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Paper Accepted*

ISSN Online 2406-0895

Review Article / Преглед литературе

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Genetic basis of otosclerosis

Генетичка основа отосклерозе

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Received: March 6, 2020

Revised: April 29, 2020

Accepted: April 30, 2020

Online First: May 7, 2020

DOI: <https://doi.org/10.2298/SARH200306026Z>

***Accepted papers** are articles in press that have gone through due peer review process and have been accepted for publication by the Editorial Board of the *Serbian Archives of Medicine*. They have not yet been copy-edited and/or formatted in the publication house style, and the text may be changed before the final publication.

Although accepted papers do not yet have all the accompanying bibliographic details available, they can already be cited using the year of online publication and the DOI, as follows: the author's last name and initial of the first name, article title, journal title, online first publication month and year, and the DOI; e.g.: Petrović P, Jovanović J. The title of the article. *Srp Arh Celok Lek*. Online First, February 2017.

When the final article is assigned to volumes/issues of the journal, the Article in Press version will be removed and the final version will appear in the associated published volumes/issues of the journal. The date the article was made available online first will be carried over.

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SUMMARY

Introduction Otosclerosis is a disorder of the bone labyrinth and stapes resulting in conductive hearing loss. The genetic basis of otosclerosis still remains unknown.

We aimed at reporting a comprehensive review of up-to-date knowledge on genetic basis of otosclerosis.

Methods Narrative literature review was undertaken to summarize the data about genetics of otosclerosis.

Results Genetics of otosclerosis has not been studied extensively and the literature on this topic is scarce. However, knowledge of genetic basis of otosclerosis is recently increasing. We have presented an overview of the knowledge of association of genetic markers with otosclerosis, gained from linkage analyses, candidate-gene studies, and modern high-throughput genomic studies.

Conclusion Due to its complex pathophysiology, otosclerosis is not a disease whose genetic base will be easily understood. Multiple omics analysis and bioinformatics will lead to elucidation of genetic basis of otosclerosis.

Keywords: otosclerosis; genetics; linkage analyses; candidate-gene studies; high-throughput genomic studies

САЖЕТАК

Увод Отосклероза је поремећај коштане капсуле лавиринта и слушних кошчица ува, који доводи до губитка слуха због немогућности провођења звука. Генетички узрок отосклерозе је непознат. Циљ овог рада је био сачинити свеобухватни преглед савремених сазнања о генетичкој основи отосклерозе.

Метод Наративни преглед литературе је коришћен за приказ података о генетици отосклерозе.

Резултати Генетика отосклерозе није много изучавана и литературни подаци о генетичкој основи отосклерозе су оскудни. Међутим, у новије време, знања о генетичкој основи отосклерозе се проширују. Овде је приказан преглед знања о асоцијацији генетичких маркера и отосклерозе, која су резултат анализа генетичке повезаности, као и асоцијативних студија гена кандидата и свеобухватних анализа генома.

Закључак Отосклероза због своје комплексности није болест чија ће генетичка основа бити лако расветљена. Анализе омика и биоинформатика ће допринети разумевању генетичке основе отосклерозе.

Кључне речи: отосклероза; генетика; анализа генетичке повезаности; студија гена кандидата; свеобухватна анализа генома

INTRODUCTION

Otosclerosis is a disorder of the bone labyrinth and stapes known to affect only humans. Unlike all other bones in the body, the human otic capsule undergoes very little remodeling following development. Otosclerosis is a process of pathologic remodeling within a bone that is normally refractory to remodeling. The foci of otosclerotic bone are silent clinically, until the movement of stapes is impaired by invasion of the stapediostapedial articulation. Otosclerosis is a disease of inflammatory character where there is abnormal bone production in the otic capsule and around the base of the stapes. It is one of the most common causes of conductive hearing loss [1]. Its etiology is not fully understood. Some of the proposed causes are genetics, viral diseases and autoimmune reactions [2]. Among viral agents, measles are distinguished.

The primary symptom produced by the otosclerotic lesions is conductive hearing loss, usually bilateral, with the onset between the age 20 and 30. The magnitude of the hearing loss is directly related to the degree of fixation of the stapes footplate [3]. The patients with otosclerosis may also exhibit vestibular disturbance.

Some studies have demonstrated the effect of vitamin D deficiency and some heavy metals, especially calcium, on the progression of otosclerosis [4]. Therefore, it is speculated that dietary regime for patients with otosclerosis could be based on fruits and vegetables containing specific nutrients [5].

