Diversity of the basic defect of homozygous CFTR mutation genotypes in humans

F Stanke,1,2 M Ballmann,2 I Bronsveld,4 T Dörk,3 S Gallati,5 U Laabs,1 N Derichs,2 M Ritzka,1,2 H-G Posselt,6 H K Harms,7 M Griese,7 H Blau,8 G Mastella,9 J Bijman,10 H Veeze,11 B Tümmler1,2

ABSTRACT

Background: Knowledge of how CFTR mutations other than F508del translate into the basic defect in cystic fibrosis (CF) is scarce due to the low incidence of homozygous index cases.

Methods: 17 individuals who are homozygous for deletions, missense, stop or splice site mutations in the CF gene were investigated for clinical symptoms of CF and assessed in CFTR function by sweat test, nasal potential difference and intestinal current measurement.

Results: CFTR activity in sweat gland, upper airways and distal intestine was normal for homozygous carriers of G342R or L907F and in the range of F508del homozygotes for homozygous carriers of E292K, W1098L, R553X, R1162X, CFTD1126insA or CFTD1232+10 kb C-T were not consistent CF or non-CF in the three bioassays. 14 individuals exhibited some chloride conductance in the airways and/or in the intestine which was identified by the differential response to cAMP and DIDS as being caused by CFTR or at least two other chloride conductances.

Discussion: CFTR mutations may lead to unusual electrophysiological or clinical manifestations. In vivo and ex vivo functional assessment of CFTR function and in-depth clinical examination of the index cases are indicated to classify yet uncharacterised CFTR mutations as either disease-causing lesions, risk factors, modifiers or neutral variants.

Cystic fibrosis (CF) is an autosomal recessive disorder of all exocrine glands and is caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene.1 CFTR is localised in the apical membrane of epithelial cells and confers cAMP-activatable transport of chloride, bicarbonate and glutathione.1 The basic defect in CF impairs the apical permeability for chloride and is assessed in humans by the increased chloride concentrations in sweat,2 the nasal potential difference (NPD)3 and CFTR function by sweat test,2 the nasal potential difference (NPD)3 and intestinal current measurement.4 The investigation of compound heterozygous individuals does not provide unequivocal information about the phenotype of the individual mutation and consequently the in vivo phenotype of a CFTR mutation can best be studied in homozygotes for a particular CFTR mutation.

This report describes the basic defect of individuals who are homozygous for non-F508del CFTR mutations. These rare index cases were identified during the last 15 years by CFTR mutation analysis of large patient populations in Germany, Italy and Israel. A cohort of F508del homozygous sibpairs from nine European countries was used as reference group. The patients’ defective chloride transport across the apical membrane of epithelial cells was measured by the physiological assays employed when diagnosing CF—that is, the sweat test,6 the nasal potential difference (NPD)7 and intestinal current measurements (ICM) of freshly excised rectal suction biopsies.6,7 CFTR function was not detectable in individuals who harbour two CFTR null alleles and close to normal in two individuals who are homozygous for non-conservative amino acid substitutions.

SUBJECTS AND METHODS

Subjects

The index cases (table 1) selected from CF centres in Germany, Italy and Israel were investigated at the CF centres in Verona (patients 3 and 4) or Hannover (all other patients). Informed consent was obtained from all patients and at least one parental guide in case of minors. This study was approved by the ethics committee of the Medizinische Hochschule Hannover (study no. 1226). All subjects except patient 2 were offspring of consanguineous marriages which was documented by the parental status of first or second generation cousins in the family tree (patients 1, 3–14) or by the overrepresentation of homozygous marker genotypes (patients 15–17). Before the date of the investigation of the basic defect by NPD and/or ICM, all index cases had been diagnosed with CF. The diagnosis had been based on clinical symptoms compatible with CF and/or by in vivo or ex vivo analysis of patients’ material, because almost all patients with CF are either homozygous for F508del or compound heterozygous for two different CFTR mutations. The investigation of compound heterozygous individuals does not provide unequivocal information about the phenotype of the individual mutation and consequently the in vivo phenotype of a CFTR mutation can best be studied in homozygotes for a particular CFTR mutation.

