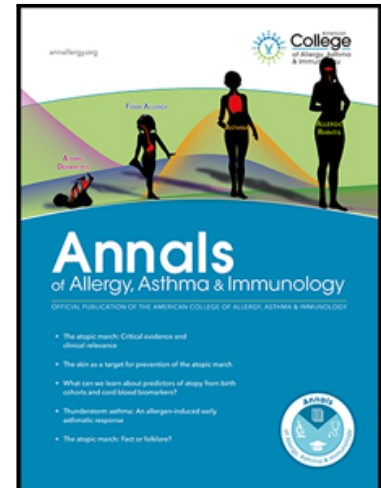


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Heterogeneous associations of polyomaviruses and herpesviruses with allergy-related phenotypes in childhood

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**Title page**

**Title:** Heterogeneous associations of polyomaviruses and herpesviruses with allergy-related phenotypes in childhood

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**Keywords:** asthma, children, eczema, herpesviruses, polyomaviruses

**Abbreviations:** IgG: immunoglobulin G; RSV, respiratory syncytial virus; MeDAL: Mechanisms of the Development of Allergy

Background. Evidence suggests a complex interplay between infections and allergic diseases.

**Objective.** To explore the association of fourteen common viruses with eczema, asthma and rhinoconjunctivitis in childhood.

**Methods.** We used cross-sectional (n=686) and prospective (n=440) data from children participating in the Rhea birth cohort. IgG to polyomaviruses (BKPyV, JCPyV, KIPyV, WUPyV, HPyV6, HPyV7, TSPyV, MCPyV, HPyV9, HPyV10) and herpesviruses (EBV, CMV, HSV-1, HSV-2) were measured at age four by fluorescent bead-based multiplex serology. Definitions of eczema, asthma and rhinoconjunctivitis at age four and six were based on questionnaires. Mediation of the associations by immune biomarkers was tested.

**Results.** Less likely to have eczema at age four were KIPyV [OR:0.47 (95% CI:0.27, 0.82)] and HPyV6 [OR:0.44 (95% CI:0.26, 0.73)] seropositives compared to their seronegative counterparts. EBV seropositivity was negatively associated with eczema at age four [OR:0.39 (95% CI:0.22, 0.67)] and six [OR:0.50 (95% CI:0.25, 0.99)]. Children with a higher burden of herpesviruses or of skin polyomaviruses had the lowest odds of eczema at age four. Higher odds for asthma at age four were found for WUPyV seropositive children [OR:3.98 (95% CI:1.38, 11.51)], and for children seropositive to both respiratory polyomaviruses (KIPyV and WUPyV) [OR:7.35 (95% CI:1.66, 32.59)] compared to children seronegative to both. No associations were observed for rhinoconjunctivitis. There was no evidence of mediation by immune biomarkers.

**Conclusion.** A heterogeneous pattern of infections and allergic diseases was observed with common infections associated to a decreased eczema risk and an increased asthma risk in children.

## Manuscript

### Introduction

The interplay between infections and allergic diseases in early life is complex<sup>1</sup>. On the one hand, human respiratory syncytial virus (RSV) and rhinovirus infections are important risk factors for asthma development<sup>2,3</sup>. However and although data are more limited, this type of association may also occur with other viral, bacterial and helminth infections<sup>4,5</sup>. Moreover, certain pathogens contribute to the clinical course of allergic diseases (e.g. *Staphylococcus aureus* in eczema flares, rhinoviruses in asthma exacerbations) and atopic children are at increased risk of severe infections (e.g. hospitalization due to influenza for asthmatics). On the other hand, a normal course of infections in early life has been hypothesized to confer protection against the development of allergic diseases (the hygiene and “old friends” hypothesis)<sup>6</sup>. This was first based on observations of a protective effect of sibship size, a farming childhood and early day care attendance in the development of allergic diseases<sup>6-8</sup>. Subsequent studies explored the contribution of specific infections or of a composite infectious index, sometimes using biological measures, but provided divergent results<sup>9-15</sup>.

New evidence might emerge if we explore the association of novel pathogens with allergic diseases. Polyomaviruses are recently identified DNA viruses, worldwide spread and acquired subclinically in childhood. KIPyV and WUPyV are found in respiratory samples but their association with disease remains speculative and no study has assessed their role in asthma<sup>16</sup>. Moreover, MCPyV (associated with Merkel cell

carcinoma, a rare skin cancer among the elderly), TSPyV (associated with trichodysplasia spinulosa, a rare skin condition among immunocompromised patients), HPyV6, HPyV7 (both recently linked to pruritic skin eruptions) and HPyV9 are all polyomaviruses who reside chronically in the skin. It would be interesting to explore the association of such innocent skin viruses to the general population with eczema, given the possible role of dysbiosis in disease manifestation and severity<sup>17</sup>. Herpesviruses are also suspected to play a role in allergic diseases because of their efficiency in establishing chronic infections, their interplay with the immune system and the recent changes in their epidemiology, which have occurred in parallel to the rise of allergies<sup>18,19</sup>. Nonetheless results are not conclusive and differ remarkably. For example, different studies have shown a negative, a positive or no association between EBV seropositivity and IgE sensitization or skin prick test<sup>14,15,20-27</sup>. None of those studies have found an association between EBV and eczema, asthma or allergic rhinitis. Moreover, mediation effects are rarely examined. Infections may have prolonged effects on the host immune system<sup>28,29</sup>, and alterations in immune biomarkers could be a potential biological mechanism explaining the association of infections with allergic diseases<sup>30</sup>.

The purpose of this study was to examine whether acquisition of polyomaviruses and herpesviruses in the first four years of life is associated with allergy related phenotypes at four and six years of age and to evaluate the role of immune biomarkers in these associations.

## Methods

The Rhea study is an ongoing birth cohort that recruited 1606 pregnant women from February 2007 to January 2008 ([www.rhea.gr](http://www.rhea.gr)) and followed-up mothers during pregnancy, delivery and children up to present<sup>31</sup>. The study was approved by the Ethical Committee of the University Hospital of Heraklion (Reference No: 96-6/2/2007) and all participants provided written informed consent.

#### **Exposure assessment**

Serology is widely used to assess previous exposure to the polyomaviruses and herpesviruses, which after primary infection have the ability to remain in a latent or persistent state in the body. Serological analysis took place in the German Cancer Research Center, Heidelberg, Germany using serum samples collected at age four. Immunoglobulin G (IgG) seroreactivity against the viral capsid protein 1 of ten polyomaviruses (BKPyV, JCPyV, KIPyV, WUPyV, HPyV6, HPyV7, TSPyV, MCPyV, HPyV9, HPyV10), four EBV antigens, five CMV antigens and one HSV-1 antigen was measured by fluorescent bead-based multiplex serology and details can be found elsewhere<sup>32</sup>. Seropositivity for a given antigen was defined as values greater than the antigen-specific cut-off<sup>32</sup>. EBV and CMV seropositivity was defined as seropositivity for at least two virus specific antigens<sup>32</sup>. The aggregate number of polyomaviruses or herpesviruses that the children were seropositive to were referred as polyomaviruses or herpesviruses burden respectively<sup>32</sup>. We examined the polyomaviruses burden in categories of “ $\leq 3$  polyomaviruses”, “4–7 polyomaviruses”, and “ $\geq 8$  polyomaviruses” based on the frequency distribution of this variable (approximately defining the lowest and highest 15%). Furthermore, we created the burden of polyomaviruses by their site of latency, revealing the burden of skin (HPyV6, HPyV7, TSPyV,

MCPyV, HPyV9), of respiratory (KIPyV, WUPyV) and of the other polyomaviruses (BKPyV and JCPyV residing in the urinary tract, HPyV10 residing in the gastrointestinal tract). The burden of skin polyomaviruses was examined in categories of “0-1 polyomavirus” and “2-5 polyomaviruses” (defining the lowest 30% and highest 70% correspondingly) based on the prevalence of eczema in these groups (hypothesis and data driven categorization)<sup>33</sup>. The herpesviruses burden was examined as ‘0 viruses’, ‘1 virus’, and ‘2–3 viruses’. The last category was a combination because few children had three viral seropositivities (n=6, 0.9%). These categories were defined so as to examine differences between children with meaningful different levels of acquisition of viruses and have been used before by our group.

