Purine degradation pathway metabolites at birth and the risk of lower respiratory tract infections in infancy

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This study reveals a potential role of the purine degradation pathway (PDP) in the pathogenesis of LRTIs in infancy and proposes PDP metabolites as biomarkers of disease risk https://bit.ly/41beelG

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Abstract

Background Lower respiratory tract infections (LRTIs) are the leading cause of infant morbidity and mortality worldwide, and altered metabolite production is recognised as a critical factor in LRTI pathogenesis.

Methods This study aimed to identify prenatal metabolic changes associated with LRTI risk in infancy, using liquid chromatography-mass spectrometry unbiased metabolomics analysis on cord blood from 810 full-term newborns.

Results We identified 22 compounds linked to LRTIs in infancy, enriched for purine degradation pathway (PDP) metabolites. High cord blood PDP metabolites, including xanthine, hypoxanthine, xanthosine and inosine, were linked to reduced LRTI risk during infancy. Notably, a low xanthine to uric acid ratio at birth predicted a four-fold increased LRTI risk.

Conclusion This study is the first to reveal that high cord blood PDP metabolites identify newborns at lower LRTI risk, stratifying disease risk at birth. Moreover, our results prompt further study on PDP enzymes as pharmacological targets to decrease LRTI morbidity and mortality for at-risk newborns.

Introduction

Lower respiratory tract infections (LRTIs), defined as pneumonia or bronchiolitis, remain the leading cause of morbidity and mortality among children under 5 years of age [1]. In the USA, bronchiolitis is the leading cause of infant hospitalisation, accounting for ~110 000 hospitalisations annually [2]. Globally, LRTIs cause over 650 000 childhood deaths each year, with children in the first year of life being the most vulnerable [1]. Early-life LRTIs are also linked to chronic respiratory morbidity in childhood [3, 4] and premature respiratory deaths in adults [5]. While risk factors for LRTIs have been identified, the pathogenesis of LRTI in infancy is highly heterogeneous, and our understanding of individual molecular pathways linked to susceptibility to early-life LRTIs is incomplete.

Altered metabolite production is increasingly recognised as a key molecular factor in the pathogenesis of LRTIs [6–8]. Previous studies have identified several metabolic pathways that regulate immune responses against viruses and other respiratory pathogens [7–9]. Specifically, there is compelling evidence in animal

models and human infants that the purine degradation pathway (PDP) is heightened during respiratory syncytial virus (RSV) infection [7, 8]. Uric acid, the PDP's final product, contributes to RSV immunopathology by activating the inflammasome and other cellular stress responses that perpetuate a non-infectious inflammatory state linked to severe respiratory disease [7]. In addition, xanthine oxidase (XO) inhibition, which blocks uric acid synthesis and increases intermediate purine metabolites, reduces pathologic responses during viral LRTI [7, 8]. These data suggest that an individual's PDP metabolic activity at birth is a hitherto unidentified important molecular determinant of susceptibility or resistance to early-life LRTIs.

In this study, we analysed molecular and clinical data from a large birth cohort of children to define specific metabolic pathways linked to early-life LRTI development. Using mass spectrometry-based cord blood metabolomics from full-term infants, we conducted unbiased discovery studies linking cord blood metabolites to LRTI risk. Thereafter, we performed pathway-specific analyses to test whether high levels of PDP intermediate metabolites, namely xanthine, xanthosine, inosine and hypoxanthine, could identify a subset of newborns with reduced risk of LRTI during the first year of life.

Methods

This study was conducted among 1000 mother–infant pairs in the Boston Birth Cohort (BBC) and examined the relationship between cord blood metabolites and LRTI during infancy (figure 1). Of the initial 1000 participant infants, 187 were excluded due to preterm birth (n=177), trisomy 21 (n=6) and incomplete data (n=4). In addition, infants lost to follow-up with unknown infancy LRTI status (n=3) were excluded from cross-sectional analyses (supplementary figure E1). The primary outcome was the occurrence of LRTIs, defined as incident bronchiolitis, bronchitis or pneumonia within the first year of life (⩽12 months). Our covariates included prenatal and perinatal characteristics linked to early-life LRTIs or conditions that may affect the fetal metabolome.

Cord blood samples were analysed using liquid chromatography–tandem mass spectrometry (LC–MS). We adhered to rigorous quality control protocols that have been previously described [10]. This analysis

