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Inheritance of a common androgen synthesis variant allele is associated with female COVID susceptibility in UK Biobank

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Abstract

Context—A sex discordance in COVID exists, with males disproportionately affected. Although sex steroids may play a role in this discordance, no definitive genetic data exist to support androgen-mediated immune suppression for viral susceptibility, nor for adrenally produced androgens.

Objective—The common adrenal-permissive missense-encoding variant *HSD3B1*(1245C) that enables androgen synthesis from adrenal precursors and that has been linked to suppression of inflammation in severe asthma was investigated in COVID susceptibility and outcomes reported in the UK Biobank.

Methods—The UK Biobank is a long-term study with detailed medical information and health outcomes for over 500,000 genotyped individuals. We obtained COVID test results, inpatient hospital records, and death records and tested for associations between COVID susceptibility or outcomes and *HSD3B1*(1245A/C) genotype. Primary analyses were performed on the UK Biobank Caucasian cohort. The outcomes were identification as a COVID case among all subjects, COVID positivity among COVID-tested subjects, and mortality among subjects identified as COVID cases.

Results—Adrenal-permissive *HSD3B1*(1245C) genotype was associated with identification as a COVID case (odds ratio 1.11 per C allele, 95% confidence interval 1.04 – 1.18, p = 0.0013) and COVID test positivity (OR 1.09, 95% CI 1.02 – 1.17, p = 0.011) in older (≥ 70 years of age)

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women. In women identified as COVID cases, there was a positive linear relationship between age and 1245C allele frequency ($p < 0.0001$). No associations were found between genotype and mortality, or between genotype and circulating sex hormone levels.

Conclusion—Our study suggests that a common androgen synthesis variant regulates immune susceptibility to COVID infection in women, with increasingly strong effects as women age.

Keywords

COVID; androgens; steroids; DHEA; immunology; susceptibility

Introduction

The COVID-19 pandemic caused by the SARS-CoV-2 virus emerged in late 2019 and in the early months of 2020 quickly became a global health emergency. Recognition that men face higher risks of hospitalization and death from COVID than women[1–3] has spurred research into whether sex hormone signaling plays a role in COVID susceptibility and/or outcomes. Although much of this research has focused on whether androgens regulate viral receptor protein ACE2 and co-receptor TMPRSS2[4–8], it is also known that sex hormone signaling can modulate immune responses[9–11]. Generally speaking, a sex discordance in inflammatory and infectious disease processes[12, 13], including COVID, is recognized. However, there is great uncertainty in attributing the difference in outcomes to androgens because there are many other physiologic differences that exist between males and females.

The enzyme 3β-hydroxysteroid dehydrogenase isotype 1 (3βHSD1) catalyzes a critical and rate-limiting step in the pathway to production in peripheral tissues of the potent androgens testosterone and dihydrotestosterone (DHT) from adrenally produced precursors dehydroepiandrosterone (DHEA) and its sulfated form DHEA-S[14]. The adrenal-permissive form of the gene for 3βHSD1, *HSD3B1*(1245C), was originally linked to faster prostate cancer progression in the face of androgen deprivation therapy (i.e., medical castration) because of adrenally derived androgen synthesis[15–20]. More recently, *HSD3B1* genotype has been shown to also affect the response to oral glucocorticoid treatment in patients with severe asthma[21], an inflammatory condition of the lungs often associated with T cell activation[22]. The 1245C allele encodes a form of the enzyme that is resistant to ubiquitination and degradation, leading to increased production of potent androgens from adrenal precursors, and can therefore be described as adrenal androgen permissive, or "adrenal-permissive"[23] for simplicity. By contrast, the 1245A, or adrenal-restrictive allele, encodes a more rapidly degraded form of the enzyme, leading to less production of potent androgens[24].

A side effect of glucocorticoid treatment is suppression of circulating DHEA and DHEA-S levels[21]. Active androgens (i.e., potent agonists of the androgen receptor) testosterone and DHT are thought to downregulate immune/inflammatory responses[25]. Experimental evidence for androgen-induced immunosuppression includes, for example, that castration of mice reduces B cell lymphopoiesis and this is reversed by androgen replacement[26] and that androgen ablation allows greater T cell response to prostate tumors in a mouse immunotherapy model[27]. In asthma patients, higher androgen receptor expression in the

bronchial epithelium has been associated with lower disease severity[28], and inherited androgen receptor deficiency has been associated with greater risk of asthma[29]. Thus, in the asthma context, even in an environment of a decreased adrenal precursor pool brought on by glucocorticoid treatment, the adrenal-permissive 1245C allele still enables sufficient downstream androgen production to reduce airway inflammation in those with severe asthma, whereas patients with the adrenal-restrictive 1245A allele experience less reduction in airway inflammation[21] (Fig. 1).

The evidence from cohorts of severe asthmatics that *HSD3B1* genotype could affect function of the immune system led us to ask whether there are links between *HSD3B1* genotype and COVID susceptibility and/or outcomes. Additionally, in both prostate cancer and asthma, the effect of the adrenal-permissive allele becomes evident specifically in contexts of decreased circulating androgen levels due to androgen deprivation therapy in prostate cancer and glucocorticoid treatment in asthma. Circulating androgen levels decline with age[30–32], so as COVID most severely impacts older patients[2, 33], this provided further impetus for determining whether a link exists between *HSD3B1* and COVID.

Materials and Methods

Analysis was performed on subjects from the UK Biobank, a long-term study tracking medical information of over 500,000 genotyped individuals. Genotyping was performed using the UK BiLEVE Axiom Array or UK Biobank Axiom Array by Applied Biosystems. Additional SNPs were imputed with the IMPUTE4 program using the Haplotype Reference Consortium, UK10K, and 1000 Genomes phase 3 panels as reference panels. Rs1047303 (i.e., *HSD3B1*(1245A/C)) was imputed with high accuracy (information score > 0.99).

Population structure and ancestry were assessed via principal component analysis on SNP data. A plot of the first two genetic principal components (PCs) along with the top level of self-identified ethnic background is shown in Fig. S1A, which illustrates the association between lower values of PC1 and self-identified White ethnic background. The UK Biobank additionally classified a cohort of genetically Caucasian individuals defined as “samples who self-identified as White British” (a sub-level of White ethnicity) “and have very similar genetic ancestry based on a principal components analysis of the genotypes”[34], illustrated in Fig. S1B. We further curated this Caucasian cohort for sex discordance, outliers in heterozygosity or missingness rate, and kinship: individuals with different values for genetic and self-identified sex were excluded, individuals identified by the UK Biobank as outliers were excluded, and for pairs of individuals with kinship scores greater than 0.18, the individual with higher genotype missingness was excluded. Additionally, the subject pool for our analyses was restricted to subjects who were alive at the beginning of 2020. The primary analyses were performed on the curated, UK Biobank defined Caucasian cohort (n = 362,441).

