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Short Communication

TNFAIP3-interacting protein *1* polymorphisms and their association with symptomatic human respiratory syncytial virus infection and bronchiolitis in infants younger than one year from South Africa: A case-control study

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ABSTRACT

Objectives: This study analyzed the association of TNFAIP3-interacting protein 1 (*TNIP1*) polymorphisms with the symptomatic human respiratory syncytial virus (HRSV) infection and bronchiolitis in infants.
Methods: A case-control study was conducted involving 129 hospitalized infants with symptomatic HRSV infection (case group) and 161 healthy infants (control group) in South Africa (2016-2018). Six *TNIP1* polymorphisms (rs869976, rs4958881, rs73272842, rs3792783, rs17728338, and rs999011) were genotyped. Genetic associations were evaluated using logistic regression adjusted by age and gender.
Results: Both rs73272842 G and rs999011 C alleles were associated with reduced odds for symptomatic HRSV infection (adjusted odd ratio [aOR] = 0.68 [95% confidence interval {CI} = 0.48-0.96] and aOR = 0.36 [95% CI = 0.19-0.68], respectively] and bronchiolitis (aOR = 0.71 [95% CI = 0.50-1.00] and aOR = 0.38 [95% CI = 0.22-0.66], respectively). The significance of these associations was validated using the BCa Bootstrap method (P <0.05). The haplotype GC (composed of rs73272842 and rs999011) was associated with reduced odds of symptomatic HRSV infection (aOR = 0.53 [95% CI = 0.37-0.77]) and bronchiolitis (aOR = 0.62 [95% CI = 0.46-0.84]), which were validated by the BCa Bootstrap method (P = 0.002 for both).
Conclusion: *TNIP1* rs73272842 G allele and rs999011 C allele were associated with reduced odds of symptomatic HRSV infection and the development of bronchiolitis in infants, suggesting that *TNIP1* polymorphisms could impact susceptibility to HRSV illness.

Introduction

Human respiratory syncytial virus (HRSV) can cause severe diseases such as bronchiolitis and pneumonia in specific vulnerable populations, such as infants [1]. Bronchiolitis is the most common severe HRSV-related disease in infants, characterized by inflammation of the bronchioles, mucus production, and subsequent airway

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Table 1
Epidemiological characteristics of RSV-infected infants and control group.

Characteristics	Controls	Cases	*P*-value	Non-bronchiolitis	Bronchiolitis	*P*-value
No.	161	129		108	21	
Male	83 (51.6)	69 (53.5)	0.743	54 (50)	15 (71.4)	0.072
Age (months)	3.5 (1.7-6.0)	3.0 (1.6-5.7)	0.343	2.9 (1.4-5.8)	3.2 (2.3-4.8)	0.754
Height (cm)	-	57 (52-63)	-	56 (51.5-62)	61 (55-64.5)	0.083
Weight (Kg)	-	5.6 (4.6-7.5)	-	5.4 (4.5-7.3)	6.5 (5.4-7.6)	0.094
Mid-upper arm circumference (cm)	-	14 (12-15)	-	14 (12-15)	14 (12.5-15.5)	0.300
RSV subtypes	-					0.137
A	-	31 (24.6)	-	25 (23.8)	6 (28.6)	-
B	-	77 (61.1)	-	65 (61.9)	12 (57.1)	-
A/B	-	1 (0.8)	-	0 (0)	1 (4.8)	-
Unsubtypable	-	17 (13.5)	-	15 (14.3)	2 (9.5)	-
Premature	-	17 (13.2)	-	13 (12.0)	4 (19.0)	0.385
HIV mother	-	38 (29.9)	-	33 (31.1)	5 (23.8)	0.503
HIV infant	-	1 (0.8)	-	1 (0.9)	0 (0)	0.658
Infant fed at 0-2 months	-					0.605
Exclusive breastfeeding	-	95 (77.2)	-	81 (78.6)	14 (70)	-
Formula feeding	-	25 (20.3)	-	19 (18.5)	6 (30)	-
Mixed (Breast + other)	-	2 (1.6)	-	2 (1.9)	0 (0)	-
Unknown	-	1 (0.8)	-	1 (0.9)	0 (0)	-
No. children <5 years living at home	-	1 (1-2)	-	1 (1-2)	1 (1-2)	0.706
No. of bedrooms	-	3 (2-4)	-	3 (2-4)	3 (2-4)	0.776
People smoking at home	-	39 (30.2)	-	35 (32.4)	4 (19.1)	0.223