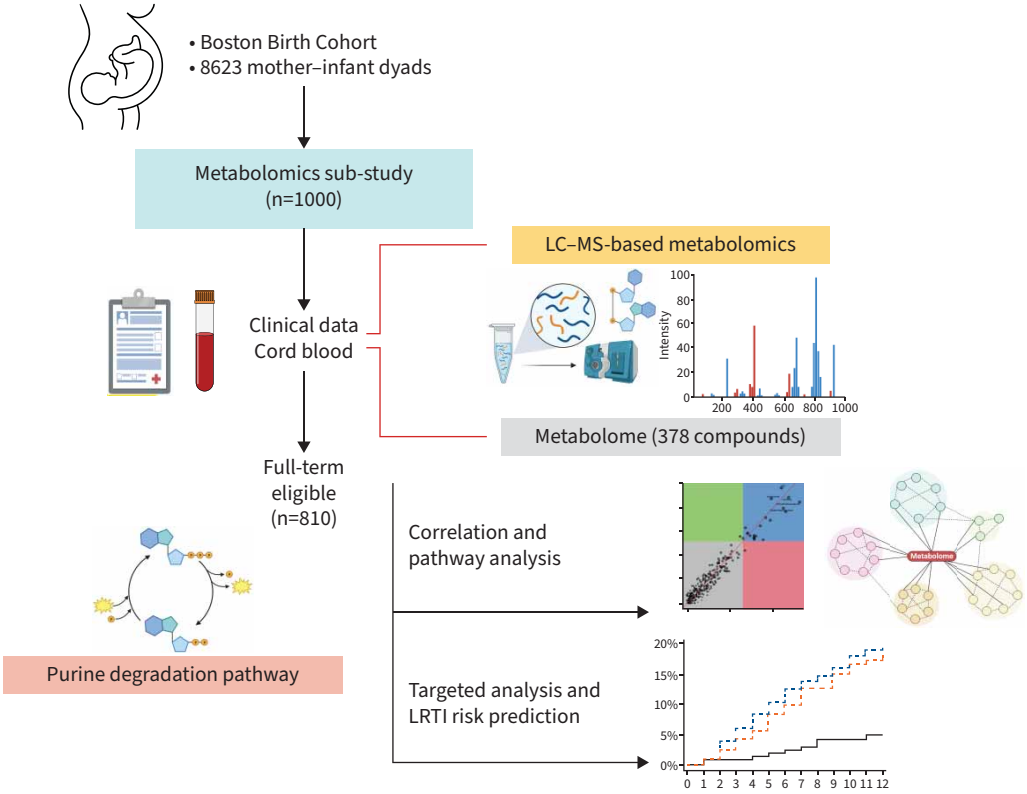

FIGURE 1 Study overview. Clinical data and cord blood metabolites (378 compounds) from 810 eligible mother–infant pairs were analysed to identify prenatal metabolic pathways linked to susceptibility to lower respiratory tract infections (LRTIs) in infancy and test early-life LRTI risk biomarkers. LC–MS: liquid chromatography–tandem mass spectrometry. Figure partially created using BioRender.com

included 378 metabolites with a coefficient of variation <20%. Non-detectable values were imputed as one-half of the minimal value of each metabolite. We used the natural logarithmic and rank-based inverse normal transformation methods to render the distributions approximately Gaussian and reduce the impact of outliers [10].

Generalised linear models were used to study prospective associations between cord blood metabolites and LRTI during infancy. This initial exploratory analysis included only infants with complete data (n=754) (supplementary figure E2 and table E1). A p-value <0.05 was considered significant, and adjustments were made for multiple comparisons using the false discovery rate (FDR). Hierarchical clustering (using the Ward method and Euclidian distance measure) and pathway analyses were performed. For pathway analysis, the Small Molecule Pathway Database (SMPDB) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) were queried using each metabolite's Human Metabolome Database (HMDB) ID number [11–13]. The enrichment method was a hypergeometric test, and the topology analysis was relative-betweenness centrality.

Additional logistic regression models assessed the link between enriched metabolites and LRTI during infancy. Specifically, we tested whether high cord blood levels of xanthine, hypoxanthine, inosine, xanthosine, 6,8-dihydroxypurine and 8-hydroxy-deoxyguanosine (defined as inverse normal transformed cord blood levels in the upper quartile) had higher odds of LRTI in infancy, compared with infants with lower cord blood levels of these metabolites (defined as inverse normal transformed levels in the 1st, 2nd and 3rd quartiles). These analyses accounted for known maternal and infant LRTI risk factors [14, 15] and included 810 infants (supplementary figure E1). Covariables with missing observations (table 1 and supplementary table E3) were imputed using the multiple imputation (mi) function in STATA 14 [16], and sensitivity analyses confirmed the comparability of imputed and non-imputed data.

We also examined enzymatic pathways and intermediate metabolites of the PDP (figure 2a and supplementary figure E4) by calculating the xanthine to uric acid ratio (XU ratio). This ratio was defined as the log ratio of log-transformed cord blood xanthine and uric acid relative intensity values (measured by LC-MS). The XU ratio evaluated xanthine oxidase (XO) as a function of its substrate (xanthine) related to its product (uric acid), providing an overall assessment of PDP activity at birth. The cumulative risk of incident LRTI was estimated for children with low XU ratio (lowest quartile of XU ratio values), mid XU ratio (XU ratio values in the second and third quartiles) and high XU ratio (XU ratio levels in the upper quartile) using Kaplan–Meier survival analysis and Cox proportional hazards models.

Maternal–fetal characteristics linked to high levels of PDP metabolites (xanthine, hypoxanthine, inosine and xanthosine) were also analysed. We used Pearson correlation analysis to evaluate links between inverse normal transformed cord blood levels of these PDP metabolites and maternal age and gestational age. Additionally, we used chi-square tests to assess associations between high levels of these PDP metabolites and chronic maternal conditions (overweight/obesity and maternal asthma), obstetric complications (gestational diabetes, preeclampsia/eclampsia and low birthweight), and newborns' low Apgar scores (defined as Apgar below 7 at 1 and 5 min). Our analyses were conducted using STATA version 14 (Stata Statistical Software: Release 14, 2015; StataCorp., College Station, TX, USA), R studio (RStudio: Integrated Development Environment for R, 2022; RStudio Team, Boston, MA, USA) and Metaboanalyst 5.0 [17].

