Array CGH in routine prenatal diagnosis practice: reply letter

We thank Dr. Cavalli et al. and Dr. Lin et al. for their interest in our paper. We welcome the opportunity to provide clarification.

We certainly agree with Cavalli et al. that an accurate evaluation of aCGH in terms of performance, quality control, effectiveness and usefulness is of paramount importance and these are precisely the aims of our study.1 Furthermore, the small size of the cohorts analysed in previously reported prospective studies,2 has necessitated our undertaking of a large-scale prospective clinical trial. Recently, other similar large-cohort prospective studies have reported results concordant with our findings.3,4 We also welcome the correspondence from Lin et al. further providing a significant amount of data supporting our conclusions.

Cavalli et al. expressed their legitimate difference of opinion on the use of aCGH in prenatal diagnosis. In contrast there are a number of papers where the authors recommend offering aCGH as a first line test.4–8 Our view in this discussion is strongly on the side of the latter position, because offering aCGH testing only as a second-line test substantially limits the diagnostic potential of this assay, missing pathogenic copy number variations (CNVs). The most relevant point in relation to this argument relates to the nine (0.9%) fetal conditions that would have remained undiagnosed if only a conventional karyotype had been performed. More important, four (0.4%) of such finding would have otherwise been overlooked following the diagnostic strategy suggested by Cavalli et al. The latest data from our still ongoing prospective trial continues to show a similar outcome, with 22/2500 (0.9%) submicroscopic chromosomal abnormalities that would have been missed, 15 (0.6%) of which if using the above proposed strategy.

At present, data available provide substantial evidence for the feasibility of introducing aCGH into routine prenatal diagnosis as a first-line test. As properly pointed out by Lin et al. the issue still to be addressed is: to which category of pregnancies? In our opinion, this technology should be available to all pregnant women undergoing invasive prenatal testing, regardless of risk factors. The updated results of our study speak clearly in favour of such a testing paradigm, showing the use of aCGH results in an increased detection rate regardless of the indication for analysis. This becomes especially evident when examining the data from high-risk groups, in which the detection rate is elevated to 6.5% (7/107), but also in groups with a priori low risk for detection of submicroscopic chromosome abnormalities, such as advanced maternal age (7/958, 0.7%) and parental anxiety groups (8/1355, 0.6%). Similar results were also reported by Armengol et al.4 and Lin et al.

We agree with Cavalli et al. that the current challenge of the application of aCGH in the routine prenatal diagnosis practice is minimising the potential to detect VOUS whilst maximising the detection of pathogenic CNV. However, it is well known that the differences in the proportion of such kind of undesirable findings is mainly related to the array platform used and its resolution. In our study, we have carefully selected a platform specifically developed for prenatal applications, with a balance with increased resolution in locations of known constitutional disorders and less coverage in polymorphic CNVs. This platform allowed us to detect a single VOUS occurrence out of 1037 samples. In a recent systematic review,2 VOUS are reported to occur in ~1% of prenatal samples. Combining the above data, the average probability of detecting such findings in prenatal samples can be estimated around 0.3%, similarly to the value reported by Lin et al. The point of debate is surely whether we are able or willing to deal with such level of variations of unclear significance (VOUS) uncertainty. We believe this ~0.3% rate of finding a VOUS is not dramatically different from what is observed with cytogenetic karyotype analysis. Hence, VOUS identified by prenatal aCGH might be approached in a similar manner and managed by providing the patients a thorough pretest and posttest counselling.9

A separate point for discussion is the issue of detection of CNVs with known pathological effects but incomplete penetrance and variable expressivity. Genetic counsellors are already familiar with this problem and preliminary guidelines are already available.10 In our opinion, in these cases, it may be preferable to explain the findings in the course of a proper posttest counselling and keep the family fully informed, doing so we would be respectful of patients’ autonomy.

Cavalli et al. also stated that in 3/9 cases the pathological CNVs should have been reliably detected by a good quality karyotyping at the 400-band level. This was true in only two samples: case 2, where the conventional karyotyping failed to detect the chromosomal abnormality; case 3, in which a cell culture failure occurred. Although we agree with this assertion, it is an ideal, because it is well known that the resolution of banding techniques is generally influenced by the quality of the chromosome preparation, especially when performing karyotyping from CVS samples, as it was for case 2. The above cases, instead, represent clear examples of inherent limitations of the use of traditional karyotyping in prenatal diagnosis.

Cavalli et al. also concerned about the real improvement in clinical sensitivity of the technique compared with the strategy they propose. It should be also remembered that, utilising
conventional karyotyping and aCGH as a second line test in high-risk pregnancies, the patients would obtain the results over 3 weeks later. The rapid confirmation of prenatal findings is essential for purposes of best practice clinical management. In addition, the logic of this approach could also be questioned because if the reporting of the aCGH results to patients occurs only after obtaining the results from conventional karyotyping, this may prolong the anxiety amongst patients because of the extended time required, instead of just offering aCGH as first line test. As well as improving reporting timescales, it is now clear that aCGH produces a substantial improvement of ~1% to 3% in detection rate of pathogenic chromosome abnormalities than conventional karyotyping.1–4 In our study reasons we believe that a figure of 0.9% represents the real improvement in detection rate of the aCGH technique, and similar results were also reported by Lin et al.

Funding sources: None

Conflicts of interest: None declared

REFERENCES
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