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Alternative splicing of *OAS1* alters the risk for severe COVID-19

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ABSTRACT

A locus containing *OAS1/2/3* has been identified as a risk locus for severe COVID-19 among Europeans ancestry individuals, with a protective haplotype of ~75 kilobases derived from Neanderthals. Here, we show that among several potentially causal variants at this locus, a splice variant of *OAS1* occurs in people of African ancestry independently of the Neanderthal haplotype and confers protection against COVID-19 of a magnitude similar to that seen in individuals without African ancestry.

MAIN TEXT

The COVID-19 pandemic has haunted the world for over a year. During this period, several large international efforts[1–4] have been launched to identify the genetic determinants of COVID-19 susceptibility and severity. These efforts have identified more than a dozen genomic regions associated with severe COVID-19. However, the causal variants in these regions are yet to be identified, hampering our ability to understand COVID-19 pathophysiology.

When risk haplotypes are long it is more challenging to disentangle causal variants due to linkage disequilibrium (LD). This is especially problematic for haplotypes derived from Neanderthals and Denisovans that often span several tens of kilobases or more. Two notable COVID-19 examples are the major risk locus on chromosome 3 (3p21.31) and the *OAS1/2/3* locus on chromosome 12 (12q24.13), which both carry haplotypes of Neanderthal origin[5,6]. The *OAS* genes encode enzymes catalyzing the synthesis of short polyadenylates, which activate ribonuclease L that in turn degrades intracellular double-stranded RNA and triggers several other antiviral mechanisms[7]. The protective Neanderthal-derived haplotype confers ~23% reduced risk of becoming critically ill upon infection with SARS-CoV-2[3]. Supporting this, a recent Mendelian randomization study found that increased circulating levels of OAS1 were associated with reduced risk of very severe COVID-19, hospitalization for COVID-19 and susceptibility to this disease[8]. However, other evidence from a transcription-wide association study, suggested a stronger association with OAS3 levels[3]. Thus, efforts are required to disentangle the causal gene, or genes, at this locus.

The *OAS* region was identified as a COVID-19 risk locus in association studies[1,3] of mainly Europeans. The protective haplotype is derived from Neanderthals, is ~75 kilobases long and covers the three genes *OAS1/2/3*[6]. A candidate causal variant in the region is rs10774671, which falls in a splice acceptor site of *OAS1* and where the protective (G) allele results in a longer and more active OAS1 enzyme[9]. However, this variant is as associated with COVID-19 severity as any of the hundreds of variants in LD. In European ancestry individuals we find 130 variants co-segregating

(r^2>0.8) with the splice-acceptor variant (**Fig. 1a**). Thus, further methods are required to disentangle the causal SNP(s) at this locus. Doing so, could help to identify the causal gene.

One method to better identify causal SNPs at an associated locus is to test associations in different ancestries, particularly when these other populations have different LD structure and shorter haplotypes. Therefore, to examine if we could identify a population with which we could test this variant independently, we investigated the presence of co-segregating variants in populations in the 1000 Genomes project[10]. In South Asians, there are 129 such variants and in East Asian 128 variants. In stark contrast, no variants co-segregate with rs10774671 in Africans at a LD of r^2>0.6 (**Fig. 1b**). Thus, the African ancestry population offers a possibility to independently test if rs10774671 is associated with COVID-19 severity.

To test the association of splice acceptor variant rs10774671 in people of African ancestry with COVID-19 outcomes we combined five studies that had assessed COVID-19 severity (UK BioBank, Penn Medicine BioBank, Columbia University COVID-19 Biobank, Biobanque Québec COVID-19 and the VA Million Veteran Program), comprising 1,842 cases and 118,631 controls of African Ancestry. We found that the rs10774671-G allele conferred a protection against COVID-19 hospitalization in this population (**Fig. 2**, p = 0.03) of similar magnitude (OR = 0.92, 95% confidence interval [CI]: 0.86-0.99) as in Europeans (OR = 0.89, 95% CI: 0.86-0.93), a population in which the rs10774671-G allele is less common (35% allele frequency versus 66% among African ancestry individuals[10]). Moreover, we find no evidence of heterogeneity accross the five studies (Cochran's Q = 2.00, p = 0.74; I^2 = 0.0% [0.0%-58.4%]; τ^2 = 0.00 [0.00-0.09], 95% CI in brackets, see Methods). Thus, rs10774671 is associated with COVID-19 severity independently of the variants with which it is associated in non-African populations.

