Department of Services and Preventive
MedicineUOCTransfusion Centre–

"GENOMIC COHORT STUDY OF THE LOMBARD HEPATIC BIOBANK: PERSONALIZED MEDICINE FOR THE MANAGEMENT OF COMP LICENSES OF HEPATIC AND CARDIOVASCULAR LICENSES IN HEPATIC STATEHUS NON-ALCOHOLIC"

(TheLiverBIoBankLombardigenomiccohortstudy(LIVER-BIBLE):personalizedmedicineforthemanagementofhepaticaand cardiovasculartromboticcomplicationsof fattyliver).

Acronym:LIVER-BIBLE

Version 3.0 of 13 July 2022

Promoter:IRCCS Ca' Granda Hospital Foundation
MaggiorePoliclinico,ViaSforza28,20122Milan,Italy

Coordinator centre: UOCTransfusion Centre
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Principal Investigator:Dr. Luca Valenti

Signature

FLOWCHART

BASIC ASSESSMENT				M AND S THE		
	0 - 6	7-12	1-6pm	19-24	25-54	55-126
Screening inclusion and exclusion criteria	- -	- -	-	-	-	
Explanation of the study to the patient and signing of the informed consent	- -	- -	-	-	-	
Clinical evaluation with collection of medical history and parameters and graphs	- -	- -	-	-	-	
Objective exam	- -	- -	-	-	-	
Collection of data on eating habits and lifestyle	- -	- -	-	-	-	
Ongoing pharmacological therapies	- -	- -	-	-	-	
Examine them (CBC, blood sugar, insulin, glycated hemoglobin, total and fractionated cholesterol, triglycerides, albumin, creatinine, transaminase, ferritin, creatine protein, microalbuminuria spot)	- -	- -	-	-	-	
Pergenetic blood sampling	- -	- -	-	-	-	
Blood sampling for studying serum and coagulation biomarkers (interleukin 32, IL-32;VWF:Ag,FVIII/PCratio,ADAMTS:Ag)	- -	- -	-	-	-	
Vibration-ControlledTransientElastography(VCTE)Fibroscan-Hepatic Stiffness Evaluation, CAP	- -	- -	- -	- -	-	-
Cardiovascular risk assessment (echo color dopper of supra-aortic trunks)	- -	- -	- -	- -	- -	- -

FOLLOW UP			M AND S THE			
	0-12	1-6pm	19-24	25-54	55-126	27-150
Update of the patient's status. Collection of data			- -	- -	-	-
on eating habits and lifestyle. Cognitive status			- -	- -	-	
questionnaire			- -	- -	- -	
Examine them			- -	- -	-	-
(CBC, blood sugar, insulin, glycated hemoglobin, total and fractionated cholesterol, triglycerides, albumin,						

creatinine, transaminase, ferritin, creatine protein, spot microalbumin, C peptide, fecal occult blood)

Vibration-ControlledTransientElastography(VCTE) Fibroscan		-	- -	- -	-	-
Characterization of the intestinal microbiome in feces		- -	- -	- -	-	-
Genetic characterization		- -	- -	- -	-	-
Genomic study (whole exome sequencing, WES)			- -	- -	- -	-
Validation of new genetic variants			- -	- -	- -	- -
Development of a genetic risk score			-	- -	- -	- -
WES analysis			-	-	-	- -
Generation of in vitro models of NAFLD	- -	- -	- -	- -	-	-

DIAGNOSISAFLD	M AND S THE					
	0-12	1-6pm	19-24	25-54	55-126	127-150
Abdomen ultrasound where clinically indicated	-	- -	- -	- -	- -	-
Hepatic histology characterization where clinically indicated	-	- -	- -	- -	- -	-
Cardiological evaluation where clinically indicated (hypertension, diabetes mellitus)	-	- -	- -	- -	- -	-
Lipidomic and metabolomic study	-	-	-	- -	- -	- -
Liver fat assessment by MRI where available in subjects with liver biopsy	- -	-	- -	-	-	-

