Published in final edited form as:

Laryngoscope. 2021 February ; 131(2): E605–E611. doi:10.1002/lary.28741.

Association of CARD8 Activating Polymorphism With Bone Erosion in Cholesteatoma Patients

Neel Sangal, MD,
Department of Otolaryngology – Head and Neck Surgery, Rutgers New Jersey Medical School, Newark, New Jersey, U.S.A

John Yan, BA,
Department of Otolaryngology – Head and Neck Surgery, Rutgers New Jersey Medical School, Newark, New Jersey, U.S.A

Jessica Pryor, BS,

Marcus L. Elias, BS,

Biju Joseph, PhD,

Luis Ulloa, PhD,
Center for Perioperative Organ Protection. Department of Anesthesiology, Duke University Medical Center, Durham, North Carolina, U.S.A

Robert W. Jyung, MD
Department of Otolaryngology – Head and Neck Surgery, Rutgers New Jersey Medical School, Newark, New Jersey, U.S.A

Abstract

Objectives: We compared the incidence of polymorphisms activating the NLRP3 inflammasome between controls and patients with cholesteatoma and its potential association with bone erosion in patients with cholesteatoma.

Methods: This is a case-control study assessing the mutation rates in genes of interest in patients with and without cholesteatoma. A total of 133 saliva samples from control (n = 65) and cholesteatoma (n = 68) patients were collected for DNA extraction. Caspase recruitment domain family member 8 (CARD8) (AA: homozygous wild type, AT: heterozygous, TT: homozygous mutant polymorphism) and NLRP3 (CC: homozygous wild type, CA: heterozygous, AA: homozygous mutant) polymorphisms were analyzed with TaqMan single-nucleotide polymorphism (SNP) quantitative polymerase chain reaction (ThermoFisher Scientific, Waltham, MA). Mutation status was correlated with a novel bone erosion scoring model developed as a part of this study. Summary statistics, including frequencies (%) and median (Q1, Q3) were used to describe the sample.

Send correspondence to Robert W. Jyung, MD, Director of Otology/Neurotology, Department of Otolaryngology–Head and Neck Surgery, Rutgers New Jersey Medical School, 90 Bergen Street, Suite 8100, Newark, NJ 07103. jyungrw@njms.rutgers.edu.

The authors have no funding, financial relationships, or conflicts of interest to disclose.

Results: The presence of CARD8 and NLRP3 homozygous wild-type polymorphisms were generally similar for the control and cholesteatoma patient groups. CARD8 homozygous TT polymorphisms were an exception, occurring more frequently in patients who developed a cholesteatoma compared to the control group (29% vs. 10%, $P = .009$). Those patients with CARD8 homozygous TT polymorphism had higher median scores of bone erosion as compared to subjects with nonhomozygous mutant genotypes (median [interquartile range]: 4.0 [3.0, 5.5] vs. 2.5 [1.0, 3.5], $P = .0142$).

Conclusion: Cholesteatoma patients have a significant, twofold higher incidence of CARD8 homozygous TT polymorphism. Furthermore, cholesteatoma patients with this homozygous polymorphism had greater bone erosion rates than controls. These findings suggest that genetic mutations may increase host susceptibility to cholesteatomas. Specifically, the CARD8 TT polymorphism may influence the severity of cholesteatoma-induced bone erosion.

Level of Evidence: 3b

Keywords

Cholesteatoma; otolaryngology; single-nucleotide polymorphism; bone erosion; CARD8

INTRODUCTION

Acquired cholesteatoma (AC) is an invasive disease that develops from the squamous epithelium of the tympanic membrane or external auditory canal and is characterized by accumulation of keratin and bone destruction. Due to its capacity for bone destruction, cholesteatoma can cause sequelae such as hearing loss by ossicular erosion, vestibular symptoms secondary to fistulae of the semicircular canals, and even intracranial complications.[1] The primary treatment strategy is surgical extirpation of the epithelial envelope, but recurrences are not uncommon.

Currently, there exist four prominent theories that explain the pathogenesis of cholesteatoma, each potentially explaining specific clinical situations. These theories include invagination of the tympanic membrane to form retraction pockets, squamous metaplasia of the inner ear mucosal lining leading to hyperproliferation, germinal skin layer proliferation of the tympanic membrane leading to keratin-filled microcyst formation, and abnormal migration patterns of tympanic membrane skin due to preexisting perforations.[2] However, clear descriptions of the step-by-step mechanisms for each of these proposed theories are lacking.

