



Why the Airway Microbiome Matters for Asthmatics

Le Bonheur researchers review the impact of the microbiome on asthma development, severity in the first review of its kind

Understanding the role of the airway microbiome in the development and progression of asthma may lead to new treatments or more effective administration of current therapeutics, says a review article from a team of investigators at Le Bonheur Children's Hospital published in *Frontiers in Pharmacology*. The review explored the relationship between the airway microbiome and asthma alongside other intrinsic and extrinsic factors, with particular emphasis on how these factors impact asthma development and severity in pediatric and Black populations as high-risk groups. This review was co-authored by Chief of Pediatric Pulmonology Patricia J. Dubin, MD, Researcher Amali E. Samarasinghe, PhD, and Emergency Medicine Physician Mark A. Snider, DO.



Review co-authors (left to right) Emergency Medicine Physician Mark A. Snider, DO, Researcher Amali Samarasinghe, PhD and Chief of Pediatric Pulmonology Patricia J. Dubin, MD

"Crosstalk between the mucosal microbiota and the immune system as well as the gut-lung axis have direct correlations to immune bias that may promote chronic diseases like asthma," said Dubin. "Asthma initiation and pathogenesis are multifaceted and complex with input from genetic and environmental components."

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Cell Connections

Le Bonheur nephrologist investigates cellular dynamics that lead to polycystic kidney disease



Le Bonheur Chief of Nephrology and Director of the Tuberous Sclerosis Center of Excellence, John Bissler, MD

John Bissler, MD, Le Bonheur chief of Pediatric Nephrology and director of the Tuberous Sclerosis Center of Excellence, recently published research in *Biology* investigating the cellular-level dynamics that lead to polycystic kidney disease (PKD) when the Pkd1 gene or the Tsc2 gene is deleted.

The results showed that deletions of either gene increased the production of extracellular vesicles (EVs), particles that provide communication between cells, which appear to recruit genetically unaltered cells to participate in the development of kidney cysts. In addition, deletion of either the Pkd1 or Tsc2 gene caused kidney cells to take up more EVs and hold onto the EVs for a longer period of time. Researchers hope that further understanding of EVs' impact on PKD will provide an avenue for new therapies.

"Patients with PKD and tuberous sclerosis complex (TSC) are born with typical kidneys, but quickly develop cysts causing a premature loss of kidney function," said Bissler. "The more we understand how EVs are involved in the development of PKD, the more potential we have for early intervention to preserve kidney function."

Results also showed that primary cilia, structures on the surface of cells that act as antennae or sensors, play a large role in transmitting and receiving EVs. When researchers deleted the Pkd1 gene and the gene responsible for the protein that builds cilia, EV production was

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Composition and Development of the Airway Microbiome

When comparing the airway microbiome, which includes bacteria, viruses and fungi, a microbiome of high density and low diversity is associated with inflammation. Historically, bacteria have been associated with asthma exacerbations, severity of disease and how individuals respond to treatments. The virome of asthma patients had a reduced abundance and diversity of the viruses that attack bacteria, and severe asthma patients had an increased virome density that correlated with poor lung function. When looking at the asthma patient lung mycobiome, research has shown that significant fluctuations, with the loss of good fungi and the proliferation of pathogenic fungi, may promote the development of asthma.

"In addition, there may be significant differences in the airway microbiome in asthma patients of different ethnicities or racial groups that may impact disease progression and may require personalized therapeutic approaches," said Samarasinghe.

The Role of the Gut-Lung Axis

Researchers also looked at the gut-lung axis and how this might impact asthma patients. It is postulated that the microbiota of the gut and lungs may cross-regulate as the respiratory and gastrointestinal tracts share a common embryonic origin. Certain gut bacteria were more abundant in Black women, showing a link between the gut microbiome and disease susceptibility and severity in terms of race. Researchers noted that the observed variation in microbiome may be a result of socioeconomic differences rather than inherent biological factors.

