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Published in:
British Journal of Dermatology

DOI:
10.1111/bjd.15191

Publication date:
2016

Document Version
Peer reviewed version

Received Date : 01-Aug-2016

Revised Date : 12-Oct-2016

Accepted Date : 26-Oct-2016

Article type : Research Letter

Atopic dermatitis patients with filaggrin loss-of-function mutations show good but lower responses to immunosuppressive treatment.

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Funding: none

Disclosure of potential conflict of interest:

W.H.I McLean has filed patents on genetic testing and treatment development related to the filaggrin gene. The rest of the authors, E. Roekevisch., Mariska M.G. Leeflang., M.E. Schram., L.E. Campbell., S. Kezic., J.D. Bos., P.I. Spuls., M.A. Middelkamp Hup have declared that they have no conflict of interest.

Key words

Atopic dermatitis, Atopic eczema, Filaggrin, Skin barrier, Mutation, Azathioprine, Methotrexate, Immunosuppressive treatment, Treatment outcome

Abbreviations used

AD	Atopic Dermatitis
AZA	Azathioprine
EASI	Eczema Area and Severity Index
FLG	Filaggrin gene
FMG	Filaggrin Mutation Group
IGA	Investigator Global Assessment
LRM	Linear Regression Model
MTX	Methotrexate
Non-FMG	Non Filaggrin Mutation Group
NMF	Natural Moisturizing Factors (NMF)
OSI	Online Supporting Information
PGA	Patient Global Assessment
POEM	Patient Oriented Eczema Measurement
SCORAD	SCOring Atopic Dermatitis
SCORAD50	Improvement of SCORAD with at least 50%
SD	Standard Deviation
VAS	Visual Analogue Scale

Research Letter;

Filaggrin (*FLG)* mutations are a strong risk factor to develop atopic dermatitis (AD). However, the relationship between *FLG* mutations and treatment outcome in AD has not been thoroughly studied. To investigate whether *FLG* mutations influence immunosuppressive treatment outcome in AD, we studied the effect of *FLG* mutations in patients with severe AD participating in a single blinded randomized controlled trial (RCT) with methotrexate (MTX) or azathioprine (AZA) during a 24 weeks treatment regimen.[(1)] Two years after randomization buccal mucosa swabs were collected from 36 of the 42 RCT patients (86%) to determine the *FLG* genotype status (R501X, 2282del4, R2447X, S3247X and 3321delA mutations).

In the original RCT both MTX and AZA induced significant reductions in SCORAD at 12 and 24 weeks, without a significant difference between treatments.[(1)] We therefore pooled all patients together for this sub-study and divided them into a *FLG* mutation group (FMG) and non-FMG. The primary outcome parameter was the SCORAD, which was obtained at baseline, week 2,4,8,12 and 24 and analysed as repeated measurements (lme4 package in R (version 3.2.5). All SCORAD values were obtained from patients receiving systemic

immunosuppressive treatment with or without topical immunosuppressive treatment, except for three values in three patients. Two patients stopped systemic treatment because of adverse events (FMG, week 2; non-FMG, week 4) and their last scores, obtained within 2 weeks of stopping systemic therapy, were under topical immunosuppressive treatment alone. One patient (FMG) stopped systemic treatment at week 12, and values at week 24 were under topical immunosuppressive treatment alone. As the mean age was significantly higher in the FMG vs. the non-FMG (P<0.01, online supporting information (OSI), Table E1), all analyses were corrected for age as a possible confounder and age was included in the models as a potential confounder variable. As treatment regimen (AZA or MTX) did not influence treatment success in FMG vs. non-FMG, we included in the mixed models as covariates visit, mutation, age and an interaction term for visit *mutation.

The main limitation of this sub-study was the small sample size and the inability to randomize on *FLG* mutation status. For details see Methods section at the OSI (www.brjdermatol.org), and our previous publication.[1]

FLG mutations were found in 13 patients (36%, OSI Tabel E2). Every single patient, both in FMG and non-FMG, showed improvement of SCORAD at week 24 compared to baseline (OSI Figure E1). The mean SCORAD in the FMG decreased with 20.7 points (95%CI 14.7-26.6, P < 0.01) and 31.3 points in the non-FMG (95%CI 26.3-36.2, P < 0.01). However, in the course of 24 weeks, the FMG showed less improvement in SCORAD compared to the non-FMG (P=0.02) and the improvement in SCORAD of patients in the FMG stagnated during the course of the treatment (Figure 1). When the three SCORAD values obtained under topical treatment alone were excluded from data analysis, the FMG showed a trend towards less improvement in the course of 24 weeks compared to non-FMG , as a significant difference was not reached (P=0.075). Median dosages of topical immunosuppressive treatment (FMG 132 gram, non-FMG 120 gram, P=0.51) and median dosages of MTX (FMG 83.8 gram, non-FMG 90 gram, P=0.26) and AZA (FMG 837.5 gram, non-FMG 825 gram P=0.75) were comparable between groups in the course of 24 weeks (Mann Whitney U test).