Important emerging data now reveals the critical role of inflammatory complexes known as inflammasomes in the pathogenesis of cholesteatoma through their activation of toll-like receptors (TLRs) and associated downstream products, often irrespective of bacterial infection.[3] Inflammasomes are multiprotein complexes that utilize caspase-1 in order to cleave pro-IL-1β and pro-IL-18 cytokine precursors into their mature proinflammatory forms IL-1β and IL-18 in response to invading pathogens and certain danger-associated molecular patterns.[4]

Studies have also shown that IL-1β acts as a primary bone resorption stimulus through the induction of osteoclast differentiation.[5] Recently, Alippe et al. suggested that products from the development of bone matrix in response to erosion activate the NLRP3 inflammasome, leading to a positive feedback loop.[6] Previous work has already demonstrated the presence of IL-1β protein in cholesteatoma specimens.[7] Therefore, evidence is now converging to implicate inflammasome-mediated production of IL-1β in the bone erosion induced by cholesteatomas.

Genetic mutations that cause dysregulation or constitutive activation of certain inflammasomes can contribute to a wide range of inflammatory conditions, including rheumatoid arthritis, gout, arteriosclerosis, and Crohn disease.[8-10] The NLRP3 inflammasome (named after one of its critical functional proteins) is the most extensively studied inflammasome to date because it plays a central role in innate immunity and induction of inflammatory cell death (pyroptosis). Mutations in two proteins essential to regulation of NLRP3 inflammasome activity, CARD8 (NCBI reference: NM_014959) and NLRP3 (NCBI reference: NM_004895.3), have also shown to be associated with increased risk for chronic inflammation.[11] CARD8, an inhibitory adapter protein, works in conjunction with the sensor protein NLRP3 to regulate inflammasome activity by binding NLRP3, thus preventing assembly of the inflammasome complex.[11] To date, no studies have assessed whether polymorphisms in these genes influence the pathogenesis of cholesteatoma. Given the recent studies implicating inflammasome-mediated inflammation in cholesteatoma, we reasoned that these mutations may contribute to cholesteatoma progression as well.

In this study, we focused on two commonly studied genetic variants: CARD8 C10X (accession number: rs2043211) and NLRP3 Q705K (rs35829419). The polymorphism C10X in CARD8 (rs2043211) causes a nonsense mutation in exon 5 that incapacitates inhibitory pathways of caspase-1 activation, causing constitutive activation of downstream NLRP3 pathways and an increase in caspase-1 production and cytokine release.[12,13] Meanwhile, the Q705K polymorphism in NLRP3 (rs35829419) achieves a similar phenotypic effect through a gain-of-function missense mutation in exon 3.[14,15]

Using the aforementioned genetic variants of NLRP3 and CARD8 as a reflection of inflammatory susceptibility within the host genetic background, we assessed the incidence of these variants in a cohort of cholesteatoma patients as compared to a control group. In addition, we assessed whether either of these gene variants are associated with a greater degree of temporal bone erosion. To our knowledge, this is the first study examining the contribution of host genetic predisposition for inflammation as a factor in the development and severity of human cholesteatoma.

MATERIALS AND METHODS

Identification of Subjects

All procedures concerning the collection and reporting of human subject data in this study are sanctioned by the Rutgers Institutional Review Board (application number: Pro201600-00599). Subjects were identified from the patient population of one surgeon

at a large academic center in an urban area (University Hospital, Newark NJ). There were 65 control patients with no middle ear pathology, and 68 patients with cholesteatoma enrolled in the study. No cholesteatoma patients exhibited intracranial complications. Patients with any of the following criteria were excluded: systemic lupus erythematosus, rheumatoid arthritis, Behcet disease, Reiter disease, immune deficiency syndrome, celiac disease, inflammatory bowel disease, tumors of oral cavity, erythema multiforme, vesiculobullous disease, major or herpetiform aphthous lesions, oral lesions due to drugs or radiation, pregnancy, and periodontal disease. Atherosclerosis, although linked to inflammasome activation, has not been utilized as an exclusion criterion in similar studies and therefore was not factored into subject recruitment. Patient self-reported parameters of ethnicity and age were collected and used to match patients in the control and cholesteatoma groups. Written informed consents were obtained from all participants according to the guidelines of the ethics committee of Rutgers New Jersey Medical School (Newark, NJ) prior to saliva sampling.

