The common adiponutrin (PNPLA3) p.I148M variant increases non-invasively measured hepatic steatosis quantified by controlled attenuation parameter and determines the fate of liver patients

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Introduction and Aim
The common variant p.I148M of the PNPLA3 gene, which encodes the enzyme adiponutrin, represents a genetic driver of severe hepatic phenotypes (Anstee et al Nat Rev Gastroenterol Hepatol 2013). These phenotypes fall into the spectrum of PNPLA3-associated steatosis/steatohepatitis (PASH, Figure 1). Here we investigate the association between the PNPLA3 variant and non-invasively quantified hepatic fat levels in patients with chronic liver diseases (CLDs).

![Figure 1: PNPLA3-associated steatosis/steatohepatitis (PASH) phenotypes associated with variant PNPLA3 (Krawczyk/Lammert, Semin Liver Dis 2013).](image1.jpg)

Patients and Methods
- Table 1 summarizes the characteristics of 234 patients with CLD who were referred to our center between 2010 and 2013 for genotyping of PNPLA3 as part of their diagnostic work-up.
- Liver steatosis was assessed non-invasively by transient elastography (FibroScan®, Echosens, Paris, France) using controlled attenuation parameter (CAP™) in a subgroup of 97 patients. The control group was represented by 279 patients who underwent a colonoscopy without abnormal findings. The PNPLA3 SNP rs738409G>C (p.I148M) was genotyped using a PCR-based assay with 5'-nuclease and fluorescence detection.
- Allele frequency differences were assessed by chi² test and genotype differences by Armitage’s trend test. Median liver steatosis values among carriers of the [II], [IM] and [MM] genotypes were compared by Kruskal-Wallis non-parametric analysis of variance (ANOVA) and Mann-Whitney U tests.

<table>
<thead>
<tr>
<th>PNPLA3 genotype frequencies</th>
<th>Cases</th>
<th>Controls</th>
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<tbody>
<tr>
<td><strong>[II]</strong></td>
<td>115 (49.1%)</td>
<td>164 (58.8%)</td>
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<tr>
<td><strong>[IM]</strong></td>
<td>88 (37.6%)</td>
<td>103 (36.9%)</td>
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<tr>
<td><strong>[MM]</strong></td>
<td>31 (13.3%)</td>
<td>12 (4.3%)</td>
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Table 1: Demographic and clinical characteristics of the study subjects and PNPLA3 genotype frequencies.

Results
- The cases displayed significantly (P=0.001) higher frequencies of the PNPLA3 risk allele [M] as compared to controls (Table 1).
- The de Finetti diagram (Figure 2) demonstrates a significant deviation from HWE towards the risk allele in patients (exact P=0.02), but not in healthy individuals (P>0.05).
- Overall, the PNPLA3 variant significantly increases the risk of presenting with severe liver phenotypes that eventually lead to referral and informed consent for genotyping (common OR=1.72, P=0.001; allele frequency difference OR=1.60, P=0.0008).

![Figure 2: De Finetti diagram for the PNPLA3 variant with HWE parabola.](image2.jpg)

Conclusions
This is the first study showing an association between hepatic steatosis quantified using CAP and the PNPLA3 variant across various liver diseases. Our results provide evidence that patients with the PNPLA3 risk variant develop more severe CLD. This supports the concept of a subgroup of liver patients who are at-risk due to PNPLA3-associated steatosis/steatohepatitis (PASH) with or without other concurrent liver diseases.