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Clinical Predictors of Olfactory Function: Insights from Inflammation and Imaging

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1.1 List of content

1.2 List of Abbreviations

ORN	olfactory receptor neurons
CRS	chronic rhinosinusitis
VAS	visual analogue scales
MRI	magnetic resonance imaging
OB	olfactory bulb
QOL	quality of life
PIOD	post-infectious olfactory dysfunction
PTOD	posttraumatic olfactory dysfunction
OC	Olfactory cleft
OERPs	olfactory event-related potentials
fMRI	functional magnetic resonance imaging
PET	positron emission tomography
ICA	isolated congenital anosmia
TDI	threshold-discrimination-identification
ROC	Receiver operating characteristic
LLCP	lateral lamella of cribriform plate

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2 Introduction of the topic

2.1 The olfactory system

2.1.1 Olfaction

Olfaction is one of the basic human senses, yet compared to other senses such as vision or hearing, its importance in daily life and clinical diagnosis is often overlooked by both the general public and physicians. Olfaction is crucial for maintaining a safe and healthy lifestyle and has a wide range of functions, including the avoidance of environmental hazards, finding and identifying food (Croy et al., 2014), spatial orientation (Dahmani et al., 2018), flavor perception, social interactions (e.g., recognition of emotions, romantic relationships)(Hofer et al., 2018). and cognitive functions (e.g., modulation of memories) (Doty, 2017).

2.1.2 Anatomy and physiology

The olfactory system can be divided into three main components: the olfactory mucosa, the olfactory bulb (OB), and the olfactory cortex. The olfactory mucosa is a specialized tissue located in the upper part of the nasal cavity, and it serves as the initial site for odor detection. The olfactory mucosa is primarily composed of several cell types, including basal cells, supporting cells, and olfactory receptor neurons (ORN). Basal cells as progenitor cells, including globose basal cells and horizontal basal cells, are capable of generating olfactory sensory neurons and supporting cells. In the mature nervous system, precursor cells gradually replace neurons. Supporting cells are dispersed around ORN and provide structural and metabolic support to the ORN. ORN are bipolar neurons, equipped with a dendrite extending towards the surface of the mucosa and an axon projecting towards the OB. ORN dendrites carry specialized immotile cilia, which are covered in mucus and contain odor receptors. When odorants bind to these receptors, they initiate a signalling cascade that generates an electrical signal, which is then transmitted to the OB. ORNs are the only neurons in direct contact the environment, rendering them subject to various insults, including inflammatory processes. As a result, conditions like chronic rhinosinusitis and allergic rhinitis can lead to impaired olfactory function (X. Han et al., 2020).

The OB, located above the cribriform plate at the anterior skull base, serves as the first relay station in the olfactory pathway, responsible for receiving and integrating sensory signals originating from ORN. The OB is a highly organized structure, composed of several distinct layers and function to facilitate odor processing. These structures (from the outer to the inner part of the OB) include the olfactory nerve layer, glomerular layer, external plexiform layer, mitral cell layer, internal plexiform layer, granule cell layer and periglomerular cell layer. The OB plays a crucial role in olfactory perception, contributing to the identification, discrimination, and encoding of odors (Witt, 2020). Magnetic resonance imaging (MRI) enables us to visualize the OB and effectively identify lesions and structural damage within this region (Chung et al., 2018).

The OB shows high plasticity. a decrease of OB volume has also been reported in patients with olfactory dysfunction due to various causes (Rombaux et al., 2010; Hummel et al., 2015; Mazal et al., 2016; Yao et al., 2018). Following a period of 4 months of olfactory training, showed that 97 healthy people exhibited an average increase of OB volume (Negoias et al., 2017). OB plasticity may depend on numerous factors which are currently discussed, for example, (1) continuous neuronal supply from the subventricular zone (SVZ), where young neurons migrate within the rostral migratory stream and replace interneurons (periglomerular cells and granular cells) in the OB (Curtis et al., 2007); (2) continuous synaptogenesis with dendrites of mitral/tufted cells occurring from incoming axonal projections of olfactory receptor neurons at the glomerular level; and (3) in a recent animal study, a new form of structural remodeling of adult-born OB neurons suggest direct neurogenesis within the OB itself (Breton-Provencher et al., 2016).

The olfactory cortex refers to a network of brain regions that are responsible for higher-level processing and interpretation of olfactory information. Anatomically, the

olfactory cortex comprises several distinct regions, including the piriform cortex, the entorhinal cortex, and the orbitofrontal cortex. The piriform cortex receives direct input from mitral and tufted cells of the OB via the lateral olfactory tract. Within this region, the initial stages of odor processing occur, involving integration and extraction of specific features from the sensory input. Preliminary investigations of the entorhinal cortex suggest that it may not only serve as an olfactory input to hippocampal structures but also as a crucial top-down regulator of olfactory cortical and bulb functions. The orbitofrontal cortex plays a significant role in odor stimulation and decision-making process related to odor cues. It is also an essential multisensory site for integration of olfaction information with

other sensory modalities, such as taste, oral sensation, and vision, all of which contribute to enhancing perception (Witt, 2020).

Figure 2-1 Anatomy of the olfactory pathway to the brain. Figure adapted based on work by Patrick J. Lynch available at https://commons.wikimedia.org/wiki/File:Head_olfactory_nerve.jpg.

2.1.3 Olfactory dysfunction

Olfactory dysfunction is a prevalent issue, affecting millions of individuals worldwide. It can manifest as quantitative or qualitative impairments, including anosmia (complete loss of smell), hyposmia (reduced sense of smell), or even parosmia (distorted sense of smell). with one study showing that Among the cohort of individuals aged 45 years and older, a notable proportion of 5% exhibited anosmia, while an additional 15% demonstrated a hyposmia (Landis & Hummel, 2006; Hummel et al., 2016). Olfaction plays an essential role in eating behavior, both predicting and stimulating appetite and perceiving flavors during

food consumption (Boesveldt & de Graaf, 2017). People with olfactory dysfunction may experience a decrease in appetite, affecting their nutritional intake. They are more likely to report difficulties with cooking. Anosmic patients are three times more likely to experience dangerous events with 25-50% of anosmic patients reporting accidental consumption of rotten or spoiled food (Santos et al., 2004). Olfactory dysfunction is associated with significant depressive symptoms and loneliness, with women more likely to report depression and anxiety associated with olfactory dysfunction compared to men (Boesveldt & de Graaf, 2017). Unexplained olfactory dysfunction has also been related to increased mortality (Croy et al., 2014; Pinto et al., 2014). All in all, olfactory dysfunction can severely affect quality of life.

2.1.4 Assessment of olfactory function

1. Subjective Assessment

Subjective testing can be easily performed in daily clinical practice using visual analogue scales (VAS) and Likert-type questionnaires. Patients are asked to rate frequency and severity of their symptoms based on their experience of smell loss, which provides insights into the real-world impact of smell loss in olfactory dysfunction patients. It has been shown to be a simple, relatively reliable method to differentiate between normosmia and hyposmia/anosmia (Frasnelli & Hummel, 2005; Zou et al., 2019). Therefore, “subjective” olfactory assessment appears to be helpful for olfaction screening when psychophysical tests are unavailable. Mattos et al. showed that olfactory-specific questionnaires can be helpful for post-treatment follow-up, they also established the minimal clinically important difference of these questionnaires which helps to gauge clinically relevant differences in olfactory function, and the impact of interventions (Mattos et al., 2018). However, Philpott et al. reported that only 28% patients in a rhinology clinic are aware of their olfactory function before being tested (Philpott et al., 2008). A survey at the clinic conducted by Lötsch et al have shown that almost 30% (355/1227) of anosmic subjects rated their ability to smell as at least "average” (Lötsch & Hummel, 2019). On an individual level, much of the literature emphasized that there are striking differences between rated and measured olfactory function(Welge-Luessen et al., 2005; Haxel et al., 2012).

2. Psychophysical assessment of olfactory function

Orthonasal tests

Orthonasal olfaction describes the perception of odors through sniffing. A number of standardized and validated orthonasal psychophysical olfactory tests have been developed. The “Sniffin’ Sticks” test (Burghart; Wedel, Germany) and the Smell Identification Test (Sensonics Inc., Haddon Heights, NJ, USA) are the two most widely used tests for clinical and research applications. While most tests focus on odor identification, the Sniffin Sticks, for example, is multicomponent and allows for the assessment of odor threshold, odor discrimination and identification. Combined testing of these components to diagnose smell loss appears to be more sensitive than individual tests, especially when including assessment of odor thresholds (Lötsch et al., 2007). In CRS patients, odor threshold appears to be more affected than odor discrimination and identification, as shown in a study examining 1226 subjects (Whitcroft et al., 2017). This is in contrast to other disease etiologies: patients with post-infectious olfactory dysfunction, for example, have relatively well preserved odor threshold and discrimination during the period of recovery, but poor odor identification (Whitcroft et al., 2017). Multicomponent olfactory tests may therefore aid in diagnosing the underlying cause of impaired olfaction.

In light of the above, validated and, if possible, multicomponent psychophysical olfactory tests can aid in diagnosis, quantitatively monitor patients’ symptom and help to evaluate the efficiency of therapy (Hummel et al., 2016).

Retronasal tests

Many patients complain of taste loss. However, apart from a relatively small number of patients with gustatory dysfunction (sweet, sour, bitter, salty and savory/umami), the symptom “taste loss” typically signals loss of flavor (Deems et al., 1991). Retronasal olfaction, well described by Rozin (Rozin, 1982) in 1982,is a critical element in flavor perception, related to smells that arise from inside the mouth during eating and drinking. Food-associated volatiles are carried by retronasal airflow reaching the olfactory epithelium upon exhalation rather than by orthonasal flow, due to the unique shape of the human oropharynx (Ni et al., 2015). Studies based on MRI and electrophysiological recordings have demonstrated the processing of retronasal odors to be distinct from orthonasal perception of the same odors (Heilmann & Hummel, 2004; Small et al., 2005).

Over the past two decades, methods for the clinical assessment of retronasal olfactory function have become available. Heilman et al. (Heilmann et al., 2002) introduced a retronasal olfactory test using “taste powders” with grocery store condiments and food items (e.g., spices, instant drinks). The taste powders are administered to the subject's oral cavity using squeezable plastic vials. Another retronasal olfactory test is “candy smell test” introduced by Renner et al. (Renner et al., 2009) comprised of 23 differently aromatized smell candies. Both tests are considered as easy to handle, reliable tools to investigate retronasal olfaction. Their results are well correlated with orthonasal function (e.g. the “Sniffin’ Sticks” scores) and differentiate normosmia, hyposmia, and anosmia. However, still some issues are present. Neitehr taste powders nor the “candies” are tasteless, other sensory modalities like the taste and/or texture may enhance the performance correctly during retronasal test. Powders are non-standardized reagents, which may affect inter-test results’ reliability. Several new tools have been proposed. For example, “Candy Smell Test” for self-testing (Besser et al., 2020b), tasteless powders (Yoshino et al., 2020), and freeze‐dried retronasal stimuli (Pal et al., 2019). What is more, currently, retronasal odor identification and thresholds were assessed (Yoshino et al., 2021). Other possibilities to assess retronasal olfactory function may be expected in the future, e.g. retronasal discrimination.

A significant correlation was reported between retronasal olfaction and olfactory-specific quality of life (QOL) which was not found to the same degree for orthonasal function (Othieno et al., 2018). Retronasal olfaction can provide additional information when evaluating changes in eating habits. Moreover, regular exposure to retronasal odors (“retronasal training”) may have the potential to improve food-related quality of life (Besser et al., 2020a).

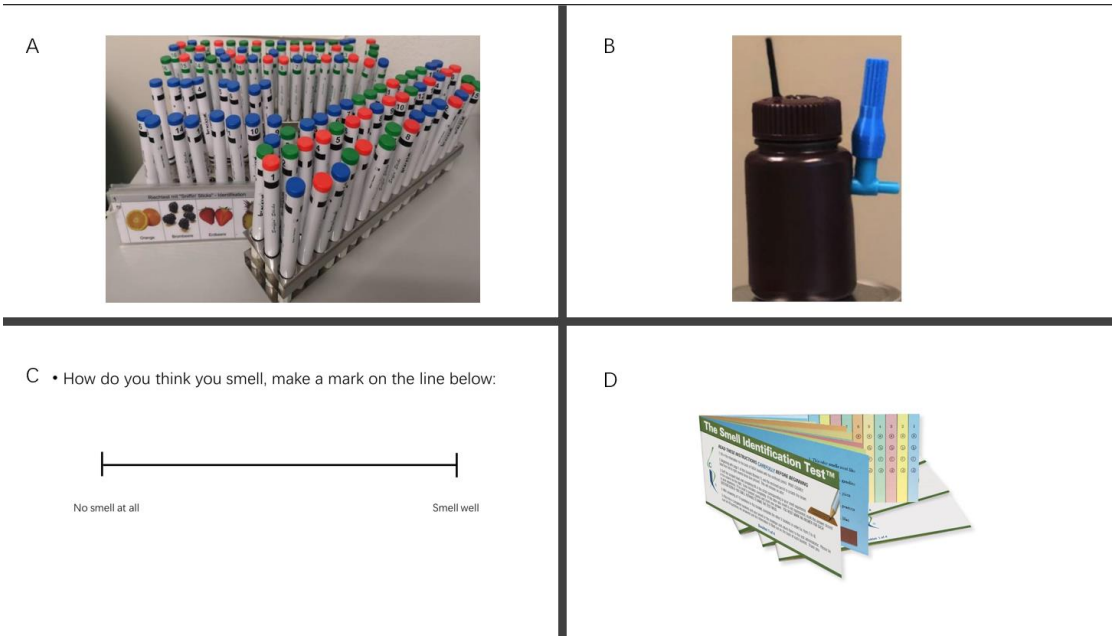

Figure 2-2 A. Sniffin' Sticks test battery. From front to back: set of 48 pens for Threshold (T) test, 16 pens for Identification (I) and 16 pens for Discrimination (D); B. Odorant delivery container for retronasal olfactory test. C. Visual analogue scales (VAS) for olfaction; D. Smell Identification Test™ (UPSIT®) is a 40-item, one-time use smell test, figure adapted from https://sensonics.com/product/smell-identification-test/.

2.2 Clinical predictors of olfactory function

Despite its prevalence and impact on the quality of life, diagnosing and treating olfactory dysfunction remain challenging for clinicians. One of the primary challenges lies in the objective assessment of olfactory function, as traditional self-report measures can be subjective and prone to biases (Welge-Luessen et al., 2005; Haxel et al., 2012). Furthermore, the underlying mechanisms of olfactory dysfunction are often complex and multifactorial, making it difficult to pinpoint a specific cause or identify an effective treatment strategy.

Developing reliable diagnostic tools and targeted therapies for olfactory dysfunction requires a comprehensive understanding of clinical predictors for olfaction. This highlights the significance of research efforts aimed at elucidating these relationships and advancing our understanding of olfactory dysfunction and its management.

While significant progress has been made in comprehending the fundamental science of olfaction, including the function of odorant receptors (Buck & Axel, 1991; Zhao et al., 1998; Malnic et al., 1999; Billesbolle et al., 2023), olfactory signaling pathways (Meyer et al., 2000), and central processing in the brain, advancements in managing clinical olfactory dysfunction have remained limited. Numerous clinical factors have been extensively investigated and found to be linked with olfactory dysfunction.

2.2.1 Age

The prevalence of olfactory dysfunction increases with age, particularly in individuals over 60 years old, resulting in a reduced ability to detect, identify, and discriminate between different odors (Adams et al., 2017). This decline in olfactory function is attributed to several factors, such as a decrease in the number of olfactory receptor neurons, changes in odorant binding proteins, and alterations in the OB (Hummel & Oleszkiewicz, 2020). aged OB shows that the decrease of the volume of the OB, the concentration of mitral cells per unit area, and both layer thickness and the number of glomeruli (Bhatnagar et al., 1987; Meisami et al., 1998).

2.2.2 Gender

gender differences in olfactory perception have been observed, with women generally demonstrating superior olfactory abilities compared to men (Croy et al., 2017). This difference is hypothesized to be due to hormonal fluctuations.

2.2.3 Toxic agent exposure

Occupational and environmental exposures to harmful substances such as tobacco smoke (Ajmani et al., 2017), air pollution (Ajmani et al., 2016), and certain chemicals (Doty & Bromley, 2004) can cause olfactory dysfunction. These substances may cause direct damage to the olfactory epithelium or lead to inflammation and oxidative stress, which can impair olfactory function, Exposure to certain chemicals or environmental pollutants can lead to olfactory dysfunction due to the direct toxic effects on olfactory neurons or indirect effects on the central nervous system.

2.2.4 Various health conditions

Chronic rhinosinusitis and allergic rhinitis are common sinonasal diseases that can lead to olfactory dysfunction (Yan et al., 2020). Inflammation and obstruction in the nasal cavity impair the passage of odorants to the olfactory epithelium. In neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis, olfactory dysfunction could potentially be attributed to a shared fundamental neuropathological substrate. For example, impairments in forebrain neurotransmitter, specifically those involving cholinergic transmission, demonstrate a correlation with quantitative smell test scores (Doty, 2017).

Understanding these predictors can provide valuable insights into the mechanisms underlying our sense of smell, as well as inform the development of potential interventions to enhance or restore olfactory function in individuals with impaired olfaction.

2.3 Clinical Predictors of Olfactory Function: Insights from Inflammation and Imaging

To expand research in this area, we are focusing on inflammation and imaging as clinical predictors of olfaction, as these factors have been shown to play a significant role in the development and progression of olfactory dysfunction. Exploring these two factors will help us better understand the underlying mechanisms and potentially improve diagnostic and treatment strategies.

2.3.1 Olfaction and inflammation

Inflammation has been implicated in several conditions that lead to olfactory dysfunction, such as chronic rhinosinusitis and allergic rhinitis. Olfactory dysfunction is among the cardinal diagnostic features (nasal blockage / obstruction / congestion, nasal discharge, facial pain/pressure and reduction or loss of smell) of chronic rhinosinusitis (CRS). Approximately 67-78% of CRS patients are affected by impaired olfaction(Kohli et al., 2017), and more and more researchers are focusing their attention on the sense of smell in CRS. Olfactory dysfunction is deemed to be a major contributor to medication use and poor quality of life in CRS patients (Katotomichelakis et al., 2013; Mattos et al., 2017), Evidence also demonstrates olfactory dysfunction, to be strongly associated with the type 2 inflammatory endotype. Patients with eosinophilic CRS complain of a stronger degree of smell loss(Thompson et al., 2016), and the degree of smell function was found to be positively correlated with the inflammatory condition of the nasal cavity (Tsybikov et al., 2016). Inflammatory components are involved in the impaired olfactory function in CRS, resulting in ORN dysfunction and death (Ge et al., 2002; Kern et al., 2004). What is more, inflammation appears to affect the entire olfactory system, from the periphery to central-nervous areas.

2.3.2 Olfaction and imaging structures

CT and MRI allow the examination of olfaction-related structures. Olfactory cleft (OC) opacification quantified using CT has been shown to be an effective method to evaluate olfactory function in patients with CRS(Soler et al., 2015), In Kohli's study, quantitative measures of OC opacification correlate with odor threshold, discrimination, and identification scores within the CRS with nasal polyps patients. Apart from correlation with

olfaction function, OC opacification may also help predict recovery of olfaction function after surgery in CRS with nasal polyps patients (Vandenhende-Szymanski et al., 2015).

Compared with CT, MRI offers unique advantages in the delineation of olfactory structures including OBs, olfactory sulcus, olfactory tract and olfactory cortex. For the OB and sulcus, this works best in T2-weighted sequences due to the bright CSF surrounding these structures. Both OB volume and olfactory sulcus depth have been shown to be of clinical relevance in various pathological conditions (Negoias et al., 2010; Rombaux et al., 2010; Hummel et al., 2015). Patients with olfactory dysfunction exhibit a reduction in OB volume (Rombaux et al., 2008; Herzallah et al., 2013). Importantly, a marked increase of OB volume was observed after treatment, concomitant with an increase in olfactory function (Gudziol et al., 2009; Alarabawy et al., 2016; Shehata et al., 2018). In keeping with this, structural alterations in gray matter volume within olfactory-related regions has also been shown in patients with olfactory impairments (Han et al., 2017). Moreover, gray matter volume within olfactory-eloquent regions increases after treatment, along with improved olfactory function (Whitcroft et al., 2018). These dynamic changes in OB and gray matter volume reflect the apparent plastic nature of the olfactory system.

Therefore, CT/MRI-based volumetric analysis would appear to be a useful objective morphological tool to assess olfactory function, particularly in longitudinally tracking patients' recovery after treatment.

Other ways to evaluate olfactory function in a relatively unbiased way include olfactory event-related potentials (OERPs), functional magnetic resonance imaging (fMRI), or positron emission tomography (PET) (Zatorre et al., 1992; Lascano et al., 2010; P. Han et al., 2020). These methods allow deeper insights into the functional characterization of the human olfactory system, with the capability to explore the pathophysiology of smell dysfunction. However, these examinations are typically limited to use in research, partly due to their relatively high cost, and the need for specialized equipment and expertise.