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LIST OF ABBREVIATIONS

THERE IS:Ethics Committee

THERE:Informed Consent

CRF:Case Report Form, collection form

dataGCP:Good Clinical Practice, good practice

clinicNAFLD:nonalcoholic fatty liver disease

WES:wholeexomesequencing

VCTE:vibration-controlledTransientElastography

GRS:genetic risk

scoreHFCS:hepatic fat

contentBMI:

bodymassindex

T2D:type 2 diabetes mellitusLSM:

liverStiffnessmeasu rementIMT:

intimate-

mediathickness

RESPONSIBILITY (role of the promoter and collaborators)

Operational unit	Participant Name	Role and functions in the practice
Department of Medicine Transfusion ediHematologia, FondazioneIRCCSCa'Granda OspedaleMaggiorePoliclinico	Dr. Daniele Prati Prof. Luca Valenti (principal investigator)	Coordination of the study Recruitment and characterization of patients Data analysis

Internal collaborations

Operational unit	Participant Name	Role and functions in the practice
UOC General Medicine – Haemostasis Thrombosis FondazioneIRCCSCa'GrandaOspedaleMaggiorePoliclinic o	Prof. FloraPeyvand the	Characterization through laboratory investigations of role from the coagulation in complications of NAFLD

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UOCGastroenterologyandHepatologyFondazione IRCCS Ca' GrandaOspedale Maggiore Policlinico	Prof. Pietro Lampertico	Characterization clinic of patients with NAFLD (ultrasound abdominal ebiopsy hepatic oveclinically indicated)

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External body that will take care of the analysis of the biological sample

Institution	Operational unit	Participant Name	Role and functions in the practice
CIC bioGUNE	Precision Medicine & Metabolism	Prof. José M. Mato	lipidomics and metabolomics study on serum samples from the entire cohort
Columbia University	Department of Medicine	Prof. Ira A. Tabas Prof. Utpal Pajvani	Immunohistochemical and biomarker analyzes on liver histological specimens
University of Helsinki	Institute for MolecularMe say Finland FIMM Technology Center	Dr. PäiviLahermo	Genotyping and genetic analysis

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- Approval of the Ethics Committee

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- Closing of the studio-
- Document archiving-
- Dissemination of information regarding scientific discovery-
- Confidentiality
- Publications

- Intellectual property rights in the study results
- Indemnity and compensation in the event of
- damages Financial agreements
- Disclosure on conflicts of interest
- References

-INTRODUCTION

-Backgrounderational

Non-alcoholic fatty liver disease (NAFLD) is causally involved in the pathogenesis of the metabolic alterations typical of the "metabolic syndrome".

The harmful effect of NAFLD is not limited to liver disease. Patients with NAFLD in fact show an increased risk of mortality in general, cardiovascular events, kidney disease and some specific tumors (1,2).

Despite recent advances, accurate non-invasive biomarkers and effective therapies to diagnose and cure progressive NAFLD are not yet available. However, inherited factors play an important role in determining predisposition to the disease. In recent years, common genetic variants in PNPLA3 (major risk variant), TM6SF2, MBOAT7 and GCKR have been demonstrated to predispose to the full spectrum of NAFLD (3).

Preliminary data from our group have shown how a new genetic risk score that predicts liver fat content (HFC-GRS) can improve risk stratification of liver disease progression in NAFLD and how new common and rare genetic variants contribute to susceptibility to NAFLD. . The same data also highlighted a possible new non-invasive circulating biomarker associated with liver fat and lipotoxicity (interleukin-32).

It is therefore hypothesized that the evaluation of complete genetic risk scores (GRS) can be used to stratify the risk of liver-related complications and to select the best pharmacological therapy.

Technological advances that enable the interrogation of the entire human genome, combined with the exploitation of bioinformatics systems approaches, are proving promising for providing patients and clinicians with unique health information from the molecular, cellular to multi-organ levels.

