PAPER

Chronic pancreatitis and cystic fibrosis

H Witt

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Recent discoveries of trypsinogen and trypsin inhibitor mutations in patients with chronic pancreatitis (CP) support the hypothesis that an inappropriate activation of pancreatic zymogens to active enzymes within the pancreatic parenchyma starts the inflammatory process. Current data suggest that CP may be inherited dominant, recessive, or complex as a result of mutations in the above mentioned or yet unidentified genes. Evaluation of patients with CP should include genetic testing. Cystic fibrosis (CF) is an autosomal recessive inherited disorder caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene and is characterised by pancreatic insufficiency and chronic bronchopulmonary infection. The progression and severity of pulmonary disease differs considerably between people with identical *CFTR* mutations and does not seem to correlate with the type or class of the *CFTR* mutation. The identification of further disease modifying genetic factors will increase the pathophysiological understanding and may help to identify new therapeutic targets.

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CHRONIC PANCREATITIS

In industrialised countries, chronic pancreatitis (CP) is caused by long term alcohol misuse in about 70% to 80% of cases.[1][2] Other causes such as anatomical anomalies, hyperlipidaemia, and hypercalcaemia are rare. In 10%–30% of patients with CP there is no apparent underlying cause, including heredity, and these cases are classified as idiopathic CP. The clinical pattern of CP is characterised by an early stage with recurrent episodes of acute pancreatitis followed by a late stage including pancreatic calcifications, exocrine insufficiency, and diabetes mellitus in most patients.[1] Clinically, chronic pancreatitis is characterised by recurrent or persisting abdominal pain, though the disease may present without pain. Morphologically, the pancreas shows an irregular sclerosis with focal, segmental, or diffuse destruction of the parenchyma.[3] Abnormalities of the pancreatic duct system such as dilatations or strictures as well as intraductal plugs containing protein or calculi are frequent findings. The reported incidence of CP in industrialised countries has been estimated to about 3.5 to 10 per 100 000 inhabitants. As most adult CP patients suffers from alcohol related disease, the epidemiological data published to date are not applicable to paediatric patients.

In 1952, Comfort and Steinberg reported that CP clusters in selected families, suggesting an inherited disease in these patients.[4] The present paragraph of this review delineates the different genes involved in the pathogenesis of hereditary or idiopathic pancreatitis, the impact of these genetic discoveries on other types of CP such as alcohol induced CP, and the implications for disease pathogenesis.

Cationic trypsinogen (*PRSS1*)

In 1896, Hans Chiari postulated that pancreatitis results from pancreatic autodigestion.[5] An inappropriate conversion of pancreatic zymogens to active enzymes within the pancreatic parenchyma was proposed to initiate the inflammatory process. A key role has been attributed to the activation of trypsinogen to trypsin, converting all proteolytic proenzymes to their active form.[6] Three different trypsinogens have been described in human pancreatic juice and have been designated, according to their electrophoretic mobility, as cationic trypsinogen, anionic trypsinogen, and mesotrypsinogen.[7] Compared with the anionic isoenzyme, the cationic trypsinogen autoactivates more easily and is more resistant to autolysis.[8][9]

By microsatellite linkage analysis, several groups located a gene for hereditary pancreatitis on the long arm of chromosome 7 (7q35).[10][12] Subsequently, a mutation in the cationic trypsinogen gene, also referred to as serine protease 1 (*PRSS1*), was identified as underlying defect by a candidate gene approach. In five families with CP, a substitution of arginine by histidine at residue 122 (R122H) segregated with the disease.[13] R122H seems to be the most common *PRSS1* mutation observed worldwide. In subsequent studies, two other *PRSS1* mutations have been frequently described: a substitution of asparagine by isoleucine at codon 29 (N29I)[14][15] and an exchange of alanine by valine at codon 16 (A16V).[16] The latter mutation is usually observed in patients with idiopathic CP.[16] In the following years, several other *PRSS1* mutations were reported: -28delTCC, D22G, K23R, N29T, P36R, G83E, K92N, L104P, R116C, R122C, V123M, and C139F.[17–25] With the exception of R116C and R122C, these variations were found in single patients or families only, and a detailed clinical background was mostly not mentioned. Thus, their pathogenic significance remains largely to be elucidated.

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Abbreviations: CP, chronic pancreatitis; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; MHC, major histocompatibility complex; TNFα, tumour necrosis factor α; TGFβ₁, transforming growth factor β₁; GSTM1, glutathione S transferase M1; NO, nitric oxide; MBL, mannose binding lectin; CBAVD; congenital bilateral absence of the vas deferens; ABPA, allergic bronchopulmonary aspergillosis

Although the precise disease mechanisms have not been unravelled, it is now a generally accepted model that an increased intrapancreatic trypsin activity results in pancreatitis. Site directed mutagenesis of recombinant human cationic trypsinogen revealed that all of the mutations studied (N29I, N29T, R122H, and R122C) increased significantly autoactivation in vitro.[26–30] Additionally, N29T, R122H, and R122C but not N29I inhibited autolysis of the enzyme.[26–30] A study using synthetic peptides showed an increased hydrolysis rate of the D22G and the K23R variants compared with wild type, indicating that these mutations might also facilitate trypsinogen autoactivation.[18] However, supporting data on recombinant enzymes are still lacking. To date, no experimental data exist on the functional consequences of the A16V mutation. In summary, increased intrapancreatic trypsinogen activation may be the common initiating step of pancreatitis caused by PRSS1 mutations, whereas stabilisation of trypsin may be an accessory mechanism.