This observation is compatible with the fact that Neanderthal haplotypes are rare or absent in African populations[11,12] and that ancestral alleles seen in Neanderthals, such as the G allele at rs10774671, can also exist today as a result of inheritance from the ancestral population common to both modern humans and Neanderthals. In the latter case, such variants have existed in modern humans in the order of half a million years ago and therefore co-segregate with different variants than when they are derived from gene flow from Neanderthals into modern humans that occurred about 60,000 years ago[13]. Here, we leverage this fact to show that the ancestral splice variant, encoding a longer and more active enzyme[9], is responsible for the protective effect associated with this locus[14]. These findings provide evidence that the splice-site variant at this locus influences COVID-19 outcomes by manipulating splicing of *OAS1*. Further, this rapid insight highlights the importance of including populations of different ancestries in genetic association studies and rapidly sharing data through large, international consortia.

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METHODS

Study participants

Our analysis pooled hospitalized COVID-19 patients of African ancestry (n = 1,842) from five cohorts. The UK BioBank cohort contained 74 cases and 9,305 controls, the Penn Medicine BioBank 114 cases and 8,901 controls, the Columbia University Biobank 304 cases and 2,246 controls, Québec Covid-19 50 cases and 50 controls, and VA Million Veteran Program 1,300 cases and 98,129 controls. All participants gave appropriate consent and ethical approvals were obtained from the relevant research ethics boards.

Summary statistics - VA *Million Veteran Program*

The VA Million Veteran Program (MVP) is a US-based longitudinal research program investigating how genes, lifestyle, and military exposures influences health and illness in Veterans, with study recruitment commencing in 2011[15]. Study participants were genotyped using a customized Affymetrix Axiom biobank array (the MVP 1.0 Genotyping Array), containing 723,305 variants[16]. Imputation was performed to a hybrid imputation panel comprised of the African Genome Resources panel (https://imputation.sanger.ac.uk/?about=1#referencepanels) and 1000G v3p5. COVID-19 cases were identified using an algorithm developed by the VA COVID National Surveillance Tool (NST)[17]. COVID-19-related hospitalizations were defined as hospital admissions between 7 days before and 30 days after an individual's positive SARS-CoV-2 test. The association of hospitalized COVID-19 cases versus all other MVP participants was tested under an additive logistic model and was corrected for age, age^2, sex, age-by-sex, and ethnicity-specific PCs. Individuals who died before March 1, 2020 were excluded as was one individual from each related pair. The analysis was restricted to only African American MVP participants (as defined by HARE[18]) resulting in 1,300 cases and 98,129 controls.

Summary statistics - Biobanque Québécoise de la COVID-19

The Biobanque Québécoise de la COVID-19 (BQC-19) is a prospective hospital-based biobank recruiting patients with proven or suspected COVID-19 (Jewish General Hospital research ethics board no. 2020-2137). Whole genome genotyping was performed for all participants, with imputation using the TOPMed server. Individuals with African ancestry were determined by projecting genetic principal components on the 1000G reference panel. Our 50 cases were defined as patients hospitalized with COVID-19 or who died from the infection. Controls were the 50 other African ancestry participants, of which 32 had a clinical presentation consistent with COVID-19, but never had a positive test. An additive logistic regression model with the first 10 genetic principal

components, age, sex, age^2, age-by-sex, and age^2-by-sex as covariates was used to determine the effect of the protective G allele on the risk of being a case.