By taking advantage of these new technologies, our study aims to allow a "personalised" or "precision" approach to medicine, based on the contemporary evaluation of genetic and non-invasive biomarkers, with the aim of improving risk stratification in the field of metabolic diseases, to implement prevention adapted to individual characteristics and start treating the causes instead the symptoms of the disease, thus making the treatment of chronic diseases more cost-effective (4).

-OBJECTIVE/HYPOTHESIS OF THE EXPERIMENTATION

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The main objective of the study is to improve the risk stratification of hepatic and cardiovascular complications in the context of non-alcoholic fatty liver disease (NAFLD), using an approach based on precision medicine.

-Primary objective -

The primary objective will be to examine whether a genetic risk score that takes into account genetic factors associated with predisposition to NAFLD combined with the evaluation of environmental triggers, imaging techniques and new circulating biomarkers can help stratify the risk of NAFLD and associated complications in a cohort of asymptomatic high-risk individuals from the general population.

We will consider blood donors aged between 40 and 65 years in the presence of overweight or obesity (body mass index-BMI > 25 kg / m_2), associated with at least two of the following characteristics: increased fasting blood glucose or T2D (fasting blood glucose ≥100mg/dl) or dyslipidemia (triglycerides≥150mg/dl, HDL <45/55 in M/F) or arterial hypertension (n=2,452 , 11.8% of the entire cohort).

This task will include the characterization of clinical risk factors, lifestyle and metabolic factors, quantification ofof liver fat and assessment of the presence of liver damage (by measurement of liver stiffness, LSM) and collection of biological samples.

Patients with increased liver enzymes (AST/ALT > 42/30 in M/F) or elevated LSM on Fibroscan (LSM ≥7.9 kPa) (5), possibly indicative of the presence of significant liver fibrosis, will also undergo:

a) Upper abdominal ultrasound, as clinically indicated

b) histological evaluation of liver damage (as clinically indicated) to confirm the stage of liver disease, and exclude concomitant liver disease and offer possible inclusion in clinical studies for the identification of new pharmacological therapies.

As regards the evaluation of cardiovascular risk, the intima-media thickness (IMT) of the carotid-communal arteries there presence of carotid plaques will be evaluated in all individuals using color Doppler ultrasound as clinically indicated. It will come Furthermore, the possible initial impact of the cardiometabolic alterations on the cognitive-neurological state of the participants was assessed by the experimenter doctor via MMSE (mini-mental state examination) questionnaire. These instrumental tests will be carried out if required by clinical practice for the clinical situation of the patient.

- Secondary objective(s).

Further aims will be to validate the causal role of HFC in the alterations of metabolism, coagulation, early of cardiovascular damage associated with NAFLD and to study the mechanism that links genetic susceptibility to liver disease and coagulation balance in in vitro models of NAFLD in hepatocytes, sinusoidal endothelial cells and hepatic stellate cells.

We expect to demonstrate that the combined evaluation of new genetic and circulating biomarkers with imaging can improve the non-invasive prediction of both liver-related damage (leading to personalized surveillance of liver disease complications) and non-hepatic complications of NAFLD, with particular attention to the role of coagulation balance, and help identify new genetic modifiers of disease progression that could be priorities for future therapeutic approaches.

-STUDYDESIGN-

-Study design -

Interventional, with the collection of biological, non-pharmacological, monocentric, prospective material.

-Inclusion criteria -

We will take into consideration, after signing informed consent, blood donors aged between 40 and 65 years in the presence of overweight or obesity (body mass index-BMI > 25 kg / m_2), associated with at least two of the following characteristics: increased fasting blood sugar or T2D (fasting blood sugar ≥100 mg/dl) or dyslipidemia (triglycerides ≥150 mg/dl, HDL <45/55 in M/F) or arterial hypertension (n = 2,452, 11.8% of the entire cohort).

- Exclusion criteria -

Subjects suffering from chronic degenerative diseases will be excluded, except for well-compensated hypertension and type 2 diabetes mellitus which does not require pharmacological therapy (as is already common practice for eligibility for blood donation) and donors aged >65 and <40 to avoid the introduction of bias.