"Differences in gut microbiota have been associated with disease susceptibility by race and sex," said Samarasinghe. "Analysis of the gut microbiome may be a simple and early biomarker for disease predisposition."

Factors Affecting the Microbiome

The review looked at additional factors that affect the airway microbiome and thereby might have an impact on asthma development and severity. These factors included:

- Genetics
- Smoking: Black smokers have more negative consequences of smoking, including asthma, compared to White smokers.
- Diet and nutrition: Incorporating fiber-rich foods led to significant alterations to the gut microbiome and improved mucosal health in Black populations. Vitamin D deficiencies are correlated with asthma in Black children.
- Drugs: Antibiotics and corticosteroids can impact the microbiome.

"The risk factors for the pathogenesis and severity of asthma are multifactorial. Whether race contributes to asthma severity and outcomes more as a biological factor or a social construct is unclear, but what is clear is that higher prevalence, higher severity and worse outcomes are associated with the Black race as defined in literature," said Snider.

Children with developmental delay undergoing tonsillectomy have heightened complication risks

Children with developmental delay may be at risk for higher complication rates following tonsillectomy compared to children without developmental delay, according to research published in *International Journal of Pediatric Otorhinolaryngology* by Anthony Sheyn, MD, FACS, chief of Otolaryngology at Le Bonheur Children's Hospital.



Le Bonheur Chief of Otolaryngology Anthony Sheyn, MD, FACS, published research showing the increased risk of complications for children with developmental delay who undergo tonsillectomy.

Developmental delay (DD) refers to a broad group of conditions that involve impairments in communication, gross and fine motor skills, problem solving and social interactions. Children with DD often have complex medical histories with comorbidities, increasing their need for more health care treatment than children without DD.

"We have a limited amount of literature studying the effect of DD in pediatric surgical patients," said Sheyn. "We wanted to further investigate the role that DD has on the outcome of our most performed pediatric surgery, tonsillectomy."

Researchers reviewed chart data from 400 tonsillectomy patients to compare post-operative complication rates between children with and without DD. Of these patients, 56 (13.9%) had a DD diagnosis.

Results showed that patients with DD had a significantly increased risk for post-operative complications and experienced significantly more respiratory complications, such as respiratory arrest and overnight oxygen desaturation. Where 32.14% of patients with DD had a complication after surgery, only 8.72% of patients without DD had a complication. Other complications included post-operative hemorrhage, dehydration and vomiting.

Furthermore, patients with moderate to severe DD had a higher risk for post-operative complications compared to patients with mild DD. Patients with Down syndrome, Global Developmental Delay or two or more delays in developmental milestones were classified as severe. Within the severe DD subgroup, nine out of 13 patients (69.23%) experienced a complication after surgery.

This study emphasizes the need for in depth pre-operative planning for patients with DD to lower risk of complications post-operation. Researchers suggest this need is heightened for patients with moderate to severe DD, but more research is needed to define DD severity and how it affects surgical outcomes.

"This elevated risk of complications should be included in pre-operative counseling and has potential implications for pre-operative decision making and treatment plans in this high-risk population," said Sheyn. "We suggest in depth counseling and close follow up after surgery for these patients to help reduce the current findings of an increased complication rate."

Critical Need: Trauma and Integrated Mental Health Care

Largest pediatric study of its kind links acute stress disorder symptoms to traumatic injuries

In the largest pediatric study to date reviewing Acute Stress Disorder (ASD) following a traumatic injury, results showed that 64.8% of children tested positive for ASD in the aftermath of a traumatic injury. ASD is the precursor to Post Traumatic Stress Disorder (PTSD). The study stems from a partnership between Le Bonheur Children's Trauma Services Division and the BRAIN Center at the University of Memphis and was published in *Trauma Surgery & Acute Care Open*. The results of the study provide evidence of the link between traumatic injury and mental health distress symptoms in children and much-needed data to advocate for integrated mental health screening in the hospital setting at the time of an injury.

