Severe COVID-19-associated variants linked to chemokine receptor gene control in monocytes and macrophages

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Abstract

Genome-wide association studies have identified 3p21.31 as the main risk locus for severe COVID-19, although underlying mechanisms remain elusive. We perform an epigenomic dissection of 3p21.31, identifying a CTCF-dependent tissue-specific 3D regulatory chromatin hub that controls the activity of several chemokine receptor genes. Risk SNPs colocalize with regulatory elements and are linked to increased expression of CCR1, CCR2 and CCR5 in monocytes and macrophages. As excessive organ infiltration of inflammatory monocytes and macrophages is a hallmark of severe COVID-19, our findings provide a rationale for the genetic association of 3p21.31 variants with elevated risk of hospitalization upon SARS-CoV-2 infection.

Keywords: SARS-CoV-2, COVID-19, 3p21.31, GWAS, Monocyte, Macrophage, Chemokine receptor, 3D genome organization, CTCF, Gene regulation

Background

Coronavirus disease 2019 (COVID-19) is a potentially life-threatening respiratory disorder caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. Clinical manifestations of SARS-CoV-2 infection range from no or mild symptoms to respiratory failure. Life-threatening disease is often associated with an excessive inflammatory response to SARS-CoV-2, involving elevated systemic cytokine levels and profound organ infiltration by monocytes and macrophages [2, 3]. Besides clinical characteristics such as age and various comorbidities [4], genetic differences play a role in predisposing individuals to progress towards severe disease [5, 6]. In genome-wide association studies (GWASs), the 3p21.31 locus was strongly associated with increased risks of morbidity and mortality - in particular for younger (≤ 60 years) individuals [7]. However, it is currently still largely unclear how variants and genes in this locus affect the immune response against SARS-CoV-2 and COVID-19 disease pathophysiology.
Results and discussion
COVID-19 GWAS meta-analyses (release 4 by the COVID-19 Host Genetics Initiative [8]) confirmed the strong association between the 3p21.31 locus and COVID-19, both when comparing hospitalized COVID-19 patients with healthy control subjects (Additional file 1: Fig.S1a) or with non-hospitalized patients (Additional file 1: Fig. S1b), indicating a stronger link with more severe disease. We focused on the former comparison (8638 hospitalized COVID-19 patients vs. 1,736,547 control subjects) to maximize the number of associated SNPs available for downstream analysis. Regional association plots generated using the Functional Mapping and Annotation (FUMA) platform [9] revealed a region of 743 kb with 21 independent significant ($P<5\times10^{-8}$) GWAS SNPs and hundreds of variants in high linkage disequilibrium (LD; $r^2>0.8$) (Fig. 1a). Approximately 96% of these SNPs fall in non-coding regions adjacent to 12 known protein-coding genes (Fig. 1a).

Common disease-associated genetic variants predominantly localize to regulatory DNA elements [11]. To identify disease-relevant candidate genes and gene regulatory regions at 3p21.31, we integrated GWAS findings with publicly available data from large-scale transcriptomics and epigenome profiling studies. Special emphasis was placed on immune cells, as detrimental hyperinflammation is characteristic of severe COVID-19 [2, 3]. Analysis of histone 3 lysine 27 acetylation (H3K27Ac) profiles from ENCODE [12] and BLUEPRINT [13] databases revealed cell type-specific active gene regulatory elements (GREs) at 3p21.31, with particularly strong activity seen in monocytes, monocyte-derived macrophages and neutrophils (Fig. 1b, c, Additional file 1: Fig.S2). The largest fraction of disease-associated SNPs overlapped with monocyte H3K27Ac$^+$ GREs, which were concentrated in three active chromatin regions (ACRs) near the CCR1, CCR2, CCR3 and CCR5 genes (Fig. 1b, c). CCR1 and CCR2 are critical mediators of monocyte/macrophage polarization and tissue infiltration [14], which are pathogenic hallmarks of severe COVID-19 [2, 3]. The three ACRs also showed substantial chromatin accessibility (as measured by DNase-Seq) in monocytes (Additional file 1: Fig.S2). Gene expression analysis using data from 6 transcriptome repositories (see the “Methods” section) confirmed strong transcriptional activity of the 3′ CCR genes in tissues containing haematopoietic cells (e.g. whole blood, spleen), with especially CCR1 and CCR2 being highly expressed in classical monocytes, macrophages and neutrophils (Fig. 1d, Additional file 1: Figs. S2-S3). Of note, several other immune cell subsets, including T cell and dendritic cell subsets, also expressed specific CCR genes (Fig. 1d, Additional file 1: Figs. S2-S3). Chromatin interaction profiles from primary immune cells (measured by promoter-capture Hi-C [15]) revealed extensive monocyte/macrophage-specific chromatin interactions between the three ACRs, as exemplified by CCR1 promoter interaction profiles in monocyte-derived macrophages and T cells (Fig. 1e, Additional file 1: Fig. S4a-b). In all immune cells profiled by Javierre et al. [15], no significant interactions were detected between 3p21.31 gene promoters and the lead SNP region or the most distal SNPs in LIMD1 (Additional file 1: Fig.S4c), although HindIII-based promoter-capture Hi-C has limited resolution very close (<20 kb) to viewpoints.

