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Research Article

Compound Heterozygosity for *CFTR* Phe508del/Pro750Leu in Two Siblings with Normal Sweat Chloride, Lung Function, Growth, and Fecal Elastase

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Abstract

Cystic Fibrosis (CF) is one of the most common autosomal recessive genetic diseases. In the United States, newborn screening programs screen for CF by measuring the level of Immunoreactive Trypsinogen (IRT). Positive IRT screens are followed by additional diagnostic testing, including sweat chloride testing and genetic testing of the *CFTR* gene. This report describes a female patient with a positive newborn screening result for CF, who was found to have one rare *CFTR* variant c.2249C>T (p.Pro750Leu, also known as P750L) in *trans* with c.1521_1523delCTT (p.Phe508del, also known as delF508), as does her sister. Clinical follow up for several years indicates that both siblings have minimal respiratory symptoms, normal growth parameters and laboratory testing. In contrast to previous reports, Pro750Leu may not be pathogenic for lung and pancreatic disease.

Keywords: Cystic fibrosis, *CFTR*, delF508, deltaF508, Genetic testing, Newborn screening, Phe508del, Pro750Leu, P750L, Variant interpretation

Introduction

As genetic testing is increasingly utilized to aid in making a diagnosis, accurate interpretation of sequence variants is critically important. This is particularly relevant for the disorders included in the newborn screen, in which a diagnosis is often suggested in an otherwise asymptomatic individual. CF is one of the most common autosomal recessive disorders, especially in the Northern European (Caucasian) population. Although the clinical spectrum of CF is highly variable, it is mainly characterized by recurrent respiratory infections, disruption of the exocrine function of the pancreas, Congenital Bilateral Absence of the Vas Deferens (CBAVD) and other clinical problems. CF mortality and morbidity are mainly due to pulmonary complications [1].

In the United States, all states screen for CF by measuring the level of IRT [2]. For patients who screen positive, additional diagnostic testing includes sweat chloride testing and genetic testing of *CFTR* (panels of common mutations or sequencing of the entire coding region). Although one pathogenic variant, p.Phe508del (legacy name is known as deltaF508), accounts for at least one mutation in over half of CF patients, the disorder exhibits significant allelic heterogeneity, with over 2,000 pathogenic variants identified to date. For many of these rare variants, phenotypic data is not readily available or is limited due to low frequency, making interpretation difficult. Of note, when two variants are on the same parental chromosome, they are called in *cis*, and when they are on different parental chromosomes, they are called in *trans*. However, two variants on the same parental chromosome (in *cis*) is called a complex allele [3-4]. CF is inherited in an autosomal recessive pattern, where the disease will manifest when patients have two pathogenic variants in *trans*. This report describes two siblings with one such rare variant, Pro750Leu, in *trans* with Phe508del (p.[(Phe508del)];[(Pro750Leu)]) who have normal phenotypes.

Methods

Sanger sequencing

The entire coding regions of all 27 exons of the *CFTR* gene (exons plus 50 bp upstream and 50 bp downstream of exon/intron boundaries) were amplified using specific forward and reverse primers, and bidirectionally sequenced using Sanger methodology.

Multiplex Ligation-dependent Probe Amplification (MLPA)

CFTR MLPA was performed using the SALSA MLPA P091 *CFTR* probe mix (MRC Holland, Netherlands), and each exon of the *CFTR* gene was targeted with at least one probe.

Results

Clinical findings

Patients A and B are full sisters. Patient A, who was 36 months old at the time of presentation to the University of Michigan C.S. Mott Children's Hospital, was diagnosed with cystic fibrosis in California by newborn screening. Genetic testing of *CFTR* revealed the presence of two variants, Phe508del and Pro750Leu. *CFTR* MLPA was negative for intragenic deletions or duplications. Patient A had two normal sweat chloride tests in early infancy, with levels of 21 and 12 mmol/L (normal <40). She had a third sweat chloride test at 21 months of age that was 27 mmol/l. Patient A, was born at term with a birth weight

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of 3.8 kg. Her fecal elastase test was normal. During early childhood, her growth was normal, and her stools were non-greasy. She was occasionally treated for respiratory infections with antibiotics, prednisone, albuterol, and chest physiotherapy. Respiratory infections were characterized primarily by wet cough. She contracted RSV infection at 25 months of age. Her throat cultures usually grew normal flora, but the culture after RSV infection grew beta-lactamase-negative *Hemophilus influenzae*. Her chest X-rays showed peribronchial cuffing. Patient A also had a history of recurrent ear infections. Laboratory studies were normal except for a 25-hydroxy vitamin D level three months prior to presentation of 12 ng/mL (normal 25-100 ng/mL). Her medications were vitamin D (2,000 IU cholecalciferol/day) and albuterol nebulizer treatments. She also performed vest physiotherapy 2-3 times per day as needed.

Patient A has been followed at the University of Michigan for four years. At 7 years of age, her weight was 46.9 kg (>99th percentile) and height was 130 cm (87th percentile). Her physical exam has remained normal. Her laboratory tests have included seven throat cultures with normal flora and three chest radiographs, each of which were read as normal except for a few streaky densities, likely secondary to the exposure being made on expiration. Two complete blood counts and metabolic panels were normal. Her most recent 25-hydroxy vitamin D level remained slightly low at 22 ng/mL. Vitamin A levels were normal. Spirometries at 62, 70 and 84 months of age (FVC 110%, FEV1 112%, FEV1/FVC 112%, FEF25-75 124%) were normal.

