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CFTR Complex Alleles in Individuals with Cystic Fibrosis Showing Limited Response to Elexacaftor/Tezacaftor/Ivacaftor (ETI) Triple Modulator Therapy

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Abstract

Background

In many people with cystic fibrosis (CF, pwCF), genetic diagnosis often detects only common *CFTR* variants. Complex alleles—multiple variants on the same allele—frequently remain undetected, yet can influence CFTR function, response to modulators such as elexacaftor/tezacaftor/ivacaftor (ETI), and disease progression. Understanding CF's genetic diversity is essential for advancing personalized therapies. We investigated whether individuals with reduced ETI response carry complex alleles and further characterized these alleles.

Methods

Next generation sequencing (NGS) enables identification of previously unknown variants and determination of their phase (*in cis* or *in trans*). Given the unclear frequency and functional role of most complex alleles, NGS was used to comprehensively screen for all variants.

Results

We recruited 42 cases from participating CF centers, including 16 high responders, 14 low responders, and 12 intermediate responders, classified based on their reduction in sweat chloride following ETI (>40%, ≤15%, and 15–40%, respectively).

Among low responders 8 of 14 individuals carried a complex allele, seven of whom harbor [F508del;L467F] and one a yet undescribed complex allele [F508del;1585-9412A>G]. Reduced response occurred when the complex allele was present on both alleles or paired with a class I variant. In each group of the intermediate and high responders one pwCF carried a complex allele.

Conclusion

Detection of complex alleles is crucial for understanding CF disease progression and therapy response. NGS is an effective method to identify complex alleles and distinguish responder classes. Further studies are planned to better characterize the frequency and variability of complex alleles across different responders in Germany.

Background

In recent years, more comprehensive molecular genetic testing, especially via Next Generation Sequencing (NGS), has led to several reports of complex alleles, indicating the presence of more than one variant on the same allele within the Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) gene (referred to as *in cis*, as opposed to *in trans*, meaning on two different alleles). For most of these complex alleles, the clinical relevance is unclear as they are rarely occurring [(1)]. Detailed studies of four complex *CFTR*-alleles ([S466X;R1070Q];[CFTRdele2,3], [E217G;G509D];[F508del], [F1052V;1367del5]; [c.1209+2T>C] and [F508del;L467F];[F508del], given as legacy names if available) that were tested *in vitro* regarding their effects on folding, CFTR function, and the efficacy of modulator therapy, were conducted in the past [(2, 3)]. The results showed, that the [F508del;L467F] allele, observed in people with CF (pwCF) from Russia, Italy and France, significantly reduces the effectiveness of modulator therapy when present either in a homozygous state or *in trans* with a non-responsive variant[(3–5)]. However, in the era prior to availability of the triple modulator therapy **e**lexacaftor/**t**ezacaftor/**i**vacaftor (ETI), these pwCF showed no observable clinical differences compared to pwCF homozygous for the F508del variant [(6)], hindering earlier detection of the allele. In the Genome Aggregation database gnomAD v4.1.0 [(7, 8)], the L467F variant (NM_000492.4(CFTR): c.1399C>T, p.(Leu467Phe)) is detected in 40 individuals of European descent (last accessed on the 15th of January 2026). For 10 of these individuals, a F508del variant (NM_000492.4(CFTR):c.1521_1523del, p.(Phe508del)) is present simultaneously, although the data do not provide information on whether it is *in cis* or *in trans* (gnomAD v2.1.1). The leucine at p.467 within the *CFTR* gene is highly conserved, the *in silico* predictions for pathogenicity are particularly conspicuous (REVEL-Score [(9)] = 0.785 (cut-off for pathogenicity > 0.7)), and another amino acid substitution at the same position has already been classified as pathogenic. It could be shown that the L467F variant itself does not result in a classical CF phenotype, but disturbs the maturation of the CFTR protein, possibly through a disrupted interaction with the cytoskeleton [(5)]. Nothing is known about the prevalence of this allele in Germany.

