



Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com



Original article

Prenatal diagnosis of cystic fibrosis: 10-years experience



Diagnostic prénatal de la mucoviscidose : 10 ans d'expérience

S. Hadj Fredj^{a,*}, F. Ouali^a, H. Siala^a, A. Bibi^a, R. Othmani^a, B. Dakhlaoui^a, F. Zouari^b,
 T. Messaoud^a

^a Biochemistry Laboratory, Bechir Hamza Children's hospital, Bab Saadoun, 1007 Tunis, Tunisia

^b Department C, Center of maternity and neonatology, Tunis, Tunisia

ARTICLE INFO

Article history:

Received 26 February 2015

Accepted 17 April 2015

Available online 20 May 2015

Keywords:

Prenatal diagnosis

Cystic fibrosis

At risk couples

Maternal cell contamination

ABSTRACT

Purpose. – We present in this study our 10 years experience in prenatal diagnosis of cystic fibrosis performed in the Tunisian population.

Patients and methods. – Based on family history, 40 Tunisian couples were selected for prenatal diagnosis. Fetal DNA was isolated from amniotic fluid collected by transabdominal amniocentesis or from chronic villi by transcervical chorionic villus sampling. The genetic analysis for cystic fibrosis mutations was performed by denaturing gradient gel electrophoresis and denaturing high-pressure liquid phase chromatography. We performed microsatellites analysis by capillary electrophoresis in order to verify the absence of maternal cell contamination.

Results. – Thirteen fetuses were affected, 21 were heterozygous carriers and 15 were healthy with two normal alleles of *CFTR* gene. Ten couples opted for therapeutic abortion. The microsatellites genotyping showed the absence of contamination of the fetal DNA by maternal DNA in 93.75%.

Conclusion. – Our diagnostic strategy provides rapid and reliable prenatal diagnosis at risk families of cystic fibrosis.

© 2015 Elsevier Masson SAS. All rights reserved.

R É S U M É

Objectif. – Nous présentons dans cette étude nos 10 ans d'expérience dans le diagnostic prénatal de la mucoviscidose dans la population tunisienne.

Patients et méthodes. – Basé sur l'histoire familiale, 40 couples à risque ont été sélectionnés pour le diagnostic prénatal. L'ADN fœtal a été isolé à partir du liquide amniotique recueilli par amniocentèse transabdominale ou de villosités chroniques par prélèvement de villosités choriales. L'analyse génétique des mutations mucoviscidiques a été effectuée par l'électrophorèse sur gel en gradient dénaturant et la chromatographie liquide à haute pression en conditions dénaturantes. Nous avons effectué une étude des microsatellites par électrophorèse capillaire afin de vérifier l'absence de contamination de l'ADN fœtal par les cellules maternelles.

Résultats. – L'étude moléculaire a montré 13 fœtus atteints, 21 porteurs sains et 15 normaux avec deux allèles normaux du gène *CFTR*. Onze couples ont opté pour l'avortement thérapeutique. L'analyse des microsatellites a montré l'absence de contamination par l'ADN maternel dans 93,75 %.

Conclusion. – Notre stratégie de diagnostic fournit un diagnostic prénatal pour les familles à risque de la mucoviscidose fiable et rapide.

© 2015 Elsevier Masson SAS. Tous droits réservés.

Mots clés :

Diagnostic prénatal

Mucoviscidose

Couples à risque

Contamination par les cellules maternelles

1. Introduction

Cystic fibrosis (CF) is the most common, inherited and life-shortening disease affecting children and adults in European-derived populations. It affects about 1 neonate in 3500 and

* Corresponding author.

E-mail address: hadjfredjsondess@yahoo.fr (S. Hadj Fredj).

approximately one individual in 30 is a CF carrier, with marked regional variations [1].

It results from mutations in the gene encoding the CF transmembrane conductance regulator (CFTR) protein, which regulates ion transport at epithelial surfaces, in particular in the airways, pancreatic ducts, intestines, bile ducts and sweat ducts [2]. More than 1900 mutations have so far been identified in the CFTR gene [3].

Due to remarkable progress in the field of molecular biology, rapid advances in the prenatal diagnosis (PND) of genetic defects are being made. PND of CF is currently recommended to those couples in which both are known to be carriers of a CF mutation. CF may also be revealed in fetuses with ultrasound digestive anomalies such as hyperechogenic bowel and dilatation, mostly during the second trimester of pregnancy.

In a pilot screening study for CF in Tunisia, we determined the most frequent mutations in our population; a panel of 17 mutations allowed us to identify about 90% of CF chromosomes [4,5]. We so characterized 420 CF families. When a CF carrier is identified, genetic counseling is offered and the partner is sequentially invited to undergo testing.

In this study, we report on our experience in prenatal diagnosis of cystic fibrosis in Tunisian population. We also investigated the informativeness of microsatellites analysis in differentiation of fetuses and excluding the maternal blood contamination in order to provide a correct genetic counseling.

2. Material and methods

2.1. Study population

From March 2003 to December 2014, based on family history, prenatal diagnosis of cystic fibrosis was performed on 49 fetuses from 40 unrelated families.

Samples analyzed for DPN were chorionic villus sample (CVS) in 10 cases and amniotic fluid in 39 cases.

A detailed description of operation flow, report time interval, risks of invasive sampling, complications and fetal loss rate, were explained clearly in the counseling process. Parents were informed about the status of the fetus after diagnosis, so that they could take the decision whether to continue the pregnancy or not.