Human Samples

DNA samples were collected using the SalivaBio Oral Swab (SOS) system (Salimetrics, State College PA), a synthetic oral swab that has been validated for analysis of single nucleotide polymorphisms. Patients were instructed to refrain from eating, chewing gum, or drinking colored liquids for 30 minutes prior to sample collection. Swabs were placed under each patient's tongue for 2 minutes, placed into collection tubes, and immediately frozen at −20°C. Within 12 hours, samples were transferred to cryovials and stored at −80°C for long-term storage.

Genotyping of Single Nucleotide Polymorphisms

A PureLink Genomic DNA extraction method (ThermoFisher, Waltham, MA) was utilized to isolate DNA from SOS. Samples were centrifuged to remove saliva, incubated with cell lysis buffer containing proteinase K, and incubated at 55°C. After cell lysis, DNA was purified on PureLink genomic silica-based membrane columns. DNA was eluted from the columns using Tris HCl-EDTA, quantitated, and then the purity and yield for the purified DNA was determined by measuring absorbance at A260 and A280 by spectrophotometry.

The NLRP3 Q705K (genotyping assay ID: C__25648615_10) and CARD8 C10X (genotyping assay ID: C_11708080_1) polymorphisms were assessed using a TaqMan single-nucleotide polymorphism (SNP) polymerase chain reaction (PCR)-based genotype assay (Applied Biosystems, Waltham, MA). Reactions were read using a QuantStudio 3 Real-Time qPCR system (ThermoFisher Scientific) according to the manufacturer's protocol using standard conditions (60°C for 30 minutes followed by, 95°C for 10 seconds, and 15 seconds at 92°C and 50 cycles at 60°C for 1 second). To control the quality of genotyping, reactions were conducted blind to case and control status. Each PCR run for NLRP3 contained a wild-type (CC), heterozygous (CA), and homozygous mutant (AA) genotype and two negative controls. Each CARD8 run contained analogous wild-type (AA), heterozygous (AT), and homozygous mutant (TT) genotypes with two negative controls.

Development of Bone Erosion Scale

A thorough literature search was performed to identify any current and validated methods for quantifying temporal bone destruction relevant to cholesteatoma. No grading methods pertaining to temporal erosion were found that were appropriate for this study. A previous study analyzed the extent of erosion of several temporal bone structures in patients with chronic suppurative otitis media (CSOM) compared to those with CSOM and cholesteatoma. The structures analyzed in that study helped guide which structures to include in this study's grading approach.[16] A straightforward grading system was devised to quantify levels of bone destruction that are directly caused by the cholesteatoma.

Primary grading analysis was reported on a detailed table depicted in Figure 1, which assigns a specific number to each structure of interest. Higher grades correspond to more severe bone destruction of that particular structure, whereas a score of 0 was designated for structures that were unmarred by the cholesteatomatous inflammation.

The grading system is based on first-hand surgical observations, as detailed in the senior author's meticulous operative notes. The grade of each structure was summated and provided each patient with a total grade indicative of the amount of temporal bone destruction caused by the cholesteatoma. If operative notes were not available or if no surgery was performed, bone erosion was quantified by review of a temporal bone high-resolution CT scan. If necessary, review of surgical videos was performed to complete the grading process.

Data Analysis

Summary statistics, including frequencies (%) and median (Q1, Q3), were used to describe the sample. Normality was assessed using the Shapiro Wilks test ($P < .05$). Fisher Exact tests were used to assess differences between the control and cholesteatoma groups in demographic characteristics and the presence of mutations. Bone erosions scores were summarized using the median with interquartile range (Q1, Q3); differences across the control and cholesteatoma groups was assessed using a Wilcoxon Rank Sum Test. Logistic regression was used to assess the odds of developing a cholesteatoma based on the presence of CARD8 (C10X) and NLRP3 (Q705K) variants. An initial alpha of 0.05 was used in significance testing; this alpha was then adjusted to reflect the effect of multiple comparisons (3) across each of the two mutation groups (($\alpha_{adjusted} = .05/3) = .0167$, for which 3 refers to the number of genotypes per gene). Therefore, all significant relationships reported have a P value that is less than .0167.