COVID test results, inpatient hospital records, and death records from the UK Biobank were downloaded. As of April 2021, COVID test results up to March 15, 2021, hospital admissions up to February 2, 2021, and death records up to March 23, 2021 were available and were included in analyses. Subjects with COVID test results were identified

and classified as having tested positive or having solely negative test results. Subjects with ICD10 codes U07.1 (i.e., laboratory-confirmed COVID) or U07.2 (i.e., clinically diagnosed COVID without laboratory confirmation) from inpatient hospital episodes were also identified and subjects with COVID ICD10 codes despite having no or solely negative COVID test results were included as additional COVID cases. For analysis of mortality among subjects who had COVID, death records were cross referenced against COVID test results and ICD10 codes and subjects who died at any time after having tested positive for COVID and/or having been diagnosed with COVID as a hospital inpatient were identified; i.e., all-cause mortality for subjects who had COVID was assessed. For analysis of COVID with respiratory failure, subjects with an ICD10 code beginning with J96 (respiratory failure) occurring simultaneous to a COVID ICD10 code, or up to two weeks prior to or one month subsequent to a COVID ICD10 code, were identified. Data on which UK Biobank subjects received glucocorticoid treatment while hospitalized with COVID were not available.

Analyses of odds of outcomes (COVID case identification, positivity on COVID tests, death among subjects with COVID, respiratory failure among hospitalized COVID patients) by rs1047303 genotype were performed using binomial logistic regression of outcome against genotype by number of 1245C alleles. Covariates used in the main analyses were age, sex (in the analyses including both sexes together), BMI, and the first ten genetic principal components. Analyses were performed on the entire Caucasian cohort and on subgroups by sex and age (below 60, 60–69, and 70 and above). Ages were determined using subjects' birth years and months and were calculated as of September 2020, the midpoint of the COVID results. To confirm the robustness of findings in women above an age threshold to variations in the selected threshold, we performed an additional analysis in which we systematically varied the lower edge of the age range in yearly intervals from age 50 to age 80 and determined regression results for women of each specified age and above.

To assess whether allele frequencies systematically varied with increasing age, subjects (female and male separately) were grouped into one-year age bins and the *HSD3B1*(1245A/C) allele frequencies were calculated for subjects within each one-year bin, both for subjects identified as COVID cases and for the entire population. Weighted linear regressions of allele frequency ~ year of age weighted by number of cases or subjects for each year of age were then performed. To additionally visualize these trends with less noise, the smoothed frequencies of adrenal-permissive (AC or CC) and adrenal-restrictive (AA) genotypes among female and male subjects who had COVID were calculated and were plotted by moving 9-year windows of age inclusion. For each year of age, the genotype frequencies and confidence intervals were calculated for the group of subjects identified as COVID cases and with age equal to the given year ± 4. For example, the data points in this visualization for age 70 included subjects from ages 66 through 74.

Blood samples were collected from UK Biobank subjects upon enrollment to the study in 2006–2010 and key biochemistry markers were measured, including testosterone and estradiol, which were measured using a Beckman Coulter DXI 800 with analysis methodology chemiluminescent immunoassay – competitive binding. The bottom of the reportable range was 0.35 nM for testosterone and 175 pM for estradiol.

Analyses were performed using R under RStudio. This study was approved by the NHS National Research Ethics Committee (REC ref 16/NW/0274) through the generic ethical approval for UK Biobank studies (approved UK Biobank application #44578).

Results

To examine whether COVID susceptibility and *HSD3B1* genotype are associated, we performed regression analyses between identification as a COVID case and genotype in genotyped subjects of the UK Biobank who were alive at the beginning of 2020. The UK Biobank is a long-term study containing genotyping results and detailed medical information from approximately 500,000 UK residents who were between the ages of 40 and 69 years when recruited to the study in 2006–2010[35]. Current ages (as of September 2020, the midpoint of the COVID results) range from 48 to 86 with a median age of 69. Fig. S2 shows a histogram of the ages of UK Biobank subjects included in the analysis; over 99.5% of subjects had ages within the range 51 – 82. As of the beginning of April 2021, COVID test results had been reported for 69,349 genotyped UK Biobank subjects, 15,812 having tested positive. An additional 513 subjects who did not have positive test results were identified as COVID cases by ICD10 codes from inpatient hospital episodes, bringing the total number of cases to 16,325.

Table 1 shows the characteristics of UK Biobank subjects who were identified as COVID cases and of the rest of the subject population, who served as controls. Frequencies of *HSD3B1*(1245C) vary by ancestry, ranging in the UK Biobank from 0.074 and 0.085 in Chinese and African subjects, respectively, to 0.193 in South Asian subjects and 0.323 in the Caucasian cohort. As shown in Table 1, in the UK Biobank population, compared to the entire cohort, subjects identified as COVID cases were more likely ($p < 0.00001$ for all comparisons) to be male (47.6% of COVID cases were male compared to 44.8% of controls), younger on average (mean age for COVID cases was 65.2 years compared to 67.9 for controls), more likely to be of non-White ethnicity (89.4% of COVID cases had White ethnicity compared to 94.3% of controls), and had higher BMI on average (mean BMI for COVID cases was 28.4 compared to 27.3 for controls). Because non-European ancestry was strongly associated with both the likelihood of identification as a COVID case and with *HSD3B1*(1245A/C) genotype, to reduce the chances of confounding by ancestry, our subsequent analyses were performed on the Caucasian cohort.

We performed logistic regressions against the number (0, 1, or 2) of adrenal-permissive *HSD3B1* 1245C alleles and calculated odds ratios for the outcome of being identified as a COVID case. We analyzed men and women of all ages together as well as men and women separately, and to determine whether there was an association specific to those of advanced age, we further broke these groups down according to age below 60, between 60 and 69, and 70 or above. The results (Fig. 2A) show that when looking at all ages together there was no apparent association between genotype and COVID susceptibility, but in women of age 70 or above, there was a statistically strong association between adrenal-permissive genotype and odds of identified COVID infection (odds ratio = 1.11 per C allele, 95% confidence interval = 1.04 – 1.18, $p = 0.0013$). In subjects of age below 60, there was a weaker association with modestly reduced odds of COVID infection with adrenal-permissive

genotype (OR = 0.94, 95% CI = 0.89 – 0.98, p = 0.010) driven by similar trends in each sex. Additionally, in women of age 70 or above, there was a step-wise increase in COVID rates with increasing copies of the C allele (AA genotype 936 cases/44,617 subjects = 2.10%, AC genotype 992/42,749 = 2.32%, CC genotype 262/10,210 = 2.57%; see Table S1 for full breakdowns by sex and age group), suggesting that a second copy of the adrenal-permissive C allele confers additional risk above a single copy.

For an alternate approach to assaying COVID susceptibility, we also calculated odds ratios for testing positive for COVID among only those subjects with COVID test results, i.e., a comparison between those who tested positive and those who tested negative. This analysis yielded similar results (Fig. 2B) to the comparison between COVID cases and the rest of the population. Notably, in women of age 70 or above there was again an association between adrenal-permissive genotype and COVID (OR = 1.09, 95% CI = 1.02 – 1.17, p = 0.011).

