

Risks for cold frequency vary by sex: role of asthma, age, TLR7 and leukocyte subsets.

## **SUPPLEMENTARY INFORMATION**

### ***Materials and methods***

#### ***Participant questionnaire***

All participants answered a questionnaire to assess their basic demographics and to assess their respiratory infection frequency they answered the following question: “How frequently have you had cold symptoms (at least one of these: fever, chills, sore throat, runny nose or cough for at least one day) per year?”, to record their ancestry the following question was asked: “Please write the country where each of your grandparents was born. Also, give their ancestry – that is, the place where most of their ancestors came from”, to assess workplace contact with children the following question was asked: “Do you have regular contact with children at your workplace?” and to record if household has children the following question was asked: “Do you have children under 15 year old in your household?”.

#### ***Data storage***

Participant information was made anonymous by assigning a study identity number. Both participant and asthma questionnaires (see appendix) were generated using the Checkbox program (Watertown, USA) online, that participants could access to complete the questionnaires online before their appointment. The opted paper versions of the questionnaires are stored in a secure location, and the electronic

copies of questionnaires are downloaded to a file and secured with a password in a restricted access server. Results from the sample measurements were recorded anonymously in a separate database.

### ***Human rhinovirus 16***

Human rhinovirus (RV)16 was grown in Ohio HeLa cells. Confluent cells were incubated with RV16 lysate at 37°C until 60% of the cells were lysed. The infected cells were disrupted with three cycles of freezing and thawing and subsequently clarified with a centrifugation step and Optiprep (Sigma Aldrich Pty Ltd, Germany) purification. Titration assay was then completed to determine the concentration of the virus.

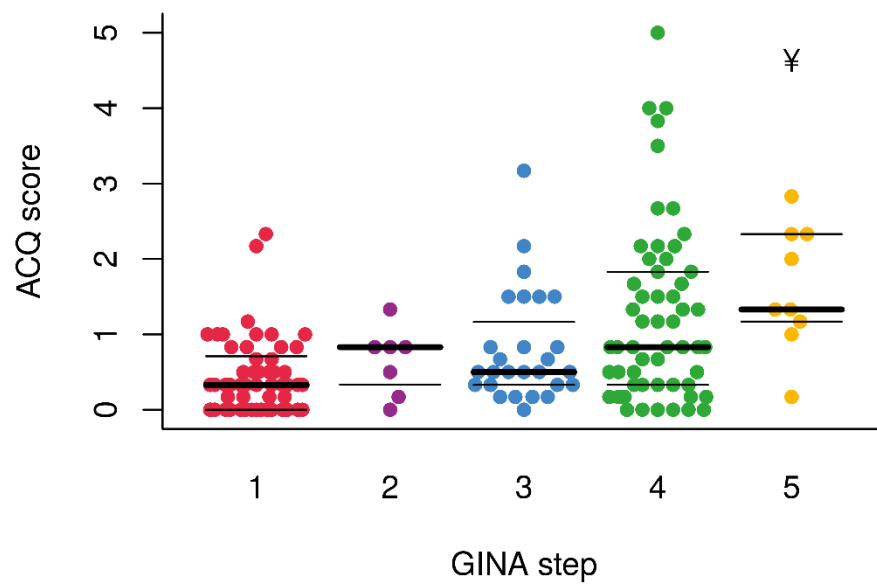
### ***PBMC isolation and culture***

PBMC were isolated with Lymphoprep (Stemcell technologies, Tullamarine Australia) density gradient centrifugation and washed twice before counting with RPMI medium 1640 (Lifetechnologies, Melbourne Australia) supplemented with 2% Foetal Calf Serum (FCS) and PSG (Lifetechnologies, Melbourne Australia, 100u/ml Penicillin, 100µg/ml Streptomycin and 0.292mg/ml L-glutamine). Freshly isolated PBMC were rested for one hour before culturing in RPMI medium 1640 supplemented with 2% FCS and PSG for 24 hours with either MOI =2.5 of RV-16 or 1µM TLR8 specific agonist VTX-2337 (Sapphire Bioscience, Waterloo Australia).

