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1 **Functional and genetic predisposition to rhinovirus lower respiratory tract**
2 **infections in prematurely born infants**

3
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33 **ABSTRACT**

34

35 Term born infants are predisposed to human rhinovirus (HRV) lower respiratory
36 tract infections (LRTI) by reduced neonatal lung function and genetic
37 susceptibility. Our aim was to investigate whether prematurely born infants
38 were similarly predisposed to HRV LRTIs or any other viral LRTIs. Infants
39 born less than 36 weeks of gestational age were recruited. Prior to
40 neonatal/maternity unit discharge, lung function (functional residual capacity by
41 helium gas dilution and multiple breath washout, lung clearance index and
42 compliance (C_{rs}) and resistance (R_{rs}) of the respiratory system) was assessed and
43 DNA samples assessed for eight single nucleotide polymorphisms (SNPs) in
44 seven genes: ADAM33, IL10, MMP16, NF κ B1A, SFTPC, VDR and NOS2A.
45 Infants were prospectively followed until one year corrected age.
46 Nasopharyngeal aspirates (NPAs) were sent whenever an infant developed a
47 LRTI and tested for 13 viruses. One hundred and thirty-nine infants were
48 included in the analysis. Infants who developed HRV LRTIs had reduced C_{rs}
49 (1.6 versus 1.2 mL/cmH₂O/kg, $p=0.044$) at 36 weeks postmenstrual age. A SNP
50 in the gene coding for the vitamin D receptor was associated with the
51 development of HRV LRTIs and any viral LRTIs ($p=0.02$).

52 *Conclusion* Prematurely born infants may have both a functional and genetic
53 predisposition to HRV LRTIs.

54

55 **Key words:** human rhinovirus; single nucleotide polymorphisms; compliance
56 and resistance of the respiratory system; functional residual capacity

57

58 **List of abbreviations**

59

60	BPD	Bronchopulmonary dysplasia
61	C_{rs}	Compliance of the respiratory system
62	FRC_{HE}	Functional residual capacity (by helium gas)
63	HRV	Rhinovirus
64	LCI	Lung clearance index
65	LRTI	Lower respiratory tract infection
66	NPA	Nasopharyngeal aspirates
67	PCR	Polymerase chain reaction
68	PMA	Postmenstrual age
69	R_{rs}	Resistance of the respiratory system
70	RSV	Respiratory syncytial virus
71	SNP	Single nucleotide polymorphisms
72	VDR	Vitamin D receptor

73

74 **AUTHORS SUMMARY**

75

76 **What is known**

- 77 • Term born infants are predisposed to rhinovirus lower respiratory tract
78 (HRV LRTIs) infection by reduced neonatal lung function.
- 79 • Term born infants requiring hospitalisation due to HRV bronchiolitis were
80 more likely to have single nucleotide polymorphism (SNP) in the IL-10
81 gene.

82

83 **What is new**

- 84 • Prematurely born infants who developed a HRV LRTI had lower C_{rs} before
85 maternity unit discharge.
- 86 • A SNP in the gene coding for the vitamin D receptor was associated with
87 the development of HRV LRTIs and overall respiratory viral LRTIs in
88 prematurely born infants.

89

90 INTRODUCTION

91

92 Human rhinoviruses (HRV) are the most common cause of respiratory tract
93 infection in infants, with almost all infants developing at least one HRV
94 infection in the first year after birth [14, 23]. Both term and prematurely born
95 infants are susceptible to developing LRTIs caused by HRV [3, 11, 13, 21,24].
96 Some term born infants may be predisposed to wheezy HRV LRTIs by reduced
97 neonatal lung function [22]. The adjusted risk of developing a wheezy HRV
98 LRTI in the first year of life was 1.8 times higher for each standard deviation
99 increase of airway resistance (R_{rs}) measured at two months of age [22]. In
100 addition, some term born infants may be genetically predisposed to HRV
101 infection. Infants developing HRV bronchiolitis requiring hospitalisation at less
102 than six months of age were more likely to have a single nucleotide
103 polymorphism (SNP) in the IL-10 gene compared to unselected blood donors
104 [9]. Other SNPs in genes coding for IL-18, TLR4 and IFN- γ did not confer
105 susceptibility to hospitalisation for HRV infection [9]. The aim of this study
106 was to determine whether prematurely born infants were functionally and
107 genetically predisposed to HRV LRTIs. An additional aim was to determine
108 whether prematurely born infants were functionally and genetically predisposed
109 overall to respiratory viral LRTIs.

