Published in final edited form as:

Ann Allergy Asthma Immunol. 2017 June ; 118(6): 737–738.e1. doi:10.1016/j.anai.2017.04.003.

Influence of *FLG* mutations and *TSLP* polymorphisms on atopic dermatitis onset age

Joy Wan, MD^{1,2}, Nandita Mitra, PhD², Ole J. Hoffstad, MA², and David J. Margolis, MD, PhD^{1,2}

¹Department of Dermatology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA

²Department of Biostatistics and Epidemiology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA

Keywords

atopic dermatitis; early onset; eczema; epidemiology; filaggrin; genetics; thymic stromal lymphopoietin

Atopic dermatitis (AD) is a chronic, inflammatory skin disorder driven by complex biologic and environmental influences. AD has been divided into early onset and late onset forms, with some speculating that early onset AD is driven predominantly by genetic factors while late onset disease is driven by environmental exposures.¹ Among many genetic factors relevant to AD are filaggrin (*FLG*), an epidermal barrier protein, and thymic stromal lymphopoietin (*TSLP*), an IL-7-like cytokine that promotes type 2 helper T cell differentiation. Loss-of-function mutations in *FLG* increase the odds of AD by more than three-fold and are more common in infantile onset AD.^{2–4} Single-nucleotide polymorphisms (SNPs) in *TSLP* have been linked to AD persistence and eczema herpeticum,^{5,6} but its impact on AD onset is unknown. We thus performed a cross-sectional study to examine the impact of *FLG* mutations and *TSLP* polymorphisms on the age of AD onset.

We used the Pediatric Eczema Elective Registry (PEER), a nationwide cohort of over 7500 subjects with pediatric onset AD, of whom 822 subjects had available genetic data. Details of the PEER cohort have been reported previously, and all subjects provided written

Corresponding author: Joy Wan, MD, Hospital of the University of Pennsylvania, Department of Dermatology, 3600 Spruce Street, 2 East Gates Building, Philadelphia, PA 19104; Tel: 215-614-1620; Fax: 215-615-3140; Joy.Wan@uphs.upenn.edu.

Trial registration: Not applicable

Conflict of interest: The data source used in this study is the Pediatric Eczema Elective Registry (PEER), which is funded by Valeant Pharmaceuticals through a grant to D.J.M. The PEER study is an FDA-mandated study as part of the FDA approval process for pimecrolimus, a medication for atopic dermatitis. However, Valeant had no role in the design and conduct of this study; collection, management, analysis and interpretation of data; preparation, review, or approval of the manuscript; or the decision to submit the manuscript for publication.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

informed consent.^{7,8} Children were 2 to 17 years old at enrollment, had a physician-confirmed diagnosis of AD, and had used pimecrolimus cream, a common treatment for AD. Exclusion criteria included lymphoproliferative disease, malignancy, and use of systemic immunosuppressants. Subjects or their caregivers reported the child's age of AD onset as: 0–6 months, 6–12 months, or the exact age in years if greater than 12 months. The onset ages of 0–6 months and 6–12 months were treated as 3 and 9 months, respectively, in the analysis. A total of 798 subjects in PEER were genotyped for the four most prevalent *FLG* null mutations in Europeans (R501X, 2282del4, R2447X, and S3247X), as detailed previously.⁸ Seven hundred seventy subjects were genotyped for the *TSLP*rs1898671 tagging SNP,⁵ where C is the major allele and T is the minor allele, the latter being associated with less persistent AD and lower risk of eczema herpeticum.^{5,6} As previous analyses using ancestry informative markers revealed tight concordance between genetically inferred and self-reported race,⁸ we used self-reported race in this study. Fisher's exact test was used to compare demographic characteristics with respect to *FLG* and *TSLP* status. In the primary analysis, the median age of AD onset was compared among subjects with wild-type, 1 *FLG* mutation (heterozygotes), and 2 *FLG* mutations (homozygotes or compound heterozygotes) and among subjects with 0, 1, and 2 minor alleles at *TSLP*rs1898671 using the Kruskal-Wallis test and test for trend. In a secondary analysis, we dichotomized AD onset age into ≤2 and >2 years old, the former being the most common definition of 'early onset' AD.^{3,9} Logistic regression was performed to compare the odds of early onset AD with respect to *FLG* and *TSLP* status under an additive model.

