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Letter to the Editor

Single nucleotide polymorphisms in key aging pathways, and phenotypic markers of frailty are associated with increased odds of hospital admission with COVID-19

Dear Editor,

The recent paper by Kundi et al. provided evidence of the increased risk of developing severe Covid-19 disease in patients that are older and frail [1]. However, the underlying mechanisms that drive the increased risk of death and hospital admission in this demographic are less obvious. We adopted a candidate gene approach to establish whether single nucleotide polymorphisms (SNPs) in several key pathways involved in the ageing process are associated with increased risk of hospital admission following Sars-Cov-2 infection. Our findings indicate that SNPs in RICTOR and the INK4/ARF loci, along with known markers of frailty, are associated with increased risk of hospital admission due to Covid-19. We also found that the role that the INK4/ARF polymorphism plays is Covid-19 hospital admission may be dependent upon ethnicity.

We investigated participants enrolled in the UKBiobank, a large multicentre prospective study, involving over 500,000 patients recruited between 2006 and 2010 [2]. Data on hospital admission episodes, including principle diagnosis, were provided by the Data Access Request Service in England, the Secure Anonymised Information Linkage databank in Wales, and the electronic Data Research and Innovation Service in Scotland. Genetic data on the 5 candidate SNPs: rs34197572 (KEAP1), rs2943641 (IRS1), rs4402960 (IGF2BP2), rs2043112 (RICTOR), and rs10757278 (INK4A/ARF) were called using the UK BiLEVE Axiom array and the UK Biobank Axiom array. These SNPs code for proteins involved in several key ageing pathways. The numbers of non-cancer illnesses and medications was obtained through nurse-led interviews.

Generalised linear models (GLM) with fixed effects were implemented using R statistical software to model hospital admissions. A binomial error distribution was assumed and logistic regression used to select the best-fitting model. The significance of each of the fixed effects was assessed by comparing the deviance values of the model before and after removal in order to arrive at the minimal adequate model [3]. Both genetic and non-genetic terms were fitted as main effects. Of the former, we included Age (years), number of medications, grip strength (kg) and number of co-morbidities. Five SNPs were included as genetic terms and coded as genotype (e.g., AA, GA or GG). The significance level of all tests was set at 0.05. The minimal adequate model, with all terms being significant, was identified and odds ratios estimated for each term (1).

We obtained the following results from our study. The UK-Biobank dataset consisted of a total of 502,505 participants. Patients who had died from a non-Covid related diagnosis were removed, leaving a total of 471,278 participants eligible for analysis. The mean age of the sample was 68.1 years old (SD=8.10, range 49–86 years). As of August 2020, a total of 705 participants (0.15%) were admitted to a UK hospital with a primary diagnosis of Covid-19. The mean age of those admitted to hospital was 71.0 years old (SD=8.10, range 51–84 years). All phenotypic markers plus two of the five SNPs (rs10757278 and rs2043112) contributed significantly to the logistic regression model. Table 1 provides a summary of the effect size of each variable, expressed as an odds ratio.

Table 1
Effect size of variables associated with hospital admission due to Covid-19. Note that OR is expressed per unit increase in years (age), number of reported non-cancer illnesses (co-morbidities), number of medicines (medicines), and kg force (grip strength), and to the AA geneotype (rs2043112 and rs10757278).

Variable	OR	2.5% CI	97.5%CI	Pr > |z|
rs2043112 GG	1.267	1.006	1.611	0.04863
rs10757278 GA	0.805	0.672	0.966	0.01875
Medications	1.116	1.084	1.148	<0.0001
Co-morbidities	1.073	1.028	1.119	0.00114
Age	1.037	1.026	1.049	<0.0001
Grip strength	1.014	1.008	1.021	<0.0001

