1 **Age at cytomegalovirus, Epstein Barr virus and varicella zoster virus infection and risk of atopy: the**

2 **Born in Bradford cohort, UK**

3 Short title: Age at infection with herpesviruses and risk of atopy

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34   **Authors' contributions**

35   LP conceived and designed the study, analysed and interpreted the data and drafted and revised the

36   manuscript. DW co-ordinated data collection, interpreted the data and revised the manuscript. PG

37   designed the study, interpreted the data and revised the manuscript. JW designed the study,

38   interpreted the data and revised the manuscript. All authors have given final approval of the version

39   to be published.

40

41 **Abstract**

43 Background: The prevalence of allergic diseases has increased in recent decades but the causes

44 remain unclear. Changes in the epidemiology of childhood infections could have contributed but the

45 current evidence is inconclusive. This study aims to investigate whether age at cytomegalovirus

46 (CMV), Epstein Barr virus (EBV) or varicella zoster virus (VZV) infection are associated with the

47 development of atopy.

48 Methods: 2559 children were enrolled in the Born in Bradford Allergy and Infection Study. Serum

49 samples collected at 12 and 24 months were tested for CMV-IgG, EBV-IgG and VZV-IgG for 1000

50 children to establish age at infection. Skin prick testing (SPT) was conducted at age 4 years.

51 Results: Serology and SPT results were available for 740 children. Of these, 135 (18%) were atopic. In

52 girls there was a strong association of CMV infection in the second year with increased odds of atopy

53 (adjusted OR 4.38, 95% CI 1.87–10.29) but this was not observed in boys. Age at EBV or VZV

54 infection were not associated with risk of atopy in unadjusted analysis, but there was effect

55 modification by sex; girls infected with VZV in the second year of life had increased odds of atopy

56 (adjusted OR 2.85, 95% CI 1.29 – 6.30).

57 Conclusions: Our results highlight potential sex-specific effects of age at CMV infection and age at

58 VZV infection on risk of atopy, which provide insight into the mechanisms involved in the

59 development of atopy.

60 233 words

63 **Key words:** age at infection, atopy, birth cohort, cytomegalovirus, Epstein Barr virus

Introduction

The hygiene hypothesis proposes that the dramatic rise in prevalence of eczema, hay fever and allergic asthma in recent decades is due to a reduction in childhood infections[1,2]. However, many studies have investigated proxy measures for childhood infections such as birth order and child care[3,4] or have focussed on symptomatic, clinically diagnosed infections[5-7].

Cytomegalovirus (CMV), Epstein Barr virus (EBV) and varicella zoster virus (VZV) are persistent herpesviruses commonly acquired in childhood. CMV and EBV are usually asymptomatic and VZV causes chickenpox. There is good evidence that CMV and EBV affect the developing immune system[8,9]. CMV impacts on T cell[10] and NK cell[11,12] differentiation and CMV seropositivity is associated with inflammation, atherosclerosis and immunosenescence[13,14]. EBV is associated with immune disorders such as multiple sclerosis and lymphoma[15,16]. The features of CMV and EBV in particular indicate that changes in the epidemiology of these infections could have contributed to the increases in eczema, hay fever and allergic asthma.

In a Swedish study no association was found between CMV or EBV seropositivity at age 4 and asthma, hay fever or eczema, although CMV positive/EBV negative children were more likely to have specific IgE to common allergens than children seronegative for both infections[17,18]. In another study, children who were EBV seropositive at 24 months were less likely to be IgE-sensitised at this age compared to EBV seronegative children and this association was enhanced among children co-infected with CMV[19]. Another analysis by the same group showed that children infected with EBV before 2 years were less likely to be IgE-sensitised at age 2 and 5 years while those infected after 2 years were more likely to be IgE-sensitised at 5 years[20].

There was no association between CMV, EBV or VZV infection at age 12 months and eczema, asthma, hay fever, total serum IgE levels or allergic sensitisation at age 7 in a German study[21]. In a UK case-control study there was no association between EBV or VZV infection and eczema in 1-4 year olds[22]. Other studies have examined the association between seropositivity to CMV, EBV, VZV

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90   and other infections and risk of atopy or eczema, hay fever, or asthma, but the combined evidence is

91   inconclusive[23-25]. In some, the findings were limited by a lack of statistical power but in most,

92   infection status was measured at only one time point, often at age 4 or older, so it was not possible

93   to pinpoint age at infection more precisely. As herpesvirus infections are common it is unlikely that

94   infection per se is associated with risk of disease, but alteration in risk could result from infection at

95   a critical period in immune development. Measuring infection status at more than one age in the

96   first years of life is therefore important. In the Born in Bradford Allergy and Infection Study, children

97   were tested for CMV-, EBV- and VZV-IgG at 12 and 24 months. We have previously reported earlier

98   infection in Pakistani compared to white British children[26]. The aim of this analysis is to investigate

99   whether earlier CMV and/or EBV infection protect against the development of atopy.

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Methods

The Allergy and Infection Study (ALL IN) is a sub-study of the Born in Bradford birth cohort (BiB),

described elsewhere[27]. Children were eligible to participate if they were enrolled in BiB with a

maternal baseline questionnaire available and were born on or after 1 March 2008. Parents were

invited to participate in ALL IN just before the child's first birthday. If they agreed, a home or clinic

visit was arranged and informed consent obtained. A blood sample was taken and a questionnaire

completed at 12 months and again at 24 months of age. Serum aliquots were stored at -80^0C. The

questionnaires included detailed information on breastfeeding and child care, and the standard

International Study of Asthma and Allergies in Childhood (ISAAC) questions on potential risk factors

for atopy (pets, damp, mould, fuel for cooking and heating, type of windows in the child's bedroom,

type of flooring, type of pillow and bedding for the child)[28].