### ***Supplementary tables and figures***

**Table E1 Primer sequences**

Gene	Direction	Primer sequence 5' – 3'
CLEC4C	forward	CCTCTGTCTGACCCTGCAT
	reverse	GCCAAGCCCTTAGATCCTT
TLR7	forward	ATGGTGTTTCCAATGTGGAC
	reverse	GTTTCGTGGGAATACCTCCAG
TLR8	forward	TCCTTCAGTCGTCAATGCTG
	reverse	CGTTTGGGGAACTTCCTGTA
UBC	forward	GCAGTTCTTGTTTGTGGATCGCT
	reverse	TGACATTCTCGATGGTGTCACTGG
B2M	forward	AGGCTATCCAGCGTACTCCAAAGA
	reverse	CGGATGGATGAAACCCAGACACAT



**Figure E1 ACQ6 score by GINA step.** *ACQ6 score distribution by GINA step. The thick vertical line represents the median ACQ6 score, and the thin lines represent the interquartile ranges. ¥ designates a significant Mann-Whitney U-test with p-value < 0.0125 vs GINA step 1.*

**Table E2 Asthma participants described by GINA step group and ACQ score**

	GINA step						ACQ6 score	
	1	2	3	4	5	p (1)	Coef	p (2)
	Mild				Severe			
<b>n</b>	52	7	27	55	9			
<b>Women – n(%)</b>	29 (55.8)	7 (100.0)	21 (77.8)	38 (69.1)	2 (22.2)	<b>0.004</b>	0.04	0.823
<b>Age at donation (years)</b>	29.0 [24.0, 39.3]	51.0 [33.5, 56.0]	31.0 [25.0, 45.5]	37.0 [29.5, 49.5]	51.0 [42.0, 61.0] ¥	<b>0.001</b>	0.01	<b>0.005</b>
<b>Smoked pack years</b>	0.0 [0.0, 0.0]	0.0 [0.0, 0.0]	0.0 [0.0, 0.0]	0.0 [0.0, 1.8]	0.0 [0.0, 0.0]	0.060	0.03	<b>0.031</b>
<b>BMI (kg/m<sup>2</sup>)</b>	23.0 [21.0, 27.2]	30.0 [26.5, 35.0]	27.0 [22.5, 29.5]	28.0 [24.5, 30.0]	29.0 [27.0, 35.0]	<b>0.001</b>	0.04	<b>0.002</b>
<b>Cold frequency</b>	3.0 [3.0, 5.0]	3.0 [3.0, 12.0]	3.0 [3.0, 5.0]	3.0 [3.0, 5.0]	5.5 [3.5, 8.2]	0.658	0.01	0.557
<b>ACQ6 score</b>	0.3 [0.0, 0.7]	0.8 [0.3, 0.8]	0.5 [0.3, 1.2]	0.8 [0.3, 1.8]	1.3 [1.2, 2.3] ¥	<b>&lt;0.001</b>		
<b>IgE kU/L</b>	104.0 [48.2, 317.2]	91.0 [49.5, 269.0]	165.0 [66.0, 517.0]	164.0 [52.5, 358.0]	217.0 [31.0, 224.0]	0.779	0.0001	0.282
<b>Eosinophils x 10<sup>9</sup>/L</b>	0.2 [0.1, 0.4]	0.2 [0.2, 0.5]	0.3 [0.2, 0.4]	0.3 [0.2, 0.4]	0.2 [0.1, 0.4]	0.146	0.75	<b>0.020</b>
<b>Neutrophil x 10<sup>9</sup>/L</b>	3.3 [2.7, 4.1]	4.4 [4.0, 4.6]	3.7 [3.0, 4.7]	4.0 [3.3, 4.7]	3.6 [2.6, 6.3]	0.055	0.08	0.133
<b>Asthma diagnosis age (years)</b>	1.0 [1.0, 2.0]	1.0 [1.0, 1.0]	1.0 [1.0, 2.5]	1.0 [1.0, 2.0]	1.0 [1.0, 3.0]	0.668	0.09	0.366
<b>In 12 months missed work for asthma</b>	8 (15.4)	0 (0.0)	11 (40.7)	26 (47.3)	4 (44.4)	<b>0.002</b>	0.39	<b>0.015</b>
<b>Ever hospitalised for asthma</b>	27 (51.9)	3 (42.9)	12 (44.4)	33 (60.0)	8 (88.9)	0.161	0.44	<b>0.004</b>
<b>In 12 months hospitalised for asthma</b>	1 (1.9)	0 (0.0)	3 (11.1)	7 (12.7)	3 (33.3)	<b>0.026</b>	0.66	<b>0.011</b>
<b>In 12 months taken OCS</b>	8 (15.4)	2 (28.6)	9 (33.3)	27 (49.1)	9 (100.0)	<b>&lt;0.001</b>	0.69	<b>&lt; 0.001</b>

