Clinica Chimica Acta 445 (2015) 2–6

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Detection of sex chromosome aneuploidies using quantitative fluorescent PCR in the Hungarian population

Balint Nagy *, Richard Gyula Nagy, Levente Lazar, Julianna Schonleber, Csaba Papp, Janos Rigo Jr.

1st Department of Obstetrics and Gynecology, Semmelweis University, Budapest, Hungary

ARTICLE INFO

Article history:
Received 2 October 2014
Received in revised form 9 March 2015
Accepted 9 March 2015
Available online 16 March 2015

Keywords:
Quantitative fluorescent-PCR
Sex chromosomes
Small tandem repeats
Aneuploidies
Prenatal diagnosis

ABSTRACT

Background: Aneuploidies are the most frequent chromosomal abnormalities at birth. Autosomal aneuploidies cause serious malformations like trisomy 21, trisomy 18 and trisomy 13. However sex chromosome aneuploidies are causing less severe syndromes. For the detection of these aneuploidies, the "gold standard" method is the cytogenetic analysis of fetal cells, karyograms show all numerical and structural abnormalities, but it takes 2–4 weeks to get the reports. Molecular biological methods were developed to overcome the long culture time, thus, FISH and quantitative fluorescent PCR were introduced. In this work we show our experience with a commercial kit for the detection of sex chromosome aneuploidies.
Methods: We analyzed 20.173 amniotic fluid samples for the period of 2006–2013 in our department. A conventional cytogenetic analysis was performed on the samples. We checked the reliability of quantitative fluorescent PCR and DNA fragment analysis on those samples where sex chromosomal aneuploidy was diagnosed.
Results: From the 20.173 amniotic fluid samples we found 50 samples with sex chromosome aneuploidy. There were 19 samples showing 46, XO, 17 samples with 46, XXY, 9 samples with 47, XXX and 5 samples with 47, XYY karyotypes. The applied quantitative fluorescent PCR and DNA fragment analyses method are suitable to detect all abnormal sex chromosome aneuploidies.
Conclusions: Quantitative fluorescent PCR is a fast and reliable method for detection of sex chromosome aneuploidies.

1. Background

Conventional karyotyping is the "gold standard" method for the prenatal detection of numerical and structural chromosomal abnormalities in diagnostic centers. The culture of the amniotic fluid cells takes time and the reporting lasts about 2–3 weeks. Alternative methods have been developed to solve this problem. Fluorescence *in situ* hybridization (FISH) was developed in 1970s, and the first centromerspecific probes were used for the detection of the most common trisomies from 1990 [1–3]. FISH probes are expensive and the method is labor intensive, whereas faster and cheaper methods such as quantitative fluorescent PCR and DNS fragment analysis (QF-PCR) were introduced in 1993 [4]. These two methods give information according to the designed probes or primer systems but not for the whole chromosomal set. Array comparative genomic hybridization (aCGH) method was introduced about ten years ago which gives information about all chromosomes with high resolution, which is better from the conventional karyotyping [5]. In the past few years the next generation sequencing and the use of massive parallel sequencing made possible the non-invasive detection of the most common trisomies. Non-invasive prenatal testing using fetal DNA from maternal plasma became very popular lately. Several tests showed that these are highly accurate for fetal trisomy evaluation in high risk pregnancies [6–9].

Nowadays the most commonly used tests in the routine prenatal diagnostic testing centers are karyotyping, QF-PCR and FISH. We started the prenatal genetic diagnosis of chromosomal abnormalities in our laboratory with karyotyping in 1991 and QF-PCR was introduced in 1997 [10,11]. This method makes reliable detection of the most common autosomal and sex chromosome trisomies, while the detection of Turner syndrome, and XXX syndrome was problematic. Recently new commercial tests were developed to solve this problem. We decided to check out the accuracy of one of these commercially available tests on our samples collected in the last 8 years.

2. Materials and methods

21.173 amniotic fluid samples were collected at the 16–20th gestational weeks during the last 8 years (from January 1st 2006, until

* Corresponding author at: 1088 Budapest, Baross u. 27, Hungary.
E-mail address: nagy.balint@noi1.sote.hu (B. Nagy).

http://dx.doi.org/10.1016/j.cca.2015.03.009
0009-8981/

Table 1
The diagnosed numerical chromosomal aneuploidies from 2006 to 2013.

Year	Number of amniocentesis	Autosomal numerical aneuploidy			X and Y chromosome numerical aneuploidy			
		Trisomy 21	Trisomy 18	Trisomy 13	XO	XXY	XXX	XYY
2006	2554	22	6	4	2	1	0	0
2007	2981	24	7	1	3	3	2	0
2008	3146	22	7	2	4	3	1	0
2009	2858	28	9	3	3	2	1	0
2010	2279	20	8	2	4	0	1	1
2011	2604	31	4	5	1	2	1	1
2012	2047	21	7	2	1	6	0	2
2013	1704	24	12	3	1	0	3	1
	20.173	192	60	22	19	17	9	5

December 31st 2013) in our department (Table 1). All patients were informed and they signed a consent.

2.1. Karyotyping

Amniotic fluid centrifugation and culture were performed immediately after sample was obtained. Briefly, 10 ml of amniotic fluid samples were centrifuged at 1200 rpm for 10 min to obtain amniocytes, and the supernatant was discarded. Cells were cultured in Chang Medium D (Irvine Scientific, Ireland) for 10–14 days [12]. Conventional cytogenetic analysis was performed on all samples by using Lucia automatic evaluation system (Prague, Czech Republic).