Patient B, the older full sister of patient A, presented to the University of Michigan, C.S. Mott Children's Hospital at 70 months of age. Her birth weight was 3.6 kg. At delivery she had an Apgar score of 10 and had no respiratory problems in the first months of life. Her development was normal. She was fed with breast milk and Similac infant formula. She did not have a neonatal screen for CF as she was born one year prior to CF newborn screening in Michigan. However, after her sister was diagnosed through newborn screening in California, Patient B underwent sweat chloride testing at 48 months of age, and the results were normal. Throughout infancy and early childhood, she had nasal congestion and drainage sometimes accompanied by coughing and vomiting especially at night. She also had frequent ear infections. She has had snoring since she was a baby. She was a gassy baby and had occasional malodorous stools that did not float. She also had frequent crampy abdominal pain (once or twice a week).

Patient B has been followed at the University of Michigan for four years. At 9 years of age, her weight was 70.4 kg (>99th percentile) and height was 152.8 cm (99th percentile). Her physical exam has remained normal. Her laboratory tests have included *CFTR* genetic testing at age 8 that showed the same genotype as her sister, Phe508del and Pro750Leu. Her spirometry has remained normal (FVC 115%, FEV1 119%, FEV1/FVC 89% and FEF25-75 133%). Chest X-ray revealed low lung volumes but otherwise was read as normal. Sinus films were normal. Two throat cultures have been obtained, each of which showed only normal flora. She also has low 25-hydroxy vitamin D levels (16 ng/mL).

Testing of the patients' parents revealed that the mother carries the Phe508del pathogenic variant and the father carries the Pro750Leu variant, confirming that both siblings inherited the variants in *trans*.

Discussion

CF is the most common autosomal recessive genetic disease in the Northern European (Caucasian) population. Allelic heterogeneity within the *CFTR* gene with more than 2,000 pathogenic variants identified, can present challenges for genotype-phenotype predictions. This report describes two siblings with two *CFTR* variants, Phe508del and Pro750Leu, with normal sweat chloride testing, minimal respiratory symptoms, normal growth parameters and laboratory testing.

There are three previous reports in the literature, with conflicting interpretations of pathogenicity of the Pro750Leu variant. The first is of a male Mexican patient with Pro750Leu in *trans* with a Phe508del allele [5]. This patient had early onset of symptoms at 2 months of age and had pancreatic insufficiency and chronic respiratory disease, leading the authors to conclude that Pro750Leu is a pathogenic variant. In contrast to our patients, the reported patient presented with a severe CF course. Of note, *CFTR* testing in that study was performed by Single-Strand Conformation Polymorphism (SSCP) and multiplex Heteroduplex (mHET) analyses, followed by sequencing of affected fragments. Thus, there is a possibility that another pathogenic variant in *cis* with Pro750Leu was not detected.

A second report is of a patient identified by newborn screening to have an elevated IRT with a borderline sweat chloride test (44 mmol/L) [6]. Sequencing identified a complex allele p.[(Arg352Trp; Pro750Leu)] in combination with a Phe508del allele in *trans*. This patient had negative stool elastase testing and is being treated prophylactically in a CF clinic [6]. The phenotype described in this report is similar to our patients.

A third report described a Chinese patient with congenital bilateral absence of the vas deferens. Sequencing revealed two variants in *trans*, Pro750Leu and Gly970Asp [7]. CBAVD is not an outcome that we can assess in our patients. Pro750Leu has also been reported, based on personal correspondence, in the Cystic Fibrosis Mutation Database (CFTR1) in 2 more patients with incomplete clinical and molecular data [8]. However, Pro750Leu is not listed in the CFTR2 mutation database [9].

Large whole exome sequencing studies reveal an allele frequency for Pro750Leu of 0.00037 (24 of 65,650 alleles) in European (non-Finnish) populations [10]. PolyPhen-2 predicts the substitution to be benign [11]. The SIFT (Sorting Tolerant from Intolerant) algorithm predicts that the substitution of leucine for proline is tolerated based on conservation of protein sequences [12]. This proline residue is not highly conserved and is substituted with leucine in other mammals such as the brush-tailed rat, guinea pig, and chinchilla [13]. ClinVar reports differing interpretations among reporting testing laboratories: uncertain significance by Emory Genetics Laboratory and likely pathogenic by the Center for Pediatric Genomic Medicine. A third entry from Invitae based on literature review offers no assertion of clinical significance [14].

Considering the small number of cases with Pro750Leu, the wide spectrum of clinical outcomes, and the potentially incomplete molecular genotypes reported previously, a clear genotype-phenotype correlation cannot be derived. Additional careful descriptions of patients with Pro750Leu or complex Pro750Leu alleles in *trans* with other pathogenic variants are needed to fully understand the molecular consequences.

With widespread adoption of newborn screening for CF and increased utilization of *CFTR* genetic testing, rare variants will be identified at an increasing rate. It is obvious that the clinical and molecular data of patients with rare *CFTR* variants are incomplete in the currently available public databases. Therefore, ongoing description of

phenotype data in cases, including normal outcomes, will be needed for accurate clinical interpretations of these variants.

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