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First report on genome wide association study in western Indian population reveals host genetic factors for COVID-19 severity and outcome

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ABSTRACT

Different human races across the globe responded in a different way to the SARS-CoV-2 infection leading to different disease severity. Therefore, it is anticipated that host genetic factors have a straight association with the COVID-19. We identified a total 6, 7, and 6 genomic loci for deceased-recovered, asymptomatic-recovered, and deceased-asymptomatic group comparison, respectively. Unfavourable alleles of the markers nearby the genes which are associated with lung and heart diseases such as Tumor necrosis factor superfamily (TNFSF4&18), showed noteworthy association with the disease severity and outcome for the COVID-19 patients in the western Indian population. The markers found with significant association with disease prognosis or recovery are of value in determining the individual's response to SARS-CoV-2 infection and can be used for the risk prediction in COVID-19. Besides, GWAS study in other populations from India may help to strengthen the outcome of this study.

1. Introduction

Host-pathogen interaction studies are pivotal in understanding infectious disease biology. The genetic interaction of host and pathogen determines response, progression and severity of the infection. COVID-19 caused by the infection of SARS-CoV-2 has shaken the human race for the past two and half years. Like other RNA viruses, it has also high mutation frequency and genetic variations which has led to rapid blow-out of virus across the globe [1–3]. One of the most perplexing features of SARS-CoV-2 infection is diverse range of clinical symptoms observed in different populations and over different waves. COVID-19 many leads to respiratory illness, blood clotting manifested by asymptomatic to moderate (fever, cough and shortness of breath) or severe symptoms (pneumonia, acute respiratory distress, and diffuse alveolar damage) as well as death in 2–3% [4] patients. The severity of disease is also positively correlated with increased age and presence of comorbidities if any [5–9]. In addition, the fatality rate also varies with age and among the different ethnic groups [10], suggesting complex interactions between virus and host genetic makeup to determine the disease outcome. Identification of populations at higher risk of developing severe disease is important for the development and implementation of effective control measures. Genome wide study (GWAS) is widely used to identify the host genetic factors involved in disease susceptibility, following suitable drug development [11,12]. Therefore, the host genetic makeup contributing to the disease resistance, susceptibility, and severity in case of COVID-19 need to be studied in detail in different human races. Till date, several genome wide association studies on COVID-19 are available [13–22]. Recently, researchers have also mapped epigenetic factors

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with COVID-19 severity [23]. However, no GWAS study has been carried out for the Indian population. Therefore, this study was undertaken to identify the host genetic factors involved in susceptibility and severity of COVID-19 patients during first wave of COVID-19 in Gujarat, India. Patients with asymptomatic to severe infection with monitoring of their final outcome as recovered or deceased were enrolled in this study. Data was further analysed using two different GWAS analysis pipelines, PLINK and Scalable and Accurate Implementation of Generalized mixed mode (SAIGE) to correlate association of key host genetic variants playing a significant role in COVID-19.

2. Results

Based on incident rate during the first wave of COVID-19 in 2020, 571 samples were collected from 25 different hospitals of 24 districts across Gujarat, India. Out of 571 samples, 172 and 399 were female and male, respectively. Median age of the patients in particular group and the percentage of patients with comorbidity increases as the disease severity increases i.e. deceased>recovered>asymptomatic and it was reversed for the ct values (viral load) as determined using the three viral genes targeted in the RT-PCR (Table 1).

2.1. Quality analysis

We used Axiom Analysis Suite for the QC of raw data. After quality filtering, 561 out of 571 patients, and 8538,78 (98.335) high resolution markers out of 8,68,298 markers were obtained. As well, based on the results of population stratification PCA plot (Fig. 1), population outliers were identified and removed. Therefore, variants from 558 (94, 317 and 147, deceased, recovered, and asymptomatic, respectively) patients were analysed using two different pipelines, PLINK and SAIGE. Finally, results of PLINK was considered and reported in this study. We found several markers at various genomic loci which are associated with different COVID-19 severity. Total 6, 7, and 6 genomic loci with $p \leq$ 10–7 for deceased-recovered, deceased-asymptomatic, and recovered-asymptomatic group, respectively were identified. Comparison of two tools i.e. PLINK and SAIGE used for GWAS analysis is shown in supplementary Table S2 and respective allele and genotype frequencies are mentioned in supplementary Table S3. Manhattan plot for SNPs with $p \leq$ 10–7 and Q-Q plots are depicted in Fig. 2, while Manhattan plots for markers with $p \leq$ 10–6 are shown in supplementary Fig. S2.

Table 1
Details of male and female patients with reference to three disease states.

	Deceased	Recovered	Asymptomatic	Total
Male	$N = 60$	$N = 228$	$N = 111$	399
Median age years (Range)	60.5 (35–86)	52 (15–90)	39 (18–84)	NA
Comorbidity	41/60 (68.33%)	102/228 (44.73%)	13/111 (11.71%)	156 (52.0%)
Median Ct of N gene	27.13	29.37	30.06	NA
Median Ct of ORF gene	26.32	28.5	29.88	
Median Ct of S gene	27.12	28.18	29.9	
Female	$N = 36$	$N = 99$	$N = 37$	172
Median age years (Range)	62 (40–86)	54 (18–90)	35 (20–76)	NA
Comorbidity	27/36 (75.0%)	56/99 (56.56%)	10/37 (27%)	151 (87.8%)
Median Ct of N gene	24.95	29.88	30.7	NA
Median Ct of ORF gene	24.5	29.9	30.13	
Median Ct of S gene	24.93	29.21	29.37	

Additionally, any markers reported for COVID-19 within the 1 MB region of the markers/loci identified in this study are shown in Supplementary Table S4.

2.2. Deceased vs recovered (mortality)

For this comparison, after imputation, we had total 29,48,95,933 SNPs out of which 63,32,698 SNPs passed the cutoff MAF > 0.05. Upon further analysis of these 63,32,698 SNPs using PLINK, we obtained a total of 6 significant markers having $p \leq$ 10–7 (Table 2). The genes associated with these six significant genomic loci are *TNFSF4*, *TNFSF18*, *DHX15*, *RP1–15D23.2*, *GOT2P2, WAC*, *PPARGC1A, CTD-2036A18.2*, *PTP4A1P4*, and LINC00540-AL354828.1. Marker, rs17300100, (chr1:173115604:T:G; 1q25.1) with *p*-value 9.14E-07 (CHISQ 24.21) has nearest genes tumor necrosis factor 4 (*TNFSF4*) (upstream, 68.127 kb), tumor necrosis factor 18 (*TNFSF18*) (downstream, 64.641 kb) and *GOT2P2* (downstream, 25.496). Here, the frequency of altered allele (G) is 9.3% higher among the deceased patients as compared to those who recovered. The regional association plot for this marker is shown in Fig. 3A. Similarly, two very nearby (1536 bp apart) markers at chromosome 4p15.2 are rs73246461 (chr4:24511798:A:G; *p*-value-4.54E-07; CHIQ:25.45; and rs12651262, (chr4:24513334:A:C; p-value- 9.84E-07; CHIQ:23.96) are located within downstream region of *DHX15*. The regional association plots for these two markers are shown in Fig. 3B&C. For these two positions, the frequency of altered allele was found to be 6.8% lesser in deceased patients as compared to those who recovered. The Manhattan plot for markers with *p*-value ≤10–6 is shown in supplementary Fig. S2A and listed in supplementary Table S6.

2.3. Recovered vs asymptomatic (susceptibility)

For this comparison, we got total 6 significant genomic loci (Table 3). Interesting marker for this comparison is rs72663004 (chr1:20166359:C:T; *p*-value 7.92E-07; CHISQ 24.38; 1p36.12) and genes nearby to this marker are *PLA2G2D*, *PLA2G2C*, *PLA2G5*, and *UBXN10*. For this marker, the frequency of altered allele is 12.27% lesser in asymptomatic patients when compared with recovered patients. A regional association plot for this marker is shown in Fig. 4A. Another two markers present on chromosome 9 (9p24.2) are rs72699049 (chr9:3320390:C:A; *p*-value 3.21E-07; CHISQ 26.12) and rs72699016 (chr9:3258654:T:G; *p*-value 3.59E-07; CHISQ 25.91) having nearby gene *RFX3*. At both these loci, the frequency of altered allele is 1.5 and 4.94%, respectively lower in the patients those who remained asymptomatic after SARS-CoV-2 infection against those who recovered after infection. When the frequency of these loci was compared with deceased patents again, it was lower in asymptomatic one. Region association plot for both these markers are shown in Fig. 4B&C. Other two nearby markers (within 874 bp region) on chromosome 4 (4q35.2) are rs72717619 (chr4:188628742:C:G; *p*-value 9.56E-07; CHISQ 24.02) and rs1734523522 (chr4:188628742:C:G; p-value 9.56E-07; CHISQ 24.02). Nearby of these loci, only pseudogenes are present. The Manhattan plots for markers with p-value ≤10–6 is shown in Fig. S2B and listed in supplementary Table S6.

