Torque Teno Virus in Nasopharyngeal Aspirate of Children With Respiratory Infections

Teresa del Rosal  
Hospital Universitario La Paz

María Luz García-García (marialuz.hso@gmail.com)  
Hospital Universitario Severo Ochoa

Inmaculada Casas  
National Center Microbiology, ISCIII, Spain

Sonia Alcolea  
Hospital Universitario La Paz

María Iglesias-Caballero  
National Center for Microbiology (ISCIII)

Francisco Pozo  
National Center for Microbiology (ISCIII)

Jose Manuel Rodrigo-Muñoz  
IIS-Fundación Jiménez Díaz, CIBER de Enfermedades Respiratorias (CIBERES)

Victoria del Pozo  
IIS-Fundación Jiménez Díaz

Cristina Calvo  
Hospital Universitario La Paz

Research Article

Keywords: Torque teno virus, infections, immune function, nasopharyngeal

Posted Date: January 5th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1197264/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Torque teno virus (TTV) is responsible for persistent infections and is considered a marker of immune function. The role of TTV as a facilitator of respiratory infections (RIs) is unknown. We aimed to estimate the prevalence of TTV in the nasopharyngeal aspirate (NPA) of hospitalized children with RIs and correlate them with outcomes and immune response. NPA was taken for testing 16 respiratory viruses by RT-polymerase chain reaction (PCR), TTV PCR, and immunological study.

Sixty hospitalized children with an RI and 3 healthy control infants were included. A total of 51/60 patients had a positive common respiratory viral (CRV) identification. A total of 24/63 (38.1%) children were TTV+ and had other CRVs in 95.8% of cases vs 74.4% in TTV- (p=0.029). TTV+ patients tended to be older, have fever, and need PICU admission more often than TTV- patients. Abnormal chest X-ray was more frequent in the TTV+ patients, OR 2.6 (95% CI: 1.3-5.2).

The genetic expression of laggrin (involved in epithelial barrier integrity) was lower in TTV+ patients; however, levels of laggrin in the NPA were increased.

In summary, TTV infection is common in children with RI and could be associated with pneumonia, greater severity, and alteration in laggrin gene expression and protein release.

Introduction

Torque teno virus (TTV) is a prototype anellovirus, a small ubiquitous DNA virus responsible for persistent asymptomatic infections\textsuperscript{1,2}. TTV is considered to be a marker of immunological status\textsuperscript{3}. An increase in TTV replication has been observed in sepsis\textsuperscript{4}, HIV infection\textsuperscript{5,6,7}, untreated cancer\textsuperscript{8}, bone marrow transplantation\textsuperscript{9,10}, and solid organ transplantation\textsuperscript{11,12,13} as a potential endogenous marker of immunosuppression. In terms of respiratory infections, TTV has been found in children with recurrent pneumonias, showing its potential to infect the respiratory tract\textsuperscript{14}. It has also been associated with bronchopneumonia, and its role in asthma is under study\textsuperscript{15,16}. As far as we know, there have been few studies of TTV in respiratory samples of children with respiratory tract infections, correlating its presence with the immunological state or its clinical evolution\textsuperscript{15}. The role of TTV as a facilitator of respiratory infections or airway inflammation remains to be determined.

The objective of this study was to determine the prevalence of TTV in hospitalized children with respiratory infections, analyzing their nasopharyngeal aspirate samples and how viral detection correlates with individual clinical evolution. We also analyzed the innate immune response of the children who were positive for TTV.

Patients And Methods

This is a substudy of an ongoing prospective investigation of respiratory tract infections in children, approved by the Medical Ethics Committee of Carlos III Health Institute; Ethics Committee of Hospital La
Paz and Ethics Committee of Hospital Severo Ochoa. All research was performed in accordance with regulations and the Declaration of Helsinki. Informed consent was obtained from all parents or legal guardians.

