Broadening our understanding by the use of molecular cytogenetic techniques: full monosomy 21

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Introduction

Multiple risk factors such as exposure to teratogens, advanced parental age, anatomic anomalies of the mother, genetic diseases and chromosomal anomalies are involved in embryo loss. Spontaneous abortions occur in approximately 10–15% of clinically recognised pregnancies. Most women who miscarry are not informed of the cause of their pregnancy loss, thereby causing them anxiety, confusion, low self-esteem and depression [1, 2]. Knowledge of the causes of spontaneous abortion reduces both stress and the feeling of culpability that occur after miscarriage [1].

Cytogenetic advances in the understanding and detection of human chromosomal rearrangements related to certain diseases have allowed for diagnosis of most cases of

miscarriage. According to the literature, some 65% of first-trimester spontaneous abortions have been shown to carry chromosomal anomalies [3], although this figure may be below the actual percentage due to culture failure since karyotyping cannot be performed on such cases [4]. Therefore, it is likely that advanced molecular techniques such as multiplex ligation-dependent probe amplification (MLPA) would permit an increase in the percentage of chromosomal anomalies detected and even make it possible to identify other full monosomies which are involved in foetal loss.

Loss of a whole chromosome may be lethal possibly because of the haploinsufficiency of essential genes. In fact, Turner syndrome (45X) is the only full monosomy that is compatible with life.

Here, we report a full monosomy 21 identified by MLPA and confirmed by array CGH in a molecular study of 310 miscarriages. Full monosomy 21 (FM21) is a very rare chromosomal anomaly and one of the few cases that have appeared in the literature to date is the first full monosomy 21 observed in Spain published by Mori MA *et al* in 2004 [5].

Capsule A full monosomy 21 was detected by MLPA in a chorionic villi sample taken during the 7th week of pregnancy and confirmed by array CGH.

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Case report

We received a miscarriage sample from a 33-year-old primigravida with a family ectopic pregnancy who aborted at 7 weeks' gestation. After counselling, the parents provided informed consent for foetal karyotyping and molecular analysis.

Due to maceration conditions cultivation was unsuccessful. Material was examined and dissected to exclude maternal

decidua remains. Foetal tissue, as well as maternal decidua were selected for molecular assay.

After dissection and chemical digestion, Genomic DNA was isolated from foetal tissue by using the Tissue Kit using the automated BioRobot EZ1 (QIAGEN, Hilden, Germany).

As reflected in the best practice guidelines for laboratory quality control established by the European Molecular Quality Network (EMQN, St Mary's Hospital, Manchester, United Kingdom) regarding prenatal diagnosis, testing for maternal contamination of prenatal samples using a minimum of three highly polymorphic microsatellite loci should be considered, particularly when the genotype is shown to be the same as the maternal one [6]. Therefore, QF-PCR for prenatal

detection of aneuploidy in chromosomes 13, 18, 21, X and Y was performed in an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and analysed with Gene Mapper v.4.0 software package (Applied Biosystems, Foster City, CA, USA) in order to confirm the foetal origin of the sample, discard the most common aneuploidies and determine the sex of the sample [7].

The results revealed that the foetus was male and with no maternal contamination. However, analysis using chromosome 21 markers showed a loss of maternal alleles or a homozygous pattern for D21S1411. In addition, the final product amplification of D21S1414 from the chorionic villi and the amplification of the maternal microsatellite do not

Fig. 1 QFPCR analysis data of the miscarriage sample (AB-1256) compared to maternal decidua's one

match each other. Therefore, further analysis was needed to interpret the results (Fig. 1).

Owing to the absence of karyotyping because of the culture failure, MLPA with SALSA P070 and SALSA P036

probe sets were purchased from MRC-Holland (MRC-Holland, Amsterdam, The Netherlands). These sets contain one probe for each subtelomeric region of every chromosome in order to detect both unbalanced chromosomal

Fig. 2 MLPA p036 and p070 SALSA kit results from miscarriage sample (AB-1256) compared to a control sample. The black arrows remark the significant decreased peaks of the probes related to chromosome 21

rearrangements and aneuploidies in the samples. MLPA analysis was performed according to the manufacturer's instructions [8]. Amplification products were electrophoresed in an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and analysed with Gene Mapper v.4.0 software package (Applied Biosystems, Foster City, CA, USA).

The remarkable decrease seen in the peaks of the 21 chromosome probes was found in both SALSA P070 and SALSA P036 test (Fig. 2). Normalisation of peak area values was achieved as recommended by the manufacturer. Since chromosome 21 probes used in both SALSA are located in the subtelomeric region of the long arm and also beneath the centromere, full monosomy 21, a marker chromosome, or any complex rearrangement could be the conclusion reached from the results.

In order to assess the validity of both causes, and due to the absence of karyotype data, further STRs assays were performed by using the following markers along chromosome 21: D21S263, D21S266, D21S1414 and D21S1411 (Fig. 3).

The results once again revealed either a loss of maternal alleles in all markers or a homozygous pattern for all the markers. Since this technique was not effective enough to distinguish between both causes, the results of QF-PCR using X-chromosome markers (DXS1214 and DXS8170 X chromosome markers in a hemizygous male pattern) were used. These results were normalised to the data from the 21

chromosome microsatellites in order to quantify relative gene dosage. Two male DNA controls with a homozygous pattern for both D21S1411 and D21S1414 21 markers were used in order to normalise 1 dose of X chromosome with 2 doses of a homozygous peak corresponding to the 21 chromosome. These two 21-chromosome STRs alleles from the foetus sample showed half of the dosage seen in the controls (Fig. 4). This finding suggested that full monosomy 21 might be involved in the phenotype of this miscarriage. Finally, 1MB resolution ARRAY-CGH, a technique that was described by Solinas-Toldo S et al. in 1977 [9], was carried out to obtain higher resolution, thereby confirming full monosomy 21 (Fig. 5).

Conclusion

Multiple explanations (e.g. markers, ring and full monosomy) could be behind the initial results obtained from MLPA and microsatellite analysis. However, certain issues led us to assume that the ring chromosome 21 and marker chromosome were the causes of the miscarriage instead of full monosomy 21: 1) the latter is an extremely rare chromosomal anomaly, and therefore few cases had been reported; 2) These cases were diagnosed as unbalanced translocations when re-evaluated by molecular techniques after the dawn of the molecular era; 3) though a rare event, most marker and ring chromosomes are formed from the acocentric chromosomes and there is a wide

Fig. 3 Four STR markers along chromosome 21 analysis

range of variability in the clinical effects of them between the normal phenotype and multiple malformations

However, after using markers along the 21-chromosome, full monosomy seemed more plausible and array CGH was the key to confirming what MLPA revealed at first.

As in the vast majority of trisomy 21 cases, maternal meiotic non-disjunction is the origin of the responsible mechanism underlying full monosomy 21 in at least four (present case included) out of the five cases reported [10].

Molecular assays must be an alternative or complement to cytogenetic studies in order to remedy culture limitations. Until now, some cell cultures failed, thus impeding the performance of karyotype studies. Cell cultures may progress either in cases of normal karyotypes or in those with certain chromosomal anomalies that allow preferential cell proliferation in vitro. Such patterns probably cause the frequency of this anomaly to be skewed. As new molecular techniques are developed, this percentage may increase.

Fig. 4 STR assay data normalization from the chromosome 21 microsatellites

Fig. 5 Array CGH results which show the full deletion of the chromosome 21. The figure below is a zoomed in versions of the deleted region shown in the figure above

The latest advances in molecular genetics have opened up new perspectives in our understanding of miscarriages.

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Conflict of interest None to report

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