2.4. Deceased vs asymptomatic (morbidity)

For this comparison, after imputation, we got a total of 30,76,54,523 variants. Out of which 64,68,284 variants passed the threshold, MAF > 0.05. Here, we found total 7 significant markers and the associated genes are *ANGEL1*, *LRRC74A*, *ANO3*, *MOK*, *CINP*, *TECPR2*, and many uncharacterized loci (Table 4). Marker rs34279101, (chr14:76832814:C:CT; *p*-value 4.12E-08; CHISQ 30.09; 14q24.3) has the nearby genes *ANGEL1* and *LRRC74A*. Here, the frequency of altered allele is 7.4% higher in deceased patients as compared to asymptomatic group. A regional association plot for rs34279101 marker is shown in Fig. 5. Two other very nearby markers present on chromosome 14 (14q32.31) are rs11160678 (chr14:102356233:A:G; *p*-value-5.23E-07; CHISQ-25.18)

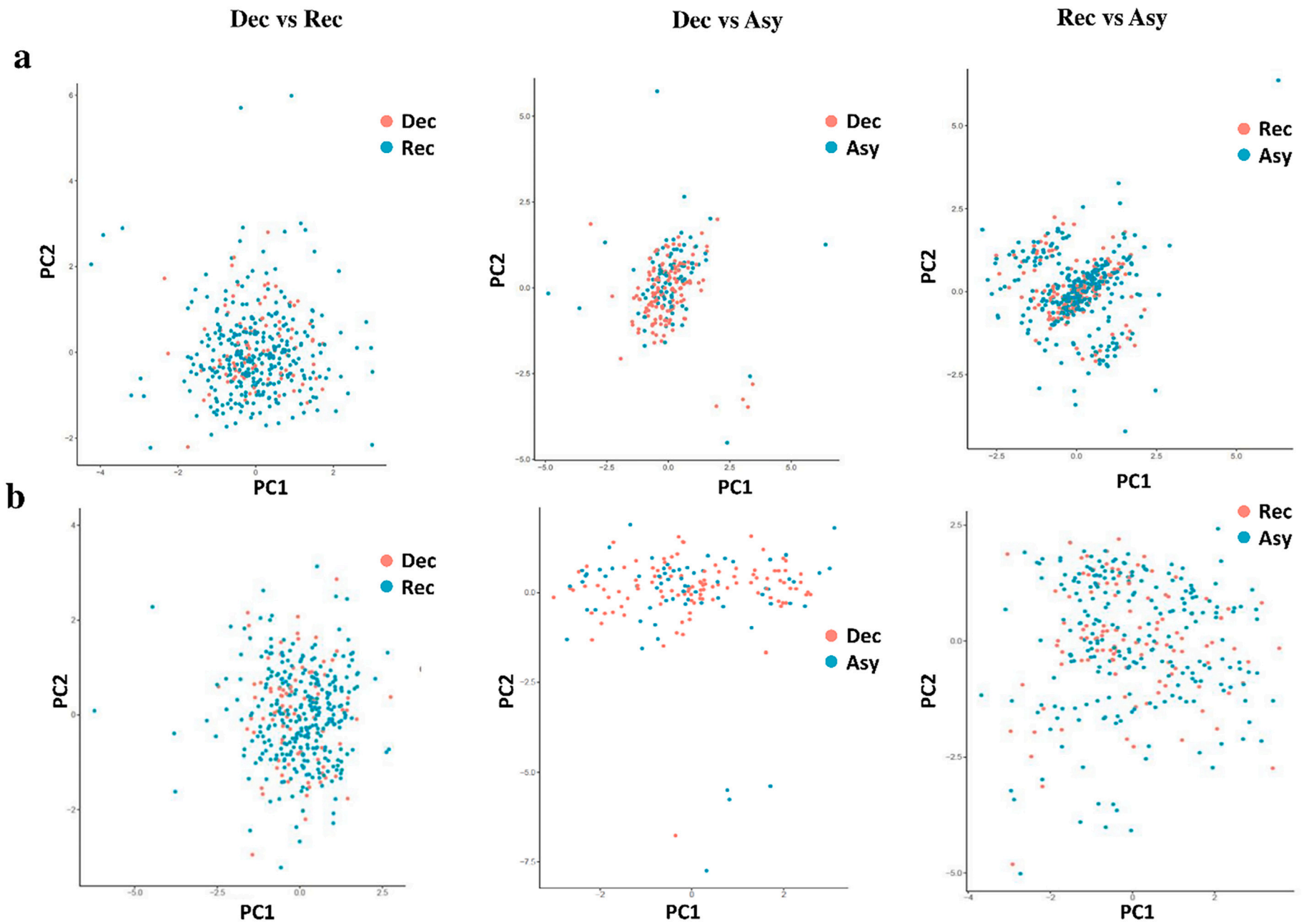

Fig. 1. Population stratification by principal component analysis using PLINK. Scatter plot depict principal component one (PC1) vs principal component two (PC2), (A) without removing outliers and (B) after removing population outliers.

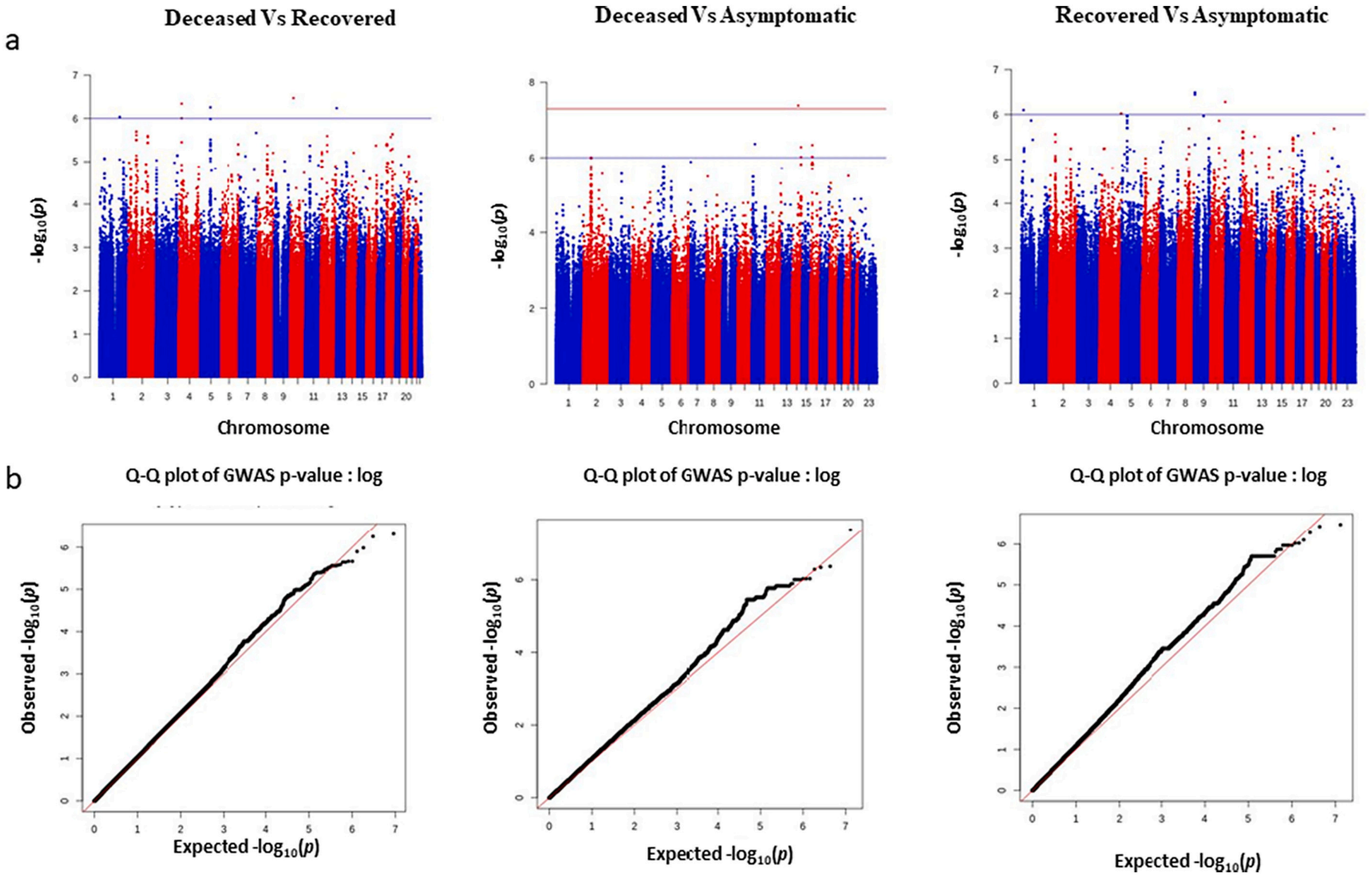

Fig. 2. Figure depicting Manhattan and Q-Q plots of the association statistics from the meta-analysis of three-group comparison using PLINK. (A) Manhattan plot and (B) Q-Q plot. For Manhattan plots, *p*-values from GWAS analysis is plotted and threshold was set $P \leq 10^{-6}$. Quantile-quantile (Q-Q) plots are showing quantile distribution of observed p-values (on the y-axis) versus the quantile distribution of expected p-values to show genomic inflation (λ) for each analysis.

