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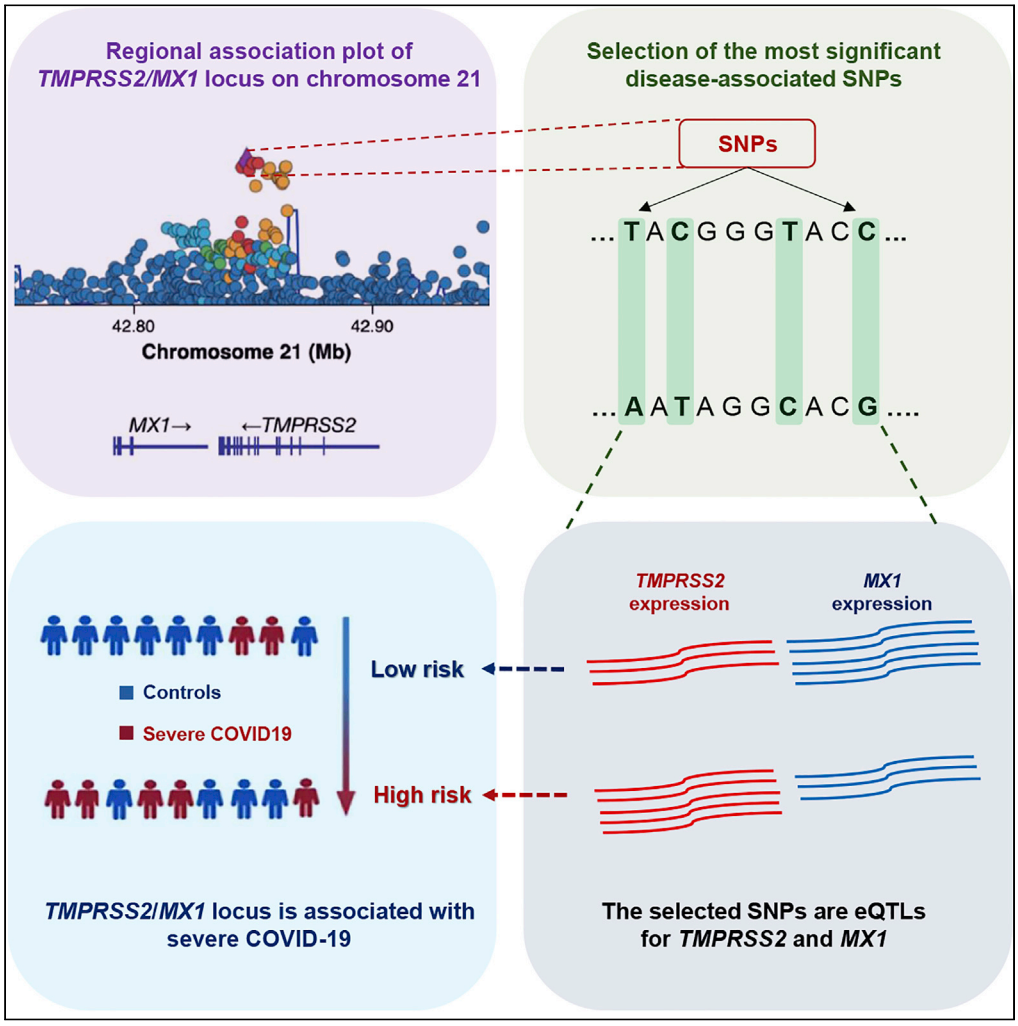

Immacolata Andolfo, Roberta Russo, Vito Alessandro Lasorsa, ..., Massimo Zollo, Achille Iolascon, Mario Capasso

mario.capasso@unina.it

Highlights

Genetic analysis was performed on 7,970 individuals hospitalized for COVID-19

Five SNPs within *TMPRSS2/MX1* locus (chr.21) are associated with severe COVID-19

The minor alleles of the five SNPs correlated with high level of *MX1* expression in blood

MX1 could be a potential therapeutic target in patients with COVID-19

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Common variants at 21q22.3 locus influence *MX1* and *TMPRSS2* gene expression and susceptibility to severe COVID-19

Immacolata Andolfo,[1,2] Roberta Russo,[1,2] Vito Alessandro Lasorsa,[1,2] Sueva Cantalupo,[1,2] Barbara Eleni Rosato,[1,2] Ferdinando Bonfiglio,[3] Giulia Frisso,[1,2] Pasquale Abete,[4] Gian Marco Cassese,[4] Giuseppe Servillo,[5] Gabriella Esposito,[1,2] Ivan Gentile,[6] Carmelo Piscopo,[7] Romolo Villani,[8] Giuseppe Fiorentino,[9] Pellegrino Cerino,[10] Carlo Buonerba,[10] Biancamaria Pierri,[10,11] Massimo Zollo,[1,2] Achille Iolascon,[1,2] and Mario Capasso[1,2,12,*]

SUMMARY

The established risk factors of coronavirus disease 2019 (COVID-19) are advanced age, male sex, and comorbidities, but they do not fully explain the wide spectrum of disease manifestations. Genetic factors implicated in the host antiviral response provide for novel insights into its pathogenesis.

We performed an in-depth genetic analysis of chromosome 21 exploiting the genome-wide association study data, including 6,406 individuals hospitalized for COVID-19 and 902,088 controls with European genetic ancestry from the COVID-19 Host Genetics Initiative. We found that five single nucleotide polymorphisms within *TMPRSS2* and near *MX1* gene show associations with severe COVID-19. The minor alleles of the five single nucleotide polymorphisms (SNPs) correlated with a reduced risk of developing severe COVID-19 and high level of *MX1* expression in blood.

Our findings demonstrate that host genetic factors can influence the different clinical presentations of COVID-19 and that MX1 could be a potential therapeutic target.

INTRODUCTION

The recent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has caused so far more than over 2.5 million deaths (https://covid19.who.int/). The coronavirus disease 2019 (COVID-19), caused by the SARS-CoV-2, is associated with diverse clinical presentations, ranging from asymptomatic or mildly symptomatic infections to respiratory failure and death (Bellani et al., 2021; Grasselli et al., 2020, 2021; Richardson et al., 2020). Advanced age is an established risk factor, as well as male sex and comorbidities such as hypertension and diabetes (Zhou et al., 2020). Since these risk factors do not fully explain the wide spectrum of disease manifestations, dissecting the genetics of the host response to SARS-CoV-2 infection may provide novel insights into its pathogenesis (Anastassopoulou et al., 2020).

A genome-wide association study (GWAS) (Ellinghaus et al., 2020) identified two susceptibility loci of severe COVID-19: the first locus on chromosome 3 harbors multiple genes (*SLC6A20*, *LZFTL1*, *CCR9*, *CXCR6*, *XCR1*, *FYCO1*) that could be functionally implicated in COVID-19 pathology; the second on chromosome 9 that defines the ABO blood groups (Ellinghaus et al., 2020). Other very recent papers reported the results from the analysis of two large independent GWASs that validated the two previous risk loci and found novel risk variants at chromosome 19p13.3, 12q24.13, and 21q22.1 associated with severe COVID-19 (Pairo-Castineira et al., 2021; Shelton et al., 2020).

Two whole-exome sequencing studies showed that inactivating rare mutations in genes belonging to the type I interferon pathway predispose to life-threatening COVID-19 pneumonia (van der Made et al., 2020; Zhang et al., 2020). Additionally, preliminary results on a small set of Italian cases suggest that coding variants in *TMPRSS2* and *PCSK3* may contribute to the variability in infection susceptibility and severity (Latini et al., 2020).

[1]Department of Molecular Medicine and Medical Biotechnologies, University of Naples, Federico II, 80145 Naples, Italy

[2]CEINGE Biotecnologie Avanzate, Via Gaetano Salvatore, 486, 80145 Napoli, Italy

[3]Dipartimento di Ingegneria chimica, dei Materiali e della Produzione industriale, Università degli Studi di Napoli Federico II, Napoli, Italy

[4]COVID Hospital, P.O.S. Anna e SS. Madonna della Neve di Boscotrecase, Ospedali Riuniti Area Vesuviana, Napoli, Italy

[5]Dipartimento di Neuroscienze e Scienze riproduttive ed odontostomatologiche, Università degli Studi di Napoli Federico II, Napoli, Italy

[6]Dipartimento di Medicina clinica e Chirurgia, Università degli Studi di Napoli Federico II, Napoli, Italy

[7]Medical and Laboratory Genetics Unit, A.O.R.N. 'Antonio Cardarelli', Napoli, Italy

[8]Poison Centre, A.O.R.N. 'Antonio Cardarelli', Napoli, Italy

[9]AORN dei Colli Presidio Ospedaliero Cotugno, Napoli, Italy

[10]Istituto Zooprofilattico Sperimentale del Mezzogiorno, Napoli, Italy

[11]Dipartimento di Medicina, Chirurgia e Odontoiatria "Scuola Medica Salernitana", Università di Salerno, Baronissi, Italy

[12]Lead contact

*Correspondence: mario.capasso@unina.it

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In our previous opinion article, based on the analysis of allele frequencies across different populations and expression quantitative trait loci (eQTLs) data, we hypothesized that common variants on chromosome 21 near *TMPRSS2* and *MX1* genes may be genetic risk factors associated with the COVID-19 different clinical manifestations (Russo et al., 2020). Both *TMPRSS2* and *MX1* are involved in the host response to SARS-CoV-2 infection. ACE2 is the main entry receptor for SARS-CoV-2 (Wang et al., 2020). Entry depends on the binding of the surface unit S1 of the spike (S) protein of the virus to the receptor. SARS-CoV-2 engages ACE2 as the entry receptor and employs the host cellular TMPRSS2 for S-protein priming (Hoffmann et al., 2020b; Matsuyama et al., 2010). Particularly, binding of SARS-CoV-2 S-protein with ACE2 receptor is then followed by host TMPRSS2-mediated cleavage of the viral S-protein. This process, defined as priming, involves cleavage of the S-protein at S1/S2 and S2 sites which is essential for the viral fusion with the host cell membrane before entry into the cell (Hoffmann et al., 2020b; Matsuyama et al., 2020). SARS-CoV-2 can use other proteases such as cathepsin B/L for S-protein in the absence of TMPRSS2 receptors. However, in the lungs (the primary organ for SARS-CoV-2 infection), cathepsin B/L cannot substitute for TMPRSS2 protease activity as the latter is indispensable for viral entry as observed for SARS-CoV and MERS-CoV (Hoffmann et al., 2020a). *MX1* is an interferon-α/β inducible gene that encodes a guanosine triphosphate metabolizing protein involved in the cellular antiviral response (Ciancanelli et al., 2016).