-PROCEDURES RELATING TO THE STUDY-

-Intervention -

1) Selection of blood donors at risk of NAFLD and metabolic diseases and collection of the blood sample

As previously described, we will consider blood donors aged between 40 and 65 years in the presence of overweight or obesity (body mass index-BMI > 25 kg / m_2), associated with at least two of the following characteristics: increased fasting blood glucose or T2D (fasting blood glucose ≥100mg/dl) or dyslipidemia (triglycerides≥150mg/dl, HDL<45/55 inM/F) or arterial hypertension (n= 2,452, 11.8% of the entire cohort).

At the time of enrollment, data will be collected on the characterization of clinical risk factors, lifestyle and metabolic factors, liver fat, liver damage (through Fibroscan measurement of liver stiffness, liver stiffness measurement (LSM) and controlled attenuation parameter (CAP)), as indicated by clinical practice in these subjects at higher metabolic risk and therefore dysteatosis. This information will be collected in the blood donor subject's management system. In this first visit, a blood sample will be carried out (13 ml in total, 2 5 ml tubes and one 3 ml tube) and a urine sample will be collected for the performance of biochemical tests (as already foreseen in the clinical practice of blood donor subjects high risk of developing metabolic and cardiovascular diseases): blood count, blood sugar, insulin, glycated hemoglobin, total and fractional cholesterol, triglycerides, albumin, creatinine, transaminase, ferritin, C-reactive protein, fibrinogen, prothrombin time, spot microalbuminuria.

On this occasion, three additional aliquots of blood will also be taken (15 ml total, 3 5 ml tubes) which

they will be used respectively for DNA extraction, the evaluation of serum biomarkers and the coagulation profile (see point 3: Genomics and biomarker study).

Follow up:

The medical history with any clinical events that have occurred and the biochemical tests (blood count, blood sugar, total and fractionated cholesterol, triglycerides, albumin, creatinine, transaminase, ferritin, normally expected in blood donors) will be updated annually.

Donors who still present at least three of the risk factors listed above approximately one year after the first check-up will be offered a follow-up which involves a partial diversification of additional biochemical tests compared to those carried out at enrollment, to collect further useful information for prognostic purposes to stratify the risk of hepatic and extrahepatic complications; albumin, C peptide, glycated hemoglobin, microalbuminuria in urine, insulin, C-reactive protein will be performed. An additional stool sample will also be collected to perform fecal occult blood testing and microbiota biobanking.

We therefore plan to characterize the intestinal microbiota in feces at the Laboratory in approximately 200 individuals through a) metagenomic analysis (taxonomic and functional) and evaluation of serum levels of TMAO and other metabolites of bacterial origin, and b) the interaction of the microbiome with classic risk factors. These analyzes will be carried out at the Genomic Platform Laboratories of the IRCCS Ca' Granda Ospedale Maggiore Policlinico Foundation, under the responsibility of Prof. Valenti.

We expect a very high rate of participation in the study thanks to the selection of highly motivated individuals, characterized by a commitment to health promotion and confidence in scientific research.

2) Evaluation of early cardiovascular damage and characterization of liver damage in patients with high probability of severe NAFLD

The intima-media thickness of the common carotid arteries (CCA-IMT) and the presence of carotid plaques will be assessed by color Doppler ultrasound in individuals at higher cardiovascular risk (impaired fasting blood sugar, blood sugar>100mg/dl, HbA1c>40mmol/mol), as indicated in the normal course of care for these subjects.

Donors with increased liver enzymes (>42/30 in M/F) and/or LSM≥7.9k will also be offered for clinical investigation:

a) Upper abdominal ultrasound in collaboration with the Gastroenterology and Hepatology UOC (Prof Pietro Lampertico and Dr. Mirella Fraquelli).

b) Where clinically indicated, a histological evaluation of liver damage will be proposed (in collaboration with the UOC Gastroenterology and Hepatology, Prof. Pietro Lampertico and Dr. Mirella Fraquelli) to confirm the staging of liver diseases, exclude concomitant liver diseases and possibly offer inclusion in clinical trials for new therapies.

