Advances in the etiology of chronic pancreatitis

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Chronic Pancreatitis - Etiology

Reasons to discriminate between different etiologies:
- Specific treatment options
- Inherent co-morbidities
- Different cancer surveillance strategies
Chronic Pancreatitis – Alcohol Cessation

Incidence of calcifications

Probability in %

Time after onset of symptoms

Layer et al, Gastroenterology 1994;107:1481-87
Chronic Pancreatitis - Alcohol Cessation

Strum et al, 1971
Gastard et al, 1973
Leger et al, 1974
Prinz et al, 1974
Bornman et al, 1980
Marks et al, 1980
Miyaka et al, 1987
Hayakawa et al, 1989
Strum et al, 1995

Patients with pain [%]

Alcohol
Stop Alcohol
Chronic Pancreatitis – Cessation of Smoking


Log rank p<0.0001
OR 2.0 [CI 1.1-3.8]

Cumulative Incidence of calcifications, %

Smokers

Non-Smokers

years after diagnosis of chronic alcoholic pancreatitis
Hereditary Pancreatitis

* Hereditary pancreatitis is clinically indistinguishable from other forms and varieties of pancreatitis.

14 year old girl with chronic pancreatitis and R122H-mutation

48 year old women with chronic pancreatitis and R122H-mutation
1952, first description of hereditary pancreatitis (autosomal dominant trait).

1996 Discovery of the first mutation associated with hereditary pancreatitis in the cationic trypsinogen gene (PRSS1) by Whitcomb et al.

Haplotype-Analysis of 10 unrelated families with a R122H mutation from an area of 100 km resulted in 7 different haplotypes. This precludes a Founder-Effect.
Cationic trypsin

Activation site
Clinical course of Hereditary Pancreatitis

Cumulative age at onset, %

Cumulative incidence of diabetes, %

50-70% increased risk for pancreatic cancer in patients with hereditary pancreatitis.
40% cumulative risk until 70. years of age.

Elimination/Treatment of causal factors:
- Smoking
- Alcohol
- Hyperlipidemia
- Hypercalcemia
- Gallstones
- Duct stricture
- Drugs and Medications

Indications for Genetic Hereditary Pancreatitis testing

- Recurrent (2 or more) episodes of *acute* Pancreatitis without identifiable cause or etiology

- Idiopathic *chronic* Pancreatitis - especially in children and young adults under the age of 25 years

- Pancreatitis in a patient with a positive family history of *Pancreatitis* (one or more first or second degree relatives)

*I. Ellis, M.M. Lerch, D.C. Whitcomb: Pancreatology 2001:1:405-415*
Pathophysiological role of two mutant trypsinogens: Decreased autoactivation

Pepsin activity (%)

PRSS1

Wild-Type

R122C

Time (h)

pH 8.0, 5 mM Ca\(^{2+}\)

Wild-Type

G191R

Time (min)

Simon et al. J. Biol. Chem. 2002; 277: 5404-5410

Witt et al Nature Genetics 2006; 38: 668-73.

Witt et al Nature Genetics 2006; 38: 668-73.
The phenotype of two novel trypsinogen mutations


Witt et al Nature Genetics 2006; 38: 668-73.
R116C Trypsin – Retention and ER Stress

### PRSS-Mutations: „gain or loss of function“?

<table>
<thead>
<tr>
<th></th>
<th>Autoactivation</th>
<th>Autolysis</th>
<th>Cathepsin B-ind. activation</th>
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<tbody>
<tr>
<td><strong>A16V</strong></td>
<td>?</td>
<td>?</td>
<td>?</td>
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<tr>
<td><strong>D19A</strong></td>
<td>▲</td>
<td></td>
<td></td>
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<tr>
<td><strong>D22G</strong></td>
<td>▲</td>
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<td><strong>K23R</strong></td>
<td>▲</td>
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<tr>
<td><strong>N29I</strong></td>
<td>▲</td>
<td>▶▶</td>
<td>▶▶</td>
</tr>
<tr>
<td><strong>E79K</strong></td>
<td>▼</td>
<td>▶▶</td>
<td>▶▶</td>
</tr>
<tr>
<td><strong>R122C</strong></td>
<td>▼</td>
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<tr>
<td><strong>R122H</strong></td>
<td>▶</td>
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<td><strong>XXX</strong></td>
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<td><strong>R116C</strong></td>
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<td>▶▶</td>
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<tr>
<td><strong>G191R</strong></td>
<td>▼</td>
<td>▶</td>
<td>?</td>
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- ▲: gain of function
- ▼: loss of function
- ▶▶: intracellular processing
Sporadic point mutations in the PRSS1-Gen in idiopathic chronic Pancreatitis

- In 5 of 50 Patients with idiopathic Pancreatitis (10%) mutations in the cationic Trypsinogen gene were found.

- Affected Patients represented 35% of all patients under 25 years.

In 78 families with hereditary pancreatitis and 200 individuals (6673 patients years) PRSS1 mutations were detected in 68%. R122H: 78%, N29I: 12% and others 10%.

