

Article type : Letter to the Editor

Editor : Claudia Traidl-Hoffmann

## Natural Moisturizing Factor as a clinical marker in atopic dermatitis

### To the Editor

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 atopic dermatitis (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 non-invasively 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 paediatric 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 non-invasively on the palm of the hand by Raman spectroscopy using an in vivo Raman skin analyser (gen2-SCA, RiverD International B.V., 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, This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/all.13942

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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 supplementary 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)(see supplementary S2). 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) (see Table 1 and supplementary table 1). 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 values had been measured rapidly and non-invasively 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 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 non-invasive 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 non-invasive measurement of NMF by Raman spectroscopy a promising approach to improve clinical stratification of endotypes in AD.

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**Table 1. Associations of NMF with clinical parameters**

| Clinical parameters                                                                                                                                                                                                                                                                           | Decreased NMF <sup>1</sup><br>(n=67) | Normal NMF<br>(n=140) | Decreased NMF <sup>2</sup>        |                                  |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|-----------------------|-----------------------------------|----------------------------------|
|                                                                                                                                                                                                                                                                                               |                                      |                       | Odds ratio (95% CI)<br>Unadjusted | Odds ratio (95% CI)<br>Adjusted* |
| 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                    | <b>2.19 (1.14 - 4.22)</b>         | <b>2.12 (1.02 - 4.43)</b>        |
| Allergic sensitization,<br>Food                                                                                                                                                                                                                                                               |                                      |                       |                                   |                                  |
| No                                                                                                                                                                                                                                                                                            | 31                                   | 94                    | Reference                         | Reference                        |
| Yes                                                                                                                                                                                                                                                                                           | 36                                   | 46                    | <b>2.37 (1.31 - 4.31)</b>         | <b>2.27 (1.21 - 4.23)</b>        |
| Allergic sensitization,<br>Inhalant                                                                                                                                                                                                                                                           |                                      |                       |                                   |                                  |
| No                                                                                                                                                                                                                                                                                            | 27                                   | 85                    | Reference                         | Reference                        |
| Yes                                                                                                                                                                                                                                                                                           | 40                                   | 55                    | <b>2.29 (1.26 - 4.15)</b>         | <b>2.22 (1.13 - 4.34)</b>        |
| Food allergy                                                                                                                                                                                                                                                                                  |                                      |                       |                                   |                                  |
| No                                                                                                                                                                                                                                                                                            | 47                                   | 122                   | Reference                         | Reference                        |
| Yes                                                                                                                                                                                                                                                                                           | 20                                   | 18                    | <b>2.88 (1.40 - 5.93)</b>         | <b>2.79 (1.33 - 5.86)</b>        |
| Asthma                                                                                                                                                                                                                                                                                        |                                      |                       |                                   |                                  |
| No                                                                                                                                                                                                                                                                                            | 47                                   | 116                   | Reference                         | Reference                        |
| Yes                                                                                                                                                                                                                                                                                           | 20                                   | 24                    | <b>2.06 (1.04 - 4.07)</b>         | 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)               |
| <sup>1</sup> Values are based on absolute numbers                                                                                                                                                                                                                                             |                                      |                       |                                   |                                  |
| <sup>2</sup> 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. Abbreviation: CI=confidence interval. *Adjusted for age during measurement and gender. |                                      |                       |                                   |                                  |

## TABLES

Table 1. Associations of NMF with clinical parameters

## FIGURE CAPTIONS

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 than in the normal NMF group ( $P < 0.05$  using Pearson's Chi-square test).

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## Author contributions

SP, PC, DK and LB developed the research question and designed the research project. DK, LB and AN performed the research, analyzed the data and wrote the manuscript. NE was involved in the analysis of the data. SP, PC, TN, NA, MP, SK and GP critically reviewed the manuscript and contributed to interpreting the data. All were involved in the revisions of the manuscript. All authors read and approved the final manuscript before submission.

