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ORIGINAL ARTICLE

Genetic variations on influenza virus infection outcomes in Moroccan patients

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ABSTRACT

Background and Objectives: The influenza infection remains a significant global health challenge, it leads to illnesses that vary from mild to severe, and in certain cases, death. The innate immune response is the first line of defense against pathogen invaders; identification of variants associated with susceptibility or protection could further elucidate immune mechanisms and provide the basis for new therapeutic targets.

Materials and Methods: We investigated four widely studied SNPs on the immune response to RNA infection in samples collected as part of sentinel influenza surveillance system. Of 1925 nasopharyngeal swabs collected from patients with Severe Acute Respiratory Infection (SARI) and Influenza-Like Illness (ILI), 115 samples were positive for ILI and 83 were positive for SARI. A third group of healthy individuals was also enrolled as a control. Genetic polymorphisms of the *OAS3* (*rs10735079*), *TYK2* (*rs74956615*) and *APOBEC3G* (*rs8177832* and *rs2294367*) genes were genotyped using Human TaqMan SNP Genotyping Assays (ThermoFisher Scientific©). The association of Single Nucleotide Polymorphisms (SNPs) with ILI and SARI was investigated using SNPStats software.

Results: The *rs2294367* in *APOBECG3* show a strong and significant association with ILI and SARI across all genetic models, with a p-value<0.001 and OR between 2 and 6, while no association was found with *rs8177832*. The results for *TYK2* suggest a potential protective effect, while the *OAS3* SNP shows a strong and significant association with a decreased risk of Influenza infection specially with ILI group (OR<1 and p < 0.0001).

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Conclusion: Our results open up a new perspective for new methods and strategies of therapy aimed to enhance the body's natural defenses against influenza virus infection.

Keywords: Influenza; SARI; ILI; *APOBEC3G*; *OAS3*; *TYK2*; Single nucleotide polymorphisms

INTRODUCTION

The infection caused by the influenza virus remains a significant global health challenge, resulting in seasonal outbreaks and sporadic pandemics. It leads to illnesses that can vary from mild to severe, and in certain cases, death, prompting us to consider that the immune response of the host is vital in influencing both the vulnerability and the intensity of influenza-related illness.

The initial host response to viral infection relies largely upon the innate immune response, which is the first line of defense against pathogen invaders. The innate response employs a broad array of pattern recognition receptors to detect conserved molecular patterns on pathogens. When viral components are detected, a cascade of signaling events is initiated leading to type I and type III interferon (IFN) production (1).

The *TYK2* gene is a critical component of Type I and Type III Interferon (IFN) signaling pathways, which are responsible for host defense against viral infections. Type I IFNs (IFN-α/β) are elicited directly after viral infection and play a key role in causing an antiviral cell state (2).

It accomplishes this by inducing the transcription of thousands of interferon-stimulated genes (ISGs), whose proteins function to directly inhibit several sites of the viral life cycle, ultimately resulting in the inhibition of viral replication and spread (2).

Among these ISGs, the 2'-5'-oligoadenylate synthetase (OAS) family of enzymes stands out as a significant component of the cell's antiviral mechanism. Within this family, the *OAS3* gene generate a key enzyme that plays a role in both antiviral function and signal transduction.

As part of the host immune defense, the *APOBEC3G* genes also play a vital role in limiting viral replication and regulating the immune response.

This enzymatic function plays a vital role in its antiviral activity, especially against retroviruses such as HIV-1, as *APOBEC3G* can trigger hypermutations in the viral genome, resulting in its inactivation (3).

Furthermore evidence supports a potential candidate link between polymorphisms in APOBEC3G and susceptibility to Human Papillomavirus (HPV) infection, as well as the progression of cervical lesions (4) and it has been demonstrated that *APOBEC3G* can also bind with and inhibit negative-strand RNA viruses, such as respiratory syncytial virus (RSV), measles, and mumps (5).

