Sex Chromosomes Mosaicism Detection by Quantitative Fluorescent PCR Based Technique

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Abstract: Background: The X and Y chromosomes may break the rules of meiosis resulting eggs or sperms with wrong number of sex chromosomes. Missing or extra copies of sex chromosomes into the zygote produced by such sperm and egg are supposed to be the cause of infertility, growth abnormalities, and, in some cases, behavioral and learning problems. Mosaics are possible for all types of sex chromosomes abnormalities (SCA). SCAs are detected before birth using different techniques, such as, combining fetal conventional cytogenetics, FISH and a new rapid method based on quantitative fluorescence polymerase chain reaction (QF-PCR) technology. Objectives: To assess low-level sex chromosome mosaicism using OF-PCR technique and to compare the data with the results of FISH and conventional cytogenetics. Methods: QF-PCR test for chromosome 13, 18, 21 trisomies and sex chromosomes aneuploidies detection was used. Results: Five sex chromosomes mosaicism cases were prenatally detected and another case of 45, X/47, XYY Turner’s syndrome was reported by karyotype technique. Conclusions: The QF-PCR assay is a prenatal diagnosis technique that allows the rapid detection of selected chromosomes aneuploidies, thus relieving the maternal anxiety and facilitating options in pregnancy. Also the results confirm the ability of prenatal diagnosis method QF-PCR to provide rapidly reliable information about fetal sex chromosome mosaic state.

Key words: QF-PCR, prenatal diagnosis, fetal karyotype, FISH, mosaicism.

1. Introduction

Sex chromosomes aneuploidies have a high incidence of occurrence affecting 1 in 400–500 live newborns mostly because of the relative less severe condition (NUSSBAUM [1]). Within this group of genetic disorders, Turner syndrome is a common one, affecting 1 in 2500 female newborns (COVIC et al. [2], BIANCO [3]) that accounts for 1–2% of all clinically recognized pregnancies (MARTINEZ [4]) and for 15% of all spontaneously aborted fetuses (DOMINO [5]). The syndrome is characterized by broad spectrum of features including short stature (98%), gonadal dysgenesis with primary amenorrhea and infertility (95%), sexual infantilism, lymphedema (70%), prominent, anomalous ears with hearing impairment (70%), high palate (82%), short neck (80%), webbing of the neck (65%), broad chest (75%), cubitus valgus (75%) (DOMINO [5]). The syndrome etiology was associated with complete or partial monosomy of the X chromosome; just half of Turner syndrome patients are pure monosomic for the X chromosome, the rest bearing a multitude of chromosomal aberrations including: the presence of an isochromosome of the long arm of the X, ring X, mosaicism for two or more normal or abnormal cell lines that in 3–6% of cells, the second sex chromosome is Y (PREMI [6]). It is estimated that more than 12% of patients with Turner syndrome are Y-positive but cytogenetic analysis failed to detect it because of the low-frequency mosaicism or restricted to certain tissues, translocation of Y material in X chromosome or autosomes (BIANCO [3]) or instability of chromosomes with structural aberrations. The presence of Y-chromosome material in Turner syndrome patients increases the risk of developing gonadal tumors like benign tumor...
gonadoblastoma, which has considerable malignant potential (GRAVHOLT [7]), dysgerminoma, or non-tumoral androgen-producing lesions (SIEGEL [8]). All these pathological aspects are dependent also on the actual Y chromosome distribution in tissues.

In this study, we attempted to determine whether the QF-PCR (quantitative fluorescence polymerase chain reaction) technologies for chromosome 13, 18, 21, X and Y aneuploidies detection can identify sex chromosomes mosaicism. We present a sex chromosome mosaicism case with an abnormal predominant 45, X cell line and an additional also abnormal 47, XYY cell line previously reported by karyotype; also we describe five cases of prenatally detected sex chromosomes mosaicism.

QF-PCR based approach with selected STR markers for detection of chromosome 13, 18, 21 trisomies and sex chromosomes aneuploidies was introduced four years ago in the Romanian prenatal diagnosis field to overcome the most important disadvantages of classical testing procedure regarding the time consuming protocol, sample quantity and quality. It is considered a reliable, efficient, low cost and rapid method because the results are obtained within 24 hours after sampling and facilitates options in pregnancy. Regarding the level of mosaicism detectable by QF-PCR technique we found references claiming that 15% is the minimum level of detection in cases of triallelic trisomies and 20% in diallelic trisomies (DONAGHUE [12]).

2. Materials and Methods

We tested 2957 samples from pregnant women who approached our laboratory for prenatal diagnosis testing between 2008 and 2011. The samples were collected using amniocentesis or chorionic villus sampling procedures. A female patient suspected of having Turner syndrome based on ambiguous phenotype was tested too by combining different techniques and comparing the QF-PCR technique’s power of discrimination of different cell lines. The blood sample was collected by venipuncture in a vacutainer with EDTA.