Together, this analysis reveals the strong transcriptional activity of a CCR gene cluster within the 3p21.31 COVID-19 risk locus in immune cells, especially in monocytes and macrophages. Activity is centred around CCR1 and its genomic surroundings, which
Fig. 1 The 3p21.31 severe COVID-19 risk locus harbours a 3D chromatin hub that controls monocyte-macrophage chemokine receptor expression. a) FUMA regional plot of the 3p21.31 locus highlighting all variants in high linkage disequilibrium ($r^2 > 0.8$, $P < 0.05$) with independent significant ($P < 5 \times 10^{-8}$) GWAS SNPs. Bar graph denotes SNP distribution. b) Number of COVID-19-associated SNPs overlapping with H3K27Ac+ regions in the indicated cell types. c) UCSC genome browser view of H3K27Ac ChIP-Seq tracks for the indicated cell or tissue types (fibro. = fibroblast, epith. = epithelial, Mph = macrophage, mem. B = memory B cell). Genes and FUMA SNPs are shown above, yellow shading indicates location monocyte/macrophage-specific active chromatin regions (ACR1-3'). d) Normalized gene expression levels (transcripts per million; TPM) of 3p21.31 candidate genes across various immune cell subsets from peripheral blood (DICE and HaemoSphere databases) and in vitro transdifferentiated induced macrophages (iMacs [10]). e) Circos plots showing significant chromatin interactions with the CCR1 promoter (green dashed line) in LPS stimulated macrophages or CD8+ T cells as measured by promoter-capture Hi-C (freq: frequency). ACRs are indicated in orange. f) Schematic indicating active chromatin hub formation involving the ACRs (enhancer; Enh.), CTCF binding sites and indicated CCR genes in monocytes/macrophages. g) Experimental scheme depicting C/EBPα-driven transdifferentiation of B cells carrying CTCF-mAID alleles into iMacs. Exposure to auxin induces rapid degradation of CTCF-mAID [10]. h) Hi-C interaction matrices (5 kb resolution, smoothened) for iMacs before (left) and after (right) auxin-inducible CTCF degradation, resulting in weaker interactions (indicated by numbers) between CCR genes and/or ACR1 (colour code as in panel f). CTCF ChIP-Seq peaks in iMacs are indicated below. i) Gene expression changes of indicated genes in iMacs after CTCF degradation.
are organized in a 3D chromatin hub involving the other active CCR genes (i.e. CCR2, CCR5) and putative enhancer elements (Fig. 1f)—a chromatin conformation often used for complex tissue-specific gene regulation [16]. To further substantiate the relevance of local 3D chromatin organization for 3p21.31 CCR gene regulation in myeloid cells, we used epigenomics data from the BLaER induced macrophage (iMac) cell line system [10]. The iMacs, which morphologically and functionally closely resemble macrophages [17], showed highly comparable H3K27Ac enrichment at the 3p21.31 ACRs and expressed high levels of CCR1, CCR2 and CCR5 (Additional file 1: Fig.S5a-b). High-resolution in-situ Hi-C data [10] of iMacs revealed that the 3p21.31 COVID-19-associated genomic block resides in the nuclear A compartment (Additional file 1: Fig.S5c), a chromosomal compartment located in the nuclear interior that groups together transcriptionally active chromatin [18]. Zooming in, we observed that most of the 3p21.31 risk variants and all associated chemokine receptor genes localize to a single topologically associating domain (TAD) (Additional file 1: Fig.S5d), representing an insulated genomic neighbourhood that promotes establishing interactions between genes and regulatory elements inside the TAD [18]. Interestingly, ACR1 and ACR3 were flanked by strong binding sites for the genome architectural CCCTC-binding factor CTCF [19] in iMacs and primary monocytes (Additional file 1: Fig.S5a). Together with the presence of additional CTCF binding sites within all three ACRs, including the CCR1 promoter region (Additional file 1: Fig.S5a), these data suggest that CTCF organizes local 3D active chromatin hub formation to insulate the CCR3-CCR1-CCR2-CCR5 gene cluster for transcriptional regulation. To test this hypothesis, we leveraged our recently developed iMac line expressing CTCF fused to an auxin-inducible degron (mAID), which allows for rapid degradation of CTCF and disruption of 3D genome architecture (Fig. 1g) [10]. Detailed Hi-C analysis confirmed the presence of strong interactions between the ACRs and 3’ CCR genes in iMacs, which were disrupted upon CTCF depletion (Fig. 1h). Importantly, chromatin hub decommissioning specifically reduced CCR1, CCR2 and CCR5 expression (Fig. 1i), revealing that CTCF-mediated 3D chromatin interactions are critical for regulating 3p21.31 CCR gene activity in macrophages. Of note, expression of the CCRL2 gene just downstream of CCR5—encoding an atypical chemokine receptor involved in macrophage polarization [20]—was only marginally affected by CTCF depletion (log2 fold change of 0.23).