RESULTS

There were 65 patients in the control group and 68 in the cholesteatoma group. The cohort was 52% (n = 69) male and 48% (n = 64) female. Among the control group population, two subjects possessed NLRP3 data (n = 133) without corresponding CARD8 data (n = 131) due to inconclusive PCR results. The self-reported racial/ethnic distribution of patients in both groups is shown in Table I. Caucasians comprised the largest racial/ethnic group across age

and treatment groups. The racial/ethnic and age distributions did not differ in the control and cholesteatoma groups ($P = .1081$ and $P = 1.0$) for adults and children, respectively.

The genotypic distribution of patients is shown in Table II. Roughly 89% of patients had the NLRP3 CC genotype irrespective of the presence of CARD8 variants. NLRP3 AA genotypes were not common (1%). Among CARD8 genotypes, the most frequent was AT (n = 58, 44%), followed by AA (n = 47, 36%). Table III shows the distribution of minor allele frequencies (MAF) by race for the entire sample and by treatment group. However, because case-control matching factored in ethnicity, MAF distributions across ethnicity did not need to be considered in the data analyses.

The distribution of CARD8 and NLRP3 genotypes in the control and cholesteatoma groups is shown in Table IV. The odds of developing a cholesteatoma were not different for patients who had any of the genotypes except for CARD8_TT. Patients with the CARD_TT genotype were roughly 2 times more likely to develop a cholesteatoma than patients without (OR = 1.95, $P = .01$). Additional regression analyses were performed to test for interactions between genotypes, that is, whether the odds of developing a cholesteatoma was different for patients who had both a CARD8 and a NLRP3 homozygous mutant genotype. All possible combinations were examined, and the interactions were deemed not significant in part because the cell size of these specific combinations became too small. Further analyses were performed comparing matching subjects based on race both with and without age considerations. The two models yielded similar results, and the CARD8_TT genotype was significantly associated with cholesteatoma incidence irrespective of age when matching subjects. Sensitivity analysis found similar results when using a higher age threshold (aged 21 years and under, aged over 21 years). This suggests that incorporating age into the matching criteria was probably not necessary.

Median bone erosion scores determined using the bone erosion scoring system (Fig. 1) varied based on the presence of the CARD8 TT genotype as displayed in Table VA. Patients who had this genotype had a median score of 4.0 (3.0, 5.5) compared to a median of 2.5 (1.0, 3.5) among subjects who did not have the genotype ($P = 0.0142$). The medians were not significantly different across any of the other genotypes. Table VB displays the median bone erosion scores after cross-tabulation of NLRP3 and CARD8 genotypes.

DISCUSSION

Inflammation has recently been implicated in cholesteatoma pathogenesis. We explored the hypothesis that genetic predisposition for inflammation can influence the development and severity of cholesteatoma. Previous studies suggested that asymptomatic carriers of one of these mutations have the same levels of IL-1β/IL-18 as wild-type individuals, whereas double carriers have significantly higher levels of these cytokines. These results suggest a synergistic effect of NLRP3 and CARD8 mutations on the development of a proinflammatory state.[10,17,18]

Our cholesteatoma patients exhibited a homozygous CARD8 C10X mutation status 1.95 times higher (29.41% vs. 9.52%) than our control population. Although this does not prove

that homozygous CARD8 mutation status is causal, it can suggest it as a predisposing factor for cholesteatoma. Likewise, homozygous CARD8 mutation status has been implicated in other inflammatory conditions such as tuberculosis and gout.[19,20] However, CARD8 status does not seem to influence other inflammatory disorders such as Crohn disease or rheumatoid arthritis.[14,21] The exact significance of homozygous CARD8 mutation status for the development of cholesteatoma remains unclear: however, it remains an intriguing finding that warrants further investigation.

In addition, we examined the potential effect of these mutations on the *progression* of cholesteatoma, that is, severity of bone erosion. When CARD8 mutation status was correlated with a bone erosion score culled from operative reports and preoperative imaging, patients with homozygous mutant CARD8 genotypes exhibited significantly greater levels of bone erosion compared to patients without CARD8 mutations. These results suggest that the host inflammatory state exerts a significant influence on the progression of cholesteatoma bone destruction. Increased severity of disease based on host inflammatory mutations for both NLRP3 and CARD8 has already been described for both Crohn disease and rheumatoid arthritis.[12,14] These results highlight the impact of the presence of the homozygous CARD8 mutant state in our study because the aforementioned studies demonstrated an impact on severity but not frequency of mutations compared to controls.

