findings and potentially inform the development of targeted therapeutics.

**Data availability statement**

Complete differentially expressed gene results are available in the Supplementary Materials and Methods.

**ORCIDs**

Leigh A. Nattkemper: http://orcid.org/0000-0003-0895-7985

Kayla Fourzali: http://orcid.org/0000-0001-8016-7166

Gil Yosipovitch: http://orcid.org/0000-0001-6303-1822

**CONFLICT OF INTEREST**

GY is a consultant and scientific advisory board member of Trevi, Pfizer, Regeneron Sanofi, Galderma, Novartis, Leo, Eli Lilly, Kinika, Bellus, and GlaxoSmithKline and received funding and participated in trials by Pfizer, Leo, Novartis, Regeneron Sanofi, Kinika, and Sun Pharma. The remaining authors state no conflict of interest.

**ACKNOWLEDGMENTS**

This work was supported by GlaxoSmithKline and Hans Hofland. Temple University’s Fox Chase Cancer Center Genomic Services core was responsible for the RNA sequencing and data analysis.

**AUTHOR CONTRIBUTIONS**

Conceptualization: LAN; Formal Analysis: LAN; Investigation: LAN; Methodology: GY, LAN; Supervision: GY, LAN; Writing — Original Draft Preparation: KF, LAN; Writing — Review and Editing: KF, LAN, GY

Leigh A. Nattkemper, Kayla Fourzali, and Gil Yosipovitch

1Miami Itch Center, Dr. Phillip Frost
Department of Dermatology and Cutaneous Surgery, University of Miami, Miami, Florida, USA

*Corresponding author e-mail: Givosipovitch@med.miami.edu

**SUPPLEMENTARY MATERIAL**

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2020.11.017.

**REFERENCES**


**Staphylococcal Hemolytic Potential Is Correlated with Increased Severity of Atopic Dermatitis in Children and Young Adults**

*Journal of Investigative Dermatology* (2021) 141, 1588–1591; doi: 10.1016/j.jid.2020.11.023

**TO THE EDITOR**

Atopic dermatitis (AD) represents a complex intersection between skin barrier dysfunction, host immunologic response, and external factors such as allergic triggers and the skin microbiome. However, the relative contributions of these factors are not clear. There is a surge in *Staphylococcus aureus* on the skin during flares of AD (Kong et al., 2012). *S. aureus* strains seen in AD flares are unique to the host and capable of eliciting varied immune responses (Byrd et al., 2017). No direct relationship between severity and in vitro toxin genotype has been established (Kim et al., 2009). *S. epidermidis* may similarly contribute to the propagation of AD through the elicitation of toxins (Martinez-García et al., 2018). Further information on strains of staphylococci found on patients with AD may aid in developing therapeutic targets.

Abbreviation: AD, atopic dermatitis

Accepted manuscript published online 25 December 2020; corrected proof published online 19 January 2021

© 2021 The Authors. Published by Elsevier, Inc. on behalf of the Society for Investigative Dermatology.
δ-Toxin (also known as δ-hemolysin) is produced by 97% of *S. aureus* strains as well as many coagulase-negative staphylococci (Dinges et al., 2000). Staphylococcal δ-toxin lyses mammalian erythrocytes and is particularly potent against rabbit erythrocytes (Kreger et al., 1971); it also induces mast cell degranulation in vitro and is capable of inducing inflammatory skin disease in mouse models (Nakamura et al., 2013). Variability in δ-toxin production by *S. aureus* strains is influenced by complex interactions between a number of genes, with the *carA* gene playing a critical role in its production (Su et al., 2020). In this study, we report data from a pilot study to begin to define the relationship between hemolytic toxin production and AD severity in pediatric patients and young adults.

The Emory University (Atlanta, GA) Institutional Review Board approved this study (Institutional Review Board #0087896 and 00093739). Written informed consent and/or assent was obtained from parents or patients aged 2–20 years with AD. The diagnosis of AD was confirmed by a board-certified pediatric dermatologist after initial screening on the basis of the criteria outlined by Hanifin and Rajka (1980). Patients were recruited from the Emory University pediatric dermatology clinics from May 2016 to May 2018 and were not asked to modify their topical or oral medications before this study to collect preliminary data on this subject. We assessed disease severity at the time of sample collection on the basis of the SCORing Atopic Dermatitis Index score, with fewer mild (*n* = 5) and severe (*n* = 4) scores (Chopra et al., 2017) (Supplementary Table S1). Samples were collected from nine patients without AD. The skin of severely affected patients had the highest staphylococcal density in all pooled samples (Supplementary Table S2) as well as relative to the affected skin in patients with mild or moderate AD (Figure 1). Density was not significantly different in nares or unaffected skin between mild, moderate, and severe patients (Figure 1).

Hemolytic potential of 1,054 isolates was assessed in a rabbit blood lysis model (Supplementary Figure S1). There was a significant increase in average hemolytic activity of staphylococcal isolates pooled from all

---

**Figure 1. CFUs per ml for isolates collected at different sites, grouped by patients with AD disease severity or control.** Each data point represents the staphylococcal load on a single swab. (a) All pooled samples from all sites. (b) Nares isolates. (c) Isolates from unaffected skin. (d) Isolates from affected skin (shown with unaffected controls for the purpose of comparison). Significance was determined by unpaired parametric two-tailed *t*-tests, with *P* < 0.05; **P** < 0.01. See Supplementary Table S2 for corresponding analyses. AD, atopic dermatitis; CFU, colony-forming unit.
collected sites from mild patients relative to those from moderate patients and those from moderate patients relative to those from severe patients (Figure 2a and Supplementary Table S3), whereas hemolysis in control isolates was not significantly different from that in mild patients. A similar stepwise increase in average hemolytic activity was demonstrated in isolates from unaffected skin of patients with AD, as well as from nares, and affected skin when analyzed independently (Figure 2b–d). Body site and patient-level data are provided in Supplementary Figures S2 and S3.