#### **Outcome assessment**

Phenotypes were determined from symptom-based questionnaires that parents completed through personal interview at ages four and six. Definitions have been developed by a panel of experts within the Mechanisms of the Development of Allergy (MeDALL) consortium<sup>34</sup>. Current eczema was defined as positive answer to both: (1) “Has your child had an itchy rash which was coming and going (intermittently) at any time in the past 12 months?”, (2) “Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes”. Current asthma was defined as a positive answer to at least two of the three following questions: (1) “Has your child ever been diagnosed by a doctor as having asthma?”, (2) “Has your child had wheezing or whistling in the chest in the past 12 months?”, (3) “Has your child taken any medicines for asthma or breathing difficulties (wheezing, chest tightness,

shortness of breath) in the last 12 months?”. Current rhinoconjunctivitis was defined as a positive answer to both (1) “Has your child had problems with sneezing or a runny or blocked nose when he/she did not have a cold or flu in the past 12 months?” (2) “If yes, in the past 12 months, were the nose symptoms accompanied by itchy, watering eyes?”.

#### **Immune biomarkers**

Several immune biomarkers were measured in blood samples collected at age four. The Milliplex Map human high sensitivity T cell magnetic bead panel (Cat. # HSTCMAG-28SK) from Millipore (Billerica, MA) was used for the simultaneous quantification of IFN- $\gamma$  IL-1 $\beta$ , IL-6, IL-8, IL-10, IL-17 $\alpha$ , MIP-1 $\alpha$  and TNF- $\alpha$  using the automated analyzer Luminex 100 connected with the Luminex xPONENT software<sup>35</sup>.

#### **Statistical analysis**

Descriptive analyses of the study population characteristics, exposures and outcomes were conducted. We used logistic regression models to examine the association (OR, 95% CI) of single infections (seropositive vs seronegative), polyomaviruses and herpesviruses burden on outcomes based on MeDALL definitions. Tests for trend were tested by assigning ordinal variables in categories of exposure as continuous variables in each model (polyomaviruses and herpesviruses burden). Interaction between EBV and CMV serostatus with outcomes<sup>25</sup>, was tested by including appropriate interaction terms in regression models.

Confounders were identified with directed acyclic graphs (eFigure1), based on background knowledge on determinants of the exposure and outcome variables<sup>32,36</sup>. We included the following confounders: maternal education (low:  $\leq 9$  years; medium:  $> 9$  years up to attending postsecondary school education; high: attending university or having a university/technical college degree), child's gender (male, female), breastfeeding (never, 1–6 months,  $>6$  months), older siblings (yes, no), day care attendance at an early age ( $< 2$  years,  $\geq 2$  years of age), passive smoking inside the home (yes, no) and residency at outcome assessment (urban, rural). Family history of allergic diseases, delivery mode, preterm birth, pets, mould, child's body mass index, frequency of handwashing and house cleaning, were considered but did not meet criteria for confounding. Missing data on selected covariates were imputed to minimize the impact of lack of data (0.6% to 8.4%). We applied logistic and ordinal-logistic chained equations to multiply impute missing values (mi impute procedure in STATA 13.0) and 20 imputed data sets were generated<sup>37</sup>. As recommended, the imputation models included the exposure and outcome variables of the analysis.

Mediation analysis was conducted to further assess whether the effects of viruses on allergy related phenotypes operated through immune biomarkers (eFigure1). Because original distributions of immune biomarkers were skewed, they were log2-transformed. Mediation analysis decomposes the total effect into direct and indirect effects<sup>38</sup>. We used the paramed command in STATA, which performs causal mediation analysis using parametric regression models<sup>39</sup>, as an extension of Baron and Kenny method. Mediation analysis was conducted in the non-imputed dataset because paramed command is not officially supported with multiple imputation.

All significant tests were two-sided using  $p < 0.05$  as the level of statistical significance. All statistical analyses were performed using STATA version 13.1 (Statacorp, College Station, TX).

## Results

### Participants

Of 1,363 singleton live births of the Rhea cohort, 879 and 607 children attended the four and six years of age follow-ups respectively. At age four, serological data were available for 690 children. Almost all children (680 to 686 children, depending on the outcome) had data at the same age on eczema, asthma or rhinoconjunctivitis whereas up to 440 children had data at age six on similar phenotypes and constitute the population of the present analyses. Children with serology at age four and outcome data at age six versus those without outcome data at age six were more likely to be boys (55% versus 48%), of mothers with higher educational level (36% versus 23%), but did not differ in any other characteristic (family history of allergic diseases, seroprevalence of viruses, environmental exposures or lifestyle characteristic).

### Descriptives

At age four, seroprevalence to polyomaviruses ranged from 21% for HPyV9 to 82% for HPyV10 [BKPyV (76%), JCPyV (34%), KIPyV (44%), WUPyV (75%), HPyV6 (62%), HPyV7 (23%), TSPyV (51%), MCPyV (68%)] whereas, 53%, 26% and 4% were EBV, CMV and HSV-1

seropositive respectively. Table 1 presents the occurrence of allergy related symptoms and phenotypes in the study population. At age four and six years, the prevalence of eczema was 9.8% and 9%, of asthma 7% and 9.8% and of rhinoconjunctivitis 5.4% and 3.7% respectively using the MeDALL definition.

#### Single viruses

Figure 1 depicts the adjusted associations (OR, 95% CI) of polyomaviruses and herpesviruses seropositivity at age four with eczema, asthma and rhinoconjunctivitis at age four and six using the imputed dataset. Children seropositive to KIPyV and HPyV6 were less likely to have eczema at age four [OR:0.49 (95% CI:0.28, 0.88)] and [OR:0.44 (95% CI:0.26, 0.73)] accordingly compared to their seronegative counterparts. Similarly, EBV seropositivity was negatively associated with eczema at age four [OR:0.39 (95% CI:0.22, 0.67)] and six [OR:0.50 (95% CI:0.25, 0.99)]. Higher odds for asthma at age four were found for WUPyV seropositive children [OR:3.98 (95% CI:1.38, 11.51)]. No evidence for a multiplicative interaction between EBV and CMV serostatus with any of the outcomes was observed (all interaction terms had p-values>0.28).

#### Multiple viruses

Table 2 presents associations between the polyomaviruses and herpesviruses burden at age four with eczema, asthma and rhinoconjunctivitis at age four and six using the imputed dataset. Children with “2-3 herpesviruses” had lower odds of eczema at age four [OR:0.37 (95% CI:0.17, 0.84)] as well as those with “1 herpesvirus ” [OR:0.57 (95% CI:0.33, 0.99)] versus those with “0 herpesviruses” with a statistically significant

dose-response association ( $p_{\text{trend}}$ : 0.007). Children with “2-3 herpesviruses” had also lower odds for eczema at age six [OR:0.26 (95% CI:0.07, 0.93)] versus those with “0 herpesviruses” ( $p_{\text{trend}}$ : 0.046). Polyomaviruses burden was not associated with allergy related phenotypes but only when we examined by site of latency (skin, respiratory tract, other). We found that children seropositive to “2-5 skin polyomaviruses” versus those with “0-1 skin polyomavirus” had lower odds of eczema at age four [OR:0.51 (95% CI:0.31, 0.88)] (table 3, using the imputed dataset). Moreover, higher odds of asthma at age four were found among children seropositive to “2 respiratory polyomaviruses” [OR:7.35 (95% CI:1.66, 32.59)] or “1 respiratory polyomavirus” [OR: 4.48 (95% CI:1.02, 19.63)] compared to children seronegative to both ( $p_{\text{trend}}$ : 0.003) (table 3).

