Pancreatic function and gene deletion F508 in cystic fibrosis

Graziella Borgo, Gianni Mastella, Paolo Gasparini, Antonella Zorzanello, Roberta Doro, Pier Franco Pignatti

Abstract
In view of the possible relation between pancreatic function and cystic fibrosis (CF) gene mutations, a detailed study on Italian patients was performed. Seventy pancreatic insufficient and 48 pancreatic sufficient patients were included after very accurate characterisation of their pancreatic and digestive function, all performed in the same CF centre. The CF gene deletion F508 was tested to define the patients' genotypes.

The results confirm that the mutation correlates with pancreatic insufficiency, and is recessive to other, as yet unreported, mutant alleles that determine pancreatic sufficiency. An indication that duodenal bicarbonate output is more severely reduced in the presence of deletion F508 is also presented. The data are discussed in relation to a hypothesis on the primary effects of CF gene deletion F508.

Cystic fibrosis (CF) is the most common genetic disease in the Caucasian population; it is inherited as an autosomal recessive trait and the estimated carrier frequency is about 1:22.

All patients show a raised electrolyte concentration in sweat, even though the clinical expression of the disease is heterogeneous. Obstructive lung disease and pancreatic insufficiency are the main clinical features. About 15% of patients have sufficient pancreatic function for normal digestion and do not require pancreatic enzyme supplementation.

Genetic linkage studies have mapped the CF locus to chromosome 7. Several DNA markers, including KM19, XV2c, and MP6d9, are tightly linked to the CF locus. The CF gene has recently been identified and a deletion of three base pairs described, which results in the loss of a phenylalanine residue at position 508 (ΔF508) of the putative product of the CF gene.

In North American CF patients, from 68% to 76% of the chromosomes show the deletion.

On the basis of extended DNA marker haplotype analysis of the CF locus, at least seven additional putative mutations have been identified and the number of identified mutations has already increased beyond this (CF Genetic Analysis Consortium, personal communication).

Determination of deletion F508 in pancreatic sufficient (PS) and pancreatic insufficient (PI) patients provided strong support for the hypothesis that pancreatic insufficiency is the result of the presence of two 'severe' alleles, deletion F508 belonging to this category.

Pancreatic sufficiency in turn has been attributed to the presence of at least one mild allele, dominant over the 'severe' ones. This hypothesis was derived from a limited number (39 PI and 21 PS) of North American patients.

Published data about Italian CF haplotypes and their relation to PS and PI are available, derived from collaborative studies from different CF centres. These data may be further expanded as follows. (1) The frequency of deletion F508 is much lower in the Italian population. (2) The haplotype frequencies vary in different Italian regions. (3) The ascertainment of the clinical condition may have been based on rather varying criteria (different criteria for determination of pancreatic function have been used in previous studies).
For all these reasons, and in order to perform a more precise clinical study, we have collected a large series of CF patients and studied the distribution of deletion F508 in subjects with pancreatic sufficiency or insufficiency, after assessment of pancreatic and digestive function performed in the same CF centre, according to strict and identical evaluation criteria.

**Subjects and methods**

**PATIENTS**

A total of 123 CF patients, 67 males and 56 females, referred to the Verona CF centre, was studied. The mean age at the time of the last follow up was 14 years (SD 8, range 0.9 to 34 years). CF diagnosis was based on sweat tests performed according to Gibson and Cooke, and confirmed by typical clinical pulmonary or gastrointestinal findings.

**ASSESSMENT OF PANCREATIC FUNCTION**

Exocrine pancreatic secretory function was assessed according to the following criteria. (1) Direct study of pancreatic function by duodenal intubation and pancreatic stimulation test (pancreozymin and secretin bolus 2 U/kg body weight). Output over 30 min/kg body weight of bicarbonate (gasanalytic method), trypsin, chymotrypsin, and lipase (titrimetric method) was determined in 89 patients, including 44 of 48 PS patients. (2) Measurement of faecal chymotrypsin (kit Boeringwerke Biochemia, Mannheim Monotest Chymotrypsin) was performed in 73 patients. (3) Determination of serum levels of immunoreactive trypsin (IRT, kit Sorin-Biomedin, Saluzzo, Italy) was performed in 48 cases.

**DIGESTIVE FUNCTION ANALYSIS**

Fat digestion was studied using the following tests. (1) Three day fat balance with analysis of faecal fat according to Van De Kamer's method, as modified by Jeejeebhoy et al for medium chain triglyceride fats. The coefficient of fat absorption was calculated in 99 cases. (2) Twenty four hour fat loss (using the method of Van De Kamer) was measured in 11 patients. (3) Steatocrit (according to Phuapradit et al) was performed on duplicate faecal specimens in 105 cases.