Serine protease inhibitor, Kazal type 1 (SPINK1)
The serine protease inhibitor, Kazal type 1 (SPINK1), also known as pancreatic secretory trypsin inhibitor (PSTI), is a potent antiprotease that is thought to be an important inactivation factor of intrapancreatic trypsin activity. SPINK1 was first isolated in the bovine pancreas by Kazal and coworkers in 1948.[31] SPINK1 possesses a reactive site that serves as a specific target substrate for trypsin.[32] However, trypsin inhibition by SPINK1 is only temporary because the trypsin-SPINK1 complex itself serves as substrate for trypsin, resulting in a subsequent degradation of the inhibitor molecule and in restoration of the original trypsin activity.[33]

A considerable number of patients with hereditary CP do not show a PRSS1 mutation,[16] suggesting that genetic defects in other genes might be involved in the disease pathogenesis. As "gain of function" mutations in the PRSS1 gene cause pancreatitis by increased tryptic activity within the pancreatic parenchyma due to a "super trypsin", it was hypothesised that pancreatitis may also be raised by "loss of function" mutations in pancreatic trypsin inhibitors. By a candidate gene approach, the serine protease inhibitor, Kazal type 1 (SPINK1) was identified as another pancreatitis gene: in 18 of 96 unrelated paediatric patients, a substitution of asparagine by serine at codon 34 (N34S) was found.[34] Six patients were homozygous for this mutation. No phenotypic differences between heterozygous and homozygous N34S patients were detected. Compound heterozygosity of the N34S heterozygotes as well as gross deletions or insertions were excluded by analysing the complete intronic sequences after long range polymerase chain reaction.[34] The association between N34S and CP was confirmed by others.[35–38] N34S is mostly found in patients without a family history of CP: 15%–40% of patients with so called idiopathic CP carry N34S on one allele or on both alleles.[35–38] Interestingly, N34S is in complete linkage disequilibrium with four other intronic sequence variants: IVS1–37 T>C, IVS2+268 A>G, IVS3–604 G>A, and IVS3–66_-65 insTTTT.[34] This finding indicates that N34S is an evolutionary ancient mutation arisen a long time ago. So far, no experimental studies have elucidated the functional consequences of N34S.

In one CP family with multiple affected members, a heterozygous mutation disrupting the start codon (M1T) was detected.[34] The mutation was found in the index patient, in the unaffected father, and in the affected grandfather. Moreover, the deceased great grandfather suffered from CP. This pedigree suggests that even a single SPINK1 mutation may strongly contribute to the disease development. Additional SPINK1 mutations have been reported, mostly in single patients or families: -215G>A, -53C>T, -41G>A, -2C>A, L14P, D50E, IVS3+2 T>C, IVS3+125 C>A and IVS3+184 T>A, R65Q, R67C.[34 35 39–43]

Cystic fibrosis transmembrane conductance regulator (CFTR)
Recently, two studies described an association between CFTR mutations and CP.[44 45] The link between CFTR and CP is supported by the findings that both conditions may show abnormal sweat chloride contents[46] as well as pancreatic ductal obstruction due to inspissated secretions.[47 48] Moreover, some patients with cystic fibrosis (CF) suffer from recurrent attacks of pancreatitis.[49 50]

Sharer and coworkers tested 134 patients with CP, including 60 cases with idiopathic disease and 71 cases with alcohol induced CP, for 22 mutations and for the 5T allele in intron 8.[44] Eighteen patients (13.4%), including 12 with idiopathic CP (20%), were heterozygous for a CFTR mutation. Fourteen patients (10.4%) had the 5T allele in intron 8. Four patients were heterozygous for both a CFTR mutation and the 5T allele. The frequency of CFTR mutations in alcohol related CP was two times and in idiopathic CP four times the expected, whereas the frequency of the 5T allele was not increased (allele frequency 0.05[51]). Cohn and coworkers investigated 17 CFTR mutations and the 5T allele in 27 patients with idiopathic CP.[45] Seven patients (25.9%) had at least one CFTR mutation and five patients (18.5%) had a 5T allele. One patient was compound heterozygous for the ΔF508 and the R117H mutation and two patients were heterozygous for the ΔF508 mutation and the 5T allele. The frequency of CFTR mutations in idiopathic CP was six times higher than expected, whereas the frequency of the 5T allele was twice as high. Both studies investigated the most common CFTR mutations only.

In more recent studies, Noone and colleagues and Audrézet and coworkers analysed the complete CFTR coding sequence and PRSS1 and SPINK1 in CP patients.[43 52] In both studies, 25% to 30% carried at least one CFTR mutation (without 5T allele), and several patients were compound-heterozygous for a CFTR mutation or were trans-heterozygous for a CFTR mutation and a mutation in SPINK1 respectively PRSS1.[43 52] These two studies illuminate the significance of a combination of different mutations in different genes in inherited pancreatitis.

α₁ Antitrypsin
α_1 Antitrypsin is a serum inhibitor of serine proteases such as neutrophil elastase, cathepsin G, and trypsin. Inherited α_1 antitrypsin deficiency is associated with pulmonary emphysema and liver disease. Two frequent genetic defects lead to an α_1 antitrypsin deficiency: a glutamine to valine substitution at codon 264 (E264V) (PiS) and a glutamine to lysine substitution at codon 342 (E342K) (PiZ).[53]

An association between α_1 antitrypsin deficiency and CP has been suggested by several case reports and two systematic studies,[54 55] but conflicting results were obtained by other authors.[56–58] All these studies, however, were performed by either α_1 antitrypsin phenotyping or by measurement of serum concentrations and focused predominantly on alcohol induced pancreatitis. In two recent studies, α_1 antitrypsin genotypes were investigated in 96 patients and 124 patients with non-alcoholic CP.[59 60] In both studies, the frequencies of the α_1 antitrypsin deficiency alleles PiS and PiZ did not significantly differ between patients and controls and were similar to the reported frequencies in Europe.[53] The lack of an association of α_1 antitrypsin deficiency and pancreatitis in these studies is in line with the results of a follow up study in 246 PiZ homozygous individuals in which no case of symptomatic pancreatitis was reported.[61]