It is interesting to speculate on mechanisms underlying lower response to immunosuppressive treatment in AD patients with a *FLG* mutation. Th2 cytokines can down-regulate filaggrin protein expression[3] and skin barrier impairment in AD patients with severe skin inflammation seems similar in patients with and without *FLG* mutations and correlated with the SCORAD.[4] As only patients with severe AD were included in our study[1], we expect that at initiation of the study-levels of skin barrier impairment and inflammation were comparable in the FMG and non-FMG, which is supported by similar mean SCORAD in both groups. When decreasing inflammation by immunosuppressive treatment, downregulation of *FLG* expression by the inflammatory infiltrate will be abrogated, therefore allowing skin barrier restoration and likely establishment of normal filaggrin protein levels in the non-FMG. In contrast, FMG patients will also experience improvement of their AD due to the immunosuppression, but their barrier function impairment and reduced filaggrin protein levels will still be present in the end because of their genetically caused filaggrin protein deficiency. This may explain the lower therapeutic responses in the FMG in the course over 24 weeks, and why the initial improvement stagnates during the treatment period. In conclusion, both groups showed a significant

improvement in SCORAD compared to baseline, however, *FLG* mutations may have a negative impact on immunosuppressive treatment success in severe AD as the improvement in the FMG stagnated during the course of the treatment. Additional research investigating the relationship between *FLG* mutation status and treatment success are necessary to confirm these findings.

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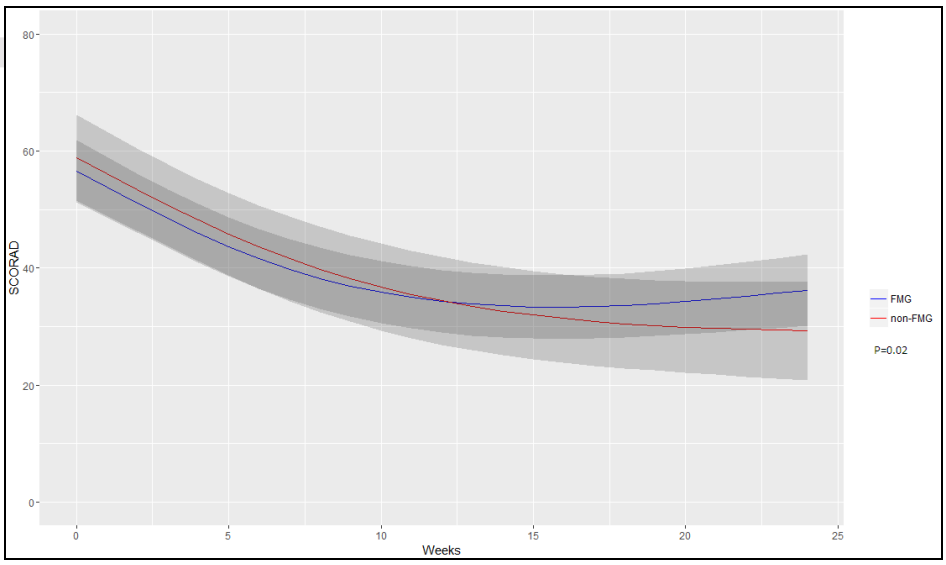
Figure1. Mean (SD) reduction in SCORAD from baseline till 24 weeks, adjusted for age



Differences in outcomes between the filaggrin mutation group (FMG, n=13) and non-FMG (n=23) when on combined systemic and topical immunosuppressive treatment, or topical immunosuppressive treatment alone. Data includes all data points. Ggplot package in R was used to draw the Figures. The gray areas r*epresents* the *95% confidence interval for the mean reduction in SCORAD in the FMG and the non-FMG*. *Dark gray represents an overlap between groups. These results show that both groups showed an improvement in SCORAD compared to baseline, however FMG patients showed less improvement in SCORAD in the course of 24 weeks compared to non-FMG (P=0.02), and improvement stagnates during the course of the treatment.*

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