A common TMPRSS2 variant has a protective effect against severe COVID-19

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ARTICLE INFO

Article History:
Received 18 October 2021
Accepted 6 January 2022
Available online 10 January 2022

Keywords:
SARS-CoV-2
COVID-19
TMPRSS2
Targeting the host to prevent COVID19 severity

ABSTRACT

Background: The human protein transmembrane protease serine type 2 (TMPRSS2) plays a key role in SARS-CoV-2 infection, as it is required to activate the virus’ spike protein, facilitating entry into target cells. We hypothesized that naturally-occurring TMPRSS2 human genetic variants affecting the structure and function of the TMPRSS2 protein may modulate the severity of SARS-CoV-2 infection.

Methods: We focused on the only common TMPRSS2 non-synonymous variant predicted to be damaging (rs12329760 C>T, p.V160M), which has a minor allele frequency ranging from 0.14 in Ashkenazi Jewish to 0.38 in East Asians. We analysed the association between the rs12329760 and COVID-19 severity in 2,244 critically ill patients with COVID-19 from 208 UK intensive care units recruited as part of the GenOMICC (Genetics Of Mortality In Critical Care) study. Logistic regression analyses were adjusted for sex, age and deprivation index. For in vitro studies, HEK293 cells were co-transfected with ACE2 and either TMPRSS2 wild type or mutant (TMPRSS2V160M). A SARS-CoV-2 pseudovirus entry assay was used to investigate the ability of TMPRSS2V160M to promote viral entry.

Results: We show that the T allele of rs12329760 is associated with a reduced likelihood of developing severe COVID-19 (OR 0.87, 95%CI:0.79–0.97, p = 0.01). This association was stronger in homozygous individuals when compared to the general population (OR 0.65, 95%CI:0.50–0.84, p = 1.3 £10−3). We demonstrate in vitro that this variant, which causes the amino acid substitution valine to methionine, affects the catalytic activity of TMPRSS2 and is less able to support SARS-CoV-2 spike-mediated entry into cells.

Conclusion: TMPRSS2 rs12329760 is a common variant associated with a significantly decreased risk of severe COVID-19. Further studies are needed to assess the expression of TMPRSS2 across different age groups. Moreover, our results identify TMPRSS2 as a promising drug target, with a potential role for camostat mesilate, a drug approved for the treatment of chronic pancreatitis and postoperative reflux esophagitis, in the treatment of COVID-19. Clinical trials are needed to confirm this.

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Introduction

The severe acute respiratory syndrome like coronavirus (SARS-CoV-2) has infected over 190 million individuals globally and has caused more than 4.2 million deaths as of August 2021 [1]. SARS-CoV-2 infection has a broad clinical spectrum, ranging from asymptomatic or
mildly symptomatic, to a life-threatening presentation requiring admission to intensive care. Age, and to a much lesser extent male gender and various underlying clinical conditions, such as cardiovascular disease, obesity and diabetes, are known risk factors associated with an increased COVID-19 morbidity and mortality [2,3]. The role of an individual's genetic background has recently emerged as an additional, yet not clearly understood, risk factor for COVID-19 [4–6]. Rare genetic variants in genes involved in the regulation of type I interferon (IFN) immunity, including autosomal recessive IRF7 and IFNAR1 deficiencies, have been identified in patients with life-threatening COVID-19 [6]. Autoantibodies to type I IFNs also account for at least 10% of cases of critical COVID-19 pneumonia [7]. Genome-wide association studies (GWAS) have discovered genetic haplotypes spanning several genes that are associated with COVID-19 severity [3,8,9].

The transmembrane protease serine type 2 (TMPRSS2) protein plays a key role in coronavirus infections [9–11], including SARS-CoV-2, as it is required for priming the virus' spike (S) glycoprotein through its cleavage, thus facilitating endosome-independent entry into target cells [12,13]. TMPRSS2, which is part of the type 2 transmembrane serine protease (TTSP) family, is characterized by androgen receptor elements located upstream to its transcription site [14]. As well as cleaving and activating viral glycoproteins of coronaviruses and influenza A and B viruses [15], TMPRSS2 is subject to autocleavage, which results in the liberation of its soluble catalytic domain [16]. The conditions under which autocleavage of TMPRSS2 and other members of the TTSPs family occurs are yet to be elucidated.

TMPRSS2 is expressed in lung and bronchial cells [17], but also in the colon, stomach, pancreas, salivary glands and numerous other tissues [18]. Moreover, it is co-expressed in bronchial and lung cells with the angiotensin-converting enzyme 2 (ACE2) [17], which is the best described SARS-CoV-2 cellular receptor [19]. In the olfactory epithelium of mice, the expression of TMPRSS2, but not ACE2, appears to be age-related and greater in older compared to younger animals [20]. Similarly, a recent study showed that expression of TMPRSS2 in mouse and human lung tissue is also age-related [21]. Studies in TMPRSS2 knock out (KO) mice reported reduced SARS-CoV and MERS-CoV replication in the lungs compared to wild-type mice, and a reduced proinflammatory viral response, especially cytokine and chemokine release via the Toll-like receptor 3 pathway [22,23]. We have recently shown that TMPRSS2 expression permits cell surface entry of SARS-CoV-2, allowing the virus to bypass potent endosomal restriction factors [24]. In vitro studies have shown that TMPRSS2 inhibitors prevent primary airway cell and organoid infection by SARS-CoV and SARS-CoV-2 [25,24,26]. In animal studies, mice infected with SARS-CoV and treated with the serine protease inhibitor camostat mesilate had a high survival rate [27]. Recently, camostat mesilate (which, in Japan, is already approved for patients with chronic pancreatitis and postoperative reflux esophagitis) was shown to block SARS-CoV-2 lung cell infection in vitro [12,24]. Furthermore, camostat mesylate and its metabolite GBP34 were shown to block SARS-CoV-2 spread in human lung tissue ex vivo [28]. Several clinical trials using camostate in COVID-19 patients are currently underway [29].

In view of the data from animal models and cell-based studies supporting a protective role of a knock out TMPRSS2 on coronavirus infection (including SARS and MERS), we hypothesized that naturally-occurring TMPRSS2 genetic variants affecting the structure and function of the TMPRSS2 protein may modulate the severity of SARS-CoV-2 infection.

Methods

TMPRSS2 three-dimensional structure and variant analysis

The recently released 3D structure of TMPRSS2 (PDB: 7meq) was used to assess the impact missense variants. The Phyre homology modelling algorithm [30] was used to resolve missing amino acid regions in the SRSC domain that were not experimentally solved (described in Supplementary material). The FASTA sequence of TMPRSS2 was obtained from the UniProt protein knowledge database [31] (UniProt Id O15393, corresponding to 492 amino acid transcript Ensembl ID ENST00000332149.10). The recently released AlphaFold model [32] was compared to the Phyre model. The impact of each missense variant on the TMPRSS2 protein structure was assessed by analysing the following 16 features, using our in-house algorithm Missense3D [33]: breakage of a disulfide bond, hydrogen bond or salt bridge, introduction of a buried proline, clash, introduction of hydrophilic residue, introduction of a buried charged residue, charge switch in a buried residue, alteration in secondary structure, replacement of a charged with uncharged buried residue, introduction of a disallowed phi/psi region, replacement of a buried glycine with any other residue, alteration in a cavity, replacement of cis proline, buried to exposed residue switch, replacement of a glycine located in a bend. In addition, we used the SIFT [34] and Polyphen2 [35] variant predictors, which mainly use evolutionary conservation to assess the effect of each variant. The effect of variant rs12329760 was further assessed using: i. CONDEL [36], which reports a weighted average of the scores from fathMM and MutationAssessor, and ii. FoldX force field [37], which calculates the stability of a protein based on the estimation of its free energy. A ΔΔG= 0.5 kcal/mol (calculated as: ΔΔG= ΔGmut− ΔGwild) was predicted to have a destabilizing effect.

