



## TLR3 (rs3775291) variant is not associated with SARS-CoV-2 infection and related mortality: a population-based correlation analysis

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To the Editor,

With great interest, we read the recent article by Dhangadamajhi et al. [1], which identified the possible association of TLR3 exonic variant (rs3775291) with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and mortality rates in global populations. The authors included TLR3 rs3775291 polymorphism data of 48,835 healthy individuals from 40 countries and detected an important correlation between the rs3775291 minor allele and susceptibility to SARS-CoV-2 infection and mortality. An earlier study [2] used minor allele frequency data from 14 different countries to show a similar correlation between the rs3775291 variant and COVID-19 susceptibility and mortality. Although the data investigation and reporting were done elegantly and scholarly, we found a few minor issues that need to be discussed further.

1. To draw a firm conclusion in a population-scale analysis, all existing studies must be included. Genotype data from 48,835 healthy controls from 40 countries were used by the authors [1]. The minor allele frequency was obtained from 1000 genome projects and gnomAD, and other databases, such as PubMed and google scholar. After searching various databases (1000 genomes, gnomAD, dbSNP, PubMed and google scholar) for minor allele frequency, we found more reports from various populations [Tunisia ( $n=5$ ), Brazil ( $n=2$ ), China ( $n=13$ ), South Korea ( $n=4$ ), Japan ( $n=4$ ), Scotland ( $n=2$ ), Spain ( $n=3$ ), Denmark ( $n=3$ ), Germany ( $n=4$ ), Poland ( $n=2$ ), Italy ( $n=2$ ), Finland ( $n=3$ ), and India ( $n=5$ )]. When compared to the included reports of Dhangadamajhi et al. [1], we were unable to trace

allele frequency data from the Bulgarian population, and a smaller number of studies from the USA ( $n=4$ ) and Sweden cohorts ( $n=2$ ) were identified.

2. For population-scale correlation analysis, excluding reports that do not obey Hardy–Weinberg equilibrium (HWE) is critical. In line with this, Dhangadamajhi et al., proposed that such reports be removed from the correlation analysis. However, they erroneously included genotype data from Barbados ( $\chi^2=4.536$ ,  $p=0.033$ ) and Bangladesh ( $\chi^2=3.775$ ,  $p=0.052$ ) populations, which were deviated or very close to the HWE deviation score.
3. Despite the authors' claim that genotype data from 40 countries were used in the study, the number of countries considered for the population-scale analysis was actually 39. For the correlation study, the authors used minor allele frequency data from Finland twice. The minor allele data of Finland must be pooled before the correlation analysis.
4. The Pearson correlation test was used to assess the relationship between the prevalence of minor allele 'T' and the SARS-CoV-2 infection and mortality rate per million subjects in different populations. The Spearman rank correlation coefficient would be the most suitable [3] to test the relationship between TLR3 variant and COVID-19 since the two variables were on different scales and the analysis was not conducted in SARS-CoV-2 infected cases. Using data from Dhangadamajhi et al. [1], a Spearman rank correlation study showed no significant association between SARS-CoV-2 and the TLR-3 rs3775291 polymorphism (infection:  $r=0.244$ ,  $p=0.128$ ; mortality:  $r=0.247$ ,  $p=0.124$ ).
5. For obtaining minor allele frequency in different populations, the authors used two different search strategies: (1) genomic databases, such as 1000 Genomes Project and gnomAD, and (2) literature databases, such as PubMed and Google Scholar. Although allele frequency and the total number of healthy subjects considered for MAF calculation have been mentioned in the manuscript's

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**Table 1** TLR3 rs3775291 minor allele frequency and SARS-CoV-2 related data of different countries

