DR. KIRSI NUOLIVIRTA (Orcid ID: 0000-0002-2612-9449)
PROF. MATTI KORPPI (Orcid ID: 0000-0001-8858-1134)
DR. EERO LAUHKONEN (Orcid ID: 0000-0003-4654-7602)

Article type: Original Articles

Original article:

*Toll-interacting protein* polymorphisms in viral bronchiolitis outcomes

Sari Törmänen¹, Johanna Teräsjärvi², Kirsi Nuolivirta³, Qiushui He²,⁴ Matti Korppi¹, Eero Laukonen¹∗

¹Center for Child Health Research, Faculty of Medicine and Life Sciences, University of Tampere and University Hospital, Tampere, Finland
²Institute of Biomedicine, University of Turku, Turku, Finland
³Department of Paediatrics, Seinäjoki Central Hospital, Seinäjoki, Finland
⁴Department of Medical Microbiology, Capital Medical University, Beijing, China

*Corresponding Author:*
Dr Eero Laukonen, Center for Child Health Research, Arvo2 building, 33014 University of Tampere, Finland. eero.laukonen@fimnet.fi

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ped.14746

This article is protected by copyright. All rights reserved
Disclosure statement: The authors declare no conflict of interest

Short title: TOLLIP and post-bronchiolitis asthma

Word count 2495 words, 7 text pages, 3 tables

Table 1. Tollip rs116938768 and rs5743854 genotypes in relation to the clinical disease severity markers in 166 children admitted due to bronchiolitis before 6 months of age.

Table 2. Tollip rs116938768 and rs5743854 genotypes in relation to clinical outcomes at 5-7 years of age in 141 children admitted due to bronchiolitis before 6 months of age.

Table 3. Tollip rs116938768 and rs5743854 genotypes in relation to clinical outcomes at 11-13 years of age in 125 children admitted due to bronchiolitis before 6 months of age.
Abstract

Background Toll-interacting protein is a key factor in regulating innate immunity responses via gate-keeping Toll-like receptors. Genetic variance in innate immunity has been linked with susceptibility to infections. Children with viral bronchiolitis in infancy are at increased risk of later asthma. Aim was to evaluate the role of toll-interacting protein gene point mutations in severity of bronchiolitis and subsequent risk of asthma.

Methods Infants less than six-month-old were recruited during hospitalization due to bronchiolitis. In all, 166 children were prospectively followed-up to age of 1.5, 6 and 11 years. Clinical data on viral aetiology and severity markers, and further post-bronchiolitis asthma and lung function outcomes were compared to genetic differences in two single-nucleotide point mutations rs116938768 and rs5743854 in the toll-interacting protein gene.

Results Toll-interacting protein rs116938768 or rs5743854 did not show significant associations with severity markers or viral aetiology of bronchiolitis. Follow-up data on current asthma or lung function at 6 or 11 years of age after bronchiolitis were not associated with the investigated mutations.

Conclusion Toll-interacting protein gene point mutations in rs116938768 or rs5743854 were not involved with the clinical course of viral bronchiolitis in early infancy, and did not predict post-bronchiolitis asthma or lung function reduction by the age of 11 years.

Keywords: Viral bronchiolitis, Toll-interacting protein, Innate Immunity, Asthma, Lung function
INTRODUCTION
Bronchiolitis in infancy has long-term consequences in later respiratory health of the child. According to long-term follow-up studies, up to 40% of children hospitalized for bronchiolitis in infancy are diagnosed with asthma by the time they reach teenage. Parental asthma, atopic eczema of the child or rhinovirus as a causative agent of bronchiolitis are well known risk factors but do not fully explain why only some children with bronchiolitis develop asthma. Therefore, host genetics, especially genes regulating innate immunity, are likely to play an important role in the disease process.