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and medication, a physical examination, documentation of meconium ileus, meconium ileus equivalent, hepatobiliary disease, diabetes mellitus, fertility, other diseases, anthropometry, determination of pancreatic elastase in stool as an index for the exocrine pancreatic status, a lung function test (the forced vital capacity and the forced expiratory volume in 1 s were determined by spirometry and expressed as a predictive value based on Knudson’s formulae), bacteriology of sputum or deep throat swab, serum IgG and IgE and antipseudomonal IgG. After the clinical examination a Gibson–Cooke pilocarpine iontophoresis sweat test, NPD and ICM measurements were performed within the next 72 h.

**Mutation analysis at the CFTR locus**

A cascade approach was taken to identify CFTR mutations in genomic DNA from nuclear blood cells. K-EDTA blood samples were taken from the patient and, at the Hannover site, also from the parents. First, frequent CFTR mutations were directly tested by established polymerase chain reaction (PCR)-based protocols guided by the parental CFTR haplotypes of the index case. Next, the 27 exons of the CFTR gene together with their flanking intron regions were amplified separately by PCR. Before 1995, sequence variations were sought by single strand conformation polymorphism (SSCP) or restriction enzyme based analysis of PCR product. All non-F508del mutations detected by direct testing or anomalous migration behaviour in SSCP were confirmed by direct genomic sequencing of the respective exon. Starting by 1995, SSCP was omitted and the whole coding and flanking intron sequences were determined by direct genomic sequencing as the second step. Finally, specimens which were refractory to amplification by PCR were scanned for genomic rearrangements by Southern hybridisation with their flanking intron regions were amplified separately by PCR. All non-F508del mutations previously by the same protocol and the same core team of investigators. All F508del homozygotes had CF-typical sweat test, NPD and ICM values (tables 2 and 3).

Expression of functional CFTR was expected to be absent in patients harbouring two CFTR null alleles. The homozygotes for the large out-of-frame 21 kb deletion of exons 2 and 3 of the CFTR gene (Fig 1B) or the nonsense mutation R553X showed indeed full-blown CFTR loss-of-function phenotypes in the bioassays. The two R1162X homozygous patients, however, had some chloride secretory activity in their intestine that consisted of both DIDS-sensitive and DIDS-insensitive components (fig 1D, tables 4 and 5).

Residual chloride conductance was detected in subjects with splice site mutations that permit production of some wild type CFTR. The mutation 3849+10 kb C-T is known to activate a cryptic splice acceptor site in intron 19 and leads to variable minute chloride secretion; (4) forskolin (10−5 M), to initiate the cholinergic Ca2+-linked chloride secretion; (5) 4,4′-disothiocyanatostilbene-2,2′-disulfonic acid (DIDS, 2.10−4 M, M), to inhibit DIDS-sensitive Cl− transporters like the Ca2+-dependent Cl− channels and the ORCC; (6) histamine (5.10−4 M, S), to reactivate the Ca2+-dependent chloride secretory pathway.4

**RESULTS**

Seventeen individuals homozygous for a non-F508del CFTR mutation were recruited for the study. The patients’ clinical characteristics and the sweat test, NPD and ICM values are shown in tables 1–3. The electrophysiological phenotype of the patients’ basic defect (table 4) was compared with that of F508del homozygous CF siblings who had been studied previously by the same protocol and the same core team of investigators. All F508del homozygotes had CF-typical sweat test, NPD and ICM values (tables 2 and 3).

Access to the subcutaneous space was obtained by a needle filled with NaCl solution inserted subcutaneously into the forearm. The lower nasal turbinate was superfused with buffer B, which typically activates CFTR chloride channels, was tested.

**Intestinal current measurements**

ICM was performed on freshly obtained rectal suction biopsies in a micro-Ussing chamber according to our published protocol. The biopsies equilibrated in Meyler’s buffer (gassed with 95% O2−5% CO2) were sequentially exposed to the mucosal (M) and/or serosal (S) side to: (1) amiloride (10−4 M, M); (2) indomethacin (10−5 M, M+S), to reduce basal chloride secretion by inhibiting the endogenous prostaglandin formation; (3) carbachol (10−4 M, S), to initiate the cholinergic Ca2+-linked chloride secretion; (4) forskolin (10−5 M, M+S) together with 8-bromo-cAMP (10−3 M, M+S), to open cAMP-dependent Cl− channels like CFTR; (5) 4,4′-disothiocyanatostilbene-2,2′-disulfonic acid (DIDS, 2.10−4 M, M), to inhibit DIDS-sensitive Cl− transporters like the Ca2+-dependent Cl− channels and the ORCC; (6) histamine (5.10−4 M, S), to reactivate the Ca2+-dependent chloride secretory pathway.