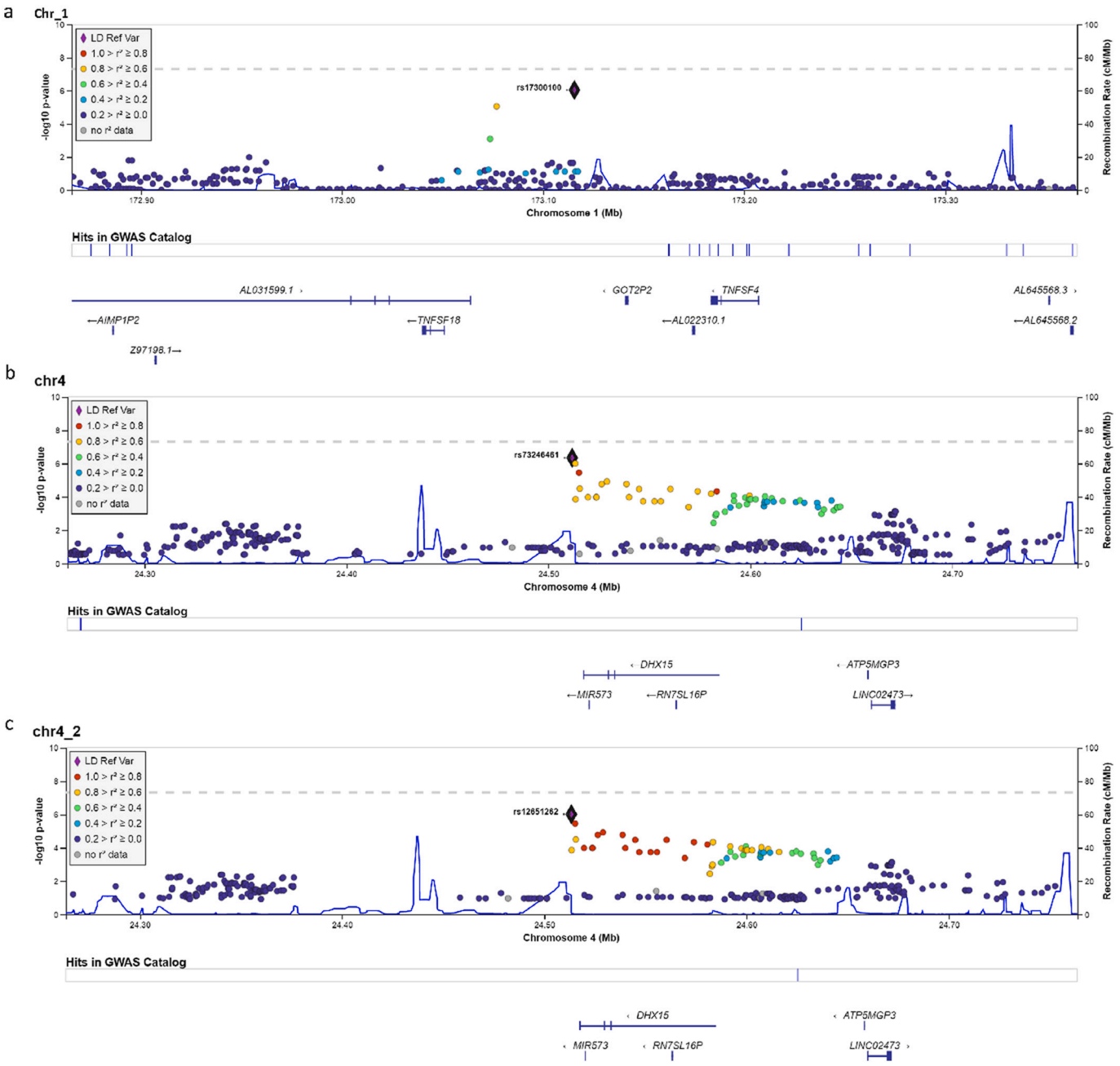

Fig. 3. Regional association plots for region around the significant loci for deceased-recovered comparison. (A) rs17300100, (B) rs73246461, and (C) rs12651262. These plots were generated using LocusZoom using all the population. The most strongly associated SNPs are highlighted as purple diamond. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and rs12323812 (chr14:102363920:C:T; p-value-9.51E-07; CHISQ-24.02). Here the nearest genes are *MOK*, *CINP* and *TECPR2*. Similarly, three significant markers on chromosome 16 (16p12.3) are, rs1453512 (chr16:16924042:C:A; p-value-4.60E-07; CHISQ-25.42), rs1597988 (chr16:16900377:T:C, p-value-9.44E-07; CHISQ-24.04), and rs4371135 (chr16:16897099:G:C, p-value-9.44E-07; CHISQ-24.04). These three markers on chromosome 16 having no any gene nearby, instead all are falling under uncharacterized loci i.e. pseudogene AC098965.1. Moreover, the difference in frequency of the altered allele is also minor. The Manhattan plot for markers with p-value ≤10–6 is shown in supplementary Fig. S2C and listed in supplementary Table S7.

3. Discussion

Currently, researchers across the globe are working on the different aspect of the SARS-CoV-2 infection through looking into epidemiology [10,24], viral mutations [25,26], host transcriptome signature [27], in silico analysis of different mutations [28,29] etc. to tackle the viral infection. Apart from this, researchers are also looking for herbal remedies [30,31] to prevent and cure SARS-CoV2 infection. While, all this information is very crucial to understand the viral transmissibility and/or severity, host genetic makeup is also one of the factors which are also equally important. Therefore, although comorbidities and age remain the major contributors for mortalities, host genetics appears as significant component for observed differences in individual response to COVID-19 infection, disease progression, as well as severity [32–34]. As

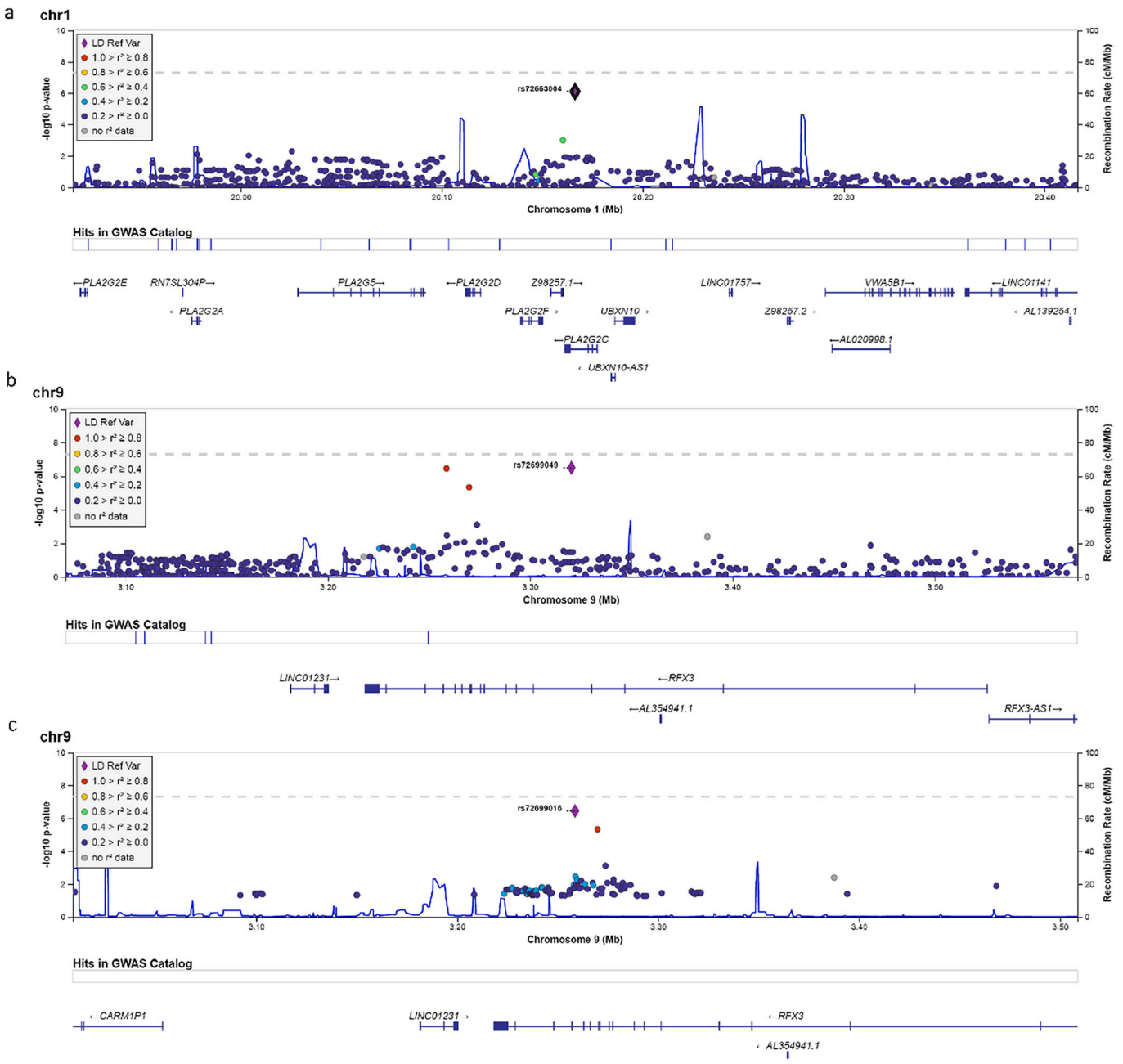

Fig. 4. Regional association plot for region around the significant loci for recovered-asymptomatic comparison. (A) rs72663004, (B) rs72699049, and (C) rs72699016. These plots were generated using LocusZoom using all the population. The most strongly associated SNPs are highlighted as purple diamond. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mentioned previously, several GWAS studies on COVID-19 patients for various ethnic groups have been carried out and identified several genes. Very recently, COVID-19 Host Genetics Initiative has published GWAS data of 46 studies across 19 countries and reported 13 significant ($P < 1.67 \times 10–8$) genomic loci for COVID-19 [17]. Similarly, [35] have reported 8 super-variants for COVID-19 mortality. Here we aimed to identify the host SNPs and genes associated with COVID-19 severity in the asymptomatic, recovered and deceased patients from Gujarat, India.

Lungs are the major organ associated with respiratory diseases including COVID-19. Thrombosis related heart and lung malfunctions are common in COVID-19 patients [36–38]. Similarly, high D-Dimer in COVID-19 patients is associated with mortality [39]. Additionally, cardiac arrest in COVID-19 patients and post COVID-19 is prominent [40,41]. Therefore, genes associated with heart and lung disease are very crucial and worth to study with prospect of COVID-19 complications.

The analysis of deceased vs recovered patients identified six genomic loci. Tumor Necrosis Factor Ligand Superfamily membrane genes, *TNFSF4* and *TNFSF18* are located in the vicinity of significantly associated marker (rs17300100) in this study and the genes associated have key role in the inflammatory disease conditions or inflammatory activation of macrophage/microglia cells [42]. Moreover, mutation in *TNFSF4* have a known role in myocardial infarction [43] and Systemic Lupus Erythematosus (SLE) [44]. Several studies have discussed the role of T cell response in COVID-19 [45–48]. *TNFSF18* is associated with T-cell responses as well, can act as a co-stimulator and lower the threshold for T-cell activation and proliferation [49–51]. Another nearest gene to this marker is *GOT2P2* i.e. Glutamic-Oxaloacetic Transaminase 2-Like 2.