Toll-like receptors (TLRs) are key molecules in detecting foreign pathogens, inducing innate immune responses and activating adaptive immunity to defend host against outside intruders. Toll-interacting protein (Tollip) is an inhibitory adaptive protein, widely expressed in the respiratory tract (1), which dampens TLR downstream signalling and controls the subsequent inflammatory responses (2). Several TLR gene polymorphisms have been associated with infectious and inflammatory diseases, including asthma and related atopic diseases, but Tollip gene is less studied. As a negative regulator of TLR signalling it is, however, an interesting molecule in the TLR pathway and therefore worth of investigating, as impairment in Tollip function might lead to excessive inflammatory responses.

Tollip rs5743854 and rs116938768 are closely located in the promoter region of the gene, and rs5743854 has been previously associated with attenuated mRNA expression in monocytes and increased inflammatory response after TLR2 stimulation (3). The carriage of Tollip rs5743854 wild allele has been linked with atopic eczema in one study (4). Data on Tollip rs116938768 functionality are lacking, but the reason to explore it was that due to apparent closeness of only 10 base-pairs it was suspected to have similar properties to rs5743854. Further support for this rationale came from a study looking at another SNP in close proximity Tollip rs5743899, where the carriage of variant allele was associated with attenuated lung function and a lower in vitro anti-viral response to rhinovirus infection in asthmatic subjects (5).
We aimed to investigate the role of two *Tollip* gene polymorphisms, rs116938768 and rs5743854 respectively, in the viral aetiology and severity of bronchiolitis under six months of age, and in the post-bronchiolitis outcome at 5-7 years and 11-13 years of ages.

**MATERIAL AND METHODS**

This study was conducted at the Department of Paediatrics, Tampere University Hospital, Finland, and the detailed design of this study has been described previously (6). In brief, 187 previously healthy infants were included in this study in 2001 to 2004 when they were hospitalized for bronchiolitis at less than six months of age. Bronchiolitis was defined as first episode of an acute lower respiratory tract infection characterized with runny nose, cough and diffuse wheezes or inspiratory crackles on auscultation. The first follow-up visit was arranged in 2003 to 2005 at the mean age of 18 months, and 139 children attended (7). The second follow-up visit was arranged in 2008 to 2010 when the children were 5 to 7 years of age and 166 children attended (8). The third follow-up visit was arranged in 2014 to 2015 when the children were 11 to 13 years of age, and 138 children attended (9). Whole blood samples were obtained for genetic studies at the first or second follow-up visit.

The viral aetiology of bronchiolitis was studied by antigen detection and polymerase chain reaction from nasopharyngeal aspirates. The studied viruses were respiratory syncytial virus (RSV), rhinovirus, human metapneumovirus, influenza A and B virus, parainfluenza type 1, 2 and 3 viruses, adenovirus and human bocavirus. The severity of bronchiolitis was evaluated as the need of supplementary oxygen, length-of-stay in hospital and the need of feeding support.

At the follow-up visits, data were collected on the occurrence of atopic eczema, wheezing episodes, asthma and allergy diagnoses and the use of asthma medications since the last follow-up visit by structured questionnaires. In addition, all follow-up visits included clinical examination and interview by a doctor. Lung function and bronchial responsiveness were evaluated by impulse oscillometry (Jaeger, Master Screen IOS, Höchberg, Germany) with exercise challenge test at the 5-7 years control visit (10), and by flow-volume spirometry (Vmax™ Carefusion, Becton, Dickinson and Company, NJ, USA) with a bronchodilation test at the 11-13 years control visit (11).
Current asthma was defined as the current use of inhaled corticosteroid (ICS) medication or having asthma-suggestive symptoms (repeated wheezing, or prolonged cough or night cough for at least 4 weeks) during the last 12 months prior to the control visit with a diagnostic finding in the exercise challenge or bronchodilation test. Previous asthma at 5-7 years control visit was defined as the previous use of ICS as continuous or intermittent maintenance medication for asthma, and if the child had previous or current asthma, the term “asthma ever” was used. Persistent asthma at 11-13 years control visit was defined as the presence of current asthma at both the 5-7 years and 11-13 years control visits. Allergic rhinitis was defined as symptoms of runny or stuffy nose or repeated sneezing outside infection during the last 12 months before the control visits. Atopic dermatitis was defined as doctor-diagnosed allergic eczema.