Of 798 subjects with *FLG* data, 14.7% had 1 null mutation and 2.6% had 2 null mutations (Table). Of 770 subjects tested for the *TSLP* polymorphism, 33.4% had 1 minor allele and 5.3% had 2 minor alleles (Table). Both *FLG* and *TSLP* status varied significantly by race ($p<0.001$), but AD onset age was not significantly associated with race or sex (Table).

The median ages of AD onset were 0.75 (IQR 0.25–2.0), 0.75 (IQR 0.25–1.0), and 0.25 (IQR 0.25–0.75) years for subjects with 0, 1, and 2 *FLG* mutations, respectively (test for trend $p<0.001$). The odds of early onset AD was significantly higher among subjects with 1 *FLG* mutation (OR 1.74, 95% CI 1.02–2.96) and was over three-fold higher, albeit not statistically significant, among subjects with 2 mutations (OR 3.03, 95% CI 0.70–13.16). Restricting the analysis to only white subjects led to similar findings (Supplementary Table 1). In multivariable models adjusted for race, race was not statistically significant and its inclusion did not alter the main effect estimates (Supplementary Table 2). The median age of AD onset was 0.75 (IQR 0.25–2.0) years for all *TSLP*rs1898671 variants, and the odds of early onset AD did not vary by *TSLP* status (OR 1.01, 95% CI 0.70–1.44 for CT variant [1 minor allele] and OR 1.07, 95% CI 0.50–2.31 for TT variant [2 minor alleles]). Among 746 subjects tested for both *FLG* and *TSLP*, there was no significant interaction between *FLG* and *TSLP* on the odds of early onset AD ($p=0.43$).

Our results indicate that *FLG* null mutations are associated with early onset AD.^{3,4} Unlike prior studies, we distinguished between subjects with 1 *FLG* mutation versus 2 mutations and found a dose-dependent relationship between the number of mutations and the timing of AD onset, with AD beginning earliest among subjects who are homozygous or compound heterozygous for *FLG* mutations. In contrast, *TSLP*rs1898671 was not associated with the

timing of AD onset and did not modify the effect of *FLG* on onset age. One possible explanation for this novel finding is that *TSLP*rs1898671 may be a weak risk factor for AD onset but instead influences disease course only after AD has developed.

A potential limitation of our study is misclassification bias in the self-reported ages of AD onset. However, our secondary analysis examining onset age as a dichotomized outcome did not alter the results. Furthermore, we examined only the most prevalent European *FLG* mutations and one *TSLP* polymorphism, limiting our ability to draw conclusions about other variants and genes. Finally, a limitation in all genetic studies is additional population stratification that is not captured by the analysis.

In conclusion, we found that among subjects with AD, common *FLG* null mutations are associated with earlier AD onset in a dose-dependent manner while *TSLP*rs1898671 appears unrelated to the timing of AD onset. Our findings support the notion that early onset and late onset AD differ in their genetic underpinnings, and future studies are needed to better distinguish these subgroups of AD. Moreover, whether preventative strategies can modulate the genetic risk of early onset AD requires further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding sources: This study was supported by grants from the National Institutes of Health T32-AR007465 (J.W.), Dermatology Foundation (J.W.), and Valeant Pharmaceuticals (D.J.M.).

Abbreviations

AD	atopic dermatitis
CI	confidence interval
IQR	interquartile range
PEER	Pediatric Eczema Elective Registry
SNP	single-nucleotide polymorphism

References

1. Loo EXL, Llanora GV, Lu Q, Aw MM, Lee BW, Shek LP. Supplementation with probiotics in the first 6 months of life did not protect against eczema and allergy in at-risk Asian infants: A 5-year follow-up. *International Archives of Allergy & Immunology*. 2014; 163(1):25–28. [PubMed: 24247661]
2. Brown SJ, McLean WH. One remarkable molecule: Filaggrin. *J Invest Dermatol*. 2012; 132(3 Pt 2): 751–762. [PubMed: 22158554]
3. Rupnik H, Rijavec M, Korosec P. Filaggrin loss-of-function mutations are not associated with atopic dermatitis that develops in late childhood or adulthood. *Br J Dermatol*. 2015; 172(2):455–461. [PubMed: 25314673]

