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Genetic basis of otosclerosis

Генетичка основа отосклерозе

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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

**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 capsula 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 stapediovestibular 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-β1, 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-β1, a significant association between variants in TGF-β1 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 \textit{COL1A1} and otosclerosis have been strongly associated [33].

Variations in \textit{COLIA2} gene could indirectly influence \textit{COL1A1} thus could have an impact on otosclerosis too. German study showed that some of the \textit{COLIA1} otosclerosis-associated variants alter binding of transcription factors that regulate transcription of \textit{COLIA2} [18]. They have confirmed that targeted deletion of \textit{COLIA2} in the mouse model leads to a mild conductive hearing loss. However, the Spanish group found no evidence in favor of \textit{COLIA2} genes association with otosclerosis [32]. Type II collagen, the main collagen of cartilage, is encoded by \textit{COL2A1} gene. Autoreactivity to \textit{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 (\textit{AGT}) and angiotensinogen converting enzyme genes (\textit{ACE}) in a French-Caucasian cohort was related to higher plasma concentrations of \textit{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 \textit{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 \textit{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 \textit{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 (Serpin 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 toll 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.

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| Gene   | Full name of the gene          | Protein function                                                                 | Type of the study                  | Reference       |
|--------|--------------------------------|----------------------------------------------------------------------------------|------------------------------------|-----------------|
| **COL1A1** | 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]            |
| **COL1A2** | 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                | [17, 18, 31, 32, 33] |
| **COL2A1** | 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]        |
| **ACE**          | 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]        |
| **AGT**          | Angiotensinogen | component of the renin–angiotensin system (RAS), a hormone system that regulates blood pressure and fluid balance | case control studies              | [30, 36]        |
| **ATII**         | 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]            |
| **ATIIIR**       | Angiotensin-II receptor | receptor for angiotensin-II | case control study                | [38]            |
| **RELN**         | Rellin | extracellular matrix protein | GWAS                              | [41]            |
| **TGFB1**        | Transforming growth factor beta 1 | multifunctional cytokine family | case control studies              | [12–15, 29]     |
| **BMP2**         | Bone morphogenetic protein 2 | multi-functional growth factors that belong to the transforming growth factor beta | case control studies              | [12–15]         |
| **BMP4**         | Bone morphogenetic protein 4 | multi-functional growth factors that belong to the transforming growth factor beta | case control studies              | [12–15]         |
| **SERPINF1**     | Serpin peptidase inhibitor, Clade F | pigment epithelium-derived factor - potent inhibitor of angiogenesis | family study - whole exome sequencing | [44]            |
| **CD46**         | Cluster of differentiation cell surface antigen 34 | measles virus receptor, transmembrane phosphoglycoprotein | case control study                | [40]            |
| **HLA-B40**      | Human leukocyte antigen | human leukocyte antigen (HLA) proteins complex | case control study                | [39]            |