To assess and compare rates of bone erosion between cholesteatoma patients and controls, a novel quantified erosion scale was developed. Meyerhoff et al. first suggested a staging system for cholesteatoma in 1986 based on location, ossicular defects, and presence of complications.[22] Since this work, there have been many efforts to characterize cholesteatoma in a standardized manner. More recently, the Japanese Otological Society validated a staging system for pars flaccida and pars tensa cholesteatoma with multiple revisions, and most recently the CHOLE staging system has been proposed.[23,24] However, on the authors' literature review, there was no system that provided a quantitative, standardized method of measuring bone erosion in this disease. Therefore, an itemized, novel scoring system was developed to allow direct comparison of clinical bone erosion in patients with cholesteatoma.

Bone is a dynamic tissue, undergoing constant remodeling, with some estimates citing a turnover rate of 15% per year. There is a careful balance struck between osteoblasts and osteoclasts by virtue of RANKL-RANK signaling such that this turnover yields relatively similar bone matrix density and volume.[5] However, inflammation has been shown to change the nature of this relationship, leading to sclerosis and erosion of bone, often simultaneously. The NLRP3 inflammasome is a known downstream component of RANKL-RANK signaling by NF-kB activation.[5] The NLRP3 inflammasome is a multiprotein oligomer that is activated by many pathogenic signals inducing the production of caspase-1.[25] This enzyme is involved in the production of inflammatory cytokines IL-1β and IL-18. IL-1β has been shown to directly increase the differentiation of naïve cells into osteoclasts and further activate them. In the setting of disease, these osteoclasts have been dubbed pathologically activated osteoclasts, and when unbalanced with osteoblasts, lead to increased production of activated metalloproteinases, directly causing bone erosion.[3] Given that inflammatory diseases are largely medically treated; our results in the context of supporting information

raise the question whether anti-inflammatory strategies could offer complementary medical management for the surgical treatment of cholesteatoma.

A potential confounder to our results is infection because it is the strongest activator of toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD) like receptors leading to NLRP3 inflammasome-mediated cytokine production. A study by Lee et al. showed that culture of cholesteatoma specimens was positive for staphlycoccal species and pseudomonas in 33.3% (n = 7) of specimens, with the other 66.6% (n = 14) being sterile.[26] This important finding suggests that the inflammation and activation of NLRP3 found through histopathologic staining in most cholesteatoma specimens is distinct from infection.[27] TLR-2 and TLR-4 have been found to be strongly expressed in the mucosal epithelium and infiltrating inflammatory cells in cholesteatoma samples, regardless of infection status. Further, a study by Jiang et al. finds that modulation of TLR4 through TREM-2 promotes bone erosion in cholesteatoma.[3] Our results warrant future studies to determine the relative impact of bacteria-mediated versus infection-independent pathways for inflammasome activation.

As a pilot inductive study, there are certainly limitations that we acknowledge. There was a limited sample size, which informed a relatively low power, making it difficult to derive conclusions regarding rare polymorphisms. Patients were derived from a single physician's patient pool, allowing for relatively uniform collection and scoring methods but limiting the enrollment of patients. Because we restricted patient sample collection to noninvasive means, no systemic blood cytokine levels were obtained; therefore, we cannot demonstrate that homozygous CARD8 mutations resulted in an elevated systemic inflammatory state. However, Sahdo et al. have demonstrated that mutations for NLRP3 or CARD8 cause systemic elevations in inflammatory cytokines, including IL-1β and IL-18.[17]

CONCLUSION

This study is the first to examine whether the presence of inflammasome-activating polymorphisms in the NLRP3 and CARD8 genes affects the severity of cholesteatoma-induced bone erosion. We found a significantly higher rate of CARD8 activating mutations in the cholesteatoma cohort when compared to the control group. Furthermore, those with these activating CARD8 mutations displayed significantly higher levels of bone erosion than the cholesteatoma patients without these mutations. Therefore, our findings support the role of inflammation in the progression of acquired cholesteatoma.

Acknowledgment

The authors gratefully acknowledge the statistical expertise and tireless efforts of Tracy Andrews, MS.