Participants

Genetics Of Mortality In Critical Care (GenOMICC) and the International Severe Acute Respiratory Infection Consortium (ISARIC) Coronavirus Clinical Characterisation Consortium (4C) (ISARIC 4C)

Cases: this cohort was established between March 2020 and July 2020 (first COVID-19 wave) and comprises of 2244 critically ill, hospitalised COVID-19 positive patients from 208 UK intensive care units (ICUs): 2109, patients were recruited as part of the GenOMICC project, and an additional 133 cases as part of the International Severe Acute Respiratory Infection Consortium (ISARIC) Coronavirus Clinical Characterisation Consortium (4C) study. The clinical characteristics and comorbidities of these patients have been extensively reported in Pairo-Castineira et al. [8]. Only unrelated individuals (up to 3rd degree, based on kinship analysis (King 2.1)) were included. Samples were excluded if the genotype-based sex inference did not match the reported sex, or if a XX karyotype was present. Moreover, patients of mixed genetic ancestry, and from ancestry groups with small numbers of cases (such as North American Indian, n = 13) defined using admixture supervised mode with 1000 genomes as reference, were excluded.

Controls: ancestry-matched controls (ratio 1 case to 5 controls) without a positive COVID-19 test were obtained from the UK BioBank population study. COVID-19 test results in BioBank are obtained from Public Health England, Public Health Scotland and SAIL for English, Scottish and Welsh data, respectively. The vast majority of results are from nose/throat swabs analysed by PCR. For patients admitted to hospital, results can also be from samples obtained from the lower respiratory tract. Only unrelated individuals (up to 3rd degree) were included. Individuals with sex mismatch were excluded. For validation, 45,875 unrelated individuals of European ancestry from the 100,000 Genomes Project were used as an alternative control group.

DNA extraction, genotyping and quality control have been described in detail previously [8]. Genetic ancestry was inferred using ADMIXTURE and reference individuals from the 1000 Genomes project. Imputation was performed using the TOPMed reference panel.

Cells, pseudovirus and plasmid

Human embryonic kidney 293T cells (293Ts; ATCC) were maintained in Dulbecco’s modified Eagle’s medium (DMEM), 10% foetal

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calf serum (FCS), 1% non-essential amino acids (NEAA), 1% penicillin-streptomycin (P/S). Human Caco-2 (ATCC HTB-37) and Calu-3 (ATCC HTB-55) were maintained in DMEM, 20% FCS, 1% NEAA, 1% P/S. All cell lines were maintained at 37 °C, 5% CO2.

Lentiviral pseudotype production was performed as previously described [24][38]. Briefly, pseudovirus was generated by co-transfecting 293Ts with lentiviral packaging constructs pCSFLW (minimal HIV genome with firefly luciferase reporter), pCAGGs-GAGPOL (HIV packing proteins) and the relevant viral glycoprotein in pcDNA3.1 – either the G glycoprotein from Indiana vesiculovirus (VSV-G) or SARS-CoV-2 spike protein. Co-transfections were performed at a plasmid ratio of 1:5:1:1 for pCSFLW:GAGPOL:glycoprotein. Pseudovirus was harvested at 48 and 72 h post-transfection, pooled, filtered, then frozen down. ACE2 FLAG was used as previously described [24]. TMPRSS2 expression plasmid was a kind gift from Roger Reeves (Addgene plasmid #53,887; http://n2t.net/addgene:53887; RRID: Addgene_53,887) [39]. Non-cleavable ACE2-FLAG and TMPRSS2 mutants were generated by overlap extension PCR or site-directed mutagenesis.

Phenotypic assays

293Ts were co-transfected with FLAG-tagged, non-cleavable ACE2 and TMPRSS2, as previously described [24]. Briefly, confluent 10cm² dishes of 293T cells were co-transfected with 1 μg each of TMPRSS2 and ACE2-FLAG. 24 h later, cells were resuspended in fresh media and either spun down for lysis and western blot or added to 96 well plates along with pseudovirus. 24 h later, media was refreshed and a further 24 h later, cells were lysed with reporter lysis buffer (Promega), and luminescence (measured as relative luminescence units, RLU) was read on a FLUOstar Omega plate reader (BMF Labtech) using the Luciferase Assay System (Promega).

Cell pellets for western blot were lysed in RIPA buffer (150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM TRIS, pH 7.4) supplemented with an EDTA-free protease inhibitor cocktail tablet (Roche). Cell lysates were combined with 4x Laemmli buffer (Bio-Rad) with 10% β-mercaptoethanol and boiled for 5 min. Membranes were probed with mouse anti-tubulin (abcam; ab7291), rabbit anti-TMPRSS2 (abcam; ab92323) and/or mouse anti-FLAG (F1804, Sigma). Near infra-red (NIR) secondary antibodies, IRDye® 680RD Goat anti-mouse (abcam; ab216776) and IRDye® 800CW Goat anti-rabbit (abcam; ab216773) were subsequently used. Blots were imaged using the Odyssey Imaging System (LI-COR Biosciences). Densitometry was performed using ImageJ.

Reagent, cell lines and antibody validation

All reagents, cell lines and antibodies used in this study are commercially available and validation data are available on the manufacturers’ websites.

Statistical analysis

Sample size: Critically ill Covid-19 patients, n = 2,244; random controls matched by ancestry from UK Biobank, n = 11,220. The sample size was determined pragmatically by the number of cases recruited during the first wave of the outbreak in the UK (as described in [8]). No randomization was performed. Blinding was not used in this study because the exposure (genotype) and outcome (ICU admission) are objective. Confounding was controlled by the use of covariates: age, sex, deprivation score and genetic ancestry [8]. The association between the TMPRSS2 rs12329760 variant and COVID-19 severity was assessed using logistic regression. Genetic associations in the GenOMICC/ISARIC 4C cohort were analysed as previously described [8]. Briefly, logistic regression with additive and recessive models was performed in PLINKv1.9, adjusting for sex, age, mean-centred age-squared, top 10 principal components (principal component analysis [PCA] performed to adjust for population stratification) and deprivation index decile based on UK postcode. Each major ancestry group alternative in the 100,000 Genomes control group was performed with mixed model association tests in SAIGE (v0.39) [40], including age, sex, age-squared, age-sex interaction and the first 20 principal components as covariates. Trans-ethnic meta-analysis of GenOMICC data for different ancestries was performed by METAL using an inverse-variance weighted method and the P-value for heterogeneity was calculated with Cochran’s Q-test for heterogeneity implemented in the same software [41].

Additional publicly available genetic data were obtained from the COVID-19 Host Genetics Initiative meta-analyses, release 6 [June 15, 2021] [42]. The COVID-19 Host genetics initiative classifies COVID-19 severity according to the use of invasive and non-invasive ventilation during hospital admission. Here we report the four different phenotype comparisons:

- **A2**: 8,779 critically ill confirmed cases (inclusion criteria: hospitalized for COVID-19 and either death or on respiratory support including intubation, CPAP, BiPAP, continue external negative pressure, Optiflow/very high flow Positive End Expiratory Pressure Oxygen) versus 1,001,875 population controls,
- **B1**: 14,480 hospitalised cases versus 73,191 non-hospitalised cases,
- **B2**: 24,274 hospitalised cases versus 2,061,529 population controls, and
- **C2**: 112,443 COVID-19 cases of unspecified severity versus 2,473,889 population controls.

Analyses used all data with the exclusion of the 23&Me study, for which full data were not publicly available. Meta-analysis in all cases was performed using a fixed effect, inverse variance-weighted model, either as a trans-ethnic meta-analysis or subsetted by ancestry group.

Data are presented as mean ± standard deviation. Log-normality was assessed using the Shapiro-Wilk test and QQ plot. A two-tailed Student’s t-test was used to compare the means of two groups. One-way ANOVA was used to compare the means of more than two groups.

Colocalisation analysis

Colocalisation analysis for genetic associations was performed by an Approximate Bayes Factor approach using the package coloc version 5, in R 4.1.0 [43]. Summary statistics (beta and variance) were from GWAS data [8] and from lung eQTL data from GTex v8 [44], in individuals of European ancestry. To reduce the likelihood of violation of the single causal variant assumption arising from multiple independent association signals, the analysis was restricted to a region extending to 5 kb upstream and downstream of the TMPRSS2 gene. With the assumption that exactly one measured SNP in the region was causal for each trait, SNP-level priors (p1 and p2) of 1/ (n SNPs) were used for the probability of association with each individual trait, with an arbitrary prior of 0.1 x p1 for p12, the SNP-level prior probability of association with both traits. Sensitivity analysis was performed to assess the impact of prior selection, comparing the selected priors to the more stringent default priors (10⁻⁴ for p1 and p2, 10⁻⁵ for p12), and varying the p12 range from p1 to p1 x p2.