For the prenatal samples we performed the DNA extraction with the DNA IQ™ System kit and the QIAamp® DNA Mini kit, according to the manufacturers’ recommendations (Promega and respectively Qiagen). The DNA elution was performed in 25–50 µl from which approximately 3–30 ng DNA were used in a QF-PCR multiplex based reaction. The blood sample was processed for genomic DNA extraction using Wizard® Genomic DNA Purification Kit according to the manufacturer recommendations (Promega, USA).

For diagnosis of chromosome 13, 18, 21, X and Y aneuploidies were used three different products: the Aneufast™ kit (Genomed Ltd, UK), the Devyser Compact (DevyserAB) and an “in house” product developed based on the published data of Kathy Mann, Erwin Petek and Barbara Perl (18). All three products were based on the quantitative fluorescent amplification principle of selected chromosome-specific short tandem repeats (STRs) and non-polymorphic markers. The QF-PCR products were analyzed by capillary electrophoresis on an ABI 310 Genetic Analyzer (Applied Biosystems) where the amplified products produce fluorescent peaks with specific arias and the final data were generated using GeneMapper ID v3.2 software (Applied Biosystems). The result interpretation is based on the peaks arias ratio. For normal heterozygous markers the alleles’ ratio should be within the range 0.8–1.4 and up to 1.5 if the alleles are separated by more than 24 pb. If the marker is homozygous, just one peak will appear in the electropherogram of the sample and the result will be non-informative because of the overlapping of the alleles (Table 1).

A trisomic marker can be diallelic or triallelic: the trisomic diallelic marker will present a ratio within the range 0.45–0.65 or 1.8–2.4 and the triallelic one a 1:1:1 ratio (Fig. 1). There is an intermediate interval between 1.4–1.8 and 0.65–0.8 that includes neither normal nor
Table 1  STR markers for the sex chromosomes included in the Aneufast™ kit (Genomed Ltd, UK), the Devyser Compact (DevyserAB) and Mann et al. method.

| Chromosome | Markers location | Aneufast | Devyser Compact | Mann et al. method |
|------------|------------------|---------|-----------------|-------------------|
| X and Y chr. Non-polymorphic markers |                   |         |                 |                   |
| Xp22.22/Yp11.2 | §               |         |                 |                   |
| Xp22.1-22.31/Yp11.2 | §               |         |                 |                   |
| Pseudoautosomal markers |                   |         |                 |                   |
| Xq21.3/Yp11.31 | §               |         |                 |                   |
| Xq21.31/Yp11.31 | §               |         |                 |                   |
| Xp22.3/Yp11.3 | §               |         |                 |                   |
| Xq22.3 | §               |         |                 |                   |
| Xq11.2-12 | §               |         |                 |                   |
| Xq12-12.33 | §               |         |                 |                   |
| Xq13.1 | §               |         |                 |                   |
| Xq21.33 | §               |         | §               |                   |
| Xq26.1 | §               |         | §               |                   |
| Xq26.2 | §               |         | §               | § §              |
| Xq28 | §               |         | §               |                   |
| Y |                   |         | §               |                   |
| Yp11.2 | §               |         | §               |                   |
| Yp11.31 | §               |         | §               |                   |
| Yq11.223 | §               |         | §               |                   |
| X/7 |                   |         | §               |                   |
| Xq13/7q34 |                   |         | §               |                   |

Fig. 1  Allelic patterns determined by QF-PCR technique. A: Normal diallelic pattern; B: Noninformative pattern; C: Trisomic diallelic pattern; D: Trisomic triallelic pattern.

trisomic alleles. The result is considered inconclusive and a retesting is recommended. This inconclusive result may be due to a primer site polymorphism. Another observation is that ranges < 0.45 and > 2.4 involving the sex chromosomes indicates the presence of more than three copies of the marker.

3. Results

SCAs found among 2957 prenatal samples showed an incidence of 1 per 591 pregnancies. All SCAs were found in singleton pregnancies. No inconclusive results
or failure of karyotype results were obtained. No correlation of maternal age and the SCAs was found.

Applying the rapid QF-PCR technique for prenatal detection of trisomy 21, 18, 13 and sex chromosomes aneuploidies we detected five cases of sex chromosomes mosaicism. We excluded the maternal cell contamination by analyzing also a maternal sample. The QF-PCR test results were confirmed in two cases by the karyotype results and for another three cases the FISH (fluorescence in situ hybridization) test was available.

The first case analyzed is an amniotic fluid sample with 45, X/47, XXX karyotype result. The QF-PCR result showed unbalanced peak ratios for all X specific markers and for the DXYS267 pseudoautosomal marker (Xq21.31/Yp11.31) (Mann et al.). The Y specific markers were absent.