In defining the cohort used for our analyses, a kinship exclusion threshold was used that resulted in one of any pair of first-degree (i.e., parent and child or sibling and sibling) relatives being excluded. To ensure that the results were not being distorted by the presence of other pairs of relatives, we tested the effects of using the most conservative exclusion criteria, excluding all subjects with at least one third-degree relative identified in the UK Biobank. This substantially reduced the sample sizes (by approximately 25 to 30%) and therefore marginally expanded the confidence intervals, but produced identical point estimates to those of our main findings in women of ages 70 and above: the COVID case identification odds ratio remained 1.11 (95% CI 1.02 – 1.19, p = 0.0098) and the COVID positivity odds ratio remained 1.09 (95% CI 1.00 – 1.18, p = 0.054), suggesting our results were not confounded by the presence of related individuals.

To test whether the association in women of elevated age might be due to selecting a cutoff age that happened to create the strongest result, we systematically varied the lower cutoff age for inclusion in the regression analysis from 50 through 80 and calculated the odds ratios and confidence intervals by year of cutoff age. Fig. 3 shows the results: for each year of age, the odds ratio and confidence interval for COVID cases among women of that age or older are shown. Note that a vertical slice through the graph at age 70 is equivalent to the “female, age ≥ 70” row in Fig. 2A. For any lower cutoff age from 59 through 80, the result that an association between the C allele and women of at least that age having elevated COVID odds, with a 95% confidence interval not overlapping an odds ratio of 1.0, would hold true. Because results similar to our initial cutoff were obtained over a wide range of cutoff ages, the association in women of elevated age is not the result of having selected a specific age. As the cutoff age increases, the odds ratio tends to become larger; at the same time, the confidence interval grows wider as the sample size decreases (e.g., at a cutoff age of 70, subjects of age 70 and up are included; at a cutoff age of 80, only subjects of age 80 and up are included). For the COVID positivity among COVID tested women odds ratios, a cutoff age ranging anywhere from 64 through 76 would result in the 95% confidence interval not overlapping 1.0, with the lower bound of the confidence interval only crossing 1.0 at some cutoff ages above 76 due to decreasing sample sizes (Fig. S3), further demonstrating that the association with genotype in women of elevated age is not an artifact of having selected a specific cutoff age.

Because age, sex, BMI, and ethnicity were all associated with likelihood of identification as a COVID case (Table 1), our main regression analyses were adjusted for age, sex (in the both sexes analyses), BMI, and the first ten genetic principal components. To test whether the BMI adjustment might have biased the results, we tested the fully adjusted model without the BMI adjustment, which produced extremely similar results to the fully adjusted model for both the COVID case vs. population (Table S2) and COVID positive vs. COVID negative (Table S3) comparisons. For example, the odds ratio for COVID case identification with the C allele for women of ages 70 and above was 1.11 (95% CI 1.04 – 1.18, p = 0.00128) in the fully adjusted model and remained 1.11 (95% CI 1.04 – 1.18, p = 0.00147) in the model without BMI adjustment.

The UK's COVID testing strategy was not constant over the course of the investigation period, due to especially limited resources at the beginning of the pandemic. According to the Oxford Covid-19 Government Response Tracker, prior to May 18, 2020, access to testing was restricted to people meeting specific criteria, whereas from that point on it was available to anyone showing symptoms[36]. To assess whether the changing availability of testing affected the apparent association between adrenal-permissive genotype and COVID susceptibility, we repeated the analyses but split into two time periods: first restricting results to testing and diagnoses that occurred prior to May 18, 2020, and then restricting results to May 18, 2020, onward. Although the sample sizes were much smaller for the first period, associations between both COVID case identification and test positivity were observed in women ages 70 and above in both periods (odds ratios for COVID case identification: early pandemic 1.21 (95% CI 1.01 – 1.45, p = 0.0348), subsequent period 1.09 (95% CI 1.02 – 1.17, p = 0.0107); odds ratios for COVID test positivity: early pandemic 1.38 (95% CI 1.08 – 1.76, p = 0.0106), subsequent period 1.08 (95% CI 1.00 – 1.16, p = 0.0431). There was some variability of odds ratios, but for all age and sex breakdowns, there was substantial overlap in the confidence intervals across the two time periods, suggesting that changing availability of testing had little effect on results (Fig. S4).

To explore whether there were associations between *HSD3B1* genotype and COVID severity or outcomes, we analyzed the UK Biobank's hospitalization and death records and calculated the odds ratios by number of C alleles for respiratory failure diagnosis among subjects who were hospitalized for COVID and for death among subjects who were identified as COVID cases. For these analyses, we analyzed men and women of all ages together, men and women separately, and age groups below 70 and 70 or above, rather than separating ages below 60 from 60 – 69, due to small numbers of events below age 60. These analyses did not reveal associations between genotype and COVID outcomes (respiratory failure: Fig. 4A; death: Fig. 4B), although the numbers of events for these analyses were considerably smaller than for the COVID cases and positivity analyses, especially for the lower age ranges. The mortality analysis included deaths with any listed cause of death subsequent to identification as a COVID case; restricting the included deaths to those with COVID listed as a cause of death similarly did not reveal associations with genotype (not shown).

To attempt to explore the mechanistic basis for the associations we found, we interrogated whether there were associations between *HSD3B1* genotype and the levels in circulation

of the steroids for which data are available in the UK Biobank: testosterone and estradiol. Concentrations of these steroids were measured in serum samples collected when participants enrolled in the study in 2006–2010. For testosterone, we performed analyses in men and women separately, as well as specifically in women who were premenopausal or postmenopausal upon entering the study. For estradiol, >90% of men and of postmenopausal women had levels below the bottom of the reportable range (175 pM), so we only analyzed the data from premenopausal women. We did not find that testosterone or estradiol levels differed by genotype (Fig. S5), which is consistent with the results from our previous study in which *HSD3B1* genotype was found to be associated with response to glucocorticoid treatment in asthma but circulating testosterone levels were not found to differ by genotype[21].

Lastly, because we observed that the COVID odds ratios for women of elevated age tended to increase with an increasing lower cutoff age, we examined the relationship between subject age and frequencies of adrenal-permissive and adrenal-restrictive genotypes among subjects identified as having had COVID. We graphed the adrenal-permissive (1245C) allele frequencies of Caucasian cohort subjects who had COVID by year of age and found that in women, there was a linear relationship between age and C allele frequency (adjusted $R^2 = 0.37$, p = 7.87e-05 [regression weighted by number of cases per year of age], Fig. 5A), whereas in men, there was no such relationship (adjusted $R^2 = -0.01$, p = 0.437, Fig. 5B). This relationship in women was specific to COVID cases and was not present for the overall population (adjusted $R^2 = 0.01$, p = 0.241 for all Caucasian women alive in 2020, Fig. 5C), nor was there a notable association in the overall male population (adjusted $R^2 = 0.02$, p = 0.184 for all Caucasian men alive in 2020, Fig. 5D). To more clearly visualize how the frequencies of adrenal-permissive vs. adrenal-restrictive genotypes varied with age, we also graphed the adrenal-permissive (AC or CC) and adrenal-restrictive (AA) frequencies of Caucasian cohort subjects who had COVID by a moving window of ages, illustrating that in women, adrenal-permissive genotype frequencies steadily increased with increasing age (Fig. 5E), whereas in men, there was no clear trend with age (Fig. 5F). In women, at the lower end of the age range the adrenal-permissive genotype frequency of COVID cases is marginally lower than the overall population frequency; at the upper end of the age range, it is substantially higher. These results imply that the association between adrenal-permissive genotype and COVID susceptibility becomes increasingly strong with increasing age in women.

