The Journal of International Advanced Otology

J Int Adv Otol 2016; 12(1): 77–81 ● DOI: 10.5152/iao.2016.1732

Original Article

Angiotensin-Converting Enzyme (ACE) I/D and Alpha-Adducin (ADD1) G460W Gene Polymorphisms in Turkish Patients with Severe Chronic Tinnitus

Salim Yüce, Enver Sancakdar, Gökhan Bağcı, Sema Koç, Hande Küçük Kurtulgan, Binnur Bağcı, Mansur Doğan, İsmail Önder Uysal

Department of Otorhinolaryngology, Cumhuriyet University School of Medicine, Sivas, Turkey (SY, MD, İÖU)
Department of Biochemistry, Cumhuriyet University School of Medicine, Sivas, Turkey (ES)
Department of Medical Genetics, Cumhuriyet University School of Medicine, Sivas, Turkey (GB, HKK)
Department of Ear Nose Throat Head and Neck Surgery, Antalya Training and Research Hospital, Antalya, Turkey (SK)
Department of Nutrition and Dietetics, Cumhuriyet University School of Health Sciences, Sivas, Turkey (BB)

OBJECTIVE: Tinnitus is described as a disturbing sound sensation in the absence of external stimulation. We aimed to investigate whether there is any relationship between severe chronic tinnitus and angiotensin-converting enzyme (ACE) I/D and α-adducin (ADD1) G460W gene polymorphisms.

MATERIALS and METHODS: The patient group and control group consisted of 89 and 104 individuals, respectively. The evaluation of tinnitus was performed using the Strukturiertes Tinnitus-Interview (STI). The Tinnitus Handicap Inventory (THI) was used to evaluate the tinnitus severity. Polymerase chain reaction (PCR) and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) techniques were used for genotyping.

RESULTS: With regard to the ACE I/D polymorphism, there was no significant difference in genotype and allele frequencies between the patient group and control group. However, a statistically significant difference was found in genotype ($p<0.01$) and allele frequencies ($p=0.021$) of the ADD1 G460W gene polymorphism. Combined genotype analysis showed that the ACE II /ADD1 GW genotype was statistically significantly higher in the patient group than in the control group ($X2: 7.15$, $p=0.007$). The odds ratio value of the GW genotype was 2.5 (95% CI=1.4–4.7) ($p<0.01$).

CONCLUSION: Our results demonstrate an association between ADD1 G460W gene polymorphism and susceptibility to severe chronic tinnitus. It was found that the GW genotype increased the disease risk by 2.5-fold compared with other genotypes. This indicates that ADD1 G460W polymorphism could be an important factor in the pathophysiology of tinnitus.

KEYWORDS: Tinnitus, ACE, ADD1, polymorphism

INTRODUCTION

Tinnitus is described as a disturbing sound sensation in the absence of external stimulation [1]. Tinnitus, which is one of the most common symptoms of the auditory system, is a symptom of many diseases. Characteristically, the prevalence of tinnitus increases by age. The estimated prevalence of tinnitus in the general population varies from 3% to 30%. Tinnitus concerns approximately 15% of the general population, 10-15% of which experience severe chronic tinnitus. The prevalence is higher in elderly patients and in workers exposed to industrial noise than in the general population [2, 3].

For tinnitus, a limited number of risk factors have been suggested, including increasing age, hypertension, hearing loss, loud noise exposure, dietary factors, ototoxic drugs, elevated blood lipids, and alcohol consumption [4-6]. Approximately 50% of patients with tinnitus are not attributed to any above mentioned causes; for this reason, inter-individual predisposition to tinnitus might be explained by the effect of genetic factors [4-8].