Complexity of the inheritance pattern
Hereditary pancreatitis is usually defined as an autosomal dominant disease with a penetrance of 80%.[62] The clinical characteristics of the most families with the R122H or the N29I mutation in the PRSS1 gene are in line with this concept. In contrast, A16V shows a low penetrance and is typically
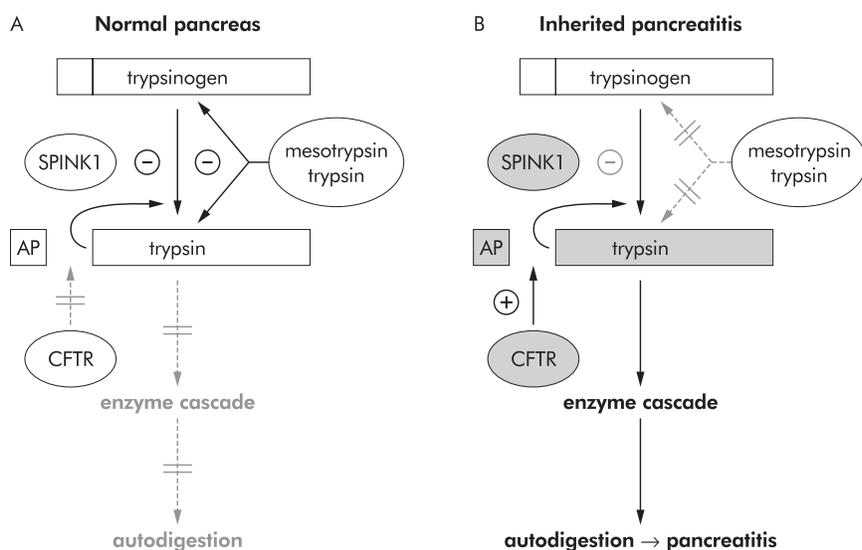
Figure 1 Model of inherited pancreatitis. (A) Condition in the normal pancreas: trypsin resulting from autoactivation of trypsinogen within the pancreatic parenchyma is inhibited by SPINK1 and in the second line by mesotrypsin or trypsin. This defence mechanism prevents the pancreas from activation of the pancreatic enzyme cascade and autodigestion. (B) Condition in inherited pancreatitis: mutations in *PRSS1* or in *SPINK1* lead to an imbalance of proteases and their inhibitors within the pancreatic parenchyma resulting in an inappropriate conversion of pancreatic zymogens to active enzymes with autodigestion and inflammation. Mutations in *CFTR* may disturb this delicate balance by intrapancreatic acidification or by a defective apical trafficking of zymogen granules and thus facilitate the intrapancreatic activation of digestive enzymes. Dark boxes represent products of mutated genes (modified from reference 34). (AP, activation peptide).

found in patients without a family history of CP, indicating that *PRSS1* mutations do not follow exclusively an autosomal dominant inheritance pattern.[16] Thus, trypsinogen mutations display a considerable variability of penetrance.

There are different points of view about the significance of *SPINK1* mutations in CP and the inheritance pattern.[35][63] In our opinion, different *SPINK1* mutations may lead to different inheritance patterns. For example, M1T mutation destroys the start codon resulting most probably in a "null" allele. The reported M1T family suggests a dominant inheritance.[34] On the other hand, N34S may decrease the SPINK1 capacity less resulting in recessive or complex trait. About 0.5% to 2% of the general population in Europe are N34S carriers and most will not develop CP. One may speculate that only the combination of N34S with other genetic defects or environmental factors results in CP. On the other hand, it is noteworthy that in two large studies about 5% to 10% of CP patients were homozygous for N34S.[34][35] According to an expected homozygote frequency of 1:10 000 to 1:40 000 in a general population, these data represent a striking enrichment of N34S homozygotes in CP patients and indicate a very strong influence of N34S on the disease pathogenesis.

We may classify *SPINK1* and *PRSS1* mutations into severe or mild with respect to the different degree in which this mutation affects protein function. Severe *PRSS1* mutations that result in a high disease penetrance such as R122H may strongly facilitate autoactivation or may exhibit additional features such as trypsin stabilisation. Other *PRSS1* mutations, such as A16V, may facilitate autoactivation less and the disease initiating threshold is only reached in combination with other genetic or environmental factors. Also the effect of *SPINK1* mutations may depend on the rate of pancreatic SPINK1 reduction. In the heterozygous state, N34S may cause only a subliminal lowering of SPINK1, but initiate pancreatitis in the homozygous state.

Although an association between *CFTR* mutations and idiopathic CP is now well established, the pathogenic mechanisms are poorly understood. It may be speculated that the combination of two mild or of one mild and a severe *CFTR* mutation predisposes to CP. However, many patients with idiopathic CP show only one mutant *CFTR* allele.[43][52] Possibly, CP is predisposed by compound heterozygosity for *CFTR* mutations or by a combination of defects in *CFTR* and other genes such as *SPINK1* as recently described.[43][52]

Disease model of inherited pancreatitis

Several mechanisms protect the pancreas from autodigestion by activation of the pancreatic digestive cascade[64]: intrapancreatic tryptic activity is prevented by the synthesis of digestive enzymes as inactive proenzymes (zymogens), by the localisation of the activating enzyme enteropeptidase outside the pancreas, and by a low calcium concentration. In the normal pancreas, small amounts of trypsinogen are hydrolysed to active trypsin in the pancreatic parenchyma, but this tryptic activity is dammed by co-synthesised protease inhibitors such as SPINK1.[65] Furthermore, trypsin itself activates trypsin-like enzymes such as mesotrypsin readily degrading trypsinogen and other zymogens[66] (fig 1A). Pancreatitis may result from an imbalance of proteases and their inhibitors within the pancreatic parenchyma (fig 1B). Gain of function mutations in the cationic trypsinogen resulting in an increased autoactivation of the enzyme or loss of function mutations in *SPINK1* resulting in a decreased inhibitory capacity may, in a similar way, disturb the delicate intrapancreatic balance of proteases and their specific inhibitors. The net effect of a single mutation may depend on the different degree in which this mutation affects protein function and on the presence of mutations in other genes such as *CFTR*. It has been suggested that pancreatic dysfunction in CF is a result of a decreased ductal pH.[67] This lowering of pH may subsequently lead to a defective solubilisation of proteins, a defective apical trafficking of zymogen granules, or to an increased autoactivation of trypsinogen. A slight reduction of CFTR function, as proposed for the heterozygous state, may raise the susceptibility for CP by facilitating the intrapancreatic activation of digestive enzymes.

The predominant feature of inherited pancreatitis is a relapsing pancreatitis. This clinical course poses the question why inherited pancreatitis is characterised by recurring instead of continuing inflammation, although the underlying genetic defect is present within the pancreatic parenchyma from the cradle to the grave. We postulate that endogenous or exogenous factors might trigger the particular event. Hereditary pancreatitis patients occasionally report that viral infections preceded the acute pancreatitis attack, suggesting that viral agents or drugs used for treatment such as salicylates initiate the acute event. In some of our patients the cause of pancreatitis was attributed to mild abdominal trauma
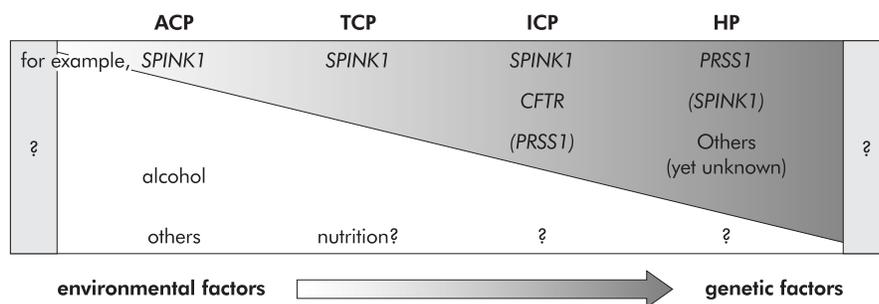
Figure 2 Schematic presentation of the influence of different genetic and environmental factors on the pathogenesis of chronic pancreatitis. ACP, alcoholic chronic pancreatitis; TCP, tropical calcific pancreatitis; ICP, idiopathic pancreatitis; HP, hereditary pancreatitis.

before genetic testing revealed a pathogenic *PRSS1* mutation (unpublished data).