Discussion

We have demonstrated that, in the UK Biobank, there is an association between possessing the adrenal-permissive *HSD3B1* 1245C allele and being identified as having COVID-19 in women of elevated age, i.e., around 70 and older. This association exists whether comparing those subjects who were identified as COVID cases to the rest of the population (Fig. 2A) or comparing those subjects who tested positive for COVID to those who tested negative (Fig. 2B). This association in older women is not an artifact of having selected a specific cutoff age, but rather is robust to using a wide range of cutoff ages (Fig. 3). On the other hand, we did not detect an association between *HSD3B1* genotype and COVID outcomes (Fig. 4). Lastly, we found that the pool of women with COVID became increasingly

enriched for adrenal-permissive genotypes with increasing age (Fig. 5), suggesting that adrenal-permissive steroid biosynthesis may have an increasing effect on immune response as women age. Although statistically well supported, the associations we observed did not have enormous effect sizes (odds ratio ~1.1 per risk allele in women of advanced age), so it does not appear that *HSD3B1* genotype is a strong determinant of an individual's COVID risk; rather, the results are of interest for the potential implications regarding the role of steroids in the immune system.

Previously reported phenotypic associations with adrenal-restrictive vs. adrenal-permissive *HSD3B1* genotype, in both prostate cancer and asthma, occur in contexts of reduced circulating androgen levels. In men, the pool of gonadally produced androgens is vastly larger than in women, so the relative contribution of adrenally produced androgens is greater in women. Furthermore, in postmenopausal women, the adrenals are the sole source of sex steroids, whereas men continue to produce androgens gonadally throughout life[37, 38]. As menopause typically occurs between ages 45 and 55 and over 90% of UK Biobank subjects are greater than 55 years old, the female cohort is very predominantly postmenopausal. Additionally, circulating levels of the adrenally produced precursor DHEA that is a 3βHSD1 substrate in the pathway to potent androgen production decrease with increasing age[39, 40]. Considering these points together, one could speculate that the adrenal-permissive genotype would have a more pronounced physiologic effect in postmenopausal women that would become increasingly strong with increasing age, which is in line with our results showing that as women's ages increase, the association between possessing the adrenal-permissive C allele and being identified as having COVID becomes increasingly strong (Fig. 5). As COVID outcomes are by far the worst in elderly patients, the existence of a germline steroid-metabolizing enzyme variant that is associated with heightened susceptibility to COVID in older women would be of physiologic and potentially pharmacologic importance.

The possibility of selection bias, which it has been argued undermines understanding of COVID risk in numerous ways[41], is an important issue to consider when interpreting any results from observational studies showing associations between genetic or other risk factors and disease susceptibility or outcomes. Although the UK Biobank subject population exhibits a "healthy volunteer" bias compared to the population at large, it is still possible to obtain valid results illuminating exposure-diseases relationships that can be generalized to a wider population[42]. In the case of *HSD3B1*(1245A/C) genotype, the most obvious potential bias comes from the much higher C allele frequencies in people of European ancestry than of other ancestries. Our use of a cohort defined as being genetically Caucasian by the UK Biobank using very restrictive genetic principal component windows largely eliminates this potential source of bias. However, due to small sample sizes for UK Biobank subjects of non-European ancestry, we cannot conclude whether the results are generalizable to non-European populations. An additional method of guarding against selection bias was our use of two separate means of assessing COVID susceptibility – identification as a COVID case among the whole study population, and test positivity among COVID tested subjects. If a putative risk factor caused a selection bias such that subjects with that risk factor were more likely to undergo COVID testing, but the factor did not actually increase COVID susceptibility, one might see higher rates of COVID case identification with the putative risk factor, but lower rates of test positivity. Therefore, looking only at case rates

or only at positive test rates could lead to the false conclusion of the factor either increasing COVID susceptibility or protecting against COVID[41]. In our study, women of elevated age had increased odds of both being identified as COVID cases and of testing positive for COVID, arguing against this hypothetical selection bias effect. The robustness of the results to inclusion or exclusion of different covariates, such as BMI[43], further supports the results not being due to selection bias.

The statistically strongest element of our results ($p < 0.0001$) is the age gradient in allele frequencies among women identified as COVID cases, with C allele frequency increasing with increasing age (Fig. 5). With the existence of such an age gradient, especially in an older cohort, it is essential to consider whether survival bias, a specific type of selection bias, is the source of this result[44]. Because the age gradient was only present among women identified as COVID cases, and was not present among the overall subject pool of women, it appears that *HSD3B1* genotype does not substantially affect risk of death and therefore the result is unlikely to be due to survival bias. An alternate explanation to the C allele being associated with increased COVID susceptibility as women age would be that the C allele, although not substantially affecting overall mortality, selects for survival of women who are more susceptible to COVID, but we are not aware of a mechanism by which this could occur. Broadly speaking, the other known associations with *HSD3B1* genotype – in outcomes of castration-resistant prostate cancer[45], in estrogen receptor positivity of postmenopausal breast cancer[46], and in response to glucocorticoid treatment in asthma[21] – seem unlikely to create a selection bias at the population level that would explain our results. We cannot rule out the possibility that there are more complex selection mechanisms at work, but the use of a study cohort defined by very similar genetic ancestry, and as a result possessing consistent *HSD3B1*(1245A/C) allele frequencies throughout the cohort, should reduce the chances of this. All these points taken together suggest that the observed associations are not due to selection bias, although the possibility of selection bias cannot be completely ruled out.