By 2020, more than 120 complex alleles have been identified in *CFTR* [(10)]. In Germany, approximately 50 different complex alleles with unknown clinical or therapeutic relevance were documented in the German CF registry (12/2024 [(11)]). The complex allele [F508del;L467F] was not among them.

To gain a complete understanding of *CFTR* variants and their clinical consequences, it is essential to implement workflows such as NGS in routine CF diagnostics to identify previously unknown variants and complex alleles. Due to its detection levels of up to 99 %, NGS is considered to provide a more precise diagnosis of CF compared to screening for the most common variants [12]. Genotyping panels are currently the method of choice when screening for *CFTR* variants, due to its robustness and easy to handle workflow [13, 14]. However, utilizing NGS at an early stage can facilitate the prompt administration of targeted therapies, as opposed to postponing intervention, administering wrong therapeutics or relying on false diagnoses, merely because NGS is not yet established as the standard diagnostic approach. Additionally, one major advantage of NGS when compared to Sanger sequencing is the possibility to detect variant phase, meaning the localisation on the same allele or on different alleles. This approach only works for variants located within a distance of ≤300 bp, corresponding to the library insert size when using short read technologies.

Material and Methods

To identify and characterize the landscape of complex alleles EDTA-Samples of 42 pwCF were collected in a multicentre manner and divided into response groups based on sweat chloride changes (in percent):

1. No or only minimal decrease in sweat chloride levels (0–15 %) following initiation of ETI, or
2. Low or no response to ETI according to the treating physician's assessment (e.g. no improvement in percent predicted FEV1 (ppFEV1) after a minimum of 12 months of ETI therapy OR no improvement in body mass index (BMI) after a minimum of 12 months of ETI therapy)

Furthermore, we formed two more response groups according to sweat chloride changes compared to a baseline sweat chloride level prior to ETI therapy:

- Intermediate Responder: > 15 %, ≤ 40 % reduction of sweat chloride values compared to baseline level
- High Responder: > 40 % reduction of sweat chloride values compared to baseline level.

Sweat chloride values were obtained from before and during ETI treatment for the entire cohort. If there was more than one measurement available, the median was determined.

All of the participants in this study had an additional tube of EDTA whole-blood collected during their regularly scheduled quarterly visit (approximately 2 mL). The blood was sent to the Institute of Human Genetics, Leipzig alongside a signed written consent form, information regarding clinical data (via the REDCap tool (Version 14.6.10; © 2025 Vanderbilt University) [15, 16]) and assessment data on the current quality of life of the participants (standardized, externally validated, and published quality of life questionnaire CFQ-R). The Cystic Fibrosis Questionnaire–Revised (CFQ-R) is the principal patient-reported outcome measure in CF research and has been widely used to assess the effects of ETI therapy in pwCF [17, 18].

From the collected EDTA whole blood, genomic DNA was isolated in the laboratory using the MagCore® HF16 Plus Nucleic Acid Extractor and 101 cartridge by RBC Bioscience Corp., New Taipei City, Taiwan, and DNA concentration was measured using the Qubit 3/4 (Thermo Scientific™, Waltham, Massachusetts, USA) and Infinite® 200 PRO (Tecan Trading AG, Switzerland). Library preparation was performed using the Twist Library Preparation EF Kit 2.0

and the Twist Universal Adapter System - TruSeq Compatible, 96 Samples Plate A-D from Twist Bioscience (San Francisco, California, USA). Targeted enrichment was conducted using probes from the previously developed Twist Custom Panel (TE-91078956), covering all *CFTR* exons, introns, and regulatory regions, before sequencing on an Illumina platform [12].