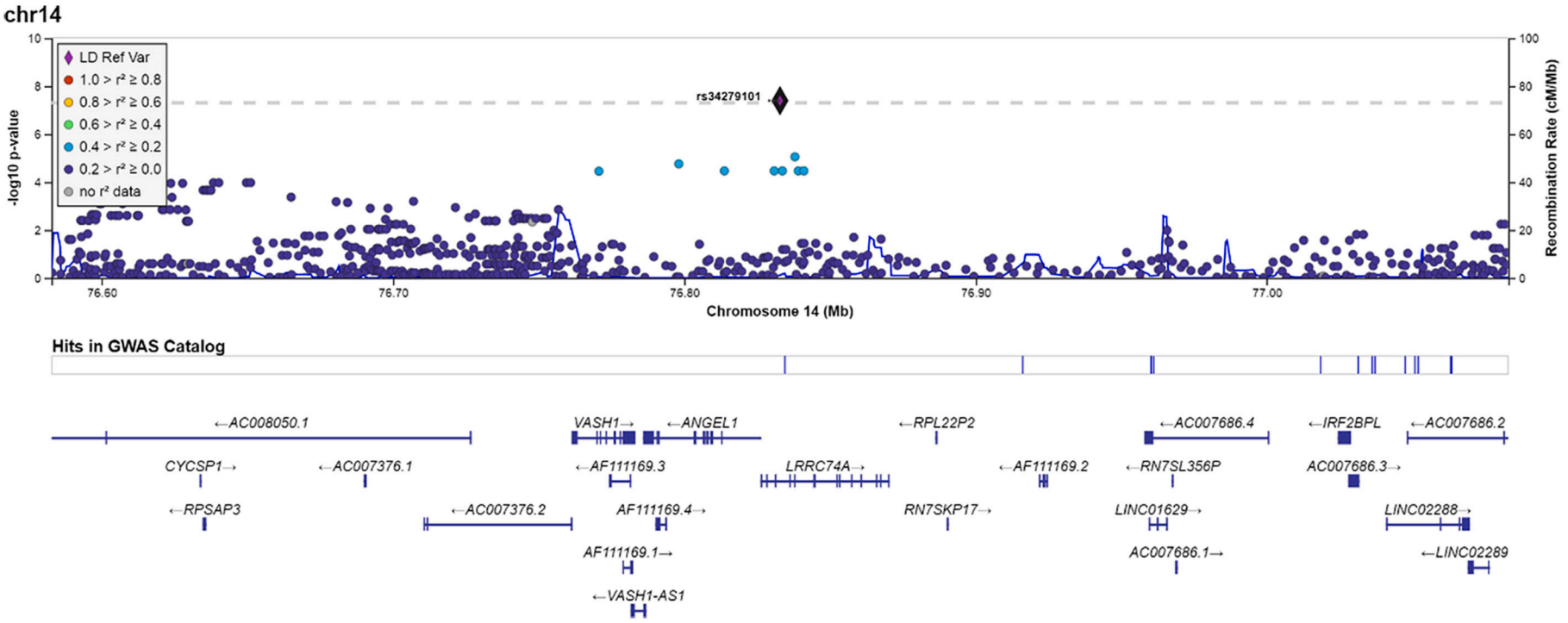

Fig. 5. Regional association plot for region around rs34279101 for deceased-asymptomatic comparison. These plot was generated using LocusZoom using all the population. The most strongly associated SNP is shown as purple diamond. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Significant markers for deceased-recovered comparison. imputed data was analysed using PLINK with MAF >0.05 and markers with p-value p ≤ 10–7 were considered significant.

CHR	SNP	BP	rsID	Band position	Ref	Alt	CHISQ	p- value	Gene
10	chr10:28606315:C:T	28,606,315	rs12773860	10p12.1	C	T	26.02	3.38E-07	WAC-AS1, BAMBI, WAC-AS1, RNU4ATAC6P, TPRKBP1, RNU6,1067P, snRNA
4	chr4:24511798:A:G	24,511,798	rs73246461	4p15.2	A	G	25.45	4.54E-07	PPARGC1A, DHX15
5	chr5:86011821:T:G	86,011,821	rs4424029	5q14.3	T	G	25.07	5.52E-07	CTD, 2036A18.2, PTP4A1P4
13	chr13:22495675:AG:A	22,495,675	rs10714879; rs398021874; rs398077102	13q12.11	AG	G	24.99	5.78E-07	LINC00540, AL354828.1
1	chr1:173115604:T:G	173,115,604	rs17300100	1q25.1	T	G	24.1	9.14E-07	TNFSF4, TNFSF18, RP1–15D23.2, GOT2P2
4	chr4:24513334:A:C	24,513,334	rs12651262	4p15.2	A	C	23.96	9.84E-07	DHX15, RN7SL16P, PPARGC1A

Table 3
Significant markers for recovered-asymptomatic comparison. imputed data was analysed using PLINK with MAF >0.05 and markers with p-value p ≤ 10–7 were considered significant.

CHR	SNP	BP	rsID	Band position	Ref	Alt	CHISQ	p-value	Gene
9	chr9:3320390:C:A	3,320,390	Rs72699049	9p24.2	C	A	26.12	3.21E-07	RFX3
9	chr9:3258654:T:G	3,258,654	rs72699016	9p24.2	T	G	25.91	3.59E-07	RFX3, LINC01231
10	chr10:130802064:T:G	130,802,064	rs12253652	10q26.3	T	G	25.17	5.24E-07	AC016816.1-MIR378C
1	chr1:20166359:C:T	20,166,359	rs72663004	1p36.12	C	T	24.38	7.92E-07	PLA2G2D, UBXN10, LINC01757, PLA2G5, UBXN10-AS1, PLA2G2F, Z98257.1, PLA2G2C
4	chr4:188628742:C:G	188,628,742	rs72717619	4q35.2	C	G	24.02	9.56E-07	AC093909.3, LINC01060, RNU7-192P, snRNA, LINC01060
4	chr4:188627868:CTCT:C	188,627,868	rs1734523522	4q35.2	CTCT	C	24.02	9.56E-07	AC093909.5, LINC01060, RNU7-192P, snRNA

Table 4
Significant markers for deceased-asymptomatic comparison. imputed data was analysed using PLINK with MAF >0.05 and markers with p-value p ≤ 10–7 were considered significant.

CHR	SNP	BP	rsID	Band position	Ref	Alt	CHISQ	p-value	Gene
14	chr14:76832814:C:CT	76,832,814	rs34279101	14q24.3	C	CT	30.09	4.12E-08	ANGEL1, VASH1-AS1, AC007376.2, RN7SKP17, misc_RNA, AF111169.1, LRRC74A, RPL22P2, AF111169.4
11	chr11:26675470:T:G	26,675,470	rs10835056	11p14.2	T	G	25.55	4.32E-07	ANO3, SLC5A12
16	chr16:16924042:C:A	16,924,042	rs1453512	16p12.3	C	A	25.42	4.60E-07	AC098965.1
14	chr14:102356233:A:G	1.02E+08	rs11160678	14q32.31	A	G	25.18	5.23E-07	MOK, TECPR2, ZNF839, CINP
16	chr16:16900377:T:C	16,900,377	rs1597988	16p12.3	T	C	24.04	9.44E-07	AC098965.1
16	chr16:16897099:G:C	16,897,099	rs4371135	16p12.3	G	C	24.04	9.44E-07	AC098965.1
14	chr14:102363920:C:T	1.02E+08	rs12323812	14q32.31	C	T	24.02	9.51E-07	MOK, CINP, TECPR2, ZNF839

Two SNPs (rs6691738 and rs10158467) nearby rs17300100 have been reported for asthma [52,53]. Additionally, five SNPs/markers (rs114680188, rs12117214, rs147327230, rs9425716, and 1:173368150) within 1 MB region of *TNFSF4* and *TNFSF18* are already reported for their association with COVID-19 [54]. For this marker, the frequency of altered allele is 9.3% higher in deceased patients as compared to those who recovered. Similarly, if we compare this frequency with other populations, it is also high in the European population (which also experienced high mortality compared to SAS) and matching with the frequency of deceased group. The frequency of this allele in South Asian population is similar to the recovered group in this study. Another two significant markers are located on 4p15.2 and nearby genes are *DHX15*, *RN7SL16P*, *PPARGC1A*. It has been reported that several members of the *DEXD/H* box helicase family including *DHX15* have key role in innate immunity against the viral infection [55–58]. Further, knockdown of *DHX15* impair the capacity of myeloid dendritic cells to synthesize *IFN-b, IL-6*, and *TNF-a* in response to dsRNA and RNA virus [55] and *NF-κB* regulation of cytokines, *ERK* and *TNF-α* signalling pathways play an important role in inflammation [59]. In the same way, *DDX1* has been reported to interact with *NSP14* of the infectious bronchitis coronavirus and enhance viral replication [60]. Moreover, in support to this study, numerous SNPs (with *p*-value $\leq 1 \times 10^{-4}$) in the 1 Mb downstream stream region of *DHX15* gene have been reported for their association with COVID-19. Similarly, another gene

nearby these two loci is *PPARGC1A* (*PGC-1α*) which is a key regulator of mitochondrial function [61,62]. Recent evidences suggest that, SARS-CoV-2 take over the mitochondrial function and specifically disrupt the immune function in COVID-19 patients [63–67]. Downregulation of *PPARGC1A* during SARS-CoV-2 infection is already reported [68]. Thus, the finding of this is comparable with the previous studies. Contrary to rs17300100, here the reference alleles were found to be associated with the mortally/severalty while, altered allele was found to be protective (recovery). Here also, the allele frequency of the European population is very close to the altered allele frequency of the deceased patients. In one the study authors have performed Transcriptome Wide Association Study (TWAS) and reported genetic regulation of *CXCR6*, and correlated it with COVID-19 severity [69]. Thus, the findings of this study is also correlating with the previously reported data. All this data, in sum suggest that, *TNFSF*, *GOT2P2, DHX15*, and *PPARGC1A* may have a vital role in COVID-19 severity and mortality.

