
QF-PCR as a Rapid Technique to be Introduced into Routine Prenatal Cytogenetic Diagnosis

Thesis

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List of contents

Introduction and aim of work	1
I. The cell division	4
A. Division of somatic cells	4
1. First stage (Gap 1 stage)	5
2. Second stage (synthesis stage)	5
3. Third stage (Gap 2 stage)	5
4. Final stage (Mitosis)	5
B. Division of gametics cells	7
1. Meiosis I	7
2. Meiosis II	8
Spermatogenesis and oogenesis	9
1. Spermatogenesis.....	9
2. Oogenesis	10
Medical relevance of meiosis and mitosis	11
II. Chromosomal abnormalities	12
A. structural abnormalities	12
1) Unbalanced abnormalities	12
2) Balanced chromosomal abnormalities.....	13
B. Numerical chromosomal abnormalities	14
Aneuploidy	15
Mechanism and etiology of aneuploidies	15
A. Autosomal aneuploidies	17
1. Chromosome 21	17
2. Chromosome 18	20
3. Chromosome 13	21
B. Sex chromosome aneuploidies	22
1. X chromosome	23
2. Y chromosome	24
Mosaicism	25
III. The human DNA	27
Structure of the DNA	28
A. Primary structure	28
B. Secondary structure	29
Replication of the DNA	30
DNA polymorphism and microsatellites	31
Selection of the STR markers	34
IV. Prenatal diagnosis of aneuploidies	37
Methods used in prenatal diagnosis	38
A. Non-invasive methods	39
1. Biochemical markers	39
a. Maternal serum alfa-feto protein	40
b. Pregnancy associated plasma protein	40
c. Inhibin A	41
d. Human chorionic gonadotropin	42
e. Unconjugated estriol	42
2. Ultrasound markers	43

a. Nuchal translucency	43
b. Absence or hypoplasia of fetal nasal bone	44
c. Echogenic bowel	44
d. Short long bones	45
e. Echogenic intracardiac foci	45
f. Mild pyelectasis	47
Protocols of screening	47
3. Fetal DNA in maternal blood	48
B. Invasive methods	50
1. Amniocentesis	50
2. Chorionic villous sampling	50
3. Cordocentesis	51
Traditional cytogenetic diagnosis	52
1. G-banding	52
2. Q-banding	53
3. R-banding	54
4. T-banding	54
5. Cd-staining	55
6. NOR-staining	55
7. High-resolution banding	56
Molecular cytogenetic diagnosis.....	57
1. Fluorescent in situ hybridization	57
2. Polymerase chain reaction	58
a) Multiplex ligation-dependent probe amplification	59
b) Multiplex amplification probe hybridization	59
c) Allele quantification	60
d) Real time quantitative PCR	61
e) Quantitative fluorescence PCR	62
V. Quantitative fluorescent PCR.....	63
Introduction	63
Results patterns	64
QF-PCR in the prenatal diagnosis of abnormalities.....	68
Diagnosis of autosomal aneuploidies	68
Diagnosis of sex chromosomal aneuploidies	72
Potential problems when using QF-PCR	74
1) Fetal mosaicism	74
2) Maternal cell contamination	76
3) Informative polymorphism	77
4) Stuttering	79
5) Special technical requirements	79
QF-PCR versus FISH	80
Subjects and Methods	82
Results	93
Discussion	115
Summary	126
Conclusion & Recommendations	128
References	130
Arabic summary	

List of tables

Table No.	Table title	Page No.
Table of Review		
1	Screening protocols of aneuploidy.....	48
2	Markers used in QF-PCR.....	70
3	Comparison between the three most commonly used techniques in prenatal diagnosis of chromosomal aneuploidy.....	81
Tables of Materials and Methods		
4	Markers used in the Aneufast kit for chromosomal aneuploidy detection	84
5	Markers included in each of the six sets.....	86
Table of Results		
6	Age distribution between the patients.....	93
7	Different causes of patients' referral.....	94
8	Different gestational age enrolled.....	95
9	Different culture times and the corresponding number of samples.....	96
10	Number of homozygous, heterozygous and heterozygous peaks	114

