FONDAZIONE IRCCS CA' GRANDA
OSPEDALE MAGGIORE POLICLINICO
DIPARTIMENTO DI FISIOPATOLOGIA E DEI TRAPIANTI
UNIVERSITA' DEGLI STUDI DI MILANO
U.O. MEDICINA INTERNA AD INDIRIZZO METABOLICO

Study for the Evaluation of Risk of hEpatocellular carcinoma in NonAlcoholic fatty liver

A multicenter prospective cohort study for the evaluation of the interaction between congenital and acquired risk factors in the pathogenesis of hepatocellular carcinoma in patients with nonalcoholic fatty liver and disease risk stratification.

Principal investigator: Luca Valenti **Co-Investigator:** Serena Pelusi
Other investigators: Silvia Fargion, coordinators of participating centers, Guido Baselli, Anna Fracanzani.

Background: Nonalcoholic fatty liver disease (NAFLD) is projected to become the leading cause of hepatocellular carcinoma (HCC) in Western countries within 2025 [1-4]. NAFLD-HCC frequently develops without overt cirrhosis suggesting that steatosis directly promotes hepatic carcinogenesis [5-8]. However, progression of liver disease to cirrhosis and HCC is more frequent in the subgroup of patients who develop non-alcoholic steatohepatitis (NASH) [9]. Established risk factors include older age, obesity, severe insulin resistance - type 2 diabetes (T2D), hypertension, non-alcoholic steatohepatitis (NASH) [9]. Due to the very high prevalence of NAFLD, the occurrence of HCC in patients without advanced fibrosis, and lack of disease awareness, classic screening strategies for the detection early HCC are ineffective [4]. As a consequence, most of patients who developed NAFLD-HCC are diagnosed at an advanced stage, when curative treatments are not anymore possible [10,11]. This renders the development of new noninvasive biomarkers able to stratify the risk of HCC development in NAFLD patients a public health priority. Genetic factors have been shown to influence disease progression in NAFLD, and family history remains the main risk factor for HCC development [9,12]. The common genetic polymorphism rs738409 C>G encoding for the I148M variant in PNPLA3 is the main common genetic determinant of hepatic fat content and of progressive NAFLD [13-17], and NAFLD-HCC [18], but it has a low sensitivity to be used as single prognostic biomarker [19]. The rs58542926 E167K variant in TM6SF2 [20-22], and the rs641738 C>T variant in MBOAT7 may also be involved [23-25]. Moreover, rare mutations inducing Mendelian diseases due to severe derangements in the function of encoded proteins may predispose to NAFLD-HCC.

Aims: The overall aim is to quantify the impact of genetic risk factors on the risk of NAFLD-HCC, as compared to other major outcomes, and their interaction with acquired triggers on disease in a prospective cohort of at risk patients.
Specific aims will be:

1. Validation of the inclusion criteria as able to identify the individuals at risk of NAFLD-HCC among NAFLD patients in follow-up.
2. Identification of the impact of single genetic variants on HCC risk, and development of a score to predict NAFLD-HCC and select patients for whom the screening may be cost-effective.
3. The study cohort will allow the detailed characterization of the natural history of advanced liver disease related to NAFLD (including patients with moderate alcohol intake) in a multicenter Italian cohort, with complete clinical, metabolic and most importantly genetic characterization, and the identification of inherited risk factors for progression to HCC and other major outcomes (in particular hepatic decompensation, cardiovascular events, cancer, and overall mortality).

Study design: To maximize the study power, we will select different categories of NAFLD patients > 45 years old at higher risk of HCC because of already advanced liver disease (Fibrosis stage F3-F4), family history and/or confirmation of rare genetic mutations with a strong impact on HCC, or with strong acquired risk factors (type 2 diabetes or obesity) carrying at least three common genetic risk variants for the disease. We plan to enroll 500 patients with advanced

NAFLD, who will be followed for 5 years, to have a >80% power to detect a significant association of the genetic score with HCC risk in the first study phase, accounting also for the occurrence of other competitive major clinical outcomes. Candidate common risk variants will be determined and their impact on clinical outcomes analyzed in a first study phase. Next, we will also consider a panel of rare variants, which are being identified as associated with NAFLD-HCC in ongoing studies (second stage by targeted resequencing of candidate genes). Finally, we will request funding to proceed to a discovery phase of new risk variants (third stage: whole exome sequencing).

Participating centers:

1. Internal Medicine and Metabolic Diseases, Policlinico, Milano; coordinating center (L Valenti): enrolling (n=35)
2. Gastroenterology and Hepatology, Policlinico, Milano (P Lampertico): enrolling (n=2)
3. Hepatology, Rozzano (A Aghemo): obtained ethical committee approval
4. Internal Medicine, Udine (G Soardo): requesting ethical committee approval
5. Internal Medicine, Roma (L Miele): requesting ethical committee approval
6. Gastroenterology, Ancona (G Svegliati-Baroni): requesting ethical committee approval
7. Internal Medicine, Bologna (F Piscaglia): requesting ethical committee approval
8. Gastroenterology, Torino (E Bugianesi): agreed
9. Gastroenterology, Palermo (S Petta): agreed

Others centers are being contacted to join the study group, and endorsement is being requested from the Italian Association for the Study of Liver Disease (AISF).