**Mutation report**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>F508del</td>
<td>Novel mutation in exon 10</td>
</tr>
<tr>
<td>R553X</td>
<td>Nonsense mutation in exon 3</td>
</tr>
<tr>
<td>3849+10 kb C-T</td>
<td>Large out-of-frame deletion</td>
</tr>
</tbody>
</table>

**REFERENCES**

1. Knudson AG. Mutation report.
<table>
<thead>
<tr>
<th>Patient number</th>
<th>CFTR genotype</th>
<th>Sex</th>
<th>Age at diagnosis</th>
<th>Symptoms at diagnosis</th>
<th>Pancreatic status</th>
<th>Meconium ileus</th>
<th>Colonisation with Pseudomonas aeruginosa</th>
<th>Age at onset of P. aeruginosa colonisation</th>
<th>Peculiar features</th>
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<tbody>
<tr>
<td>Out-of-frame deletion</td>
<td>CFTRdele2,3(21 kb)/CFTRdele2,3(21 kb)</td>
<td>F</td>
<td>Birth</td>
<td>Meconium ileus</td>
<td>PI</td>
<td>Yes</td>
<td>Yes</td>
<td>6 y 6 mo</td>
<td>Diabetes mellitus</td>
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<tr>
<td>2</td>
<td>R553X/R553X</td>
<td>F</td>
<td>16 mo</td>
<td>Steatorrhea, failure to thrive</td>
<td>PI</td>
<td>No</td>
<td>Yes</td>
<td>10 y</td>
<td>Diabetes mellitus, atopy</td>
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<tr>
<td>3</td>
<td>R1162X/R1162X</td>
<td>F</td>
<td>5 mo</td>
<td>Malnutrition</td>
<td>PI</td>
<td>No</td>
<td>Yes</td>
<td>18 y 4 mo</td>
<td>Atopy</td>
</tr>
<tr>
<td>4</td>
<td>R1162X/R1162X</td>
<td>M</td>
<td>18 d</td>
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<td>PI</td>
<td>No</td>
<td>No</td>
<td></td>
<td></td>
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<tr>
<td>Splice site mutation</td>
<td>1898+3 A-G/1898+3 A-G</td>
<td>F</td>
<td>3 mo</td>
<td>Pneumonia</td>
<td>PS</td>
<td>No</td>
<td>No</td>
<td></td>
<td>Repetitive episodes of salt loss and dehydration</td>
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<tr>
<td>6</td>
<td>3849+10 kb C-T/3849+10 kb C-T</td>
<td>M</td>
<td>20 y 5 mo</td>
<td>Pneumonia, dyspnoea, hypoxaemia</td>
<td>PS</td>
<td>No</td>
<td>Yes</td>
<td>26 y 6 mo</td>
<td>Lung transplant, splenomegaly</td>
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<td>7</td>
<td>3849+10 kb C-T/3849+10 kb C-T</td>
<td>M</td>
<td>11 y 2 mo</td>
<td>Bronchitis, positive family anamnesis</td>
<td>PS</td>
<td>No</td>
<td>Yes</td>
<td>12 y 3 mo</td>
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<td>F</td>
<td>Birth</td>
<td>Meconium ileus</td>
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<td>Yes</td>
<td>Yes</td>
<td>9 y</td>
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<tr>
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<td>CFTRdele2(ins186)/CFTRdele2(ins186)</td>
<td>M</td>
<td>2 y</td>
<td>Bronchitis</td>
<td>PI</td>
<td>No</td>
<td>Yes</td>
<td>4 y</td>
<td></td>
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<td>Missense mutation</td>
<td>E92K/E92K</td>
<td>M</td>
<td>8 mo</td>
<td>Salt loss, muscle weakness</td>
<td>PS</td>
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<td>Yes</td>
<td>11 y 9 mo</td>
<td>Fatigue, muscle weakness and salt loss during exercise</td>
</tr>
<tr>
<td>11</td>
<td>G314E/G314E</td>
<td>F</td>
<td></td>
<td>Bronchitis</td>
<td>PS</td>
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<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
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<td>F</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>W1098L/W1098L</td>
<td>M</td>
<td>2 mo</td>
<td>Salt loss, failure to thrive</td>
<td>PS</td>
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<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>M1101K/M1101K</td>
<td>F</td>
<td>5y 9 mo</td>
<td>Pneumonia</td>
<td>PI</td>
<td>No</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
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<td>F</td>
<td>4 y 6 mo</td>
<td>Airways infections</td>
<td>PS</td>
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<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>M1101K/M1101K</td>
<td>M</td>
<td>1 mo</td>
<td>Positive family anamnesis</td>
<td>PI</td>
<td>No</td>
<td>No</td>
<td></td>
<td></td>
</tr>
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</table>

