Introduction: A significant number of sensorineural hearing loss (SSNHL) patients had no noticeable hearing improvement after glucocorticoid (GC) treatment. In the present study, we examined expression of the nuclear factor erythroid 2-related factor 2 (NRF2) and histone deacetylase 2 (HDAC2) in peripheral blood mononuclear cells (PBMCs) of refractory SSNHL patients to study the role of NRF2-HDAC2 pathway in GC insensitivity hearing improvement after GC treatment, which is usually referred to as refractory SSNHL or GC insensitivity.

Materials and Methods: Forty-four refractory SSNHL patients were treated by intratympanic GC infusion. Hearing was tested in all patients before and after treatment by pure tone hearing test. NRF2/HDAC2 mRNA and protein levels were examined in PBMCs of refractory SSNHL patients before and after treatment. PBMCs from healthy volunteers were used as normal controls. Results: According to the hearing improvement after treatment, patients were assigned into 2 groups: the intratympanic GC sensitive (IGCS) group (hearing recovery ≥15 dB HL) and the intratympanic GC insensitive (IGCI) group (hearing recovery <15 dB HL). Before treatment, the NRF2 mRNA level was lower in all patients than the normal control group. After treatment, NRF2 and HDAC2 mRNA and protein levels were increased in the IGCS group, while no significant change was observed in the IGCI group.

Conclusion: Low response of NRF2/HDAC2 proteins is associated with GC insensitivity in SSNHL. We speculate that the NRF2-HDAC2 pathway affects GC sensitivity in SSNHL patients.

Keywords
Hearing loss · Nuclear factor erythroid 2-related factor 2 · Histone deacetylase 2 · Glucocorticoids

Abstract
Introduction: Sudden sensorineural hearing loss (SSNHL) is a common inner ear disease that is defined as an unexpected (less than 3 days) and unexplained hearing loss of more
than 30 dB HL in at least consecutive 3 frequencies [1]. Although its etiology remains unclear, glucocorticoid (GC) administration is currently recommended as the first-line treatment and salvage therapy in the clinic, primarily due to its anti-inflammatory effects [2]. However, approximately 20% of SSNHL patients have no noticeable hearing improvement after GC treatment [3], a phenomenon which is called GC insensitivity [4]. Although the mechanisms are uncertain, our previous study has indicated that GC insensitivity is associated with a reduction of histone deacetylase2 (HDAC2) protein level in refractory SSNHL patients [5]. Consistent with our prior studies, reduced HDAC2 activity is also associated GC insensitivity in inflammatory diseases [6]. HDAC2 is an enzyme that removes acetyl groups from heterochromatin, allowing chromosome condensation and inhibiting the transcription of inflammatory genes that regulated by nuclear factor κB, allowing GC to carry out its anti-inflammatory functions [6, 7]. HDAC2 is normally regulated by post-translational modifications in response to oxidative or nitric stress stimuli [8]. However, it is currently unknown what regulatory mechanism leads to the reduction of HDAC2 protein activity in SSNHL.

Nuclear factor erythroid 2-related factor 2 (NRF2) is a leucine zipper transcription factor of the “cap ‘n’ collar” family that plays a key role in regulating steroid sensitivity via HDAC2 in the mouse lung and human keratinocytes [9]. Normally, NRF2 mRNA is constitutively expressed [10]. Under quiescent conditions, NRF2 is sequestered by the Kelch-like ECH-associated protein 1 (Keap1) in the cytoplasm in a ubiquinated, inactivated status. When challenged by reactive oxygen species, NRF2 is phosphorylated and uncoupled from Keap1, allowing for its translocation into the nucleus. In the nucleus, NRF2 combines with the antioxidant response element in DNA sequences to activate a series of phase II detoxification and antioxidant enzymes to remove reactive oxygen species and fight oxidative stress (OS) [11]. To date, it is unclear what role NRF2 activity plays in SSNHL, although OS may be one of the underlying mechanisms of SSNHL [12]. Based on these observations, we hypothesized that the NRF2-HDAC2 pathway may be involved in refractory SSNHL patients and may affect GC insensitivity. In the present study, we first examined the levels of NRF2/HDAC2 in refractory SSNHL patients to discern if its expression patterns might correlate with GC sensitivity.

