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Supplementary information

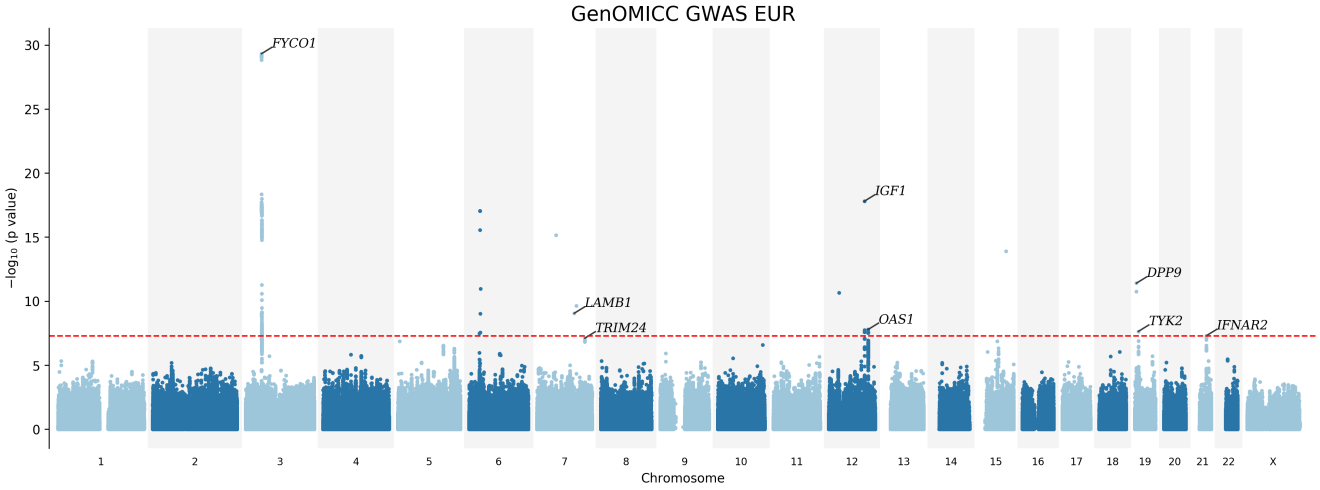
Genetic mechanisms of critical illness in COVID-19

In the format provided by the authors and unedited

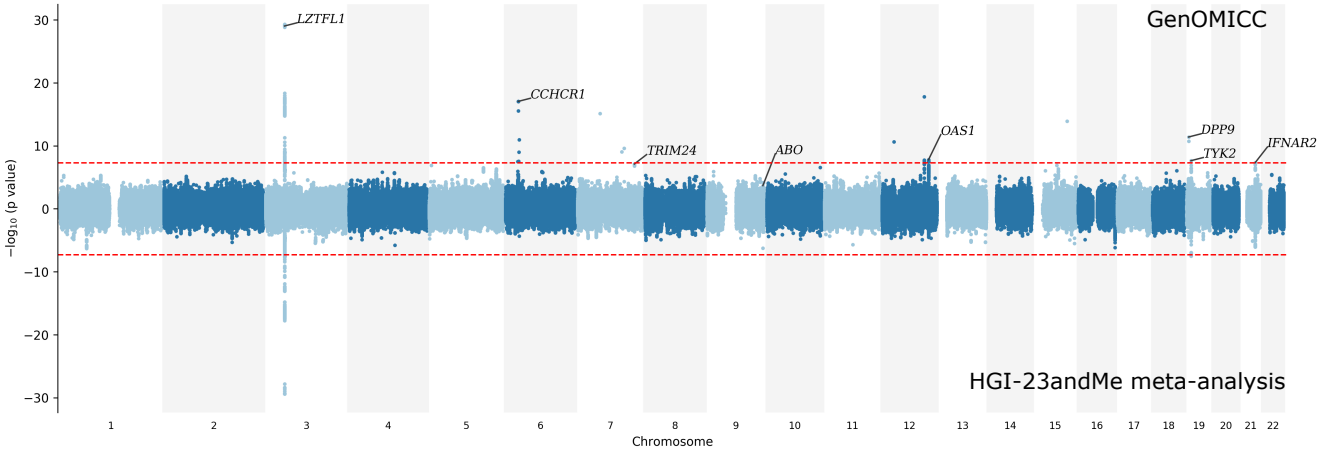
Genetic mechanisms of critical illness in Covid-19 Supplementary Information

Contents

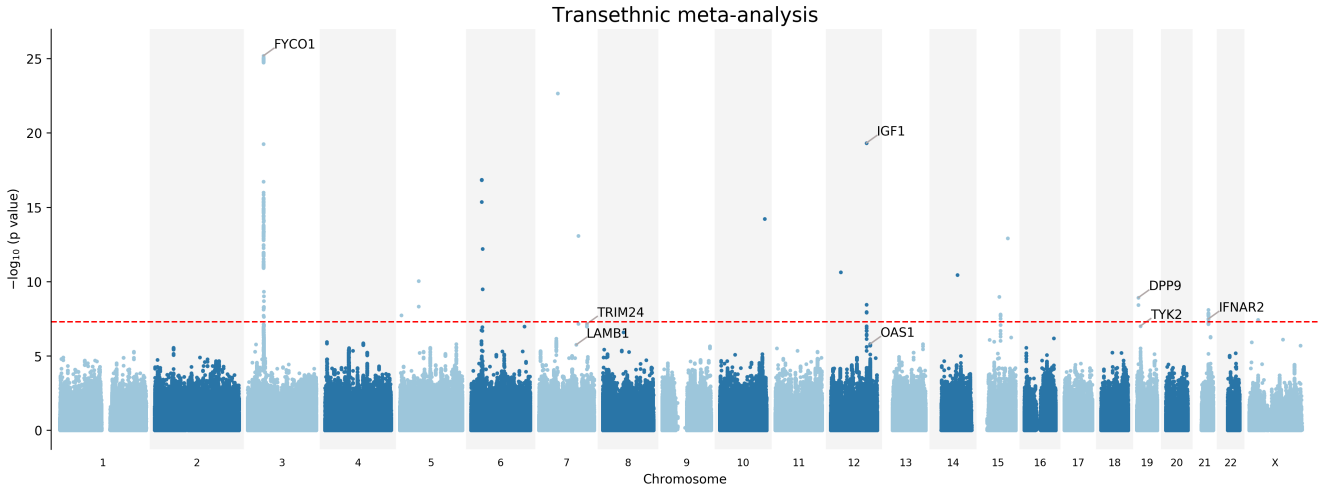
GWAS details



Supplementary Figure 1: Manhattan plot showing single nucleotide polymorphism (SNP)-level uncorrected p-values from GWAS analysis (before validation vs Generation Scotland and 100,000 genomes controls) in largest ancestry group, EUR (red horizontal line shows genome-wide significance at $-log_{10}(5 \times 10^{-8})$)



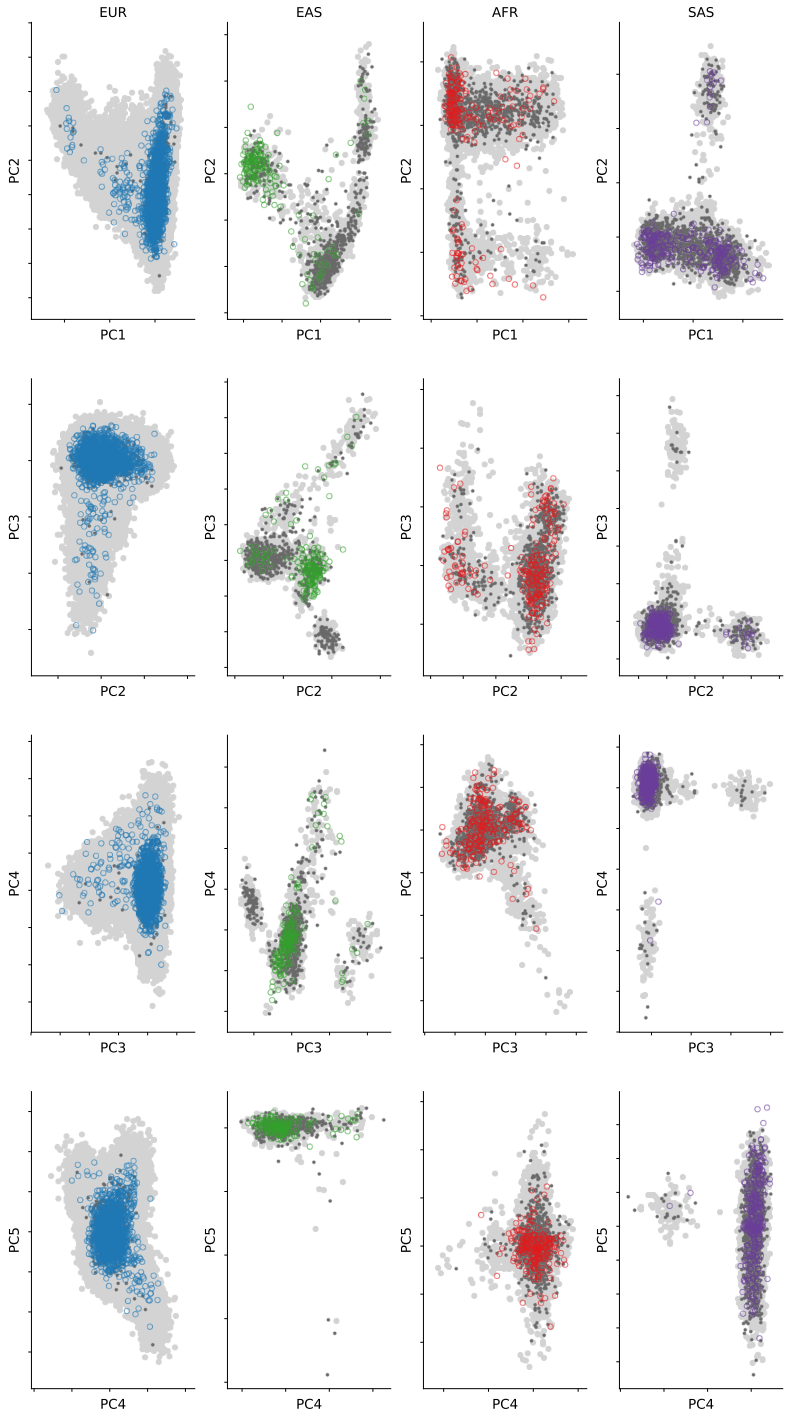
Supplementary Figure 2: Miami plot showing relationship between the EUR GenOMICC GWAS vs UK Biobank (before validation vs Generation Scotland and 100,000 genomes controls) and a restricted meta-analysis including only patients from the Covid-19 Host Genetics Initiative and 23andMe. Raw (uncorrected) p-values from GWAS analysis are shown. In upper (GenOMICC) panel, red horizontal line shows genome-wide significance for common variants at $-log_{10}(5 \times 10^{-8})$; in lower (meta-analysis) panel, red horizontal line shows a more stringent genome-wide significance threshold for meta-analysis variants at $-log_{10}(10^{-8})$



