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Additional evidence on the phenotype produced by combination of *CFTR* 1677delTA alleles and their relevance in causing CFTR-related disease

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Abstract

Cystic fibrosis is the most common, life-threatening, autosomal recessive disease in the Caucasian population. It is caused by mutations in the cystic fibrosis transmembrane conductance regulator gene, which encodes a chloride ion channel expressed on the surface of epithelial cells. There are more than 2000 variants of the cystic fibrosis transmembrane conductance regulator gene reported worldwide. Some of these variants cause classic cystic fibrosis, while others are labeled as variants of unknown significance or variants of varying clinical consequences alleles and associated with atypical disease or cystic fibrosis transmembrane conductance regulator-related disorders. Although these alleles do not directly cause cystic fibrosis, they may predispose compound heterozygous patients to certain clinical phenotypes. Specifically, 1677delTA has been reported as a pathogenic allele in homozygous state or in combination with other cystic fibrosis-causing alleles. However, the L997F allele is considered to be benign or causative of non-classic cystic fibrosis or cystic fibrosis transmembrane conductance regulator-related disorders in combination with other pathogenic alleles. In this case series, we describe three cases with 1677delTA and L997F genotype, and speculate that a co-concurrence of these two alleles in *trans* does not cause classic cystic fibrosis symptoms; however, because the late-onset of cystic fibrosis is possible in the presence of rare alleles, such as L997F, longer follow-up of these patients and identification of a greater number of adults with 1677delTA/L997F genotype are necessary to make final conclusion about the nature of this genotype.

Keywords

cystic fibrosis transmembrane conductance regulator, cystic fibrosis, L997F, 1677delTA

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Introduction

Cystic fibrosis (CF), an autosomal recessive disease, is the most common life-limiting condition in Caucasians, with a frequency of 1 in 2000–3000 live births.[1] This ratio varies among different groups and geographical regions.[2] CF is caused by mutations in the cystic fibrosis transmembrane regulator (*CFTR*) gene on the long arm of chromosome 7 (7q31.2 region) comprising 27 exons.[3] Manifestations of CF include meconium ileus, recurrent pulmonary infections, bronchiectasis, pancreatic insufficiency, biliary cirrhosis, and poor weight gain, among others.[4,5]

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Table 1. Demographics and clinical data of cases.

Case	Age	Allele 1	Allele 2	IRT, ng/mL	SCL, mmol/L	Pancreatic status	Weight
1	26 months	c.1545_1546del p.(Tyr515fs*)	c.2991G > C p.(Leu997Phe)	88	25	PS	N
2	32 months	c.1545_1546del p.(Tyr515fs*)	c.2991G > C p.(Leu997Phe)	108	59, 39, 20	PS	N
3	7.5 years	c.1545_1546del p.(Tyr515fs*)	c.2991G > C p.(Leu997Phe)	N	N	PS	N

IRT: immunoreactive trypsinogen; N: normal; PS: pancreatic sufficient; SCL: sweat chloride level.

Currently there are over 2000 variants of the *CFTR* gene reported worldwide and not all of them are CF-causing.[6] Some of these variants cause classic CF, while others are associated with atypical CF or CFTR-related disorders (CFTR-RD).[7,8] F508del is the most common CF-causing mutation, with a prevalence of 30%–80% depending on the ethnic group.[6] In Georgia, molecular genetic analyses of CF patients revealed a relatively high frequency of 1677delTA (c.1545_1546delTA) mutation,[9] classified as Class I mutation, caused by frameshift and nonsense mutations.[10] Clinical presentation includes impaired lung function, pancreatic insufficiency, and persistent infection with *Pseudomonas aeruginosa*.[11]

Although CF is considered monogenic, the correlation between CF genotype and phenotype is complex,[12] creating a challenge not only for the geneticists and pediatricians but also for adult pulmonologists or other specialists, who are obligated to diagnose patients and predict the phenotypic consequences of new mutations.[8] Multiple genotype–phenotype studies have shown that other factors, such as modifier genes and environmental components, should be considered to accommodate a complex spectrum of disease phenotypes and provide the best possible care for the patients.[13,14]

In this article, we present the retrospective analysis of three patients with 1677delTA/L997F genotype (Table 1). This work is a continuation of the case described by Tkemaladze et al.,[15] in which the researchers presented a familial case of CF with a rare combination of alleles.

Case series

Case 1: 26-month-old boy

G1P1, born term, with birth weight 4000 g. The perinatal period was uneventful. Newborn screening (NBS) showed an increased level of immunoreactive trypsinogen (IRT; 88 ng/mL). Genetic testing was performed at 1 month, which revealed two heterozygous variants 1677delTA and L997F. Subsequent parental segregation analysis confirmed the *trans* phase of the variants. Afterward, a sweat test was performed and it showed normal chloride levels (25 mmol/L). Stool pancreatic elastase was normal as well. He was breastfed until 9 months with introduction of the solid food later on. His weight gain was always normal. Currently, at the age of 22 months, the child never had any respiratory infections and his weight is within normal range (12 kg).