### Materials and Methods

#### Recruitment of Refractory SSNHL Patients

Forty-four SSNHL patients were recruited from March 2015 to July 2016 at the Department of Otorhinolaryngology-Head and Neck Surgery, Nanjing Drum Tower Hospital, Medical School of Nanjing University, Nanjing, China. All patients had failed a conventional treatment regimen and were diagnosed as refractory SSNHL [1, 5]. All patients had severe or profound hearing loss with a pure-tone average (PTA, 0.25-0.5-1-2-4-8 kHz) greater than 60 dB in the affected ears (Table 1). The exclusion criteria included 1) patients with systemic inflammatory, immunological, or metabolic diseases; 2) patients with a family history of SSNHL; 3) patients with retro-cochlear diseases; and 4) loss of follow-up or withdrawal in the middle of study. Twenty healthy volunteers were recruited as a control group. All procedures were carried out in accordance with SSNHL guideline of Chinese Medical Association [13].

#### Treatment and Follow-Up

All patients who previously received a systemic GC treatment for at least 7 days with an average PTA improvement less than 15 dB HL (0.25–8 kHz) [13]. All patients in the present investigation were treated with intratympanic methylprednisolone perfusion (20 mg/infusion/day for 10 days, Pfizer, Inc, USA), Ginkgo biloba extract injection (105 mg/day, intravenously), and monosialotetrahexosylganglioside sodium (a neurotrophic drug, 40 mg/day, intravenously) for 10 consecutive days according to the guideline published by Chinese Medical Association [13]. The systemic GC treatment had been stopped for 3–8 days in all patients before enrollment in the present study so that there was no overlap of the 2 treatments. All patients were followed up for PTA for 3 months or more. According to the hearing improvement at 3 months after onset, patients were assigned into the intratympanic GC sensitive (IGCS) group (hearing recovery ≥15 dB HL) and the intratympanic GC insensitive (IGCI) group (hearing recovery <15 dB HL) [13].

#### Plasma and PBMC Collection

Twenty milliliters of peripheral venous blood were collected from SSNHL patients before and after 10-day intratympanic methylprednisolone perfusion and from healthy volunteers. After centrifugation, PBMCs were collected using the Ficoll method. PBMCs were stored at −80°C until further use.

### Table 1. General clinical data of SSNHL patients (number of patients or mean ± SD)

| Parameter                              | Value                              |
|----------------------------------------|------------------------------------|
| Age, years                             | 44.5±12.9                          |
| Gender (M:F)                           | 20:24                              |
| Interval from onset to intratympanic GC (d) | 21.7±13.3                        |
| Affected ear (L&R)                     | 16:28                              |
| Patients with tinnitus                 | 32                                 |
| Patients with dizziness/vertigo        | 13                                 |
| PTA (dB, 0.25–8 kHz) before treatment  | 85.4±14.2                          |
| PTA (dB, 0.25–8 kHz) after treatment   | 73.6±20.4                          |

A, atretic; SSNHL, sensorineural hearing loss; M, male; F, female; d, day; L, left ear; R, right ear; GC, glucocorticoid; PTA, pure-tone average.
NRF2/HDAC2 mRNA Expression in PBMCs