In this study, to further support our hypothesis, we exploited GWAS meta-analysis data from the COVID-19 Host Genetics Initiative (COVID-19 Host Genetics Initiative, 2020) and performed an in-depth genetic analysis of chromosome 21 using summary statistics where common variants at this chromosome were associated with severe COVID-19 at the genome-wide significance level ($p \leq 5 \times 10^{-8}$). Using the cohort of 908,494 subjects with European origins, we found five SNPs at the *TMPRSS2/MX1* locus showing suggestive association with the disease. All five SNPs replicated the association in two independent cohorts of Asian subjects, whereas two SNPs confirmed the association in African and one SNP in the Italian cohort. Significant eQTLs signals were found for the *MX1* gene in blood.

RESULTS

TMPRSS2/MX1 locus is associated with severe COVID-19

To prove that common variants at *TMPRSS2/MX1* (21q22.3) locus may affect the susceptibility to severe COVID-19 onset, we analyzed the summary statistics of a large available GWAS dataset released by the COVID-19 Host Genetics Initiative (COVID-19 Host Genetics Initiative, 2020). The data set includes 6,406 hospitalized cases and 902,088 controls with European ancestry (Table S1). A region on chromosome 21 appears to be significantly associated with severe COVID-19 at the genome-wide level (https://www.covid19hg.org/results/) as also demonstrated in a recently published GWAS study (Pairo-Castineira et al., 2021). To investigate whether more than one association signals may exist at chromosome 21, we selected 74 SNPs showing a $p \leq 1 \times 10^{-5}$ and we identified 3 independent loci among them (Table S2). The most significant signal was represented by rs13050728 ($p = 2.76 \times 10^{-12}$, OR = 0.83, Figure 1A) that maps within the *INFRA2* gene. The other two signals showed a suggestive significance level ($p \leq 1 \times 10^{-5}$) and were tagged by rs111783124 ($p = 2.39 \times 10^{-6}$, OR = 1.17, Figure 1B) and rs3787946 ($p = 2.73 \times 10^{-6}$, OR = 0.87, Figure 1C), respectively. The rs3787946 maps in an intronic region of *TMPRSS2* and the first closest gene was *MX1* (Figure 1C); herein, we named this locus as "*TMPRSS2/MX1*". An in-depth inspection of the *TMPRSS2/MX1* locus showed that 13 SNPs were in linkage disequilibrium (LD) with the lead rs3787946 ($r^2 > 0.8$, Table 1) and that the 5 most significant SNPs (p values ranging from 2.7×10^{-6} to 5.8×10^{-6}, Table 1) were in strong LD with each other ($r^2 \geq 0.90$, Figure S1). The other 9 SNPs showed an LD with the lead SNP rs3787946 ranging from 0.8 to 0.9 and p values ranging from 6×10^{-4} to 0.04 (Table 1). We then sought to replicate the associations of the 14 SNPs in three independent cohorts of cases and controls of GenOMMIC GWAS (Pairo-Castineira et al., 2021) with non-European ancestry. All the 11 available SNPs replicated in the east asian (EAS) population; the top five SNPs replicated in the South Asian (SAS) ancestry population, whereas two of five SNPs in the African (AFR) one (Table 1). By using the TaqMan assay, we typed the rs12329760 variant in samples from 226 hospitalized COVID-19 patients (Table S3) and 1848 controls from Southern Italy collected in our Institute. An additional Italian cohort of 1915 controls and 770 cases, typed for rs12329760 by whole-exome sequencing, was obtained from the Network for Italian Genomes (NIG) database (Daga et al., 2021). After combining the two cohorts, we confirmed the minor allele as a protective factor against the aggressive form of the disease (Table 2, $OR_{allele} = 0.89$, $P_{allele} = 0.07$; $OR_{dominant} = 0.57$, $p = 0.01$; $OR_{CCvsTT} = 0.57$, $p = 0.01$). The results of our case-control study suggest that the protective effect against the severity of COVID-19 is mainly due to the TT genotype.

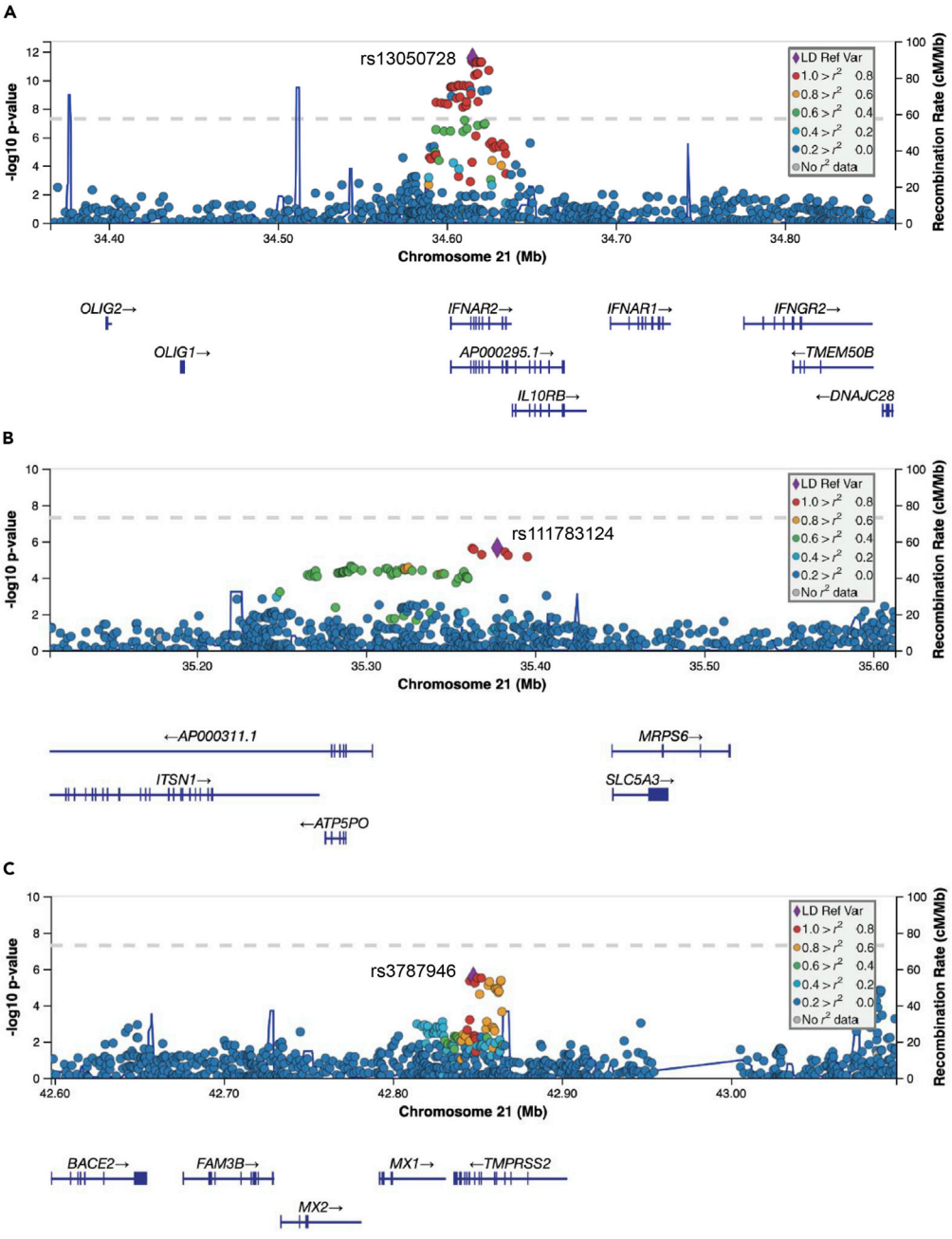

Figure 1. Regional association plots of the SNPs at three independent association signals of chromosome 21

Plots were generated using LocusZoom. Y axes represent the significance of association (−log10 transformed p values) and the recombination rate. SNPs are color-coded based on pairwise linkage disequilibrium (r^2) with indicated lead SNPs: rs13050728 (A), rs111783124 (B) and rs3787946 (C).

SNPs at *TMPRSS2/MX1* locus are enriched in regulatory regions active in the thymus

We tested if the 14 SNPs (Table 1) and their proxy SNPs ($r^2 > 0.8$) were significantly over-represented in active enhancers and promoters in multiple cell types and tissues by using HaploReg v4.1. These SNPs were enriched in the regulatory regions of several tissues (Table S4) but the best enrichment was found in induced pluripotent stem cells and thymus (Figure 2A).

Table 1. Associations of SNPs at *TMPRSS2/MX1* risk locus in linkage disequilibrium with the lead rs3787946 in different populations and prioritization scores

RS number	EA	OA	MAF	r^2	OR	P_EUR	OR	P_EAS	OR	P_SAS	OR	P_AFR	[a]Region score	[a]TSS score	[b]Predicted function	[b]Score	[c]Combined score
rs3787946	C	G	0.23	1.00	0.87	**2.73×10^{-6}**	0.63	**0.026**	0.71	**0.02**	0.74	**0.07**	0.16	0.29	INTRONIC	2	6
rs9983330	G	A	0.23	0.91	0.88	**3.12×10^{-6}**	0.54	**0.004**	0.73	**0.04**	0.79	0.16	0.31	0.64	REGULATORY	4	26
rs12329760	T	C	0.24	0.90	0.88	**3.13×10^{-6}**	0.64	**0.029**	0.76	**0.08**	0.78	0.14	0.32	0.41	MISSENSE	7	23
rs2298661	A	C	0.23	0.99	0.88	**4.51×10^{-6}**	0.63	**0.030**	0.67	**0.01**	0.60	**0.01**	0.18	0.35	INTRONIC	2	9
rs9985159	T	C	0.23	0.98	0.88	**5.80×10^{-6}**	0.61	**0.018**	0.75	**0.06**	0.98	0.89	0.16	0.46	INTRONIC	2	15
rs2298660	T	C	0.20	0.82	0.88	0.001	NA	NA	NA	NA	NA	NA	0.12	0.28	INTRONIC	2	4
rs7364088	A	G	0.26	0.84	0.91	0.002	NA	NA	NA	NA	NA	NA	0.19	0.23	INTRONIC	2	6
rs2298663	T	C	0.25	0.87	1.08	**0.005**	1.49	**0.052**	1.12	0.40	0.94	0.66	0.26	0.37	REGULATORY	4	15
rs2094881	C	T	0.25	0.87	1.08	**0.005**	1.47	**0.058**	1.10	0.47	0.93	0.60	0.29	0.26	REGULATORY	4	13
rs8131649	T	C	0.25	0.85	0.92	**0.007**	0.64	**0.035**	0.90	0.46	1.01	0.93	0.26	0.35	REGULATORY	4	12
rs8134203	T	C	0.26	0.85	1.08	**0.007**	1.49	**0.058**	1.09	0.54	0.91	0.50	0.26	0.41	REGULATORY	4	17
rs8134216	T	C	0.26	0.85	1.08	**0.007**	1.54	**0.038**	1.11	0.43	0.91	0.49	0.28	0.4	REGULATORY	4	19
rs2104810	A	G	0.26	0.85	1.08	**0.008**	1.54	**0.040**	1.10	0.47	0.90	0.48	0.23	0.35	REGULATORY	4	11
rs8131648	C	T	0.26	0.85	1.07	0.036	NA	NA	NA	NA	NA	NA	0.33	0.42	REGULATORY	4	26

In bold the SNPs that replicated in at least one cohort.

