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Case Study

A CVS was received at 14 weeks of gestation from a 31 year old woman with a first trimester Downs screening risk of 1:62. The QFPCR result of the uncultured dissociated material showed 4 markers on chromosome 21 having ratios consistent with trisomy 21. Subsequent G banded analysis of cultured cells showed a normal karyotype with no evidence of mosaicism.

No material was available for follow up.

Method

All CVS samples at the Bristol Genetics Laboratory have Quantitative Fluorescent Polymerase Chain Reaction (QFPCR) for the detection of trisomies 13, 18 and 21. After the removal of any maternal tissue the sample is finely chopped and then digested with trypsin in accordance with ACC Best Practice Guidelines (2007)⁽¹⁾. The dissociated material is then used for both the DNA extraction for QFPCR and the initiation of long term cell cultures. Using this method the aliquot of cellular material used for the QFPCR should represent all the villi present in the sample and contain both cytotrophoblast and mesodermal tissues minimising the risk of misdiagnosis due to confined placental mosaicism. A single frond of villi with the tips removed is reserved for confirmation of abnormal results. All samples have G banded chromosome analysis of cultured cells.

Uncultured QFPCR results

Chromosome 21: 4 markers gave a biallelic trisomic ratio, a further marker gave an inconclusive ratio.

Analysis was confirmed on single frond of villi (all informative markers giving trisomic results).

A result consistent with trisomy 21 was reported.

Results of cultured cells

A primary culture was harvested at 12 days; 5 colonies showed a 46,XX result.

Subsequent harvests of coverslips sub-cultured from 2 different primary cultures all showed a 46,XX result (total 20 cells analysed)

In situ hybridisation with Abbot /Vysis Aneuvysion 13/21 probe (21q21.12-q22.2) showed two copies of the probes in 40 metaphase and 200 interphase cells from 2 sub-cultures from different primaries.

Cells from a total of 4 initial cultures were analysed with no evidence of mosaicism, the FISH analysis also excluded trisomy 21 as a result of a cryptic chromosome rearrangement.

QFPCR of cultured cells

QFPCR analysis of sub-cultured cells from 4 initial cultures showed normal ratios for all informative chromosome 21 markers and confirmed all allele sizes present were the same as that observed in the uncultured material.

It is laboratory practice to make a back up culture from the supernatants from the primary cultures at the first change of media. This 'supernatant' culture still had small pieces of floating uncultured villi material in it. This material was removed and analysed by QFPCR, the results of this material confirmed the original trisomy 21 result.

These results confirmed that both cultured and uncultured cells were from the same individual (excluding sample mix up) and showed that maternal cell contamination of the cultured cells was highly unlikely.

Marker	Chromosome location	Ratio on uncultured dissociated material	Ratio on cultured material
D21S11	21q21.1	Uninformative	Uninformative
D21S1437	21q21.1	Uninformative	Uninformative
D21S1435	21q21.2	1.93	1.08
D21S226	21q21.3	0.62	1.10
D21S1280	21q22.11	0.67 (inconclusive) (0.62 on single frond)	0.93
D21S2055	21q22	0.65	1.01
D21S1411	21q22.3	2.14	1.07
D21S1446	21q22.3	Uninformative	Uninformative

Discussion

In this case, as no follow up material was available it is not known whether the foetus had full or mosaic trisomy 21 or whether the trisomic cells were confined to the placental tissue. It is possible that the trisomy 21 cells were at a selective disadvantage and did not grow in culture, this is a very unusual finding for which there is limited evidence in the literature^(2,3).

Previously reported cases with discrepancy of results for trisomy 21 in CVS between QFPCR and cultured cells were processed by QFPCR studies carried out using individual undissociated fronds of CVS⁽⁴⁾. It is suggested that analysis of dissociated villi material will minimise complete discrepant results between QFPCR and karyotype analysis⁽⁵⁾. An ACC audit⁽⁶⁾ showed that discrepant results are rare and more likely to be reported when undissociated fronds are used and the trisomic markers are biallelic; no discrepant cases of trisomy 21 were reported with QFPCR of dissociated villi. Information from the audit was passed to the ACC professional standards committee for review of the best practice guidelines. Following this result this laboratory has adopted the practice of reporting trisomies in CVS where all markers are biallelic such that karyotype confirmation is recommended as the abnormal cell line may have arisen as a post-zygotic event and could represent confined placental mosaicism.

It is understood that best practice guidelines are in the process of revision and that further guidance will be issued. A further ACC audit is underway to ascertain whether changes in sample preparation have reduced the number of discrepant results.

References

- ACC Best Practice Guidelines for QF-PCR diagnosis of Aneuploidy (2007). http://www.cytogenetics.org.uk/prof_standards/professional_standards.htm
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