2.2. DNA isolation

DNA was isolated from 1.5 ml amniotic fluid samples by using High Pure PCR Template Preparation kit (Roche, Penzberg, Germany) according to the manufacturer's instructions [13].

2.3. Quantitative fluorescent PCR and DNA fragment analyses (QF-PCR)

QF-PCR was performed on those selected samples where we detected sex chromosome related numerical abnormality. We used the Chromoquant QF-PCR STaR kit according to the manufacturer's instructions (CyberGene AB, Stockholm, Sweden). This kit contains small tandem repeat (STR) sequences for five chromosomes, 4 STRs for chromosome X, 3 STRs for chromosome Y, 6 STRs for chromosome 21, 5 STRs for chromosome 18 and 5 STRs for chromosome 13. PCR was performed on an Applied Biosystems 9800 (Perkin Elmer, USA) thermal cycler. The obtained PCR products were analyzed on an Applied Biosystems Genetic Analyzer 3130 (ABI, USA) with a molecular weight standard ABI LIZ 500 (ABI, USA). The analyzed fragments were divided to normal euploid or triploid according to the peak ratios (euploid > 0.8 < 1.4, while triploid >1.8; or < 0.65).

3. Results

During the past 8 years we performed 20.173 amniocenteses in our department and we found 274 fetuses with autosomal and 50 fetuses with sex chromosome aneuploidies (Table 1). We checked out the reliability using a commercially available QF-PCR kit for detection of sex chromosome aneuploidies on these samples. We found 19 samples with 45, XO, 17 samples with 47, XXY, 9 samples with 47, XXX and 5 samples with 47, XYY karyotypes (Table 1). Fig. 1 shows a typical electrophoretogram of a sample with 45, XO karyotype. The TAF9B peaks (3p24.2/Xq13.1–q21.1) are showing 1:0.5 ratio. Fig. 2 is an electrophoretogram of a sample having a 47, XXY karyotype. The AMEL gene peaks are showing 1:0.5 ratio. Fig. 3 shows a sample with 47, XYY karyotype. The AMEL gene peaks are present in 1:2 ratio. Fig. 4 is a typical electrophoretogram of a sample having a 47, XXX karyotype. All X chromosome related marker shows 1:1:1 or 1:0.5 or 0.5:1 ratio. The QF-PCR kit is able to detect all observed sex chromosomal aneuploidies.

Based on our results the occurrence of 45, XO is 0.0009; 47, XXY is 0.0008; 47, XXX is 0.0004 and 47, XYY is 0.00002 in the Hungarian population.

4. Discussion

The most common numerical chromosomal abnormalities observed in the liveborns are trisomy 21, 18, 13, and sex chromosomes [14–16]. The most widely used method for prenatal detection for these is the cytogenetic analyses of fetal cells obtained by amniocentesis or

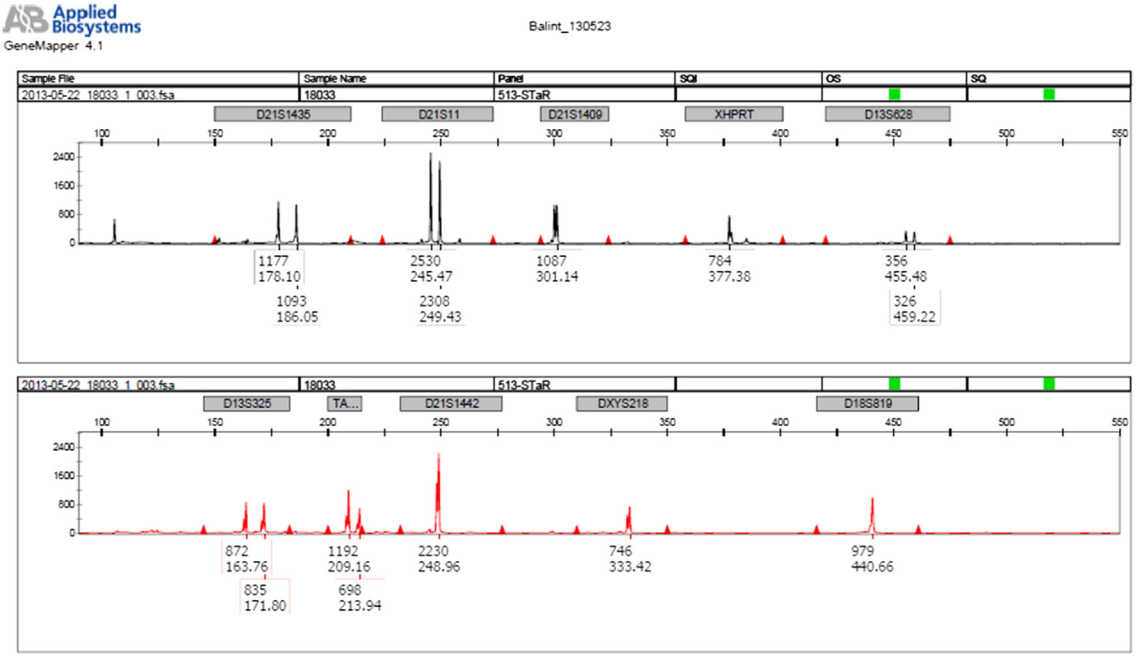

Fig. 1. Electrophoretogram of a fetus with 45, XO karyotype. The AMEL, DXS6854, DX6803, X22, XHPRT, DXYS218 markers are present with one peak. The SRY marker does not show any signal, while TAF9B marker shows up with 1:0.5 ratio, the GT10_STS47 signal is also missing.