Although *APOBEC3G* is recognized as a restriction factor for many viruses, its function regarding RNA viruses such as influenza remains ambiguous. Research has indicated that infection with influenza A virus can lead to an increase in *APOBEC3G* expression; however, this increase does not automatically result in effective antiviral responses against the virus (6).

In fact, genetic differences in all these genes, like single nucleotide polymorphisms (SNPs), may impact the function of the protein and, as a result, influence how an individual responds to viral infections (7).

Context of the study. All these finding results point to the need to consider the functional implications of the *APOBEC3G*, *OAS3* and *TYK2* SNPs in the scope of host-virus interactions and disease outcomes. It is possible that these genetic variances have direct or indirect effects on the progression of influenza infection by several possible mechanisms, which led us to study these SNPs on positive cases detected in sentinel influenza surveillance cases to see if there is a relationship with susceptibility and or severity with influenza infection on the Moroccan population.

Identification of variants associated with increased susceptibility or protection could further elucidate immune mechanisms and potentially provide the basis for new therapeutic targets.

MATERIALS AND METHODS

Study participants. In Morocco, influenza surveillance is carried out by a sentinel system whose objectives are to monitor the evolution of seasonal influenza, estimate its burden of disease, identify circulating viruses and monitor their sensitivity to antivirals.

The Virological surveillance system is based on a public network of 8 first health care for moderate cases ILI (Illness Like Influenza) and 8 hospital centers for the severe acute respiratory infection (SARI), plus a second private network of 30 volunteer doctors from the liberal sector (especially general practitioners, pediatricians and pneumo-physiologists) for ILI cases also.

A total of 1925 cases, either ILI or SARI, that met the WHO definition for suspected cases were collected in this study. The study time frame was in the season 2023-2024 from August 25, 2023, to April 17, 2024. Nasopharyngeal (NP) and oropharyngeal (OP) specimens were collected in tubes containing 3ml of VTM, and transported to the National Influenza Center within 48 hours and stabilized at 4°C temperature.

All samples were tested by multiplex RT-PCR for the screening of Influenza A, B, and SARS-CoV-2 viruses according to WHO recommendations, using kits supplied by the CDC Atlanta. Subsequently, 115 ILI samples and 83 SARI samples tested positive and were included in our study.

A third group of healthy people collected through the blood transfusion center in Rabat was enrolled in this study as a control case.

Genotyping. Patients with influenza infection were included for the genetic analysis. The cohort included 83 SARI,115 ILI and 60 Healthy group. Human DNA for ILI and SARI was extracted from 400 µL of nasopharyngeal swabs using the EZ 1 Qiagen automated system, and 60 µL of the eluate was collected.

For the healthy group, extraction was made from 300 µl of EDTA anticoagulant blood, DNA using MagPurix®Blood DNA Extraction Kit with the Zinexts MagPurix EVO 24 CE IVD system (Zinexts Life Science Corp., New Taipei City, Taiwan).

Genetic polymorphisms of the *OAS3* (*rs10735079*), *TYK2* (*rs74956615*) and *APOBEC3G* (*rs8177832* and *rs2294367*) genes were genotyped using specific Human TaqMan SNP Genotyping Assays 40x (ThermoFisher Scientific©). Real-time PCR reactions were performed using the TaqMan Genotyping Master Mix Multiplex Master mix (ThermoFisher Scientific©) and performed in the Quant studio 5 Real-Time PCR System 0.2 mL.

Statistical analysis. We performed Hardy-Weinberg Equilibrium (HWE) tests to test the genetic equilibrium of the study population. Genotypic and allelic frequencies were also calculated in both ILI and SARI cases and controls using SNPStats software. Deviations from HWE were checked for each SNP using a chi-squared test.

Association between ILI, SARI and SNPs for response was investigated by using SNPStats software, Odds ratio (OR) and confidence intervals (CI) at 95% were calculated to estimate the associations of infection with all the SNPs polymorphisms (if the confidence interval includes 1, the association is statistically not significant). Logistic regression analyses were subsequently applied to all the models, including codominant, dominant, and recessive models. We considered a p-value <.05 to be statistically significant.