Results

Clinical characteristics of newborns in the BBC metabolome sub-study

Of the 810 full-term eligible newborns, 126 infants had at least one LRTI during infancy (supplementary figure E1) with a median age of 5.5 months (IQR 6) for the first LRTI. The LRTI group had a higher proportion of males (n=80, 63.5%) and slightly lower gestational age (39.2 weeks (IQR 1.6) *versus* 39.4 weeks (IQR 1.9)) than the non-LRTI group. No significant differences were found between the LRTI and non-LRTI groups in terms of other infant characteristics (mode of delivery, birthweight, proportion of low-birthweight infants and breastfeeding status), maternal characteristics (race, age at delivery, education level, parity, pre-pregnancy weight and smoking in the third trimester), chronic maternal conditions (asthma, diabetes, hypertension), or gestational complications such as diabetes or preeclampsia/eclampsia (table 1).

Identification of metabolites associated with LRTI in the first year of life

We tested associations between LRTI and 378 cord blood metabolites and included relevant factors (sex, gestational age, maternal obesity, maternal history of gestational diabetes, low birthweight and maternal smoking in the third trimester) in the adjusted models (supplementary figure E2 and table E1). There were 22 metabolites linked to LRTI before applying multiple testing correction (supplementary figure E2 and

TABLE 1 Summary of baseline characteristics by study group (lower respiratory tract infection (LRTI) and no LRTI during the first year of life)

Variable	All infants	Infants with LRTI	Infants without LRTI	p-value
Infants, n	810	126	684	
Sex, n (%)				**0.013**
Female	378 (46.7)	46 (36.5)	332 (48.5)	
Male	432 (53.3)	80 (63.5)	352 (51.5)	
Gestational age weeks, median (IQR)	39.4 (1.9)	39.2 (1.6)	39.4 (1.9)	**0.044**
Type of delivery, n (%)				0.201
Vaginal	552 (68.2)	78 (61.9)	474 (69.3)	
C-section	256 (31.6)	48 (38.1)	208 (30.4)	
Unknown[#]	2 (0.25)	0	2 (0.25)	
Birthweight g, median (IQR)	3260 (680)	3300 (645)	3255 (680)	0.981
Low birthweight, n (%)	62 (7.7)	11 (8.7)	51 (7.5)	0.621
Breastfeeding, n (%)				0.065
Breastfed infant	583 (72.0)	84 (66.7)	499 (73.0)	
Unknown[#]	36 (4.4)	3 (2.4)	33 (4.8)	
Maternal race, n (%)				0.536
White	39 (4.8)	6 (4.8)	33 (4.8)	
Black	469 (57.9)	72 (57.1)	397 (59.0)	
Hispanic	192 (23.7)	27 (21.4)	165 (24.1)	
Other/mixed race	110 (13.6)	21 (16.7)	89 (13.0)	
Maternal age years, median (IQR)	27.6 (10.2)	26.1 (10.6)	27.8 (10.1)	0.196
Maternal education, n (%)				0.700
No school/elementary	28 (3.5)	4 (3.2)	24 (3.5)	
Some secondary school	192 (23.7)	37 (29.4)	155 (22.7)	
High school graduate/GED	310 (38.3)	47 (37.3)	263 (38.5)	
Some college	174 (21.5)	23 (18.3)	151 (22.1)	
College degree and above	100 (12.4)	14 (11.1)	86 (12.6)	
Unknown[#]	6 (0.7)	1 (0.8)	5 (0.7)	
Multiparous mother, n (%)	478 (59.1)	74 (58.7)	404 (59.0)	0.944
Maternal pre-pregnancy weight, n (%)				0.207
BMI <25 $kg \cdot m^{-2}$	382 (47.2)	52 (41.3)	330 (48.3)	
BMI ⩾25 $kg \cdot m^{-2}$ (overweight or obese)	379 (46.8)	68 (54.0)	311 (45.5)	
Unknown[#]	49 (6.1)	6 (4.8)	43 (6.3)	
Maternal diabetes, n (%)				0.143
Gestational	62 (7.7)	15 (11.9)	47 (6.9)	
Chronic	30 (3.7)	5 (4.0)	25 (3.7)	
Preeclampsia, eclampsia, HELLP, n (%)				0.593
Yes	43 (5.3)	8 (6.4)	35 (5.1)	
Unknown[#]	4 (0.6)	0	4 (0.5)	
Maternal chronic hypertension, n (%)				0.736
Yes	38 (4.7)	7 (5.6)	31 (4.5)	
Unknown[#]	2 (0.25)	0	2 (0.29)	
Maternal asthma, n (%)				
Yes	107 (13.2)	24 (19.1)	83 (12.1)	0.089
Unknown[#]	70 (8.6)	12 (9.5)	58 (8.5)	
Maternal smoking 3rd trimester, n (%)				0.624
Yes	66 (8.2)	13 (10.3)	53 (7.8)	
Unknown[#]	7 (0.9)	1 (0.8)	6 (0.9)	