Statistics: Values were expressed as absolute numbers (percentages) and medians (percentile 25; percentile 75). *P*-values were calculated by chi-square and Mann-Whitney test.
Abbreviations: RSV, respiratory syncytial virus.

obstruction. An excessive inflammatory response has been related to HRSV pathogenesis, but also a robust innate immune response is needed to control virus replication and protect against severe HRSV disease [2].

Viral infection, including HRSV, triggers intracellular signaling, activating transcription factors and subsequent expression of inflammatory and antiviral genes [3]. These signaling pathways are tightly regulated to prevent excessive inflammation and tissue damage while ensuring virus clearance. TNFAIP3-interacting protein 1 (TNIP1) downregulates the activation of NF-κB and IRF3/7 transcription factors, negatively modulating the inflammatory response and positively the innate response against viruses [4]. Thus, TNIP1 expression is related to the response against HRSV [5] and *TNIP1* single-nucleotide polymorphisms (SNPs) are linked to septic shock-related death [6].

We aimed to analyze the association of *TNIP1* polymorphisms with symptomatic HRSV infection and bronchiolitis in infants.

Methods

We conducted an unmatched case-control study nested within a prospective surveillance program in South Africa. From these participants, we selected 129 infants who attended the hospital with an acute medical illness and HRSV infection (case group) and 161 healthy infants (control group) who were less than 1 year old; both groups had a blood sample stored at −80°C. All babies included in the current study were enrolled in four hospitals (case group) and two Health Centres (control group) across three provinces of South Africa (Full description in Supplementary file). All participants had informed consent signed by their parents or guardians. The University of the Witwatersrand Human Research Ethics Committee approved this study (reference number M190216).

The main outcome was the presence of symptomatic HRSV infection (hospital admission with confirmed HRSV PCR). The secondary outcome was the bronchiolitis diagnosis at admission with confirmed RSV infection (controls, cases without bronchiolitis, and cases with bronchiolitis). The main exposure variables were six *TNIP1* SNPs (rs869976, rs4958881, rs73272842, rs3792783, rs17728338, and rs999011). HRSV infection diagnosis, SNPs selection, and DNA genotyping are described in the Supplementary file.

Several in silico analyses were performed using online bioinformatics tools: GTEx PORTAL Release V8 (https://gtexportal.org/), rVarBase (http://rv.psych.ac.cn/), and predictSNP2 (https://openebench.bsc.es/tool/predictsnp2). The statistical analysis used Stata 17.0 (StataCorp, Texas, USA). Two-tailed *P*-values ≤0.05 were considered significant. Categorical variables were compared with the chi-square test. Continuous variables compared with the Mann-Whitney test. Hardy-Weinberg equilibrium (HWE) was evaluated using the Stata's *genhwi* package. The genetic association was evaluated for three inheritance models (additive, recessive and dominant). Logistic regressions (binomial and ordinal) were used to analyze the association between *TNIP1* polymorphisms and the outcome variables (symptomatic HRSV infection and bronchiolitis, respectively). The significance level was defined as a p-value <0.05 (two-tailed) and a False Discovery Rate (q-value) <0.2. For significant SNPs, logistic regressions were adjusted by age and gender. Stata's *Haplologit* package was used to evaluate haplotype associations. The BCa Bootstrap method (Jackknife replications (290) and Bootstrap replications (1,000)) was used to validate significant results.