**Clinical assessment**

This was a multicenter study performed in Madrid, Spain, between January 2021 and June 2021. The study population was comprised of children younger than 5 years of age with acute respiratory infection (ARI) admitted to either of the participating hospitals, Severo Ochoa (Leganés) or La Paz University Hospital (Madrid). The exclusion criterion was a refusal to participate in the study. Patients were evaluated by an attending physician, and the patients’ clinical characteristics were analyzed. During their hospital stay, and as part of the study, a physician completed a questionnaire with the following variables: age; sex; month of admission; clinical diagnosis; history of prematurity or underlying chronic diseases; need for oxygen therapy, evaluated via transcutaneous oxygen saturation; fever; maximum axillary temperature; presence of infiltrates and/or atelectasis in chest X-rays; administration of antibiotic therapy; length of hospital stay; need for admission to a Pediatric Intensive Care Unit (PICU); total white blood cell count; serum C-reactive protein levels (mg/L); and blood culture results (in those cases for which such tests had been performed). As part of our global study of respiratory infections, a small number of healthy children attended as outpatients for non-infectious causes (e.g., vaccines, minor surgery) were included as controls.

Acute expiratory wheezing was considered to be bronchiolitis when it occurred for the first time in children aged younger than 2 years, following the classic criteria of McConnochie\(^{17}\). All other episodes of acute expiratory wheezing were considered to be recurrent wheezing. Laryngotracheobronchitis was associated with inspiratory stridor and wheezing. Laryngitis was associated with inspiratory stridor without wheezing. Cases with both focal infiltrates and consolidation in chest X-rays were, in the absence of wheezing, classified as pneumonia. However, those cases with wheezing were classified as bronchiolitis or recurrent wheezing as appropriate.

**Viral studies**

Specimens consisted of nasopharyngeal aspirates (NPAs) that were obtained from each patient at admission. NPAs and nasopharyngeal swabs were sent for virological investigation to the Respiratory Viruses and Influenza Unit at the National Center for Microbiology (ISCIII), Madrid, Spain. Samples were stored at 4 °C in a refrigerator and were processed within 24 hours after collection. Upon reception, 3 aliquots were prepared and stored at −80 °C. Both the reception and the NPA sample processing areas are separated from those defined as working areas.

RNA and DNA from 200-µl aliquots of NPA were extracted with the QIAamp Mini Elute Virus spin kit in an automated extractor (QIAcube, Qiagen, Valencia, CA, USA).
Detection of respiratory virus was performed by 4 independent multiplex reverse transcription-polymerase chain reaction (RT-PCR) assays. The first assay detected Influenza A, B, and C viruses; the second was used to detect parainfluenza viruses 1 to 4, human rhinoviruses (HRVs), and enteroviruses; and the third assay detected the presence of respiratory syncytial virus (RSV) types A and B, human metapneumovirus, human bocavirus, and human adenoviruses (HAdVs). These 3 assays were real time multiplex RT-PCRs and used the SuperScript™ III Platinum® One-Step Quantitative RT-PCR System (Invitrogen). A fourth multiplex RT-PCR was used for investigation of human coronavirus (HCoV), using generic primers that were able to detect both alpha and beta coronavirus. Typing of HCoV was performed using a reverse specific primer for detection of HCoV 229E, HCoV NL63, HCoV OC43, and HCoV HKU1. Primers and Taqman probes for the 3 independent multiplex real time RT-PCRs were based on previously published designs by our group\textsuperscript{18}, and the HCoV primers are available on request.

**Torque teno virus study**

A generic PCR assay was designed for the detection of the TTV diverse group. Previous studies had described a variation in the rate of positive detection depending on the target site of amplification in the PCR. Given that the untranslated regions (UTRs) of the genome are more conserved as compared to the open reading frames, primers designed in the UTR will cross-match a large number of genotypes and increase the rate of detection\textsuperscript{19,20}.

The new screening primers designed were the forward primer U5F (5'-YKTCGTICACTTCCTGGGC-3') and the reverse primer U5R (5'-CGAGCCCGAATTGCCCC-3'), used at a 10-pmol concentration in the reaction mixture.

This method involved cycling consisting of a denaturation cycle of 95 °C for 5 minutes, followed by 40 cycles with a denaturation step of 30 seconds at 95 °C, an annealing step of 30 seconds at 57 °C and an extension step of 1 minute at 72 °C. The final extension step was 72 °C for 5 minutes.

Amplified products (~180 bp) were visualized by 2% agarose gel electrophoresis containing 5 mg/ml of GreenGel in 1x Tris borate buffer.