Cumulative risk of pancreatic cancer was 11% at the age of 50 and 49% at the age of 75.

Smoking and diabetes mellitus are the main risk factors.

Trypsinogen copy number variations are present in 6% of idiopathic chronic pancreatitis cases but are unrelated to familial chronic or tropical calcifying pancreatitis.
Chymotrypsin C (CTRC) variants that diminish activity or secretion are associated with chronic pancreatitis
Rosendahl et al Nature Genetics 2008; 40: 78-82

- Chymotrypsin C is a trypsin-degrading enzyme. Alterations in the CTRC Gene at position p.R254W and p.K247_R254del are present in 30 out of 901 (3.3%) pancreatitis individuals but only in 21 of 2804 controls (0.7%).

- Functional analysis showed impaired activity or reduced secretion indicating that loss of function alterations in CTRC predispose to pancreatitis.
Pancreatic secretory Trypsin Inhibitor (PSTI, SPINK-1)

Witt et al, *(Nat. Genet., 2000)*  
Mutations in 23% of children with idiopathic chronic Pancreatitis  
☞ autosomal-recessive disorder

Pfützer et al, *(Gastroenterology, 2000)*  
Mutations in idiopathic chronic Pancreatitis (25%), hereditary Pancreatitis and in the healthy population (2%).  
☞ Modifier - Gene, risk of pancreatitis < 1%

Bhatia et al, Schneider et al, *(Gastroenterology, 2002)*  
Mutations in tropical calcifying pancreatitis (up to 44%) and in ‘Fibrocalculous Pancreatic Diabetes mellitus‘ (55%).  
☞ Risk factor for tropical Pancreatitis and Diabetes mellitus
SPINK1 Mutations (N34S) are found among Pancreatitis patients as well as among healthy carriers of Trypsinogen mutations.

SPINK1 Mutations in Hereditary Pancreatitis

Cumulative Incidence of Pancreatitis

SPINK1 Mutations have no influence on the severity of clinical disease course of hereditary pancreatitis

# Association of CTSB Polymorphisms with Tropical Calcific Pancreatitis

<table>
<thead>
<tr>
<th></th>
<th>Hyderabad</th>
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<tbody>
<tr>
<td></td>
<td>Patients/Controls</td>
<td>Patients/Controls</td>
<td>Patients/Controls</td>
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<tr>
<td></td>
<td>(n=140)</td>
<td>(n=155)</td>
<td>(n=166)</td>
<td>(n=175)</td>
<td>(n=306)</td>
<td>(n=330)</td>
<td></td>
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<tr>
<td>Leu26Val</td>
<td>0.48</td>
<td>0.30</td>
<td>0.45</td>
<td>0.28</td>
<td>0.46*</td>
<td>0.29*</td>
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<tr>
<td>Ser53Gly</td>
<td>0.10</td>
<td>0.06</td>
<td>0.09</td>
<td>0.04</td>
<td>0.09*</td>
<td>0.04*</td>
<td></td>
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</tbody>
</table>

* p = 0.013


eastic origin: Dravidian

[Image: Association of CTSB Polymorphisms with Tropical Calcific Pancreatitis]
## CTSB Val26 mutation in German ICP Patients

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>C/C</th>
<th>C/G</th>
<th>G/G</th>
<th>pG (Mahurkar)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients</strong></td>
<td>64</td>
<td>23</td>
<td>31</td>
<td>10</td>
<td>0.398 (51/128)</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>100</td>
<td>30</td>
<td>44</td>
<td>26</td>
<td>0.480 (96/200)</td>
</tr>
<tr>
<td>X² p-Value</td>
<td>0.428</td>
<td>0.578</td>
<td>0.117</td>
<td>0.147</td>
<td>0.013</td>
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<tr>
<td>OR</td>
<td>0.764</td>
<td>0.836</td>
<td>1.897</td>
<td>0.718</td>
<td>2.09</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.372 - 1,569</td>
<td>0.424 - 1,648</td>
<td>0.792 - 4,621</td>
<td>0.45 - 1.15</td>
<td>1.55 - 2.81</td>
</tr>
</tbody>
</table>

## Cathepsin B mutation Leu26Val in pancreatitis

<table>
<thead>
<tr>
<th>Population</th>
<th>Ethnic origin</th>
<th>(n)</th>
<th>pG (Val26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEPH</td>
<td>Mixed Caucasian</td>
<td>92</td>
<td>0.320</td>
</tr>
<tr>
<td>Pooled_CEPH</td>
<td>Caucasian</td>
<td>94</td>
<td>0.323</td>
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<tr>
<td>HapMap-CEU</td>
<td>European</td>
<td>55</td>
<td>0.355</td>
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<tr>
<td>SC_95_C</td>
<td>Caucasian</td>
<td>45</td>
<td>0.367</td>
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<tr>
<td>HapMap-YRI</td>
<td>Sub-Saharan African</td>
<td>52</td>
<td>0.394</td>
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<tr>
<td>TSC_42_C</td>
<td>Caucasian</td>
<td>41</td>
<td>0.430</td>
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<tr>
<td>JBIC-allele</td>
<td>Japanese</td>
<td>732</td>
<td>0.493</td>
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<tr>
<td>HapMap-JPT</td>
<td>Asian</td>
<td>44</td>
<td>0.523</td>
</tr>
<tr>
<td>HapMap-HCB</td>
<td>Asian</td>
<td>43</td>
<td>0.547</td>
</tr>
</tbody>
</table>

