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LETTERS 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 1 A 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 allergics 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 sputum 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 resilience [4] —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 relation 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 resilience[4] and *Ruminococcus*, which was also relatively enriched in nonallergics, were linked to gut iron and reduced clinical reactivity.

Clinical trials registration # NCT03815981 and NCT03816800

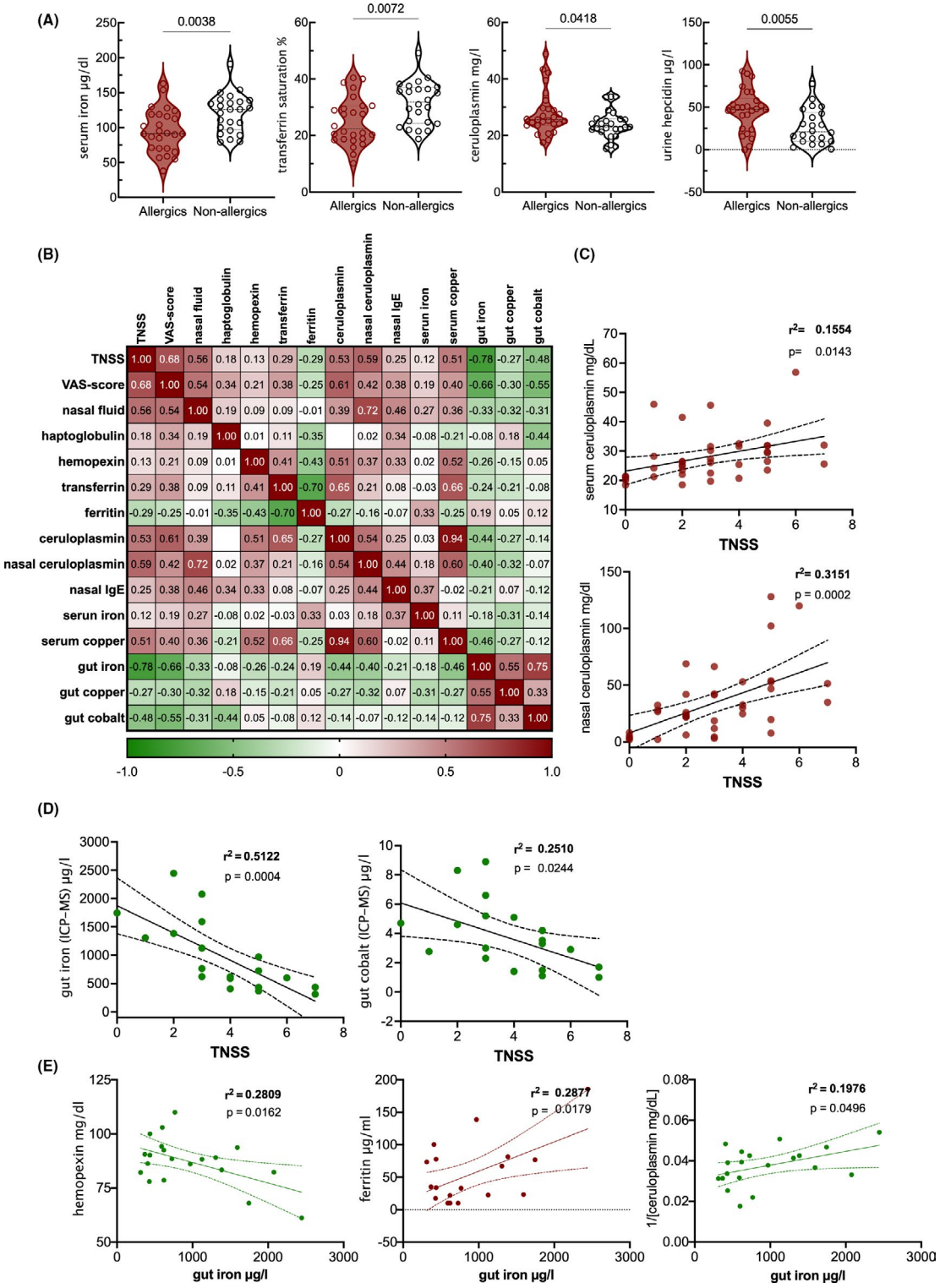

FIGURE 1 Functional iron deficiency and elevated ceruloplasmin as a marker for clinical reactivity in allergic women. A, Serum iron, transferrin saturation, serum ceruloplasmin, and urinary hepcidin levels of sera from allergic (n=27) and nonallergic (n=24) premenopausal women. B, Spearman correlation 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. $^{*}p < 0.05$, $^{**}p < 0.01$