Summary statistics - Penn Medicine Biobank

The Penn Medicine Biobank (PMBB) contains ~60,000 prospectively consented participants, all patients of Penn Medicine hospitals, for whom DNA samples have been obtained and on whom extensive phenotypic information has been generated from the electronic health record (EHR). 20,079 participants were genotyped using the Illumina Global Screening Array (Version 2) and further imputed using the TOPMed imputation server. SNPs with a call rate less than 1%, minor allele frequency less than 1%, or imputation info score less than 0.3 were excluded from further analysis. To define each ancestral group, principal component analysis was performed after merging the PMBB data with the 10000 Genomes Project reference dataset using the smartpca module of the Eigensoft package. We performed quantitative discriminant analysis (QDA) on all samples using the 1000 Genomes Project samples as a training sets to generate ancestry calls for all PMBB samples included in the analysis. Ultimately 9,015 African-ancestry genotyped samples were identified and included in our association study. All PMBB participants were followed for SARS-CoV-2 infection and hospitalization, with COVID-19 infection defined as any patient with a positive SARS-CoV-2 nasal swab or for whom the ICD billing code U07.1 had been coded in the EHR, and with COVID-19-related hospitalizations defined as the subset of these patients who had been admitted to hospital in the previous year with U07.1 as the admission diagnosis code, or who had been admitted for COVID-19-related symptoms as determined by manual chart review. Association analyses were performed using the Firth logistic regression test as implemented in REGENIE, including as covariates age, age^2, sex, age-by-sex, and the first six ancestry-specific principal components of the genomic data. The PMBB is approved under Institutional Review Board of Perelman School of Medicine at University of Pennsylvania.

Summary statistics - Columbia University Biobank

The Columbia University COVID-19 Biobank was established in response to the New York City infection surge in March 2020. The biobank recruited COVID-19 cases of diverse ancestry among all patients who were treated at Columbia University Irving Medical Center between March and May 2020. All cases were diagnosed by positive SARS-CoV-2 PCR test based on nasopharyngeal samples. The mean age of cases was 62.89 years, and the percentage of females was 43%. DNA of whole blood samples was extracted using standard procedures and genotyping was performed using the Illumina Global Diversity Array (GDA) chip. The controls were genotyped using the Illumina Multiethnic Global Ancestry (MEGA) chip. The analysis of intensity clusters and genotype calls

were performed in Illumina Genome Studio software; all SNPs were called on forward DNA strand and standard quality control (QC) filters were applied, including per-SNP genotyping rate > 95%, per-individual genotyping rate > 90%, minor allele frequency (MAF) > 0.01, and Hardy–Weinberg equilibrium (HWE) test p-value > 10-08 in controls. The duplicates and cryptic relatedness in the given cohort were determined and excluded based on the estimated pairwise kinship coefficients > 0.0884. After QC, the dataset consisted of 6,757 individuals (1,029 cases and 5,728 controls) genotyped for 1,096,321 SNPs with overall genotyping rate of 99.9%. The imputation analysis was performed using TopMed imputation server. A total of 13,439,413 common markers imputed at high quality (R2 > 0.8 and MAF > 0.01) were used in downstream analyses. To define the African ancestry cluster, we used PCA against 1000 Genomes reference populations followed by k-means clustering on significant PCs of ancestry. The African ancestry cluster contained 332 cases positive for SARS-CoV-2 and 2,246 population controls. Of the 332 African ancestry cases, 304 had severe COVID-19 requiring hospitalization. Among the 304 cases included, 78 (26%) had respiratory failure requiring intubation and invasive ventilatory support, and 86 (28%) died due to COVID-19. We then tested the effect of rs10774671-G on the risk of hospitalization using SAIGE (Scalable and Accurate Implementation of GEneralized mixed model), after adjustment for sex and five principal components of ancestry. The collection of samples to the Columbia University COVID-19 Biobank was approved by the Institutional Review Board (IRB) of Columbia University (IRB protocol number AAAS7370), while the genetic analyses were approved under Columbia University IRB protocol number AAAS7948.

Summary statistics - UK BioBank

Association analyses were performed using the Firth logistic regression test implemented in `REGENIE`, including as covariates age, age^2, sex, age-by-sex, age^2-by-sex, and ten ancestry-informative principle components. Association analyses were performed using the Firth logistic regression test implemented in REGENIE, including as covariates age, age2, sex, age-by-sex, age2-by-sex, and ten ancestry-informative principle components. The data was downloaded at https://rgc-covid19.regeneron.com/results [2021-02-02].

Meta-analysis

The meta-analysis was done using inverse-variance weighting in the R-package `meta`. Heterogeneity was measured using Cochran's Q, Higgin's & Thompson's I^2, and τ^2 using the DerSimonian-Laird estimator.

Linkage disequilibrium

Linkage disequilibrium was calculated using LDlink[19] 4.1 in the genomic region 113.30-113.45 Mb (*hg19*) using data from the 1000 Genomes Project[10].

