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Letter to the Editor

Naringerin as candidate drug against SARS-CoV-2: The role for TPC2 genomic variants in COVID-19

Dear Editor,

We read with interest the recent article in Pharmacological Research reporting that the flavanone Naringenin, which targets the endo-lysosomal Two-Pore Channels (TPCs), exhibits antiviral activity against human Coronaviruses (CoVs) [1]. Naringenin inhibits TPC2 activity by impairing intracellular calcium signalling [2]. After the recent review which listed candidate drugs against SARS-CoV-2 [3], the work by Clementi and co-workers further supports that Naringenin could be added to the list of potential therapeutics against SARS-CoV-2 infection and COVID-19 disease. This observation raises new interest in the role for TPC2 in human diseases.

The human gene encoding TPC2, *TPCN2*, has been studied in human phenotypes. *TPCN2* single nucleotide variants (SNVs) had been found to be associated with a shift from brown to blond hair colour; the functional role of TPC2 variants was further demonstrated by the mean of endolysosomal patchclamp techniques. The rs1551305 variant in the *TPCN2* gene was found to be associated with increased risk for type 2 diabetes in a case-control study; a recent Genome-Wide Association Study (GWAS) meta-analysis confirmed the association in a very large cohort of individuals from different ancestries.

So far, there was no rationale to consider *TPCN2* among candidate genes associated to the phenotype variability of COVID-19. A recent systematic review did not detect any study investigating *TPCN2* as candidate gene associated with CoVs infection and outcomes [4]. A large genome-wide association study (GWAS) investigated the genomic variants associated with COVID-19. Two loci provided significant signal of association applying the GWAS constraints. At locus 3p21.31, the association signal spanned the genes *SLC6A20*, *LZTFL1*, *CCR9*, *FYCO1*, *CXCR6* and *XCR1*; the association signal at locus 9q34.2 coincided with the ABO blood group locus [5].

Thanks to the public availability of the complete set of results from this GWAS, it has been possible to inspect the region encompassing *TPCN2*. Six SNVs that were analysed in the GWAS span the *TPCN2* gene. For none of them the p value reached the stringent threshold for the GWAS. However, three SNVs (rs72917317, rs150527451, rs72928978) showed a signal of association with OR > 1.3 and $p < 0.01$.

We targeted the three SNVs on the linkage disequilibrium (LD) map of the *TPCN2* locus by examining the 1000 Genomes project data set (CEU Population, phase 3, inspected on Nov 4, 2020 through the Ensembl web tool at www.ensembl.org). All three SNVs are in LD with each other and with the tagging SNV flanking the *TPCN2* locus (rs72930659), which also showed a nominally significant association signal (OR = 1.34, p = 0.002). If the most conservative Bonferroni correction for multiple testing is applied, the p value remains below the alpha threshold 0.05 for to the SNVs spanning the gene and the tagging SNV downward, i.e. rs72917317, rs150527451, rs72928978 and rs72930659.

The rs72928978 single nucleotide substitution is a missense coding variant, which was predicted to exert a moderate impact according to the Variant Effect Predictor (VEP) tool (as implemented in the Ensembl web site: www.ensembl.org). The *TPCN2* variants named rs72917317 rs150527451, and the downstream tagging variant rs72930659, are labelled as modifier (i.e., the impact of the predicted consequence type) and predicted to act on non-sense mediated decay, as well as six additional intronic variants within the *TPCN2* locus (rs3019757, rs145602179, rs57833364, rs57307280, rs10896434, rs147953094).

Moreover, we examined the single-cell RNA sequencing data set obtained from patients with COVID-19 (Single-cell atlas of the peripheral immune response to severe COVID-19, available from the Single Cell Expression Atlas repository – www.ebi.ac.uk/gxa/sc/home) and found that *TPCN2* is widely expressed in peripheral blood mononuclear cells.

It is noteworthy that *TPCN2* variants were found to be associated with increased risk of type 2 diabetes. Age, cardiovascular disease and metabolic disorders have been recognised as the predominant determinants of COVID-19 severe outcome; diabetes, in particular, is a major comorbidity factor. It might be speculated that the differential distribution of *TPCN2* genotypes in COVID-19 patients is determined by an overrepresentation of individuals with diabetes in the GWAS cohort. This connection could be further explored by stratifying the data sets according to the presence of endophenotypes such as type 2 diabetes.

Though the GWAS results on COVID-19 are not a proof *per se*, the biological role of TPC2 demonstrated by Clementi et al. [1], coupled with its expression in patients' blood cells and the putative functional role of *TPCN2* genomic variants, would increase the prior probability that TPC2 plays a role in SARS-CoV-2 infection. Based on this ground, the hypothesis as to whether *TPCN2* genomic variants influence the COVID-19 phenotypes should be further investigated.

Though the biological effects of Naringenin were established in multiple *in vitro* investigations, few studies are available on its use as a therapeutic molecule. Notably, Naringenin has been proposed as a novel therapeutic agent for hepatitis C virus (HCV) infection treatment, since it has been described to reduce HCV secretion in infected primary human hepatocytes and in mice [6].

In the pharmacogenetics perspective, the effect of *TPCN2* genomic variants might be of primary interest if Naringenin is tested as experimental drug against SARS-CoV-2 in humans. *TPCN2* gene variants may be associated with variable response to the drug and should be considered as relevant variable in clinical studies.

With reference to the aforementioned manuscript, I hereby declare that the work has not been published previously and is not under consideration for publication elsewhere, and that its publication is approved by all authors.

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Declaration of Competing Interest

The authors report no declarations of interest.

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