Surgical treatment, such as stapedectomy, is used in the treatment of otosclerosis, but the disease can also be managed through hearing amplification with hearing aids [6].

The genetic cause underlying otosclerosis development still remains unknown. The fact that otosclerosis affects multiple members in large families indicates its strong genetic foundation. Also, sporadic forms of otosclerosis have been identified. Linkage analysis have been used to identify genetic loci/genes responsible for otosclerosis in families segregating autosomal dominant form of the disease [7]. So far, eight genetic loci have been mapped, designated *OTSC1-5*, *OTSC7-8* and *OTSC10* loci [8, 9]. Although a genetic cause is evident in otosclerosis, none of the genes involved in otosclerosis development have been proven in these loci. Several genetic association studies were conducted to determine genetic background of the sporadic forms of otosclerosis. Quite a few genes and molecular pathways have been implicated in otosclerosis development playing roles in bone metabolism, immune system, inflammation and endocrine system [8, 10, 11, 12]. Candidate gene studies were directed by considering physiologic processes that could be important for otosclerosis development. For that purpose, genes and proteins involved in bone remodeling were among first to be analyzed [12–15]. Also, the building “blocks” of the skeletal system were legitimate candidate for investigation [16, 17, 18].

We aimed at reporting a comprehensive review of up to date knowledge on genetic basis of otosclerosis.

SEARCH STRATEGY

We used an approach characteristic for a narrative review, consisting of critical analysis of the literature published in electronic or paper-based journal articles. We searched the PubMed database [19] from 1980 to December 2018. using the terms “otosclerosis” AND “gene” OR “genetic” OR “familial”. Studies were identified from the titles and abstracts by the primary reviewers (BZ, JG) and the secondary reviewer (SP). The ethical norms of the Declaration of Helsinki were followed.

GENETIC BASIS OF OTOSCLEROSIS: AN OVERVIEW

Here we present an overview of the knowledge of association of genetic markers with otosclerosis, gained from the association studies of genetic markers and otosclerosis using diverse methodology, starting from linkage analyses, through candidate-gene studies, to modern high-throughput genomic studies, such as genome-wide association studies (GWAS), microarray and next generation sequencing (NGS).

LINKAGE ANALYSES

Linkage analysis have contributed to identification of genetic loci associated with otosclerosis. Eight genetic loci have been mapped, designated OTSC1-5, OTSC7-8 and OTSC10 loci. *OTSC1* locus was mapped on chromosome 15q25-q26 and contains 33 genes. It was identified in large Indian [20] and Tunisian families [21]. *OTSC2* locus with 152 genes was located on chromosome 7q34-36 and found in a Belgian and British families [22]. *OTSC3* locus was mapped to 6p21.3-22.3 and identified in Cypriot and Tunisian families [23]. This locus contains 488 genes and maps to MHC locus. One study revealed that MHC locus have been associated with otosclerosis in Greek patients with otosclerosis [24]. *OTSC4* locus comprising 74 genes was located on chromosome 16q21-23.2 and described in Israeli family [25]. *OTSC5* locus with 59 genes was defined in a Dutch family and located on chromosome 3q22-24 [26]. *OTSC7* locus containing 66 genes was mapped to chromosome 6q13-16.1 and identified in a Greek family [27]. *OTSC8* locus with 24 known and 121 predicted genes was mapped in a Tunisian family to chromosome 9p13.1-9q21.11 [28].

OTSC10 locus was identified in a Dutch family on 1q41–44 chromosome and it comprised 306 genes/predicted genes [9]. Loci *OTSC6* and *OTSC9* have been reserved by the Human Genome Organization Gene Nomenclature Committee but still not published. Although a genetic cause is evident in otosclerosis, none of the genes involved in otosclerosis development have been proven in these loci.