We next sought to directly link COVID-19-associated genetic variants to altered 3’ CCR gene expression in myeloid immune cells. To this end, we used FUMA to systematically analyze previously reported expression quantitative trait loci (eQTLs) overlapping with the 958 COVID-19-associated (P<5e−8) SNPs. As eQTL sources, we focused on disease-relevant tissues rich in monocytes/macrophages (i.e. whole blood and lung tissue) and studies using purified monocytes or in vitro differentiated macrophages (see the “Methods” section). The 3’ 3p21.31 CCR genes showed highly significant eQTL associations (FDR <0.05) with COVID-19-associated variants, especially in monocytes and macrophages (Fig. 2a, b). Multiple risk SNPs were identified as eQTLs for CCR1, CCR2, CCR3 and CCR5 in monocytes/macrophages, with the majority correlating with increased gene expression (Fig. 2c, d). No eQTL associations were detected for CCRL2. To further prioritize variants with potential biological significance we used RegulomeDB [21] and CADD [22] SNP annotations.
Stringent filters for both scores were combined with localization within a putative monocyte regulatory region (H3K27Ac+ and DNAse+), yielding four unique candidate causal SNPs of which three were associated with increased CCR1, CCR2 and/or CCR5 expression (Fig. 2e, f). These variants did not engage in significant interactions with sequences far outside the susceptibility region, e.g. beyond the CCR gene cluster (Additional file 1: Fig.S6). Candidate causal variants mostly clustered within ACR2 and altered putative transcription factor binding motifs, readily providing testable hypotheses for future investigations (Additional file 1: Fig.S7). For example, two SNPs within the CCR1 promoter affected binding motifs of known regulators of the macrophage inflammatory expression programme (Additional file 1: Fig.S7a-b). Variant
rs3181080 optimizes a composite Interferon Regulatory Factor (IRF)-Activator Protein 1 (AP1) motif, which is used for cooperative binding of IRF and AP1 family transcription factors that promote monocyte/macrophage activation [23]. In line with CCR1 activation by IRF/AP1 factors, binding of AP1 proteins and IRF4 to rs3181080 was detected in CCR1-expressing GM12878 lymphoblastoid cells (Additional File 1: Fig.S7c). Previous experiments in mouse macrophages [24] confirmed IRF binding to the Ccr1 promoter (Additional file 1: Fig.S7d). The second CCR1 promoter variant, rs34919616, disrupts a critical nucleotide in a motif for BCL6 (Additional file 1: Fig. S7a-b), a suppressor of inflammatory gene expression in macrophages [25].

Taken together, these data show that the COVID-19-associated 3p21.31 locus harbours a CTCF-dependent tissue-specific 3D chromatin hub that controls chemotactic receptor expression in monocytes and macrophages. Several 3p21.31 variants localize to gene regulatory elements within this chromatin hub and are associated with elevated CCR1, CCR2, CCR3 and CCR5 expression, which is further supported by a recent transcriptome-wide association study in lung tissue [6]. Mechanistically, these risk variants may modulate transcription factor binding at CCR gene regulatory elements. CCR1, CCR2 and CCR5 upregulation could enhance lung infiltration by monocytes and macrophages upon viral infection [14], contributing to the rapid and deleterious hyperinflammation observed in COVID-19 patients suffering from severe disease [2, 3] (Fig. 2g).