BIBLIOGRAPHY

1. Semaan MT, Megerian CA. The pathophysiology of cholesteatoma. Otolaryngol Clin North Am 2006;39:1143–1159. [PubMed: 17097438]
2. Tinling SP, Chole RA. Gerbilline cholesteatoma development part I: epithelial migration pattern and rate on the gerbil tympanic membrane: comparisons with human and guinea pig. Otolaryngol Head Neck Surg 2006;134:788–793. [PubMed: 16647536]

3. Jiang H, Si Y, Li Z, et al. TREM-2 promotes acquired cholesteatoma-induced bone destruction by modulating TLR4 signaling pathway and osteoclasts activation. Sci Rep 2016;6:38761. [PubMed: 27934908]
4. Yang Y, Wang H, Kouadir M, Song H, Shi F. Recent advances in the mechanisms of NLRP3 inflammasome activation and its inhibitors. Cell Death Dis 2019;10:128. [PubMed: 30755589]
5. Lorenzo J The many ways of osteoclast activation. J Clin Invest 2017;127:2530–2532. [PubMed: 28530641]
6. Alippe Y, Wang C, Ricci B, et al. Bone matrix components activate the NLRP3 inflammasome and promote osteoclast differentiation. Sci Rep 2017;7:6630. [PubMed: 28747793]
7. Ahn JM, Huang CC, Abramson M. Third place–resident basic science award 1990. Interleukin 1 causing bone destruction in middle ear cholesteatoma. Otolaryngol Head Neck Surg 1990;103:527–536. [PubMed: 2174138]
8. Kastbom A, Johansson M, Verma D, Soderkvist P, Rantapaa-Dahlqvist S. CARD8 p.C10X polymorphism is associated with inflammatory activity in early rheumatoid arthritis. Ann Rheum Dis 2010;69:723–726. [PubMed: 19443463]
9. Roberts RL, Van Rij AM, Phillips LV, et al. Interaction of the inflammasome genes CARD8 and NLRP3 in abdominal aortic aneurysms. Atherosclerosis 2011;218:123–126. [PubMed: 21621776]
10. Schoultz I, Verma D, Halfvarsson J, et al. Combined polymorphisms in genes encoding the inflammasome components NALP3 and CARD8 confer susceptibility to Crohn's disease in Swedish men. Am J Gastroenterol 2009;104:1180–1188. [PubMed: 19319132]
11. Mao L, Kitani A, Similuk M, et al. Loss-of-function CARD8 mutation causes NLRP3 inflammasome activation and Crohn's disease. J Clin Invest 2018;128:1793–1806. [PubMed: 29408806]
12. Asfaw Idosa B, Sahdo B, Balcha E, et al. C10X polymorphism in the CARD8 gene is associated with bacteraemia. Immun Inflamm Dis 2014;2:13–20. [PubMed: 25400921]
13. Paramel GV, Sirsjo A, Fransen K. Role of genetic alterations in the NLRP3 and CARD8 genes in health and disease. Mediators Inflamm 2015;2015:846782. [PubMed: 25788762]
14. Jenko B, Praprotnik S, Tomsic M, Dolzan V. NLRP3 and CARD8 polymorphisms influence higher disease activity in rheumatoid arthritis. J Med Biochem 2016;35:319–323. [PubMed: 28356883]
15. Kastbom A, Klingberg E, Verma D, et al. Genetic variants in CARD8 but not in NLRP3 are associated with ankylosing spondylitis. Scand J Rheumatol 2013;42:465–468. [PubMed: 23547871]
16. Gul A, Akdag M, Kinis V, et al. Radiologic and surgical findings in chronic suppurative otitis media. J Craniofac Surg 2014;25:2027–2029. [PubMed: 25377960]
17. Sahdo B, Fransen K, Asfaw Idosa B, et al. Cytokine profile in a cohort of healthy blood donors carrying polymorphisms in genes encoding the NLRP3 inflammasome. PLoS One 2013;8:e75457. [PubMed: 24098386]
18. Verma D, Sarndahl E, Andersson H, et al. The Q705K polymorphism in NLRP3 is a gain-of-function alteration leading to excessive interleukin-1beta and IL-18 production. PLoS One 2012;7:e34977. [PubMed: 22529966]
19. Assefa A, Gelaw B, Getnet G, Yitayew G. The effect of incident tuberculosis on immunological response of HIV patients on highly active anti-retroviral therapy at the university of Gondar hospital, north-west Ethiopia: a retrospective follow-up study. BMC Infect Dis 2014;14:468. [PubMed: 25164855]
20. Chen Y, Ren X, Li C, et al. CARD8 rs2043211 polymorphism is associated with gout in a Chinese male population. Cell Physiol Biochem 2015;35:1394–1400. [PubMed: 25790751]
21. Zhang ZT, Ma XJ, Zong Y, Du XM, Hu JH, Lu GC. Is the CARD8 rs2043211 polymorphism associated with susceptibility to Crohn's disease? A meta-analysis. Autoimmunity 2015;48:524–531. [PubMed: 26462578]
22. Meyerhoff WL, Truelson J. Cholesteatoma staging. Laryngoscope 1986;96:935–939. [PubMed: 3747693]
23. Roosli C, Baechinger D, Linder T, Huber A. First experience with the ChOLE classification in combination with a QoL questionnaire. J Laryngol Otol 2017;130:75.