**Genetic studies**

In the present study, we determined two single nucleotide polymorphisms (SNPs) of the *Tollip* gene, rs5743854 and rs116938768, in samples from 166 children. PCR-based sequencing was used for the SNP detection and following primers were designed: (forward) 5’-TTCGGACGTGCGACCC-3 and (reverse) 5’-AACCGCGCCCCATCTTTA-3 and purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). The sequencing was performed at the Institute for Molecular Medicine Finland (FIMM) laboratories, Helsinki, Finland.

Population-based data on the *Tollip* rs116938768 and rs5743854 gene polymorphisms were available from the 1000 Genomes Project for 99 (12), and from the Genome Aggregation Database for 1738 Finnish subjects (3476 alleles) (13). The minor allele frequencies (MAFs) were compared between the study population and the Finnish data.

**ETHICS**

The study was carried out in accordance with the World Medical Association’s (WMA) declaration of Helsinki. We obtained informed parental consent, including the use of samples for genetic studies on bronchiolitis and asthma risk both during hospitalization and at the control visits. The protocol of the study was approved by the Ethics committee of the Tampere University.
Hospital district, Tampere, Finland. The personal data of the study subject were not given to the laboratories that performed the genetic studies, the Department of Medical Microbiology and Immunology, Turku, Finland, or the Institute for Molecular Medicine Finland laboratories, Helsinki, Finland.

Statistics
Statistical analyses were carried out with SPSS version 25.0 software (IBM Corp, NY, USA). The Chi-square test and Fisher’s exact tests were used, as appropriate, for categorized variables and pairwise comparison of two groups with Student’s t-test for continuous variables.

RESULTS

Hospitalization data
There were 166 children with genetic and clinical data available during hospitalization and 84 (50.6%) of them were boys. RSV was the causative agent in 114 (68.7%) cases, rhinovirus in 19 cases and other viruses in 20 cases (human metapneumovirus in 6, influenza A virus in 9, parainfluenza type 3 virus in 4 and adenovirus in 1 cases). The virus was not identified in 13 samples. Fifty-seven (34.3%) children needed feeding support, 31 (18.7%) needed supplemental oxygen and the mean length of hospital stay was 4.49 (range 0-22, SD 3.19) days.

The Tollip rs116938768 genotype was CC (wild) in 150 (90.4%) cases and variant CT in 16 (9.6%) cases. The variant TT genotype was not detected. The Tollip rs5743854 genotype was CC (wild) in 118 (71.1%) cases, variant CG in 41 (24.7%) cases and variant GG in 7 (4.2%) cases. There were no significant associations between the wild and variant genotypes and the virus aetiology of bronchiolitis nor the severity markers of bronchiolitis (Table 1).

Comparison with population genetic data
The minor allele frequency (MAF) of Tollip rs116938768 (allele T) in this study population was 0.05 and the MAF (allele G) of Tollip rs5743854 was 0.17. The MAFs in the bronchiolitis group did not differ from the general Finnish population according to FIN data in the 1000 Genomes project.
(p=0.48 and p=0.56, respectively), nor from the Finnish data in Genome Aggregation Database (p=0.67 and p=0.88, respectively)

5-7-year follow-up data

There were 141 children with clinical and genetic data available from the second follow-up visit at 5-7 years of age. In all, 18 (12.8%) of the children had continuous ICS medication for asthma and in addition, one child was diagnosed with asthma at the control visit based on asthma presumptive symptoms during the last 12 months and a positive finding in the exercise challenge test. Therefore, 19 (13.5%) of the children were regarded to have current asthma. Altogether 38 (27.0%) of the children had been diagnosed with current or previous asthma and according to the definition, had asthma ever. In addition, 42 (29.8%) children had current atopic dermatitis and 40 (28.4%) had current allergic rhinitis.

