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Reporting three rare pathogenic variants at the *CFTR* gene in two unrelated Iranian Azeri children with cystic fibrosis

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Abstract

Background Cystic fibrosis (CF) is an autosomal recessive inherited life-threatening disease that causes changes in the electrolyte transport system, leading to high absorption of sodium and water. Disease-causing variants of the *CFTR* gene are responsible for this disease. In the present study, three rare pathogenic variants were identified in two unrelated Iranian Azeri children with CF.

Case presentation One child affected with CF was found to have two rare variants, c.1545_1546delTA and c.3196C > T, detected through Sanger sequencing of the entire coding region and promoter of the *CFTR* gene. Another patient was identified as compound heterozygous for the variant c.1545_1546delTA and a rare variant, c.2998delA, which has not been previously reported. The variants were found to be in trans, as both parents were heterozygous for the variants.

Conclusion The rare variant c.1545_1546delTA has been previously reported as a known variant in certain populations, including Azeris in northwest Iran. Our results, along with previous findings, suggest that this variant may be considered a founder mutation in specific geographical regions. The variant c.2998delA has not been previously reported as pathogenic. Following the guidelines of the American College of Genetics and Genomics and considering the proband's symptoms, we classified this variant as pathogenic based on criteria PVS1, PS4, PM3, PP1, and PM2. The identified variants in the *CFTR* gene as well as the previously reported variants could serve as a basis for future genetic counseling and prenatal diagnosis in Iran.

Keywords Cystic fibrosis, *CFTR*, Rs121908776, Rs397508475, Rs78194216, Ardabil, Iran

Background

CFTR, as a member of the ATP-binding cassette (ABC) transporter superfamily, functions as a chloride channel that controls ion and water secretion and absorption in epithelial tissues. More than 2000 pathogenic variants in the *CFTR* gene (OMIM ID: 602,421) have been reported in patients affected with cystic fibrosis (CF; OMIM ID: 219,700) [1]. These disease-causing variants disrupt the function of the chloride channels, preventing them from regulating the flow of chloride ions and water across the cell membranes. As a result, the lung and pancreas produce unusually sticky mucus, which clogs the airways and various ducts [2].

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In the current study, our aim was to report three rare pathogenic variants found in two Iranian children with CF. The ethical and regulatory issues related to the collection of human specimens for research purposes have been approved by the Ardabil University of Medical Sciences (Approval ID: IR.ARUMS.MEDICINE.REC.1401.143).

Case presentation

Two boys, aged 2 and 3, with a clinical diagnosis of cystic fibrosis (CF) and no family history of the disease, were referred to the Homa Genetic Lab in Ardabil, Iran. They had unrelated healthy parents. Sanger sequencing was used to determine pathogenic and likely pathogenic variants in the coding regions, splicing sequences, and the promoter of the *CFTR* gene using previously reported amplification primers [2]. The results showed compound heterozygosity in both cases.

In proband 1, the variants NM_000492.4(*CFTR*):c.1545_1546delTA (p.Tyr515Ter) (dbSNP code: rs121908776) and NM_000492.4(*CFTR*):c.2998delA (p.Ile1000LeufsTer2) (dbSNP code: rs397508475) were identified. Both variants are frame-shift variants that result in premature termination of protein translation. The normal translated protein of the CFTR gene has 1480 amino acids, while the resulting products of the noted variants are 515 and 1002 amino acids for the variants c.1545_1546delTA and c.2998delA, respectively. However, the variant c.2998delA had previously been reported as identified in the homozygous state (without evidence) in a Turkish neonate diagnosed with CF after neonatal screening [3]. Proband 2 had two pathogenic variants: c.1545_1546delTA and NM_000492.4(*CFTR*):c.3196C > T (p.Arg1066Cys) (dbSNP code: rs78194216).

The parental origin of the variants of proband 1 was determined to be paternal at position 1545 and maternal at position 2998 (Fig. 1). For proband 2, the variants were paternal at position 3196 and maternal at position 1545 (Fig. 2). There was no genetic relationship between the father of proband 1 and the mother of proband 2, even though they shared the same genotype.