**Ethics**

Research ethics committees (Scotland 15/SS/0110, England, Wales and Northern Ireland: 19/WM/0247). Current and previous versions of the study protocol are available at genomicsc.org/protocol. All participants gave informed consent.
We first analysed the relation between TMPRSS2 rs12329760 and life-threatening SARS-CoV-2 infection in 2,244 critically ill, hospitalised, COVID-19 positive patients from 208 UK intensive care units (ICUs) (Table 1) recruited as part of the GenOMICC (genomicc.org) and ISARIC 4C (isaric4c.net) projects. These patients were representative of critically ill patients with COVID-19 in the UK population during the first Sars-CoV-2 outbreak of 2020[8]. Patients were treated in intensive care units (ICU/ITU) because of their propensity to critical respiratory failure due to COVID-19. Within the GenOMICC cohort (n = 2,109), mean age was 57.3 ± 12.1, 624 (30%) patients were females, and 396(19%) had comorbidities; 1,557 (74%) required invasive ventilation and 459 (22%) died within 60 days. Within the ISARIC 4C cohort (n = 135), mean age was 57.3 ± 2.9, 46 (34%) were females, and 40 (30%) had comorbidities; 25 (19%) required invasive ventilation and 22 (16%) died within 60 days, as described in[8], 11,220 ancestry-matched individuals without a COVID-19-positive PCR test from the UK Biobank, acted as controls. Under an additive model, we found that the minor T allele of rs12329760 was significantly associated with a protective effect against severe COVID-19 in individuals of European ancestry (1,676 cases, 8,379 controls) with an OR of 0.87 (95%CI:0.79–0.97, p = 0.01). A protective effect was also observed in individuals of East Asian ancestry (149 cases, 745 controls; OR 0.64, 95%CI:0.43–0.95, p = 0.03). Similar effect sizes were observed in South Asians and Africans, but did not reach statistical significance, most likely as a result of the small sample size (Fig. 2). We further confirmed this protective effect on a trans-ethnic meta-analysis, using the entire cohort of 2,244 patients (OR 0.84, 95%CI:0.77–0.93, p = 5.8 × 10⁻⁴, Fig. 2, panel A). A heterogeneity analysis suggested that the T allele has a similar effect across different ethnicities (p = 0.47). To ascertain that this association was not an artefact due to population bias in the UK Biobank controls, the results from the European cohort were confirmed on an independent control population (45,875 unrelated individuals of European ancestry from 100 K Genomes [48]; OR 0.89, 95%CI:0.81–0.89, p = 0.02). Under a recessive model (i.e. individuals homozygous for the T allele), the trans-ethnic meta-analysis on 2,244 critically ill COVID-19 patients estimated an OR of 0.65 for TT homozygotes (95%CI:0.50–0.84, p = 1.3 × 10⁻⁴). In subset analyses, the OR was estimated at 0.70 (95%CI:0.52–0.95, p = 0.024) in Europeans, and 0.28 (95%CI:0.09–0.82, p = 0.019) in East Asians versus their corresponding ancestry-matched controls (Fig. 2, panel B).

To assess whether the rs12329760 could be a proxy for an association with a nearby expression quantitative trait locus (eQTL), colocalisation analysis was performed to compare the GWAS signal at the locus to eQTL associations for TMPRSS2 and neighbouring gene MX1 in GTex version 8 [44] (Figure S 3 A-C), using an Approximate Bayes Factor approach [43]. Under an assumption of a single causal variant within the locus for each transcript, the posterior probability of a common causal variant was 1.1% for TMPRSS2 expression and 2.0% for MX1 expression, compared to posterior probabilities of 67% and 42% respectively for independent associations. Sensitivity analysis showed that the analysis was robust to choice of prior probabilities: more stringent software-default single-trait priors increased the posterior probabilities of null or single-trait-only association hypotheses, but had little impact on the colocalisation probability (1.1% for TMPRSS2 and 0.2% for MX1); varying prior probability for colocalisation (Figure S 3 D-F) had an impact only when approaching the prior for single-trait associations, and did not result in posterior probabilities for colocalisation exceeding those for separate associations. Although independent contributions from multiple variants towards the genetic association cannot be excluded, this indicates that any genetic association between rs12329760 and severe COVID-19 is unlikely to be attributable to linkage disequilibrium with an eQTL and, thus, modification of protein function is more likely.

For additional corroboration of the genetic signal, we investigated the results of large GWAS meta-analyses performed in the context of
the COVID-19 Host Genetics Initiative (COVID-19hg, available at https://www.COVID-19hg.org/, release 6, June 2021) [42]. Compared with the general population, in a trans-ethnic meta-analysis, the minor T allele of rs12329760 was associated with a significantly protective effect against severe COVID-19 (patients requiring hospitalization for COVID-19): 24,274 cases versus 2,061,529 controls; OR 0.95, 95%CI:0.92−0.97, \( p=4.72 \times 10^{-6} \). In ancestry-specific subgroup analyses, this effect was significant for a European population (OR 0.94, 95%CI:0.91−0.96, \( p=5.66 \times 10^{-6} \)), but not for individuals of African, Hispanic-American, or admixed African/Hispanic/American ancestry; however, lower sample sizes for these groups limited study power, and subgroup analyses were not available for Asian populations. The protective effect was particularly evident in confirmed, critically ill cases (8,779 cases versus 1,001,875 population controls; OR 0.91, 95%CI:0.87−0.95, \( p=8.18 \times 10^{-6} \)). Furthermore, the rs12329760 T allele was associated with reduced risk of hospitalization after confirmed infection (14,480 hospitalised versus 73,191 cases not requiring hospitalization within 21 days after the test): OR 0.96, 95%CI:0.92−0.99, \( p=0.012 \). Finally, there was no significant difference (\( p=0.056 \)) in the prevalence of the T allele between the

Fig. 1. The TMPRSS2 protein and the p.Val160Met variant
The TMPRSS2 protein is composed of a cytoplasmic region (residues 1–84), a transmembrane region (TM, residues 85–105) and an extracellular region (residues 106–492). The latter is composed of three domains: the LDLR class A (residues 112–149), the scavenger receptor cysteine-rich domain (SRCR) (residues 150–242) and the Peptidase S1 (residues 256–489), which contains the protease active site: residues His296, Asp345 and Ser441. The three-dimensional structure of the extracellular region residues 145–491 corresponding to domains SRCR-2 (in green) and Peptidase S1 (in blue) is presented. Valine 160 (Val 160, depicted as a red sphere on the cartoon), which harbours variant p.Val160Met, occurs in the SRCR domain and spatially far from the TMPRSS2 catalytic site (mapped onto the surface of TMPRSS2).
general population \((n = 2,473,889)\) and pooled individuals with a laboratory-confirmed SARS-CoV-2 infection (including hospitalized and life-threatening COVID-19 cases from the meta-analyses previously described) or with a self-reported or physician-confirmed COVID diagnosis (total \(n = 112,443\) cases, Fig. 2, panel A).

Although these meta-analyses include UK Biobank data and all except the hospitalised versus non-hospitalised comparison include data from the GenOMICC/ISARIC 4C cohort, and thus do not provide completely independent replication, these cohorts only comprise less than 25\% of the total cases, limiting the impact of this single study on the overall results. These data therefore provide further support for our hypothesis that the TMPRSS2 rs12329760 variant has a protective effect against severe and/or life-threatening COVID-19. However, studies examining the prevalence of this variant in SARS-CoV-2 infected asymptomatic or pauci-symptomatic individuals are needed to ascertain its protective effect against mild viral infection.