A significantly higher proportion of males developed a LRTI, and gestational age was slightly lower in the LRTI group. No significant differences were observed in maternal characteristics (age at delivery, race, parity, pre-pregnancy body mass index (BMI), smoking in the 3rd trimester), maternal conditions (asthma, diabetes, preeclampsia/eclampsia and chronic hypertension), infant characteristics (type of delivery) or breastfeeding status. Statistically significant p-values are in bold. IQR: interquartile range; C-section: caesarean section; GED: General Educational Development test; HELLP: haemolysis, elevated liver enzymes, low platelet count. [#]: missing observations.

table E1). Hierarchical clustering analysis showed that these 22 metabolites clustered in two main groups (figure 3). The first group consisted of metabolites in the PDP (xanthine, hypoxanthine, inosine and xanthosine) and two related compounds (6,8-dihydroxypurine and 8-hydroxy-deoxyguanosine). The remaining 16 molecules were in the second group, which had two distinct subgroups. The first subgroup comprised glycerolipids (C46:0 TAG, C47:0 TAG, C44:1 TAG, C42:0 TAG, C44:0 TAG). The second subgroup included smaller subgroups of sphingolipids (C22:0 ceramide, C16:0 ceramide), other complex

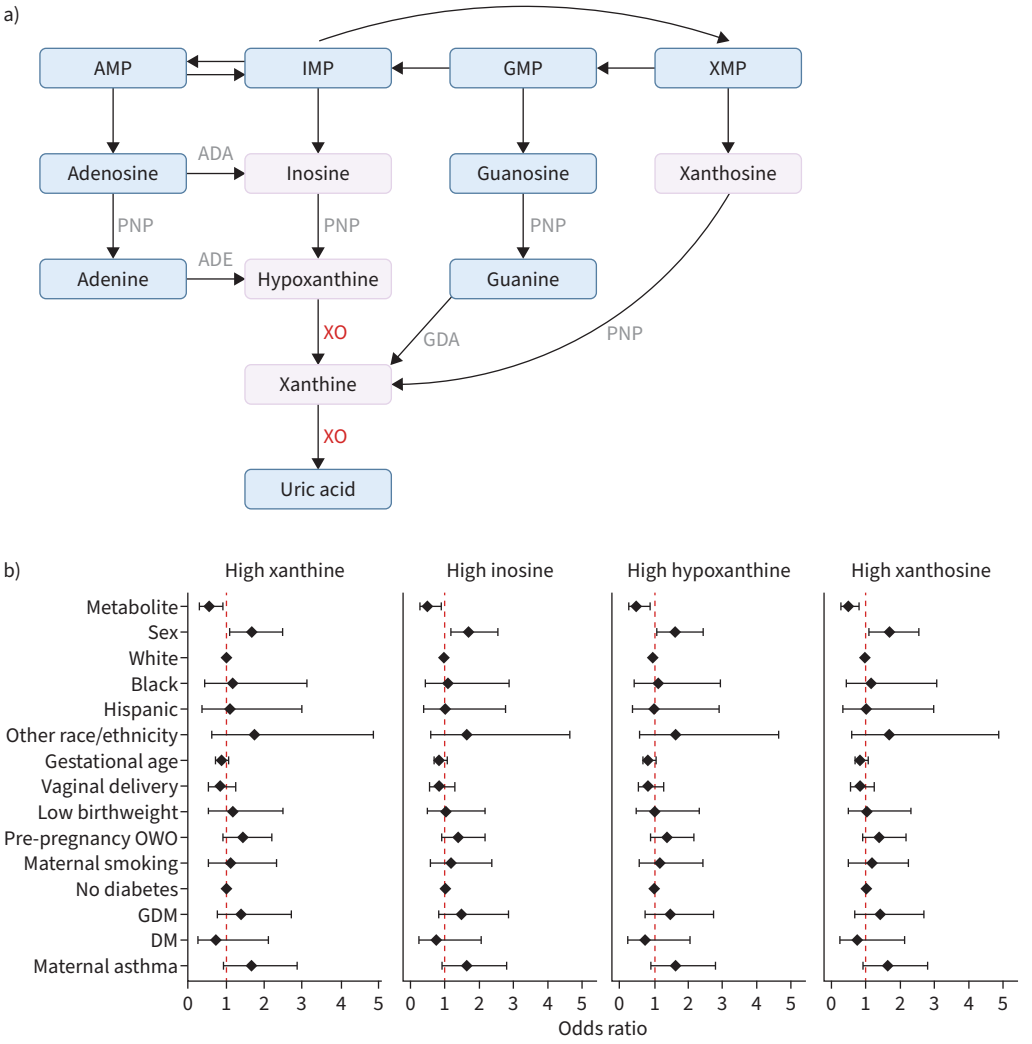

FIGURE 2 Purine degradation pathway (PDP) cord blood metabolites associated with lower respiratory tract infections (LRTIs) during infancy. a) High cord blood levels of four intermediate purine degradation metabolites (in pink) were associated with a lower likelihood of LRTI in infancy. These metabolites are upstream of the oxidation of hypoxanthine to xanthine and xanthine to uric acid, two reactions catalysed by the xanthine oxidase (XO). b) Forest plot diagrams of LRTI associations with high PDP cord blood levels (defined as inverse normal transformed levels in the upper quartile) are shown. A total of 810 eligible full-term newborns were included. Associations were tested using multivariate logistic regression models adjusted by clinically relevant covariables. Odds ratios with 95% confidence intervals below 1.0 indicate a lower likelihood of LRTI in infancy. AMP: adenosine monophosphate; IMP: inosine monophosphate; GMP: guanosine monophosphate; XMP: xanthosine monophosphate; ADA: adenosine deaminase; PNP: purine nucleoside phosphorylase; ADE: adenine deaminase; GDA: guanine deaminase; OWO: overweight or obesity; GDM: gestational diabetes; DM: diabetes mellitus. Figure partially created using BioRender.com