Analysis of recovered vs asymptomatic patients revealed significant association of six genomic loci with SARS-CoV-2 infection. Among these, rs72663004 (chr1:20166359:C:T) has nearby genes are secretory calcium-dependent phospholipase group A2 (*PLA2G5*, *PLA2G2D*, *PLA2G2F*, and *PLA2G2C*) and *UBXN10*. Lipid metabolism plays an important role in viral endocytosis, exocytosis and also act as a putative target for antiviral therapy [70,71]. Up regulation of sphingomyelins, GM3s, and glycerophosphocholines have been reported in COVID-19 patients [72,73]. Function of phospholipase A2 group IID in age related susceptibility for SARS-CoV infection is already reported [74]. Moreover, it has been also anticipated that, inhibition of phospholipases A2 may help in treatment of COVID-19 patients. Another nearby gene to marker rs72663004 is ubiquitin regulatory X (*UBX*) and members of this family have been reported to inhibit the viral life cycle of retrovirus and lentivirus via regulation of genes involve in the pathways related to cell adhesion and immune system signalling. Innate immune system plays an important role during an early stage of infection for any pathogen. Cilia in the respiratory track play an essential role in innate immune system for the respiratory infections via removing the gasped elements [75,76]. *RFX* family of transcription factors including regulatory factor X3 (*RFX3*) is indispensable for ciliogenesis [77–79]. It has been also reported that *RFX3* complement with *FOXJ1* for cilia formation in the human airway epithelium and any mutation in this gene may cause Primary ciliary dyskinesia [75,79,80]. In this study, we also found two markers (rs72699049 & rs72699016) on chromosome 9p24.2 both are located near *RFX3*. If we compare the allele frequencies for the markers rs72699016, rs12253652, rs72717619, and rs1734523522, frequency of altered allele is very high both in recovered and asymptomatic group as compared to the frequency in the European and South Asian populations. And if, we compare only recovered and asymptomatic group for this study, among the above mentioned locations, rs72699016, rs12253652, rs72663004 has higher altered allele frequency in the patients those who recovered. This data altogether suggests that, the altered allele at these genomic positions in the Western Indian population might have a protective role against COVID-19, i.e. even if they are infected with SARS-CoV-2, they may have remained asymptomatic or recovered.

For the analysis of deceased vs asymptomatic patients, seven significant loci are identified. Here, the important one is rs34279101and nearby genes are *ANGEL1*, *LRRC74A*, *VASH1-AS1*, and uncharacterized genes. As mentioned earlier, respiratory distress syndrome and lung pathology is commonly observed in severe cases of COVID-19. While comparing deceased and asymptomatic patients in the present study, we found two putative genes *LRRC74A* and *ANGEL1* near rs34279101 which may have role in COVID-19 severity in deceased patients. In support to this study, five locations within 1 Mb region of rs34279101 are already reported for COVID-19 however, with leaser p-vale. Function of leucine rich repeat such as *LRRC10* in cardiomyopathy [81] and primary ciliary dyskinesia [82] has already been reported. Similarly, allelic variants in *LRRC56* are associated with primary ciliary dyskinesia, a disorder associated with chronic respiratory tract infections [83]. Another nearby gene to this locus is *ANGEL1/Ccr4e*. Again, this gene in either way associated with cardiac disease such as loss of myocardial cells. At this locus, the frequency of altered allele is higher in deceased individuals as compared to asymptomatic patients. However, allele frequency in other populations is quite different. rs10835056 on 11p14.2 has nearby genes *ANO3* and *SLC5A12*. Elevated level of lactate and lactate dehydrogenase may be because of hypoxia [84,85] or inflammation, is reported in the COVID-19 patients and also associated with the mortality in the septic patients [86–89]. Low and high affinity *SLC5A12* transporters transport the lactate [90]. Previous studies suggest that *SLC5A12* transport lactate into the T cells at the site of inflammation and control its function [91,92]. At this locus, the reference allele was found to be associated with mortality as its frequency was higher than the asymptomatic patients group.

4. Conclusion

In summary, the present study suggests that polymorphic loci around genes involved in lung and heart diseases such as Tumor necrosis factor superfamily *TNFSF4&18*, *GOT2P2*, and *LRRC74A* as well as genes connected with innate immune system (*DHX15*), and mitochondrial function (*PPARGC1A*) are significantly associated with COVID-19 severity in Western India population. Whereas, altered allele near *RFX3 and UBXN10* genes are found to be protective in COVID-19 patients in the study population. Our findings suggest that, identified genomic markers may be decisive for the COVID-19 progression and severity in the Western Indian population. Therefore, the unfavourable alleles of the markers showing association with the disease severity and outcome can be used for risk prediction during the SARS-CoV-2 infections.

5. Material and methods

5.1. Recruitment of patients

In this study, we recruited 571 COVID-19 patients with different stages of disease severity. Samples were collected from 25 different hospitals of 24 districts across the Gujarat state of India (Supplementary Fig. S1). All the metadata information such as age, sex, and comorbidity if any, were recorded (Supplementary Table S1). All the patients were confirmed for SARS-CoV-2 infection using RT-PCR of nasopharyngeal swab samples using TaqPath™ 1-Step RT-qPCR kit on Applied Biosystems 7500 Fast Dx Real-Time PCR system (Thermo Fisher Scientific). Based on the clinical manifestations and disease severity, all the patients were broadly categorized as either symptomatic or asymptomatic. Asymptomatic patients are those who experienced very mild symptoms such as cough, body aches, etc. but did not required hospitalisation. Symptomatic patients had major symptoms including cold, fever, breathlessness, sore throat etc. and importantly they required ventilation or oxygenation in the intensive care unit (ICU). Symptomatic patients were further followed for the final outcome and further divided into two groups i.e. recovered and deceased. Therefore, in the final analysis, comparison was made among three groups i.e. asymptomatic, symptomatic but recovered and deceased. With these criteria, total 148, 327 and 96 patients were considered as asymptomatic, recovered and deceased, respectively.

5.2. Sample processing, genotyping, imputation and data GWAS analysis

DNA from blood samples was isolated using John's method [93]. Quantity of extracted DNA was estimated using DNA High sensitivity assay kit on Qubit fluorimeter v 4.0 (Thermo Fisher Scientific). Quality of extracted DNA was assessed using agarose gel electrophoresis and QIAxpert system (QIAGEN). For genotyping, we used Axiom™ Precision Medicine Diversity Array (PMDA) Plus Kit, 96-format containing 8,68,298 markers selected for high genomic coverage (Thermo Fisher

Scientific) on GeneTitan Multi-Channel (MC) Instrument (Thermo Fisher Scientific). Best markers were selected using Axiom Analysis Suite following the best practices workflow with the following parameters: sample QC Threshold; QC call_rate: ≥97, SNP QC Threshold; scr-cutoff: ≥95, and therefore, markers with high-resolution were analysed further. For imputation, we used TOPMed Imputation Server (https://imputation.biodatacatalyst.nhlbi.nih.gov/). To perform GWAS, imputed chromosome files were merged and VCF format files were further converted to plink format. The population stratification was performed using PLINK v1.9. GWAS analysis was performed using PLINK v1.9 [94] and SAIGE v 0.44.5 [95] at minor allele frequency (MAF) >0.05. Comparison between different groups was done as; deceased vs recovered, deceased vs asymptomatic and recovered vs asymptomatic patients.

Declaration of Competing Interests

All the authors of this manuscript declare no competing interests.

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Ethical approval

The present study involving human participants were reviewed and approved by the Institutional Ethical Committee of Gujarat Biotechnology Research Centre (GBRC), Gandhinagar, B. J. Medical College and Civil hospital, Ahmedabad, reference No. EC/Approval/38/2020 and GMERS medical College Gandhinagar, reference No. GMERS/MCG/IEC/06/2020.

Availability of data

The analysed data from the current study is submitted to https://www.covid19hg.org/.

Author contributions

Chaitanya Joshi: Conceptualization, Funding acquisition, Project administraion, Methodology, Editing manuscript, Supervision. Madhvi Joshi: Funding acquisition, Investigation, Review and editing manuscript, Supervision, Project administration. Ramesh Pandit: Formal analysis, Writing original draft. Indra Singh and Afzal Ansari: Formal analysis. Janvi Raval and Zarna Patel: Data curation. Raghav Dixit, Pranay Shah, Kamlesh Upadhyay, Naresh Chauhan, Kairavi Desai, Meenakshi Shah, and Bhavesh Modi: Resources, Review manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygeno.2022.110399.

References

[1] N. Sapoval, M. Mahmoud, M.D. Jochum, Y. Liu, R.A. Leo Elworth, Q. Wang, D. Albin, H. Ogilvie, M.D. Lee, S. Villapol, K.M. Hernandez, I.M. Berry, J. Foox, A. Beheshti, K. Ternus, K.M. Aagaard, D. Posada, C.E. Mason, F. Sedlazeck, T. J. Treangen, Hidden genomic diversity of SARS-CoV-2: implications for qRT-PCR diagnostics and transmission, bioRxiv (2020), https://doi.org/10.1101/2020.07.02.184481.

[2] A. Gomez-Carballa, X. Bello, J. Pardo-Seco, F. Martinon-Torres, A. Salas, Mapping genome variation of SARS-CoV-2 worldwide highlights the impact of COVID-19 super-spreaders, Genome Res. 30 (2020) 1434–1448.

[3] N. De Maio, C.R. Walker, Y. Turakhia, R. Lanfear, R. Corbett-Detig, N. Goldman, Mutation rates and selection on synonymous mutations in SARS-CoV-2, Genome Biol. Evol. 13 (2021).

[4] Y. Cao, A. Hiyoshi, S. Montgomery, COVID-19 case-fatality rate and demographic and socioeconomic influencers: worldwide spatial regression analysis based on country-level data, BMJ Open 10 (2020), e043560.

[5] A. Sanyaolu, C. Okorie, A. Marinkovic, R. Patidar, K. Younis, P. Desai, Z. Hosein, I. Padda, J. Mangat, M. Altaf, Comorbidity and its impact on patients with COVID-19, SN Compr. Clin. Med. (2020) 1–8.

[6] X. Liang, L. Shi, Y. Wang, W. Xiao, G. Duan, H. Yang, The association of hypertension with the severity and mortality of COVID-19 patients: evidence based on adjusted effect estimates, J. Inf. Secur. 81 (2020) e44–e47.

[7] X. Liang, J. Xu, W. Xiao, L. Shi, H. Yang, The association of diabetes with COVID-19 disease severity: evidence from adjusted effect estimates, Hormon. (Athens) 20 (2021) 409–414.

