How can whole *CFTR* genotyping contribute in genetically unsolved Cystic Fibrosis cases?

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Background/Objectives

Genotyping Cystic Fibrosis (CF) patients is crucial for diagnosis confirmation and treatment options. Recent modulator therapies allow for correction of malfunctioning CFTR, but depend on the underlying genotype. Still 5.4% of patients [1] remain undiagnosed after conventional genetic testing and can therefore benefit from whole *CFTR*-genotyping.

Methods

731 patients with clinically confirmed CF-diagnosis, but ambiguous genotype were assembled using the German CF Registry. 508 variants were identified and re-classified, if required using ClinVar, HGMD and CFTR1/2 and corrected in the registry database. Genetic testing was offered to all patients whose variants were inconclusive and further patients lacking genetic CF-confirmation were called for testing. Patient samples were analysed using a Next-Generation-Sequencing-custom-design-panel covering all 27 exons including intronic and regulatory regions.

[1] German CF Registry annual report 2020 (https://www.muko.info/fileadmin/user_upload/angebote/qualitaetsmanagement/register/berichtsbaende/Berichtsband_2020.pdf)

Table 1: Mutation distribution in the German CF registry [1]

Mutation combinations	Frequency	Percent
F508del homozygot	3,040	45.7
F508del heterozygous: Second mutation identified	2,472	37.2
F508del heterozygous: Second mutation not identified	187	2.8
No verification of F508del: Both mutations identified	779	11.7
No verification of F508del: Only one mutation identified	66	1.0
No verification of F508del: No mutations identified	104	1.6
Total	6,648	100.0

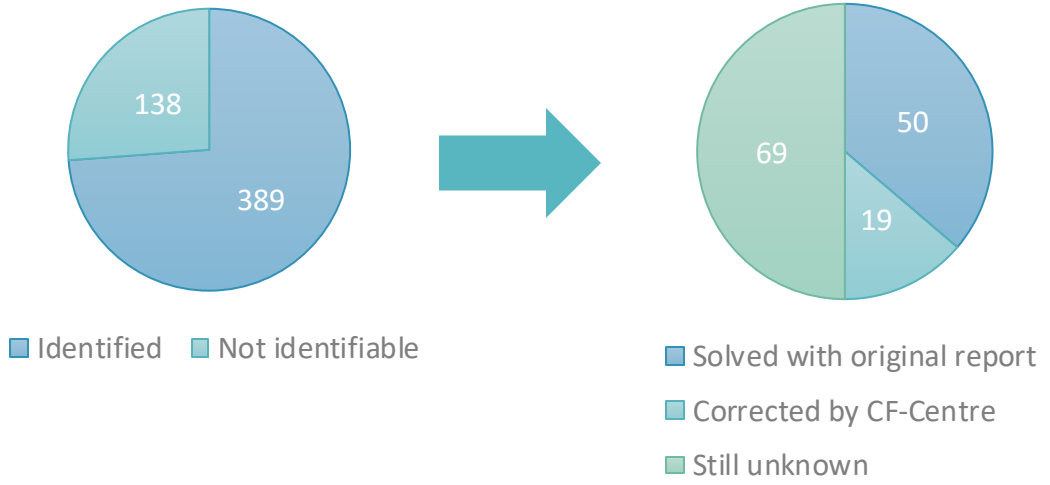


Figure 1: left: Ambiguos registry entries were re-analysed. ¾ remain still unclear. right: From these, 50 cases were documentation errors and could be solved with the original lab report, 19 cases were clarified by the supervising CF centre.

Results

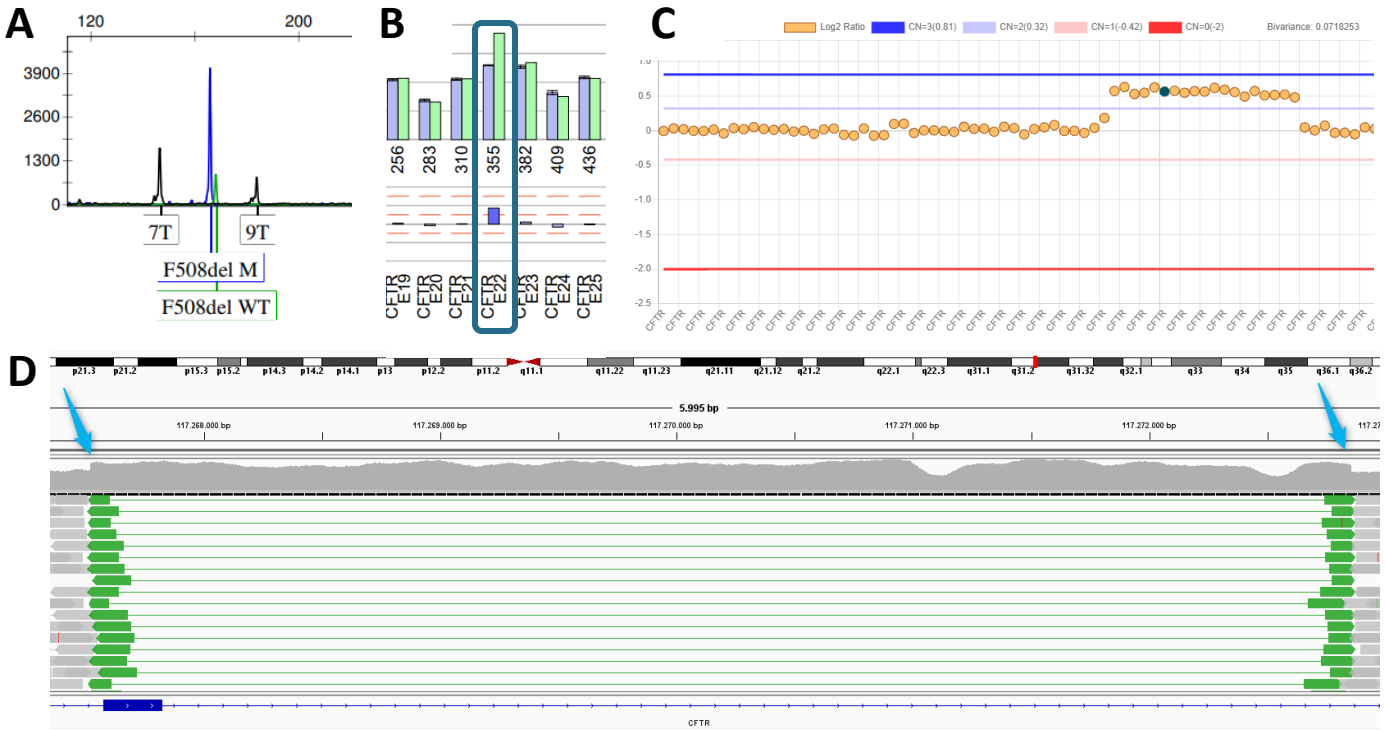
Identification of inconclusive variants led to the discovery of 48 variants not formerly reported in the context of CF.
20 samples with previously unknown or incomplete *CFTR* genotype were sequenced via NGS with an overall success rate of 70%. In 4 cases, deep intronic variants were identified which are undetectable using conventional sequencing (+/- 20 bp from exonic borders).

