Department of Services and Preventive Medicine UOC
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The Foundation Genomic SARS-CoV-2 Study

Acronym: FoGS

STUDY SPONSOR:IRCCS Ca' Granda Foundation Ospedale Maggiore Policlinico Milan.

STUDY COORDINATION:Department of Transfusion Medicine and Hematology, IRCCS Ca' Foundation Granda Ospedale Maggiore Policlinico, University of Milan, Marangoni Pavilion, via Francesco Sforza 35, 20122, Milan, Italy, Head: Dr. Daniele Prati

PRINCIPAL INVESTIGATOR:Dr. Luca Vittorio Valenti

Type of study:Spontaneous, biological, cross-sectional, monocentric

Version: 1.0

Dates: 04/08/2020

DECLARATION OF CONFIDENTIALITY

RESPONSIBILITIES (role of promoter and collaborators)

InternalCollaborations

Department	Participant'sname	Role in the study
Department Of Medicine Transfusion and Hematology, IRCCS Ca' Granda Foundation Maggiore Policlinico Hospital, University of Milan, directed by dr. Daniele Prati	Daniele Prati Guido Baselli Serena Pelusi Giuseppe Lamorte	study coordination, data analysis and interpretation, recruitment and characterization of controls
Scientific Direction, IRCCS Ca' Granda Foundation Ospedale Maggiore Policlinico	Filippo Martinelli-Boneschi Monica Miozzo, Luigia Scudeller, Silvano Bosari	genotyping, data analysis and interpretation, biobanking, study funding.
UOC Adult Resuscitation and Intensive Care, IRCCS Ca' Granda Foundation Ospedale Maggiore Policlinico and University of Milan	Alberto Zanella Giacomo Grasselli Antonio Pesenti	patients recruitment and characterization substudy management.
IRCCS Ca' Granda Maggiore Hospital Foundation “COVID-19 Network” Polyclinic	Valter Monzani, Francesco Blasi, Andrea Gori, Alessandra Bandera, Flora Peyvandi, George Constantine, Anna Ludovica Fracanzani, Marina Baldini Leonardo Terranova	patientsrecruitment and characterization.
UOC High Intensity Pediatrics	Paola Marchisio	patientsrecruitment and

Treatment, IRCCS Ca' Granda Ospedale Maggiore Policlinico Foundation		characterization.
UOC General Medicine, IRCCS Ca' Granda Ospedale Maggiore Policlinico Foundation	Maria Carrabba	patients recruitment and characterization, substudy management.
UOC Laboratory Medicine, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico	Ferruccio Ceriotti	samplescollection, laboratorycharacterization.

External Collaborations (biological samples analysis, data analysis, diagnostic procedures)

Institution	Department	Participant'sname	Role in the study
University of Oslo	Department of Gastroenterology,	Tom Hemming Karlsen	study funding, data analysis
University of Kiel	Department of Molecular Medicine	Andre Franke	genotyping and genetic analysis
Humanitas University and Research Hospital		Stefano Duga, Rossana Asselta	data analysis, controls for collaborative studies
San Gerardo Hospital, Bicocca University		Pietro Invernizzi, Andrea Blonde	controls for collaborative studies