Alcohol related chronic pancreatitis

The association between alcohol misuse and CP is well established. There is a wide variation between people in their susceptibility to alcohol and only 5%–10% of heavy drinkers develop CP.[68] The role of genetic factors in the development of alcoholic CP is mostly unknown.

Several studies investigating *PRSS1*,[69] pancreatitis associated protein (PAP),[70] α_1 antitrypsin, CFTR,[44 71] alcohol dehydrogenase and MHC antigens[72] gave negative or conflicting results. Recently, an association between the *SPINK1* N34S mutation and alcoholic CP in a large cohort of patients originating from three different countries has been described: a heterozygous N34S mutation was detected in 16 of 274 patients with alcoholic CP (5.8%), but in only 4 of 540 healthy control individuals (0.8 %) and in 1 of 98 alcoholic controls without CP (1.0%).[73] Two subsequent studies observed also an N34S frequency in alcohol related CP of 6.0% and 5.6% respectively.[37 38] However, one study failed to find a significant association between N34S and alcoholic CP because of the high N34S frequency in their control subjects (4 of 100).[38]

Analysis of pancreatic juice showed an increase of the trypsinogen/trypsin inhibitor ratio in alcoholics compared with non-alcoholic controls, indicating a weakening of the defence mechanism provided by the trypsin inhibitor against premature zymogen activation.[74] As most alcoholics do not suffer from CP, the association between *SPINK1* mutations and alcoholic CP underlines the relevance of the pancreatic protease/protease inhibitor system in the disease pathogenesis and supports the hypothesis that a combination of exogenous and genetic factors may predispose to alcoholic CP.

Indications for genetic analysis

Testing for *PRSS1* and *SPINK1* mutations is indicated in all patients with acute or chronic pancreatitis and a family history of CP or pancreatic cancer as well as in all patients with chronic CP without a family history after exclusion of predisposing factors such as alcohol misuse or hypertriglyceridaemia. In the presence of a family history, genetic testing should be started with analysis for the *PRSS1* mutations R122H or N29I, which will be most probably found in these cases, whereas in the absence of family history most probably a *SPINK1* N34S mutation, a *PRSS1* A16V mutation, or a *CFTR* mutation will be identified. So far, only limited data are given about the occurrence of *PRSS1* or *SPINK1* mutations in patients with secondary CP—that is, alcohol misuse, anatomical anomalies, etc. Future studies will clarify if clinical testing is appropriate in these patients. A negative genetic test result does not exclude a genetic basis of the disease: in our study population more than 50% of patients with primary CP do not show a mutation in one of the two above mentioned genes. As genetic analyses are expensive and time consuming, genetic testing should not be performed in patients with acute pancreatitis without a family history of CP. Because of the lack of therapeutic consequences, asymptomatic relatives should

be screened after detailed counselling only. As patients with CF may suffer from recurrent attacks of pancreatitis without significant pulmonary disease or malabsorption, all patients with raised or borderline sweat chloride concentrations should be tested for *CFTR* mutations. In our opinion, prenatal testing for *PRSS1* or *SPINK1* mutations is not indicated.

Rethinking chronic pancreatitis

The genetic studies about inherited pancreatitis have substantially changed our disease understanding. For a long time, hereditary pancreatitis was thought to be a rare disorder. The recent findings of *PRSS1*, *SPINK1,* and *CFTR* mutations in patients with so called idiopathic CP, however, demonstrate that inherited cases of CP are much more common than originally envisioned. These data challenge the differentiation between "hereditary" and "idiopathic" pancreatitis. Different mutations in different genes might lead to different phenotypic presentations and inheritance pattern and even the same mutation in the same gene might have different consequences depending on the individual's genetic background and environmental factors. The discovery of *SPINK1* mutations in other types of CP such as tropical calcific pancreatitis (unpublished data) and alcohol induced CP further blur the borders between the particular CP subtypes. Future research will most probably reveal a very complex interaction between various environmental and genetic factors with flowing transitions among these subtypes (fig 2). This hypothesis is supported by the observation of *SPINK1* mutations in individuals in which the cause of CP was attributed to metabolic disorders or anatomical anomalies.[37]

In several symposiums held in Marseilles, Cambridge, and Rome, experts tried to define and to classify inflammatory pancreatic diseases. According to these classification systems, CP is currently defined as an *continuing* inflammatory disease characterised by the destruction of pancreatic parenchyma leading to progressive or permanent impairment of exocrine function, endocrine function, or both.[3 75 76] It is important to note that these classifications scarcely consider aetiological factors. According to this classification, acute pancreatitis has been defined "not as a disease but as a spectrum of inflammatory lesions".[76] Furthermore, most participants agreed that CP is a cause and not a consequence of acute pancreatitis and that acute pancreatitis only rarely progresses to CP.[3 77] According to this concept, acute and chronic pancreatitis are two separate diseases that rarely merge.

The current definition of chronic pancreatitis, however, is not compatible with the presentation of inherited pancreatitis. The predominant feature of inherited pancreatitis is a relapsing pancreatitis starting in childhood with initially absent signs of functional or morphological pancreatic damage. During the course of the disease, most of the patients with acute relapsing pancreatitis develop all morphological and functional signs of CP. Consequently, acute and chronic pancreatitis have to be considered as different stages of one dynamic disease process. The different concepts of CP in the paediatric and non-paediatric literature reflect the fact that the paediatrician is confronted with the early stage of disease,

whereas the internist is usually dealing with its late stage. The findings of the same genetic defects in different types of acute and chronic pancreatitis support the old concept that all intermediate stages between acute pancreatitis and chronic calcifying pancreatitis may exist as pristinely postulated by Comfort and coworkers.[78]

Summary

Since the first description of inherited pancreatitis reported an autosomal dominant trait, hereditary CP was defined as an rare dominant inherited disease. Subsequently, the fact of familial clustering in one generation only, which indicates other inheritance pattern such as recessive or complex trait, was blinded out in the disease concept of hereditary CP for a long time. The identification of *PRSS1*, *SPINK1*, and *CFTR* mutations in patients with so called idiopathic chronic pancreatitis, however, shows that inherited cases of CP are much more frequent and that different mutations in different genes might lead to different inheritance pattern. Evaluation of patients with CP without an obvious predisposing factor should include genetic testing for mutations in the above mentioned genes even in the absence of a family history of pancreatitis. The finding of *SPINK1* mutations in alcohol induced pancreatitis indicates that genetic factors may increase disease susceptibility to primary non-hereditary CP types. Thus, the identification of further genes involved into the pathogenesis of inherited CP probably will also improve our knowledge about more common types of CP such as alcoholic or tropical CP.