REFERENCES

1. Bugianesi E, Leone N, Vanni E, et al. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology.* 2002;123(1):134-140.
2. Baffy G, Brunt EM, Caldwell SH. Hepatocellular carcinoma in non-alcoholic fatty liver disease: An emerging menace. *J Hepatol.* 2012;56(6):1384-1391.
3. Dyson J, Jaques B, Chattopadyhay D, et al. Hepatocellular cancer: the impact of obesity, type 2 diabetes and a multidisciplinary team. *Journal of hepatology.* 2014;60(1):110-117.
4. Dongiovanni P, Romeo S, Valenti L. Hepatocellular carcinoma in nonalcoholic fatty liver: role of environmental and genetic factors. *World J Gastroenterol.* 2014;20(36):12945-12955.
5. Torres DM, Harrison SA. Nonalcoholic steatohepatitis and noncirrhotic hepatocellular carcinoma: fertile soil. *Seminars in liver disease.* 2012;32(1):30-38.
6. Kawada N, Imanaka K, Kawaguchi T, et al. Hepatocellular carcinoma arising from non-cirrhotic nonalcoholic steatohepatitis. *J Gastroenterol.* 2009;44(12):1190-1194.
7. Ertle J, Dechene A, Sowa JP, et al. Non-alcoholic fatty liver disease progresses to hepatocellular carcinoma in the absence of apparent cirrhosis. *International journal of cancer Journal international du cancer.* 2011;128(10):2436-2443.
8. Chagas AL, Kikuchi LO, Oliveira CP, et al. Does hepatocellular carcinoma in non-alcoholic steatohepatitis exist in cirrhotic and non-cirrhotic patients? *Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas / Sociedade Brasileira de Biofisica [et al].* 2009;42(10):958-962.
9. Dongiovanni P, Valenti L. Genetics of nonalcoholic fatty liver disease. *Metabolism: clinical and experimental.* 2015.
10. Piscaglia F, Svegliati-Baroni G, Barchetti A, et al. Clinical patterns of hepatocellular carcinoma in nonalcoholic fatty liver disease: A multicenter prospective study. *Hepatology.* 2016;63(3):827-838.
11. Younossi ZM, Otgonsuren M, Henry L, et al. Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009. *Hepatology.* 2015;62(6):1723-1730.
12. Turati F, Edefonti V, Talamini R, et al. Family history of liver cancer and hepatocellular carcinoma. *Hepatology.* 2012;55(5):1416-1425.
13. Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet.* 2008;40:1461-1465.
14. Valenti L, Al-Serri A, Daly AK, et al. Homozygosity for the PNPLA3 / adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology.* 2010;51:1209-1217.
15. Sookoian S, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. *Hepatology.* 2011;53(6):1883-1894.
16. Dongiovanni P, Donati B, Fares R, et al. PNPLA3 I148M polymorphism and progressive liver disease. *World J Gastroenterol.* 2013;19(41):6969-6978.
17. Yuan X, Waterworth D, Perry JR, et al. Population-based genome-wide association studies reveal six loci influencing plasma levels of liver enzymes. *Am J Hum Genet.* 2008;83(4):520-528.
18. Liu YL, Patman GL, Leathart JB, et al. Carriage of the PNPLA3 rs738409 C >G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. *J Hepatol.* 2013;61(1):75-81.
19. Anstee QM, Liu YL, Day CP, Reeves HL. Reply to: HCC and liver disease risk in homozygous PNPLA3 p.I148M carriers approach monogenic inheritance. *J Hepatol.* 2015;62(4):982-983.
20. Kozlitina J, Smagris E, Stender S, et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet.* 2014;46(4):352-356.
21. Liu YL, Reeves HL, Burt AD, et al. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. *Nature communications.* 2014;5:4309.
22. Dongiovanni P, Petta S, Maglio C, et al. Transmembrane 6 superfamily member 2 gene variant disentangles nonalcoholic steatohepatitis from cardiovascular disease. *Hepatology.* 2015;61(2):506-514.
23. Buch S, Stickel F, Trepo E. A genome-wide association study confirms PNPLA3 and identifies TM6SF2 and MBOAT7 as risk loci for alcohol-related cirrhosis. 2015;47(12):1443-1448.
24. Mancina RM, Dongiovanni P, Petta S, et al. The MBOAT7-TMC4 Variant rs641738 Increases Risk of Nonalcoholic Fatty Liver Disease in Individuals of European Descent. *Gastroenterology.* 2016;150(5):1219-1230 e1216.
25. Donati B, Dongiovanni P, Romeo S, et al. MBOAT7 rs641738 variant and hepatocellular carcinoma in non-cirrhotic individuals *Sci Rep.* 2017:in press.