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Cystic Fibrosis Cascade Genetic Testing in Families from Goiás, Brazil: A Pilot Study

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Abstract

Cascade genetic testing may extend the benefits of a cystic fibrosis (CF) diagnosis to relatives, yet its application in Brazilian families remains insufficiently documented, particularly for uncommon *CFTR* variants. We evaluated family-based carrier identification, reproductive risk assessment, and a targeted assay for the rare S4X (p.Ser4Ter) variant in Goiás, Brazil. In this descriptive cross-sectional pilot study, four individuals with CF and 51 relatives from four families were enrolled. Relatives underwent pre-test genetic counseling and targeted qPCR testing for familial F508del and, in one family, S4X; pedigrees informed segregation analysis. All 51 invited relatives were tested, of whom 23 (45.1%; 95% CI 32.3–58.6%) carried an investigated variant (19 F508del; four S4X). Carrier proportions ranged from 23.8% to 71.4% across families. S4X was traced across generations within one family, and a custom qPCR assay consistently distinguished noncarriers from heterozygotes across five runs, although comprehensive analytical validation was not achieved. Both partners were genotyped in only six of 15 eligible couples (40.0%), limiting reproductive risk assessment. These findings illustrate the potential of family-centered, variant-targeted testing to inform genetic counseling in a Brazilian regional setting while exposing critical implementation needs. Wider partner testing, clear communication of residual risk following negative targeted results, and independent assay validation are necessary before broader integration into genetic services.

INTRODUCTION

Cystic fibrosis (CF; OMIM #219700) is a multisystemic autosomal recessive disorder caused by biallelic pathogenic variants in the *CFTR* (Cystic Fibrosis Transmembrane Conductance Regulator) gene (Shteinberg et al. 2021). The *CFTR* gene, located in the cytogenetic region 7q31.2, encodes the CFTR protein, a transmembrane protein expressed on the apical surface of epithelial cells in various organs (Riordan et al. 1989). Among its main functions is the regulation of chloride (Cl^-) and bicarbonate (HCO_3^-) ion transport in the lungs, pancreas, liver, intestine and reproductive tract. Consequently, alterations in CFTR protein function impair ion transport and the hydration of secretions, disrupting mucociliary clearance and promoting chronic bacterial colonisation, which contributes to the establishment of a persistent cycle of inflammation and tissue damage (Shteinberg et al. 2021; Morrison et al. 2019).

As of October 12, 2026, a total of 3,733 *CFTR* variants were identified in the ClinVar database using the “<1 kb, single gene” filter, of which 874 were classified as pathogenic or likely pathogenic. This total represents the number of unique variants across both categories, including those assigned to both classifications (668 pathogenic and 361 likely pathogenic), thereby avoiding double counting (NCBI n.d.). According to their functional consequences, *CFTR* variants can be grouped into six (or, in more recent classifications, seven) classes (De Boeck and Amaral 2016).

Among this broad spectrum of genetic alterations, NM_000492.4:c.1521_1523del (p.Phe508del; rs113993960), legacy name F508del, is the most common CF-causing variant, accounting for

approximately 65–70% of CF-associated alleles worldwide, according to the CFTR2 database (last updated January 30, 2026) (CFTR2 2026). The deletion of phenylalanine at position 508 impairs the folding and processing of CFTR, reducing its expression in the plasma membrane and classifying it as a class II variant (Cheng et al. 1990).

Although F508del is the predominant variant worldwide, other pathogenic variants occur at extremely low frequencies and remain poorly characterised. The *CFTR* variant NM_000492.4.c.11C > A (p.Ser4Ter; ClinVar Variation ID: 53211; rs397508173), legacy name S4X, is rare worldwide, accounting for approximately 0.0535% of CF-associated alleles reported in the CFTR2 database (CFTR2 2026). This is a nonsense variant that introduces a premature stop codon at the start of translation, compromising the production of functional CFTR protein and is classified as a class I variant (Glavac et al. 1993). However, in the Brazilian CF Registry, S4X ranks as the 11th most frequent *CFTR* variant, accounting for 1.3% of genotyped alleles (141 alleles) in the 2024 report, indicating a higher frequency in the Brazilian cohort than that reported in the CFTR2 database. Despite this, no commercial genotyping assay is available for S4X (GBEFC 2024).