In support of this notion, single cell transcriptomics revealed increased levels of CCR1 and CCR5 as well as their ligands CCL2/CCL3 specifically in pulmonary macrophages from critical COVID-19 patients [26, 27]. Additionally, CCL2 plasma levels showed the highest predictive value for mortality in a COVID-19 patient cohort [28]. These findings are in line with excessive pulmonary influx of monocytes and subsequent differentiation into inflammatory tissue macrophages as a hallmark of severe COVID-19 (Fig. 2g) [26].

Our analysis has several limitations. Although we provide compelling evidence for monocyte-macrophage 3’ CCR gene activity linked to 3p21.31 risk variants, several other immune cell types involved in antiviral immunity also express some of these chemokine receptors (e.g. CCR1 on neutrophils, CCR5 on T cell subsets) and may therefore also be affected by the genetic variants. Moreover, although our analysis detected fewer non-coding regulatory activity in the 5’ part of the 3p21.31 COVID-19-associated genomic block, this region harbours the lead SNP and several actively transcribed genes with more housekeeping-like expression patterns, which may also be relevant for COVID-19 pathophysiology. Indeed, Downes et al. recently reported that a variant in high LD with the lead SNPs affects an enhancer of LZTFL1 in non-immune cells, with potential implications for anti-viral responses [29]. Although variants in the lead SNP region were also reported as eQTLs for CCR2 and CCR5 in monocytes and macrophages, this likely reflects the high LD ($r^2$>0.8) of these variants with the 3’ CCR SNPs (Fig. 1a). In support of this notion, genetic deletion of a 68kb region around the lead SNP in a myeloid cell line did not affect 3’ 3p21.31 CCR gene expression [30] and Downes et al. found no evidence of these SNPs disrupting gene regulatory mechanisms in immune cells [29]. Another study integrating loss-of-function experiments in an airway epithelial carcinoma cell line with eQTL data implicated SLC6A20 and CXCR6 in COVID-19 pathophysiology [31], whereas deleting the lead SNP region resulted in reduced CCR9 and SLC6A20 expression in leukemic T cells [30]. Future investigations including additional (non-immune)
cell types are required to further elucidate the candidate causal genes operating in different cell types and/or under different microenvironmental circumstances.

Conclusions
Our data support a scenario in which common genetic variants increase susceptibility to develop severe COVID-19 by affecting gene regulatory control of monocyte-macrophage chemotactic receptor expression. As a consequence, elevated migratory capacity of monocytes and macrophages could contribute to aggravated inflammatory responses and more severe disease. These data add to our understanding of the genetic basis of COVID-19 disease heterogeneity and support exploring therapeutic targeting of monocyte-macrophage 3p21.31 CCR activity in hospitalized COVID-19 patients.

Methods
GWAS data retrieval
Version 4 COVID-19 GWAS meta-analysis data was retrieved from The COVID-19 Host Genetics Initiative at https://www.covid19hg.org/. GWAS data (GRCh37/hg38 genome build) was obtained from two studies: B1_ALL (hospitalized COVID-19 vs. non-hospitalized COVID-19; 2430 cases versus 8478 controls) and B2_ALL (hospitalized COVID-19 vs. population; 8638 cases versus 1,736,547 controls). GWAS summary statistics files were used to generate input files for FUMA using standard data frame processing functions in Rstudio v.1.3.

Identification of a high LD block of COVID-19 associated SNPs
FUMA [9] was performed for both B1_ALL and B2_ALL GWASs (version 4 summary statistics downloaded from https://www.covid19hg.org/) using default settings, with exception of the $r^2$ (LD) used to define independent significant SNPs, which was set to $\geq 0.8$. Manhattan and regional plots were generated by FUMA's SNP2GENE function. Significant FUMA SNPs were converted to GRCh38/hg38 using UCSC LiftOver (https://genome.ucsc.edu/cgi-bin/hgLiftOver) to allow aligning variants to the epigenomic profiles.

