Evidence for impaired olfactory function and structural brain integrity in a disorder of ciliary function, Usher syndrome

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ABSTRACT

Diseases involving cilia dysfunction, such as Usher Syndrome (USH), often involve visual and auditory loss. Psychophysical evidence has suggested that this may also hold true for the peripheral olfactory domain. Here we aimed to go a step further by attempting to establish relations between the integrity of cortical structures and olfactory function in this condition. We investigated olfactory function for USH types 1 (USH1) and 2 (USH2). Bilateral olfactory bulb (OB) volume and olfactory sulcus (OS) depth were also analysed.

Thirty-three controls with no previous olfactory deficits were age, sex and handedness-matched to 32 USH patients (11 USH1, 21 USH2). A butanol detection threshold test was performed to measure olfactory function. For OB volume and OS depth, morphometric measurements were performed using magnetic resonance imaging (MRI) based on detailed segmentation by three independent operators. Averaged values across these were used for the statistical analyses. Total intracranial volume was estimated using Freesurfer to account for head size variability.

Olfactory threshold was significantly lower in controls when compared to USH, USH1, and USH2. OS depth was found to be shallower in both hemispheres in USH patients when compared with the control group. OB volume was not significantly different between control and USH groups, or respective subgroups. Nevertheless, butanol threshold was negatively correlated with the left OB volume for the USH type 1 subgroup. The main effect of OS depth reduction was found to be mainly due to the comparison between USH2 and controls.

Our results provide evidence for morphometric changes and olfactory dysfunction in patients with USH. This correlated with a reduction in left OB volume in the USH1 subgroup, the most severe USH phenotype. The main effect of reduced OS depth was found to stem mainly from USH2 raising questions regarding a possible complex interaction between sensory olfactory loss and central cortical changes in this disease.

1. Introduction

Usher Syndrome (USH) is an heterogeneous and severely debilitating genetic disease of autosomal recessive nature. It is considered to be the most common cause of inherited deaf-blindness with a worldwide prevalence of 3–8:100000 (Mathur and Yang, 2015; Toms et al., 2015).

USH is thought to be a ciliopathy characterized by sensorineural hearing loss and progressive retinal degeneration in the form of Retinitis Pigmentosa (RP), featuring night blindness and loss of peripheral vision in early stages, with progression to blindness (Mathur and Yang, 2015; Toms et al., 2015). USH accounts for 18% of RP and 5% of inherited deafness cases (Toms et al., 2015).

USH may be subdivided in three types (Mathur and Yang, 2015;

Toms et al., 2015). USH type 1 (USH1) is characterized by onset of RP during infancy along with congenital profound deafness and vestibular impairment (Mathur and Yang, 2015). USH type 2 (USH2) is the most common type and has a later onset of RP during puberty or early adulthood and shows a milder hearing loss with no vestibular dysfunction. In rare cases, a progressive loss of hearing along with variable expression of both vestibular dysfunction and RP onset lead to a diagnosis of USH type 3 (USH3). The cases with additional atypical features are described as atypical USH (Mathur and Yang, 2015; Toms et al., 2015; Yan and Liu, 2010).

Thirteen genes have been associated with USH so far (Mathur and Yang, 2015), mainly comprising functions regarding cell adherence and protein scaffolding and signalling. Proteins encoded by these genes may participate in a multiprotein complex responsible for the development and maintenance of the hair bundles of the inner ear and photoreceptors in the retina. This may imply that multiple splice isoforms of USH genes play similar roles in different tissues, such as the outer segment and calyceal processes of photoreceptors in the retina, microvilli in the intestine, and the cilia in olfactory epithelium (Jansen et al., 2016; Toms et al., 2015; Yan and Liu, 2010).

Olfactory involvement has long been suspected in this syndrome (Arden and Fox, 1979; Jansen et al., 2016; Seeliger et al., 1999; Zrada et al., 1996), though conclusions have been controversial. A recent animal study demonstrated USH proteins' expression in the olfactory epithelium (Jansen et al., 2016) and a human-based USH study reporting accelerated psychophysical olfactory loss with age in USH patients led to a renewed interest in studying olfaction in USH (Ribeiro et al., 2016).