It is planned to share part of the samples resulting from liver biopsies for analysis at the Department of Medicine, Columbia University Irving Medical Center, with the collaboration of Prof. Ira A. Tabas and prof. Utpal Pajvani, for the evaluation and validation of markers associated with liver disease, after preparation of appropriate MTA.

The study will collect the data and results of these tests if already foreseen for the clinical situation of the enrolled patient.

3) Study of genomics and biomarkers

The study cohort will be genotyped for the following genetic factors: rs738409 C>G (PNPLA3 I148M), rs58542926C>T(TM6SF2E167K), rs1260326C>T(GCKRP446L), and rs641738C>TnellocusMBOAT7.

In addition to characterizing genetic variants in known associated genes, whole-exome sequencing (WES) can also comprehensively screen all coding parts of the genome (exons) without any a priori knowledge, enabling the identification of potentially novel candidate variants.

IL-32 and other biomarkers will be measured by ELISA.

At the end of examine if a low one activation of haemostasis can protect the liverfrom damage induced from metabolic dysmetabolism, we will further analyze the hemostatic profile as described below: measurement of endogenous thrombin potential (ETP) ± thrombomodulin, FII, antithrombin (AT), VWF: RCo, VWF multimeters, ADAMTS activity.

The analysis regarding the coagulation profile will be carried out in collaboration with the UOC MedicinaGenerale – Hemostasis and Thrombosis of the IRCCS Ca' Granda Ospedale Maggiore Policlinico Foundation (Prof. Flora Peyvandi).

Genotyping and genetic analysis of the samples will be carried out at the Institute for Molecular MedicineFinland FIMM Tecnology Center (University of Helsinki) with the collaboration of Dr. Päivi Lahermo. The identified samples will be sent in groups of approximately 500 and any residues not used for analysis will be destroyed.

4) Generation of an invitro genetic model of NAFLD

We will provide functional validation on the impact of the new, stronger genetic variant identified in the epidemiological study as associated with HFC in cellular models.

5) Metabolomics and lipidomics analysis

The genetic variants identified to have an impact on the onset and progression of NAFLD are all associated with genes involved in biological processes of lipid droplet (LD) remodeling, intracellular lipid synthesis and degradation, and low-density lipoprotein (VLDL) secretion. (8, 9). Therefore, in order to increasingly understand metabolic and lipid alterations in relation to the genetic profile of each individual, the serum samples of the individuals

enrolled in the entire study cohort, upon stipulation of a specific MTA, they will be transferred to the Precision Medicine & Metabolism Laboratory of the CIC bioGUNE in Derio (Bilbao), under the responsibility of Prof. José M. Mato, in order to carry out lipidomics and metabolomics analyses. , using respectively nuclear magnetic resonance (NMR) for the former and liquid chromatography coupled to mass spectrometry (LC-MS) for the latter.

-ENDPOINT-

a) Examine whether the genetic risk profile of the individual, together with the evaluation of environmental factors, imaging techniques and new circulating biomarkers, can help in risk stratification of NAFL and associated complications.

b) Validate the causal role of liver fat in alterations of metabolism, coagulation and early cardiovascular damage associated with NAFLD.

c) Identify new genetic and hereditary risk variants associated with NAFLD.

d) Investigate the mechanism underlying genetic susceptibility to liver disease and coagulation imbalances in in vitro models of NAFLD.

- PrimaryEndpoint

In order to test the causal association of liver fat accumulation (HFC) with liver disease and cardiovascular complications, the primary endpoint will be to examine the association between HFC-genetic risk score (GRS) and:

a) measurement of CAP by Fibroscan (controlled attenuation parameter, parameter used for the quantification of liver fat)

b) liver disease (LSM) at Fibroscan, estimation of liver damage

- Secondary Endpoint(s).