Results

No significant differences were found between the epidemiological characteristics of 129 infants in the case group (108 without bronchiolitis and 21 with bronchiolitis) and 161 infants in the control group (Table 1). Most infants (282/297; 97%) in the study population belonged to the Black ethnic group.

All *TNIP1* polymorphisms (rs869976, rs4958881, rs73272842, rs3792783, rs17728338, and rs999011) were in HWE (Supplementary Table 1). D' and r^2 values were lower than 0.60, except for rs73272842 and rs3792783 (D' = 0.87 and r^2 = 0.75), the only two *TNIP1* polymorphisms that tended to be inherited together (Supplementary Figure 1).

The genetic association of *TNIP1* polymorphisms with clinical outcomes using univariate (unadjusted) models (Supplementary Table 2) showed that only rs73272842 and rs999011 were significantly associated with symptomatic HRSV infection and bronchiolitis (*P*-value <0.05; q-value <0.2). In adjusted regression models (Table 2), rs73272842 GG/GA genotype and rs73272842 G al-

Table 2
Genetic adjusted association of TNFAIP3-interacting protein *1* polymorphisms with outcome variables.

Single-nucleotide polymorphisms	Genotypes	Adjusted odds ratio (95% confidence interval)	*P*-value	*P*-value[c]
A) Symptomatic respiratory syncytial virus infection[a]				
rs73272842	GG/GA vs AA	0.55 (0.33-0.92)	**0.024**	**0.033**
	G vs A allele	0.68 (0.48-0.96)	**0.030**	**0.046**
rs999011	CC vs TT/CT	0.36 (0.18-0.71)	**0.003**	**0.007**
	C vs T allele	0.36 (0.19-0.68)	**0.002**	**0.002**
B) Bronchiolitis[b]				
rs73272842	GG/GA vs AA	0.59 (0.36-0.97)	**0.038**	**0.047**
	G vs A allele	0.71 (0.50-1.00)	0.051	0.070
rs999011	CC vs TT/CT	0.36 (0.19-0.67)	**0.001**	**0.003**
	C vs T allele	0.38 (0.22-0.66)	**0.001**	**0.001**

Statistics: Data were calculated by logistic binomial regressions[a] and logistic ordinal regressions[b], which were adjusted by sex and age.[c] *P*-values were validated by BCa Bootstrap. Significant *P*-values are shown in bold.

lele were associated with reduced odds of symptomatic HRSV infection (adjusted odd ratio [aOR] = 0.55 [95% confidence interval {CI} = 0.33-0.92] and aOR = 0.68 [95% CI = 0.48-0.96]; respectively) and rs73272842 GG/GA genotype with bronchiolitis (aOR = 0.59 [95% CI = 0.36-0.97]). The rs999011 CC genotype and rs999011 C allele were associated with reduced odds of symptomatic HRSV infection (aOR = 0.36 [95% CI = 0.18-0.71] and aOR = 0.36 [95% CI = 0.19-0.68), respectively) and bronchiolitis (aOR = 0.36 [95% CI = 0.19-0.67] and aOR = 0.38 [95% CI = 0.22-0.66]; respectively). The BCa Bootstrap method validated all significant *P*-values ($P < 0.05$).

The *TNIP1* haplotype GC (composed of rs73272842 and rs999011) was associated with reduced odds of symptomatic HRSV infection (OR = 0.53 [95% CI = 0.37-0.77] and bronchiolitis OR = 0.62 [95% CI = 0.46-0.84]), being also validated by the BCa Bootstrap method ($P = 0.002$ and $P = 0.002$; respectively) (Supplementary Table 3).

Discussion

Our data show that *TNIP1* rs73272842 G allele and rs999011 C allele were associated with reduced odds of symptomatic HRSV infection and bronchiolitis in infants. Furthermore, the *TNIP1* haplotype GC, formed by rs73272842 and rs999011, was associated with a reduced likelihood of experiencing symptomatic HRSV infection and bronchiolitis.

