

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

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32 **ABSTRACT**

33

34 Term born infants are predisposed to human rhinovirus (HRV) lower respiratory
35 tract infections (LRTI) by reduced neonatal lung function and genetic
36 susceptibility. Our aim was to investigate whether prematurely born infants
37 were similarly predisposed to HRV LRTIs. Infants born less than 36 weeks of
38 gestational age were recruited. Prior to neonatal/maternity unit discharge, lung
39 function (functional residual capacity by helium gas dilution and multiple breath
40 washout, lung clearance index and compliance (C_{rs}) and resistance (R_{rs}) of the
41 respiratory system) was assessed and DNA samples assessed for eight single
42 nucleotide polymorphisms (SNPs) in seven genes: ADAM33, IL10, MMP16
43 NF κ B1A, SFTPC, VDR and NOS2A. Infants were prospectively followed until
44 one year corrected age. Nasopharyngeal aspirates (NPAs) were sent whenever
45 an infant developed a LRTI and tested for 13 viruses. One hundred and six
46 infants were included in the analysis. Infants who developed HRV LRTIs had
47 reduced C_{rs} (1.6 versus 1.2 mL/cmH₂O/kg, $p=0.044$) at 36 weeks postmenstrual
48 age. A SNP in the gene coding for the vitamin D receptor was associated with
49 the development of HRV LRTIs ($p=0.047$) and other respiratory viral infections
50 ($p=0.02$).

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

53

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

56

57 **List of abbreviations**

58

59 BPD Bronchopulmonary dysplasia

60 C_{rs} Compliance of the respiratory system

61 FRC_{He} Functional residual capacity (by helium gas dilution)

62 HRV Rhinovirus

63 LCI Lung clearance index

64 LRTI Lower respiratory tract infection

65 NPA Nasopharyngeal aspirate

66 PCR Polymerase chain reaction

67 PMA Postmenstrual age

68 R_{rs} Resistance of the respiratory system

69 RSV Respiratory syncytial virus

70 SNP Single nucleotide polymorphism

71 VDR Vitamin D receptor

72

73 **AUTHORS SUMMARY**

74

75 **What is known**

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

81

82 **What is new**

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

88

89 INTRODUCTION

90

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

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110

111

112

113 MATERIALS AND METHODS

114

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

122

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

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

139

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

153

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

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

167

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

180

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

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

187

188 **Statistical Analysis**

189

190 The infants were divided into two groups depending on their HRV LRTI status.

191 The “no LRTI group” consisted of infants who did not develop a viral LRTI

192 throughout the study period and the “HRV LRTI group” consisted of infants

193 who developed at least one HRV LRTI during the study period. The infants in

194 the HRV LRTI group may also have had other viral LRTIs. We also undertook

195 a subsidiary analysis of all infants who had LRTIs with NPAs positive for all

196 respiratory viruses. We assessed and compared their outcomes to infants who

197 had no viral LRTI. Infants who had LRTIs in whom no virus was detected

198 from the NPA were excluded from the analysis.

199

200 Data were tested for normality using the Shapiro-Wilk test. Data were analysed

201 using either the independent T-test, the Mann-Whitney U test, the Chi-squared

202 test or the Fisher’s exact test as appropriate. A multivariable regression model

203 was used to examine whether lung function at 36 weeks PMA was a predictor of

204 HRV LRTI, independent of other variables associated with respiratory function

205 which in the univariate analysis were significant at $p \leq 0.1$. Statistical analysis

206 was carried out with IBM SPSS Statistics (version 22, New York, USA).

207

208 Sample size

209

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

215

216 **RESULTS**

217

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

231

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

236

237 The HRV LRTI group were more immature (36 weeks versus 37 weeks PMA,
238 $p=0.031$) and of lower weight (1908 versus 2113 g, $p=0.007$) when their lung
239 function was measured. The HRV LRTI group had a smaller FRC_{He}
240 uncorrected for weight ($p=0.004$), although this was no longer significantly
241 different after correcting for weight ($p=0.13$), a smaller FRC_{MBW} uncorrected for
242 weight ($p=0.001$) which remained significantly different when corrected for
243 weight ($p=0.042$), a lower C_{rs} uncorrected for weight ($p=0.001$) which remained
244 significantly different when corrected for weight ($p=0.005$) and a higher R_{rs}
245 ($p=0.028$) (Table 3). Multivariate analysis revealed that after correcting for
246 significant differences in the demographic data the only difference in lung
247 function between the groups that remained significant was in the C_{rs} corrected
248 for weight (Table 3). There were no significant differences in the lung function
249 results of the infants who had any respiratory virus LRTI compared to those who
250 had no LRTI after correcting for differences in the demographics (Appendix
251 Table 2).

252

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

256 level. Infants with the G allele were significantly more likely (OR 2.07 (95% CI
257 [0.98-3.13], $p=0.047$) to develop HRV LRTIs than those with the A allele
258 (Table 4). Similarly, there was a significant difference in the SNP in the VDR
259 gene at the allele level between infants who did and did not develop a viral
260 LRTI ($p=0.02$) (Appendix Table 3).