Angiotensin-converting enzyme (ACE) is an enzyme that plays a crucial role in the renin-angiotensin system. The ACE-coding gene is located on chromosome 17q and has two alleles: deletion (D) and insertion (I). ACE I/D polymorphism has been associated with vascular disorders such as hypertension, coronary artery disease, diabetic or non-diabetic nephropathy, and cerebrovascular disease [9]. Cardiovascular and body water regulation are regulated by the renin-angiotensin system, which is a hormone system. One of the reasons for pulsatile or non-pulsatile tinnitus may be the cardiovascular and body water

Presented in: This manuscript was presented on The European Human Genetics Conference, 31 May - 3 June 2014, Milan, Italy.

Corresponding Address: Gökhan Bağcı E-mail: gokhanbagci@hotmail.com.tr

Submitted: 14.10.2015 **Revision received:** 17.03.2016 **Accepted:** 18.03.2016

Table 1. PCR and RFLP conditions of ACE (I/D) and ADD1 G460W polymorphisms

Genes and polymorphisms	Primers and/or restriction endonuclease	Annealing temperature for PCR	Fragments and genotypes
ACE (I/D)	Forward primer: 5′-CTG GAG ACC ACT CCC ATC CTT TCT-3′	59°C	II: 490 bp
	Reverse primer: 5′-GAT GTG GCC ATC ACA TTC GTC AGA-3′		ID: 490, 190 bp
			DD: 190 bp
ADD1 G460W	Forward primer: 5′-CTC CTT TGC TAG TGA CGG TGA TTC-3′	62°C	GG: 122, 25 bp
	Reverse primer: 5′-GAC TTG GGA CTG CTT CCA TTC GGCC-3′.		GW: 147, 122, 25 bp
	Sau961 (New England Biolabs; Hitchin, UK)		WW: 147 bp

ACE: angiotensin-converting enzyme; ADD1: α-Adducin; I: insertion; D: deletion; G: glycine; W: tryptophan

regulation alterations related to ACE via cerebral blood flow and flow-related noise alterations [10].

By interacting with the epithelial sodium channel, α-adducin (ADD1) exerts crucial and complex biological effects on volume and sodium homeostasis. An ADD1 polymorphism of codon 460, coding the substitution of tryptophan (Trp) in place of glycine (Gly) (Gly460Trp), has been reported to be linked to elevated blood pressure, sensitivity to salt, and cardiovascular diseases. Adducin is involved in signal transduction mechanisms by modulating the actin cytoskeleton at cell-cell contact sites. There is a lot of experimental evidence indicating that adducin may be a candidate protein in primary or essential hypertension [11, 12]. It has been shown that the organ of corti and stria vascularis are the primary lesion sites in hypertension [13].

Endolymph, perilymph, and intrastrial spaces play important role in ion and fluid balance in the inner ear. The Na^+-K^+-ATPase transporter is need for K^+ secretion into the endolymph because the intrastrial space has a lower K^+ concentration than endolymph. Na^+-K^+ transporters play an important role in K^+ secretion into the endolymph and the accumulation of K^+ in the intrastrial fluid [14]. The K^+ inside the intrastrial space and perilymph is absorbed by strial marginal cells and vestibular dark cells via the Na^+-K^+-ATPase pump and the Na^+-2Cl-K^+ transporter [15]. The absence of Na^+-K^+ transporters creates an interruption in secretion of K^+ into the endolymph and generates a failure in the endocochlear potential [14]. Kuijper and Bonting [16] demonstrated that ouabain causes an interruption of K^+ secretion and disrupts the endocochlear potential by blocking the Na^+-K^+-ATPase transporter. α-Adducin increases Na^+-K^+-ATPase pump activity [17]. This increased activity may result in an increased volume and endolymph osmolarity [17, 18].

Angiotensin-converting enzyme I/D polymorphism and (ADD1) G460W polymorphism have been previously associated to hypertension and there may be a possible association with severe chronic tinnitus. In the light of this information, we aimed to investigate the relationship between (ACE) I/D and (ADD1) G460W gene polymorphisms and severe chronic tinnitus.