Magnetic resonance imaging (MRI) and other imaging methods such as positron emission tomography and functional MRI have enabled additional progress in the study of olfaction in several disorders. Neural mechanisms of olfactory function have been studied not only in healthy subjects (Seubert et al., 2013a; Seubert et al., 2013b), but also in Parkinson's disease (Mueller et al., 2005; Wang et al., 2011) and schizophrenia (Nguyen et al., 2011; Turetsky, 2000; Yousem et al., 2001), among other pathologies with known olfactory deficits. Moreover, in Alzheimer's disease (Thomann et al., 2009) and Bardet-Biedl Syndrome (BBS) (Braun et al., 2014, 2016), olfactory bulb (OB) volume and olfactory sulcus (OS) depth have been shown to be correlated with olfactory function. To the best of our knowledge, there is no published study correlating morphometric and olfactory functional changes regarding Usher syndrome.

Here we aimed to evaluate putative morphometric changes in OB volume and OS depth for USH patients and correlate such alterations with olfactory function.

2. Methods

2.1. Participants

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Faculty of Medicine of University of Coimbra. Written informed consent was obtained from all participants after research procedures had been fully explained.

Control subjects were enlisted from the local population while patients were recruited from the Otorhinolaryngology consultation of *Centro Hospitalar e Universitário de Coimbra*, Coimbra, Portugal. USH patients were clinically diagnosed as defined by the USH consortium (Smith et al., 1994; Steiner and Abraham, 1978). All subjects were subjected to a thorough clinical examination and an extensive review of clinical history.

Exclusion criteria were previously documented olfactory impairment or neurodegenerative disease, abnormal neuroradiological findings, and diagnosis of diabetes mellitus as this common disease is involved in atrophy of several brain regions (Wang et al., 2014).

Table 1
Age and gender descriptive statistics regarding groups and subgroups, all matched between groups and subgroups ($p > 0.05$).

	Age	Gender
	Mean ± standard deviation (years-old)	Male:Female ratio
Control	42.76 ± 10.51	21: 12
USH	46.59 ± 13.54	20: 12
USH1	45.55 ± 16.27	8: 3
USH2	47.14 ± 12.28	21: 9

The final control group consisted of 33 healthy subjects [21 males, 12 females; 42.76 ± 10.51 years-old (mean ± standard deviation)]. The patient's group included 32 subjects (20 males, 12 females; 46.59 ± 13.54 years-old).

The USH group was further divided into 2 subgroups: USH1 [11 patients (8 males, 3 females); 45.55 ± 16.27 years-old] and USH2 [21 patients (12 males, 9 females); 47.14 ± 12.28 years-old]. All groups (Control, USH) and subgroups (Control, USH1, USH2) were age, gender, and handedness-matched (Table 1).

2.2. Psychophysics and brain imaging

A binasal butanol olfactory threshold test was executed using a staircase procedure with a set of 8 solutions of n-butanol ranging from 4.000% to 0.002% following a 1:3 dilution with water as a solvent (Seeliger et al., 1999). Given this method, the lower the concentration detected by the subject – olfactory threshold – the better the olfactory performance (Cain, 1983, b; Cain and Gent. 1988; Toledano et al. 2003).

Scanning was performed on a 3-Tesla scanner (Magneton TrioTim, Siemens AG, Germany) at the Portuguese Brain Imaging Network, using a 12-channel birdcage head coil. Two T1-weighted Magnetization-Prepared Rapid Acquisition with Gradient Echo sequences were acquired from each participant [$1 \times 1 \times 1 \text{ mm}^3$ voxel size; Repetition Time (TR) 2.53 s; Echo Time (TE) 3.42 ms; Flip Angle (FA) 7°; Field of View (FOV) $256 \times 256 \text{ mm}^2$; 176 slices]. Additionally, one T2-weighted sequence was acquired for each subject ($0.4 \times 0.4 \times 2.0 \text{ mm}^3$ voxel size; TR 6.4 s; TE 148 ms; FA 150°; FOV $230 \times 230 \text{ mm}^2$; 30 slices) (Burmeister et al. 2011).

