LETTER TO THE EDITOR

Functional iron-deficiency in women with allergic rhinitis is associated with symptoms after nasal provocation and lack of iron-sequestering microbes

To the Editor,

It is still unclear which underlying factors in atopic subjects contribute to their heightened immune response and lead to increased IgE production. Iron deficiency and anemia are clearly associated with the onset of allergic diseases with a poor iron status at birth increasing the risk of developing an atopic disease, whereas an improved iron status seems to prevent the onset of allergy.1 Importantly, in our preclinical studies, we were able to confirm that iron can be selectively delivered to immune cells with beta-lactoglobulin carrying iron complexes as ligands, resulting in immune resilience and allergy prevention.2 As a lack of iron is also a danger signal for immune cells leading to Th2 activation,2 we hypothesized that atopy may be linked with functional iron deficiency, in which the iron stores are not accessible, thus facilitating allergy development and amplifying the clinical symptom burden in allergy sufferers. As iron parameters and reference ranges differ between gender and with age, we eliminated confounding factors (Supplementary Materials, exclusion criteria) and this study focused only on premenopausal women with or without respiratory allergies.

Here, we show for the first time that women with allergic rhinitis and without anemia—though having similar dietary habits as nonallergic ones (Table S1, Figure S1)—have decreased levels of metabolically active iron in the circulation with lower serum iron, transferrin saturation, and elevated ceruloplasmin levels compared with nonallergic women, while significantly more hepcidin is produced (Figure 1A and more parameters on Table S2 and Figure S2; sensitization profile is listed in Table S3 and Table S4). Despite increased iron mobilization efforts by the copper-containing ferroxidase ceruloplasmin, which also acts as an acute-phase reactant, allergic women had lower serum iron. However, greater amount of urinary hepcidin, which blocks cellular iron release and mobilization, was present in our allergic rhinitis cohort and may point toward greater immune activity inherent in allergies compared with nonallergic individuals. The increased hepcidin excretion also suggests that in women with allergic rhinitis, dietary intestinal iron absorption and/or cellular iron release are inhibited to a greater degree than in nonallergic individuals.

Furthermore, for the first time, ceruloplasmin levels in serum (r = 0.53, p=0.0007) and nasal secretions (r = 0.59, p=0.000089) were linked with symptoms after nasal provocation (Figure 1B, C and Table S5, S6, S7). Gut iron appeared to be beneficial and was associated with reduced symptom burden (r=−0.78, p= 0.000042) (Figure 1B,D) that even showed linearity in regression analysis in allergic rhinitis subjects that underwent graded nasal provocation. Gut iron also seemed to contribute to an improved iron status in women (Figure 1E).

Indeed, elevated levels of ceruloplasmin have already been described in atopic dermatitis and asthma patients and were suggested as a putative marker for plasma protein leakage in subjects suffering from asthma and chronic obstructive pulmonary disease.3 Our data also link ceruloplasmin with clinical reactivity and thus with the allergy effector cells, the mast cells.

The presence of iron in stool extracts reflects the occurrence of commensal microbial communities relying on and solubilizing iron. Bacteria associated with gut iron (green), such as members of the genus Bacteroides—that also support immune iron repletion and resilience4—and Ruminococcus were associated with reduced symptom burden (amplicon sequence variants, ASV_qzd_als, r=−0.61, p=0.00007; ASV_jhl_q2w, r=−0.51, p=0.0017) were negatively associated with serum ceruloplasmin (Figure 2). Importantly, the same ASV of the genus Ruminococcus was also relatively enriched in our nonallergic cohort (Figure 2E), emphasizing its role to a healthy gut.5 Similar to a study with atopic dermatitis subjects,6 only one ASV of the genus Faecalibacterium (red, ASV_k3p_vr6, r=0.34 m p=0.04) showed an association with symptom severity and ceruloplasmin (r=0.3) and was negatively associated with gut iron (r=−0.45).

In summary, in this association study, we linked the hyper-active state in allergic rhinitis subjects with decreased iron mobilization. Ceruloplasmin was the sole protein marker that was linked with clinical reactivity probably due to its increased release by mast cells, whereas gut iron levels seemed beneficial to reduce symptom burden. The microbial taxa Bacteroides, which contribute in immune iron repletion and resilience4 and Ruminococcus, which was also relatively enriched in nonallergics, were linked to gut iron and reduced clinical reactivity.
**Figure 1** Functional iron deficiency and elevated ceruloplasmin as a marker for clinical reactivity in allergic women. A. Serum iron, transferrin saturation, serum ceruloplasmin, and urinary hepcidin levels of sera from allergic (n=27) and nonallergic (n=24) premenopausal women. B. Spearman correlation matrix of total nasal symptom score (TNSS), visual analogue scale (VAS), weight of nasal secretions—as a measure of clinical reactivity—with blood, nasal fluid parameters as well as gut iron, gut copper, and gut cobalt of stool extracts of women that underwent nasal provocation with grass or birch pollen extract (n=38). C. Linear regression analysis of TNSS with ceruloplasmin from serum and nasal secretions. D. Linear regression analysis of TNSS with gut iron and gut cobalt. D. Linear regression of gut iron with hemopexin, ferritin, and the reciprocal value of ceruloplasmin. Positive and significant dependencies in linear regression analysis are depicted in red; significant negative ones in green dots. Prior analysis normality and lognormality tests were employed. Student’s t test was used for parametric; Mann-Whitney U test was used for nonparametric data. *p < 0.05, **p < 0.01
Members of the genera *Ruminococcus* and *Bacteroides* show a negative correlation with clinical reactivity with *Ruminococcus* ASV being decreased in allergics. A, Side-by-side comparison of Spearman correlations of gut microbiome ASVs to total nasal symptom score (TNSS), serum ceruloplasmin, and gut iron (dark green and dark red, significant after FDR adjustments $q<0.05$; light green and red are not significant trends). B, Linear regressions to TNSS ($n=38$), C, to serum ceruloplasmin ($n=38$), and D, to gut iron ($n=20$) of the relative abundance of ASV of the genera *Bacteroides*, *Ruminococcus*, and *Alistipes* negatively associated with TNSS as well as relative abundance of ASV of *Faecalibacterium* positively associated with TNSS (green when negatively associated and significant, gray when not significant, red when positively associated and significant). E, Comparison of relative abundance of ASVs from the genera *Ruminococcus*, *Bacteroides*, *Alistipes*, and *Faecalibacterium* in allergics ($n=26$) and nonallergics ($n=22$) as well as the summarized relative abundance of ASVs positively associated with TNSS. Data were nonparametric and Mann-Whitney $U$ test were used for statistical analysis. $^{**}p<0.01$
Our findings underline the existence of functional iron deficiency in subjects with allergy. The knowledge may be exploited to combat the allergy epidemic by developing a new generation of therapeutic and prophylactic approaches. To replenish immune iron may thus become a way to achieve immune resilience.

**KEYWORDS**
allergic rhinitis, ceruloplasmin, clinical reactivity, functional iron deficiency, microbes

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**CONFLICT OF INTEREST**
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