Raw data analysis was carried out using Varfeed software (Limbus, Rostock), and variants (SNVs and CNVs) were classified according to the latest criteria of the American College of Medical Genetics and Genomics [19], as well as ACGS Best Practice Guidelines for Variant Classification [20] using Varvis software (Limbus, Rostock, Germany). *CFTR* variants were annotated using the MANE transcript NM_000492.4 to ensure consistent variant nomenclature aligned to the human genome assembly GRCh38 (hg38) [21]. Data analysis, visualization and Chi-square statistics were calculated in Microsoft Excel (Version 2408; Microsoft Corporation, Redmond, WA, USA, Figure 1) [22] and in R (Version 4.4.2; R Foundation for Statistical Computing, Vienna, Austria) [23] using RStudio (Version 2024.04.2+764; Posit Software, PBC, Boston, MA, USA, Figure 2 and 3) [24]. Pairwise comparisons were performed using two-sided Fisher's exact test [25]. To confirm the phase of the variants the Integrative Genomics Viewer (IGV) was used as a tool for visualization [26]. Phasing was determined by short-read sequencing based on variant proximity, and alternatively by segregation analysis of parental samples. Treating physicians received a report on the results after validation and interpretation of the sequencing data.

Results

A total of 42 cases from participating CF clinics were included, comprising 16 high responders and 26 cases whose response were not as expected after ETI initiation (all summarized in Suppl. Table S1). 14 of these showed no or only a slight drop in sweat chloride value (low responder) and 12 pwCF could be sorted into the intermediate group (Figure 1). We used NGS to investigate the genotype of the collected cases.

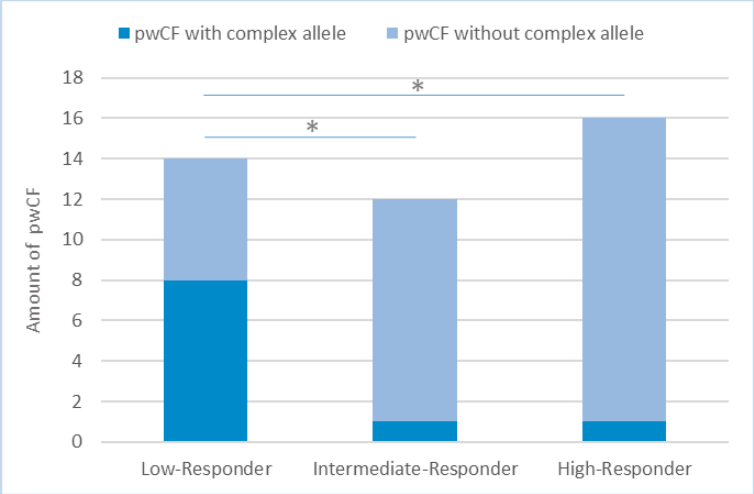


Figure 1: Distribution of pwCF with (dark blue) and without (light blue) complex alleles in the three response groups. In the low responder group 8 out of 14 carried a complex allele. In the intermediate and high responder groups only 1 pwCF respectively carried a complex allele (low to intermediate responder p=0,014; low to high responder p=0,0043).

Eight of the 14 low responders carried a complex allele, seven of which the previously known [F508del;L467F] complex allele and one a previously undescribed [F508del;1585-9412A>G];[1898+1G->A] allele. The [F508del;L467F] variant occurred in homozygous state in three individuals, while in four cases it was heterozygous, with a class I variant *in trans* (Figure 2, Table 1). The sweat chloride values differed between 87 and 110 mmol/L before and 86 and 116 mmol/L during ETI treatment. On average, sweat chloride changed by ±9 % in the low responder cohort. 11 improved slightly, while 3 worsened in their sweat chloride values. One individual, carrying the [F508del;L467F] complex allele did not receive ETI therapy yet, justifying absent sweat chloride values during ETI therapy. Another case did not provide medical reports of sweat chloride values before ETI therapy due to a move to Germany. This individual is homozygous for F508del and does not carry a complex allele. Both cases were not included in the subsequent analysis.

