Minso et al., Intestinal current measurement and nasal potential difference to make a diagnosis of cases with inconclusive CFTR genetics and sweat test'

Supplementary Information

Uncommon genotype – phenotype associations

The investigated patient population inherently consists of individuals who are neither healthy nor show the full-blown clinical picture of CF but rather presented symptoms compatible with CFTR dysfunction that alerted the expert to ask for a more thorough assessment by CFTR biomarkers. Of the whole cohort listed in Table S1 we exemplify a few insightful cases to illustrate the association between CFTR activity, basic defect and manifestation of disease:

Compound heterozygosity of the missense mutation p.Thr1299Ile together with p.Phe508del was associated with an intermediary sweat test, pathological NPD and normal ICM consistent with the patient’s history of chronic pulmonary disease, i.e. non-allergic asthma, bronchiectasis and recurrent airway infections with *Staphylococcus aureus*.

A young CF adult homozygous for the p.Glu831Ter mutation showed pathological sweat test and NPD but was clinically almost asymptomatic in accordance with the analysis of this mutation that alternative splicing at the affected NAGNAG tandem site can remove the deleterious premature UAG stop codon and lead to the synthesis of a functional CFTR-ΔGlu831 protein isoform.\(^5\)

A heterozygous carrier of the c.489+1G>T splice mutation showed a NPD in the CF range. Chloride concentrations in sweat tests performed within two years on five separate occasions were 40, 23, 91, 22 and 78 mmol/L. The variability of sweat chloride within this subject was much higher than the 95% limits of between test repeatability reported in the literature for CF patients and non-CF controls of maximally about ± 20 mmol/L.\(^5\) We hypothesize that an intermittently acting modifier of CFTR expression may account for this unusual phenotype. The patient’s major complaints prior to diagnosis had been episodes of abdominal pain and pancreatitis, but after start of athletic activities recommended by the CF physicians the young adult gained weight (BMI 28 kg/m\(^2\)) and normal lung function (FEV1 98% pred.) and has yet not experienced any further episode of CF illness despite being a chronic carrier of *S. aureus* in his airways.

CF had been diagnosed in a currently 53-year old man as an infant on the occasion of an episode of salt depletion and metabolic alkalosis and subsequent sweat tests in the borderline range of 60 – 70 mmol/L. Since he did not develop any typical signs of CF pulmonary or intestinal disease during his 20-year attendance of the CF clinic, numerous further sweat tests were performed yielding chloride concentrations in the normal, borderline and pathological range (Table S1). The NPD performed by the age of 48 years revealed an extremely low basic potential, no response to amiloride and a small depolarization potential. The diagnosis ‘pseudohypoaldosteronism’ was corroborated by the detection of the hypoactive p.Phe61Leu missense variant in the alpha-subunit of the epithelial sodium channel ENaC.\(^5\)

The two CF patients with the highest age at diagnosis of 43 and 65 years had experienced chronic CF-typical symptoms since early childhood. All tested CFTR biomarkers were consistently in the CF range. Never seen by a physician at a CF centre, they managed the disease for decades with endurance sports and a high-calorie low fat diet with high-dose vitamin and mineral supplements.
Table S2. Outcome of NPD and ICM measurements in CFTR genotypes of unknown or variable clinical significance

| HGVS                      | Legacy name                                      |
|---------------------------|-------------------------------------------------|
| **a. Recordings in CF range**                                      |
| p.Phe508del / p.[Arg74Trp-Val201Met-Asp1270Asn] (n = 2) | F508del / R74W-V201M-D1270Q$^a$                  |
| p.Phe508del / p.Arg117His- c.1210-12T(7) (n = 5)        | F508del / R117H-7T                               |
| p.Phe508del / p.Leu206Trp                                     | F508del / L206W                                  |
| p.Phe508del / p.Gly314Glu                                     | F508del / G314E                                  |
| p.Phe508del / p.Asp579His$^a$                                 | F508del / D579H$^a$                             |
| p.Phe508del / p.Ser977Phe- c.1210-12T(5)                    | F508del / S977F-5T                               |
| p.Phe508del / p.Asp1152His (n = 2)                           | F508del / D1152H                                 |
| p.Phe508del / p.Thr1299Ile$^a$                                | F508del / T1299I$^a$                             |
| p.Phe508del / c.1210-12T(5) (n = 3)                          | F508del / 5T                                    |
| p.Phe508del / c.2620-15C>G$^a$                               | F508del / 2752 − 15C>G$^a$                      |
| p.Leu159Ser$^a$ / c.1210-12T(5)                              | L159S$^a$ / 5T                                  |
| p.Asp1152His / p.Asp1152His                                   | D1152H / D1152H                                 |
| p.Ala1285Gln$^a$ / c.1210-12T(5)                             | A1285Q$^a$ / 5T                                 |
| **b. Recordings in the normal range**                         |
| p.Phe508del / p.Leu997Phe                                      | F508del / L997F                                 |
| p.Arg75Gln / p.Val201Met                                      | R75Q / V201M                                   |
| p.Arg75Gln / p.Val769Ile$^a$                                  | R75Q / V769I$^a$                               |
| p.Arg117His - c.1210-12T(7) / p.Arg117His - c.1210-12T(7)$^b$ | R117H − 7T / R117H − 7T$^b$                     |
| p.Arg117His - c.1210-12T(7) / p.Leu997Phe                    | R117H / L997F                                  |
| p.Gly437Asp / p.Val1293Ile$^a$                                | G437D / V1293I$^a$                             |
| p.Gln1035Ter$^a$ / p.Asp1152His                               | Q1035X$^a$ / D1152H                             |
| c.-8G>C (5′UTR) / c.-8G>C (5′UTR)$^a$                        |                                                   |

$^a$ rare sequence variant not listed in the CFTR2 database by June 24$^{th}$, 2020

$^b$ The 3-year old child was classified as CFTR-RD despite normal recordings in the ICM.
Intra-subject variability of NPD measurements

- Intrasubject variation of basic potential [mV]
- Intrasubject variation of amiloride response [mV]
- Intrasubject variation of response to chloride-free and iso solutions [mV]
Figure S1. Intra-subject variation of the nares in the basal PD (top), hyperpolarization upon superfusion with amiloride (middle) and the cumulative depolarization upon superfusion with chloride-free solution and isoproterenol (bottom).

The tracings of the respiratory epithelium were similar in both nostrils for the vast majority of the examined persons, as shown below in Table S3 and above in Figures S1.

Table S3. Intra-subject difference between NPD measurements of nostrils

| Potential difference       | Basal PD | Δ amiloride | Δ (low Cl⁻ + isoproterenol) |
|---------------------------|----------|------------|-------------------------------|
| Median [mV]               | 4        | 2          | 4                             |
| Range of 95% of data [mV] | 0 – 16   | 0 – 14     | 0 – 19                        |

Intra-subject variability was similar for basal PD and the responses to superfusion with amiloride or chloride-free solution in contrast to a recent report on NPD measurements in PI CF patients who are compound heterozygous for a nonsense mutation. Tracings of this PI CF cohort showed a smaller variability in Δ (low Cl⁻ + isoproterenol) than in basal PD and Δ amiloride. These differences make sense because a PI CF cohort expresses none or very low CFTR-mediated chloride conductance giving rise to nil or very small depolarization whereas our study population exhibited residual or normal CFTR activity and hence produced depolarization potentials similar to the basal PD.

References

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