To examine NRF2/HDAC2 mRNA expression in PBMCs, total RNA was extracted by using TRIZol (Invitrogen, Waltham, MA, USA). cDNA was generated using PrimeScript™ RT Master Mix kit (TAKARA, Dalian, China). RT-qPCR reactions were performed using SYBR® PremixExTaq™ II (TAKARA, Dalian, China) according to the manufacturer’s guidelines. The primer (TAKARA, Dalian, China) sequences were as follows: NRF2 (forward – 5′-TGAGGTTTCTTCGCTACGGT-3′; reverse – 5′-CTTCTGTCAGTTTCGCTGG-3′), HDAC2 (forward – 5′-ATGGCGTACAGTCAAGGAGG-3′; reverse – 5′-TGCGGATTCTATGAGGCTTCAA-3′), and β-Actin (forward – 5′-GTCCACCGAAATGCTTCTA-3′; reverse – 5′-TGCTGTCACCTTCACCGTTC-3′). Samples were incubated at 95°C for 30 s for initial denaturation cycle, followed by 40 cycles of 95°C for 5 s and 60°C for 34 s to amplify the cDNA. Triplicate reactions were run for each gene. 2−ΔΔCT method was used to calculate the fold changes in expression of NRF2/HDAC2 [14].

Determination of NRF2/HDAC2 Protein Level in PBMCs

To examine NRF2/HDAC2 protein levels in PBMCs, nuclear proteins were extracted from PBMCs of SSNHL patients and normal controls using the nuclear protein extraction kit (Beyotime, Nanjing, China). HDAC2 protein levels were measured with a commercially available HDAC2 assay kit (EpiQuik, Farmingdale, New York). NRF2 protein levels were measured in nuclear extracts by following the manufacturer’s instructions of a commercially ELISA-based kit (Cayman Europe). Statistical Analyses

All data were analyzed with the SPSS 19.0 software and presented as the mean ± standard deviation (x ± s) or the median (the smallest percentage and the highest percentage for inhibition of PBMC proliferation). The distribution of all data was tested first. Non-normality test (Wilcoxon signed ranks test) was used if the distribution was not normal. Independent sample t-test was used in comparison between 2 groups. Singe factor variance analysis was used in comparison among 3 groups, and paired t-test was used in comparisons before and after treatment in each group. p < 0.05 was considered to be statistically significant.

Results

General Clinical Data of SSNHL Patients

In the present study, clinical data were collected from 44 refractory SSNHL patients. All patients were followed up for 3 months. The average PTA at 0.25–8 kHz after treatment was 73.6 ± 20.4 dB HL, which was significantly decreased relative to the average PTA measured before treatment (85.4 ± 14.2 dB HL, t = 5.333, df = 43, paired test, p < 0.001). Therefore, the overall averaged hearing improvement in all patients was 11.8 dB HL (85.4–73.6 dB HL), which represents a 4x the just noticeable difference in discriminating a difference in sound intensity lies between 2 and 3 dB [15]. According to hearing recovery after intratympanic treatment, patients were assigned into 2 groups: the IGCS group (hearing recovery ≥15 dB HL) and the IGCI group (hearing recovery <15 dB HL). The general clinical information of all SSNHL patients is shown in Table 1. Comparison of the general clinical data between the IGCS and IGCI groups is shown in Table 2. There were no significant differences between the 2 groups in patient age, the number of patients with tinnitus or dizziness/vertigo, the prevalence of affected ear, or average PTA before the intratympanic GC treatment (all p > 0.05, Table 2). However, the IGCI group had a longer interval from onset to the intratympanic GC treatment and more male patients than the IGCS group (all p < 0.05, Table 2). The shorter interval in the IGCS group indicates that intratympanic GC treatment should be initiated as soon as possible once patients failed a systemic treatment.

Table 2. General clinical data in the IGCS and IGCI groups

| Characteristic                          | IGCS (n = 16) | IGCI (n = 28) | p value |
|----------------------------------------|---------------|---------------|---------|
| Age, years                             | 42.4±13.1     | 45.7±12.8     | 0.519   |
| Number of male patients (%)            | 4 (25)        | 16 (57.1)     | 0.039*  |
| Interval from onset to intratympanic GC (d) | 15.8±6.8       | 25.1±15.0     | 0.024*  |
| Distribution of affected ears (L: R)   | 6/10          | 8/20          | 0.541   |
| Number of patients with tinnitus (%)   | 11 (68.6)     | 21 (75)       | 0.654   |
| Number of patients with dizziness (%)  | 4 (25)        | 9 (32.1)      | 0.617   |
| PTA before treatment (dB, 0.25–8 kHz)  | 84.3±12.3     | 86.1±15.3     | 0.693   |
| PTA after treatment (dB, 0.25–8 kHz)   | 55.6±14.1     | 83.6±16.6     | 0.000*  |