To evaluate the role of liver fat (HFC) in coagulation alterations and in influencing cardiovascular risk as a secondary point, the association between HFC-RBC will be evaluated if:

c) the presence of pro-coagulant factors (e.g. VWF:Ag, FVIII/PCratio, ADAMTS:Ag)

d) vascular damage (estimated by color Doppler ultrasound of the supra-aortic trunks)

e) renal damage (estimated glomerular filtration and microalbuminuria)

f) the risk of colon polyposis

g) The cognitive state

-DURATION/TIMELINE OF THE STUDY

Start of study: June 2020

End of enrollment: December 2024 (indicative date, updated based on the rescheduling of activities linked to the COVID-19 health emergency and the impact of the study on the scientific productivity of the UOC (10-13,1-4,1) Start of Follow-up: June 2021 (indicative date) End of Follow-up: December 2030 (indicative date) Finedellostudio: December 2032 (indicative date)

The study will last 150 months. In the first 54 months we expect to enroll approximately 2500 subjects in accordance with the inclusion criteria.

On an annual basis from the date of enrollment we plan to maintain the follow-up activity constant with repetition and expansion of the clinical-laboratory and instrumental evaluation, according to normal clinical practice, as illustrated in the flowchart.

-STATISTICAL ANALYSIS

- Sample size

Considering preliminary data from our group (6, 7) indicating that an HFC-GRS above the median value is associated with at least a 3-fold higher risk of developing NASH and clinically significant fibrosis, the power of the study to detect an impact of the genetic score on the risk of liver disease (NASH or clinically significant fibrosis) is > 95% ($p < 0.05$, two-sided).
Regarding the possibility of prospectively evaluating hepatic and extrahepatic complications, given the age range and the presence of metabolic risk factors, we expect that in our cohort during the 3-year study period the cumulative incidence of major cardiovascular thrombotic events (death, myocardial infarction or cerebrovascular events, venous thromboembolism) will be 3-4%, while that of cancer will be around 2-3%. The sample size has >80% power to detect a hazard ratio of 1.8 regarding extrahepatic complications associated with genetically determined hepatic fat accumulation, in agreement with literature data.

- Data analysis

In order to test the causal association of liver fat (HFC) accumulation with liver disease and cardiovascular complications, we will examine the association between HFC-genetic risk score (GRS) and:

a) measurement of CAP by Fibroscan (controlled attenuation parameter, parameter used for the quantification of liver fat)

b) liver disease (LSM) at Fibroscan, estimation of liver damage

c) the presence of pro-coagulant factors (e.g. VWF:Ag, FVIII/PCratio, ADAMTS:Ag)

d) vascular damage (estimated by color Doppler ultrasound of the supra-aortic trunks)

e) renal damage (estimated glomerular filtration and microalbuminuria)

f) the risk of colon polyposis

g) the cognitive state of the enrolled subjects.

Analyzes will be performed by fitting the data to generalized linear regression models. In particular, general linear models will be used to examine continuous traits, logistic regression models for binary traits and ordinal regression models for ordinal traits. Confounding factors and the interaction between genetic, epigenetic and environmental triggers will be evaluated by inserting them into the models.

The association between hepatic fat content (HFC) and NAFLD-related outcomes will be assessed using linear and logistic regression models, with HFC as the explanatory variable, and each trait as the outcome. The causal effect of HFC will be estimated using the Wald (ratio) method, with standard errors approximated by the delta method.

Everyone

The models will be adjusted for clinically relevant confounders, including age, gender and behavioral risk factors, diet, body weight, abdominal circumference, insulin resistance and physical activity.

Statistical analyzes will be performed using version 3.3.2 of the R statistical analysis software (http://www.R-project.org/). P values <0.05 will be considered statistically significant.

-ADVERSE EVENTS-

The project does not involve the administration of drugs or other substances or invasive clinical practices. Therefore, no adverse events are expected.

