Alpha-1:
Testing your liver the gentle way

Pavel Strnad

Department of Internal Medicine III, University Hospital Aachen
European center for AAT deficiency-associated liver disease
Lisbon, April 7th 2017
My way into the Alpha-1 world

Broad Spectrum of Hepatocyte Inclusions in Humans, Animals, and Experimental Models

Pavel Strnad, Renwar Nuraldeen, Nurdan Guldiken, Daniel Hartmann, Vineet Mahajan, Helmut Denk, and Johannes Haybaeck
My way into the Alpha-1 world

Hepatic inclusions

Human

- Primary liver inclusions
  - P62-containing
    - Mallory-Denk bodies (MDB)
    - Intracytoplasmic hyaline bodies (IHB)
  - Porphyrin-containing
    - Needle-like cytoplasmic inclusions (NLI)
    - NLI-like inclusions
  - ER storage disorders
    - α1-Antitrypsin inclusions
    - α1-Antichymotrypsin inclusions
    - Ground glass inclusions
    - Fibrinogen inclusions
    - Pale bodies

- Inclusions in multiple tissues
  - Viral
    - Cytomegalovirus (CMV)
    - Herpes simplex virus (HSV)
    - Marburg virus
    - Ebola virus
    - Yellow fever virus
    - Rift valley fever virus

- Carbohydrate-containing
  - Lafora bodies
  - Inclusions in glycosylation type I disorders
  - Inclusions in Andersen disease
  - Inclusions in adult polyglucosan body disease

Animal

- IB disease (snakes)
- IB hepatitis (chickens)

- Polyglutamine IBs
  - Polyglutamine aggregates

- Inclusions in sarcoidosis
  - Schaumann bodies
  - Asteroid bodies
  - Centrospheres
My way into the Alpha-1 world

**Hepatic inclusions**

**Human**
- Primary liver inclusions
  - Porphyrin-containing
    - Needle-like cytoplasmic inclusions (NLI)
    - NLI-like inclusions
  - Carbohydrate-containing
    - Lafora bodies
    - Inclusions in glycosylation type I disorders
    - Inclusions in Andersen disease
    - Inclusions in adult polyglucosan body disease

**Inclusions in multiple tissues**
- Viral
  - Cytomegalovirus (CMV)
  - Herpes simplex virus (HSV)
  - Marburg virus
  - Ebola virus
  - Yellow fever virus
  - Rift valley fever virus

**Animal**
- IB disease (snakes)
- IB hepatitis (chickens)

**Polyglutamine IBs**
- Polyglutamine aggregates

**Inclusions in sarcoidosis**
- Schaumann bodies
- Asteroid bodies
- Centrospheres
My way into the Alpha-1 world

Hepatic inclusions

Human

Primary liver inclusions

Inclusions in multiple tissues

Animal

IB disease (snakes)

IB hepatitis (chickens)

Polyglutamine IBs
- Polyglutamine aggregates

Carbohydrate-containing
- Lafora bodies
- Inclusions in glycosylation type I disorders
- Inclusions in Andersen disease
- Inclusions in adult polyglucosan body disease

Inclusions in sarcoidosis
- Schaumann bodies
- Asteroid bodies
- Centrospheres
My way into the Alpha-1 world
My way into the Alpha-1 world
What have we done so far?

- HBs-PiZ mice as a proteotoxic liver model
- Liver disease in PiZZ
- PiMZ as a disease modifier
HBs-PiZ mice

ER storage disorders
- a1-Antitrypsin inclusions
- a1-Antichymotrypsin inclusions
- Ground glass inclusions
- Fibrinogen inclusions
- Pale bodies

There is no existing therapy suppressing HBs production!
HBs-PiZ mice
HBs-PiZ mice

2 months

10 months

14 months

ALT

Fibrosis

Tumor load

Tumor number
HBs-PiZ mice

2 months

ER overload response

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>AAT</th>
<th>HBS</th>
<th>DOUBLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NfKB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAMIN B1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHOP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β actin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Activation of mTOR signalling, autophagy overload?
HBs-PiZ mice

2 months
What have we done so far?

- HBs-PiZ mice as a proteotoxic liver model
- Liver disease in PiZZ
- PiMZ as a disease modifier
Aachen Alpha1 study

Time to care for AATD livers!

- **Objective:**
  To systematically evaluate the extent of liver disease and contributing factors in patients with the homozygous PiZZ genotype.