Supplementary Figure 3: Manhattan plot showing SNP-level p-values from trans-ethnic meta-analysis including European (EUR), African (AFR), East Asian (EAS), and South Asian (SAS) PCA-determined ancestry groups in GenOMICC. (red horizontal line shows genome-wide significance at $-log_{10}(5 \times 10^{-8})$)

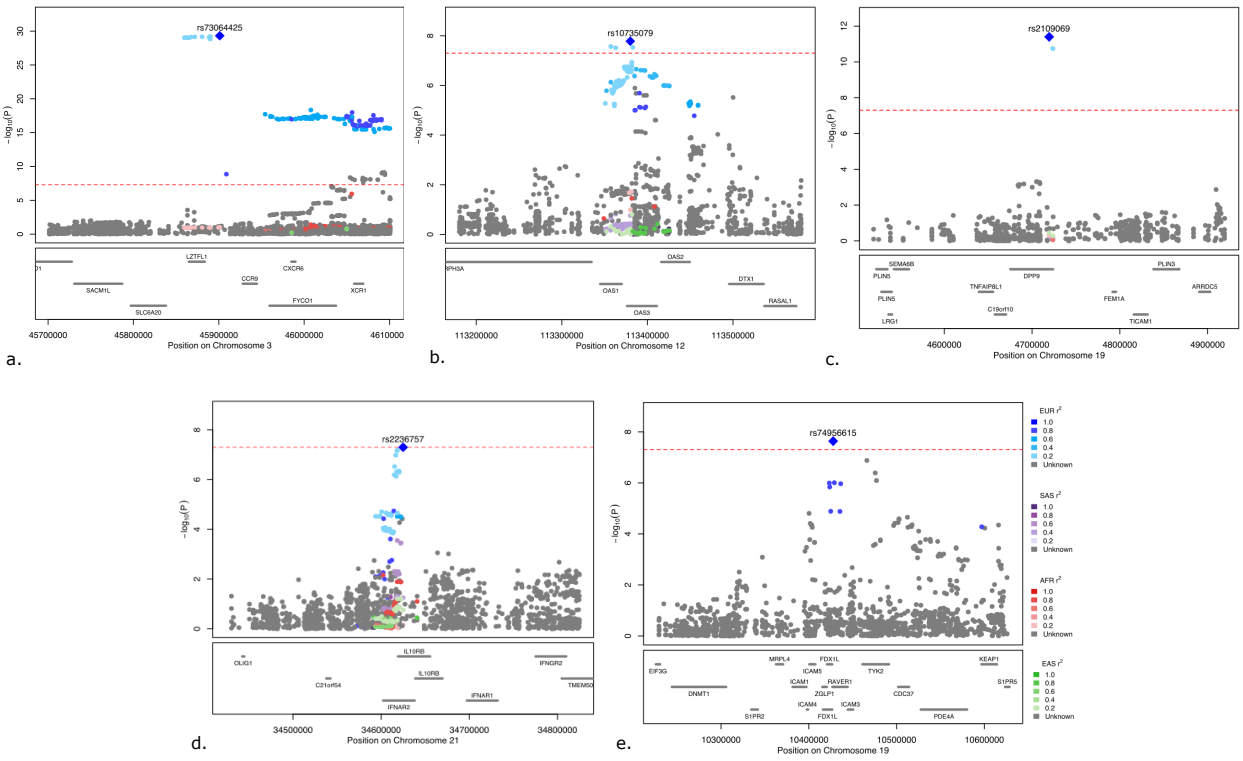
Supplementary Table 1: Lead variants from sex-specific genome-wide significant regions in GenOMICC European ancestry group. chr:pos - chromosome and position of the top SNP (build 37); Risk – risk allele; Other - other allele; RAF - risk allele frequency; OR - effect size (odds ratio) of the risk allele; locus – gene nearest to the top SNP.

snps	chr.pos.b37.	Risk	Other	RAF.male	OR.male	P.male	RAF.female	OR.female	P.female
rs73064425	3:45901089	T	C	0.083	2.3	3.2e-28	0.083	2	1.3e-07
rs143334143	6:31121426	A	G	0.076	1.9	4.7e-15	0.073	2.3	1.1e-09
rs3131294	6:32180146	G	A	0.86	1.5	4.2e-08	0.86	1.5	0.00081

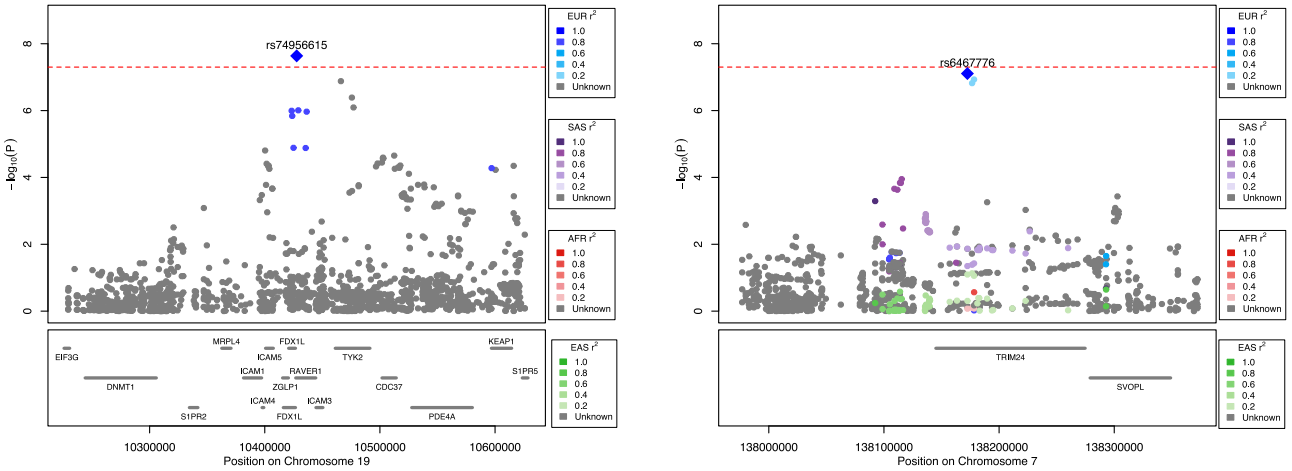


Supplementary Figure 4: PCA plots showing the distribution of all cases and controls for the first 5 principal components for each ancestry group. Cases are shown as coloured open circles: European (EUR, blue), African (AFR, red), East Asian (EAS, green), and South Asian (SAS, purple). Controls are dark grey closed circles. UK Biobank population background is shown as light grey closed circles.