CYSTIC FIBROSIS

CF is an autosomal recessive inherited disorder characterised by chronic obstructive pulmonary disease with proximal bronchiectasis often resulting in lung failure, by exocrine pancreatic insufficiency with maldigestion, and by increased sweat chloride concentration. Other clinical features include meconium ileus, liver fibrosis, and male infertility due to obstructive azoospermia. The incidence of CF in the white population is about 1:2500. The present review focuses mainly on the *CFTR* genotype phenotype correlation with respect to disease involvement of different organs, the impact of genetic modifying factors on the phenotype, and the impact of *CFTR* changes on diseases other than CF.

In 1989, *CFTR* was identified as the CF gene by linkage analysis and chromosome walking (positional cloning).[79][80] Human *CFTR* is located on the long arm of chromosome 7 (7q31), spans about 250 kb, and is separated into 27 exons.[80][81] *CFTR* belongs to the ATP binding cassette (ABC) superfamily and encodes a transmembrane protein present at the surface of most epithelial cells functioning as a cyclic adenosine monophosphate (cAMP) responsive chloride channel.[82] CFTR has also been implicated in several other processes such as regulation of other ion channels and membrane trafficking. CFTR comprises two membrane spanning domains (MSD1 and MSD2), two nucleotide binding domains (NBD1 and NBD2), and a central, intracellulary regulatory domain (R region) with multiple phosphorylation sites.[79][83]

So far, more than 1000 *CFTR* mutations have been identified. The most common *CFTR* mutation is a deletion of three nucleotides in exon 10 abolishing a phenylalanine residue at position 508 (F508del). Worldwide, this mutation accounts for 66% of all CF chromosomes.[84] About 50% of CF patients are homozygous for this mutation. The impact of a specific *CFTR* mutation on the disease severity depends on various factors such as type of mutation (missense, frameshift deletion, etc), the consequences of the mutation for structure and function (mutation class) and the position of mutation within the gene (location in functionally or structurally relevant regions). Also, the presence of other genetic alterations within the same allele can significantly influence the phenotypic effect as in the case of the R117H mutation.[51]

CFTR mutations can be divided into five or six general classes that reflect their known or predicted molecular dysfunction[85–87] (fig 3). Mutations resulting in defective transcription of mRNA (class I) lead to virtually no functional protein and are equivalent to a "null" mutation. Class II mutations are characterised by defect processing or trafficking of the CFTR protein. To this class belongs the most common *CFTR* mutation, F508del, which leads to degradation of the protein in the endoplasmic reticulum.[88] Mutations leading to a defective activation (class III) because of alteration of ATP binding or hydrolysis at the nucleotide binding domains (NBD) result in a normal but non-functional amount of CFTR protein at the cell membrane. Class IV mutations such R117H and R347P affect the chloride conductive function and are associated with decreased but residual CFTR function. Class V
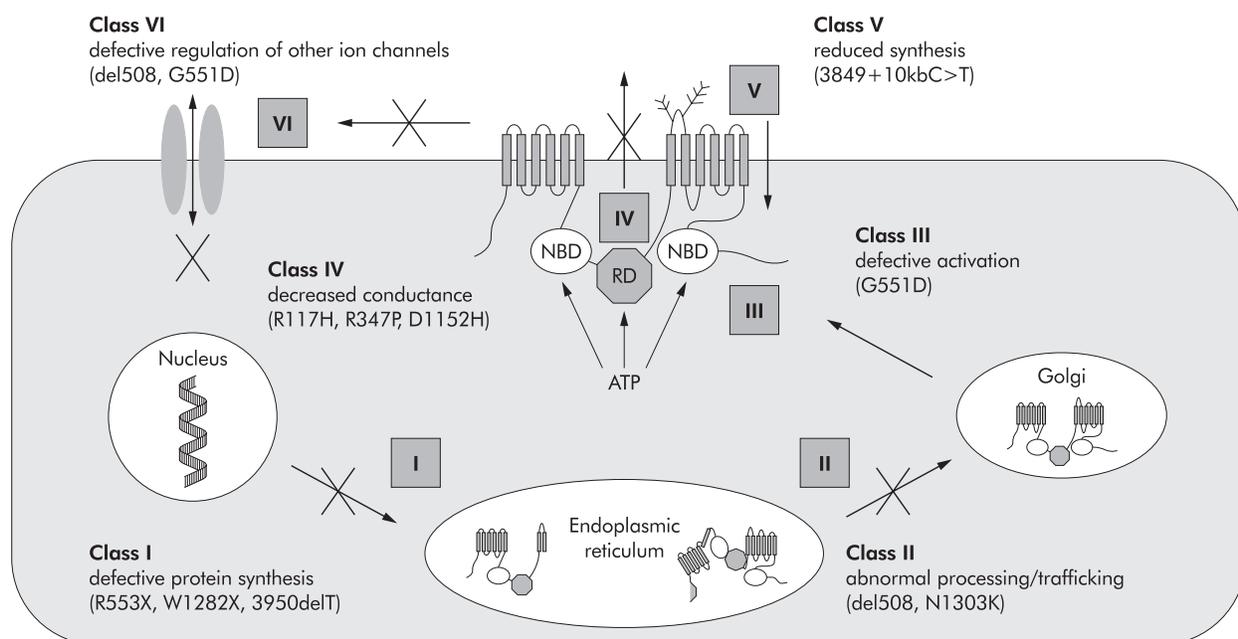

Figure 3 *CFTR* mutations classes. The subdivision reflects the known or predicted biosynthetic and functional consequences.

mutations result in a decreased amount of functional protein by abnormal splicing or reduced trafficking. Some mutations may also affect the regulatory function of CFTR on other ion channels (class VI). Class I-III mutations generally affects the amount of functional CFTR more seriously than class IV and V mutations and are usually associated with a classic CF. It is worthwhile to note that a specific mutation can be associated with more than one of the above mentioned mechanisms: the G551D mutation affects activation (class III) as well as the regulatory property of CFTR on other ion channels ("class VI").[89]

Genotype phenotype correlation and modifier genes
The course of disease differs between individuals with identical *CFTR* mutations even in the same family suggesting that environmental and inherited factors may modify the disease phenotype. Pancreatic function is strongly determined by the CFTR genotype, whereas the course of pulmonary disease seems to be largely dependent on secondary factors.