110

111

112

113

114 MATERIALS AND METHODS

115

116 Analysis was undertaken of the results of infants entered into a study
117 investigating the risk factors for viral LRTIs in prematurely born infants [7].
118 Infants were eligible for recruitment into the study if they were born prior to the
119 onset of the RSV season (1st October to 31st March in the UK) in 2008 or 2009
120 and were born at less than 36 weeks of completed gestation. Ethical approval
121 was obtained from King's College Hospital NHS Foundation Trust Research
122 Ethics Committee.

123

124 Prior to neonatal/maternity unit discharge either blood or buccal swabs were
125 obtained from infants and stored at -20°C until tested. The samples were then
126 sent on dry ice to the National Institute for Public Health and the Environment
127 (RIVM) in Bilthoven, The Netherlands for testing. DNA was isolated from the
128 blood samples or buccal swabs and then stored at -20°C at the RIVM until
129 analysed [6]. Eight single nucleotide polymorphisms (SNPs) were chosen to be
130 tested. The chosen SNPs had previously been associated with HRV infection in
131 term born infants less than six months old [9]. Nuclear factor- κ -B activity has
132 been associated with steroid resistant airway epithelium in HRV infection *in*
133 *vitro* and thus the SNP NF κ B1A rs2233409 was also included [15]. In addition,
134 we have studied SNPs associated with reduced lung function in previously
135 healthy children at three and five years of age [20], RSV infection in
136 prematurely born infants [19], prematurity [10] or bronchopulmonary dysplasia
137 (BPD) [8]. We also included SNPs associated with RSV infection in

138 prematurely born infants as they may be associated with other viral causes of
139 bronchiolitis (i.e. HRV) in prematurely born infants.

140

141 The extracted DNA samples were diluted with TE Buffer to 7 ng/μL and sent to
142 KBioscience (Herts, UK) for genotyping. Six SNPs (ADAM33 rs2787094,
143 IL10 rs1800872, MMP16 rs2664349, MMP16 rs2664352, NFκB1A rs2233409
144 and SFTPC rs1124) were tested at KBioscience with the KASPar technology
145 and two further SNPs (vitamin D receptor [VDR rs10735810] and nitric oxide
146 synthase 2A [NOS2A rs1060826]) were tested at the RIVM in the Netherlands.
147 Genotyping of VDR rs10735810 was performed by a custom TaqMan SNP
148 genotyping assay (Applied Biosystems, Carlsbad, USA) and genotyping of
149 NOS2A rs1060826 was performed by using TaqMan SNP genotyping assay
150 C_9458082_10. Genotyping of both SNPs tested at the RIVM was carried out
151 on a 7500 Fast Real-Time PCR system (Applied Biosystems) as previously
152 described [6]. The genotype distributions of the eight SNPs were in Hardy-
153 Weinberg equilibrium [6].

154

155 Lung function was assessed at 36 weeks postmenstrual age (PMA) whilst infants
156 were still inpatients on the neonatal or maternity unit. Infants were not sedated
157 or ventilated during lung function testing. Lung volume was assessed by
158 measurement of functional residual capacity (FRC_{He}), using a commercially
159 available helium gas dilution system (EBS 2615, Equilibrated Bio Systems,
160 New York) as previously described [5]. Lung volume was also assessed by the
161 measurement of FRC (FRC_{MBW}) using the commercially available open circuit

multiple breath wash-in/out system (Exhalyzer D, Ecomedics, Duernten, Switzerland) and using sulphur hexafluoride as a tracer gas as previously described [6]. The MBW technique also measures ventilation inhomogeneity (VI), measured as lung clearance index (LCI) as previously described [6]. Compliance (C_{rs}) and resistance (R_{rs}) of the respiratory system were measured using the single breath occlusion technique as previously described [7].

Following neonatal or maternity unit discharge, infants were followed prospectively until one year corrected age. Whenever an infant developed an LRTI, regardless of whether the child remained at home or required hospitalisation a nasopharyngeal aspirate (NPA) was taken. An infant was diagnosed with a viral LRTI if they had coryzal symptoms together with a respiratory examination demonstrating either a raised respiratory rate for their age, crackles or wheeze or respiratory distress (e.g. tracheal tug or intercostal or subcostal recession). NPAs were tested for 11 viruses (rhinovirus, RSV A and B, human metapneumovirus, influenza A and B, parainfluenza 1-3, enterovirus and parechovirus) using real time reverse transcription polymerase chain reaction (PCR) and for adenovirus and bocavirus using real time PCR as previously described [5].

The neonatal notes were reviewed to document demographic and clinical data and to document the duration of the infants' admission on the neonatal and/or maternity unit. Antenatal, perinatal and postnatal data collected included that on maternal infections, antenatal steroid use, use of surfactant, duration of

186 respiratory support, development of bronchopulmonary dysplasia (BPD),
187 postnatal infant sepsis, breast/formula feeding and use of palivizumab [7].