Of the 2559 children enrolled in ALL IN, serum samples were tested for CMV-IgG, EBV-IgG and VZV-

IgG for 1000 children to establish age at infection; infected by 12 months, infected between 12 and

24 months; or uninfected at 24 months[26]. Samples with concentration values of ≥6.0 AU/mL were

considered as positive for CMV-IgG, and VCA IgG concentrations of ≥20 U/mL were considered as

positive for EBV-IgG. For VZV, samples containing 160 mIU/ml were considered positive, those

between 140 and 160 were equivocal and those less than 140 mIU/ml were deemed negative.

When the children were 4 years old the parents were re-contacted and invited for a visit including a

questionnaire and skin prick test (SPT), as part of the MeDALL (Mechanisms of the Development of

Allergy) study[29,30]. The SPT was performed according to a standard protocol using the following

allergens: cat hair, dog hair, grass mix, house dust mites *Dermatophagoides pteronyssinus* and

Dermatophagoides farinae plus a positive control (histamine 1%) and a negative saline control

(Allergopharma kits supplied by Diagenics Ltd). The mean wheal diameter was recorded for each

allergen (and the diameter of any wheal from the negative control subtracted). A wheal diameter of

126 >=3mm was considered a positive reaction. Children with a positive reaction to at least one allergen

127 were defined as atopic.

128 The community research staff were trained in SPT, with quality control procedures to assess intra-

129 observer variability; all staff had to perform two-monthly quality checks and achieve a coefficient of

130 variation of less than 20 in their SPT series.

131 Parents were given the results of their child's SPT with written guidance on allergen avoidance for

132 those with positive tests.

133 This study had ethical approval from the London School of Hygiene & Tropical Medicine ethics

134 committee (refs: 5320 and 6249) and the Bradford Research Ethics committee (refs: 08/H1302/21

135 and 12/YH/0252).

136 Statistical analysis

137 Data analysis was conducted using Stata version 14[31]. Frequency distributions for key variables

138 described the children and their mothers. Cross-tabulations showed associations between these

139 variables and atopic status. Age at CMV, EBV and VZV infection were the key exposures of interest.

140 The outcome for this analysis was measured at age 4 years only and in all children at the same age.

141 Our data therefore provide an estimate of prevalence, not risk, of atopy so it is appropriate to

142 present prevalence odds ratios from logistic regression analysis. If univariable analysis demonstrated

143 an association with atopy, the relevant variable was included as a confounder in the multiple logistic

144 regression analysis. The findings for age-at-CMV infection were compared between the univariable

145 and multiple logistic regression analyses, and no evidence of multicollinearity was found, so the

146 multiple regression analysis was accepted as the final model. Birth order and duration of

147 breastfeeding were included in the CMV model *a priori* as these are strongly associated with age at

148 CMV infection[26] and associated with atopy in other studies[32-34]. Effect modification by sex and ethnic

149 group were investigated[35].

150   Previous studies have indicated interaction between EBV and CMV infection on atopy/IgE

151   sensitisation[18,19] so we compared the proportion of children who were atopic for combinations of

152   age at CMV/EBV/VZV infection.

153   The number and type of positive allergens per child were described and examined by ethnic group

154   and sex.

Results

SPT was completed at the 4-year visit for 740 children with serological data. Of these, 135 (18%) were atopic (95% CI 16–21).

Table 1 shows the characteristics of the children and their mothers.

<u>Unadjusted analysis</u>

Among the children who were CMV infected by 12 months and those who remained uninfected at 24 months, 17% were atopic (31/181 and 82/485, respectively). However, of the 74 children who were CMV infected between 12 and 24 months, 30% (22) were atopic. There were no differences in the proportion of children atopic by age at EBV infection or by age at VZV infection (18 or 19% in each group).

Boys were twice as likely to be atopic than girls and Pakistani children were twice as likely to be atopic than White British children. Childcare attendance by age 24 months, reporting pets in the home and central heating at the 12-month visit, and smoking in pregnancy were associated with reduced risk of atopy. Low birth weight and damp reported at 12 months were associated with an increased risk of atopy. There was not strong evidence of an association with pets and damp reported at the 24-month visit (Table 1). There was no evidence of associations between atopy and mould in the home, type of windows in child's bedroom, gas for cooking, gas fire, wood or coal fire with chimney, type of flooring or bedding (data not shown).

Only nine respondents reported making changes due to asthma/allergies (mainly changing to hard floors or new carpets, different bedding) at the 12-month questionnaire (3 were atopic) and only 17 reported making changes at the 24-month questionnaire (5 were atopic).

The associations of atopy with breastfeeding, childcare, pets and smoking in pregnancy may reflect differences in the prevalence of these factors between white British and Pakistani children; Pakistani

178 women were more likely to breastfeed, less likely to use childcare and far less likely to smoke than

179 white British women. Pakistani families were less likely to have pets than white British families.

180 The 740 children included here were generally similar to the total 1000 children with serological

181 data, although there was a slightly higher proportion of Pakistani children.