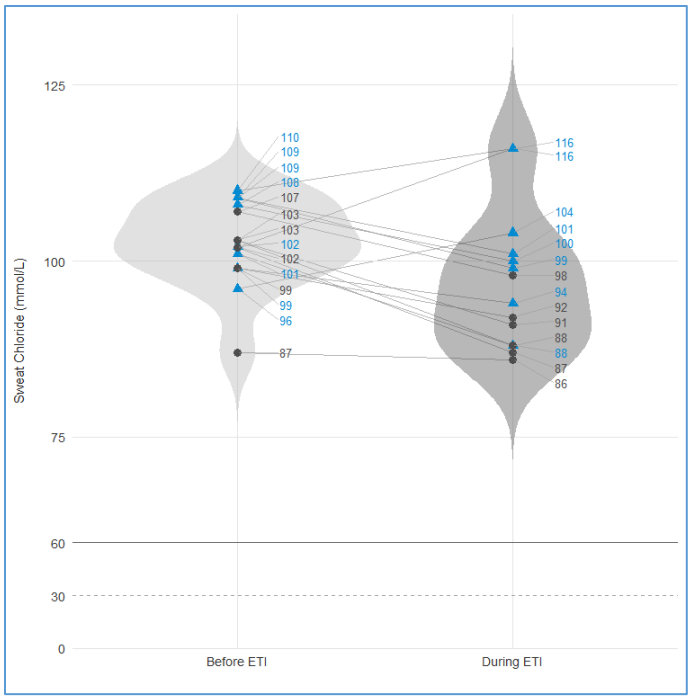


Figure 2: Sweat chloride reduction in the course of before and during treatment with ETI therapy for 14 low responders with known sweat chloride values. The light grey plot represents the sweat chloride values before ETI treatment, the dark grey plot during ETI treatment. The solid line indicates 60 mmol/L, the cutoff for pathogenicity in CF. The dashed line indicates 30 mmol/L. Marked in blue are the cases identified with a complex allele.

Table 1: Overview of pwCF with a complex allele and short description of their *CFTR* genotype given as legacy name. Shown are the eight pwCF from the low responder (LR) group, seven of which carry the [F508del;L467F] complex allele and one of which carries the [F508del;1585-9412A>G] complex allele, as well as the two pwCF from the intermediate (IR) and high-responder (HR) groups with their respective genotypes. Further details on all participants in Suppl. Table S1.

Group	Legacy Name
LR	[F508del;L467F];[F508del;L467F]
LR	[F508del;1585-9412A>G];[1898+1G->A]
LR	[F508del;L467F];[N1303K]
LR	[F508del;L467F];[F508del;L467F]
LR	[F508del;L467F];[CFTRdele2,3]
LR	[F508del;L467F];[2184delA]
LR	[F508del;L467F];[F508del;L467F]
LR	[F508del;L467F];[G542X]
IR	[F508del;L467F];[F508del;L467F]
HR	[F508del;1874insT];[F508del]

In the intermediate group we could identify one case carrying the complex allele [F508del;L467F] (Figure 3, Table 1). The sweat chloride values differed between 94 and 110 mmol/L before and 62 and 86 mmol/L during ETI treatment. On average, sweat chloride

changed by 28 % in the intermediate responder cohort. All of them improved in their sweat chloride values.