A, atretic; IGCS, the intratympanic GC sensitive; IGCI, the intratympanic GC insensitive; GC, glucocorticoid; d, day; L, left ear; R, right ear; PTA, pure-tone average. * p < 0.05.
Increased HDAC2 Protein and mRNA Levels in the IGCS Group

Before treatment, HDAC2 mRNA expression in the IGCS and IGCI groups was significantly higher than the control group (independent sample t-test, *p < 0.05) before treatment. After treatment, significantly increased HDAC2 mRNA expression was observed in the IGCS group compared to the level before treatment (paired t-test, *p < 0.05) while no significant change was observed in the IGCI group (*p > 0.05). Before treatment, HDAC2 protein level was significantly lower in the IGCS and IGCI groups than the control group (independent sample t-test, *p < 0.05). After treatment, significantly increased HDAC2 protein level was observed in the IGCS group compared to the level before treatment (paired t-test, *all p < 0.05) while no change was observed in the IGCI group (*p > 0.05). Before treatment, significantly lower NRF2 mRNA levels were observed in the IGCS and IGCI groups compared to the control group (independent sample t-test, *p < 0.05). The treatment significantly increased NRF2 mRNA levels in the IGCS group (paired t-test, *p < 0.05) but not in the IGCI group (*p > 0.05). Before treatment, NRF2 protein levels were not significantly changed in the IGCS and IGCI groups compared to the control group (paired t-test, all p > 0.05). After treatment, NRF2 protein levels were increased in the IGCS group (paired t-test, *p < 0.05) but not in the IGCI group compared to the levels before treatment (*p > 0.05). * indicates p < 0.05 compared to the normal group and * indicates p < 0.05 compared to the levels before treatment. NRF2, nuclear factor erythroid 2-related factor 2; HDAC2, histone deacetylase 2; IGCS, intratympanic glucocorticoid sensitive; IGCI, intratympanic glucocorticoid insensitive.

Decreased NRF2 mRNA and Protein Expression in PBMCs of SSNHL Patients

NRF2 mRNA and protein levels were examined in PBMCs of 44 SSNHL patients. Before treatment, significantly lower NRF2 mRNA levels were detected by RT-qPCR in all SSNHL patients (0.51 ± 0.055) compared to the normal control group (0.67 ± 0.111, t = −9.457, inde-
NRF2-HDAC2 Pathway in Refractory SSNHL

The pathogenesis of SSNHL is widely divergent, and the possible mechanisms include vasospasm, vascular embolism or thrombosis, stria dysfunction, membrane labyrinth hydrops, and hair cell injury in the inner ear [13]. In accordance with the complex etiology of the disorder, GCs, antioxidants, vasodilators, and neurotrophins have all been used to treat SSNHL. Among these drugs, GCs are currently recommended as the first line of treatment for SSNHL, due to their effects in mitigating inflammation, microcirculation improvement, and ionic balance in the inner ear [17]. However, clinical studies have found that some SSNHL patients are insensitive or resistant to GCs, as seen in some patients with severe asthma or chronic obstructive lung disease [18]. Because of this heterogeneous GC insensitivity, some SSNHL patients have a poor prognosis [4]. Although the mechanisms of GC insensitivity are unclear, our earlier work and the present studies have suggested that the reduction of HDAC2 protein level may be involved in GC resistance in refractory SSNHL patients [5]. We have also observed that HDAC2 is broadly expressed in the cochlea, including basilar membrane, stria vascularis, and spiral ganglion [19]. Furthermore, HDAC2 expression in the cochlea is negatively correlated with GC effectiveness in an acute cochlear injury model, and a HDAC2 activator, trichostatin A (TSA), has been shown to improve GC effectiveness [17]. All these results indicated the importance of HDAC2 in GC sensitivity in SSNHL.