Although it is well established that men are at greater risk of severe disease and death from COVID than women, existing data make it less clear whether there is any disparity between men and women in susceptibility to infection. A large meta-analysis covering studies from around the globe reported that the overall proportion of male COVID cases was exactly half[47], and The Sex, Gender, and COVID-19 Project, which tracks all sex-disaggregated COVID cases reported worldwide, similarly shows as of April 2021 that male cases account for 49.2% of the 73,793,217 sex-disaggregated cases globally[48]. These results indicate that men and women are infected at the same rate, but other studies suggest that the lack of observed sex discordance is an artifact of all ages being grouped together. Multiple studies have reported that case rates are higher in women from roughly ages 20 to 60 with a reversal to being higher in men above age 60[49–51]. This has been attributed to women being overrepresented among high-exposure occupations such as health care workers[49, 51]. Evidence for sex hormone mediated modulation of immune responses, the precise mechanisms of which are still the subject of much ongoing research[10, 13], raises the question of whether pre vs. postmenopausal hormone environments could also be a factor. Our results, from a cohort predominantly above age 60, are in agreement with this observed trend, as COVID case rates in the UK Biobank subject population are higher

among men than women (Table 1), with this difference being driven by the 60 and up portion of the population (Table S1). Taken together, this body of evidence indicates that men, or at the very least older men, may in fact be more susceptible to COVID infection than women, although disentangling the contributions of gender-based differences in occupational risk of exposure, gender-based differences in compliance with protective measures such as social distancing and mask-wearing[52], and sex-based differences in biological susceptibility to infection is difficult. An additional caveat is the question of whether differences are being measured in total infection rates or in rates of symptomatic infection. Nonetheless, our finding that women of elevated age who inherited the variant form of 3βHSD1 conferring increased androgen synthesis have higher COVID rates is consistent with androgen-mediated immune suppression being a contributing factor to the higher COVID rates in older men vs. women.

Whereas our results suggest that increased androgen-mediated immune suppression in older women of adrenal-permissive genotype could lead to increased susceptibility to COVID infection, we did not find evidence for a similar effect in outcomes (respiratory failure or death) among COVID patients (Fig. 4). The analyses for outcomes may have been underpowered; with 80% power and alpha level 0.05, in Caucasian females the respiratory failure analysis was powered to detect a 1.51 odds ratio and the mortality analysis a 1.25 odds ratio, so these should be considered exploratory analyses. It was shown in a large, randomized trial that treatment with the glucocorticoid dexamethasone strongly reduced mortality in COVID patients on ventilation but not in patients receiving no respiratory support[53], and dexamethasone has since become a mainstay treatment for hospitalized COVID patients with severe disease. Taken together with the findings we report here and our previous finding that *HSD3B1* genotype affects response to glucocorticoid treatment in asthma with the adrenal-permissive 1245C allele associated with reduced airway inflammation[21], this raises the question of whether *HSD3B1* genotype could affect response to dexamethasone in COVID patients, and whether adrenal-permissive genotype could be beneficial in such patients, as it is in the asthma setting. This question could not be addressed using the data available in the UK Biobank and should be the subject of future studies. The differential effects of dexamethasone in COVID patients who were or were not receiving respiratory support also highlight how the results of steroid-induced immune suppression could be context-dependent: immune suppression could make it harder to ward off an initial infection, but could be beneficial in a patient with an advanced infection and out-of-control inflammatory response.

The finding of increased COVID rates in older women with adrenal-permissive genotype has the strongest statistical support of our results, but we also found some evidence that in younger (below age 60) subjects of both sexes, adrenal-permissive genotype was associated with modestly reduced COVID rates, i.e., an association in the opposite direction (Fig. 2). The statistical support for this finding is not nearly as strong, so this result should be interpreted with caution and discussion of its implications should be regarded with this caution in mind. The products of 3βHSD1-catalyzed reactions include immediate precursors of both androgens and estrogens, and estrogens have been reported to bolster immune response[9–11], including in a mouse study of the SARS-CoV-1 virus that caused the 2003 SARS outbreak[54] and is closely related to the SARS-CoV-2 virus that causes COVID. If

the modestly reduced COVID rates in younger subjects with adrenal-permissive genotype represent a real trend, this suggests the possibility that the interplay of androgens and estrogens in regulating immune response could vary in both age and sex-dependent ways; i.e., cells expressing *HSD3B1*(1245C) could potentially have heightened production of both androgens and estrogens, the effects of which might vary with age and/or sex.

In women identified as COVID cases, we found a strong linear relationship between age and adrenal-permissive *HSD3B1*(1245C) allele frequencies (Fig. 5). This linear relationship covering the range of roughly 50 through 80 years of age raises the question of whether the same relationship would continue over a wider age range. It is tempting to speculate that adrenal-permissive genotype would continue to become more strongly associated with susceptibility to infection if the age range were expanded upward but that there would be a plateau rather than a continued decrease if the age range were expanded downward to premenopausal ages. In postmenopausal women, the adrenals are the sole source of sex steroids, and DHEA levels continue to decline with increasing age. By contrast, in premenopausal women, the adrenally produced sex steroid pool is in addition to the gonadally produced pool. However, it is not possible to address this question using the UK Biobank; women's menopausal statuses when they enrolled in the study are available, but not menopausal statuses as of 2020. In the under 60 age range contained in the UK Biobank (i.e., 50–59), most but not all women would be postmenopausal, and menopausal statuses of individual women not previously identified as having undergone menopause cannot be ascertained. To address the question of whether the association is different in pre vs. postmenopausal women, a subject population with sufficient sample sizes over a wider range of ages is required.

The sex-specific and age-specific nature of our results raises the question of whether there are sex-specific or age-specific associations between other germline variants and COVID susceptibility. The COVID-19 Host Genetics Initiative (HGI), in a genome-wide association meta-analysis including nearly 50,000 patients, reported 13 associations with genome-wide significance for COVID infection or severity, but no sex-specific associations with genome-wide significance[55]. Given the very low significance thresholds required for genome-wide significance, the lack of such findings does not rule out the possibility of sex-specific associations. In publicly available results from the HGI, we found no association with *HSD3B1* genotype, but this is expected given that our UK Biobank analyses that pooled all ages and both sexes found no associations. In contrast to the lack of sex-specific findings in the COVID HGI's published results, another genome-wide association study (GWAS) using UK Biobank data reported a difference in the effect of an *ABO* variant between sexes, as well as a male-specific association with *ANKS1A* variants, the latter of which the authors reported reached nominal significance in the COVID HGI[56]. Whereas these other studies have examined sex-specific associations, we are not aware of age-stratified GWAS results for COVID susceptibility or outcomes. Our results, showing an association between adrenal-permissive *HSD3B1* genotype and female COVID susceptibility that grows stronger with increasing age, should be validated in an independent cohort with sufficient sample sizes and results that are stratified by sex and age.

To attempt to explore the mechanistic basis for the association, we analyzed circulating testosterone levels in men and women as well as circulating estradiol levels in premenopausal women and did not find that levels differed by genotype. This is consistent with a previous study that did not find differences in testosterone levels by genotype in male or female asthma patients[21]; the results we report here come in a general population with much larger sample sizes. 3βHSD1 is also directly upstream of potent estrogen production; another study similarly did not find an association between genotype and circulating estrogen levels in postmenopausal breast cancer patients, but found that estrogen-driven postmenopausal breast cancer was enriched for adrenal-permissive genotype[46]. Despite the apparent lack of an appreciable effect of *HSD3B1* genotype on circulating steroid levels, clear phenotypes with *HSD3B1* genotype have been observed in two asthma cohorts[21], at least eight prostate cancer cohorts[45], and three postmenopausal breast cancer cohorts[46]. Taken together, these results suggest that the effects of *HSD3B1* genotype are mediated largely independent of circulating sex steroid levels. Steroid levels in tissues can differ profoundly from those in circulation[57–59], so *HSD3B1* genotype could potentially affect concentrations at the cell or tissue level without measurably affecting concentrations in circulation, which should be further studied.