MATERIALS and METHODS

Patients

The total number of individuals included in this study was 193. The patient group and control group consisted of 89 and 104 individuals, respectively. Between 2006-2013, patients suffering from severe chronic tinnitus for more than 6 months were retrieved from the medical records and the patients were invited to the Hospital, and informed consent forms were taken at Department of Otorhinolaryngology, Gaziosmanpaşa University, Tokat. The control group was selected from individuals without any otologic or systemic diseases. The patients and controls in the current study were from the same geographic region (central Anatolia region of Turkey) and the same ethnic origin (Turkish-Caucasian). Moreover, approval of the ethical committee was taken from Clinical Research Ethics Committee of Gaziosmanpaşa University.

Study Design

This study was conducted in patients who had been diagnosed with tinnitus for more than 6 months and who were older than 18 years. In the present study, exclusion criteria were determined as follows: patients having acute and objective tinnitus, sensorineural hearing loss, ear infection, external or middle ear problem, chronic otitis media, otosclerosis, vestibular schwannoma, Ménière's disease, otorrhea, temporal bone trauma history, psychiatric disorder, previous ear surgery history, previous neurotologic surgery history, ototoxicity history, smoking habit, diabetes mellitus, hypertension, cardiovascular disease, hypothyroidism, hypercholesterolemia, hyperlipidemia, head trauma, and known hyperacusia.

The evaluation of tinnitus was performed using the Strukturiertes Tinnitus-Interview (STI); a decrease of at least 35% of the initial score was considered clinically significant. The Tinnitus Handicap Inventory (THI) was used to evaluate the tinnitus severity. Patients were assessed on the basis of 100 points; 58-76 points were accepted as severe tinnitus.

Genotyping

Peripheral blood samples obtained both from patients with tinnitus and from the control groups were collected in tubes with K_3EDTA and stored at −20°C until the study time. Total genomic DNA was extracted from peripheral blood samples using the Invisorb Spin Blood extraction kit (Invitek; Berlin, Germany). PCR and RFLP conditions, using primers and/or restriction endonucleases and fragment lengths of the detected genotypes, were shown in Table 1.

Statistical Analysis

Statistical analyses in the present study were performed using the Statistical Package for the Social Sciences version 15.0 (SPSS; Chicago, IL, USA) package program. The Chi-square test was used to compare the differences between the groups in terms of frequencies of genotypes and alleles. The Student-t test was performed to compare the demographic data of the groups. Allele frequencies were assessed by the gene counting method. The odds ratio (OR) values were given with 95% confidence intervals. $P<0.05$ was regarded as significant.

RESULTS

The study group consisted of 89 individuals: 48 women (53.9%) and 41 men (46.1%), with a mean age of 48.1±13.5 years. The control group consisted of 104 individuals: 50 women (48.1%) and 54 men (51.9%), with a mean age of 45.0±16.0 years.

There was no difference between the groups in terms of sex and age. When the patient and control groups were compared in terms of ACE genotype distribution, no significant difference was observed (X^2=1.586, p=0.452) (Table 2). On the other hand, in terms of, the GW genotype of ADD1 G460W was found to be significantly higher in the patient group than that of control group (X^2=9.4, p=0.009) (Table 2). In terms of ACE allele frequencies, there was no significant difference between the patient and control groups (X^2=0.7, p=0.4) (Table 2). On the other hand, in terms of ADD1 G460W alleles, the W allele was found to be significantly higher in the patient group than that of the control group (X^2=5.3, p=0.021) (Table 2). It was found that the GW genotype increased the disease risk by 2.5-fold compared with the other genotypes (Table 3).

When ACE I/D and ADD1 G460W genotypes were evaluated together, the II/GW genotype was found to be statistically significantly higher in the patient group than that of the control group (X^2=7.15, p=0.007). II/WW and DD/WW genotypes were observed neither in the patient group nor in the control group. The distributions of other combined genotypes of ACE I/D and ADD1 G460W were shown in Table 4.