Categorical variables are expressed as frequency (percentage), and continuous variables are expressed as median [interquartile range] for each GINA step group. Variables are tested for an association with ACQ6 score with univariate linear regression. BMI = body mass index, n = study group size, BMI = body mass index, ACQ = asthma control questionnaire, mo. = months, OCS = oral

*corticosteroid medication, cold frequency = frequency of self-reported respiratory infections.  $p$  (1 = statistical significance of the chi-square test for categorical variables or Kruskal-Wallis test for continuous variables between the GINA step groups.  $p$  (2 = statistical significance of univariate test with ACQ6 score as an outcome variable. Coef =  $\beta$ -coefficient for association.  $\forall$  = post-hoc test  $p < 0.05 / 4$  tests = 0.0125 vs GINA step 1.*

**Table E3 Multivariable linear regression models for respiratory infection frequency by asthma status**

***Non-asthmatic only***

Dependent variable: Cold frequency per year								
	Univariable models				Multivariable model			
	Est. Coef	CI 2.5%	CI 97.5%	p	Est. Coef	CI 2.5%	CI 97.5%	p
Sex	0.016	-0.17	0.20	0.87				
Age at donation	-0.02	-0.027	-0.013	<b>&lt; 0.001</b>	-0.008	-0.015	- 0.000 4	<b>0.037</b>
BMI (kg/m <sup>2</sup> )	0.005	-0.018	0.028	0.66				
Has children	0.15	-0.099	0.40	0.23				
Children at work	0.094	-0.29	0.48	0.63				
Neutrophil count x 10 <sup>9</sup> /L	0.047	-0.028	0.12	0.22	0.075	0.007	0.14	<b>0.030</b>
Eosinophil count x 10 <sup>9</sup> /L	0.52	-0.31	1.4	0.22				
<i>CLEC4C</i> mRNA	0.54	-0.098	1.2	0.097	0.55	-0.18	1.3	0.14
<i>TLR7</i> mRNA	-0.097	-0.71	0.51	0.75				
<i>TLR8</i> mRNA	-0.16	-0.80	0.47	0.61				
IFN $\alpha$ ng/ml	0.03	-0.037	0.096	0.38				
TNF ng/ml	-							
	0.000	-0.028	0.027	0.95				
	8							
IL12 ng/ml	0.38	-0.023	0.78	0.064				

Multivariable model included n = 133 with 13 missing observations deleted.  
Adjusted R<sup>2</sup>: 0.058, p = 0.012.

***Asthmatic only only***

Dependent variable: Cold frequency per year								
	Univariable models				Multivariable model			
	Est. Coef	CI 2.5%	CI 97.5%	p	Est. Coef	CI 2.5%	CI 97.5 %	p
Sex	0.0064	-0.19	0.21	0.95				
Age at donation	-0.009	-0.016	-0.002	<b>0.015</b>	-0.022	-0.030	- 0.016	<b>&lt; 0.001</b>
BMI (kg/m <sup>2</sup> )	0.0055	-0.012	0.022	0.52	0.029	0.008	0.050	<b>0.008</b>
Has children	0.08	-0.13	0.29	0.46				
Children at work	0.11	-0.16	0.38	0.43				
Neutrophil count x 10 <sup>9</sup> /L	0.059	-	0.13	0.083				
		0.0079						

<b>Eosinophil count x 10<sup>9</sup>/L</b>	0.0046	-0.42	0.43	0.98				
<b>CLEC4C mRNA</b>	0.57	-0.16	1.3	0.12	0.70	0.13	1.26	<b>0.016</b>
<b>TLR7 mRNA</b>	-0.17	-0.87	0.53	0.63				
<b>TLR8 mRNA</b>	0.12	-0.60	0.84	0.75				
<b>IFN<math>\alpha</math> ng/ml</b>	0.025	-0.062	0.11	0.57				
<b>TNF ng/ml</b>	0.002	-0.023	0.026	0.88				
<b>IL12 ng/ml</b>	-0.051	-0.33	0.23	0.72				

**Multivariable model included n = 142 with 5 missing observations deleted. Adjusted R<sup>2</sup>: 0.23, p < 0.001.**

*Cold frequency was natural log transformed, CLEC4C, TLR7 and TLR8 gene expression at baseline were measured in resting whole blood, IFN $\alpha$  production stimulated with RV16, TNF and IL12 production stimulated with TLR8 agonist were measured in PBMC. Est. coef = estimated coefficient, CI = confidence interval, p = p-value.*