Population	Dhangadamajhi et al. [1]		Present study		Data assessed on 18th January 2021 (Dhangadamajhi et al. 2021 [1])		Data assessed on 1st April 2021		Data sources/remarks		
	Number of studies	MAF	Total cases	Number of studies	MAF	Total number of Healthy controls	SARS CoV-2 infection / million	SARS-CoV-2 death/million		SARS CoV-2 infection / million	SARS-CoV-2 death/million
Barbados	1	4.2	96	1	4.2	96	3603	24	12,697	146	1000 Genomes, this study was excluded for deviation of genotypes distribution from HWE
Nigeria	2	0.7	207	1	0.926	108	514	7	776	10	1000 Genomes
Gambia	1	1.8	113	1	1.8	113	1589	52	2213	67	1000 Genomes
Luhya, Kenya	1	3.5	99	1	3.5	99	1821	32	2453	39	1000 Genomes
Sierra Leone	1	1.2	85	1	1.2	85	367	10	492	10	1000 Genomes
Tunisia	1	16.45	158	5	12.0	532	14,729	465	21,327	740	Moumad et al., 2013, Abida et al., 2020
Morocco	1	17.64	204	1	17.7	204	12,319	212	13,323	237	Moumad et al., 2013
USA	7	29.73	3677	4	29.4	3105	72,592	1210	93,747	1700	Dhiman et al., 2008, Edwards et al., 2008, Slattery et al., 2012, Resler et al., 2013
Colombia	2	27.88	459	2	27.9	459	36,544	935	46,920	1237	1000 Genomes, Allikmets et al., 2009
Peru	1	35.3	85	1	35.3	85	31,789	1164	46,491	1561	1000 Genomes
Brazil	1	33.1	299	2	35.4	760	39,340	976	59,682	1506	Assmann et al., 2014, Sa et al., 2015
Nicaragua	1	21.21	132	1	21.2	132	923	25	999	27	Lundkvist et al., 2020
China	8	31.86	3361	13	27.3	5089	61	3	63	3	1000 Genomes, Li et al., 2017, Pang et al., 2014, Chen et al., 2015, Cheng et al., 2014, Ye et al., 2020, Rong et al., 2013, Chen et al., 2017, Wang et al., 2015, Rong et al., 2013
South Korea	2	25.85	756	4	29.9	5114	1400	24	2020	34	Korean Genome Projec, KOREAN population from KRGDB, Cho et al., 2017, Hwang et al., 2009
Taiwan	3	35.31	2281	3	34.6	1713	35	0.3	43	0.4	Yang et al., 2013, Yang et al., 2014
Japan	3	27.27	264	4	32.6	422	2449	34	3741	72	1000 Genomes, Ueta et al., 2007, Ikezoe et al., 2015, Matsuo et al., 2016
Vietnam	1	38.9	99	1	38.9	99	16	0.4	27	0.4	1000 Genomes
Finland	1	33.3	99	DNA	DNA	DNA	7231	111	13,692	152	-
Scotland, UK	1	32.4	91	2	30.3	249	30,330	990	63,766	1859	1000 Genomes, Dwyer et al., 2013,
Spain	2	30.40	472	3	30.1	430	48,160	1140	70,226	1613	1000 Genomes, Sironi et al., 2012, Matas-Cobos et al., 2015
Denmark	2	28.35	1280	3	27.9	1696	32,430	301	39,708	417	Laska et al., 2014, Enevold et al., 2014, Laska et al., 2014

Table 1 (continued)

