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GENETIC INSIGHT INTO COVID-19 RELATED LIVER INJURY

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Metabolic dysfunction and fatty liver disease (FLD) are epidemiologically associated with increased risk of severe COVID-19 requiring hospitalization (1, 2). The mechanism may encompass promotion of inflammation by facilitation of liver injury, which is a frequent feature of severe COVID-19 (3). However, metabolic dysfunction rather than FLD itself may induce disease progression (2). Indeed, we previously showed that a genetic FLD-risk score does not predispose to severe COVID-19 in UK Biobank population-based cohort (UKBB) (4).

In a recent study, Zhou *et al*. reported that in case-control study in hospitalized Asian patients younger than 60 years that metabolic dysfunction-associated FLD (MAFLD), was associated with a four-fold higher probability of severe disease (5). Importantly, Authors adjusted the association for confounders matching the controls for age, sex, and adiposity (5). Therefore, data may suggest that hepatic fat, inflammation and fibrosis, are involved in mediating the effect of FLD on COVID-19 outcomes in hospitalized patients (3). However, the severity of insulin resistance and gut-liver axis alterations may alternatively account for the epidemiological association. Furthermore, increased liver fat may be a consequence rather than the cause of severe COVID-19 due to the cytokine storm, procoagulant status, hypoxia, drug-induced liver injury, and direct liver infection (3).

To gain insight into the relationship among FLD, liver damage and COVID-19, here we exploited robust genetic predictors of hepatic fat and fibrosis, namely the *PNPLA3* I148M variant and polygenic risk score of hepatic fat content (PRS-HFC), which are inherited independently of dysmetabolism at conception (6). These were assessed in a case-control cohort of European unrelated individuals (Fondazione Ca' Granda Milan Genomic Study, FOGS), a subset of genomewide association study identifying the first main genetic predictors of severe COVID-19, whose baseline features are shown in Table 1 (7). We also examined variants at the chromosome 3 gene cluster (C3), *ABO* blood locus and *FUT2* encoding the ABO non-secretor phenotype, as proxies for the direct impact of COVID-19 severity on liver damage in hospitalized patients (7, 8).

Results are shown in Table 2. We first confirmed that C3 and *ABO* variation are associated with COVID-19 risk (1, 7). C3 variation was also associated with more severe outcome in hospitalized patients, while *ABO* was not (1, 7). ABO non-secretor phenotype was not associated with COVID-19, but protected against severe outcomes (8). In line with previous evidence in UKBB, genetic predisposition to FLD did neither increase the risk of COVID-19 nor of severe outcome (4). On the contrary, if ever, as in UKBB higher PRS-HFC tended to be protective (*P*=NS; Table 2, upper panel).

Indeed, despite during COVID-19 genetic predisposition to FLD tended to be associated with higher ALT (Table 2, bottom panel), it concomitantly resulted in an attenuation of systemic inflammation (CRP levels), which was paralleled by a relative preservation of hepatic synthesis (circulating albumin) in *PNPLA3* I148M variant carriers. On the other hand, C3 variation predisposed to severe COVID-19 independently of any effect on CRP levels and on liver injury. Finally, ABO non-secretor phenotype was

associated with increased liver enzymes (AST and possibly ALT levels). It remains to be determined whether alterations of membrane glycans shedding underpin differential tissue susceptibility to SARS-CoV-2 infection (e.g. lung vs. liver), thereby accounting for these findings. Aminotransferases were independently associated with male sex and younger age, but not with obesity/hypertension, while diabetes with lower AST (not shown).

In conclusion, we found that a genetics-based instrument, a robust unconfounded lifelong proxy of predisposition to and progression of FLD, did not increase the risk of severe COVID-19 in hospitalized patients. Therefore, FLD predisposition does not invariably result in increased inflammation, but despite facilitating liver injury genetic predisposition to accumulate liver fat may paradoxically protect during COVID-19. In addition, genetic predisposition to the development and progression of COVID-19 is not mediated through increased liver injury. Additional studies are required to confirm these findings.

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Competing interest statement:

Authors declare that they do not have any conflict of interest relevant to the present study.

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TABLES

Table 1. Clinical features of the Fondazione IRCCS Ca' Granda cohort of 508 cases with severe COVID-19 with respiratory failure and 889 healthy controls (blood donors) with available genetic data. All were unrelated individuals of European ancestry included in the previous GWAS study (7). Biochemical data were available for 360 and 646 individuals, respectively.