By investigating the predictors of olfaction, we can gain insights into the complex interplay between them and possibly help in the design of more effective and targeted interventions, as well as contribute to the novel therapeutic strategies for individuals with impaired olfactory function.

The overall aim of this thesis was to investigating the association between olfactory dysfunction and the clinical predictors, with a focusing on inflammation, imaging index. Specifically, we summarized the association between olfactory dysfunction and inflammation in chronic rhinosinusitis (CRS) patients. And investigated the relation between olfaction and shape of OB, anterior skull base. We hypothesis that overall intensity of the inflammatory response in CRS, the shape of the OB and the morphology of the anterior skull base can predict the olfaction function.

3 Method

3.1 Method1: Publication 1 Olfaction: sensitive indicator of inflammatory burden in chronic rhinosinusitis

In study 1, we reviewed olfactory dysfunction with regard to its clinical evaluation, pathophysiological mechanisms, relation with inflammatory burden and anti-inflammatory responses in CRS patients. The first section of this paper describes the assessment of olfactory function using various measures, from ratings to MR based imaging. Then, we discuss the conductive and inflammatory mechanisms related to olfactory dysfunction in CRS: olfaction is associated with certain inflammatory patterns and is potentially a marker of CRS subtype. Finally, we review anti-inflammatory therapies including conservative and surgical approaches, and their effectiveness in olfactory dysfunction in CRS.

3.2 Method2: Publication 2 The shape of the OB predicts olfactory function

In study 2, a total of 192 patients (86 men) complaining of smell loss with age ranging from 24 to 81 years (mean±standard deviation; 57±12.8 years), and 77 healthy subjects (39 men), with age ranging from 25 to 82 years (51±15.5 years) were included in this cross-sectional study. Patient group consisted of people visiting the Smell and Taste Clinic of the Department of Otorhinolaryngology at TU Dresden starting from 2016 until 2020. Diagnosis of acquired olfactory dysfunction was made according to the recent Position Paper on Olfactory Dysfunction.(Hummel et al., 2016) As part of their assessment, all patients underwent an evaluation that included an endoscopical examination of the nasal cavity, structured history collection, olfactory testing and structural T2-weighted MRI (Welge-Luessen et al., 2013). Classification of olfactory function was based on the underlying etiological conditions, i.e., olfactory dysfunction secondary to CRS, post-infectious olfactory dysfunction (PIOD), posttraumatic olfactory dysfunction (PTOD), olfactory dysfunction associated with neurological disease, and idiopathic olfactory dysfunction. A high-resolution structural T2 weighted MRI scan was obtained for all. Patients with congenital anosmia were excluded based on their history and brain MRI examinations. General exclusion criteria were major hearing or vision problems and history of a psychiatric disorder. All healthy subjects had no history of an underlying or preceding major medical condition and reported a normal sense of smell. This retrospective study was approved by the TU Dresden, Medical Faculty Ethics Review

Board. All subjects provided written informed consent before their detailed evaluation. Subjects' demographic information and olfactory function are shown in Table 1.

MRI Acquisition

MRI acquisitions were performed on a 3T scanner (Siemens, Erlangen, Germany) with an 8-channel phase-array head coil. Standardized MRI for structural analyses was performed for all subjects, targeting structures that consisted of the left and right OB with a coronal T2-weighted fast spin-echo sequence: TR/TE = 4800/152 ms; slice thickness 2 mm; matrix size 256x256; 30 slices; averages 2; in-plane resolution 0.4x0.4 mm and no intersection gap) covering the anterior and middle segments of the base of the skull.

Olfactory testing

Quantitative testing of olfactory function was performed using the ''Sniffin' Sticks'' test (Burghart GmbH, Holm, Germany) (Hummel et al., 1997). Odorants were presented with pen-like devices. It was comprised of three tests, namely odor threshold with 16 increasing concentrations of phenyl ethyl alcohol using a single staircase, 3-alternative forced choice (3AFC) procedure (range of scores: 1 to 16). Odor discrimination was determined with a 3AFC procedure and 16 triplets (range of scores 0 to 16). Odor identification was tested using a 16-item multiple forced choice test (range of scores 0 -16). The three subsets of this test were summated into the so-called "threshold-discrimination-identification (TDI) score", leading to a maximum score of 48. For odor presentation, the pen's cap was removed by the experimenter for approximately 3s and the felt tip was placed approximately 2 cm in front of the subjects' nostrils.

Evaluation of the volume of the OB

We randomly chose 90 subjects (45 patients and 45 age and sex matched healthy control) from the cohort investigated here to evaluate the OB volume. The OB volume was measured on coronal T2-weighted MR images with ITK-SNAP software (Version 3.8.0, Pennsylvania, PA, USA). The planimetric contours (surface in mm^2) of OB were delineated manually by an experienced observer blinded to the subjects. Then all surfaces were added and multiplied to obtain the volume of OB segment in mm^3. This approach of calculating OB volumes has been shown to be highly reliable and accurate (Yousem et al., 1998; Rombaux et al., 2010; Mazal et al., 2016).

Evaluation of the shape of the OB

MRI scans were examined using ITK-SNAP software (Version 3.8.0, Pennsylvania, PA, USA) that allows images to be viewed in three orthogonal planes. Configurations of cross-sectional areas of OB were visually inspected in the slice through the most posterior coronal plane tangent to the eyeballs, in accordance to previous research (Hummel et al., 2015). Heuristically, based on opinions from a small group of experts, we chose to look at the shape of the OB according to its integrity and divided OBs into those homogeneous and scattered OBs. In homogeneous OB contours, the shape was considered by its outline, as convex, concave, and plane. Convex OBs curve outward; concave OBs curve inward, plane OBs are flat on both sides. The convex OB shape is then subdivided further into olive shape, circle or a plano-convex shape. For the concave OB shape, either banana shape or an irregular shape is determined (Schneider & Floemer, 2009; Burmeister et al., 2012). Images were assessed by a trained clinical expert (YPZ) who was blinded to other clinical data of the respective cases. To assess inter-observer reliability, 107 cases were randomly analyzed by the observers (YPZ and AJ). The inter-rater agreement between two independent persons analyzing the OB shape was substantial (Cohen's Kappa = 0.73, 95% CI = 0.60 - 0.86, $P<0.001$).

Statistical analysis

Seventy-seven patients were randomly selected and individually matched by age (within ± 2years) and gender with the healthy group. OB shapes distribution were compared between patients and healthy controls using chi-square tests. Chi- square test was performed to compare the two OB shape patterns for gender differences. ANOVA and the Student's t-test were computed to examine the relation between OB shapes, age and causes of smell loss. A non-parametric Mann-Whitney U test was applied to compare olfactory function among groups with different OB shape patterns. Multivariate linear regression analyses were performed using TDI, T, D and I as dependent variables, with shape of OB, age, and gender defining the set of independent variables. Moreover, differences for OB shapes, age, gender, and olfactory function were compared separately in patients and healthy controls. Statistics were performed using SPSS version 26 for Windows (SPSS Inc., Chicago, Illinois, USA). A *p*-value threshold of 0.05 was used to define statistical significance.

3.3 Method3: Publication 3 (third study) Anterior skull base abnormalities in congenital anosmia

Study design and Setting

This was a retrospective study conducted by reviewing medical records of patients who received treatment in the Smell and Taste Clinic of the Department of Otorhinolaryngology at the TU Dresden. All participants provided written informed consent. They participated in studies that had been approved by the Ethics Committee at the Medical Faculty at the "Technische Universität Dresden".

Participants

Inclusion criteria

Congenital anosmia was diagnosed according to the "position paper on olfactory dysfunction"(Hummel et al., 2016) based on **1.** detailed structured medical history; participants reported that they never experienced any olfactory perception(Hummel et al., 2013a); **2**. psychophysical examination was assessed with the extended Sniffin' Sticks olfactory test (Burghart, Wedel, Germany) with participants's TDI (Threshold-Discrimination-Identification) score in the range of anosmia (score ≤16)(Oleszkiewicz et al., 2019); **3.** electrophysiological measurements; participants did not exhibit an EEG response following olfactory stimulation(Hummel et al., 1991). **4.** MRI; the OB or olfactory tract was aplastic or hypoplastic (according to literature, ICA when both OB and olfactory tract are normal, is very rare)(Yousem et al., 1996). Randomly selected healthy controls with no known history of olfactory dysfunction were recruited by advertisement and were matched to the participants by age (±2 years) and gender.

Exclusion criteria were: **1.** signs and symptoms of different conditions possibly causing anosmia (severe CRS, traumatic brain injury etc.); **2.** altered anatomy of anterior skull base or paranasal sinus due to iatrogenic causes or other pathology, e.g. tumors; **3.** obscured ethmoid sinus pathology; 4. congenital anosmia combined with other anomalies, e.g. Kallmann syndrome, CHARGE syndrome.

Data sources/ measurement

MRI acquisition

MRI was performed using a 3-Tesla scanner (model Prisma; Siemens, Erlangen, Germany) and a 32-channel coil. Images were acquired with T2 weighted sequence, covering the anterior and middle segments of the head. The scanning parameters were: 30-46 slices, slice thickness 1mm, no gap, echo time (TE) = 78 ms, repetition time (TR) = 1500 ms, flip angle = 150˚, field of view matrix = 256 x 320.

Structural assessment

For delineating and measuring the anterior skull base, we used ITK-SNAP, which provided tools to measure lengths and angles of anatomical structures. Measurements were made in the coronal plane of the posterior tangent through the eyeballs (Abolmaali et al., 2002). The following parameters were recorded on both sides: **1,** Depth of the olfactory fossa (in mm), measured as the vertical height of the olfactory fossa and classified by the Keros' classification system into type 1 (depth 1-3 mm), type 2 (depth 4-7 mm) or type 3 (depth more than 8 mm)(Keros, 1962). Asymmetry in the depth (difference of more than 1 mm) between the right and left olfactory fossa were also examined; **2,** Width of olfactory fossa (in mm), defined as the distance between the crista galli and the lateral wall of the olfactory fossa through the depth of olfactory fossa medially and perpendicularly; **3**, Angle of LLCP (in degrees), measurement was calculated at the angle formed by the LLCP and the horizontal line drawn through the cribriform plate. This was further classified into 3 classes depending on the hypothetical risk of iatrogenic injuries: class I (>80 degrees, low risk), class II (45 to 80 degrees, medium risk) and class III (<45 degrees, high risk)
(Keros, 1962); **4,** Angle of fovea ethmoidalis (in degrees), calculated as the angle formed by the fovea ethmoidalis and LLCP; 5, depth of the olfactory sulcus.

For describing the status of OB concerning the development, we divided it into 2 phenotypes: ICA with OB present and ICA with absence of OB.

Statistical analysis

SPSS version 26 (SPSS INC, Illinois, USA) was used for data analysis, graphical visualization were performed using GraphPad Prism 9.3.1 (GraphPad Software, Inc., La

Jolla, CA, USA). Descriptive statistics were performed for all study participants. Student's t-test and $\chi 2$ were used for comparisons between individuals with ICA and healthy controls. Receiver operating characteristic (ROC) curves were used to establish sensitivity and specificity of the depth of respective deepest olfactory fossa, angle of respective largest LLCP, angle of respective largest FE. The Optimal cutoff values were chosen based on the highest Youden index (sensitivity + specificity − 1). Cutoff values with the highest Youden index (optimal combination of sensitivity and specificity) have the least amount of overlap between groups, represent a clinically relevant cutoff. The area under the ROC curve was used to quantify diagnostic accuracy of these measurements. The association between measurements were estimated through Pearson's correlation coefficient. We further compared the measurements between individuals with ICA regarding absence or presence of OB. P value < 0.05 was considered as statistically significant.

4 Contributions in the publications

Publication 1

The study concept was planned together with Thomas Hummel. I prepared all final figures, wrote the original draft of the manuscript and was responsible for all submission steps.

Publication 2

The study concept was planned together with Thomas Hummel. I did data analysis and prepared all final figures, wrote the original draft of the manuscript and was responsible for all submission steps

Publication 3

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5 Publications list

The following publications are included in this cummulative thesis:

Publication 1

Title	Olfaction: Sensitive indicator of inflammatory burden in chronic rhinosinusitis.
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Publication 2

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Publication 3

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Journal	ORL
Submitted	2023
Impact Factor	1.919 (InCites Journal Citation Report, 2021)
Quartile (Field)	Q2 (Otorhinolaryngology)
Rank	30/62 (Otorhinolaryngology)

6 Publication 1 (first study) Olfaction: sensitive indicator of inflammatory burden in chronic rhinosinusitis (Yan et al., 2020)

6.1 Abstract 1

Olfactory dysfunction has a high prevalence in chronic rhinosinusitis (CRS) patients and significantly affects quality of life. CRS is recognized as a complex disorder encompassing heterogeneous inflammatory processes in the nose and paranasal sinuses. Olfactory dysfunction in CRS patients is associated with the level of inflammatory mediators and the efficiency of inflammatory control. Learning about the association between CRS-related inflammation and olfactory function will provide clues to the pathogenesis of CRS. The first section of this review describes the assessment of olfactory function using various measures, from ratings to MR based imaging. Then, we discuss the conductive and inflammatory mechanisms related to olfactory dysfunction in CRS: olfaction is associated with certain inflammatory patterns and is potentially a marker of CRS subtype. Finally, we review anti-inflammatory therapies including conservative and surgical approaches, and their effectiveness in olfactory dysfunction in CRS. In conclusion, assessment of olfactory function should be considered in the clinical evaluation of CRS patients, not only for detecting and quantifying patients' symptom but also because it appears to be useful to objectively assess the efficacy of CRS treatment over time. In addition, olfaction can be expected to expand the library of CRS phenotypes and endotypes and, hence, pave the way for more precise, tailored treatment options.

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REVIEW

Laryngoscope Investigative Otolaryngology

Olfaction: Sensitive indicator of inflammatory burden in chronic rhinosinusitis

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Abstract

Background and Objective: Olfactory dysfunction has a high prevalence in chronic rhinosinusitis (CRS) patients and significantly affects quality of life. CRS is recognized as a complex disorder encompassing heterogeneous inflammatory processes in the nose and paranasal sinuses. Olfactory dysfunction in CRS patients is associated with the level of inflammatory mediators and the efficiency of inflammatory control. Learning about the association between CRS-related inflammation and olfactory function will provide clues to the pathogenesis of CRS.

Structure: The first section of this review describes the assessment of olfactory function using various measures, from ratings to MR based imaging. Then, we discuss the conductive and inflammatory mechanisms related to olfactory dysfunction in CRS: olfaction is associated with certain inflammatory patterns and is potentially a marker of CRS subtype. Finally, we review anti-inflammatory therapies including conservative and surgical approaches, and their effectiveness in olfactory dysfunction in CRS.

Conclusion: Assessment of olfactory function should be considered in the clinical evaluation of CRS patients, not only for detecting and quantifying patients' symptom, but also because it appears to be useful to objectively assess the efficacy of CRS treatment over time. In addition, olfaction can be expected to expand the library of CRS phenotypes and endotypes and, hence, pave the way for more precise, tailored treatment options.

KEYWORDS
anosmia, chronic rhinosinusits, inflammation, nose, olfaction, smell

1 | INTRODUCTION

Olfaction, one of the basic human senses, has a wide range of functions, including the avoidance of environmental hazards, finding and identifying food,[1] spatial orientation,[2] flavor perception, social interactions (eg, recognition of emotions and romantic relationships),[3] and cognitive functions (eg, modulation of memories).[4] Patients with olfactory dysfunction are more likely to report difficulties with cooking, feeling less safe, and depression and anxiety. Unexplained olfactory dysfunction has also been related to increased mortality.[1,5] All in all, olfactory dysfunction can severely affect quality of life (QOL).

Olfactory dysfunction is among the cardinal diagnostic features (nasal blockage/obstruction/congestion, nasal discharge, facial pain/

pressure, and reduction or loss of smell) of chronic rhinosinusitis (CRS), which is defined as an inflammatory disease of the nasal cavities and paranasal sinuses lasting 12 weeks or longer.[6] Approximately 67% to ~78% of CRS patients are affected by impaired olfaction,[7] and more and more researchers are focusing their attention on the sense of smell in CRS. (Figure 1) Olfactory dysfunction is deemed to be a major contributor to medication use and poor QOL in CRS patients.[8,9]

To date CRS is mainly clinically classified into the two main phenotypes: CRS without nasal polyps (CRSsNP) and with nasal polyps (CRSwNP). Over the past decade, research has begun to explore the heterogeneity of CRS by finding immuno-pathophysiologic mechanisms and defining inflammatory endotypes, In fact, the recent EPOS2020 position paper has divided CRS into: *Type 2* presenting with high level of Th2 cytokines, such as IL-4, IL-5, and IL-13, and infiltrating eosinophils, which is more resistant to therapies and exhibits a high rate of recurrence, while *nontype2* is related to Th1/Th17 immune responses characterized by cytokine IL-17A and IL-8 as well as excess neutrophilic inflammation and interferon-gamma (IFN-γ).[10] Biologic agents targeting type 2 inflammation hold great promise in providing targeted therapies in severe and recalcitrant CRS patients. At present, Dupilumab, a humanized monoclonal IgG4 antibody directed against the interleukin-4 receptor α (IL-4Rα) subunit, is the first biological to be approved by the Food and Drug Administration (FDA) of the USA and the European Medicines Agency (EMA) in 2019 for use in CRSwNP. Dupilumab shows significant improvement in almost all clinical symptoms of CRSwNP.[11] In light of such results, it has been suggested that biologicals as novel therapies in nonadequate disease control may potentially revolutionize CRSwNP treatment.[12]

Research efforts have been made to investigate the relation between endotype and phenotype of CRS. CRSwNP is known as a type 2 reaction, while CRSsNP is linked with predominantly Th-1 cell response.[13] Type 2 endotypes tend to be more resistant to current therapies and are associated with asthma.[14] Evidence also demonstrates olfactory dysfunction, a cardinal CRS symptom, to be strongly associated with the type 2 inflammatory endotype. Patients with eosinophilic CRS complain of a stronger degree of smell loss,[15] and the degree of smell function was found to be positively correlated with the inflammatory condition of the nasal cavity.[16] Given that the overall intensity of the inflammatory response is closely associated with the severity of disease and carries prognostic information, it seems important to understand the role of olfaction in CRS and its association with the inflammatory status.[17] In this paper, we aim to review olfactory dysfunction with regard to its clinical evaluation, pathophysiological mechanisms, relation with inflammatory burden and anti-inflammatory responses in CRS patients.

2 | ASSESSMENT OF OLFACTORY FUNCTION

2.1 | Subjective assessment

Subjective testing can be easily performed in daily clinical practice using visual analogue scales (VAS) and Likert-type questionnaires. Patients are asked to rate frequency and severity of their symptoms based on their experience of smell loss, which provides insights into the real-world impact of smell loss in CRS patients. It has been shown to be a simple, relatively reliable method to differentiate between normosmia and hyposmia/anosmia.[18,19] Therefore, "subjective" olfactory assessment appears to be helpful for olfaction screening when psychophysical tests are unavailable. Mattos et al showed that olfactory-specific questionnaires can be helpful for post-treatment follow-up, they also established the minimal clinically important difference of these questionnaires which helps to gauge clinically relevant differences in olfactory function, and the impact of interventions.[20] However, Philpott et al reported that only 28% patients in a rhinology clinic are aware of their olfactory function before being tested.[21] A survey at the clinic conducted by Lötsch et al have shown that almost 30% (355/1227) of anosmic subjects rated their ability to smell as at least "average".[22] On an individual level, much of the literature

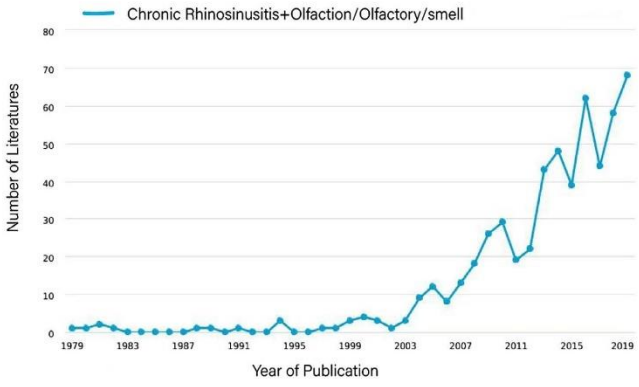

FIGURE 1 Number of literatures related to CRS and olfactory function published between the year 1979 and 2019. The key terms (["chronic rhinosinusitis"] and ["olfaction" or "olfactory" or "smell"]) were used to search relevant articles in Scopus (documents by year)

Figure 6-1

emphasized that there are striking differences between rated and measured olfactory function.[23,24]

Similarly, in a recent study of 109 patients with CRS, only a weak relationship between olfactory-specific QOL and psychophysical measures of olfactory function was found.[8] Interestingly, considering the nature of the sense of smell as determinant of flavor perception, it needs to be further investigated to what degree flavor loss is among the main drivers of CRS patients to seek medical counseling.[25,26] Indeed, increasing evidence shows low correlation between subjective symptoms and objective findings in CRS.[27] The gradual onset of smell loss in CRS may explain why olfactory loss often goes unnoticed.[28] Given this discrepancy, assessment of olfactory function based on self-reports is recommended to be performed in conjunction with psychophysical methods allowing for a more objective characterization of the sense of smell.[29]

2.2 | Psychophysical assessment of olfactory function

2.2.1 | Orthonasal tests

Orthonasal olfaction describes the perception of odors through sniffing. A number of standardized and validated orthonasal psychophysical olfactory tests have been developed. The "Sniffin' Sticks" test (Burghart; Wedel, Germany) and the Smell Identification Test (Sensonics Inc., Haddon Heights, New Jersey) are the two most widely used tests for clinical and research applications. While most tests focus on odor identification, the Sniffin Sticks, for example, is multicomponent and allows for the assessment of odor threshold, odor discrimination and identification. Combined testing of these components to diagnose smell loss appears to be more sensitive than individual tests, especially when including assessment of odor thresholds.[30] In CRS patients, odor threshold appears to be more affected than odor discrimination and identification, as shown in a study examining 1226 subjects.[31] This is in contrast to other disease etiologies: patients with postinfectious olfactory dysfunction, for example, have relatively well preserved odor threshold and discrimination during the period of recovery, but poor odor identification.[31] Multicomponent olfactory tests may therefore aid in diagnosing the underlying cause of impaired olfaction.