EA: Effect Allele; OA: Other Allele; EUR: European; EAS: East Asian; SAS: South Asian; AFR: African; ITA: Italian; MAF: minor allele frequency; OR: odds ratio.

[a]Scores from GWAVA predictor tool.

[b]Scores from CADD predictor tool.

[c]GWAVA and CADD scores were ranked from the smallest to largest and the obtained values were summed.

Table 2. Association of rs12329760 SNP with severe COVID-19 in Italian population

	SI cases n = 226		SI controls n = 1848		NIG cases n = 770		NIG controls n = 1915		All cases n = 996		All controls n = 3763							
Genotype	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	P_{SI}	OR (CI: 95%)	P_{NIG}	OR (CI: 95%)	P_{All}	OR (CI: 95%)
CC	164	72.6	1274	68.9	532	69.1	1289	67.3	696	69.9	2563	68.1	–		–		–	
CT	57	25.2	497	26.9	220	28.6	554	28.9	277	27.8	1051	27.9	0.47	0.89 (0.64–1.22)	0.68	0.96 (0.79–1.15)	0.71	0.97 (0.83–1.13)
TT	5	2.2	77	4.2	18	2.3	72	3.8	23	2.3	149	4.0	0.14	0.50 (0.20–1.26)	0.06	0.60 (0.35–1.02)	**0.01**	**0.57 (0.36–0.89)**
Allele																		
C	385	85.2	3045	82.4	1284	83.4	3132	81.8	1669	83.8	6177	82.1	–		–		–	
T	67	14.8	651	17.6	256	16.6	698	18.2	323	16.2	1349	17.9	0.14	0.81 (0.62–1.07)	0.16	0.89 (0.76–1.04)	**0.07**	**0.89 (0.78–1.01)**
Dominant																		
CC/CT	221	97.8	1771	95.8	752	97.7	1843	96.2	973	97.7	3614	96.0	–		–		–	
TT	5	2.2	77	4.2	18	2.3	72	3.8	23	2.3	149	4.0	0.15	0.52 (0.20–1.30)	0.06	0.61 (0.36–1.03)	**0.01**	**0.57 (0.37–0.89)**
Recessive																		
CC	159	70.4	1274	68.9	532	69.1	1289	67.3	691	69.4	2563	68.1	–		–		–	
CT/TT	62	27.4	574	31.1	238	30.9	626	32.7	300	30.1	1200	31.9	0.26	0.84 (0.61–1.14)	0.37	0.92 (0.76–1.10)	0.28	0.92 (0.79–1.07)

NIG, Network for Italian Genomes; OR, odds ratio; CI, confidence interval; SI, Southern Italy.
In bold are highlighted the statistically significant results.

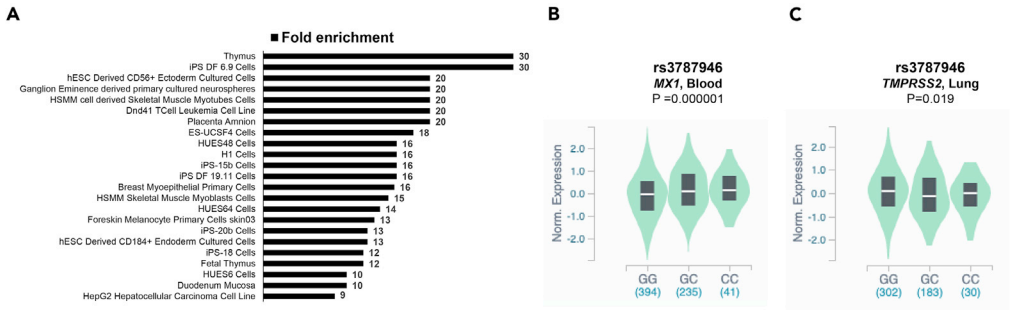

Figure 2. Enrichment of SNPs in regulatory regions and eQTL analyses

The statistically significant fold enrichments ($p < 0.05$ after Bonferroni correction) of SNPs in regulatory DNA regions active in different tissues are shown (A). eQTL violin plots between genotypes of rs3787946 (B) and rs3787946 (C) with *MX1* and *TMPRSS2* expression from the Genotype-Tissue Expression (GTEx). The significance threshold adjusted for multiple comparisons is equal to 0.000055.

Functional role of the most significant SNPs at *TMPRSS2/MX1* locus

We then investigated the predicted functional role of the 14 SNPs by GWAVA and CADD tools. We found that two of the five most significant SNPs, i.e. rs9983330 and rs12329760, showed the first (combined score = 26) and second (combined score = 23) most significant score (Table 1). The rs12329760 was classified as a coding variant (p.Val197Met) localized in the exon 6 of the *TMPRSS2* gene and was predicted to be pathogenic (PolyPhen-2 = probably damaging and SIFT = deleterious).

The most significant disease-associated SNPs are eQTLs for *MX1* in blood

We verified if the top five SNPs (Table 1) might cause gene expression alterations interrogating the GTEx portal for all the common variants within *TMPRSS2/MX1* locus. We found that all the top five SNPs had eQTL signals for *MX1* exclusively in blood tissue. Particularly, the minor alleles of these SNPs correlated with higher expression of *MX1* compared to the major alleles (Figures 2B and S2A). Of note, all the other SNPs, except for rs2298660, did not have eQTL signals for *MX1* in the blood (Table S5). The two SNPs rs12329760 and rs2298660 were confirmed as eQTLs for *MX1* in the blood ($p = 1.79 \times 10^{-6}$ and 2.8×10^{-6}, minor alleles correlated with a higher expression compared to the major alleles) by interrogation of another independent publicly available data set (Westra et al., 2013). *TMPRSS2* is highly expressed in lung (Russo et al., 2020), so we investigated if the top five SNPs were eQTLs for *TMPRSS2* in lung tissues at a nominally statistically significant level ($p \leq 0.05$). We found that the minor alleles of four out of five SNPs correlated with lower expression of *TMPRSS2* compared to the major alleles (Figures 2C and S2B). Notably, rs12329760 is also an eQTL for *TMPRSS2* in osteoblasts treated with dexamethasone (Grundberg et al., 2011).

DISCUSSION

Despite the substantial advances made in recent months in the field of SARS-CoV-2 infection, the major question remains about the identification of the factors that modulate the variable clinical spectrum of COVID-19.

Host genetic risk factors are emerging as a potential explanation for the clinical heterogeneity of COVID-19 and are also crucial to find new druggable therapeutic targets (Asselta et al., 2020; Beck and Aksentijevich, 2020; Benetti et al., 2020; Pairo-Castineira et al., 2021; Singh et al., 2020). The main host cell entry factors of SARS-CoV-2 are ACE2 and TMPRSS2 (Asselta et al., 2020; Benetti et al., 2020). The spike (S) glycoprotein of the virus binds to the ACE2 making it essential for the entry of the virus into the host cell. S-protein priming by the serine protease TMPRSS2 allows the fusion of viral and cellular membranes, resulting in virus entry and replication in the host cells (Singh et al., 2020). TMPRSS2 is emerging as a host cell factor that is critical for SARS-CoV-2 infection (Hoffmann et al., 2020b).

In our previous study, we hypothesized that common variants at chromosome 21, driving *TMPRSS2* and *MX1* expression, might have a mild-to-moderate effect on the susceptibility to SARS-CoV-2 infection. Particularly, genetic variants associated with reduced *TMPRSS2* and elevated *MX1* expression might confer

less individual susceptibility to SARS-CoV-2 infection and favor a better outcome (Russo et al., 2020). Here, to further support our hypothesis, we exploited GWAS data of a cohort of 908,494 subjects with European origins from the COVID-19 Host Genetics Initiative (COVID-19 Host Genetics Initiative, 2020) and performed an in-depth genetic analysis of chromosome 21. We identified five common variants (rs3787946, rs9983330, rs12329760, rs2298661, and rs9985159) at locus 21q22.3 within *TMPRSS2* and near the *MX1* gene that showed suggestive associations with severe COVID-19. In particular, we found that the alleles with minor frequency were less recurrent among hospitalized patients when compared to the control individuals, suggesting their protective role against the progression of the disease. Interestingly, all five SNPs were replicated in two cohorts of Asian origin, whereas two SNPs replicated in a case series of African ancestry. Additionally, we replicated the association of the rs12329760 SNP in an independent case-control cohort of Italian origin. As "proof of concept", the rs12329760 SNP was also detected in recent studies (Hou et al., 2020; Vargas-Alarcon et al., 2020). It was demonstrated that the SNP, in addition to its eQTL role, decreased the stability of the protein, which might impede viral entry (Vishnubhotla et al., 2020); moreover, *in silico* analysis demonstrated that it created a *de novo* pocket protein (Paniri et al., 2020). These results confirm 21q22.3 as a novel susceptibility locus to unfavorable outcome of COVID-19. Furthermore, molecular mechanisms underlying this genetic predisposition may be common among individuals with different ethnicity.

The results from our enrichment analysis for regulatory genomic regions suggested that the identified SNPs and other proxy SNPs located at 21q22.3 locus can be associated with different outcomes of COVID-19 by altering DNA elements that regulate the transcription of *MX1* and likely of other genes relevant to the thymus functions. The thymus plays a significant role in the regulation of adaptive immune responses. The effect of aging on the thymus and immune senescence is well established, and the resulting inflammaging is found to be implicated in the development of many chronic diseases (Gunes et al., 2020; Kellogg and Equils, 2020). Both aging and diseases of inflammaging are associated with severe COVID-19, and a dysfunctional thymus may be implicated in the unfavorable outcome of disease (Gunes et al., 2020; Kellogg and Equils, 2020). Of note, MX1 plays an important role in the thymus as part of the innate antiviral immune response. Indeed, it is exclusively expressed after engagement of the type I interferon receptor by interferon-α/β in normal fetal and post-natal human thymus, but not in the periphery. The highest level of MX1 is properly found in mature thymocytes (Colantonio et al., 2011).