	COVID-19	**Healthy controls**	***P*-value**
Age, years	64.5±15.1	41.2±13.2	<0.0001
Sex, M	346 (68.0)	585 (65.7)	0.42
Smoking, yes	59 (11.6)	224 (25.2)	<0.0001
Hypertension, yes	167 (32.8)	65 (7.3)	<0.0001
Type 2 diabetes, yes	63 (12.4)	0	<0.0001
Severe outcome ^	137 (26.9)	NA	-
Hb, g/dl	12.5±1.9	14.4±1.2	<0.0001
NLR, ratio	7.1±7.5	1.9±0.7	<0.0001
Platelets, 10^3/mm^3	259±124	232±49	<0.0001
creatinine	1.1±0.8	0.9±0.2	<0.0001
CRP, mg/dl	9.8±8.1	NA	-
Bilirubin, mg/dl	1.1±5.3	NA	-
Albumin, g/l	3.4±0.5	NA	-
Ferritin, ng/ml	984 (475-1758)	74 (37-138)	<0.0001
ALT, IU/l	58 (37-86)	22 (17-30)	<0.0001
AST, IU/l	40 (24-67)	21 (17-25)	<0.0001
GGT, IU/l	42 (21-82)	14 (10-21)	<0.0001
HSI, score	40.4±9.9	NA	-
FIB-4, score	2.0 (1.3-2.8)	0.7 (0.5-1.0)	<0.0001
***PNPLA3* I148M, distribution**	262/208/38 (51.6/40.9/7.5)	448/357/84 (50.4/40.2/9.4)	0.46
PRS-HFC, score	0.3±0.2	0.3±0.2	0.20
rs58542926 C>T at chr3 cluster, distribution	366/137/5 (72.0/27.0/1.0)	734/147/8 (82.6/16.5/0.9)	<0.0001
rs657152 C>A at *ABO*, distribution	172/243/93 (33.8/47.9/18.3)	367/420/102 (41.2/47.3/11.5)	0.0004

rs601338 G>A at *FUT2*, distribution	153/254/101 (30.1/50.3/19.6)	268/447/174 (30.0/50.1/19.9)	0.99

Data are shown as mean±SD, median (IQR), n (%) values as required. NA: not available, NLR: neutrophils/lymphocytes ratio, CRP: C reactive protein, PRS-HFC: polygenic risk score of hepatic fat content, HSI: hepatic steatosis index, chr: chromosome. ^ Defined as need for mechanical ventilation and/or in-hospital mortality. Data were compared by generalized linear models.

Table 2. Impact of genetic predisposition to FLD and COVID-19 on the risk of severe COVID-19 with respiratory failure, clinical outcomes and liver damage in the Fondazione Ca' Granda Genomic Study cohort (FOGS).

	PNPLA3 I148M		PRS-HFC		rs58542926 C>T at chr3		rs657152 C>A *ABO*		rs601338 G>A *FUT2*	
	OR, 95% CI	***P*-value**	**OR, 95% CI**	***P*-value**	**OR, 95% CI**	***P*-value**	**OR, 95% CI**	***P*-value**	**OR, 95% CI**	***P*-value**
COVID-19°	0.87, 0.70-1.09	0.23	0.58, 0.30-1.18	0.08	1.88, 1.36-2.60	0.0001	1.29, 1.04-1.59	0.019	1.00, 0.81-1.22	0.98
Severe outcome^	0.88, 0.70-1-10	0.27	0.57, 0.28-1.18	0.13	1.90, 1.37-2.63	0.0001	0.62, 0.30-1.31	0.22	0.73, 0.55-0.96	0.026
	Estimate, SE	***P*-value**	**Estimate, SE**	***P*-value**	**Estimate, SE**	***P*-value**	**Estimate, SE**	***P*-value**	**Estimate, SE**	***P*-value**
CRP, log mg/dl	-0.28, 0.13	0.006	-1.00, 0.31	0.001	0.13, 0.13	0.13	0.01, 0.08	0.88	0.01, 0.09	0.99
AST, log IU/l	0.06, 0.06	0.29	0.11, 0.18	0.57	0.04, 0.08	0.59	0.02, 0.05	0.72	0.13, 0.05	0.012
ALT, log IU/l	0.14, 0.07	0.050	0.36, 0.21	0.09	-0.02, 0.09	0.82	-0.06, 0.06	0.33	0.11, 0.05	0.05
GGT, log IU/l	0.04, 0.09	0.64	0.08, 0.30	0.78	0.10, 0.12	0.41	-0.012, 0.08	0.13	0.06, 0.08	0.48
Albumin, g/l	0.08, 0.05	0.042	0.18, 0.15	0.20	-0.08, 0.06	0.18	-0.05, 0.04	0.20	0.07, 0.04	0.10
Bilirubin, mg/dl	0.87, 0.48	0.07	0.29, 0.50	0.40	-0.06, 0.62	0.92	0.15, 0.41	0.70	-0.70, 0.43	0.10
HSI, score	0.00, 1.43	0.99	2.00, 4.58	0.66	-0.65, 1.76	0.71	-2.91, 1.28	0.025	0.24, 1.19	0.83
FIB-4, score	0.27, 0.20	0.17	0.96, 0.62	0.12	0.03, 0.26	0.89	-0.12, 0.18	0.52	0.07, 0.17	0.69

At multivariate generalized linear models adjusted for age, sex, presence of arterial hypertension, smoking. Logistic models were fitted for clinical outcomes, and the impact of genetic variants examined under additive models. Non-normally distributed variables were log-transformed before entering the models. ° Risk of hospitalization due to severe COVID-19 with respiratory failure. ^ Defined as need for mechanical ventilation and/or in-hospital mortality. Chr: chromosome, CRP: C reactive protein, PRS-HFC: polygenic risk score of hepatic fat content, HSI: hepatic steatosis index.