The high molecular heterogeneity of CF becomes particularly significant when considered alongside the global scale of the disease. A 2024 study by Guo, King, and Hill estimated that 188,336 people across 96 countries were living with CF, of whom only about 60% had been diagnosed (Guo et al. 2024). In the Brazilian context, the 2024 Brazilian Cystic Fibrosis Registry (REBRAFC) reported 6,194 patients with CF, of whom 154 reside in state of Goiás, Brazil (GBEFC 2024). These data underscore the importance of understanding not only the most common variants, but also rare variants present in local populations.

Given the autosomal recessive inheritance of CF, the implications of a molecular diagnosis extend beyond the affected individual to their relatives. In this context, genetic counseling plays a central role by integrating clinical, molecular, and family history information to support the interpretation of genetic findings, assessment of recurrence risks, and identification of relatives who may be carriers or at increased genetic risk. Genetic counseling also provides individuals and families with information regarding inheritance, diagnosis, disease management, and reproductive implications, while supporting informed and autonomous decision-making (Resta et al. 2006; Biesecker 2020). This family-centered approach provides the basis for the systematic identification and testing of relatives at risk.

One of the strategies used to extend this investigation to family members is cascade screening, defined as the systematic and targeted testing for variants previously identified in an index case. This approach constitutes an efficient and cost-effective strategy for identifying carriers, as it allows molecular analysis to be targeted at specific variants already known to be present in the family, thereby avoiding, in many cases, the need for comprehensive and expensive molecular panels (Roberts et al. 2018; George et al. 2015).

In Brazil, next-generation sequencing (NGS) has been increasingly used for molecular characterization of CF and assessment of eligibility for *CFTR* modulator therapies. However, standardized approaches to cascade genetic testing of relatives remain limited, and rare variants such as S4X are not routinely

included in commercially available genotyping panels. In this context, this pilot study aimed to (i) evaluate the yield of cascade carrier testing in four families of CF probands from Goiás, Brazil; (ii) characterize the intrafamilial segregation of F508del and S4X; and (iii) develop and preliminarily evaluate a custom allele-specific quantitative polymerase chain reaction (qPCR) assay for S4X detection.

METHODS

Design, participants and ethics

This observational, descriptive, cross-sectional study included four probands with cystic fibrosis (CF) referred from HC-UFG to CEGH-UFG and 51 relatives recruited for cascade genetic screening. Probands from Families 1, 2, and 4 were homozygous for *CFTR* F508del, whereas the proband from Family 3 was compound heterozygous for F508del and S4X. These genotypes were established through previous molecular testing. Screening uptake was calculated as the proportion of invited relatives who underwent genetic testing.

The study was approved by the Research Ethics Committee of UFG (approval no. 5,027,099; CAAE 51765421.0.0000.5083). Written informed consent was obtained from adult participants or the legal guardians of minors. All participants received pre-test genetic counselling, and post-test counselling was offered following disclosure of genetic results. Further details on recruitment, molecular testing, ethical procedures, and genetic counselling are provided in the Supplementary Methods. The study workflow is illustrated in Supplementary Figure S1.

Clinical and pedigree data

Clinical data from probands were retrospectively extracted from medical records covering the three years preceding data collection. Variables included age at diagnosis and enrollment, body mass index (BMI), metabolic and bone abnormalities, gastrointestinal and respiratory manifestations, pancreatic insufficiency, bacterial airway colonization, pulmonary exacerbations, hospitalizations, antibiotic use, pulmonary function (FEV1, when available), and treatments, including *CFTR* modulators and supportive therapies.