Ethical considerations. The protocol is classified as a non-interventional study according to Moroccan law 28-13, which is focused on the protection of individuals participating in biomedical research. For this reason, it does not require approval from an ethics committee or IRB. Verbal informed consent was obtained with caution from all study patients and the control group to align with the ethical standards.

Limits of study. There are some limitations to this study. Due to the relatively small sample size, the generalizability of the results was limited, indicating that larger cohorts are needed. Furthermore, we analyzed all influenza positive cases combined without stratifying by virus type (A/H1N1, A/H3N2, and influenza B) or age group, which may have hidden important differences in subgroups.

RESULTS

The study sample involved 1925 patients collected during the season 2024-2025 and among the recruited cases a total of 198 were confirmed positive to Influenza virus by RT PCR, with 83 SARI cases and 115 ILI cases. The average of age was 44 years in the SARI group and 23 years in the ILI group. As for healthy controls, 60 subject were enrolled in this study, and their mean age was 35 years, ranging from 18 to 59 years.

Genotype frequencies: *OAS3* genotype frequencies. According to the *OAS3* frequency, as shown in Fig. 1, the A/A genotype is significantly more frequent in infected cases (ILI 80% and SARI 64%) than in

controls (48%). In case of the heterozygous A/G genotype, the frequency was the highest in controls cases (around 42%) and it is less represented in other cases, particularly in ILIs (around 17%). Moreover, the frequency of the G/G genotype was relatively rare in all groups, but slightly more frequent in controls (around 10%), which may also be associated with a possible protective effect.

The G allele (present in A/G and especially G/G genotypes) appears to be more frequent in the ILI group.

***TYK2* genotype frequencies.** The frequency of the A/A genotype is very low in all groups (around 3% in control and ILI and 0% in SARI), and also low for the T/A genotype in all three groups (around 2% in control and SARI, 7% in ILI), while the T/T genotype is the most common in all three groups, with the highest frequency in the SARI group (around 98%), followed by the control group (around 95%), and then the ILI group (around 90%) (Fig. 2).

The very low frequencies of the A/A and T/A genotypes make it difficult to draw strong conclusions visually. The high prevalence of T/T across all groups, especially SARI, aligns with the lack of significant association found in the table.

***APOBEC3G rs8177832* genotype frequencies.** Concerning *rs8177832* the A/A genotype is the most frequent in all three groups, with similar high percentages (around 79-82%), while the A/G genotype has a lower frequency (around 17-20%) across the groups, with a slightly higher percentage in the SARI group, and the G/G genotype is rare in all three groups (around 1-2%) (Fig. 3).

The relatively similar genotype frequencies across the control, ILI, and SARI groups visually reinforce the lack of significant association found in the tables for this SNP with either condition.

***APOBEC3G rs2294367* genotype frequencies.** The frequency of the C/C genotype is highest in the control group (around 60%), followed by ILI (around 27%), and then SARI (around 28%), and the C/G genotype is more frequent in the SARI group (around 48%), followed by ILI (around 43%), and then the control group (around 32%), while the G/G genotype is highest in the ILI (around 30%), followed by SARI group (around 24%), and it is the lowest in the control group (around 8%) (Fig. 4).