DISCUSSION

The etiology of chronic tinnitus remains poorly understood. There is lots of evidence that genetic factors are predisposed to chronic tinnitus [19]. In the present study, we studied ACE and ADD1 gene polymorphisms to show any possible role on the pathophysiology of severe chronic tinnitus.

Our study is the first to show that there is a possible association between G460W polymorphism and the pathophysiology of tinnitus. A number of studies have suggested that ADD1 Trp allele poses a higher risk for cardiovascular and renal diseases [20-22]. Morrison et al. [23] claimed that ADD1 Trp allele increases the coronary heart disease risk by a factor of 2-fold and Sugimoto et al. [24] reported that Gly460Trp polymorphism is a marker of deteriorating health. Tinnitus often presents in adults with Ménière's disease [25]. Teggi et al. [26] found similar results to our study. They reported that the Trp allele frequency of ADD1 G460W polymorphism was significantly increased in patients with Ménière's disease compared to controls.

There have been many studies showing a strong relationship between α-adducin gene G460W polymorphism and essential hypertension. It has been shown that especially individuals with the W allele have an increased risk for hypertension [27, 28]. In our study, having a significantly higher GW genotype and W allele in the patient group compared to the controls suggests that α-adducin G460W polymorphism plays a role in the pathophysiology of tinnitus.

Although a wide array of compounds is recommended to treat tinnitus patients, no drug used in the treatment of tinnitus patients on the market has yet been approved by US Food and Drug Administration or European Medicines Agency [29]. Elgoyhen et al. [30] suggested that

Table 2. Genotype distribution and allele frequencies of ACE I/D and ADD1 G460W polymorphisms

ACE I/D genotypes	Patients, n(%)	Controls, n(%)	p
II	18 (20.2)	14 (13.5)	0.452
ID	41 (46.1)	52 (50)	
DD	30 (33.7)	38 (36.5)	
Alleles			
I	77 (43.25)	80 (38.4)	0.394
D	101 (56.75)	128 (61.6)	
ADD1 G460W genotypes			
GG	47 (52.8)	76 (73.1)	0.009
GW	41 (46.1)	26 (25.0)	
WW	1 (1.1)	2 (1.9)	
Alleles			
G	135 (75.8)	178 (85.6)	0.021
W	43 (24.2)	30 (14.4)	

ACE: angiotensin-converting enzyme; ADD1: α-Adducin; I: insertion; D: deletion; G: glycine; W: tryptophan

Table 3. Odds ratio values for ADD1 G460W genotypes

		95% Confidence Interval		
ADD1 G460W	OR	Lower	Upper	p
WW vs. GG+GW	1.7	0.16	19.3	>0.05
GG vs. GW+WW	0.4	0.22	0.75	>0.05
GW vs. GG+WW	2.5	1.4	4.7	**0.0025**

ADD1: α-Adducin; OR: odds ratio; G: glycine; W: tryptophan

Table 4. Combined genotype frequencies of ACE I/D and ADD1 G460W polymorphisms

ACE/ADD1	Patients, n%	Controls, n%	p
II/GG	7 (7.9)	12 (11.5)	>0.05
II/GW	11 (12.4)	2 (1.9)	x^2: 7.15; **p: 0.007**
ID/GG	27 (30.3)	40 (38.5)	>0.05
ID/GW	13 (14.6)	10 (9.6)	>0.05
ID/WW	1 (1.1)	2 (1.9)	>0.05
DD/GG	13 (14.6)	24 (23.0)	>0.05
DD/GW	17 (19.1)	14 (13.5)	>0.05

ACE: angiotensin-converting enzyme; ADD1: α-Adducin; I: insertion; D: deletion; G: glycine; W: tryptophan

ACE is a significant target for tinnitus, and to the best of the authors' knowledge, this was the first time it was determined to be related to tinnitus. According to the authors, the potential role of the brain renin-angiotensin system has been recommended for auditory attention. In this way, this system might attend in tinnitus-related sound and auditory attention and affective brain circuits.