* d, days; F, female, M, male; mo, months; PI, exocrine pancreatic insufficient; PS, exocrine pancreatic sufficient; y, years
* Sibpairs: patients 3 & 4, 6 & 7, 9 & 10, 15, 16 & 17.
Seven study participants were homozygous for a missense mutation in the $CFTR$ gene (table 4). By the time of writing, none of the 5- to 23-year-old individuals had yet experienced any severe exacerbation of CF pulmonary or gastrointestinal disease. The homozygotes for E92K, W1098L or M1101K showed highly elevated sweat chloride concentrations in the CF range on several occasions, whereas the homozygotes for G314E or L997F had normal sweat electrolytes like non-CF (the other two molecular level such as nonsense mediated decay and exon skipping may distinguish in their clinical phenotypes from those of F508del homozygotes, although the consequences at the molecular level such as nonsense mediated decay and exon skipping or defective maturation and trafficking of protein are different. All other $CFTR$ mutations investigated in this study translated into facets of disease and basic defect that are atypical for the most common genotype. Splice site mutations, for example, were associated with progressive lung disease and a positive family anamnesis or suggestive respiratory symptoms, was withdrawn for these two individuals.

### DISCUSSION

This first study of how non-F508del homozygous $CFTR$ genotypes translate into the basic defect in the major affected organs in vivo, uncovered the full range from complete loss-of-function to normal non-CF phenotypes. All patients except patient 2 descended from consanguineous marriages characterised by an overrepresentation of homozygous genotypes. Hence we expected the sibpairs in our cohort to be concordant in their manifestations of aberrant epithelial chloride conductance, but this was not the case. The siblings showed an individual rather than a shared electrophysiological signature in NPD and ICM (tables 2 and 3), suggesting that individual factors modify the CF phenotype at the level of the basic defect even though it is more closely related to the mutation genotype than any clinical symptom. More than 90% of CF patients are homozygous or compound heterozygous for F508del, and consequently our perception of CF disease is dominated by this major mutation. Loss-of-function mutations such as $CFTRdele2,3(21$ kb) or R553X were not distinguishable in their clinical phenotypes from those of F508del homozygotes, although the consequences at the molecular level such as nonsense mediated decay and exon skipping defective maturation and trafficking of protein are different. All other $CFTR$ mutations investigated in this study translated into facets of disease and basic defect that are atypical for the most common genotype. Splice site mutations, for example, were associated with progressive lung disease and a
<table>
<thead>
<tr>
<th>Patient number</th>
<th>CFTR genotype</th>
<th>Carbachol (reverse signal)</th>
<th>Response to histamine (reverse signal)</th>
<th>DIDS (reverse signal)</th>
<th>Sensitivity to DIDS</th>
<th>cAMP response</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>CFTRdele2 (21 kb)/CFTRdele2 (21 kb)</td>
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<td>2</td>
<td>R553X/R553X</td>
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<td>100</td>
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<td>3</td>
<td>R1162X/R1162X</td>
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<td>40</td>
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<td>Yes</td>
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<td>5</td>
<td>188I-3 A-G/188I-3 A-G</td>
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<td>Intermediate</td>
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<tr>
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<td>Minute</td>
<td>70</td>
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<td>3849-10 kb C/3849-10 kb C</td>
<td>100</td>
<td>Minute</td>
<td>100</td>
<td>Yes</td>
<td>Yes</td>
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<td>100</td>
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<td>40</td>
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<td>Yes</td>
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<tr>
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<td>11</td>
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<td>Yes</td>
<td>Yes</td>
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<td>12</td>
<td>G111R/G111R</td>
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<td>Intermediate</td>
<td>0</td>
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<td>13</td>
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<td>14</td>
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<tr>
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<td>Yes</td>
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<tr>
<td>17</td>
<td>M1101K/M1101K</td>
<td>50</td>
<td>Small</td>
<td>50</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**Table 3. Assessment of basic defect (B): intestinal current measurements (ICM)**