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FIGURES

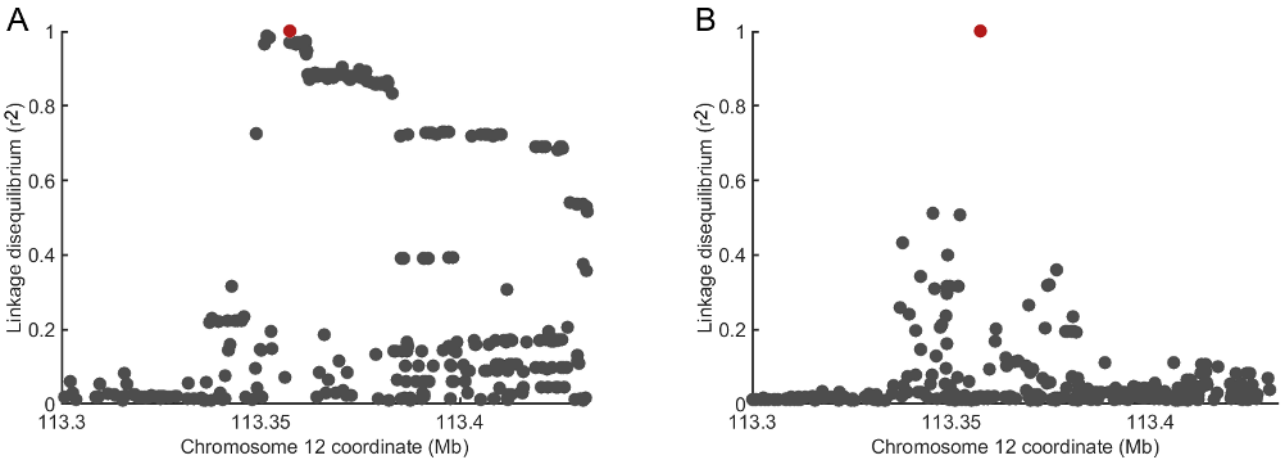


Fig 1. Linkage disequilibrium of the splice acceptor variant in individuals of European and African ancestries. A) Linkage decay in individuals European ancestry (n = 503). 130 variants are in LD ($r^2 > 0.8$) with the splice acceptor variant rs10774671 (marked in red). **B**) Same as in A) but for African ancestry (n = 661). No variants were found to be in LD with the splice acceptor variant. Data from the 1000 Genomes project[10]. *X*-axis gives *hg19* coordinates.