In the present investigation, we compared the expression of NRF2 mRNA and protein level in PBMCs from refractory SSNHL patients with normal controls. We observed a lower expression of NRF2 mRNA in all refractory SSNHL patients before treatment. NRF2 is considered as a switch to initiate the internal antioxidant system. In the normal condition, Keap1 works as a negative regulator of NRF2 to sequester it in the cytoplasm and mediate its ubiquitination [20]. OS causes NRF2 to dissociate from Keap1 and to subsequently translocate into the nucleus, resulting in the transcription of its target antioxidant and anti-inflammation genes [21]. Our current research is suggestive of an attenuated OS defense system in refractory SSNHL patients. This result is consistent with an unbalanced oxidation status in SSNHL patients reported previously [11, 22], and the central role that OS has been suggested to play in the pathophysiology of SSNHL [23].

However, an important issue is that all NRF2 variables evaluated prior to intratympanic treatment had systemic corticoid use interference. The levels of NRF2 expression could be altered by the initial systemic corticoid use; therefore, they may not truly reflect their levels in refractory SSNHL patients. In the present study, the interval between peripheral blood collection and last systemic corticoid dose was more than 3 days in all patients, more than 4 days in 70% of patients, and more than 8 days in 19% of patients. Up to date, there are no reports about NRF2 expression in SSNHL patients before GC treatment in the literature. However, the biological half-life of dexamethasone is 2.4 ± 0.6 h and of methylprednisolone is 4.6 ± 1.2 h [24]. Methylprednisolone is not detectable in the blood 18 h after intramuscular or intravenous injection [25]. Furthermore, NRF2 protein has a very short half-life (of about 15 min) [9]. Therefore, the interference of the systemic corticoid use on the changes of NRF2 expression in PMBCs, at least in the protein level, could be ruled out. Nevertheless, NRF2 expression should be examined in SSNHL patients before GC treatment in the future.
In the current study, we observed that nuclear NRF2 protein level was increased in the body of the IGCS group patients after treatment, indicating that NRF2 translocated, and consequently activated the OS defense system [26], under conditions in which hearing was improved. However, in contrast to IGCS patients, nuclear NRF2 expression decreased in the IGCI group, possibly through NRF2 phosphorylation [27], miRNA inhibition [28], or Keap1-mediated inactivation, indicating that reduced NRF2 also may be a factor in GC resistance in SSNHL as seen in mice and human keratinocytes [11].

In the present study, the pattern of NRF2 expression before and after treatment was consistent with the changes in HDAC2 expression in our previous study [5]. Considering the close relationship between NRF2 and HDAC2 [11], a possible explanation is that NRF2 expression in the IGCS group was induced by GC treatment, which, in turn, induced a higher level of HDAC2 in these patients [29]. In the IGCI group, however, decreased NRF2 expression inhibited HDAC2 activity and then caused GC resistance. This positive relationship between NRF2 and HDAC2 expression was confirmed by previous reports [8,29]. Our current results indicate that the NRF2-HDAC pathway is involved in the resistance of GC in refractory SSNHL.

We speculate that OS might contextually overwhelm the intrinsic antioxidant system in refectory SSNHL, thus inhibiting the expression of NRF2 and promoting GC insensitivity and poor hearing improvement. Promoting the expression of NRF2 may improve the antioxidant defense system, elevate HDAC2 activity, increase the sensitivity to GC, and thus improve their hearing prognosis in SSNHL patients.

Our current and previous studies have indicated that the interval from onset to the intratympanic treatment is an independent risk factor that correlated with the prognosis of SSNHL [30]. This phenomenon was also reported by other groups [31]. However, in the current study, both GC sensitive and insensitive groups had similar mRNA/protein levels of NRF2 and HDAC2 in PBMCs before treatment (all \(p > 0.05\)), and the changes in NRF2 and HDAC2 expression were observed only in the GC sensitive group after treatment (all \(p < 0.05\)). Therefore, we believe the effect of interval on the expression of NRF2 and HDAC2 is limited.