182 <u>Adjusted analysis</u>

183 There was some evidence of effect modification of the association between age at CMV infection

184 and atopy by sex (LRT x^2=4.80, p=0.09), adjusting for ethnic group, birth order, duration of

185 breastfeeding, regular childcare attendance by 24 months, low birth weight, pets in the home (12

186 months), damp (12 months), smoking pregnancy and central heating (12 months). In boys there was

187 no association between CMV infection in the second year and odds of atopy (adjusted OR (aOR)

188 1.20, 95% CI 0.52–2.79) and weak evidence of a protective effect of infection before 12 months (aOR

189 0.57, 95% CI 0.31–1.04). In girls there was a strong association of CMV infection in the second year

190 with increased odds of atopy (aOR 4.38, 95% CI 1.87–10.29) and no association with infection in the

191 first year (aOR 0.97, 95% CI 0.40–2.33) (Table 2).

192 There was also evidence of effect modification by sex of the association between age at EBV

193 infection and atopy (LRT x^2=5.87, p=0.05) and age at VZV infection and atopy (LRT x^2=8.52, p=0.01);

194 the sex-specific estimates are presented in Table 2. Girls infected with VZV in the second year of life

195 were at increased odds of atopy (aOR 2.85, 95% CI 1.29 – 6.30). There was no evidence of effect

196 modification by ethnic group of the association between age at CMV, EBV or VZV infection and

197 atopy.

198 Table 1 shows the adjusted estimates (from the model for age at CMV infection) for the other

199 variables associated with atopy. Boys had three times the odds of atopy overall compared to girls.

200 The increased risk of atopy among Pakistani compared to White British children attenuated in

201 multivariable analysis. There was weak evidence that children of low birth weight, those breastfed

202   for more than 12 months and children living in homes without central heating had around twice the

203   odds of atopy. There was good evidence that children living in damp homes were more likely to be

204   atopic.

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206   <u>Infection with more than one virus in the second year is associated with greater risk of atopy</u>

207   Of the total 740 children, 23 were infected with CMV and EBV between 12 and 24 months; 8 of 23

208   (35%) were atopic. Among 33 children who were CMV and EBV infected by 12 months, 6 (18%) were

209   atopic. Similarly, among 121 children who were CMV and EBV infected by 24 months, 25 (21%) were

210   atopic. Of 20 children who were CMV and VZV infected between 12 and 24 months, 9 (45%) were

211   atopic and of 5 children who were infected with all three viruses between 12 and 24 months, 4

212   (80%) were atopic. The final model for age at CMV infection, stratified by EBV infection status at 24

213   months gave similar estimates to the overall results (data not shown).

214   <u>Ethnic group differences in atopy</u>

215   Among 386 Pakistani mothers, 250 were born outside, and 136 were born in, the UK. 64 (26%)

216   children of those born outside the UK were atopic compared to 25 (18%) children of women born in

217   the UK (χ^2=2.59, p=0.11). 27 (11%) children of White British women born in the UK were atopic.

218   Only two of the White British women were born abroad (and one child was atopic).

219   <u>Which allergens were positive?</u>

220   There were five allergens in the panel. Most of the 135 atopic children were positive for one (n=56)

221   or two (n=52) allergens (Table 3). Positive reactions to house dust mites (HDM) were most common,

222   followed by grass mix with positive cat and dog results the least common. The number of positive

223   allergens per child did not vary substantially by ethnic group or sex.

224   Pakistani children were more likely to have a positive test for HDM than White British children.

225   Among 56 children who reacted to one allergen, 33 (83%) of 40 Pakistani children were positive to

226   HDM compared to 5 (50%) of the 10 White British children (χ^2=4.63, Fisher's exact p=0.05). All

227   except two children (1 WB, 1 Pakistani) with two positive reactions and all except one child (WB)

228   with three positive reactions were positive for at least one HDM.

229   Among 56 children who reacted to one allergen, a higher proportion of boys were positive to HDM

230   than girls but there was no evidence of a true difference (χ^2=1.38, p=0.24). Among 52 children who

231   reacted to two allergens, all except one boy and one girl were positive for one or both HDM, and

232   among 21 children with three positive reactions, all except one boy were positive for one or both

233   HDM.

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Discussion

Our main finding is that, among the girls, CMV infection in the second year of life is associated with a 4–fold increased odds of atopy compared to acquiring it in the first year or remaining uninfected at 2 years. Among the boys there was no association between infection at 12-24 months and atopy, but weak evidence of a protective effect of CMV infection in the first year of life on odds of atopy. Although age at EBV infection and age at VZV infection were not associated with risk of atopy in unadjusted analyses, there was good evidence of effect modification by sex. Stratified, adjusted estimates revealed an increased odds of atopy among girls infected with VZV in the second year of life compared to those still uninfected at 2 years.

Traditionally, until 50-70 years ago in the UK and in low income countries today, most children became infected with CMV by the age of 12 months[36,37]. One explanation for our findings is that 'delayed' infection, coinciding with immune development in the second year of life, may disrupt the balance of immune responses, leading to atopy. A second potential explanation is that CMV infection acquired when maternal antibody titres are declining might present a lower initial viral load to the immune system than found with perinatal infection from breast milk or infection later in life. Further studies are needed on this and should be linked to our observation of increased atopy in those of low birth weight who would have reduced transplacental passage of IgG.

The consistent direction of the sex-specific estimates for CMV, EBV and VZV, with increased odds of atopy among girls infected in the second year with CMV and with VZV, supports the biological plausibility of a sex-specific mechanism in the development of atopy. The 'critical window' seems to be the second year of life when also considering combinations of CMV, EBV and VZV infection; the highest proportion of atopic children were among those infected in the second year of life with one or more of the viruses. Some evidence of a protective effect of CMV infection in the first year of life, at least in boys, fits with the traditional age at infection and corresponding low prevalence of atopy, and demonstrates the importance of differentiating between infection in the first and second years.

