

# Molecular genetics of patatin-like phospholipase domain-containing 3 and chronic liver disease

Carlo Pirazzi

Department of Molecular and Clinical Medicine, Institute of Medicine  
Sahlgrenska Academy at University of Gothenburg, Göteborg, Sweden

## ABSTRACT

Chronic liver disease is a major health burden worldwide. Major determinants of this condition are viral infections, alcohol abuse and obesity. Genetic background modulates the effect of damaging agents on the liver. The genetic variant rs738409 in the patatin-like phospholipase domain-containing 3 (PNPLA3) gene associates with increased susceptibility to the entire spectrum of chronic liver disease, and in particular with non-alcoholic fatty liver disease. The variant results in an isoleucine to methionine substitution at position 148 (I148M) of the amino acid sequence and was first associated with increased hepatocyte fat content. Despite the strength of the genetic association, the mechanisms causing liver fat accumulation and hepatocyte damage are not yet understood.

In this thesis, we tested the following hypotheses: 1) PNPLA3 is involved in hepatic very low density lipoprotein secretion 2) the protein acts as a glycerolipid hydrolase and the 148M mutation is a loss of function 3) PNPLA3 has a specific role in retinol metabolism in hepatic stellate cells. We tested the first hypothesis measuring VLDL secretion in a cohort of 55 individuals genotyped for the I148M variant and we found that carriers of the 148M allele secret less VLDL for a given amount of liver fat. We confirmed this result in vitro by measuring APOB secretion in cell lines stably overexpressing the 148I or the 148M PNPLA3. We tested the second hypothesis performing enzymatic activity assays using purified 148I and 148M recombinant proteins. The wild type protein had glycerolipid hydrolase activity and the 148M mutation induced a loss of function. Finally, we tested the third hypothesis assessing the effect of PNPLA3 up- and down-regulation on hepatic stellate cell retinyl palmitate content and retinol release. We found that PNPLA3 insulin-mediated up-regulation induces retinol release from hepatic stellate cells and that this effect is abolished by PNPLA3 silencing. We confirmed this finding by looking at human circulating levels of RBP4, a reliable marker of retinol plasma levels, in 146 individuals genotyped for the I148M variant. We found carriers of the M allele to have lower RBP4 plasma levels, confirming the role of PNPLA3 in retinol metabolism.

In conclusion, we identified two possible mechanisms underlying the susceptibility to chronic liver disease in carriers of the PNPLA3 mutation: 1) reduced intracellular triglyceride mobilization leading to hepatocyte damage 2) impaired hepatic stellate cell retinol metabolism causing abnormal response of hepatocytes to damaging agents.

**Keywords:** NAFLD, PNPLA3, VLDL, retinol, hepatic stellate cells

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av **Carlo Pirazzi**

**Fakultetsopponent: Professor Frank Lammert**

Department of Medicine II, Saarland University Hospital, Homburg, Germany

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- I. **C Pirazzi**, M Adiels, MA Burza, RM Mancina, M Levin, M Ståhlman, MR Taskinen, M Orho-Melander, J Perman, A Pujia, L Andersson, C Maglio, T Montalcini, O Wiklund, J Borén, S Romeo

**Patatin-like phospholipase domain-containing 3 (PNPLA3) I148M (rs738409) affects hepatic VLDL secretion in humans and in vitro**

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- II. P Pingitore\*, **C Pirazzi**\*, RM Mancina, BM Motta, C Indiveri, A Pujia, T Montalcini, K Hedfalk, S Romeo  
\*equal contribution

**Recombinant PNPLA3 protein shows triglyceride hydrolase activity and its I148M mutation results in loss of function**

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- III. **C Pirazzi**, L Valenti, BM Motta, P Pingitore, K Hedfalk, RM Mancina, MA Burza, C Indiveri, Y Ferro, T Montalcini, C Maglio, P Dongiovanni, S Fargion, R Rametta, A Pujia, L Andersson, S Ghosal, M Levin, O Wiklund, M Iacovino, J Borén, S Romeo

**PNPLA3 has retinyl-palmitate lipase activity in human hepatic stellate cells**

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