Although severe patients were most heavily colonized with staphylococci in affected skin (Supplementary Table S2), our study aligns with previous studies showing that staphylococcal load alone does not consistently correlate with the severity of AD (Byrd et al., 2017; Totte et al., 2019). Mild and moderate patients in our study did not have significantly higher staphylococcal colonization load than control patients (Supplementary Table S2) but did show a stepwise increase in hemolytic activity of staphylococcal isolates (Supplementary Table S3). Our results build on previous studies to suggest that a more precise understanding of staphylococcal strains is necessary to understand this interaction.

In this study, we provide strong preliminary data that individual isolate level differences in staphylococcal virulence is the agr system, and research aimed at the identification of small molecule inhibitors of this system in efforts to diminish S. aureus virulence has emerged over the past decade (Salam and Quave, 2018). In some cases, these studies have progressed toward acute and inflammatory models of skin disease. For example, solanamide B was recently reported to suppress δ-toxin–induced disease in a modified epicutaneous murine disease model of AD (Baldry et al., 2018). Future studies on the association demonstrated in this study may provide insight into potential therapeutics targeting staphylococcal virulence.

Although our study design was selective for staphylococci, our methods did not differentiate between hemolysis caused by distinct coagulase-negative species (Supplementary Figure S4). S. epidermidis strains may be important drivers of the association noted in this study. In vivo interactions between S. epidermidis and S. aureus are complex and warrant future investigation.

Figure 2. Zone of hemolysis for isolates collected at different sites, grouped by AD disease severity or control. Each data point represents the hemolytic activity of a single staphylococcal isolate. Multiple isolates were sampled from each swab. (a) All pooled samples from all sites. (b) Nares isolates. (c) Isolates from unaffected skin. (d) Isolates from affected skin (shown with unaffected controls for the purpose of comparison). Significance was determined by unpaired parametric two-tailed t-tests, with *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. See Supplementary Table S3 for corresponding analyses and Supplementary Figure S4a–d for a breakdown of hemolytic activity between Staphylococcus aureus and CoNS. Of note, S. aureus isolates from nares exhibited significantly greater hemolytic activity in patients with severe and moderate AD than CoNS. Likewise, S. aureus isolates from unaffected skin exhibited larger zones of hemolysis than CoNS in mild and severe AD cases. Regarding the isolates from lesional skin, only severe AD cases exhibited greater hemolysis among S. aureus isolates than among CoNS. AD, atopic dermatitis; CoNS, coagulase-negative staphylococci.
Although we noted a correlation between AD severity and staphylococcal hemolysis in this study, this study does not address the causation of this factor. Our study design did not control for all potential confounders such as immunosuppression, topical product use, or bathing practices, but we plan to address these factors in future studies. Topical steroid use may reduce the level of colonization with S. aureus on atopic skin (Nilsson et al., 1992), which may have influenced the density of colonization in some of our patients because we did not control for this factor. Clearly, the relationship between these factors is complex and warrants dedicated study. This was a single-site pilot study, and further research is required to define the generalizability of these results. We plan to explore the relative contribution of individual staphylococcal isolates in the severity of AD in future studies.

Data availability statement
Data are available within the article or its supplementary materials.

ORCID
Emily A. Gurnee: http://orcid.org/0000-0002-6450-7826
Mengqing Xu: http://orcid.org/0000-0002-0599-7888
Caitlin J. Risener: http://orcid.org/0000-0002-4824-6064
Kelly Lehman: http://orcid.org/0000-0001-8946-0342
Kate Nelson: http://orcid.org/0000-0001-8783-2157
Robert A. Swerlick: http://orcid.org/0000-0002-9802-4144
Cassandra L. Quave: http://orcid.org/0000-0001-9615-7886

CONFLICT OF INTEREST
The authors state no conflict of interest.

ACKNOWLEDGMENTS
We acknowledge the Department of Dermatology Emory University (Atlanta, GA) development funds for financial support. This work has been performed in Atlanta, Georgia.

AUTHOR CONTRIBUTIONS
Conceptualization: CLQ, RAS; Data Curation: EAG, CLQ; Formal Analysis: CLQ; Investigation: EAG, MX, CJR, KL, KN; Methodology: CLQ, EAG, RAS; Project Administration: CLQ; Resources: CLQ, EAG; Supervision: CLQ, EAG; Validation: CLQ, EAG; Visualization: CLQ, EAG; Writing - Original Draft Preparation: EAG; Writing - Review and Editing: EAG, MX, CJR, KL, KN, RAS, CLQ

Emily A. Gurnee1, Mengqing Xu2, Caitlin J. Risener3, Kelly Lehman4, Kate Nelson5, Robert A. Swerlick6 and Cassandra L. Quave1,7

1Department of Dermatology, Emory University School of Medicine, Atlanta, Georgia, USA; 2Center for the Study of Human Health, Emory University, Atlanta, Georgia, USA; 3Molecular and Systems Pharmacology Graduate Program, Emory University, Atlanta, Georgia, USA; 4Microbiology and Molecular Genetics Graduate Program, Emory University, Atlanta, Georgia, USA; and 5Corresponding author e-mail: cquave@emory.edu

SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2020.11.023.

REFERENCES


Salam AM, Quave CL. Targeting virulence in Staphylococcus aureus by chemical inhibition of the accessory gene regulator system in vivo. mSphere 2018;3:e00500–17.