Pancreas
Pancreatic damage in CF is characterised by widespread loss of acinar cells with fatty replacement and interstitial fibrosis. Pancreatic disease in CF varies from complete loss of exocrine and endocrine function to nearly normal pancreatic function. About 90% of the CF patients show exocrine pancreatic insufficiency. The type respectively class of *CFTR* mutation correlates closely with the pancreatic function: the vast majority of patients homozygous for F508del are pancreatic insufficient. However, a small percentage of F508del homozygotes has preserved pancreatic function.[91]

Liver
Chronic fibrotic liver disease accounts for virtually all non-pulmonary causes of mortality in patients with CF. Histopathologically, CF related liver disease is characterised by focal biliary fibrosis. Clinically significant liver disease develops in 2%–5 % of the patients.

Several studies failed to find any significant association between a specific *CFTR* mutation or mutation class and the development of liver disease.[91–94] Colombo and coworkers analysed 189 CF patients for clinical and genetic risk factors of liver disease and did not find any genotype phenotype correlation. However, the risk of acquiring liver disease was increased about fourfold in patients with a history of meconium ileus or distal intestinal obstruction syndrome (DIOS) in comparison with CF patients unaffected by these complications.[93] Others investigated 288 CF patients and found no difference in the CFTR genotype distribution or a history of meconium ileus or DIOS in patients with and without liver disease.[94] In both studies, pancreatic insufficiency was present in all patients with liver disease. However, liver fibrosis has also been reported in patients with pancreatic sufficiency.[95]

Only two studies investigated the role of genetic modifying factors in CF related liver disease. Duthie and coworkers analysed major histocompatibility complex (MHC) genes and found a significant difference of the *DQ6* allele in patients with liver disease compared with those without liver disease or healthy controls.[96] Another group found a significant association of mannose binding lectin (*MBL2*) (for *MBL2* see paragraph pulmonary disease) deficiency and liver cirrhosis in 216 F508del homozygous CF patients: 5.4% with wild type or one mutated *MBL2* allele showed evidence of cirrhosis compared with 30.8% of those patients with two deficiency alleles.[97] So far, the relation between liver disease and genetic variations of MHC genes or *MBL2* has not been investigated by other groups and remains to be confirmed.

Meconium ileus
About 10% to 20% of neonates with CF will present with meconium ileus, an intestinal obstruction attributable to inspissation of meconium in the terminal ileum.[98 99] Almost all patients with meconium ileus have pancreatic insufficiency. Meconium ileus seems to be independent from the type or class of the underlying *CFTR* mutation.[90 100] Kerem and coworkers observed a recurrence rate of meconium ileus in 29% of subsequent siblings in families in which the first child had meconium ileus compared with 6% of siblings born to families in which the first child did not have meconium ileus.[99] Both, the poor genotype phenotype correlation and the familial clustering indicate that other genetic factors might be involved in the pathogenesis of neonatal intestinal obstruction. Recently, a CF modifier locus for meconium ileus on the long arm of the human chromosome 19 (19q13) has been identified.[101] However, there was no single haplotype associated with meconium ileus suggesting multiple mutations in the modifier gene or multiple modifier loci. So far, the underlying genetic defect or defects have not been identified.

A candidate gene study investigated the role of haemochromatosis (HFE) gene mutations as modifier for meconium ileus in CF patients.[102] The carrier frequency of the C282Y mutation in the *HFE* gene among 89 F508del homozygotes was significantly higher in those with meconium ileus if compared with the unaffected controls (19.4% versus 7.7%), but not if compared with CF patients without meconium ileus (19.4% versus 10.3%).[102] These findings remain to be confirmed in larger study populations.

Pulmonary disease
Pulmonary disease is the primary cause of death in CF and accounts for more than 90% of the mortality. It is proposed that abnormal electrolyte transport because of a defect CFTR causes altered mucus viscosity and decreased mucosal defence against infection, which lead to airway plugging, chronic inflammation, and progressive destruction of the lung.

CF patients demonstrate a high variability in pulmonary disease that poorly correlates with the type of *CFTR* mutation.[90 103 104] This is in contrast with pancreatic involvement: pancreatic sufficiency is frequently associated with mild *CFTR* mutations.[90] In the past years, polymorphisms in various candidate genes have been investigated by several groups.

MHC genes play an important part in immune defence and allergic reactions. MHC class II alleles have been implicated as possible modifiers of the pulmonary phenotype by influencing specific IgE levels and susceptibility to chronic *Pseudomonas aeruginosa* colonisation.[105] Among CF patients, there was a higher probability of colonisation with *Pseudomonas aeruginosa* in those with the *DR7* allele, and a lower probability of colonisation in those with the *DR4* allele. Twelve of 98 patients were not yet colonised with *Pseudomonas aeruginosa*: none of the 12 were *DR7+* and 6 were *DR4+*.[105]

Deficient α_1 antitrypsin variants also have been associated with earlier onset of lung colonisation with *Pseudomonas aeruginosa* and higher total IgG levels.[106] However, these findings could not be confirmed by others. Instead, Mahadeva and coworkers found a significantly better lung function in CF patients with an α_1 antitrypsin deficiency allele.[107 108]

The same group also investigated α_1 antichymotrypsin in 157 CF patients and found that patients with α_1 antichymotrypsin deficiency have less, rather than more severe pulmonary disease.[109] The authors hypothesised that a genetic deficiency of proteinase inhibitors might permit downregulation of the inflammatory response and thereby reducing the severity of pulmonary damage.

Tumour necrosis factor α (TNFα) is a potent immunomodulator and proinflammatory cytokine. TNFα polymorphisms have been implicated in the pathogenesis of various autoimmune and infectious diseases. A biallelic promoter polymorphism (-308 G>A) influences the level of TNFα transcription.[110] Hull and Thomson investigated 53 CF patients and found a significant association between the high expression TNFα allele (TNF2, -308 A) and reduced pulmonary

function as determined by FEV$_1$.[111] The TNFα gene is located between the MHC class I and MHC class II loci. Thus, the observed association may be attributable to linkage disequilibrium between the *TNF2* allele and MHC alleles.