24. Tono T, Sakagami M, Kojima H, et al. Staging and classification criteria for middle ear cholesteatoma proposed by the Japan otological society. Auris Nasus Larynx 2017;44:135–140. [PubMed: 27616746]

25. Kariya S, Okano M, Zhao P, et al. Activation of NLRP3 inflammasome in human middle ear cholesteatoma and chronic otitis media. Acta Otolaryngol 2016;136:136–140. [PubMed: 26457439]

26. Lee SH, Jang JH, Lee D, Lee HR, Lee KY. Surgical outcomes of early congenital cholesteatoma: minimally invasive transcanal approach. Laryngoscope 2014;124:755–759. [PubMed: 23877960]

27. Hirai H, Kariya S, Okano M, et al. Expression of toll-like receptors in chronic otitis media and cholesteatoma. Int J Pediatr Otorhinolaryngol 2013;77:674–676. [PubMed: 23380629]

Structure/Score	1	2	3
Scutum	Limited < or = 2mm	Extensive >2mm	Posterior bony canal wall
Incus	Long process OR body	Long process AND part of body	Absent
Stapes	Partial superstructure	Entire superstructure	Footplate
Malleus	Partial head OR partial manubrium	Partial head AND partial manubrium	Absent malleus
Facial Nerve	Limited <5mm length	Extensive, >5mm in length	-
Otic Capsule	Blue line of lateral SCC	Full fistula of lateral SCC	-
Cochlea	Blue line of cochlea	Full fistula of cochlea	-
Tegmen	Thin but not dehiscent	Limited, <5mm diameter	Extensive, >5mm diameter
Mastoid	Limited, medial to Koerner's septum	Extensive, lateral to Koerner's septum	Cortex
Sigmoid	Limited, <5mm	Extensive, >5mm	-
Carotid	Limited, <5mm	Extensive, >5mm	-

Fig. 1.
Bone erosion scoring system.

TABLE I.

Race and Age of Control and Cholesteatoma Cases.

	Age: Over 18 Years		Age: 18 Years and Under	
Race/ethnicity	**Control n = 56**	**Cholesteatoma n = 64**	**Control n = 9**	**Cholesteatoma n = 4**
Caucasian	22 (39.29)	30 (46.88)	3 (33.33)	1 (25.00)
African American	12 (21.43)	5 (7.81)	1 (11.11)	1 (25.00)
East Asian	19 (33.93)	19 (29.69)	3 (33.33)	2 (50.00)
Latin American	2 (3.57)	4 (6.25)	1 (11.11)	0 (0.00)
South Asian/Pacific Islander	1 (1.79)	6 (9.38)	1 (11.11)	0 (0.00)

TABLE II.

Genotypic Distribution Among the Study Population.

	CARD8 AA		CARD8 AT		CARD8 TT	
NLRP3*	**No n = 84**	**Yes n = 47**	**No n = 73**	**Yes n = 58**	**No n = 105**	**Yes n = 26**
CC (n = 119)	75 (89.3)	43 (91.5)	64 (87.7)	54 (93.1)	97 (92.4)	21 (80.8)
CA (n = 13)	8 (9.5)	4 (8.5)	8 (11.0)	4 (6.9)	8 (7.6)	4 (15.4)
AA (n = 1)	1 (1.2)	0 (0.0)	1 (1.4)	0 (0.0)	0 (0.0)	1 (3.9)

* Two control subjects possessed NLRP3 data of CC and CA genotype without corresponding CARD8 data: respective n's total 133 and 131. The two subjects have been omitted from the table, except for the far left total NLRP3 column, which reflects the total number of NLRP3 entries (n = 133).

AA = homozygous wild type; AT = heterozygous; CARD8 = caspase recruitment domain family member 8; TT = homozygous mutant polymorphism.