Genetic studies conducted on tinnitus are very limited and are particularly focused on candidate-gene association studies. In a study,

Hwang et al. [31] demonstrated that salicylate-induced tinnitus correlated with the increased gene expression of TNF-α and IL-1β. Deniz et al. [32] investigated the possible role of serotonin transporter gene (SLC6A4) polymorphisms in tinnitus. They did not find any difference between the allele and genotype frequencies of the patients with tinnitus and with the controls regarding VNTR and 5-HTTLPR polymorphisms. Also they found no association between SLC6A4 polymorphism and the psychoacoustic parameters of tinnitus. On the other hand, they found a significant relationship between 5-HTTLPR polymorphism and the visual analog scale scores of the patients. In another study, authors investigated the effect of acute and chronic salicylate treatment on the expression of NR2B, Arg3.1, and Egr-1 in an animal model of tinnitus, and they found both mRNA levels and the protein expression of NR2B and Arg3.1 increased in rats that were chronically administered salicylate [33]. In a study conducted with inner ear potassium recycling genes, significant associations were observed for two variants in KCNE1 and one SLC12A2 gene [34]. Furthermore, Sand et al. [35] reported that GDNF genotypes together with BDNF genotypes predict tinnitus severity in women but not in men.

Voltage-gated ion channels are known to be strong candidates related with the studies of the pathophysiology of tinnitus [36, 37]. It has been shown that α-adducin increased the Na^+-K^+-ATPase pump activity [17]. Consequently, this increased activity may result in an increased volume and endolymph osmolarity [17, 18]. The identification of possible contribution of α-adducin to the activity of Na^+-K^+-ATPase pump may help to clarify the pathogenesis of tinnitus and possibly may lead to improved diagnosis and treatment of tinnitus.

Hereditary and environmental factors play important roles in the etiology of tinnitus. Further studies are necessary for clarifying genetic contributions to the pathophysiology of tinnitus. Furthermore, genetic tests can be useful when individual genetic variants are found to determine responsiveness to pharmacological therapy [38]. Our results indicate that additional studies must be undertaken to determine whether one or both genes have any role in the pathogenesis of tinnitus.

In conclusion, although ACE I/D polymorphism did not show any difference between the patient group and control group, there was a significant difference in terms of genotype distribution and allele frequencies of ADD1 G460W gene polymorphism. Further studies should be carried out to explain the combined effect of gene-gene interactions, environmental factors, and individual biological characteristics for understanding the potential mechanism of the pathophysiology of tinnitus in humans. The efficiency and accuracy of our results should be increased and supported by results obtained in studies on larger populations.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gaziosmanpaşa University