The rs73272842 SNP is situated within an intronic region of the *TNIP1* gene, whereas the rs999011 SNP is located upstream of *TNIP1*. SNPs outside the coding region do not modify the protein's primary sequence. However, they can reduce the binding of a transcription factor to a gene promoter, alter mRNA splicing and/or stability, protein translation, and/or co-translational protein folding, among other effects. These changes may alter protein expression and/or function [7].

Using the GTEx Portal, we found that the G allele is associated with lower *TNIP1* expression levels than the A allele. Additionally, we observed that both rs73272842 and rs999011 polymorphisms are involved in changes in the chromatin state across various tissues and cell lines as described by rVarBase, an updated database for regulatory features of human variants. Chromatin modifications potentially affect DNA accessibility to transcription factors, thereby influencing the regulation of *TNIP1* expression. Similarly, using predictSNP2, a unified platform for predicting SNP effects in distinct genomic regions, the rs999011 polymorphism was identified as a deleterious variant. This finding supports that the rs999011 polymorphism could influence disease susceptibility. In addition, our findings are consistent with previous articles, in which the rs73272842 G allele has been associated with a lower risk of septic shock in patients undergoing major surgery [6], systemic lupus erythematosus [8], and Sjogren's syndrome [9]. This suggests that this SNP may have an impact on various inflammatory-related conditions.

Therefore, a plausible scenario is that both *TNIP1* polymorphisms could impact HRSV disease by modifying *TNIP1* gene expression. In this context, the rs73272842 G and rs999011 C alleles could potentially decrease *TNIP1* expression, subsequently enhancing NF-κB-mediated gene transcription, including several genes that encode diverse effectors of host antiviral immunity. Consequently, this mechanism would lead to more effective control of viral replication and a reduction in severe disease manifestation [10]. Consistent with this hypothesis, we have shown in another article that siRNA-mediated downregulation of *TNIP1* increases the immune response and apoptosis in HRSV-infected cells, resulting in decreased virus production [5].

However, it is also important to note that the rs73272842 and rs999011 polymorphisms are in high linkage disequilibrium with several other SNPs, potentially affecting *TNIP1* expression regulation. Consequently, we cannot rule out that other SNPs in high LD could be the causal polymorphism. Additional studies would be required to corroborate the functional role of these *TNIP1* polymorphisms.

This study has some limitations. Firstly, the sample size is small, especially for infants with bronchiolitis, which could potentially impact the validity of the results. Secondly, the retrospective design may have introduced biases. Thirdly, the diagnosis of bronchiolitis relied on clinical notes, which could occasionally lack precision.

In conclusion, *TNIP1* rs73272842 G and rs999011 C alleles were associated with reduced odds of symptomatic HRSV infection and the development of bronchiolitis in infants. Our preliminary study suggests that *TNIP1* polymorphisms could affect susceptibility to HRSV illness.

Declaration of Competing Interest

The authors have no competing interests to declare.

CRediT authorship contribution statement

María Martin-Vicente: Data curation, Investigation, Methodology, Writing – review & editing. **Hloni Mthiyane:** Data curation. **María A Jiménez-Sousa:** Formal analysis, Investigation, Writing – original draft. **Kathleen Subramoney:** Data curation. **Orienka Hellferscee:** Data curation. **Nicole Wolter:** Data curation. **Sibongile Walaza:** Data curation. **Cheryl Cohen:** Data curation. **Anne von Gottberg:** Data curation. **Salvador Resino:** Conceptualization, Formal analysis, Funding acquisition, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing –

original draft. **Isidoro Martínez:** Conceptualization, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft. **Florette K Treurnicht:** Conceptualization, Data curation, Funding acquisition, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

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Ethics approval and consent to participate

The research was done according to the Declaration of Helsinki, and the Human Research Ethical Committee Certificate approved this study (Ref.: M190216). All participants had an informed consent signed by their parents or guardians.

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Consent for publication

Not applicable.

Availability of data and materials

The datasets analyzed during the current study may be available upon reasonable request.

Authors' information (optional)

Not applicable.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2023.09.013.

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