Manual segmentation of the OB was performed moving distally from the abrupt change in diameter at the beginning of the olfactory tract (Mueller et al. 2005). OS depth (Fig. 1) was measured in the plane of the posterior tangent through the eyeballs (Duprez and Rombaux 2010; Nguyen et al. 2011). Both were completed in Osirix v6.5.2 (Pixmeo SARL, Geneva, Switzerland) by three operators (JNR, ACP, SF) using the T2-weighted sequence. Since intraclass coefficient (95% confidence interval) were of 0.924 (0.891–0.949) for OB and 0.960 (0.942–0.973) for OS, all measurements were averaged in the following statistical analyses.

Freesurfer v5.3 software package (http://surfer.nmr.mgh.harvard.edu/) was used to perform cortical reconstruction and volumetric segmentation using the averaged T1-weighted scans for all subjects. The total intracranial volume values were extracted for each participant to account for head size variability.

2.3. Statistical analysis

Statistical analyses were performed between main groups (Control and USH) and between subgroups (Control, USH1, and USH2). Normality and variance homogeneity tests were performed when necessary to select either parametric or non-parametric statistical tests. For MRI structural measurements (OB volume, OS length), we used repeated-measures Analysis of Covariance (ANCOVA) with group or subgroups as the 'between-subjects' factor. Side [left (L), right (R)] was

Fig. 1. Example of the implementation of olfactory sulcus (OS) depth measurement, in this case on the right side. It is performed using Osirix by first finding the plane of the posterior tangent through the eyeballs. Afterwards a virtual tangent to the inferior gyrus rectus and internal orbital gyrus is obtained. Using this line as a starting point, another one is then drawn to measure the OS. Both lines are represented in red in Osirix (see orange arrow). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

defined as the only 'within-subjects factor'. Both age and estimated total intracranial volume were included as covariates. Despite age-matching, we still included age as a covariate because of the observed intragroup correlations (age × ROS, $r = -0.289$, $p = 0.020$; Age × LOS $r = -0.401$, $p = 0.001$).

Regarding the butanol threshold, we performed Mann-Whitney or Kruskal-Wallis H tests (with Bonferroni correction for multiple comparisons for post-hoc tests) for group or subgroup analyses, respectively. In addition, non-parametric Spearman correlations were used to assess the association between butanol threshold and OB, OS, and age.

All analyses were performed using SPSS Statistics v23.0 (SPSS Inc., Chicago, IL, USA) with statistical significance set at $p < 0.05$.

3. Results

3.1. Olfactory threshold

The butanol threshold was significantly higher ($U = 237$, $p = 9.700 \times 10^{-5}$) in USH patients (median = 0.140%, interquartile range [IQR] = 0.047–0.430%) when compared with the control group (median = 0.016%, IQR = 0.016–0.047%).

Furthermore, a Kruskal-Wallis H test showed a statistically significant difference between subgroups [$\chi^2(2) = 19.469$, $p = 5.900 \times 10^{-5}$]. Both USH1 (median = 0.430%, IQR = 0.140–0.430%; adjusted $p = 7.100 \times 10^{-5}$) and USH2 (median = 0.047%, IQR = 0.016–0.430%; adjusted $p = 0.036$) had higher butanol thresholds than the control group. No differences were found between USH1 and USH2 (adjusted $p = 0.116$).

3.2. Olfactory bulb and sulcus morphometry

Although a lower mean OB volume was found in USH patients, no overall main effect was found between groups[$F(1,61) = 0.099$, $p = 0.754$], nor between subgroups[$F(2,60) = 0.261$, $p = 0.771$], and therefore no post-hoc tests were performed. Descriptive statistics for OB volumes are provided in Table 2.

Regarding OS depth, we found a statistically significant main effect

Table 2
Olfactory bulb volume for Control and USH groups and its subgroups. No overall main effect was found between groups $F(1,61) = 0.099$, $p = 0.754$] nor between subgroups[$F(2,60) = 0.261$, $p = 0.771$]. ROB – Right olfactory bulb. LOB – Left olfactory bulb.