Asthma ever was somewhat more common in children with the Tollip rs5743854 variant CG or GG genotypes when compared together to wild CC genotype (38.8 vs. 23.3%, p=0.11) (Table 2). Further, the Tollip rs5743854 variant homozygous GG genotype alone compared to other genotypes combined (CC or CG) showed a weak association with asthma ever (66.7% vs. 25.2%, p=0.045). Tollip rs116938768 wild and variant genotypes did not show any significant association with asthma ever. There were no significant associations between the Tollip rs5743854 or rs116938768 wild and variant genotypes and ICS use, current asthma or presence of atopic dermatitis or allergic rhinitis (Table 2).

Lung function data were available from 100 cases with genetic data available. Neither Tollip rs5743854 or Tollip rs116938768 showed any significant difference between wild and variant genotypes in baseline lung function by IOS (data not shown). Tollip rs5743854 variant CG or GG genotypes as combined (n=75) showed on average less airways reactivity (increase in Rrs5 +3.4% vs. +10.4%, p=0.04) in response to exercise challenge compared to children with wild CC genotype (n=25). Tollip rs116938768 did not show any significant differences between wild and variant genotypes in the exercise challenge test (data not shown).
11-13-year follow-up data

There were 125 children with clinical and genetic data available from the third follow-up visit at 11-13 years of age. In all, 11 (8.9%) of the children had continuous ICS medication for asthma and in addition, four children were diagnosed with asthma at the control visit based on asthma presumptive symptoms during the last 12 months and a positive finding in spirometry. Therefore, 15 (12.0%) of the children were regarded to have current asthma. Nine children (7.2%) had had current asthma at both the 5-7 years and 11-13 years control visits and were regarded as having persistent asthma. In addition, 31 (24.8%) children had current atopic dermatitis and 19 (15.2%) had current doctor-diagnosed allergic rhinitis. There were no significant associations between the Tollip rs5743854 or rs116938768 wild and variant genotypes and ICS use, current asthma, persistent asthma or presence of atopic dermatitis or allergic rhinitis (Table 3).

In addition, lung function by spirometry and bronchodilation test were available from 84 cases with genetic data. There were no significant differences between Tollip rs5743854 or Tollip rs116938768 wild and variant genotypes in baseline spirometry or bronchial reversibility to bronchodilation (data not shown).

DISCUSSION

This long-term follow-up study did not find any significant associations between Tollip rs5743854 or rs116938768 polymorphisms and bronchiolitis in infancy or post-bronchiolitis outcome at 5-7 or 11-13 years of age. Early-life contacts with microbes and subsequent activation of innate immunity via TLRs are thought to be important for the normal maturation of adaptive immunity. Lack of microbial contacts or impaired function or defect in downstream signalling of TLRs may lead to sustained Th2-cytokine milieu that is characteristic for asthmatic airways. Tollip is a negative regulator of TLR-induced inflammatory responses, and a mutation in the encoding gene may therefore attenuate its anti-inflammatory function and lead to excessive inflammatory responses.
Tollip gene polymorphisms have previously been associated e.g. with the susceptibility to sepsis (14) and tuberculosis (15). In a mouse study Tollip deficiency was associated with enhanced lung eosinophilia (16). Only one previous study has investigated the connection between asthma and Tollip gene polymorphisms being located in close proximity to the now studied SNPs. Tollip rs5743899 in adult asthmatic subjects with variant genotype was associated with attenuated lung function indicated by lower FEV1/FVC-ratio when compared to subjects with wild genotype. In addition, this variant genotype was also associated with lower anti-viral response to rhinovirus infection in vitro (5).