Whilst odor threshold may carry diagnostic information, in patients who have undergone treatment for CRS, it has been suggested that odor discrimination best reflects overall change in olfactory function.[32] In addition, odor discrimination has the strongest correlation with olfactory-specific QOL in CRS.[8]

In light of the above, validated and, if possible, multicomponent psychophysical olfactory tests can aid in diagnosis, quantitatively monitor patients' symptom and help to evaluate the efficiency of CRS therapy.[29]

2.2.2 | Retronasal tests

Many patients complain of taste loss. However, apart from a relatively small number of patients with gustatory dysfunction (sweet, sour, bitter, salty, and savory/umami), the symptom "taste loss" typically signals loss of flavor.[33] Retronasal olfaction, well described by Rozin[34] in 1982,is a critical element in flavor perception, related to smells that arise from inside the mouth during eating and drinking. Food-associated volatiles are carried by retronasal airflow reaching the olfactory epithelium upon exhalation rather than by orthonasal flow, due to the unique shape of the human oropharynx.[35] Studies based on magnetic resonance imaging (MRI) and electrophysiological recordings have demonstrated the processing of retronasal odors to be distinct from orthonasal perception of the same odors.[36,37]

Over the past two decades, methods for the clinical assessment of retronasal olfactory function have become available. Heilman et al[38] introduced a retronasal olfactory test using "taste powders" with grocery store condiments and food items (eg, spices and instant drinks). The taste powders are administered to the subject's oral cavity using squeezable plastic vials. Another retronasal olfactory test is "candy smell test" introduced by Renner et al,[39] comprised of 23 differently aromatized smell candies. Both tests are considered as easy to handle, reliable tools to investigate retronasal olfaction. Their results are well correlated with orthonasal function (eg, the "Sniffin' Sticks" scores) and differentiate normosmia, hyposmia, and anosmia. However, still some issues are present. Neither taste powders nor the "candies" are tasteless, other sensory modalities like the taste and/or texture may enhance the performance correctly during retronasal test. Powders are nonstandardized reagents, which may affect intertest results' reliability. Several new tools have been proposed. For example, "Candy Smell Test" for self-testing,[40] tasteless powders,[41] and freeze-dried retronasal stimuli.[42] What is more, currently, only retronasal odor identification is assessed which is strongly dependent on cultural backgrounds. Other possibilities to assess retronasal olfactory function may be expected in the future, for example, retronasal discrimination, or assessment of retronasal thresholds.

Retronasal and orthonasal odor identification are correlated in CRS patients.[43] Completely obstructing the olfactory cleft (OC) can significantly decrease orthonasal and retronasal olfactory function.[44] However, retronasal olfaction is more often preserved to a higher degree in CRSwP compared to orthonasal olfaction, supporting the idea that polyps mechanically change airflow to the OC.[45] A significant correlation was reported between retronasal olfaction and olfactory-specific QOL which was not found to the same degree for orthonasal function.[43] Retronasal olfaction can provide additional information when evaluating changes in eating habits. Moreover, regular exposure to retronasal odors ("retronasal training") may have the potential to improve food-related QOL.[46]

2.3 | Assessment of olfactory-related parameters using imaging and electrophysiological tools

CT and MRI allow the examination of olfaction-related structures. CT of the sinuses remains the modality of choice to confirm or exclude diagnosis and evaluate the severity of CRS. OC opacification quantified using CT has been shown to be an effective method to evaluate

olfactory function in patients with CRS.[47] It seems the association between OC opacification and olfactory function based on the nasal polyps. In 148 CRS patients, Catherine el al[48] found OC opacification only correlated with olfactory function in CRSwNP, whereas not in CRSsNP. Similarly, in Kohli's study, quantitative measures of OC opacification correlate with odor threshold, discrimination, and identification scores within the CRSwNP patients. However, in CRSsNP subgroup, odor thresholds correlate with OC opacification, while odor discrimination/identification do not.[49] In addition, whether retronasal olfactory function linked to CT opacification of the OC have been unclear in current literature. Apart from correlation with olfaction function, OC opacification may also help predict recovery of olfaction function after surgery in CRSwNP patients.[50]

Compared with CT, MRI offers unique advantages in the delineation of olfactory structures including olfactory bulbs, olfactory sulcus, olfactory tract, and olfactory cortex. For the olfactory bulb and sulcus, this works best in T2-weighted sequences due to the bright CSF surrounding these structures. Both olfactory bulb volume and olfactory sulcus depth have been shown to be of clinical relevance in various pathological conditions.[51-53] CRS patients exhibit a reduction in OB volume.[54,55] Importantly, a marked increase of OB volume was observed after treatment, concomitant with an increase in olfactory function.[56-58] In keeping with this, structural alterations in gray matter volume within olfactory-related regions has also been shown in CRS patients with olfactory impairments.[59] Moreover, grey matter volume within olfactory-eloquent regions increases after surgical treatment for CRS, along with improved olfactory function.[60] These dynamic changes in OB and gray matter volume reflect the apparent plastic nature of the olfactory system.

Therefore, CT/MRI-based volumetric analysis would appear to be a useful objective morphological tool to assess olfactory function in CRS patients, particularly in longitudinally tracking patients' recovery after treatment.

Other ways to evaluate olfactory function in a relatively unbiased way include olfactory event-related potentials (OERPs), functional magnetic resonance imaging (fMRI), or positron emission tomography (PET).[61-63] These methods allow deeper insights into the functional characterization of the human olfactory system, with the capability to explore the pathophysiology of smell dysfunction. However, these examinations are typically limited to use in research, partly due to their relatively high cost, and the need for specialized equipment and expertise.

3 | MECHANISMS FOR OLFACTORY LOSS IN CRS

The mechanisms underlying CRS-associated olfactory loss are not fully known. It has traditionally been assumed to be of a conductive origin, with the OC being mechanically obstructed by polyps/edematous mucosal tissue, leading to impaired airflow, and reduced odorant access to the olfactory epithelium. Recently, Besser et al effectively established a hyposmia model obstructed the OC with dissolvable nasal dressing.[44] In keeping with this, nasal polyps can affect orthonasal olfactory function more strongly than retronasal olfactory function, emphasizing conductive mechanisms in smell dysfunction.[64] While there is no doubt that nasal patency is essential for olfactory perception, observations in patients with CRS show that smell loss is possible even when the OC is nonobstructed and no changes in nasal airflow are present. Furthermore, removal of nasal polyps does not always increase olfactory function in CRS.[65,66] Hence, olfactory loss in CRS can also be attributed to inflammatory processes.

The histologic changes of olfactory mucosa in CRS patients include—goblet cell hyperplasia, squamous metaplasia, and more commonly, erosion, which is characterized by severe olfactory epithelial layers loss and a higher prevalence of inflammatory cell infiltration.[67-69] Inflammation within the olfactory epithelium may directly or indirectly decrease the quantity of olfactory neurons. Biopsies specimen of the olfactory epithelium in cases of olfactory dysfunction secondary to CRS showed apoptotic changes.[67] In the mouse, progressive inflammatory infiltration in the olfactory epithelium triggers caspase-3 activity, known as the executioner caspase in apoptosis, leading to olfactory neuron death in CRS.[70,71] Eosinophilic inflammation directly impairs or even kills olfactory sensory neurons, resulting possibly from neurotoxic effects from the release of eosinophilic granule proteins and eosinophil-related cytokine damage.[72-74] Compared with the noneosinophilic CRS group, eosinophilic CRS patients have a more pronounced smell loss, fewer OMP (olfactory marker protein: a protein that marks mature olfactory neurons used in immunohistochemistry) positive cells and greater epithelial erosion.[75] Recent work in mice suggests that type 2 inflammation decreases the number of immature olfactory neurons, but not the mature olfactory neurons, indicating that it may interfere with the process of olfactory neurogenesis.[76] After some time, this may lead to reduced mature olfactory neuron populations, resulting in reduced OMP+ cells, as mentioned above.

Inflammation can also have a negative impact on the overlying mucus layer of the respiratory and olfactory epithelium. Mucus is secreted by respiratory submucosal glands (SMGs) and Bowman's glands.[77] Inflammation may lead to hypersecretion, in turn leading to altered potassium and sodium concentrations within the olfactory mucus. Accordingly, disruption of the ionic milieu may interfere with olfactory receptor activation and downstream transduction cascades.[78,79] Furthermore, there is evidence from animal models of CRS showing that olfactory stem cell proliferation and differentiation are arrested with local inducible expression of TNF-a and interferon-c.[80-83] Importantly, olfactory receptor neurons can reverse these changes when such inflammation subsides.[81,84]

It is believed that the immune system plays a major role in CRS development. Emerging evidence suggests olfactory stem cell to be involved in crosstalk between CRS inflammatory microenvironment and immune cells, which switch from their regenerative state to immune defense, resulting in impaired neurogenesis and olfactory neuron replacement.[85] This work has expanded the underlying mechanisms of CRS-related smell loss and the possibility of developing novel therapies.

As mentioned above, changes in the central olfactory system are associated with smell loss in CRS. Reduced olfactory bulb volume has been demonstrated in CRS patients with smell loss.[55,56] In rodents, recent studies suggest that persistent nasal inflammation induces glial activation, damage of the olfactory bulb circuit, and ultimately atrophy of the olfactory bulb.[86] Of particular interest, change in olfactory bulb volume is related to change in odor threshold, which is considered to partly reflect peripheral olfactory function. Given that olfactory bulb directly receives axons from the olfactory receptor neurons, the decrease of olfactory bulb volume in CRS might be due to decreasing input from the olfactory epithelium. In addition, structural and integrity changes in white matter and gray matter related to the olfactory areas have also been demonstrated.[59,87,88] It indicates that an inflammatory state in the nasal cavity is sufficient to produce a gradual and accumulated effect in central areas, which may contribute to the smell loss in CRS patients. It remains unknown which specific processes are responsible for these changes in central-nervous structures, what these changes mean for prognosis and how they could be utilized in the clinical treatment of CRS related olfactory loss. It appears that smell loss in CRS is one of the best example for central changes due to peripheral inflammation and obstruction- and that timely therapy (and associated diagnosis) of CRS is crucial.

Hence, impaired olfactory function in CRS patients is multifactorial. It not only results from physical obstruction of the nasal cavity, but also involves an inflammatory component resulting in olfactory receptor neuron dysfunction and death. What is more, CRS appears to affect the entire olfactory system, from the *periphery to central-nervous areas*. Although many recent advances remain in preclinical stages, knowledge of CRS-associated olfactory loss contributes to the development of future therapeutic approaches.

4 | OLFACTION INDICATES THE DEGREE OF INFLAMMATION AND MAY BE A MARKER OF CRS SUBTYPE

Previous histological studies of olfactory tissue in CRS demonstrated that CRS patients with olfactory deficit exhibited a higher degree of inflammation with an influx of lymphocytes, macrophages, and eosinophils.[67] An increasing number of studies support the idea that olfactory function is quantitatively associated with the level of inflammatory mediators. In a cross-sectional analysis of 34 patients with olfactory loss due to CRS, Schlosser et al found that scores of olfactory function were inversely correlated with levels of IL-5 in the OC.[89] Furthermore, one prospective case-control study showed that odor identification scores in CRS patients related to the degree of IL-2, IL-5, IL-6, IL-10, and IL-13 collected from olfactory mucus.[90] Of note, a direct correlation was found between the cytokine levels in the OC and levels in the middle meatus,[90] which suggests that cytokine changes are found simultaneously in the respiratory mucosa and the olfactory mucosa. These results are consistent with the previous observation of a significant correlation between the eosinophil counts in the respiratory and olfactory mucosa.[67,75]

Olfactory loss seems to be more severe and occurs at earlier stages of the disease in patients with eosinophilic infiltration.[9] The smell reduction of CRS has been reported to show correlations with blood and nasal mucosa eosinophil count.[91,92] The eosinophilic marker, Charcot-Leyden crystal (CLC) gene expression, has been found to be significantly correlated with olfactory threshold, even when confounders that is, the presence of nasal polyps, radiographic and endoscopic findings, were controlled.[93] In addition, in both the CRSsNP and CRSwNP groups, olfactory test scores have been shown to be negatively correlated with neuron-specific enolase (NSE) levels.[16] Given this evidence, it appears that the severity of olfactory function serves as a surrogate marker of inflammation within the entire nasal cavity, not just the local environment in the area of the OC.

Multicomponent psychophysical olfactory testing (ie, assessment of odor threshold, discrimination, identification) increases sensitivity in diagnosing olfactory dysfunction. In a study involving 1226 hyposmic patients, Whitcroft et al found patients with CRS related smell loss to have particularly impaired odor threshold (ie, they had a low sensitivity), relative to odor identification and discrimination.[31] This study suggested that the pattern of subtest scores provides diagnostic benefit regarding the underlying pathology. In line with this evidence, Lavin et al showed that threshold scores are associated with eosinophilia, as measured by CLC, more closely than discrimination score.[93] In contrast, data from Schlosser et al, in a much smaller group, indicate that olfactory identification rather than threshold correlates best with OC mucus IL-5,[89] which is one of the essential cytokines that activate type 2 helper T-cell (Th2) inflammation. Considering the limited number of studies, the present interpretation is that different patterns of smell test scores may be associated with different CRS endotypes, with the Th2 predominant inflammatory profile producing injury to the olfactory epithelium. More studies are needed to clarify this.

The evolving classification of CRS helps to successfully tailor patient care. The division of CRS into two major phenotypes, CRSsNP and CRSwNP, is widely accepted. Smell loss has been described as a major feature of CRSwNP affecting 83% to 91% of patients. What is more, olfactory function is more severe and more frequent as reported by patients with CRSwNP compared to CRSsNP patients.[7,94] Recently, there has been effort to identify phenotypic subgroups by using clustering methods, based on common clinical symptoms. In these studies, olfactory function seems to be a stable and valuable factor in the various clusters. In a multi-institutional prospective study of 690 patients in CRS, separating patients into five clusters, olfactory function at baseline was significantly different between clusters. Moreover, patient clusters with the best olfactory function experience the greatest benefit with surgery.[95] Cole et al identified five clusters depending on the severity and frequency of symptoms using 37 questions from 3535 subjects. The results indicated that self-reported smell loss is a factor with little longitudinal change.[96] In Morse et al's study, the majority of anosmic patients were found in a specific CRS cluster characterized by nasal polyposis (100%), allergic fungal rhinosinusitis (50%), and aspirin-exacerbated respiratory disease (AERD) (33%).[97] These results suggest that olfactory function is a

cardinal symptom that can be used to identify sub-phenotypes of CRS, which may provide prognostic information. Subclassification of CRS is underway for better understanding of CRS endotypes. Studies point out that smell loss may also be closely related to Th2-skewed inflammatory CRS endotype.[97]

Taken together, these results provide important insights into olfactory function in CRS which is linked to the sinonasal inflammatory response: it appears to be an indicator of CRS severity. Accurate, quantitative assessment of smell function, with well-established and reliable tools, should continue to aid in the establishment of CRS phenotypes and endotypes, as well as the optimization of available treatments.

4.1 | Therapies of olfactory function in CRS

The plasticity of the olfactory system allows recovery after treatment of CRS related olfactory dysfunction.[98] The first-line therapy of olfactory function in CRS is to treat the underlying sinonasal condition.[29] Accordingly, olfactory function has been shown to respond to both medical and surgical interventions in CRS (Table 1).

4.1.1 | Conservative approaches

Standard conservative treatment of CRS is based on glucocorticosteroids, administered orally, for example, prednisolone, or topically via nasal spray/drops, for example, fluticasone, mometasone, or beclametasone. Both administration forms have been shown to be effective in improving olfactory function in CRS patients: A meta-analysis showed that orally administered glucocorticosteroids improve both self-rated and psychophysically assessed function, compared to placebo, included studies used prednisolone (30-50 mg/day) for 14 days and 32 mg methylprednisolone tapered off over 20 days compared to placebo.[99,100] However, oral steroids seem to improve olfaction only for a short period of time (8-12 weeks).[101] Topical steroids have also been shown to be effective in terms of subjectively rated olfactory function. However, as discussed above, subjective and objective testing have been shown to correlate poorly—with subjective ratings being confounded by numerous factors including nasal airflow and the patients expectations.[27] Yousefi et al's study revealed there are not significantly improvement in olfactory threshold among 16 CRSsNP patients after 3 months topical corticosteroids and nasal irrigation of normal saline ($P = .311$ for men and $P = .139$ for women).[102]

The therapeutic efficacy of topical steroids seems to depend strongly on the mode of application.[103] Steroid nasal drops administered in the supine position with the head tilted back in patients with CRS and nasal polyps (CRSwNP) was prospectively shown to improve olfactory threshold and identification scores on the Conneticut Chemosensory Clinical Research Center (CCCRC) test.[104] In addition, work by Shu and colleagues showed that the application of nasal spray more directly to the OC using a longer applicator provides

TABLE 1 Anti-inflammatory approaches regarding olfactory function outcome to CRS patients (heterogeneous approaches in terms of methods used and groups of patients studied)

Reference	Anti-inflammatory approach (conservative, surgical, biologicals)	EG/ controls (n)	Patients phenotype	Follow-up	Measure of dfaction	Outcome (significance)
Vaidyanathan et al[100]	Oral corticosteroids	29/29	CRSwNP	2 wk	VAS for smell (scale 0-100)	Predicted mean difference = −28.3 ($P = .002$)
Yousefi et al[102]	topical corticosteroids and nasal irrigation of normal saline	16/17	CRSsNP	1, 3 mo	smell threshold test	Mean difference = .33 ($P = .31$ for men, $P = .14$ for women)
Dadgarnia et al[110]	ESS	40	CRSwNP	3 mo	Smell identification test (range 0-24)	Mean difference = 2.3 ($P = .001$)
Paksoy et al[111]	Surgical and standardized medical treatment	30	CRSwNP	3 mo	Sniffin' Sticks (range 1-48)	Mean difference = 6.8 ($P < .001$)
Wong et al[112]	Draf III surgery Frontal Sinus Surgery	104	recalcitrant CRS	30.6 mo	Likert scale for smell (range 0-5)	Significant improvement (71% vs 28%, $P < .01$)
Bachert et al[11]	Dupilumab	438/286	CRSwNP	24 wk	UPSIT (range 0-40)	Predicted mean difference = 11.3 ($P < .0001$)

Abbreviations: CRS, chronic rhinosinusitis; CRSsNP, chronic rhinosinusitis without nasal polyposis; CRSwNP, chronic rhinosinusitis with nasal polyposis; EG, experimental group; ESS, endoscopic sinus surgery; UPSIT, The University of Pennsylvania Smell Identification Test; VAS, visual analogue scale.

table 6-1

significantly better results in terms of olfactory outcome compared to conventional nasal sprays.[105]

Separate from anti-inflammatory effects, evidence from animal work shows that glucocorticoids can upregulate cyclic nucleotide-gated (CNG) channels, so potentially enhancing olfactory receptor response (olfactory receptors are G-protein coupled receptors that involve the activation of CNG during their transduction cascades). In addition, glucocorticoids can increase the production of intracellular cAMP within olfactory receptor neurons, again enhancing transduction cascades.[106] Finally, glucocorticoids can induce the apoptosis of mature olfactory receptor neurons, and cytokines released after apoptosis can support the regeneration of olfactory receptor neurons.[107]

When systemic steroids are considered, there is no widely accepted agreement regarding the dose, frequency and duration of use. Notably, there is little research where patients have been followed up for a year or longer, there is still a lack of knowledge about the long-term efficacy of steroids in the treatment of smell loss secondary to CRS. Furthermore, the association of osteonecrosis with the use of systemic steroids cannot be ignored by doctors and patients.[108]

Currently existing evidence does not provide support for improvement of olfactory function with antibacterial and antifungal treatment.[99]

4.1.2 | Surgical approaches

Endoscopic sinus surgery (ESS) for patients with CRS is the most common and important therapy for medically refractive patients.[6] The goal of ESS for CRS is to clear polyps and excess polypoid tissue, optimize sinus function, and facilitate use of topical treatments, all of which help improve the inflammatory response and might restore olfactory function. CRS associated olfactory function has been shown to benefit from ESS.[94,109-111] Significant olfactory improvement was also observed in recalcitrant CRS by Draf III sinus surgery.[112] Revision ESS has been shown to restore odor identification abilities in nearly half of patients studied.[113] However, some patients experience no improvement or even deterioration in olfactory function after surgery. For example, Jiang et al showed that surgery seems to have no improvement on olfactory function for patients with medically refractory symptoms.[114] One large prospective study (n = 775) using standardized odor identification tests demonstrated that only 23% of CRS patients received olfactory improvement, 68% no improvement, and 9% deterioration after ESS.[115] In contrast, in a 5 year follow-up study in 34 patients, 9% of CRS patients had no improvement and 6% had deterioration after ESS, based on measures of odor thresholds.[116] Therefore, olfactory outcomes after ESS are variable, and it remains challenging to predict surgical outcome in individuals.