The five SNPs here identified had eQTL signals for *MX1* exclusively in blood tissue. Particularly, the minor allele of these SNPs correlated with higher expression of *MX1* and associated with a minor risk of developing severe COVID-19. These results support the evidence that *MX1* can play a relevant role in determining less severe forms of disease and are in line with a recent study that suggests *MX1* as an antiviral effector against SARS-CoV-2 (Bizzotto et al., 2020). Indeed, the expression of *MX1* was found to be high in SARS-CoV-2 positive subjects, negatively correlated with age, and independently associated with increased viral load (Bizzotto et al., 2020). MX1 is part of the antiviral response induced by type I and III interferons (Zav'yalov et al., 2019). Inactivating mutations in genes belonging to type I interferon pathway and the consequently decreased levels of proteins have been shown to occur in patients with severe COVID-19 (Zhang et al., 2020).

Of note, within the region on chromosome 21, significantly associated with severe COVID-19 at the genome-wide level, the most significant signal was represented by rs13050728 that maps within the *INFRA2* gene. Particularly, *INFRA2* gene encodes for the type I membrane protein that forms the interferon-α/β receptor, involved in the canonical host antiviral signaling mediators (Duncan et al., 2015), so associated with interferon signaling like MX1. The SNP rs13050728 was previously identified as lead variant from the meta-analysis of overlapping SNPs between GenOMICC, The COVID-19 Host Genetics Initiative and 23andMe studies and its allele C was reported to reduce the odds of severe COVID-19 as associated with an increased expression of *IFNAR2* (Pairo-Castineira et al., 2021). These findings, along with ours, further strength the protective role of IFN pathway against severe COVID-19.

We also report that the minor allele of four of the top five SNPs might reduce the expression of *TMPRSS2* in lung tissues. In particular, the rs12329760 coding variant (p.Val197Met) is predicted to decrease the TMPRSS2 protein stability and ACE2 binding, thus decreasing virus entry into the cells (Vishnubhotla et al., 2020). Of note, this variant was recently found to be less frequent among Chinese patients with critical COVID-19 disease (Wang et al., 2020). Additionally, it correlates with lower expression of *TMPRSS2* in

osteoblast treated with dexamethasone (Grundberg et al., 2011), a drug currently used to inhibit an excessive inflammation response (Group et al., 2020). Together, these data suggest that even the functions of *TMPRSS2* may be affected by the occurrence of protective variants against severe COVID-19.

Finally, we want to point out that our findings highlight the effectiveness of investigating other independent (putative) risk loci, when they do not pass genome-wide significance levels. These loci, usually overlooked in extensive meta-analysis and multi-cohorts efforts, might indeed contain important genetic variants associated with severe COVID-19 and map genes relevant to the pathogenesis of this disease. We then encourage post-GWAS genetic (re)analyses using multiple data sources to unravel novel COVID-19 risk loci and possible insights on the underlying biology.

In conclusion, our results provide evidence that common variants, regulating the expression of *MX1*, can predispose to the risk of developing severe COVID-19. Unraveling the role of regulatory variants at the *TMPRSS2/MX1* locus could represent an important starting point for the treatment of COVID-19.

Limitations of the study

The data on eQTLs related to *TMPRSS2* must be interpreted with caution as these eQTL signals in the lung ($p = 0.019$) do not pass the GTEx significance threshold adjusted for multiple comparisons (0.000055). Additional studies are required to further verify the role of genetic variants at *TMPRSS2/MX1* locus in modulating the *TMPRSS2* expression. Furthermore, the statistical approach adopted in this study did not include multivariate analyses to take into account confounding factors. Although this limitation does not affect the robustness of the presented genetic associations as replicated in multiple independent cohorts, we believe that future studies will help to better define the effect of genetic variants at *TMPRSS2/MX1* locus on the clinical subgroups of COVID-19 disease; for instance, performing association analyses on patients stratified by disease aggressiveness or controlled for comorbidities in larger cohorts.

METHODS

All methods can be found in the accompanying transparent methods supplemental file.

Resource availability

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Prof. Mario Capasso, mario.capasso@unina.it.

Material availability

This study did not generate nor use any new or unique reagents.

Data and code availability

Manhattan plot and QQ plot of the results from the large GWAS "The COVID-19 Host Genetics Initiative website" are available at the website (https://www.covid19hg.org/results/). The 770 hospitalized COVID-19 cases and 1915 controls typed for rs12329760 by whole-exome sequencing were retrieved from the web database Network for Italian Genomes (NIG) available at the website (http://nigdb.cineca.it/index.php).

Prediction of the functional impact of 14 SNPs at TMPRSS2/MX1 locus was assessed by Genome Wide Annotation of VAriants (GWAVA) tool available at the website (https://www.sanger.ac.uk/sanger/StatGen_Gwava) and by Combined Annotation Dependent Depletion (CADD) tool at (https://cadd.gs.washington.edu/).

The Blood eQTL Browser is available at (https://www.genenetwork.nl/bloodeqtlbrowser/).

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.102322.

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AUTHOR CONTRIBUTIONS

I.A., R.R., and M.C. designed and conducted the study, and prepared the manuscript; M.C., V.A.L., and F.B. analyzed the data; B.E.R. sampled genomic DNA from COVID-19 patients; S.C. genotyped COVID-19 patients and in-house controls; G.F., A.P., G.M.C., G.S., G.E., I.G., C.P., R.V., G.P., P.C., C.B., and B.P. cared for COVID-19 patients; M.Z. and A.I. provided a critical review of the manuscript. All the authors read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare that there are no competing interests.

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Supplemental information

Common variants at 21q22.3 locus influence *MX1* and *TMPRSS2* gene expression and susceptibility to severe COVID-19

Immacolata Andolfo, Roberta Russo, Vito Alessandro Lasorsa, Sueva Cantalupo, Barbara Eleni Rosato, Ferdinando Bonfiglio, Giulia Frisso, Pasquale Abete, Gian Marco Cassese, Giuseppe Servillo, Gabriella Esposito, Ivan Gentile, Carmelo Piscopo, Romolo Villani, Giuseppe Fiorentino, Pellegrino Cerino, Carlo Buonerba, Biancamaria Pierri, Massimo Zollo, Achille Iolascon, and Mario Capasso

Supplemental Information

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Common variants at 21q22.3 locus influence *MX1* and *TMPRSS2* gene expression and susceptibility to severe COVID-19

Authors:

Immacolata Andolfo[1,2], Roberta Russo[1,2], Alessandro Vito Lasorsa[1,2], Sueva Cantalupo[1,2], Barbara Eleni Rosato[1,2], Ferdinando Bonfiglio[3], Giulia Frisso[1,2], Abete Pasquale[3], Gian Marco Cassese[4], Giuseppe Servillo[5], Gabriella Esposito[1,2], Ivan Gentile[6], Carmelo Piscopo[7], Romolo Villani[8], Giuseppe Fiorentino[9], Pellegrino Cerino[10], Carlo Buonerba[10], Biancamaria Pierri[10,11], Massimo Zollo[1,2], Achille Iolascon[1,2], Mario Capasso[1,2]

[1]Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, Napoli, Italy
[2]CEINGE Biotecnologie Avanzate, Napoli, Italy
[3]Dipartimento di Ingegneria chimica, dei Materiali e della Produzione industriale, Università degli Studi di Napoli Federico II, Napoli, Italy
[4]COVID Hospital, P.O.S. Anna e SS. Madonna della Neve di Boscotrecase, Ospedali Riuniti Area Vesuviana, Napoli, Italy
[5]Dipartimento di Neuroscienze e Scienze riproduttive ed odontostomatologiche, Università degli Studi di Napoli Federico II, Napoli, Italy
[6]Dipartimento di Medicina clinica e Chirurgia, Università degli Studi di Napoli Federico II, Napoli, Italy
[7]Medical and Laboratory Genetics Unit, A.O.R.N. 'Antonio Cardarelli', Napoli, Italy
[8] Poison Centre, A.O.R.N. 'Antonio Cardarelli', Napoli, Italy
[9]AORN dei Colli Presidio Ospedaliero Cotugno, Napoli, Italy
[10]Istituto Zooprofilattico Sperimentale del Mezzogiorno, Napoli, Italy
[11]Dipartimento di Medicina, Chirurgia e Odontoiatria "Scuola Medica Salernitana", Università di Salerno, Baronissi, Italy

Corresponding author and lead contact

Mario Capasso, Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, Napoli, Italy; CEINGE Biotecnologie Avanzate, Napoli, Italy; mario.capasso@unina.it

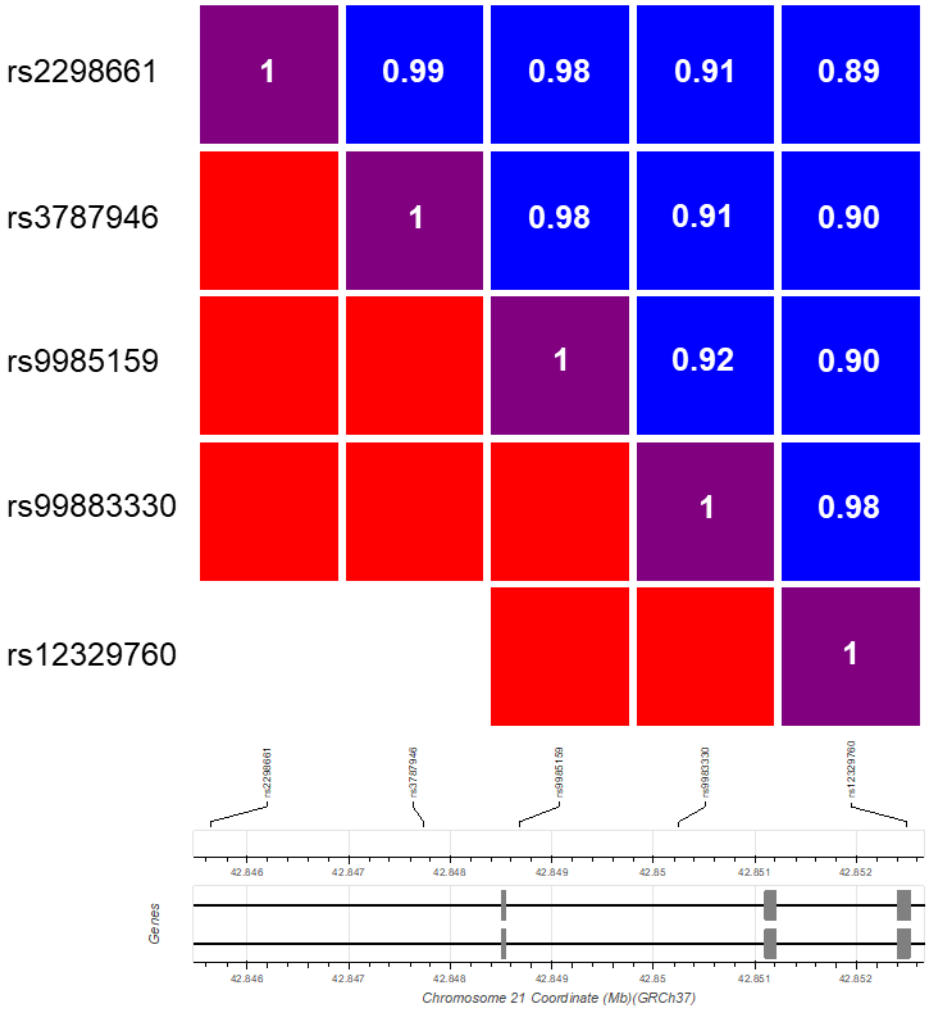

Figure S1. Linkage disequilibrium block at *TMPRSS2/MX1* locus. Related to Figure 1.
Linkage disequilibrium of the 5 most significant SNPs (P-values ranged from 2.7×10^{-6} to 5.8×10^{-6}) with the lead rs3787946 at *TMPRSS2/MX1* locus. The D' and r^2 data are computed with the genetic information from the European population by using the web tool LD-link (https://ldlink.nci.nih.gov/?tab=home).