Regions in detail



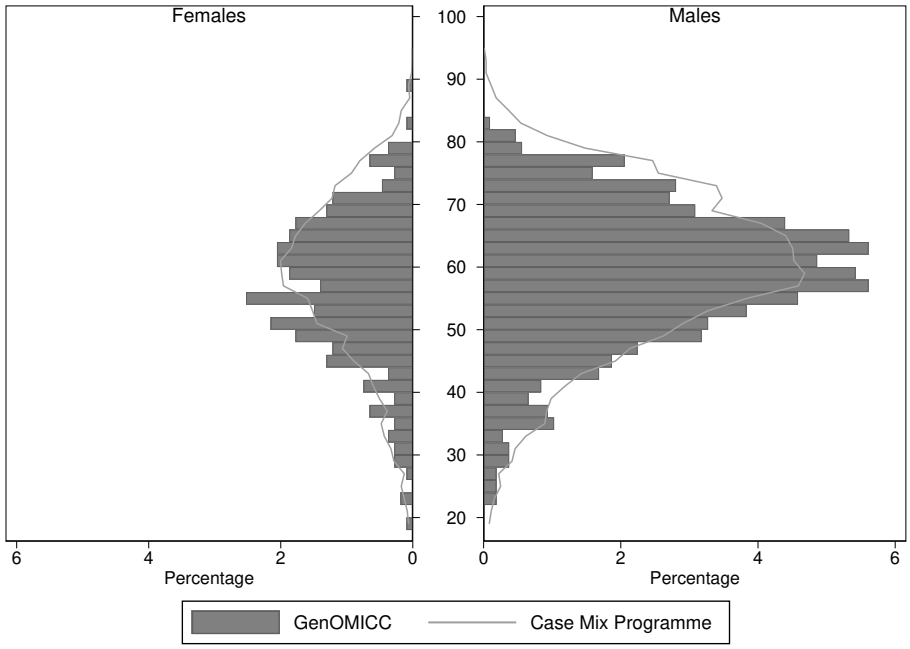
Supplementary Figure 5: Genomic region plots showing ancestry-specific p-values and LD structure. Each variant is plotted at the y-axis position for GWAS within a given ancestry group in GenOMICC vs UK Biobank analysis. A variant is plotted for an ancestry group only if it has MAF>5% in that group in UK Biobank. Colour depicts genetic ancestry group (EUR = blue, EAS = green, AFR = red, SAS = purple); shading shows linkage disequilibrium value (LD, r^2) within a given ancestry group with the lead SNP; variants with unknown LD for each ancestry group are shown in grey; lead SNP for EUR is shown in each plot as a blue diamond. LD reference is calculated using PLINK from 5 cases randomly selected unrelated matched-ethnicity subjects in UK Biobank.



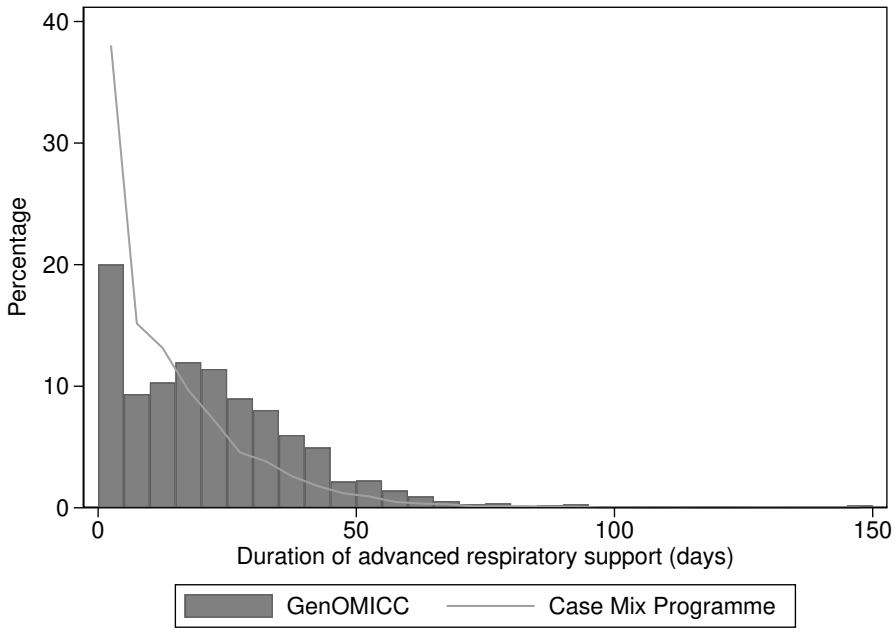
Supplementary Figure 6: Regional plots showing the TYK2, TRIM24, IGF1, and HLA loci. Red horizontal line shows genome-wide significance at $-log_{10}(5 \times 10^{-8})$). Colour depicts linkage disequilibrium (LD, r^2) with lead SNP (purple diamond) in each plot. LD reference is calculated using PLINK from 10,000 randomly-selected unrelated European subjects in UK Biobank.

Demographics, recruitment and severity

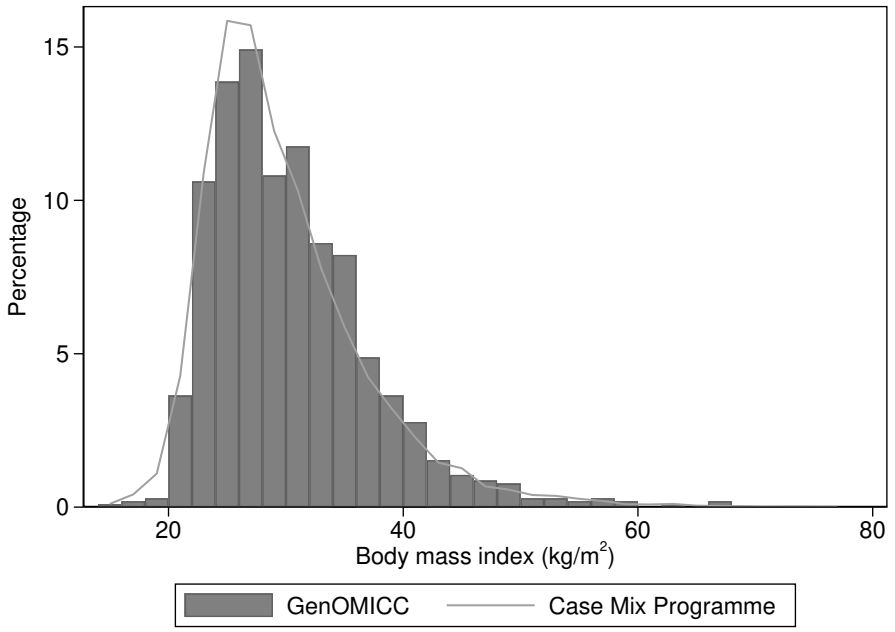
Cases were broadly representative of the UK critically-ill population. For 1069 GenOMICC cases, matched clinical data could be obtained from the Intensive Care National Audit and Research Centre (ICNARC) Case Mix Programme (CMP), a national audit of critical care admission in England, Wales and Northern Ireland.



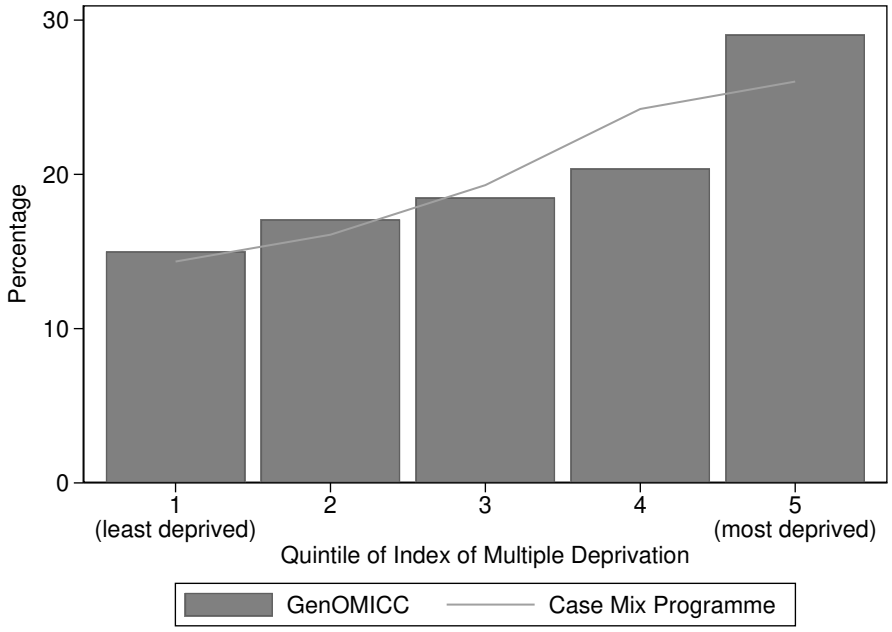
Supplementary Figure 7: Histogram showing age (in 2 year bins; first column shows lower end of band) and sex for 1069 patients recruited to GenOMICC for whom case mix programme data were available (bars), and all critically ill Covid-19 patients from the ICNARC Case Mix Programme (lines).



