CASE REPORT

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Impact of genetic testing and family health history of cystic fibrosis in the early prenatal diagnosis and prevention of a new case of genetic disorder

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Abstract

Cystic fibrosis (CF) is a multi-system autosomal recessive disorder, results of mutations in the CF transmembrane conductance regulator (*CFTR*) gene, located on the long arm of chromosome 7. We present a special family couple with particular medical history of CF, who comes to our Clinic for genetic tests and a prenatal genetic counseling, to prevent the birth of a new affected CF child. Genetic analysis showed that the first affected child, a daughter, is compound heterozygous for two clinically significant recessive mutations: *c.1521_1523delCTT*; *p.Phe508del*, inherited from her mother, who carries the same *CFTR* mutation, and *c.1853_1863delTTTTGCATGAA*; *p.Ile618Argfs 2*, inherited from her father, who is heterozygous, healthy carrier, for the same *CFTR* mutation. In our case report, early prenatal genetic testing, pre- and post-test genetic counseling was crucial in the management of the present pregnancy, to prevent the birth of a new affected CF child.

Keywords: cystic fibrosis, regulator gene, family history, prenatal diagnosis, genetic testing.

Introduction

Among Caucasians one of the most frequent conditions is cystic fibrosis (CF), an autosomal recessive disease, affecting about 36 000 individuals in the European Union [1]. CF is a chronic disease that currently has no cure. This disease is determined by mutations in the CF transmembrane conductance regulator (*CFTR*) gene (7q31.2) [2]. The early diagnosis and the emerging therapies that target the basic genetic defect are the two main reasons that the current median survival rate is 41.1 years [3]. Europe presents a different situation because of limited available resources and facilities, causing a considerable morbidity and early mortality, despite the positive outlook [4]. The *CFTR* gene has 27 exons and a total of 250 000 bases of deoxyribonucleic acid (DNA), which suffers post-transcriptional modifications, forming a messenger ribonucleic acid (mRNA) of 6500 base pairs [5]. *CFTR* gene is predominantly expressed in the apical membrane of epithelial cells and alterations of this gene cause a complex phenotype, affecting multiple organs [6], especially the production and flow of mucus within the respiratory system, sweat glands and digestive system. The major cause of morbidity and mortality in CF disease is the chronic bacterial infection of the lung, one of the most common pulmonary complications caused by mucous plugging [6]. This gene is coding for a transmembrane protein with the same name, a tightly regulated anion channel whose function is to conduct through the plasma membrane chloride and bicarbonate ions. Also, the CFTR transmembrane protein has important role in the activity of other transmembrane proteins [7]. In this gene, it have been identified over 2000 mutations [single nucleotide polymorphisms (SNPs) or insertions or deletions (InDels)], 70% of the patients present the major mutation, the deletion of phenylalanine at codon 508, Δ*F508* (a 3-bp deletion) [8]. The most frequent 38 pathogenic mutations, including *df508*, can be detected through a standard screening panel of the principal variants of *CFTR* gene through the amplification of target sequences, reverse-hybridization and color development. Different mutations in this gene have varying effects and can lead to different phenotypes [9]. Because of this variety, it was necessary to be classified in six classes by the effect on the protein function. The mutations from class one to three determinates a nonfunctional CFTR protein, causing a severe phenotype, whereas the mutations from class four to six are associated with a mild phenotype, where the CFTR protein has a residual function [10]. In order to identify the remaining *CFTR* mutations from the coding regions, the most reliable and cost effective method is Sanger sequencing.

The aim of the present case report is to highlight the importance of genetic testing in the early prenatal diagnosis and prevention of a new case of CF.

Case presentation

A 32-year-old pregnant woman and her husband, 33 years old, presented to the Maternal Fetal Medicine Unit, Alco San Medical Center, Bucharest, Romania, in December 2018, for prenatal ultrasound (US) investigation, genetic tests and a prenatal genetic counseling based on family health history.

