

# Prenatal diagnosis in a cystic fibrosis family: a combined molecular strategy for a precise diagnosis

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## ABSTRACT

**Introduction.** The high genetic heterogeneity in populations with a wide spectrum of mutations in the CF transmembrane conductance regulator gene (*CFTR*), makes the detection of mutations a very hard and difficult task, thereby limiting the accurate diagnosis of the disease, mainly in patients with uncharacterized mutations. **Material and methods.** Molecular strategies, like targeted identification of the most frequent *CFTR* mutations in Mexican population combined with linkage analysis using markers, is very useful for carrier detection and for prenatal diagnosis in affected families with CF. In this paper we show that the combination of methodologies was a crucial alternative to reach a precise prenatal CF diagnosis. We documented CF diagnosis in a 14th-week fetus combining the screening of the most common mutations in Mexican population with linkage analysis of two extragenic polymorphisms (*XV2C/TaqI* and *KM19/PstI*). **Results.** We determined that the fetus inherited the P.G542X mutation from its mother and an unknown mutation from its father through the chromosomal phases analysis.

**Key words.** Cystic fibrosis. RFLPs. Haplotypes. *CFTR* mutations. Prenatal diagnosis.

## BACKGROUND

In Mexico we documented a total of 46 different mutations in 77% of chromosomes of 230 unrelated Mexican patients diagnosed with cystic fibrosis (CF, MIM# 219700).<sup>1</sup> These results show that Mexico has one of the widest spectrums of *CFTR* mutations

**Diagnóstico prenatal en una familia con fibrosis quística: combinación de estrategias moleculares para un diagnóstico preciso**

## RESUMEN

**Introducción.** La gran heterogeneidad genética en poblaciones con un amplio espectro de mutaciones en el gen regulador de la conductancia transmembranal de la fibrosis quística (*CFTR*) hace que la detección de mutaciones sea una tarea ardua y difícil, lo que limita el diagnóstico preciso de la enfermedad, principalmente en pacientes con mutaciones no caracterizadas. La combinación más frecuente, en la población mexicana, de estrategias moleculares (como la búsqueda dirigida de las mutaciones *CFTR*) junto con el análisis de ligamiento con marcadores es muy útil para la detección de portadores y diagnóstico prenatal en las familias afectadas con fibrosis quística. **Material y métodos.** En este trabajo se muestra que la combinación de metodologías es una alternativa fundamental para llegar a un diagnóstico prenatal preciso de fibrosis quística. Se documenta el diagnóstico prenatal de fibrosis quística en un producto de 14 semanas de gestación, combinando el tamizaje de las mutaciones más frecuentes en la población mexicana con el análisis de ligamiento de dos polimorfismos extragénicos (*XV2C/TaqI* y *KM19/PstI*). **Resultados.** Se determinó que el feto hereda la mutación G542X de su madre y una mutación desconocida de su padre a través del análisis de las fases de los cromosomas.

**Palabras clave.** Fibrosis quística. RFLPs. Haplotipos. Mutaciones *CFTR*. Diagnóstico prenatal.

worldwide. This great genetic heterogeneity makes the detection of mutations very burdensome, and limits their accurate diagnosis in patients with unknown mutations. In some cases, combination of strategies such as directed identification of the most common mutations<sup>2</sup> and linkage analysis using polymorphic markers is ideal.<sup>3</sup>

Apart from its low cost, the method is quick and easy to handle, and it is very suitable for both carrier detection and prenatal diagnosis. This work shows the usefulness of combining directed screening of the most common mutations in Mexican population with polymorphism analysis in prenatal diagnosis in a family with cystic fibrosis.

## MATERIAL AND METHODS

### Family

The index case (II-1) was a 9-years-old female with clinical diagnosis of CF. She was referred for genetic counseling from the Mexican Association of Cystic Fibrosis. The 36-year-old mother (I-1) was in the 14th week of her second pregnancy (II-2) (Figure 1A). Prenatal molecular diagnosis of this product was performed of the request of the mother.

### DNA samples

Genomic DNA was extracted from peripheral blood leukocytes of I-1, I-2 and II-1 and from the amniotic fluid cells (II-2) (Figure 1A), according to standard protocols (QIA-AMP DNA blood mini kit, Qiagen, Vienna, Austria).<sup>4</sup> This study was approved by the respective local ethics and research committees and all individuals signed an informed consent.<sup>5,6</sup>

### Molecular study

The most common mutations in the Mexican population (P.F508, del P.G542X, P.N1303K, P.I507 and S549N) were screened by PCR-mediated site-directed mutagenesis (PMS) in the index case and her parents as previously described by Orozco, *et al.*<sup>3</sup>

After screening the mutations, prenatal molecular diagnosis was performed by combining two strategies: directed mutation screening and linkage analysis using two extragenic Restriction Fragments Lengths Polymorphisms (RFLPs) (XV2C/*TaqI* and KM19/*PstI*).<sup>7,8</sup> The haplotypes derived from these polymorphisms were defined as:<sup>9</sup>

- A(X1-K1).
- B(X1-K2).
- C(X2-K1).
- D(X2-K2).

The chromosomal phases were determined by allele segregation analysis from the parents to children in combination with the mutation identified in the family.

