DOI: 10.1111/all.13942

LETTER TO THE EDITOR

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI WILEY

Natural moisturizing factor as a clinical marker in atopic dermatitis

To the Editor

Atopic dermatitis (AD) is a heterogeneous disease with various biological origins and clinical appearances. It is likely that different therapies or treatment intensities are not equally effective for all AD endotypes. The strongest genetic risk factor for AD is a null mutation in the filaggrin gene (*FLG*).[1] Patients with eczema who carry a FLG null mutation are also prone to more persistent, severe eczema, and earlier onset of AD compared to patients without a FLG null mutation.

Stratification of patients based on the FLG null endotype could enable more targeted treatment. Methods to determine FLG null mutations based on genotyping are time consuming and require specialized laboratory infrastructure, further complicated by the existence of over 50 different polymorphisms with widely varying prevalences between ethnic groups.[2] In the stratum corneum (*SC*) filaggrin is enzymatically degraded into its constituting amino acids and their derivatives, together with specific salts and sugars collectively named natural moisturizing factor (NMF). Decreased NMF provides an accurate surrogate marker for the presence of FLG null polymorphisms.[3] This can be measured rapidly and noninvasively by Raman spectroscopy in a clinically compatible test.

We have assessed the potential of NMF as a novel clinical marker in AD by examining the association of clinically measured NMF values with severity of AD, early onset of AD, and the co-morbidities of AD: allergic sensitization, food allergy, bronchial hyperreactivity (BHR), asthma, and allergic rhinitis.

Of 207 children with AD (0-18 years of age), NMF values had been measured routinely during a visit to the pediatric atopy center KinderHaven-Sophia Children's Hospital-Erasmus MC University Medical Center Rotterdam in The Netherlands. The retrospective study protocol was approved by the medical ethics committee of Erasmus MC (MEC-2016-244). AD was diagnosed by a dermatologist according to the UK Working Party's Diagnostic Criteria for Atopic Dermatitis.[4] NMF had been measured noninvasively on the palm of the hand by Raman spectroscopy using an in vivo Raman skin analyzer (gen2-SCA, RiverD International BV, Rotterdam). NMF values were classified as *normal NMF* (>1.14 arbitrary units) or *decreased NMF* (<0.995 arbitrary units), using a 0.07 confidence interval around the threshold of 1.07 as established by O'Regan et al.[3] Patients with a NMF value between 0.995-1.14 were excluded. The interval was the estimated 95% confidence interval, calculated as the standard error (SE) of the NMF value, averaged over the entire cohort, and multiplied by 1.96. Disease characteristics and comorbidity status were retrieved from the electronic medical patient files by two independent researchers (see Appendix S1). Severity (*mild to moderate or severe*) of AD was measured by proxy of therapy based on the criteria as described by Wollenberg et al[5] (Appendix S1). Associations between NMF status and the clinical parameters were tested by univariate and multivariate logistic regression models with adjustment for age and gender.

Sixty-seven out of 207 (32.4%) patients had decreased NMF. Figure 1 shows the distribution of disease severity in relation to the groups *normal NMF* and *decreased NMF*. Patients with decreased NMF had increased risk of severe AD, OR 2.12 (95% CI 1.02-4.43), sensitization for food allergens, OR 2.27 (95%CI 1.21-4.23), sensitization for inhalation allergens, OR 2.22 (95%CI 1.13-4.34), and food allergies, OR 2.79 (95% CI 1.33-5.86; Table 1 and Table S1). Having decreased NMF did not show an association with early-onset AD, allergic rhinitis, BHR, asthma and combined asthma, and/or BHR.