To investigate the phenotypic effect of the TMPRSS2 V160M variant, we co-transfected 293Ts cells, which we have previously confirmed that they do not endogenously express ACE2 or TMPRSS2 [24], with ACE2 and either TMPRSS2 wild type (TMPRSS2_{WT}) or V160M (TMPRSS2_{V160M}), as previously described [24]. We and others previously observed that co-expression of TMPRSS2 and ACE2 results in rapid cleavage of ACE2. We, therefore, used a mutant ACE2 that is more poorly degraded by TMPRSS2 [49]. Two additional TMPRSS2 variants were included as controls: the catalytically inactive S441A (TMPRSS2_{S441A}) and the catalytically active R255Q (TMPRSS2_{R255Q}), that is unable to autocleave [16]. First, we investigated the autocleavage pattern of the different TMPRSS2 variants. The N-terminal membrane-bound part of TMPRSS2 can exist as different cleaved intermediates: a full-length uncleaved form of approximately 55 kDa, a partially cleaved form, and a fully cleaved form of 20 kDa. The latter is the product of TMPRSS2 autocleavage at arginine 255, which results in the liberation of the catalytically active protease domain in the extracellular space, leaving a small transmembrane N-terminal domain [16]. Wild type TMPRSS2 is expressed as roughly equal amounts of full-length and fully cleaved forms, with a small amount of partially cleaved product. As expected, the catalytically inactive TMPRSS2_{S441A} and the non-autocleavable TMPRSS2_{R255Q} resulted in only the full-length TMPRSS2 being expressed. However, TMPRSS2_{V160M} resulted in a significantly higher proportion of full-length (55 kDa), and a significantly lower proportion of fully cleaved protein (20 kDa) \((p < 0.05, \text{Student's t-test})\). This difference was clear across a range of TMPRSS2 concentrations, with TMPRSS2 showing a concentration-dependent autocleavage phenotype: the higher the concentration of TMPRSS2, the higher the amount of autocleavage. Overall, these data suggest the V160M substitution exerts a partial inhibitory effect on the proteolytic autocleavage of TMPRSS2 (see Fig. 3A-D, Supplementary Figure S4).

Subsequently, we investigated the effect of TMPRSS2_{V160M} on promoting viral entry, using a previously described SARS-CoV-2 pseudovirus entry assay [24]. Pseudovirus expressing the glycoprotein from the vesicular stomatitis virus (VSV-G) was used as a control, as this virus enters cells in a TMPRSS2-independent manner [24]. Briefly, cells co-transfected with ACE2 and TMPRSS2 wild type or variants were incubated with the pseudovirus (as described in [24,38]) and, after 48 h, luminescence was measured. TMPRSS2_{WT} enhanced viral...
entry by ~5-fold compared to empty vector, while the catalytically dead TMPRSS2S441A showed no enhancement (Fig. 3E). The non-auto-cleavable mutant TMPRSS2R255Q showed similar enhancement, suggesting that autocleavage is dispensable for optimal TMPRSS2-mediated enhancement. TMPRSS2V160M showed no significant difference in viral entry compared to the TMPRSS2WT. Overall, the expression of catalytically active TMPRSS2 proteins only slightly inhibited VSV-G mediated entry (Fig. 3F).

The partial inhibitory effect exerted by the V160M variant on the proteolytic autocleavage of TMPRSS2 resulted in a far greater proportion of uncleaved, surface-expressed TMPRSS2V160M compared to TMPRSS2WT. We compared this autocleavage seen in transfected 293T cells, to that seen in several epithelial cell lines that naturally express endogenous ACE2 and TMPRSS2: the human lung cell line, Calu-3, and the human colorectal adenocarcinoma cell line, Caco-2, both of which are extensively used for SARS-CoV-2 research. Interestingly, no fully cleaved TMPRSS2 could be detected as opposed to 293T cells, while both cell lines expressed mostly full length or partially cleaved TMPRSS2. This again suggests that the high levels of autocleavage seen in 293T cells may be, in part, an artefact of overexpression (Supplementary Figure S5). Therefore, we re-assessed whether TMPRSS2V160M affects SARS-CoV-2 S-expressing pseudovirus entry by using the double mutant TMPRSS2R255Q/V160M (which does not autocleave and is, therefore, more similar to endogenous TMPRSS2 in Calu-3 and Caco-2 cells), to control for protein cell-surface expression. Under these conditions, and across a range of plasmid titrations of both TMPRSS2 mutants and ACE2, TMPRSS2R255Q/V160M showed a significantly reduced ability to promote SARS-CoV-2 S-expressing pseudovirus compared to TMPRSS2R255Q alone, despite equal protein expression (Fig. 4A,C,D,F). Again, TMPRSS2R255Q/V160M had no effect on VSV-G-mediated entry (Fig. 4B,E).

**Discussion**

Overall, our results suggest that the rs12329760 C>T variant results in a moderately less catalytically active TMPRSS2, which is less able to autocleave and prime the SARS-CoV-2 spike protein. This may explain the protective effect against life-threatening COVID-19 observed in our cohort of patients admitted to ICU, compared to the general population. Such an effect was more prominent in homozygotes (recessive model) for the rs12329760 variant in whom a 30% (OR 0.70) risk reduction was observed. Unfortunately, we did not have samples from asymptomatic/pauci symptomatic patients, but data from COVID-19 meta-analyses appear to suggest that the rs12329760 variant has no protective effect against SARS-CoV-2 infection per se.

The allele frequency of TMPRSS2 rs12329760 (data from GnomAD population database) varies across different populations and is higher...
in East Asian and Finnish individuals (MAF 0.38 and 0.37, respectively) compared to south Asians (MAF 0.25) and non-Finnish Europeans (MAF 0.23). The lowest frequency of the T allele is reported in Latino and Jewish-Ashkenazi individuals (MAF 0.15). However, in our study, the small sample size of populations of non-European ancestry does not allow conclusions on the effect size of TMPRSS2 rs12329760 in different ethnicities. Genotyping of the TMPRSS2 rs12329760 variant on large COVID-19 cohorts of patients of non-European genetic ancestry is, therefore, needed to assess its role in determining the differences in the severity of COVID-19 across various populations (e.g. between East Asia and Europe [50]). Indeed, a recent study showed a lower T allele frequency in a small cohort of Chinese patients with life-threatening COVID-19 compared to the population frequency [51]. Although the differences in the proportion of SARS-CoV-2 patients who develop severe COVID-19 across different populations [50] are more likely to be explained by social behaviour, public health measures to curb outbreaks, exposure to other viruses and immunological factors, human genetic variation across different populations may also contribute to the observed differences.

The pharmacological inhibition of TMPRSS2 using serine protease inhibitors, such as camostat and nafamostat, has been proposed as a pharmacological treatment of COVID-19 patients. In vitro [12] and animal studies have demonstrated that camostat can block viral entry (reviewed in [52]), and initial reports on the repurposing of camostat in COVID-19 patients have provided promising results [53]. However, a recently completed clinical trial using camostat in patients hospitalized for severe COVID-19, did not demonstrate a significant reduction in time-to-clinical improvement compared to placebo [54]. As the authors suggest, these patients were likely to have passed the most active stage of viral replication at the time of treatment and were in the hyper-inflammatory stage of the COVID-19, thus possibly explaining the lack of camostat efficacy. Several additional clinical trials of camostat in COVID-19 are currently underway [29]. Recently, the placebo-controlled phase III trial conducted in Japan on pauci-symptomatic COVID-19 patients administering camostat mesilate 600 mg 4 times a day did not meet its primary end point of time to negative Sars-CoV-2 test [55], however data on secondary end points, such as progression to severe or life-threatening COVID-19, are still not publicly available.

Very little is known on TMPRSS2 and further extensive in vitro and in vivo studies on its pathophysiology are necessary. Since the beginning of the COVID-19 pandemic, the interest in TMPRSS2 has focused only on its role as a serine protease involved in the activation of the SARS-CoV-2 spike protein. However, as a soluble protease, TMPRSS2 may have additional substrates, and in vitro studies have demonstrated that PAR2 is one of these substrates [56,57]. PAR2 is expressed in several tissues, including lung, vascular endothelial and vascular smooth muscle cells [58,59] and its protease-mediated activation promotes inflammation by inducing prostaglandin synthesis and cytokine production in the lungs and other organs [60–64]. An intriguing hypothesis is that, similar to other soluble serine proteases, such as the human airway trypsin-like protease HAT (also known as

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**Fig. 4. Phenotypic impact of the TMPRSS2 non-autocleavable version of the V160M variant on SARS-CoV-2 spike-mediated entry**

Entry of lentiviral pseudotypes expressing (A,D) SARS-CoV-2 spike glycoprotein or (B,E) vesicular stomatitis virus glycoprotein (VSV-G) into HEK293Ts co-expressing ACE2-FLAG and either empty vector or TMPRSS2 variants. Data shows mean±standard deviation of 3 independent repeats from different weeks, normalised to empty vector. A-C shows titrations of mutant TMPRSS2 with constant ACE2 expression, while D-E show titrations of ACE2 with constant levels of TMPRSS2 expressed.