lipids (C52:0 TAG, C38:4 DAG, C34:1 DAG or TAG fragment) and other compounds (7-imidazole propionate, C7 carnitine, cinnamoyl glycine, 7-dehydrodesmosterol, 2-amino octanoate) (figure 3, supplementary table E1). Notably, even though linear associations did not withstand FDR adjustment, enrichment analyses revealed a significant concentration of compounds in the purine metabolism pathway (FDR=0.007547) (supplementary figure E3). The enriched metabolites were identified as purine nucleosides (xanthosine and inosine) or imidazopyridines (xanthine and hypoxanthine), which serve as uric acid precursors in the PDP (figure 2a and supplementary figure E4 and table E1). Of note, TAGs without KEGG or HMDB identifiers (C42:0, C:44:1, C34 DAG or TAG fragment) or not present in the queried databases (C44:0 TAG, C47:0 TAG) were not represented in our pathway analysis (supplementary table E2).

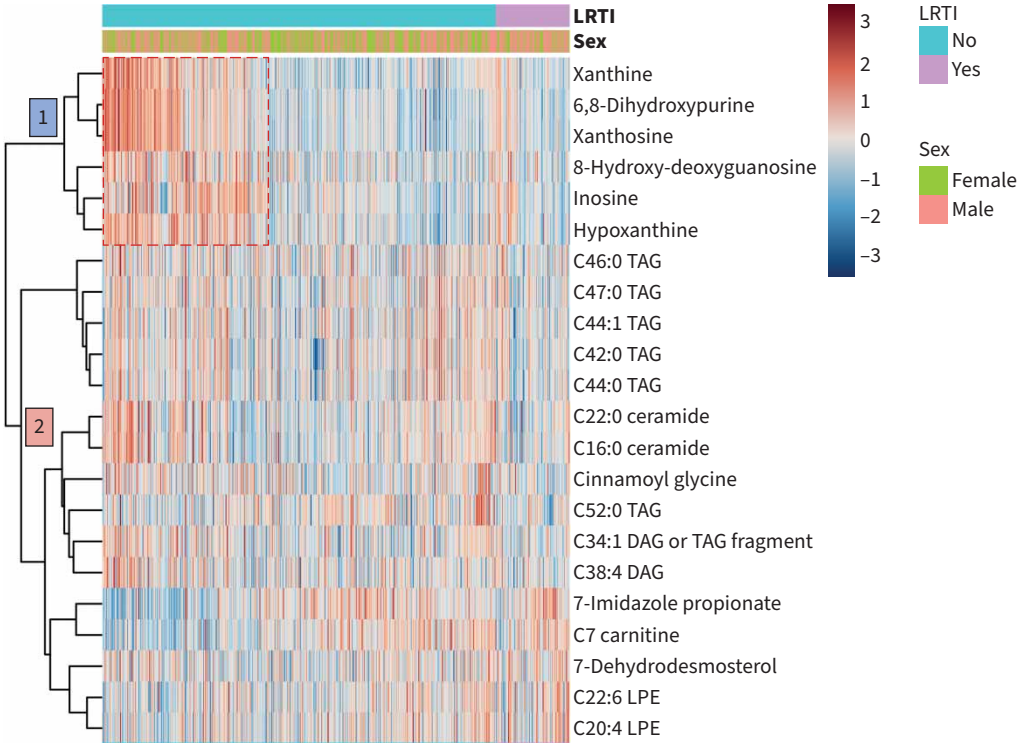

FIGURE 3 Heat map and dendrogram of metabolites associated with lower respiratory tract infections (LRTIs) during infancy. LRTI associations with 378 metabolites were tested in 754 infants using generalised linear regression. 22 metabolites significantly associated with LRTIs in the adjusted models are displayed. The metabolites are represented in rows, and the individual infant samples are represented in columns. The colour of each cell in the heat map represents the level of the corresponding metabolite in each infant's cord blood (blue, lower abundance; red, higher abundance), which has been inverse normal transformed. The dendrogram is a hierarchical clustering diagram that groups metabolites based on their similarity in association with LRTI. The upper part of the dendrogram (cluster 1) includes purine degradation pathway (PDP) metabolites (xanthine, xanthosine, inosine and hypoxanthine) and related compounds (6,8-dihydroxypurine and 8-hydroxy-deoxyguanosine). The red dotted square shows a subset of infants with higher levels of PDP metabolites who did not have a LRTI in the first year of life. Cluster 2 includes subgroups of glycerolipids (C46:0 TAG, C47:0 TAG, C44:1 TAG, C42:0 TAG, C44:0 TAG), sphingolipids (C22:0 ceramide, C16:0 ceramide), other complex lipids (C52:0 TAG, C38:4 DAG, C34:1 DAG or TAG fragment) and other compounds (7-imidazole propionate, C7 carnitine, cinnamoyl glycine, 7-dehydrodesmosterol, 2-amino octanoate).