ChIP-Seq, DNAse-Seq and (promoter-capture) Hi-C data analysis
ChIP-Seq and DNAse-Seq epigenomic data used were retrieved from public ENCODE [12] and BLUEPRINT [13] databases. Data were visualized in the UCSC Genome Browser (https://genome.ucsc.edu). The intersect function of BEDTools [32] was used to determine the number of FUMA SNPs overlapping with H3K27Ac$^+$ regions in the indicated cell types. Peak calling files for each H3K27Ac dataset were directly obtained from the ENCODE website (https://www.encodeproject.org/). Circos plots visualizing promoter-capture HiC data from the BLUEPRINT consortium [15] were generated using https://www.chicp.org/chicp/, with a threshold normalized interaction value of 7. ChIP-Seq and in-situ Hi-C data from in vitro transdifferentiated macrophages (induced macrophages or iMacs), both prior to and after auxin-inducible CTCF degradation, were obtained from GSE140528 and analysed as previously described [10].
Gene expression analysis
RNA-Seq profiles from a broad spectrum of selected relevant cell types were obtained from public ENCODE [12] and BLUEPRINT [13] databases and visualized in the UCSC Genome Browser. Expression value heatmaps from various collection of (immune) cell types were obtained from DICE [33] (https://dice-database.org/), GTEx v8 [34] (via FUMA’s GENE2FUNCTION function), BioGPS [35] (http://biogps.org/), Haemosphere [36] (https://www.haemosphere.org/) and Monaco et al. [37] (GSE107011). Transcripts per million (TPM) values were visualized as averaged values using Morpheus (https://software.broadinstitute.org/morpheus/). RNA-Seq and TPM values for iMacs were obtained from GSE140528 and analysed as previously described [10].

Candidate causal variant filtering
The Combined Annotation Dependent Depletion (CADD [22]) and RegulomeDB [21] scores for all significantly associated SNPs were also obtained from FUMA. As thresholds to identify candidate causal variants, we used CADD scores >14 and RegulomeDB scores <3. SNPs were further filtered based on their combined overlap with H3K27Ac ChIP-Seq and DNAse-seq peaks in monocytes (data obtained from ENCODE [12]). Transcription factor binding motifs were obtained using HOMER [38].

Expression quantitative trait locus (eQTL) analysis
eQTL analysis was performed using FUMA, focusing on tissues relevant for COVID-19 pathophysiology and enriched for monocytes/macrophages (i.e. whole blood and lung from GTEx v8 [34]) or studies using monocytes and/or in vitro differentiated macrophages [39-41]. Thresholds for statistical significance were set to FDR<0.05.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s13059-022-02669-z.

Additional file 1: Figure S1. Overview of genome-wide genetic associations with severe COVID-19. Figure S2. Transcriptional and epigenomic activity at 3p21.31 in selected cell types. Figure S3. Gene expression analysis of 3p21.31 candidate genes across various nonimmune and immune cells types. Figure S4. Regulatory chromatin interactions across the 3p21.31 COVID-19 risk locus. Figure S5. Epigenomic landscape and 3D genome folding at the 3p21.31 COVID-19 risk locus in iMacs. Figure S6. Chromatin interactions with prioritized 3p21.31 COVID-19 risk variants. Figure S7. 3p21.31 COVID-19 risk variants disrupt putative transcription factor binding sites.

Additional file 2: Table S1. Database accession numbers of individual datasets used and accompanying citations used throughout this study.

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Availability of data and materials
All datasets generated and/or analysed during the current study are available in the public repositories and/or using the persistent web links (also see Methods section): GWAS data was retrieved from https://www.2019hg.org/ (version 4, B1_ALL & B2_ALL); FUMA analysis (including eQTL analysis) was performed using https://fuma.ctglab.nl/; ChIP-Seq, DNase-Seq and RNA-Seq data used were retrieved from public ENCODE (https://www.encodeproject.org/), BLUEPRINT (http://dcp.blueprint-epigenome.eu/#/home), DICE (https://dice-database.org/), GTEx (https://gtexportal.org/home/), BioGPS (http://biogps.org/) and Haemosphere (https://www.haemosphere.org/) databases; epigenomics data was visualized in the UCSC Genome Browser (https://genome.ucsc.edu); promoter-capture Hi-C data from the BLUEPRINT consortium was visualized using https://www.chicp.org/chicp/; iMac Hi-C and RNA-Seq data was obtained from GSE140528; heatmaps were generated using Morpheus (https://software.broadinstitute.org/morpheus/). Database accession numbers of individual datasets used and accompanying citations can be found in Additional file 2: Table S1.

Declarations
Ethics approval and consent to participate
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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