**DEFINITION OF PANCREATIC SUFFICIENCY**

For the purpose of this study, patients were defined as pancreatic sufficient (PS) if they presented with: pancreatic enzyme output greater than the first centile of our own laboratory reference values; faecal chymotrypsin > 5 U/g; fat absorption coefficient >92%; 24 hour steatorrhoea <4g; IRT at normal or high levels (normal value 12 to 24 μg/l); steatocrit ≤3%.

As pancreatic dysfunction in CF is partly age related, and in most patients pancreatic structure and function is well established after the second year of life, only patients in whom pancreatic sufficiency was present after this age were entered into the study.

**DEFINITION OF PANCREATIC INSUFFICIENCY**

Pancreatic insufficiency (PI) was defined on the basis of: at least two determinations of pancreatic enzyme output lower or equal to the first centile of the reference values; faecal chymotrypsin absent or lower than 3 U/g faeces (duplicate specimens from at least two samples); fat absorption coefficient <90%; 24 hour steatorrhoea ≥4 g; serum IRT absent or lower than the first centile of the reference values; steatocrit ≥10%. The ascertainment of pancreatic insufficiency was considered at whatever age it was performed, on the assumption that the pancreatic insufficiency in CF is irreversible.

**FINAL CLASSIFICATION OF PANCREATIC FUNCTION**

In the evaluation of pancreatic sufficiency or insufficiency in each case, all the available results were considered. A decreasing value was assigned to the above criteria according to their order.

Patients assumed to have pancreatic sufficiency on the basis of retrospective data were all checked between October and December 1989 before being entered into the study.

The final judgement on pancreatic function was then based on the comprehensive evaluation of both pancreatic and digestive parameters. Patients were defined as pancreatic sufficient only if they showed retrospective normal values, confirmed by at least two tests of pancreatic function (usually faecal chymotrypsin and serum immunoreactive trypsin) and at least one test of digestive function (usually steatocrit or 24 hour steatorrhoea) at the latest check up.

**DNA ANALYSIS**

Genomic DNA was prepared from whole blood by standard methods. Deletion F508 was detected, after DNA amplification, by polyacrylamide gel electrophoresis or by oligonucleotide hybridisation or both.

**STATISTICAL EVALUATION**

The difference in distribution of deletion F508 among PS and PI patients was evaluated by the $\chi^2$ test. The means of the results of pancreatic bicarbonate output in duodenal fluid, assuming the distribution of
bicarbonate values in PI and PS groups to be non-parametric, were compared using the Mann–Whitney test.25

Results
A total of 123 patients was initially included in the study, as detailed in the Methods section. On retrospective analysis, 64 were defined as pancreatic insufficient and 59 as pancreatic sufficient. Six of the latter had developed pancreatic insufficiency within the period of time from the first evaluation to the follow up (from 2.2 to 8.6 years), so they were transferred to the pancreatic insufficient group.

Two pancreatic sufficient patients did not present to follow up and were excluded from the study. Three subjects who did not have pancreatic enzyme replacement showed conflicting laboratory results, and were thought to have borderline pancreatic sufficiency, so were excluded. Therefore, the final total in the study included 48 confirmed pancreatic sufficient and 70 pancreatic insufficient patients.

Table 1 Distribution of ΔF508 in CF–PS and PI patients.

<table>
<thead>
<tr>
<th>Total chromosomes</th>
<th>Chromosomes with the deletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=236)</td>
<td>(n=83)</td>
</tr>
<tr>
<td>PS patients (n=48)</td>
<td>96 (26%)</td>
</tr>
<tr>
<td>PI patients (n=70)</td>
<td>140 (58%)</td>
</tr>
</tbody>
</table>

ΔF508=deletion phenylalanine 508. PS=pancreatic sufficient. PI=pancreatic insufficient. χ²=5.91, p=0.015.

Table 2 Distribution of ΔF508 genotypes in CF–PS and PI patients.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>PS patients (n=48)</th>
<th>PI patients (n=70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔF508/ΔF508</td>
<td>0 (0%)</td>
<td>11 (16%)</td>
</tr>
<tr>
<td>ΔF508/U</td>
<td>25 (52%)</td>
<td>36 (51%)</td>
</tr>
<tr>
<td>U/U</td>
<td>23 (48%)</td>
<td>23 (35%)</td>
</tr>
</tbody>
</table>

U=other uncharacterised mutation.

