Expert Review 02

Detecting mosaicism with QF-PCR

Authored by Dr. Kathy Mann, Guy's Hospital, London, UK

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About the author

Kathy Mann, PhD, is a Principal Clinical Scientist within the Cytogenetics Department, GSTS Pathology, based at Guy's Hospital, London UK. With colleagues she developed and established the first QF-PCR service for the rapid detection of prenatal aneuploidy in the UK National Health Service (NHS) in 2000, a service which now processes >4,000 samples/year. The laboratory has since published more than 15 peer-reviewed papers related to the field of QF-PCR including new service developments such as stand-alone testing, which have subsequently been adopted by other laboratories, and their recent report detailing the results of the more than 40,000 prenatal samples. Other publications detail QF-PCR assays and best practice and describe phenomena such as mosaicism, maternal cell contamination, CNV and somatic microsatellite mutations. Kathy co-authored the first draft of the UK QF-PCR Best Practice Guidelines (2005) and has edited subsequent versions including the 2012 Guidelines. She has been an assessor for the Molecular Rapid Aneuploidy (MRA) UK NEQAS since its inception in 2004 and she was a speaker and joint organiser of the International QF-PCR Workshops from 2003-2008.

Introduction

Mosaicism was first described by Curt Stern in the 1930s and denotes the presence of two or more populations of cells with different genotypes that have developed from a single fertilised egg. This phenomenon is a widely recognised complication of prenatal diagnosis with between 1 and 2% of prenatal karyotypes found to be mosaic (ACC Working Party, 1994), usually manifesting as the presence of normal and aneuploid cell lines with an individual-specific distribution within the placenta and/or fetus. Although mosaicism is confined to the placenta in more than 80% of cases, it is important to identify the presence of both cell lines; either or both cell lines may be present in the fetus, modifying phenotype expression, whilst an abnormal cell line confined to the placenta may result in placental insufficiency and growth restriction in the fetus.

It follows that mosaicism is more frequently found in chorionic villi (CV) samples than amniotic fluid (AF). CV are made up of two distinct cell lineages, chorionic ectoderm (trophoblast) and chorionic mesoderm (mesenchymal core), both of which are embryonically distinct from the fetus. Traditionally, karyotype analysis of CV samples involved analysis of both lineages with the direct or short-term culture investi-gating the more embryologically distinct cytotrophoblast whilst karyotype analysis of long term cultures looked at the mesenchymal core. Cytotrophoblast results that are discordant with mesoderm and/or fetal status are widely reported; in the case of trisomy 21, false negative results are more prevalent in direct or short-term culture (STC), consistent with the absence of trisomic cells from the embryologically remote cytotrophoblast layer (Sikkema-Raddatz, 1997; Saura, 1998). However, non-mosaic results obtained from the mesoderm are considered to be a reliable indicator of fetal abnormality (Smith, 1999), and few rare cases of mesoderm results that are discrepant with the fetal karyotype have been reported (Pindar, 1992; Pittalis, 1994; Phillips, 1997; Brun, 2003; Riegel, 2006). If trisomy mosaicism is detected in a CV sample, the fetus may have non-mosaic or mosaic trisomy, or may be normal with the abnormal cell line confined to the placenta. Amniotic fluid contains fetal cells and therefore mosaicism detected in amniotic fluid samples indicates true fetal mosaicism, although the distribution of cell lines within fetal tissues cannot be determined.

Origin of mosaicism

An aneuploid cell line may be generated by either a meiotic or mitotic nondisjunction event. Mosaicism arises either from a trisomy conception which subsequently undergoes a mitotic rescue event generating a normal cell line, or from a normal conception followed by a mitotic nondisjunction event in a single cell which gives rise to a trisomy cell line. The timing and location of these events determines the distribution of the cell lineages in the placenta and/or fetus and therefore the phenotype.

Detection of mosaicism by QF-PCR

Given the significance of mosaicism to prenatal diagnosis, it is clear that any technique that is applied to the diagnosis of aneuploidy in prenatal samples should be able to detect aneuploid mosaicism and that for CV samples the mesoderm must be represented. With these criteria in mind, the performance of quantitative fluorescence-PCR (QF-PCR) analysis can be considered (the principle and application of QF-PCR analysis for the detection of aneuploidy in prenatal samples is described in Expert Review 01: An introduction to QF-PCR).