LIST OF ABBREVIATIONS

IEC:IndependentEthics Committee

THERE:Informed Consent

CRF:Case Report Form

GCP:Good Clinical Practice

GWAS:Genome-wide association studies

PNS:single nucleotide polymorphisms

GRS:genetic risk score

Summary

BACKGROUND AND OVERVIEW OF THE PROJECT

The Lombardy region in Northern Italy is now in the midst of an outbreak of severe acute respiratory disease syndrome coronavirus 2 (SARS-CoV-2), the virus that causes coronavirus disease 2019 (COVID-19)[1]. The COVID-19 epidemic situation needs little introduction and represent a global world-wide emergency with mortality rates rapidly increasing in Europe and the US. Evidence is accumulating that the majority of individuals infected by SARS-CoV-2 are asymptomatic and the major source of viral spread[3], and that a considerable fraction of these has active viral replication[4,5].Furthermore,disease behavior is variable, with the majority of patients experiencing only mild symptoms or no symptoms at all. Some patients develop severe pulmonary affection, with aggressive and extensive inflammatory destruction of lung parenchyma and associated inflammatory responses and superinfections, driving large fractions of the COVID-19 related mortality. What exactly drives this development of severe lung disease remains unknown, but old age, obesity, diabetes and other co-morbidities increase the risk, while the role played by specific medications is still uncertain. Variation in virus genetics and patient immunology are also likely . As to the latter point, we hypothesize that host genetics may play a role in determining development of severe lung disease in SARS-CoV-2 infection.

Genome-wide association studies (GWAS) have been applied to decipher the genetic predisposition in thousands of disease traits since the study design was invented in 2005. The genetic signals detected vary from very strong effects that can be detected in a few hundred individuals, to very weak effects requiring cohorts of tens of thousands for detection. By 2020, the study design is now a robust, off-the-shelf, easy-to-perform industry-standard screening tool for genetic predisposition, even available through "consumer genetics" online-based companies. The study design is simple: testing for genetic variants throughout the genome (single nucleotide polymorphisms, SNPs) using SNP microarrays, comparing their frequencies in patients versus controls (or across other variables). For inflammatory phenotypes in particular, GWAS has proven an efficient tool, delineating hundreds of susceptibility loci in many conditions, some of which has provided novel and surprising disease insights.

GWAS serves two purposes. Most importantly they allow to determine biological factors involved in disease development, thus potentially guiding drug development and therapy. This would be particularly relevant during the current COVID-19 emergency, when hundreds of trials have begun and there is an urgent need to prioritize well-conducted collaborative studies based on robust pathophysiological data. Secondly, and increasingly popular, they allow for the calculation of a "polygenic risk score" to predict disease development. Both aspects appear crucial to clarify for COVID-19 lung disease: (a) are there genetic signatures suggesting which biological mechanisms are involved that may suggest relevant therapeutic approaches, and (b) can we predict those at risk (or those with very low risk)?

The Fondazione IRCCS Ca' GrandaOspedale Maggiore Policlinico is the main public clinical research institute in Italy and has taken a leading role in the medical management of the COVID-19 epidemics in Milan and the Lombardy region. Due to the high volume of patients treated, the ongoing characterization of a large cohort of individuals who acquired the infection without developing symptoms (CoDS study), and scientific leadership in the field of human genetics, the Foundation is in a unique position to conduct a genomewide association study for the identification of the inherited determinants of the susceptibility to severe COVID-19.

Hypothesis

Host genetic factors contribute to development of severe pulmonary disease in patients with SARS-CoV-2 infection.

Purpose and aims

The purpose of the entire project is to identify genetic determinants of the predisposition to develop respiratory failure in persons infected by SARS-CoV-2; knowledge of these factors may educate the biological understanding of the development of severe lung disease in SARS-CoV-2 infection. Such an understanding may inform treatment trials of potential utility towards more effective management of this patient group. A polygenic risk score may also be useful in identifying high/low risk patients, eg among health-care providers and vulnerable individuals with significant comorbidities.

Specific aims of the project are:

1. To identify the main common genetic determinants of severe COVID-19 by conducting a GWAS: in first phase we will compare the first 1,000 cases (SARS-CoV-2 positive patients with severe lung affection)to 5,000 historical healthy controls from the same geographic area, in order to provide initial data informing the research in the field in a timely fashion. This will be conducted in close collaboration with the University of Kiel COVID-19 genomic initiative. Later on, we will proceed to assess objectives 1 and 2 in the full cohort, including also infected controls who did not developed clinically significant symptoms.

2. To provide extensive genetic characterization of all patients managed at the Foundation in order to facilitate other groups working on specific projects (eg on candidate genes such as SERPINA1andACE2by the Intensive Care and Cardiology Units and others that may emerge from upcoming projects), in a large population and in a very timely fashion, and without incurring in any additional expenses for the Fondazione research network, and coordinate research efforts.