260   Our results highlight potential sex-specific effects of age at CMV infection on risk of atopy. Overall,

261   boys had a greater risk of atopy than girls, as reported in other studies[38,39], but differences in risk of

262   atopy by age at CMV infection were only observed for girls. So why is 'delayed' CMV infection

263   associated with atopy in girls but not in boys?

264   There are other examples of sex differences in response or susceptibility to infections early in life[40,41]

265   and neonatal vitamin A supplementation (NVAS) was associated with increased atopy in girls but not

266   boys in long-term follow-up of children enrolled in a previous randomised controlled trial in Guinea-

267   Bissau[42]. As in our study, females were less likely to be atopic than males overall, but those receiving

268   NVAS were at increased risk of atopy (adjusted RR=1.78, 95% CI 1.17–2.71) and wheeze during

269   childhood (adjusted RR=1.78, 95% CI 1.02–3.09) compared to those receiving placebo. Possible

270   mechanisms to explain these associations are that retinoic acids from vitamin A can lead to an

271   enhanced Th2 response and a reduced Th1 response. High oestrogen levels can also promote a Th2

272   response and girls experience surges in oestrogen levels in early infancy as well as in puberty. A

273   further study by the group demonstrates that NVAS is associated with differences in response to

274   BCG vaccination by sex and increased pro- to anti-inflammatory cytokine ratios in females but

275   decreased ratios in males, at 4- 6 months of age[43].

276   In the Generation R cohort (population-based cohort from fetal life onwards, in Rotterdam, the

277   Netherlands) no interaction of sex and CMV infection was observed in relation to immune

278   phenotypes but the children were tested at age 6 years[35], so differences by age at infection could

279   not be investigated. In exploratory studies of the same cohort, an impact of female sex and CMV

280   infection on immune cell dynamics in early childhood has been demonstrated[44].

281   The combined evidence indicates that the sex-specific associations we have observed may provide

282   clues about the mechanisms involved in the development of atopy. There is evidence that CMV

283   influences anti-inflammatory mediators (viral and host IL-10)[45]. CMV infection may therefore

284   influence the Th1/Th2 balance particularly in the second year of life, and this, combined with

285   hormonal effects in girls, could increase the risk of atopy. It is also possible that the reverse is true:

286   early immune changes which lead to atopy may be associated with delayed CMV infection.

287   The group of children who were CMV infected in the second year is relatively small, only 10% of the

288   total, and the majority of children in this cohort remained uninfected at age 2 years. Their risk of

289   atopy was similar to those infected in the first year and we do not know the age distribution of

290   subsequent CMV infection in this group. However, we assume that CMV infection during early

291   immune development, i.e. the first 2-3 years, influences risk of atopy at age 4 years, and later

292   infection has less impact.

293   We did not observe any differences in risk of atopy by age at EBV infection, in contrast to the

294   Swedish studies[19], although there was evidence of effect modification by sex. EBV infection tends to

295   be acquired later than CMV; in this cohort most of the CMV infections occurred in the first year,

296   usually through contact with the mother whereas most EBV infections occurred in the second year

297   via contact with siblings and other children[26]. Delayed infection for EBV is therefore around age 3 or

298   4 years and older. A comparison of children infected by age 2 years (the 'traditional' age at infection)

299   with those infected between 2 and 4 years (delayed infection) would demonstrate any effect on risk

300   of atopy and we did not carry out serological testing at age 4 years. However, an effect on atopy risk

301   would only be expected if the delayed infection coincided with a critical period of immune

302   development. Similarly, VZV infection is usually acquired around age 3 or 4 years through contact

303   with other children and we show that infection during the second year of life, a likely critical period

304   of immune development, is associated with increased odds of atopy.

305   The 18% prevalence of atopy in our study is generally consistent with results from other European

306   birth cohorts[19,46] and UK studies[47,48]. Some of these measured allergen specific IgE (sIgE) in serum

307   rather than SPT and the children included ranged from age 2 to 7 years with all primary school-aged

308   children included in one study.

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309   The higher odds of atopy among Pakistani children, although attenuated in multivariable analysis,

310   especially when including childcare in the model, is of interest and there was some evidence of a

311   higher risk of atopy in children of women born abroad and recently migrated to the UK. In the US

312   NHANES study allergic sensitisation was associated with ethnicity and among those aged 1 to 5 years

313   was less prevalent in non-Hispanic white children than other ethnic groups[39]. The number of

314   positive allergens per child did not vary by ethnicity but there was weak evidence that Pakistani

315   children were more likely to have a positive test to HDM than White British children. Higher levels of

316   HDM and sensitisation to HDM have been associated with damp in the home[49]. In our study a higher

317   proportion of Pakistani children had damp in the home at 12 months than White British children,

318   which might partly explain the higher odds of atopy in Pakistani children.

319   The key strength of our study is having infection status at both 12 and 24 months. To our knowledge,

320   this is the first study to measure CMV- and EBV-IgG at 12 and 24 months in relation to atopic status.

321   The benefits of the cohort design included risk factor data collected at 12 and 24 months, and at 4

322   years, whereas cross-sectional studies may not capture earlier exposures accurately either due to

323   recall bias or if there are changes between age one and four years, which may have been made in

324   response to allergic symptoms. There were few reported changes due to asthma or allergies at the

325   12 and 24-month questionnaire.