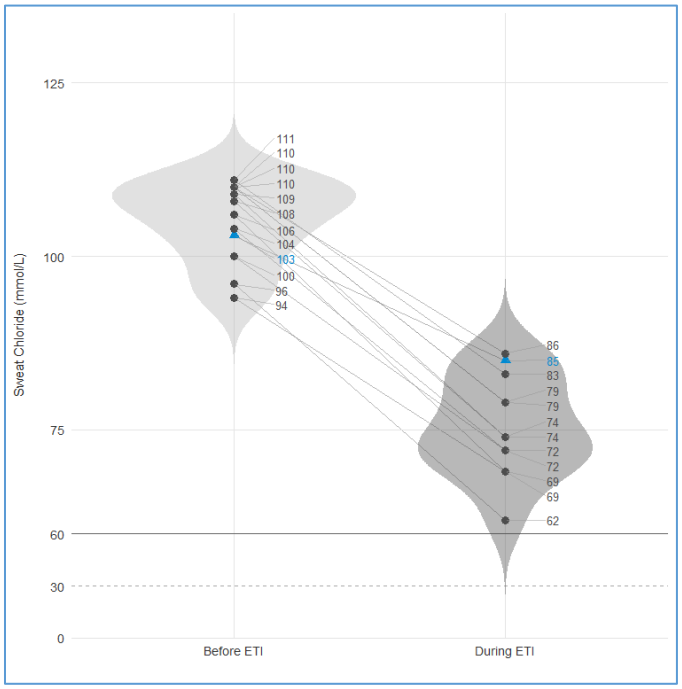


Figure 3: Sweat chloride reduction in the course of before and during treatment with ETI therapy for the group of the 12 intermediate responders. The light grey plot represents the sweat chloride values before ETI treatment, the dark grey plot during ETI treatment. The solid line indicates 60 mmol/L, the cutoff for pathogenicity in CF. The dashed line indicates 30 mmol/L. Marked in blue is the one case identified with a complex allele.

All high responder improved through ETI treatment and showed reduced sweat chloride levels by over 40 mmol/L (Figure 4). In seven cases, the sweat chloride was reduced to below 30 mmol/l, which corresponds to values observed in healthy individuals [27]. The sweat chloride values differed between 74 and 108 mmol/L before and 9 to 60 mmol/L during ETI treatment. On average, sweat chloride changed by ±70 % in the high responder cohort.

While sequencing the 16 high responders as controls, we identified an additional heterozygous *CFTR* variant in a patient previously thought to only be homozygous for F508del. This complex allele [F508del;1874insT] (Table 1) has not been described in the literature to date, but has been published by us recently [28]. Because the diagnosis had originally been made through targeted CF screening, the additional variant was missed, leading to the incorrect assumption that the individual carried two class II alleles. In fact, the second variant c.1742dup, p.(Leu581Phefs*8), 1874insT likely causes a frameshift and most likely a subsequent nonsense-mediated mRNA decay, classifying it as a class I mutation. Despite only carrying one “true” F508del allele, the child was treated with lumacaftor/ivacaftor – a CFTR

modulator approved for individuals homozygous for the F508del variant – regardless of limited evidence of its effectiveness in pwCF heterozygous for F508del [29], before switching to ETI. Notably, her clinical response to lumacaftor/ivacaftor was suboptimal, whereas her response to ETI was within the expected range, indicating no effect on ETI responsiveness of this identified complex allele [28]. We could not identify any additional carriers of complex alleles in the high responder cohort.

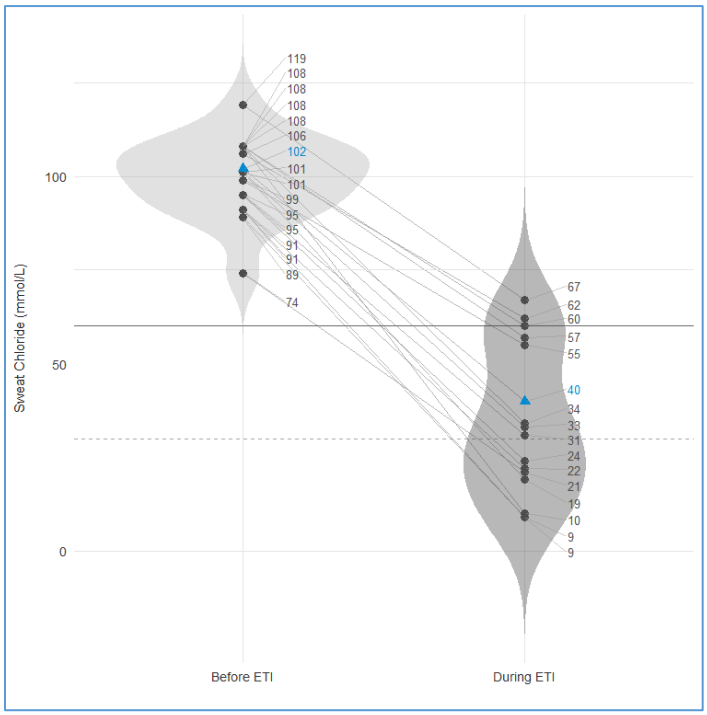


Figure 4: Sweat chloride reduction in the course of before and during treatment with ETI therapy for the group of the 16 high responders. The light grey plot represents the sweat chloride values before ETI treatment, the dark grey plot during ETI treatment. The solid line indicates 60 mmol/L, the cutoff for pathogenicity in CF. The dashed line indicates 30 mmol/L. Marked in blue is the one case identified with a complex allele.