The presence of deletion F508 was investigated in all patients. It was present in 26% of CF–PS chromosomes, and in 41% of CF–PI chromosomes, in agreement with our previous reports.15 16 Different, still uncharacterised, mutations must therefore be carried by 74% of PS and by 59% of PI chromosomes (table 1). This difference is significant (χ²=5.91, p=0.015). Among PS patients, 52% were heterozygous and none homozygous for deletion F508. Among PI patients, 51% were heterozygous and 16% homozygous for the mutation (table 2).

We then considered the hypothesis of Kerem et al6 that pancreatic sufficiency may be the result of the presence of 'mild' (M) alleles which produce a dominant phenotype with residual exocrine function. ‘Severe’ alleles, including deletion F508, would on the other hand express little or no pancreatic function.

The observed genotype frequencies of CF–PI and CF–PS patients are shown in table 3, together with the frequencies predicted on the basis of the model mentioned above, and on the assumption of Hardy-Weinberg equilibrium in the population. The total frequency of severe alleles was deduced from the observed proportion of CF–PI patients in our centre: (0.85)½/0.922.

The observed frequency of deletion F508 in the total CF population attending the centre is 0.404. The frequency of S alleles is therefore 0.922–0.404=0.518. The frequency of M is 1–0.922=0.078. The expected numbers of CF–PI and CF–PS patients were calculated after normalisation within each group. As shown in table 3, the observed frequencies are in agreement with the hypothesis, thus indicating that CF–PI patients carry two ‘severe’ alleles, while CF–PS patients carry at least one copy of a ‘mild’ mutation. These data also explain the higher frequency of deletion F508 in PI patients. The model of Kerem et al6 is therefore confirmed in the Italian population, where the frequency of deletion F508 is lower than in North American CF patients.15

Ductal pancreatic function of PS and PI patients

Table 3 Mild and severe alleles in CF–PI and PS patients according to Hardy–Weinberg equilibrium.

<table>
<thead>
<tr>
<th>Pancreatic function</th>
<th>Assumed genotype</th>
<th>Predicted frequency</th>
<th>Observed No of patients</th>
<th>Expected No of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI</td>
<td>F,F</td>
<td>0.163</td>
<td>11</td>
<td>13.4</td>
</tr>
<tr>
<td></td>
<td>F,S</td>
<td>0.419</td>
<td>36</td>
<td>34.5</td>
</tr>
<tr>
<td></td>
<td>S,S</td>
<td>0.268</td>
<td>23</td>
<td>22.1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.85</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>PS</td>
<td>M,F</td>
<td>0.063</td>
<td>25</td>
<td>20.2</td>
</tr>
<tr>
<td></td>
<td>M,S</td>
<td>0.081</td>
<td>23</td>
<td>25.9</td>
</tr>
<tr>
<td></td>
<td>M,M</td>
<td>0.006</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.150</td>
<td>48</td>
<td>48</td>
</tr>
</tbody>
</table>

S=uncharacterised severe mutant alleles. M=uncharacterised mild mutant alleles.

The hypothesis6 and the calculations are detailed in the text.

χ² PI patients=0.532 (NS).
χ² PS patients=1.97 (NS).
χ² PI+PS patients=2.502 (NS).
was evaluated by measuring the duodenal bicarbonate output. A wide range of values resulted from the study of 45 PI and 44 PS. The mean value in PI was 6·24 (SD 6·59) mEq/kg/30 min and in PS 29·35 (SD 29·22).

Bicarbonate output was compared with genotypes (table 4). No significant difference was evident, even though it was observed that in the PI patient group deletion F508 homozygotes had a mean value of 4·19, deletion F508 heterozygotes 5·24, and patients with an unknown mutation(s) 9·2. Comparison between ΔF508 homozygotes and other genotypes with the Mann-Whitney test gave p = 0·12.

The observation that deletion F508 heterozygotes had a mean value of bicarbonate output of 34·8 in PS patients and 5·2 in PI patients indicates that uncharacterised mild mutant alleles show dominant effects on duodenal bicarbonate output in the same way as indicated above for pancreatic enzyme secretion.

**Discussion**

The accurate characterisation of pancreatic function is an important aspect of this report. Unfortunately, the exocrine pancreas is difficult to study, because the organ and its secretions are inaccessible for anatomical reasons. For this reason many previous studies have been performed using patients assessed by indirect tests of pancreatic function, since they are less (or non) invasive and easier to perform. However, they are generally less specific and less sensitive, and measure features secondary to the pancreatic insufficiency which arise only when almost all the organ is destroyed. It has been shown that very little residual pancreatic function is sufficient to prevent fat mal-absorption and that non-pancreatic factors (mainly lingual lipase) may control the degree of steatorrhoea.