Relatives were characterized by sex, age, birthplace, genotype, carrier status, and reported CF-related manifestations. Pedigrees were constructed to document familial relationships, variant segregation, and reproductive risks. Reproductive risk assessment excluded probands' parents and couples with a deceased partner and considered the availability of genetic testing results for both partners. Further details are provided in the Supplementary Methods.

DNA extraction and quality assessment

Germline DNA was extracted from peripheral blood leukocytes; detailed extraction and quality-control procedures are provided in the Supplementary Methods.

qPCR genotyping and assay validation

Genotyping of *CFTR* F508del and S4X variants was performed using TaqMan-based allelic discrimination assays on a QuantStudio™ 5 Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific, USA). F508del was analysed using a commercial assay (C_151693869_10), internally verified against four previously NGS-characterised samples, whereas S4X was investigated using a custom-designed assay developed in accordance with applicable MIQE recommendations (Bustin et al. 2009). All reactions were performed in duplicate with appropriate genotype controls and no-template controls.

The S4X assay was evaluated for allelic discrimination, intra-assay repeatability, consistency across five independent runs, and performance at decreasing DNA inputs (20, 2, and 0.2 ng per reaction). Validation included comparison with the previously characterised S4X-positive proband and was restricted to WT/WT and WT/S4X genotypes because no S4X/S4X reference sample was available. S4X screening was performed exclusively in Family 3. Detailed assay design, reaction conditions, and validation procedures are provided in the Supplementary Methods.

Genotype calling and quality control

Fluorescence data were analysed using QuantStudio™ Design and Analysis Software v1.5.3. Genotypes were initially assigned automatically from allelic discrimination plots and subsequently reviewed using amplification and multicomponent plots. Samples were considered valid only when technical replicates were concordant, genotype clusters were unambiguous, genotype controls showed the expected profiles, and no amplification was observed in the NTC. All genotype calls were independently reviewed by two assessors. Samples with discordant replicates, ambiguous cluster positioning, atypical amplification profiles, or inadequate fluorescence signals were reanalysed.

Statistical analysis

Descriptive statistics were used to summarize demographic, clinical, and genetic data. Categorical variables were expressed as absolute and relative frequencies, with 95% confidence intervals (Wilson method) for the carrier yield, whereas continuous variables were summarized using mean, median, standard deviation, and range, as appropriate.

RESULTS

Sample composition

The sample comprised 55 individuals, including 4 probands (4/55, 7.3%; all female) and 51 relatives (51/55, 92.7%), distributed across four families ranging from 7 to 21 members. All 51 invited individuals underwent cascade genetic testing, corresponding to an uptake rate of 100% among those invited. Overall, 34 participants were female (34/55, 61.8%) and 21 were male (21/55, 38.2%). Among relatives, 30 were female (30/51, 58.8%) and 21 were male (21/51, 41.2%). Age data were available for 47 of the 51 relatives (47/51, 92.2%). Among these, six (6/47, 12.8%) were younger than 18 years, 37 (37/47, 78.7%) were aged 18–59 years, and four (4/47, 8.5%) were aged 60 years or older. The overall mean age was 36.8 years (SD 16.74; median 36; range 5–73). Geographically, relatives were predominantly from Goiânia (23/51, 45.1%) and Nova Veneza (16/51, 31.4%), followed by Goiatuba (7/51, 13.7%), Inhumas (2/51, 3.9%), Itapirapuã (1/51, 2.0%), Varjão (1/51, 2.0%), and Nazareno (1/51, 2.0%). All municipalities were located in Goiás, except Nazareno, which is located in Minas Gerais, Brazil.

qPCR assay standardization for S4X

Internal verification of the commercial qPCR assay for the *CFTR* F508del variant showed complete agreement between qPCR genotype calls and genotypes previously determined by NGS. The three F508del-homozygous samples and the F508del-heterozygous sample used for verification were correctly classified, corresponding to 100% concordance. Technical duplicates yielded concordant genotype calls, genotype controls showed the expected fluorescence profiles, and no amplification was observed in the no-template controls.