A significant benefit of the genotyping aspect of QF-PCR is that it provides information regarding the origin of a trisomy cell line which can be of some clinical significance. Trisomy results that exhibit at least one short tandem repeat (STR) with three different length length alleles representing three different chromosome homologues indicate a meiotic error and trisomy conception (Figure 1a).

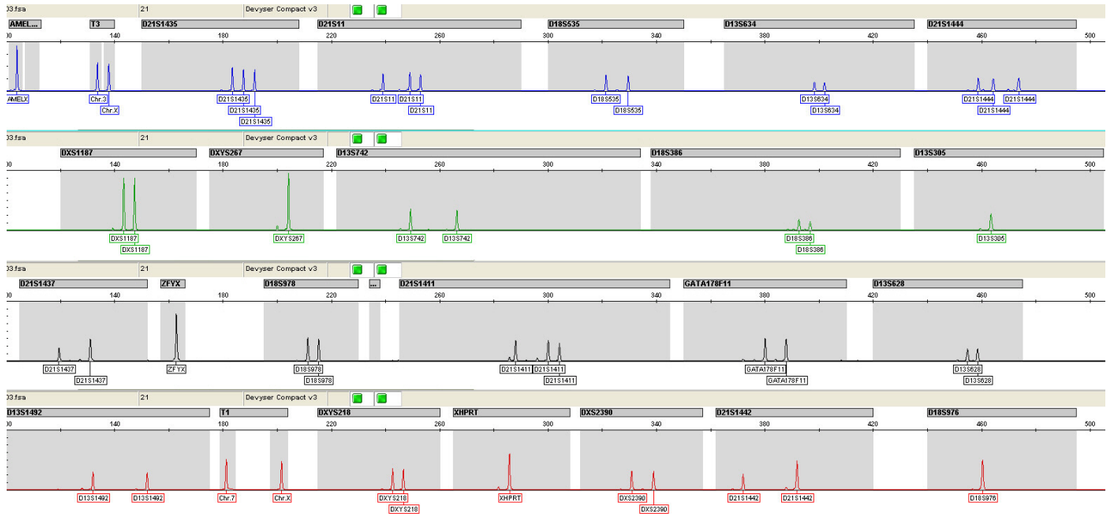

Fig. 1a. QF-PCR trace showing trisomy 21 as represented by six markers, four of which are triallelic (D21S1435, D21S11, D21S1444 and D21S1411), consistent with a trisomy conception.

Trisomy results that consist of only 2:1 and 1:2 biallelic STRs represent either meiotic or mitotic nondisjunction errors (Figure 1b). In a UK audit 7.4%, 17% and 10.3% of trisomy 13, 18 and 21 results respectively, had no triallelic STRs (Waters, 2008) and may therefore represent mitotic nondisjunction events (the number of tested STRs determines the proportion of abnormal biallelic samples; 6.7 and 13% of trisomy 21 results with 10 and 5 STRs, respectively). In summary, the QF-PCR allele pattern in both mosaic and non-mosaic samples is determined to some extent by the origin of the abnormal cell line.

Mosaicism is represented by skewed biallelic ratios and/or additional allele peaks for all informative STRs on a single chromosome (Figure 2), or in the case of mosaic triploidy on every chromosome. Triallelic results exhibit a standard pattern of two alleles of equal proportions and a third smaller allele representing the trisomy cell line (Figure 3). The degree of skewing of biallelic STRs and size of the additional allele peak in the case of triallelic STRs represents the proportion of abnormal cell line in the tested sample.

The amount of each cell line can be estimated from either the biallelic ratios or, more easily, from the triallelic results (Figure 3). Our experience has shown that the use of a QF-PCR strategy combined with experienced analysis is consistently able to detect mosaicism for chromosome aneuploidy if the minority cell line contributes at least 20% of cells to the sample. However, it is possible to detect mosaicism at a level as low as 15% if the abnormal cell line is meiotic in origin and exhibits at least one triallelic result (Donaghue, 2005). This can be compared with estimates for karyotype analysis where analysis of eleven cells excludes 25% mosaicism with a confidence interval of 95%.