For the above reasons, the attribution of normal pancreatic function to CF patients not requiring pancreatic enzyme supplementation, or having normal digestive function tests, may lead to an inaccurate assessment of the patients. In the present study, these criteria were therefore never used alone to classify CF patients, as they are insufficient for an accurate evaluation of pancreatic function.

Another aspect to be underlined is the progression of pancreatic damage with age. Waters et al have shown that up to 38% of CF infants screened in the neonatal period show pancreatic sufficiency and evidence of normal fat digestion, while in many of them pancreatic insufficiency is expected to develop with advancing age. Cross sectional analysis of large CF populations in fact usually reveals pancreatic sufficiency only in 15% of CF patients. For this reason, we excluded from our study patients evaluated for pancreatic function under 2 years of age. No patient was assigned to the pancreatic sufficient group without a recent (less than three months) reassessment. In spite of our efforts to separate strictly pancreatic sufficient from insufficient patients, three of them remained unclassified, as conflicting results were obtained from pancreatic and digestive function tests.

In well characterised patients, deletion F508 has a different frequency in pancreatic sufficient and insufficient patients. Our data confirm the hypothesis that it is a mutation conferring a 'severe' pancreatic phenotype, and that it is recessive to 'mild' mutations conferring normal or slight pancreatic function. Only pancreatic insufficient patients carry two copies of the deletion while pancreatic sufficient patients always carry at least one 'mild' unknown CF allele.

It is noteworthy that the hypothesis of Kerem et al agrees in particular with this sample of the Italian population, in which the frequency of deletion F508 is much lower than in the North American population they studied. Our opinion about the reported cases of deletion F508 homozygotes among PS patients is that their pancreatic function was probably questionably evaluated as normal.

Finally, it is interesting to consider in what way deletion F508 may influence pancreatic function. There is evidence that the basic alteration in CF is related to defective chloride transport. The CF gene predicted protein product CFTR is likely to be involved in the transport of ions across the cell membrane, as an ion channel itself or as a regulatory protein which controls the opening and 'gating' of an apical plasma membrane chloride channel.

When the chloride channel opens, chloride exits through the membrane, followed by sodium, and finally by water secretion. A defective chloride channel would therefore lead to a diminished secretory volume.

Chloride dependent bicarbonate secretion was shown in CF pancreas, to which a deficiency of fluid
Pancreatic function and gene deletion F508 in cystic fibrosis

secretion could be correlated. The diminished fluid secretion is present in the pancreas of CF patients at any given level of enzyme secretion, even those with good enzymatic output. Bicarbonate secretion might therefore be considered a primary phenomenon related to deletion F508.

However, there is some conflict in this sense in our study as in CF–PI patients there is less bicarbonate output when deletion F508 is present, and PI homozygotes for the deletion have the least output; such a tendency is not shown in PS patients. A possible bias in evaluating bicarbonate output is that it was measured in patients at different ages. Finally, the role of degenerative factors in local tissue in determining pancreatic disease, and possibly its rate of progression, is unknown. These factors may be related to the decreased fluid output and the consequent increased protein concentration, which may in turn predispose to ductal obstruction and progressive acinar destruction.33

In order to establish further how genetic factors may influence the degree of pancreatic disease in CF, the effects of the still uncharacterised CF mutations need to be assessed.

We wish to thank Drs A Bonizzato and A Savoia for helpful collaboration, P Faggionato, A Facchin, and R Galavotti for skilful technical assistance and Drs S Lorenzini and L Zanolla for advice on statistical evaluations.

Pancreatic function and gene deletion F508 in cystic fibrosis.

G Borgo, G Mastella, P Gasparini, A Zorzanello, R Doro and P F Pignatti

doi: 10.1136/jmg.27.11.665

Updated information and services can be found at: 
[http://jmg.bmj.com/content/27/11/665](http://jmg.bmj.com/content/27/11/665)

**Email alerting service**

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Notes**

To request permissions go to: 
[http://group.bmj.com/group/rights-licensing/permissions](http://group.bmj.com/group/rights-licensing/permissions)

To order reprints go to: 
[http://journals.bmj.com/cgi/reprintform](http://journals.bmj.com/cgi/reprintform)

To subscribe to BMJ go to: 
[http://group.bmj.com/subscribe/](http://group.bmj.com/subscribe/)