No association was found between polyomaviruses and herpesviruses with rhinoconjunctivitis.

#### **Additional analyses**

Mediation effects were examined in the statistically significant associations identified above. There was no evidence of mediation by levels of immune biomarkers at age four (non-statistically significant natural indirect effects, all p-values >0.11, eTables 1 and 2). When we restricted the analyses at age four only in children that were also assessed at age six, we observed no noteworthy changes in the results (data not shown). When we repeated analysis in the non-imputed dataset, results were materially unchanged. Moreover, in 60 children of the present analyses we had available serological data at age three<sup>32</sup>. We used these data to explore whether timing of exposure is important. Results did not indicate that children who seroconverted and those that were stable seropositive had different seroprevalence of allergy related phenotypes but we

acknowledge that we lack power to identify statistical significant results (data not shown). Lastly, we combined outcome data at ages four and six and created the longitudinal phenotypes of eczema, asthma and rhinoconjunctivitis (“never”: not at age four not at age six; “transient”: either at age four or at age six; “persistent”: at both ages four and six). We observed that children seronegative to HPyV6 and EBV had statistically significant higher prevalence of both persistent and transient eczema versus those seropositive with differences being more evident for persistent than transient eczema (Figure 2). Also children seropositive to 2-3 herpesviruses were more likely to never had eczema (93% of them versus 84.2% among those with 1 herpesvirus seropositivity and 80.7% among those with 0 herpesvirus seropositivity), whereas children seronegative to all herpesviruses had the highest prevalence of persistent eczema among the three groups (Figure 2).

## Discussion

This study shows heterogeneous results in the association of polyomaviruses and herpesviruses with eczema and asthma in childhood. Acquisition of KIPyV, HPyV6, EBV, a higher burden of herpesviruses as well as of skin polyomaviruses was inversely related to eczema. On the other hand, WUPyV seropositivity and a higher burden of respiratory polyomaviruses were associated with higher odds for asthma. No significant associations were found between viruses and rhinoconjunctivitis.

A systematic review in 2011, concluded that apart from helminth infections there are no consistent findings for other pathogens to be linked with eczema<sup>40</sup>. Most recent data though point to a role of skin commensal microbes, including coagulase negative staphylococci, in eczema risk and

severity<sup>41</sup>. Nevertheless, no previous study has investigated the association of polyomaviruses with eczema and only scarce data exist for herpesviruses. Four studies explored the association of EBV with eczema showing null results but two of those studies found increased levels of EBV antibodies in children and adults with atopic dermatitis<sup>42-45</sup>. We observed a very robust negative association between EBV acquisition in the first four years of life and eczema manifestation up to age six. Support for an atopy-protective role of EBV is found in several studies using markers of allergic sensitization (total IgE, allergen specific IgE, skin prick tests) but contradictory results exist as well<sup>14,15,20-27</sup>. Characteristically a study in Swedish children showed that those infected with EBV <2 years of age were less likely to be persistently IgE sensitized whereas children who were infected between 2-5 years of age were more likely to be IgE sensitized at 5 years of age<sup>20</sup>. In our study, timing of exposure did not change results but we have only limited serological data at an earlier time point (3 years of age). We should note that we did not observe any association between HSV-1, which is the most common pathogen in eczema herpeticum, and eczema.

Eczema risk decreased, increased or did not change with a higher number of clinically evident infections in different studies. In contrast, we examined the effect of common “innocent” infections serologically confirmed and found that a higher burden of herpesviruses infections and of skin polyomaviruses decreased the odds for eczema in childhood. This finding is in accordance with the hygiene hypothesis, which proposes that experience of common infections in childhood is a natural process and exerts allergy-protective effects<sup>6</sup>. If this is true for eczema, then why results are not replicated for asthma or rhinoconjunctivitis in our study? A possible explanation is that eczema is a disease perhaps more clearly atopic than asthma or rhinoconjunctivitis, of which only a proportion of symptoms at this young age are attributable to atopy<sup>46</sup>. Moreover, we

observed that lack of exposure specifically to skin polyomaviruses – and not in generally to all polyomaviruses- was positively associated with eczema. Skin polyomaviruses are shed chronically from the skin of healthy individuals and are considered members of the skin microbiome<sup>47</sup>. Increasing evidence suggests that eczema manifestation and progression might be related to an imbalance in acquisition of normal flora<sup>48</sup>. As an example, children colonized with commensal staphylococci, other than staphylococcus aureus, at 2 months of age had lower risk of eczema at one year of age<sup>49</sup>. Promising results come from clinical trials trying to reconstruct the skin microbiome of patients with eczema<sup>50</sup>. Nonetheless, viruses are the least studied of all members of the skin microbiota.

Respiratory viruses mostly of RNA genome likely contribute to the pathogenesis of asthma<sup>5,51</sup>. Trials have shown for first time that modification of clinical evident infections with RSV or rhinovirus may change the natural course of asthma<sup>52</sup>. Pediatric population is exposed to a variety of other respiratory viruses. Wesolowska-Andersen et al showed that even clinically silent respiratory viruses that are present in the respiratory tract may promote profound biological response in the airway<sup>53</sup>. WUPyV and KIPyV are DNA viruses detected in respiratory samples of asymptomatic children at varying frequencies<sup>16</sup>. Based on existing literature, respiratory polyomaviruses are not likely contributing to severe respiratory illness (e.g. requiring hospitalization) even among immunocompromised<sup>54,55</sup> but it is possible that they play a role in mild respiratory disease experienced by young children<sup>56,57</sup>. We are the first to explore and find a positive association between WUPyV and a higher burden of respiratory polyomaviruses, serologically confirmed, with asthma. Again, this was a tissue-specific effect for polyomaviruses. These

associations are novel and need to be further clarified. For example, they might be dependent on co-infection of WUPyV and KIPyV with other viruses such as rhinovirus or RSV.