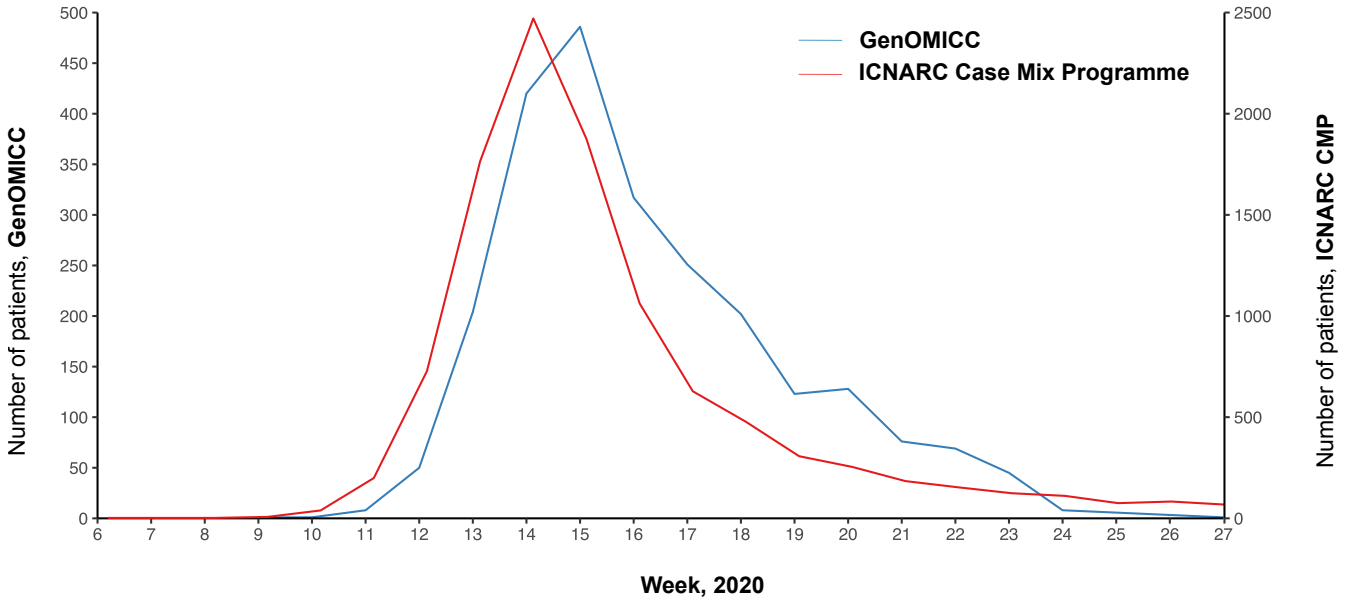
Supplementary Figure 8: Histogram showing duration of advanced respiratory support (invasive ventilation) for 1069 patients recruited to GenOMICC for whom case mix programme data were available (bars), and all critically ill Covid-19 patients from the ICNARC Case Mix Programme (lines). Patients who required a very short period of invasive ventilation are more likely to be discharged early from the ICU, and hence may have been disproportionately missed by the research teams from recruitment to GenOMICC.



Supplementary Figure 9: Histogram showing body mass index (BMI, kg/m^2; 2 kg/m2 bands; first column shows lower end of band) for 1069 patients recruited to GenOMICC for whom case mix programme data were available (bars), and all critically ill Covid-19 patients from the ICNARC Case Mix Programme (lines).

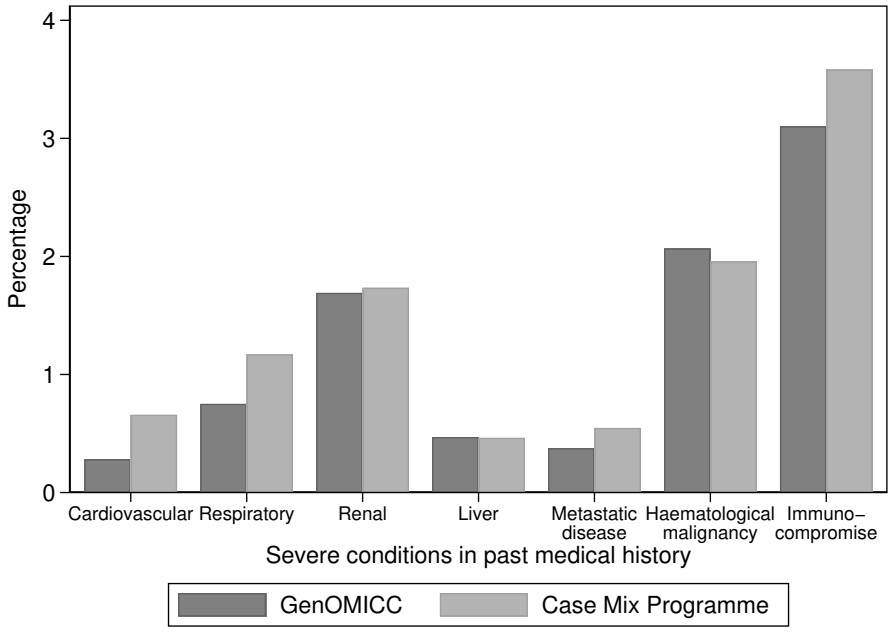


Supplementary Figure 10: Percentage of patients in Index of Multiple Deprivation 2019[1] quintiles (a government statistic measuring deprivation in small local units across the country). Data are shown for 1069 patients recruited to GenOMICC for whom case mix programme data were available (bars), and all critically ill Covid-19 patients from the ICNARC Case Mix Programme (lines).



Supplementary Figure 11: Rate of recruitment showing the GenOMICC cases included in the current analysis (UK-wide), and the overall rate of ICU admissions with Covid-19 in the ICNARC Case Mix Programme (England, Wales and Northern Ireland only).

Comorbidity



Supplementary Figure 12: Past medical history recorded for 1069 patients recruited to GenOMICC for whom case mix programme data were available (dark grey bars), and all critically ill Covid-19 patients from the ICNARC Case Mix Programme (light grey bars).

Supplementary Table 2: Comorbid illness in subsets of cases for whom detailed information are available. †n = number of participants with available data. *The specific co-morbidities recorded, and how they were defined, differed between the Intensive Care National Audit & Research Centre (ICNARC) database (for GenOMICC participants) and the ISARIC4C database. ICNARC used more stringent definitions to capture "very severe" co-morbidities whereas ISARIC4C recorded any chronic disease affecting an organ system, even if less severe.

Co-morbidity	*n* (%)	Definition*
GenOMICC (n=1064†)		
Cardiovascular	3 (0.3)	Cardiovascular symptoms at rest
Pulmonary	8 (0.8)	Dyspnoea with light activity or requires home ventilation
Renal	18 (1.7)	Requires renal replacement therapy (RRT)
Liver	5 (0.5)	Liver cirrhosis (biopsy-proven), portal hypertension or hepatic encephalopathy

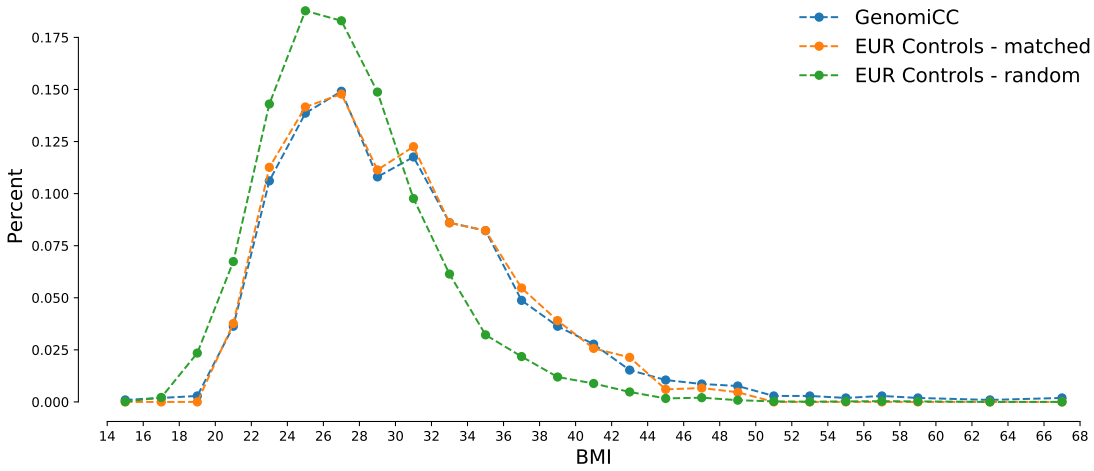
Co-morbidity	*n* (%)	Definition*
Malignant neoplasm	4 (0.4)	Malignant neoplasm with distant metastases
Haematologic neoplasm	22 (2.1)	Leukaemia, lymphoma or multiple myeloma
Immunocompromise	33 (3.1)	Acquired (iatrogenic, HIV/AIDS) or congenital
ISARIC4C (n=108†)		
Cardiovascular	31 (28.7)	Chronic heart disease (excluding hypertension)
Pulmonary	9 (8.3)	Chronic lung disease (excluding asthma)
Renal	8 (7.4)	Chronic kidney disease, irrespective of requirement for RRT
Liver	0 (0)	Moderate or severe liver disease
Malignant neoplasm	6 (5.6)	Local or metastatic neoplasm
Haematologic disease	0 (0)	Chronic haematologic disease of any aetiology