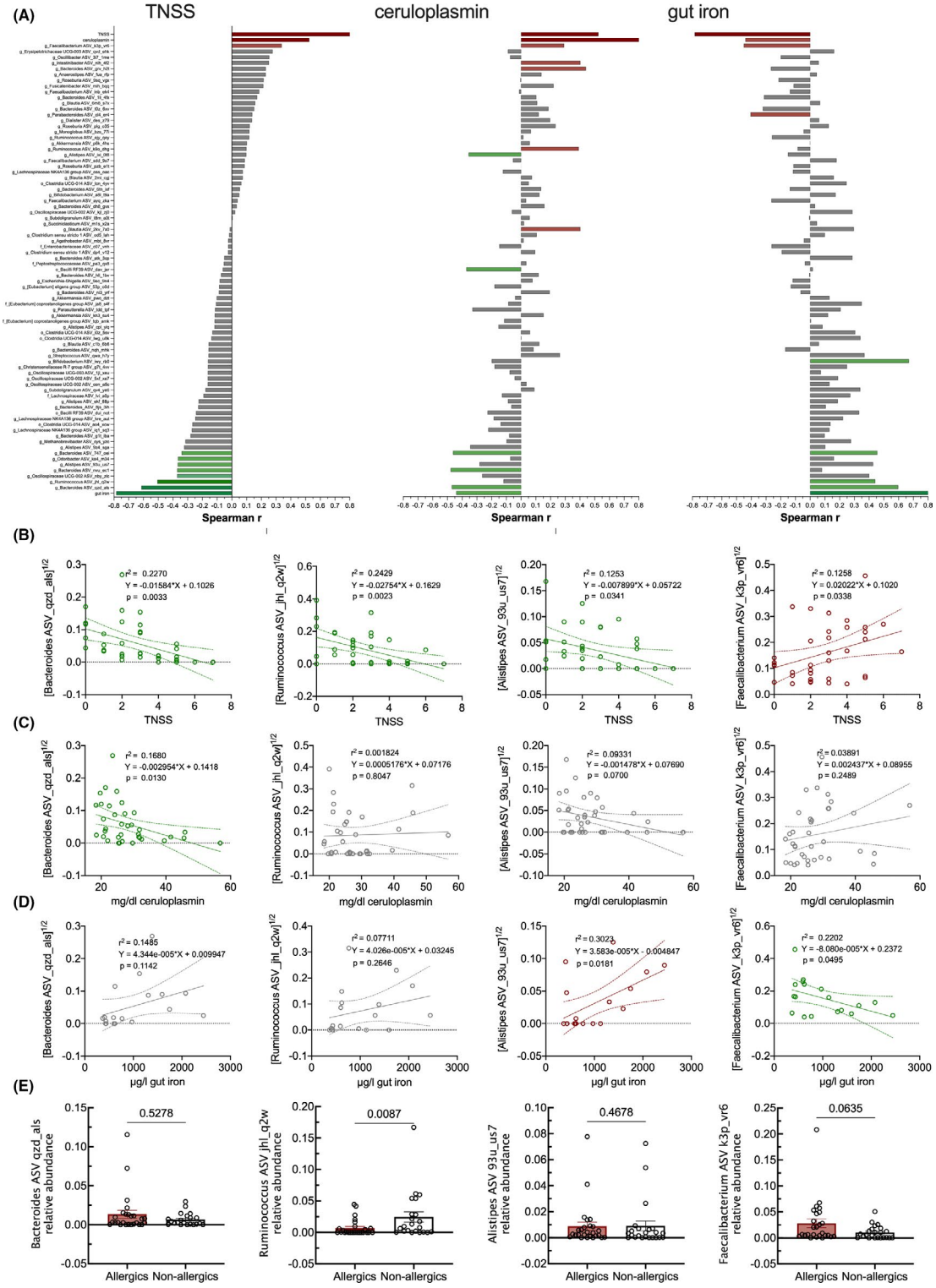

FIGURE 2 Members of the genera *Ruminococcus* and *Bacteroides* show a negative correlation with clinical reactivity with *Ruminococcus* ASV being decreased in allergics A, Side-by-side comparison of Spearman correlations of gut microbiome ASVs to total nasal symptom score (TNSS), serum ceruloplasmin, 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