**Table E4 Multivariable logistic regression models for asthma fitted in women or men only**

<i>Women only</i>								
Dependent variable: Asthma in women								
	Univariable models				Multivariable model			
	OR	CI 2.5%	CI 97.5%	p	OR	CI 2.5%	CI 97.5%	p
<b>CLEC4C mRNA</b>	0.75	0.052	1.1	0.83				
<b>TLR7 mRNA</b>	0.16	0.022	1.0	<b>0.060</b>				
<b>TLR8 mRNA</b>	0.15	0.016	1.3	0.092	0.15	0.014	1.6	0.12
<b>IFN<math>\alpha</math> ng/ml</b>	0.89	0.71	1.1	0.29				
<b>TNF ng/ml</b>	0.98	0.89	1.1	0.64				
<b>IL12 ng/ml</b>	5.1	0.93	33	0.074	3.8	0.57	30	0.19
<b>Age at donation</b>	1.0	0.99	1.0	0.28				
<b>BMI (kg/m<sup>2</sup>)</b>	1.2	1.1	1.2	<b>&lt; 0.001</b>	1.2	1.1	1.2	<b>&lt; 0.001</b>
Multivariable model included n = 184 with 3 missing observations deleted. Pseudo R <sup>2</sup> : 0.19, p < 0.001.								

<i>Men only</i>								
Dependent variable: Asthma in men								
	Univariable models				Multivariable model			
	OR	CI 2.5%	CI 97.5%	p	OR	CI 2.5%	CI 97.5%	p
<b>CLEC4C mRNA</b>	0.33	0.027	3.0	0.35				
<b>TLR7 mRNA</b>	0.067	0.003	1.4	0.091	0.058	0.002	1.3	0.083
<b>TLR8 mRNA</b>	0.56	0.041	6.7	0.65				
<b>IFN<math>\alpha</math> ng/ml</b>	1.1	0.70	1.6	0.78				
<b>TNF ng/ml</b>	1.1	1.0	1.3	<b>0.049</b>	1.1	1.0	1.3	<b>0.027</b>
<b>IL12 ng/ml</b>	1.5	0.57	4.8	0.44				
<b>Age at donation</b>	1.0	0.98	1.0	0.84				
<b>BMI (kg/m<sup>2</sup>)</b>	1.1	0.96	1.2	0.28	1.1	0.98	1.2	0.14
Multivariable model included n = 113 with 1 missing observation deleted. Pseudo R <sup>2</sup> : 0.12, p = 0.015.								

*CLEC4C, TLR7 and TLR8 gene expression at baseline were measured in resting whole blood, IFN $\alpha$  production stimulated with RV16, TNF and IL12 production stimulated with TLR8 agonist were measured in PBMC. OR = odds ratio, CI = confidence interval, p = p-value.*

**Table E5 Multivariable linear regression model for cold frequency in people with BMI < 25**

**Dependent variable: Cold frequency per year**

	Univariable models				Multivariable model			
	Est. Coef	CI 2.5%	CI 97.5%	p	Est. Coef	CI 2.5%	CI 97.5%	p
<b>Asthma</b>	0.39	0.19	0.58	<b>&lt; 0.001</b>	0.37	0.18	0.56	<b>&lt; 0.001</b>
<b>Age at donation</b>	-0.015	-0.024	-0.007	<b>&lt; 0.001</b>	-0.01	-0.02	-0.005	<b>0.002</b>
<b>BMI (kg/m<sup>2</sup>)</b>	0.017	-0.038	0.072	0.53				
<b>Sex</b>	0.024	-0.19	0.23	0.82				
<b>Has children</b>	0.04	-0.22	0.30	0.76				
<b>Children at work</b>	0.17	-0.15	0.49	0.30				
<b>Neutrophil count x 10<sup>9</sup>/L</b>	0.076	0.0005	0.15	<b>0.049</b>	0.082	0.013	0.15	<b>0.02</b>
<b>Eosinophil count x 10<sup>9</sup>/L</b>	0.45	-0.15	1.0	0.14				
<b>CLEC4C mRNA</b>	0.90	0.26	1.5	<b>0.006</b>	1.0	0.42	1.6	<b>&lt; 0.001</b>
<b>TLR7 mRNA</b>	0.042	-0.62	0.70	0.90				
<b>TLR8 mRNA</b>	0.44	-0.33	1.2	0.26				
<b>IFN<math>\alpha</math> ng/ml</b>	0.033	-0.038	0.10	0.36				
<b>TNF ng/ml</b>	0.012	-0.011	0.035	0.32				
<b>IL12 ng/ml</b>	0.18	-0.11	0.47	0.22				