For the S4X variant, a custom allele-discrimination qPCR assay was developed. The assay generated a 108-bp amplicon spanning chr7:117480031–117480138 (GRCh38), with the forward and reverse primers binding at chr7:117480031–117480049 and chr7:117480120–117480138, respectively, and both allele-specific probes spanning chr7:117480095–117480115. The primers and allele-specific probes showed physicochemical and structural characteristics compatible with their application in allele-discrimination qPCR. Oligonucleotide sequences, length, melting temperature, GC content, and predicted hairpin free-energy values are presented in Table 1, while the positions of the primers and allele-specific probes relative to the c.11C > A discriminating nucleotide are illustrated in Supplementary Figure S2.

Table 1
Physicochemical, structural and sequence parameters of oligonucleotides used in the qPCR assay for the S4X variant.

Oligonucleotide	Sequence	Length (bp)	Tm (°C)	GC (%)	ΔG hairpin (kcal/mol)
Primer forward	GGTCTTTGGCATTAGGAGC	19	60.0	52.6	0.00
Primer reverse	AGTTTGGAGACAACGCTGG	19	62.0	52.6	−1.1
Wild-type probe (FAM)	5'-FAM-ATGCAGAGGTCGCCTCTGGAA-MBQ-1-3’	21	67.6	57.1	−3.9
Mutant probe (HEX)	5'-HEX-ATGCAGAGGTAGCCTCTGGAA-MBQ-1-3'	21	64.6	52.4	−3.9

Note: Tm and hairpin ΔG were calculated with Primer3 using SantaLucia (1998) nearest-neighbour parameters (50 mM Na^+, 3 mM Mg^{2+}, 0.8 mM dNTPs, 250 nM oligonucleotide); probe Tm values exclude the contribution of the MGB moiety. The discriminating nucleotide (c.11C > A) is at position 11 of each probe.

An additional substitution at the S4X discriminating nucleotide was identified in gnomAD v4.1.2: *CFTR* c.11C > T (p.Ser4Leu; rs397508173), with an allele frequency of 2.231×10^{-5} (36/1,613,664 alleles) and no homozygous individuals reported. Unlike the pathogenic S4X variant (c.11C > A), this alternative substitution is classified as a variant of uncertain significance in ClinVar. Two further rare variants immediately adjacent to the discriminating nucleotide were identified: rs775329266 (chr7:117480104T > C; AF = 6.198×10^{-7}) and rs1158877973 (chr7:117480106G > T; AF = 6.196×10^{-7}). Both were observed in a single allele, with no homozygous individuals reported.

Additionally, 74 other alternative alleles were identified within the primer- and probe-binding regions. Of these, 29 had available population allele frequencies in gnomAD v4.1.2, ranging from 6.20×10^{-7} to 2.01×10^{-4}, whereas 45 were not reported in the queried dataset. The observed variants were predominantly represented in European and Middle Eastern genetic ancestry groups. Their identifiers and population allele frequencies are provided in Supplementary Table S1. The potential effects of these variants on primer and probe hybridisation, amplification efficiency, and genotype discrimination were not experimentally evaluated.

Under the established reaction conditions, the custom assay produced clearly distinguishable WT/WT and WT/S4X genotype clusters. Amplification and multicomponent plots showed stable fluorescence profiles for the allele-specific probes, with no evidence of nonspecific amplification or contamination in the no-template controls. The S4X-positive proband, previously characterized by sequencing, was correctly classified as WT/S4X by the custom qPCR assay.