Case 2: 32-month-old boy

G3P3, born term, with birth weight of 3800 g. The perinatal period was uneventful. NBS showed an increased level of IRT (108 ng/mL). Sweat test was performed three times: at the age of 1 month—59 mmol/L, at 1.5 months—39 mmol/L, and at 6 months—20 mmol/L. In infants (up to 6 months of age), $Cl^- \leqslant 29$ mmol/L is considered as normal; 30–59 mmol/L as intermediate, and $\geqslant 60$ mmol/L indicative of CF.[8] Genetic testing was performed at 6 months, which revealed two heterozygous variants: 1677delTA and L997F. Subsequent parental segregation analysis confirmed the *trans* phase of the variants. Stool elastase was normal. Until now the child has good weight gain, he never had any respiratory infections, and is in good general health.

Case 3: 7.5-year-old girl

G1P1, older sister of Case 2. Born term with birth weight 2500 g. NBS was negative. Genetic analysis was performed at the age of 6.5 years because of familiarity and it showed two heterozygous variants: 1677delTA and L997F. Subsequent analysis of sweat chloride and stool elastase showed normal results. She is apparently healthy, never had any episode of upper respiratory infection, her weight is within normal range.

Discussion

In this study, we analyzed three cases of compound heterozygotes with rare genotype: 1677delTA/L997F. NBS showed mildly increased level of IRT in Case 1 and Case 2 (108 and 88 ng/mL, respectively; reference range of IRT is < 55 ng/mL) and it was normal in Case 3. The sweat chloride levels were within reference ranges ($\leqslant 29$ mmol/L) in all three cases. No functional tests, such as nasal potential difference/intestinal current measurements, were available for these patients. Importantly, at the given age, none of the patients have any upper respiratory system involvement, gastrointestinal issues, or problems with weight gain.

This study is a continuation of our previous study where we described the familial case of CF with rare genotype.[15] Family members included two children with classic CF (12-year-old boy and 9-year-old girl with the same I1234V/1677delTA genotype), one child with atypical CF (7-year-old girl with I1234V/L997F alleles) and their apparently healthy mother (43-year-old female with 1677delTA/L997F). In this family, the mother and her daughter were compound heterozygotes and shared L997F allele in combinations with different pathogenic alleles—1677delTA for the mother and I1234V for the child. Of note, the mother with the 1677delTA/L997F genotype was asymptomatic, while her daughter with the I1234V/L997F genotype had atypical CF with recurrent pneumonia that improved after treatment with pancreatic enzyme replacement therapy. This case demonstrates that L997F allele causes phenotypic variability when combined with other pathogenic alleles.[15] It should be noted that L99F variant is considered a non-CF causing variant according to CFTR2[6] and reported as a CFTR-RD-causing[16] or as a variant of variable clinical consequence.[17] The clinical phenotype of the 1677delTA/L99F genotype can therefore be unpredictable.

In the present case series, we describe three additional pediatric patients who share the same 1677delTA/L997F genotype. Even though their young age does not allow us to evaluate the full phenotypic spectrum of the condition, the fact that all of them have normal sweat chloride concentration and stool elastase, as well as adequate weight gain and so far, no evidence of recurrent bronchopulmonary infections or bronchiectasis gives us strong bases to suspect that this genotype does not cause classical CF.

This brings the question of the prevalence of the 1677delTA/L99F genotype among healthy populations. Moreover, we may also suspect there are several reasons why CFTR2 database does not contain such allele combination. First, 1677delTA mutation is one of the prevalent mutations in the Caucasus region, including in Georgia,[9,18,19] but it is rare in European populations. Second, the database is updated through the published literature of those affected with CF or CFTR-RD and the reason why 1677delTA/L997F genotype is absent in the database is because individuals with this genotype might be asymptomatic or healthy carriers.

Conclusion

Our study confirms once again that the diagnoses of CF are complex and it relies on NBS results, measurement of sweat chloride level, functional tests, where available, genetic alterations, and most importantly—the clinical manifestation. The described case series gives further insight into understanding the implications of various *CFTR* alleles and broadens the knowledge related to the complex phenotype produced by the *CFTR* gene. In the era of cystic fibrosis transmembrane conductance regulator (CFTR) modulators, accuracy of CF diagnosis is important, as it has significant therapeutic implications. In addition, because the late-onset of manifestations of CF is possible in the presence of rare alleles, such as L997F, longer follow-up of these patients, and identification and phenotypical characterization of a greater number of adults with 1677delTA/L997F genotype are necessary to make final conclusion about the nature of the above-mentioned genotype.

Author contributions

All authors contributed to the study conception and design. T.T., E.K., and M.G. contributed to conceptualization and clinical monitoring of patients; V.S. contributed to methodology; Z.Z. and N.N. contributed to methodology and original draft preparation; M.J.L., A.R., and E.A. contributed to methodology, review, and editing. All authors read and approved the final manuscript.

Data availability

The datasets used and analyzed during the current report are available from the corresponding author (E.K.) on request.

Declaration of conflicting interests

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