In summary, the results of this investigation demonstrate that attenuation of the NRF2-HDAC2 pathway can lead to the generation of GC resistance in SSNHL patients, thus impacting their prognosis. Therefore, therapeutically increasing NRF2 levels may have merit in counteracting CG resistance and improve the prognosis. The current study provides a new way of thinking about patient-tailored treatments for SSNHL. However, more clinical and mechanistic studies are needed to address the specific role that the NRF2-HDAC2 regulatory pathway plays in modulating the cochlear response to GC treatment in SSNHL patients.

Acknowledgements

The authors wish to thank Drs. Xiaoping Du, Matthew B. West, and Zachary Yokell in Hough Ear Institute (Oklahoma City, OK, United States) for critically reviewing the manuscript.

Statement of Ethics

The study protocol and informed consent form were approved by the Ethics Committee of Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School (2016-194-01). The informed consent forms were obtained from all enrolled patients. The study protocol was approved by the institute’s committee on human research. The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

Conflict of Interest Statement

No conflict of interest relevant to this article was reported.

Funding Sources

This study was supported by grants from the National Natural Science Funds of China (81670931); from the Department of Science and Technology of Jiangsu Province (BL2014002); from the Six Talent Peaks Project of Jiangsu Province (WSW-075); from Nanjing Medical Science and Technology Development Key Projects (ZKK17019); and Jiangsu Provincial Key Medical Discipline (ZDXKB2016015), China.

Author Contributions

W.D.S. planned the investigation and revised the manuscript critically. H.Q. carried out the experiments, analyzed the data, and drafted and revised the manuscript with the guidance of W.D.S., Z.W.G., and Y.H.D. collected clinical data partially, preformed the experiments partially, and interpreted the clinical and experimental data partially, reviewed the manuscript. J.H., Q.Q.Z., and W.M. collected clinical data and assisted the experiments partially. All authors read and commented on the final draft, agreed to be accountable for all aspects of the work.
References

1. Stachler RJ, Chandrasekhar SS, Archer SM, Rosenfeld RM, Schwartz SR, Barrs DM, et al. Clinical practice guideline: sudden hearing loss. Otolaryngol Head Neck Surg. 2012; 146(3 Suppl):S1–35.

2. Crane RA, Camilon M, Nguyen S, Meyer TA. Corticosteroid resistance in patients with asthma and chronic obstructive pulmonary disease. J Allergy Clin Immunol. 2013;131(3):636–45.

3. Segre CV, Chiocca S. Regulating the regulators: the post-translational code of class I HDACs. J Biomed Biotechnol. 2011;2011:690848.

4. Adenuga D, Caiito S, Yao H, Sundar IK, Hwang JW, Chung S, et al. Nrf2 deficiency influences susceptibility to steroid resistance via HDAC2 reduction. Biochem Biophys Res Commun. 2010;403(3–4):452–6.

5. Stewart D, Killeen E, Naquin R, Alam S, Alam M. Degradation of transcription factor Nrf2 via the ubiquitin-proteasome pathway and stabilization by cadmium. J Biol Chem. 2003;278(4):2396–402.

11. Chen B, Lu Y, Chen Y, Cheng J. The role of Nrf2 in oxidative-stress-induced endothelial injuries. J Endocrinol. 2015;225(3):R83–99.

12. Capaccio P, Pignataro L, Gaini LM, Sigismund PE, Novembrino C, De Giuseppe R, et al. Unbalanced oxidative status in idiopathic sudden sensorineural hearing loss. Eur Arch Otorhinolaryngol. 2012;269(2):449–53.

13. Editorial board of Chinese journal of Otorhinolaryngology head and Neck surgery, society of Otorhinolaryngology head and Neck surgery, Chinese medical association. [Guideline of diagnosis and treatment of sudden deafness]. Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi. 2015;50(6):443–7.