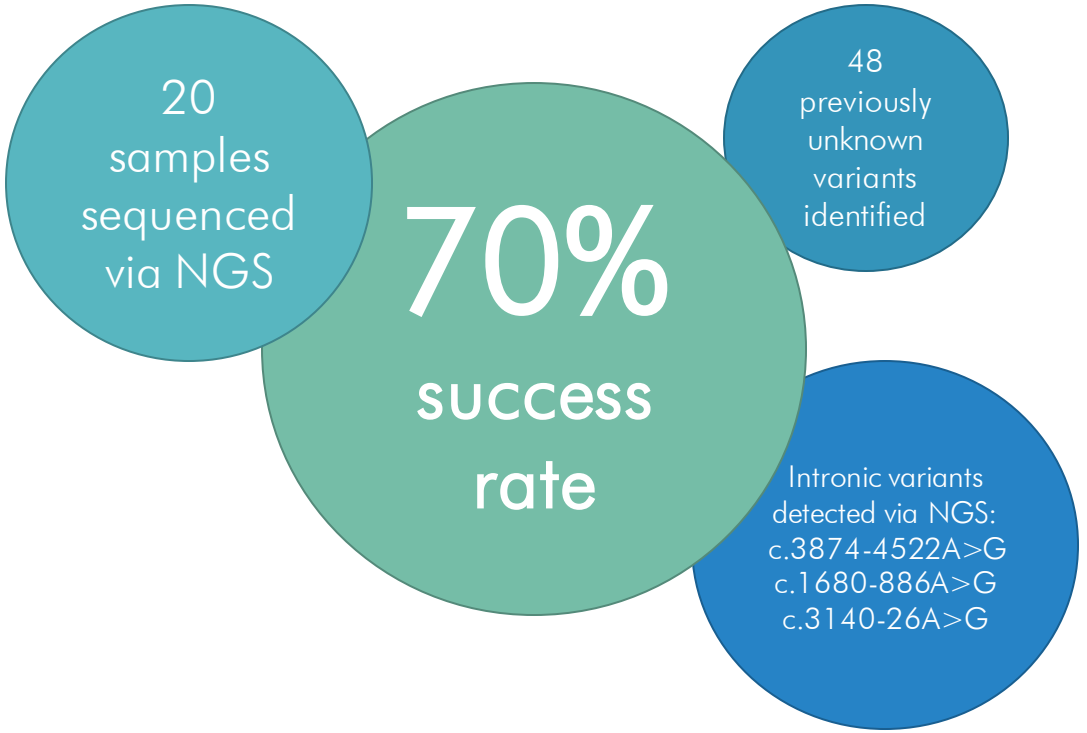
All results were uploaded to ClinVar and previously unknown variants were reported to CFTR1/2 for database completion.

Whole *CFTR*-NGS-analyses can significantly contribute to the diagnostic yield of CF cases and enable access to modulator therapy.

Case report:

Girl, age at diagnosis: 2 months, positive in newborn screening, elevated sweat chloride
(A) screening: F508del heterozygous (maternal), (B) full analysis: duplication exon 22 (paternal)
(C and D) NGS: duplication with exact breakpoints and localisation:
chr7:g.117267524_117272856dup





Outlook

Whole CFTR-genotyping can greatly increase the genetic diagnostic rate of CF-patients and should therefore be considered as a replacement for the current strategies in routine diagnostics. Unsolved cases might further benefit from transcriptome sequencing on nasal epithelial cells to analyse the CFTR-mRNA and better assess intronic variants.

Interested in participating?

We are still looking for further patients to participate. Please contact us: *simone.ahting@medizin.uni-leipzig.de* or visit
https://www.uniklinikum-leipzig.de/einrichtungen/humangenetik/forschung/cftr-register
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