"Optimal use of QF-PCR will identify most clinically significant cases of mosaicism"

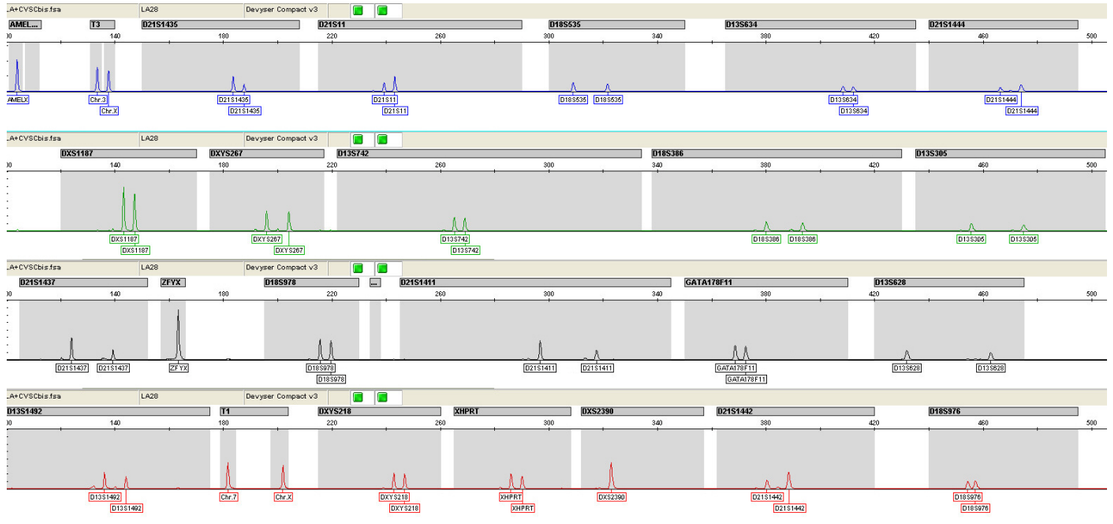

Fig. 1b. QF-PCR trace showing trisomy 21 as represented by six biallelic 2:1 and 1:2 results, consistent with either a meiotic or mitotic nondisjunction event generating the trisomy cell line and an increased risk of mosaicism.

If both normal and abnormal cell lines can be confidently identified then these should be reported (UK Best Practice Guidelines, 2012). However, the interpretation of such results should consider the sample type, origin of the abnormal cell line, level of the abnormal cell line and the referral indication. With any CV mosaic result it is important to note on the report that the abnormal cell line may be confined to the placenta and that follow-up studies are recommended which may include analysis of cultured cells, AF or fetal blood.

Incidence of mosaicism for the common aneuploidies

This depends on the sample type, sample preparation and referral indication and will therefore vary between centres. Mosaicism for trisomies 13, 18 and 21 identified by karyotype analysis was reported to occur in 0.26% of CVS samples (Smith et al., 1999) and in 0.016% (Worton and Stern, 1984) and 0.007% (Bui et al., 1984) of AF samples.

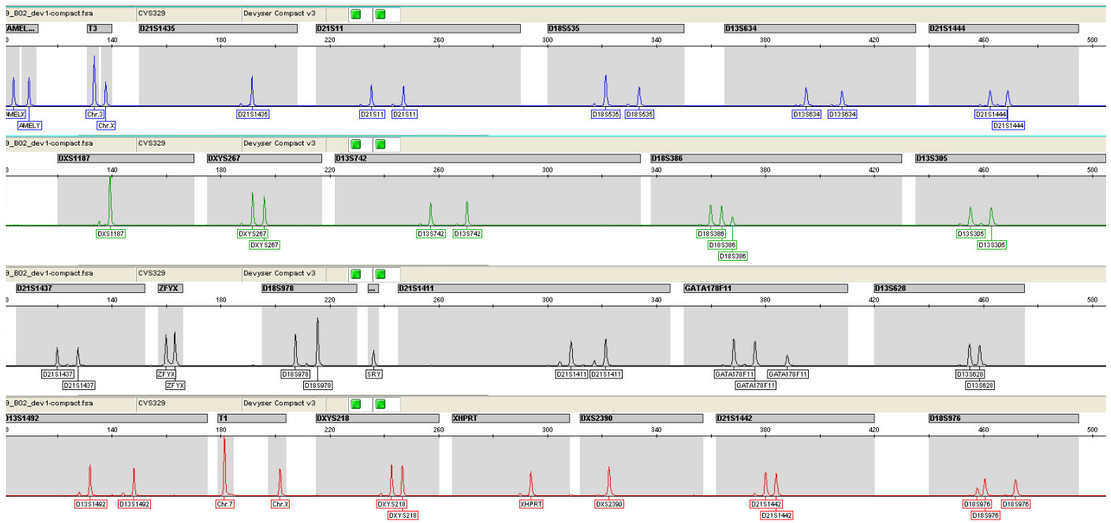

Fig. 2. QF-PCR trace showing mosaicism for trisomy 18. Triallelic results indicate a meiotic non-disjunction event and a trisomy conception followed by a mitotic rescue event. The triallelic results (D18S386, GATA178F11 and D18S976) show the expected pattern of two higher but equal peaks and one smaller peak which represents the trisomy cell line.