List of Figures

Fig. No.	Title	Page No.
1.	The cell cycle.....	4
2.	Schematic representation of mitosis.....	6
3.	Schematic representation of meiosis I.....	8
4.	Schematic representation of meiosis II.....	9
5.	Spermatogenesis and oogenesis.....	11
6.	Structural rearrangements of chromosomes.....	15
7.	Chromosome 21.....	18
8.	Chromosome 18.....	21
9.	Chromosome 13.....	22
10.	X chromosome.....	24
11.	Y chromosome.....	25
12.	Chemical structures of the principal bases in nucleic acids.....	30
13.	DNA replication.....	31
14.	G-banding.....	53
15.	R-banding.....	54
16.	Nor-banding.....	56
17.	Diallelic and uninformative patterns.....	64
18.	Triallelic and diallelic patterns in trisomic subjects.....	65
19.	Trisomy 21 by QF-PCR.....	66
Figures of Results		
20.	Pie chart of comparison between different causes of patients' referral.....	95
21.	The culture time needed to get the cytogenetic result.....	97
22.	A metaphase of normal female.....	98
23.	A case of normal female karyotype.....	99
24.	A metaphase of normal male.....	100
25.	A normal male karyotype.....	101
26.	A metaphase of trisomy 21.....	102
27.	Trisomy 21 karyotype.....	103
28.	DNA concentration in different samples.....	104
29.	Normal female pattern by QF-PCR.....	106
30.	Normal male pattern by QF-PCR.....	108
31.	Trisomy 21 male pattern by QF-PCR.....	110
32.	Inconclusive results.....	112
33.	Extra markers done for the same sample.....	113

List of abbreviations

AFP	Alfa-fetoprotein
AMXY	Amelogenin locus on X and Y chromosomes
bp	Base pairs
CVS	Chorionic villous sampling
DNA	deoxyribonucleic acid
DSCR	Down syndrome critical region
E3	Unconjugated estriol
FISH	Fluorescent in situ hybridization
G banding	Giemsa banding
hCG	Human chorionic gonadotropin
IAM	infinite allele model
MAPH	Multiplex amplifiable probe hybridization
MBP	Mylein basic protein
MLPA	multiplex ligation-dependent probe amplification
MoM	Multiples of median
NOR-staining	Silver staining for nucleolar organizer regions
NT	Nuchal translucency
PAPP-A	Pregnancy associated plasma protein A
PCR	Polymerase chain reaction
Q- banding	Quinacrine banding
QF-PCR	quantitative fluorescent polymerase chain reaction assay
q-PCR	quantitative polymerase chain reaction
R- banding	Reverse banding
S1	Markers Multiplex set 1
S2	Marekers Multiplex set 2
SNP	Single-nucleotide polymorphism
STR	Short tandem repeats
T- banding	Telomere banding
Ta	Annealing temperature
β-hCG	Beta subunit-human chorionic gonadotropin

Introduction

Chromosomal aberrations are relatively uncommon, but because of their intractability they are among the most important causes of congenital malformation and mental handicap. They also contribute to many other problems including infertility, abnormal sexual development, and spontaneous abortion. This makes the diagnosis of these diseases before birth an aim of a lot of centers and physicians (<http://www.emro.who.int/Publications>).

The majority of chromosome abnormalities identified in prenatal samples are trisomy for chromosomes 13, 18 or 21 and sex chromosome aneuploidies. These are associated with the newborn phenotypes, Patau syndrome, Edwards syndrome and Down syndrome (trisomy 13, 18 and 21, respectively), and the less severe Turner (monosomy X) and Klinefelter (XXY) syndromes (*Mann et al., 2004*).

Prenatal diagnosis for women at increased risk for chromosome abnormalities is routinely undertaken for karyotype analysis. While standard cytogenetic studies detect structural and copy number changes of chromosomes, it requires prolonged cell culture resulting in average reporting times of about two to three weeks (*Rooney and Czepulkowski 2001*).

Rapid methods, such as quantitative fluorescent polymerase chain reaction assay (QF-PCR) by using small tandem repeat (STR) markers, were developed for prenatal detection of sex and aneuploidies involving chromosomes 21, 18 and 13. This method can relieve parental anxiety, and improve the quality of pregnancy management (*Mann et al., 2001*).

The use of QF-PCR analysis of short tandem repeats (STR) for the detection of aneuploidy was first reported by Mansfield (1993). It has

since been validated and successfully applied for the rapid diagnosis of prenatal aneuploidy by a number of European labs (*Cirigliano et al., 2001; Levett et al., 2001 and Mann et al., 2004*). The rapid result is issued to clinicians and is followed by full karyotype analysis in all cases where cultured cells are available. The technique has -been approved by the New York State Clinical Laboratory Evaluation Program (*Brown et al., 2006*). Another advantage of QF-PCR over other methods of prenatal diagnosis is that it is feasible on a few cells. Also, since the analysis can easily be automated, many samples can be processed at the same time (*Cirigliano et al., 2001*). Molecular cytogenetic testing by QF-PCR for pregnant women at increased risk of chromosome aneuploidy leads to rapid reassurance for those with normal results and earlier decisions on pregnancy management in the case of abnormality (*Ogilvie et al., 2005*).