-RISK/BENEFIT ASSESSMENT-

This study will provide us with the tools to highlight the possible causal association between hepatic fat accumulation and extrahepatic disease associated with NAFLD, regardless of numerous confounding factors.
This could have important practical implications for predicting the long-term outcomes of therapies aimed at reducing liver fat with enormous relevance for the management of patients and the prioritization of new therapeutic approaches (e.g. therapies aimed at counteracting the deleterious effect of specific genetic mutations on fat metabolism, reducing the accumulation of fatty liver etc.).
The study will also allow us to provide proof of principle of the usefulness of measuring CAP and LSM using Fibroscan for the clinical characterization of patients at risk in the general population.
The development of a GRS combined with clinical-instrumental evaluation could finally be implemented in the general population and prove to have great relevance for the initiation of programs aimed at stratifying the risk of hepatic and extrahepatic complications in NAFLD, the selection of patients for screening

Of

such complications, at the start of lifestyle change programs, as well as pharmacological and primary prevention approaches.

-STUDY MANAGEMENT-

For data collection, the Emonet 15 software will be used, already currently used to manage blood donor selection. The data will be de-identified by assigning a unique code to each subject.

-REGULATORY ASPECTS AND ETHICAL CONSIDERATIONS-

- Approval of the Competent Authority

This study will be conducted in accordance with the rules of the ICH/GCP (International Conference of Harmonization/Good Clinical Practice) and all applicable laws, including the Declaration of Helsinki of June 1964, as amended by the latest WorldMedical Association General Assembly in Seoul, 2008.

- Approval of the Ethics Committee

The investigator must ensure that the protocol has been seen and approved by the local Independent Ethics Committee (EC) before starting the study. The EC must also review and approve the informed consent (IC) form and all written information received from the patient prior to enrollment in the study.
Should it be necessary to modify the protocol and/or the IC during the study, the investigator will be the guarantor and therefore the person in charge of ensuring the review and approval of this modified document according to the EC's request.
The content of these changes will be implemented only after the CE has approved them. Until that time, it will be necessary to refer to the previous version of the already approved document.

- Informed Consent (IC)

The investigator or other personnel appointed by him has the task of informing people about all aspects and procedures of the procedure
study. The informed consent of the participants will be collected by the doctor in charge of the metabolic evaluation of the subject located there.

The process for obtaining informed consent must comply with current regulatory procedures. The investigator (or designated collaborator) and the subject must date and sign the informed consent form before the patient initiates any study-related procedures. The subject will receive a copy of the IC dated and signed by both parties; the original copy will be kept in the archives designated for study. Neither the investigator nor designated personnel should in any way exert any coercion or influence on a subject to participate or continue to participate in the study. A subject's decision to participate in the study must be completely voluntary. The investigator and designated personnel should emphasize to the subject that he or she may withdraw consent at any time without penalty or loss of any benefits to which he or she may be entitled.
Written or oral information relating to the study, including the written consent form, must not contain any linguistic expression that forces the subject to (even apparently) renounce his legal rights, or that would exonerate the investigator, the holosponsor from responsibility for negligence.

- Duties of the experimenter -

In accordance with applicable local regulations, the investigator must send periodic reports regarding the progress of the study in his center to the EC and notify the EC of the closure of the study. Periodic reports and closure notifications are part of the investigator's responsibilities.

- Study monitoring

In accordance with applicable regulations and good clinical practice (GCP), the study monitoring staff will check the data entered in the CRF while maintaining its confidentiality.

In accordance with applicable regulations and good clinical practice (GCP), the monitor must visit or contact the center The periodically. The duration, nature and frequency of such visits/contacts depend on the frequency of recruitment, the quality of the documents held by the center and their adherence to the protocol.