The frequency of complex alleles differed significantly between the responder groups ($\chi^2(2) = 12{,}88$, p=0,0016). Low responders more often carried a complex allele (8/14), while only 1/12 intermediate responders (p-value: 0,014) and 1/16 high responders were found to harbour a complex allele (p-value: 0,0043). Low response only occurred when the complex allele was present on both alleles or when a class I variant was present *in trans* to the complex allele.

Discussion

The incidence of complex alleles in the context of CF in Germany, Europe and worldwide has not been investigated to date and these alleles cannot be detected in most routinely performed genetic analyses for CF diagnosis. Therefore, we aimed at understanding and better characterising the occurrence and frequency of complex alleles in Germany using NGS.

Complex alleles have been described in several genes associated with monogenic diseases. For example, a complex allele in the β-globin locus consisting of a deletion in *HBD* together with a splice variant in *HBB* forms the so-called Corfu $\delta^0\beta^+$ thalassemia allele and results in a distinct hematological phenotype [30]. Likewise, in the retinal disease gene *ABCA4*, cis-acting variants within the same allele can modify the penetrance of pathogenic variants and thereby influence disease manifestation in Stargardt disease [31]. Although these and other reports demonstrate that complex alleles are not restricted to a single gene, the phenomenon has been studied most extensively in the *CFTR* gene, where numerous complex alleles have been described and shown to influence disease severity and therapeutic response [32].

Previous studies have investigated the occurrence and functional consequences of *CFTR* complex alleles in several European populations. In a French study, Baatallah et al. identified multiple cis-variants associated with the common F508del variant and demonstrated that some of these can affect *CFTR* maturation or splicing, thereby modifying the response to CFTR modulators [5]. Similarly, Sondo et al. showed that the complex allele [F508del;L467F] markedly reduces the pharmacological rescue of CFTR by elexacaftor/tezacaftor/ivacaftor [4]. Studies from Russia further suggest that complex alleles are not rare, with frequencies of approximately 8 % among F508del homozygous carriers reported in CF cohorts [3]. Taken together, these findings indicate that complex *CFTR* alleles occur across multiple European populations and may represent an important source of genetic and therapeutic variability. Compared to the literature, we identified a complex allele in 23.8 % of our total cohort. When focusing on low responders, the [F508del;L467F] complex allele was detected in 30.8 % of cases (8 out of 26), 15.4 % (4) of which carried [F508del;L467F] in a homozygous state. The p-value comparing the low responder to each the intermediate and high responder cohort indicates that the presence of a complex allele is associated with a lower response to ETI.

The one case found in the intermediate group with the [F508del;L467F] complex allele shows sweat chloride value reduction of 17 %, which is only 2 % above the threshold for low responders. We selected the thresholds for our three groups according to literature data. A publication from the German CF registry and a real world study from the Italian SITTMA group state that sweat chloride values change in the course of ETI treatment by 43.7 to 50.9 mmol/L, with many patients reaching intermediate or even normal ranges (<60 or <30 mmol/L) [33, 34]. Given the known intra-individual variability of sweat chloride measurements (limits of repeatability up to approximately ±20 mmol/L), we defined low responders as individuals with a sweat chloride reduction of ≤15 %, as changes within this range may reflect measurement variability rather than a true treatment response [35]. Whenever possible, multiple sweat chloride measurements were obtained and the median value was used for comparison. However, for patients with only a single measurement available, measurement variability may have influenced the classification, and some individuals categorized as intermediate responders might actually belong to the low or high responder groups.