14. McShefferty D, Whitmer WM, Akeroyd MA. The just-meaningful difference in speech-to-noise ratio. Trends Hear. 2016;20:233121651626570.

15. Zhou QQ, Dai YH, Du XP, Hou J, Qi H, She W. Aminophylline restores glucocorticoid sensitivity in a guinea pig model of sudden sensorineural hearing loss. Otolaryngol Head Neck Surg. 2016;154(1):164–70.

16. Shen Y, Xu C, Xue L, Zhou Q, Hou J, Qi H, She W. Histone deacetylase 2 in sudden sensorineural hearing loss patients in response to intratympanic methylprednisolone perfusion. Otolaryngol Head Neck Surg. 2016;154(1):164–70.

17. Barnes PJ, Adcock IM. Glucocorticoid resistance in inflammatory diseases. Lancet. 2009;373(9678):1905–17.

18. Weinberg R, Weinberg K, Weinberg J. Role of Nrf2-regulated miRNAs in human malignancies. Oncotarget. 2013;4(8):1130–42.

19. Jain AK, Jaiswal AK. GSK-3beta acts upstream of Fyn kinase in regulation of nuclear export and degradation of NF-E2 related factor 2. J Biol Chem. 2007;282(22):16502–10.

20. Shah NM, Rushworth SA, Murray MY, Bowles KM, MacEwan DJ. Understanding the role of NRF2-regulated miRNAs in human malignancies. Oncotarget. 2013;4(8):1130–42.

21. Cullinan SB, Zhang D, Hannink M, Arvisais E, Kaufman RJ, Diehl JA. Nrf2 is a direct PERK substrate and effector of PERK-dependent cell survival. Mol Cell Biol. 2003;23(20):7196–209.

22. Guly J, Muderris T, Yalciner G, Sevil E, Ber- cin S, Ergin M, et al. A comprehensive study of oxidative stress in sudden hearing loss. Eur Arch Otorhinolaryngol. 2017;274(3):1301–8.

23. Olivetto E, Simoni E, Guarani V, Astolfi I, Martini A. Sensorineural hearing loss and ischemic injury: Development of animal models to assess vascular and oxidative effects. Hear Res. 2015;327:58–68.

24. Czock D, Keller F, Rasche FM, Häussler U. Pharmacokinetics and pharmacodynamics of systemically administered glucocorticoids. Clin Pharmacokinet. 2005;44(1):61–98.

25. Antal EJ, Wright CE, Gillespie WR, Albert KS. Influence of route of administration on the pharmacokinetics of methylprednisolone. J Pharmacokinet Biopharm. 1983;11(6):561–76.

26. Hine CM, Mitchell JR. NRF2 and the phase II response in acute stress resistance induced by dietary restriction. J Clin Exp Pathol. 2012;54(4):7329.

27. Jain AK, Jaiswal AK. GSK-3beta acts upstream of Fyn kinase in regulation of nuclear export and degradation of NF-E2 related factor 2. J Biol Chem. 2007;282(22):16502–10.

28. Shah NM, Rushworth SA, Murray MY, Bowles KM, MacEwan DJ. Understanding the role of NRF2-regulated miRNAs in human malignancies. Oncotarget. 2013;4(8):1130–42.

29. Wiegman CH, Li F, Clarke CJ, Jazrawi E, Kirkham P, Barnes PJ, et al. A comprehensive analysis of oxidative stress in the ozone-induced lung inflammation mouse model. Clin Sci. 2014;126(6):425–40.

30. She W, Dai Y, Du X, Yu C, Chen F, Wang J, et al. Hearing evaluation of intratympanic methylprednisolone perfusion for refractory sudden sensorineural hearing loss. Otolaryngol Head Neck Surg. 2010;Feb;142(2):266–71.

31. Qian Y, Zhong S, Hu G, Kang H, Wang L, Lei Y. Sudden sensorineural hearing loss in children: a report of 73 cases. Otol Neurotol. 2018;39(8):1018–24.