**Immunological study**

A portion of each NPA sample was centrifuged to obtain the cellular pellet and supernatant. Samples with mucus were filtered with a 40-µm nylon filter. The pellet was resuspended in 0.7 mL Qiazol Lysis Reagent (Qiagen, Hilden, Germany) and frozen at −80 °C. The supernatants were also frozen at −80 °C.

We purified 500 ng of RNA from the NPA cell pellet, quantified by a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) with phenol-chloroform. It was then reverse-transcribed with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA), and analyzed by semi-quantitative real-time PCR on a StepOnePlus Real-Time PCR System with TaqManTM probes for gene expression (Applied Biosystems) detection of 18s, *IFNG*, *TLR3*, *FLG*, *AREG*, interleukin (*IL*)-13, *IL*-33, *IL*-10, and TaqManTM Gene Expression MasterMix (Applied Biosystems),
following the manufacturer’s guidelines. Relative gene expression was calculated using the Cycle Threshold (Ct) and the $2^{-\Delta\Delta\text{Ct}}$ method\textsuperscript{21}, where:

$$\Delta\Delta\text{Ct} = \Delta\text{Ct}_{\text{TTV}^+} - \Delta\text{Ct}_{\text{TTV}^-}$$

and

$$\Delta\text{Ct} = \Delta\text{Ct}_{\text{gene}} - \Delta\text{Ct}_{\text{Housekeeping gene}}$$

In the NPA supernatant, filaggrin was analyzed by an enzyme-linked immunosorbent assay (ELISA) kit (Cloud-Cone Corp., TX, USA), following the manufacturer’s instructions.

**Statistical analysis**

The descriptive data were expressed as mean and first and third quartile (interquartile range [IQR]) for the continuous variables, and through counts and percentages for the categorical variables.

The continuous variables that followed a normal distribution were compared using a one-way analysis of variance with Bonferroni correction, or through T tests. When the distribution was not normal, we used the Mann–Whitney U test or Kruskal–Wallis test with Dunn correction. The categorical variables were compared using a chi-squared test or Fisher’s exact test, and results were expressed as odds ratios (ORs).

P-values <0.05 were considered statistically significant, and confidence intervals were calculated at 95% for all the estimations. The analyses were performed using SPSS software (version 21; SPSS Inc, Chicago, IL, USA) and with Graph-Pad Prism 8 (GraphPad Software Inc., San Diego, CA, USA).

**Results**

The study population consisted of 60 hospitalized children with a diagnosis of respiratory infection and 3 healthy control infants. A total of 51/60 (85%) patients had a positive common respiratory virus (CRV) identification, and 24 (47%) were coinfected with more than one virus. The median age was 11.8 months (IQR 1.4-23.5), 66.7% were male, and 9 patients had been born preterm (14.3%). The children were mainly recruited (75%) in April and May of 2021, coinciding in this pandemic year with a higher incidence of RSV respiratory infections during those months. The most commonly identified viruses were HRV and RSV in the same proportion (27/63; 42.9%), followed by human bocavirus (14; 32.2%) and HAdV (9; 14.3%), 2 parainfluenza, 1 HCoV, and 1 metapneumovirus.

TTV was identified in a total of 24/63 (38.1%) children, 23 in the case group and one in the healthy control group, who interestingly also had an asymptomatic HRV detection. No respiratory virus was detected in the two remaining children in the control group.

Regarding clinical data, 28/51 (55%) children had fever, with a median maximum temperature of 38.5 ºC (IQR 38-39.1), and 44/60 (73.3%) had hypoxia, with a median duration of 3 days of oxygen supplementation (IQR 2-4). Eleven patients had infiltrates or atelectasis on X-ray (18%). Bronchiolitis was the most frequent diagnosis (29/60; 48.3%), followed by 22/60 (36.6%) recurrent wheezing episodes. There were also 3 febrile syndromes, 3 upper respiratory infections (one with otitis), and 1 pneumonia, 1
laryngitis, and 1 laryngotracheobronchitis. The median hospital stay was 4 days (IQR 2-6.5), and 7/60 (11.6%) required PICU admission.