There are strengths and limitations in our study. Strengths include the population-based prospective design and the large number of common infections assessed with multiplex serology. In contrast to previous studies, that grouped infections according to mode of transmission, we grouped polyomaviruses according to their site of latency which showed tissue-specific effects<sup>9,10,12</sup>. We acknowledge that we did not have direct measures of the presence of polyomaviruses on the skin or respiratory tract, but such invasive procedures in children would have increased non-participation rates and introduce significant selection-bias. There is only one study assessing the correlation between polyomaviruses serology and presence of the virus in the skin (MCPyV) showing good correlation<sup>58</sup>. Polyomaviruses and herpesviruses examined in this study are mostly acquired subclinically, thus serology has been widely used to determine the prevalence of those infections and explore disease associations. Although not supported by the literature, we cannot exclude the possibility that children with allergic diseases may have not seroconverted to the specific viruses after exposure. This would account for reverse causality regarding the negative associations with eczema. Outcome misclassification is a potential concern, nonetheless MeDALL questionnaires were developed from former well-validated questionnaires and definitions combined several features. Although our prospective design reduced the possibility of reverse-causality, inferences should be cautious. For example, we lack knowledge on how many children seroconverted from four to six years, which might have affected the observed associations at age six. As in all observational studies, there is the possibility of unmeasured residual confounding (e.g.

genes). Other reasons that might explain our findings are that viral infections may be surrogate markers for some other infections or environmental factors. Chance findings are always of concern when multiple comparisons are performed but we drew our conclusions on the basis of consistency of results. Correction for multiple testing could be very restrictive because the outcomes were not independent and our analysis was considered exploratory especially for polyomaviruses<sup>59</sup>. Nonetheless, after accounting for multiple testing of all statistical tests between exposures and outcomes by controlling the false discovery rate at 0.25 based on the method by Benjamini and Hochberg, we still observed statistically significant associations for EBV, HPyV6 and skin polyomaviruses burden with eczema at age 4 as well as for WUPyV and respiratory polyomaviruses burden with asthma at age 4. Seroprevalence of herpesviruses and polyomaviruses in our study is comparable those reported in Europe and the United States. The prevalence of eczema, asthma and rhinoconjunctivitis in our study is in accordance with results from the most recent studies in the Greek population<sup>60</sup>.

To conclude, we present new evidence suggesting that acquisition of polyomaviruses and herpesviruses in the first years of life is heterogeneously linked with allergic phenotypes in childhood. Particularly, acquisition of KIPyV, HPyV6, EBV, and of a higher herpesviruses and skin polyomaviruses burden were negatively associated with eczema. On the other hand, respiratory polyomaviruses and particularly WUPyV were associated with the presence of asthma phenotype. Further research in different populations and setting is necessary to clarify these associations and the underlying mechanisms.

## References

1. Grayson MH. The Complicated Dance of Infections and Asthma. *Immunol Allergy Clin North Am.* 2019;39(3):xv-xvi.
2. Esteban I, Stein RT, Polack FP. A Durable Relationship: Respiratory Syncytial Virus Bronchiolitis and Asthma past Their Golden Anniversary. *Vaccines.* 2020;8(2).
3. Liu L, Pan Y, Zhu Y, et al. Association between rhinovirus wheezing illness and the development of childhood asthma: a meta-analysis. *BMJ Open.* 2017;7(4):e013034.
4. Coverstone AM, Wang L, Sumino K. Beyond Respiratory Syncytial Virus and Rhinovirus in the Pathogenesis and Exacerbation of Asthma. *Immunol Allergy Clin North Am.* 2019;39(3):391-401.
5. Bønnelykke K, Vissing NH, Sevelsted A, Johnston SL, Bisgaard H. Association between respiratory infections in early life and later asthma is independent of virus type. *J Allergy Clin Immunol.* 2015;136(1):81-86.e4.
6. Strachan D. Family size, infection and atopy: the first decade of the “hygiene hypothesis.” *Thorax.* 2000;55(90001):2S - 10.
7. Cullinan P. Childhood allergies, birth order and family size. *Thorax.* 2005;61(1):3-5.
8. von Mutius E. Allergies, infections and the hygiene hypothesis – The epidemiological evidence. *Immunobiology.* 2007;212(6):433-439.
9. Michos A, Terzidis A, Kanariou M, et al. Association of allergic sensitization with infectious diseases burden in Roma and non-Roma children: Infections and atopy in children. *Pediatr Allergy Immunol.* 2011;22(2):243-248.
10. Linneberg A, Østergaard C, Tvede M, et al. IgG antibodies against microorganisms and atopic disease in Danish adults: The Copenhagen Allergy Study. *J Allergy Clin Immunol.* 2003;111(4):847-853.
11. Matricardi PM, Rosmini F, Ferrigno L, et al. Cross sectional retrospective study of prevalence of atopy among Italian military students with antibodies against hepatitis A virus. *BMJ.* 1997;314(7086):999-1003.

12. Matricardi PM, Rosmini F, Riondino S, et al. Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. *BMJ*. 2000;320(7232):412-417.
13. Flohr C, Pascoe D, Williams HC. Atopic dermatitis and the “hygiene hypothesis”: too clean to be true? *Br J Dermatol*. 2005;152(2):202-216.
14. Pembrey L, Waiblinger D, Griffiths P, Wright J. Age at cytomegalovirus, Epstein Barr virus and varicella zoster virus infection and risk of atopy: The Born in Bradford cohort, UK. *Pediatr Allergy Immunol Off Publ Eur Soc Pediatr Allergy Immunol*. 2019;30(6):604-613.
15. Janson C, Asbjornsdottir H, Birgisdottir A, et al. The effect of infectious burden on the prevalence of atopy and respiratory allergies in Iceland, Estonia, and Sweden. *J Allergy Clin Immunol*. 2007;120(3):673-679.
16. Wattier RL, Vázquez M, Weibel C, et al. Role of human polyomaviruses in respiratory tract disease in young children. *Emerg Infect Dis*. 2008;14(11):1766-1768.
17. Williams MR, Gallo RL. Evidence that Human Skin Microbiome Dysbiosis Promotes Atopic Dermatitis. *J Invest Dermatol*. 2017;137(12):2460-2461.
18. Sehrawat S, Kumar D, Rouse BT. Herpesviruses: Harmonious Pathogens but Relevant Cofactors in Other Diseases? *Front Cell Infect Microbiol*. 2018;8:177.
19. Platts-Mills TAE. The allergy epidemics: 1870-2010. *J Allergy Clin Immunol*. 2015;136(1):3-13.
20. Saghafian-Hedengren S, Sverremark-Ekström E, Linde A, Lilja G, Nilsson C. Early-life EBV infection protects against persistent IgE sensitization. *J Allergy Clin Immunol*. 2010;125(2):433-438.
21. Alcantara-Neves NM, Veiga RV, Dattoli VCC, et al. The effect of single and multiple infections on atopy and wheezing in children. *J Allergy Clin Immunol*. 2012;129(2):359-367, 367.e1-3.
22. Nilsson C, Larsson Sigfrinius A-K, Montgomery SM, et al. Epstein-Barr virus and cytomegalovirus are differentially associated with numbers of cytokine-producing cells and early atopy. *Clin Exp Allergy J Br Soc Allergy Clin Immunol*. 2009;39(4):509-517.

23. Hesla HM, Gutzeit C, Stenius F, et al. Herpesvirus infections and allergic sensitization in children of families with anthroposophic and non-anthroposophic lifestyle - the ALADDIN birth cohort. *Pediatr Allergy Immunol Off Publ Eur Soc Pediatr Allergy Immunol*. 2013;24(1):61-65.
24. Veiga RV, Cunha SS, Dattoli VCC, et al. Chronic virus infections suppress atopy but not asthma in a set of children from a large Latin American city: a cross-section study. *BMC Pulm Med*. 2011;11:24.
25. Meel ER, Jaddoe VWV, Reiss IKM, et al. The influence of Epstein-Barr virus and cytomegalovirus on childhood respiratory health: A population-based prospective cohort study. *Clin Exp Allergy*. 2020;50(4):499-507.
26. Laske N, Volk H-D, Liebenthal C, et al. Infantile natural immunization to herpes group viruses is unrelated to the development of asthma and atopic phenotypes in childhood. *J Allergy Clin Immunol*. 2002;110(5):811-813.
27. Okudaira R, Mukoyama T, Suzuki N, et al. Epstein-Barr Virus Infection in Childhood May Precipitate Atopic Diseases. *Allergol Int*. 2005;54(3):483-490.
28. Muehling LM, Heymann PW, Wright PW, et al. Human TH1 and TH2 cells targeting rhinovirus and allergen coordinately promote allergic asthma. *J Allergy Clin Immunol*. Published online April 2020:S0091674920304942.
29. Edwards MR, Strong K, Cameron A, Walton RP, Jackson DJ, Johnston SL. Viral infections in allergy and immunology: How allergic inflammation influences viral infections and illness. *J Allergy Clin Immunol*. 2017;140(4):909-920.
30. Bush A. Cytokines and Chemokines as Biomarkers of Future Asthma. *Front Pediatr*. 2019;7:72.
31. Chatzi L, Leventakou V, Vafeiadi M, et al. Cohort Profile: The Mother-Child Cohort in Crete, Greece (Rhea Study). *Int J Epidemiol*. 2017;46(5):1392-1393k.
32. Karachaliou M, Waterboer T, Casabonne D, et al. The Natural History of Human Polyomaviruses and Herpesviruses in Early Life—The Rhea Birth Cohort in Greece. *Am J Epidemiol*. Published online March 10, 2016:kwv281.

