Clinical Letter

Analysis of genetic impact on smell impairment in patients with hereditary angioedema type 1 and 2

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Dear Editors,

Hereditary angioedema (HAE) type 1/2 is a rare genetic disorder caused by *SERPING1* mutations on chromosome 11. It is characterized by recurrent attacks of severe swelling. Hereditary angioedema is underdiagnosed, and patients frequently have a protracted clinical course from the first symptoms to correct diagnosis and treatment. This is challenging for practitioners and can lead to significant health problems and impaired quality of life in patients. Various prophylactic and on demand treatment options exist for managing HAE [1, 2].

The burden of HAE affects patients in many aspects of their daily lives. This includes smell impairment, as recently described by Perricone et al., albeit without age-standardized smell-scores [3]. It is known that chromosome 11 also contains the highest number of olfactory receptor genes (ORGs) and the largest olfactory receptor clusters of the genome [4, 5]. This study aimed to compare the sense of smell of HAE patients and healthy controls using age-standardized smell-scores, and to examine whether ORG gene expression of normosmic and hyposmic HAE patients differs.

We tested HAE patients and sex- and age-matched controls for their olfactory ability using Sniffin' Sticks threshold, discrimination, identification (TDI)-test and age-standardized smell-scores. Participants were divided into three groups, and total scores for normosmia were adapted accordingly, i.e. group 1: 18–35 years, normosmia ≥ 31; group 2: 36–55 years, normosmia > 29; group 3: > 55 years, normosmia > 28 [6]. Participants were aged ≥ 18 years and provided written informed consent. All HAE patients agreed in a separate declaration of consent to genetic analyses (Ethics committee approval EA1/197/14 Charité – Universitätsmedizin Berlin). Exclusion criteria included pregnancy, rhinosinusitis, allergic rhinitis with acute complaints, infectious diseases, history of nose surgery, head injuries, and the use of antihistamines or nasal sprays (xylometazoline or corticoid) up to seven days before the examination. The study protocol included a general patient history and rhinosinusitis questionnaire. Hereditary angioedema patients also completed a short questionnaire on their disease history and therapy. Ear, nose, and throat (ENT)-examinations were performed by nasal endoscopy. Blood samples from HAE patients and controls were analyzed for HAE-specific parameters (C1-INH concentration and activity and C4-complement). In all HAE patients, the exonic regions and exon/intron boundaries of the *SERPING1* gene were examined for mutations by polymerase chain reaction followed by Sanger sequencing. When no mutation was detected, a Multiplex Ligation-dependent Probe Amplification (SALSA MLPA® probemix P243-A3 *SERPING1*) was performed to test for deletions or duplications. Array-based gene expression analysis of all functioning ORGs on chromosome 11 was performed using the Illumina HT12v4 Expression Bead Chip. Categorical data of patients and controls were compared, and statistical analysis was performed using the R-system. Overall, *P*-values ≤ 0.05 were determined to be significant.

In total, 30 HAE patients and 30 controls were included in this study. The clinical data are shown in Table S1 (online Supporting Information). Hereditary angioedema patients exhibited hyposmia significantly more often than healthy controls, in 33 % vs. 6.7 % of cases, respectively ($P = 0.006$) (Figure 1). We observed no significant differences in smell (TDI)-scores. All participants showed no significant differences and no nasal polyps in nasal examinations. None of the HAE-defining parameters correlated with the olfactory-test results. Nevertheless, patients and controls with normosmia had significantly higher levels of C1-INH activity ($P < 0.01$), concentration ($P = 0.026$) and C4 ($P < 0.01$) than those with hyposmia. In one of 30 tested HAE patients with clear evidence of HAE type 1 from laboratory chemistry as well as patient history and pedigree analysis we did not detect mutations of the *SERPING1* gene. The array-based gene expression analysis for ORGs on chromosome 11 revealed expression (detection *P*-value < 0.05) of six out of 165 ORGs in either HAE patients with hyposmia (*OR5D18*), HAE patients with normosmia (*OR2AG1*, *OR4C45*) or across both groups (*OR51T1, OR56B1, ORAOV1*). None of the ORGs expressed in the hyposmia and normosmia group showed significant differences in expression levels that would explain the hyposmia (Figure 2).