188

189 **Statistical Analysis**

190

191 The infants were divided into two groups depending on their HRV LRTI status.
192 The “no LRTI group” consisted of infants who did not develop a viral LRTI
193 throughout the study period and the “HRV LRTI group” consisted of infants
194 who developed at least one HRV LRTI during the study period. The infants in
195 the HRV LRTI group may also have had other viral LRTIs. We also undertook
196 a subsidiary analysis of all infants who had LRTIs with NPAs positive for
197 respiratory viruses and compared their outcomes to infants who had no LRTI.
198 Infants who had LRTIs but no virus was detected from the NPA were excluded
199 from the analysis.

200

201 Data were tested for normality using the Shapiro-Wilk test. Data were analysed
202 using either the independent T-test, the Mann-Whitney U test, the Chi-squared
203 test or the Fisher’s exact test as appropriate. A multivariable regression model
204 was used to examine whether lung function at 36 weeks PMA was a predictor of
205 HRV LRTI or respiratory viral LRTIs, independent of other variables which in
206 the univariate analysis were significant at $p \leq 0.1$. Statistical analysis was
207 carried out with IBM SPSS Statistics (version 19, New York, USA).

208

209

210 Sample size

211

212 A sample size of 28 infants in each group allowed the detection of a difference
213 in the premorbid lung function results equivalent to one standard deviation, with
214 90% power and two-sided 5% significance. A previous study [2], demonstrated
215 a significant difference in lung function (R_{rs}) equivalent to one standard
216 deviation between the groups.

217

218 **RESULTS**

219

220 During the study period two hundred and fifty one infants met the eligibility
221 criteria for recruitment into the study (Figure 1). One hundred and thirty-nine
222 infants were included in the overall analysis. Their median gestational age (GA)
223 was 34 (range 23-35) weeks and median birth weight 1904 (range 610-3610) g.
224 Four infants received palivizumab of which one was admitted to hospital due to
225 an RSV LRTI. There were significant differences when comparing the
226 demographic data of the HRV group and the no LRTI group. The HRV group
227 were more immature and lighter at birth, more received surfactant, had a longer
228 duration of supplemental oxygen, developed BPD, received palivizumab,
229 developed postnatal sepsis and had a longer duration of neonatal/maternity unit
230 stay (Table 1). Comparison of those infants who developed any respiratory
231 virus LRTI compared to no LRTI is shown in Appendix Table 1. Some infants
232 developed more than one viral LRTI or had more than one virus detected from
233 an NPA during a HRV LRTI (Table 2).

234 Eight (25%) infants in the HRV LRTI group required hospitalisation (six due to
235 a viral LRTI [two HRV]), one due to a minor head injury and one due to
236 gastroenteritis. Nine (12%) infants in the no LRTI group required
237 hospitalisation (all due to non-respiratory causes).

238

239 The HRV LRTI group were more immature (36 weeks versus 37 weeks PMA,
240 $p=0.031$) and of lower weight (1908 versus 2113 g, $p=0.007$) when their lung
241 function was measured. The HRV LRTI group had a smaller FRC_{He}
242 uncorrected for weight ($p=0.004$), although this was no longer significantly
243 different after correcting for weight ($p=0.13$), a smaller FRC_{MBW} uncorrected for
244 weight ($p=0.001$) which remained significantly different when corrected for
245 weight ($p=0.042$), a lower C_{rs} uncorrected for weight ($p=0.001$) which remained
246 significantly different when corrected for weight ($p=0.005$) and a higher R_{rs}
247 ($p=0.028$) (Table 3). Multivariate analysis revealed that after correcting for
248 significant differences in the demographic data the only difference in lung
249 function between the groups that remained significant was in the C_{rs} corrected
250 for weight (Table 3). There were no significant differences in the lung function
251 results of the infants who had any respiratory virus LRTI compared to those who
252 had no LRTI after correcting for differences in their demographics (Appendix
253 Table 2).

254

255 There were no significant differences at the genotype level in any of the SNPs
256 between the HRV LRTI and no LRTI groups (data not shown). There was a
257 significant difference in the SNP (rs10735810) in the VDR gene at the allele

level. Infants with the G allele were significantly more likely (OR 2.07 (95% CI [0.98-3.13], $p=0.047$) to develop HRV LRTIs than those with the A allele (Table 4). Similarly there was a significant difference in the SNP in the VDR gene at the allele level between infants who did and did not develop a respiratory viral LRTI ($p=0.02$) (Appendix Table 3).

DISCUSSION

We have demonstrated that prematurely born infants who developed HRV LRTIs had reduced premorbid lung function, that is they had significantly lower C_{rs} than those who did not develop an HRV LRTI. In addition, a SNP in the G allele of the vitamin D receptor gene was associated with an increased risk of developing HRV LRTIs and respiratory viral LRTIs overall.