This visualization strongly supports the association

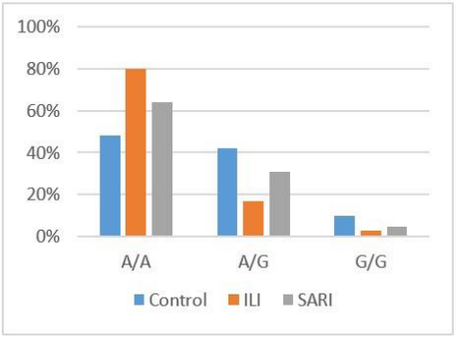

Fig. 1. *OAS3* genotype frequencies

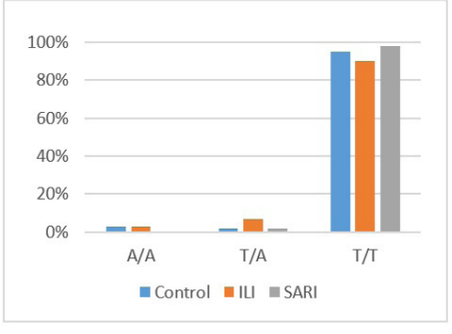

Fig. 2. *TYK2* genotype frequencies

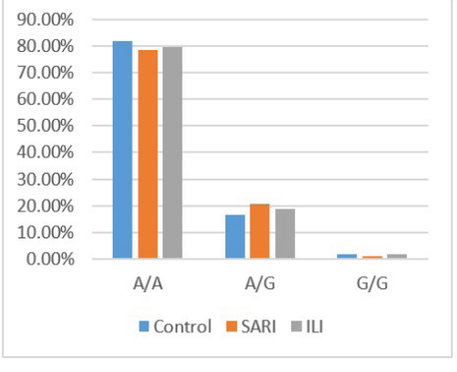

Fig. 3. Apobec *rs8177832* genotype frequencies

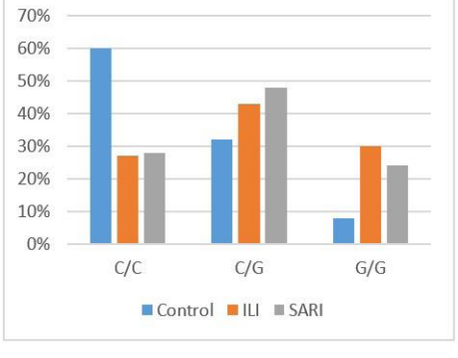

Fig. 4. Apobec *rs229367* genotype frequencies

between our findings. The frequency of the G allele (present in C/G and G/G genotypes) increases in both the ILI and SARI groups, with a more pronounced rise in the latter, compared to the control group.

Association with response. The *APOBEC3G* SNP *rs2294367* shows a significant association with SARI across all three genetic models tested: Codominant, Dominant, and Recessive with a significant p-value and a higher odd (Table 1). The presence of the G allele is strongly associated with an increased risk of SARI. The association is statisti- cally significant across all tested genetic models. The codominant model suggests a dose-dependent effect, with the G/G genotype conferring the highest risk.

In contrast to *rs2294367*, the *rs8177832* SNP does not show a statistically significant association with either SARI or ILI in any of the tested genetic models ($p > 0.05$), and the genotypes appear to be similarly distributed between controls and SARI (Table 2).

Same result for ILI group, the association is statistically significant with *rs2294367* across all tested genetic models, p-value is very significate and or vary between 2 and 8, with the homozygous G/G genotype showing the strongest effect with an increased risk of ILI.

The analysis of *TYK2* association with SARI and ILI (Table 3) shows that the A/A genotype is particularly rare in this population (ILI and SARI), while the T/T genotype being more representative.

For SARI group in codominant model, individuals with the A/A genotype have an odds ratio of 0.00 (95% CI: 0.00-NA). This result is based on 2 individuals with A/A in the control group and none in the SARI group. The p-value is not reported here, likely due to the issue with calculating the odds ratio. In the dominant model, individuals with at least one A allele (T/A or A/A) have an odds ratio of 0.47, and the p-value is not significant (95% CI: 0.08–2.90, $p = 0.41$). The small number of individuals with the A allele likely influences this result.

The Recessive Model (A allele recessive) is similar to the codominant model. This suggests a potential protective effect, but the lack of A/A individuals in the SARI group makes the estimate unreliable. The p-value of 0.061 is close to the conventional significance threshold of 0.05 but should be interpreted cautiously given the data limitations.