33. Mabikwa OV, Greenwood DC, Baxter PD, Fleming SJ. Assessing the reporting of categorised quantitative variables in observational epidemiological studies. *BMC Health Serv Res*. 2017;17(1):201.
34. Pinart M, Benet M, Annesi-Maesano I, et al. Comorbidity of eczema, rhinitis, and asthma in IgE-sensitised and non-IgE-sensitised children in MeDALL: a population-based cohort study. *Lancet Respir Med*. 2014;2(2):131-140.
35. Kyriklaki A, Margetaki K, Kampouri M, et al. Association between high levels of inflammatory markers and cognitive outcomes at 4 years of age: The Rhea mother-child cohort study, Crete, Greece. *Cytokine*. 2019;117:1-7.
36. Wahn U, von Mutius E. Childhood risk factors for atopy and the importance of early intervention. *J Allergy Clin Immunol*. 2001;107(4):567-574.
37. White IR, Royston P, Wood AM. Multiple imputation using chained equations: Issues and guidance for practice. *Stat Med*. 2011;30(4):377-399.
38. VanderWeele TJ, Vansteelandt S. Odds Ratios for Mediation Analysis for a Dichotomous Outcome. *Am J Epidemiol*. 2010;172(12):1339-1348.
39. Valeri L, VanderWeele TJ. Mediation analysis allowing for exposure-mediator interactions and causal interpretation: Theoretical assumptions and implementation with SAS and SPSS macros. *Psychol Methods*. 2013;18(2):137-150.
40. Flohr C, Yeo L. Atopic Dermatitis and the Hygiene Hypothesis Revisited. In: Shiohara T, ed. *Current Problems in Dermatology*. Vol 41. KARGER; 2011:1-34.
41. Alexander H, Paller AS, Traidl-Hoffmann C, et al. The role of bacterial skin infections in atopic dermatitis: expert statement and review from the International Eczema Council Skin Infection Group. *Br J Dermatol*. 2020;182(6):1331-1342.
42. Calvani M, Alessandri C, Paolone G, Rosengard L, Caro A, Franco D. Correlation between Epstein Barr virus antibodies, serum IgE and atopic disease. *Pediatr Allergy Immunol*. 1997;8(2):91-96.
43. Gibbs S. Atopic dermatitis and the hygiene hypothesis: a case-control study. *Int J Epidemiol*. 2004;33(1):199-207.

44. Rystedt I, Strannegård IL, Strannegård O. Increased serum levels of antibodies to Epstein-Barr virus in adults with history of atopic dermatitis. *Int Arch Allergy Appl Immunol*. 1984;75(2):179-183.
45. Strannegård I-L, Strannegård Ö. Epstein-Barr Virus Antibodies in Children with Atopic Disease. *Int Arch Allergy Immunol*. 1981;64(3):314-319.
46. Owens L, Laing IA, Zhang G, Turner S, Le Souëf PN. Prevalence of allergic sensitization, hay fever, eczema, and asthma in a longitudinal birth cohort. *J Asthma Allergy*. 2018;11:173-180.
47. Nguyen KD, Chamseddin BH, Cockerell CJ, Wang RC. The Biology and Clinical Features of Cutaneous Polyomaviruses. *J Invest Dermatol*. 2019;139(2):285-292.
48. Paller AS, Kong HH, Seed P, et al. The microbiome in patients with atopic dermatitis. *J Allergy Clin Immunol*. 2019;143(1):26-35.
49. Kennedy EA, Connolly J, Hourihane JO, et al. Skin microbiome before development of atopic dermatitis: Early colonization with commensal staphylococci at 2 months is associated with a lower risk of atopic dermatitis at 1 year. *J Allergy Clin Immunol*. 2017;139(1):166-172.
50. Myles IA, Earland NJ, Anderson ED, et al. First-in-human topical microbiome transplantation with *Roseomonas mucosa* for atopic dermatitis. *JCI Insight*. 2018;3(9).
51. Jartti T, Bønnelykke K, Elenius V, Feleszko W. Role of viruses in asthma. *Semin Immunopathol*. 2020;42(1):61-74.
52. Leino A, Lukkarinen M, Turunen R, et al. Pulmonary function and bronchial reactivity 4 years after the first virus-induced wheezing. *Allergy*. 2019;74(3):518-526.
53. Wesolowska-Andersen A, Everman JL, Davidson R, et al. Dual RNA-seq reveals viral infections in asthmatic children without respiratory illness which are associated with changes in the airway transcriptome. *Genome Biol*. 2017;18(1):12.
54. Subramoney K, Hellferscee O, Pretorius M, et al. Human bocavirus, coronavirus, and polyomavirus detected among patients hospitalised with severe acute respiratory illness in South Africa, 2012 to 2013. *Health Sci Rep*. 2018;1(8):e59.

55. Hansen-Estruch C, Coleman KK, Thoon KC, Low JG, Anderson BD, Gray GC. Prevalence of Respiratory Polyomaviruses Among Pediatric Patients With Respiratory Symptoms in Singapore. *Front Pediatr*. 2018;6:228.
56. Kuypers J, Campbell AP, Guthrie KA, et al. WU and KI Polyomaviruses in Respiratory Samples from Allogeneic Hematopoietic Cell Transplant Recipients. *Emerg Infect Dis*. 2012;18(10):1580-1588.
57. van der Zalm MM, Rossen JWA, van Ewijk BE, et al. Prevalence and Pathogenicity of WU and KI Polyomaviruses in Children, the Netherlands. *Emerg Infect Dis*. 2008;14(11):1787-1789.
58. Pastrana DV, Wieland U, Silling S, Buck CB, Pfister H. Positive correlation between Merkel cell polyomavirus viral load and capsid-specific antibody titer. *Med Microbiol Immunol (Berl)*. 2012;201(1):17-23.
59. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiol Camb Mass*. 1990;1(1):43-46.
60. Anthracopoulos MB, Fouzas S, Pandiora A, Panagiotopoulou E, Liolios E, Priftis KN. Prevalence trends of rhinoconjunctivitis, eczema, and atopic asthma in Greek schoolchildren: four surveys during 1991-2008. *Allergy Asthma Proc*. 2011;32(6):56-62.