Term born infants have been shown to have reduced lung function prior to developing HRV LRTIs [22]. Although, in that study overall there were no significant differences in lung function between the infants who did and did not develop an HRV infection, those infants who wheezed with an HRV infection had significantly reduced lung function (C_{rs} and R_{rs}) compared with those infants who had HRV infections but did not wheeze [22]. In this study, initial analysis demonstrated several differences in lung function between infants who did and did not develop an HRV LRTI. After adjusting for differences in the demographic data, however, the only significant difference that remained was in

282 C_{rs} corrected for weight. A possible explanation is that infants with a low C_{rs}
283 may have less lung distensibility leading to poorer clearance of respiratory
284 secretions. In term born infants, a reduced C_{rs} was associated with an increased
285 susceptibility to hospitalisation with RSV LRTIs as well as post RSV
286 bronchiolitis wheezing [25]. Although there were significant differences in lung
287 function between the all virus LRTI and the no LRTI group, these disappeared
288 after adjusting for confounding factors.

289

290 Vitamin D deficiency has been associated with an increased risk of developing
291 viral LRTIs in infants, in particular RSV LRTIs [1, 18]. In addition, SNPs in the
292 VDR gene have been associated with severe RSV bronchiolitis and other viral
293 LRTIs in infants [12, 17] but no previous study has investigated the role of the
294 VDR in HRV infection. In this study a SNP in the gene coding for VDR was
295 associated with the development of HRV LRTIs in prematurely born infants
296 and, in addition, in infants overall with respiratory viral LRTIs. Vitamin D has
297 an important role in innate immunity [4] it is thus plausible that defects in the
298 VDR will increase an infant's susceptibility to HRV infections. Only one
299 previous study [9] has investigated the genetic susceptibility of infants to HRV
300 infection. In that study [9], term born infants with the A allele of a SNP (at -
301 1082) in the gene coding for IL-10 were more likely to be hospitalised for HRV
302 bronchiolitis at less than six months of age than those with the G allele. In this
303 study a different SNP (rs10735810) in the IL-10 gene was not associated with
304 HRV LRTI. The difference in those results suggest that genetic susceptibility to
305 HRV infection is different in term and prematurely born infants. The other SNPs

306 tested in this study have been associated with severe RSV infection, prematurity
307 or BPD in prematurely born infants but not HRV infection and did not appear to
308 influence the development of HRV LRTIs, suggesting they may not have a role
309 in prematurely born infants' susceptibility to HRV LRTI. We also did not find
310 any significant association between the SNPs tested and respiratory viral
311 infections overall. The numbers of infants with each viral infection precluded
312 subanalysis at that level.

313

314 The current study has strengths and some weaknesses. A large cohort of
315 prematurely born infants from a variety of ethnic backgrounds was prospectively
316 followed. Lung function was assessed before neonatal or maternity unit
317 discharge, that is prior to any of the infants being infected with any viral
318 infection. The wide range of ethnicities in the study may have affected the
319 results, as genotype differences in various ethnic groups may increase the
320 likelihood of associations occurring by chance [16]. No correction was made for
321 multiple testing of the genetics data; it is, therefore, possible the significant
322 differences we demonstrate with respect to VDR could be attributable to chance.
323 Nevertheless, we demonstrate a significant relationship not only with HRV
324 LRTIs but any respiratory viral LRTIs. Although infants born at less than 36
325 weeks GA were eligible for entry into the study most of the infants recruited
326 were born moderately prematurely (median gestational age 34 weeks) and thus
327 the results of this study may not be generalisable to all infants born extremely
328 prematurely.

329 In conclusion, prematurely born infants may be predisposed to HRV LRTIs by
330 both reduced premorbid lung function and genetic susceptibility. A SNP in the
331 gene coding for VDR was associated with the development overall of
332 respiratory viral LRTIs.
333

334 **Compliance with ethical standards**

335

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343 **Conflict of interest:** There is no conflict of interest to declare from all authors.

344 **Compliance and ethical standards:** All procedures performed in studies
345 involving human participants were in accordance with the ethical standards of
346 the institutional and/or national research committee and with the 1964 Helsinki
347 declaration and its later amendments or comparable ethical standards.

348 **Informed consent:** Infants whose parents gave informed written consent were
349 recruited.

350 **Contributor statement:** AG, SLJ and LB designed the study. MS and MZ
351 undertook the virological analyses. SBD undertook the lung function
352 assessments. MA, TW and SBD were responsible for the follow up of the
353 patients. SBD, HMH, RJ and LB undertook the genetic analyses. All authors
354 were involved in the preparation of the manuscript.