Same result for the ILI group; there is no statistically significant association found between this *TYK2* SNP and the risk of Influenza-like Illness in this study. While some odds ratios suggest potential trends (increased risk with the T/A genotype in the codominant model OR=4.38), these are not supported by statistically significant p-value (P=0.25) and are often based on small numbers of individuals with the minor allele (1 in control, 8 in ILI).

Table 4 shows that the A/A genotype in the control group was about 48.3% compared to 63.9% for the SARI group and 80% in the ILI group. In the SARI

Table 1. Association between the 2 *APOBEC3G* SNPs for SARI cases

rs2294367	**Association with response Patient: SARI**				
Model	**Genotype**	**Control**	**SARI**	**OR (95%)**	**P-value**
Codominant	C/C	36 (60%)	23 (27.7%)	1	3.00E-04
	C/G	19 (31.7%)	40 (48.2%)	3.30 (1.55-7.02)	
	G/G	5 (8.3%)	20 (24.1%)	6.26 (2.06-19.02)	
Dominant	C/C	36 (60%)	23 (27.7%)	1	1.00E-04
	C/G-G/G	24 (40%)	60 (72.3%)	3.91 (1.93-7.92)	
Recessive	C/C-C/G	55 (91.7%)	63 (75.9%)	1	0.011
	G/G	5 (8.3%)	20 (24.1%)	3.49 (1.23-9.93)	
rs8177832					
Codominant	A/A	49 (81.7%)	65 (78.3%)	1	0.83
	A/G	10 (16.7%)	17 (20.5%)	1.28 (0.54-3.04)	
	G/G	1 (1.7%)	1 (1.2%)	0.75 (0.05-12.35)	
Dominant	A/A	49 (81.7%)	65 (78.3%)	1	0.62
	A/G-G/G	11 (18.3%)	18 (21.7%)	1.23 (0.53-2.85)	
Recessive	A/A-A/G	59 (98.3%)	82 (98.8%)	1	0.82
	G/G	1 (1.7%)	1 (1.2%)	0.72 (0.04-11.74)	

Table 2. Association between the 2 *APOBEC3G* SNPs for ILI cases

rs2294367	Association with response Patient: ILI				
Model	**Genotype**	**Control**	**ILI**	**OR (95% CI)**	**P-value**
Codominant	C/C	36 (60%)	31 (27%)	1	<0.0001
	C/G	19 (31.7%)	49 (42.6%)	2.99 (1.47-6.12)	
	G/G	5 (8.3%)	35 (30.4%)	8.13 (2.84-23.30)	
Dominant	C/C	36 (60%)	31 (27%)	1	<0.0001
	C/G-G/G	24 (40%)	84 (73%)	4.06 (2.10-7.87)	
Recessive	C/C-C/G	55 (91.7%)	80 (69.6%)	1	4.00E-04
	G/G	5 (8.3%)	35 (30.4%)	4.81 (1.77-13.06)	
rs8177832					
Codominant	A/A	49 (81.7%)	91 (79.1%)	1	0.92
	A/G	10 (16.7%)	22 (19.1%)	1.18 (0.52-2.70)	
	G/G	1 (1.7%)	2 (1.7%)	1.08 (0.10-12.18)	
Dominant	A/A	49 (81.7%)	91 (79.1%)	1	0.69
	A/G-G/G	11 (18.3%)	24 (20.9%)	1.17 (0.53-2.60)	
Recessive	A/A-A/G	59 (98.3%)	113 (98.3%)	1	0.97
	G/G	1 (1.7%)	2 (1.7%)	1.04 (0.09-11.76)	

Table 3. Association between *TYK2* SNP and Influenza status (ILI, SARI)