Figure S2.

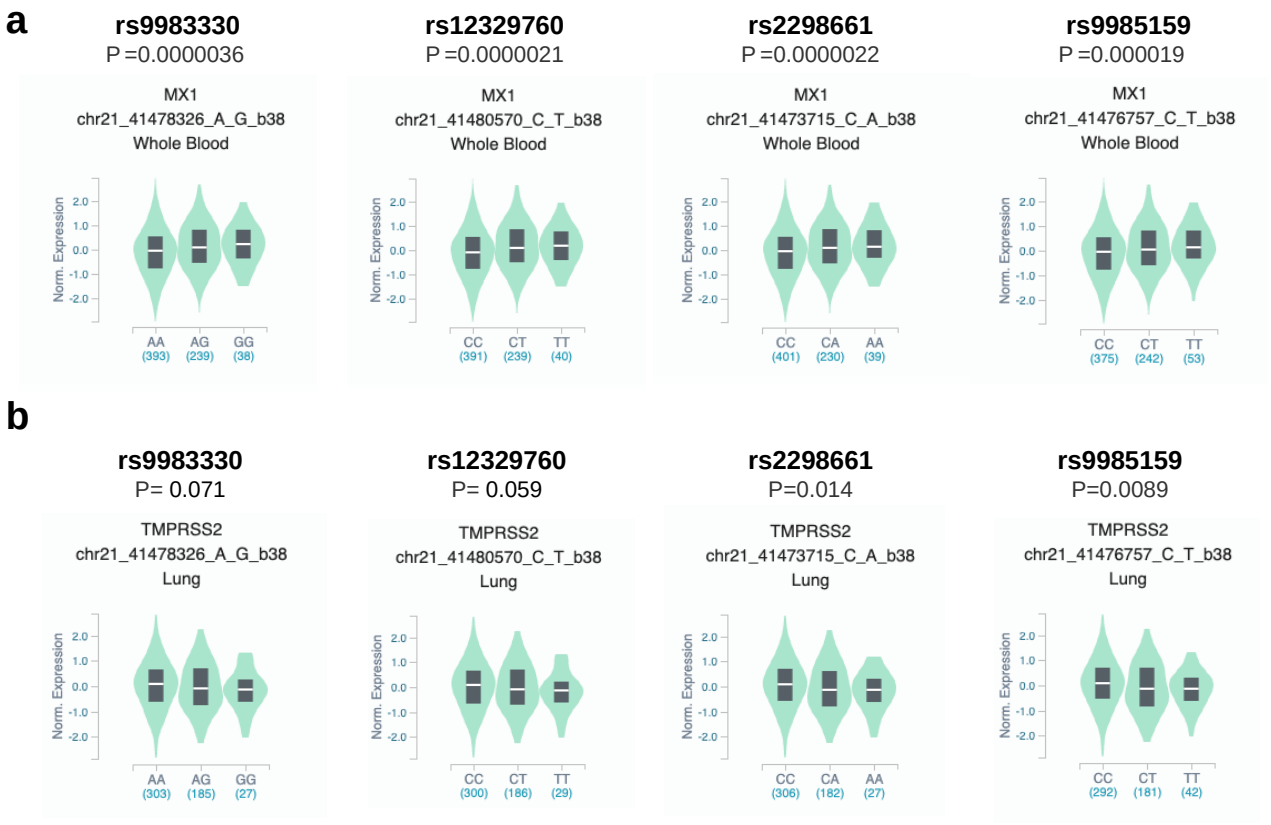

Figure S2. Analysis of the eQTL signals of the top four disease-associated SNPs in LD with the lead SNP rs3787946. Related to Figure 2.

Violin plots showing the eQTL signals for the rs9983330, rs12329760, rs2298661, and rs9985159 on *MX1* expression in whole blood (a) and on *TMPRSS2* expression in lung (b). The significance threshold adjusted for multiple comparisons is 0.000055.

Table S1. Study groups that have contributed to GWAS meta-analyses of the COVID-19 Host Genetics Initiative. Related to Figure 1.

Name	n_cases	n_controls
Amsterdam_UMC_COVID_study_group_EUR	108	1413
DECODE_EUR	89	274322
BelCovid_EUR	109	1484
GENCOVID_EUR	571	2472
FinnGen_FIN	83	238628
SPGRX_EUR	311	302
HOSTAGE_EUR	1610	2205
BQC19_EUR	181	354
UKBB_EUR	765	364341
MVP_EUR	436	2180
BoSCO_EUR	139	262
Ancestry_EUR	250	1967
SweCovid_EUR	78	3778
genomicc_EUR	1676	8380
	6406	902088

EUR: individuals have European origins

FIN: individuals with Finnish origins

Table S2. Summary statistics at chromosome 21 from GWAS dataset (COVID-19 Host Genetics Initiative, "B2_ALL_eur_leave_23andme"). Related to Figure 1.

CHR	POS	REF	ALT	SNP	all_meta_N	all_inv_var_meta_beta	all_inv_var_meta_sebeta	all_inv_var_meta_p	all_inv_var_het_p	all_meta_sample_N	all_meta_AF	rsid	OR	CI95L	CI95U
21	34615210	T	C	21:34615210:T:C	13	-1.82E-01	2.60E-02	2.76E-12	3.46E-02	905878	6.56E-01	rs13050728	0.834	0.791	0.876
21	34614834	G	A	21:34614834:G:A	13	-1.80E-01	2.60E-02	4.61E-12	3.53E-02	905878	6.56E-01	rs9976829	0.835	0.793	0.878
21	34617729	A	G	21:34617729:A:G	13	-1.80E-01	2.60E-02	4.98E-12	4.11E-02	905878	6.57E-01	rs2252639	0.836	0.793	0.878
21	34620451	G	A	21:34620451:G:A	13	-1.80E-01	2.60E-02	5.18E-12	3.23E-02	905878	6.57E-01	rs2834163	0.836	0.793	0.878
21	34619445	A	G	21:34619445:A:G	13	-1.80E-01	2.60E-02	5.38E-12	3.35E-02	905878	6.57E-01	rs2073361	0.836	0.793	0.878
21	34620207	C	T	21:34620207:C:T	13	-1.79E-01	2.60E-02	5.66E-12	3.15E-02	905878	6.57E-01	rs2834161	0.836	0.793	0.879
21	34616923	C	A	21:34616923:C:A	13	-1.79E-01	2.60E-02	5.90E-12	3.47E-02	905878	6.56E-01	NA	0.836	0.794	0.879
21	34617950	A	T	21:34617950:A:T	13	-1.79E-01	2.60E-02	5.95E-12	5.02E-02	905878	6.57E-01	rs2252650	0.836	0.793	0.879
21	34624917	A	G	21:34624917:A:G	13	-1.79E-01	2.67E-02	2.01E-11	2.69E-02	905878	6.93E-01	rs2236757	0.836	0.793	0.880
21	34618043	A	T	21:34618043:A:T	13	-1.77E-01	2.66E-02	3.38E-11	3.41E-02	905878	6.93E-01	rs2284549	0.838	0.794	0.882
21	34618313	A	G	21:34618313:A:G	13	-1.76E-01	2.66E-02	3.53E-11	3.29E-02	905878	6.93E-01	rs2284551	0.838	0.795	0.882
21	34616545	A	G	21:34616545:A:G	13	-1.75E-01	2.66E-02	4.50E-11	2.85E-02	905878	6.93E-01	rs2834157	0.839	0.796	0.883
21	34606634	A	C	21:34606634:A:C	13	1.65E-01	2.60E-02	2.22E-10	6.95E-02	905878	3.43E-01	rs2834154	1.179	1.119	1.239
21	34609944	A	G	21:34609944:A:G	14	1.57E-01	2.48E-02	2.31E-10	7.43E-02	908494	3.43E-01	rs9636867	1.170	1.113	1.227
21	34607436	G	A	21:34607436:G:A	13	1.65E-01	2.60E-02	2.35E-10	6.71E-02	905878	3.43E-01	rs6517153	1.179	1.119	1.239
21	34613301	A	G	21:34613301:A:G	14	1.57E-01	2.47E-02	2.39E-10	8.60E-02	908494	3.43E-01	rs17860169	1.170	1.113	1.226
21	34611571	C	G	21:34611571:C:G	13	1.58E-01	2.50E-02	2.58E-10	5.65E-02	634083	3.37E-01	NA	1.171	1.114	1.229
21	34603249	C	G	21:34603249:C:G	13	1.64E-01	2.60E-02	2.79E-10	5.72E-02	905878	3.43E-01	NA	1.178	1.118	1.238
21	34604557	G	A	21:34604557:G:A	13	1.64E-01	2.60E-02	2.87E-10	6.01E-02	905878	3.43E-01	rs2300370	1.178	1.118	1.238
21	34602934	T	C	21:34602934:T:C	13	1.64E-01	2.60E-02	2.88E-10	5.30E-02	905878	3.43E-01	rs12482556	1.178	1.118	1.238
21	34602305	C	A	21:34602305:C:A	13	1.64E-01	2.60E-02	3.01E-10	8.97E-02	905878	3.43E-01	NA	1.178	1.118	1.238
21	34623919	A	G	21:34623919:A:G	14	2.59E-01	4.17E-02	4.86E-10	9.05E-02	908494	7.73E-02	rs17860220	1.296	1.190	1.402
21	34614250	T	C	21:34614250:T:C	14	2.53E-01	4.08E-02	5.19E-10	7.12E-02	908494	7.77E-02	rs2229207	1.288	1.185	1.391