**Notes:**
- **Out-of-frame deletion:**
  - Patient 6: 188I-3 A-G
  - Patient 7: 3849-10 kb C
- **Nonsense mutation:**
  - Patient 2: R553X
  - Patient 3: R1162X
- **Splice-site mutation:**
  - Patient 5: 188I-3 A-G
- **In-frame deletion:**
  - Patient 8: CFTRdele2
  - Patient 9: CFTRdele2
- **Missense mutation:**
  - Patient 11: E90K
  - Patient 12: G34E
- **Non-CF***
  - Patient 17: M1101K

**DIDS (reverse signal):**
- Patient 1: 100%
- Patient 2: 100%
- Patient 3: 100%
- Patient 4: 100%
- Patient 5: 100%
- Patient 6: 100%
- Patient 7: 100%
- Patient 8: 100%
- Patient 9: 100%
- Patient 10: 100%
- Patient 11: 100%
- Patient 12: 100%
- Patient 13: 100%
- Patient 14: 100%
- Patient 15: 100%
- Patient 16: 100%
- Patient 17: 100%

**Sensitivity to DIDS:**
- Patient 1: No
- Patient 2: No
- Patient 3: No
- Patient 4: No
- Patient 5: No
- Patient 6: No
- Patient 7: No
- Patient 8: No
- Patient 9: No
- Patient 10: No
- Patient 11: No
- Patient 12: No
- Patient 13: No
- Patient 14: No
- Patient 15: No
- Patient 16: No
- Patient 17: No

**Carbachol (% reverse signal):**
- Patient 1: 100%
- Patient 2: 100%
- Patient 3: 100%
- Patient 4: 100%
- Patient 5: 100%
- Patient 6: 100%
- Patient 7: 100%
- Patient 8: 100%
- Patient 9: 100%
- Patient 10: 100%
- Patient 11: 100%
- Patient 12: 100%
- Patient 13: 100%
- Patient 14: 100%
- Patient 15: 100%
- Patient 16: 100%
- Patient 17: 100%

**Response to histamine (% reverse signal):**
- Patient 1: None
- Patient 2: None
- Patient 3: None
- Patient 4: None
- Patient 5: None
- Patient 6: None
- Patient 7: None
- Patient 8: None
- Patient 9: None
- Patient 10: None
- Patient 11: None
- Patient 12: None
- Patient 13: None
- Patient 14: None
- Patient 15: None
- Patient 16: None
- Patient 17: None

**Response to cAMP (reverse signal):**
- Patient 1: None
- Patient 2: None
- Patient 3: None
- Patient 4: None
- Patient 5: None
- Patient 6: None
- Patient 7: None
- Patient 8: None
- Patient 9: None
- Patient 10: None
- Patient 11: None
- Patient 12: None
- Patient 13: None
- Patient 14: None
- Patient 15: None
- Patient 16: None
- Patient 17: None

*Range and threshold of values for the different categories are based on previously published data (summarised in De Jonge et al.*

*Subgroups:*
- **CF***: 0–100
- **CF* (alternative chloride secretion):** 0–10
- **CF* (residual chloride secretion):** 0–100

*CF* response sensitive to DIDS in patient 8.
Table 4  Evaluation of the basic defect in individuals with homozygous CFTR mutation genotypes

<table>
<thead>
<tr>
<th>Patient number</th>
<th>CFTR mutation</th>
<th>Values within the range of F508del homozygotes</th>
<th>Values within the range of Intermediate</th>
<th>Values within the range of Non-CF healthy controls</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Sweat chloride*</td>
<td>ICM†</td>
<td>NPD‡ Basal</td>
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<td>Out-of-frame deletion</td>
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<td>CFTIRdele2,3(21 kb)</td>
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<td>Non-sense mutation</td>
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<tr>
<td>5</td>
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<td>6</td>
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<td>In-frame deletion</td>
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<td>8</td>
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<td>9</td>
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<td>CFTIRdele2(ins186)</td>
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<td>Missense mutation</td>
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<tr>
<td>11</td>
<td>E92K</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>12</td>
<td>G334E</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>13</td>
<td>L997F</td>
<td>X</td>
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<td>W1098L</td>
<td>X</td>
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<td>15</td>
<td>M1101K</td>
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<td>M1101K</td>
<td>X</td>
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<tr>
<td>17</td>
<td>M1101K</td>
<td>X</td>
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</tbody>
</table>

Sibpairs: patients 3 & 4, 6 & 7, 9 & 10, 15, 16 & 17.
Consanguineous parents of patients: 1, 3–14.