ESS for CRS can also help to improve overall QoL, and smell improvement is positively associated with patients' QoL changes. An increase in olfactory function of 4.75 points on the composite threshold + discrimination + identification Sniffin' Sticks score is considered the cutoff point for clinically significant QoL recovery.[117] Notably, olfactory dysfunction before ESS has been described as a helpful predictor for postoperative QoL outcomes.[118] Thus, olfactory assessment is an important preoperative step for case selection and counseling regarding expected surgical outcomes.

Many studies have attempted to find reliable predictors of olfactory outcome after ESS. Nasal polyposis seems to play a key role in olfactory function in CRS patients.[94,115,119] Previous work has shown only 13.5% of CRSwNP patients to be normosmic, and about half of such patients to be anosmic, in this study, ESS significantly improved severely impaired olfactory function in CRSwNP patients at 6 months after treatment.[120] Polyposis of the OC is crucial and should receive special attention.[121] Of note, co-existent pathology in CRSwNP, for example, respiratory epithelial adenomatoid hamartomas (REAH), a benign tumor, present in 48% of biopsied oedematous OCs in the study by Lorentz et al, should be included in the differential diagnosis of nasal polyposis.[122] Nasal polyposis is significantly associated with better outcomes in postoperative olfactory function.[94] Other research has indicated the following predictors for a positive surgical outcome: anosmia, no prior surgery, opacification of the OC, and favorable wound healing status.[123-127] Furthermore, olfactory changes after administration of systemic glucocorticosteroid therapy predicts the olfactory outcome after sinus surgery in CRSwNP.[128]

ESS with steroid nasal spray has been compared to steroid nasal spray alone in a recent prospective study. In both groups olfactory function improved after treatment. However, remission rate was greater in the ESS group (60%) compared to the conservative group (20%).[129] In a prospective, multi-center study, CRS patients treated with ESS had better olfactory function than who were treated with medication.[130] in contrast, some studies revealed that there were no significant differences in olfactory function between ESS and standard medical therapy groups.[102,131] These differences in outcome may be due in part to the heterogeneity in patients involved and to methods used for assessment. Further studies involving larger samples of participants and more sensitive, unified measures of olfaction are required.

Finally, olfactory deterioration has been considered the most sensitive indicator for CRS recurrence.[132,133]

4.1.3 | Use of biologicals

If surgical and medical treatment fails, biologicals can be considered for a growing number of CRS patients. Dupilumab, a humanized monoclonal IgG4 antibody directed against the interleukin-4 receptor α (IL-4Rα) subunit, is the first targeted biologic therapy for the treatment of CRSwNP, which was approved in the European Union and the USA in 2019. Significant loss of smell has become one of six criteria needed to use biologicals.[6] Placebo-controlled clinical studies have demonstrated efficacy of dupilumab in improving clinical aspects of CRS, based on endoscopy, radiography and measures of QOL. The sense of smell improved from baseline rapidly (within the first 4 weeks) and significantly (by more than 10 points after 24 weeks, using the 40-item Smell Identification Test).[11] Omalizumab, another antibody targeting IgE, has also be shown to improve olfactory

awareness scores in comparison to a control group.[134] Cavaliere reported a case study of a patient with olfactory dysfunction secondary to CRSwNP, the patient experienced complete recovery from anosmia with the anti-IL-5 monoclonal antibody mepolizumab treatment.[135] The range of antibodies targeted in the treatment of CRS can be expected to continue to expand. Furthermore, biological treatments may be combined with surgery or glucocorticosteroids in future care pathways.

As discussed above, olfactory function in CRS is significantly associated with the sinonasal inflammatory response, as well as response to anti-inflammatory treatments: e.g. application of glucocorticosteroids directly to the OC, polyp removal from the OC, or biological treatment. Olfaction can therefore be used as a marker for inflammatory state and to predict response to treatment.

5 | CONCLUSION

Olfactory dysfunction, with a high prevalence in CRS patients, has a significant impact on health and QOL. Detailed assessment of olfactory function should be considered in the clinical evaluation of CRS patients, especially with well-established and reliable psychophysical testing, not only for detecting and quantifying patients' symptom, but also because it is useful to objectively assess the efficacy of CRS treatment over time. In particular, olfactory function seems to be a stable and valid factor in the various clusters of clinical presentations, linked with certain inflammatory patterns and reflective of the response to anti-inflammatory treatment. Accordingly, olfaction may act as a marker in the progression of chronic sinonasal inflammation, help to differentiate CRS phenotypes and endotypes and ultimately aid in the development of tailored treatment regimens.

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CONFLICT OF INTEREST

Xiaoguang Yan, Katherine Lisa Whitcroft, and Thomas Hummel declare that they have no conflict of interest.

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7 Publication 2 (second study) The shape of the OB predicts olfactory function (Yan et al., 2022)

7.1 Abstract 2

Objectives: The olfactory bulb (OB) plays a key role in the processing of olfactory information. A large body of research has shown that OB volumes correlate with olfactory function, which provides diagnostic and prognostic information in olfactory dysfunction. Still, the potential value of the OB shape remains unclear. Based on our clinical experience we hypothesized that the shape of the OB predicts olfactory function, and that it is linked to olfactory loss, age, and gender. The aim of this study was to produce a classification of OB shape in the human brain, scalable to clinical and research applications.

Methods: Patients with the 5 most frequent causes of olfactory dysfunction (n= 192) as well as age/gender-matched healthy controls (n= 77) were recruited. Olfactory function was examined in great detail using the extended “Sniffin’ Sticks” test. A high-resolution structural T2 weighted MRI scan was obtained for all. The planimetric contours (surface in mm^2) of OB were delineated manually and then all surfaces were added and multiplied to obtain the OB volume in mm^3. OB shapes were outlined manually and characterized on a selected slice through the posterior coronal plane tangent to the eyeballs. We looked at OB shapes in terms of convexity and defined 7 categories based on OB contours: olive, circle, plano-convex, banana, irregular, plane and scattered. Categorization of OB shapes is possible with a inter-rater agreement (Cohen’s Kappa = 0.73).

Results: Our results suggested that OB shapes in patients with olfactory dysfunction are significantly different from healthy individuals. They were correlated with olfactory function in the whole group, independent of age, gender and OB volume. Moreover, OB shapes seemed to change with age in healthy subjects. Importantly, we found that OB shapes were also associated with different causes of olfactory disorders.

Conclusion: Our study provides first evidence that the shape of the OB may be used as a biomarker for olfactory dysfunction.

brain sciences

MDPI

Article

The Shape of the Olfactory Bulb Predicts Olfactory Function

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Abstract: The olfactory bulb (OB) plays a key role in the processing of olfactory information. A large body of research has shown that OB volumes correlate with olfactory function, which provides diagnostic and prognostic information in olfactory dysfunction. Still, the potential value of the OB shape remains unclear. Based on our clinical experience we hypothesized that the shape of the OB predicts olfactory function, and that it is linked to olfactory loss, age, and gender. The aim of this study was to produce a classification of OB shape in the human brain, scalable to clinical and research applications. Results from patients with the five most frequent causes of olfactory dysfunction ($n = 192$) as well as age/gender-matched healthy controls ($n = 77$) were included. Olfactory function was examined in great detail using the extended "Sniffin' Sticks" test. A high-resolution structural T2-weighted MRI scan was obtained for all. The planimetric contours (surface in mm^2) of OB were delineated manually, and then all surfaces were added and multiplied to obtain the OB volume in mm^3. OB shapes were outlined manually and characterized on a selected slice through the posterior coronal plane tangential to the eyeballs. We looked at OB shapes in terms of convexity and defined two patterns/seven categories based on OB contours: convex (olive, circle, and plano-convex) and non-convex (banana, irregular, plane, and scattered). Categorization of OB shapes is possible with a substantial inter-rater agreement (Cohen's Kappa = 0.73). Our results suggested that non-convex OB patterns were significantly more often observed in patients than in controls. OB shapes were correlated with olfactory function in the whole group, independent of age, gender, and OB volume. OB shapes seemed to change with age in healthy subjects. Importantly, the results indicated that OB shapes were associated with certain causes of olfactory disorders, i.e., an irregular OB shape was significantly more often observed in post-traumatic olfactory loss. Our study provides evidence that the shape of the OB can be used as a biomarker for olfactory dysfunction.

Keywords: olfactory bulb shape; deformation; olfaction; plasticity; MRI; anosmia; smell

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1. Introduction

The olfactory bulb (OB) plays an important role in the processing of olfactory information. From an evolutionary perspective, the OB is one of the earliest structures of the vertebrate brain to develop [1,2]. The OB has also been suggested to serve as a repository of resident progenitor cells in the mature human brain, receiving neuroblasts migrating to it via a lateral ventricular extension [3,4]. It is widely accepted that odor coding is based on the spatiotemporal pattern of activation of the olfactory glomeruli in the OB [5,6].

OB has gained attention in the field of clinical research. Using magnetic resonance imaging (MRI), olfactory dysfunction can be gauged based on OB volumetrics [7,8]. Cutoff

volumes separating normal volumes from hypoplastic ones have been established [9]. Results based on longitudinal studies showed how changes in OB volumes associate with improved olfactory function secondary to treatment [10]. Therefore, the assessment of OB structure is considered to provide diagnostic and prognostic information on a morphological basis. However, all the previous studies focused on the size and volume of the OB. They neglected the relation between OB shape and olfactory function.

To address this gap, the current study aimed (1) to explore potential differences in OB shapes between patients with the five most frequent etiologies of olfactory dysfunction and healthy controls and (2) to examine the relationship between OB shape and olfactory function, age, gender, and OB volume.

2. Methods

In this study, we used the data from datasets acquired within the context of six different studies. All these studies had been approved by the TU Dresden, Medical Faculty Ethics Review Board (EK96032015, EK348092018, EK56022016, EK262082010, EK203052017, EK 235072018). All subjects provided written informed consent before their detailed evaluation. A total of 192 patients (86 men) complaining of smell loss, with ages ranging from 24 to 81 years (mean ± standard deviation; 57 ± 12.8 years) and 77 healthy subjects (39 men), with ages ranging from 25 to 82 years (51 ± 15.5 years), were included in this cross-sectional, retrospective study. Patient groups consisted of subjects visiting the Smell and Taste Clinic of the Department of Otorhinolaryngology at TU Dresden starting from 2016 until 2020. Diagnosis of acquired olfactory dysfunction was made according to the recent Position Paper on Olfactory Dysfunction [11]. As part of their assessment, all patients underwent an evaluation that included an endoscopical examination of the nasal cavity, structured history collection, olfactory testing, and structural T2-weighted magnetic resonance imaging (MRI) [12]. Classification of the olfactory dysfunction was based on the underlying etiological conditions, i.e., olfactory dysfunction secondary to chronic rhinosinusitis (CRS), post-infectious olfactory dysfunction (PIOD), posttraumatic olfactory dysfunction (PTOD), olfactory dysfunction associated with neurological disease (patients with olfactory dysfunction related to Parkinson's disease were recruited in this study), and idiopathic olfactory dysfunction. A high-resolution structural T2-weighted MRI scan was obtained for all. Patients with congenital anosmia were excluded based on their history and brain MRI examinations. All healthy subjects had no history of an underlying or preceding major medical condition and reported a normal sense of smell. Subjects' demographic information and olfactory function are shown in Table 1.

Table 1. Demographic characteristics of patients, results from olfactory testing.

Characteristic	Before Matching	After Matching			
	Patients	Patients	Healthy Control	Statistics	*p*
Age (mean [SD])	57 [12.8]	51 [15.2]	51 [15.5]	$t = 0.07$	NS
Gender (number (%))					
Male (*n* (%))	86 (45)	39 (49)	39 (49)	$\chi^2 = 0$	NS
Female (*n* (%))	106 (55)	38 (51)	38 (51)		
Total (*n*)	192	77	77		
Smell function (mean [SD])					
TDI	18.1 [7.6]	18.6 [8.5]	33.5 [4.2]	$t = 13.7$	$p < 0.001$
T	2.7 [2.7]	3.2 [3.1]	7.4 [2.9]	$t = 8.6$	$p < 0.001$
D	8.2 [3.0]	8.4 [3.0]	12.5 [2.1]	$t = 10.2$	$p < 0.001$
I	7.4 [3.5]	7.4 [4.1]	13.6 [1.5]	$t = 12.3$	$p < 0.001$

TDI = Threshold-Discrimination-Identification; T = odor threshold; D = odor discrimination; I = odor identification; NS = not significant; SD = standard deviation.

table 7-1

2.1. MRI Acquisition

MRI acquisitions were performed on a 3T scanner (Siemens, Erlangen, Germany) with an 8-channel phase-array head coil. A standardized MRI for structural analyses was performed for all subjects, targeting structures that consisted of the left and right OB with a coronal T2-weighted fast spin-echo sequence: TR/TE = 4800/152 ms; slice thickness 2 mm; matrix size 256 × 256; 30 slices; averages 2; in-plane resolution 0.4 × 0.4 mm and no intersection gap) covering the anterior and middle segments of the base of the skull.

2.2. Olfactory Testing

Quantitative testing of olfactory function was performed using the "Sniffin' Sticks" test (Burghart GmbH, Wedel, Germany) [13]. Odorants were presented with pen-like devices. It was comprised of three tests, namely odor threshold with 16 increasing concentrations of phenyl ethyl alcohol using a single staircase, 3-alternative forced choice (3AFC) procedure (range of scores: 1 to 16). Odor discrimination was determined with a 3AFC procedure and 16 triplets (range of scores 0 to 16). Odor identification was tested using a 16-item multiple forced choice test (range of scores 0–16). The three subsets of this test were summated into the so-called "threshold-discrimination-identification (TDI) score", leading to a maximum score of 48. For odor presentation, the pen's cap was removed by the experimenter for approximately 3s and the felt tip was placed approximately 2 cm in front of the subjects' nostrils.

2.3. Evaluation of the Volume of the OB

We randomly chose 90 subjects (45 patients and 45 age- and sex-matched healthy control) from the cohort investigated here to evaluate the OB volume. The OB volume was measured on coronal T2-weighted MR images with ITK-SNAP software (Version 3.8.0, Pennsylvania, PA, USA). The planimetric contours (surface in mm^2) of OB were delineated manually by an experienced observer blinded to the subjects. Then, all surfaces were added and multiplied to obtain the volume of OB segment in mm^3. This approach of calculating OB volumes has been shown to be highly reliable and accurate [7,14,15].

2.4. Evaluation of the Shape of the OB

MRI scans were examined using ITK-SNAP software (Version 3.8.0, Pennsylvania, PA, USA), which allows images to be viewed in three orthogonal planes. Configurations of cross-sectional areas of OB were visually inspected in the slice through the most posterior coronal plane tangent to the eyeballs, in accordance to previous research [16]. Heuristically, based on opinions from a small group of experts, we chose to look at the shape of the OB according to its integrity and divided OBs into those homogeneous and scattered OBs. In homogeneous OB contours, the shape was considered by its outline as convex, concave, or plane. Convex OBs curve outward; concave OBs curve inward; and plane OBs are flat on both sides. The convex OB shape is then subdivided further into an olive shape, circle, or a plano-convex shape. For the concave OB shape, either the banana shape or an irregular shape is determined (Figures 1 and 2) [17,18]. Images were assessed by a trained clinical expert (YZ) who was blinded to other clinical data of the respective cases. To assess inter-observer reliability, 107 cases were randomly analyzed by the observers (YZ and AJ). The inter-rater agreement between two independent persons analyzing the OB shape was substantial (Cohen's Kappa = 0.73, 95% CI = 0.60–0.86, $p < 0.001$).

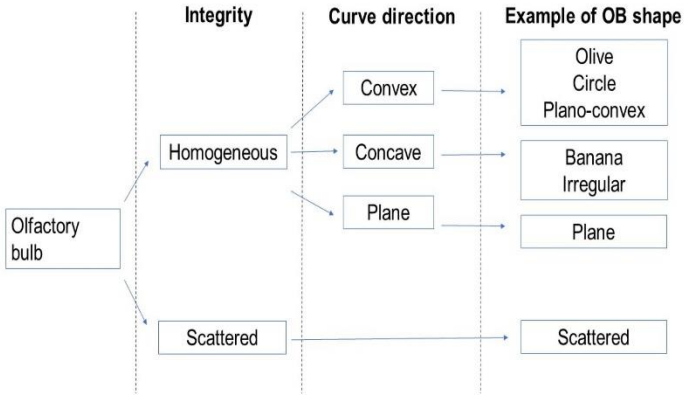

Figure 1. Classification of OB shapes.

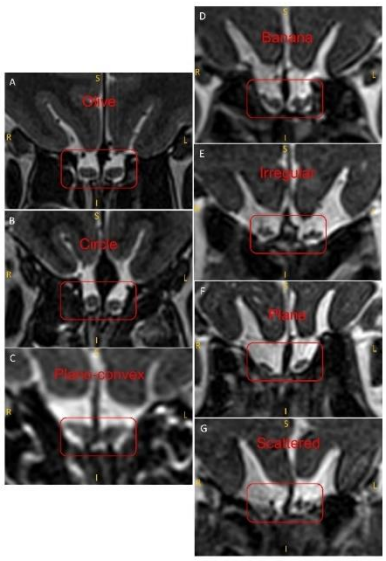

Figure 2. OB shapes are shown in the coronal T2-weighted MR image (red box) (R-Right, L-Left, S-Superior/Dorsal, I-Inferior/Ventral). (**A**) 23-year-old female healthy control (TDI = 37.25) with an olive-shaped OB; (**B**) 31-year-old healthy male control (TDI = 33.5) with circular OB; (**C**) 55-year-old woman with post-viral olfactory function (TDI = 12) demonstrating plano-convex OB; (**D**) 55-year-old man with post-viral olfactory function (TDI = 15) demonstrating banana-shaped OB; (**E**) 57-year-old woman with post-traumatic olfactory function (TDI = 23) showing irregular OB; (**F**) 54-year-old woman with post-viral olfactory function (TDI = 15) demonstrating plane OB; (**G**) 89-year-old woman with post-viral olfactory function (TDI = 20.5) demonstrating scattered OB. TDI = Threshold-Discrimination-Identification.

Figure 7-1

Figure 7-2

2.5. Statistical Analysis

Seventy-seven patients were randomly selected and individually matched by age (within ± 2 years) and gender with the healthy group. OB shapes distribution were compared between patients and healthy controls using chi-squared tests. A chi-squared test was performed to compare the two OB shape patterns for gender differences. ANOVA and Student's *t*-test were computed to examine the relation between OB shapes, age, and causes of smell loss. A non-parametric Mann–Whitney U test was applied to compare olfactory function among groups with different OB shape patterns. Multivariate linear regression analyses were performed using TDI, T, D, and I as dependent variables, with the shape of OB, age, and gender defining the set of independent variables. Moreover, differences for OB shapes, age, gender, and olfactory function were compared separately in patients and healthy controls. Statistics and graphical visualization were performed using GraphPad Prism 9.3.1 (GraphPad Software, Inc., La Jolla, CA, USA). A *p*-value threshold of 0.05 was used to define statistical significance. As the olfactory system tends to exhibit a right-sided dominance in processing, in our study statistical analyses were only performed based on the data from the right OB [19].

3. Results

3.1. Participant Demographics

A total of 192 patients with five different causes of olfactory dysfunction and 77 healthy controls were included in this study. Then, 77 patients were individually matched with the control group in term of age and gender. See Table 1 for the subjects' demographic characteristics and olfactory function scores. We found no significant differences in age ($t = 0.07$, $p > 0.05$) and gender ($\chi^2 = 0$, $p > 0.05$) between cohorts with patients or healthy controls. As expected, significant differences were found in olfactory function between the patients and healthy controls ($t = 13.7$, $p < 0.05$).

3.2. Comparison of Patients and Controls in Terms of OB Shapes

Across matched groups (patients, $n = 77$; healthy control, $n = 77$), the data showed that olive-shaped (probability of occurrence (PO) = 28%) and banana-shaped OBs (PO = 21%) were the two most common types of OB shapes (Figure 3). Among patients, the banana shape (PO = 32%) was the most common shape, while in controls the olive-shaped (PO = 44%) was most frequent. The frequency of OBs with non-convex shape was significantly higher in patients with olfactory dysfunction (PO = 66%) than in healthy controls (PO = 29%) ($\chi^2 = 21.9$, $p < 0.001$) (Figure 4).