## RESULTS

The analysis of the most common CF mutations in Mexican population showed that the index case is a compound heterozygous harboring P.G542X mutation inherited from her mother and an unknown mutation (X) inherited from her father (P.G542X/X) (Figure 1B). Prenatal diagnosis was performed from amniotic fluid, revealing a fetus carrying the P.G542X mutation. To identify if the fetus was bearing the unknown mutation, prenatal diagnosis was completed using a linkage analysis with two polymorphisms (KM19/*PstI* and XV2C/*TaqI*).

XV-2C/*Taq* polymorphism analysis was informative for the index case and her father (X1/X2), but not for her mother, who was homozygous for allele 1 (X1/X1). Since the mother was carrier of the P.G542X mutation, thus, uncharacterized CF mutation inherited from the father, linked to the X2 allele (Figure 1C). Molecular analysis of the DNA obtained from amniotic fluid showed that the fetus, besides of the P.G542X mutation, carried the X2 allele harboring the uncharacterized mutation inherited through its father (Figure 1C). So, the fetus was diagnosed with CF.

KM-19/*PstI* marker and haplotype analyses were not informative, since all family members were heterozygous for this polymorphism.

## DISCUSSION

In recent years, molecular diagnosis has become a very important component of timely and accurate diagnosis, as well as genetic counseling. In addition, it is the most precise approach for carrier detection and prenatal diagnosis in a wide variety of Mendelian diseases. In Mexico, a total of 46 different mutations affecting 77% of the CF chromosomes have been identified. Actually, only 3 mutations display a frequency > 2% and their identification permits the characterization of near to 54% of chromosomes.<sup>1</sup> Moreover, most alterations were found as private mutations, suggesting that some rare mutations remain unidentified yet.

Molecular diagnosis of cystic fibrosis becomes a very hard and difficult task in populations with a wide spectrum of *CFTR* mutations. However, when mutations cannot be identified, it could be improved combining a set of strategies.

Here, we report a prenatal diagnosis in a family with an affected CF girl, who was a compound heterozygous for P.G542X mutation and an unknown mutation. A molecular combined strategy allowed us the prenatal diagnosis in a 14th week fetus. After

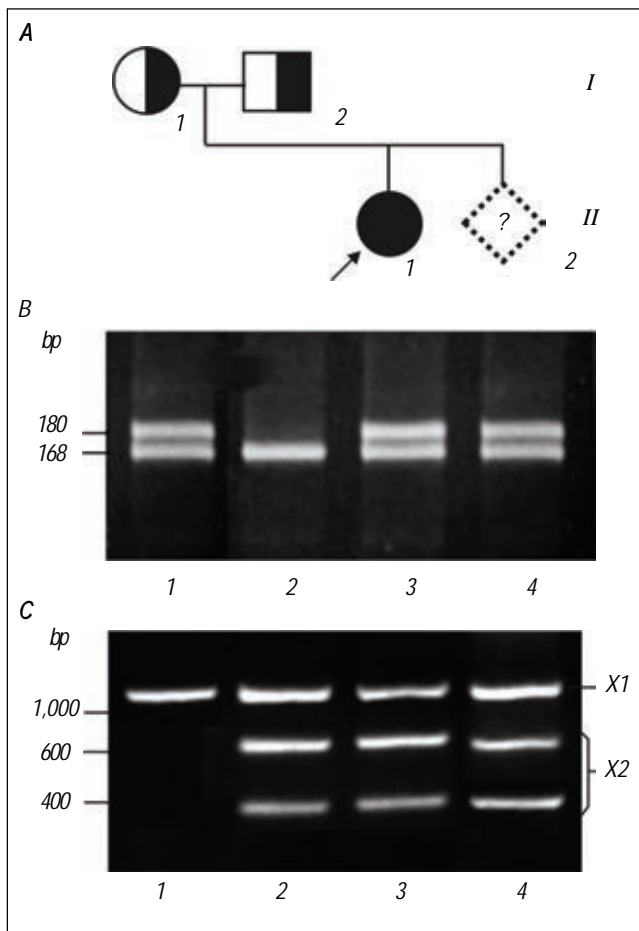


Figure 1. A. Pedigree of the family studied. B. Analysis of G542X mutation by PSM. The gel shows amplification products digested with enzyme *HphI*, which resolves normal alleles (band of 168 bp) and mutated alleles (band of 180 bp). Therefore compound heterozygote individuals with CF and healthy carriers of the mutation show both bands of 180 and 168 bp., line 1: mother of the index case, carrier of the mutation G542X/N, line 2: father of the index case, without the mutation G542X; line 3: index case, compound heterozygote G542X/X; and line 4: fetus with the mutation G542X. C. Analysis of marker XV-2C/*TaqI*. 2% Agarose gel, amplification products digested are shown, indicating the type of alleles present in the family, line 1: homozygote mother for the allele 1 (X1/X1), line 2: heterozygote father (X1/X2), line 3 heterozygote patient (X1/X2) and line 4 heterozygote fetus (X1/X2).

direct detection of the P.G542X mutation in the fetus, we analyzed two extragenic polymorphisms: XV2C/*TaqI* and KM19/*PstI*, in which recombination has not been reported. We were able to determine the segregation of both *CFTR* mutant alleles in the affected family, including the fetus (II -2), using only the XV2C/*TaqI* RFLP analysis, since KM-19/*PstI* was not informative. Given that the fetus inherited the same genotype as her affected sister (II-1),

this analysis allowed us to give an accurate prenatal diagnosis and genetic counseling to this family.

It is necessary to emphasize that combination of methodologies is a crucial alternative to reach a precise CF diagnosis, in populations with a high genetic heterogeneity.

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