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - S.Y., G.B., M.D., İ.Ö.U.; Design - E.S., B.B., S.K., H.K.K.; Supervision - M.D., İ.Ö.U.; Resources - S.Y., S.K.; Materials - S.K., M.D.; Data Collection and/or Processing - S.K., S.Y.; Analysis and/or Interpretation - G.B., H.K.K., B.B.; Literature Search - G.B., B.B., M.D.; Writing Manuscript - S.Y., G.B., B.B.; Critical Review - S.Y., İ.Ö.U.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Chan Y. Tinnitus: Etiology, classification, characteristics, and treatment. Discov Med 2009; 8: 133-6.
2. Xu X, Bu X, Zhou L, Xing G, Liu C, Wang D. An epidemiologic study of tinnitus in a population in Jiangsu Province, China. J Am Acad Audiol 2011; 22: 578-85. [CrossRef]
3. Seidman MD, Jacobson GP. Update on tinnitus. Otolaryngol Clin North Am 1996; 29: 455-65.
4. Shargorodsky J, Curhan GC, Farwell WR. Prevalence and characteristics of tinnitus among US adults. Am J Med 2010; 123: 711-8. [CrossRef]
5. Axelsson A, Ringdahl A. Tinnitus—a study of its prevalence and characteristics. Br J Audiol 1989; 23: 53-62. [CrossRef]
6. Nondahl DM, Cruickshanks KJ, Wiley TL, Klein R, Klein BE, Tweed TS. Prevalence and 5-year incidence of tinnitus among older adults: the epidemiology of hearing loss study. J Am Acad Audiol 2002; 13: 323-31.
7. Heller AJ. Classification and epidemiology of tinnitus. Otolaryngol Clin North Am 2003; 36: 239-48. [CrossRef]
8. Burns EM. A comparison of variability among measurements of subjective tinnitus and objective stimuli. Audiology 1984; 23: 426-40. [CrossRef]
9. Fatini C, Guiducci S, Abbate R, Matucci-Cerinic M. Vascular injury in systemic sclerosis: angiotensin-converting enzyme insertion/deletion polymorphism. Curr Rheumatol Rep 2004; 6: 149-55. [CrossRef]
10. Wright JW, Harding JW. The brain renin-angiotensin system: a diversity of functions and implications for CNS diseases. Pflugers Arch 2013; 465: 133-51. [CrossRef]
11. Tripodi G, Valtorta F, Torielli L, Chieregatti E, Salardi S, Trusolino L, et al. Hypertension-associated point mutations in the adducin alpha and beta subunits affect actin cytoskeleton and ion transport. J Clin Invest 1996; 97: 2815-22. [CrossRef]
12. Staessen JA, Bianchi G. Adducin and hypertension. Pharmacogenomics 2005; 6: 665-9. [CrossRef]
13. Gates GA, Cobb JL, D'Agostino RB, Wolf PA. The relation of hearing in the elderly to the presence of cardiovascular disease and cardiovascular risk factors. Arch Otolaryngol Head Neck Surg 1993; 119: 156-61. [CrossRef]
14. Marcus DC, Wu T, Wangemann P, Kofuji P. KCNJ10 (Kir4.1) potassium channel knockout abolishes endocochlear potential. Am J Physiol Cell Physiol 2002; 282: 403-7. [CrossRef]
15. Wangemann P, Liu J, Marcus DC. Ion transport mechanisms responsible for K+ secretion and the transepithelial voltage across marginal cells of striavascularis in vitro. Hear Res 1995; 84: 19-29. [CrossRef]
16. Kuijper W, Bonting SL. The cochlear potentials. I. The effect of ouabain on the cochlear potentials of the guinea pig. Pflugers Arch 1970; 320: 348-58.
17. Ferrandi M, Salardi S, Tripodi G, Barassi P, Rivera R, Manunta P, et al. Evidence for an interaction between adducin and Na+-K+-ATPase: relation to genetic hypertension. Am J Physiol 1999; 277: 1338-49.
18. Lang F, Vallon V, Knipper M, Wangemann P. Functional significance of channels and transporters expressed in the inner ear and kidney. Am J Physiol Cell Physiol 2007; 293: 1187-208. [CrossRef]
19. Sand PG, Langguth B, Kleinjung T, Eichhammer P. Genetics of chronic tinnitus. Prog Brain Res 2007; 166: 159-68. [CrossRef]
20. Balkestein EJ, Wang JG, Struijker-Boudier HA, Barlassina C, Bianchi G, Birkenhäger WH, et al. Carotid and femoral intima-media thickness in relation to three candidate genes in a Caucasian population. J Hypertens 2002; 20: 1551-61. [CrossRef]