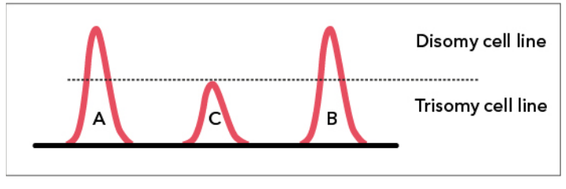

Fig. 3. A typical triallelic mosaic genotype showing two larger alleles of equal height (A and B) and a smaller third allele (C). The trisomy cell line is represented by the genotype beneath the dashed line (alleles A, B and C) whilst the disomy cell line is represented by alleles above the line (A and B). The proportion of trisomy cells can be calculated as area of allele C/area of either allele A or B.

Interestingly, of fifteen samples with a mosaic result following QF-PCR, nine (60%) exhibited a biallelic STR pattern compared to an average of 19% in non-mosaic cases, suggesting that in many cases the abnormal cell line was generated from a mitotic event. Cirigliano et al., 2009, report 0.17% of 43,000 samples (of which 83% were AFs) tested by QF-PCR showed mosaicism for trisomies 13, 18, 21 and sex chromosome aneuploidies. Mann et al., 2012, detected 84 cases of mosaicism for trisomy 13, 18 or 21 (0.21% of all samples) using QF-PCR, the majority (68) of which were CV samples.

Discrepant QF-PCR/karyotype results

There are a number of reports that detail completely discrepant QF-PCR and karyotype results in CV samples due to placental mosaicism; the majority of these have an allele pattern consistent with mitotic generation of the abnormal cell line (Allen, 2006; Waters, 2006; Waters, 2007; Lau, 2009; Holgado, 2011). The incidence of discrepant results is likely to be determined by the quality and size of the original CV biopsy, sampling of the biopsy, DNA preparation method and analysis experience, and will therefore vary between centres. The effect of sample preparation was discussed by Waters et al, 2007, who detail three discrepant cases during the testing of 3,700 CV samples. At this time most laboratories tested two or more whole villi taken from different regions of the sample. Following review, it was proposed that a more representative sample should be tested; dissociation of at least 5mg of CV by either enzymatic digestion or mechanical disruption results in a pool of cells which can be used for QF-PCR analysis, aCGH analysis and/or to establish cultures for karyotype analysis. Representation of the mesenchyme in the dissociated cell pool was subsequently demonstrated to be between 40 and 50% (Mann, 2007). Since this protocol change, our laboratory has had one case of a discrepant result between PCR analysis of uncultured cells and karyotype anaylsis of cultured cells in more than 10,000 CV samples (<0.01%) (Mann, 2012). The use of dissociated cells is now recommended as part of UK best practice (UK Best Practice Guidelines, 2012).

The number of completely discrepant results in CV samples varies significantly between centres: Holgado et al., 2011, report a 1/815 incidence whilst our laboratory reports <1/10,000 (Mann, 2012). All of the reasons detailed above may account for this difference but the sample preparation protocol may be the most significant factor; Holgado et al. report starting with 1mg of CV tissue for QF-PCR analysis and that samples for QF-PCR and karyotype analysis are taken from different cell pools.

"The use of dissociated cells is now recommended as part of UK best practice"

As all four of our discrepant cases exhibited a biallelic abnormal cell line (Waters, 2007), we reviewed the implications for non-mosaic abnormal results that show no evidence of a meiotic nondisjunction event; as the abnormal cell line may be mitotic in origin there is an increased risk of mosaicism compared to abnormal results that clearly demonstrate a meiotic origin. Although mosaicism in these samples is still a rare event we have changed our practice so that reports for CV samples with biallelic abnormal results state that there is no evidence of meiotic nondisjunction and that in the absence of ultrasound abnormalities, any decision regarding pregnancy termination should await the karyotype result. For AF results the origin of the trisomy has little significance as the abnormal result is derived in part from fetal cells.

Summary

Mosaicism in prenatal samples is a complex but important phenomenon. Optimal use of QF-PCR will identify most clinically significant cases of mosaicism, and also provide additional information regarding the origin of the abnormal cell line.

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