		MEAN (mm^3)	SD (mm^3)
Control	ROB	60.07	14.38
	LOB	61.37	13.88
USH	ROB	57.64	16.17
	LOB	54.96	14.38
USH1	ROB	55.13	14.98
	LOB	51.51	15.39
USH2	ROB	58.95	16.98
	LOB	56.77	13.79

on overall OS depth between groups[$F(1,61) = 8.924$, $p = 0.004$], with post-hoc tests showing a significant reduction for USH for both right OS [$t(63) = 2.996$, adjusted $p = 0.004$] and left OS [$t(63) = 2.510$, adjusted $p = 0.015$].

An overall effect was also present among subgroups [$F(2, 60) = 4.801$, $p = 0.012$], with a significant reduction of OS on both sides only in the USH2 subgroup [right OS: $t(52) = 3.060$, $p = 0.003$; left OS: $t(52) = 2.571$, $p = 0.013$] when compared with controls. Type 1 USH patients did not have significant differences regarding OS when compared with both USH2 [right OS: $t(30) = 0.836$, $p = 0.406$; left OS: $t(30) = 0.728$, $p = 0.470$] nor with controls [right OS: $t(42) = 2.014$, $p = 0.051$; left OS: $t(42) = 1.405$, $p = 0.168$]. Descriptive measures for OS are provided in Table 3 and a visualisation of the means across groups and subgroups can be seen in Fig. 2. Given that these patients had visual and auditory loss, it was expected that these structures were also affected. This was indeed the case for the visual areas ($p < 0.001$ on t-tests between groups).

3.3. Correlations with butanol thresholds

We found a correlation between the butanol threshold and age ($r_s = 0.246$, $p = 0.048$) only in healthy controls ($r_s = 0.440$, $p = 0.010$) and neither in the USH group ($r_s = 0.015$, $p = 0.934$) nor in its subgroups (USH1: $r_s = 0.280$, $p = 0.405$; USH2: $r_s = 0.022$, $p = 0.925$).

The butanol threshold was found to be negatively correlated with the left OB volume ($r_s = -0.249$, $p = 0.045$) for all subjects, but not with the right OB ($r_s = -0.059$, $p = 0.642$). In addition of this effect being derived from the USH group ($r_s = -0.393$, $p = 0.026$), this result was mainly explained by the USH1 subgroup ($r_s = -0.692$, $p = 0.018$).

Butanol threshold showed no correlation with OS depth on either side for neither healthy controls nor USH patients and its subgroups.

Table 3
Olfactory sulcus depths for Control and USH groups and its subgroups. An overall main effect was found between groups, $F(1,61) = 8.924$, $p = 0.004$, and subgroups, $F(2, 60) = 4.801$, $p = 0.012$, with a decrease in the depth of the sulci only for the USH group and USH2 subgroup, respectively. ROS – Right olfactory sulcus. LOS – Left olfactory sulcus.