3. To provide a coordination to manage the collaboration with other research consortia in the field, eg the "An anonymized GWAS to urgently query host genetic predisposition to severe COVID-19 (SARS-CoV-2 infection) lung disease, and the COVID-19 Host Genetic Initiative (https://www.covid19hg.org). Indeed, collaboration among large networks analyzing several thousand cases will be ultimately necessary to clarify the genetic basis of COVID-19 susceptibility, and large collaborative networks are essential instruments for conducting human genetic research.

Secondary aims:

1. To develop a genetic risk score (GRS) to stratify disease risk, based on both genomewide significant loci and candidate variants (including those regulating immune response and viral receptors).

2. To assess the impact of genetic risk variants on disease outcomes, specific clinical characteristics, and response to specific therapeutic approaches (pharmacogenomic analysis) in patients who developed respiratory failure.

3. To examine the possible role of rare genetic variants in determining the predisposition to severe COVID-19 in individuals who developed severe respiratory failure requiring ventilation despite age < 50 years and lack of comorbidities and risk factors (in collaboration with COVID-19 Host genetic initiative and other international research networks).

4. To analyze the predisposition to develop severe COVID-19 in pediatric patients.

Project timeline

Milestones	Planning
Basal samples collection	03/23/2020
Ethical committee submission	04/09/2020
Phase 1 analysis - start	04/05/2020
Phase 1 analysis – initial report	01/06/2020
Phase 2 analysis	01/09/2020 - 06/09/2021
Final study report (main outcomes)	09/31/2021
Additional analyzes (secondary outcomes), and planning of additional steps	03/06/2020 – 06/02/2022

STUDY PROTOCOL

We will conduct a genomewide association study to identify common genetic variants predisposing to the development of severe SARS-CoV-2 infection.

Objectives

1)To identify differences in genetic background between patients with severeSARS-CoV-2 infection and healthy controls from the same geographic area

ENDPOINTS: SNPs as identified by GWAS

2) To identify differences in genetic background between patients with severe versus mild or moderate SARS-CoV-2 infection

ENDPOINTS: SNPs as identified by GWAS

Study Design

Cross-sectional study, with non-standard study-specific biological testing.

The study consists of two phases as reported in the project timeline:

1. In the first phase of the study, we will compare the first 1,000 cases (SARS-CoV-2 positive patients with severe lung affection) to 5,000 historical healthy controls from the same geographic area, in order to provide initial data informing the research in the field in a timely fashion. This will be conducted in close collaboration with the University of Kiel COVID-19 genomic initiative.
2. In the second phase of the study, we will proceed to analyze the full cohort, including also infected controls who did not develop clinically significant symptoms.

Participants (eligibility)

1. Cohort 1: Cases

SARS-CoV-2 positive patients (no age limit) with severe lung affection defined by hospitalization and respiratory failure requiring support of any kind (ranging from O_2support via non-invasive ventilation to full respirator/ECMO) at the Fondazione IRCCS Ca' Granda. These patients will be selected among those enrolled in the "COVID-19 Network", "EPIDEMIOLOGICAL AND CLINICAL CHARACTERISTICS OF PATIENTS HOSPITALIZED IN ITALIAN INTENSIVE THERAPY AFFECTED BY 2019-NCOV: COHORT STUDY MULTI-CENTRIC RETROSPECTIVE-PROSPECTIVE: 2019-nCoV_ICU",and other COVID-19 research registries sponsored by the IRCCS Ca' Granda Foundation.