The existence of a differential immune response based on differential androgen metabolism occurring specifically within one sex (i.e., women) could help shed light on the role of androgens in immune responses in general. Comparisons between men (higher androgen levels) and women (lower androgen levels) are confounded by numerous other physiologic differences between the sexes; in a comparison between women with adrenal-permissive and women with adrenal-restrictive androgen synthesis, these confounding factors are absent. The mechanisms underlying associations between immune responses and inherited adrenal androgen metabolism by way of *HSD3B1* genotype are subjects for future study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data and materials availability:

Data are regulated by UK Biobank’s policies on access.

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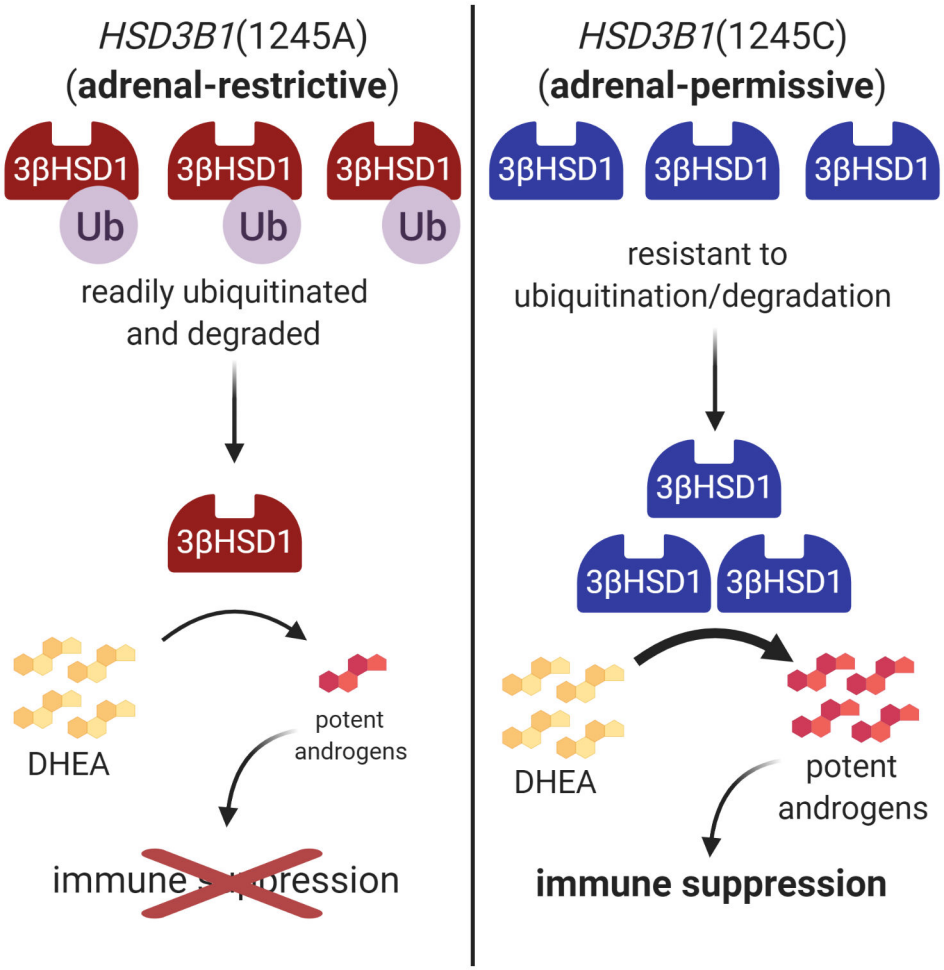

Figure 1. Hypothesized mechanism: adrenal-permissive *HSD3B1* genotype leads to greater androgen synthesis and immune suppression.

Depending on genotype, *HSD3B1* codes for an enzyme 3βHSD1 that is either readily ubiquitinated and degraded (adrenal-restrictive form, left) or is resistant to ubiquitination and degradation (adrenal-permissive form, right) and as a result accumulates at higher levels in cells. The higher levels of the adrenal-permissive enzyme could enable greater conversion of DHEA to downstream potent androgens at sufficient levels to suppress immune responses, whereas these levels would not be reached with the adrenal-restrictive form. Created with BioRender.com.

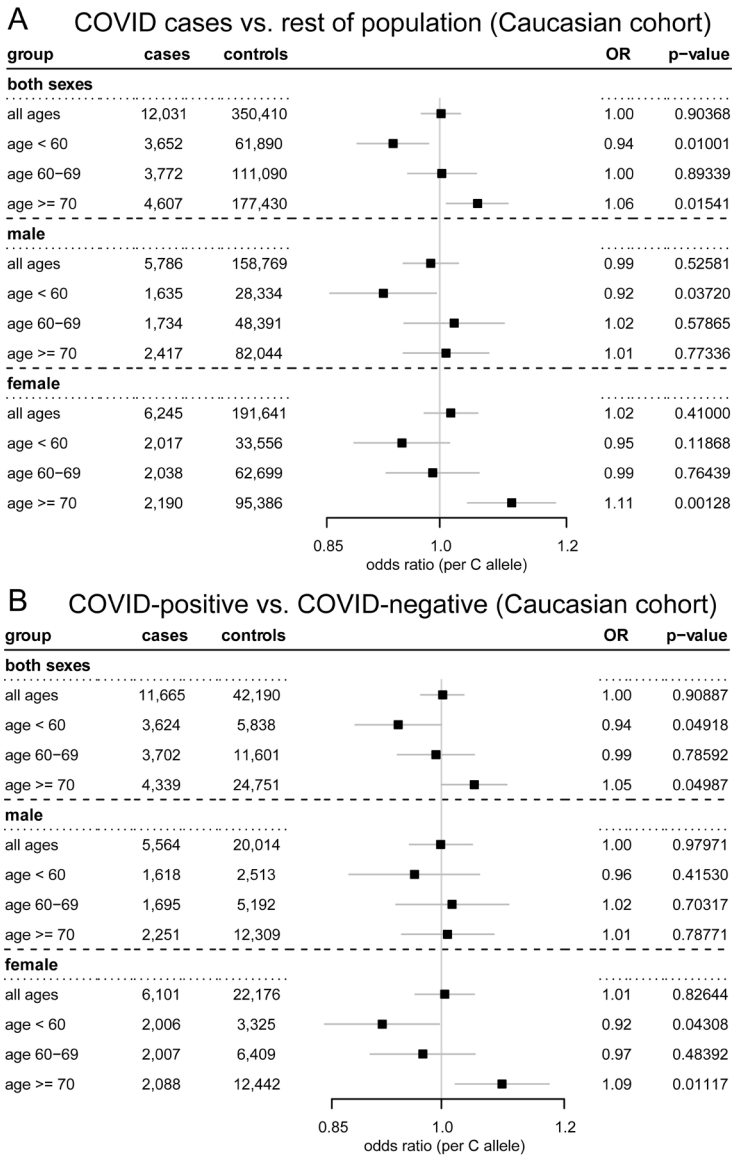

Figure 2. In older women, the adrenal-permissive *HSD3B1*(1245C) allele is associated with increased odds of COVID.