21	34620801	A	G	21:34620801:A:G	14	2.56E-01	4.14E-02	5.56E-10	8.71E-02	908494	7.81E-02	rs2073362	1.292	1.188	1.397
21	34614255	T	G	21:34614255:T:G	13	1.52E-01	2.48E-02	9.15E-10	1.37E-01	907881	3.43E-01	NA	1.164	1.108	1.221
21	34602794	G	T	21:34602794:G:T	14	2.50E-01	4.12E-02	1.34E-09	4.25E-02	908494	7.74E-02	rs17860118	1.284	1.180	1.388
21	34607870	C	G	21:34607870:C:G	13	1.61E-01	2.66E-02	1.53E-09	8.03E-02	905878	3.07E-01	rs17860142	1.174	1.113	1.235
21	34605778	C	T	21:34605778:C:T	13	1.60E-01	2.66E-02	1.83E-09	8.61E-02	905878	3.07E-01	rs2248420	1.174	1.112	1.235
21	34618439	G	A	21:34618439:G:A	14	1.50E-01	2.54E-02	3.17E-09	6.95E-02	908494	3.09E-01	rs12053666	1.162	1.104	1.220
21	34611730	C	T	21:34611730:C:T	14	1.50E-01	2.54E-02	3.23E-09	7.79E-02	908494	3.06E-01	rs17860165	1.162	1.104	1.220
21	34593710	T	A	21:34593710:T:A	13	1.57E-01	2.66E-02	3.53E-09	1.12E-01	905878	3.10E-01	rs62226132	1.170	1.109	1.231
21	34596750	C	T	21:34596750:C:T	13	1.57E-01	2.66E-02	3.84E-09	9.87E-02	905878	3.10E-01	rs62226152	1.170	1.109	1.231
21	34599084	G	A	21:34599084:G:A	13	1.56E-01	2.66E-02	4.17E-09	1.02E-01	905878	3.10E-01	NA	1.169	1.108	1.230
21	34600508	G	T	21:34600508:G:T	13	1.56E-01	2.66E-02	4.84E-09	9.04E-02	905878	3.09E-01	NA	1.168	1.108	1.229
21	34611318	C	T	21:34611318:C:T	14	1.48E-01	2.54E-02	5.76E-09	7.94E-02	908494	3.07E-01	rs12482014	1.159	1.102	1.217
21	34611545	T	C	21:34611545:T:C	14	1.47E-01	2.53E-02	5.92E-09	5.32E-02	908494	3.07E-01	rs12482193	1.159	1.101	1.216
21	34609505	G	A	21:34609505:G:A	13	1.48E-01	2.55E-02	7.50E-09	7.10E-02	907881	3.06E-01	rs3153	1.159	1.101	1.217
21	34610487	T	C	21:34610487:T:C	12	1.34E-01	2.47E-02	6.37E-08	2.79E-01	905265	5.74E-01	rs1131964	1.143	1.088	1.199
21	34622536	A	G	21:34622536:A:G	13	-1.31E-01	2.48E-02	1.11E-07	2.66E-02	905878	4.86E-01	rs2834165	0.877	0.834	0.919
21	34621948	A	C	21:34621948:A:C	13	-1.31E-01	2.47E-02	1.26E-07	2.47E-02	905878	4.86E-01	rs2834164	0.877	0.835	0.920
21	34618285	G	A	21:34618285:G:A	13	-1.30E-01	2.47E-02	1.47E-07	1.98E-02	905878	4.88E-01	rs2284550	0.878	0.836	0.921
21	34611992	A	G	21:34611992:A:G	12	1.29E-01	2.50E-02	2.42E-07	3.00E-01	667167	5.86E-01	NA	1.138	1.082	1.193
21	34593574	G	C	21:34593574:G:C	13	1.27E-01	2.47E-02	2.91E-07	1.76E-01	905878	5.67E-01	NA	1.135	1.080	1.190
21	34602246	T	G	21:34602246:T:G	11	1.28E-01	2.52E-02	3.72E-07	1.56E-01	392756	5.55E-01	NA	1.137	1.081	1.193
21	34598385	A	C	21:34598385:A:C	12	1.26E-01	2.48E-02	3.82E-07	1.38E-01	905265	5.67E-01	rs1476415	1.134	1.079	1.189
21	34609596	T	G	21:34609596:T:G	12	1.26E-01	2.48E-02	4.00E-07	2.83E-01	905265	5.74E-01	NA	1.134	1.079	1.189
21	34617213	T	C	21:34617213:T:C	12	-1.57E-01	3.18E-02	7.99E-07	3.58E-02	895822	6.56E-01	rs2834158	0.855	0.801	0.908
21	34626854	C	T	21:34626854:C:T	12	-1.52E-01	3.21E-02	2.04E-06	6.00E-02	895822	6.64E-01	rs9975538	0.859	0.805	0.913
21	35377591	C	T	21:35377591:C:T	14	1.62E-01	3.43E-02	2.25E-06	1.81E-01	908494	1.29E-01	NA	1.176	1.097	1.255
21	35362848	G	A	21:35362848:G:A	14	1.56E-01	3.30E-02	2.39E-06	2.15E-01	908494	1.33E-01	rs111783124	1.169	1.093	1.244
21	34649337	A	G	21:34649337:A:G	13	2.07E-01	4.40E-02	2.58E-06	2.73E-02	898438	1.25E-01	NA	1.230	1.124	1.336

21	42847735	G	C	21:42847735:G:C	14	-1.34E-01	2.86E-02	2.73E-06	8.59E-01	908494	2.64E-01	rs3787946	0.875	0.825	0.924
21	35363759	T	G	21:35363759:T:G	14	1.55E-01	3.30E-02	2.79E-06	2.15E-01	908494	1.33E-01	rs11088268	1.167	1.092	1.243
21	34625413	A	G	21:34625413:A:G	12	-1.49E-01	3.19E-02	2.90E-06	5.10E-02	895822	6.64E-01	rs2236758	0.861	0.808	0.915
21	34632316	T	C	21:34632316:T:C	13	1.40E-01	2.99E-02	2.90E-06	1.71E-01	898438	3.36E-01	rs2250226	1.150	1.083	1.217
21	42850253	A	G	21:42850253:A:G	14	-1.32E-01	2.84E-02	3.12E-06	9.08E-01	908494	2.76E-01	rs9983330	0.876	0.827	0.925
21	42852497	C	T	21:42852497:C:T	14	-1.32E-01	2.83E-02	3.13E-06	8.87E-01	908494	2.75E-01	rs12329760	0.876	0.828	0.925
21	38577172	A	C	21:38577172:A:C	8	7.37E-01	1.58E-01	3.29E-06	2.81E-01	380965	1.53E-02	rs56309117	2.089	1.441	2.738
21	35382261	G	A	21:35382261:G:A	14	1.59E-01	3.44E-02	3.85E-06	2.81E-01	908494	1.26E-01	NA	1.172	1.093	1.251
21	42864074	C	T	21:42864074:C:T	14	-1.23E-01	2.67E-02	4.25E-06	8.86E-01	908494	3.23E-01	rs9305745	0.884	0.838	0.931
21	34629175	A	G	21:34629175:A:G	13	1.37E-01	2.98E-02	4.40E-06	2.11E-01	898438	3.36E-01	rs11911133	1.147	1.080	1.214
21	34592463	T	C	21:34592463:T:C	14	2.64E-01	5.76E-02	4.45E-06	7.03E-01	908494	4.03E-02	rs112268545	1.302	1.155	1.449
21	42845642	C	A	21:42845642:C:A	14	-1.32E-01	2.87E-02	4.51E-06	8.81E-01	908494	2.64E-01	NA	0.877	0.827	0.926
21	34631133	A	G	21:34631133:A:G	13	1.36E-01	2.97E-02	4.62E-06	2.02E-01	898438	3.36E-01	rs17860241	1.146	1.079	1.212
21	42863723	T	A	21:42863723:T:A	14	-1.22E-01	2.67E-02	4.76E-06	9.23E-01	908494	3.22E-01	rs10154090	0.885	0.839	0.931
21	34634045	C	G	21:34634045:C:G	12	1.47E-01	3.23E-02	4.94E-06	1.04E-01	895822	3.34E-01	rs6517156	1.159	1.085	1.232
21	42857322	C	G	21:42857322:C:G	14	-1.26E-01	2.75E-02	5.00E-06	9.18E-01	908494	3.10E-01	rs9983252	0.882	0.834	0.929
21	34590250	G	A	21:34590250:G:A	14	2.60E-01	5.71E-02	5.25E-06	7.12E-01	908494	4.02E-02	rs79997810	1.297	1.152	1.442
21	35368402	G	T	21:35368402:G:T	13	1.52E-01	3.35E-02	5.25E-06	1.42E-01	634083	1.25E-01	rs12627254	1.165	1.088	1.241
21	34627774	C	G	21:34627774:C:G	11	-1.48E-01	3.27E-02	5.57E-06	9.49E-02	621411	6.72E-01	rs11701402	0.862	0.807	0.917
21	42848684	C	T	21:42848684:C:T	14	-1.29E-01	2.85E-02	5.80E-06	9.14E-01	908494	2.64E-01	NA	0.879	0.829	0.928
21	35383937	A	G	21:35383937:A:G	14	1.55E-01	3.43E-02	5.83E-06	3.00E-01	908494	1.27E-01	rs11088269	1.168	1.090	1.247
21	35395439	G	C	21:35395439:G:C	14	1.55E-01	3.45E-02	7.06E-06	2.50E-01	908494	1.24E-01	rs11702497	1.168	1.089	1.247
21	42856544	T	C	21:42856544:T:C	14	-1.23E-01	2.75E-02	7.80E-06	9.20E-01	908494	3.11E-01	rs2838039	0.884	0.837	0.932

The lead SNPs of the 3 independent loci are colored in yellow

Table S3. Characteristics of Italian patients recruited by our research group. Related to Table 1.

Characteristic	Severe cases	%
	N=226	
Age		
Years, mean (standard deviation)	62.3 (16.6)	
Unknown	5	
Sex - no. (%)		
Male	142	62.8
Female	74	32.8
Unknown	10	4.4
Previous coexisting disease - no. (%)		
0-2	136	60.2
>=3	41	18.1
Unknown	49	21.7
Oxigen Therapy		
No Mechanical ventilation or Intubation	105	46.5
Mechanical ventilation or Intubation	81	35.8
Unknown	40	17.7

Table S4. Results of SNP enrichment analysis in regulatory elements in different tissues and cell types. Related to Figure 2.