4. Greisenegger E, Novak N, Maintz L, et al. Analysis of four prevalent filaggrin mutations (R501X, 2282del4, R2447X and S3247X) in Austrian and German patients with atopic dermatitis. *J Eur Acad Dermatol Venereol*. 2010; 24(5):607–610. [PubMed: 19874431]
5. Margolis DJ, Kim B, Apter AJ, et al. Thymic stromal lymphopoietin variation, filaggrin loss of function, and the persistence of atopic dermatitis. *JAMA Dermatology*. 2014; 150(3):254–259. [PubMed: 24401911]
6. Gao PS, Rafaels NM, Mu D, et al. Genetic variants in thymic stromal lymphopoietin are associated with atopic dermatitis and eczema herpeticum. *J Allergy Clin Immunol*. 2010; 125(6):1403–1407. e4. [PubMed: 20466416]
7. Kapoor R, Menon C, Hoffstad O, Bilker W, Leclerc P, Margolis DJ. The prevalence of atopic triad in children with physician-confirmed atopic dermatitis. *J Am Acad Dermatol*. 2008; 58(1):68–73. [PubMed: 17692428]
8. Margolis DJ, Apter AJ, Gupta J, et al. The persistence of atopic dermatitis and filaggrin (FLG) mutations in a US longitudinal cohort. *J Allergy Clin Immunol*. 2012; 130(4):912–917. [PubMed: 22951058]
9. Carlsten C, Dimich-Ward H, Ferguson A, et al. Atopic dermatitis in a high-risk cohort: Natural history, associated allergic outcomes, and risk factors. *Ann Allergy Asthma Immunol*. 2013; 110(1): 24–28. [PubMed: 23244654]

TABLE

Subject demographic characteristics, age of AD onset, and *FLG* and *TSLP* status

	Full cohort	Age of AD onset (N=818)				<i>FLG</i> mutation status (N=798)				<i>TSLP</i> rs1898671 variant ^Δ (N=770)			
		Median (IQR), y	2 years old	> 2 years old	* <i>p</i>	Wild-type	1 null mutation	2 null mutations	* <i>p</i>	CC	CT	TT	* <i>p</i>
N (%)	822	0.75 (0.25–2.0)	633 (77.4%)	185 (22.6%)	-	660 (82.7%)	117 (14.7%)	21 (2.6%)	-	472 (61.3%)	257 (33.4%)	41 (5.3%)	-
Sex													
Male	394 (47.9%)	0.75 (0.25–2.0)	312 (79.6%)	80 (20.4%)	0.16	309 (80.5%)	66 (17.2%)	9 (2.3%)	0.14	221 (59.4%)	128 (34.4%)	23 (6.2%)	0.44
Female	428 (52.1%)	0.75 (0.25–2.0)	321 (75.4%)	105 (24.7%)		351 (84.8%)	51 (12.3%)	12 (2.9%)		251 (63.1%)	129 (32.4%)	18 (4.5%)	
Race / ethnicity (self-reported)													
White (non-Hispanic)	361 (43.9%)	0.75 (0.25–2.0)	284 (78.9%)	76 (21.1%)	0.32	241 (68.7%)	92 (26.2%)	18 (5.1%)	<0.001	138 (40.1%)	169 (49.1%)	37 (10.8%)	<0.001
Black/African American (non-Hispanic)	333 (40.5%)	0.75 (0.25–3.0)	248 (74.9%)	83 (25.1%)		308 (95.4%)	14 (4.3%)	1 (0.3%)		254 (82.5%)	51 (16.6%)	3 (1.0%)	
Hispanic	68 (8.3%)	0.75 (0.25–2.0)	54 (79.4%)	14 (20.6%)		62 (93.9%)	3 (4.6%)	1 (1.5%)		42 (64.6%)	23 (35.4%)	0 (0%)	
Asian / Pacific Islander	26 (3.2%)	0.25 (0.25–0.75)	22 (88.0%)	3 (12.0%)		24 (96.0%)	1 (4.0%)	0 (0%)		18 (81.8%)	4 (18.2%)	0 (0%)	
American Indian / Alaskan Native	6 (0.7%)	1.9 (0.25–7.0)	3 (50.0%)	3 (50.0%)		3 (60.0%)	2 (40.0%)	0 (0%)		5 (83.3%)	1 (16.7%)	0 (0%)	
Multiracial	28 (3.4%)	0.75 (0.25–2.0)	22 (78.6%)	6 (21.4%)		22 (78.6%)	5 (17.9%)	1 (3.6%)		15 (60.0%)	9 (36.0%)	1 (4.0%)	

* Fisher's exact test

^Δ C = major allele; T = minor allele

Abbreviations: AD, atopic dermatitis; *FLG*, filaggrin; IQR, interquartile range; *TSLP*, thymic stromal lymphopoietin