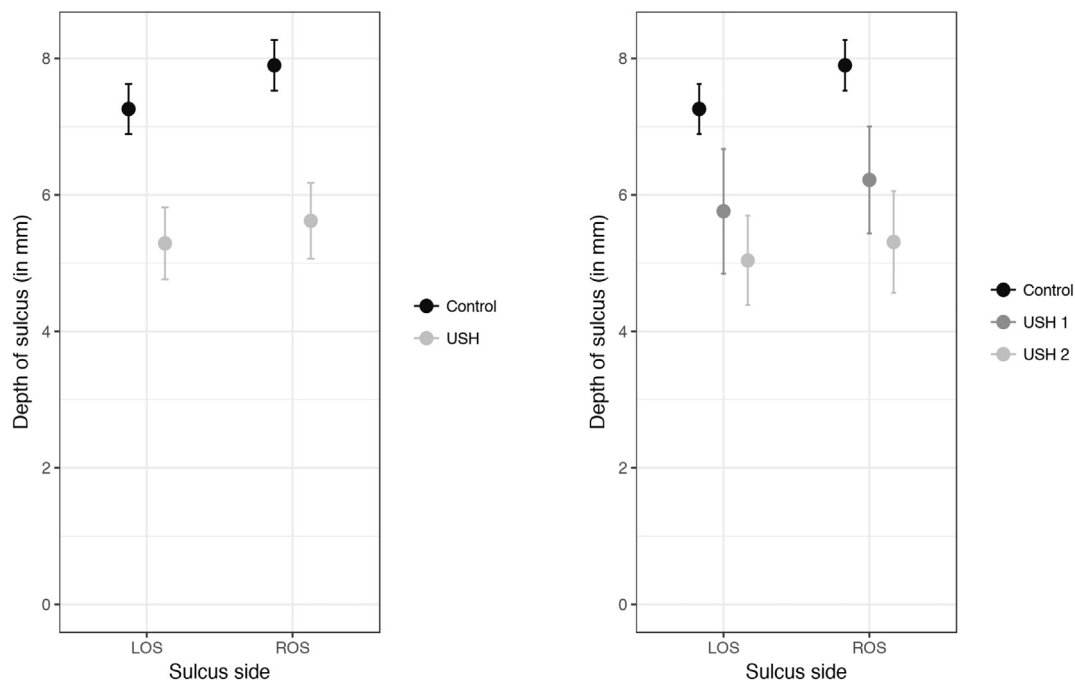|		MEAN (mm)	SD (mm)
Control	ROS	7.90	2.14
	LOS	7.26	2.11
USH	ROS	5.62	3.14
	LOS	5.29	2.98
USH 1	ROS	6.22	2.60
	LOS	5.76	3.03
USH 2	ROS	5.31	3.41
	LOS	5.04	3.00

Fig. 2. Mean and standard errors of mean of both groups and subgroups for left and right olfactory sulci. ROS – Right olfactory sulcus. LOS – Left olfactory sulcus.

4. Discussion

This study provides novel insights into structure-function correlations in USH, concerning psychophysical function and central olfactory structures, as assessed by psychophysics and MRI, respectively.

Although there is some uncertainty regarding olfactory deficits in USH patients (Seeliger et al. 1999), our study corroborates this notion in line with previous functional findings such as sensory deficits identified in 22 USH patients (Zrada et al. 1996) and studies in other ciliopathies as BBS (Braun et al. 2014). Furthermore, in our group of subjects, we were able to confirm the functional relevance and dominance of olfactory deficits in USH1 patients (see also our previous psychophysical work in USH1 patients (Ribeiro et al. 2016)).

Jansen et al., 2016 correlated olfactory deficits in USH mice models with the presence of USH olfactory neuroepithelium protein changes. These changes seem to affect the development of stereocilia and stereocilia-like structures in not only the inner ear and in the retina, but also in the olfactory cilia as previously suggested (Arden and Fox 1979).

We further confirmed the well described age-related decrease in olfactory psychophysical function in healthy subjects possibly justified by repeated viral infections of the upper airway, a decrease in neurotransmitters, and degeneration of the olfactory epithelium, among others (Huart et al. 2013; Yousem 1989). In our USH patient group, no such age dependent correlation was found, suggesting that disease-related factors play a more dominant role in the olfactory phenotype than age. Yet, another study from our research group (Ribeiro et al. 2016) – with a total of 65 USH patients (22 USH1, 43 USH2) – showed that an age-related olfactory decline in both USH1 and USH2 is also present, though the latter did not reach statistical significance.

With a reduction in olfactory function we would have expected to also find a reduction in OB volume, as it has been found to positively correlate with olfactory performance in healthy subjects (Buschhüter et al. 2008; Huart et al. 2013; Nguyen et al., 2016; Seubert et al., 2013b; Turetsky, 2000; Yousem 1989) and several diseases (Nguyen et al. 2011; Rombaux et al., 2009; Thomann et al. 2009). This could be explained by the OB' role in connecting the peripheral and central nervous system via the olfactory tract making this region a primary place for processing olfactory information (Buschhüter et al. 2008; Huart et al. 2013). The OB volume is thus generally correlated with the olfactory function.