In the present study, we found that Tollip rs5743854 variant genotypes showed a trend with having had an asthma diagnosis ever by 5-7 years of age, but this was no longer present at the 11-13 years visit. Further, the Tollip rs5743854 variant homozygous GG genotype did show a weak association with asthma ever at 5-7 years of age, when analysed as a subgroup against other genotypes. In line, Tollip rs5743854 variation was associated with decreased Tollip messenger ribonucleic acid expression in monocytes of infants vaccinated with BCG only if the variant genotype was homozygous (3).

The group defined as “asthma ever” consisted of 19 children with current asthma and 19 children with previous asthmatic symptoms before 5-7 years of age. In subgroup analyses, cases with rs5743854 variant genotypes, now analysed as combined, showed less reactivity in response to exercise challenge compared to wild genotype at preschool age. This suggests that Tollip rs5743854 variant genotypes may be associated with transient asthmatic symptoms after bronchiolitis, remitting by school-age and showing less airway reactivity in response to exercise. This is interesting in the light that majority of the study population presented RSV as a causative agent of bronchiolitis. RSV infections before the age of three years have been associated with non-atopic asthma phenotype characterized by wheezing up to age of 11 years and lower lung function at age 13 years (17). In the current study, baseline lung function testing did not show any significant associations with the investigated Tollip SNPs at 5-7 or 11-13 years of age. In addition, the investigated SNPs in this study were not associated with the viral aetiology or the severity outcomes of bronchiolitis in infancy. Thus, as the Tollip rs5743854 variant genotype may
be associated with transient wheezing symptoms, evidence for persistent post-bronchiolitis asthma or lower lung function was not found at the age of 11-13 years.

The main strengths of our study are the prospective design with carefully collected data during more than ten years of follow-up time, and the depth of phenotyping clinical outcomes. The study population is of Finnish origin and therefore highly homogeneous, which is a clear benefit in genetic studies enabling reliable comparisons to population-based databases. We also acknowledge a few limitations in our study. First, the sample size is relatively small for genetic studies which may have led to false negative results. Second, we did not study the functionality of the Tollip SNPs. Tollip rs5743854 is located in the non-coding promoter region of the gene and has been shown to be functional in previous studies (3). Data on the functionality of Tollip rs116938768, however, is lacking. Third, in terms of outcomes, such as post-bronchiolitis asthma, the results are applicable mainly for children, who have experienced moderate-to-severe bronchiolitis. Instead, in terms of bronchiolitis susceptibility, the negative results are applicable to population, but only to the Finnish population, since bronchiolitis cases were compared with two sets of Finnish population data.

The negative results of this study implicate, that the investigated polymorphisms in the Tollip gene are not involved in the severity or viral aetiology of bronchiolitis nor in the development of post-bronchiolitis asthma. However, other SNPs in the Tollip gene are still worth to be investigated in the future as Tollip clearly plays an important role in modulating inflammatory responses induced by TLRs.

Acknowledgements

ST declares receiving post-doctoral grants from Tampere tuberculosis foundation and Research Foundation of Pulmonary Diseases Finland.

Disclosure statement

This article is protected by copyright. All rights reserved
The authors declare no conflict of interest

**Author contributions**

ST was responsible for analyses and wrote the first draft, JT participated in the analyses and writing, KN and MK, attended to the design of the study and writing, QH was responsible for the genetic analysis and participated in the writing, EL was responsible for the final paper. All authors read and approved the final manuscript.
(1) Moncayo-Nieto OL, Wilkinson TS, Brittan M, McHugh BJ, Jones RO, Conway Morris A, et al. Differential response to bacteria, and TOLLIP expression, in the human respiratory tract. BMJ Open Respir Res 2014 September 11;1(1):e000046-000046. eCollection 2014.

(2) Zhang G, Ghosh S. Negative regulation of toll-like receptor-mediated signaling by Tollip. J Biol Chem 2002 March 01;277(9):7059-7065.