**Comparison between TTV-positive and -negative patients**

The 24 positive TTV patients were compared with the 39 TTV-negative children. The clinical data are shown in Table 1. Viral coinfection with another CRV was present in 95.8% (23/24) of the TTV-positive patients vs 74.4% (29/39) in the TTV-negative patients, p=0.029. No specific CRV was significantly associated with the presence of TTV. The two positive parainfluenza cases were associated with detection of TTV in NPA. Viral CRV coinfections were not associated with TTV presence.

The TTV-positive patients tended to be older, to have fever, and to need more frequent PICU admission than the negative ones, although the differences did not reach statistical significance. The TTV-positive children were also more likely to have radiological abnormalities, OR 2.6 (95% CI 1.3-5.2), p=0.030.

**Evaluation of the immune response and barrier integrity in children with and without TTV infection**

An aliquot of NPA was available in 25 patients, 12 with positive TTV (one was a healthy control) and another 13 with negative TTV (two were healthy controls).

Interestingly, filaggrin mRNA (a gene involved in epithelial barrier integrity) was found more often expressed in TTV- infants compared to the TTV+ (40% vs. 0%; p=0.04; Figure 1A). When we analyzed the filaggrin protein by ELISA in the NPA supernatants, we found mildly increased levels of filaggrin in the TTV+ group (33.1±2.8 ng/ml vs. 25.3±3.3 ng/ml; p=0.08; Figure 1B). When we evaluated other genes involved in the immune response (TLR3, IL-33, IL-10, IFNG, and IL-13), we did not find significant differences between the TTV- and TTV+ (p>0.05), although more TTV+ infants expressed TLR3 and IL33 compared with the TTV- (45% vs. 20% and 45% vs. 10%, respectively) (Figure 1A).

**Discussion**

The prevalence of TTV infection in children with ARI was high in our study, reaching 38% of cases. It was associated with an infection by another respiratory virus in practically all cases. The children with detection of TTV in NPA more frequently had pneumonia on radiography, and a clinical course with fever and frequent admission to the PICU. Our results appear to indicate that TTV infection is a marker of impaired immune response and a facilitator of respiratory infections and greater severity. The study of the immune response of our patients has shown that TTV+ children have a higher abundance of the filaggrin protein in NPA, with a lower gene expression. This result could mean that there is an alteration of the epithelial barrier in the TTV+ group, which is associated with liberation of this molecule to the supernatant of their NPA. Filaggrin is a protein involved in the integrity of the epithelial barrier and could play a role in allowing or favoring these respiratory infections\(^{22}\).
Chronic TTV infections have been reported in healthy individuals, and colonization is considered to begin very early in life, possibly transplacental in some cases, or in the family environment by the fecal-oral route\textsuperscript{23,24}. Colonization increases with age. Although the prevalence of TTV in blood samples has been more often studied than in respiratory secretions, the respiratory route is considered to be a frequent path of dissemination and contagion\textsuperscript{15}. The role of TTV in pediatric respiratory infections is poorly understood. Maggi F et al\textsuperscript{15}, in the most important study in this regard, found that the presence of TTV was associated with bronchopneumonia and other respiratory viral infections, similarly to our study. They hypothesized that coinfection could increase the severity of other respiratory infections. Our study supports this hypothesis, and we observed that our TTV+ patients had coinfection with other CRVs and a more severe clinical outcome.

To study the respiratory secretions of these children in terms of their immune response is tempting, to better understand the pathophysiology of this association. The TTV+ patients had higher expression of $\text{TLR3}$ and $\text{IL33}$ in the NPA. Although they were not statistically significant results, this lack of significance might be due to the small sample analyzed; however, it suggests that in the group infected by TTV there is a higher detection of genes upregulated by viral infection. The increased expression of $\text{TLR3}$ and $\text{IL-33}$ in NPA has been previously described by our group, primarily in children with bronchiolitis, and this was the main diagnosis in our cohort\textsuperscript{25}. They have also been overexpressed in animal models of asthma exacerbation\textsuperscript{26}. Some studies have shown that viruses like RSV or HRV induce IL-33 synthesis by damaging epithelium in pulmonary diseases\textsuperscript{27}.