261

262

263 **DISCUSSION**

264

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

270

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

280 C_{rs} corrected for weight. A possible explanation is that infants with a low C_{rs}
281 may have less lung distensibility leading to poorer clearance of respiratory
282 secretions. In term born infants, a reduced C_{rs} was associated with an increased
283 susceptibility to hospitalisation with RSV LRTIs as well as post RSV
284 bronchiolitis wheezing [25].

285

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

304 the development of HRV LRTIs, suggesting they may not have a role in
305 prematurely born infants' susceptibility to HRV LRTI.

306

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

320 In conclusion, prematurely born infants may be predisposed to HRV LRTIs by
321 both reduced premorbid lung function and genetic susceptibility.

322

323 **Compliance with ethical standards**

324

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

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

337 **Informed consent:** Infants whose parents gave informed written consent were
338 recruited.

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

344

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446 and post-respiratory syncytial virus wheeze in term infants. *Eur*
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- 448

449 Table 1: Demographic data

450 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

451

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

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

454 more than one viral LRTI.

455

	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

456

457

458

459 Table 3: Lung function results

460 Data are shown as median (range).

461

	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

462

463 *Univariate analysis comparing the two groups

464 **Multivariate analysis adjusting for confounding factors including gestational

465 age, birth weight, bronchopulmonary dysplasia, antenatal steroids, receipt of

466 surfactant, postnatal sepsis, duration of supplemental oxygen, receipt of

467 Palivizumab and PMA and weight at lung function testing.

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

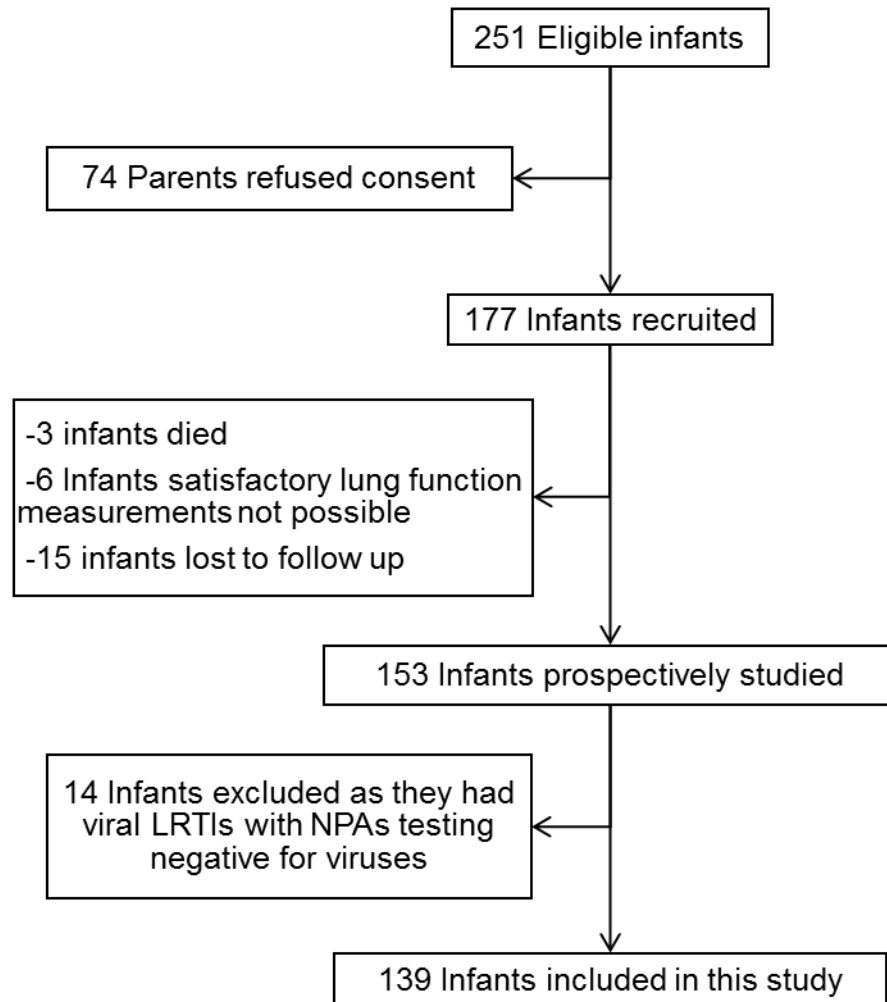
469 Data are shown as n (%).

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)

470 **FIGURE LEGEND**

471

472 Figure 1: Flow diagram of eligibility



473

474

475 APPENDIX

476

477 Table 1: Demographic data

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

	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

479

480 Table 2: Lung function results

481 Data are shown as median (range).

	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

482 *Univariate analysis comparing the two groups

483 **Multivariate analysis adjusting for confounding factors including gestational
 484 age, birth weight, bronchopulmonary dysplasia, antenatal steroids, receipt of
 485 surfactant, duration of supplemental oxygen, receipt of Palivizumab and weight
 486 at lung function testing.

Table 3: Associations at the allele level by HRV status
Data are shown as n (%).

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) rs2664352	C	61 (50%)	69 (50%)	>0.99	1.0 (0.61-1.62)
	T	61 (50%)	69 (50%)		1.0 (0.61-1.62)
MMP16 rs2664349	G	75 (64%)	86 (60%)	0.84	0.95 (0.57-1.57)
	A	43 (36%)	52 (40%)		1.05 (0.63-1.75)