- Informed consent

2. Cohort 2: Mild/moderate infection controls

- blood donors (age 18-70 years) donating between 24/02/2020 and 18/05/2020(~7,500 donors) which will be confirmed to have been infected with SARS-CoV-2 without developing clinically significant symptoms, identified in the CoDS project "COVID-19 Donor Study" sponsored by the Foundation
- confirmed to be exposed to the virus (by means of positive viremia and/or IgM/G antibodies).
- informed consent

3. Cohort 3: Healthy controls (general population controls)

Healthy control data of Italian individuals available from previous multi-ethnic assessments (n=5,000, age 14-80 years). These data are already present within a database at the University of Kiel comprising more than 50,000 cases and are available for the analysis of this study.

Study flowchart

	Baselines
Timing	04/01/2020 – 09/30/2020
ROUTINE ASSESSMENT:	
Clinical evaluation	X
Medical history[to]	X
Clinical outcomes[b]	X
Basic biochemical panel[b]	X
Collection of blood sample for DNA extraction	X

[to]Including family history, lifestyle evaluation, physical activity, alcohol and smoking, risk factors for communicable disorders, comorbidities.

[b]as specified in the research registries in which the patients are enrolled.

Genomic study, laboratory methods

In the first phase of the study, identified peripheral blood samples from severely affected cases (cases, cohort 1) will be sent to the Department of Molecular Medicine of the University of Kiel: Dr.Andre Franke will be responsible for storage and analysis of the samples. Genotyping will be performed by Illumina GlobalScreeningArray-24 v3.0 + Multi-Disease array (Illumina), at the Department of Molecular Medicine of the University of Kiel. At the end of the analyses, any leftover of biological samples will be sent back to IRCCS Ca' Granda Foundation.

In the second phase of the study, DNA will be extracted from identified peripheral blood samples from mild/moderate infection controls (cohort 2) at the Genomic platform of the Fondazione IRCCS Ca' Granda. Deidentified DNA samples will be sequenced at the Fondazione IRCCS Ca' Granda by using the same approach. Prof. Luca Valenti in collaboration with Monica Miozzo (Scientific direction) will be responsible for the genomic analyzes of these samples at the Fondazione IRCCS Ca' Granda.

The genetic risk profile (common inherited variants) will be evaluated at genomewide level by Illumina Infinium Global screening array (Illumina), and frequency distributions of common genetic variants will be compared between donors who cleared the infection without developing severe symptoms and patients admitted for respiratory failure (enrolled by the COVID-19 research networks of the Fondazione IRCCS Ca' Granda).

Substudy:to examine the possible role of rare genetic variants in determining the predisposition to severe COVID-19, DNA (isolated from the same biological samples of the main study) of individuals who developed severe respiratory failure requiring ventilation despite age < 50 years and lack of comorbidities and risk factors will be further sequenced and analyzed by Whole exome sequencing (WES) at the Department of Molecular Medicine of the University of Kiel (Dr.Andre Franke).

Any leftover of biological samples will be stored atBiobank POLI-MI for additional 15 years under the responsibility of Dr.Luca Valenti.

Potential biases and pitfalls

Samples of insufficient quality due to low call rate (<0.95%) or outlying heterozygosity will be excluded. TO set of LD-pruned SNPs with MAF>40% were used to estimate identity by descent (IBD) and ancestry and any closely related individuals excluded. Principal component analysis will be used to exclude samples with non-European ancestry. SNPs with a minor allele frequency less than 0.1%, Hardy-Weinberg equilibrium P < 10^{-7}, call rate lower than 95% will be excluded as well.

In order to be able to compare data to other cohorts to increase the sample power and allow replication, we will proceed to single nucleotide polymorphisms (SNP) imputation in the cases was carried out using the Michigan Imputation Server (https://imputationserver.sph.umich.edu/start.html#!pages/home). Each cohort was uploaded to the Michigan Imputation Server and imputed to the Haplotype Reference Consortium (HRC 1.1r 2016) reference panel. Imputed genotypes will be filtered by an r^2score of at least 0.8, and only overlapping well-imputed SNPs between the two cohorts will be retained.

In case during the planned analyzes we will need a larger sample size to answer the research questions, we will actively involve cases from collaborating hospitals and the international collaborative network.