CANDIDATE-GENE STUDIES

Additionally, several genes have been shown to be disease-causing/ disease-related for otosclerosis. Genes influencing dysregulation of bone remodeling within the otic capsule were investigated as otosclerosis has been identified as primary disorder of bone remodeling. Bone morphogenetic proteins (BMPs) and TGF- β 1, members of TGF- β superfamily, are critical regulators of bone turnover. These proteins work as specific growth factors and also as inflammatory cytokines. Out of 20 different BMPs identified so far, only BMPs 2–7 have a central role in the embryonic endochondral bone development and later in new bone formation and repair [13]. Elevated expression levels of BMPs contribute to the increased bone turnover in active phases of otosclerosis. A significant association between clinical otosclerosis and variants in *BMP2* and *BMP4* genes was demonstrated. However, it is concluded that rare variants in *BMP2* and *BMP4* are not a major genetic component in the otosclerosis, at least in German otosclerosis population [14]. Nonfunctional variants influence reduced downstream *BMP* signaling, namely phosphorylation of Smad1/5/8 [14]. As for the other gene involved in the regulation of bone turnover, *TGF- β 1*, a significant association between variants in *TGF- β 1* and clinically and histologically confirmed otosclerosis in Hungarian and in British populations was found [15, 29].

Type I collagen *COL1A1* gene was already shown to be involved in development of osteogenesis imperfecta, a disease similar to otosclerosis in the means of its major characteristics, namely conductive hearing loss [30]. An American study demonstrated a significant association of otosclerosis and *COL1A1* [16]. The same group further revealed a significant association between clinical otosclerosis and the Sp1 binding site variant in the first intron of *COL1A1* [17]. A replication study on German patients with otosclerosis identified haplotypes including the Sp1 binding site variants associated with otosclerosis [18]. A significant association between otosclerosis and *COL1A1* were additionally confirmed in

Egyptian, Turkish [31] and Tunisian studies. Despite finding that in Spanish samples of 100 cases and 100 matched controls the previous findings were not replicated [32], results of a comprehensive meta-analysis are supporting the fact that *COL1A1* and otosclerosis have been strongly associated [33].

Variations in *COL1A2* gene could indirectly influence *COL1A1* thus could have an impact on otosclerosis too. German study showed that some of the *COL1A1* otosclerosis-associated variants alter binding of transcription factors that regulate transcription of *COL1A2* [18]. They have confirmed that targeted deletion of *COL1A2* in the mouse model leads to a mild conductive hearing loss. However, the Spanish group found no evidence in favor of *COL1A2* genes association with otosclerosis [32]. Type II collagen, the main collagen of cartilage, is encoded by *COL2A1* gene. Autoreactivity to *COL2A1* was proposed as a cause of otosclerosis [34], nevertheless this finding has been revisited [35].

Association with genes in the renin-angiotensin-aldosterone system and otosclerosis was also demonstrated [36]. Variants in angiotensinogen (*AGT*) and angiotensinogen converting enzyme genes (*ACE*) in a French-Caucasian cohort was related to higher plasma concentrations of *ACE* and also associated with a higher risk of otosclerosis development. A replication study done in a larger Belgian-Dutch population was unable to confirm these findings [37]. Also, four members of the activation pathway of *AGT* cascade were not expressed at protein level in otosclerotic stapes footplates [38]. However, it is possible that other variants in renin-angiotensin-aldosterone system could be associated with otosclerosis.

Class I major histocompatibility complex (MHC) has been described in otosclerosis when *OTSC3* was mapped [28] and the frequency of HLA-B40 was significantly lower in patients with otosclerosis than in healthy blood donors [39].

Additionally, an environmental factor, namely persistent measles infection, was implicated in the development of otosclerosis [2]. A novel splice variant in the *CD46* receptor gene was detected and a potential association between measles virus infection and otosclerosis was demonstrated [40].

HIGH-THROUGHPUT GENOMIC STUDIES

Genomic studies performed using high-throughput methodology are not numerous, but their contribution to the elucidation of genetic basis of otosclerosis is significant. One of these studies represents the first successful genome-wide association study for a hearing impairment [41].

A genome-wide association study recognized a significant association of the *RELN* gene, coding for an extracellular matrix protein important in brain development and synaptic plasticity, and the pathogenesis of otosclerosis [41]. This association was observed in various European and non-European populations [29, 42, 43]. Nevertheless, variants in *RELN* gene might be associated with a specific otosclerosis-like phenotype, clinically not distinguishable of otosclerosis [38]. *RELN* gene is not actively expressed in adult stapes footplates [13] and further studies are needed to determine the role of *RELN* in the pathophysiology of otosclerosis.

A whole-exome sequencing study in four families with inherited otosclerosis identified several rare heterozygous *SERPINF1* variants suggesting that it may be a common pathogenic pathway in the otosclerosis development [44]. *SERPINF1* (Serpine Peptidase Inhibitor, Clade F) gene encodes PEDF (pigment epithelium-derived factor), which is shown to be involved in the regulation of bone density and inhibition of angiogenesis.

