CASE REPORT

Prenatal diagnosis of i(18q) and dup(18q) cases by quantitative fluorescent PCR

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SUMMARY

Particular sonographic fetal malformations are common in chromosome 18 aberrations, requiring invasive prenatal tests to confirm the diagnosis. Karyotyping is the gold standard assay in these cases, although it is a high complexity, expensive and approximately 2 weeks turnaround time test. On the contrary, quantitative fluorescent PCR is considered an accurate, simple, low cost and rapid assay, particularly useful for the diagnosis of aneuploidies of chromosomes 13, 18 and 21 and for the detection of maternal cell contamination of the sample. Clinical presentation of two cases of rare chromosome 18 defects, diagnosed using both techniques. One case was an isochromosome and the other was a partial duplication. Quantitative fluorescent PCR was an invaluable tool for the cytogenetics laboratory

BACKGROUND

Although trisomy 18 (Edwards syndrome) is relatively common, isochromosome 18q as well as duplication 18q are uncommon cytogenetic mutations that have many phenotypic features in common with trisomy 18. The incidence of trisomy 18 is estimated at around 1/6000 and 1/8000 births.¹ Isochromosomes can be formed by different pathways. Isochromosomes contain a mirror duplication of one chromosome arm. An isochromosome for the long arm (q) is a chromosome with two long arm and no short arm. A duplication of part of the long-arm entitles a partial trisomy of the chromosome affected. For duplications of autosomal segments, the degree of growth and mental retardation and the incidence of malformations usually correlate positively with the size of the duplicated segment up to a size which, except for the few autosomal trisomies that sometimes survive to term (for chromosomes 13, 18 and 21), is incompatible with life. Both mutations involve partial aneuploidy without changing the normal chromosome number.²

We described two de novo cases prenatally diagnosed by conventional cytogenetics and by quantitative fluorescent PCR (QF-PCR). This later technique has many advantages over fetal karyotype diagnosis, since it is a rapid, simple and accurate prenatal diagnostic test. It allows the detection of the great majority of clinically important chromosomal pathologies. The automation of the procedure easily allows a single operator to handle up to 40 samples at the same time and the results made available 24 h later, at a very low cost.³

CASE PRESENTATION

Case 1

A pregnant woman had an amniocentesis at 29 weeks of gestation according to last menstrual period (LMP) and 27 weeks of gestation according to ultrasound (US) due to a fetal US showing hypoplastic left heart syndrome, mild hydrops and intra-uterine growth restriction.

Cytogenetic studies

Our cytogenetic assays are accredited (ISO/IEC 17025 : 2005). Three independent amniotic fluid open cultures were set up that were mass harvested. The fetal karyotype obtained (figure 1) was 46,XX,i(18)(q10). In this case, the female fetus had trisomy of the long arm (q) of 1 of her chromosomes 18 due to the isochromosome and at the same time, she had monosomy of the short arm (p), since this arm is lost in the isochromosome formation. Both parental karyotypes were normal.

QF-PCR study

It was performed as validated in our laboratory⁴ according to professional guidelines,⁵ using an ABI 3130 Genetic Analyzer (Applied Biosystems, Carlsbad, California, USA) and the results of the electrophoretogram showed trisomy for the short tandem repeats (STR) tested corresponding to the long arm or 18q and monosomy for the STR located in the short arm of chromosome 18 (figure 2 and table 1).

A female baby was born by caesarean section at 38 weeks of gestation, at term but small for gestational age, height 40 cm and weight 2270 g. Apgar scores were 4–4–6. She had microcephaly, micrognathia, low set ears, short neck, cleft palate, clenched fingers, rocker bottom feet and congenital cardiopathy with cyanosis and dyspnoea. She died the day after due to cardiac failure and the autopsy revealed balanced atrioventricular canal defect and left ventricle hypoplasia.

Case 2

A pregnant woman had an amniocentesis at 24 weeks of gestation (LMP and US), due to a fetal US showing neural tube defect, hydrocephaly and cardiopathy.

Cytogenetic studies

Two independent amniotic fluid open cultures were set up that were mass harvested. The fetal karyotype (figure 3) was mos46,XX,dup(18)(q21→qter)/46,XX. This means that the female fetus had two

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Figure 1 Partial karyotype and ideograms showing both chromosomes 18, the isochromosome i(18)(q10) is on the left side.

cell lines, one cell line had a partial trisomy of the long arm of one chromosome 18 due to a segmental duplication, and the other cell line had a normal female karyotype. Both parental karyotypes were normal.