Transforming growth factor β$_1$ (TGFβ$_1$) is a multifunctional cytokine that promotes fibroblast proliferation and collagen deposition.[112] TGFβ$_1$ production shows an interindividual variability that partly depends on polymorphisms in the promoter and in the signal peptide region. Arkwright and coworkers studied 171 *CFTR* F508del homozygotes for the TGFβ$_1$ L10P and R25P polymorphisms and found an accelerated decline of pulmonary function in patients with a leucine allele than in those with a proline allele at codon 10. The authors implied that a high TGFβ$_1$ producer genotype predisposes CF patients to more rapidly development of pulmonary disease.[113] However, no association with the codon 25 allele, which is known to influence TGFβ$_1$ production,[114] was found, and no data exist about the impact of the L10P polymorphism on TGFβ$_1$ synthesis.

Glutathione S transferase M1 (GSTM1) belongs to a multigene family of enzymes involved in detoxification and cellular protection against oxidative stress by removal of reactive oxygen species and regeneration of S-thiolated proteins. Three *GSTM1* alleles have been identified: *GSTM1*A*, *GSTM1*B*, and *GSTM1*0*. Some 40% to 65% of the human population are homozygous for a deletion of the entire gene (*GSTM1*0*)[115] and thus do not produce the protein. The *GSTM1*0* allele has been associated with an increased risk for various cancers.[116 117] Baranov and coworkers investigated 194 CF patients for the *GSTM1*0* allele and reported a significant association between homozygosity for the null genotype and earlier diagnosis of CF.[118] However, no data about pulmonary function or *Pseudomonas aeruginosa* colonisation have been given. In a subsequent study, a lower Shwachman score and a higher Chrispin-Norman chest radiographic score in patients with the null allele have been found.[111] However, the number of investigated patients was small and no differences of FEV$_1$ were observed between homozygous and non-homozygous patients. Both studies implied that the observed associations might be attributable to the reduced ability to deal with oxidant stress, particularly within the lung. It might be worthwhile to note that *GSTM1* is expressed at high levels in the liver but is only weakly synthesised in lung tissue.[119]

Nitric oxide (NO) is an important factor in cell signalling, pathogen killing, and smooth muscle relaxation. NO is synthesised by a group of enzymes called NO synthases (NOS). As NO is important in bactericidal action, lowered levels of NO might lead to an increased risk of infection. Exhaled NO levels are reduced in CF.[120] One group investigated the size of an AAT trinucleotide repeat in intron 20 of the *NOS1* gene in CF patients.[121] Although no differences between FEV$_1$ and FVC were observed in patients with different trinucleotide sizes, expired NO was significantly lower in patients who harboured two alleles with a high number of repeats (>12) than in those who showed alleles with fewer repeats. Moreover, colonisation with *Pseudomonas aeruginosa* and *Aspergillus fumigatus* was significantly more common in patients with high repeat numbers.[121] The authors supposed that lowered NO formation attributable to high repeat numbers in *NOS1* might render CF patients more susceptibly to airway colonisation.

More recently, mutations in the gene encoding for mannose binding lectin 2 (MBL2) have been associated with the severity of lung disease and mortality in patients with CF.[122 123] MBL is a serum protein that belongs to the collectin subgroup of the C type lectin superfamily.[124] Collectins bind specifically to oligosaccharides on the surface of microbial pathogens, thereby initiating agglutination, neutralising or opsonisation of the micro-organisms for phagocytosis. It has been shown that *MBL2* alleles causing low MBL serum concentrations are associated with an increased risk of different types of infections.[125–128] Three missense mutations in the exon 1 of

MBL2 independently cause low MBL serum concentrations: Gly 54 Asp (G54D), Gly 57 Glu (G57E), and Arg 52 Cys (R52C).[125 129 130] Several nucleotide substitutions in the promoter region may also affect the MBL serum level.[131 132] Garred and coworkers analysed 149 CF patients for *MBL2* mutations and found a significant reduction of pulmonary function (FEV$_1$ and FVC) in patients carrying a variant *MBL2* allele. Moreover, death and lung transplantation were increased threefold in patients with variant MBL.[122] Gabolde and colleagues compared the clinical characteristics of 11 patients homozygous or compound heterozygous for *MBL2* mutations with 11 sex and age matched CF patients with wild type *MBL2* and also found a significant decrease of pulmonary function in those with mutant *MBL2*.[123]

We have to point out that most of reported pulmonary modifiers in CF were analysed by one group only and remain to be confirmed in future studies. Because spurious results in genetic association studies are common, the obtained data should be interpreted warily. As yet, the association between *MBL2* deficiency alleles and decreased lung function only have been reported by two independent groups. However, MBL2 mutations explain only a small part of clinical variability. Therefore, other genetic variations probably contribute to the disease course and the host susceptibility to colonisation with *Pseudomonas aeruginosa* and other pathogens. The detection of further pulmonary modifier genes in the near future is probable and will have an important impact on new therapeutic strategies by influencing the identified proteins or their pathways.

CFTR mutations in other diseases than CF

Mutations in the *CFTR* gene not only cause CF but also have been associated with other diseases such as idiopathic chronic pancreatitis (see paragraph chronic pancreatitis above), male infertility due to congenital bilateral absence of the vas deferens (CBAVD), disseminated bronchiectasis, allergic bronchopulmonary aspergillosis (ABPA), asthma, chronic rhinosinusitis, and sarcoidosis.

Congenital bilateral absence of the vas deferens (CBAVD)

It is common knowledge that most men with CF are infertile because of obstructive azoospermia. Histological studies have demonstrated absence of the vas deferens and a rudimentary or absent epididymis.[133] CBAVD occurs in 1%–2% of the infertile male population. Dumur and colleagues described an increased frequency of F508del heterozygotes in a group of 17 CBAVD patients.[134] The increased frequency of *CFTR* mutations has been confirmed by many others.[135–140] About 60% to 70% of CBAVD patients show at least one known CFTR mutation. It has been hypothesised that *CFTR* mutations cause defective chloride transport in the epididymis and possible early regression of the mesonephric duct.