PDP metabolites identify a subset of infants with a lower likelihood of LRTIs during the first year of life

Our initial analyses identified a subset of infants in the non-LRTI group noted to have higher cord blood levels of purine metabolites (figure 3 and supplementary figure E5). Accordingly, we tested whether infants born with high levels of xanthine, hypoxanthine, inosine, xanthosine, 6,8-dihydroxypurine and 8-hydroxy-deoxyguanosine (defined as inverse normal transformed levels in the upper quartile) were associated with decreased LRTI frequency in infancy. Our multivariate analyses included 810 infants and revealed that newborns with high cord blood levels of xanthine (OR 0.54, 95% CI 0.32–0.90, p=0.0019), hypoxanthine (OR 0.50, 95% CI 0.29–0.85, p-value=0.010), inosine (OR 0.53, 95% CI 0.32–0.89, p=0.016) and xanthosine (OR 0.48, 95% CI 0.28–0.81, p=0.006) had decreased odds of LRTI during the first year of life after adjusting by maternal race/ethnicity, maternal body mass index, smoking in the 3rd trimester, history of asthma and diabetes, low birthweight, gestational age, type of delivery and infant's sex and breastfeeding status (figure 2b and supplementary tables E4 to E7). Similarly, a negative association was noted between high cord blood levels of 6,8-dihydroxypurine (OR 0.38, 95% CI 0.22–0.66, p=0.001) and 8-hydroxy-deoxyguanosine (OR 0.54, 95% CI 0.32–0.91, p=0.021) and LRTI in infancy (supplementary tables E8 to E9). There were no associations between infancy LRTI and other PDP metabolites (uric acid, adenosine or guanine).

TABLE 2 Adjusted Cox proportional hazard model assessing the relation between xanthine to uric acid (XU) ratio and lower respiratory tract infections (LRTIs) in infancy

Variable	Hazard ratio	p-value	95% CI	
			Lower limit	Upper limit
XU ratio				
High	Ref			
Mid	**4.328803**	**0.0000**	**2.232059**	**8.395177**
Low	**3.916139**	**0.0000**	**1.924946**	**7.967052**
Sex	**1.574584**	**0.015**	**1.091282**	**2.271927**
Race/ethnicity				
White	Ref			
Black	1.075354	0.869	0.4541628	2.546191
Hispanic	0.986087	0.976	0.3959693	2.455664
Other/mixed race	1.458029	0.43	0.5710306	3.722828
Gestational age	0.8880001	0.132	0.7607904	1.03648
Vaginal delivery	0.8952258	0.564	0.6148932	1.303363
Low birthweight	1.276473	0.482	0.6462273	2.521379
Pre-pregnancy overweight/obesity	1.398311	0.097	0.9411889	2.07745
Smoking 3rd trimester	0.9908371	0.977	0.5324306	1.843918
Maternal diabetes				
None	Ref			
Gestational	1.332663	0.311	0.764698	2.322472
Chronic	0.7265918	0.497	0.289092	1.826185
Maternal asthma	1.472438	0.116	0.9076483	2.388673
Breastfeeding	0.7250254	0.106	0.4911927	1.070175

Of 813 infants included in the adjusted model, infants with low and intermediate XU ratios had an increased risk of LRTI during infancy compared with infants with high XU ratios (reference group). Male sex was also significantly associated with an increased LRTI hazard in infancy. Statistically significant hazard ratios and p-values are in bold.

Newborns with a low XU acid ratio have a higher risk of LRTI during infancy

We next examined the clinical relevance of the XU ratio as a marker of XO and PDP activity at birth. Log-rank tests and Cox regression analyses showed that infants with low and mid XU ratios had a significantly elevated risk of LRTI in infancy compared with those with high XU ratios (figure 4 and table 2). Specifically, low XU ratio infants had over a four-fold higher risk (HR 4.33, 95% CI 2.23–8.40, p<0.001), and mid XU ratio infants had nearly a four-fold elevated risk (HR 3.92, 95% CI 1.93–7.97, p<0.001) of LRTI in the first year of life.

Maternal–fetal features associated with high levels of PDP metabolites in cord blood

Having identified that the PDP at birth is associated with LRTI risk during infancy, we next examined maternal–fetal factors linked to cord blood PDP metabolite levels. We found that high levels of xanthine (p<0.0001) and hypoxanthine (p=0.0059) were associated with low birthweight, as there was a subset of full-term infants with high xanthine (29 out of 203, 14.3%) and high hypoxanthine (25 out of 203, 12.3%), who weighed <2500 g at birth. Nonetheless, most children with high purine metabolites were in the normal weight group (supplementary figure E6). Similarly, high xanthine levels were associated with preeclampsia/eclampsia (p=0.023), and high xanthosine levels were related to maternal pre-pregnancy overweight/obesity (p=0.029) (supplementary figure E7). Notably, an inverse association was observed between gestational diabetes and high xanthine levels (p=0.0041). No significant associations were found between high PDP metabolite levels and other maternal or infant characteristics (maternal age, history of maternal asthma, gestational age) or Apgar scores at 1 and 5 min.