Matched controls

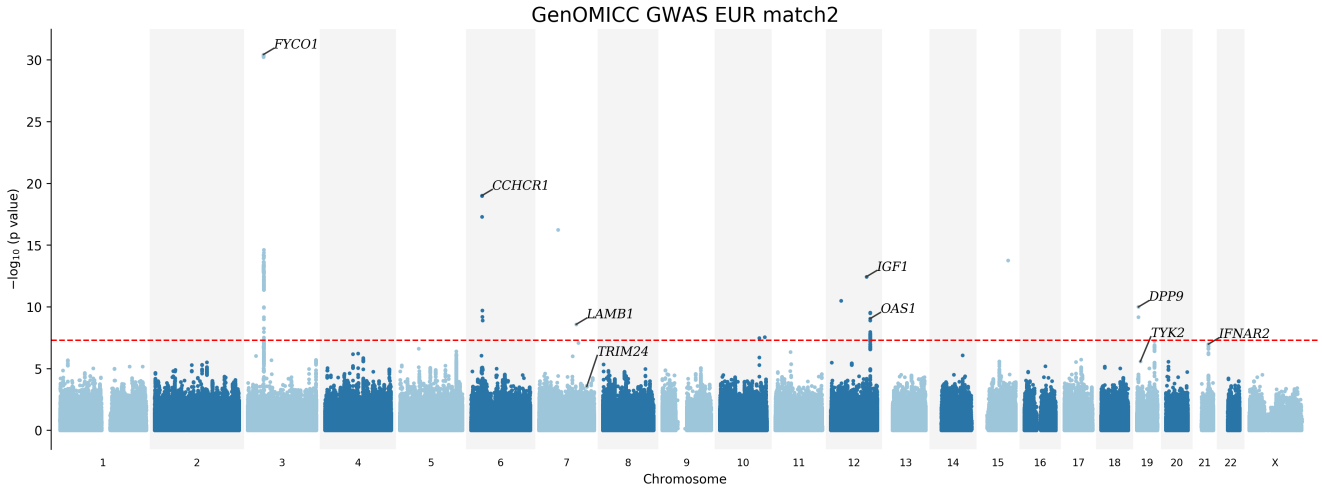
Because of the evidence of residual inflation in the GenOMICC EUR UK Biobank analysis (Supplementary Figure **??**), and the genetic correlations with obesity and educational attainment (Supplementary Figure 19a), we undertook further analysis to examine the effect of additional correction for population structure. We performed a GWAS in which we restricted the analysis to cases for whom UK Biobank controls could be identified according to the following rules:

- individual matches by ancestry, sex, age, and deprivation quintile
- BMI sampled from a distribution that parallels the ICNARC CMP BMI distribution for the GenOMICC cases (Supplementary Figure 9)

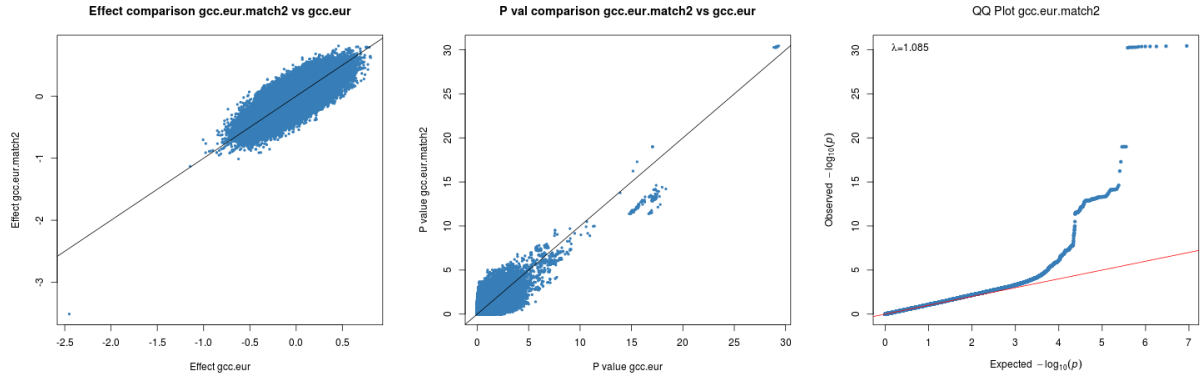
Applying these rules produced a smaller comparison than the primary analysis: $n_{cases} = 1260$; $n_{controls} = 6300$.



Supplementary Figure 13: Distribution of body mass index (BMI, kg/m^2; 2 kg/m2 bands) for GenOMICCcases (blue), UK Biobank random controls (primary analysis, green) and UK Biobank BMI-matched controls (orange)



(a)



(b) (c) (d)

Supplementary Figure 14: Results from secondary analysis with matched controls. (a) Manhattan plot showing raw (uncorrected) single nucleotide polymorphism (SNP)-level p-values for genome-wide significant associations (red horizontal line shows genome-wide significance at $-log_{10}(5 \times 10^{-8})$). (b) Correlation with primary analysis in effect sizes (β), and raw p-values (c). (d) QQ plot of raw (uncorrected) p-values.

Mendelian Randomisation

Supplementary Table 3: *a priori* core set genes that are potentially directly informative for prioritisation of drugs in trials. MOA - mechanism of action. MAIC - meta-analysis by information content.

Gene symbol	Rationale
ACE2	Core viral entry mechanism
TMPRSS2	Core viral entry mechanism
CTSL	Core viral entry mechanism
IL1A	MOA anakinra, MAIC top hit 2020-9-1[2]
IL1B	MOA anakinra
IL1R1	MOA anakinra
IL1R2	MOA anakinra
IL6	MOA tocilizumab/sarilumab
IL6R	MOA tocilizumab/sarilumab
CSF1	MAIC Covid 2020-9-1[2], Differential levels in severe/fatal cases
CSF2	MOA mavrilimumab,[3] other monoclonal ABs[4]
CSF3	MAIC Covid 2020-9-1[2], Differential levels in severe/fatal cases
PPIA	MAIC Covid 2020-9-1[2], MOA cyclophilin inhibitors[5]
IFNA1	MOA interferon α
IFNB1	MOA interferon β
IFNAR1	MOA interferon α/β
IFNAR2	MOA interferon α/β, MAIC Influenza[6]
IFNG	MOA interferon γ, MAIC Covid 2020-9-1[2]
IFNGR1	MOA interferon γ, MAIC Influenza[6]
IFNGR2	MOA interferon γ
FCGR1A	MOA fostamatinib
SYK	MOA fostamatinib
JAK1	MOA baricitinib/ruxolitinib
JAK2	MOA baricitinib/ruxolitinib
TNF	MOA anti-TNF drugs (e.g. infliximab, etanercept)
BTK	MOA Bruton's tyrosine kinase inhibitors (e.g. acalabrutinib)

Supplementary Table 4: Mendelian randomisation of gene expression of genes selected *a priori* as potential therapeutic targets for COVID-19. Gene: gene name; SNPchr: chromosome (GRCh37) of the SNP used as the instrumental variable; SNPpos: position (GRCh37) of the SNP; ALT: GRCh37 alternate allele; REF: GRCh37 reference allele; b_SMR: Mendelian Randomisation effect-size estimate; p_SMR: p-value of the Mendelian Randomisation effect-size estimate; p_HEIDI: p-value of the HEIDI test; nsnp_HEIDI: number of SNPs used in the calculation of p_HEIDI; † is Bonferroni significant.

Gene	SNPchr	SNPpos	ALT	REF	b_SMR	p_SMR	p_HEIDI	nsnp_HEIDI
IFNAR2	21	34610487	C	T	-1.491	0.0043†	0.0150	6
IFNAR1	21	34670914	T	A	-0.782	0.0341	0.1984	10
IL6R	1	154405058	G	T	0.839	0.0362	0.1937	6

Gene	SNPchr	SNPpos	ALT	REF	b_SMR	p_SMR	p_HEIDI	nsnp_HEIDI
JAK1	1	65513680	T	C	0.360	0.4199	0.1094	6
CTSL	9	90355073	A	G	-0.196	0.5021	0.6468	3
IFNGR2	21	34782395	C	A	-0.231	0.5037	0.9570	14
CSF3	17	39156499	G	A	0.012	0.9629	-	-