EJJ declares shareholdership in Biomedical Int. R+D GmbH, Vienna, Austria. FRW and EJJ are inventors of EP2894478, owned by Biomedical International R+D GmbH, Vienna, Austria. The other authors declare no relevant conflict of interest in relation to this publication.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Expression of histamine receptors H2R and H4R are predominantly regulated via the IL-4/IL-13 receptor type II on human M2 macrophages

To the Editor,

The pathophysiology of allergic skin diseases such as atopic dermatitis (AD) is characterized by complex interactions between susceptibility genes of the disease, a defect skin barrier and aberrant immune responses. Among other immune cells, monocytes differentiating to macrophages are attracted into inflamed skin and are exposed to Th2 cytokines and to histamine detectable in high concentrations in AD patients' skin contributing to immunomodulation and pruritus.[1,2] Increasing expression levels of functional histamine receptors, in particular of the histamine H4 receptor (H4R), on immune cells within the inflammatory infiltrate of eczematous skin lesions may exacerbate inflammation by mediating cytokine and chemokine release in contact to local histamine.[3] Knowledge of these mechanisms may result in therapeutic strategies to alleviate inflammation.

Two specific antagonists of the H4R were studied in phase II studies with AD patients.[2,S1] A number of other substances, particularly dupilumab[S2], currently approved for treatment of type II inflammatory diseases, have shown meaningful reduction of pruritic symptoms and clinical AD severity scores.

Since little is known about the cross talk of type II cytokines and histamine receptors on macrophages known to be increased in the dermis of inflamed skin in AD [S3], we investigated in this study if the Th2 cytokines IL-4 and IL-13 influence the histamine receptor mRNA expression levels in human monocyte-derived M2 macrophages differentiated in the presence of M-CSF for 10 days (see additional information in the Material and Methods section and Figure S1 in this article's Online Repository).

As shown previously, the H1R-, H2R- and H4R- but not H3R mRNA expressions are detectable in fully differentiated human M2 macrophages.[4]

The stimulation of M2 macrophages with IL-4 led to an up-regulation of H2R and H4R, whereas IL-13 selectively up-regulated the H4R at mRNA level in M2 macrophages (Figure 1B,C,E,F). The H1R mRNA expression was neither up-regulated by IL-4 nor by IL-13 (Figure 1A,D).

Macrophage M2 marker CD206, also known as mannose receptor C-type I, expressing cells were detected in the dermis of AD patients pointing to a possible role for macrophages in disease pathology[S3]. Interestingly, M2 macrophages generated from monocytes from patients with moderate-to-severe AD showed higher constitutive expression levels of the H4R mRNA when compared to healthy controls, which was not seen for the H2R (Figure 1G,H). Since it is suggested that serum IgE levels correlate with disease severity of AD[S4], we also measured total IgE levels in the serum of respective patients by ImmunoCap, Thermo Fisher Scientific. AD patients with elevated H4R mRNA expression levels showed also high total IgE levels in their serum as shown in Table S1 in this article´s Online Repository. These data obtained in human macrophages strongly fit observations in human eosinophils in a recently published work.[5] In human eosinophils, H4R mRNA expression was also up-regulated by IL-4 and IL-13 whereas up-regulation of H2R mRNA expression was, just as in macrophages, mediated via IL-4 only.[5] Similar to M2 macrophages, eosinophils from AD patients constitutively express high levels of H4R when compared to cells from healthy controls.[5]

The up-regulation of H4R mRNA expression also in response to IL-13 as well as the high levels of constitutive mRNA expression of the