Odds ratios (per C allele) and 95% confidence intervals for COVID in different age and sex breakdowns of the UK Biobank Caucasian cohort. **(A)** Subjects who were identified as COVID cases by positive test or ICD10 diagnosis code during inpatient hospital episode (or both) vs. all other UK Biobank subjects who were alive at the beginning of 2020. **(B)** Subjects who tested positive for COVID vs. subjects with solely negative COVID test results. Results were adjusted for sex, age, BMI, and the first ten genetic principal components (regressions including both sexes) or for age, BMI, and the first ten genetic principal components (regressions limited to one sex).

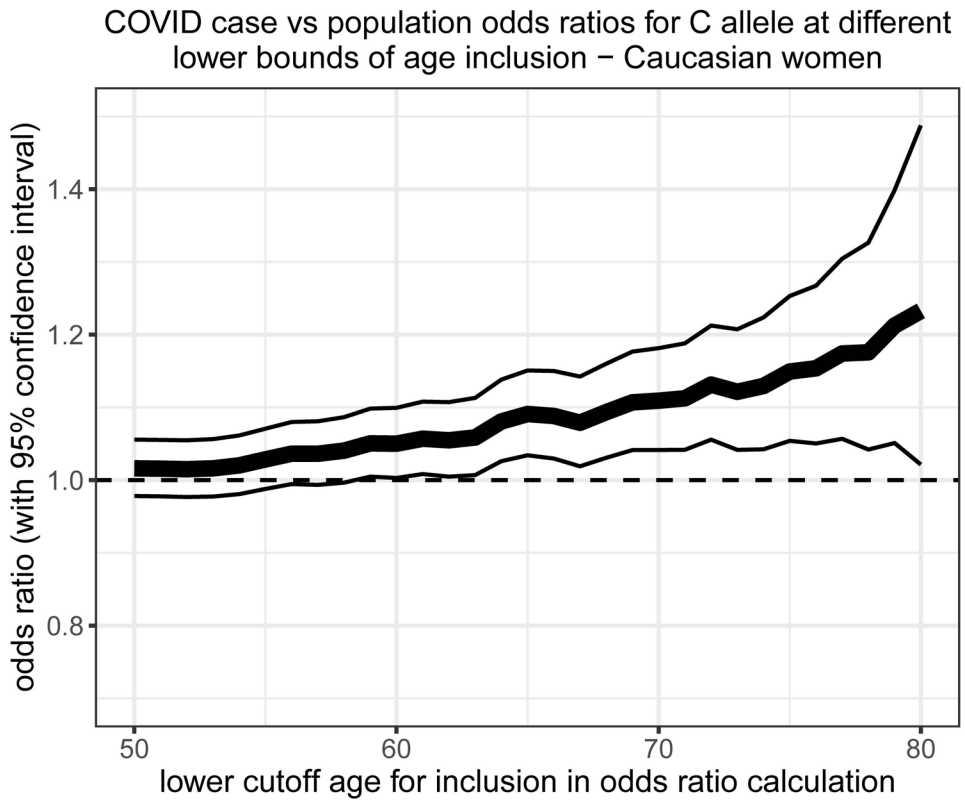

Figure 3. Adrenal-permissive genotype associates with higher COVID odds in older women regardless of the lower cutoff age for inclusion in the analysis.

Odds ratios per C allele (thick line) and 95% confidence intervals (thin lines) for being identified as a COVID case in Caucasian cohort women at least [cutoff age] years old, by cutoff age. For each year of age, the odds ratio and confidence interval in women that age or older were calculated. Note that the result at a lower cutoff age of 70 is the same as the "female, age ≥ 70" result in Fig. 2A.

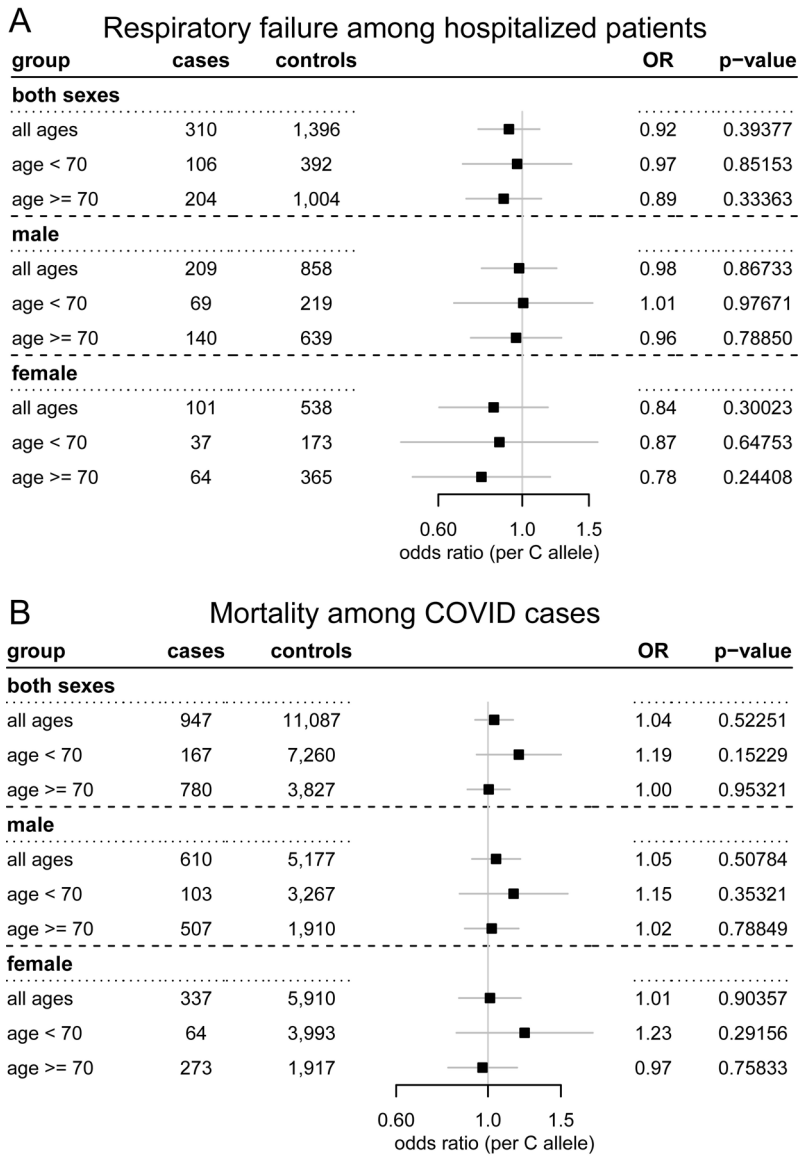

Figure 4. *HSD3B1* genotype does not appear to be associated with COVID outcomes.