Aim of the Work

In this study we aim to evaluate QF-PCR as a rapid technique to diagnose trisomy of chromosomes number 13, 18, 21 and sex chromosomes in comparison with the traditional karyotyping to be introduced into routine prenatal diagnosis.

I. The cell division

The understanding of the cell division is essential to understand cytogenetics and cytogenetic abnormalities resulting from errors during division. Two types of cell division are known: mitosis and meiosis. Mitosis is the division of somatic cells, while meiosis is a special type of division that occurs only in gametic cells (Gersen and Keagle, 2005).

A. Division of somatic cells

The average mammalian cell cycle lasts about 17-18 hours. It is the transition of a cell from one interphase through cell division and back to the interphase (Therman and Susman, 1993).

The cell cycle (Fig.1) is divided into four major stages variable in their length; the first three stages gap1 (G1), synthesis (S) and gap2 (G2) comprise the interphase. The fourth and final stage is mitosis (M) (Gersen and Keagle, 2005).

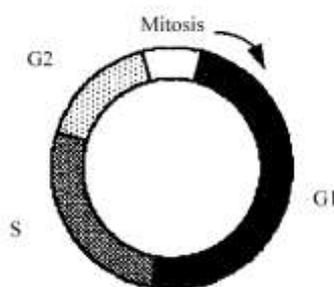


Fig (1): The cell cycle: G1, S, G2 and Mitosis (Gersen and Keagle, 2005).

- 1. First stage (Gap 1 stage, G1):** Chromosomes exist as single chromatids. Cells are metabolically active during this stage; this is the stage when the protein synthesis takes place and cell prepares for DNA synthesis (Barch et al., 1997).
- 2. Second stage (Synthesis stage, S):** It is when the DNA synthesis occurs. The DNA replicates itself and then forms chromosomes consisting of two identical chromatids (Therman and Susman, 1993).
- 3. Third stage (Gap 2 stage, G2):** The cell prepares to undergo the cell division. The completion of G2 represents the end of the interphase (Therman and Susman, 1993).
- 4. Final stage (Mitosis, M):** This stage lasts for only 1-2 hours in most mammalian cells. Mitosis is the process by which the cells reproduce themselves, creating two daughter cells that are genetically identical to one another and to the original parent cell (Gersen and Keagle, 2005).

It is further divided into stages (fig. 2):

a) Prophase: This is when chromosomes begin to coil, become more condensed and begin to become visible as discrete structures (Nussbaum et al., 2004).

b) Prometaphase: The nuclear membrane disappears and spindle fibers begin to appear. Chromosomes attach to the spindle fiber at their kinetochores (Nussbaum et al., 2004).

c) Metaphase: The mitotic spindle is completed, their centrioles divide, move to opposite poles and chromosomes line up on the equatorial plate. It is the metaphase chromosomes that are traditionally studied in cytogenetics (Murray and Hunt, 1993).

d) Anaphase: Centromeres divide longitudinally and the chromatids separate during this stage. Sister chromatids migrate to opposite poles (Miller and Therman, 2001).

e) Telophase: The chromosomes uncoil and become indistinguishable again, the nuclear membrane is reconstructed (Miller and Therman, 2001).

f) Cytokinesis: it is the process by which the cytoplasm cleaves.

g) Interphase: Following completion of cell division, the cell remains in this stage until the next cell division.