Discussion

Loss of function (LOF) is a known mechanism of CF, with approximately 850 reported pathogenic LOF variants at the *CFTR* gene. The previously reported pathogenic variant c.1545_1546delTA (rs121908776) has a very low population frequency. According to the Iranome database (http://iranome.ir/), it was reported in only one Iranian individual (1/1600 = 0.000625 for the general population). The reported allele did not originate from the Azeri population (Ardabil is located in this region). However, in a previous report on the Azeri population, this variant was detected in 4.5 percent of the mutant alleles responsible for affecting cystic fibrosis [4]. Furthermore, its worldwide frequency is very low (GnomAD: 0; ALFA: 1/11862 = 0.00008; TOPMED: 2/264690 = 0.000008).

Based on the American College of Medical Genetics and Genomics (ACMG) guidelines, this variant is classified as pathogenic (Varsome criteria (https://varsome.com/): PVS1 (null variant (nonsense) in gene *CFTR*), PP5 (ClinVar classifies this variant as Pathogenic, citing 11 articles), PM2 (variant not found in GnomAD genomes and Gnomad exomes). ClinVar also classified this variant as pathogenic (3 stars; reviewed by expert panel) (https://www.ncbi.nlm.nih.gov/clinvar/). The sources cited for classifying this variant as pathogenic include the following: (i) causing a premature termination codon that is expected to be targeted by nonsense-mediated mRNA decay; (ii) reported in individuals with the cystic fibrosis phenotype; (iii) associated with elevated sweat chloride levels, pancreatic insufficiency, and Pseudomonas infection; (iv) absence of the variant in 121,248 control chromosomes from ExAC; (v) being a common variant in the Black Sea region, particularly in Georgia [4–12].

The present study and previous reports indicate that this variant could be considered a common pathogenic variant or founder variant in populations originating from the Black Sea region, such as the Turkish, Georgian, and Iranian Azeri populations.

The variant c.3196C > T (rs78194216) is a rare pathogenic variant for cystic fibrosis. The frequency of this variant is 0.000064 as shown in the GnomAD variant database (https://gnomad.broadinstitute.org). It was not reported in the Iranian population (based on the Iranome database). According to ACMG guidelines (Varsome criteria: PP5 (ClinVar classifies this variant as Pathogenic, citing 11 articles), PM5 (two pathogenic alternative variants identified), PP3 (MetaRNN = 0.957), PM1 (hot-spot of length 17 amino acids has 25 missense/in-frame variants (13 pathogenic variants, 12 uncertain variants, and no benign), which qualifies as moderate pathogenic), PM2 (variant not found in GnomAD genomes)), ClinVar classifies this variant as pathogenic. Like variant rs121908776, this is a 3-star pathogenic variant (reviewed by expert panel). Several factors contribute to this variant being classified as pathogenic: (i) located in the ABC transporter type 1, transmembrane domain (via InterPro); (ii) altering a conserved nucleotide; (iii) predicted to be damaging by 4/4 in silico tools; (iv) reported in CF patients; (v) reported in a large study including patients from Europe and North America (122/79392 CF alleles = 0.0015); (vi) other missense pathogenic variants reported in the same residue