Population	Dhangadamajhi et al. [1]		Present study		Data assessed on 18th January 2021 (Dhangadamajhi et al. [1])		Data assessed on 1st April 2021		Data sources/remarks		
	Number of studies	MAF	Total cases	Number of studies	MAF	Total number of Healthy controls	SARS CoV-2 infection / million	SARS-CoV-2 death/million	SARS-CoV-2 infection / million	SARS-CoV-2 death/million	
Germany	2	28.96	1034	4	28.8	1291	24,132	555	33,701	917	Yang et al., 2012, Gast et al., 2011, Allikmets et al., 2009, Ye et al., 2020
Poland	1	25.64	78	2	27.0	150	37,796	878	61,396	1403	Studzińska et al., 2017, Grygorczuk et al., 2017
Ireland	1	26.61	263	1	26.2	263	33,527	511	47,371	941	Cooke et al., 2018
Lithuania	1	34.0	135				61,701	894	80,231	1327	Two studies were excluded for HWE deviation
Russia	1	32.6	269	1	34.6	269	24,283	446	31,135	677	Barkhash et al., 2013
Sweden	3	30.43	14,186	2	30.1	1109	51,659	1019	79,328	1327	Günaydin et al., 2014, Svensson et al., 2012
Iceland	1	27.5	169	1	27.5	169	17,393	85	18,096	85	Allikmets et al., 2009
Netherlands	2	29.53	1107	2	29.5	1107	52,560	750	74,148	964	Allikmets et al., 2009
Serbia	1	33.17	104	1	33.1	104	42,420	425	68,947	609	Stanimirovic et al., 2013
Italy	1	30.8	107	2	27.1	345	38,939	1346	59,357	1811	1000 Genomes, Sironi et al., 2012
Finland	1	32.68	12,549	3	32.4	16,110	7231	111	13,962	152	1000 Genomes
Estonia	1	32	2412	1	31.9	4480	27,649	241	80,187	680	Genetic variation in the Estonia population
Bulgaria	1	29.94	1335	DNA	DNA	DNA	30,565	1222	49,591	1910	-
Bangladesh	1	27.3	86	1	27.3	86	3183	48	3684	55	1000 Genomes This study was excluded for deviation of genotypes distribution from HWE
India	2	24.14	205	5	12.1	766	7600	110	8791	117	1000 Genomes, Alagarasu et al., 2014, Biyani et al., 2015, Meena et al., 2015
Pakistan	1	24	96	1	23.9	96	2315	49	3003	65	1000 Genomes
Sri Lanka	1	31.9	102	1	31.9	102	2420	12	4316	26	1000 Genomes
Iran	1	29.66	118	1	29.6	118	15,660	671	22,237	739	Habibadi et al., 2020
Australia	1	33.7	163	1	33.7	163	1118	35	1140	35	Allikmets et al., 2009

COVID-19 related data were obtained from article Dhangadamajhi et al and worldometer assessed on 1st April 2021. TLR3 rs3775291 polymorphism genotype or allele data were obtained from 1000 genomes, dbSNP, PubMed and Google Scholar  
DNA data not available

supplementary table, a piece of additional information on references and data sources would be more beneficial for the researchers.

6. On 18 January 2020, the authors collected SARS-CoV-2 data from various countries, including infections, mortality, and recovery rates. The first cases of SARS-CoV-2 infection were identified in Wuhan, China, in December 2019, and the World Health Organization declared COVID-19 a pandemic on 11 March 2020. The date listed in the paper and the supplementary Table 1 (18th January 2020) may be a typographical error.

The infection and mortality status of SARS-CoV-2 on 18 January 2021 were obtained from Dhangadamajhi et al. supplementary dataset. Data on minor allele frequency were gathered from a variety of databases, as shown in Table 1. Reports with HWE deviated genotype distributions were omitted from the present analysis [Barbados ( $n = 1$ ), Bangladesh ( $n = 1$ ), China ( $n = 2$ ), India ( $n = 1$ ), Lithuania ( $n = 2$ ), Nigeria ( $n = 1$ )] and a total of 47,136 healthy subjects from 35 different populations were taken into account. Using the modified minor allele frequency data, a reanalysis of the association between rs3775291 minor allele frequency and COVID-19 showed no significant correlation between rs3775291 minor allele 'T' and SARS-CoV-2 infection (Spearman  $r = 0.181$ ,  $p = 0.295$ ,  $n = 35$ ) or mortality (Spearman  $r = 0.146$ ,  $p = 0.402$ ). Up-to-date data of SARS-CoV-2

infection and mortality rate per million were obtained from the Worldometer website (assessed on 1st April 2021). The spearman rank correlation study of rs3775291 minor allele frequency (T) with SARS-CoV-2 infection rate (Spearman  $r = 0.212$ ,  $p = 0.221$ ,  $n = 35$ ) and mortality rate (Spearman  $r = 0.143$ ,  $p = 0.412$ ,  $n = 35$ ) also failed to show a potential association of rs3775291 polymorphism with COVID-19, bolstering the absence of an association between rs3775291 and related mortality. However, case-control studies in different populations are needed to confirm our findings.

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