Indeed, we did not find evidence for significant reduction in OB volume for both hemispheres in USH and for both of the patients' subgroups, in contrast with the effects observed in OS. This is intriguing because OB has been shown to suffer significant volumetric differences in as short periods of time as of 3 months after olfactory training therapy (Gudziol et al. 2009). This is generally explained by the occurrence of neuroplasticity, previously reported to occur in the OB (Buschhüter et al. 2008; Turetsky, 2000), more patent in younger people (Rombaux et al. 2010). Correlation analysis suggests that OB may play a role in functional olfactory reserve in this condition.

Accordingly, a negative correlation was found to exist between olfactory thresholds (worse performance) and left OB volume for USH patients, namely USH1. In addition, taking into account the premise of a brain right-side dominance in olfactory function (Abolmaali et al. 2002; Hummel et al. 2003; Rombaux et al., 2009), we were surprised to find that akin to BBS patients (Braun et al. 2016) this correlation was present in the left hemisphere. Contrary to healthy subjects and patients with other diseases, this could imply that when faced with an olfactory deficit, the left OB may be more vulnerable in these specific pathologic entities, USH and BBS, both grouped as ciliopathies. Given that olfactory function presents the above mentioned right-hemisphere dominance the observation of a correlation specifically for the left side requires additional explanation. Given that this was also observed in other conditions such as BBS this maybe due to the lower functional reserve and consequent higher disease susceptibility of the left side.

Interestingly enough, with OS depth also having been linked with olfactory performance, a bilateral reduction was present in USH. This main effect was found to be due mainly to the USH2 group. Given that OS reduction does not necessarily mirror changes in OB (Hummel et al. 2015), this suggests a complex interaction between peripheral neurosensory deficits and cortical changes.

These findings suggest that olfactory threshold testing should be included in the standard clinical assessment of USH. More specifically, in the early diagnosis of USH it could be used prior to genetic testing, or at least as additional information because of the clinical implications in daily life functioning.

Moreover, other functional imaging studies(e.g. functional MRI can potentially provide a more comprehensive understanding of the role of

OB, OS, and other olfactory-related brain regions in the sensory phenotype of USH.

5. Conclusion

We found a reduction in OS depth, mostly in USH2 patients, and decreased olfactory performance. In addition, the left OB volume correlated with USH patients olfactory function. Our study also raises relevant questions for future longitudinal studies regarding the effect that multiple and early sensory deficits may have in brain structures over disease progression. The structure-function correlations highlighted in this study suggest the need for future research based on functional imaging approaches such as functional MRI.

Conflicts of interest

The authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nicl.2019.101757.

References

Abolmaali, N.D., Hietschold, V., Vogl, T.J., Hüttenbrink, K.B., Hummel, T., 2002. MR evaluation in patients with isolated anosmia since birth or early childhood. Am. J. Neuroradiol. https://doi.org/10.1016/j.neuroscience.2010.05.012.

Arden, G.B., Fox, B., 1979. Increased incidence of abnormal nasal cilia in patients with retinitis pigmentosa [17]. Nature. https://doi.org/10.1038/279534a0.

Braun, J.-J., Noblet, V., Durand, M., Scheidecker, S., Zinetti-Bertschy, A., Foucher, J., Marion, V., Muller, J., Riehm, S., Dollfus, H., Kremer, S., 2014. Olfaction evaluation and correlation with brain atrophy in Bardet-Biedl syndrome. Clin. Genet. https://doi.org/10.1111/cge.12391.

Braun, J.J., Noblet, V., Kremer, S., Molière, S., Dollfus, H., Marion, V., Goetz, N., Muller, J., Riehm, S., 2016. Value of MRI olfactory bulb evaluation in the assessment of olfactory dysfunction in Bardet–Biedl syndrome. Clin. Genet. https://doi.org/10.1111/cge.12697.

Burmeister, H.P., Baltzer, P.A.T., Möslein, C., Bitter, T., Gudziol, H., Dietzel, M., Guntinas-Lichius, O., Kaiser, W.A., 2011. Reproducibility and repeatability of volumetric measurements for olfactory bulb volumetry: which method is appropriate? An update using 3 tesla MRI. Acad. Radiol. https://doi.org/10.1016/j.acra.2011.02.018.