"We have a critical need for mental health screening and counseling services at the time of an acute pediatric trauma — these findings further highlight that need," said Le Bonheur's Medical Director of Trauma Services Regan Williams, MD. "Traumatic experiences can negatively affect long-term function and increase overall mental health distress in these kids without the proper mental health intervention."

Investigators were awarded a grant to embed trauma mental health counselors in the Trauma division at Le Bonheur Children's, integrating mental health care with medical care at the bedside. This study was a needs assessment to identify the incidence and establish a baseline understanding of children who experience symptoms of acute stress disorder (ASD) after a traumatic injury. Previous pediatric studies of mental health and traumatic injury have focused on the development of post-traumatic stress disorder (PTSD), which requires symptoms to be present for at least one month for diagnosis. Screening for symptoms of ASD — avoidance, negative mood, hyperarousal and intrusive symptoms — allows for earlier intervention and prevention of the development of PTSD.

In the eight months of the study, 617 patients ages 2-18 years were screened using the Child Stress Disorders Checklist-Screening Form (CSDC-SF) — a four item self-report assessment adapted from the Child Stress Disorders Checklist (CSDC). The questions rate a child's behaviors of avoidance, emotional distress and hyperarousal, with a score higher than one indicative of acute stress disorder. All

patients received emotional support, psychoeducation and free outpatient counseling resources. Patients scoring one or higher were educated on coping mechanisms and potential risk factors of PTSD and were recommended for outpatient mental health services.

The average ASD score was 1.71 with 64.8% of children scoring one or higher. The percentages found in this study are significantly higher than previous studies, which researchers say may be attributed to the prolonged heightened stress response due to the COVID-19 pandemic.

Older participants, females, motor vehicle collision patients and weapons-related patients experienced higher levels of

ASD symptoms in the study. Discrepancies in scores between older and younger patients may reflect the elevated risk for exposure to traumatic events for adolescents and the concern of under reporting among caregivers for children in early childhood. Furthermore, the higher level of ASD in motor vehicle collision and weapons-related injury patients show a link between injury severity and increased mental distress.

"Our findings further support literature indicating that ASD is a prevalent reaction that children experience following an injury," said Williams. "Proper screening, early identification and treatment can lead to improved mental health outcomes among children post-injury."

Providing timely mental health intervention is crucial for several reasons, researchers say. Prolonged exposure to stress can lead to long-term negative impacts on a child's mental health. It is critical to develop mental and behavioral health counseling that is integrated with medical care at the time of the traumatic injury. This allows for proper screening, early identification and prompt treatment to mitigate the consequences of ASD and PTSD. This study provides evidence needed to advocate for these types of services in pediatric hospitals.

Future research will examine ASD symptoms three months after injury to see if PTSD diagnoses were reduced with mental health intervention and assess the efficacy of various counseling interventions after traumatic injury.



Trauma Services providers round with counselors from the Hospital-Based Violence Intervention Program — a group of counselors providing mental health interventions for children who have experienced a traumatic injury. This is a partnership with the BRAIN Center at the University of Memphis.

Le Bonheur researchers publish 233 articles in 2022

CFRI researchers continue to publish cutting-edge studies that help children with serious diseases who depend on Le Bonheur. In 2022, Le Bonheur-affiliated authors and researchers published 233 articles. All of these articles can be found on PubMed.gov. This volume of peer-reviewed research builds upon the astounding publishing output of 317 articles seen in 2021.

Please remember to include Le Bonheur in your authorship affiliation. CFRI's goal is to publish more than 200 manuscripts per year, and affiliation is one way we track our progress.

lowered and PKD was less severe.