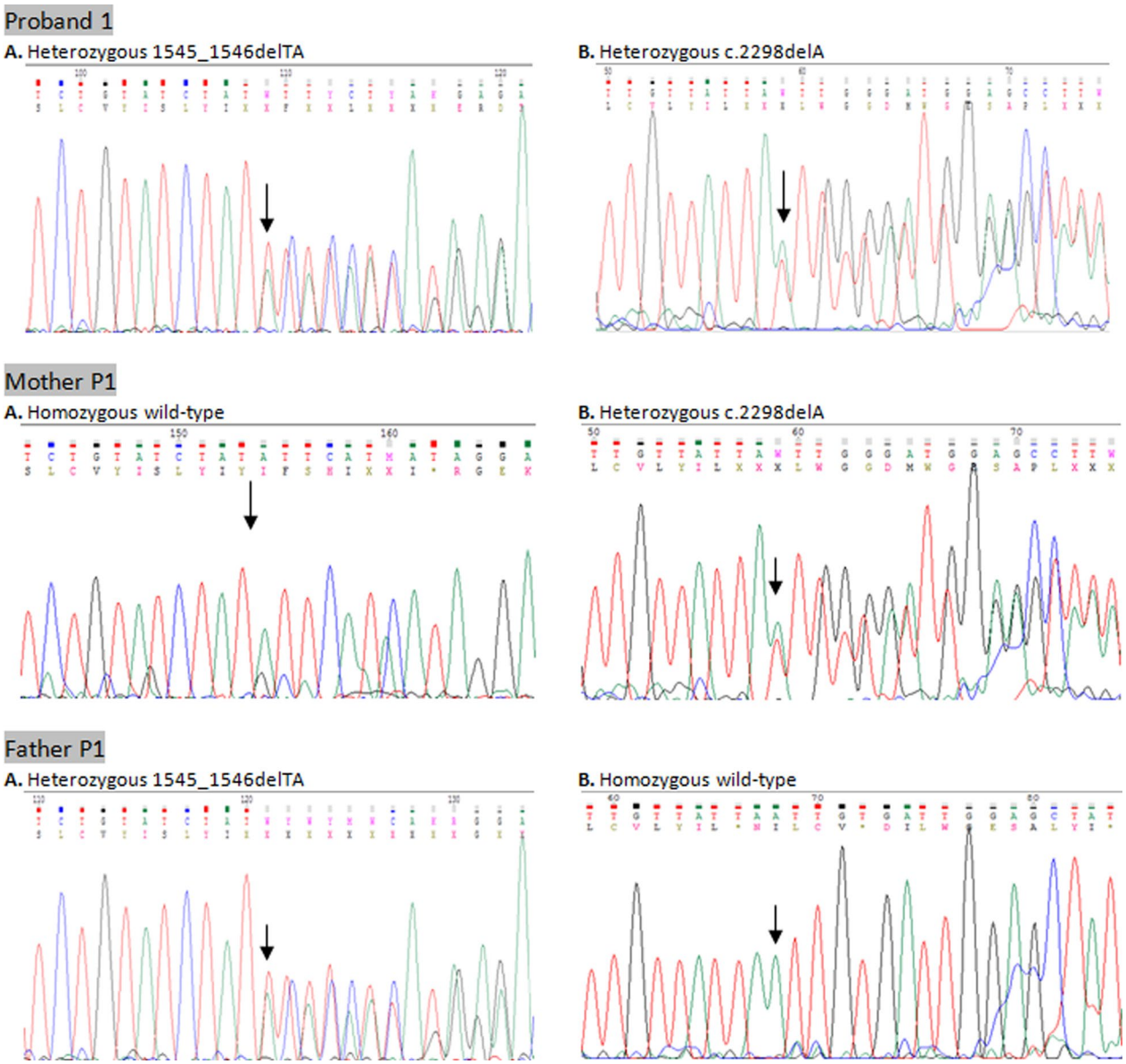

Fig. 1 Compound heterozygosity of the proband 1 and the parental origins of the variants c.1545_1546delTA and C.2998delA

(R1066H, R1066S, R1066L), indicating this residue as a mutational hot-spot region; (vii) support of a damaging effect on the gene product by well-established in vitro or in vivo functional studies; (viii) co-segregating with the disease in multiple affected family members [5, 13–22].

The variant rs397508475 (c.2998delA:p.Ile1000LeufsTer2) has not been previously documented for its pathogenicity. Due to its frame-shift mutation leading to loss-of-function (LOF) changes, which is a known mechanism of CF disease, affecting one functional domain by the mutation in the exon, including 35 pathogenic variants in this exon, containing 362 pathogenic variants in the truncated region, and not found in the GnomAD genomes and exomes, this variant is classified as likely pathogenic by Varsome with criteria PVS1 (null variant (frame shift) in gene *CFTR*) and PM2 (variant not found in GnomAD genomes and Gnomad exomes). Based on the ACMG guidelines with the criteria PVS1 for being a frame-shift variant, PS4 for detecting an individual with symptoms of CF disease, PM3 for detection as compound heterozygous in trans with a known pathogenic variant, PP1 for being well segregated with the disease, and PM2 for rarity in genomic databases, we classify this variant as pathogenic.