The most important finding is that the frequency of TTV+ children that expressed filaggrin mRNA was lower than in the TTV-, and that the filaggrin protein was nevertheless increased in NPA from the TTV+. Filaggrin is a protein involved in the integrity of the epithelial barrier and its mutations have been associated with atopic dermatitis or ichthyosis\textsuperscript{28,29}. In our TTV+ patients, filaggrin is likely underexpressed, and the increase of filaggrin in NPA is part of the ongoing repair mechanism to maintain barrier function, triggered to respond to the epithelial damage caused by viral infection. Filaggrin release from keratohyalin granules into the keratinocyte cytoplasm is a main event in the cornification process, and it is critical for skin barrier function. The increase of filaggrin observed in the supernatant might be explained by damage to the cellular integrity and the consequent liberation of intracellular filaggrin to the extracellular medium\textsuperscript{30}.

Whether the presence of TTV in respiratory samples is a marker of a deficient immune response or is a consequence of it is difficult to elucidate. We cannot rule out that the previous presence of chronic TTV infection predisposes patients to infection by other CRVs and to a greater severity.

On the other hand, the association of altered filaggrin with the possible development of asthma, as well as increased Th-2 immune response in our patients who clinically had a diagnosis of bronchiolitis or recurrent wheezing, suggest that the presence of TTV infection could have a role in respiratory diseases. Several studies have suggested that TTV plays a role in the development and/or exacerbation of
respiratory diseases in childhood such as asthma\textsuperscript{31}. It has been postulated that TTV has a role in respiratory dysfunction, either alone or synergistically with other viruses, and could act as an enhancer of inflammation systemically or at specific body sites, such as the upper and lower airways\textsuperscript{32}.

A limitation of our study is that it was performed during a pandemic. Thus, and as has been previously mentioned, the sample size was small, and although plausible, it does not allow for firm conclusions to be drawn about the immune response. However, it provides a comprehensive view of TTV respiratory infection, which has been little studied so far, in a homogeneous population of infants.

In conclusion, TTV infection is common in children with viral respiratory infections and could be associated with pneumonia and greater severity, as well as an alteration in the epithelial barrier due to low filaggrin gene expression. Larger prospective studies will be able to unravel whether TTV favors respiratory viral infections or is a marker of impaired immune response.

Declarations

ACKNOWLEDGMENTS

The authors acknowledge the excellent technical assistance provided by Noelia Reyes; Vanessa Montero and Diana K Santos in the Respiratory Viruses and Inuenza Unit, CNM, ISCIII.

Funding

Funded by FIS (Fondo de Investigaciones Sanitarias – Spanish Health Research Fund- Grants: PI18CIII/00009 and PI18/00167. It has also been co-financed by a research grant from the Alfonso X el Sabio University (2018).

Competing interests

The authors declare no competing interests.

CONTRIBUTIONS

Research idea and study design: C.C and M.L.G-G; Data acquisition: C.C, T.R, S.A, M.I-C, F.P, J.M.R-M; Data analysis and interpretation: T.R, C.C, I.C, V.P, J.M.R-M, F.P, M.I-C; Original draft preparation: C.C, T.R, V.P; Funding acquisition: C.C., M.L.G-G, I.C. All authors have reviewed the manuscript.

References
1. Nishizawa, T., Okamoto, H., Konishi, K., Yoshizawa, H., Miyakawa, Y., & Mayumi, M. A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology. *Biochem Biophys Res Commun, 241*, 92–97 (1997). https://doi.org/10.1006/bbrc.1997.7765

2. Naoumov, N. V., Petrova, E. P., Thomas, M. G., & Williams, R. Presence of a newly described human DNA virus (TTV) in patients with liver disease. *Lancet, 352*, 195–197. (1998). https://doi.org/10.1016/S0140-6736(98)04069-0

3. Focosi, D., Antonelli, G., Pistello, M., & Maggi, F. Torquetenovirus: the human virome from bench to bedside. *Clin Microbiol Infect 22*, 589–593. (2016). https://doi.org/10.1016/j.cmi.2016.04.007

4. Walton, A. H., Muenzer, J. T., Rasche, D., Boomer, J. S., Sato, B., Brownstein, B. H., Pachot, A., Brooks, T. L., Deych, E., Shannon, W. D., Green, J. M., Storch, G. A., & Hotchkiss, R. S. Reactivation of multiple viruses in patients with sepsis. *PloS one, 9*, e98819 (2014). https://doi.org/10.1371/journal.pone.0098819