	***TYK2* association with response Patient**				
Model	**Genotype**	**Control**	**SARI**	**OR (95% CI)**	**P-value**
Codominant	T/T	57 (95%)	81 (97.6%)	1	0.17
	T/A	1 (1.7%)	2 (2.4%)	1.41 (0.12-15.90)	
	A/A	2 (3.3%)	0 (0%)	0.00 (0.00-NA)	
Dominant	T/T	57 (95%)	81 (97.6%)	1	0.41
	T/A-A/A	3 (5%)	2 (2.4%)	0.47 (0.08-2.90)	
Recessive	T/T-T/A	58 (96.7%)	83 (100%)	1	0.061
	A/A	2 (3.3%)	0 (0%)	0.00 (0.00-NA)	
Model	**Genotype**	**Control**	**ILI**	**OR (95% CI)**	**P-value**
Codominant	T/T	57 (95%)	104 (90.4%)	1	0.25
	T/A	1 (1.7%)	8 (7%)	4.38 (0.53-35.94)	
	A/A	2 (3.3%)	3 (2.6%)	0.82 (0.13-5.06)	
Dominant	T/T	57 (95%)	104 (90.4%)	1	0.27
	T/A-A/A	3 (5%)	11 (9.6%)	2.01 (0.54-7.50)	
Recessive	T/T-T/A	58 (96.7%)	112 (97.4%)	1	0.79
	A/A	2 (3.3%)	3 (2.6%)	0.78 (0.13-4.78)	

group, the A/G and G/G genotypes in the codominant model show odds ratios of 0.57 and 0.36, respectively; these suggest a potentially decreased risk of SARI, although the p-value is not statistically significant. Furthermore, the G/G genotype has an odds ratio of 0.46 (95% CI: 0.12-1.69, p = 0.23).

We observed that the G allele in the dominant model has an odds ratio of 0.53 (95% CI: 0.27-1.04, p = 0.064). This odds ratio suggests a potentially decreased risk of SARI, and the p-value is approaching statistical significance (typically < 0.05), though it does not cross the conventional threshold.

For the ILI group, in both the codominant and dominant models, the A/G and G/G genotypes show significantly lower odds ratios for having ILI (0.25, 0.16, and 0.23), and the p-value is highly significant (p < 0.0001). This indicates a strong protective effect of the A/G and G/G genotype against ILI.

Table 4. Association between *OAS3* SNP and Influenza status (ILI, SARI)

OAS3 association with response Patient					
Model	**Genotype**	**Control**	**SARI**	**OR (95% CI)**	**P-value**
Codominant	A/A	29 (48.3%)	53 (63.9%)	1	0.15
	A/G	25 (41.7%)	26 (31.3%)	0.57 (0.28-1.16)	
	G/G	6 (10%)	4 (4.8%)	0.36 (0.10-1.40)	
Dominant	A/A	29 (48.3%)	53 (63.9%)	1	0.064
	A/G-G/G	31 (51.7%)	30 (36.1%)	0.53 (0.27-1.04)	
Recessive	A/A-A/G	54 (90%)	79 (95.2%)	1	0.23
	G/G	6 (10%)	4 (4.8%)	0.46 (0.12-1.69)	
Model	**Genotype**	**Control**	**ILI**	**OR (95% CI)**	**P-value**
Codominant	A/A	29 (48.3%)	92 (80%)	1	1.00E-04
	A/G	25 (41.7%)	20 (17.4%)	0.25 (0.12-0.52)	
	G/G	6 (10%)	3 (2.6%)	0.16 (0.04-0.67)	
Dominant	A/A	29 (48.3%)	92 (80%)	1	<0.0001
	A/G-G/G	31 (51.7%)	23 (20%)	0.23 (0.12-0.46)	
Recessive	A/A-A/G	54 (90%)	112 (97.4%)	1	0.042
	G/G	6 (10%)	3 (2.6%)	0.24 (0.06-1.00)	

DISCUSSION

Infectious disease development is significantly influenced by genetic variations in human host genes. The impact of host genetics on disease susceptibility has been the subject of extensive research during the COVID-19 pandemic, and rare genetic variations linked to innate immunity that increase the risk of severe COVID-19 have been discovered by genetic research (8).