355

356 **REFERENCES**

- 357 1 Belderbos ME, Houben ML, Wilbrink B, Lentjes E, Bloemen EM,
358 Kimpfen JL, Rovers M, Bont L (2011) Cord blood vitamin D
359 deficiency is associated with respiratory syncytial virus bronchiolitis.
360 Pediatrics 127:e1513–20.
- 361 2 Broughton S, Bhat R, Roberts A, Zuckerman M, Rafferty GF,
362 Greenough A (2006) Diminished lung function, RSV infection, and
363 respiratory morbidity in prematurely born infants. Arch Dis Child
364 91:26–30.
- 365 3 Chidekel AS, Rosen CL, Bazzzy AR (1997) Rhinovirus infection
366 associated with serious lower respiratory illness in patients with
367 bronchopulmonary dysplasia. Pediatr Infect Dis J 16:43–7.
- 368 4 Chun RF, Liu PT, Modlin RL, Adams JS, Hewison M (2014) Impact of
369 vitamin D on immune function: lessons learned from genome-wide
370 analysis. Front Physiol 5:151.
- 371 5 Drysdale SB, Lo J, Prendergast M, Alcazar M, Wilson T, Zuckerman M,
372 Smith M, Broughton S, Rafferty GF, Peacock JL, Johnston SL,
373 Greenough A (2014) Lung function of preterm infants before and
374 after viral infections. Eur J Pediatr 173:1497–504.
- 375 6 Drysdale SB, Prendergast M, Alcazar M, Wilson T, Smith M,
376 Zuckerman M, Broughton S, Rafferty GF, Johnston SL,
377 Hodemaekers HM, Janssen R, Bont L, Greenough A (2014) Genetic
378 predisposition of RSV infection-related respiratory morbidity in
379 preterm infants. Eur J Pediatr 173:905–912.

- 380 7 Drysdale SB, Wilson T, Alcazar M, Broughton S, Zuckerman M, Smith
381 M, Rafferty GF, Johnston SL, Greenough A (2011) Lung function
382 prior to viral lower respiratory tract infections in prematurely born
383 infants. *Thorax* 66:468–473.
- 384 8 Hadchouel A, Decobert F, Franco-Montoya M-L, Halphen I, Jarreau PH,
385 Boucherat O, Martin E, Benachi A, Amselem S, Bourbon J, Danan
386 C, Delacourt C (2008) Matrix metalloproteinase gene polymorphisms
387 and bronchopulmonary dysplasia: identification of MMP16 as a new
388 player in lung development. *PLoS One* 3:e3188.
- 389 9 Helminen M, Nuolivirta K, Virta M, Halkosalo A, Korppi M, Vesikari T,
390 Hurme M (2008) IL-10 gene polymorphism at -1082 A/G is
391 associated with severe rhinovirus bronchiolitis in infants. *Pediatr*
392 *Pulmonol* 43:391–5.
- 393 10 Lahti M, Marttila R, Hallman M (2004) Surfactant protein C gene
394 variation in the Finnish population - association with perinatal
395 respiratory disease. *Eur J Hum Genet* 12:312–20.
- 396 11 Lemanske RF, Jackson DJ, Gangnon RE, Evans MD, Li Z, Shult PA,
397 Kirk CJ, Reisdorf E, Roberg KA, Anderson EL, Carlson-Dakes KT,
398 Adler KJ, Gilbertson-White S, Pappas TE, Dasilva DF, Tisler CJ,
399 Gern JE (2005) Rhinovirus illnesses during infancy predict
400 subsequent childhood wheezing. *J Allergy Clin Immunol* 116:571–7.
- 401 12 McNally JD, Sampson M, Matheson L, McNally JD, Sampson M
402 (2014) Vitamin D receptor (VDR) polymorphisms and severe RSV

403 bronchiolitis: a systematic review and meta-analysis. *Pediatr*
404 *Pulmonol* 49:790–9.

405 13 Miller EK, Bugna J, Libster R, Shepherd BE, Scalzo PM, Acosta PL,
406 Hijano D, Reynoso N, Batalle JP, Coviello S, Klein MI, Bauer G,
407 Benitez A, Kleeberger SR, Polack FP (2012) Human rhinoviruses in
408 severe respiratory disease in very low birth weight infants. *Pediatrics*
409 129:e60–7.

410 14 Müller L, Mack I, Tapparel C, Kaiser L, Alves MP, Kieninger E, Frey U,
411 Regamey N, Latzin P (2015) Human Rhinovirus Types and
412 Association with Respiratory Symptoms During the First Year of
413 Life. *Pediatr Infect Dis J* 34:907–909.