**Table 1.** Occurrence (n, %) of allergy related symptoms and phenotypes in the study population.

|                                                                                                                                                                                                                  | Mother         | Father        | Child           |                 |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|---------------|-----------------|-----------------|
|                                                                                                                                                                                                                  |                |               | 4 years         | 6 years         |
| <b>Eczema</b>                                                                                                                                                                                                    |                |               |                 |                 |
| Ever diagnosed by a physician                                                                                                                                                                                    | 84/624 (13.5%) | 35/604 (5.8%) | 111/683 (16.3%) | 70/438 (16%)    |
| Parental report “Has your child had an itchy rash which was coming and going (intermittently) at any time in the past 12 months?”                                                                                | NA             | NA            | 91/685 (13.3%)  | 92/439 (21%)    |
| Parental report “Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes?” | NA             | NA            | 67/683 (9.8%)   | 39/435 (9%)     |
| MeDALL definition- current eczema (positive answer to both parental reports)                                                                                                                                     | NA             | NA            | 67/683 (9.8%)   | 39/435 (9%)     |
| <b>Asthma</b>                                                                                                                                                                                                    |                |               |                 |                 |
| Ever diagnosed by a physician                                                                                                                                                                                    | 26/609 (4.3%)  | 21/610 (3.4%) | 33/684 (4.8%)   | 54/451 (12%)    |
| Parental report “Has your child had wheezing or whistling in the chest in the past 12 months?”                                                                                                                   | NA             | NA            | 27/685 (3.9%)   | 35/440 (7.9%)   |
| Parental report “Has your child taken any medicines for asthma or breathing difficulties (wheezing, chest tightness, shortness of breath) in the last 12 months?”                                                | NA             | NA            | 410/681 (60.2%) | 121/439 (27.6%) |
| MeDALL definition-current asthma (positive answer to two out of three criteria)                                                                                                                                  | NA             | NA            | 48/686 (7%)     | 43/440 (9.8%)   |
| <b>Rhinoconjunctivitis</b>                                                                                                                                                                                       |                |               |                 |                 |
| Ever diagnosed by a physician                                                                                                                                                                                    | 87/614 (14.2%) | 72/600 (12%)  | 54/673 (8%)     | NA              |
| Parental report “Has your child had problems with sneezing or a runny or blocked nose when he/she did not have a cold or flu in the past 12 months?”                                                             | NA             | NA            | 157/683 (23%)   | 122/436 (28%)   |

|                                                                                                              |    |    |               |               |
|--------------------------------------------------------------------------------------------------------------|----|----|---------------|---------------|
| Parental report “If yes, in the past 12 months, were the nose symptoms accompanied by itchy, watering eyes?” | NA | NA | 37/680 (5.4%) | 16/431 (3.7%) |
| MeDALL definition- current rhinoconjunctivitis (positive answer to both parental reports)                    | NA | NA | 37/680 (5.4%) | 16/431 (3.7%) |

Abbreviations: MeDALL, Mechanisms of the Development of Allergy; NA, non applicable

Table 2. Adjusted associations (OR, 95% CI) between polyomaviruses and herpesviruses burden and allergy related phenotypes at age four and six.

|                            | Polyomaviruses burden <sup>†</sup> |              |         |                   |              |         | Herpesviruses burden <sup>‡</sup> |              |         |             |              |         |
|----------------------------|------------------------------------|--------------|---------|-------------------|--------------|---------|-----------------------------------|--------------|---------|-------------|--------------|---------|
|                            | Medium (4-7 viruses)               |              |         | High (≥8 viruses) |              |         | 1 virus                           |              |         | 2-3 viruses |              |         |
|                            | OR                                 | 95%CI        | P-value | OR                | 95%CI        | P-value | OR                                | 95%CI        | P-value | OR          | 95%CI        | P-value |
| <b>Eczema</b>              |                                    |              |         |                   |              |         |                                   |              |         |             |              |         |
| 4 years                    | 0.64                               | [0.34, 1.19] | 0.16    | 0.48              | [0.17, 1.31] | 0.15    | 0.57                              | [0.33, 0.99] | 0.047   | 0.37        | [0.17, 0.84] | 0.02    |
| 6 years                    | 0.79                               | [0.33, 1.87] | 0.59    | 0.76              | [0.23, 2.56] | 0.66    | 0.83                              | [0.41, 1.67] | 0.60    | 0.26        | [0.07, 0.93] | 0.04    |
| <b>Asthma</b>              |                                    |              |         |                   |              |         |                                   |              |         |             |              |         |
| 4 years                    | 1.31                               | [0.52, 3.29] | 0.57    | 1.39              | [0.43, 4.49] | 0.59    | 0.95                              | [0.49, 1.82] | 0.87    | 0.67        | [0.28, 1.61] | 0.37    |
| 6 years                    | 2.26                               | [0.83, 6.14] | 0.11    | 0.95              | [0.21, 4.30] | 0.95    | 1.34                              | [0.66, 2.71] | 0.42    | 0.75        | [0.27, 2.04] | 0.57    |
| <b>Rhinoconjunctivitis</b> |                                    |              |         |                   |              |         |                                   |              |         |             |              |         |
| 4 years                    | 1.10                               | [0.45, 2.72] | 0.83    | 0.67              | [0.16, 2.83] | 0.59    | 0.93                              | [0.45, 1.94] | 0.85    | 0.59        | [0.21, 1.70] | 0.33    |
| 6 years                    | 0.74                               | [0.19, 2.93] | 0.67    | 1.09              | [0.19, 6.12] | 0.93    | 0.62                              | [0.21, 1.85] | 0.39    | 0.40        | [0.08, 1.99] | 0.26    |

All models are logistic regression adjusted for maternal education, child's gender, breastfeeding, older siblings, area of residency, passive smoking, early daycare

attendance. <sup>†</sup>Reference group is that of low polyomaviruses burden (seropositivity to ≤3 polyomaviruses), <sup>‡</sup>Reference group is that of 0 herpesviruses burden

Abbreviations: CI, confidence interval; OR, odds ratio

Table 3. Adjusted associations (OR, 95% CI) between polyomaviruses burden by site of latency and allergy related phenotypes at age four and six.

|                     | Skin<br>(HPyV6, HPyV7, TSV,<br>MCPyV, HPyV9) |              |         | Respiratory tract<br>(KIPyV, WUPyV) |               |         |                        |               |         | Other<br>(BKPyV, JCPyV, HPyV10) |              |         |
|---------------------|----------------------------------------------|--------------|---------|-------------------------------------|---------------|---------|------------------------|---------------|---------|---------------------------------|--------------|---------|
|                     | 2-5 viruses <sup>†</sup>                     |              |         | 1 virus <sup>‡</sup>                |               |         | 2 viruses <sup>‡</sup> |               |         | 2-3 viruses <sup>†</sup>        |              |         |
|                     | OR                                           | 95%CI        | P-value | OR                                  | 95%CI         | P-value | OR                     | 95%CI         | P-value | OR                              | 95%CI        | P-value |
|                     |                                              |              |         |                                     |               |         |                        |               |         |                                 |              |         |
| Eczema              |                                              |              |         |                                     |               |         |                        |               |         |                                 |              |         |
| 4 years             | 0.51                                         | [0.31, 0.88] | 0.01    | 0.96                                | [0.50, 1.85]  | 0.90    | 0.58                   | [0.27, 1.26]  | 0.17    | 1.04                            | [0.58, 1.87] | 0.89    |
| 6 years             | 0.84                                         | [0.41, 1.70] | 0.63    | 0.66                                | [0.26, 1.65]  | 0.37    | 0.83                   | [0.32, 2.17]  | 0.71    | 0.87                            | [0.42, 1.82] | 0.71    |
| Asthma              |                                              |              |         |                                     |               |         |                        |               |         |                                 |              |         |
| 4 years             | 0.69                                         | [0.37, 1.28] | 0.24    | 4.48                                | [1.02, 19.63] | 0.05    | 7.35                   | [1.66, 32.59] | 0.009   | 1.11                            | [0.55, 2.24] | 0.78    |
| 6 years             | 0.88                                         | [0.44, 1.75] | 0.71    | 1.36                                | [0.51, 3.63]  | 0.54    | 1.75                   | [0.63, 4.83]  | 0.28    | 0.72                            | [0.37, 1.44] | 0.36    |
| Rhinoconjunctivitis |                                              |              |         |                                     |               |         |                        |               |         |                                 |              |         |
| 4 years             | 0.66                                         | [0.33, 1.33] | 0.24    | 1.07                                | [0.42, 2.72]  | 0.88    | 1.04                   | [0.37, 2.89]  | 0.94    | 1.04                            | [0.48, 2.24] | 0.92    |
| 6 years             | 1.31                                         | [0.41, 4.26] | 0.65    | 3.69                                | [0.45, 30.55] | 0.23    | 2.28                   | [0.25, 21.18] | 0.47    | 1.14                            | [0.34, 3.78] | 0.83    |