It is a Caucasian couple, non-consanguineous, with a family history of CF (Figure 1).

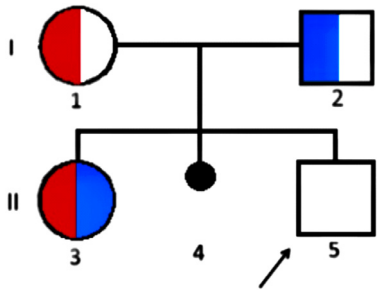

Figure 1 – ***Family tree: I 1 – Mother is heterozygous, healthy carrier, for the clinically significant mutation: c.1521_1523delCTT; p.Phe508del; I 2 – Father is heterozygous, healthy carrier, for the clinically significant mutation: c.1853_1863delTTTTGCATGAA; p.IIe618Argfs 2; II 3 – First child of the couple (II 5 and II 6), an affected CF girl, is compound heterozygous for two clinically significant recessive mutations, inherited from her parents; II 4 – Second pregnancy of the couple (II 5 and II 6), stopped in evolution; II 5 – Proband. CF: Cystic fibrosis.***

The couple has one child, an affected CF girl. The second pregnancy was stopped in evolution at 11 weeks of gestation, for unknown causes. The current pregnancy is the third pregnancy of the couple.

The US examination was performed using a Voluson E10 US, which shows a single pregnancy, six weeks of gestation, normally developed.

Knowing the couple's family history with high genetic risk, following a discussion with a maternal–fetal medicine specialist, it was decided to perform additional specific prenatal genetic investigations, including: CF genetic testing for the affected girl, CF genetic carrier testing for both parents, chorionic villus sampling (CVS) at 10 to 12 weeks or amniocentesis at 14 to 16 weeks for the actual pregnancy, to determine if the fetus is affected by CF or not, and to prevent a new affected child.

The workflow for the detection of mutations in the 27 exons of *CFTR* gene, including the intron/exon borders, was: DNA isolation, polymerase chain reaction (PCR) amplification, post-PCR purification, PCR sequencing, clean-up of PCR sequencing products, capillary electrophoresis and data analysis (Figure 2).

All subjects provided informed consent to use the de-identified biological samples for the scientific study.

DNA isolation

For CF genetic testing of the parents and of the affected CF girl, genomic DNA from peripheral blood samples, collected in ethylenediaminetetraacetic acid (EDTA)-collection tubes, was extracted from venous blood.

For CF genetic testing of the fetus, genomic DNA was extracted from chorionic villi using a commercial kit (ReliaPrep™ Blood gDNA Miniprep System – Promega, USA), following the manufacturer's protocol. The DNA concentration was measured using a Life Science UV/Vis spectrophotometer (Beckman Coulter, USA) and stored at -20ºC for future analysis.

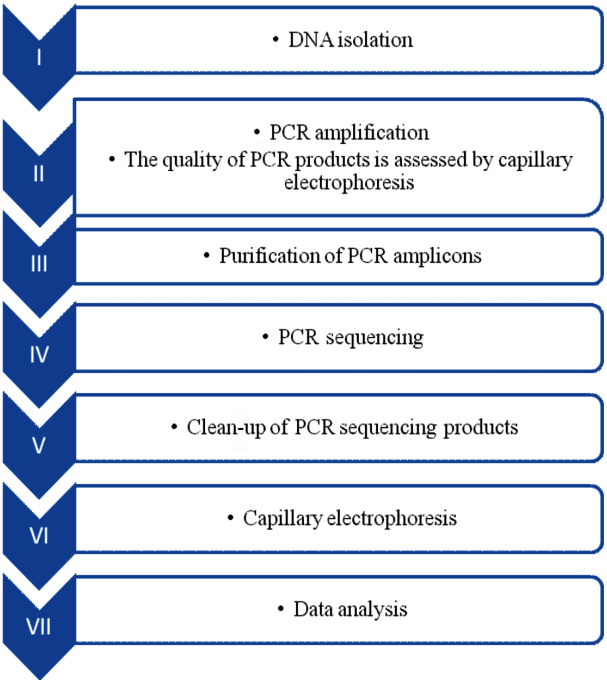

Figure 2 – ***Sanger sequencing workflow. DNA: Deoxyribonucleic acid; PCR: Polymerase chain reaction.***