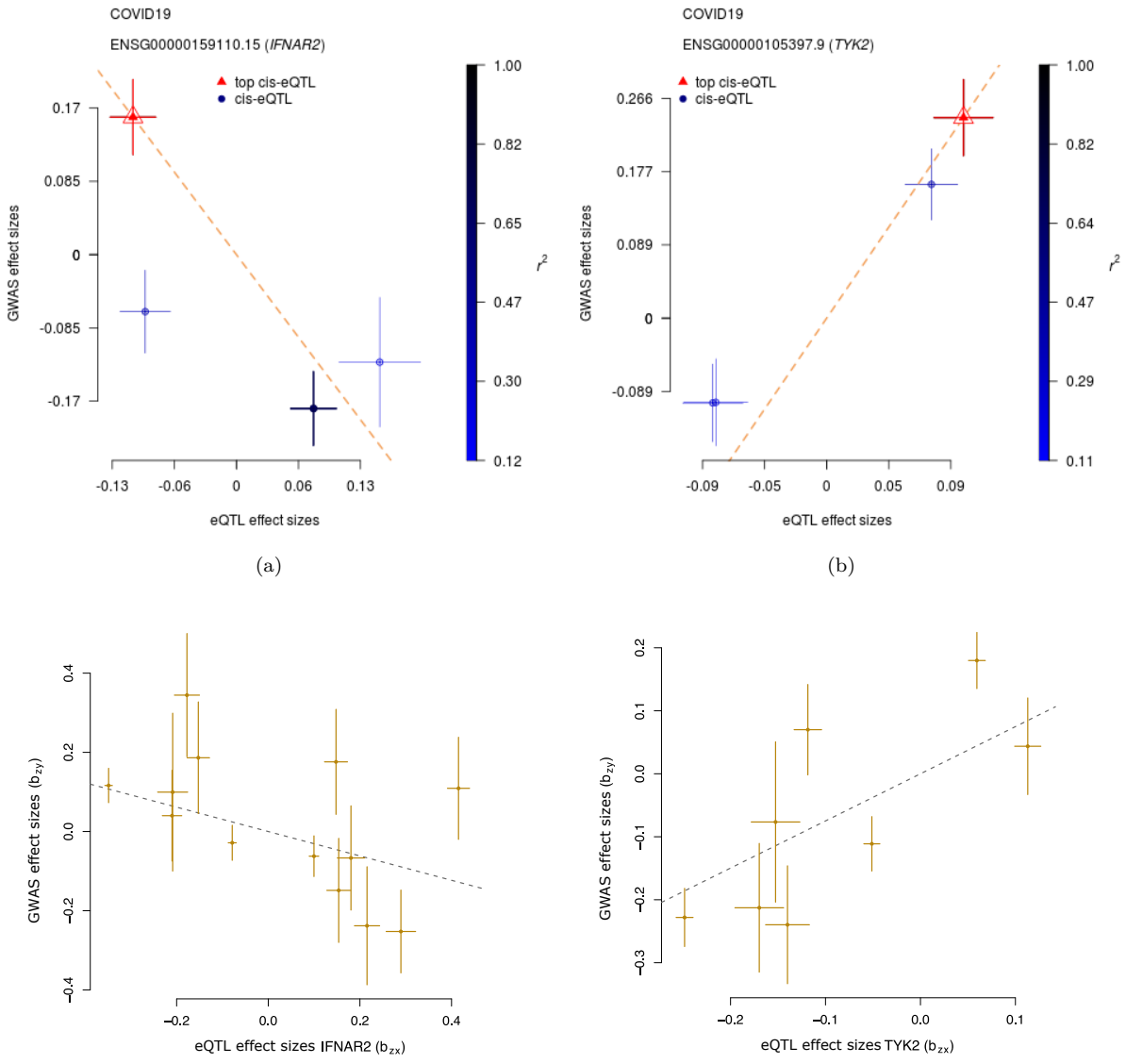
Supplementary Table 5: External replication (using Covid-19 HGI, UK Biobank excluded, and eQTLgen) of 9 genes with suggestive Mendelian randomisation associations from genome-wide analysis in GenOMICC (4,614 unique Ensembl gene IDs included in original analysis, genes selected had MR $p < 0.05/9$ and HEIDI $p > 0.05$). Gene: gene name; SNPchr: chromosome (GRCh37) of the SNP used as the instrumental variable; SNPpos: position (GRCh37) of the SNP; ALT: GRCh37 alternate allele; REF: GRCh37 reference allele; dir_SMR: direction of effect of the Mendelian Randomisation effect-size estimate; p_SMR: p-value of the Mendelian Randomisation effect-size estimate. † is Bonferroni significant.

Gene	SNPchr	SNPpos	ALT	REF	dir_SMR	p_SMR
TYK2	19	10466123	T	C	+	5.53E-05†
IGSF9B	11	133812299	C	T	+	1.90E-01
CCDC28A	6	139085617	G	C	+	2.69E-01
ORMDL3	17	38073968	C	G	-	3.85E-01
GNLY	2	85934499	A	C	-	6.99E-01
S100A13	1	153673034	G	A	-	7.04E-01
MMP25	16	3099335	A	G	-	7.84E-01
TOMM7	7	22860474	A	G	+	9.10E-01
TNFSF15	9	117579457	A	G	NA	NA

In order to further validate the key Mendelian randomisation findings, generalized summary-data Mendelian randomisation (GSMR)[7] was performed using multiple quasi-independent SNPs for TYK2 and IFNAR2 (Methods). Using data from eQTLgen[8], 8 and 13 quasi-independent SNPs after HEIDI-outlier filtering test were identified for each gene respectively. The GSMR results replicated the SMR results, with both a consistent direction of effect and a significant p-value in both cases (Supplementary Table 6, Supplementary Figure 15).

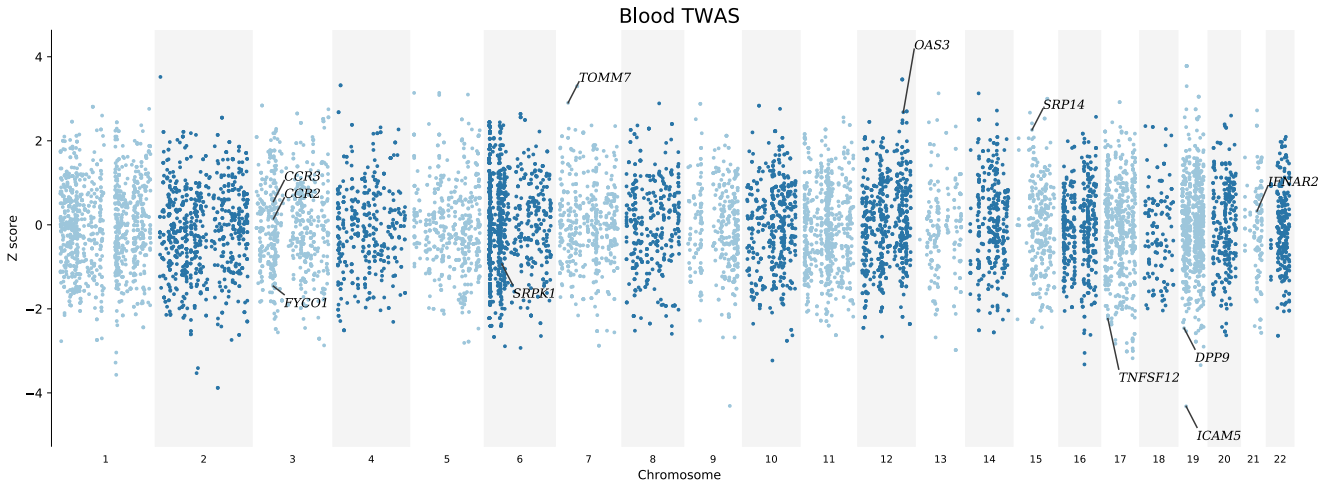
Supplementary Table 6: Results of GSMR analysis using GenOMICC GWAS EUR as outcome, for IFNAR2 and TYK2. β_{xy} - effect size; se - standard error for β_{xy}; p - Mendelian randomisation p-value; nsnp - number of SNPs included.{#tbl:mr.cojo}

Exposure	β_{xy}	se	p	nsnp
IFNAR2	-0.307834	0.115121	0.0075	13
TYK2	0.74874	0.168939	9.33e-06	8



Supplementary Figure 15: Exposure and outcome effect-size estimate ($\beta_{exposure}$ vs $\beta_{outcome}$) plots for IFNAR2 (a,c) and TYK2 (b,d). (a,b): SNPs included in the HEIDI tests for IFNAR2 and TYK2 using exposure data from GTEx v7 Whole Blood and outcome data from GenOMICC. Top SNPs are rs1131964 (chr21:34610487) and rs11085727 (chr19:10466123), respectively. Color bars show r^2 with top SNP. The orange dashed line represents the SMR effect-size estimates of the top cis-eQTL (i.e. it is not a regression line). (c,d) Generalized summary-data-based Mendelian randomisation (GSMR) for IFNAR2 and TYK2 using exposure data from eQTLgen and outcome data from GenOMICC. SNPs are quasi-independent, following LD and HEIDI-outlier filtering. Error bars show standard errors centred on the mean.

Transcriptome-wide association study



Supplementary Figure 16: Z-scores showing direction of effect for genotype-inferred expression of transcripts encoding protein-coding genes in blood (GTEXv8).