21. Wang JG, Staessen JA, Tizzoni L, Brand E, Birkenhäger WH, Fagard R, et al. Renal function in relation to three candidate genes. Am J Kidney Dis 2001; 38: 1158-68. [CrossRef]
22. Beeks E, van der Klauw MM, Kroon AA, Spiering W, Fuss-Lejeune MJ, de Leeuw PW. α-Adducin Gly460Trp polymorphism and renal hemodynamics in essential hypertension. Hypertension 2004; 44: 419-23.[CrossRef]
23. Morrison AC, Bray MS, Folsom AR, Boerwinkle E. ADD1 460W allele associated with cardiovascular disease in hypertensive individuals. Hypertension 2002; 39: 1053-7. [CrossRef]
24. Sugimoto K, Hozawa A, Katsuya T, Matsubara M, Ohkubo T, Tsuji I, et al. Alpha-adducin Gly460Trp polymorphism is associated with low renin hypertension in younger subjects in the Ohasama study. J Hypertens 2002; 20: 1779-84. [CrossRef]
25. Verstreken M, Declau F, Wuyts FL, D'Haese P, Van Camp G, Fransen E, et al. Hereditary otovestibular dysfunction and Meniere's disease in a large Belgian family is caused by a missense mutation in the COCH gene. Otol Neurotol 2001; 22: 874-81. [CrossRef]
26. Teggi R, Lanzani C, Zagato L, Delli Carpini S, Manunta P, Bianchi G, et al. Gly460Trp alpha-adducin mutation as a possible mechanism leading to endolymphatic hydrops in Ménière's syndrome. Otol Neurotol 2008; 29: 824-8. [CrossRef]
27. Barlassina C, Norton GR, Samani NJ, Woodwiss AJ, Candy GC, Radevski I, et al. Alpha-adducin polymorphism in hypertensives of South African ancestry. Am J Hypertens 2000; 13: 719-23. [CrossRef]
28. Ju Z, Zhang H, Sun K, Song Y, Lu H, Hui R, et al. Alpha-adducin gene polymorphism is associated with essential hypertension in Chinese: A case-control and family-based study. J Hypertens 2003; 21: 1861-8. [CrossRef]
29. Elgoyhen AB, Langguth B. Pharmacological approaches to the treatment of tinnitus. Drug Discov Today 2010; 15: 300-5. [CrossRef]
30. Elgoyhen AB, Langguth B, Nowak W, Schecklmann M, De Ridder D, Vanneste S. Identifying tinnitus-related genes based on a side-effect network analysis. CPT Pharmacometrics Syst Pharmacol 2014; 3: 1-10. [CrossRef]
31. Hwang JH, Chen JC, Yang SY, Wang MF, Chan YC. Expression of tumor necrosis factor-a and interleukin-1b genes in the cochlea and inferior colliculus in salicylate-induced tinnitus. J Neuroinflammation 2011; 8: 30. [CrossRef]
32. Deniz M, Bayazit YA, Celenk F, Karabulut H, Yilmaz A, Gunduz B, et al. Significance of serotonin transporter gene polymorphism in tinnitus. Otol Neurotol 2010; 31: 19-24. [CrossRef]
33. Hu SS, Mei L, Chen JY, Huang ZW, Wu H. Expression of immediate-early genes in the dorsal cochlear nucleus in salicylate-induced tinnitus. Eur Arch Otorhinolaryngol 2015: DOI 10.1007/s00405-014-3479-3. [CrossRef]
34. Pawełczyk M, Rajkowska E, Kotyło P, Dudarewicz A, Camp G, Śliwińska-Kowalska M. Analysis of inner ear potassium recycling genes as potential factors associated with tinnitus. Int J Occup Environ Med 2012; 25: 356-64. [CrossRef]
35. Sand PG, Langguth B, Schecklmann M, Kleinjung T. GDNF and BDNF gene interplay in chronic tinnitus. Int J Mol Epidemiol Genet 2012; 3: 245-51.
36. Wangemann P. K+ cycling and the endocochlear potential. Hear Res 2002; 165: 1-9. [CrossRef]
37. Hibino H, Nin F, Tsuzuki C, Kurachi Y. How is the highly positive endocochlear potential formed? The specific architecture of the stria vascularis and the roles of the ion-transport apparatus. Pflugers Arch 2010; 459: 521-33. [CrossRef]
38. Martin DM, Raphael Y. Gene-based diagnostic and treatment methods for tinnitus. Int Tinnitus J 2002; 9: 3-10.