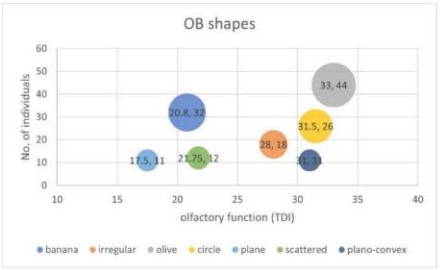

Figure 3. Bubble chart shows median TDI olfactory scores and number of investigated participants for OB shapes within each bubble (TDI scores; number). The size of the bubble indicates the number of individuals with the respective characteristic. Olive-shaped (28%, 44/154) and banana-shaped OBs (21%, 32/154) were the two most common types of OB shapes. Olive-shaped, circle, and plano-convex OB shapes had relatively higher median TDI total scores than other OB shapes. TDI = Threshold-Discrimination-Identification.

Figure 7-3

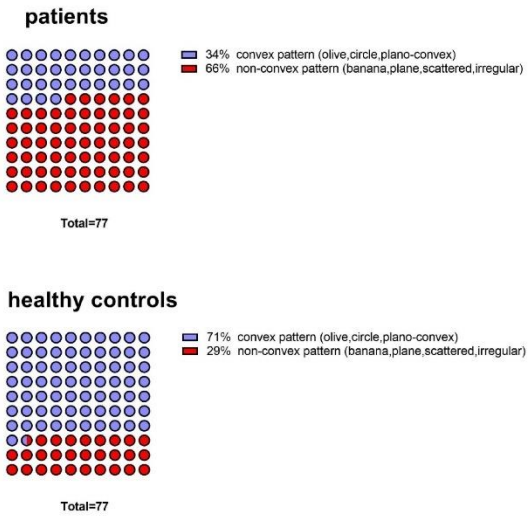

Figure 4. Pattern of OB shapes in patients and healthy controls.

3.3. *Correlation of OB Shapes with the Olfactory Function*

Our results indicated an association between specific OB shapes and olfactory function. Across matched subjects, olive-shaped OB had the highest median TDI score (TDI = 33) (Figure 3). The plane OB shape had the lowest median TDI score (TDI = 17.5) (Figure 3). More interestingly, our results suggest that the TDI scores of the convex OB patterns, i.e., olive, circle, and plano-convex OBs, were associated with relatively higher olfactory scores than the other OB types. Statistical analysis showed that the non-convex OB pattern, in comparison to the convex OB pattern, came with a significantly lower olfactory function, defined by olfactory threshold, odor discrimination, odor identification, and the composite olfactory test score (U = 1644.50, U = 1967.50, U = 1869.00, and U = 1703.50, respectively; $p < 0.001$) (Table 2 and Figure 5). Results from multiple regression analyses showed that differences in the shape of the OB, observed in relation to olfactory function, was independent of age, gender, and OB volume ($p < 0.05$). Multiple logistic regression adjusted for age, gender, and OB volume indicated an association with olfactory dysfunction for OB shape (odds ratio [OR] convex vs non-convex, 4.5 [95% CI, 2.0–6.5]).

Table 2. Comparison between convex pattern and non-convex pattern across matched groups.

Characteristic	Convex Pattern	Non-Convex Pattern	Statistic	*p*
Age (mean [SD])	51 [15.2]	51 [15.5]	$t = 0.106$	NS
Gender, *n* (%)				
Male (*n* (%))	44 (54)	34 (47)	$\chi^2 = 0.922$	NS
Female (*n* (%))	37 (46)	39 (53)		
Total (*n*)	81	73		
Smell function (median)				
TDI	32.8	21.5	U = 1703.500	$p < 0.001$
T	6.5	2.3	U = 1644.500	$p < 0.001$
D	12	10	U = 1967.500	$p < 0.001$
I	13	9	U = 1869.000	$p < 0.001$

NS = not significant; TDI = Threshold-Discrimination-Identification; T = odor threshold; D = odor discrimination; I = odor identification; SD = standard deviation.

table 7-2

Figure 7-4

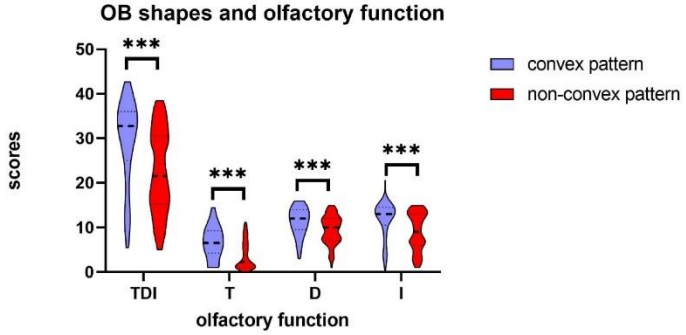

Figure 5. Violin plot comparisons of olfactory scores between subjects with convex pattern OB and subjects with non-convex pattern OB showing the median (a black dash line in the center of violin), and interquartile range (the black dot line on the violin plot). Subjects with non-convex pattern OB had significantly lower TDI total scores and T, D, and I component scores compared to subjects with convex pattern OB (all *P*'s < 0.001), *** $p < 0.001$. TDI = Threshold-Discrimination-Identification. T = odor threshold; D = odor discrimination; I = odor identification.

Neither in the group of patients nor in the group of healthy controls did we observe significant differences in the TDI score between convex and non-convex OB pattern groups (patients: t = 0.69, $p = 0.49$, controls: t = 1.91, $p = 0.06$).

3.4. OB Shape and OB Volume

Compared with subjects with non-convex OB shape, subjects with convex OB shape had a significantly higher OB volume (47.0 ± 16.0 mm^3 vs. 37.8 ± 18.3 mm^3, $p < 0.05$). There were no significant differences among the seven types of OB shape and OB volume ($F = 1.77$, $p > 0.05$).

3.5. Correlation of OB Shape with Age

Across matched subjects, there was no significant difference in age between the convex and non-convex OB pattern group (t = 0.10, $p = 0.92$).

Subjects with scattered a OB shape had the highest average age (75 ± 9.1 years, $n = 3$) compared to other OB shapes (olive: 48 ± 13.9 years, $n = 33$; circle: 55 ± 14.8 years, $n = 16$; triangle: 43 ± 15.2 years, $n = 6$; banana: 55 ± 14.7 years, $n = 8$; strip: 42 ± 3.8 years, $n = 3$; irregular:51 ± 21.7 years, $n = 8$). Indeed, the frequency of non-convex OB patterns was significantly higher in the group of older healthy subjects (age > 60 years, $n = 21$, 9/21 = 43%) compared to younger subjects (age ≤ 60 years, $n = 56$, 11/56 = 20%) ($\chi^2 = 4.28$, $p < 0.05$). However, the mean age of subjects with a convex pattern (50 ± 14.3 years, $n = 57$) and a non-convex pattern (54 ± 18.6 years, $n = 20$) was statistically not different between groups (t = 1.17, $p = 0.25$).

3.6. OB Shapes Associated with the Causes of Olfactory Loss in Patients

According to the causes of smell impairment, patients were categorized into five groups. Group 1 (G1) consisted of 57 patients with an idiopathic smell disorder. Group 2 (G2) contained 31 patients with smell impairment second to CRS, group 3 (G3) 58 patients with postinfectious olfactory loss, group 4 (G4) 45 patients with post traumatic olfactory loss, and group 5 (G5) with 11 patients with idiopathic Parkinson syndrome. Prevalence of OB shapes in patients with different causes were shown (Table 3). Additionally, we found that an irregular OB shape was significantly more often observed in post-traumatic olfactory loss ($n = 45$, 12/45 = 26.7%) than in other etiologies of olfactory loss (idiopathic: $n = 57$, 9/57 = 15.8%, $\chi^2 = 1.82$; postinfectious: $n = 58$, 9/58 = 15.5%, $\chi^2 = 1.94$; CRS:

Figure 7-5

$n = 21$, 3/21 = 14.3%, $\chi^2 = 1.25$; $p < 0.05$). There is no significant difference for the number of patients with convex and those with non-convex patterns among different causes of olfactory dysfunction ($\chi^2 = 5.5$, $p = 0.237$).

Table 3. Prevalence of OB shapes in patients with different causes.

Causes, OB Shape	Olive, no. (%)	Circle, no. (%)	Plano-Convex, no. (%)	Banana, no. (%)	Irregular, no. (%)	Plane, no. (%)	Scattered, no. (%)	Total
Sinonasal	4 (19.0%)	3 (14.3%)	2 (9.5%)	6 (28.6%)	3 (14.3%)	1 (4.8%)	2 (4.8%)	21
idiopathic	6 (10.5%)	5 (8.8%)	1 (1.8%)	18 (31.6%)	9 (15.8%)	4 (7.0%)	14 (24.6%)	57
Parkinson's disease	1 (9.1%)	4 (36.4%)	0 (0.0%)	3 (27.3%)	3 (27.3%)	0 (0.0%)	0 (0.0%)	11
PIOD	3 (5.2%)	9 (15.5%)	7 (12.1%)	15 (25.9%)	9 (15.5%)	11 (19.0%)	7 (12.1%)	58
PTOD	9 (20.0%)	0 (0.0%)	4 (8.9%)	11 (24.4%)	12 (26.7%)	3 (6.7%)	4 (8.9%)	45
Total, no.	53	36	23	21	19	26	14	192

PIOD = post-infectious olfactory dysfunction, PTOD = posttraumatic olfactory dysfunction.

4. Discussion

Our study proposes a new framework to classify the shape of the human OB and provides first evidence of the association between OB shape and olfactory dysfunction, using a large sample size of healthy controls and patients with different types of olfactory dysfunction. Our observations indicate the presence of morphological changes in the OB, from regular to irregular and from integrity to disintegration. These changes are linked to human olfactory function, even when adjusting for age, gender, and OB volume. In addition, we found that the OB exhibits increased deformation with age across healthy subjects. Furthermore, we found that an irregular OB shape was significantly more often observed in post-traumatic olfactory loss.

In addition to the typical olive-shaped OB, which reflects a normal, healthy status of the OB, various other OB shapes were also observed in this study, for example, the banana-shaped OB with the convexity of the walls. These forms are common in patients with smell deficits and are also found in healthy subjects. Such an overlap in OB shapes between patients and controls could be at least partly explained by the fact that some controls may have olfactory dysfunction without being aware of it [20,21]. Consistent with this, previous studies have described the variability of the OB structure, and especially the OB volume. Buschhüter et al. [9] reported that OB volumes decrease with age in healthy people. They proposed normative data of OB volume in relation to age based on results from 125 patients. Furthermore, a decrease of OB volume has also been reported in patients with olfactory dysfunction due to various causes [14–16,22]. Moreover, in a longitudinal study, Gudziol et al. [10] described an OB change post treatment in 19 CRS patients, which was correlated with olfactory function. Another study, following a period of 4 months of olfactory training, showed that 97 healthy people exhibited an average increase of OB volume [23].

At a macroscopic level, Burmeister et al. [18] depicted laminar patterns of the human OBs ($n = 24$). They identified three layers in 8.3%, two layers in 83.3%, and one layer in 8.3% of their subjects using high resolution 3T MRI. At a cellular level, the spatial distribution of glomeruli in the human OB is irregular and complex [24] and shows higher variability than what is seen in animals [25,26].

The structural variability in OB shapes and volumes appears to be an expression of OB plasticity. As the OB receives olfactory input from the olfactory epithelium, it is natural to think that OB structure is altered secondary to reduced sensory input. In fact, OB volume has been observed to be associated with nasal septal deviation, with OB volume being significantly lower at the narrower side, which may indicate a "bottom-up modulation" of the OB [27]. Importantly, the OB also appears to be subject to "top-down modulation", showing in some neurodegenerative diseases as well as mental disorders, which are associated with a smaller OB volume [28–30]. Top-down modulation also became evident in an experiment on lateralized olfactory exposure ("olfactory training") in healthy

table 7-3

subjects who exhibited an increased OB volume not just on the side exposed to odors but on the ipsi- and contralateral sides [31].

OB plasticity may depend on numerous factors that are currently discussed, for example, (1) continuous neuronal supply from the subventricular zone (SVZ), where young neurons migrate within the rostral migratory stream and replace interneurons (periglomerular cells and granular cells) in the OB [3]; (2) continuous synaptogenesis with dendrites of mitral/tufted cells occurring from incoming axonal projections of olfactory receptor neurons at the glomerular level; and (3) in a recent animal study, a new form of structural remodeling of adult-born OB neurons suggest direct neurogenesis within the OB itself [32]. Emerging evidence for OB plasticity indicates that OB is not just a static relay station, but dynamically processes olfactory information based on experience and context [33].

Considering that pronounced morphological changes of the olfactory epithelium are found with anosmia or aging, and that information regarding individual odorant processed by olfactory sensory neurons (OSNs) in the olfactory epithelium (OE) is integrated into distinct subsets of glomeruli in a stereotyped region of the OB, we hypothesize that changes of patterns at the level of the OE may induce specific changes in OB shape [34–36].

The association between shape changes of sensory related regions and function have been reported, for example for the auditory system or the visual systems [37,38]. For the olfactory system, findings from the present study are in line with prior evidence suggesting that the OB structure is associated with olfactory function. Such correlation has been shown not only for overall olfactory ability, but also for the subcomponent's odor identification and odor threshold [15,39,40]. Even dynamic changes in odor threshold were significantly and positively correlated with changes in OB volume [10]. However, the volumetric evaluation of OB is not yet a routine procedure for patients with olfactory dysfunction in a typical outpatient clinic, since it requires special software and some time needed for OB segmentation. Hence, our study presents an easier, simpler method to visually classify and assess OB deformation, with a large potential for clinical application as a biomarker for patients with olfactory dysfunction. For example, our study shows that a "plane" and "scattered" OB shape is generally associated with decreased olfactory function.

To note, the present results suggested that OB morphology and olfactory function correlate, but that a reliable individual diagnosis based on OB morphology alone is not possible. Although the group differences are clear, any one OB shape can be found in both patients and healthy controls. This overlap can partly explain that the banana OB shape is associated with lower olfactory function than what is found in subjects with an irregular OB shape, although both are concave shapes. Specifically, the irregular OB shape (44%, 8/18) is more often found in healthy controls than is the banana OB shape (25%, 8/18).

A clinically relevant finding was that we provided a new perspective to look at the OB shape in terms of the convexity—either a convex pattern or non-convex pattern. Non-convex OB patterns were significantly more often observed in patients than in controls. Therefore, the convexity of OB shape appears to reflect pathological changes of patients with olfaction dysfunction and can be a useful additional morphological parameter in the assessment of olfactory function.

The plano-convex OB shape was attributed to the convex OB shape pattern. Even if this subgroup is classified as a non-convex pattern, the difference between patients and healthy controls is still present in terms of the ratio between convex and non-convex patterns (in patients, convex:non-convex = 27:73; in healthy controls, convex:non-convex = 64:36, $\chi^2 = 27.6$, $p < 0.001$).

We found differences in age-related OB shape in healthy participants. In humans, an aged OB shows a decrease of the volume of the OB, the concentration of mitral cells per unit area, and both layer thickness and the number of glomeruli [41,42]. However, the results in rodents are more complex. In mice, OB volume does not change [43], or may even increase with age [44]. In the present study, the subjects with scattered OB shape were the oldest, suggesting an age-related degeneration of OB structures. It is unclear whether this change in OB shape is related, for example, to the OB microstructure or the turnover of

interneurons. Like other sensory systems, olfactory function decreases with increasing age, due to numerous reasons [45].

Also, in the current study, non-convex OB patterns occurred in the five most common etiologies for olfactory dysfunction with a high percentage. The highest prevalence of the irregular OB shapes was observed in post-traumatic olfactory dysfunction. This may result from the specific mechanisms, with a traumatic injury leading to the direct, sudden disruption of olfactory pathways, for example, direct shearing of the olfactory nerves and focal contusion within the OB. Of note, the olfactory system is among the earliest affected structures in neurodegenerative disease, such as Alzheimer's disease and Parkinson's disease. In the present study, the prevalence of non-convex OB shapes pattern was higher for subjects with neurodegenerative diseases in comparison to healthy controls.

Results from the current study support the idea of an association between OB shapes and olfactory function. Our findings raise numerous questions that future studies should address, namely (1) whether OB shapes can be complementary to volumetric assessment of longitudinal changes, (2) whether OB shapes are helpful in terms of prognostic information in patients with olfactory dysfunction, and (3) whether, and if so, how treatment induces changes in OB morphology. Overall, in this study, we visually classified and qualitatively assessed OB shapes, which is easy and simple in order to screen OB deformations. We deliberately pursued this path in order to provide a simple clinical metric to obtain more detailed information on olfactory dysfunction. Still, the current results suggest that OB morphology contains information on olfactory function, and possibly also on the prognosis of the disorder. Further research should provide more objective measures to characterize the OB shape and increase the inter-rater agreement.

5. Conclusions

The variability of OB shape was examined in humans. The categorization of OB shapes is possible with a good inter-rater agreement. Non-convex OB patterns were significantly more often observed in patients than in controls. Additionally, there is an association between OB shape and olfactory function. In healthy subjects, OB shape seemed to change with age. The results also indicated that OB shapes were associated with certain causes of olfactory disorders, i.e., an irregular OB shape was significantly more often observed in post-traumatic olfactory loss.

Author Contributions: Conceptualization, X.Y. and T.H.; methodology, X.Y. and T.H.; software, X.Y.; validation, X.Y., A.J., Y.Z., F.A., H.M.F. and T.H.; formal analysis, X.Y.; investigation, X.Y, A.J. and Y.Z.; resources, T.H.; data curation, T.H.; writing—original draft preparation, X.Y. and T.H.; writing—review and editing, X.Y., A.J., Y.Z., F.A., H.M.F. and T.H.; visualization, X.Y. and T.H.; supervision, T.H.; project administration, T.H.; funding acquisition, T.H. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: All subjects provided written informed consent before their detailed evaluation.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author (Xiaoguang YAN, M.D., Smell and Taste Clinic, Department of Otorhinolaryngology, TU Dresden, Fetscherstrasse 74, 01307 Dresden, Germany; Tel.: +49-351-458-4189; Fax: +49-351-458-4326; xiaoguang.yan@tu-dresden.de). The data are not publicly available due to their containing information that could compromise the privacy of research participants.

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Conflicts of Interest: All authors declare that they have no conflict of interest.

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8 Publication 3 (third study) Anterior skull base abnormalities in congenital anosmia (Yan et al., 2023)

8.1 Abstract 3

Introduction: The structures of the skull and the brain are related to each other. Prior work in individuals with isolated congenital anosmia (ICA) showed that these individuals were characterized by olfactory bulb (OB) defects. The aim of this study was to compare the morphological pattern of anterior skull base surrounding the OB between individuals with ICA and normosmic controls. We meant to investigate whether these features can help to distinguish abnormalities from normal variation.

Methods: We conducted a retrospective study to acquire T2 weighted magnetic resonance images from individuals diagnosed with ICA (n=31) and healthy, normosmic controls matched for age and gender (n=62). Between both groups, we compared the depth and width of the olfactory fossa, angle of ethmoidal fovea as well as angle of lateral lamella of cribriform plate. Within the ICA group we further performed subgroup analyses based on the presence or absence of the OB, to investigate whether the morphology of the anterior skull base relates to the presence of OBs. The diagnostic performance of these parameters was evaluated using receiver operating characteristic analysis.

Results: Individuals with ICA exhibited a flattened ethmoid roof and shallower olfactory fossa when compared to controls. Further, the absence of the OB was found to be associated with a higher degree of flattening of the ethmoid roof and a shallow olfactory fossa. We reached the results in the following areas under the receiver operating characteristic curves (AUC): 0.80 - angle of fovea ethmoidalis, 0.76 - depth of olfactory fossa, 0.70 - angle of lateral lamella of cribriform plate for significant differentiation between individuals with ICA and normosmic controls.

Conclusion: Individuals with ICA exhibited an unusual anterior skull base surrounding the OB. This study supports the idea of an integrated development of OB and anterior skull base. Hence, the morphological pattern of the anterior skull base surrounding the OB helps to distinguish individuals with ICA from normosmic

controls, and may therefore be useful for the diagnosis of ICA, although it is certainly not an invariable sign of congenital anosmia.

Running title:
anterior skull base in congenital anosmia

TYPE OF ARTICLE
ORIGINAL CONTRIBUTION

Anterior skull base abnormalities in congenital anosmia

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ABSTRACT

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Key words：Anterior skull base, morphology, isolated congenital anomia, MRI

INTRODUCTION

The intimate structural relationship between the skull and the brain has long been recognized in both evolutionary biology and clinical medicine. Research has consistently shown skull-brain morphological integration during hominin evolution[1,2]. Skull development is highly coordinated. Unique changes in skull morphology, including a domed cranial vault, highly flexed cranial base, and retracted facial skeleton, are believed to be a direct result of a dramatic increase in brain size[3,4]. Data by Trygve and his colleagues obtained in a European population suggested a tight correspondence between skull and brain morphology[5]. In fact, skull malformations (e.g., craniosynostosis) and brain malformations (e.g., holoprosencephaly) can affect skull development and brain growth and vice versa.

The olfactory bulb (OB), located at the base of the anterior cranial fossa, is the critical first relay of the olfactory system. Congenital absence of the OB may co-occur with other brain malformations, for example holoprosencephaly which is the most common abnormality of brain development affecting the forebrain and the facial features in liveborn infants[6]. OB defect can also present as an isolated finding or as a component of chromosomopathies (e.g. CHARGE syndrome) or endocrinopathies (e.g. Kallmann syndrome)[7,8]. What's more, absence of OBs occurs frequently as an isolated finding.

