. Taking into consideration all these findings, and reviewing the data reported in all these prospective studies, the additional detection rate of CMA in the advanced maternal age/parental anxiety population is 33/7973 (0.4%), thus confirming the data of Ogilvie *et al.*[4] that we cited in our position statement.

Secondly, the frequency of variations of unclear significance (VOUS) depends on which platform is used for prenatal diagnosis; a range of 0–4% has been found for targeted/low-resolution (0.5–1.0 Mb) platforms. This is clearly stated in the SIGU position paper. The study by Armengol *et al.*[3] provides further evidence of the frequencies of VOUS using different techniques. These authors analyzed a total of 900 pregnant women with four different techniques (karyotyping, quantitative fluorescent polymerase chain reaction, multiplex ligation-dependent probe amplification, CMA) and a total of 22 VOUS were identified. Using a targeted microarray, 17 VOUS were detected by CMA (1.9%) and only three (0.3%) by karyotyping. In the studies cited by Fiorentino and Baldi, the reported VOUS frequencies were significantly lower: zero in the study of Fiorentino *et al.*[5], 0.1% in that of Lee *et al.*[2] and the frequency was not specified by Park *et al.*[1]. In all these studies targeted/low-resolution CMA platforms were used, making it difficult to compare results with those of the genome-wide platform recommended in our position statement.

Moreover, our extensive experience in prenatal testing and genetic counseling leads us to emphasize that simplifying the discussion about the best management of prenatal diagnostic genetic testing to a sequence of percentages and technical performances is misleading.

It appears that the incidence of VOUS is also strictly dependent on the criteria of interpretation of CMA results and on the level of expertise involved in database consultation. An accurate review of the cases reported in the above papers supports this consideration and, more generally, the importance of genetic counseling. In pregnant women undergoing CMA for advanced maternal age or anxiety, one of four cases (Table 3, Fiorentino *et al.*[5]), six of 15 cases (Table 3, Lee *et al.*[2]) and five of five cases (Table 3, Armengol *et al.*[3]) with inherited VOUS and/or associated with variable, hard-to-predict, phenotype, decided to continue the pregnancy. Surprisingly, Case 9 of Fiorentino *et al.*[5] opted to terminate the pregnancy in the presence of a 22q11.2 duplication inherited from the normal mother; while in Case 11 of Armengol *et al.*[3] the decision was to continue the pregnancy in the presence of the same duplication, despite the fact that it occurred *de novo*. This duplication is associated with inter- and intrafamilial phenotype variability, and a reliable phenotype–genotype correlation cannot be done. In addition, the two cases with PMP22 deletion and duplication identified by CMA in the study of Fiorentino *et al.* (their Cases 1 and 4), both inherited from affected mothers, should have been suspected during pretest genetic counseling. Finally in 3/3 cases of VOUS reported by Lee *et al.*[2] (their Table 3) the women opted to terminate the pregnancy. All these data strongly support, according to SIGU guidelines, the necessity, when performing CMA, of pre- and post-test genetic counseling by trained geneticists. Finally, Fiorentino and Baldi pointed out the issue of the best diagnostic strategy. It is obvious that the translation into clinical practice of a diagnostic test is a critical point in the prenatal setting. In the SIGU position statement, CMA has been recommended as a second-level analysis after karyotyping in cases with single/multiple ultrasound fetal malformations and an apparently normal karyotype, and in cases with supernumerary chromosomal markers or *de novo* chromosomal aberrations. A different strategy, using 'first-tier' CMA analysis in all pregnant women, would consistently anticipate the cytogenetic diagnosis, providing a better time frame for genetic counseling and the parental decision-making process. However, while respecting the legitimate commercial interests of many operators involved in prenatal diagnosis, we still believe that the analytical sensitivity or the earliness of the test should not be the sole basis for introducing a genetic test into clinical practice. We would like to underline the fact that in the recent European Cytogeneticists Association (ECA) newsletter[6] (29 January 2012), the Permanent Working Group for Cytogenetics and Society reached, independently, similar conclusions to those reported in our document and included in the Clinical Indications For Investigation Of Array-CGH in the prenatal setting only a few selected conditions (Appendix, page 23). The ECA conclusions, as did ours, take into consideration the whole benefit of the test rather than single features such as sensitivity or earliness. Neither association has any conflict of interest and both evaluated the indication of CMA in terms of performance, quality control, effectiveness and usefulness. Starting from 2000, a model was developed by the CDC's (Centers for Disease Control and Prevention) Office of Public Health Genomics (OPHG) as a process for assessing the safety and effectiveness of DNA-based genetic tests[7]. This model, the ACCE model, took its name from the terms Analytical validity, Clinical validity, Clinical utility and associated Ethical, legal and social implications. We still believe that, for the good of our patients, this approach is mandatory.

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References

1. Park JH, Woo JH, Shim SH, Yang SJ, Choi YM, Yang KS, Cha DH. Application of a target array comparative genomic hybridization to prenatal diagnosis. *BMC Med Genet* 2010; Jun 24; **11**: 102.
2. Lee CN, Lin SY, Lin CH, Shih JC, Lin TH, Su YN. *BJOG*. 2012; Feb 7. doi: 10.1111/j.1471-0528.2012.03279.x. [Epub ahead of print].
3. Armengol L, Nevado J, Serra-Juhé C, Plaja A, Mediano C, García-Santiago FA, García-Aragonés M, Villa O, Mansilla E, Preciado C, Fernández L, Mori MA, García-Pérez L, Lapunzina PD, Pérez-Jurado LA. Clinical utility of chromosomal microarray analysis in invasive prenatal diagnosis. *Hum Genet* 2011; Oct 6. [Epub ahead of print].
4. Ogilvie CM, Yaron Y, Beaudet AL. Current controversies in prenatal diagnosis 3: For prenatal diagnosis, should we offer less or more than metaphase karyotyping? *Prenat Diagn* 2009; **29**: 11–4.
5. Fiorentino F, Caiazzo F, Napolitano S, Spizzichino L, Bono S, Sessa M, Nuccitelli A, Biricik A, Gordon A, Rizzo G, Baldi M. Introducing array comparative genomic hybridization into routine prenatal diagnosis practice: a prospective study on over 1000 consecutive clinical cases. *Prenat Diagn* 2011; **31**: 1270–82. doi: 10.1002/pd.2884. Epub 2011 Oct 28.
6. Hastings R, Howell R, Dagna Bricarelli F, Kristoffersson U, Cavani S. General Guidelines and Quality Assurance for Cytogenetics. European Cytogeneticists Association Newsletter, No. 29, January 2012.
7. Centers for Disease Control and Prevention. ACCE Model Process for Evaluating Genetic Tests. http://www.cdc.gov/genomics/gtesting/ACCE/index.htm [Accessed 17 February 2012].