2.2. Methods

DNA was extracted from blood cells from index and their parents by the salt precipitation methods [6]. The fetal DNA is extracted from amniotic fluid by addition of a lyses solution then subjected to a temperature of 95 °C for 10 minutes followed by 10 minutes on ice.

The chorionic villus (2–15 mg) are washed in sterile saline solution and then, the fetal tissue is dissected carefully to remove maternal tissue. DNA was extracted from the tissue/cell samples by a simple salting out technique after digestion with proteinase K at 55 °C.

2.3. Mutation analysis

The molecular study was first performed in the parents and the index. The fetus was studied when a mutation was identified in at least one parent.

As a first step, all DNA samples were screened for the F508del mutation by a heteroduplex assay in 10% polyacrylamide gel electrophoresis. Sample was further analyzed by DGGE [7] (denaturing gradient gel electrophoresis) for the exons 5, 11, 19, 20 and 21 and by DHPLC [8] (denaturing high-pressure liquid phase chromatography), on a Transgenomic WAVE DNA Fragment Analysis System (TransgenomicTM, Crewe, UK), for the remaining exons. The DNA samples that showed abnormal profiles were sequenced using the Big Dye terminator cycle sequencing kit (Perkin-Elmer, Applied Biosystems, Foster City, CA, USA) and analyzed on an ABI Prism 310 DNA sequencer (Perkin-Elmer, Applied Biosystems) according to the manufacturer's protocols. The sequencing data were analyzed with ABI DNA sequencing analysis software (Version 3.4.1). Each single stranded product was concentrated using a centri-sep columns (Applied Biosystems, Foster City, CA, USA).

2.4. Microsatellites analysis

Haplotypes of all samples from fetus and their parents were analyzed using polymorphic microsatellite markers. The markers used were D7S486, D8S285, IFN1,

D9S171, D12S391 and D21S226. Based on these genotypes, haplotypes were constructed and the fetal phenotypes were estimated.

PCR were performed with 200 ng of DNA, 1X Master Mix (Promega) and 10 pmol of each primer with Hex or Fam fluorescent in a total volume of 14 µL. Thirty-five PCR cycles for 1 min each at 90 °C, 57 °C and 72 °C were performed. Amplified products (1 µL) were mixed with 20 µL deionised formamide and 1 µL of 500 TAMRA Size standards (Applied Biosystems, CA, USA). Samples were denatured at 92 °C for 3 min, then injected in ABI Prism 310 (Applied Biosystems, CA, USA), and analysed by GeneScan analysis software version 3.1.2.

2.5. Mutation nomenclature

Nucleotide numbers are derived from cDNA CFTR sequences (GenBank accession no.NM_000492). Mutations are named according to the numbering used in the CFTR Mutation Database (<http://www.genet.sickkids.on.ca/cftr/>).

3. Results

Since 2003, a total of 49 pregnancies at CF risk were made in 40 families, one of whom had a twin pregnancy. These families with a first CF child were fully informative for direct DNA analysis performed by heteroduplex analysis, DGGE and DHPLC techniques. Ten different CFTR mutations were identified, including F508del (51.28%), E1104X (12.82%), N1303K (8.97%), G542X (8.97%), 711 + 1 G→T (6.41%), W1282X (5.12 %), R785X (1.28 %) and V754M (1.28%). Two novel mutations were detected in this study: 4268 + 2 T→G (2.56%) and 3729 delA insTCT (1.28%) located respectively.

Among the 49 tested fetuses, 13 were affected, 21 were heterozygous carriers and 15 were healthy with two normal alleles of CFTR gene (Table 1). Eleven couples have decided to stop pregnancy. Only two couples refused termination of pregnancy for ethical reasons.

We noted two fetal loss after prenatal sampling by transcervical chorionic villus. Maternal contamination was tested by microsatellites analysis when the fetus was diagnosed as a carrier. The used markers were D7S486, D8S285, IFN1, D9S171, D12S391 and D21S226. When one of the polymorphic alleles of the mother was absent in fetal DNA, the fetal sample was considered as free from maternal contamination.

The microsatellites genotyping results showed the presence of contamination with maternal DNA in 1 case from the 21 tested cases.

4. Discussion

The first possibility of prenatal diagnosis for cystic fibrosis has been proposed in 1980 by Brock [9] to couples at risk of 1 in 4. The test was based on the measurement of amniotic fluid at 17 weeks of gestation of alkaline phosphatase, enzymes from the brush border of the intestine, but this test has 10% false positives and 5% false negatives.

Table 1
Different genotypes finding in the 49 fetus analyzed.

Fetus genotype	Number	Percentage (%)
F508del/-	14	28.57
F508del/F508del	6	12.24
E1104X/-	3	6.12
N1303K/-	3	6.12
E1104X/N1303K	2	4.08
F508del/711 + 1 G→T	1	2.04
E1104X/E1104X	1	2.04
W1282X/W1282X	1	2.04
711 + 1 G→T/711 + 1 G→T	1	2.04
4268 + 2 T→G/4268 + 2 T→G	1	2.04
G542X/-	1	2.04
-/-	15	30.61

“-”: absence of mutation.

Download English Version:

<https://daneshyari.com/en/article/4135888>

Download Persian Version:

<https://daneshyari.com/article/4135888>

[Daneshyari.com](https://daneshyari.com)