Across five independent qPCR runs performed under equivalent experimental conditions, the assay consistently produced distinguishable WT/WT and WT/S4X genotype clusters. Although different

samples were analysed across runs, the overall allelic-discrimination patterns and fluorescence profiles remained consistent. Minor dispersion of individual data points was observed in some runs but did not interfere with genotype assignment, which was supported by multicomponent-plot analysis.

Analytical sensitivity was evaluated using total genomic DNA inputs of 20ng, 2ng, and 0.2ng per reaction. Clear discrimination between WT/WT and WT/S4X genotypes was maintained at all three DNA inputs, including the lowest amount tested. Triplicate reactions remained consistently grouped within their respective genotype clusters, with no overlap between WT/WT and WT/S4X samples. The analytical sensitivity experiment is shown in Supplementary Figure S3.

All samples analysed in duplicate showed concordant genotype calls, supporting intra-assay repeatability. Because an S4X/S4X reference sample was unavailable, validation was restricted to discrimination between WT/WT and WT/S4X genotypes.

Clinical characterization of probands

Clinical characteristics are detailed in Supplementary Table S2. All four probands had pancreatic insufficiency. The two adult probands (26 and 23 years) were receiving elexacaftor/tezacaftor/ivacaftor and had FEV1 values of 57% and 65% predicted, respectively. Pulmonary exacerbations occurred only in Proband 3, and no hospitalizations were documented during the three-year evaluation period.

Genotype distribution and allele frequency

Among 51 relatives, 23 (45.1%; 95% CI, 32.3–58.6%) carried an investigated *CFTR* variant, including 19 F508del and four S4X carriers (Fig. 1).

Familial segregation

Carrier frequencies varied across families: 71.4% (5/7), 60.0% (9/15), 23.8% (5/21), and 50.0% (4/8) in Families 1–4, respectively. In Family 3, S4X was identified in four relatives across generations within a specific pedigree branch (Supplementary Figure S4).

Among 15 eligible couples, both partners were genotyped in six (40%). No additional couples carrying the investigated variants in both partners were identified. Genotypes and reproductive probabilities are detailed in Table 2. Residual reproductive risk could not be excluded because testing was restricted to familial variants.

Table 2
CFTR genotypes and estimated reproductive probabilities among six couples from four families affected by CF.

Family	Couple (Pedigree ID)	Genotypes	Recurrence probability (%)
1	IV-6 × IV-7	F508del/F508del × F508del (−)	100% (carrier offspring); residual risk of affected offspring cannot be quantified
2	I-1 × I-2	F508del (−) × F508del/WT	50% (carrier offspring); residual risk of affected offspring cannot be quantified
2	I-3 × I-4	F508del (−) × F508del/WT	50% (carrier offspring); residual risk of affected offspring cannot be quantified
3	IV-1 × IV-2	F508del (−), S4X (−) × F508del (−), S4X (−)	0% (carrier offspring for either tested variant); residual risk of affected offspring cannot be quantified
4	II-5 × II-6	F508del (−) × F508del/WT	50% (carrier offspring); residual risk of affected offspring cannot be quantified
4	III-5 × III-6	F508del/WT × F508del (−)	50% (carrier offspring); residual risk of affected offspring cannot be quantified

Note: Carrier probabilities were estimated according to Mendelian inheritance and refer exclusively to the investigated familial *CFTR* variants (F508del and S4X). Negative results indicate that the tested variants were not detected and do not exclude the presence of other pathogenic *CFTR* variants. Therefore, the residual risk of having offspring affected by cystic fibrosis could not be quantified.

DISCUSSION

This pilot study demonstrated the feasibility of combining cascade genetic screening with targeted qPCR genotyping in four families affected by CF in Goiás, Brazil. Carrier identification and characterization of familial variant segregation provided information relevant to genetic counselling and reproductive risk assessment. Targeting known familial variants may also reduce the need for broader molecular testing in relatives (Biesecker 2020; George et al. 2015; de Wert et al. 2026).