Through these contacts, the monitor must:

• monitor and evaluate the progress of the study

• examine the collected data

• conduct verification of the source document

• identify each problem and its solutions

The aims of the monitoring activity are to verify that:

• the rights and well-being of the individual are respected

• the study's data is accurate, complete and verifiable from the original documents

• the study will be conducted in accordance with the protocol and any approved amendments, GCP and applicable regulations

The experimenter must:

• give the monitor direct access to all relevant documentation

• dedicate part of your time and staff to the monitor to discuss the monitoring results and any other possible aspects.

The monitor must also contact the center prior to the start of the study to discuss the protocol and data collection procedures with the staff.

- Quality assurance of the study

As promoter, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico can carry out a quality control of the study at its own discretion. In this case, the investigator must allow the monitor to have direct access to all relevant documentation and dedicate part of his time and staff to the reviewer to discuss the monitoring results and any other aspects of the study.

Furthermore, Regulatory Authorities may carry out inspections. In this case, the investigator must authorize the inspector

direct access to all relevant documentation, and dedicate part of its time and staff to the inspector himself to discuss the results of the monitoring and any other aspects of the study.

- Closing of the studio

When the study is closed, the monitor or experimenter must activate a series of procedures:

-review all study documentation -

-reconcilestudy data-

-reconcile all declaration relationships.-

- Document archiving -

In accordance with applicable national regulations, the investigator must keep a copy of all documentation and store it in a dry and safe place after the study is closed.

- Dissemination of information regarding scientific discovery -

- Confidentiality

The investigator and other personnel involved in the study must process all information relating to the study (including the protocol, data obtained and all documentation produced during the study) and must not use such information, data or reports for purposes other than those described in the protocol. These restrictions do not apply to:

-information that becomes publicly available, not due to negligence on the part of the investigator or his staff;-

-information that requires confidential disclosure to CE for the sole purpose of evaluating the study;-

-information that must be disclosed in order to obtain appropriate medical treatment for a study subject.-

- Publications -

The IRCCS Ca' Granda Foundation, OspedaleMaggiore Policlinico is the sole owner of the data. Communications, reporting and publication of study results will be the responsibility of the principal investigator.

-INTELLECTUAL PROPERTY RIGHTS IN THE STUDY RESULTS-

The Parties mutually acknowledge from now on that during the implementation of the Project, within the limits of what is strictly necessary for the carrying out of this, they could be used to various extentsknow-how, technical material and/or goods protected by industrial and/or intellectual property rights or susceptible to protection, developed before the start of the Project by the Parties which remain in the ownership of each of them and on which the other Party will not acquire

any rights of any kind.

All interested parties mutually indemnify each other since in the course of the implementation of the Project they will Between and/or may be used in collaboration to varying degreesknow-howand/or goods covered by industrial and/or intellectual property rights owned by each of them on which the other Party will not acquire any industrial and/or commercial exploitation rights in any capacity whatsoever.

The results of the research activities will be the property of the Parties in proportion to their respective inventive contribution and their use, it being understood that the making available of biological samples at the basis of the research project research, related clinical information and related medical know-how from the study sponsor will be considered an essential contribution. In cases of innovative Results, susceptible to patent protection (or similar proprietary rights) and/or economic exploitation, the co-owner Parties undertake to regulate the methods of protection and valorisation of said Results in a specific agreement. In this case, any publications will be subject to the completion of all procedures aimed at patent protection of the results.

-INDEMNITY AND COMPENSATION IN CASE OF DAMAGES-

In the event of unwanted events or any damage that may arise from participation in the research, the Insurance Policy of our Institute is also extended to cover the subjects participating in the research projects.

-FINANCIAL AGREEMENTS-

The costs of study procedures exceeding normal clinical practice (genotyping, genetic analysis and dosage of plasma biomarkers) will be covered by the following funding:

- Fondazione Patrimonio Ca' Granda: funded in the context of the call “The Liver BIoBank Lombardia gEnomiccohort study (LIVER-BIBLE): personalized medicine for the management of hepatic and cardiovascular thrombotic complications of liver disease”

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-DISCLOSURES ON CONFLICTS OF INTEREST-

The investigators declare no conflicts of interest.

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