NGS allows for the determination of variant phase, which is critical when examining complex alleles. Additionally, it enables the detection of previously unknown or rare *CFTR* variants that would be missed by targeted approaches. Finally, NGS offers a more cost-effective solution compared to Sanger sequencing of all 27 *CFTR* exons, particularly when applied at scale. Given these advantages, the use of NGS represents a more comprehensive, efficient, and economically viable strategy for *CFTR* variant analysis, with direct impact on diagnosis and treatment decisions, as demonstrated clearly through the case report described above [28].

Recent work by Kondratyeva and colleagues [36] reported that treatment with the recently approved triple modulator Vanzacaftor/Tezacaftor/Deutivacaftor (VTD, Alyftrek) in two patients carrying the [F508del;L467F] complex allele led to a marked reduction in sweat chloride and improvement in lung function. These results suggest that VTD may offer therapeutic benefit for patients with complex alleles, especially [F508del;L467F], known to respond less strongly to other CFTR modulators. Rather, the therapeutic response appears to depend on the specific variant combination and its functional consequences for CFTR. However, current evidence remains limited to small case series, and larger prospective studies are required to confirm these observations and to guide genotype-specific treatment strategies.

Outlook

After characterizing the nature and frequency of complex alleles, *in silico* modelling of *CFTR* variants carrying complex alleles may provide insights into structural alterations and their impact on ETI responsiveness. Furthermore, electrophysiological analyses, including intestinal current measurement (ICM) [37], nasal potential difference (nPD) [38], and nasal brushing, could assess residual channel function and modulator efficacy. Finally, genome-wide sequencing of collected samples may help identify genetic modifiers outside *CFTR*, and a complementary pharmacogenetic analysis could be performed in pwCF who show limited or no response to therapy despite the absence of complex *CFTR* alleles.

Limitations

A key limitation of this study is that our analysis focused primarily on sweat chloride levels. While these measurements provide important insights, they do not capture the full spectrum of clinical outcomes. Future studies should therefore include additional parameters such as lung function and BMI to obtain a more comprehensive understanding of patient responses [33].

Moreover, other clinical variables were not considered due to the relatively low return rate of patient questionnaires, which limited our ability to perform broader analyses. Another limitation is that we did not take ethnicity into account. Incorporating ethnic background in future research could help to better characterize the distribution of complex alleles and their potential impact across diverse populations. Addressing these points in subsequent studies will be crucial to provide a more complete picture of genotype–phenotype correlations and treatment responses.

Conclusion

Overall, the results of this study contribute to deepening our understanding of individual variations in treatment response in CF and could potentially help to improve clinical care and quality of life of affected pwCF. Through this work, NGS workflows should be implemented in routine diagnostics of CF, to help pwCF receive the best therapeutic options. Costs can be saved by the application of NGS, while screening for SNVs and CNVs in parallel [12] and by and by reducing the use of costly modulator therapies in patients with limited expected benefit.

Abbreviations

pwCF	person/people with CF
i.e.	in example
SNV	Single Nucleotide Variant
CNV	Copy number Variant
ETI	Elexacaftor/Tezacaftor/Ivacaftor
NGS	Next Generation Sequencing
CF	Cystic Fibrosis
bp	base pair
VTD	Vanzacaftor/Tezacaftor/Deutivacaftor

Declarations

Ethics Approval

We confirm that we have read and understood the journal's ethical publication policies and that this report adheres to those standards. Ethical approval for this research was obtained from the Ethics Committee of the University of Leipzig under the signature 123/24-ek.

Consent for publication

Written informed consent for publication of their clinical data was obtained from all participants included in this study.

Availability of data and materials

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Authors' contributions

MK analyzed and interpreted the patient data with respect to the presence of complex alleles. SA and JH reviewed the sequencing data. MK, SA, and JH drafted the manuscript. All authors critically revised the manuscript and approved the final version.

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

During the preparation of this work the authors used ChatGPT in order to improve language and readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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