All models are logistic regression adjusted for maternal education, child's gender, breastfeeding, older siblings, area of residency, passive smoking, early daycare

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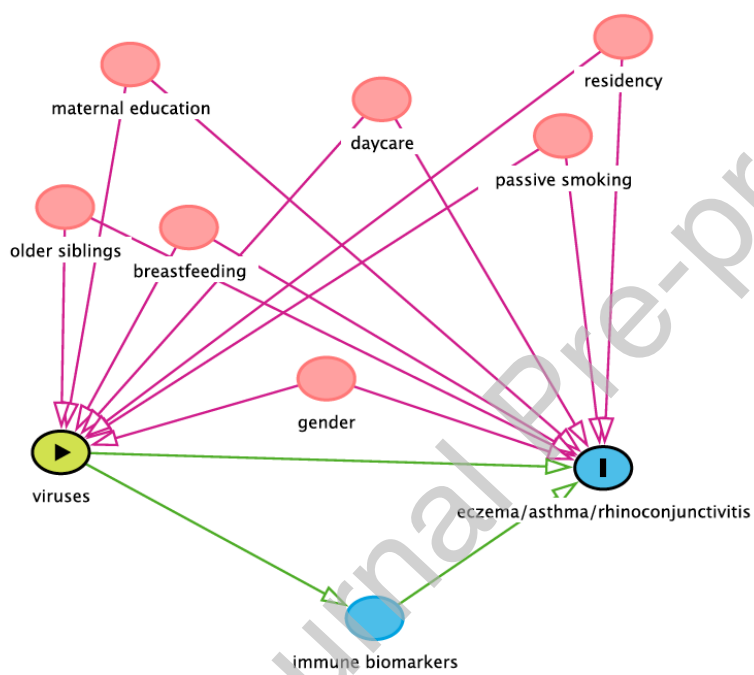
attendance. <sup>†</sup>Reference group is that of 0-1 virus, <sup>‡</sup>Reference group is that of 0 virus

Abbreviations: CI, confidence interval; OR, odds ratio

**Figure legend**

Figure 1. Associations (OR, 95% CI) of polyomaviruses and herpesviruses seropositivity at age four with allergic phenotypes at age four and six adjusted for maternal education, gender, breastfeeding, older siblings, passive smoking, residency and early daycare attendance. None HSV-1 seropositive child had rhinoconjunctivitis at age four. Abbreviations: OR, odds ratio; rc, rhinoconjunctivitis.

Figure 2. Prevalence of eczema longitudinally by EBV, herpesviruses burden and HPyV6 serostatus at age four.



eFigure1. Directed acyclic graphs (DAGs) to identify potential confounders and mediators of the association between viral infections (polyomaviruses and herpesviruses) and allergic diseases (eczema, asthma, rhinoconjunctivitis).

eTable1. Mediation effects of the association between polyomaviruses and herpesviruses on eczema at four years of age, the Rhea birth cohort

|                     | KIPyV             | HPyV6             | EBV               | HHVs burden       | Skin HPyVs burden |
|---------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                     | OR, 95% CI        | OR, 95% CI        | OR, 95% CI        | OR, 95% CI        | OR, 95% CI        |
| IFN $\gamma$        |                   |                   |                   |                   |                   |
| Direct effect       | 0.39 [0.19, 0.79] | 0.64 [0.35, 1.16] | 0.34 [0.18, 0.66] | 0.47 [0.30, 0.76] | 0.55 [0.30, 1.02] |
| Indirect effect     | 0.94 [0.85, 1.03] | 0.98 [0.89, 1.06] | 0.94 [0.86, 1.03] | 0.98 [0.92, 1.03] | 1.02 [0.93, 1.11] |
| Total effect        | 0.37 [0.18, 0.75] | 0.62 [0.33, 1.14] | 0.33 [0.17, 0.63] | 0.46 [0.29, 0.75] | 0.56 [0.30, 1.04] |
| IL1b                |                   |                   |                   |                   |                   |
| Direct effect       | 0.41 [0.21, 0.82] | 0.62 [0.34, 1.12] | 0.34 [0.18, 0.66] | 0.49 [0.31, 0.78] | 0.51 [0.28, 0.95] |
| Indirect effect     | 0.98 [0.89, 1.07] | 0.94 [0.85, 1.04] | 0.91 [0.81, 1.02] | 0.95 [0.88, 1.02] | 0.99 [0.90, 1.09] |
| Total effect        | 0.41 [0.21, 0.81] | 0.58 [0.31, 1.06] | 0.32 [0.16, 0.61] | 0.47 [0.29, 0.75] | 0.51 [0.27, 0.95] |
| IL6                 |                   |                   |                   |                   |                   |
| Direct effect       | 0.43 [0.22, 0.84] | 0.59 [0.32, 1.06] | 0.33 [0.17, 0.62] | 0.48 [0.31, 0.77] | 0.53 [0.29, 0.97] |
| Indirect effect     | 0.99 [0.96, 1.02] | 0.99 [0.97, 1.02] | 0.99 [0.96, 1.02] | 0.99 [0.96, 1.01] | 0.99 [0.97, 1.02] |
| Total effect        | 0.43 [0.22, 0.84] | 0.59 [0.32, 1.06] | 0.32 [0.17, 0.62] | 0.48 [0.31, 0.76] | 0.53 [0.29, 0.97] |
| IL8                 |                   |                   |                   |                   |                   |
| Direct effect       | 0.44 [0.22, 0.87] | 0.61 [0.34, 1.10] | 0.32 [0.17, 0.61] | 0.48 [0.31, 0.76] | 0.54 [0.29, 0.98] |
| Indirect effect     | 0.95 [0.88, 1.03] | 0.99 [0.96, 1.03] | 1.00 [0.96, 1.04] | 1.00 [0.97, 1.03] | 1.00 [0.95, 1.04] |
| Total effect        | 0.43 [0.22, 0.84] | 0.61 [0.34, 1.10] | 0.32 [0.17, 0.61] | 0.48 [0.31, 0.77] | 0.54 [0.29, 0.98] |
| IL10                |                   |                   |                   |                   |                   |
| Direct effect       | 0.42 [0.21, 0.81] | 0.60 [0.33, 1.07] | 0.32 [0.17, 0.61] | 0.48 [0.31, 0.76] | 0.53 [0.29, 0.97] |
| Indirect effect     | 1.00 [0.98, 1.02] | 0.99 [0.97, 1.02] | 1.00 [0.98, 1.02] | 1.00 [0.98, 1.03] | 0.99 [0.98, 1.01] |
| Total effect        | 0.42 [0.21, 0.82] | 0.60 [0.33, 1.07] | 0.32 [0.17, 0.61] | 0.49 [0.31, 0.77] | 0.53 [0.29, 0.97] |
| IL17a               |                   |                   |                   |                   |                   |
| Direct effect       | 0.43 [0.22, 0.86] | 0.59 [0.32, 1.07] | 0.34 [0.17, 0.64] | 0.49 [0.31, 0.78] | 0.50 [0.27, 0.92] |
| Indirect effect     | 0.98 [0.91, 1.05] | 0.98 [0.91, 1.05] | 0.96 [0.89, 1.03] | 0.98 [0.94, 1.03] | 1.05 [0.96, 1.14] |
| Total effect        | 0.43 [0.22, 0.84] | 0.58 [0.32, 1.06] | 0.32 [0.17, 0.62] | 0.48 [0.31, 0.77] | 0.53 [0.28, 0.97] |
| MIP1a               |                   |                   |                   |                   |                   |
| Direct effect       | 0.42 [0.21, 0.81] | 0.63 [0.34, 1.13] | 0.33 [0.17, 0.63] | 0.48 [0.31, 0.77] | 0.55 [0.30, 1.01] |
| Indirect effect     | 0.99 [0.93, 1.05] | 0.99 [0.93, 1.05] | 0.98 [0.93, 1.04] | 0.99 [0.95, 1.03] | 1.00 [0.94, 1.06] |
| Total effect        | 0.41 [0.21, 0.81] | 0.62 [0.34, 1.12] | 0.32 [0.17, 0.62] | 0.48 [0.31, 0.77] | 0.55 [0.30, 1.01] |
| TNFa                |                   |                   |                   |                   |                   |
| Direct effect       | 0.47 [0.24, 0.94] | 0.61 [0.33, 1.10] | 0.33 [0.17, 0.63] | 0.51 [0.32, 0.81] | 0.53 [0.29, 0.99] |
| Indirect effect     | 0.95 [0.88, 1.03] | 1.05 [0.97, 1.14] | 1.00 [0.94, 1.08] | 0.99 [0.95, 1.04] | 0.99 [0.93, 1.06] |
| Total effect        | 0.45 [0.23, 0.91] | 0.64 [0.34, 1.16] | 0.33 [0.17, 0.64] | 0.51 [0.32, 0.81] | 0.53 [0.28, 0.99] |
| Infancy antibiotics |                   |                   |                   |                   |                   |
| Direct effect       | 0.53 [0.29, 0.98] | 0.47 [0.26, 0.82] | 0.32 [0.17, 0.58] | 0.57 [0.38, 0.86] | 0.57 [0.32, 1.00] |
| Indirect effect     | 0.99 [0.89, 1.11] | 1.01 [0.88, 1.14] | 0.99 [0.89, 1.10] | 0.99 [0.92, 1.07] | 1.00 [0.88, 1.12] |
| Total effect        | 0.53 [0.28, 0.99] | 0.47 [0.26, 0.83] | 0.32 [0.17, 0.59] | 0.57 [0.37, 0.87] | 0.57 [0.32, 1.01] |