The differences between our results and those of Perricone et al. [3] may be explained by low case numbers. Also, inclusion criteria differed considerably. Perricone et al. included patients who had no HAE symptoms in the previous three months and were treated with danazol alone. Since danazol is not a standard HAE treatment in Germany and can only be used off-label, we included only six patients using danazol. All other HAE patients received on demand treatment

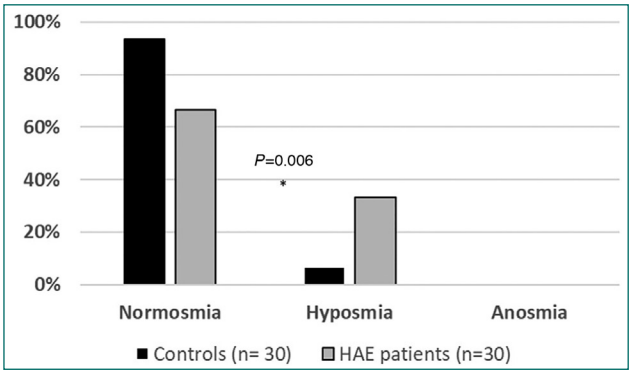

Figure 1 Proportion of HAE patients (n = 30) and healthy controls (n = 30) with normosmia, hyposmia or anosmia measured by the Sniffin' Sticks Test. HAE, hereditary angioedema.

with icatibant or C1-INH or prophylactic treatment with the latter. In this study drug intake did not affect olfactory ability, three of six patients treated with danazol had normosmia and three had hyposmia. It is known, however, that many drugs can alter olfaction, even years after treatment is discontinued [7]. The effects of danazol treatment as well as other HAE therapies on hyposmia should be examined in larger cohorts.

Since a significant physiological reduction in olfaction occurs from the age of 50, smell-test values should be adjusted for different age-groups [6]. Interestingly, higher numbers of hyposmia in HAE patients were found in both studies, which suggests that hyposmia is a common symptom in HAE patients and can contribute to QoL impairment. Dermatologists should consider a poor correlation between subjective self-reported smell-ratings and

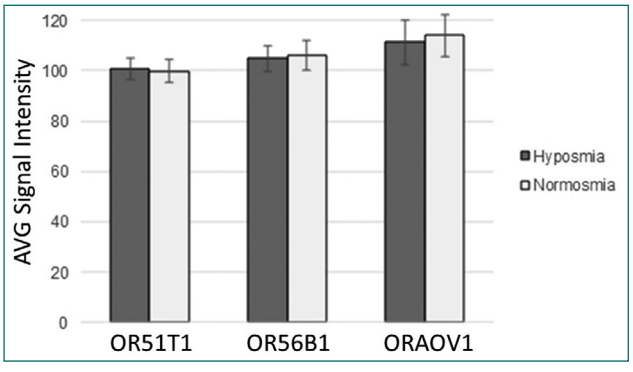

Figure 2 Result of ORG gene expression analysis in HAE patients. Bars indicate the average expression signal intensity for patients with hyposmia (n = 10; dark grey) and normosmia (n = 20; light grey). Error bars indicate the array standard deviation. No significant differences in expression levels of ORGs were observed between the groups.

standardized smell-tests [8] and monitor HAE patients together with ENT-specialists. We implemented criteria to exclude hyposmia caused by comorbidities other than HAE. ENT-specialists performed nasal endoscopies, and all patients completed questionnaires to exclude rhinosinusitis, which is a major cause of olfactory loss. Although allergies to aeroallergens were no exclusion criteria, we did exclude participants showing acute symptoms of allergic rhinitis or endonasal swelling.

Hereditary angioedema patients exhibit elevated bradykinin levels due to C1-INH deficiency. Tsai et al. investigated the influence of bradykinin on nasal fibroblasts in patients with chronic rhinosinusitis [9]. Interestingly, bradykinin was found to increase the proliferation of fibroblasts, the release of proinflammatory cytokines, the expression of adhesion molecules and cyclooxygenases 1 and 2 and subsequent monocyte adhesion [9]. Furthermore, changes in the periceptor-space, which surrounds olfactory receptor neurons, can affect olfactory perception [10]. It is, therefore, conceivable that similar pathomechanisms occur in the nasal mucosa of HAE patients. Recurrent nasal swelling could damage the mucosa. In our study, no differences were found in the smell (TDI)-subtests between patients and controls, which argues against peripheral damage alone.

In this study no alterations of ORG expression levels on chromosome 11 were detected that could explain reduced olfaction in HAE patients. For future studies, the analysis and further genetic examination of small biopsies from the nasal mucosa/olfactory epithelium of HAE patients could help to elucidate the smell impairment in these patients and reinforce the results of smell-tests.

A variety of internal diseases can lead to olfactory disorders although the pathomechanisms are not yet fully understood in some cases. Possibly, higher incidences of comorbidities affecting olfactory function in the Perricone et al. patient population led to higher rates of hyposmia and anosmia. Of note, our study population was small due to HAE being a rare condition. Only two of the patients had HAE type 2, and 28 had HAE type 1. Future studies should include a larger number of patients, and involve both HAE patients with normal C1-INH and patients with recurrent angioedema due to acquired C1-INH deficiency, to explore the possible role of C1-INH in smell impairment. Another limitation of this study was the heterogeneity in therapies used, thus making it challenging to investigate influences of therapeutics on olfactory function.

Taken together, one in three patients with HAE has hyposmia. The olfactory impairment in HAE patients appears to be independent of ORG expression levels. Further studies should investigate non-genetic underlying mechanisms of impaired smell in HAE patients and the impact of different therapeutic approaches.

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Conflict of interest

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