In this retrospective study, we examined the associations between NMF values and the clinical parameters of the atopic syndrome. NMF

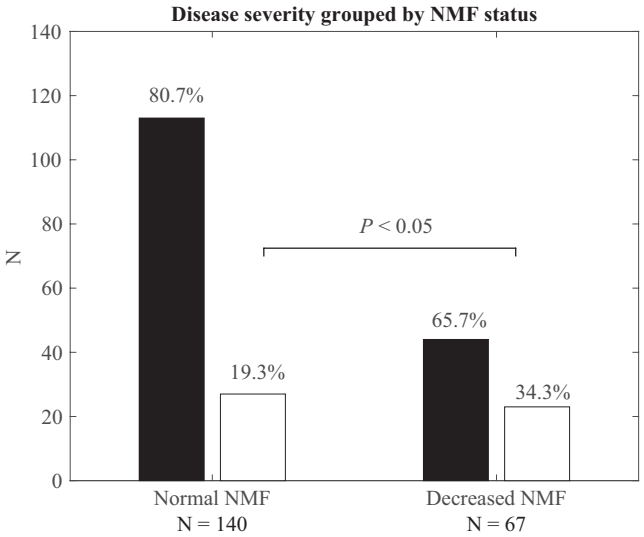

FIGURE 1 Distribution of disease severity and NMF status (n = 207). Black: mild to moderate AD; White: severe AD. The percentage of patients with severe AD is significantly higher in the decreased NMF group then in the normal NMF group ($P < 0.05$ using Pearson's chi-square test)

Anouk E. M. Nouwen and Dilara Karadavut contributed equally to the work.

TABLE 1 Associations of NMF with clinical parameters

Clinical parameters	Decreased NMF[a] (n = 67)	Normal NMF[a] (n = 140)	Decreased NMF[b] Odds ratio (95% CI) Unadjusted	Decreased NMF[b] Odds ratio (95% CI) Adjusted[c]
Onset of AD ≤6 months				
No	13	42	Reference	Reference
Yes	51	93	1.77 (0.87-3.60)	1.81 (0.89-3.70)
Disease severity of AD				
Mild and moderate	44	113	Reference	Reference
Severe	23	27	**2.19 (1.14-4.22)**	**2.12 (1.02-4.43)**
Allergic sensitization, food				
No	31	94	Reference	Reference
Yes	36	46	**2.37 (1.31-4.31)**	**2.27 (1.21-4.23)**
Allergic sensitization, inhalant				
No	27	85	Reference	Reference
Yes	40	55	**2.29 (1.26-4.15)**	**2.22 (1.13-4.34)**
Food allergy				
No	47	122	Reference	Reference
Yes	20	18	**2.88 (1.40-5.93)**	**2.79 (1.33-5.86)**
Asthma				
No	47	116	Reference	Reference
Yes	20	24	**2.06 (1.04-4.07)**	1.93 (0.92-4.09)
BHR				
No	57	127	Reference	Reference
Yes	10	13	1.71 (0.71-4.14)	1.85 (0.74-4.64)
Asthma and/or BHR				
No	42	104	Reference	Reference
Yes	25	36	1.72 (0.92-3.21)	1.69 (0.85-3.06)
Allergic rhinitis				
No	32	83	Reference	Reference
Yes	35	57	1.59 (0.8-2.86)	1.40 (0.71-2.73)

Abbreviation: CI, confidence interval.
[a]Values are based on absolute numbers
[b]Values are unadjusted odds ratios (95% confidence interval) from logistic regression models based on observed data. Bold values indicate statistical significance at $\alpha = 0.05$ level.
[c]Adjusted for age during measurement and gender.
Bold values indicate statistical significance at $\alpha=0.05$ level.

values had been measured rapidly and noninvasively by Raman spectroscopy. The results show a strong association between AD disease severity based on prior therapy, and NMF value. Decreased NMF also shows associations with the co-morbidities allergic sensitization for food and inhalant allergens and food allergy. These findings are in concordance with the reported and widely replicated associations between FLG null mutations and AD severity, as well as associations between FLG null mutations and AD co-morbidities.[2,6,7] A direct association between (clinically measured) NMF and AD disease severity and co-morbidities has, to our knowledge, not been demonstrated before.

This study supports the hypothesis that patients with a decreased NMF value might benefit from different treatment regimens than patients with normal NMF value. Future research should focus on the development and implementation of more personalized therapeutic approaches for the different endotypes within the AD population.

The results of the current study did not show an association with asthma and allergic rhinitis. The relationship between AD, asthma, and FLG mutations is complex. FLG mutations are considered a risk factor for asthma, but only in the presence of AD.[8] Children must be at least 6 years old for a diagnosis of asthma. In this study, a limited amount of patients was aged above 6 years. Future research on the association between NMF and asthma should include a larger population of patients >6 years of age.