Cell	Observed	Expected	Fold	Binomial p	^adjusted_P
E112 THYM (Thymus)	6	0.2	30.0	0	0
E021 IPSC.DF.6.9 (iPS DF 6.9 Cells)	6	0.2	30.0	0	0
E012 ESDR.CD56.ECTO (hESC Derived CD56+ Ectoderm Cultured Cells)	8	0.4	20.0	0	0
E054 BRN.GANGEM.DR.NRSPHR (Ganglion Eminence derived primary cultured neurospheres)	8	0.4	20.0	0	0
E099 PLCNT.AMN (Placenta Amnion)	6	0.3	20.0	0	0
E115 BLD.DND41.CNCR (Dnd41 TCell Leukemia Cell Line)	6	0.3	20.0	0	0
E121 MUS.HSMMT (HSMM cell derived Skeletal Muscle Myotubes Cells)	8	0.4	20.0	0	0
E024 ESC.4STAR (ES-UCSF4 Cells)	9	0.5	18.0	0	0
E014 ESC.HUES48 (HUES48 Cells)	8	0.5	16.0	0	0
E003 ESC.H1 (H1 Cells)	8	0.5	16.0	0	0
E018 IPSC.15b (iPS-15b Cells)	8	0.5	16.0	0	0
E022 IPSC.DF.19.11 (iPS DF 19.11 Cells)	8	0.5	16.0	0	0
E027 BRST.MYO (Breast Myoepithelial Primary Cells)	11	0.7	15.7	0	0
E120 MUS.HSMM (HSMM Skeletal Muscle Myoblasts Cells)	6	0.4	15.0	3.00E-06	0.000381
E008 ESC.H9 (H9 Cells)	3	0.2	15.0	0.001534	0.194818
E016 ESC.HUES64 (HUES64 Cells)	7	0.5	14.0	0	0
E061 SKIN.PEN.FRSK.MEL.03 (Foreskin Melanocyte Primary Cells skin03)	8	0.6	13.3	0	0
E020 IPSC.20B (iPS-20b Cells)	5	0.4	12.5	3.30E-05	0.0042
E011 ESDR.CD184.ENDO (hESC Derived CD184+ Endoderm Cultured Cells)	5	0.4	12.5	5.20E-05	0.0066
E019 IPSC.18 (iPS-18 Cells)	6	0.5	12.0	6.00E-06	0.0008
E093 THYM.FET (Fetal Thymus)	6	0.5	12.0	6.00E-06	0.0008
E015 ESC.HUES6 (HUES6 Cells)	6	0.6	10.0	9.00E-06	0.0011
E077 GI.DUO.MUC (Duodenum Mucosa)	4	0.4	10.0	0.000381	0.0484
E098 PANC (Pancreas)	4	0.4	10.0	0.000738	0.0937
E094 GI.STMC.GAST (Gastric)	3	0.3	10.0	0.002056	0.2611
E007 ESDR.H1.NEUR.PROG (H1 Derived Neuronal Progenitor Cultured Cells)	3	0.3	10.0	0.002349	0.2983
E075 GI.CLN.MUC (Colonic Mucosa)	2	0.2	10.0	0.01355	1.7209
E101 GI.RECT.MUC.29 (Rectal Mucosa Donor 29)	2	0.2	10.0	0.021315	2.7070
E118 LIV.HEPG2.CNCR (HepG2 Hepatocellular Carcinoma Cell Line)	6	0.7	8.6	3.20E-05	0.0041
E074 BRN.SUB.NIG (Brain Substantia Nigra)	3	0.4	7.5	0.008348	1.0602
E059 SKIN.PEN.FRSK.MEL.01 (Foreskin Melanocyte Primary Cells skin01)	2	0.3	6.7	0.044405	5.6394
E090 MUS.LEG.FET (Fetal Muscle Leg)	5	0.8	6.3	0.000715	0.0908
E071 BRN.HIPP.MID (Brain Hippocampus Middle)	3	0.5	6.0	0.011249	1.4286

E053 BRN.CRTX.DR.NRSPHR (Cortex derived primary cultured neurospheres)	3	0.5	6.0	0.014938	1.8971
E001 ESC.I3 (ES-I3 Cells)	3	0.5	6.0	0.014978	1.9022
E088 LNG.FET (Fetal Lung)	3	0.6	5.0	0.016702	2.1212
E102 GI.RECT.MUC.31 (Rectal Mucosa Donor 31)	2	0.4	5.0	0.049562	6.2944
E013 ESDR.CD56.MESO (hESC Derived CD56+ Mesoderm Cultured Cells)	2	0.4	5.0	0.071638	9.0980
E002 ESC.WA7 (ES-WA7 Cells)	1	0.2	5.0	0.175795	22.3260
E109 GI.S.INT (Small Intestine)	1	0.2	5.0	0.181152	23.0063
E110 GI.STMC.MUC (Stomach Mucosa)	2	0.5	4.0	0.085095	10.8071
E066 LIV.ADLT (Liver)	2	0.5	4.0	0.099955	12.6943
E089 MUS.TRNK.FET (Fetal Muscle Trunk)	2	0.6	3.3	0.119051	15.1195
E070 BRN.GRM.MTRX (Brain Germinal Matrix)	1	0.3	3.3	0.28264	35.8953
E072 BRN.INF.TMP (Brain Inferior Temporal Lobe)	1	0.4	2.5	0.322209	40.9205
E068 BRN.ANT.CAUD (Brain Anterior Caudate)	1	0.4	2.5	0.335088	42.5562
E069 BRN.CING.GYR (Brain Cingulate Gyrus)	1	0.4	2.5	0.337134	42.8160
E116 BLD.GM12878 (GM12878 Lymphoblastoid Cells)	1	0.4	2.5	0.343155	43.5807
E026 STRM.MRW.MSC (Bone Marrow Derived Cultured Mesenchymal Stem Cells)	1	0.5	2.0	0.394234	50.0677
E005 ESDR.H1.BMP4.TROP (H1 BMP4 Derived Trophoblast Cultured Cells)	1	0.5	2.0	0.40053	50.8673
E084 GI.L.INT.FET (Fetal Intestine Large)	1	0.5	2.0	0.404646	51.3900
E129 BONE.OSTEO (Osteoblast Primary Cells)	1	0.5	2.0	0.41587	52.8155
E085 GI.S.INT.FET (Fetal Intestine Small)	1	0.5	2.0	0.418745	53.1806
E057 SKIN.PEN.FRSK.KER.02 (Foreskin Keratinocyte Primary Cells skin02)	1	0.5	2.0	0.425214	54.0022
E006 ESDR.H1.MSC (H1 Derived Mesenchymal Stem Cells)	1	0.5	2.0	0.426514	54.1673
E119 BRST.HMEC (HMEC Mammary Epithelial Primary Cells)	1	0.6	1.7	0.449466	57.0822
E028 BRST.HMEC.35 (Breast variant Human Mammary Epithelial Cells (vHMEC))	1	0.6	1.7	0.458849	58.2738
E091 PLCNT.FET (Placenta)	1	0.6	1.7	0.481212	61.1139
E017 LNG.IMR90 (IMR90 fetal lung fibroblasts Cell Line)	0	0.6	0.0	1	1
E009 ESDR.H9.NEUR.PROG (H9 Derived Neuronal Progenitor Cultured Cells)	0	0.4	0.0	1	1
E010 ESDR.H9.NEUR (H9 Derived Neuron Cultured Cells)	0	0.5	0.0	1	1
E004 ESDR.H1.BMP4.MESO (H1 BMP4 Derived Mesendoderm Cultured Cells)	0	0.3	0.0	1	1
E062 BLD.PER.MONUC.PC (Primary mononuclear cells from peripheral blood)	0	0.2	0.0	1	1
E034 BLD.CD3.PPC (Primary T cells from peripheral blood)	0	0.5	0.0	1	1
E045 BLD.CD4.CD25I.CD127.TMEMPC (Primary T cells effector/memory enriched from peripheral blood)	0	0.2	0.0	1	1
E033 BLD.CD3.CPC (Primary T cells from cord blood)	0	0.3	0.0	1	1
E044 BLD.CD4.CD25.CD127M.TREGPC (Primary T regulatory cells from peripheral blood)	0	0.3	0.0	1	1
E043 BLD.CD4.CD25M.TPC (Primary T helper cells from peripheral blood)	0	0.5	0.0	1	1
E039 BLD.CD4.CD25M.CD45RA.NPC (Primary T helper naive cells from peripheral blood)	0	0.4	0.0	1	1

E041 BLD.CD4.CD25M.IL17M.PL.TPC (Primary T helper cells PMA-I stimulated)	0	0.5	0.0	1	1
E042 BLD.CD4.CD25M.IL17P.PL.TPC (Primary T helper 17 cells PMA-I stimulated)	0	0.4	0.0	1	1
E040 BLD.CD4.CD25M.CD45RO.MPC (Primary T helper memory cells from peripheral blood 1)	0	0.4	0.0	1	1
E037 BLD.CD4.MPC (Primary T helper memory cells from peripheral blood 2)	0	0.5	0.0	1	1
E048 BLD.CD8.MPC (Primary T CD8+ memory cells from peripheral blood)	0	0.3	0.0	1	1
E038 BLD.CD4.NPC (Primary T helper naive cells from peripheral blood)	0	0.4	0.0	1	1
E047 BLD.CD8.NPC (Primary T CD8+ naive cells from peripheral blood)	0	0.4	0.0	1	1
E029 BLD.CD14.PC (Primary monocytes from peripheral blood)	0	0.6	0.0	1	1
E031 BLD.CD19.CPC (Primary B cells from cord blood)	0	0.4	0.0	1	1
E035 BLD.CD34.PC (Primary hematopoietic stem cells)	0	0.4	0.0	1	1
E051 BLD.MOB.CD34.PC.M (Primary hematopoietic stem cells G-CSF-mobilized Male)	0	0.6	0.0	1	1
E050 BLD.MOB.CD34.PC.F (Primary hematopoietic stem cells G-CSF-mobilized Female)	0	0.6	0.0	1	1
E036 BLD.CD34.CC (Primary hematopoietic stem cells short term culture)	0	0.5	0.0	1	1
E032 BLD.CD19.PPC (Primary B cells from peripheral blood)	0	0.5	0.0	1	1
E046 BLD.CD56.PC (Primary Natural Killer cells from peripheral blood)	0	0.5	0.0	1	1
E030 BLD.CD15.PC (Primary neutrophils from peripheral blood)	0	0.4	0.0	1	1
E049 STRM.CHON.MRW.DR.MSC (Mesenchymal Stem Cell Derived Chondrocyte Cultured Cells)	0	0.6	0.0	1	1
E025 FAT.ADIP.DR.MSC (Adipose Derived Mesenchymal Stem Cell Cultured Cells)	0	0.8	0.0	1	1
E023 FAT.MSC.DR.ADIP (Mesenchymal Stem Cell Derived Adipocyte Cultured Cells)	0	0.6	0.0	1	1
E052 MUS.SAT (Muscle Satellite Cultured Cells)	0	0.6	0.0	1	1
E055 SKIN.PEN.FRSK.FIB.01 (Foreskin Fibroblast Primary Cells skin01)	0	0.6	0.0	1	1
E056 SKIN.PEN.FRSK.FIB.02 (Foreskin Fibroblast Primary Cells skin02)	0	0.4	0.0	1	1
E058 SKIN.PEN.FRSK.KER.03 (Foreskin Keratinocyte Primary Cells skin03)	0	0.6	0.0	1	1
E067 BRN.ANG.GYR (Brain Angular Gyrus)	0	0.3	0.0	1	1
E073 BRN.DL.PRFRNTL.CRTX (Brain Dorsolateral Prefrontal Cortex)	0	0.3	0.0	1	1
E082 BRN.FET.F (Fetal Brain Female)	0	0.2	0.0	1	1
E081 BRN.FET.M (Fetal Brain Male)	0	0.5	0.0	1	1
E063 FAT.ADIP.NUC (Adipose Nuclei)	0	0.5	0.0	1	1
E100 MUS.PSOAS (Psoas Muscle)	0	0.3	0.0	1	1
E108 MUS.SKLT.F (Skeletal Muscle Female)	0	0.6	0.0	1	1
E107 MUS.SKLT.M (Skeletal Muscle Male)	0	0.6	0.0	1	1
E083 HRT.FET (Fetal Heart)	0	0.7	0.0	1	1
E104 HRT.ATR.R (Right Atrium)	0	0.4	0.0	1	1
E095 HRT.VENT.L (Left Ventricle)	0	0.5	0.0	1	1
E105 HRT.VNT.R (Right Ventricle)	0	0.4	0.0	1	1
E065 VAS.AOR (Aorta)	0	0.1	0.0	1	1