Supplementary Table 7: Meta-analysis of the TWAS from whole blood and lung using MultiXcan. Gene names are provided, with p value for the meta-analysis. n tissues is the number of tissues in which the orginal TWAS was performed; best tissue indicates the tissue with lower p value in the original TWAS (Lung or Whole Blood); p best tissue is the p value of the TWAS in the best tissue.

gene name	p.val meta	n tissues	p.val best tissue	best tissue	FDR p.val	Bonferroni p.val
CXCR6	2.4e-15	1	2.4e-15	Lung	2.8e-11	2.8e-11
OAS3	4e-09	2	0.0074	Whole_Blood	2.3e-05	4.6e-05
CCR3	2.7e-07	2	5e-08	Lung	0.0011	0.0032
MAT2B	9.6e-07	1	9.6e-07	Lung	0.0028	0.011
CCR2	1.6e-06	2	6.4e-07	Lung	0.0036	0.018
TNFSF15	1.3e-05	2	1.6e-05	Whole_Blood	0.025	0.15
ICAM5	1.6e-05	2	1.6e-05	Whole_Blood	0.026	0.18
FYCO1	2e-05	2	1.6e-05	Lung	0.029	0.24

Supplementary Table 8: Genes with false discovery rate (FDR)<0.05 from TWAS analysis of imputed summary statistics for European individuals GWAS and Lung gene expression in GTExV8.

gene name	z.score	p.val twas	FDR p.val	Bonferroni p.val
CXCR6	-7.9	2.4e-15	2.3e-11	2.3e-11
CCR3	-5.4	5e-08	0.00024	0.00048
CCR2	5	6.4e-07	0.002	0.006

gene name	z.score	p.val twas	FDR p.val	Bonferroni p.val
MAT2B	-4.9	9.6e-07	0.0023	0.0091
FYCO1	4.3	1.6e-05	0.024	0.15
ICAM5	-4.3	1.6e-05	0.024	0.15
TNFSF15	-4.3	1.8e-05	0.024	0.17

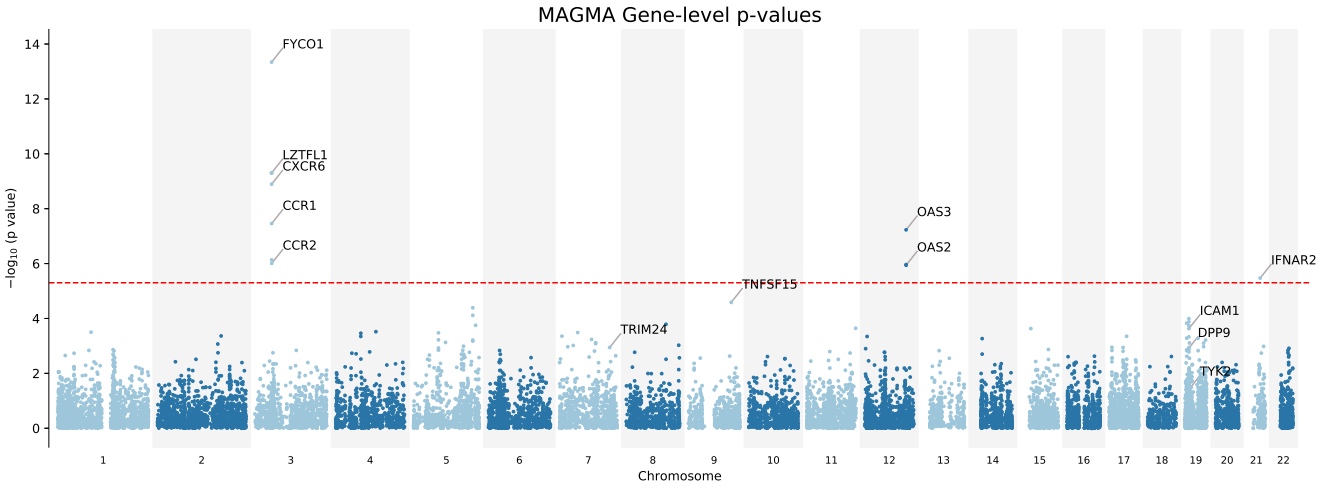
Supplementary Table 9: Genes with false discovery rate (FDR)<0.05 from TWAS analysis of imputed summary statistics for European individuals GWAS and Whole Blood gene expression in GTExV8.

gene name	z.score	p.val twas	FDR p.val	Bonferroni p.val
AC009961.3	-4.3	1.5e-05	0.044	0.12
ICAM5	-4.3	1.6e-05	0.044	0.13
TNFSF15	-4.3	1.6e-05	0.044	0.13

Gene-level burden of significance

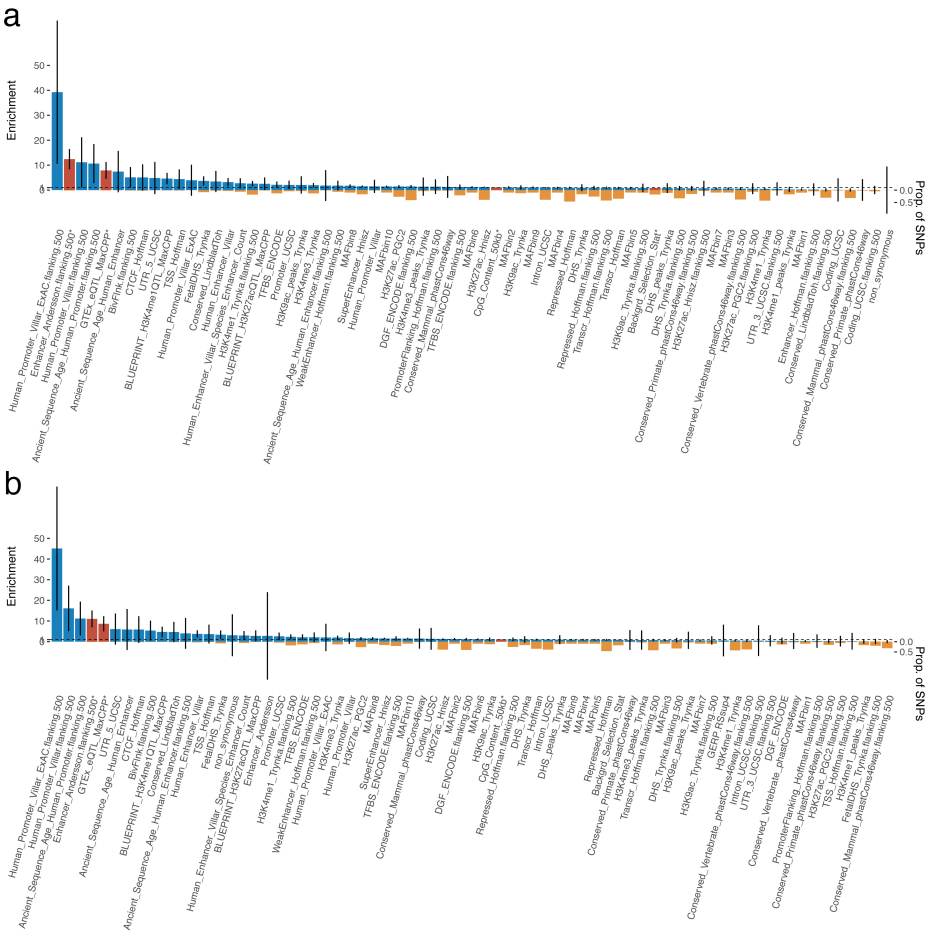
Supplementary Table 10: Protein-coding genes with $< 5 \times 10^{-6}$ in gene-level burden of significance testing with MAGMA in GenOMICC GWAS (European ancestry). These 12 genes had a gene level p-value $< 5 \times 10^{-6}$. Of these 12 genes, 7 are found in the 3p21.31 locus: *LZTFL1*, *FYCO1*, *XCR1*, *CXCR6*, *CCR1*, *CCR3* & *CCR2*. The genes *OAS1*, *OAS2*, *OAS3* are grouped in locus q24.13 on chromosome 12. Gene set analysis of gene-level burden of significance did not identify any significantly enriched pathways or gene ontology terms after correction multiple comparisons (FDR<0.05).[9]

Gene	P	Number of variants
LZTFL1	7.9751E-14	103
FYCO1	1.7685E-13	191
XCR1	3.5541E-13	46
CXCR6	1.5365E-09	34
OAS3	8.6688E-09	111
CCR1	2.1795E-07	32
OAS2	4.3267E-07	55
IFNAR2	6.253E-07	90
CCR3	7.046E-07	52
CCR2	1.2877E-06	27
OAS1	2.1406E-06	36
TNFSF15	5.3835E-06	22

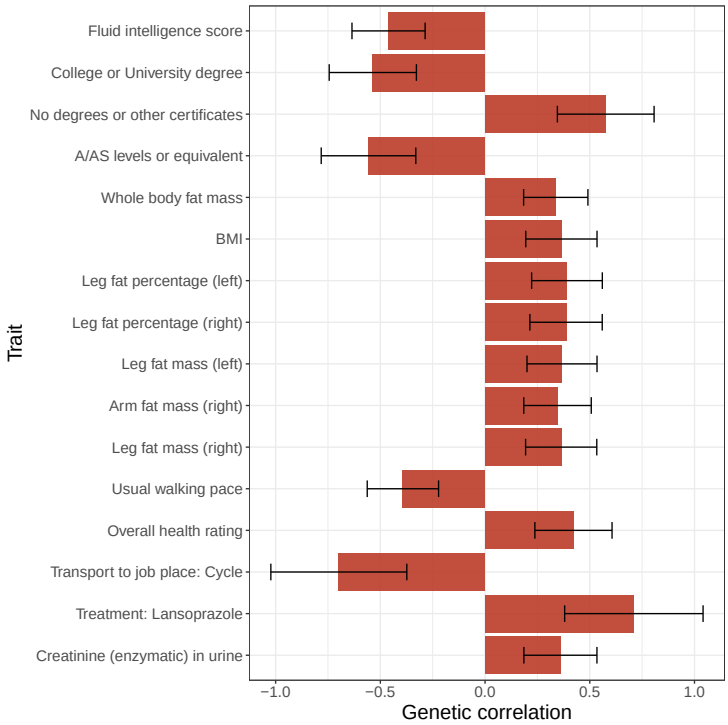


Supplementary Figure 17: Manhattan plot of gene-level burden of significance tests (MAGMA)