OB defect largely is found in congenital anosmia, characterized by a complete lack of olfactory perception since birth and aplasia or hypoplasia of the OB[9]. Accordingly, congenital anosmia can be combined with other anomalies, with Kallmann syndrome being the best documented, or without evidence of other defects, termed isolated congenital anosmia (ICA). The estimated prevalence of ICA is between 1 in 2000 to 1 in 8000, and it is now thought to be the most common form of congenital anosmia[10–12]. Brain structural changes, such as reduced depth of the olfactory sulcus, regularly accompanies OB defects, and are considered a useful clinical indicator of congenital anosmia[13]. Structural alterations are not limited to the OB and the olfactory sulcus, as the entorhinal and piriform cortices appear to be thicker in congenital

anosmia[14]. Given the close relationship between skull and brain form, it follows that the nearby skull may also differ in size and shape in individuals with OB defect. However, not much attention has been paid to changes of bony structures neighboring the OB due to congenital factor, even though alterations of the anterior skull base have been reported in Kallmann Syndrome [15] .

The anterior skull base consists of the cribriform plate and fovea ethmoidalis, an extension of the frontal bone orbital plate, separating the anterior cranial fossa superiorly and paranasal sinuses ventrally. The lateral lamella joins the cribriform plate to the fovea ethmoidalis, forms the lateral wall of the olfactory fossa. The configuration of the anterior skull base is highly variable. The depth of the olfactory fossa was described and classified by Keros into three main types: Keros type 1 (<3 mm), type 2 (4-7 mm), type 3 (8-16 mm)[16]. The fovea ethmoidalis can also vary considerably with intraindividual asymmetry. In fact, Lebowitz et al. reported that 9.5% (19/200) CT scans show an asymmetry of the height of the fovea ethmoidalis[17].

To date, there has been no research investigating the relationship between an OB defect and the structure of the surrounding skull base in the context of ICA. The presence of skull base anomalies in ICA might contribute to the understanding of its pathogenesis; the existence or lack of a structural association between skull base and OB may also provide more information as to the etiology of this process. Our hypothesis was that the morphology of the anterior skull base, as measured in terms of the depth as well as width of olfactory fossa, angle of lateral lamella of cribriform plate (LLCP), and angle of fovea ethmoidalis, is different between ICA participants and healthy controls. We further hypothesized that in ICA the morphology of the anterior skull base is correlated to OB abnormalities, and that the morphological parameters are correlated with each other.

The aim of this study was to identify and compare the morphological pattern of the anterior skull base surrounding the olfactory bulb between individuals with ICA and normosmic controls using MRI. Specifically, we aimed to investigate the diagnostic value of surrounding skull base structure and their relationship with the olfactory bulb defects, which has not been

fully explored in previous studies. By using MRI as the imaging modality, we aimed to provide a more detailed and accurate assessment of these abnormalities, which could potentially lead to improved diagnostic and treatment strategies for patients with ICA.

Materials and Methods

Study design and Setting

This was a retrospective study conducted by reviewing medical records of patients who received treatment in the Smell and Taste Clinic of the Department of Otorhinolaryngology at the TU Dresden. All participants provided written informed consent. They participated in studies that had been approved by the Ethics Committee at the Medical Faculty at the "Technische Universität Dresden".

Participants

Inclusion criteria

Congenital anosmia was diagnosed according to the "position paper on olfactory dysfunction"[18] based on **1.** detailed structured medical history; participants reported that they never experienced any olfactory perception[19]; **2**. psychophysical examination was assessed with the extended Sniffin' Sticks olfactory test (Burghart, Wedel, Germany) with participants's TDI (Threshold-Discrimination-Identification) score in the range of anosmia (score ≤16)[20]; **3.** electrophysiological measurements; participants did not exhibit an EEG response following olfactory stimulation[21]. **4.** magnetic resonance imaging (MRI); the OB or olfactory tract was aplastic or hypoplastic (according to literature, ICA when both OB and olfactory tract are normal, is very rare)[9]. Randomly selected healthy controls with no known history of olfactory dysfunction were recruited by advertisement and were matched to the participants by age (±2 years) and gender.

Exclusion criteria were: **1.** signs and symptoms of different conditions possibly causing anosmia (severe chronic rhinosinusitis, traumatic brain injury etc.); **2.** altered anatomy of anterior skull base or paranasal sinus due to iatrogenic causes or other pathology, e.g. tumors;

3. obscured ethmoid sinus pathology; 4. congenital anosmia combined with other anomalies, e.g. Kallmann syndrome, CHARGE syndrome.

Data sources/ measurement

MRI acquisition

MRI was performed using a 3-Tesla scanner (model Prisma; Siemens, Erlangen, Germany) and a 32-channel coil. Images were acquired with T2 weighted sequence, covering the anterior and middle segments of the head. The scanning parameters were: 30-46 slices, slice thickness 1mm, no gap, echo time (TE) = 78 ms, repetition time (TR) = 1500 ms, flip angle = 150˚, field of view matrix = 256 x 320.

Structural assessment

For delineating and measuring the anterior skull base, we used ITK-SNAP, which provided tools to measure lengths and angles of anatomical structures. Measurements were made in the coronal plane of the posterior tangent through the eyeballs[12]. The following parameters were recorded on both sides: **1,** Depth of the olfactory fossa (in mm), measured as the vertical height of the olfactory fossa and classified by the Keros' classification system into type 1 (depth 1-3 mm), type 2 (depth 4-7 mm) or type 3 (depth more than 8 mm)[16]. Asymmetry in the depth (difference of more than 1 mm) between the right and left olfactory fossa were also examined; **2,** Width of olfactory fossa (in mm), defined as the distance between the crista galli and the lateral wall of the olfactory fossa through the depth of olfactory fossa medially and perpendicularly; **3**, Angle of LLCP (in degrees), measurement was calculated at the angle formed by the LLCP and the horizontal line drawn through the cribriform plate. This was further classified into 3 classes depending on the hypothetical risk of iatrogenic injuries: class I (>80 degrees, low risk), class II (45 to 80 degrees, medium risk) and class III (<45 degrees, high risk)[22]; **4,** Angle of fovea ethmoidalis (in degrees), calculated as the angle formed by the fovea ethmoidalis and LLCP; 5, depth of the olfactory sulcus(Fig 1)

For describing the status of OB concerning the development, we divided it into 2 phenotypes: ICA with OB present and ICA with absence of OB.

Statistical analysis

SPSS version 26 (SPSS INC, Illinois, USA) was used for data analysis, graphical visualization were performed using GraphPad Prism 9.3.1 (GraphPad Software, Inc., La Jolla, CA, USA). Descriptive statistics were performed for all study participants. Student's t-test and $\chi 2$ were used for comparisons between individuals with ICA and healthy controls. Receiver operating characteristic (ROC) curves were used to establish sensitivity and specificity of the depth of respective deepest olfactory fossa, angle of respective largest LLCP, angle of respective largest FE. The Optimal cutoff values were chosen based on the highest Youden index (sensitivity + specificity − 1). Cutoff values with the highest Youden index (optimal combination of sensitivity and specificity) have the least amount of overlap between groups, represent a clinically relevant cutoff. The area under the ROC curve was used to quantify diagnostic accuracy of these measurements. The association between measurements were estimated through Pearson's correlation coefficient. We further compared the measurements between individuals with ICA regarding absence or presence of OB. P value < 0.05 was considered as statistically significant.

Results

Participants

We included a total of 93 participants: 31 individuals diagnosed with congenital anosmia (21 women; age 17-75 years, mean age 48.7±16.8 years) and 62 healthy control (42 women, age 18-76 years, mean age 48.1±17.3 years), matched in terms of gender and age. (Table 1) No significant differences were found between the ICA group and the control group in terms of age ($t=0.16$, $p=0.87$) and gender distribution ($p = 1$).

Smell assessment for ICA and healthy participants

By comparing TDI scores, the smell function of ICA participants (11.4±3.4, range: 2.0-16.0) was significantly lower than that of healthy controls (34.4±4.1, range: 24.5- 41.75) ($t = 26.77$, $p < 0.001$).

Comparison between ICA and healthy participants

A total of 93 MR slices (186 sides) was evaluated.

Depth of the olfactory fossa

In ICA participants, the mean depth of the olfactory fossa was 3.5±1.5mm (range: 1.3-8.0mm) on the left side and 3.4±1.4mm (range: 1.3-7.0mm) on the right side. In controls, the mean depth of olfactory fossa was significantly deeper (4.9±1.6mm (range: 2.4 -8.6mm) on the left side and 4.7±1.6mm (range: 1.9-7.6mm) on the right side) (left: $t = 3.95$, $p < 0.001$; right: $t = 3.79$, $p < 0.001$). (Fig. 2) No significant differences in the depth of olfactory fossa were observed between ICA with OB and control (left: $p = 0.15$; right: $p = 0.12$). The most common Keros type was type 1 (41/62, 66%) in ICA participants, followed by type 2 (20/62, 32%) and type 3(1/62, 2%). This distribution of Keros type was significantly different in healthy controls (Keros type 1: 41/124, 33%; Keros type 2: 79/124, 63%; Keros type 3: 4/124, 3%) (left: $\chi^2 = 7.12$, $p = 0.03$; right: $\chi^2 = 11.44$, $p = 0.001$). Asymmetry in the depth of the olfactory fossa was reported in 10% (3/31) of ICA participants, which was significantly different from controls (19/62, 31%) ($\chi^2 = 5.03$, $p = 0.02$). (Table 1)

Width of the olfactory fossa

The width of the left olfactory fossa was smaller in individuals with ICA compared to healthy controls ($p = 0.03$). This difference was not significant for the right olfactory fossa ($p = 0.21$).(Fig 2) No significant differences in the width of olfactory fossa were observed between ICA with OB and control (left: $p = 0.20$; right: $p = 0.99$).

Angle of LLCP

In ICA participants, the mean degree of the angle of LLCP was 39.4±17.5° (range:12.0-84.0°) on the left side and 49.4±20.0° (range:15.6-85.8°) on the right side. These measures were significantly lower compared to controls (left: 53.0±17.3°, range: 16.6-88.7°; right: 64.9±18.6°, range: 22.9-111.2°) ($p < 0.001$). (Fig 2) Relative to controls, the ICA with OB group had significantly lower angle of LLCP at the left side($p = 0.04$), but not at the right side ($p = 0.13$). Class III (<45°, high risk) was found in 58% (36/62) ICA participants, class I (>80°, low risk) in 4% (3/62) of cases, class II (45 to 80°, medium risk) in 37% (23/62) of ICA participants. In

healthy controls, while 25% (31/124) of class III , 14% (17/124) of class I and 61% (76/124) of class II were found. The distribution of angle classification was different between ICA participants and controls ($\chi 2=11.95$, $p < 0.001$). (Table 1)

Angle of fovea ethmoidalis

The angle of the fovea ethmoidalis on both sides was greater in individuals with ICA compared to healthy controls (all $p < 0.0001$). (Fig 2) In the ICA with OB group, the angle of the right fovea ethmoidalis was smaller compared to healthy controls ($p < 0.001$). This difference was not significant for the left angle of fovea ethmoidalis ($p = 0.09$)

Predictors for individuals with ICA

The ROC curve analysis of predictors for ICA is summarized (Fig 3). The following parameters had good accuracy as predictors for individuals with ICA: angle of fovea ethmoidalis (AUC = 0.80), angle of LLCP (AUC = 0.70), depth of olfactory fossa (AUC = 0.76), keros types (AUC = 0.65). Width of olfactory fossa had no ability to distinguish between individuals with ICA and healthy control ($p = 0.11$). Furthermore, the angle of fovea ethmoidalis can distinguish ICA with OB from normosmic controls (AUC = 0.70, $p = 0.02$). Three cutoff values were determined from the ROC analyses for each parameter for predicting ICA: 1 for optimal test efficiency (defined by the highest Youden index)-which shown 71-84% and 63-73% in terms of sensitivity and specificity respectively, 1 for maximum sensitivity (least number of false negatives), and 1 for maximal specificity (least number of false positives) (table 2).

To further validate the accuracy of the clinical scenario application. We selected another dataset, containing T2-weighted MRI images of 9 participants with congenital anosmia and 15 normosmic control**.** Based on the methodology of this study, five ENT doctors, who were blinded to other clinical data, were asked to make a diagnosis visually based on morphological features of the anterior skull base only, with the olfactory bulb and olfactory sulcus masked. The results showed that the diagnostic accuracy is more than random chance (rater 1: Sensitivity [SE] = 0.89, Specificity [SP] = 0.93; rater 2: SE = 0.67, SP = 1; rater 3: SE = 0.67, SP = 0.73; rater 4: SE = 0.89, SP = 0.80; rater 5: SE = 0.78, SP = 0.87).

Correlations of skull base parameters

The results of the correlation analysis are reported in Figure 4. Correlation analysis revealed moderate positive correlations between angle of LLCP and depth of olfactory fossa. Significant negative correlations were also found between right sided angle of fovea ethmoidalis and angle of LLCP (r between -0.63 to -0.74). To the contrary, no relevant correlation was found between width of olfactory fossa and depth of olfactory fossa.

Correlations between olfactory sulcus and skull base parameters

The results of the correlation analysis are reported in Figure 4. In the ICA group, the left depth of olfactory fossa correlated positively with depth of the olfactory fossa, angle of LLCP, negatively with right angle of fovea ethmoidalis. A similar trend was observed at the right side. In the control group, the left depth of olfactory showed a positive relationship with left depth of olfactory fossa, width of olfactory fossa.

Relationship between parameters and presence of OB

ICA participants with presence of OB showed a deeper olfactory fossa (left: $t = 2.77$, $p = 0.01$; right: $t = 3.55$, $p = 0.001$) and larger left sided angle of LLCP ($t = 2.21$, $p = 0.04$) when compared with ICA participants without OB. Further, participants with presence of OB showed, on average, relatively greater width of olfactory fossa as well as right sided angle of LLCP, and lower fovea ethmoidalis. However, these differences were not statistically significant. (Fig 5)

Discussion

Key finding

In participants with ICA, small or absent OBs are associated with morphological changes of the olfactory sulci, especially their depth[13]. Here, we investigated whether ICA is linked to altered skull structure by comparing the size and shape of ethmoid roof and olfactory fossa

between individuals with ICA and matched control. In particular, the angle of fovea ethmoidalis and LLCP as well as the depth and width of olfactory fossa were assessed. To our knowledge, this is the first investigation to suggest that ICA is strongly linked to a “flattening” of the fovea ethmoidalis, shallow olfactory fossa, and low angle of LLCP compared with matched controls, with the three parameters also being correlated to each other. Indeed, the abnormalities of ethmoid roof and olfactory fossa seem to be related to the morphological status of OB. Our findings imply that ICA participants have an unusual pattern of structural variation in anterior skull base. This pattern is characterized by a shallower olfactory fossa and larger slope of the anterior skull base. (Fig 1)

interpretation

The present results suggest that morphological changes in the anterior skull base are a clinical indicator of ICA. For example, the depth of the olfactory fossa had the ability to differentiate ICA from healthy participants. Indeed, the optimal cutoff point of less than 2.25 mm depth of the olfactory fossa was demonstrated to exhibit a specificity of 100%. Several studies investigated the normal depth of olfactory fossa and corresponding distribution of Keros type. Elwany et al. evaluated 300 participants and found Keros type II to be the most common (57%), followed by type I (42%) and type III (1%)[23]. Gera et al. in a study of 190 adult participants, observed that Keros type II was most frequent (65 %), followed by type I (20%) and Keros type III (15%), with a mean depth of 5.4mm[22]. Meloni et al. (1992) determined the depth of the cribriform plate to be 5.9 mm on average (range 1.3-17 mm)[24]. Also, in our healthy sample a higher frequency of Keros type II (63%) was found, 33% were classified as type I and 3% were type III. In our study, the mean depth of olfactory fossa was 4.7 mm at right side, and 4.9 mm at left side, which is very similar to previous reports. However, individuals with ICA had a shallower olfactory fossa, Keros type I (66%) is the most common, with an average depth of 3.4 mm.

In the present study, asymmetry in the depth of the cribriform was found in 12% of the cases. In addition, the width of the olfactory fossa in ICA was significantly lower than in matched controls. Prior observations by Keros suggested that the average width of the olfactory fossa is

2.1mm in the anterior portion, and that it gradually widens to 4.2 mm towards the posterior part. This was confirmed by Güldner et al. who obeserved a mean width of the olfactory fossa of 2.0mm in the anterior part and 3.25 mm in the posterior part[25]. The present finding suggests that individuals with ICA have a significantly different anatomic structure of the olfactory fossa than normosmic controls.

We further observed a significant difference between individuals with ICA and matched healthy individuals in the slope of the anterior skull base. In our sample with ICA, the fovea ethmoidalis had an average angle of 163° at the right side, and 154° at the left side. However, in the control group, the fovea ethmoidalis was found with an average angle of 122° at the right side, and 127° at the left side. In the literature review, few studies were found which reported a flattened fovea ethmoidalis. Lebowitz et al. analyzed the flattening of the fovea ethmoidalis on 200 CT scans, and observed 23% at the right side and 25% at the left side[17]. Souza et al., in a review of 200 CT scans, reported that flattening of fovea ethmoidalis occurred in 19% on the right and 30% on the left[26].However, these studies did not quantitatively evaluate the angle of the fovea ethmoidalis as defined in this study. Angle of LLCP, formed by the LLCP and horizontal line, can also be used to describe the slope of the anterior skull base. Data from 150 CT scans of healthy individuals show the mean degree of the angle of LLCP was 70.1°, ranging from 28 to 88°[27]. This is in line with the finding of Gera et al. who observed that the mean degree of the angle of LLCP was 71.7°, ranging from 27 to 89°[22]. Interestingly, the current study found that the mean angle in ICA is 39°, which appears to fall outside the normal range in matched controls and healthy participants from previous studies. Hence, individuals with ICA exhibit a more pronounced slope of the anterior skull base.

Several studies investigated the relationship among these morphological features of the anterior skull base. The depth of the olfactory fossa is associated with the angulation of the LLCP[22,26]. However, one study reported that there was no significant correlation between the depth of olfactory fossa and the angle of the LLCP[27] In addition, the length of the LLCP was negatively correlated with the length of the fovea ethmoidalis and the angle of the LLCP[22]. Further, the angulation of the lateral lamella is also related to asymmetry of the

fovea ethmoidalis[26]. In our study, significant correlations among morphological parameters of anterior skull base were found both in ICA participants and healthy group (Fig 4).

As opposed to the lack of research on skull abnormalities in ICA, relatively more is known about the association between brain structure and congenital olfactory deficits[28]. Distinct features include aplastic or hypoplastic OB and tract. Several studies showed a significantly shallower olfactory sulcus in ICA, believed to be a direct consequence of an increased thickness of corresponding cortex area (e.g.,[14]).

The fact that individuals with ICA characterized by small or absent OBs clearly show altered skull base in the present study raises interesting questions about the integrated development of brain and skull, and especially the OB and the surrounding bony structures. Similar to our finding that the structure of the anterior skull base can reflect the presence of an OB, studies on eyeballs show that the size of the orbital reflects the size of the eye in adult humans[29]. A significant positive linear relationship was found between obit and eyeball volumes[30]. Anophthalmia (congenital absence of the eyes) is accompanied by hypoplasia of the orbit, because the eyeball is considered to be the main endogenous factor stimulating the form of the orbital cavity[31]. From an evolutionary perspective, the prevailing notion is that in humans the morphology of the cranial base is tightly related to the brain size[4]. Primordial OB is present by 41 days gestation[32]. The OBs can be recognized in pre-natal MRI after 30 weeks gestation[33]. The anterior skull base is formed primarily of cartilage during the second fetal month, and is still largely cartilaginous at birth, being gradually ossified over the first 2 years of life[34]. However, there are few direct investigations regarding the exact signals and signaling sources crucial for the interplay between OB and anterior skull base over the course of development. Generally, hypotheses regarding factors responsible for that skull morphogenesis have been proposed, which are mostly associated with brain pressure distribution during growth and development[2]. Collectively, based on our results, we can speculate that OBs play a pivotal role in developing and maintaining a normal structure of the

anterior skull base.

limitations

A limitation of this study is the absence of syndromic forms of congenital anosmia, such as Kallmann syndrome, various ciliopathies and congenital insensitivity to pain, which is due to the retrospective cross sectional study character. This assessment would help us to use these anterior skull base abnormalities to be widely applicable. A second limitation is that we did not include genetic diagnostics of the participants, due to the retrospective cross sectional study character. This would have been interesting especially when considering the differences in genetic deficits involved in the various forms of congenital anosmia (e.g. mutation in the cyclic nucleotide-gated gene CNGA2[35]), genetic abnormalities are often associated with morphogenetic processes, resulting in altered pattern of skull base.