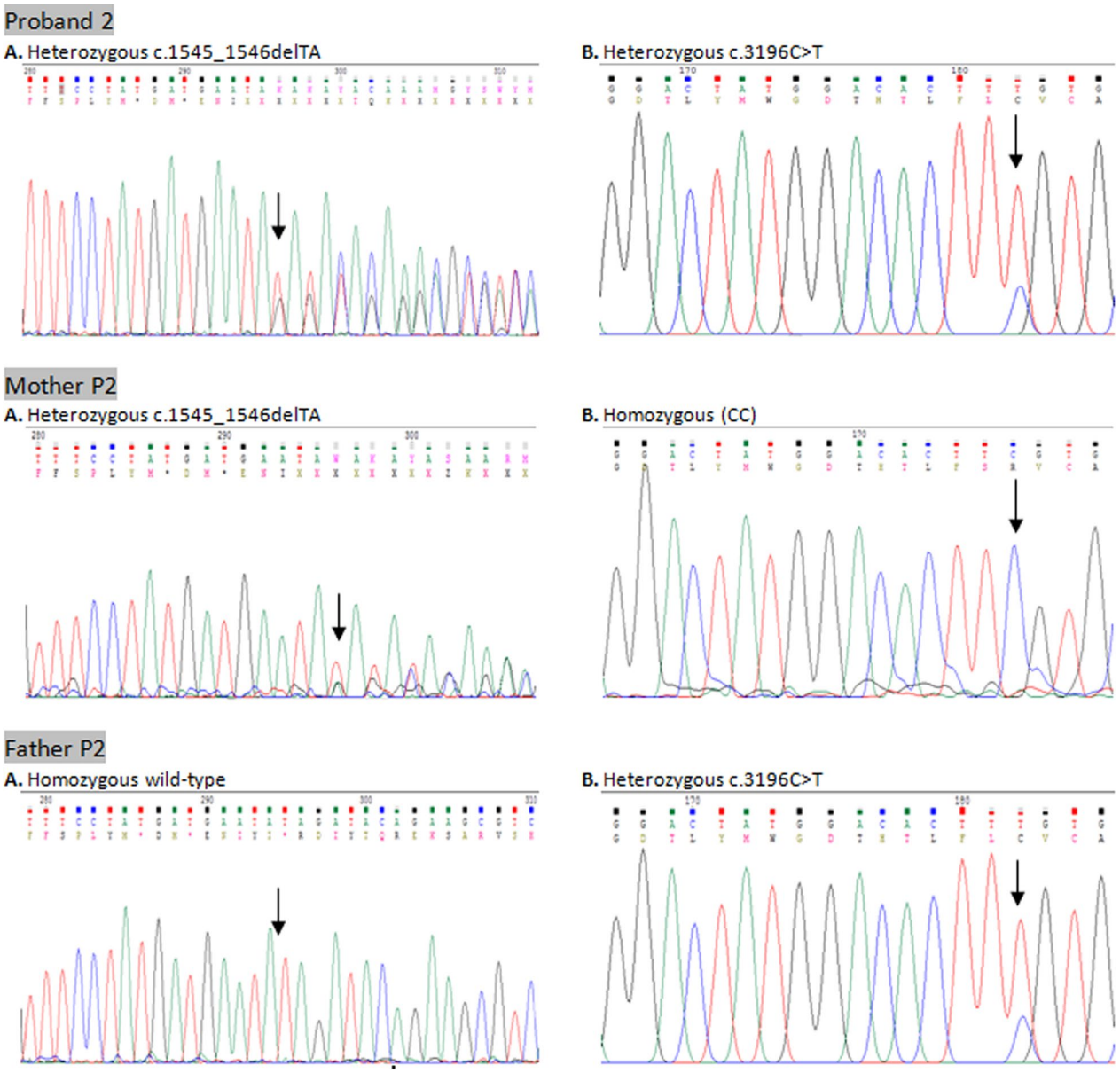

Fig. 2 Compound heterozygosity of the proband 2 and the parental origins of the variants c.1545_1546delTA and C.3196C > T

Conclusion

According to the obtained results, the rare variants c.3196C > T, c.2998delA, and c.1545_1546delTA in the *CFTR* gene, as well as the previously reported pathogenic variants could serve as a basis for future genetic counseling and prenatal diagnosis in Iran.

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Author contributions
J Khalafi and R Farajollahi performed patient recruitment and clinical assessment. H. Akhavan and E Seyedhashemi performed patient recruitment and data analysis. S.E. Hosseini-Asl performed validation of the results through laboratory methods and wrote the article. S.S. Hosseini-Asl analyzed the WES results and concluded the pathogenic status of the variants.

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Availability of data and materials
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The ethical and regulatory issues related to human specimen collection for research purposes have been approved by the Ardabil University of Medical

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Consent for publication
Written informed consent was obtained from the family for this publication.

Competing interests
The authors declare no conflict of interest.

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