Study variables and data sources

A biorepository (serum samples and buffy coats for DNA extraction) is being established and maintained within the Fondazione IRCCS PoliclinicoPOLI-MI Biobank (at the coordinating Unit).

Cohort 1: Cases

Extensive clinical data with prospective follow-up and samples of these individuals are alreadybeingcollectedwithin the "Register for the evaluation of epidemiological characteristics and patient clinicshospitalizedat the COVID-19 units: COVID-19 network" and "characteristics epidemiological and clinical findings of patients hospitalized in Italian intensive care units affected by 2019-nCoV: multicenter retrospective-prospective cohort study: 2019-nCoV-ICU". We will also involve collaborating centers in Lombardy and Northern Italy.

Cohort 2: mild/moderate infections controls

All donors undergo clinical evaluation, including updating of medical history, and biochemical testing at each blood donation. For each blood donation, the possible development of flu-like symptoms in the two weeks following blood donation will be recorded as well. All donors are indeed asked to report the development of any symptom potentially related to COVID-19 in the 14 days after the procedure, by contacting a dedicated phone number, and all calls are managed by medical personnel of the Transfusion center. All these events, as well as all the other data that will be collected for this study, are registered in the EMONET 15 informatics system.

Cohort 3: healthy controls

> 5,000 historical healthy controlsfromthe same geographic area (data derived from previous studies conducted at other institutions), in order to provide initial data informing the research in the field in a timely fashion. This will be conducted in close collaboration with the University of Kiel and Oslo COVID-19

genomic initiative, which includes collaborators from the Milan area (San Gerardo and HumanitasHospitals).

Statistical issues

Sample size

Group	Sample size	Source/notes
Cases	2000	Ca' Granda ICU and medicine departments
Mild/moderate controls	2000	based on an estimated cumulative prevalence of SARS-CoV-2 infection >10% in Lombardy at the time of project writing, we estimate to enroll at least 1,000 controls for the study (blood donors).
Healthy controls	5000 (including collaborating centres)	Historic data

The study is originally scoped toward 2,000 cases, but if more samples are collected, the experiment will be expanded (for which there is both capacity and funding).

The study is a case-control cross-sectional study. Two main comparisons will be performed:

1) cases vs healthy controls (phase 1)

2) cases vs mild/moderate infections (phase 2)

In the hypothesis that we will recruit 2,000 cases and consider 5,000 controls, we will have >99% power to detect a 4-fold increase in the risk of severe COVID-19 for variants that have a minor allele frequency >30% in the population (two sided, for a significance threshold of P values $< 10^{-8}$ generally employed in GWAS), and a good power (>80%) to detect an impact of rarer variants or of variant with smaller size effects.

Associations with very weak effect sizes may be missed with the crude approach, but for this study this compromise is acceptable to achieve feasibility and speed.

Statistical analysis plan

Statistical association analysis will be performed using logistic regression / linear mixed models, based association methods with standard tools (eg SAIGE, BOLT-LMM, PLINK etc.)) and appropriate correction for population stratification confounders, and incorporating patient age and gender as co-variables. For strong genetic signals (egHLAin many immune mediated diseases,IL28Bin HCV infection,complement factor Hpolymorphisms in age-related macular degeneration) only a few hundred patients have been required to achieve genome-wide significant findings.

The comparison will be carried out on imputed genotypes, firstly from the set obtained from imputing both cases and controls together, and then again from the set obtained by imputing all cohorts separately and then combining stringently filtered genotypes. LocusZoom plots will be examined for visualization of the signal around significantly associated SNPs.

For each trait the analysis will be performed with no clinical covariates and then again with the following additional covariates: presence of arterial hypertension, diabetes, chronic obstructive pulmonary disease, type of respiratory support.

Ethical considerations

Regulatory standards

This study will be conducted by Good Clinical Practice (GCP) rules; in accordance with the ethical principles that have their origin in the Declaration of Helsinki and with the respect to the European clinical practice, in compliance with all international guidelines and national law regulation in Italy.