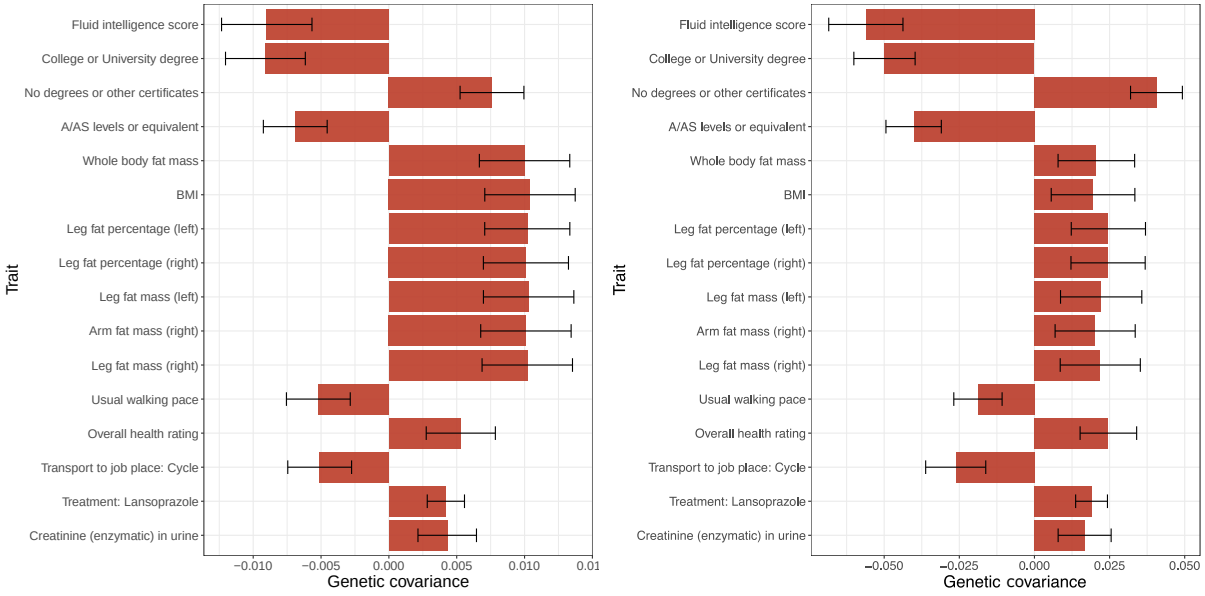
Heritability and high-definition likelihood (HDL)



Supplementary Figure 18: Heritability enrichment analysis of COVID-19 across functional genomic annotations. a) EUR ancestry; b) Meta-analysis. Enrichment of COVID-19 heritabilty was performed using stratified LD score regression by estimating the heritability among the SNPs annotated within the functional regions. The error bars represent standard errors centred around mean enrichment estimates. Enrichment estimates with Bonferroni-corrected P < 0.05 are highlighted in red.

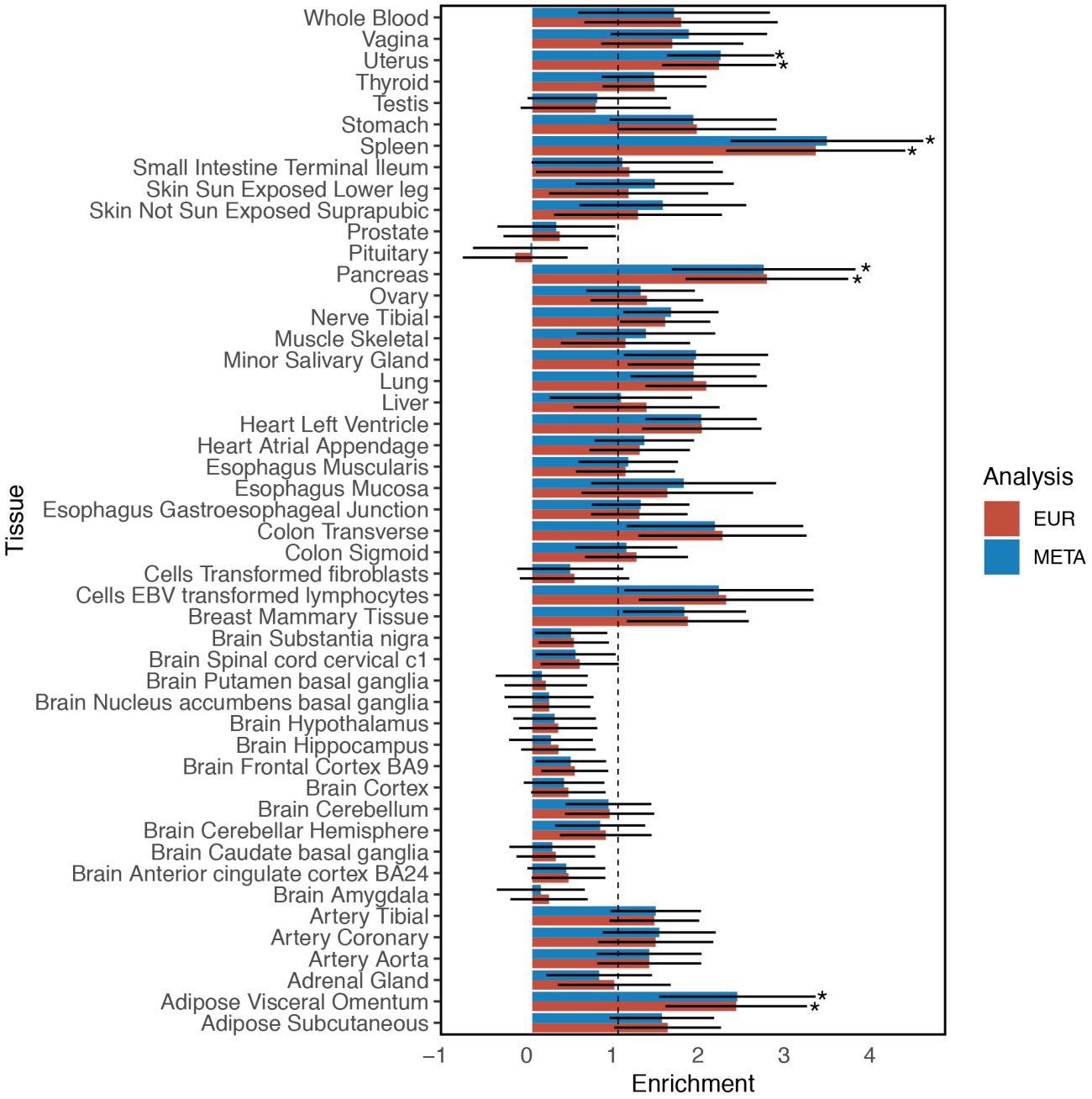


(a)



(b) (c)

Supplementary Figure 19: Genetic correlation and covariance analyses. (a) Significant genetic correlations between GenOMICC primary analysis (EUR vs UK Biobank) of severe COVID-19 and complex traits. Genetic correlations were tested via genetic covariances using high-definition likelihood (HDL) between COVID-19 EUR ancestry GWAS and 818 complex traits GWAS summary statistics. Displayed are significant discoveries at 5% level after Bonferroni correction for 818 tests. Whiskers represent 95% confidence intervals. (b,c) Validation of discovered genetic correlations between severe COVID-19 and complex traits based on GWAS with different control groups: (b) using controls from 100,000 genomes project and (c) closely-matched controls from UK Biobank (see Supplementary Figure 14). HDL was used to estimate the genetic covariances. Genetic correlations are not reported due to estimated COVID-19 heritabilities being close to zero.



Supplementary Figure 20: Heritability enrichment analysis of COVID-19 across human tissues. Enrichment of COVID-19 heritabilty in 48 tissues was performed using stratified LD score regression by estimating the heritability among the top 10% specifically expressed genes in each tissue. The enrichment statistics represent whether the observed heritability on tissue-specific genes was higher than expected (Enrichment = 1). The error bars represent standard errors centred around mean enrichment estimates. Results are shown for GenOMICC EUR vs UK Biobank (primary analysis) (EUR) and GenOMICC trans-ethnic meta-analysis (META).

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Liverpool Heart and Chest Hospital, Liverpool, UK D Wright[166], L Georg[166], S Twiss[166].

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