**Leu26Val**

- N34S positive: 0.45
- N34S negative: 0.46
- Controls: 0.29

**Population**

- **CEPH**
- **Pooled_CEPH**
- **HapMap-CEU**
- **SC_95_C**
- **HapMap-YRI**
- **TSC_42_C**
- **JBIC-allele**
- **HapMap-JPT**
- **HapMap-HCB**

**Controls**

- **Mahurkar et al. Gut 2006 55:1270-5**

**Weiss et al. Gut 2007 56:1322-3**

- **1198**
- **0.452 ± 0.042**

Chronic Pancreatitis and CFTR Mutations

One third of patients (n=27) with idiopathic pancreatitis carry CFTR-Mutations (Risk x 80).


CFTR Mutations represent a risk factor for chronic pancreatitis in patients without a history of alcohol abuse (19% of n = 60), but not for those with alcoholic pancreatitis (8.5% of n = 72). N. Sharer et al. New Engl J Med 1998;339:645-52

| CFTR allele frequency | 12/66 Patients with CFTR Mutations, 8/66 Patients with T5 Alleles |

| Abnormal CFTR alleles (affected subjects) | 25/134 (18.7%) | 11/120 (9.2%) | 0.047* (0.14) |
| Compound heterozygous alleles | 8/134 | 0/120 | 0.018* |
| Severe CFTR mutations | 4/134 | 3/120 | 0.88 |
| Mild/uncommon CFTR mutations (excluding 5T alleles) | 13/134 | 4/120 | 0.08 |
| Simple 5T alleles | 8/134 | 4/120 | 0.49 |
| 5T-12TG combination | 6/134 | 1/120 | 0.16 |
| Mild/uncommon CFTR mutations including 5T-12TG, excluding mere 5T alleles | 19/134 | 5/120 | 0.012* |
| All CFTR mutations, including 5T-12TG, excluding mere 5T alleles | 23/134 | 8/120 | 0.018* |

*p values below 0.05 are considered statistically significant.

Weiss FU Gut 2005; 54: 1456-1460
Prevalence of gene mutations in chronic pancreatitis

- **Chymotrypsin C mutations**: 45.5%
- **Idiopathic pancreatitis**: 10%
- **Trypsinogen mutations**: 18.2%
- **SPINK-1 mutations**: 15.2%
- **T5 Allels**: 12.1%
- **CFTR mutations**: 10%
Metabolic Chronic Pancreatitis – Causal treatment

Hyperlipidemia
(apoCII deficiency, lipoprotein lipase deficiency)

Serum triglyceride levels > 1000 mg/dl
Incidence extremely low
Treatment which maintains TG below 500 mg/dl leads to resolution of symptoms.


Hyperparathyroidism:
Hyperthyreoidisms leads to increased serum calcium levels, what is associated with an increased risk of pancreatitis.

The incidence of chronic pancreatitis is between 1.5 - 7%.

Early parathyreoidectomy leads to resolution of symptoms.

Hereditary chronic and idiopathic chronic pancreatitis are associated with mutations in the trypsinogen gene, the SPINK-1 Gene, the chymotrypsin C gene and the CFTR gene.

More genes will surely be identified.

The pathophysiological impact of these gene mutations are not completely understood and further experiments are warranted.

Etiologies of pancreatitis already treatable (such as autoimmune pancreatitis etc.) need to be distinguished from pancreatitis varieties that are not yet treatable – but may become so.

In hereditary pancreatitis (trypsin mutations) smoking cessation may reduce the risk of pancreatic cancer. Other surveillance strategies are, however, urgently needed.
Chronic Pancreatitis - Alcohol Cessation

Gullo et al, Gastroenterology 1988;95:1063-68

Output in % of initial

- Bicarbonate
- Lipase
- Chymotrypsin

Stop Alcohol
Alcohol

n= 18 vs. 14 pt
Δ 4-11 years
p<0.01
Today, restriction enzyme digest with **BstU I** represents the most extensive initial screening test for hereditary pancreatitis.

**Sequence in Exon 3**

- **Arg**
  - RSS1 wt: AAC-GCC-CGC-GTG-T
  - R-122-C: AAC-GCC-TGC-GTG-T
- **Cys**

*P. Simon, F.U. Weiss et al. J Biol Chem. 2002;277:5404-10*
Natural Course of Alcoholic Chronic Pancreatitis -

Stage 1 (early)  Stage 2  Stage 3

Pain

Serum Enzyme Elevation

Exocrine Pancreatic Function

5  10  15 years