Institutional review board / independent ethics committee

The protocol and the informed consent document must be submitted to the Independent Ethics Committee (IEC) for review and will receive IEC approval/favourable opinion before initiation of the study. During the study, any amendments to the protocol must also be approved by IEC. A progress report is sent to the IEC at least annually, and a summary of the study's outcome is sent at the end of the study.

Patient information

Participants enrolled will be required to consent to the deidentification of personal data for the study. Information form and the module for the acquisition of informed consent for the handling of sensitive data will be given to the patient.Specific written informed consents will be obtained by all included participants, who will be confirmed to be exposed to SARS-CoV-2 (as detected by the presence of viremia and/or seroconversion), to participate in genetic and biomarkers studies.

The investigator will fulfill the current regulations for research and documentation of informed consent, the standards of Good Clinical Practice and the ethical principles derived from the Declaration of Helsinki. The approval by the Ethics Committee will be required whether an update of the informed consent form will be needed during the study.

According to the recommendations of the Declaration of Helsinki and local regulations, each patient will be adequately informed about the aims, methods, expected benefits, potential risks and related problems to the study. Furthermore, patients will be informed of their right to refuse consent to the use of their sensitive data or to withdraw it at any time, without having any effect on their medical care.

The patient will have all the time necessary for the evaluation of the information received before providing their informed consent to the use of sensitive data. The investigator will have to obtain spontaneously informed consent in writing by the patient, before using them in any way for the study. The written consent to the handling of sensitive data must be subscribed by the date and signature of the patient and by the investigator's or his representative's ones.

The investigator has to give to the patient a signed copy of informed consent; the original form will be retained with the other documents of the study protocol; the module for the acquisition of informed consent to the treatment of sensitive data will be attached to the clinical folder. The collaborator will be appointed to review the original forms of all patients' informed consent.

Funding

The study will be funded by Institutional funding dedicated to testing the safety of blood products, and the specific research funding dedicated to the COVID-19 emergency from the Scientific Direction, Foundation IRCCS Ca' GrandaOspedale Maggiore Policlinico.

Genotyping and genetic analyzes performed at the Department of Molecular Medicine of the University of Kielwill be supported by funding by University of Oslo.

Insurance

No additional blood sampling (samples will derive from leftovers of donations) or clinical data will be required in addition to those already collected during the regular clinical practice. The internal institutional insurance policy will cover for any undesirable effects due to the participation in the study.

Confidentiality

According to the ICH guidelines for the Good Clinical Practice, the monitoring team must check the CRF entries against source documents. The personnel bound by professional secret must maintain the confidentiality of all personal identity or personal medical information (according to the confidentiality and personal data protection rules). The confidentiality of records that could identify subjects should be protected, only initials of the name and the first name will be registered with an inclusion coded number for the study (no name nor address nor identifying data).

Publications

Communications, reports, and publication of the results of the study will be under the responsibility of the principal investigator of the study. A summary of the results of the study will be written and provided on request of the participating patients.

Intellectual property rights of study results

The Promoter of the study and the Participating Centers acknowledge and accept that during the implementation of the Project, to the extent strictly necessary for the performance of the Project, know-

how, technical material and/or goods protected by industrial and/or intellectual property rights or susceptible to protection, developed prior to the commencement of the Project by the Promoter and the Participating Centres, shall be and shall remain their exclusive ownership. Nothing in this protocol shall be constructed as a grant of rights under such intellectual property.

The results arising from the research activities will be jointly owned by the Promoter of the study and the Participating Centers in proportion to their respective contribution, being understood that the provision of biological samples underlying the research project, related clinical information and related medical know-how by the Study Promoter will be considered as an essential contribution.
In cases of innovative results, which are subject to patent protection (or similar rights) and/or economic exploitation, ownership of such Invention shall be owned based on inventorship contribution. In case of joint ownership, the Promoter of the study and the Participating Centers will regulate, in fair and good condition, the protection and the exploitation of the results.

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