The noninvasive and rapid measurement of NMF by Raman spectroscopy makes the method suitable for use in children of all ages. The results of the NMF measurements are directly available

and therefore enable to act upon the result without delay. These characteristics as well as similar associations with AD as the widely replicated associations between FLG null mutations and AD make noninvasive measurement of NMF by Raman spectroscopy a promising approach to improve clinical stratification of endotypes in AD.

CONFLICT OF INTEREST

PJ Caspers and GJ Puppels are employed with RiverD International BV No potential conflicts of interest were disclosed by the other authors.

FUNDING INFORMATION

The gen2-SCA Skin Composition Analyzer used study was funded by a grant from the Stichting Jacoba. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Anouk E. M. Nouwen[1]
Dilara Karadavut[1]
Suzanne G. M. A. Pasmans[1]
Niels J. Elbert[2]
Lawrence D. N. Bos[1]
Tamar E. C. Nijsten[2]
Nicolette J. T. Arends[3]
Mariëlle W. H. Pijnenburg[3]
Senada Koljenović[4]
Gerwin J. Puppels[5,6]
Peter J. Caspers[5,6]

[1]*Department of Dermatology, Erasmus MC-Sophia Children's Hospital-Kinderhaven, Rotterdam, The Netherlands*
[2]*Department of Dermatology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands*
[3]*Department of Pediatrics, Pediatric Respiratory Medicine and Allergology, Erasmus MC-Sophia Children's Hospital-Kinderhaven, Rotterdam, The Netherlands*
[4]*Department of Pathology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands*
[5]*Center for Optical Diagnostics and Therapy, Department of Dermatology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands*
[6]*RiverD International B.V., Rotterdam, The Netherlands*

Correspondence
Suzanne G. M. A. Pasmans, Department of Dermatology, Center of Paediatric Dermatology, Sophia Children's Hospital Sp-1540, Erasmus MC, University Medical Center Rotterdam, Wytemaweg 80, 3015 CN, Rotterdam, The Netherlands.
Email: s.pasmans@erasmusmc.nl

ORCID

Anouk E. M. Nouwen https://orcid.org/0000-0003-3841-7103

REFERENCES

1. Palmer CN, Irvine AD, Terron-Kwiatkowski A, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet*. 2006;38(4):441-446.
2. Irvine AD, McLean WH, Leung DY. Filaggrin mutations associated with skin and allergic diseases. *N Engl J Med*. 2011;365(14):1315-1327.
3. O'Regan GM, Kemperman PM, Sandilands A, et al. Raman profiles of the stratum corneum define 3 filaggrin genotype–determined atopic dermatitis endophenotypes. *J Allergy Clin Immunol*. 2010;126(3):574-580.e1.
4. Williams HC, Jburney PG, Hay RJ, et al. Working party's diagnostic criteria for atopic dermatitis. I. Derivation of a minimum set of discriminators for atopic dermatitis. *Br J Dermatol*. 1994;131(3):383-396.
5. Wollenberg A, Oranje A, Deleuran M, et al. ETFAD/EADV Eczema task force 2015 position paper on diagnosis and treatment of atopic dermatitis in adult and paediatric patients. *J Eur Acad Dermatol Venereol*. 2016;30(5):729-747.
6. Rodriguez E, Baurecht H, Herberich E, et al. Meta-analysis of filaggrin polymorphisms in eczema and asthma: Robust risk factors in atopic disease. *J Allergy Clin Immunol*. 2009;123(6):1361-1370.e7.
7. Marenholz I, Kerscher T, Bauerfeind A, et al. An interaction between filaggrin mutations and early food sensitization improves the prediction of childhood asthma. *J Allergy Clin Immunol*. 2009;123(4):911-916.
8. Weidinger S, O'Sullivan M, Illig T, et al. Filaggrin mutations, atopic eczema, hay fever, and asthma in children. *J Allergy Clin Immunol*. 2008;121(5):1203-1209.e1.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.