Nonalcoholic Fatty Liver Disease (NAFLD) represents one of the leading causes of cirrhosis and hepatocellular carcinoma (HCC) in the western countries. Despite this disease frequently represents the hepatic feature of the metabolic syndrome, to date the contribution of genetic background as a modifier of both its onset and progression is well-established. Next Generation Sequencing (NGS) technology to date allow the characterization of patients’ genetic background at the omic level. However, data are often difficult to interpret, at least in part, due to the large number of variants leading to lack of study power due to high multiplicity of testing problem.

AIM

- To identify novel candidate genes associated with NAFLD progression leveraging an integrative prioritization approach
- To validate such candidates leveraging large biobanked cohort and population databases
- To provide insights about the molecular mechanisms underlying the genotype to phenotype association

### METHODS

- **Variant prioritization**: Nonsynonymous/synonymous variants were selected using a bioinformatic pipeline. Functional annotation was performed using the professional website of MetaScape (www.metascape.org). Our validation cohort included 198 Italian patients were evaluated by WES (PERCEPTION cohort), control group was represented by 50 Italian blood donors and the non-Finnish (NEF) group from the gnomAD_v2.1 (N=56,885) and the ENCODE project (N=13,540) represented by 56,885 Italian donors from the 1000 Genomes project of which individual genetic data were available. Genotype to phenotype association studies was performed using the classic Mendelian model. Our discovery WES cohort (198 Italian patients) and the validation WES cohort included 484 Italian patients were evaluated by WES (PERCEPTION cohort), control group was represented by 50 Italian blood donors and the non-Finnish (NEF) cohort included 322 healthy blood donors without NAFLD. Genotyping by Probe Extension Assay was used for all SNPs.
- **Liver biopsy cohort**: Discovery cohort analysis was performed in: firstly, we selected 200 F3/F4 samples of 200 F3/F4 liver biopsy patients with a diagnosis of nonalcoholic steatohepatitis (NASH) and a sampling from the National Institute of Health (NIH) biobank cohort (N=404). Second, we selected 125 patients with a diagnosis of NASH from the University of Gothenburg biobank cohort (N=125).
- **Transcriptomic analysis**: Genotyping of variants was performed at the DNA level and rare variants were selected using genetic association analysis. The liver Transcriptomic cohort (N=125) were used to refine the expression profile of ATG7 and the genes involved in autophagy and lipid droplet remodeling.
- **Immunohistochemistry**: A total of 195 liver samples were analyzed in the different cohort (Discovery cohort, Validation cohort, and Biobank cohort) using a clinical liver biopsy diagnostic panel, including CD68, CD3, CD8, CD4, CD163, CD20 and GLUT 1 as a positive control. Slides were evaluated by an expert pathologist. Immunohistochemistry was performed using standard procedures at the Department of Pathology.

### RESULTS

Our integrative analytical approach to WES data allowed us to link the rare variant p.V471A to the common ATG7 variants predisposing to severe fatty liver disease.

- **Pathway analysis of WES data**: We performed an integrative approach to WES data. Our integrative analytical approach to WES data allowed us to link the rare variant p.V471A to the common ATG7 variants predisposing to severe fatty liver disease.

- **Association analysis**: Highlighted the association of the common ATG7 p.V471A variant with NAFLD at the population level. In the Liver Biopsy Cohort we observed a strong association between the p.V471A genotype and grade of Hepatocellular Ballooning. Association with fibrosis was observable only in patients with severe steatosis.

- ** concession analysis**: The results from the association analysis of the p.V471A variant with NAFLD at the population level in the Liver Biopsy Cohort also showed a strong association between the p.V471A genotype and grade of Hepatocellular Ballooning. Association with fibrosis was observable only in patients with severe steatosis.

- **Functional validation experiments**: ATG7 role in lipophagy was noticed in steatotic hepatocytes in the whole cohort but we detected a significant association in patients with severe steatosis and in those carrying the p.V471A variant.

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