In this study, we focused on SNPs that had been examined in the context of infectious diseases and were associated with susceptibility to, and severity of, public health–relevant conditions. *APOBEC3G* belongs to the APOBEC family of cytidine deaminases, recognized for their capability to facilitate the conversion of cytosine to uracil in single-stranded DNA through the process of deamination (5), This protein consists of two domains of cytidine deaminase, with the C-terminal domain exhibiting catalytic activity, whereas the N-terminal domain plays a role in RNA binding and the incorporation of virions (9).

A study in Moroccan subjects by our team in HIV lab have indicated that the *rs2294367* CG genotype was associated with protection against HIV-1 infection, particularly in older individuals and females, and the GG genotype in females showed susceptibility to HIV-1 (10).

We found in our study that the *rs2294367* in *APOBECG3* has a strong and significant association with ILI across all genetic models, with even higher odds ratios than observed for SARI.

The G allele is strongly and significantly associated with an increased risk of ILI and SARI across all genetic models. For both the ILI and SARI groups, the G/G genotype shows the highest odds ratio.

This suggests that this SNP might have a stronger influence on the development of any influenza-like illness compared to specifically severe cases. This significant association of *rs2294367* with SARI and ILI suggests that this genetic variant might play a role in the susceptibility of Influenza infection. Research has indicated that infection with influenza A virus can lead to an increase in *APOBEC3G* expression; however, this increase does not automatically result in effective antiviral responses against the virus (6).

APOBEC3G does not exhibit direct antiviral activity against influenza, the role of *rs2294367* in influenza susceptibility or severity might be related to its potential influence on *APOBEC3G* expression or other indirect effects on the host immune response to the virus.

Further research is needed to understand the biological mechanisms through which this SNP influences the susceptibility and progression to severe outcomes.

Regarding the *rs8177832* polymorphism in the same gene, which is referred to H186R, this variant

is among the most thoroughly examined variations in the *APOBEC3G* gene, This SNP leads to a non-synonymous substitution, replacing histidine (H) with arginine (R) at the 186 amino acid position in the protein (3). In our study no statistically significant association was found between this SNP and the risk of Influenza-like Illness and Severe Acute Respiratory Illness in any of the tested genetic models. This reinforces the idea that this particular SNP might not be involved in susceptibility to either general influenza-like illness or severe acute respiratory illness in this studied population. Although this variant 186R (*rs8177832*) has been associated with accelerated HIV disease progression in studies with diverse ethnicities, it was not associated in a French cohort from Montreal (5). This finding is consistent with our study, which demonstrated no association with influenza infection outcomes.

For the 2 other SNPs involved in innate immunity, there was no statistically significant correlation found between the presence of the A allele and disease susceptibility when the *TYK2* SNP was analyzed in relation to respiratory influenza infections (ILI and SARI). Although the A/A genotype was relatively uncommon in the ILI group and absent in the SARI group, an odds ratio of zero suggests this genotype might be protective against SARI in this small sample. However, the confidence interval cannot be reliably estimated (NA) due to the zero count in the SARI group. This finding is based on extremely small numbers and lacks statistical power. The ILI group, on the other hand, had a slightly higher frequency of the T/A genotype, which led to wide confidence intervals and elevated but non-significant odds ratios, suggesting significant uncertainty in the estimate.

TYK2 plays a specifically crucial role for full activation of transcription factors like STAT1, STAT2, and STAT4 on Type I IFN response to ensure powerful antiviral protection (11). Likewise, Type III Interferons (IFN-λ) are crucial in the defense of mucosal surfaces like the respiratory and gastrointestinal tracts against viral infection, and TYK2 is also involved in signaling for them (12).

The proper activity of TYK2 is therefore crucial for the host to identify and eliminate viral invaders by mediating the crucial interferon-induced antiviral state.

Experiments have shown that a lack of TYK2 can lead to increased susceptibility to certain viral infections, highlighting its function in antiviral immunity (13).

Similarly, in humans, TYK2 deficiency has been associated with increased susceptibility to diverse microorganisms, including viruses, fungi, and mycobacteria (14).