- **Prospective examination** of PiZZ patients and controls without AATD:

  - **Questionnaire**
  - **Blood analysis**
  - **Elastography**
PiZZ cohort:
~300 adults with homozygous AATD

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>PiZZZ</td>
<td>300</td>
</tr>
<tr>
<td>PiMZ</td>
<td>150</td>
</tr>
<tr>
<td>PiSZ</td>
<td>25</td>
</tr>
<tr>
<td>Rare genotypes</td>
<td>40</td>
</tr>
<tr>
<td>PiMM</td>
<td>120</td>
</tr>
</tbody>
</table>
PiZZ patients have higher transaminases but usually within normal limits

**ALT ≥ ULN = 19%**
OR=2.7 [1.2, 5.6] *

**AST ≥ ULN = 11%**
OR=5.7 [1.5, 24.5] **

**GGT ≥ ULN = 22%**
OR=3.2 [1.5, 6.8] **

**AP ≥ ULN = 5%**
OR=2.7 [0.6, 10.7]

* p<0.05 - ** p<0.01
PiZZ genotype causes the development of metabolic liver disease

- Nearly 30% display at least moderate liver scarring
- 70% harbor liver steatosis
What have we done so far?

- HBs-PiZ mice as a proteotoxic liver model
- Liver disease in PiZZ
- PiMZ as a disease modifier
PiMZ in alcoholic liver disease

- Heavy drinkers with/without presence of liver cirrhosis were examined

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Non-Cirrhotic</th>
<th>Cirrhotic</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>German-Swiss</td>
<td>864</td>
<td>798</td>
<td>1662</td>
</tr>
<tr>
<td>UK</td>
<td>347</td>
<td>317</td>
<td>664</td>
</tr>
</tbody>
</table>

Buch et al., Nat Genet 2015
PiMZ in alcoholic liver disease

- Heavy drinkers with/without presence of liver cirrhosis were examined

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Non-Cirrhotic</th>
<th>Cirrhotic</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>German-Swiss</td>
<td>864</td>
<td>798</td>
<td>1662</td>
</tr>
<tr>
<td>UK</td>
<td>347</td>
<td>317</td>
<td>664</td>
</tr>
</tbody>
</table>

Buch et al., Nat Genet 2015

A) Unadjusted OR in alcohol misusers for cirrhosis development

- Germany: \(5.20\) [2.41, 11.22] \((p=2.7 \times 10^{-5})\)
- UK: \(4.88\) [1.42, 16.76] \((p=.012)\)
- Overall: \(5.11\) [2.66, 9.81] \((p=9.7 \times 10^{-7})\)

B) Adjusted OR in alcohol misusers for cirrhosis development

- Germany: \(7.44\) [2.95, 18.77] \((p=2.2 \times 10^{-5})\)
- UK: \(5.11\) [1.47, 17.81] \((p=.010)\)
- Overall: \(6.51\) [3.10, 13.70] \((p=7.8 \times 10^{-7})\)
### PiMZ in non-alcoholic fatty liver disease

- Biopsy-proven NAFLD cases

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total n=741</th>
<th>F0 n=374</th>
<th>F1-4 n=367</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PiMZ genotype (n, %)</td>
<td>40 (5.4)</td>
<td>9 (2.4)</td>
<td>31 (8.4)</td>
<td>.00031</td>
</tr>
<tr>
<td>PiZZ genotype (n, %)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>NA</td>
</tr>
<tr>
<td>PiMS genotype (n, %)</td>
<td>33 (4.7)</td>
<td>15 (4.0)</td>
<td>18 (5.6)</td>
<td>.337</td>
</tr>
<tr>
<td>PiSS genotype (n, %)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>NA</td>
</tr>
<tr>
<td>PiSZ genotype (n, %)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>NA</td>
</tr>
</tbody>
</table>
## PiMZ in non-alcoholic fatty liver disease

**Biopsy-proven NAFLD cases**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total n=741</th>
<th>F0 n=374</th>
<th>F1-4 n=367</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PiMZ genotype (n, %)</td>
<td>40 (5.4)</td>
<td>9 (2.4)</td>
<td>31 (8.4)</td>
<td>.00031</td>
</tr>
<tr>
<td>PiZZ genotype (n, %)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>NA</td>
</tr>
<tr>
<td>PiMS genotype (n, %)</td>
<td>33 (4.7)</td>
<td>15 (4.0)</td>
<td>18 (5.6)</td>
<td>.337</td>
</tr>
<tr>
<td>PiSS genotype (n, %)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>NA</td>
</tr>
<tr>
<td>PiSZ genotype (n, %)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>NA</td>
</tr>
</tbody>
</table>

### A) Unadjusted OR in NAFLD patients for fibrosis/cirrhosis development

- **Fibrosis (y/n)**  
  - OR: 3.71 [1.74, 7.90] (p=0.0007)

- **Cirrhosis (y/n)**  
  - OR: 2.84 [1.07, 7.57] (p=0.037)

### B) Adjusted OR in NAFLD patients for fibrosis/cirrhosis development

- **Fibrosis (y/n)**  
  - OR: 3.36 [1.48, 7.63] (p=0.004)

- **Cirrhosis (y/n)**  
  - OR: 4.91 [0.96, 24.99] (p=0.056)
Where do we stand?

- HBs-PiZ mice as a proteotoxic liver model
- Liver disease in PiZZ
- PiMZ as a disease modifier

There is still a lot of work to do!!!

EASL Registry Grant Kick-off meeting
Amsterdam, April 19th 2017, 6pm
pstrnad@ukaachen.de