326   SPT is widely used and accepted as a valid tool to define atopy[28]. Several members of the team

327   carried out the tests and there may have been variations in technique between them, despite

328   training and quality control checks. However, any measurement error and subsequent

329   misclassification of atopic status would have been non-differential, as the research team were

330   unaware of the infection status of the children. Some studies use sIgE to define atopic status, instead

331   of, or in addition to, SPT. There is generally good correlation between sIgE and SPT (>70%

332   concordance), at least in high income country settings[50,51].

333   Not all children with a positive SPT will develop clinical symptoms of eczema, hay fever or allergic

334   asthma. Some non-atopic children may develop different types of asthma caused by non-atopic

335   mechanisms. Further work will investigate whether the higher odds of atopy among girls infected

336   with CMV in the second year translates to an increased risk of eczema, hay fever, wheeze or asthma

337   among these children.

338   In conclusion, our results highlight potential sex-specific effects of age at CMV infection and age at

339   VZV infection on risk of atopy, which may provide insight into the mechanisms involved in the

340   development of atopy.

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350   **References**

351   1.   Strachan DP. Family size, infection and atopy: the first decade of the "hygiene hypothesis".

352   *Thorax.* 2000;55 Suppl 1:S2-10.

353   2.   Strachan DP. Hay fever, hygiene, and household size. *BMJ.* 1989;299(6710):1259-1260.

354   3.   McKeever TM, Lewis SA, Smith C, et al. Siblings, multiple births, and the incidence of allergic

355   disease: a birth cohort study using the West Midlands general practice research database.

356   *Thorax.* 2001;56(10):758-762.

357   4.   Burbank AJ, Sood AK, Kesic MJ, Peden DB, Hernandez ML. Environmental determinants of

358   allergy and asthma in early life. *The Journal of allergy and clinical immunology.*

359   2017;140(1):1-12.

360   5.   Forastiere F, Agabiti N, Corbo GM, et al. Socioeconomic status, number of siblings, and

361   respiratory infections in early life as determinants of atopy in children. *Epidemiology.*

362   1997;8(5):566-570.

363   6.   Bodner C, Godden D, Seaton A. Family size, childhood infections and atopic diseases. The

364   Aberdeen WHEASE Group. *Thorax.* 1998;53(1):28-32.

365   7.   von Mutius E, Illi S, Hirsch T, Leupold W, Keil U, Weiland SK. Frequency of infections and risk

366   of asthma, atopy and airway hyperresponsiveness in children. *Eur Respir J.* 1999;14(1):4-11.

367   8.   La Rosa C, Diamond DJ. The immune response to human CMV. *Future Virol.* 2012;7(3):279-

368   293.

369   9.   Hatton OL, Harris-Arnold A, Schaffert S, Krams SM, Martinez OM. The interplay between

370   Epstein-Barr virus and B lymphocytes: implications for infection, immunity, and disease.

371   *Immunol Res.* 2014;58(2-3):268-276.

372   10.   Miles DJ, van der Sande M, Jeffries D, et al. Maintenance of large subpopulations of

373   differentiated CD8 T-cells two years after cytomegalovirus infection in Gambian infants. *PloS*

374   *one.* 2008;3(8):e2905.

375   11.   Guma M, Budt M, Saez A, et al. Expansion of CD94/NKG2C+ NK cells in response to human

376   cytomegalovirus-infected fibroblasts. *Blood.* 2006;107(9):3624-3631.

377   12.   Lee J, Zhang T, Hwang I, et al. Epigenetic modification and antibody-dependent expansion of

378   memory-like NK cells in human cytomegalovirus-infected individuals. *Immunity.*

379   2015;42(3):431-442.

380   13.   Simanek AM, Dowd JB, Pawelec G, Melzer D, Dutta A, Aiello AE. Seropositivity to

381   cytomegalovirus, inflammation, all-cause and cardiovascular disease-related mortality in the

382   United States. *PloS one.* 2011;6(2):e16103.

383   14.   Pawelec G, Derhovanessian E. Role of CMV in immune senescence. *Virus research.*

384   2011;157(2):175-179.

385   15.   Leray E, Moreau T, Fromont A, Edan G. Epidemiology of multiple sclerosis. *Revue*

386   *neurologique.* 2015.

387   16.   Cohen JI, Fauci AS, Varmus H, Nabel GJ. Epstein-Barr virus: an important vaccine target for

388   cancer prevention. *Science translational medicine.* 2011;3(107):107fs107.

389   17.   Sidorchuk A, Lagarde F, Pershagen G, Wickman M, Linde A. Epstein-Barr virus infection is not

390   associated with development of allergy in children. *The Pediatric infectious disease journal.*

391   2003;22(7):642-647.

392   18.   Sidorchuk A, Wickman M, Pershagen G, Lagarde F, Linde A. Cytomegalovirus infection and

393   development of allergic diseases in early childhood: interaction with EBV infection? *The*

394   *Journal of allergy and clinical immunology.* 2004;114(6):1434-1440.

395   19.   Nilsson C, Linde A, Montgomery SM, et al. Does early EBV infection protect against IgE

396   sensitization? *The Journal of allergy and clinical immunology.* 2005;116(2):438-444.

397   20.   Saghafian-Hedengren S, Sverremark-Ekstrom E, Linde A, Lilja G, Nilsson C. Early-life EBV

398   infection protects against persistent IgE sensitization. *The Journal of allergy and clinical*

399   *immunology.* 2010;125(2):433-438.

400 21. Laske N, Volk HD, Liebenthalb C, et al. Infantile natural immunization to herpes group viruses

401 is unrelated to the development of asthma and atopic phenotypes in childhood. *The Journal*

402 *of allergy and clinical immunology.* 2002;110(5):811-813.

