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).

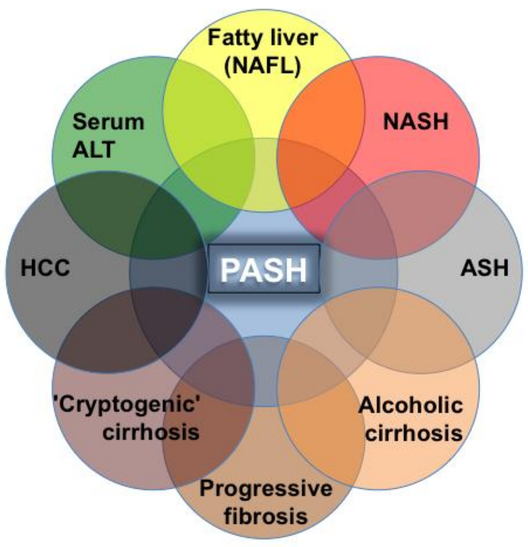

Figure 1: PNPLA3-associated steatosis/steatohepatitis (PASH) phenotypes associated with variant *PNPLA3 (Krawczyk/Lammert, Semin Liver Dis 2013).*

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 chi^2 test and genotype differences by Armitrage'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 1: Demographic and clinical characteristics of the study subjects and *PNPLA3* genotype frequencies.

	Cases	Controls
N	234	279
Men	133	114
Age (years)	51 (14-77)	72 (32-98)
Aetiology	223 non-viral[&] 11 HBV, HCV	Healthy controls
PNPLA3 genotype frequencies [II] [IM] [MM]	 115 (49.1%) 88 (37.6%) 31 (13.3%)	 164 (58.8%) 103 (36.9%) 12 (4.3%)

[&] AFL, AIH, ASH, BRIC, Budd-Chiari syndrome, cholestasis, FNH, haemochromatosis, idiopathic, NAFL, NASH, PBC, PSC

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).

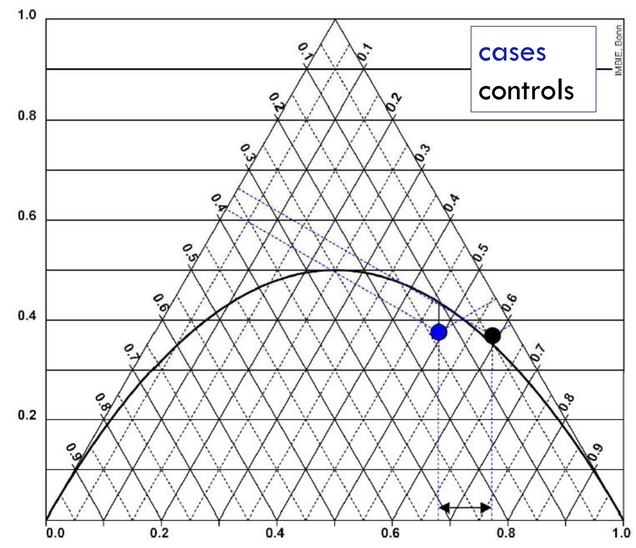

Figure 2: De Finetti diagram for the *PNPLA3* variant with HWE parabola.

- As presented in **Figure 3**, median CAP [dB/m] levels differed significantly (ANOVA P=0.003) between carriers of genotypes [II] (n=46), [IM] (n=40) and [MM] (n=11), and were 246.0, 277.0 and 320.0 (dB/m), respectively. Carriers of the prosteatotic [M] allele had higher (P=0.004) median CAP values as compared to individuals with genotype [II]. Overall, carriers of the prosteatotic [M] allele demonstrated higher (P=0.0043) median CAP levels as compared to the [II] individuals (297 dB/m *vs*. 246 dB/m).

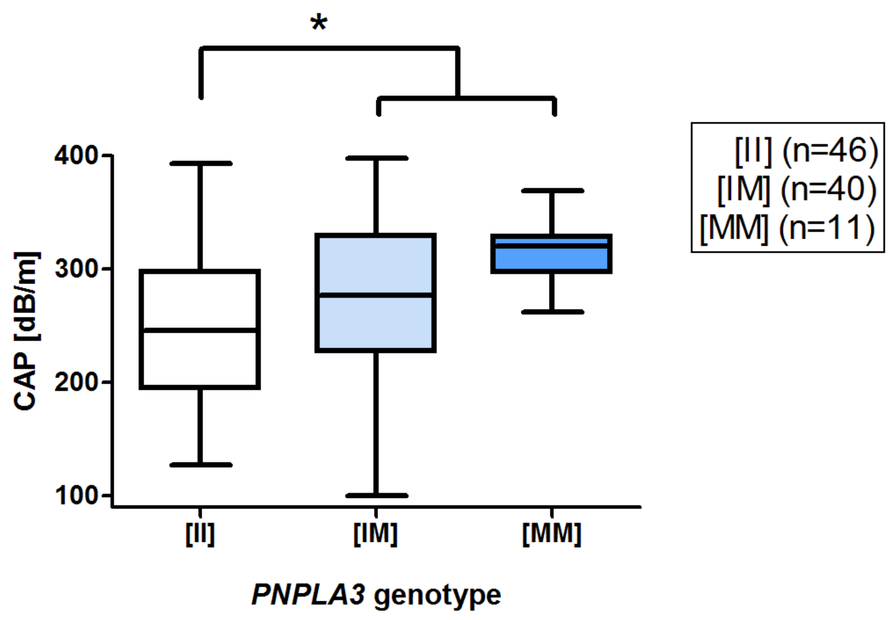

Figure 3: Box-and-Whisker plots illustrating liver steatosis (CAP [dB/m]) in carriers of distinct *PNPLA3* genotypes (P=0.004).

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.