414 15 Papi A, Contoli M, Adcock IM, Bellettato C, Padovani A, Casolari P,
415 Stanciu LA, Barnes PJ, Johnston SL, Ito K, Caramori G. (2013)
416 Rhinovirus infection causes steroid resistance in airway epithelium
417 through nuclear factor κ b and c-Jun N-terminal kinase activation. *J*
418 *Allergy Clin Immunol* 132:1075–1085.e6.

419 16 Park J, Hwang S, Lee YS, Kim SC, Lee D (2007) SNP@Ethnos: A
420 database of ethnically variant single-nucleotide polymorphisms.
421 *Nucleic Acids Res* 35:D711–5.

422 17 Roth DE, Jones AB, Prosser C, Robinson JL, Vohra S (2008) Vitamin D
423 receptor polymorphisms and the risk of acute lower respiratory tract
424 infection in early childhood. *J Infect Dis* 197:676–80.

425 18 Roth DE, Shah R, Black RE, Baqui H (2010) Vitamin D status and acute
426 lower respiratory infection in early childhood in Sylhet, Bangladesh.

- 427 Acta Paediatr 99:389–93.
- 428 19 Siezen CLE, Bont L, Hodemaekers HM, Ermers MJ, Doornbos G, Van't
429 Slot R, Wijmenga C, Houwelingen HC, Kimpen JL, Kimman TG,
430 Hoebee B, Janssen R (2009) Genetic susceptibility to respiratory
431 syncytial virus bronchiolitis in preterm children is associated with
432 airway remodeling genes and innate immune genes. *Pediatr Infect*
433 *Dis J* 28:333–5.
- 434 20 Simpson A, Maniatis N, Jury F, Cakebread JA, Lowe LA, Holgate ST,
435 Woodcock A, Ollier WE, Collins A, Custovic A, Holloway JW, John
436 SL (2005) Polymorphisms in a disintegrin and metalloprotease 33
437 (ADAM33) predict impaired early-life lung function. *Am J Respir*
438 *Crit Care Med* 172:55–60.
- 439 21 Tran DN, Trinh QD, Pham NTK, Pham TM, Ha MT, Nguyen TQ,
440 Okitsu S, Shimizu H, Hayakawa S, Mizuguchi M, Ushijima H (2015)
441 Human rhinovirus infections in hospitalized children: clinical,
442 epidemiological and virological features. *Epidemiol Infect* 1–9.
- 443 22 van der Zalm MM, Uiterwaal CSPM, Wilbrink B, Koopman M, Verheij
444 TJ, van der Ent CK (2011) The Influence of Neonatal Lung Function
445 on Rhinovirus-associated Wheeze. *Am J Respir Crit Care Med*
446 183:262–7.
- 447 23 van der Zalm MM, Wilbrink B, van Ewijk BE, van Ewijk BE, Overduin
448 P, Wolfs TF, van der Ent CK (2011) Highly frequent infections with
449 human rhinovirus in healthy young children: a longitudinal cohort
450 study. *J Clin Virol* 52:317–20.

- 451 24 van Piggelen RO, van Loon AM, Krediet TG, Verboon-Maciolek M
452 (2010) Human rhinovirus causes severe infection in preterm infants.
453 *Pediatr Infect Dis J* 29:364–5.
- 454 25 Zomer-Kooijker K, Uiterwaal CSPM, van der Gugten AC, van der
455 Gugten AC, Wilbrink B, Bont LJ, van der Ent CK (2014) Decreased
456 lung function precedes severe respiratory syncytial virus infection
457 and post-respiratory syncytial virus wheeze in term infants. *Eur*
458 *Respir J* 666–674.
- 459

460 Table 1: Demographic data

461 Data are shown as median (range) or n (%)

	No LRTI	HRV LRTI	P value
N	74	32	
Gestational age (weeks)	34 (25-35)	33 (23-35)	0.03
Birth weight (g)	2070 (895-3610)	1558 (610-2546)	<0.001
Males	49 (53%)	14 (44%)	0.53
Ethnicity:			
Caucasian	23 (31%)	8 (25%)	0.53
Black African	17 (23%)	9 (28%)	0.57
Black Caribbean	15 (20%)	6 (19%)	0.75
Asian	3 (4%)	2 (6%)	0.62
Hispanic	1 (1%)	2 (6%)	0.16
Mixed ethnicity	15 (21%)	5 (16%)	0.49
Antenatal smoking	11 (15%)	6 (19%)	0.78
Antenatal steroids	42 (57%)	24 (75%)	0.09
Maternal sepsis	14 (19%)	4 (13%)	0.58
Surfactant	11 (15%)	13 (41%)	0.006
Duration of ventilation (days)	0 (0-82)	1 (0-103)	0.10
Duration of supplemental oxygen (days)	0 (0-118)	1.5 (0-458)	0.041
Bronchopulmonary dysplasia	4 (5%)	8 (25%)	0.006
Breastfed	62 (84%)	23 (72%)	0.19
Postnatal sepsis	20 (27%)	17 (53%)	0.014
Parental atopy	52 (70%)	20 (63%)	0.50
Number of siblings	0 (0-5)	0 (0-5)	0.64
Palivizumab	0 (0%)	4 (13%)	0.007
Neonatal/maternity unit stay (days)	16 (2-118)	28 (5-276)	0.003