403 22. Gibbs S, Surridge H, Adamson R, Cohen B, Bentham G, Reading R. Atopic dermatitis and the

404 hygiene hypothesis: a case-control study. *International journal of epidemiology.*

405 2004;33(1):199-207.

406 23. Alcantara-Neves NM, Veiga RV, Dattoli VC, et al. The effect of single and multiple infections

407 on atopy and wheezing in children. *The Journal of allergy and clinical immunology.*

408 2012;129(2):359-367, 367 e351-353.

409 24. Janson C, Asbjornsdottir H, Birgisdottir A, et al. The effect of infectious burden on the

410 prevalence of atopy and respiratory allergies in Iceland, Estonia, and Sweden. *The Journal of*

411 *allergy and clinical immunology.* 2007;120(3):673-679.

412 25. Veiga RV, Cunha SS, Dattoli VC, et al. Chronic virus infections supress atopy but not asthma

413 in a set of children from a large Latin American city: a cross-section study. *BMC Pulm Med.*

414 2011;11:24.

415 26. Pembrey L, Waiblinger D, Griffiths P, Patel M, Azad R, Wright J. Cytomegalovirus, Epstein-

416 Barr virus and varicella zoster virus infection in the first two years of life: a cohort study in

417 Bradford, UK. *BMC Infect Dis.* 2017;17(1):220.

418 27. Wright J, Small N, Raynor P, et al. Cohort Profile: The Born in Bradford multi-ethnic family

419 cohort study. *International journal of epidemiology.* 2013;42(4):978-991.

420 28. Weiland SK, Bjorksten B, Brunekreef B, Cookson WO, von Mutius E, Strachan DP. Phase II of

421 the International Study of Asthma and Allergies in Childhood (ISAAC II): rationale and

422 methods. *Eur Respir J.* 2004;24(3):406-412.

423 29. Bousquet J, Anto J, Auffray C, et al. MeDALL (Mechanisms of the Development of ALLergy):

424 an integrated approach from phenotypes to systems medicine. *Allergy.* 2011;66(5):596-604.

425   30.   Hohmann C, Pinart M, Tischer C, et al. The development of the MeDALL Core Questionnaires

426   for a harmonized follow-up assessment of eleven European birth cohorts on asthma and

427   allergies. *Int Arch Allergy Immunol.* 2014;163(3):215-224.

428   31.   *Stata Statistical Software: Release 14* [computer program]. College Station, TX: StataCorp LP;

429   2015.

430   32.   Upchurch S, Harris JM, Cullinan P. Temporal changes in UK birth order and the prevalence of

431   atopy. *Allergy.* 2010;65(8):1039-1041.

432   33.   Oddy WH, Sherriff JL. Breastfeeding, body mass index, asthma and atopy in children. *Asia*

433   *Pac J Public Health.* 2003;15 Suppl:S15-17.

434   34.   Mihrshahi S, Ampon R, Webb K, et al. The association between infant feeding practices and

435   subsequent atopy among children with a family history of asthma. *Clin Exp Allergy.*

436   2007;37(5):671-679.

437   35.   Jansen MAE, van den Heuvel D, Jaddoe VWV, Moll HA, van Zelm MC. No Interactive Effects

438   of Sex and Persistent Cytomegalovirus on Immune Phenotypes in Young Children: The

439   Generation R Study. *The Journal of infectious diseases.* 2017;215(6):883-888.

440   36.   Holder B, Miles DJ, Kaye S, et al. Epstein-Barr virus but not cytomegalovirus is associated

441   with reduced vaccine antibody responses in Gambian infants. *PloS one.* 2010;5(11):e14013.

442   37.   Grose C, Johanson DC. Transmission of Cytomegalovirus, Epstein-Barr Virus, and Herpes

443   Simplex Virus Infections: From the Lucy Australopithecus Epoch to Modern-Day Netherlands.

444   *The Journal of pediatrics.* 2016;170:9-10.

445   38.   Uekert SJ, Akan G, Evans MD, et al. Sex-related differences in immune development and the

446   expression of atopy in early childhood. *The Journal of allergy and clinical immunology.*

447   2006;118(6):1375-1381.

448   39.   Salo PM, Arbes SJ, Jr., Jaramillo R, et al. Prevalence of allergic sensitization in the United

449   States: results from the National Health and Nutrition Examination Survey (NHANES) 2005-

450   2006. *The Journal of allergy and clinical immunology.* 2014;134(2):350-359.

451   40.   Thorne C, Newell ML, European Collaborative Study. Are girls more at risk of intrauterine-

452   acquired HIV infection than boys? *AIDS.* 2004;18(2):344-347.

453   41.   European Paediatric Hepatitis C Virus Network. A significant sex--but not elective cesarean

454   section--effect on mother-to-child transmission of hepatitis C virus infection. *The Journal of*

455   *infectious diseases.* 2005;192(11):1872-1879.

456   42.   Aage S, Kiraly N, Da Costa K, et al. Neonatal vitamin A supplementation associated with

457   increased atopy in girls. *Allergy.* 2015;70(8):985-994.

458   43.   Jensen KJ, Sondergaard MJ, Andersen A, et al. Long-term sex-differential effects of neonatal

459   vitamin A supplementation on in vitro cytokine responses. *Br J Nutr.* 2017;118(11):942-948.