*Sweat chloride concentrations determined in Gibson–Cooke pilocarpine iontophoresis sweat tests.
†DICD-insensitive cAMP-activatable chloride secretory responses in rectal suction biopsies as surrogate parameter for CFTR function.
‡NPD measurements of the basal PD (basal), the response to amiloride (amil) and the cumulative response to chloride-free solution plus isoprotenerol (Cl−-free).
Missense mutations are a guide to dissect the function of CFTR motifs and domains. E92K and D110H (data not shown) are located in the first ectoplasmic loop. The index cases were prone to severe salt loss upon exposure to heat or exercise, but otherwise are overweight and have normal lung function. Hence, the integrity of the loop at these positions seems to be essential for the reabsorption of salt from the sweat duct, but less relevant for ion secretion in airways and intestine.

The mutations W1098L and M1101K reside in the cytoplasmic loop 4 (residues 1035–1102). Heterologously expressed, recombinant mutants W1098R and M1101K were defective in CFTR maturation and non-functional in anion efflux assays.6 In contrast, the four homozygous patients unequivocally demonstrated substantial residual chloride conductance in the NPD and ICM bioassays (table 4). In vitro and in vivo CFTR mutant phenotypes do not match. Rescue mechanisms are probably operating in the patients which were absent in the heterologous host cells in vitro. This example makes the point that in vitro findings on recombinant mutants should be interpreted with caution when utilised for counselling of patients and their relatives.

The non-conservative amino acid substitutions L997F and G314E did not impair chloride conductance in sweat glands, airways and intestine. The normal phenotype of the glycine-to-glutamate substitution is particularly striking. The CFTR mutation database lists 20 missense mutations with a change of glycine to an acidic amino acid or vice versa, three of which are classified as benign sequence variants and eight of which as disease-causing. Several well characterised severe mutations occur in the evolutionarily conserved Walker (G1244E, G1249E) or dodecapeptide motifs (G551D, G1349D) of the ABC transporter CFTR.1 The missense mutants G622D23 in the regulatory domain and G314E in the fifth transmembrane region led to no clinical symptoms of CF. The pathogenetic role of nine mutations is still unresolved. The same amino acid substitution thus affects CFTR function to a variable extent depending on its localisation within the protein. In other words, the molecular pathology of a missense mutant is hard to predict, because CFTR topology, function and processing are complex. If mutation analysis uncovers an uncommon and/or yet uncharacterised missense mutation, diagnostic bioassays and in-depth clinical examination of the index cases need to be pursued in order to classify the clinical impact of an amino acid substitution as either neutral, benign or severe.

Our highly selected study cohort of individuals with rare homozygous CFTR genotypes showed an association with mild or asymptomatic clinical manifestation if CFTR function was in the intermediate or normal range in both NPD and ICM. This positive correlation between the amount of residual CFTR function and milder disease did not, however, apply to the individual case, if NPD and/or ICM values were within the CF range. The two 3849+10 kb C-T homozygotes who started to develop symptoms of CF respiratory disease during adolescence,

<table>
<thead>
<tr>
<th>Chloride secretory response</th>
<th>Sensitivity to cAMP</th>
<th>DIDS</th>
<th>Ion channel</th>
<th>Patient number</th>
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<tr>
<td>Yes</td>
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<td>1, 2, 9</td>
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</table>
and were diagnosed at the oldest age in our cohort, showed the most severe progression of disease during adulthood leading to lung transplantation and death; however, patients 1 and 2, with loss-of-function electrophysiological signature, experienced at the same age a comparatively high quality of life with no significant deterioration of their respiratory or gastrointestinal disease.

In summary, while our study could clearly distinguish individuals with asymptomatic or atypical CF, no prognostic value could be derived for the individual case from the amount of residual CFTR-mediated chloride secretion if the results were within the CF range. This conclusion should be borne in mind during genetic counselling of patients or parental guides that typically takes place early in the life of a CF patient.

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REFERENCES