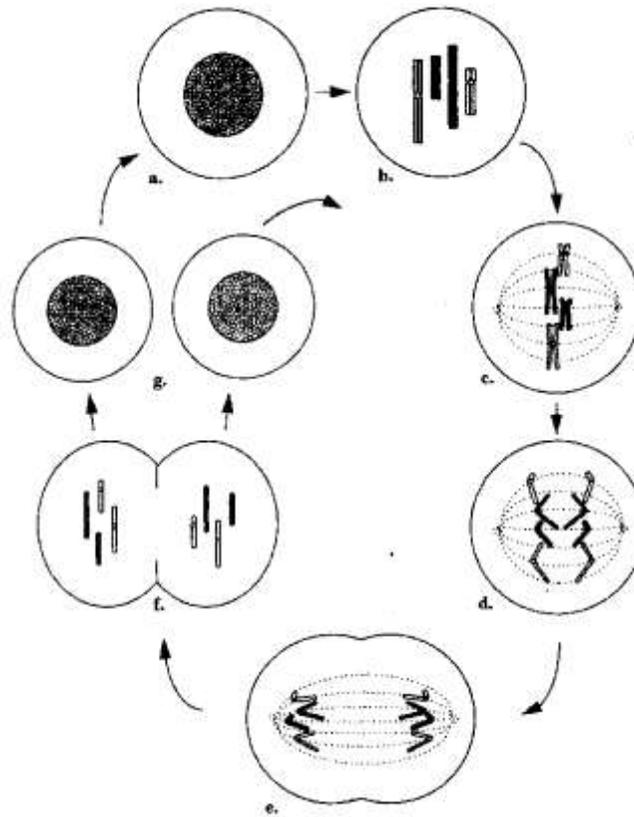


Fig. (2): Schematic representation of two pairs of chromosomes undergoing mitosis.

a: interphase, b: prophase, c: metaphase, d: anaphase, e: telophase, f: cytokinesis, g: interphase of the next cycle.

(Gersen and Keagle, 2005)

B. Division of gametic cells

Meiosis is the type of cell division by which the diploid gametic cells ($2n = 46$ chromosomes) of the germline give rise to haploid gametes ($n=23$ chromosomes). It consists of one round of DNA synthesis followed by two rounds of chromosome segregation and cell division. It occurs in the primary spermatocytes or primary oocytes (Nussbaum et al., 2004).

It is further divided into meiosis I and II

1. Meiosis I :

Throughout this stage the chromosomes condense and become shorter and thicker (Nussbaum et al., 2004).

It is a complicated process with the following stages: (Fig. 3)

- a) **Prophase I:** (subdivided into leptotene, zygotene, pachytene, diplotene, diakinesis) all through the prophase stage the chromosomes shorten and thicken (Handel, 1998).
- b) **Metaphase I:** The bivalents line up on the equatorial plate with their centromere randomly oriented toward opposite poles (Handel, 1998).
- c) **Anaphase I:** The centromeres separate and migrate to opposite poles (Handel, 1998).
- d) **Telophase I:** The haploid sets of chromosomes reach opposite poles and the cytoplasm divides. The result is two cells with 23 chromosomes (Handel, 1998).

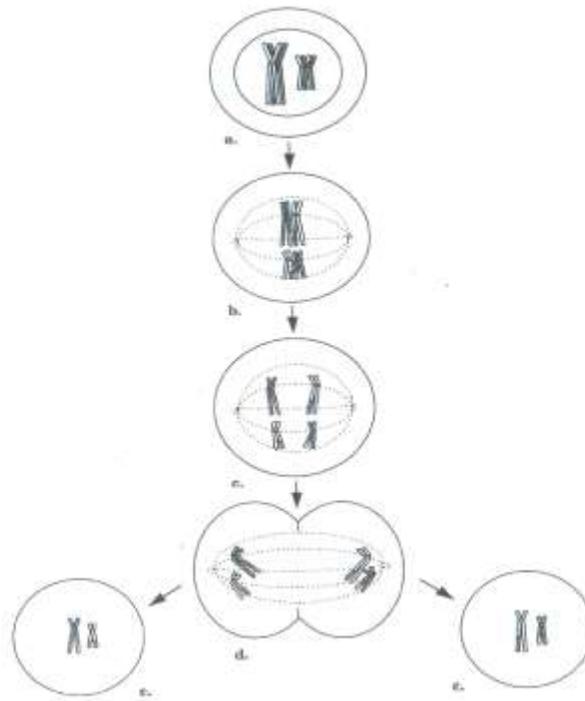


Fig. (3): schematic representation of chromosome pairs undergoing meiosis I.
 a: prophase I, b: metaphase I, c: anaphase I, d: telophase I, e: products of meiosis I.
 (Gersen and Keagle, 2005)

2. Meiosis II: (Fig.4)

The 23 chromosomes line up on the equatorial plate. The chromatids separate and move to opposite poles in anaphase II, and cytokinesis occurs in telophase II. The net result is four cells, each containing 23 chromosomes each consisting of a single chromatid. The effects of crossing-over and random assortment of homologs, each of the new cells differ genetically from one another and from the original cell (Gersen and Keagle, 2005).