460   44.   van den Heuvel D, Jansen MAE, Nasserinejad K, et al. Effects of nongenetic factors on

461   immune cell dynamics in early childhood: The Generation R Study. *The Journal of allergy and*

462   *clinical immunology.* 2017;139(6):1923-1934 e1917.

463   45.   Avdic S, McSharry BP, Steain M, et al. Human Cytomegalovirus-Encoded Human Interleukin-

464   10 (IL-10) Homolog Amplifies Its Immunomodulatory Potential by Upregulating Human IL-10

465   in Monocytes. *Journal of virology.* 2016;90(8):3819-3827.

466   46.   Gruzieva O, Gehring U, Aalberse R, et al. Meta-analysis of air pollution exposure association

467   with allergic sensitization in European birth cohorts. *The Journal of allergy and clinical*

468   *immunology.* 2014;133(3):767-776 e767.

469   47.   Perkin MR, Strachan DP. Which aspects of the farming lifestyle explain the inverse

470   association with childhood allergy? *The Journal of allergy and clinical immunology.*

471   2006;117(6):1374-1381.

472   48.   Collin SM, Granell R, Westgarth C, et al. Pet ownership is associated with increased risk of

473   non-atopic asthma and reduced risk of atopy in childhood: findings from a UK birth cohort.

474   *Clin Exp Allergy.* 2015;45(1):200-210.

475 49. Weinmayr G, Gehring U, Genuneit J, et al. Dampness and moulds in relation to respiratory

476 and allergic symptoms in children: results from Phase Two of the International Study of

477 Asthma and Allergies in Childhood (ISAAC Phase Two). *Clin Exp Allergy.* 2013;43(7):762-774.

478 50. Knight V, Wolf ML, Trikha A, Curran-Everett D, Hiserote M, Harbeck RJ. A comparison of

479 specific IgE and skin prick test results to common environmental allergens using the HYTEC

480 288. *J Immunol Methods.* 2018.

481 51. Ewan PW, Coote D. Evaluation of a capsulated hydrophilic carrier polymer (the ImmunoCAP)

482 for measurement of specific IgE antibodies. *Allergy.* 1990;45(1):22-29.

483

484 **Table 1. Characteristics of the 740 children and their mothers and associations with atopy at age 4 years.**

485 Adjusted ORs only presented for variables included in the multiple logistic regression model for age at CMV infection.

	N (%)	Number of children atopic (%)	Unadjusted OR, 95% CI, p	Adjusted OR, 95% CI, p
Age at CMV infection				
By 12 months	181 (24%)	31 (17)	1.02 (0.65 – 1.60), 0.95	See table 2 for sex-specific
12-24 months	74 (10%)	22 (30)	2.08 (1.20 – 3.61), 0.009	estimates
Uninfected at 24 months	485 (66%)	82 (17)	1.0	
Age at EBV infection				
By 12 months	112 (15%)	21 (19)	1.07 (0.63 – 1.83), 0.80	See table 2 for sex-specific
12-24 months	193 (26%)	37 (19)	1.10 (0.71 – 1.70), 0.66	estimates
Uninfected at 24 months	435 (59%)	77 (18)	1.0	
Age at VZV infection				
By 12 months	66 (9%)	12 (18)	1.00 (0.52 – 1.95), 0.99	See table 2 for sex-specific
12-24 months	128 (17%)	24 (19)	1.04 (0.64 – 1.71), 0.87	estimates
Uninfected at 24 months	546 (74%)	99 (18)	1.0	
Mother's ethnic group				
White British	258 (35%)	28 (11%)	1.0	1.0
Pakistani	386 (52%)	89 (23%)	2.46 (1.56 – 3.89), <0.001	1.78 (0.96 – 3.31), 0.07
Other	96 (13%)	18 (19%)	1.90 (0.99 – 3.61), 0.05	1.36 (0.65 – 2.85), 0.41
Sex				
Female	343 (46%)	40 (12%)	1.0	1.0
Male	397 (54%)	95 (24%)	2.38 (1.59 – 3.56), <0.001	3.28 (1.87 – 5.76), <0.001*
Birth order				
1	257 (35%)	47 (18%)	1.0	1.0
2	208 (28%)	31 (15%)	0.78 (0.48 – 1.28), 0.33	0.73 (0.43 – 1.25), 0.25
3+	275 (37%)	57 (21%)	1.17 (0.76 – 1.80), 0.48	0.86 (0.53 – 1.41), 0.56
Duration of breastfeeding				
Never	126 (17%)	20 (16%)	1.0	1.0
0-12 months	510 (69%)	88 (17%)	1.11 (0.65 – 1.88), 0.71	1.19 (0.66 – 2.13), 0.56
>12 months	102 (14%)	26 (25%)	1.81 (0.94 – 3.48), 0.07	1.93 (0.93 – 4.00), 0.08