5. Thom, K., & Petrik, J. Progression towards AIDS leads to increased Torque teno virus and Torque teno minivirus titers in tissues of HIV infected individuals. *J Med Virol, 79*, 1–7 (2007). https://doi.org/10.1002/jmv.20756

6. Fogli, M., Torti, C., Malacarne, F., Fiorentini, S., Albani, M., Izzo, I., et al. Emergence of exhausted B cells in asymptomatic HIV-1-infected patients naïve for HAART is related to reduced immune surveillance. *Clin Dev Immunol, 2012*, 829584 (2012). https://doi.org/10.1155/2012/829584

7. Li, L., Deng, X., Da Costa, A. C., Bruhn, R., Deeks, S. G., & Delwart, E. Virome analysis of antiretroviral-treated HIV patients shows no correlation between T-cell activation and anelloviruses levels. *J Clin Virol, 72*, 106–113 (2015). https://doi.org/10.1016/j.jcv.2015.09.004

8. Zhong, S., Yeo, W., Tang, M. W., Lin, X. R., Mo, F., Ho, W. M., et al. Gross elevation of TT virus genome load in the peripheral blood mononuclear cells of cancer patients. *Ann N Y Acad Sci. 945*, 84–92. (2001). https://doi.org/10.1111/j.1749-6632.2001.tb03868.x

9. Maggi, F., Ricci, V., Bendinelli, M., Nelli, L. C., Focosi, D., Papineschi, F., et al. Changes In CD8+57+ T lymphocyte expansions after autologous hematopoietic stem cell transplantation correlate with changes in torquetenovirus viremia. *Transplantation, 85*, 1867–1868. (2008) https://doi.org/10.1097/TP.0b013e31817615e6

10. Masouridi-Levrat, S., Pradier, A., Simonetta, F., Kaiser, L., Chalandon, Y., & Roosnek, E. Torque teno virus in patients undergoing allogeneic hematopoietic stem cell transplantation for hematological malignancies. *Bone Marrow Transpl, 51*, 440–442. (2016). https://doi.org/10.1038/bmt.2015.262

11. Béland, K., Dore-Nguyen, M., Gagné, M. J., Patey, N., Brassard, J., Alvarez, F., et al. Torque Teno virus in children who underwent orthotopic liver transplantation: new insights about a common pathogen. *J Infect Dis, 209*, 247–254 (2014). https://doi.org/10.1093/infdis/jit423

12. Görzer, I., Haloschan, M., Jaksch, P, Klepetko, W., & Puchhammer-Stöckl, E. Plasma DNA levels of Torque teno virus and immunosuppression after lung transplantation. *Transplantation, 33*, 320–323 (2014). https://doi.org/10.1016/j.healun.2013.12.007
13. Rezahosseini, O., Drabe, C. H., Sørensen, S. S., Rasmussen, A., Perch, M., Ostrowski, S. R., et al. Torque-Teno virus viral load as a potential endogenous marker of immune function in solid organ transplantation. Transplant Rev (Orlando, Fla.); 33, 137–144 (2019). https://doi.org/10.1016/j.tre.2019.03.004

14. Pifferi, M., Maggi, F., Di Cristofano, C., Cangiotti, A. M., Nelli, L. C., Bevilacqua, G., et al. Torquetenovirus infection and ciliary dysmotility in children with recurrent pneumonia. The Pediatr Infect Dis J, 27, 413–418 (2008). https://doi.org/10.1097/INF.0b013e318162a14f

15. Maggi, F., Pifferi, M., Fornai, C., Andreoli, E., Tempestini, E., Vatteroni, M., et al. TT virus in the nasal secretions of children with acute respiratory diseases: relations to viremia and disease severity. J Virol, 77, 2418–2425 (2003). https://doi.org/10.1128/jvi.77.4.2418-2425.2003

16. Freer, G., Maggi, F., Pifferi, M., Di Cicco, M. E., Peroni, D. G., & Pistello, M. The Virome and Its Major Component, Anellovirus, a Convoluted System Molding Human Immune Defenses and Possibly Affecting the Development of Asthma and Respiratory Diseases in Childhood. Front Microbiol 9:686 (2018). https://doi.org/10.3389/fmicb.2018.00686.