Discussion

Our analysis revealed that newborns with high levels of intermediate metabolites of the PDP (xanthosine, inosine, hypoxanthine and xanthine) and two associated metabolites (6,8-dihydroxypurine and 8-hydro-deoxyguanosine) [18–20] had approximately half the odds of experiencing an LRTI in infancy in comparison with newborns with levels in the lower quartiles, independently of other factors knowingly associated with LRTI risk in early life. To our knowledge, this is the first study to show that high levels of cord blood PDP metabolites identify a subset of full-term newborns with a decreased likelihood of LRTIs during infancy.

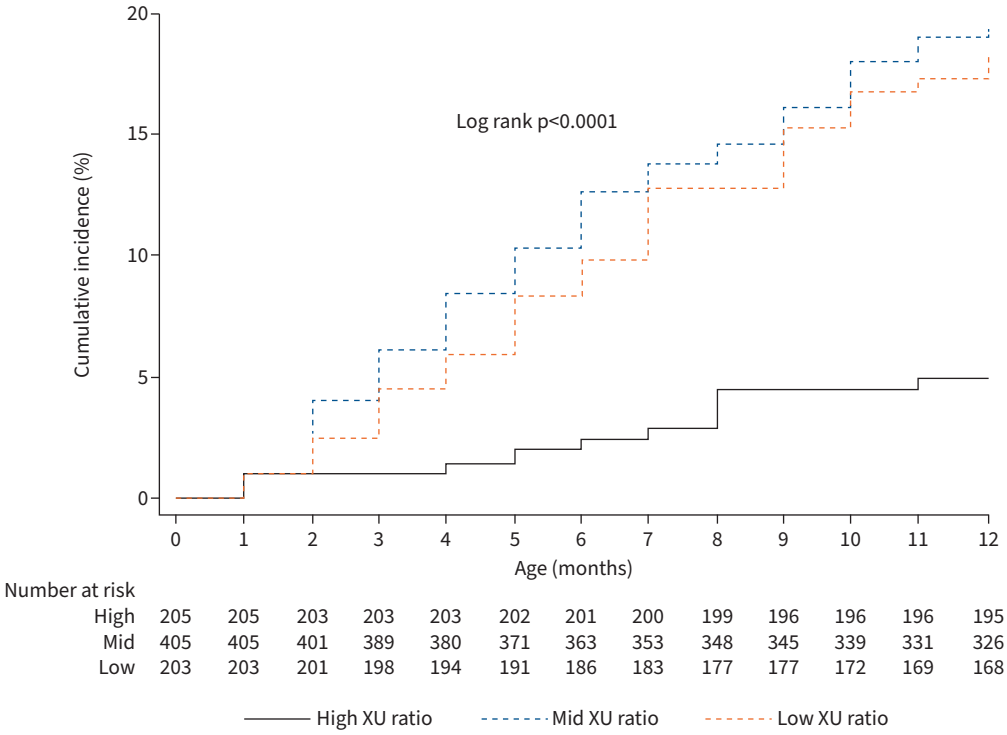

FIGURE 4 Infancy lower respiratory tract infection (LRTI) risk by xanthine to uric acid (XU) ratio. Cumulative risk of LRTI during the first year of life by XU ratio cord blood levels. Kaplan–Meier comparison of the survival function of the 813 full-term newborns included demonstrates a significant difference in incident LRTI between infants with a high XU ratio at birth and those with intermediate and low XU ratio levels (log rank p<0.0001).

The PDP is crucial in cellular processes like nucleotide synthesis and energy homeostasis, and purinergic signalling regulates critical aspects of cell behaviour [18, 21, 22]. In the respiratory system, the PDP modulates responses to infections and environmental stimuli, such as pollutants, allergens and hypoxia [23–27]. PDP metabolites also play an essential role in maintaining mucus production, ciliary mobility and the production of alveolar surfactant in the lungs [28–30]. The PDP regulates airway smooth muscle reactivity *via* adenosine-mediated bronchoconstriction and the bronchodilator effect of xanthine and its derivates (*e.g.* methylxanthines) mediated by adenosine receptor antagonism and phosphodiesterase (PDE) inhibition [31]. The PDP additionally controls the balance between prooxidative and antioxidative processes [32–34], and is essential for tissue homeostasis and adaptive cellular responses, including autophagy, cytoprotection and resolution of inflammation [24, 35].

The effects of the PDP are regulated by several enzymes and intermediary products (figure 2a). One of the key enzymes in the PDP is the xanthine oxidase (XO), which catalyses the production of uric acid from purine derivatives and generates reactive oxygen species. In turn, PDP metabolites such as inosine and uric acid are antioxidants [36, 37]. Immunologically, metabolites of the PDP have immunomodulatory effects. For example, uric acid (the final PDP product) is a DAMP (Danger Associated Molecular Pattern) with pro-inflammatory effects on airway epithelial cells *via* NLRP3 inflammasome activation [7]. Uric acid precursors, such as inosine, enhance Th1 differentiation of naïve T-cells and interferon (IFN)-γ release, critical for host defence against intracellular pathogens, including viruses [36, 38]. In contrast, adenosine inhibits monocyte and T-cell proliferation and function through the generation of intracellular cyclic AMP (cAMP) in acute inflammatory conditions [39].