Adjusted models for maternal education, child's gender, breastfeeding duration, older siblings, residency at 4y, passive smoking at 4y, early daycare attendance.  
 Direct effect is the effect of these viruses on outcomes through pathways other than immune biomarkers; while indirect effect is the effect mediated through change in levels of immune biomarkers.

eTable2. Mediation effects of the association between polyomaviruses on asthma at four years of age, the Rhea birth cohort.

|                     | WUPyV              | Respiratory HPyVs burden |
|---------------------|--------------------|--------------------------|
|                     | OR, 95% CI         | OR, 95% CI               |
| IFN $\gamma$        |                    |                          |
| Direct effect       | 3.62 [1.04, 12.56] | 2.06 [1.14, 3.74]        |
| Indirect effect     | 1.00 [0.92, 1.09]  | 1.03 [0.97, 1.08]        |
| Total effect        | 3.65 [1.05, 12.68] | 2.12 [1.16, 3.86]        |
| IL1b                |                    |                          |
| Direct effect       | 3.44 [1.00, 11.84] | 2.12 [1.16, 3.84]        |
| Indirect effect     | 0.99 [0.97, 1.03]  | 1.00 [0.98, 1.01]        |
| Total effect        | 3.44 [1.00, 11.84] | 2.12 [1.17, 3.85]        |
| IL6                 |                    |                          |
| Direct effect       | 3.75 [1.09, 12.86] | 2.16 [1.20, 3.88]        |
| Indirect effect     | 0.99 [0.95, 1.04]  | 0.99 [0.98, 1.01]        |
| Total effect        | 3.72 [1.09, 12.77] | 2.16 [1.20, 3.88]        |
| IL8                 |                    |                          |
| Direct effect       | 3.77 [1.10, 12.94] | 2.32 [1.27, 4.25]        |
| Indirect effect     | 1.00 [0.96, 1.05]  | 0.97 [0.92, 1.02]        |
| Total effect        | 3.80 [1.10, 13.07] | 2.25 [1.24, 4.11]        |
| IL10                |                    |                          |
| Direct effect       | 3.55 [1.03, 12.17] | 2.10 [1.17, 3.79]        |
| Indirect effect     | 0.99 [0.99, 1.00]  | 0.99 [0.99, 1.01]        |
| Total effect        | 3.55 [1.03, 12.17] | 2.10 [1.17, 3.79]        |
| IL17a               |                    |                          |
| Direct effect       | 3.39 [0.98, 11.68] | 2.18 [1.19, 3.99]        |
| Indirect effect     | 1.00 [0.98, 1.02]  | 1.00 [0.98, 1.02]        |
| Total effect        | 3.40 [0.99, 11.70] | 2.19 [1.20, 3.99]        |
| MIP1a               |                    |                          |
| Direct effect       | 3.67 [1.07, 12.63] | 2.13 [1.18, 3.82]        |
| Indirect effect     | 0.99 [0.94, 1.03]  | 0.99 [0.98, 1.01]        |
| Total effect        | 3.63 [1.05, 12.46] | 2.12 [1.18, 3.81]        |
| TNF $\alpha$        |                    |                          |
| Direct effect       | 3.63 [1.05, 12.48] | 2.39 [1.30, 4.42]        |
| Indirect effect     | 0.99 [0.96, 1.03]  | 0.99 [0.98, 1.01]        |
| Total effect        | 3.63 [1.05, 12.46] | 2.39 [1.30, 4.41]        |
| Infancy antibiotics |                    |                          |
| Direct effect       | 2.93 [0.99, 8.66]  | 2.10 [1.20, 3.68]        |
| Indirect effect     | 1.05 [0.93, 1.19]  | 1.03 [0.95, 1.11]        |
| Total effect        | 3.08 [1.04, 9.17]  | 2.17 [1.24, 3.83]        |

Adjusted models for maternal education, child's gender, breastfeeding duration, older siblings, residency at 4y, passive smoking at 4y, early daycare attendance.  
 Direct effect is the effect of these viruses on outcomes through pathways other than immune biomarkers; while indirect effect is the effect mediated through change in levels of immune biomarkers.

Figure 1

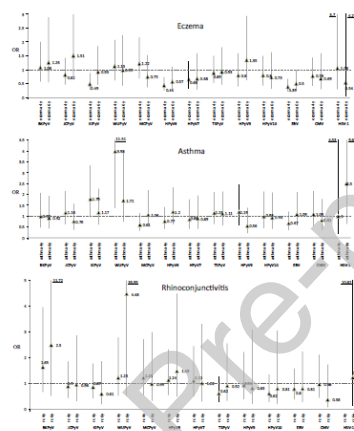


Figure 2