Odds ratios (per C allele) and 95% confidence intervals for **(A)** respiratory failure among hospitalized COVID patients and **(B)** death among subjects with identified COVID cases in different age and sex breakdowns of the UK Biobank Caucasian cohort. Results were adjusted for sex, age, BMI, and the first ten genetic principal components (regressions including both sexes) or for age, BMI, self-reported ethnicity, and the first ten genetic principal components (regressions limited to one sex).

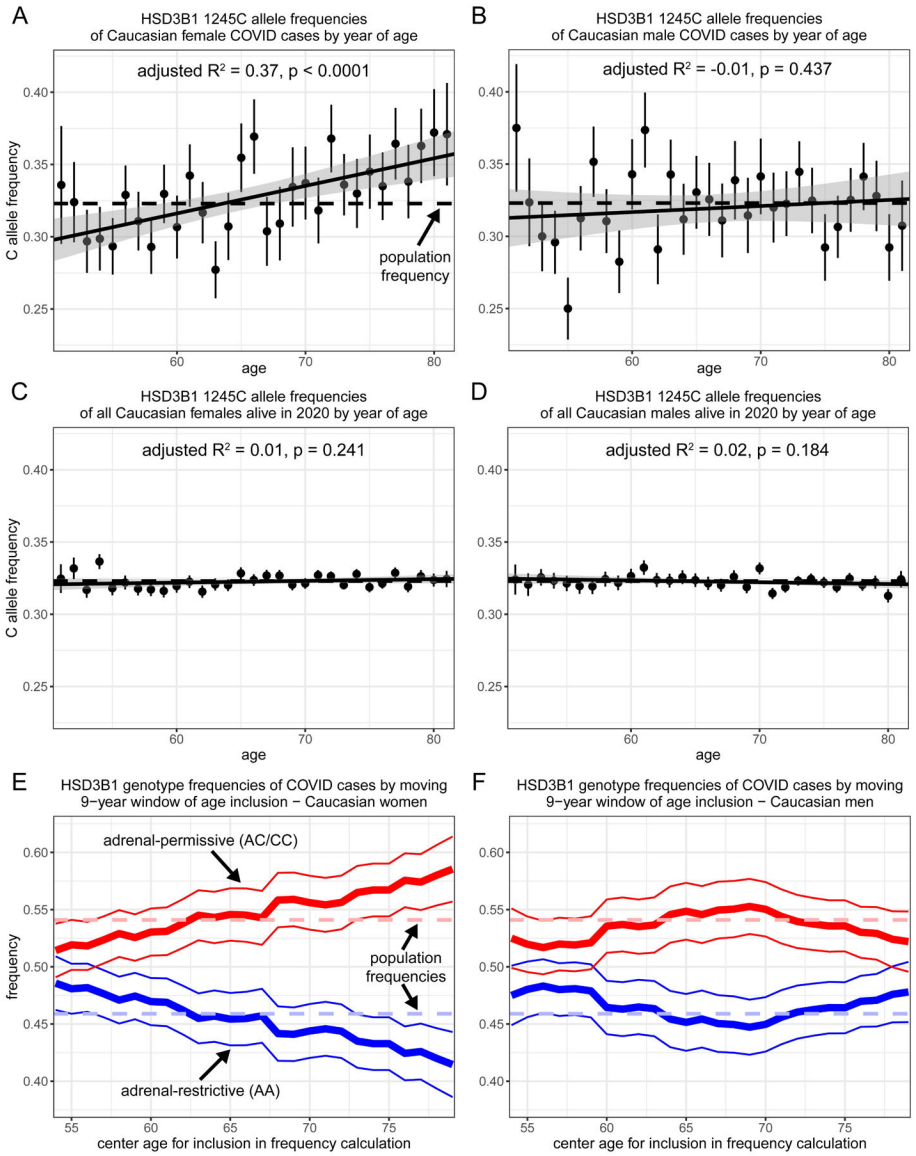

Figure 5. There is a positive linear relationship between age and adrenal-permissive genotype frequency in women identified as COVID cases.

(A-B) The C allele frequencies (± SEM) for female **(A)** and male **(B)** subjects in the UK Biobank Caucasian cohort who had COVID for each year of age, along with the lines of best fit and 95% confidence intervals from weighted linear regressions of allele frequency ~ year of age weighted by number of cases for each year of age. The dashed horizontal lines indicate the C allele frequencies of the overall cohorts. Ages 50, 82, and 83 had just 11, 17, and 4 cases, respectively, out of a total of 6,245 cases for women, and 11, 36, and 0 cases out of a total of 5,786 cases for men, and are not shown in the graphs but were included in the weighted linear regressions. **(C-D)** The C allele frequencies (± SEM) for all female **(C)** and male **(D)** subjects in the UK Biobank Caucasian cohort who were alive in 2020 for each year of age, along with the lines of best fit and 95% confidence intervals from weighted linear regressions of allele frequency ~ year of age weighted by number of cases for each year of age. **(E-F)** The smoothed frequencies of adrenal-permissive (AC or CC)

and adrenal-restrictive (AA) genotypes among female **(E)** and male **(F)** subjects in the UK Biobank Caucasian cohort who had COVID by moving 9-year windows of age inclusion. Thick lines indicate the genotype frequencies and thin lines indicate the 95% confidence intervals. Dashed horizontal lines indicate the overall population frequencies for AC/CC (upper) and AA (lower) genotypes. For each year of age, the genotype frequencies and confidence intervals were calculated for the group of subjects identified as COVID cases and with age equal to the given year ± 4. For example, the data points for age 70 include subjects from ages 66 through 74.

Table 1.

Characteristics of UK Biobank subjects identified as COVID cases and subjects who served as controls.

	All subjects alive in 2020		Caucasian cohort subjects alive in 2020	
	COVID cases	**Controls**	**COVID cases**	**Controls**
n	16,325	443,579	12,031	350,410
Sex				
Male	7,763 (47.6%) *	198,890 (44.8%)	5,786 (48.1%) *	158,769 (45.3%)
Female	8,562 (52.4%) *	244,689 (55.2%)	6,245 (51.9%) *	191,641 (54.7%)
Age (years)	65.2 (50 – 83) *	67.9 (48 – 86)	65.8 (50 – 83) *	68.3 (50 – 86)
Ethnicity				
White	14,588 (89.4%) *	418,342 (94.3%)	12,031 (100%)	350,410 (100%)
Other	1,737 (10.6%) *	25,237 (5.7%)	0 (0%)	0 (0%)
BMI (kg/m^2)	28.4± 5.1 *	27.3 ± 4.7	28.3± 5.1 *	27.3± 4.7

Age is shown as mean (range). (Note that although the overall age range was 48–86, only seven total subjects, or < 0.002%, had ages outside the range 50–83.) BMI is shown as mean (SD).

* indicates different from control population with $p < 0.00001$.