**Multivariable model included n = 145 with 10 missing observations deleted.**

**Adjusted R<sup>2</sup>: 0.22, p < 0.001.**

*Cold frequency was natural log transformed, CLEC4C, TLR7 and TLR8 gene expression at baseline were measured in resting whole blood, IFN $\alpha$  production stimulated with RV16, TNF and IL12 production stimulated with TLR8 agonist were measured in PBMC. Est. coef = estimated coefficient, CI = confidence interval, p = p-value.*

**Table E6 IFN $\alpha$  production pg/ml**

	<b>Median</b>	<b>1<sup>st</sup> quartile</b>	<b>3<sup>rd</sup> quartile</b>
<b>Unstimulated</b>	25	25	25
<b>RV16- stimulated</b>	970	530	1600

*IFN $\alpha$  production in unstimulated PBMC vs RV16 stimulated PBMC after 24h in pg/ml.*

**Table E7 Comparison of variables between men and women**

	Women	Men	p
<b>n</b>	187	114	
<b>Asthma (%)</b>	97 (51.9)	53 (46.5)	0.431
<b>Cold frequency</b>	3.00 [2.00, 5.00]	3.00 [1.00, 4.00]	0.525
<b>Has children (%)</b>	144/43 (77.0/23.0)	90/23 (79.6/20.4)	0.696
<b>Children at work (%)</b>	160/27 (85.6/14.4)	107/6 (94.7/5.3)	<b>0.024</b>
<b>Age at donation (years)</b>	34.00 [27.00, 46.00]	33.00 [24.00, 49.75]	0.550
<b>BMI (kg/m<sup>2</sup>)</b>	24.00 [21.00, 29.00]	25.00 [23.00, 28.00]	0.132
<b>Blood count x 10<sup>9</sup>/L</b>			
White blood cells	6.40 [5.50, 7.60]	5.90 [4.80, 6.68]	<b>&lt;0.001</b>
Platelets	262.50 [232.75, 298.25]	234.50 [205.25, 269.00]	<b>&lt;0.001</b>
Neutrophils	3.64 [2.96, 4.46]	3.10 [2.57, 3.95]	<b>&lt;0.001</b>
Lymphocytes	1.96 [1.68, 2.31]	1.79 [1.43, 2.14]	<b>0.001</b>
Monocytes	0.48 [0.39, 0.60]	0.51 [0.39, 0.63]	0.240
Eosinophils	0.17 [0.11, 0.30]	0.16 [0.10, 0.31]	0.797
Basophils	0.03 [0.02, 0.04]	0.02 [0.01, 0.03]	<b>0.009</b>
<b>IgE kU/L</b>	51.00 [19.50, 164.50]	117.00 [32.50, 350.50]	<b>&lt;0.001</b>
<b>TLR7 mRNA</b>	0.27 [0.20, 0.37]	0.28 [0.21, 0.38]	0.390
<b>TLR8 mRNA</b>	0.28 [0.21, 0.38]	0.30 [0.21, 0.39]	0.590
<b>CLEC4C mRNA</b>	0.11 [0.07, 0.19]	0.11 [0.07, 0.19]	0.725
<b>IFN<math>\alpha</math> ng/ml</b>	1.01 [0.60, 1.71]	0.78 [0.47, 1.42]	0.050
<b>TNF ng/ml</b>	1.97 [1.07, 3.32]	2.40 [1.62, 4.66]	<b>0.003</b>
<b>IL12 ng/ml</b>	0.13 [0.05, 0.25]	0.21 [0.10, 0.35]	<b>0.001</b>

*n* = study group size, has children = household with children under 15 years of age, work with children = work involving contact with children, over 50 = over 50 years of age, BMI = body mass index.

Continuous variables are expressed as median [interquartile range] and categorical as frequency (percentage). *p*-value is shown for Mann-Whitney U-test between the two groups for continuous variables and Chi-square test for the categorical variables. Bolded *p*-values are statistically significant.