The review of the literature that covers candidate genes associated with otosclerosis is presented in Table 1.

CONCLUSION

Otosclerosis is a complex genetic disorder, with no clearly defined genetic basis. Genetics of otosclerosis has not been studied extensively and the literature on this topic is scarce. High-throughput methodology, such as NGS, has transformed genetic and biomedical research, enabling genetic profiling of each patient. Nowadays it is widely used for diagnostics, monitoring and administering appropriate molecular-targeted and gene therapy for many diseases. As for research, NGS has become a powerful tool in GWAS. It contributes to the discovery of new disease-causing and disease-related genetic markers of complex rare

diseases, which could be implemented in clinical practice [45]. Using NGS in families affected with otosclerosis, analyzing disease-affected and disease-non affected family members, sharing the same genetic background, can add to understanding the molecular basis of the disease [46].

However, due to its complex pathophysiology, otosclerosis is not a disease whose genetic base will be easily understood. High-throughput genotyping will provide a vast amount of data, but more effective data analysis tools are missing [47, 48]. Databases that contain genomic variant data, collected using an microattribution approach principle, could assist in elucidation the genetic basis of complex rare diseases [49, 50]. Unquestionably, bioinformatics is a bottleneck of research in finding reliable genetic associations with otosclerosis. Also, gene-gene and gene-environmental interactions should be considered. Multiple omics analysis, including epigenomics, transcriptomics, proteomics and radiomics, together with powerful bioinformatics will eventually lead to implementation of the knowledge on genetic basis of otosclerosis in clinical practice.

ACKNOWLEDGMENT

This work was supported by Ministry of Education, Science and Technological Development Republic of Serbia, EB: 451-03-68/2020-14/ 200042 and COST Action BM1306.

Conflict of interest: None declared.

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Table 1. Candidate genes associated with otosclerosis

Gene	Full name of the gene	Protein function	Type of the study	Reference
<i>COL1A1</i>	Type I collagen A1	strengthens and supports many tissues in the body, including cartilage, bone, tendon, skin, and the white part of the eye (the sclera)	association study	[16]
			case control studies	[17, 18, 31, 32, 33]
<i>COL1A2</i>	Type I collagen A2	encodes the pro-alpha2 chain of type I collagen whose triple helix comprises two alpha1 chains and one alpha2 chain	case control study	[32]
<i>COL2A1</i>	Type II collagen A1	encodes the alpha-1 chain of type II collagen, a fibrillar collagen found in cartilage and the vitreous humor of the eye.	animal studies, case reports	[34, 35]
<i>ACE</i>	Angiotensin converting enzyme	central component of the renin-angiotensin system (RAS), which controls blood pressure by regulating the volume of fluids in the body. It converts the hormone angiotensin I to the active vasoconstrictor angiotensin II	case control studies	[36, 37]
<i>AGT</i>	Angiotensinogen	component of the renin-angiotensin system (RAS), a hormone system that regulates blood pressure and fluid balance	case control studies	[30, 36]
<i>ATII</i>	Angiotensin-II	hormone that may act on the central nervous system to regulate renal sympathetic nerve activity, renal function and blood pressure	case control study	[38]
<i>ATIIR</i>	Angiotensin-II receptor	receptor for angiotensin-II	case control study	[38]
<i>RELN</i>	Rellin	extracellular matrix protein	GWAS	[41]
			case control study	[13, 15, 29, 42, 43]
<i>TGFB1</i>	Transforming growing factor beta 1	multifunctional cytokine family	case control studies	[12–15, 29]
<i>BMP2</i>	Bone morphogenetic protein 2	multi-functional growth factors that belong to the transforming growth factor beta	case control studies	[12–15]
<i>BMP4</i>	Bone morphogenetic protein 4	multi-functional growth factors that belong to the transforming growth factor beta	case control study	[12–15]
<i>SERPINF1</i>	Serpin peptidase inhibitor, Clade F	pigment epithelium-derived factor - potent inhibitor of angiogenesis	family study - whole exome sequencing	[44]
<i>CD46</i>	Cluster of differentiation cell surface antigen 34	measles virus receptor, transmembrane phosphoglycoprotein	case control study	[40]
<i>HLA-B40</i>	Human leukocyte antigen	human leukocyte antigen (HLA) proteins complex	case control study	[39]