[8] H. Yang, J. Xu, X. Liang, L. Shi, Y. Wang, Autoimmune diseases are independently associated with COVID-19 severity: evidence based on adjusted effect estimates, J. Inf. Secur. 82 (2021) e23–e26.

[9] J. Xu, W. Xiao, X. Liang, P. Zhang, L. Shi, Y. Wang, H. Yang, The association of cerebrovascular disease with adverse outcomes in COVID-19 patients: a meta-analysis based on adjusted effect estimates, J. Stroke Cerebrovasc. Dis.: Off. J. Natl. Stroke Assoc. 29 (2020), 105283.

[10] C. Wang, Z. Wang, G. Wang, J.Y. Lau, K. Zhang, W. Li, COVID-19 in early 2021: current status and looking forward, Signal Transduct. Target. Ther. 6 (2021) 114.

[11] C.A. Winkler, Identifying host targets for drug development with knowledge from genome-wide studies: lessons from HIV-AIDS, Cell Host Microbe 3 (2008) 203–205.

[12] S.Y. Liou, [use of GWAS for drug discovery and development], Yakugaku zasshi, J. Pharm. Soc. Jpn 134 (2014) 485–490.

[13] C. Anastassopoulou, Z. Gkizarioti, G.P. Patrinos, A. Tsakris, Human genetic factors associated with susceptibility to SARS-CoV-2 infection and COVID-19 disease severity, Human genomics 14 (2020) 40.

[14] M.K. Smatti, Y.A. Al-Sarraj, O. Albagha, H.M. Yassine, Host genetic variants potentially associated with SARS-CoV-2: a multi-population analysis, Front. Genet. 11 (2020), 578523.

[15] D. Ellinghaus, F. Degenhardt, L. Bujanda, M. Buti, A. Albillos, P. Invernizzi, J. Fernandez, D. Prati, G. Baselli, R. Asselta, M.M. Grimsrud, C. Milani, F. Aziz, J. Kassens, S. May, M. Wendorff, L. Wienbrandt, F. Uellendahl-Werth, T. Zheng, X. Yi, R. de Pablo, A.G. Chercoles, A. Palom, A.E. Garcia-Fernandez, F. Rodriguez-Frias, A. Zanella, A. Bandera, A. Protti, A. Aghemo, A. Lleo, A. Biondi, A. Caballero-Garralda, A. Gori, A. Tanck, A. Carreras Nolla, A. Latiano, A.L. Fracanzani, A. Peschuck, A. Julia, A. Pesenti, A. Voza, D. Jimenez, B. Mateos, B. Nafria Jimenez, C. Quereda, C. Paccapelo, C. Gassner, C. Angelini, C. Cea, A. Solier, D. Pestana, E. Muniz-Diaz, E. Sandoval, E.M. Paraboschi, E. Navas, F. Garcia Sanchez, F. Ceriotti, F. Martinelli-Boneschi, F. Peyvandi, F. Blasi, L. Tellez, A. Blanco-Grau, G. Hemmrich-Stanisak, G. Grasselli, G. Costantino, G. Cardamone, G. Foti, S. Aneli, H. Kurihara, H. ElAbd, I. My, I. Galvan-Femenia, J. Martin, J. Erdmann, J. Ferrusquia-Acosta, K. Garcia-Etxebarria, L. Izquierdo-Sanchez, L. R. Bettini, L. Sumoy, L. Terranova, L. Moreira, L. Santoro, L. Scudeller, F. Mesonero, L. Roade, M.C. Ruhlemann, M. Schaefer, M. Carrabba, M. Riveiro-Barciela, M.E. Figuera Basso, M.G. Valsecchi, M. Hernandez-Tejero, M. Acosta-Herrera, M. D'Angio, M. Baldini, M. Cazzaniga, M. Schulzky, M. Cecconi, M. Wittig, M. Ciccarelli, M. Rodriguez-Gandia, M. Bocciolone, M. Miozzo, N. Montano, N. Braun, N. Sacchi, N. Martinez, O. Ozer, O. Palmieri, P. Faverio, P. Preatoni, P. Bonfanti, P. Omodei, P. Tentorio, P. Castro, P.M. Rodrigues, A. Blandino Ortiz, R. de Cid, R. Ferrer, R. Gualtierotti, R. Nieto, S. Goerg, S. Badalamenti, S. Marsal, G. Matullo, S. Pelusi, S. Juzenas, S. Aliberti, V. Monzani, V. Moreno, T. Wesse, T.L. Lenz, T. Pumarola, V. Rimoldi, S. Bosari, W. Albrecht, W. Peter, M. Romero-Gomez, M. D'Amato, S. Duga, J.M. Banales, J.R. Hov, T. Folseraas, L. Valenti, A. Franke, T.H. Karlsen, Genomewide association study of severe Covid-19 with respiratory failure, N. Engl. J. Med. 383 (2020) 1522–1534.

[16] E. Pairo-Castineira, S. Clohisey, L. Klaric, A.D. Bretherick, K. Rawlik, D. Pasko, S. Walker, N. Parkinson, M.H. Fourman, C.D. Russell, J. Furniss, A. Richmond, E. Gountouna, N. Wrobel, D. Harrison, B. Wang, Y. Wu, A. Meynert, F. Griffiths, W. Oosthuyzen, A. Kousathanas, L. Moutsianas, Z. Yang, R. Zhai, C. Zheng, G. Grimes, R. Beale, J. Millar, B. Shih, S. Keating, M. Zechner, C. Haley, D. J. Porteous, C. Hayward, J. Yang, J. Knight, C. Summers, M. Shankar-Hari, P. Klenerman, L. Turtle, A. Ho, S.C. Moore, C. Hinds, P. Horby, A. Nichol, D. Maslove, L. Ling, D. McAuley, H. Montgomery, T. Walsh, A. Pereira, A. Renieri, X. Shen, C.P. Ponting, A. Fawkes, A. Tenesa, M. Caulfield, R. Scott, K. Rowan, L. Murphy, P.J.M. Openshaw, M.G. Semple, A. Law, V. Vitart, J.F. Wilson, J. K. Baillie, Genetic mechanisms of critical illness in COVID-19, Nature 591 (2020).