17. McConnochie K. Bronchiolitis. What's in the name? Am J Dis Child; 137: 11-13 (1983).

18. Garcia-Garcia, M. L., Calvo, C., Ruiz, S., Pozo, F., Del Pozo, V., Remedios, L., et al. Role of viral coinfections in asthma development. PloS one, 12, e0189083 (2017). https://doi.org/10.1371/journal.pone.0189083

19. Hussain, T., Hussain, T., Manzoor, S., Waheed, Y., Tariq, H., & Hanif, K. Phylogenetic analysis of Torque Teno Virus genome from Pakistani isolate and incidence of co-infection among HBV/HCV infected patients. Virol J, 9, 320 (2012). https://doi.org/10.1186/1743-422X-9-320

20. Hino S. TTV, a new human virus with single stranded circular DNA genome. Rev Med Virol;12:151–158 (2002). doi:10.1002/rmv.351

21. Livak, K. J., & Schmittgen, T. D.. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods (San Diego, Calif.); 25, 402–408 (2001). https://doi.org/10.1006/meth.2001.1262

22. Armengot-Carbo, M., Hernández-Martín, Á., Torrelo, A. The role of filaggrin in the skin barrier and disease development. Acta dermo-sifiliograficas; 106, 86–95 (2015). https://doi.org/10.1016/j.ad.2013.10.019

23. Gerner, P., Oettinger, R., Gerner, W., Falbrede, J., & Wirth, S. Mother-to-infant transmission of TT virus: prevalence, extent and mechanism of vertical transmission. Pediatr Infect Dis J. 19:1074–7 (2001). doi: 10.1097/00006454-200011000-00009.

24. Sugiyama, K., Goto, K., Ando, T., Mizutani, F., Terabe, K., Kawabe, Y., et al. Route of TT virus infection in children. J Med Virol; 59:204–7 (1999). doi: 10.1002/(sici)1096-9071(199910)59:2<204::aid-jmv13>3.0.co;2-t.

25. Sastre, B., García-García, M. L., Cañas, J. A., Calvo, C., Rodrigo-Muñoz, J. M., Casas, I., et al. Bronchiolitis and recurrent wheezing are distinguished by type 2 innate lymphoid cells and immune response. Pediatr Allergy Immunol;32:51–59 (2021). doi: 10.1111/pai.13317.
26. Mahmutovic Persson, I., Akbarshahi, H., Menzel, M., Brandelius, A., & Uller. Increased expression of upstream TH2-cytokines in a mouse model of viral-induced asthma exacerbation. *J Transl Med.* **16;**14:52 (2016). doi: 10.1186/s12967-016-0808-x.

27. Jackson, D. J., Makrinioti, H., Rana, B. M., Shamji, B. W., Trujillo-Torralbo, M. B., Footitt, J., et al. IL-33-dependent type 2 inflammation during rhinovirus-induced asthma exacerbations in vivo. *Am J Respir Crit Care Med* **190;**1373–1382 (2014). https://doi.org/10.1164/rccm.201406-1039OC

28. Smith, F. J., Irvine, A. D., Terron-Kwiatkowski, A., Sandilands, A., Campbell, L. E., Zhao, Y., et al. Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. *Nat Genet,* **38,** 337–342 (2006). https://doi.org/10.1038/ng1743

29. Cabanillas, B., & Novak, N. Atopic dermatitis and filaggrin. *Curr Opin immunol,* **42,** 1–8 (2016). https://doi.org/10.1016/j.coi.2016.05.002

30. Gutowska-Owsiak, D., de La Serna, J. B., Frizische, M., Naeem, A., Podobas, E. I., Leeming, M., et al. Orchestrated control of filaggrin–actin scaffolds underpins cornification. *Cell Death Dis* **9,** 412 (2018). doi: 10.1038/s41419-018-0407-2.