The inclusion of minors warrants ethical consideration. Although carrier testing for autosomal recessive conditions is generally discouraged in asymptomatic children without immediate medical benefit, testing was conducted at the families' request, with parental or guardian consent, to investigate familial segregation. These circumstances highlight the importance of genetic counselling and respect for minors' future autonomy (Ross et al. 2013).

The commercial F508del assay showed complete agreement with previously established NGS genotypes, while the custom S4X assay demonstrated promising preliminary performance. Its oligonucleotides exhibited suitable predicted thermodynamic characteristics, and experimental evaluation showed consistent discrimination between WT/WT and WT/S4X genotypes across technical replicates, independent runs, and decreasing DNA inputs (Bustin et al. 2009; Dieffenbach et al. 1993;

Livak 1999). Nevertheless, the absence of an S4X/S4X reference sample and a broader independent validation panel precluded comprehensive assessment of analytical performance.

Clinical heterogeneity was observed among the four probands, including those sharing the F508del/F508del genotype. All presented pancreatic insufficiency, and airway colonisation included *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Differences in age, diagnostic history, treatment, and potential genetic or environmental modifiers may contribute to clinical variability (Blanchard and Waters 2019; Alvarez et al. 2004; Butnariu et al. 2021). Proband 1, diagnosed at 19 years of age, was born before the establishment of Brazil's National Neonatal Screening Program in 2001, highlighting the importance of early detection (Athanazio et al. 2017; Brazil 2001).

The carrier yield reflects targeted cascade screening rather than population prevalence. Carrier frequencies ranged from 23.8% to 71.4% across families, consistent with differences in family structure and Mendelian segregation. Reproductive risk assessment was limited by incomplete partner genotyping and the inability of targeted assays to exclude other pathogenic *CFTR* variants. Broader molecular testing may therefore be appropriate when indicated during reproductive genetic counselling (de Wert et al. 2026; Bienvenu et al. 2020).

S4X is rare internationally, representing approximately 0.0535% of CF-associated alleles in CFTR2. It was reported in only three alleles among more than 1.6 million evaluated in gnomAD and was absent from the ABraOM dataset (CFTR2, 2026; Broad Institute n.d; Naslavsky et al. 2022). In contrast, Brazilian studies have documented higher representation among individuals with CF. Silva Filho et al. reported a national frequency of 0.8%, reaching 2.5% in the Midwest, while the 2024 Brazilian CF Registry reported 1.3% (GBEFC 2024; da Silva Filho et al. 2021). Regional variation in *CFTR* genotypes has also been documented by Mota et al (2018). These findings support consideration of S4X in regionally adapted molecular screening strategies.

Within Family 3, S4X was identified in four relatives (4/21, 19.0%), segregating across generations within a specific pedigree branch. This pattern is consistent with familial transmission but cannot be extrapolated to Goiás or the general population. The comparatively higher representation of S4X in Brazilian CF cohorts raises the hypothesis of a founder effect, although population-based investigations and haplotype analyses involving unrelated carriers are required to evaluate a potential shared ancestral origin (da Silva Filho et al. 2021; Mota et al. 2018).

The main limitations include the small number of families, targeted rather than comprehensive genotyping, restriction of S4X screening to Family 3, incomplete partner genotyping, and limited independent assay validation. Further studies, particularly those including S4X/S4X reference samples, are needed before routine diagnostic implementation. These findings should therefore be interpreted as preliminary.

CONCLUSIONS

This pilot study demonstrated the feasibility of combining cascade genetic screening with targeted qPCR genotyping in four families affected by CF in Goiás, Brazil. Carrier identification and familial segregation analysis supported genetic counselling and reproductive risk assessment. The custom S4X assay showed consistent allelic discrimination, concordant technical replicates, and reproducible performance across independent runs. However, the small sample size and limited validation, particularly the absence of S4X/S4X reference samples, restrict the generalisability of these preliminary findings. Larger studies with independent validation are required before routine implementation.