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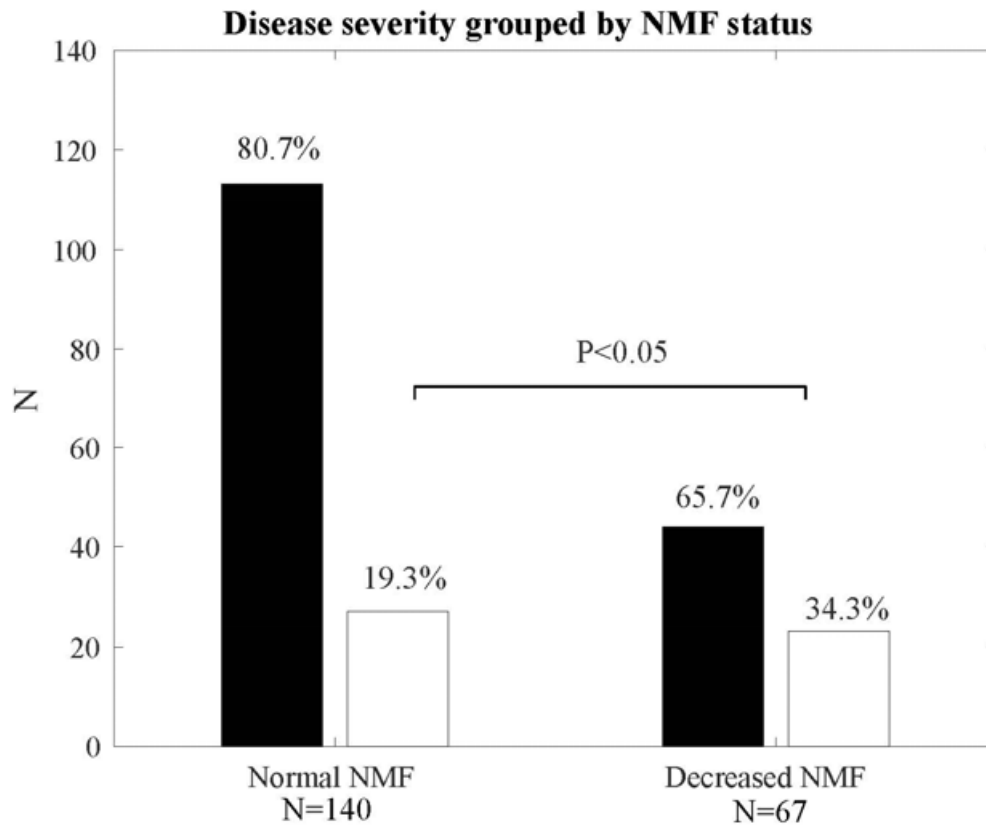
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## Supplementary

### Supplementary S1: definitions of disease characteristics and comorbidity status

Disease characteristics and comorbidity status were defined as follows: Early onset of AD was defined as first expression of AD below the age of 6 months based on medical history (1). Allergic sensitizations for food- and inhalation allergies were defined as the presence of specific levels of IgE antibodies to any food - or inhalant allergen ever measured in serum or by performing a positive skin prick test. Food allergy was defined as a positive history of a food allergic reaction to at least one food allergen within 2 hours and/or a positive double-blind placebo-controlled food challenge for a specific food allergen. No distinction was made between different food allergens. Asthma (patients  $\geq 6$  years) was defined as: doctor-diagnosed asthma at any point in time with or without a positive spirometry test and/or anamnestic data collected by questioning parent(s) or child ever. Bronchial hyperreactivity (BHR) (patients  $< 6$  years) and allergic rhinitis was also defined doctor-diagnosed at any point in time using anamnestic data.

### Supplementary S2: AD disease severity classification (mild to moderate and severe)

Patients with mild to moderate AD had received at least one of the following treatments: reactive therapy with topical glucocorticosteroids classes 1, 2 or 3; proactive therapy with topical glucocorticosteroids class 1, 2 or 3 and/or tacrolimus or psychosomatic counselling by an experienced psychologist.

Patients with severe AD had received at least one of the following treatments: hospitalization or therapy with systemic immunosuppression and/or therapy with topical glucocorticosteroids class 4 (2,3).

**Supplementary Table 1: Characteristics of the study population**

|                                                                                                           | Decreased NMF<br>n=67 | Normal NMF<br>n=140 |
|-----------------------------------------------------------------------------------------------------------|-----------------------|---------------------|
| Gender, female (%)                                                                                        | 30 (44.8%)            | 65 (46.4%)          |
| Age at examination, years (median, IQR) †                                                                 | 6.0(3.0-10.0)         | 5.0(1.25-9.0)       |
| Onset of AD, years (median, IQR) †                                                                        | 0.25(0.0-0.5)         | 0.25(0.13-0.92)     |
| Values are †medians (interquartile range (IQR)) or absolute numbers (percentages) based on observed data. |                       |                     |

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