Buschhüter, D., et al., 2008. Correlation between olfactory bulb volume and olfactory function. NeuroImage 42 (2), 498–502.

Cain, W., 1983. Clinical evaluation of olfaction. Dysphagia Clin. Manag. Adults Child. https://doi.org/10.1016/B978-0-323-18701-5.00007-5.

Cain, W.S., Goodspeed, R.B., Gent, J.F., Leonard, G., 1988. Evaluation of olfactory dysfunction in the connecticut chemosensory clinical research center. Laryngoscope. https://doi.org/10.1288/00005537-198801000-00017.

Duprez, T.P., Rombaux, P., 2010. Imaging the olfactory tract (cranial nerve #1). Eur. J. Radiol. https://doi.org/10.1016/j.ejrad.2009.05.065.

Gudziol, V., Buschhüter, D., Abolmaali, N., Gerber, J., Rombaux, P., Hummel, T., 2009. Increasing olfactory bulb volume due to treatment of chronic rhinosinusitis-a longitudinal study. Brain. https://doi.org/10.1093/brain/awp243.

Huart, C., Rombaux, P., Hummel, T., 2013. Plasticity of the human olfactory system: the olfactory bulb. Molecules. https://doi.org/10.3390/molecules180911586.

Hummel, T., Damm, M., Vent, J., Schmidt, M., Theissen, P., Larsson, M., Klussmann, J.P., 2003. Depth of olfactory sulcus and olfactory function. Brain Res. https://doi.org/10.1016/S0006-8993(03)02589-7.

Hummel, T., Urbig, A., Huart, C., Duprez, T., Rombaux, P., 2015. Volume of olfactory bulb and depth of olfactory sulcus in 378 consecutive patients with olfactory loss. J. Neurol. https://doi.org/10.1007/s00415-015-7691-x.

Jansen, F., Kalbe, B., Scholz, P., Mikosz, M., Wunderlich, K.A., Kurtenbach, S., Nagel-Wolfrum, K., Wolfrum, U., Hatt, H., Osterloh, S., 2016. Impact of the usher syndrome on olfaction. Hum. Mol. Genet. https://doi.org/10.1093/hmg/ddv490.

Mathur, P., Yang, J., 2015. Usher syndrome: hearing loss, retinal degeneration and associated abnormalities. Biochim. Biophys. Acta-Mol. Basis Dis. https://doi.org/10.1016/j.bbadis.2014.11.020.

Mueller, A., Abolmaali, N.D., Hakimi, A.R., Gloeckler, T., Herting, B., Reichmann, H., Hummel, T., 2005. Olfactory bulb volumes in patients with idiopathic Parkinson's disease - a pilot study. J. Neural Transm. https://doi.org/10.1007/s00702-005-0280-x.

Nguyen, A.D., Pelavin, P.E., Shenton, M.E., Chilakamarri, P., McCarley, R.W., Nestor, P.G., Levitt, J.J., 2011. Olfactory sulcal depth and olfactory bulb volume in patients with schizophrenia: an MRI study. Brain Imag. Behav. https://doi.org/10.1007/s11682-011-9129-0.

Nguyen, D.T., Rumeau, C., Gallet, P., Jankowski, R., 2016. Olfactory exploration: state of the art. Eur. Ann. Otorhinolaryngol. Head Neck Dis. https://doi.org/10.1016/j.anorl.2015.08.038.

Ribeiro, J.C., Oliveiros, B., Pereira, P., António, N., Hummel, T., Paiva, A., Silva, E.D., 2016. Accelerated age-related olfactory decline among type 1 usher patients. Sci. Rep. https://doi.org/10.1038/srep28309.

Rombaux, P., Duprez, T., Hummel, T., 2009. Olfactory bulb volume in the clinical assessment of olfactory dysfunction. Rhinology 47, 3–9.

Rombaux, P., Huart, C., De Volder, A.G., Cuevas, I., Renier, L., Duprez, T., Grandin, C., 2010. Increased olfactory bulb volume and olfactory function in early blind subjects. Neuroreport. https://doi.org/10.1097/WNR.0b013e32833fcb8a.