PCR amplification

The PCR reactions were run on a Veriti™ 96-Well Thermal Cycler (Applied Biosystems, USA). For the amplification of all the exons and exon/intron borders of *CFTR* gene, we used the primer pairs described by Zielenski *et al.* (2002) [8].

Each PCR reaction was performed in a 20 µL final volume, using 2.5 µL of DNA template, 1 µM of each primer, 1X Buffer (Promega), 3 mM $MgCl_2$ (Promega), 0.3 mM deoxyribonucleotide triphosphates (dNTPs) (Promega), 1 U PQ Taq polymerase (BioTeke Corporation, China) and nuclease-free water. Cycling conditions were as it follows: 1× (95°C – 10 minutes), 35× (95°C – 30 seconds, 55°C – 45 seconds, 72°C – one minute), 1× (72°C – 10 minutes), followed by a 4°C hold. PCR products specificity was evaluated through capillary electrophoresis with a QIAxcel system (Qiagen, USA).

Post-PCR purification

The resulting PCR products were purified for removing unused primers and nucleotides using the FavorPrep PCR Clean-up Mini Kit (Favorgen), according to the protocol recommended by the manufacturer.

PCR sequencing

PCR sequencing was performed using Methods Development Kit (Beckman Coulter) procedure, according to manufacturer's recommendations.

Clean-up of PCR sequencing products, capillary electrophoresis and data analysis

The amplification products obtained consecutive to

PCR sequencing were purified using ethanol and glycogen-mediated precipitation. The resulting DNA pellets were resuspended in sample loading solution (Beckman Coulter) and run on the CEQ 8800 Genetic Analysis System (Beckman Coulter). Data analysis was performed with CEQ 8800 dedicated software.

One of the most accurate methods of detecting gene mutations is Sanger sequencing. Employing this sequencing method, we sequenced all the *CFTR* gene exons and neighboring intronic regions.

Genetic analysis showed that the affected CF girl is compound heterozygous for two clinically significant mutations: *c.1521_1523delCTT*; *p.Phe508del* (heterozygous) and *c.1853_1863delTTTTGCATGAA*; *p.IIe618 Argfs 2* (heterozygous), and one mutation with unknown significance: *c. 1399C>T*; *Leu467Phe* (heterozygous).

The mother was found to carry the one clinically significant mutation: *c.1521_1523delCTT*; *p.Phe508del* (heterozygous) and one mutation with unknown significance: *c. 1399C>T*; *Leu467Phe* (heterozygous), mutations located within exon 11.

The father was found to carry the one clinically significant mutation: *c.1853_1863delTTTTGCATGAA*; *p.IIe618Argfs 2* (heterozygous) located within exon 11.

For the present pregnancy, CVS was performed transabdominally, at the 12th gestational week, under continuous US visualization. The intervention proceeded normally, without post-procedural complications.

DNA quality obtained from the chorionic villi biological sample was low, affecting the subsequent analysis for exons 1, 3, 7 and 26. For exons 1, 3 and 7, sequencing was not possible due to the lack of amplification of the targeted regions (*i.e.*, two failed PCR replicas for each exon). For exon 26, we obtained the targeted amplicon in the PCR step of the analysis. Although we could not identify any mutation in exon 26, the quality of sequencing did not reach the necessary threshold for an accurate analysis (*i.e.*, two technical replicas exhibited low fluorescence intensity).

The nucleotide sequences corresponding to the *CFTR* amplicons were aligned against the human reference genome (human *GRCh38/hg38*), in order to screen for mutations. In the analyzed sample, excepting for exon 11, we did not identify any pathogenic mutation associated with CF.