Generalizability

A variety of congenital malformations of skull may be encountered. Many clinical presentations - including hearing loss, impaired vision, cognitive disability and mental disease – manifest in children or adults in association with such malformations.[36–38] Early detection of pathological changes and further differential diagnosis and therapy decisions are ascertained with evaluation of the skull. Standard MRI procedures allow for the noninvasive assessment of the skull, which showed perfect agreement with the results from direct measurements of the skull ($r>0.99$). [39] Our data showed that ICA is associated with morphological changes including not only the abnormality of olfactory bulb - the current "gold standard" – but also changes in cranial bone structures, specific to the anterior skull base. This helps us to further understand the pathophysiology of this developmental disorder. In addition, the present study supports the idea that the OB plays a role in the development of the anterior skull base.

Structural assessments of olfaction relevant regions, e.g. olfactory cleft, OB, anterior skull base, or cribriform plate can be used to identify morphological differences when comparing these regions between patients and healthy controls. These morphological characteristics have been

shown to be different in various causes of olfactory dysfunction, e.g., inflammation and idiopathic or traumatic olfactory loss [40–42]. They can also be used to advance our understanding of aging [43]. Assessment of the OB is recommended during the clinical investigation of participants with suspected congenital anosmia. Our study demonstrated that the bony structures surrounding the OB can help to differentiate abnormalities from normal variation (ROC area was 0.70-0.80). It may add future additional complementary diagnostic insights for the assessment of congenital anosmia.

Although the configuration of the anterior skull base, like Keros classification is generally assessed with Computed Tomographic (CT) imaging, which is not justifiable due to ionizing radiation concerns when we included healthy control, we instead used MRI to evaluate both the OB and surrounding bony structures, as the latter modality is superior for soft tissue evaluation. A previous study had shown that MRI provides sufficient resolution to assess the anterior skull base[44]. Previous work showed that measurements of skull performed on MR images are highly reliable and show an excellent agreement with CT-based measurements.[45] Thus, while the measurements that were employed in this study can be implemented on both CT and MRI scans, and CT scans may provide more detailed bony information, we believe that our MRI measurements are still valuable in assessing the overall morphology of the skull base in relation to olfactory bulb defects. Our findings may be used in a clinical setting for the neuroradiological evaluation of individuals with ICA.

This approach may be of use in certain clinical situations where patients are not entirely sure whether or not they smelled anything in their lives, e.g., in children, or in older participants who sometimes have difficulties reporting their symptoms adequately. In addition, the use of electrophysiological measures as an objective confirmation of the absence of olfactory function can be problematic, because olfactory event-related potential measurements are not widely performed. Taking all that together, assessment of the morphology of the bony structures surrounding the OB provides an additional clue on the diagnosis in terms of presence of absence of olfactory function which may be useful for the diagnosis of ICA.

Despite these notable results, the sample of normosmic participant was rather small and it is

not possible to state how frequent abnormal anterior skull base anatomy is in the normosmic population. Furthermore, it is worth mentioning that an abnormal OB or abnormalities of surrounding regions are not absolute sign of congenital anosmia [46,47]. Thus, although the presently described findings may predict congenital anosmia, this is certainly not always the case. We recommend the assessment of the anterior skull base in addition to – and not instead of – a detailed history collection and thorough olfactory testing.

Conclusion

This study provides baseline morphometric data of the anterior skull base in individuals with ICA. This is the first study to demonstrate that ICA participants have an abnormal OB as well as an abnormal anterior skull base, characterized by a shallow olfactory fossa and flattened ethmoid roof. This study supports the idea of an integrated development of OB and anterior skull base. These morphological patterns of the anterior skull base surrounding the OB are applicable in distinguishing individuals with ICA from normosmic controls, and may therefore be useful for the diagnosis of ICA. However, given the absence of other common causes of congenital anosmia, the high degree of variation in the measurements and large overlaps with the normosmic controls suggest that these findings should be interpreted with caution. Future studies with larger sample sizes and more comprehensive assessments of genetic are needed to validate and expand upon our results.

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Statement of Ethics: In this study, we used the data from datasets acquired within the context of four approved studies. All these studies had been reviewed and approved by the TU Dresden, Medical Faculty Ethics Review Board(EK348092018, EK235072018, EK96032015, EK 262082010), for each study, written informed consent was obtained from participants.

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Figure 1: "a representative MRI photo" for each of the MRI structural outcomes and the morphological pattern for both ICA participants [(51y, female, TDI=13) 5 images] and healthy controls [72y, female, TDI = 34.25) 5 images].width of olfactory fossa (in mm, orange), depth of olfactory fossa (in mm, yellow), angle of fovea ethmoidalis (in °, blue), angle of the lateral lamella of cribriform plate (in °, red).

Figure 2: Structures of anterior skull base according to the groups and sides: A&E)Mean of the depth of OF for ICA group and healthy control group at both sides; B&F) Mean of the width of OF for ICA group and healthy control group at both sides; C&G) Mean of angle of LLCP for ICA group and healthy control group at both sides; D&H) Mean of angle of FE for ICA group and healthy control group at both sides. FE= fovea ethmoidalis, LLCP = lateral lamella of cribriform plate, OF = olfactory fossa, L = left, R = right. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 ns: p > 0.05.

Figure 3: Receiver operating characteristic (ROC) curve of FE (AUC=0.73), angle of LLCP (AUC= 0.73), depth of OF (AUC=0.74), Keros types. when the cutoff values were determined by optimal test efficiency (defined by the highest Youden index), which shown 68-87% and 52-76% in terms of sensitivity and specificity respectively. FE= fovea ethmoidalis, LLCP = lateral lamella of cribriform plate, OF = olfactory fossa.

Figure 4: Correlation matrix between measurements. The number represents Pearson's r values, positive correlations are displayed in blue and negative correlations in red color, color intensity is proportional to Pearson's r values.FE= fovea ethmoidalis, LLCP = lateral lamella of cribriform plate, OF= olfactory fossa , OS = olfactory sulcus.

Figure 5: structures of anterior skull base in ICA group according to the OBs status and sides with OB agenesis and ICA with OB hypogenesis: A&E) Mean of the depth of OF for OB agenesis and OB hypogenesis at both sides; B&F) Mean of the width of OF for OB agenesis and OB hypogenesis at both sides; C&G) Mean of angle of LLCP OB agenesis and OB hypogenesis at both sides; D&H) Mean of angle of FE for OB agenesis and OB hypogenesis at both sides. FE= fovea ethmoidalis, LLCP = lateral lamella of cribriform plate, OF= olfactory fossa, L = left, R = right. *p < 0.05, **p < 0.01, ns: p > 0.05.

Table 1. Demographic, smell assessment and radiologic characteristics. Data are presented as means (standard deviation) or percentage (%).

characteristic	Congenital patients	Healthy control	statistics	p value
		demographics		
Age in years [mean (SD)]	48.7 (16.8)	48.1(17.3)	t=0.16	0.87
gender	21F, 10M	42F, 20M	χ^2=0	1.00
		Development of OB		
Development of OB				
left	18A,13H *	-	-	-
right	19A,12H	-	-	-
		Depth of the olfactory fossa (in mm)		
Keros type [N (%)]				
Kero type 1	41 (66%)	41 (33%)	χ^2=7.166	p < 0.05
Kero type 2	20 (32%)	79 (63%)		
Kero type 3	1 (2%)	4 (3%)		
		smell assessment		
TDI [mean (SD)]	11.7 (3.0)	34.4 (4.1)	t =26.77	p < 0.05
		Angle of lateral lamella of cribriform plate		
Angle of LLCP classification [N (%)]				
Class I	3 (4%)	17 (14%)	χ^2=11.945	p < 0.05
Class II	23 (37%)	76 (61%)		
Class III	36 (58%)	31 (25%)		

* A = aplasia; H = hypoplasia; TDI = Threshold-Discrimination-Identification.

table 8-1

450 Table 2. Sensitivity, specificity of cutoff points of 3 morphological indices of anterior skull base for prediction
451 of ICA.

Variables	AUC (95%CI)	P	Cut-off	Sensitivity(95%CI)	Specificity(95%CI)
Depth of olfactory fossa	0.74(0.62-0.84)	<0.001	<4.65mm	0.87(0.71-0.95)	0.52(0.39-0.64)
			<2.25mm	0.26(0.14-0.43)	1.00(0.94-1.00)
			<8.45mm	1.00(0.89-1.00)	0.03(0.006-0.11)
Angle of lateral lamella of cribriform plate	0.73(0.62-0.84)	<0.001	<46.4°	0.77(0.60-0.89)	0.67(0.55- 0.78)
			<15.0°	0.06(0.01-0.21)	1.00(0.94-1.00)
			<84.1°	1.00(0.89-1.00)	0.05(0.01-0.13)
Angle of fovea ethmoidalis	0.73(0.63-0.85)	<0.001	>139.5°	0.68(0.50-0.81)	0.76(0.64-0.85)
			>191.2°	0.16(0.07-0.33)	1.00(0.91-1.00)
			>97.0°	1.00(0.89-1.00)	0.11(0.06-0.22)

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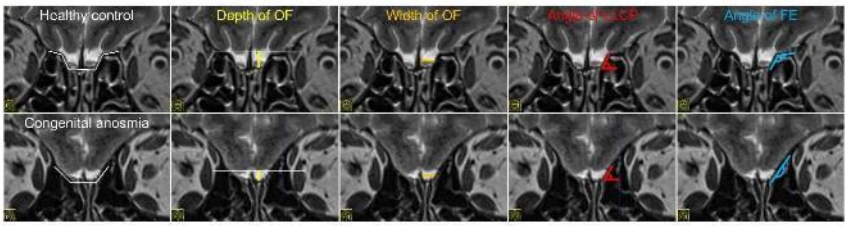
table 8-2

Figure 1: "a representative MRI photo" for each of the MRI structural outcomes and the morphological pattern for both ICA patients [(51y, female, TDI=13) 5 images] and healthy controls [72y, female, TDI = 34.25) 5 images].width of olfactory fossa (in mm, orange), depth of olfactory fossa (in mm, yellow), angle of fovea ethmoidalis (in °, blue), angle of the lateral lamella of cribriform plate (in °, red).



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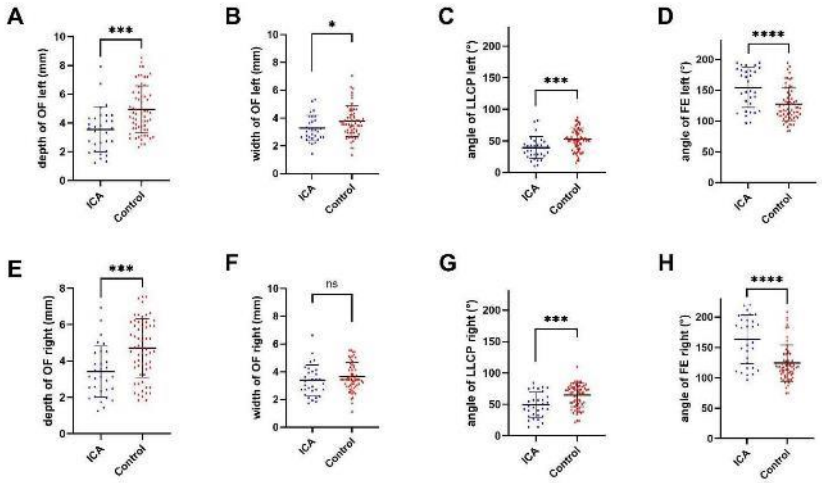

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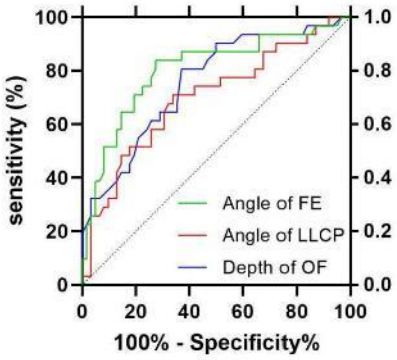
Figure 8-1

Figure 8-2



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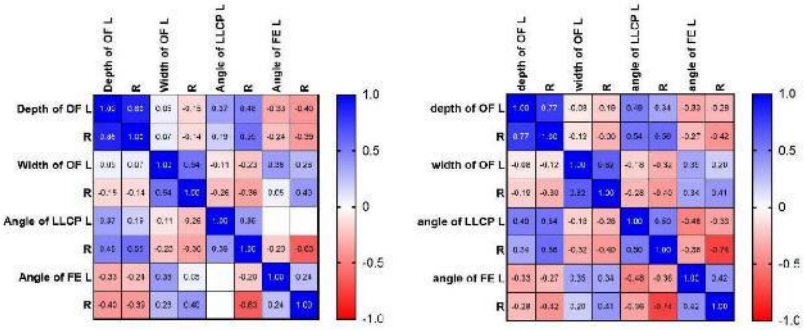

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Figure 8-3

Figure 8-4

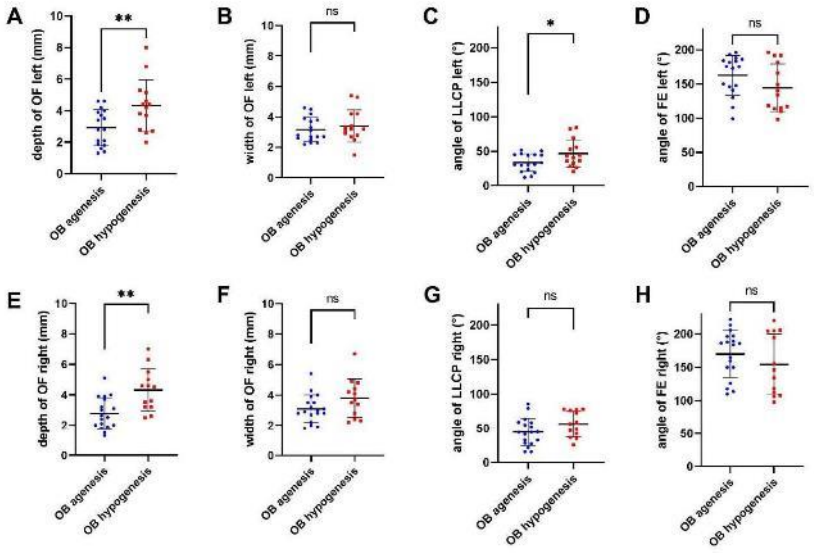

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Figure 8-5

9 Discussion and Outlook

In our first study (Publication 1), The article provides insights into the association between olfactory dysfunction and inflammation in chronic rhinosinusitis (CRS) patients. The paper suggests that olfactory function assessment should be included in the clinical evaluation of CRS patients to objectively evaluate the efficacy of treatment over time and to expand the library of CRS phenotypes and endotypes.

we summarized olfactory dysfunction with regard to its clinical evaluation, pathophysiological mechanisms, relation with inflammatory burden and anti-inflammatory responses in CRS patients. We noticed that, Olfactory dysfunction, with a high prevalence in CRS patients, has a significant impact on health and quality of life. Detailed assessment of olfactory function should be considered in the clinical evaluation of CRS patients, especially with well-established and reliable psychophysical testing, not only for detecting and quantifying patients' symptom but also because it is useful to objectively assess the efficacy of CRS treatment over time. In particular, olfactory function seems to be a stable and valid factor in the various clusters of clinical presentations, linked with certain inflammatory patterns and reflective of the response to anti-inflammatory treatment. Accordingly, olfaction may act as a marker in the progression of chronic sinonasal inflammation, help to differentiate CRS phenotypes and endotypes and ultimately aid in the development of tailored treatment regimens.

In our second study (Publication 2), The study introduces a novel method for classifying the shape of the OB in the human brain, scalable to clinical and research applications. The research suggests that the shape of the OB can be used as a biomarker for olfactory dysfunction and is associated with certain causes of olfactory disorders.

Our study proposes a new framework to classify the shape of the human OB and provides first evidence of the association between OB shape and olfactory dysfunction, using a large sample size of healthy controls and patients with different types of olfactory dysfunction. Our observations indicate the presence of morphological changes in the OB, from regular to irregular and from integrity to disintegration. These changes are linked to human olfactory function, even when adjusting for age, gender and OB volume. In addition, we found that the OB exhibits increased deformation with age across healthy subjects. Furthermore, we found that,

different causes of smell loss seem to be associated with specific signatures of OB shape.

In addition to the typical olive-shaped OB, which reflects a normal, healthy status of the OB, various other OB shapes were also observed in this study, for example, the banana-shaped OB with convexity of the walls. These forms found in healthy subjects, are also common in patients with smell deficits. Consistent with this, previous studies have described the variability of the OB structure, and especially the OB volume. Buschhüter et al (Buschhüter et al., 2008). reported that OB volumes decrease with age in healthy people. They proposed normative data of OB volume in relation to age based on results from 125 patients. Furthermore, a decrease of OB volume has also been reported in patients with olfactory dysfunction due to various causes (Rombaux et al., 2010; Hummel et al., 2015; Mazal et al., 2016; Yao et al., 2018). Moreover, in a longitudinal study, Gudziol et al (Gudziol et al., 2009). described an OB change post treatment in 19 CRS patients which was correlated with olfactory function. Another study, following a period of 4 months of olfactory training, showed that 97 healthy people exhibited an average increase of OB volume (Negoias et al., 2017).

At a macroscopic level, Burmeister et al. (Burmeister et al., 2012) depicted laminar patterns of the human OBs (n=24). They identified three layers in 8.3%, two layers in 83.3% and one layer in 8.3% of their subjects using high resolution 3T MRI. At a cellular level, the spatial distribution of glomeruli in the human OB is irregular and complex (Maresh et al., 2008). and shows higher variability than what is seen in animals (Hoogland et al., 2003; Zapiec et al., 2017).

The structural variability in OB shapes and volumes appears to be an expression of OB plasticity. As the OB receives olfactory input from the olfactory epithelium, it is natural to think that OB structure is altered secondary to reduced sensory input. In fact, OB volume has been observed to be associated with nasal septal deviation, with OB volume being significantly lower at the narrower side which may indicate a “bottom-up modulation” of the OB (Altundag et al., 2014). Importantly, the OB also appears to be subject to “top-down modulation” showing in some neurodegenerative diseases, as well as mental disorders, which are associated with a smaller OB volume (Haehner et al., 2011; Ennis & Holy, 2015; Croy & Hummel, 2017). Top-down modulation also became evident in an experiment on lateralized olfactory exposure (“olfactory training”) in healthy subjects who exhibited an increased OB volume not

just on the side exposed to odors but on the ipsi- and contralateral sides (Hummel et al., 2013b).

OB plasticity may depend on numerous factors which are currently discussed, for example, (1) continuous neuronal supply from the subventricular zone (SVZ), where young neurons migrate within the rostral migratory stream and replace interneurons (periglomerular cells and granular cells) in the OB (Curtis et al., 2007); (2) continuous synaptogenesis with dendrites of mitral/tufted cells occurring from incoming axonal projections of olfactory receptor neurons at the glomerular level; and (3) in a recent animal study, a new form of structural remodeling of adult-born OB neurons suggest direct neurogenesis within the OB itself (Breton-Provencher et al., 2016). Emerging evidence for OB plasticity indicates that OB is not just a static relay station, but dynamically processes olfactory information based on experience and context (Wu et al., 2020).

Findings from the present study are in line with prior evidence suggesting that the OB structure is associated with olfactory function (Mazal et al., 2016). Such correlation has been shown not only for overall olfactory ability, but also for the subcomponent's odor identification and odor threshold (Yousem et al., 1999; Rombaux et al., 2006). Even dynamic changes in odor threshold were significantly and positively correlated with changes in OB volume (Gudziol et al., 2009). However, volumetric evaluation of OB is not yet a routine procedure for patients with olfactory dysfunction in a typical outpatient clinic, since it requires special software and some time needed for OB segmentation. Hence, our study presents an easier, simpler method to visually classify and assess OB deformation, with large potential for clinical application as a biomarker for patients with olfactory dysfunction. For example, our study shows that a "plane" and "scattered" OB shape is generally associated with decreased olfactory function.

We found differences in age-related OB shape in healthy participants. In human, aged OB shows that the decrease of the volume of the OB, the concentration of mitral cells per unit area, and both layer thickness and the number of glomeruli (Bhatnagar et al., 1987; Meisami et al., 1998). However, the results in rodents are more complex. In mice, OB volume does not change (Richard et al., 2010), or may even increase with age (Mirich et al., 2002). In the present study, the subjects with scattered OB shape were oldest, suggesting an age-related degeneration of OB structures. It is unclear whether this change in OB shape is related, for example, to

the OB microstructure or the turnover of interneurons. Like other sensory systems, olfactory function decreases with increasing age, due to numerous reasons (Hummel & Oleszkiewicz, 2020).

Also, in the current study, non-convex OB patterns occurred in the 5 most common etiologies for olfactory dysfunction with a high percentage. The highest prevalence of the irregular OB shapes was observed in post-traumatic olfactory dysfunction. This may result from the specific mechanisms, with traumatic injury leading to direct, sudden disruption of olfactory pathways, for example, direct shearing of the olfactory nerves and focal contusion within the OB. Of note, the olfactory system is among the earliest affected structures in neurodegenerative disease, such as Alzheimer's disease and Parkinson's disease. In the present study, the prevalence of non-convex OB shapes pattern was higher for subjects with neurodegenerative diseases in comparison to healthy controls.