[17] COVID-19 Host Genetics Initiative, Mapping the human genetic architecture of COVID-19, Nature 600 (2021).
[18] J.H. Oh, A. Tannenbaum, J.O. Deasy, Identification of biological correlates associated with respiratory failure in COVID-19, BMC Med. Genet. 13 (2020) 186.
[19] J.F. Shelton, A.J. Shastri, C. Ye, C.H. Weldon, T. Filshtein-Sonmez, D. Coker, A. Symons, J. Esparza-Gordillo, S. Aslibekyan, A. Auton, Trans-ancestry analysis reveals genetic and nongenetic associations with COVID-19 susceptibility and severity, Nat. Genet. 53 (2021) 801–808.
[20] R.L. Prentice, S.G. Self, Aspects of the use of relative risk models in the design and analysis of cohort studies and prevention trials, Stat. Med. 7 (1988) 275–287.
[21] T.H. Karlsen, Understanding COVID-19 through genome-wide association studies, Nat. Genet. 54 (2022) 368–369.
[22] A. Baranova, H. Cao, J. Chen, F. Zhang, Causal association and shared genetics between asthma and COVID-19, Front. Immunol. 13 (2022), 705379.
[23] M. Castro de Moura, V. Davalos, L. Planas-Serra, D. Alvarez-Errico, C. Arribas, M. Ruiz, S. Aguilera-Albesa, J. Troya, J. Valencia-Ramos, V. Velez-Santamaria, A. Rodriguez-Palmero, J. Villar-Garcia, J.P. Horcajada, S. Albu, C. Casasnovas, A. Rull, L. Reverte, B. Dietl, D. Dalmau, M.J. Arranz, L. Llucia-Carol, A.M. Planas, J. Perez-Tur, I. Fernandez-Cadenas, P. Villares, J. Tenorio, R. Colobran, A. Martin-Nalda, P. Soler-Palacin, F. Vidal, A. Pujol, M. Esteller, Epigenome-wide association study of COVID-19 severity with respiratory failure, EBioMedicine 66 (2021), 103339.
[24] B. Ganesh, T. Rajakumar, M. Malathi, N. Manikandan, J. Nagaraj, A. Santhakumar, A. Elangovan, Y.S. Malik, Epidemiology and pathobiology of SARS-CoV-2 (COVID-19) in comparison with SARS, MERS: an updated overview of current knowledge and future perspectives, Clin. Epidemiol. Glob. Health 10 (2021), 100694.
[25] M. Joshi, A. Puvar, D. Kumar, A. Ansari, M. Pandya, J. Raval, Z. Patel, P. Trivedi, M. Gandhi, L. Pandya, K. Patel, N. Savaliya, S. Bagatharia, S. Kumar, C. Joshi, Genomic variations in SARS-CoV-2 genomes from Gujarat: underlying role of variants in disease epidemiology, Front. Genet. 12 (2021), 586569.
[26] F. Yuan, L. Wang, Y. Fang, Global SNP analysis of 11,183 SARS-CoV-2 strains reveals high genetic diversity, Transbound. Emerg. Dis. 68(6) (2020).
[27] H.S. Wong, C.L. Guo, G.H. Lin, K.Y. Lee, Y. Okada, W.C. Chang, Transcriptome network analyses in human coronavirus infections suggest a rational use of immunomodulatory drugs for COVID-19 therapy, Genomics 113 (2021) 564–575.
[28] W.T. Harvey, A.M. Carabelli, B. Jackson, R.K. Gupta, E.C. Thomson, E.M. Harrison, C. Ludden, R. Reeve, A. Rambaut, S.J. Peacock, D.L. Robertson, SARS-CoV-2 variants, spike mutations and immune escape, Nat. Rev. Microbiol. 19 (2021) 409–424.
[29] A. Chaudhari, M. Chaudhari, S. Mahera, Z. Saiyed, N.M. Nathani, S. Shukla, D. Patel, C. Patel, M. Joshi, C.G. Joshi, In-silico analysis reveals lower transcription efficiency of C241T variant of SARS-CoV-2 with host replication factors MADP1 and hnRNP-1, Inform. Med. Unlocked 25 (2021), 100670.
[30] P. Muthuramalingam, R. Jeyasri, A. Valliammai, A. Selvaraj, C. Karthika, S. Gowrishankar, S.K. Pandian, M. Ramesh, J.T. Chen, Global multi-omics and systems pharmacological strategy unravel the multi-targeted therapeutic potential of natural bioactive molecules against COVID-19: an in silico approach, Genomics 112 (2020) 4486–4504.
[31] C. Joshi, A. Chaudhari, M. Joshi, S. Bagatharia, Repurposing of the herbal formulations: molecular docking and molecular dynamics simulation studies to validate the efficacy of phytocompounds against SARS-CoV-2 proteins, J. Biomol. Struct. Dyn. (2021) 1–15.
[32] J. Taneera, M.Y. Hachim, I.Y. Hachim, S. Al Heialy, N. Sulaiman, Cellular exocytosis gene (EXOC6/6B): a potential molecular link for the susceptibility and mortality of COVID-19 in diabetic patients, bioRxiv (2020).
[33] A. Elhabyan, S. Elyaacoub, E. Sanad, A. Abukhadra, V. Dinu, The role of host genetics in susceptibility to severe viral infections in humans and insights into host genetics of severe COVID-19: a systematic review, Virus Res. 289 (2020), 198163.
[34] I.G. Ovsyannikova, I.H. Haralambieva, S.N. Crooke, G.A. Poland, R.B. Kennedy, The role of host genetics in the immune response to SARS-CoV-2 and COVID-19 susceptibility and severity, Immunol. Rev. 296 (2020) 205–219.
[35] J. Hu, C. Li, S. Wang, T. Li, H. Zhang, Genetic Variants Are Identified to Increase Risk of COVID-19 Related Mortality from UK Biobank Data, medRxiv : The Preprint Server for Health Sciences, 2020.
[36] S. Biswas, V. Thakur, P. Kaur, A. Khan, S. Kulshrestha, P. Kumar, Blood clots in COVID-19 patients: simplifying the curious mystery, Med. Hypotheses 146 (2021), 110371.
[37] M.B. Malas, I.N. Naazie, N. Elsayed, A. Mathlouthi, R. Marmor, B. Clary, Thromboembolism risk of COVID-19 is high and associated with a higher risk of mortality: a systematic review and meta-analysis, EClinicalMedicine 29 (2020), 100639.
[38] N. Tang, D. Li, X. Wang, Z. Sun, Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia, J. Thromb. Haemost.: JTH 18 (2020) 844–847.
[39] S. Shah, K. Shah, S.B. Patel, F.S. Patel, M. Osman, P. Velagapudi, M.K. Turagam, D. Lakkireddy, J. Garg, Elevated D-Dimer Levels Are Associated With Increased Risk of Mortality in Coronavirus Disease, A systematic review and Meta-analysis, Cardiol. Rev. 28 (2020) (2019) 295–302.
[40] R. Yadav, R. Bansal, S. Budakoty, P. Barwad, COVID-19 and sudden cardiac death: a new potential risk, Indian Heart J. 72 (2020) 333–336.
[41] P. Sultanian, P. Lundgren, A. Stromsoe, S. Aune, G. Bergstrom, E. Hagberg, J. Hollenberg, J. Lindqvist, T. Djarv, A. Castelheim, A. Thoren, F. Hessulf, L. Svensson, A. Claesson, H. Friberg, P. Nordberg, E. Omerovic, A. Rosengren, J. Herlitz, A. Rawshani, Cardiac arrest in COVID-19: characteristics and outcomes of in- and out-of-hospital cardiac arrest, Report Swedish Registry Cardiopulm. Resuscitat., Eur. Heart J. 42 (2021) 1094–1106.
[42] J. Xu, Y. He, J. Wang, X. Li, L. Huang, S. Li, X. Qin, Influence of the TNFSF4 rs1234315 polymorphism in the susceptibility to systemic lupus erythematosus and rheumatoid arthritis, Hum. Immunol. 80 (2019) 270–275.
[43] M. Ria, J. Lagercrantz, A. Samnegard, S. Boquist, A. Hamsten, P. Eriksson, A common polymorphism in the promoter region of the TNFSF4 gene is associated with lower allele-specific expression and risk of myocardial infarction, PLoS One 6 (2011), e17652.
[44] J.M. Wang, Z.C. Yuan, A.F. Huang, W.D. Xu, Association of TNFSF4 rs1234315, rs2205960 polymorphisms and systemic lupus erythematosus susceptibility: a meta-analysis, Lupus 28 (2019) 1197–1204.
[45] A. Kusnadi, C. Ramirez-Suastegui, V. Fajardo, S.J. Chee, B.J. Meckiff, H. Simon, E. Pelosi, G. Seumois, F. Ay, P. Vijayanand, C.H. Ottensmeier, Severely ill COVID-19 patients display impaired exhaustion features in SARS-CoV-2-reactive CD8(+) T cells, Sci. Immunol. 6 (2021).
[46] J.Y. Koh, E.C. Shin, Landscapes of SARS-CoV-2-reactive CD8(+) T cells: heterogeneity of host immune responses against SARS-CoV-2, Signal Transduct. Target. Ther. 6 (2021) 146.
[47] Z. Chen, E. John Wherry, T cell responses in patients with COVID-19, nature reviews, Immunology 20 (2020) 529–536.
[48] N. Le Bert, A.T. Tan, K. Kunasegaran, C.Y.L. Tham, M. Hafezi, A. Chia, M.H. Y. Chng, M. Lin, N. Tan, M. Linster, W.N. Chia, M.I. Chen, L.F. Wang, E.E. Ooi, S. Kalimuddin, P.A. Tambyah, J.G. Low, Y.J. Tan, A. Bertoletti, SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls, Nature 584 (2020) 457–462.
[49] K.L. Chu, N.V. Batista, K.C. Wang, A.C. Zhou, T.H. Watts, GITRL on inflammatory antigen presenting cells in the lung parenchyma provides signal 4 for T-cell accumulation and tissue-resident memory T-cell formation, Mucosal Immunol. 12 (2019) 363–377.
[50] H. Hwang, S. Lee, W.H. Lee, H.J. Lee, K. Suk, Stimulation of glucocorticoid-induced tumor necrosis factor receptor family-related protein ligand (GITRL) induces inflammatory activation of microglia in culture, J. Neurosci. Res. 88 (2010) 2188–2196.
[51] T.H. Watts, TNF/TNFR family members in costimulation of T cell responses, Annu. Rev. Immunol. 23 (2005) 23–68.
[52] J.K. Pickrell, T. Berisa, J.Z. Liu, L. Segurel, J.Y. Tung, D.A. Hinds, Detection and interpretation of shared genetic influences on 42 human traits, Nat. Genet. 48 (2016) 709–717.
[53] M.A.R. Ferreira, R. Mathur, J.M. Vonk, A. Szwajda, B. Brumpton, R. Granell, B. K. Brew, V. Ullemar, Y. Lu, Y. Jiang, P.K.E. Magnusson, R. Karlsson, D.A. Hinds, L. Paternoster, G.H. Koppelman, C. Almqvist, Genetic architectures of childhood- and adult-onset asthma are partly distinct, Am. J. Hum. Genet. 104 (2019) 665–684.
[54] The COVID-19 Host Genetics Initiative, The COVID-19 host genetics initiative, a global initiative to elucidate the role of host genetic factors in susceptibility and severity of the SARS-CoV-2 virus pandemic, Eur. J. Human Genetics: EJHG 28 (2020) 715–718.
[55] H. Lu, N. Lu, L. Weng, B. Yuan, Y.J. Liu, Z. Zhang, DHX15 senses double-stranded RNA in myeloid dendritic cells, J. Immunol. 193 (2014) 1364–1372.
[56] S. Pattabhi, M. Gale, Y.-M. Loo, 145: DHX15: a novel regulator of innate immune defense to RNA virus infections, Cytokine 70 (2014) 63.
[57] J. Xing, X. Zhou, M. Fang, E. Zhang, L.J. Minze, Z. Zhang, DHX15 is required to control RNA virus-induced intestinal inflammation, Cell Rep. 35 (2021), 109205.
[58] G. Wang, X. Xiao, Y. Wang, X. Chu, Y. Dou, L.J. Minze, R.M. Ghobrial, Z. Zhang, X. C. Li, The RNA helicase DHX15 is a critical regulator of natural killer-cell homeostasis and functions, Cell. Mol. Immunol. 19(6) (2022).
[59] N. Hemmat, Z. Asadzadeh, N.K. Ahangar, H. Alemohammad, B. Najafzadeh, A. Derakhshani, A. Baghbanzadeh, H.B. Baghi, D. Javadrashid, S. Najafi, M. Ar Gouilh, B. Baradaran, The roles of signaling pathways in SARS-CoV-2 infection; lessons learned from SARS-CoV and MERS-CoV, Arch. Virol. 166 (2021) 675–696.
[60] L. Xu, S. Khadijah, S. Fang, L. Wang, F.P. Tay, D.X. Liu, The cellular RNA helicase DDX1 interacts with coronavirus nonstructural protein 14 and enhances viral replication, J. Virol. 84 (2010) 8571–8583.
[61] F. Sanchis-Gomar, J.L. Garcia-Gimenez, M.C. Gomez-Cabrera, F.V. Pallardo, Mitochondrial biogenesis in health and disease, Mol. Ther. Appr. Curr. Pharm. Des. 20 (2014) 5619–5633.
[62] G.W. Dorn 2nd, R.B. Vega, D.P. Kelly, Mitochondrial biogenesis and dynamics in the developing and diseased heart, Genes Dev. 29 (2015) 1981–1991.
[63] R. Ganji, P.H. Reddy, Impact of COVID-19 on mitochondrial-based immunity in aging and age-related diseases, Front. Aging Neurosci. 12 (2020), 614650.
[64] K.K. Singh, G. Chaubey, J.Y. Chen, P. Suravajhala, Decoding SARS-CoV-2 hijacking of host mitochondria in COVID-19 pathogenesis, Am. J. Phys. Cell Physiol. 319 (2020) C258–C267.
[65] S. Ajaz, M.J. McPhail, K.K. Singh, S. Mujib, F.M. Trovato, S. Napoli, K. Agarwal, Mitochondrial metabolic manipulation by SARS-CoV-2 in peripheral blood mononuclear cells of patients with COVID-19, Am. J. Phys. Cell Physiol. 320 (2021) C57–C65.
[66] J.S. Ayres, A metabolic handbook for the COVID-19 pandemic, Nature Metab. 2 (2020) 572–585.
[67] Z. Yildirim, O.S. Sahin, S. Yazar, V., Bozok Cetintas, genetic and epigenetic factors associated with increased severity of Covid-19, Cell Biol. Int. 45 (2021) 1158–1174.
[68] M.A. Khan, A. Islam, SARS-CoV-2 proteins exploit Host's genetic and epigenetic mediators for the annexation of key host signaling pathways, Front. Mol. Biosci. 7 (2020), 598583.