(3) Shah JA, Musvosvi M, Shey M, Horne DJ, Wells RD, Peterson GJ, et al. A Functional Toll-Interacting Protein Variant Is Associated with Bacillus Calmette-Guerin-Specific Immune Responses and Tuberculosis. Am J Respir Crit Care Med 2017 August 15;196(4):502-511.

(4) Schimming TT, Parwez Q, Petrasch-Parwez E, Nothnagel M, Epplen JT, Hoffjan S. Association of toll-interacting protein gene polymorphisms with atopic dermatitis. BMC Dermatol 2007 March 16;7:3-3.

(5) Huang C, Jiang D, Francisco D, Berman R, Wu Q, Ledford JG, et al. Tollip SNP rs5743899 modulates human airway epithelial responses to rhinovirus infection. Clin Exp Allergy 2016 December 01;46(12):1549-1563.

(6) Nuolivirta K, Hurme M, Halkosalo A, Koponen P, Korppi M, Vesikari T, et al. Gene polymorphism of IFNG +874 T/A and TLR4 +896 A/G and recurrent infections and wheezing in toddlers with history of bronchiolitis. Pediatr Infect Dis J 2009 Dec;28(12):1121-1123.

(7) Nuolivirta K, Koponen P, He Q, Halkosalo A, Korppi M, Vesikari T, et al. Bordetella pertussis infection is common in nonvaccinated infants admitted for bronchiolitis. Pediatr Infect Dis J 2010 Nov;29(11):1013-1015.

(8) Koponen P, Helminen M, Paassilta M, Luukkaala T, Korppi M. Preschool asthma after bronchiolitis in infancy. Eur Respir J 2012 Jan;39(1):76-80.

(9) Tormanen S, Lauhkonen E, Riikonen R, Koponen P, Huhtala H, Helminen M, et al. Risk factors for asthma after infant bronchiolitis. Allergy 2018 April 01;73(4):916-922.

This article is protected by copyright. All rights reserved
(10) Lauhkonen E, Koponen P, Nuolivirta K, Paassilta M, Toikka J, Korppi M. Lung function by impulse oscillometry at age 5-7 years after bronchiolitis at age 0-6 months. Pediatr Pulmonol 2015 Apr;50(4):389-395.

(11) Riikonen R, Lauhkonen E, Tormanen S, Backman K, Koponen P, Helminen M, et al. Prospective study confirms that bronchiolitis in early infancy increases the risk of reduced lung function at 10-13 years of age. Acta Paediatr 2019 January 01;108(1):124-130.

(12) 1000 Genomes Project Consortium, Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, et al. An integrated map of genetic variation from 1,092 human genomes. Nature 2012 Nov 1;491(7422):56-65.

(13) Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature 2016;536:285.

(14) Song Z, Yin J, Yao C, Sun Z, Shao M, Zhang Y, et al. Variants in the Toll-interacting protein gene are associated with susceptibility to sepsis in the Chinese Han population. Crit Care 2011;15(1):R12.

(15) Wu S, Huang W, Wang D, Wang Y, Wang M, Zhang M, et al. Evaluation of TLR2, TLR4, and TOLLIP polymorphisms for their role in tuberculosis susceptibility. APMIS 2018 June 01;126(6):501-508.

(16) Ito Y, Schaefer N, Sanchez A, Francisco D, Alam R, Martin RJ, et al. Toll-Interacting Protein, Tollip, Inhibits IL-13-Mediated Pulmonary Eosinophilic Inflammation in Mice. J Innate Immun 2018;10(2):106-118.