E078 GI.DUO.SM.MUS (Duodenum Smooth Muscle)	0	0.3	0.0	1	1
E076 GI.CLN.SM.MUS (Colon Smooth Muscle)	0	0.4	0.0	1	1
E103 GI.RECT.SM.MUS (Rectal Smooth Muscle)	0	0.3	0.0	1	1
E111 GI.STMC.MUS (Stomach Smooth Muscle)	0	0.3	0.0	1	1
E092 GI.STMC.FET (Fetal Stomach)	0	0.5	0.0	1	1
E106 GI.CLN.SIG (Sigmoid Colon)	0	0.3	0.0	1	1
E079 GI.ESO (Esophagus)	0	0.3	0.0	1	1
E086 KID.FET (Fetal Kidney)	0	0.2	0.0	1	1
E097 OVRY (Ovary)	0	0.4	0.0	1	1
E087 PANC.ISLT (Pancreatic Islets)	0	0.2	0.0	1	1
E080 ADRL.GLND.FET (Fetal Adrenal Gland)	0	0.7	0.0	1	1
E096 LNG (Lung)	0	0.3	0.0	1	1
E113 SPLN (Spleen)	0	0.4	0.0	1	1
E114 LNG.A549.ETOH002.CNCR (A549 EtOH 0.02pct Lung Carcinoma Cell Line)	0	0.4	0.0	1	1
E117 CRVX.HELAS3.CNCR (HeLa-S3 Cervical Carcinoma Cell Line)	0	0.4	0.0	1	1
E122 VAS.HUVEC (HUVEC Umbilical Vein Endothelial Primary Cells)	0	0.5	0.0	1	1
E123 BLD.K562.CNCR (K562 Leukemia Cells)	0	0.4	0.0	1	1
E124 BLD.CD14.MONO (Monocytes-CD14+ RO01746 Primary Cells)	0	0.4	0.0	1	1
E125 BRN.NHA (NH-A Astrocytes Primary Cells)	0	0.4	0.0	1	1
E126 SKIN.NHDFAD (NHDF-Ad Adult Dermal Fibroblast Primary Cells)	0	0.6	0.0	1	1
E127 SKIN.NHEK (NHEK-Epidermal Keratinocyte Primary Cells)	0	0.5	0.0	1	1
E128 LNG.NHLF (NHLF Lung Fibroblast Primary Cells)	0	0.4	0.0	1	1

^P-values corrected according to Bonferroni method

Table S5. Results of eQTL analysis for the common variants at *TMPRSS2/MX1* locus. Related to Figure 2.

Gene	SNP	GWAS_P	eQTL_P	eQTL_P Threshold	*Statistically significant eQTL	NES	T-statistic	Tissue
MX1	rs3787946	2.73E-06	0.0000011	0.000064	YES	0.17	4.9	Whole Blood
MX1	rs12329760	3.13E-06	0.0000021	0.000064	YES	0.17	4.8	Whole Blood
MX1	rs2298661	4.51E-06	0.0000022	0.000064	YES	0.17	4.8	Whole Blood
MX1	rs9983330	3.12E-06	0.0000036	0.000064	YES	0.16	4.7	Whole Blood
MX1	rs2298660	6.28E-04	0.0000140	0.000064	YES	0.15	4.4	Whole Blood
MX1	rs9985159	5.80E-06	0.0000190	0.000064	YES	0.15	4.3	Whole Blood
MX1	rs2094881	5.17E-03	0.0000660	0.000064	0	-0.14	-4	Whole Blood
MX1	rs7364088	2.27E-03	0.0000760	0.000064	0	0.13	4	Whole Blood
MX1	rs8131648	3.58E-02	0.0001100	0.000064	0	-0.13	-3.9	Whole Blood
MX1	rs8131649	6.55E-03	0.0001100	0.000064	0	-0.13	-3.9	Whole Blood
MX1	rs8134216	7.14E-03	0.0001300	0.000064	0	-0.13	-3.9	Whole Blood
MX1	rs8134203	7.10E-03	0.0001400	0.000064	0	-0.13	-3.8	Whole Blood
MX1	rs2298663	4.65E-03	0.0001600	0.000064	0	-0.13	-3.8	Whole Blood
MX1	rs2104810	7.86E-03	0.0007000	0.000064	0	-0.12	-3.4	Whole Blood

*Only SNPs with corrected P are considered statistically singifcant eQTLs

Transparent methods supplemental file

Phenotype definition

Patients with severe COVID-19: laboratory confirmed SARS-CoV-2 infection (RNA and/or serology based), hospitalization due to coronavirus-related symptoms.
Controls: Individuals from the general population not notified as cases.

GWAS

The summary statistics, P-value, odds ratio (OR), and 95% confidence interval (CI), of chromosome 21 were obtained from the GWAS dataset "B2_ALL_eur_leave_23andme" deposited in the COVID-19 Host Genetics Initiative website (COVID-19 Host Genetics Initiative, 2020). It includes 6,406 laboratory-confirmed SARS-CoV-2 infections and hospitalized for COVID-19 cases and 902,088 controls from the general population with European genetic ancestry (**Table S2**). Manhattan plot and QQ plot of the results from this large GWAS are available at the website (https://www.covid19hg.org/results/).

Replication

The summary statistics of the SNPs used for the replication study were retrieved from the GenOMICC study (Pairo-Castineira et al., 2020). Three independent cohorts of cases and controls with different ethnicity were available throughout GenOMMIC GWAS study (Pairo-Castineira et al., 2020): 182 individuals from African ancestry, 149 of East Asian ancestry (EAS), 237 of South-Asian ancestry (SAS). Moreover, 226 hospitalized COVID-19 cases and 1848 controls (**Table S3**) enrolled from public hospitals located in Campania (Southern Italy) were typed fort the rs12329760 variant by TaqMan® SNP Genotyping (Applied Biosystems by Thermo Fisher Scientific). We selected rs12329760 SNP as it appears to has the most relevant functional role among the others, indeed, it is predicted to damage TMPRSS2 protein and to be an eQTL for *TMPRSS2* in osteoblasts treated with dexamethasone (Grundberg et al., 2011). Additionally, 770 hospitalized COVID-19 cases and 1915 controls typed for rs12329760 by whole-exome sequencing were retrieved from the web database Network for Italian Genomes (NIG) (http://nigdb.cineca.it/index.php) (Daga et al., 2021). The 1915 controls included 1685 unrelated Italian healthy controls and 230 unrelated individuals with asymptomatic SARS-CoV-2 infection who did not need hospitalization.

Definition of independent genome-wide associated loci

Using the 74 significant SNPs with $P \leq 1x10^{-5}$ of chromosome 21, we defined three independent associated loci by the following computational process. The SNPs were first sorted according to their association P-value. Then, the lead SNP, considered as the most significant SNP in a given genomic locus, was removed from this list and assigned to an independent locus together with all other SNPs which have an r^2 value less than or equal to 0.01 with this SNP. This procedure was recursively applied to the remaining SNPs in the list so that each SNP could be assigned to a locus and no SNPs were left in the original list.

Assessment of the functional role of the SNPs

Candidate regulatory SNPs were explored by HaploReg (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) (Ward and Kellis, 2012). In this analysis we included the 14 selected SNPs in addition to their proxy SNPs ($r^2>0.8$). Prediction of the functional impact of 14 SNPs at *TMPRSS2/MX1* locus was assessed by Genome Wide Annotation of VAriants (GWAVA) tool (htts://www.sanger.ac.uk/sanger/StatGen_Gwava) (Ritchie et al., 2014) and by Combined Annotation Dependent Depletion (CADD) tool (https://cadd.gs.washington.edu/) (Rentzsch et al., 2019). The scores assigned to each variant by the two tools were combined as follows: GWAVA and CADD scores were ranked from the smallest to largest and the obtained values were summed. PolyPhen-2 (Adzhubei et al., 2010) and SIFT (Sim et al., 2012) scores were used to predict the impact of the missense rs12329760 variant on TMPRSS2 protein function.

We used published data on eQTL in relevant tissues to help explain how observed genetic associations may affect gene expression levels. In particular, the selected top 5 SNPs were examined for eQTLs by screening the GTEx database containing precomputed eQTL data for ~70M significant associations between SNP markers and 49 human tissues (Data Source: GTEx Analysis Release V8, dbGaP Accession phs000424.v8.p2) (Consortium et al., 2017). The significance threshold adjusted for multiple comparisons is equal to 0.000055. The Blood eQTL Browser (https://www.genenetwork.nl/bloodeqtlbrowser/) was also queried to confirm the signals for *MX1* in an independent dataset of eQTL from blood (Westra et al., 2013). eQTLs violin plots (Figure 2b-c) were obtained from the GTEx web portal.

Statistical analysis

Allele frequencies for rs12329760 SNP were compared using the Chi-square test. A two-sided $P \leq 0.05$ was considered statistically significant.

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