Our data demonstrate that high levels of PDP metabolites like inosine, hypoxanthine, xanthosine and xanthine have a protective effect against LRTIs. One such mechanism for this protective effect could be a build-up of uric acid precursors due to decreased XO activity, leading to less damage due to oxidative stress, enhanced antiviral and anti-inflammatory immune responses, and decreased smooth muscle reactivity. Alternatively, there could be an increased conversion of adenosine to inosine and its downstream metabolites with an enhancement of their antioxidant, antiviral, immunomodulatory and bronchodilator

effects. Although the underlying cause of our findings is not yet clear, this study motivates additional research to determine whether PDP's modulation of oxidative stress, innate and adaptive immune responses, and smooth muscle reactivity in the respiratory tract confer protection to LRTIs in infancy.

To further study XO activity as a key step of the PDP, we quantified xanthine to uric acid (XU) ratios in cord blood and identified that high XU ratios (a marker of decreased XO activity) predicted a reduced risk of LRTI in infancy. This finding suggests that the underlying protective mechanism of uric acid precursors in the PDP pathway may stem from decreased XO activity, leading to an accumulation of upstream PDP metabolites and enhancement of their protective effect against symptomatic or severe LRTI. This notion is supported by previous work demonstrating that XO inhibition during neonatal murine RSV infection decreases mucus production, reduces cellular infiltrates and decreases the airways' thymic stromal lymphopoietin (TSLP), IL-33 and Th2 immune responses [8]. Moreover, since the accumulation of intermediate PDP metabolites is detected in the cord blood of newborns at birth, without active LRTI, our findings suggest that differences in XO activity are intrinsic and occur at baseline. Alternatively, there could be intrinsic overactivation of the PDP in newborns with an influx of high levels of PDP intermediate metabolites, enhancing their antioxidant, immunomodulatory and bronchodilator properties and contributing to milder LRTIs. In any case, our findings highlight the XU ratio as a biomarker for LRTI in infancy and prompt further investigation into whether XO and PDP activity are mechanisms mediating LRTI susceptibility in early life. Moreover, PDP metabolites may allow better stratification of infant LRTI risk, and XO inhibition may be a targeted intervention to decrease LRTI morbidity and mortality in newborns at risk.

Given the importance of the PDP in LRTI risk, we also studied potential maternal–fetal factors associated with PDP activity and companion metabolite levels at birth. Previous studies have described neonatal stress and perinatal hypoxia as a cause of elevation of these PDP metabolites in cord blood [40]. Although we did not find associations with Apgar scores at 1 and 5 min, some newborns with high xanthine and hypoxanthine had low birthweight (supplementary figures E6 and E7). There was also a positive association between high cord blood xanthine levels and preeclampsia/eclampsia (supplementary figure E7). It is possible that some prenatal causes of low birthweight (*e.g.* placental insufficiency) cause metabolic disturbances involving the PDP. Notably, elevated cord blood xanthine levels were inversely associated with gestational diabetes mellitus (GDM) (supplementary figure E7), and about nine out of 10 infants born to mothers with GDM had mid or low cord blood xanthine levels. This finding may indicate that dysregulation of the PDP in maternal diabetes (*e.g.* oxidative stress) [41] may affect the offspring of diabetic mothers prenatally and requires further investigation. In summary, elevated levels of PDP metabolites were linked to low birthweight and preeclampsia/eclampsia and inversely associated with gestational diabetes, three major prenatal disorders. We did not find associations between maternal diabetes, preeclampsia/eclampsia or low birthweight and LRTI in infancy, suggesting that the PDP is independently associated with those conditions.

Our study has limitations, including focusing only on physician-diagnosed cases, possibly missing milder or asymptomatic LRTI episodes. The use of ICD9/10 codes, although it allowed us to include many newborns, limits the depth of clinical assessment. As our sample is an inner-city, predominantly minority population in the USA, our findings may not be generalisable. The study also concentrates on term births, and the role of the PDP in preterm infants remains to be studied. Finally, LRTIs in infancy are multifactorial, metabolic risk is only one of their determinants and additional unmeasured factors may impact LRTI risk. However, considering the importance of the PDP in multiple cellular processes, its metabolites are promising biomarkers for risk stratification at birth and potential therapeutic targets.

In summary, our study suggests that PDP metabolic activity at birth can influence susceptibility to LRTIs during infancy and cord blood levels of intermediate purine metabolites (*e.g.* xanthine, xanthosine, inosine and hypoxanthine), and the XU ratios may serve as biomarkers for prenatal risk stratification of early LRTI risk. Moreover, as high XU ratios (a marker of low xanthine oxidase activity) predict a lower risk of LRTI, our results suggest a point of potential therapeutic intervention for LRTI prevention and/or treatment (*e.g.* XO inhibition) in newborns at risk. Further research is needed to elucidate the role of the PDP in LRTI pathogenesis and other respiratory diseases during and beyond childhood.

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E. Mondell, E. Chorvinsky, S. Bhattacharya, B.S. Bera, A. Welham, X. Hong and X. Wang. All authors provided critical feedback and approved the final version of the manuscript.

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