QF-PCR study

It was performed as described and the results of the electrophoretogram showed triplication of the STR located on the long arm of chromosome 18 and normal results for the STR of 18p (figure 4 and table 2).

Table 1 Markers and peak sizes in trisomy 18q due to i(18q)

N°	Marker	Localisation	Allelic range (bp)	Size	Area
1	D18S391	18p11.31	140-180	154.9	1896
2	D18S380	18q22.3	160-200	179.8	1970
3	D18S380	18q22.3	160-200	184.7	4040
4	D18S386	18q21.1	330-400	355.3	2934
5	D18S386	18q21.1	330-400	369.4	2541
6	D18S386	18q21.1	330-400	384.2	2250

The woman had a caesarean delivery due to premature rupture of membranes. A female baby was born alive, weight 3 455 g, height 46 cm, head circumference 53 cm. She was classified as a preterm baby adequate for gestational age. Apgar at first minute and at 5 min was 2. She had several malformations, retarded respiratory efforts, bradycardia and died 45 min later. The autopsy findings were: bilateral severe hydrocephaly, holoprosencephaly, polymicrogyria, intact myelomeningocele, left pulmonary isomerism, left pulmonary artery hypoplasia, left ventricle hypoplasia, single dilated right atrium, patent arterial duct, absence of mitral valve, malformation of hepatic lobulation, gallbladder agenesis, ring pancreas, left ureter hypoplasia, radial aplasia, arthrogryposis, chronic passive splenic congestion, pulmonary congestion, extramedullary haematopoiesis and chronic passive hepatic congestion.

DISCUSSION

In trisomy 18 cases intrauterine death occurs in more than 95% of fetuses with this chromosome anomaly. For unknown reasons, the rate of survival is higher in females than in males, leading to a female predominance among live-born trisomy 18 infants,¹ as it was the case in this report. One of the hypotheses for this approximately 5% survival rate, is the normal karyotype mosaic status in some areas of the placenta in aneuploidy cases, leading to an improved fetal blood supply in contrast with fully trisomic placentas.² Our mosaic case might thus be a true mosaic or might reflect the presence of normal karyotype

Figure 2 Quantitative fluorescent PCR showing trisomy 18q due to i(18q). Trisomy is observed as a diallelic trisomic pattern (1 : 2) of the marker D18S380, and a triallelic trisomic pattern (1 : 1 : 1) of the marker D18S386. i(18q) is observed as a monoallelic pattern in D18S391 (located in 18p), showing the absence of alleles in the p arm of one of the chromosomes 18.

Figure 3 Partial karyotype and ideograms showing both chromosomes 18, the partial duplication chromosome is on the right side.

placental cells growing in culture and ‘contaminating’ it. Unfortunately we had not the opportunity of repeating the chromosome studies in these newborns.

Another important issue is the ever growing possibility of prenatal diagnosis at any maternal age, independent of biochemical prenatal screening results, due to the abundance of sonographic abnormalities present in trisomy 18 and trisomy 18-like cases, as our report cases show (table 3).

All the listed structural abnormalities can be detected when targeted genetic sonography is performed by experienced personnel at 19–20 weeks’ gestation.⁶ The detection rate of the US scan at 18–21 weeks to detect trisomy 18 in a series of 69 women was 100%, and 92.7% in 14 or less weeks of pregnancy. A total of 80% and 87% of fetuses had two or more US abnormalities detected in the ≤ 14 and 18–21 weeks anomaly scans, respectively.⁷ Many other reports support the conclusion that in experienced hands, the sensitivity of detecting fetal trisomy 18 on prenatal sonography is 100%, and all cases will have multiple anomalies visualised.^{8–15}

The trisomy 18 phenotype appears to be associated with the presence of three copies of two critical regions, in the q arm, one proximal (18q12.1→q21.2) and one distal (18q22.3→qter), which may work in cooperation to produce the typical trisomy 18 phenotype. Severe mental retardation in these patients may

Table 2 Markers and peak sizes in dup (18) (q21→qter)

N°	Marker	Localisation	Allelic range (bp)	Size	Area
1	D18S391	18p11.31	140-180	154.9	15 332
2	D18S391	18p11.31	140-180	159.3	13 886
3	D18S380	18q22.3	160-200	177.8	6220
4	D18S380	18q22.3	160-200	181.3	6175
5	D18S380	18q22.3	160-200	184.6	6644
6	D18S386	18q21.1	330-400	363	6597
7	D18S386	18q21.1	330-400	365.9	12 767

be associated with trisomy of the proximal critical region.¹⁶ Our second case exhibits duplication of the distal critical region, in similar cases the clinical picture is reminiscent of trisomy 18, however, dysmorphisms and malformations are milder and life expectancy is enhanced.² Our case is inconsistent with these reports, since the fetus showed many malformations, including those related to trisomy 18.