In exon 11, we detected two heterozygous mutations, namely *c.1399 C>T*; *p46 Leu467Phe*, and *c.1522-1524 delTTT*; *p.Phe408del* (Figures 3 and 4).

In addition to mutation identification, we characterized the *IVS8 (TG)n – (T)n – 1540A/G* polymorphic set, which has the following nucleotide structure: *TG11 – T7/T9 – 1540G/A*. As far as we know, there are no associations between this particular polymorphic set and CF or impairment of fertility.

About fetal chromosomes, quantitative fluorescent polymerase chain reaction (QF-PCR) analysis *via* CVS did not indicate the presence of any aneuploidy of fetal chromosomes 13, 18 and 21.

Cytogenetic analysis of fetal chromosomes had revealed a 46,XY karyotype (Figures 5 and 6). No abnormalities of the number or structure of fetal chromosomes have been detected.

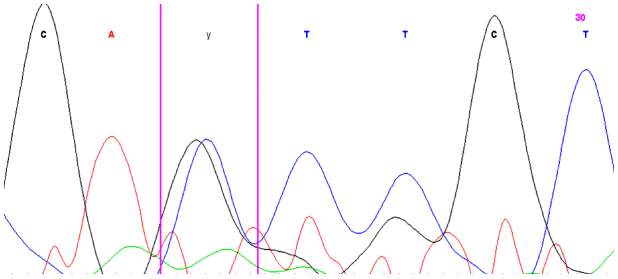

Figure 3 – ***Sequencing chromatogram showing the c.1399 C>T heterozygous mutation. The forward strand of exon 11 is displayed. The heterozygous mutation is highlighted by two pink vertical lines and is designated by the letter Y, according to the IUB ambiguity code. IUB: International Union of Biochemistry.***

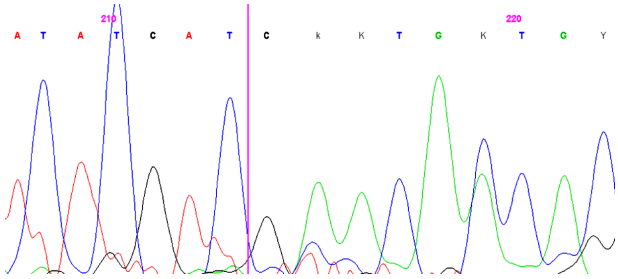

Figure 4 – ***Sequencing chromatogram showing the mixed nucleotide profiles determined by the c.1522–1524delTTT heterozygous mutation. The forward strand of exon 11 is displayed. The start of the region with overlapping amplicon profiles caused by the heterozygous mutation is highlighted by the pink vertical line.***

Figure 5 – ***Fetal karyotype: 46,XY (Slide: 1). Specimen type: chorionic villi, 12 weeks of pregnancy.***

Figure 6 – ***Fetal karyotype: 46,XY (Slide: 3). Specimen type: chorionic villi, 12 weeks of pregnancy.***

The US examination was repeated at 20 weeks of gestation, and confirmed a presence of a single fetus with normal growth parameters. No other abnormalities were detected.

After the first pregnancy, a girl with CF, and the second pregnancy, which was stopped in evolution for unknown causes, in the current pregnancy, at the third pregnancy of the couple, the fetus growed and developed normally. Also, the fetus was predicted by DNA analysis to be unaffected by CF.

The prenatal diagnosis was confirmed postnatally.

Discussions

CF is considered one of the most commonly occurring fatal genetic disorders [11]. It has an incidence of 1:2500 newborns and has an average life span of 28 years [12].

The most frequent mutation in CF patients is represented by a three base pairs deletions. This deletion leads to the loss of an amino acid (phenylalanine) in the 508 position of the CFTR protein [13].

The mutations results in the production of an abnormal CFTR protein. CF disease is caused by the loss of functional CFTR chloride (Cl^-) channels [14]. CF genetic disorder causes decreased Cl^- transport in several epithelia [15].