(17) Stein RT, Sherrill D, Morgan WJ, Holberg CJ, Halonen M, Taussig LM, et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. Lancet 1999 Aug 14;354(9178):541-545.
Table 1. *Tollip* rs116938768 and rs5743854 genotypes in relation to the clinical disease severity markers in 166 children admitted due to bronchiolitis before 6 months of age.

|                     | *Tollip* rs116938768 |                    | *Tollip* rs5743854 |                    |
|---------------------|----------------------|--------------------|---------------------|--------------------|
|                     | Genotype CC (wild)   | Genotype CT (variant) | Genotype CC (wild)   | Genotype CG or GG (variant) |
|                     | n=150                | n=16               | n=118               | n=48               |
| **Clinical finding**| **n** | **%** | **n** | **%** | **p-value*** | **n** | **%** | **n** | **%** | **p-value*** |
| Feeding support (n=57) | 52 | 34.7 | 5 | 31.3 | 1.00 | 43 | 36.4 | 14 | 29.2 | 0.47 |
| Oxygen supplementation (n=31) | 29 | 19.3 | 2 | 12.5 | 0.74 | 25 | 21.2 | 6 | 12.5 | 0.27 |
| RSV positive (n=114) | 103 | 68.7 | 11 | 68.8 | 1.00 | 82 | 69.5 | 32 | 66.7 | 0.72 |
| Length-of-stay (mean, SD) | 4.57 (SD 3.26) | 3.69 (SD 2.14) | 0.30 | 4.64 (SD 3.05) | 4.10 (SD 3.5) | 0.33 |

*p pairwise comparison wild vs. variant*
Table 2. *Tollip* rs116938768 and rs5743854 genotypes in relation to clinical outcomes at 5-7 years of age in 141 children admitted due to bronchiolitis before 6 months of age.

| Genotype | rs116938768 | rs5743854 |
|----------|-------------|-----------|
| CC (wild)| n=128       | n=103     |
| CT (variant)| n=13       | n=38      |

| Clinical finding | Genotype | n | % | Genotype | n | % | p-value* |
|------------------|----------|---|---|----------|---|---|---------|
| ICS use (n=18)   | CC       | 17| 13.3 | CT       | 1 | 7.7| 1.00    |
| Current asthma (n=19) | CC | 19| 14.8 | CT | 0 | 0.0| 0.22    |
| Asthma ever (n=38) | CC | 35| 27.3 | CT | 3 | 23.1| 1.00    |
| Allergic rhinitis (n=40) | CC | 35| 27.3 | CG or GG | 5 | 38.5| 0.29    |
| Atopic dermatitis (n=42) | CC | 40| 31.3 | CG or GG | 2 | 15.4| 0.27    |

*pairwise comparison wild vs. variant*
Table 3. *Tollip* rs116938768 and rs5743854 genotypes in relation to clinical outcomes at 11-13 years of age in 125 children admitted due to bronchiolitis before 6 months of age.

| *Tollip* rs116938768 | Genotype CC (wild) | Genotype CT (variant) |
|----------------------|-------------------|----------------------|
|                      | n=115             | n=10                 |
| **Clinical finding** |                   |                      |
| ICS use (n=11)       | 9                 | 2                    | 0.21 |
| Current asthma (n=15)| 13                | 2                    | 0.34 |
| Persistent asthma (n=9) | 8                | 1                    | 0.54 |
| Atopic dermatitis (n=31) | 30             | 1                    | 0.45 |
| Allergic rhinitis (n=19) | 18             | 1                    | 0.63 |

| *Tollip* rs5743854 | Genotype CC (wild) | Genotype CG or GG (variant) |
|---------------------|-------------------|----------------------------|
|                     | n=92              | n=33                       |
| **Clinical finding** |                   |                            |
| ICS use (n=11)      | 8                 | 3                         | 1.00 |
| Current asthma (n=15)| 10               | 5                         | 0.54 |
| Persistent asthma (n=9) | 6             | 3                         | 0.70 |
| Atopic dermatitis (n=31) | 22             | 9                         | 0.82 |
| Allergic rhinitis (n=19) | 14             | 5                         | 1.00 |

*pairwise comparison wild vs. variant*