(A,B,D,E) statistics determined by two-tailed Student’s t-test. (C,F) Western blot analysis of TMPRSS2 autocleavage mutant (R255Q) titration with or without the V160M substitution. µg values indicate the amount of TMPRSS2 or ACE2 plasmid added to each condition. RLU, relative luminescence units.

* *0.05 ≥ P > 0.01; **0.01 ≥ P > 0.001; ***0.001 ≥ P.
TMPRS11D), the soluble wild type TMPRSS2 protease may also have a role in promoting inflammation in the lungs and other tissues.

Since May 2020, when we reported the TMPRSS2 variant rs12329760 as possibly damaging to protein structure/function and raised the possibility that it could partly explain host susceptibility to COVID-19 severity [65], several other studies have also supported this hypothesis [66−71]. In this study we have confirmed our initial hypothesis and provided a mechanistic effect to explain how this variant may contribute to the host susceptibility to severe COVID-19.

As previously discussed, one limitation of our study was the lack of access to a cohort of asymptomatic/pauci symptomatic COVID-19 patients. In the absence of such a cohort, we considered the general population as a good proxy and used this for comparison with COVID-19 severe cases. Indeed, a recent systematic review and meta-analysis shows that one third of COVID-19 positive cases do not develop symptoms [72]. When well-characterized cohorts of asymptomatic/pauci symptomatic COVID-19 patients become available, it will be possible to further investigate the role of TMPRSS2 variant rs12329760 on Sars-CoV-2 infection. Another limitation of this study is that we did not directly validate our results in endogenously expressing cell lines, such as like Calu-3, as this would require gene editing the endogenous TMPRSS2. Calu-3 cells are extremely slow growing and highly resistant to single cell cloning, thus making this cell line not particularly suitable for gene editing.

In conclusion, the T allele of the common TMPRSS2 variant rs12329760 confers a reduced risk of severe COVID-19. Similar to what observed in the TMPRSS2 KO mouse, the Val160Met substitution, which exerts a partial inhibitory effect on the proteolytic auto-cleavage of TMPRSS2 and the priming of the SARS-CoV-2 spike protein, is associated with a milder COVID-19 infection compared to the wild type. Differences in population frequency of this genetic variant may contribute to the reported variability in COVID-19 severity across various ethnicities and studies on large COVID-19 cohorts of patients of non-European genetic ancestry are needed to clarify this. Further studies are needed to assess the expression of TMPRSS2 across different age groups; indeed a reduced TMPRSS2 expression in younger compared to older individuals, as observed in mice and in preliminary human studies, could help explain age-related differences in COVID-19 morbidity. Moreover, TMPRSS2 could be a viable drug target in COVID-19 patients, and camostat mesilate, or other novel TMPRSS2 inhibitors, may have a role in the treatment of COVID-19. Clinical trials are needed to confirm this.

**Funding**

Wellcome Trust, BBSRC, UKRI Future Leader’s Fellowship, Health Data Research UK

AD and NP were supported by the Wellcome Trust (grants 104,955/Z/14/Z, 218,242/Z/19/Z and 211,496/Z/18/Z) and TK by the BBSRC (grants BB/P011705/1 and BB/P023959/1), VSS is supported by UKRI Future Leader’s Fellowship [MR/S032304/1], J-LC is supported by Howard Hughes Medical Institute, Rockefeller University, St. Giles Foundation, Fisher centre for Alzheimer’s Research Foundation, Meyer Foundation, Square Foundation, Grandir - Fonds de solidarité pour l’enfance, SCOR Corporate Foundation for Science, Institut National de la Santé et de la Recherche Médicale (INSERM), University of Paris, National Institutes of Health (R01AI088364), French Foundation for Medical Research (EQUOR2100307798), FRM and French National Research Agency (ANR) GENCovid project (ANR-20-C0V1-0003); LA is supported by the Agence Nationale de la Recherche (ANR-10-JAHU-01, ANR-10-LABX-62-IBEID), TPP and WSB are supported by BBSRC grants BB/R013071/1 and BBSRC and the G2P-UK National Virology consortium (funded by MRC/UKRI, grant ref: MR/W005611/1), AT was supported by Roslin Institute Strategic Programme Grants from the BBSRC (BBS/E/D/10,002,070 and BBS/E/D/10,002,275) and Health Data Research UK (references HDR-9004 and HDR-9003).

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NP, EP-C, AT and JKB contributed to population data analysis. AD, TK and MJES contributed to 3D modelling and structural analysis. TPP and WSB contributed to laboratory work. NP, EP-C, TPP and AD contributed to data analysis. NP, TPP, WSB, AD contributed to study design. NP, TPP, EP-C, AC, VS-S, J-LC, LA, WSB, JKB, MJES and AD contributed to interpretation of findings and manuscript preparation. AD conceived the study, contributed to study coordination and wrote the first draft of the manuscript. All authors approved the final version of the manuscript.

**Data availability**

Full summary-level data in support of the findings of this study are available for download from https://genomic.org/data. Individual level data can be analysed by qualified researchers in the ISARIC 4C/GenOMICC data analysis platform by application at https://genomic.org/data. BioBank data and Genomics England data are available to registered researchers at https://www.ukbiobank.ac.uk/ and https://www.genomicsengland.co.uk/. The COVID-19 Host Genetics Initiative2 (COVID-19hg) summary statistics are available at https://www.COVID-19hg.org/.

Supplementary Information is available for this paper.

**Declaration of Competing Interest**

Dr. David reports grants from Wellcome Trust during the conduct of the study; Dr. Parkinson reports grants from Wellcome Trust during the conduct of the study; Dr. Peacock reports grants from MRC/UKRI, grants from BBSRC during the conduct of the study; Dr. Pairo-Castineira has nothing to disclose. Dr. Khanna reports grants from BBSRC during the conduct of the study; Dr. Cobat has nothing to disclose. Dr. Tenesa reports grants from BBSRC, grants from Health Data Research UK during the conduct of the study. Dr. Sancho-Shimizu reports grants from UKRI Future Leader’s Fellowship during the conduct of the study; Dr. Casanova reports other from Howard Hughes Medical Institute, other from Rockefeller University, other from St. Giles Foundation, other from Fisher centre for Alzheimer’s Research Foundation, other from Meyer Foundation, other from Square Foundation, other from Grandir - Fonds de solidarité pour l’enfance, other from SCOR Corporate Foundation for Science, other from Institut National de la Santé et de la Recherche Médicale (INSERM), other from University of Paris, other from National Institutes of Health (NIH), other from French Foundation for Medical Research (FRM), other from FRM and French National Research Agency (ANR) GENCOVID project during the conduct of the study; Dr. Abel reports other from Agence Nationale de la Recherche during the conduct of the study; Dr. Casanova reports other from Howard Hughes Medical Institute, other from Rockefeller University, other from St. Giles Foundation, other from Fisher centre for Alzheimer’s Research Foundation, other from Meyer Foundation, other from Square Foundation, other from Grandir - Fonds de solidarité pour l’enfance, other from SCOR Corporate Foundation for Science, other from Institut National de la Santé et de la Recherche Médicale (INSERM), University of Paris, National Institutes of Health (R01AI088364), French Foundation for Medical Research (EQUOR2100307798), FRM and French National Research Agency (ANR) GENCOVID project (ANR-20-C0V1-0003); LA is supported by the Agence Nationale de la Recherche (ANR-10-JAHU-01, ANR-10-LABX-62-IBEID), TPP and WSB are supported by BBSRC grants BB/R013071/1 and BBSRC and the G2P-UK National Virology consortium (funded by MRC/UKRI, grant ref: MR/W005611/1), AT was supported by Roslin Institute Strategic Programme Grants from the BBSRC (BBS/E/D/10,002,070 and BBS/E/D/10,002,275) and Health Data Research UK (references HDR-9004 and HDR-9003).

**Acknowledgement**

This research was conducted using the UK BioBank Resource under project 788

**Supplementary materials**

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.retram.2022.103333.
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Elena Aperi, Cathrine Basikolo, Bethan Blackledge, Laura Catlow, Bethan Charles, Paul Dark, Reece Doonan, Jade Harris, Alice Harvey, Daniel Horner, Karen Knowles, Stephanie Lee, Diane Lomas, Chloe Lyons, Tracy Marsden, Danielle McLaughlan, Liam McMorrow, Jessica Pendlebury, Jane Perez, Maria Poulakia, Nicola Proudfoot, Melanie Sloughton, Kathryn Slevin, Melanie Taylor, Vicky Thomas, Danielle Walker, Angiy Michael, Matthew Collis.