The first CF clinical phenotype description occurred in 1939 and the CF causative gene was cloned in 1989 [13].

The disease is characterized by chronic pulmonary obstruction, recurrent and chronic bacterial infections and inflammation such as bronchitis and pneumonia, and by digestive disorders, such as intestinal obstruction, pancreatic insufficiency with malnutrition and poor growth [16].

The main cause of morbidity and precocious mortality of these individuals is the lung disease, and the most common cause of death in children with CF is respiratory failure [17]. The processes responsible for the initiation of CF lung disorder and early CF pulmonary morbidity are not well understood [18].

The condition can be diagnosed by various procedures including blood immunoreactive trypsin test (IRT), sweat chloride test, transepithelial nasal potential difference test (TEPD), and molecular genetic testing methods [19].

According to the data available in the *Cystic Fibrosis Mutation Database*, about 70% of the mutations identified in the *CFTR* gene have exonic localization. The distribution of mutations in the 27 exons of the *CFTR* gene is not uniform, with exons presenting over one hundred distinct mutations (*e.g.*, exon 4 and exon 14) and exons where their number is relatively low (*e.g.*, exon 16 and exon 18). Of particular interest are exons 4, 8, 10, 11, 12, 14, 17, 20, 22, 23 and 24, which harbor over 880 mutations, representing more than 63% of the exonic mutations. In these exons and in their immediate vicinity, 32 of the most common *CFTR* mutations were identified [20–22].

Although prenatal genetic testing is not recommended in the early stages of pregnancy, future parents who have an increased genetic risk for transmission of pathogenic variants, fetal testing is crucial [22]. CF management warrants an interdisciplinary team because the complexity of this disorder [11].

In our case report, prenatal genetic testing was mandatory for all members of the family to prevent the birth of a new affected CF child.

We start the genetic investigations from the affected CF girl. CF is present at the first infant of the couple, because both parents carried a CF recessive gene, and their baby girl inherited a CF recessive gene from each parent.

Genetic analysis showed that the affected CF girl is compound heterozygous for two clinically significant recessive mutations: *c.1521_1523delCTT*; *p.Phe508del* and *c.1853_1863delTTTTGCATGAA*; *p.IIe618Argfs 2*.

The *CFTR* recessive mutation, *c.1521_1523delCTT*; *p.Phe508del*, was inherited from her mother, who carries the same clinically significant mutation, and the *CFTR* recessive mutation, *c.1853_1863delTTTTGCATGAA*; *p.IIe 618Argfs 2*, was inherited from her father, who is heterozygous, healthy carrier, for the same mutation.

A healthy carrier has one copy of the mutated recessive gene. In our case, each parent of the couple carries one different *CFTR* recessive gene and one normal dominant gene. They have no symptoms and no diseases.

But, not every offsprings from this special couple will necessarily have CF. With each pregnancy, the chance of having a child affected by an autosomal recessive disorder, in the case of parental carriers, is 25% (or 1 in 4) [17–24]. Other offsprings, 50%, could inherit a single *CFTR* mutant recessive gene from just one parent, and thus become a healthy carrier for CF.

Other offsprings, 25%, could inherit no *CFTR* gene and be completely free from CF, unaffected non-carrier, like our proband.

Conclusions

A heterozygous couple, healthy carriers for *CFTR* mutations requires specific multidisciplinary management and psychological support to prevent the birth of a new affected CF child. In our family case report, prenatal genetic testing was mandatory for all members of the family. Genetic analysis showed that, the first affected child, a daughter, is compound heterozygous for two clinically significant recessive mutations, inherited from her parents, who are heterozygous, healthy carrier for *CFTR* mutations. Early prenatal genetic testing, pretest and posttest genetic counseling is crucial in the management of future pregnancies of a heterozygous couple, healthy carriers for *CFTR* mutations.

Conflict of interests

The authors declare that they have no conflict of interests.

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