In contrast with classic CF, patients with CBAVD predominantly exhibit "mild" *CFTR* mutations (class IV or V) with residual functional CFTR. One characteristic of CBAVD is the high frequency of the *5T* allele, which is present in 40% to 50% of the patients.[139 140] Three length variants of a polypyrimidine tract within the splice acceptor site in intron 8 of the *CFTR* gene have been found to be associated with varying degrees of exon 9 splicing.[141 142] Of the three alleles with variable numbers of thymidines (5T, 7T, or 9T), the *9T* allele is associated with more efficient normal splicing of exon 9, whereas the *5T* allele leads to inefficient splicing with skipping of exon 9 and a defective chloride channel function.[143] The *5T* allele can also modify the phenotypic effect of other mutations. For instance, the R117H mutation in exon 4 of the *CFTR* gene may be located in *cis* (on the same chromosome) with *5T*. The R117H mutation on the *5T* background shows a cumulative effect and is associated with pulmonary disease in CF, whereas R117H

combined with the more common *7T* allele is typically observed in CBAVD without lung disease.[51]

Disseminated bronchiectasis

Poller and coworkers described an increased frequency for the F508del mutation in patients with disseminated bronchiectasis.[144] Subsequent studies confirmed the association between *CFTR* variants and disseminated bronchiectasis.[145–149] Pignatti and coworkers found a *CFTR* mutation or rare variant in 37.5%[145] and the *5T* allele in 31% of bronchiectasis patients.[146] Several CFTR variants were also detected in French patients with idiopathic bronchiectasis, however, the *5T* allele was not detected in any patient.[147] Other studies described a *CFTR* mutation in 5 of 16 patients (22%) respectively in 5 of 19 patients (26%).[148 149]

Asthma

Conflicting data exist about the influence of *CFTR* mutations on the risk of asthma. In one study a protective effect of F508del against asthma was observed,[150] but could not confirmed by others.[151] In a questionnaire survey, Dahl and coworkers reported asthma in 23 of 150 F508del heterozygotes (9%) compared with 529 of 8891 non-carriers (6%). Among the individuals with asthma 4.2% were F508del carriers compared with 2.7% of individuals without asthma.[152] A Spanish study detected in 21 of 144 asthma patients (15%) a *CFTR* missense mutation.[153] The distribution of FEV_1/FVC ratio and the predicted $FEV_1\%$ were similar in people with asthma with and without a *CFTR* variation.[153] However, investigating the four more common *CFTR* alterations found in asthmatics in an extended control sample, no significant difference was observed. Moreover, two of the *CFTR* mutations found were previously reported as non-disease causing polymorphisms (R1162L, T1220I), five alterations were described for the first time and have not been demonstrated in a previous study of 640 Spanish CF patients.[154] In summary, only 4 of 144 asthmatic patients (2.8%) possessed a verified CF causing mutation (R74W, I148T, T582R, and R1066C). A French group did not detect a statistically significant difference of *CFTR* variations between patients with asthma and controls: two of 43 patients (4.6%) and 6 of 142 control subjects (4.2%) were heterozygous for a *CFTR* mutation.[155] In another study, a group of 261 obligate *CFTR* heterozygotes and a control group, composed of 201 people negative for a standard mutation panel, were surveyed for possibly CF related conditions by a questionnaire. There was no difference between heterozygotes and controls for asthma, bronchiectasis, pneumothorax, allergic bronchopulmonary aspergillosis, sinusitis, and nasal polyps.[156]

Allergic bronchopulmonary aspergillosis (ABPA)

ABPA, a hypersensitivity disorder caused by chronic endobronchial infection with *Aspergillus fumigatus*, was first described in asthmatic patients.[157] In subjects with impaired mucus clearance and airway obstruction, the spores may germinate and release an array of antigens, resulting in a complex immune response by the host. ABPA is also a complication of CF observed in 2% to 8% of patients.[158 159] Familial occurrence of ABPA has been reported, suggesting a genetic contribution to the disease.[160 161] Miller and coworker investigated 11 patients with isolated ABPA and normal sweat electrolytes for *CFTR* mutations and detected in six patients at least one mutation.[162] Others found a heterozygous *CFTR* mutation in 6 of 21 ABPA patients (28.5%).[155]

Chronic sinusitis

CF patients commonly suffer from chronic sinusitis. Wang and coworkers analysed 147 patients with isolated chronic sinusitis and 123 controls for *CFTR* mutations and found a significant difference between both groups: eleven patients

(7%) showed at least one *CFTR* mutation, whereas two control subjects (2%) only were *CFTR* heterozygotes.[163] Another group investigated 58 children with chronic rhinosinusitis and detected in seven patients (12.1%) a *CFTR* mutation. Only one of the seven children showed a borderline abnormal sweat test. Two of the 58 patients experienced recurrent *Pseudomonas aeruginosa* sinusitis, and both were F508del heterozygotes.[164] In both studies, the frequency of the *5T* allele in the patient group was similar to that in the control group respectively to that reported for the general population.[163 164] In contrast, a Finnish study investigated 127 chronic sinusitis patients for the F508del and 394delTT mutations representing 75% of the CF chromosomes in Finland, and found only one *CFTR* heterozygote.[165]

Sarcoidosis

Recently, Bombieri and coworkers observed a significantly increased frequency of *CFTR* mutations in patients with sarcoidosis.[166] The authors detected in 8 of 26 patients a *CFTR* variation, but only in 9 of 89 control subjects. There was also a trend towards disease progression in patients with a *CFTR* mutations compared with patients without a mutation. However, some of the reported *CFTR* alterations have been found frequently in healthy individuals such as R75Q or L997F and might represent polymorphisms. Further studies are needed to elucidate the role of *CFTR* mutations in the predisposition to sarcoidosis.

Summary

The course of CF shows differences between patients even with the same *CFTR* mutation, suggesting that environmental and inherited factors modify the phenotype. Pancreatic function is strongly determined by the *CFTR* genotype, whereas the course of liver fibrosis and especially of pulmonary disease seem to be largely dependent on secondary factors. Thus, the phenotype is set by *CFTR* mutations as well as by the individual genetic background and environmental factors and varies between different organs depending on the specific organ susceptibility for defect CFTR. The identified association of *MBL2* mutations with the severity of pulmonary disease and mortality in CF patients is a first clue in understanding how genetic alterations do modify the pulmonary phenotype. The identification of further disease modifying genetic factors will contribute to our pathophysiological understanding and may open new ways of therapeutic strategies in the future. The increased frequency of *CFTR* mutations in diseases other than CF such as CBAVD or idiopathic pancreatitis contributes to the understanding of these entities, but also gives difficulties in nosological demarcations between CF and these disorders and indicates a smooth passage between CF and CFTR related diseases.

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