[69] Y. Dai, J. Wang, H.H. Jeong, W. Chen, P. Jia, Z. Zhao, Association of CXCR6 with COVID-19 severity: delineating the host genetic factors in transcriptomic regulation, Human Genetics 140(9) (2021).
[70] M. Marsh, A. Helenius, Virus entry: open sesame, Cell 124 (2006) 729–740.
[71] S. Yuan, H. Chu, J.F. Chan, Z.W. Ye, L. Wen, B. Yan, P.M. Lai, K.M. Tee, J. Huang, D. Chen, C. Li, X. Zhao, D. Yang, M.C. Chiu, C. Yip, V.K. Poon, C.C. Chan, K.H. Sze, J. Zhou, I.H. Chan, K.H. Kok, K.K. To, R.Y. Kao, J.Y. Lau, D.Y. Jin, S. Perlman, K. Y. Yuen, SREBP-dependent lipidomic reprogramming as a broad-spectrum antiviral target, Nat. Commun. 10 (2019) 120.
[72] J.W. Song, S.M. Lam, X. Fan, W.J. Cao, S.Y. Wang, H. Tian, G.H. Chua, C. Zhang, F. P. Meng, Z. Xu, J.L. Fu, L. Huang, P. Xia, T. Yang, S. Zhang, B. Li, T.J. Jiang, R. Wang, Z. Wang, M. Shi, J.Y. Zhang, F.S. Wang, G. Shui, Omics-driven systems interrogation of metabolic dysregulation in COVID-19 pathogenesis, Cell Metab. 32 (2020) 188–202 e185.
[73] E. Barberis, S. Timo, E. Amede, V.V. Vanella, C. Puricelli, G. Cappellano, D. Raineri, M.G. Cittone, E. Rizzi, A.R. Pedrinelli, V. Vassia, F.G. Casciaro, S. Priora, I. Nerici, A. Galbiati, E. Hayden, M. Falasca, R. Vaschetto, P.P. Sainaghi, U. Dianzani, R. Rolla, A. Chiocchetti, G. Baldanzi, E. Marengo, M. Manfredi, Large-scale plasma analysis revealed new mechanisms and molecules associated with the host response to SARS-CoV-2, Int. J. Mol. Sci. 21 (2020).
[74] R. Vijay, X. Hua, D.K. Meyerholz, Y. Miki, K. Yamamoto, M. Gelb, M. Murakami, S. Perlman, Critical role of phospholipase A2 group IID in age-related susceptibility to severe acute respiratory syndrome-CoV infection, J. Exp. Med. 212 (2015) 1851–1868.
[75] L. Didon, R.K. Zwick, I.W. Chao, M.S. Walters, R. Wang, N.R. Hackett, R.G. Crystal, RFX3 modulation of FOXJ1 regulation of cilia genes in the human airway epithelium, Respir. Res. 14 (2013) 70.
[76] X. Sun, Transcriptional Networks of Lung Airway Epithelial Ciliogenesis, 2009.
[77] J. Thomas, L. Morle, F. Soulavie, A. Laurencon, S. Sagnol, B. Durand, Transcriptional control of genes involved in ciliogenesis: a first step in making cilia, Biol. Cell. 102 (2010) 499–513.
[78] B. Chen, J. Niu, J. Kreuzer, B. Zheng, G.K. Jarugumilli, W. Haas, X. Wu, Auto-fatty acylation of transcription factor RFX3 regulates ciliogenesis, Proc. Natl. Acad. Sci. U. S. A. 115 (2018) E8403–E8412.
[79] L. El Zein, A. Ait-Lounis, L. Morle, J. Thomas, B. Chhin, N. Spassky, W. Reith, B. Durand, RFX3 governs growth and beating efficiency of motile cilia in mouse and controls the expression of genes involved in human ciliopathies, J. Cell Sci. 122 (2009) 3180–3189.
[80] X. Yu, C.P. Ng, H. Habacher, S. Roy, Foxj1 transcription factors are master regulators of the motile ciliogenic program, Nat. Genet. 40 (2008) 1445–1453.
[81] M.J. Brody, Y. Lee, The role of leucine-rich repeat containing protein 10 (LRRC10) in dilated cardiomyopathy, Front. Physiol. 7 (2016) 337.
[82] E. Kott, P. Duquesnoy, B. Copin, M. Legendre, F. Dastot-Le Moal, G. Montantin, L. Jeanson, A. Tamalet, J.F. Papon, J.P. Siffroi, N. Rives, V. Mitchell, J. de Blic, A. Coste, A. Clement, D. Escalier, A. Toure, E. Escudier, S. Amselem, Loss-of-function mutations in LRRC6, a gene essential for proper axonemal assembly of inner and outer dynein arms, cause primary ciliary dyskinesia, Am. J. Hum. Genet. 91 (2012) 958–964.
[83] J.S. Lucas, S.D. Davis, H. Omran, A. Shoemark, Primary ciliary dyskinesia in the genomics age, the lancet, Respir. Med. 8 (2020) 202–216.
[84] A. Rahman, T. Tabassum, Y. Araf, A. Al Nahid, M.A. Ullah, M.J. Hosen, Silent hypoxia in COVID-19: pathomechanism and possible management strategy, Mol. Biol. Rep. 48 (2021) 3863–3869.
[85] M. Jahani, S. Dokaneheifard, K. Mansouri, Hypoxia: a key feature of COVID-19 launching activation of HIF-1 and cytokine storm, J. Inflamm. (Lond) 17 (2020) 33.
[86] U.W. Iepsen, R.R. Plovsing, K. Tjelle, N.B. Foss, C.S. Meyhoff, C.K. Ryrso, R.M. G. Berg, N.H. Secher, The role of lactate in sepsis and COVID-19: perspective from contracting skeletal muscle metabolism, Exp. Physiol. (2021), https://doi.org/10.1113/EP089474.
[87] H. Yan, X. Liang, J. Du, Z. He, Y. Wang, M. Lyu, L. Yue, F. Zhang, Z. Xue, L. Xu, G. Ruan, J. Li, H. Zhu, J. Xu, S. Chen, C. Zhang, D. Lv, Z. Lin, B. Shen, Y. Zhu, B. Qian, H. Chen, T. Guo, Proteomic and metabolomic investigation of serum lactate dehydrogenase elevation in COVID-19 patients, Proteomics 21 (2021), e2100002.
[88] B.M. Henry, G. Aggarwal, J. Wong, S. Benoit, J. Vikse, M. Plebani, G. Lippi, Lactate dehydrogenase levels predict coronavirus disease, (COVID-19) severity and mortality: a pooled analysis, Am. J. Emerg. Med. 38 (2020) (2019) 1722–1726.
[89] Y. Lu, K. Sun, S. Guo, J. Wang, A. Li, X. Rong, T. Wang, Y. Shang, W. Chang, S. Wang, Early warning indicators of severe COVID-19: a single-center study of cases from Shanghai, China Front. Med. 7 (2020) 432.
[90] E. Gopal, N.S. Umapathy, P.M. Martin, S. Ananth, J.P. Gnana-Prakasam, H. Becker, C.A. Wagner, V. Ganapathy, P.D. Prasad, Cloning and functional characterization of human SMCT2 (SLC5A12) and expression pattern of the transporter in kidney, Biochim. Biophys. Acta 1768 (2007) 2690–2697.
[91] R. Haas, J. Smith, V. Rocher-Ros, S. Nadkarni, T. Montero-Melendez, F. D'Acquisto, E.J. Bland, M. Bombardieri, C. Pitzalis, M. Perretti, F.M. Marelli-Berg, C. Mauro, Lactate regulates metabolic and pro-inflammatory circuits in control of T cell migration and effector functions, PLoS Biol. 13 (2015), e1002202.
[92] M. Certo, C.H. Tsai, V. Pucino, P.C. Ho, C. Mauro, Lactate modulation of immune responses in inflammatory versus tumour microenvironments, Nat. Rev. Immunol. 21 (2021) 151–161.
[93] S.W. John, G. Weitzner, R. Rozen, C.R. Scriver, A rapid procedure for extracting genomic DNA from leukocytes, Nucleic Acids Res. 19 (1991) 408.
[94] S. Purcell, B. Neale, K. Todd-Brown, L. Thomas, M.A. Ferreira, D. Bender, J. Maller, P. Sklar, P.I. de Bakker, M.J. Daly, P.C. Sham, PLINK: a tool set for whole-genome association and population-based linkage analyses, Am. J. Hum. Genet. 81 (2007) 559–575.
[95] W. Zhou, J.B. Nielsen, L.G. Fritsche, R. Dey, M.E. Gabrielsen, B.N. Wolford, J. LeFaive, P. VandeHaar, S.A. Gagliano, A. Gifford, L.A. Bastarache, W.Q. Wei, J. C. Denny, M. Lin, K. Hveem, H.M. Kang, G.R. Abecasis, C.J. Willer, S. Lee, Efficiently controlling for case-control imbalance and sample relatedness in large-scale genetic association studies, Nat. Genet. 50 (2018) 1335–1341.