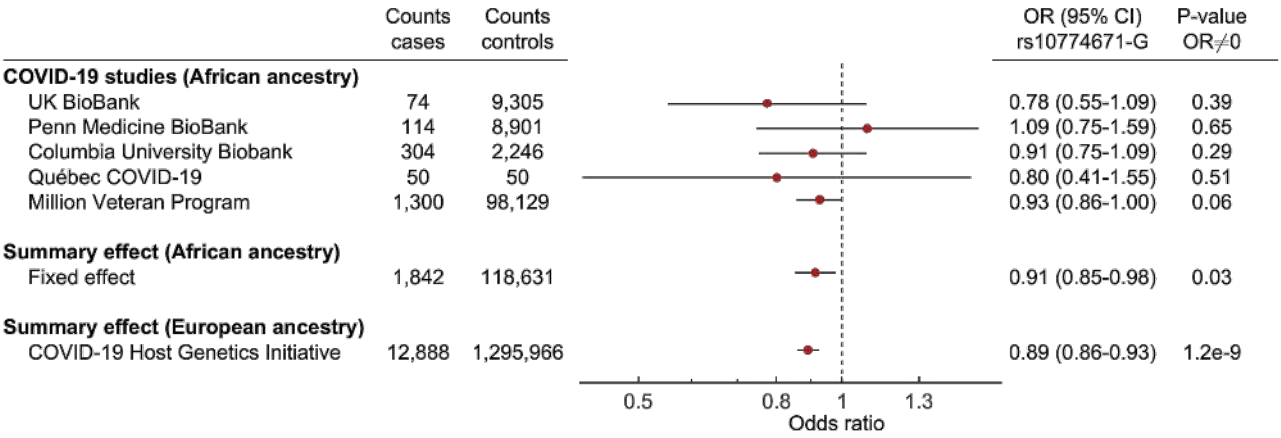
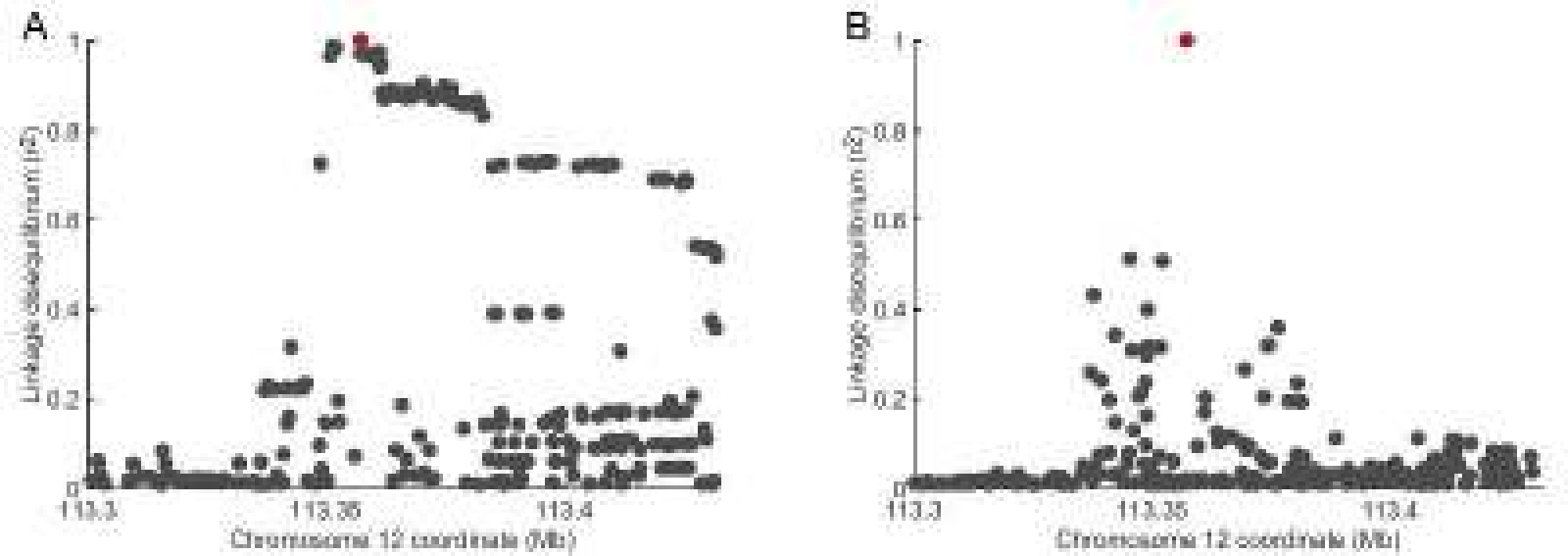


Fig 2. Odds ratios for COVID-19 hospitalization for African ancestry carriers of the ancestral splice variant. Summary effect in African ancestry individuals by a fixed effect meta-analysis of the five cohorts. Error bars give 95% confidence intervals.


A
B
Linkage disequilibrium (r2)
0
0.2
0.4
0.6
0.8
1
113.3
113.35
113.4
Chromosome 12 coordinate (Mb)

	Counts cases	Counts controls	OR (95% CI) rs10774671-G	P-value OR≠0
COVID-19 studies (African ancestry)				
UK BioBank	74	9,305	0.78 (0.55-1.09)	0.39
Penn Medicine BioBank	114	8,901	1.09 (0.75-1.59)	0.65
Columbia University Biobank	304	2,246	0.91 (0.75-1.09)	0.29
Québec COVID-19	50	50	0.80 (0.41-1.55)	0.51
Million Veteran Program	1,300	98,129	0.93 (0.86-1.00)	0.06
Summary effect (African ancestry)				
Fixed effect	1,842	118,631	0.91 (0.85-0.98)	0.03
Summary effect (European ancestry)				
COVID-19 Host Genetics Initiative	12,888	1,295,986	0.89 (0.86-0.93)	1.2e-9