William Harvey Hospital, Ashford, UK
Tracey Cosier, Gemma Millen, Neil Richardson, Natasha Schumacher, Heather Weston, James Rand.

Queen Elizabeth University Hospital, Glasgow, UK
Nicola Baxter, Steven Henderson, Sophie Kennedy-Hay, Christopher McParland, Laura Rooney, Malcolm Sim, Gordan McCreath.

Bradford Royal Infirmary, Bradford, UK
Louise Akerozy, Shereen Bang, Matt Bromley, Lucy Gurn, Tom Lawton, James Morgan, Kirsten Sellick, Deborah Warren, Brian Wilkinson, Janet McGowan, Camilla Ledgard, Amelia Stacey, Kate Pye, Ruth Bellwood, Michael Bentley.

Bristol Royal Infirmary, Bristol, UK
Jeremy Bewley, Zoe Garland, Lisa Grimmer, Bethany Gumbrill, Rebekah Johnson, Katie Sweet, Denise Webster, Georgia Efford.

Norfolk and Norwich University hospital (NNUH), Norwich, UK
Karen Convery, Deirdre Fottrell-Gould, Lisa Hudig, Jocelyn Keshet-Price, Georgina Randell, Katie Stammers.

Queen Elizabeth Hospital Gateshead, Gateshead, UK
Maria Bokhari, Vanessa Linnett, Rachael Lucas, Wendy Queen Elizabeth Hospital Gateshead.

Sunderland Royal Hospital, Sunderland, UK
Anthony Rostron, Alistair Roy, Lindsey Wood, Sarah Corney, Fiona Wakinshaw, Kimberley Rogerson, Jordan Jarmain.

Aintree University Hospital, Liverpool, UK
Robert Parker, Amie Reddy, Ian Turner-Bone, Laura Wilding, Peter Harding.

Hull Royal Infirmary, Hull, UK
Caroline Abernathy, Louise Foster, Andrew Gratrix, Vicky Martinson, Priyai Parkinson, Elizabeth Stones, Lucía Carbral-Efford.

University College Hospital, London, UK
Georgia Bercades, David Brealey, Ingrid Has, Niall MacCallum, Gladys Martir, Eamon Raith, Anna Reyes, Deborah Smyth.

Royal Devon and Exeter Hospital, Exeter, UK
Letizia Zitter, Sarah Benyon, Suzie Marriott, Linda Park, Samantha Keenan, Elizabeth Gordon, Helen Quinn, Kizzy Baines.

The Royal Papworth Hospital, Cambridge, UK
Lenka Cagova, Adama Fofano, Lucie Garner, Helen Holmes, Sue Mepham, Alice Michael Mitchell, Lucy Mwaura, Krithivasan Praman, Alain Vuylsteke, Julie Zamikula.

Ipswich Hospital, Ipswich, UK
Bally Purewa, Vanessa Rivers, Stephanie Bell.

Southend Hospital, Southend, UK
Hayley Blakemore, Borislav Borislavova, Beverley Faulkner, Emma Gendall, Elizabeth Goff, Kati Hayes, Matt Thomas, Ruth Worner, Kerry Smith, Deanna Stephens.

Milton Keynes University Hospital, Milton Keynes, UK
Louise Mew, Esther Mwaura, Richard Stewart, Felicity Williams, Lynn Wren, Sara-Beth Sutherland.

Royal Hampshire County Hospital, Hampshire, UK
Emily Bevan, Jane Martin, Dawn Trodd, Geoff Watson, Caroline Wrey Brown.

University Hospital of Wales, Cardiff, UK
Jade Cole, Michelle Davies, Rhys Davies, Donna Duffin, Helen Hill, Ben Player, Emma Thomas, Angharad Williams.

Basingstoke and North Hampshire Hospital, Basingstoke, UK
Denise Griffin, Nycola Muchenje, Mcdonald Mupudzi, Richard Partridge, Jo-Anna Conyngham, Rachel Thomas, Mary Wright, Maria Alvarez Corral.

Arrowe Park Hospital, Wirral, UK
René Jacob, Cathy Jones, Craig Denmade.

Chesterfield Royal Hospital Foundation Trust, Chesterfield, UK
Sarah Beavis, Katie Dale, Rachel Gascoyne, Joanne Hawes, Kelly Pritchard, Lesley Stevenson, Amanda Whiteman.

Musgrove Park Hospital, Taunton, UK
Patricia Doble, Joanne Hutter, Corinne Pawley, Charmaine Shovelton, Marius Vaida.

Peterborough City Hospital, Peterborough, UK and Hinchingbrooke Hospital, Huntingdon, UK
Deborah Butcher, Susie O’Sullivan, Nicola Butterworth-Cowin.

Royal Hallamshire Hospital and Northern General Hospital, Sheffield, UK
Norfanizan Ahmad, Joanne Barker, Kris Bauchmüller, Sarah Bird, Kay Cawthon, Kate Harrington, Yvonne Jackson.
Faith Kibutu117, Becky Lenagh117, Shamiso Masuko117, Gary H Mills117, Ajay Raithatha117, Matthew Wiles117, Jayne Willson117, Helen Newell117, Alison Lye117, Lorenza Nwafor117, Claire Jarman117, Sarah Rowland-Jones117, David Foote117, Jody Cole117, Roger Thompson117, James Watson117, Lisa Hessedon117, Irene Macharia117, Luke Chetan117, Jacqui Smith117, Amber Ford117, Samantha Anderson117, Kathryn Birchall117, Kay Housley117, Sara Walker117, Leanne Milner117, Helena Hanratty117, Helen Trower117, Patrick Phillips117, Simon Oxspring117, Ben Donne117.

Dumfries and Galloway Royal Infirmary, Dumfries, UK
Catherine Jardine118, Dewi Williams118, Alasdair Hay118.

Royal Bolton Hospital, Bolton, UK
Rebecca Flanagan119, Gareth Hughes119, scott Latham119, Emma McKenna119, Jennifer Anderson119, Robert Hull119, Kat Rhead119.

Lister Hospital, Stevenage, UK
Carina Cruz120, Natalie Pattison120.

Craigavon Area Hospital, County Armagh, NI
Rob Charnock121, Denise McFarland121, Denise Cosgrove121.

Southport and Formby District General Hospital, Ormskirk, UK
Ashar Ahmed122, Anna Morris122, Srinivas Jakkula122.

Calderdale Royal Hospital, Halifax, UK and Huddersfield Royal Infirmary, Huddersfield, UK
Asifa Ali123.

Calderdale Royal Hospital, Halifax, UK
Megan Brady123, Sam Dalo123, Annalisia Dance123, Lisa Gleadhill123, Jill Greig123, Kathryn Hanson123, Kelly Holdroyd123, Marie Home123, Diane Kelly123, Ross Kitson123, Lear Matapure123, Deborah Melia123, Samantha Mellor123, Tonicha Norcliffe123, Jez Pinnell123, Matthew Robinson123, Lisa Shaw123, Ryan Shaw123, Lesley Thomis123, Alison Wilson123, Tracy Wood123, Lee-Ann Bayo123, Ekta Merwaha123, Tahira Ishaq123, Sarah Hanley123.

Prince Charles Hospital, Merthyr Tydfil, UK
Bethan Deacon124, Megan Hibbert124, Carla Pothecary124, Dariusz Tetla124, Christopher Woodford124, Latha Durga124, Gareth Kennard-Holden124.

Royal Bournemouth Hospital, Bournemouth, UK
Debbie Branney125, Jordan Frankham125, Sally Pitts125, Nigel White125.

Royal Preston Hospital, Preston, UK
Shondipon Laha126, Mark Verlander126, Alexandra Williams126.

Whittington Hospital, London, UK
Abdelhamik Altalib127, Ana Alvaro127, Kayleigh Gilbert127, Louise Ma127, Loreta Mostoles127, Chetan Parmar127, Kathryn Simpson127, Champa Jetha127, Lauren Booker127, Aneeka Pratley127.