462

463 Table 2: Number of viruses detected by real-time PCR in the HRV LRTI group

464 Data shown are the number of times a virus was detected. Some infants had

465 more than one viral LRTI.

466

	Viruses detected
Rhinovirus	40
RSV A	7
RSV B	7
Adenovirus	11
Human metapneumovirus	3
Influenza A	1
Influenza B	3
Parainfluenza 1	3
Parainfluenza 2	0
Parainfluenza 3	4
Enterovirus	14
Parechovirus	3
Bocavirus	4
Dual infections	24
Triple infections	4

467

468

469

470 Table 3: Lung function results

471 Data are shown as median (range).

472

	No LRTI	HRV LRTI	P value*	P value after correcting for confounding factors**
N	74	32		
Postmenstrual age (PMA) (weeks)	36 (34-42)	37 (35-43)	0.031	N/A
Weight (g)	2113 (1362-3360)	1908 (1200-2640)	0.007	N/A
FRC _{He} (mL)	55 (30-99)	49 (10-68)	0.004	0.55
FRC _{He} (mL/kg)	25 (17-34)	24 (8-35)	0.13	0.59
FRC _{MBW} (mL)	57 (30-91)	44 (13-64)	0.001	0.16
FRC _{MBW} (mL/kg)	27 (16-35)	23 (10-34)	0.042	0.10
LCI	9.8 (7.0-13.6)	10.3 (7.7-13.8)	0.066	0.60
C _{rs} (mL/cmH ₂ O)	3.2 (1.7-5.8)	2.5 (1.0-5.4)	0.001	0.21
C _{rs} (mL/cmH ₂ O/kg)	1.6 (0.7-2.3)	1.2 (0.4-2.1)	0.005	0.044
R _{rs} (cmH ₂ O/L/s)	69 (48-144)	76 (49-199)	0.028	0.85

473

474 *Univariate analysis comparing the two groups

475 **Multivariate analysis adjusting for confounding factors

476 Table 4: Associations at the allele levels by HRV status

477 Data are shown as n (%).

Gene	Allele	Association at the allele level			
		HRV LRTI	No LRTI	P	OR (95% CI)
Vitamin D receptor (VDR)	A	13 (22%)	51 (36%)	0.047	0.48 (0.22-1.03)
	G	47 (78%)	89 (64%)		2.07 (0.98-3.13)
Nitric oxide synthase type 2A (NOS2A)	T	43 (72%)	97 (69%)	0.87	1.12 (0.55-2.31)
	C	17 (28%)	43 (31%)		0.89 (0.43-1.82)
A disintegrin and metalloprotease 33 (ADAM33)	C	17 (28%)	43 (31%)	0.87	0.89 (0.43-1.82)
	G	43 (72%)	97 (69%)		1.12 (0.55-2.31)
NFκB1A	C	50 (86%)	108 (83%)	0.83	1.21 (0.50-2.90)
	T	8 (14%)	22 (17%)		0.82 (0.34-1.99)
IL10	A	19 (32%)	45 (34%)	0.87	0.90 (0.88-1.81)
	C	41 (68%)	87 (66%)		1.11 (0.55-2.26)
Pulmonary surfactant protein C (SFTPC)	A	12 (20%)	33 (24%)	0.71	0.81 (0.36-1.80)
	G	48 (80%)	107 (76%)		1.23 (0.56-2.78)
Matrix metalloproteinase-16 (MMP16) rs2664352	C	31 (52%)	69 (50%)	0.88	1.07 (0.56-2.05)
	T	29 (48%)	69 (50%)		0.94 (0.49-1.79)
MMP16 rs2664349	G	39 (65%)	86 (60%)	0.75	1.12 (0.57-2.22)
	A	21 (35%)	52 (40%)		0.89 (0.45-1.76)