Results from the current study support the idea of an association between OB shapes and olfactory function. Our findings raise numerous questions which future studies should address, namely (1) whether OB shapes can be complementary to volumetric assessment of longitudinal changes, (2) whether OB shapes are helpful in terms of prognostic information in patients with olfactory dysfunction, and (3) whether, and if so, how treatment induces changes in OB morphology.

In our third study (Publication 3), The study compares the morphological pattern of the anterior skull base surrounding the OB between individuals with isolated congenital anosmia (ICA) and normosmic controls. The research finds that the absence of the OB is associated with a higher degree of flattening of the ethmoid roof and a shallow olfactory fossa. The study suggests that these parameters can be used to differentiate between individuals with ICA and normosmic controls.

we investigated whether ICA is linked to altered skull structure by comparing the size and shape of ethmoid roof and olfactory fossa between individuals with ICA and matched control. In particular, the angle of fovea ethmoidalis and LLCP as well as the depth and width of olfactory fossa were assessed. To our knowledge, this is the first investigation to suggest that ICA is strongly linked to a "flattening" of the fovea ethmoidalis, shallow olfactory fossa, and low angle of LLCP compared with matched controls, with the three parameters also being correlated to each other. Indeed, the abnormalities of ethmoid roof and olfactory fossa seem to be related to the morphological status of OB. Our findings imply that ICA participants have an unusual

pattern of structural variation in anterior skull base. This pattern is characterized by a shallower olfactory fossa and larger slope of the anterior skull base.

The present results suggest that morphological changes in the anterior skull base are a clinical indicator of ICA. For example, the depth of the olfactory fossa had the ability to differentiate ICA from healthy participants. Indeed, the optimal cutoff point of less than 2.25 mm depth of the olfactory fossa was demonstrated to exhibit a specificity of 100%. Several studies investigated the normal depth of olfactory fossa and corresponding distribution of Keros type. Elwany et al. evaluated 300 participants and found Keros type II to be the most common (57%), followed by type I (42%) and type III (1%) (Elwany et al., 2010). Gera et al. in a study of 190 adult participants, observed that Keros type II was most frequent (65 %), followed by type I (20%) and Keros type III (15%), with a mean depth of 5.4mm (Gera et al., 2018). Meloni et al. (1992) determined the depth of the cribriform plate to be 5.9 mm on average (range 1.3-17 mm) (Meloni et al., 1992). Also, in our healthy sample a higher frequency of Keros type II (63%) was found, 33% were classified as type I and 3% were type III. In our study, the mean depth of olfactory fossa was 4.7 mm at right side, and 4.9 mm at left side, which is very similar to previous reports. However, individuals with ICA had a shallower olfactory fossa, Keros type I (66%) is the most common, with an average depth of 3.4 mm.

In the present study, asymmetry in the depth of the cribriform was found in 12% of the cases. In addition, the width of the olfactory fossa in ICA was significantly lower than in matched controls. Prior observations by Keros suggested that the average width of the olfactory fossa is 2.1mm in the anterior portion, and that it gradually widens to 4.2 mm towards the posterior part. This was confirmed by Güldner et al. who obeserved a mean width of the olfactory fossa of 2.0mm in the anterior part and 3.25 mm in the posterior part (Savvateeva et al., 2010). The present finding suggests that individuals with ICA have a significantly different anatomic structure of the olfactory fossa than normosmic controls.

We further observed a significant difference between individuals with ICA and matched healthy individuals in the slope of the anterior skull base. In our sample with ICA, the fovea ethmoidalis had an average angle of 163° at the right side, and 154° at the left side. However, in the control group, the fovea ethmoidalis was found with an average angle of 122° at the right side, and 127° at the left side. In the literature

review, few studies were found which reported a flattened fovea ethmoidalis. Lebowitz et al. analyzed the flattening of the fovea ethmoidalis on 200 CT scans, and observed 23% at the right side and 25% at the left side (Lebowitz et al., 2001). Souza et al., in a review of 200 CT scans, reported that flattening of fovea ethmoidalis occurred in 19% on the right and 30% on the left (Souza et al., 2008).However, these studies did not quantitatively evaluate the angle of the fovea ethmoidalis as defined in this study. Angle of LLCP, formed by the LLCP and horizontal line, can also be used to describe the slope of the anterior skull base. Data from 150 CT scans of healthy individuals show the mean degree of the angle of LLCP was 70.1°, ranging from 28 to 88° (Abdullah et al., 2020). This is in line with the finding of Gera et al. who observed that the mean degree of the angle of LLCP was 71.7°, ranging from 27 to 89° (Gera et al., 2018). Interestingly, the current study found that the mean angle in ICA is 39°, which appears to fall outside the normal range in matched controls and healthy participants from previous studies. Hence, individuals with ICA exhibit a more pronounced slope of the anterior skull base.

Several studies investigated the relationship among these morphological features of the anterior skull base. The depth of the olfactory fossa is associated with the angulation of the LLCP (Souza et al., 2008; Gera et al., 2018). However, one study reported that there was no significant correlation between the depth of olfactory fossa and the angle of the LLCP (Abdullah et al., 2020). In addition, the length of the LLCP was negatively correlated with the length of the fovea ethmoidalis and the angle of the LLCP (Gera et al., 2018). Further, the angulation of the lateral lamella is also related to asymmetry of the fovea ethmoidalis (Souza et al., 2008). In our study, significant correlations among morphological parameters of anterior skull base were found both in ICA participants and healthy group.

As opposed to the lack of research on skull abnormalities in ICA, relatively more is known about the association between brain structure and congenital olfactory deficits. Distinct features include aplastic or hypoplastic OB and tract. Several studies showed a significantly shallower olfactory sulcus in ICA, believed to be a direct consequence of an increased thickness of corresponding cortex area.

A limitation of this study is the absence of syndromic forms of congenital anosmia, such as Kallmann syndrome, various ciliopathies and congenital insensitivity to pain, which is due to the retrospective cross sectional study character. This assessment would help us to use these anterior skull base abnormalities to be widely applicable. A second limitation is that we did not include genetic diagnostics of the participants, due to the retrospective cross sectional study character. This would have been interesting especially when considering the differences in genetic deficits involved in the various forms of congenital anosmia (e.g. mutation in the cyclic nucleotide-gated gene CNGA2), genetic abnormalities are often associated with morphogenetic processes, resulting in altered pattern of skull base.

Structural assessments of olfaction relevant regions, e.g. olfactory cleft, OB, anterior skull base, or cribriform plate can be used to identify morphological differences when comparing these regions between patients and healthy controls. These morphological characteristics have been shown to be different in various causes of olfactory dysfunction, e.g., inflammation and idiopathic or traumatic olfactory loss (Mahmut et al., 2020; Schlosser et al., 2021). They can also be used to advance our understanding of aging (Ganjaei et al., 2019). Assessment of the OB is recommended during the clinical investigation of participants with suspected congenital anosmia. Our study demonstrated that the bony structures surrounding the OB can help to differentiate abnormalities from normal variation (ROC area was 0.70-0.80). It may add future additional complementary diagnostic insights for the assessment of congenital anosmia.

This approach may be of use in certain clinical situations where patients are not entirely sure whether or not they smelled anything in their lives, e.g., in children, or in older participants who sometimes have difficulties reporting their symptoms adequately. In addition, the use of electrophysiological measures as an objective confirmation of the absence of olfactory function can be problematic, because olfactory event-related potential measurements are not widely performed. Taking all that together, assessment of the morphology of the bony structures surrounding the OB provides an additional clue on the diagnosis in terms of presence of absence of olfactory function which may be useful for the diagnosis of ICA.

Despite these notable results, the sample of normosmic participant was rather small and it is not possible to state how frequent abnormal anterior skull base anatomy is in

the normosmic population. Furthermore, it is worth mentioning that an abnormal OB or abnormalities of surrounding regions are not absolute sign of congenital anosmia (Rombaux et al., 2007; Weiss et al., 2020). Thus, although the presently described findings may predict congenital anosmia, this is certainly not always the case. We recommend the assessment of the anterior skull base in addition to – and not instead of – a detailed history collection and thorough olfactory testing.

These above studies emphasized that olfactory function is closely associated with overall intensity of the inflammatory response in CRS, shape of OB and anterior skull base. Thus, we recommend that assessment of olfactory function should be considered in the clinical evaluation of CRS patients, and assessment of shape of OB and anterior skull base may be useful for the diagnosis of olfactory dysfunction.

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11 Summary in German

Hintergrund

Riechstörungen sind ein weit verbreitetes Problem und können die Lebensqualität erheblich beeinträchtigen. Patienten mit Riechstörungen berichten häufiger von Schwierigkeiten beim Kochen, einem größeren Maß an Unsicherheit, und außerdem über Depressionen und Ängste. Die Diagnose und Behandlung von Riechstörungen stellen für Ärzte nach wie vor eine Herausforderung dar. Ein besseres Verständnis der prädiktiven Faktoren des Riechvermögens trägt zweifelsohne zur Entwicklung gezielter Interventionen und Therapien für Patienten mit Riechstörungen bei. Zahlreiche klinische Faktoren wurden eingehend untersucht und in Verbindung mit Riechstörungen gebracht, wie zum Beispiel Alter, Geschlecht und Exposition gegenüber toxischen Substanzen. Das übergeordnete Ziel dieser Arbeit bestand darin, die klinischen Prädiktoren des Riechvermögens zu untersuchen, wobei der Schwerpunkt auf Entzündungen und bildgebenden Verfahren lag.

Hypothese / Frage

In Publikation 1 stellten wir die Hypothese auf, dass das Riechvermögen eng mit der Gesamtintensität der entzündlichen Reaktion bei CRS verbunden ist. Unser Ziel in der Literaturübersicht war, vorliegende Daten zu Riechstörungen hinsichtlich ihrer klinischen Bewertung, pathophysiologischen Mechanismen, Beziehung zur entzündlichen Belastung und anti-entzündlichen Reaktionen bei CRS-Patienten zu sichten und zu bewerten.

In Publikation 2 vermuteten wir, dass die Form des Riechkolbens (OB) die Riechfunktion vorhersagt und dass sie mit Riechverlust, Alter und Geschlecht in Verbindung steht. Ziel dieser Studie war es, eine Klassifikation der OB-Form im menschlichen Gehirn zu erstellen, die für klinische und wissnschaftliche Anwendungen brauchbar ist.

In Publikation 3 lautete unsere Hypothese, dass die Morphologie der vorderen Schädelbasis, gemessen in Bezug auf die Tiefe und Breite der Fossa olfactoria, den Winkel der lateralen Lamelle der Siebbeinplatte (LLCP) und den Winkel der Fovea ethmoidalis, bei ICA-Teilnehmern und gesunden Teilnehmenden unterschiedlich ist. Wir vermuteten weiterhin, dass bei ICA die Morphologie der vorderen Schädelbasis mit OB-Anomalien korreliert ist und dass die morphologischen Parameter miteinander korreliert sind. Ziel dieser Studie war es, das morphologische Muster der vorderen Schädelbasis, das den Riechkolben umgibt, bei Personen mit ICA und normosmischen Kontrollen mittels MRT zu untersuchen.

Material und Methoden

In Publikation 1 beschreibt der erste Abschnitt des Artikels die Beurteilung der Riechfunktion mit verschiedenen Methoden, von Bewertungen bis hin zu MR-basierten Bildgebungsverfahren. Anschließend diskutieren wir v.a. die entzündlichen Mechanismen, die mit Riechstörungen bei CRS in Zusammenhang stehen: Die Riechfunktion ist mit bestimmten Entzündungsmustern assoziiert und könnte ein Marker für CRS-Subtypen sein. Schließlich geben wir einen Überblick über entzündungshemmende Therapien, einschließlich konservativer und chirurgischer Ansätze, und deren Wirksamkeit bei Riechstörungen bei CRS.

In Publikation 2 wurden Patienten mit den 5 häufigsten Ursachen für Riechstörungen (n=192) sowie alters-/geschlechtsangepasste gesunde Kontrollpersonen (n=77) rekrutiert. Die Riechfunktion wurde mit dem erweiterten "Sniffin' Sticks"-Test detailliert untersucht. Für alle wurde eine hochauflösende strukturelle T2-gewichtete MRT-Aufnahme erstellt. Die planimetrischen Konturen (Fläche in mm^2) der OB wurden manuell abgegrenzt, und alle Flächen wurden addiert und multipliziert, um das OB-Volumen in mm^3 zu erhalten. Die OB-Formen wurden manuell umrissen und auf einer ausgewählten Schicht in der hinteren koronalen Ebene, tangential zu den Augäpfeln, charakterisiert. Wir untersuchten die OB-Formen hinsichtlich ihrer Konvexität und definierten 7 Kategorien basierend auf den OB-Konturen: Olive, Kreis, plano-konvex, Banane, unregelmäßig, Ebene und inhomogen.

In Publikation 3 führten wir eine retrospektive Studie durch, um T2-gewichtete Magnetresonanzbilder von Personen mit ICA (n=31) und gesunden, normosmischen

Kontrollpersonen, die hinsichtlich Alter und Geschlecht angepasst waren (n=62), zu erhalten. Zwischen beiden Gruppen verglichen wir die Tiefe und Breite der Fossa olfactoria, den Winkel der Siebbeinzelle sowie den Winkel der lateralen Lamelle der Lamina cribrosa. Innerhalb der ICA-Gruppe führten wir weitere Untergruppenanalysen basierend auf dem Vorhandensein oder Fehlen des OB durch, um zu untersuchen, ob die Morphologie der vorderen Schädelbasis mit dem Vorhandensein von OBs zusammenhängt. Die diagnostische Brauchbarkeit dieser Parameter wurde mit Hilfe der Receiver-Operating-Characteristic-Analyse ausgewertet.

Ergebnisse

Für Publikation 1 stellten wir fest, dass die Beurteilung der Riechfunktion nicht nur dazu dient, die Symptome der Patienten zu erkennen und zu quantifizieren, sondern auch, weil mit ihr die Wirksamkeit der CRS-Behandlung im Laufe der Zeit objektiv beurteilt werden kann. Insbesondere scheint die Riechfunktion ein reliabler und valider Faktor in den verschiedenen klinischen Bildern zu sein, der mit bestimmten Entzündungsmustern verbunden ist und die Reaktion auf entzündungshemmende Behandlung widerspiegelt. Daher könnte die Riechfunktion als Marker für das Fortschreiten der chronischen sinonasalen Entzündung dienen, bei der Differenzierung von CRS-Phänotypen und Endotypen bedeutsam sein und letztendlich die Entwicklung individualisierter Behandlungspläne unterstützen.

Für Publikation 2 ist eine Kategorisierung der OB-Formen mit einer guten „Interrater“-Übereinstimmung (Cohen's Kappa = 0,73) möglich. Unsere Ergebnisse legen nahe, dass die OB-Formen bei Patienten mit Riechstörungen signifikant von denen gesunder Personen abweichen. Sie korrelierten mit der Riechfunktion in der gesamten Gruppe, unabhängig von Alter, Geschlecht und OB-Volumen. Darüber hinaus schienen sich die OB-Formen bei gesunden Probanden mit dem Alter zu verändern. Bestimmte OB-Formen sind zumindest teilweise mit verschiedenen Ursachen von Riechstörungen assoziiert.

Für Publikation 3 zeigten Personen mit ICA im Vergleich zu Kontrollpersonen ein abgeflachtes Siebbeindach und eine flachere Fossa olfactoria. Darüber hinaus wurde das Fehlen des OB mit einem höheren Grad an Abflachung des Siebbeindachs und

einer flacheren Fossa olfactoria in Verbindung gebracht. Personen mit ICA konnten von und normosmischen Personen anhand der mporphologsichen Parameter gut unterschieden werden (Receiver-Operating-Characteristic-Kurven (AUC): 0,80 - Winkel der Fovea ethmoidalis, 0,76 - Tiefe der Fossa olfactoria, 0,70 - Winkel der lateralen Lamelle der Lamina cribrosa).

Schlussfolgerungen

In der vorliegenden Dissertation wird gezeigt, dass die Riechfunktion eng mit der Gesamtintensität der Entzündungsreaktion bei CRS, der Form des BO und der vorderen Schädelbasis verbunden ist. Daher wird empfohlen, dass die Beurteilung der Riechfunktion bei der klinischen Bewertung von CRS-Patienten berücksichtigt werden sollte, und die Beurteilung der Form des Riechbulbus und der vorderen Schädelbasis in der Diagnositk von Riechstörungen mitgeführt werden sollte.

12 Summary in English

Background

Olfactory dysfunction is a prevalent issue and can severely affect quality of life. Patients with olfactory dysfunction are more likely to report difficulties with cooking, feeling less safe, depression and anxiety. diagnosing and treating olfactory dysfunction remain challenging for clinicians. A better understanding of predict factors of olfaction will help in the development of targeted interventions and therapies for patients with olfactory dysfunction. Numerous clinical factors have been extensively investigated and found to be linked with olfactory dysfunction, such as age, gender and toxic agent exposure. The overall aim of this thesis was to investigate the clinical predictors of olfaction, with a focus on inflammation and imaging.

Hypothesis/question

In Publication 1, we hypothesized that olfaction is closely associated with the overall intensity of the inflammatory response in CRS, we aim to review olfactory dysfunction with regard to its clinical evaluation, pathophysiological mechanisms, relation with inflammatory burden and anti-inflammatory responses in CRS patients.

In Publication 2, we hypothesized that the shape of the OB predicts olfactory function, and that it is linked to olfactory loss, age, and gender. The aim of this study was to produce a classification of OB shape in the human brain, scalable to clinical and research applications.

In Publication 3, Our hypothesis was that the morphology of the anterior skull base, as measured in terms of the depth as well as width of olfactory fossa, angle of lateral lamella of cribriform plate (LLCP), and angle of fovea ethmoidalis, is different between ICA participants and healthy controls. We further hypothesized that in ICA the morphology of the anterior skull base is correlated to OB abnormalities, and that the morphological parameters are correlated with each other. The aim of this study was to identify and compare the morphological pattern of the anterior skull base surrounding the OB between individuals with ICA and normosmic controls using MRI.

Material and methods

In Publication 1, The first section of this paper describes the assessment of olfactory function using various measures, from ratings to MR based imaging. Then, we discuss the conductive and inflammatory mechanisms related to olfactory dysfunction in CRS: olfaction is associated with certain inflammatory patterns and is potentially a marker of CRS subtype. Finally, we review anti-inflammatory therapies including conservative and surgical approaches, and their effectiveness in olfactory dysfunction in CRS.

In Publication 2, Patients with the 5 most frequent causes of olfactory dysfunction (n= 192) as well as age/gender-matched healthy controls (n= 77) were recruited. Olfactory function was examined in great detail using the extended “Sniffin’ Sticks” test. A high-resolution structural T2 weighted MRI scan was obtained for all. The planimetric contours (surface in mm^2) of OB were delineated manually and then all surfaces were added and multiplied to obtain the OB volume in mm^3. OB shapes were outlined manually and characterized on a selected slice through the posterior coronal plane tangent to the eyeballs. We looked at OB shapes in terms of convexity and defined 7 categories based on OB contours: olive, circle, plano-convex, banana, irregular, plane and scattered

In Publication 3, We conducted a retrospective study to acquire T2 weighted magnetic resonance images from individuals diagnosed with ICA (n=31) and healthy, normosmic controls matched for age and gender (n=62). Between both groups, we compared the depth and width of the olfactory fossa, angle of ethmoidal fovea as well as angle of lateral lamella of cribriform plate. Within the ICA group we further performed subgroup analyses based on the presence or absence of the OB, to investigate whether the morphology of the anterior skull base relates to the presence of OBs. The diagnostic performance of these parameters was evaluated using receiver operating characteristic analysis.

Results

For Publication 1, we noticed that to assessment olfaction function is not only for detecting and quantifying patients’ symptom but also because it is useful to objectively assess the efficacy of CRS treatment over time. In particular, olfactory function seems to be a stable and valid factor in the various clusters of clinical presentations, linked with certain inflammatory patterns and reflective of the response to anti-inflammatory treatment. Accordingly, olfaction may act as a marker in the progression of chronic sinonasal inflammation, help to differentiate CRS

phenotypes and endotypes and ultimately aid in the development of tailored treatment regimens.

For Publication 2, Categorization of OB shapes is possible with an inter-rater agreement (Cohen's Kappa = 0.73). Our results suggested that OB shapes in patients with olfactory dysfunction are significantly different from healthy individuals. They were correlated with olfactory function in the whole group, independent of age, gender and OB volume. Moreover, OB shapes seemed to change with age in healthy subjects. Importantly, we found that OB shapes were also associated with different causes of olfactory disorders.

For Publication 3, Individuals with ICA exhibited a flattened ethmoid roof and shallower olfactory fossa when compared to controls. Further, the absence of the OB was found to be associated with a higher degree of flattening of the ethmoid roof and a shallow olfactory fossa. We reached the results in the following areas under the receiver operating characteristic curves (AUC): 0.80 - angle of fovea ethmoidalis, 0.76 - depth of olfactory fossa, 0.70 - angle of lateral lamella of cribriform plate for significant differentiation between individuals with ICA and normosmic controls.

Conclusions

Olfactory function is closely associated with overall intensity of the inflammatory response in CRS, shape of OB and anterior skull base. Thus, assessment of olfactory function should be considered in the clinical evaluation of CRS patients, and assessment of shape of OB and anterior skull base may be useful for the diagnosis of olfaction dysfunction.