31. Pifferi, M., Maggi, F., Caramella, D., De Marco, E., Andreoli, E., Meschi, S., et al. High torque tenovirus loads are correlated with bronchiectasis and peripheral airflow limitation in children. *Pediatr Infect Dis J;* **25;** 804–8 (2006). doi: 10.1097/01.inf.0000232723.58355.f4.

32. Maggi, F., & Bendinelli, M. Immunobiology of the Torque teno viruses and other anelloviruses. *Curr Top Microbiol Immunol,* **331;**65–90 (2009). doi: 10.1007/978-3-540-70972-5_5.

### Tables

**Table 1. Comparison between TTV-positive and TTV-negative children**
| Clinical feature                  | TTV-positive (n=24) | TTV-negative (n=39) | OR (95% CI) | p    |
|----------------------------------|---------------------|---------------------|-------------|------|
| Male                             | 15/24 (62.5%)       | 27/39 (69.2%)       | 0.582       |      |
| Age (months)                     | 12.1 (2.5-21.4)     | 11.1 (1.3-31.4)     | 0.068       |      |
| Temperature ≥38 °C               | 14/23 (50.8%)       | 14/37 (37.8%)       | 0.068       |      |
| Highest temperature (IQR)        | 38.4 (37.9-38.9)    | 38.8 (38-39.4)      | 0.208       |      |
| Hypoxia (SatO2<93%)              | 19/23 (62.6%)       | 25/37 (67.5%)       | 0.156       |      |
| Abnormal X-ray                   | 8/23 (34.8%)        | 3/37 (8.1%)         | 2.6 (1.3-5.2) | 0.030|
| Antibiotic treatment             | 6/23 (26%)          | 8/37 (21.6%)        | 0.479       |      |
| Diagnosis:                       |                     |                     | 0.413       |      |
| Wheezing episode                 | 4/23 (17.4%)        | 8/37 (21.6%)        |             |      |
| Bronchiolitis                    | 13/23 (56.5%)       | 12/37 (32.4%)       |             |      |
| Pneumonia                        | 0                   | 1 (2.7%)            |             |      |
| Healthy control                  | 1/24 (4.2%)         | 2/39 (5.1%)         | 0.862       |      |

Blood test:

| Parameter                        | TTV-positive (IQR) | TTV-negative (IQR) | OR (95% CI) | p    |
|----------------------------------|---------------------|---------------------|-------------|------|
| Leukocytes (cells/mm³) (IQR)     | 10500 (8080-16750)  | 13260 (8520-16420)  | 0.649       |      |
| C-reactive protein (mg/L) (IQR)  | 11 (3-22)           | 7.9 (3.6-51)        | 0.984       |      |
| CRV in NPA                       | 23/24 (95.8%)       | 29/39 (74.4%)       | 0.61 (0.45-0.83)* | 0.029|

Outcomes:

| Parameter                        | TTV-positive (IQR) | TTV-negative (IQR) | OR (95% CI) | p    |
|----------------------------------|---------------------|---------------------|-------------|------|
| Hospital stay (days) (IQR)       | 5 (3-7)             | 3 (2-5)             | 0.948       |      |
| Fever duration (days) (IQR)      | 2 (1-4)             | 2 (1-4)             | 0.856       |      |
| Hypoxia duration (days) (IQR)    | 3 (2-5)             | 3 (2-4)             | 0.158       |      |
| PICU admission                   | 5/23 (21.7%)        | 2/37 (5.4%)         | 0.001       |      |
| Stay >7 days                     | 7/23 (30.4%)        | 8/37 (21.6%)        | 0.410       |      |

TTV: torque teno virus; OR: odds ratio, CI: confidence interval; CRV: common respiratory viruses; IQR, interquartile range; NPA: nasopharyngeal aspirate; SatO2: oxygen saturation.

* Negative TTV was a protective factor for CRV identification. Significant differences are in bold

**Figures**

**Figure 1**

Filaggrin expression is more frequent in infants without TTV, whereas its quantity in the supernatant is higher in the TTV+ group. (A) Frequency (%) of infants expressing the selected mRNA transcripts (TLR3,
IL-33, IL-10, IFNG, IL-13 and FLG) detected by qPCR. (B) Quantity of filagrin (ng/ml) in the supernatants of infants infected or not with TTV measured by ELISA. *p<0.05. TTV, torque teno virus; ND, not detected.