Seeliger, M., Pfister, M., Gendo, K., Paasch, S., Apfelstedt-Sylla, E., Plinkert, P., Zenner, H.P., Zrenner, E., 1999. Comparative study of visual, auditory, and olfactory function in usher syndrome. Graefes Arch. Clin. Exp. Ophthalmol. https://doi.org/10.1007/s004170050237.

Seubert, J., Freiherr, J., Djordjevic, J., Lundström, J.N., 2013a. Statistical localization of human olfactory cortex. Neuroimage. https://doi.org/10.1016/j.neuroimage.2012.10.030.

Seubert, J., Freiherr, J., Frasnelli, J., Hummel, T., Lundström, J.N., 2013b. Orbitofrontal cortex and olfactory bulb volume predict distinct aspects of olfactory performance in healthy subjects. Cereb. Cortex. https://doi.org/10.1093/cercor/bhs230.

Smith, R.J.H., Berlin, C.I., Hejtmancik, J.F., Keats, B.J.B., Kimberling, W.J., Lewis, R.A., Moller, C.G., Pelias, M.Z., Tranebjaerg, L., 1994. Clinical diagnosis of the usher syndromes. Am. J. Med. Genet. https://doi.org/10.1002/ajmg.1320500107.

Steiner, J.E., Abraham, F.A., 1978. Gustatory and olfactory function in patients affected by usher's syndrome. Chem. Senses. https://doi.org/10.1093/chemse/3.1.93.

Thomann, P.A., Dos Santos, V., Toro, P., Schönknecht, P., Essig, M., Schröder, J., 2009. Reduced olfactory bulb and tract volume in early Alzheimer's disease-a MRI study. Neurobiol. Aging. https://doi.org/10.1016/j.neurobiolaging.2007.08.001.

Toledano, A., González, E., Onrubia, T.J., Herráiz, C., Mate, M.A., García, M., Navarro, M., Plaza, G., Aparicio, J.M., De Los Santos, G., Galindo, N., 2003. Test de olfato de connecticut (cccrc): valores en voluntarios sanos. Acta Otorrinolaringol. Esp. https://doi.org/10.1016/S0001-6519(03)78467-2.

Toms, M., Bitner-Glindzicz, M., Webster, A., Moosajee, M., 2015. Usher syndrome: a review of the clinical phenotype, genes and therapeutic strategies. Expert Rev. Ophthalmol. https://doi.org/10.1586/17469899.2015.1033403.

Turetsky, B.I., 2000. Reduced olfactory bulb volume in patients with schizophrenia. Am. J. Psychiatry. https://doi.org/10.1176/appi.ajp.157.5.828.

Wang, J., You, H., Liu, J.F., Ni, D.F., Zhang, Z.X., Guan, J., 2011. Association of olfactory bulb volume and olfactory sulcus depth with olfactory function in patients with Parkinson disease. Am. J. Neuroradiol. https://doi.org/10.3174/ajnr.A2350.

Wang, C.X., Fu, K.L., Liu, H.J., Xing, F., Zhang, S.Y., 2014. Brain structural changes and their correlation with vascular disease in type 2 diabetes mellitus patients: a voxel-based morphometry study. Neural Regen. Res. https://doi.org/10.4103/1673-5374.139482.

Yan, D., Liu, X.Z., 2010. Genetics and pathological mechanisms of usher syndrome. J. Hum. Genet. https://doi.org/10.1038/jhg.2010.29.

Yousem, D.M., 1989. Olfactory bulb and tract and temporal lobe volumes. J. Chem. Inf. Model.

Yousem, D.M., Geckle, R.J., Bilker, W.B., & Doty, R.L., 1998. Olfactory bulb and tract and temporal lobe volumes: Normative data across decades. Annals of the New York Academy of Sciences 855, 546–555.

Zrada, S.E., Braat, K., Doty, R.L., Laties, A.M., 1996. Olfactory loss in usher syndrome: another sensory deficit? Am. J. Med. Genet. https://doi.org/10.1002/ajmg.1320640402.