478 **FIGURE LEGEND**

479

480 Figure 1: Flow diagram of eligibility

481

482 APPENDIX

483

484

485 Table 1: Demographic data

486

487 Data are shown as median (range) or n (%)

488

	No LRTI	All virus LRTI	P value
N	74	65	
Gestational age (weeks)	34 (25-35)	33 (23-35)	0.11
Birth weight (g)	2070 (895-3610)	2000 (1440-3154)	0.001
Males	39 (53%)	37 (57%)	0.73
Ethnicity:			
Caucasian	23 (31%)	14 (22%)	0.25
Black African	17 (23%)	19 (29%)	0.44
Black Caribbean	15 (20%)	16 (25%)	0.55
Asian	3 (4%)	3 (5%)	>0.99
Hispanic	1 (1%)	2 (3%)	0.60
Mixed ethnicity	15 (21%)	11 (14%)	0.67
Antenatal smoking	11 (15%)	11 (17%)	0.82
Antenatal steroids	42 (57%)	52 (80%)	0.004
Maternal sepsis	14 (19%)	16 (25%)	0.54
Surfactant	11 (15%)	20 (31%)	0.04
Duration of ventilation (days)	0 (0-82)	0.5 (0-103)	0.12
Duration of supplemental oxygen (days)	0 (0-118)	1 (0-458)	0.06
Bronchopulmonary dysplasia	4 (5%)	11 (17%)	0.052
Breastfed	62 (84%)	58 (89%)	>0.99
Postnatal sepsis	20 (27%)	23 (35%)	0.27
Parental atopy	52 (70%)	42 (65%)	0.59
Number of siblings	0 (0-5)	1 (0-5)	0.78
Palivizumab	0 (0%)	5 (8%)	0.02
Neonatal/maternity unit stay (days)	16 (2-118)	25 (3-276)	0.001

489

490 Table 2: Lung function results

491

492 Data are shown as median (range).

493

	No LRTI	All virus LRTI	P value*	P value after correcting for confounding factors**
N	74	65		
Postmenstrual age (PMA) (weeks)	36 (34-42)	36 (34-43)		N/A
Weight (g)	2113 (1362-3360)	1000 (1440-3154)		N/A
FRC _{He} (mL)	55 (30-99)	51 (22-99)	0.008	0.98
FRC _{He} (mL/kg)	25 (17-34)	24 (14-35)	0.27	0.94
FRC _{MBW} (mL)	57 (30-91)	53 (16-111)	0.02	0.28
FRC _{MBW} (mL/kg)	27 (16-35)	26 (10-42)	0.21	0.25
LCI	9.8 (7.0-13.6)	9.8 (6.0-14.1)	0.18	0.56
C _{rs} (mL/cmH ₂ O)	3.2 (1.7-5.8)	3.1 (1.0-6.7)	0.004	0.96
C _{rs} (mL/cmH ₂ O/kg)	1.6 (0.7-2.3)	1.3 (0.4-2.4)	0.018	0.55
R _{rs} (cmH ₂ O/L/s)	69 (48-144)	77 (43-199)	0.03	0.50

494

495 *Univariate analysis comparing the two groups

496 **Multivariate analysis adjusting for confounding factors

Table 3: Associations at the allele level by HRV status

498

499 Data are shown as n (%).

500

Gene	Allele	All virus LRTI	No LRTI	Association at the allele level	
				P	OR (95% CI)
Vitamin D receptor (VDR)	A	28 (23%)	51 (36%)	0.02	0.52 (0.30-0.90)
	G	94 (77%)	89 (64%)		1.92 (1.12-3.32)
Nitric oxide synthase type 2A (NOS2A)	T	88 (72%)	97 (69%)	0.68	1.15 (0.67-1.96)
	C	34 (28%)	43 (31%)		0.87 (0.51-1.49)
A disintegrin and metalloprotease 33 (ADAM33)	C	41 (34%)	43 (31%)	>0.99	1.01 (0.59-1.77)
	G	81 (56%)	97 (69%)		0.99 (0.56-1.72)
NFκB1A	C	99 (84%)	108 (83%)	0.87	1.06 (0.54-2.08)
	T	19 (16%)	22 (17%)		0.94 (0.48-1.84)
IL10	A	42 (34%)	45 (34%)	>0.99	1.02 (0.60-1.71)
	C	80 (66%)	87 (66%)		0.99 (0.59-1.66)
Pulmonary surfactant protein C (SFTPC)	A	19 (16%)	33 (24%)	0.12	0.60 (0.32-1.11)
	G	103 (84%)	107 (76%)		1.67 (0.89-3.13)
Matrix metalloproteinase- 16 (MMP16)	C	61 (50%)	69 (50%)	>0.99	1.0 (0.61-1.62)
	T	61 (50%)	69 (50%)		1.0 (0.61-1.62)
rs2664352					
MMP16	G	75 (64%)	86 (60%)	0.84	0.95 (0.57-1.57)
rs2664349	A	43 (36%)	52 (40%)		1.05 (0.63-1.75)

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