25

missing	2			
Regular childcare attendance by 24 months				
Ever	293 (40%)	41 (14%)	0.61 (0.41 – 0.91), 0.02	0.79 (0.49 – 1.29), 0.35
Never	447	94 (21%)	1.0	1.0
Low birth weight (<2500g)				
Yes	63 (9%)	16 (25%)	1.60 (0.88 – 2.91), 0.13	1.90 (1.00 – 3.62), 0.05
No	677	119 (18%)	1.0	1.0
Pets in the home (at 12 months)				
Yes	185 (25%)	25 (14%)	0.63 (0.39 – 1.01), 0.06	0.87 (0.50 – 1.49), 0.60
No	550	109 (20%)	1.0	1.0
missing	5			
Damp (damp spots on walls or ceilings) at 12 months				
Yes	168 (23%)	42 (25%)	1.70 (1.12 – 2.57), 0.01	1.68 (1.08 – 2.62), 0.02
No	567	93 (16%)	1.0	1.0
missing	5			
Smoking in pregnancy (asked at maternal baseline questionnaire)				
Yes	85 (12%)	9 (11%)	0.50 (0.24 – 1.02), 0.06	0.72 (0.33 – 1.57), 0.41
No	654	126 (19%)	1.0	1.0
missing	1			
Central heating at 12 months				
Yes	706 (96%)	126 (18%)	1.0	1.0
No	30	9 (30%)	1.97 (0.88 – 4.41), 0.10	2.20 (0.93 – 5.19), 0.07
missing	4			
Maternal education				
5 GCSE equivalent or less	334 (45%)	67 (20%)	1.0	
A level equiv & higher	341 (46%)	60 (18%)	0.85 (0.58 – 1.25), 0.41	
other/DK/foreign unknown	64 (9%)	8 (13%)	0.57 (0.26 – 1.25), 0.16	
missing	1			
Home ownership				
Yes	470 (64%)	85 (18%)	1.0	
No	269	50 (19%)	1.03 (0.70 – 1.52), 0.87	
missing	1			

Maternal history of eczema, asthma or hay fever**			
Yes	284 (38%)	47 (17%)	0.83 (0.56 – 1.22), 0.35
No	456	88 (19%)	1.0
Paternal history of eczema, asthma or hay fever**			
Yes	231 (31%)	45 (19%)	1.13 (0.76 – 1.68), 0.56
No	509	90 (18%)	1.0
Term delivery (≥37 weeks)			
Yes	696 (94%)	127 (18%)	1.00 (0.46 – 2.21), 0.99
No	44	8 (18%)	1.0
Mode of delivery			
Vaginal	589 (80%)	106 (18%)	1.0
Caesarean section	146	28 (19%)	1.08 (0.68 – 1.72), 0.74
missing	5		

486

487   * Adjusted OR shows main effect of sex (i.e. among CMV uninfected) from the model including interaction term between sex and age at CMV infection

488   ** Similar results if limited to maternal/paternal history of eczema or hay fever (as not all asthma is atopic)

489

Table 2. Sex-specific estimates for the association between age at CMV, EBV and VZV infection and atopy.

			n	No. atopic (%)	Unadjusted OR, 95% CI, p	Adjusted OR*, 95% CI, p n=732
Girls	**Age at CMV infection**	By 12 months	77	9 (12)	1.43 (0.62 – 3.31), 0.41	0.97 (0.40 – 2.33), 0.94
		12-24 months	42	12 (29)	4.32 (1.90 – 9.78), <0.001	4.38 (1.87 – 10.29), 0.001
		Uninfected at 24 months	224	19 (8)	1.0	1.0
Boys	**Age at CMV infection**	By 12 months	104	22 (21)	0.84 (0.49 – 1.46), 0.54	0.57 (0.31 – 1.04), 0.07
		12-24 months	32	10 (31)	1.43 (0.64 – 3.18), 0.38	1.20 (0.52 – 2.79), 0.67
		Uninfected at 24 months	261	63 (24)	1.0	1.0
Girls	**Age at EBV infection**	By 12 months	47	3 (6)	0.60 (0.17 – 2.09), 0.42	0.42 (0.12 – 1.53), 0.19
		12-24 months	91	16 (18)	1.87 (0.92 – 3.78), 0.08	1.64 (0.79 – 3.39), 0.18
		Uninfected at 24 months	205	21 (10)	1.0	1.0
Boys	**Age at EBV infection**	By 12 months	65	18 (28)	1.19 (0.64 – 2.21), 0.58	0.99 (0.52 – 1.90), 0.98
		12-24 months	102	21 (21)	0.81 (0.46 – 1.42), 0.45	0.72 (0.40 – 1.32), 0.29
		Uninfected at 24 months	230	56 (24)	1.0	1.0
Girls	**Age at VZV infection**	By 12 months	32	2 (6)	0.57 (0.13 – 2.51), 0.45	0.52 (0.11 – 2.42), 0.41
		12-24 months	64	12 (19)	1.96 (0.93 – 4.14), 0.08	2.85 (1.29 – 6.30), 0.009
		Uninfected at 24 months	247	26 (11)	1.0	1.0
Boys	**Age at VZV infection**	By 12 months	34	10 (29)	1.29 (0.59 – 2.82), 0.52	1.60 (0.70 – 3.67), 0.27
		12-24 months	64	12 (19)	0.71 (0.36 – 1.41), 0.33	0.79 (0.39 – 1.61), 0.52
		Uninfected at 24 months	299	73 (24)	1.0	1.0

* adjusted for ethnic group, birth order, duration of breastfeeding, regular childcare attendance by 24 months, low birth weight, pets in the home (12 months), damp (12 months), smoking pregnancy and central heating (12 months).

507 **Table 3. Number of positive reactions by allergen**

	Number of positive reactions >=3mm				
	1	**2**	**3**	**4**	**total**
Cat	1 (2%)	5 (5%)	5 (8%)	3 (12%)	
Dog	2 (4%)	0	2 (3%)	3 (13%)	
Grass mix	13 (23%)	6 (6%)	16 (25%)	6 (25%)	
Mite 1 (D. Pter)	27 (48%)	48 (46%)	20 (32%)	6 (25%)	
Mite 2 (D. Farin)	13 (23%)	45 (43%)	20 (32%)	6 (25%)	
	56	104 (52 children)	63 (21 children)	24 (6 children)	135

508