Declarations

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Competing interests

The authors declare no conflicts of interest.

Consent to participate

Written informed consent for publication of the relevant participant information and pedigrees was obtained from the participants or their legal guardians, as applicable.

Data availability

De-identified genotype data and raw qPCR files (.eds) are available from the corresponding author upon reasonable request, subject to ethical approval.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used AI-assisted tools to support language editing, improve clarity and readability, and assist with bibliographic verification. All AI-assisted suggestions were critically reviewed and independently verified by the authors, who retain full responsibility for the accuracy, integrity, and final content of the manuscript. AI tools were not used for data collection, experimental procedures, data analysis, or interpretation of the study findings.

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Ethics approval

Approved by the Research Ethics Committee of the Federal University of Goiás (protocol 5,027,099; CAAE 51765421.0.0000.5083).

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Figures

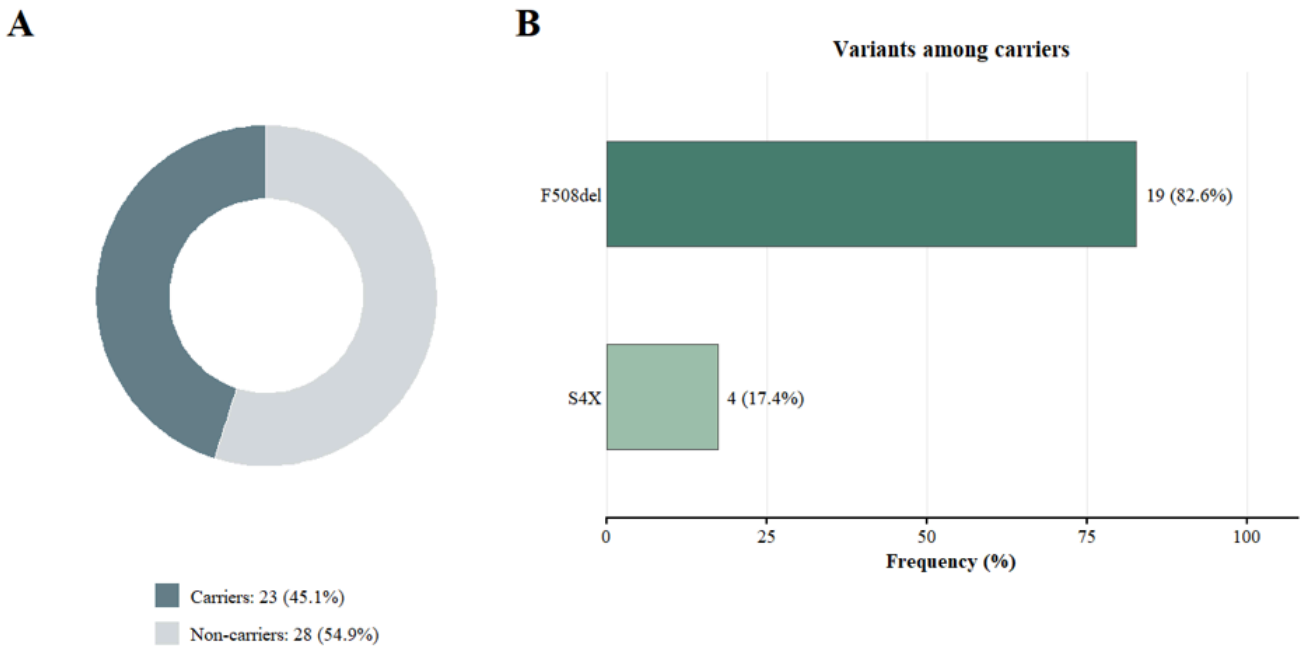


Figure 1

(A) Classification of relatives according to carrier status for the investigated *CFTR* variants (F508del and S4X). (B) Distribution of F508del and S4X among carrier relatives (n = 23), expressed as counts and percentages.

Supplementary Files

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