An unexpected finding was that the effect of the rs10757278 SNP appears to be dependent upon ethnicity. The population as a whole was not in Hardy-Weinberg equilibrium on account of population stratification (p=0.006), where the Afro-Caribbean population showed markedly different allele frequencies from the Caucasian population (F_{ST}=0.0385, $p < 0.01$). In addition, a heterozygote deficit was noted in the Afro-Caribbean group (F_{IS}=0.208, $p < 0.01$). This deficit was more marked amongst those admitted (F_{IS}=0.415, $p < 0.01$). Although the Asian group was in Hardy-Weinberg equilibrium (p=0.411), like the Afro-Caribbean group, it also showed a significant difference between the proportion of heterozygotes that were admitted compared to those that were not: Non-Caucasian individuals who are heterozygote at this particular gene have a substantially lower risk of hospital admission with Covid-19 compared to both homozygote genoptypes (OR 0.56 [0.37–0.85], p=0.006; AA/GG vs. GA). However, in Caucasians, neither rs10757278 genoptype conferred an increased/decreased risk of hospital admission with Covid-19 (Table 2).

As in the study by Kundi et al. we found that patients displaying markers of frailty are at greater risk of severe Covid-19 disease. Interestingly, we found that markers, such as polypharmacy, carry more risk of Covid-19 hospitalisation than age. However, our analysis also identified two SNPs that were significantly associated with Covid-19 hospital admission. The rs2043112 polymorphism is found in the gene that codes for the RICTOR protein, which is an integral part of the mammalian Target of Rapamycin Complex 2. This complex can regulate a number of proteins linked to lifespan/healthspan [4]. Indeed, increased activity of mTORC2 is found in aged mice CD4 T-cells, which are associated with reduced func-

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Table 2
Risk of hospital admission based on genotype of the rs10757278 polymorphism, according to ethnicity.

Ethnicity and genotype	No admission (%)	Admission (%)	OR (Pr(>Chi))
Black Afro-Caribbean			
AA/GG	6740 (64)	56 (74)	
GA	3751 (36)	20 (26)	
Asian			
AA/GG	4321 (49)	16 (62)	
GA	4450 (51)	10 (38)	
Mixed Black			
AA/GG	1581 (54)	3 (60)	
GA	1353 (46)	2 (40)	
Non-Caucasian (Total)			
AA/GG	12,642 (57)	75 (70)	
GA	9554 (43)	32 (30)	0.56 (p=0.006)
Caucasian			
AA/GG	213,435 (50)	276 (50)	
GA	212,679 (50)	273 (50)	1.00 (p>0.999)

tional activity [5]. The GG homozygotes were significantly in excess within the Covid-19 hospital admission group relative to the non-Covid-19 hospital admission group (41.2% vs. 35.9%, p=0.0181), with the heterozygotes in deficit.

The second SNP that showed an association with hospital admission is located in the INK4/ARF locus. This region produces several tumor suppressor genes, such as $P16^{INK4a}$, whose cellular expression increases with age [6]. The G allele for the rs10757278 SNP leads to lower expression of $P16^{INK4a}$ in peripheral blood T-cells, and is associated with increased risk of atherosclerosis and cardiovascular disease [7], implying lower $P16^{INK4a}$ expression, which is seen in younger more resilient individuals, may be protective. Findings from a recent case report, in which a patient treated with an CDK4/6 inhibitor had an unusual Covid-19 disease course may support this hypothesis [8]% vs. 44.7%, p=0.0442), with the AA homozygotes in deficit. In Afro-Caribbean and Asian individuals, the protection from hospital admission seen in the heterozygote group was unexpected, but marked. Why heterozygotes appear at reduced risk is unclear, but may be related to the role that $P16^{INK4a}$ plays in ageing and cancer: decreased expression is a risk for cancer, and too much is a trigger for cell senescence. Heterozygotes may have the right balance to mount an appropriate response to infection with Sars-Cov-2.

In conclusion, our observation that the G allele in the rs10757278 locus offers some degree of protection when heterozygous, together with data showing that this SNP has previously been associated with lower $P16^{INK4a}$ expression, suggests that it might be pertinent for future research to investigate whether higher expression of this marker is associated with poor Covid-19 outcomes.

Declaration of Competing Interest

Both authors declare no conflicts of interest.

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