Princess Royal Hospital, Telford and Royal Shrewsbury Hospital, Shrewsbury, UK
Colene Adams128, Anita Agasu128, Tracie Arden128, Amy Bowes128, Pauline Boyle128, Mandi Behan128, Heather Button128, Nigel Capps128, Mandy Carnahan128, Anne Carter128, Daniella Child128, Denise Donaldson128, Kelly Hard128, Fran Hurford128, Yasmin Hussain128, Ayesha Javaid128, James Jones128, Sanal Jose128, Michael Leigh128, Terry Martin128, Helen Milward128, Nichola Motherwell128, Rachel Rikunenko128, Jo Stickley128, Julie Summers128, Louise Ting128, Helen Tivenan128, Louise Tonks128, Rebecca Wilcox128.

Macclesfield District General Hospital, Macclesfield, UK
Maureen Holland129, Natalie Keenan129, Marc Lyons129, Helen Wassall129, Chris Marsh129, Mervin Mahenthran129, Emma Carter129, Thomas Kong129.

Royal Surrey County Hospital, Guildford, UK
Helen Blackman130, Ben Creagh-Brown130, Sinead Donlon130, Natalia Michalak-Glinska130, Sheila Mtuwa130, Veronika Pristopan130, Armorel Salberg130, Eleanor Smith130, Sarah Stone130, Charles Piercy130, Jerik Verula130, Dorota Burda130, Rugia Montase130, Lesley Harden130, Irving Mayango130, Cheryl Marriott130, Paul Bradley130, Celia Harris130.

Hereford County Hospital, Hereford, UK
Susan Anderson131, Eleanor Andrews131, Janine Birch131, Emma Collins131, Kate Hammerton131, Ryan O'Leary131.

University Hospital of North Tees, Stockton on Tees, UK
Michele Clark132, Sarah Purvis132.

Lincoln County Hospital, Lincoln, UK
Russell Barber133, Claire Hewitt133, Annette Hilldith133, Karen Jackson-Lawrence133, Sarah Shepardson133, Maryanne Wills133, Susan Butler133, Silvia Tavares133, Amy Cunningham133, Julia Hindale133, Sarwat Arif133.

Royal Cornwall Hospital, Truro, UK
Sarah Bean134, Karen Burt134, Michael Spivey134.

Royal United Hospital, Bath, UK
Carrie Demetriou135, Charlotte Eckbad135, Sarah Hierons135, Lucy Howie135, Sarah Mitchard135, Lidia Ramos135, Alfredo Serrano-Ruiz135, Katie White135, Fiona Kelly135.

Royal Brompton Hospital, London, UK
Daniele Cristiano136, Natalie Dormand136, Zoheeh Farzaad136, Mahitha Gunnadi136, Kamal Lijanage136, Brijesh Patel136, Sara Salmi136, Geraldine Slovak136, Vicky Thwaites136, Mathew Varghese136, Anelise C Zborowski136.

University Hospital of North Midlands, Stoke on Trent, UK
Louise Clifford137, Samuel J Turner137, Paul O'Brien137.

Basildon Hospital, Basildon, UK
Miranda Forsey138, Agilan Kaliappan138, Anne Nicholson138, Joanne Riches138, Mark Vertue138, Miranda Forsey138, Agilan Kaliappan138, Anne Nicholson138, Joanne Riches138, Mark Vertue138.

Gloucestershire Royal Hospital, Gloucester, UK
Elizabeth Allan139, Kate Darlington139, Flyon Davies139, Jack Easton139, Sumit Kumar139, Richard Lean139, Daniel Menzies139, Richard Pugh139, Xinyi Qiu139, Llinos Davies139, Hannah Williams139, Jeremy Scanlon139, Gwyneth Davies139, Callum Mackay139, Joanne Lewis139, Stephanie Rees139.

West Middlesex Hospital, Isleworth, UK
Metod Oblak140, Monica Popescu140, Mini Thankachen140.

Royal Lancaster Infirmary, Lancaster, UK
Andrew Higham141, Kerry Simpson141, Jayne Craig141.
Western General Hospital, Edinburgh, UK
Rosie Baruah142, Sheila Morris142, Susie Ferguson142, Amy Shepherd142.

Chelsea & Westminster NHS Foundation Trust, London, UK
Luke Stephen Prockter Moore143, Marcela Paola Vizzaychipi143, Laura Gomes de Almeida Martins143, Jaime Carungcong143.

The Queen Elizabeth Hospital, King’s Lynn, UK
Inthakab Ali Mohamed Ali144, Karen Beaumont144, Mark Blunt144, Zoe Coton144, Hollie Curgenven144, Mohamed Elsaadany144, Kay Fernandes144, Sameena Mohamed Ally144, Harini Rangarajan144, Varun Sarathy144, Sivarupan Selvanayagam144, Dave Vedage144, Matthew White144.

King’s Mill Hospital, Nottingham, UK
Mandy Gill145, Paul Paul145, Valli Ratnam145, Sarah Shelton145, Inez Wynter145.

Watford General Hospital, Watford, UK
Siobhain Carmody146, Valerie Joan Page146.

University Hospital Wishaw, Wishaw, UK
Claire Marie Beith147, Karen Black147, Suzanne Clements147, Alain Morrison147, Dominic Strachan147, Margaret Taylor147, Michelle Clarkson147, Stuart D’Sylva147, Kathryn Norman147.

Forth Valley Royal Hospital, Falkirk, UK
Fiona Auld148, Joanne Donnachie148, Ian Edmond148, Lynn Prestice148, Nikole Runciman148, Dario Salutous148, Lesley Symon148, Anne Todd148, Patricia Turner148, Abigail Short148, Laura Sweeney148, Euan Murdoch148, Dhaneesha Senaratne148.

George Eliot Hospital NHS Trust, Nuneaton, UK
Michaela Hill149, Thogulava Kannan149, Wild Laura149.

Barnsley Hospital, Barnsley, UK
Rikki Crawley150, Abigail Crew150, Mishell Cunningham150, Allison Daniels150, Laura Harrison150, Susan Hope150, Ken Inweregbu150, Sian Jones150, Nicola Lancaster150, Jamie Matthews150, Alice Nicholson150, Gemma Wray150.

The Great Western Hospital, Swindon, UK
Helen Langton151, Rachel Prout151, Malcolm Watters151, Catherine Novis151.

 harefield Hospital, London, UK
Anthony Barron152, Ciara Collins152, Sundeep Kaul152, Heather Passmore152, Claire Prendergast152, Anna Reed152, Paula Rogers152, Rajvinder Shokkar152, Meriel Woodruiff152, Hayley Middleton152, Oliver Polgar152, Claire Nolan152, Vicky Thwaites152, Kanta Mahay152.

Rotherham General Hospital, Rotherham, UK
Dawn Collier153, Anil Hormis153, Victoria Maynard153, Cheryl Graham153, Rachel Walker153, Victoria Maynard153.

Ysbyty Gwynedd, Bangor, UK
Ellen Knights154, Alicia Price154, Alice Thomas154, Chris Thorpe154.

Diana Princess of Wales Hospital, Grimsby, UK
Teresa Behan155, Caroline Burnett155, Jonathan Hatton155, Elaine Heeney155, Atidebe Mitra155, Maria Newton155, Rachel Pollard155, Rachael Stead155.

Russell’s Hall Hospital, Dudley, UK
Vishal Amin156, Elena Anastasescu156, Vikram Anumakonda156, Komala Karthik156, Rizwana Kausar156, Karen Reid156, Jacqueline Smith156, Janet Imeson-Wood156.

Princess Royal Hospital
Denise Skinner157, Jane Gaylard157, Dee Mullan157, Julie Newman157.

Princess Royal Hospital, Haywards Heath, UK
Denise Skinner157, Jane Gaylard157, Dee Mullan157, Julie Newman157.

St Mary’s Hospital, Newport, UK
Alison Brown158, Gabor Debreceni158, Joy Watkins158, Liz Nicoll158.

University Hospital Lewisham, London, UK
Waqas Khalid159, Rosie Rees-Anderson159, Mark Birt159.

Colchester General Hospital, Colchester, UK
Alison Ghosh160, Emma Williams160.

Queen Elizabeth the Queen Mother Hospital, Margate, UK
Louise Allen161, Eva Beranova161, Nikki Crisp161, Joanne Deeny161, Tracey Hazelton161, Alicia Knight161, Carly Price161, Sorrell Tilley161, Salah Turki161, Sharon Turner161.