Furthermore, therapy with JAK inhibitors, including those against TYK2, has the potential to impair antiviral immunity through suppression of interferon signaling pathways, which can increase the risk of viral infections in treated individuals (12).

However, in a specific context, pharmacological inhibition of TYK2 in an influenza A virus (IAV) linked pneumonia model was found to reconstitute the virally suppressed immune response to secondary bacterial infections (15).

In summary for TYK2 the lack of significant evidence for either increased risk or protection conferred by the A allele is indicated by the fact that the p-values remained above the 0.05 threshold across all models and infection types, and the confidence intervals consistently included 1. Overall, these results indicate that there is no apparent correlation between this SNP and the population under study's susceptibility to ILI or SARI; however, the potential protective effect of the A/A genotype against SARI merits additional research and investigation in larger cohorts.

And for the *OAS3* SNP, in ILI group, the G allele was strongly and significantly associated with a decreased risk of Influenza-like Illness across all genetic models, showing significantly lower odds. This suggests a protective effect of the G allele against moderate respiratory infections, the dominant model highlights this protective effect most clearly. We can say that the G allele might be associated with a more effective *OAS3* function in response to the influenza viruses that cause ILI. Perhaps this allele leads to a quicker or stronger activation of RNase L, resulting in more efficient viral clearance and less severe symptoms.

In a study conducted by Yize Li et al., it was demonstrated that *OAS3*, but not *OAS1* or *OAS2*, is required to activate RNase L and to restrict the replication of four different human viruses: West Nile virus, Sindbis virus, and influenza virus (1).

Activation of this enzyme which is the RNase L, produces a potent antiviral action by degrading viral and cellular single-stranded RNA (ssRNA), ultimately leading to the inhibition of protein synthesis and

restriction of viral replication (1).

The OAS3-RNase L pathway has demonstrated antiviral effects against several viruses such as SARS CoV 2, Chikungunya virus, Semliki forest virus, Sindbis virus (SINV) and Hepatitis C virus(16); and for Dengue virus certain OAS3 variants are associated with reduced risk of severe dengue infection, it exhibits anti-DENV activity via an RNase L-dependent mechanism (17).

Different alleles of this SNP can lead to subtle changes in the *OAS3* gene, potentially affecting the protein produced, it might lead to higher or lower levels of the OAS3 enzyme, and perhaps can affect the activity of the OAS3 protein making the enzyme more or less efficient at synthesizing 2-5As in response to viral dsRNA.

For SARI group, we observed an odds ratios (OR) less than 1 for the genotypes carrying the G allele (A/G and G/G in the codominant model, and the combined A/G-G/G in the dominant model), this results show a trend towards the G allele being protective, but this association did not reach statistical significance at the conventional $p < 0.05$ level. The dominant model shows the strongest trend with a p-value of 0.064, suggesting that further investigation with a larger sample size might be warranted.

The trend for SARI is less clear but suggests potential protection with the G allele. While not statistically significant at the conventional threshold, the odds ratios for SARI also pointed towards a protective effect of the G allele, particularly in the dominant model. It's possible that the effect of this SNP on severe outcomes is subtler or requires a larger sample size to detect. Perhaps the OAS3 pathway is important in the initial response to the virus (hence the strong association with ILI). However, other factors may become more critical in determining the severity of the illness, or this could depend on the specific subtype of Influenza A (e.g., H1N1, H3N2) or Influenza B. More research with a larger sample size is needed to confirm these possibilities.

CONCLUSION

These results highlight the complex interplay of genetics in influencing the susceptibility and severity of respiratory illnesses. Some genetic variants might predispose individuals to both moderate and severe forms, while others might have more specific effects. The *OAS3* result is particularly interesting as it suggests a potential genetic factor that might help in fighting off milder infections.

Our results open a new perspective for therapeutic methods aimed at boosting human immunity against the influenza virus. This approach is particularly timely in an era characterized by emerging pathogens and the long development timelines required for new, pathogen-specific treatments.

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