In the isochromosome cases, the fetal abnormalities related to 18q duplication prevail.² Consistent with the reported clinical picture, our patient reveals hypoplastic left heart, intrauterine growth restriction, facial dysmorphism, overlapping fingers and cleft lip.

Conventional fetal cytogenetics has been the gold standard in the prenatal diagnosis of chromosomal defects. However, this test might fail to give results due to culture failure, the sample might be contaminated with maternal cells, aberrations of less than 5 Mb are undetectable, the turnaround time is about 2 weeks, and many women experience anxiety during this waiting time for results. Fluorescent in situ hybridisation is a rapid test since it does not necessarily require metaphases but cells in interphase; however, it is a cumbersome and expensive technique. On the other hand, QF-PCR is also a rapid molecular test; it is very accurate, detects maternal cells contamination and the cost is very low. QF-PCR has become an invaluable tool in the cytogenetics laboratory.

Figure 4 Quantitative fluorescent PCR showing a partial trisomy of 18q. Duplication is observed as a triallelic trisomic pattern (1 : 1 : 1) of the marker D18S380, and a diallelic trisomic pattern (1 : 2) of the marker D18S386. Marker D18S391 (located in 18p), shows a normal diallelic pattern.

Table 3 Perinatal and sonogram examination abnormal findings⁶

Features of trisomy 18	Dup(18)(q) case	Iso(18)(q) case
Increased maternal age	19 years	32 years
Intrauterine growth restriction	—	+
Polyhydramnios	—	—
Cardiac malformations	+	+
Strawberry-shaped skull	—	—
Microcephaly	—	+
Abnormal profile	—	—
Micrognathia	—	+
Cleft lip/palate	—	+
Low set ears	—	+
Cystic hygromas	—	—
Neural tube defects	+	—
Ventriculomegaly	+	—
Agenesis of the corpus callosum	—	—
Bilateral choroid plexus cysts	—	—
Cerebellar hypoplasia	—	—
Dandy-Walker syndrome	—	—
Short ear length	—	—
Holoprosencephaly	+	—
Underdeveloped brain	+	—
Hypotelorism	—	—
Increased nuchal fold	—	—
Renal and genital abnormalities	+	—
Pancreas anomalies	+	—
Overlapping fingers	—	+
Polydactyly	—	—
Rocker bottom feet	—	+
Radial aplasia/hypoplasia	+	—
Clubfeet	—	—
Omphalocele	—	—
Arthrogryposis or contractures	+	—
Diaphragmatic hernia	—	—
Hydrops	—	+
Pleural effusions	—	—
Tracheoesophageal fistula	—	—
Absent stomach	—	—
Echogenic bowel	—	—
Cystic umbilical cord	—	—
Single umbilical artery	—	—

Learning points

- Although trisomy 18 (Edwards syndrome) is relatively common, isochromosome 18q as well as duplication 18q are uncommon cytogenetic mutations which have many phenotypic features in common with trisomy 18.
- Conventional fetal cytogenetics has been the gold standard in the prenatal diagnosis of chromosomal defects. However, this test might fail to give results due to culture failure, the sample might be contaminated with maternal cells, aberrations of less than 5 Mb are undetectable, the turnaround time is about 2 weeks, and many women experience anxiety during this waiting time for results.
- Fluorescent in situ hybridisation is a rapid test since it does not necessarily require metaphases but cells in interphase; however, it is a cumbersome and expensive technique. On the other hand, QF-PCR is also a rapid molecular test, it is simple, very accurate, detects maternal cells contamination and the cost is very low.

Contributors All the authors participated sufficiently in the intellectual content, the analysis of information, and the writing of the manuscript, to take public responsibility for it. All authors reviewed the final version of the manuscript.

Competing interests None.

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