Royal Albert Edward Infirmary, Wigan, UK
Joshua Cooper162, Cheryl Finch162, Sarah Liderth162, Alison Quinn162, Natalia Waddington162.

Victoria Hospital, Kirkcaldy, UK
Tina Coventry163, Susan Fowler163, Michael MacMahon163, Amanda McGregor163.

Eastbourne District General Hospital, East Sussex, UK and Conquest Hospital, East Sussex, UK
Anne Cowley164, Judith Highgate164, Anne Cowley164, Judith Highgate164.

Cumberland Infirmary, Carlisle, UK
Alison Brown165, Jane Gregory165, Susan O’Connell165, Tim Smith165, Luigi Barberis165.

New Cross Hospital, Wolverhampton, UK
Shameer Gopal166, Nichola Harris166, Victoria Lake166, Stella Metherell166, Elizabeth Radford166.

The Princess Alexandra Hospital, Harlow, UK
Amelia Daniel167, Joanne Finn167, Rajnish Saha167, Nikki White167, Amy Easthope167.

Salisbury District Hospital, Salisbury, UK
Phil Donnison168, Fiona Trim168, Beena Eapen168.

Dorset County Hospital, Dorchester, UK
Jenny Birch169, Laura Bough169, Josie Goodsell169, Rebecca Tutton169, Patricia Williams169, Sarah Williams169, Barbara Winter-Goowin169.
north Middlesex University Hospital NHS trust, London, UK
33The Royal Liverpool University Hospital, Liverpool, UK
34King’s College Hospital, London, UK
35Charing Cross Hospital, St Mary’s Hospital and Hammersmith Hospital, London, UK
36Nottingham University Hospital, Nottingham, UK
37John Radcliffe Hospital, Oxford, UK
38Kingston Hospital, Surrey, UK
39Kingston Hospital, Surrey, UK
40Royal Infirmary of Edinburgh, Edinburgh, UK
41Queen Alexandra Hospital, Portsmouth, UK
42Morriston Hospital, Swansea, UK
43Addenbrooke’s Hospital, Cambridge, UK
44BHRUT (Barking Havering) - Queens Hospital and King George Hospital, Essex, UK
45Royal Sussex County Hospital, Brighton, UK
46Queen Elizabeth Hospital, Birmingham, UK
47St George’s Hospital, London, UK
48Stepping Hill Hospital, Stockport, UK
49Countess of Chester Hospital, Chester, UK
50Royal Blackburn Teaching Hospital, Blackburn, UK
51The Tunbridge Wells Hospital and Maidstone Hospital, Kent, UK
52Royal Gwent Hospital, Newport, UK
53Pinderfields General Hospital, Wakefield, UK
54Royal Berkshire NHS Foundation Trust, Berkshire, UK
55Broomfield Hospital, Chelmsford, UK
56Northumbria Healthcare NHS Foundation Trust, North Shields, UK
57Whiston Hospital, Prescot, UK
58Croydon University Hospital, Croydon, UK
59York Hospital, York, UK
60Heartlands Hospital, Birmingham, UK
61Ashford and St Peter’s Hospital, Surrey, UK
62Barnet Hospital, London, UK
63East Surrey Hospital, Redhill, UK
64Ninehills Hospital, Dundee, UK
65Worthing Hospital, Worthing, UK and St Richard’s Hospital, Chichester, UK
66Southampton General Hospital, Southampton, UK
67The Alexandra Hospital, Redditch and Worcester Royal Hospital, Worcester, UK
68Sandwell General Hospital and City Hospital, Birmingham, UK
69Blackpool Victoria Hospital, Blackpool, UK
70Royal Glamorgan Hospital, Pontyclun, UK
71The Royal Oldham Hospital, Manchester, UK
72Glasgow Royal Infirmary, Glasgow, UK
73St James’s University Hospital and Leeds General Infirmary, Leeds, UK
74University Hospital North Durham, Darlington, UK and Darlington Memorial Hospital, Darlington, UK
75Fairfield General Hospital, Bury, UK
76Wythenshawe Hospital, Manchester, UK
77Royal Alexandra Hospital, Paisley, UK
78Good Hope Hospital, Birmingham, UK
79Tameside General Hospital, Ashton Under Lyne, UK
80Royal Derby Hospital, Derby, UK
81Medway Maritime Hospital, Gillingham, UK
82Royal Victoria Infirmary, Newcastle Upon Tyne, UK
83Poole Hospital, Poole, UK
84Bedford Hospital, Bedford, UK
85Queens Hospital Burton, Burton-On-Trent, UK
86North Manchester General Hospital, Manchester, UK
87Aberdeen Royal Infirmary, Aberdeen, UK
88Derriford Hospital, Plymouth, UK
89Manchester Royal Infirmary, Manchester, UK
90Salford Royal Hospital, Manchester, UK
91William Harvey Hospital, Ashford, UK
92Queen Elizabeth University Hospital, Glasgow, UK
93Bradford Royal Infirmary, Bradford, UK
94Bristol Royal Infirmary, Bristol, UK
95Norfolk and Norwich University hospital (NNUH), Norwich, UK
96Queen Elizabeth Hospital Gateshead, Gateshead, UK
97Sunderland Royal Hospital, Sunderland, UK
98Aintree University Hospital, Liverpool, UK
99Hull Royal Infirmary, Hull, UK
100Hull Royal Infirmary, Hull, UK
101University College Hospital, London, UK
102Royal Devon and Exeter Hospital, Exeter, UK
103The Royal Papworth Hospital, Cambridge, UK
104 Ipswich Hospital, Ipswich, UK
105Southmead Hospital, Bristol, UK
106 Milton Keynes University Hospital, Milton Keynes, UK
107Royal Hampshire County Hospital, Hampshire, UK
108Queen Elizabeth Hospital, Woolwich, London, UK
109Great Ormond St Hospital and UCL Great Ormond St Institute of Child Health NIHR Biomedical Research Centre, London, UK
110Stoke Mandeville Hospital, Buckinghamshire, UK
111University Hospital of Wales, Cardiff, UK
112Basingstoke and North Hampshire Hospital, Basingstoke, UK
113Arrowe Park Hospital, Wirral, UK
114Chesterfield Royal Hospital Foundation Trust, Chesterfield, UK
115Musgrove Park Hospital, Taunton, UK
116Peterborough City Hospital, Peterborough, UK and Hinchingbrooke Hospital, Huntingdon, UK
117Royal Hallamshire Hospital and Northern General Hospital, Sheffield, UK
118Dumfries and Galloway Royal Infirmary, Dumfries, UK
119Royal Bolton Hospital, Bolton, UK
120Lister Hospital, Stevenage, UK
121Craigavon Area Hospital, County Armagh, NI
122Southport and Formby District General Hospital, Ormskirk, UK
123Caldeready Royal Hospital, Halifax, UK and Huddersfield Royal Infirmary, Huddersfield, UK
124Prince Charles Hospital, Merthyr Tydfil, UK
125Royal Bournemouth Hospital, Bournemouth, UK
126Royal Preston Hospital, Preston, UK
127Whittington Hospital, London, UK
128Princess Royal Hospital, Telford and Royal Shrewsbury Hospital, Shrewsbury, UK
129Macclesfield District General Hospital, Macclesfield, UK
130Royal Surrey County Hospital, Guildford, UK
131Hereford County Hospital, Hereford, UK
132University Hospital of North Tees, Stockton on Tees, UK
133Lincoln County Hospital, Lincoln, UK
134Royal Cornwall Hospital, Truro, UK
135Royal United Hospital, Bath, UK
136Royal Brompton Hospital, London, UK
137University Hospital Crosshouse, Kilmarnock, UK
138Basildon Hospital, Basildon, UK
139Glann Clwyd Hospital, Bodelwyddan, UK
140University Hospital of North Tees, Stockton on Tees, UK
141Lincoln County Hospital, Lincoln, UK
142Bristol Royal Infirmary, Bristol, UK
143Chelsea & Westminster NHS Foundation Trust, London, UK
144The Queen Elizabeth Hospital, King’s Lynn, UK
145King’s Mill Hospital, Nottingham, UK
146Watford General Hospital, Watford, UK
147University Hospital Wishaw, Wishaw, UK
148Forth Valley Royal Hospital, Falkirk, UK
149George Eliot Hospital NHS Trust, Nuneaton, UK
150Barnsley Hospital, Barnsley, UK
151The Great Western Hospital, Swindon, UK
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