The second case presented four uninformative X specific markers, five X specific markers exhibited unbalanced peak ratios and the Y specific markers were absent. In this case was available also the FISH test that showed a 45, X result.

The third case presented five unbalanced markers, three X specific markers and two pseudoautosomal markers (Xp22.32/Yp11.3 and Xp28/Yq). The Y specific markers were absent. In this case was available also the FISH test that showed a 46, XX/47, XXX result (12%/88%).

For the fourth case the only available result was released by QF-PCR test: the amelogenin nonpolymorphic marker exhibited a 3:1 peak ratio meaning that there were three X chromosomes and one Y chromosome; six X specific markers were noninformative, the pseudoautosomal marker exhibited a 1:1 peak ratio and the Y specific markers were present. We conclude that this pattern is in accordance with a sex chromosomes mosaicism case.

The fifth mosaicism was a 45, X/46, XY, del (Y) case. The QF-PCR showed a 46, XY pattern for six markers; the pseudoautosomal marker DXYS267 had an abnormal 0.61 ratio and the Y specific marker DYS448 (Yq11.223) was absent.

Another challenging case was the woman suspected having Turner syndrome. The patient addressed to our laboratory for complete chromosome analysis by karyotype. She was 32-year-old and presented primary amenorrhea. She exhibited normal stature with skeletal abnormalities: dorsal kyphosis and scoliosis, genu varum, cubitus valgus, shortening of the 4th metacarpals and shortening of the metatarsals; also she presented low hairline at the back of the neck.

We analyzed the Aneufast’s kit basic markers sets and also the back-up markers set for sex chromosomes aneuploidies detection. Normal results for chromosomes 13, 18 and 21 were found. Regarding the sex chromosomes we analyzed 9 STR markers including one chromosome Y specific marker — SRY, one non-polymorphic marker present on both sex chromosomes — AMXY, two pseudoautosomal markers — X22 (PAR2), DXYS218 (PAR1) and five X chromosome specific markers — HPRT, DXS6803, DXS6809, DXS8377, SBMA.

The AMXY marker showed two fluorescence peaks, one for X and respectively one for Y chromosome. The two allele peak ratio (X:22827/Y:8322) was 2,74 and showed a male unbalanced result with more than 3 copies interpreted as three X chromosomes and one Y — 3:1 (XXXXY genotype). The presence of Y material was sustained also by the SRY marker included in the QF-PCR kit. The Y chromosome integrity was accomplished by the analysis of 20 STS fragments in the Y chromosome structure with the Promega’s Y Chromosome Deletion Detection System kit. We detected normal signals for all 20 STS sequences included in the kit.

The other seven STR markers showed uninformative results. The karyotype result being 45, X/47, XXY we conclude that QF-PCR based technique was able to detect the abnormal status till to a point where we couldn’t release the assumption that this is a sex chromosome mosaicism case.
4. Discussion

The QF-PCR based technique is considered a good method in detecting mosaicism cases when the second cell line exceed the minimum level of 15% in cases of triallelic trisomies and 20% in diallelic trisomies (DONAGHUE [12]). The proportion between the cells line in these six mosaicism cases exceeded 10% level of mosaicism.

We also want to point out that when performing the QF-PCR based test the final diagnosis should be taken with care in mosaics cases because is quite difficult to determine the type and ratio of various subpopulations of cells, sometimes a mosaic result can produce the same fluorescent peaks as a normal sample (CIRIGLIANO [13]). We recommend that the QF-PCR result should be confirmed by karyotype because in sex chromosome mosaicism cases we couldn’t establish the actual cell lines status.

QF-PCR technique was introduced in Romania four years ago and it was well received by medical specialists from prenatal diagnosis field thanks to advantages offered to the patients. The most important aspect is the relieving of maternal anxiety caused by prolonged period of time of fetal karyotyping technique which is significantly reduced by QF-PCR method that provide a diagnosis report in maximum 24 hours after sampling. QF-PCR assay is also efficient in detecting maternal cell contamination of fetal sample, submicroscopic duplication and somatic microsatelite mutation. In chromosomal mosaicism the rapid prenatally detection is highly necessary because this genetic status has variable phenotypic outcome and complicates genetic counseling. In our experience the QF-PCR assay is of great value considering the rapid release of the result and the 100% accuracy in detecting non-mosaic aneuploidies of chromosomes 13, 18, 21, X and Y. The data obtained from this study regarding the sex chromosomes mosaicism cases sustained also the efficiency of OF-PCR based approach with the remark that the minimum level of second cell line should above be the threshold of 15–20%.

At the moment the QF-PCR as a stand-alone prenatal test it’s preferred in carefully monitored pregnancies.
and we expect this year that the QF-PCR request to exceed the all others prenatal tests put together. We recognize that this approach has its limitations but its advantages makes the QF-PCR test the preferential choice in prenatal diagnosis in Romania.

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