TABLE III.

Minor Allele Frequency by Race and Treatment Group.

		NLRP3 Mutations				CARD8 Mutations			
Race	**n**	**CC**	**CA**	**AA**	**MAF**	**AA**	**AT**	**TT**	**MAF**
All patients	133	119	13	1	0.0564	47	58	26	0.4135
Caucasian	56	49	7	0	0.0625	24	20	12	0.3929
African American	19	18	1	0	0.0263	6	8	4	0.4211
Latin American	43	39	3	1	0.0581	12	23	7	0.4302
East Asian	7	7	0	0	0.0000	3	3	1	0.3571
South Asian/Pacific Islander	8	6	2	0	0.1250	2	4	2	0.5000
Control	65	61	4	0	0.0308	27	30	6	0.3231
Caucasian	25	22	3	0	0.0600	13	10	2	0.2800
African American	13	13	0	0	0.0000	5	5	3	0.3462
Latin American	22	21	1	0	0.0227	7	12	2	0.3636
East Asian	3	3	0	0	0.0000	2	1	0	0.1667
South Asian/Pacific Islander	2	2	0	0	0.0000	0	2	0	0.5000
Cholesteatoma	68	58	9	1	0.0809	20	28	20	0.5000
Caucasian	31	27	4	0	0.0645	11	10	10	0.4839
African American	6	5	1	0	0.0833	1	3	2	0.5833
Latin American	21	18	2	1	0.0952	5	11	5	0.5000
East Asian	4	4	0	0	0.0000	1	2	1	0.5000
South Asian/Pacific Islander	6	4	2	0	0.1667	2	2	2	0.5000

AA = homozygous wild type; AT = heterozygous; CARD8 = caspase recruitment domain family member 8; TT = homozygous mutant polymorphism; MAF = minor allele frequency.

TABLE IV.

Odds of Developing a Cholesteatoma by Genetic Mutations.

Mutation	Control n (%)	Cholesteatoma n (%)	Odds Ratio	95% Confidence Interval		*P* Value
				Lower	Upper	
NLRP3						
CC	61 (93.85)	58 (85.29)	0.7	0.374	1.296	0.254
CA	4 (6.15)	9 (13.24)	1.33	0.707	2.512	0.375
AA	0 (0.00)	1 (1.47)	–	–	–	–
CARD8						
AA	27 (42.86)	20 (29.41)	0.71	0.486	1.035	0.075
AT	30 (47.62)	28 (41.18)	0.96	0.669	1.381	0.829
TT	6 (9.52)	20 (29.41)	1.95	1.184	3.215	**0.009****

Bold signifies $p < 0.0167$.

AA = homozygous wild type; AT = heterozygous; CARD8 = caspase recruitment domain family member 8; TT = homozygous mutant polymorphism.

TABLE V.

A and B Median Bone Erosion Scores for Cholesteatoma by Genotype, With IQR.

A. Median Bone Erosion Scores Based on Genotype			
	Patient Has the Genotype		
Genotype	**No**	**Yes**	***P* Value**
NLRP3			
CC (58)	3.0 (3.0, 6.0)	3.0 (2.0, 4.0)	.2533
CA (9)	3.0 (2.0, 4.0)	3.0 (3.0, 6.0)	.2533
AA (1)**	3.0 (2.0, 4.5)	–	
CARD8			
AA (20)	3.0 (2.0, 5.0)	2.5 (1.5, 3.0)	.1716
AT (28)	3.0 (2.0, 5.0)	2.5 (1.0, 4.0)	.3375
TT (20)	2.5 (1.0, 3.5)	4.0 (3.0, 5.5)	**.0142****

B. Median Bone Erosion Scores Based on CARD8 and NLRP3 Cross-Tabulation			
	CARD8		
NLRP3	**AA**	**AT**	**TT**
CC (58)	2.5 (2.0, 3.0)	2.0 (1.0, 4.0)	4.0 (3.0, 5.0)
CA (9)	1.5 (.00, 3.0)	4.5 (3.0, 6.0)	5.0 (3.0, 7.0)
AA (1)**	–	–	–

* Note: Only 1 patient was AA for NLRP3.

IQR = interquartile range.

Bold signifies p < 0.0167.

AA = homozygous wild type; AT = heterozygous; CA: heterozygou; CARD8 = caspase recruitment domain family member 8; CC: homozygous wild type; TT = homozygous mutant polymorphism.