Other results included:

- Cells without the Pkd1 gene produced more EVs than cells without the Pkd2 gene.
 - Previously published studies from Bissler's group showed that Tsc2 gene deletion greatly increased EV production compared to Tsc1 deletion. The same proved to be true when comparing Pkd1 and Pkd2 deletion – with the former producing twice as many EVs.
 - In addition, EVs from cells without Pkd2 had changes on the surface, which raised the possibility that they may interact or bind differently with cells.
- Removing primary cilia lowers EV production.
 - Researchers added a treatment to kidney cells to remove primary cilia. This caused a significant reduction in EVs, showing that primary cilia are important contributors to the production of EVs.
- Deletion of Tsc2 gene leads to EVs that migrate to the kidney at high rates.
 - To understand how EVs move through the body, researchers used a mouse model to isolate EVs from kidney cysts and inject them into the peritoneal

cavity. Mice without a functioning Tsc2 gene had EVs that migrated to the kidney at a significantly greater amount than those with the Tsc2 gene. EVs from cells with mutations in Pkd1 or Pkd2 also had significantly increased migration to the kidneys.

- Researchers posited that kidney cyst EVs must have a method to conscript cells to migrate to the kidneys.
- Gene deletions in Pkd1, Pkd2, Tsc1 and Tsc2 caused cells to take in EVs faster and hold them longer.
 - EVs from cells with Pkd1 deletion had an uptake process 14 times faster and clearance five times slower than those from the cells with Pkd2 deleted. These results suggest that polycystin-1, a protein produced by the Pkd1 gene, regulates EV trafficking.

All of these results provide insight into the cellular-level mechanisms that lead to PKD, particularly the role that EVs play in this process.

"Our study suggests the possibility that EV production rates, tissue half-life, target homing and cargo differences may be involved in the disease process," said Bissler. "Additional studies can help us identify diagnostic and prognostic biomarkers and ultimately reveal novel therapies to reduce cystic disease."

Cost-Effective Care

High-flow nasal cannula usage outside of intensive care associated with longer hospital stays, higher costs



High-flow nasal cannula (HFNC) treatment for patients with mild to moderate bronchiolitis may be associated with longer hospital stays and higher cost of care, according to a study led by Le Bonheur Hospitalist Jeffrey C. Winer, MD, MA, MSHS, and published in the American Academy of Pediatrics (AAP) journal *Hospital Pediatrics*.

"Historically, the relationship between HFNC usage outside of the intensive care unit and outcomes including hospital length of stay and cost is unclear. Our study sought to see if HFNC itself may be partially responsible for these outcomes," said Winer.

HFNC is a treatment that delivers oxygen to a patient at a high rate, reducing the work of breathing. It is widely used for treating bronchiolitis, a lower respiratory tract infection and a leading cause of emergency room visits for children younger than 2. Recent studies on HFNC have shown variable results across patient populations, particularly regarding outcomes such as hospital length of stay (LOS) and cost of care. While severe cases

of bronchiolitis where HFNC was used were associated with symptom improvement and decreased LOS and cost, HFNC use in mild to moderate cases has been associated with less symptom improvement, along with increased LOS and cost.

The purpose of this study was to explore the relationship between HFNC usage for non-intensive care unit (ICU) patients and patient outcomes, LOS and cost of care. Researchers reviewed data from 20 hospitals in the Pediatric Health Information System. The data included patients younger than 2 years with a principal or secondary diagnosis of acute bronchiolitis. Researchers categorized these hospitals based on the proportion of non-ICU patients treated with HFNC into low, moderate or high non-ICU HFNC usage (NIHU).

Results showed that LOS was longer for patients in moderate to high NIHU hospitals compared to low NIHU hospitals. Analysis of cost of care showed similar results, where moderate and high NIHU hospitals showed higher costs compared to low NIHU hospitals, even after adjusting for clinical and demographic characteristics. Based on their analyses, researchers estimate that high NIHU hospitals have 5% to 30% longer LOS and 2% to 31% higher cost of care than low NIHU hospitals.

"The large number of patients admitted to children's hospitals means that even small relative changes in these values may lead to large burdens to hospitals and the health care system," said Winer.

This study suggests that higher NIHU may lead to increased overall resource utilization for pediatric bronchiolitis patients. More research is needed to assess how HFNC usage affects LOS and cost of care across pediatric hospitals and if HFNC should be limited in non-ICU patients.

Contact us

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