

## ORIGINAL ARTICLE

# The genetics of cholesteatoma. A systematic review using narrative synthesis

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**Objective:** A cholesteatoma is a mass of keratinising epithelium in the middle ear. It is a rare disorder that is associated with significant morbidity, and its causative risk factors are poorly understood; on a global scale, up to a million people are affected by this each year. We have conducted a systematic literature review to identify reports about the heritability of cholesteatoma or any constitutional genetic factors that may be associated with its aetiology.

**Data Sources:** A systematic search of MEDLINE (EBSCO) and two databases of curated genetic research (OMIM and Phenopedia) was conducted.

**Study Selection:** The participants and populations of interest for this review were people treated for cholesteatoma and their family members. The studies of interest reported evidence of heritability for the trait, or any association with congenital syndromes and particular genetic variants.

**Data Extraction:** The searches identified 449 unique studies, of which 35 were included in the final narrative synthesis.

**Data Synthesis:** A narrative synthesis was conducted, and data were tabulated to record characteristics, including study design, genetic data and author conclusions. Most of the studies identified in the literature search, and described here, are case reports and so represent the lowest level of evidence. In a few case reports, congenital and acquired cholesteatomas have been shown to segregate within families in the pattern typical of a monogenic or oligogenic disorder with incomplete penetrance. Evidence from syndromic cases could suggest that genes controlling ear morphology may be risk factors for cholesteatoma formation.

**Conclusions:** This is the first systematic review about the genetics of cholesteatoma, and we have identified a small body of relevant literature that provides evidence of a heritable component for its aetiology. Cholesteatoma is a complex and heterogeneous clinical phenotype, and it is often associated with chronic otitis media and with some rare congenital syndromes known to affect ear morphology and related pathologies.

Barbara A. Jennings and Peter Prinsley contributed equally to the manuscript.

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## 1 | INTRODUCTION

A cholesteatoma is a self-perpetuating erosive lesion composed of stratified keratinising squamous epithelium in the middle ear.<sup>1</sup> Cholesteatoma has both an acquired and a congenital form. It activates osteoclasts and so will erode through bone, which may include the endocranium, with an attendant risk of life-threatening intracranial infection.

The acquired form of cholesteatoma originates as an inward growth from the lateral epithelium of the tympanic membrane. A typical sequence of events in the onset of the disease includes a history of chronic otitis media (COM) in childhood, subsequent development of retraction of the tympanic membrane and then a cholesteatoma developing within and perforating through this retraction. This seems to particularly occur if the retraction is located in the superior tympanic membrane (pars flaccida).<sup>2-4</sup> In children with a history of chronic otitis media with effusion (COME), 15-35% will develop a retraction of the pars flaccida (at up to 25 years of follow-up), but only 0.1-2% will develop a cholesteatoma (at up to 8 years of follow-up).<sup>4-7</sup> Both presence and duration of COM are predictive of tympanic membrane retraction,<sup>3,4</sup> but tympanic retraction has been documented to occur in the absence of preceding COM.<sup>4</sup> However, histological studies suggest that in such cases there is nevertheless chronic middle ear inflammation, it is just not clinically apparent.<sup>8</sup> Thus, cholesteatoma is often preceded by COM, but only a small proportion of those with COM will develop cholesteatoma. What determines the transition from COM to cholesteatoma is not known, but could be due to environmental factors, heritable factors or random effects. But those who develop cholesteatoma have been reported to have between a 7% and a 20% chance of developing disease in the contralateral ear,<sup>9,10</sup> highlighting the importance of shared genes and shared environments.

Cholesteatoma can also be found behind an intact tympanic membrane.<sup>11</sup> This form is thought to be congenital, and may result from persistence of the foetal epidermoid formation, a small collection of squamous epithelial cells in the middle ear that normally undergoes apoptosis before or shortly after birth. Congenital cholesteatoma can grow laterally and erode through the tympanic membrane, and at that point, it can be difficult to differentiate congenital from acquired disease.

Cholesteatoma is a rare disorder (1:10 000 per year),<sup>1</sup> and therefore, epidemiological studies are difficult to conduct, and causative risk factors are still poorly understood. The citations about cholesteatoma in the definitive catalogue of genes and genetic diseases, *Online Mendelian Inheritance in Man*,<sup>12</sup> document minimal evidence for the Mendelian inheritance of this disorder.<sup>13</sup> However, reports of familial clustering of disease and of association with genetic syndromes (reviewed here) suggest underlying, but as yet unidentified genetic risk factors. Identifying these could enhance our understanding of disease biology, and open up pathways for diagnostic, screening and therapeutic interventions.

### Keypoints

- We have synthesised data from 35 published studies in the first systematic review about the genetics of cholesteatoma.
- Only low quality evidence from case reports, case series, and small epidemiological studies was identified.
- Familial clustering suggests a possible genetic component to risk of cholesteatoma, and evidence from congenital syndromes suggests this could relate to loci regulating ear embryology.

One way to identify candidate genetic factors is through analysis of products of gene expression in pathological specimens. There are two published large-scale analyses comparing RNA transcript expression in cholesteatoma to that in skin of the external auditory canal. These have shown several hundred genes are differentially regulated in cholesteatoma samples, including genes with products involved in growth, differentiation, signal transduction, cell communication, protein metabolism and cytoskeleton formation.<sup>14,15</sup> However, the results from these studies are inconsistent, and are measuring gene expression once cholesteatoma has formed, and so have failed to significantly further our understanding of constitutional risk.

Here, we describe findings from a systematic review of the genetics of congenital and acquired cholesteatoma. Our aims from this review were to describe how susceptibility is transmitted within families showing disease clustering, to better understand the genetic architecture of disease, and to document any genotypes shown to co-segregate with the cholesteatoma phenotype. We also aimed to classify genetic syndromes associated with increased risk of cholesteatoma, which may implicate candidate genetic loci for further investigation.

## 2 | MATERIALS AND METHODS

### 2.1 | Objectives

To synthesise published evidence that addresses the following questions:

1. Can the development of a cholesteatoma be described as a heritable trait, or is there a genetic predisposition to cholesteatoma within some families?
2. Have any genetic alterations or congenital syndromes been associated with cholesteatoma?

### 2.2 | Registration of systematic review method

The protocol was registered with the PROSPERO International prospective register of systematic reviews database in June 2015.<sup>16</sup>

## 2.3 | Search strategy

We searched the MEDLINE (EBSCO), OMIM (<http://www.ncbi.nlm.nih.gov/omim>) and Public Health genomics Knowledge Base (<https://phgkb.cdc.gov/HuGENavigator/startPagePhenoPedia.do>) from 1980 to July 2015 using the terms “Cholesteatoma” AND “famil\* (OR Gene\* OR hered\* OR inherit\* OR syndrom\* OR kindred OR pedigree OR oncogene\* OR tumour suppressor OR tumor suppressor OR epigenetic\* OR mutat\* OR somatic OR homeobox).” We supplemented the search with relevant references identified in the citation lists at the article review stage.

## 2.4 | Inclusion and exclusion criteria

Studies were identified from the titles and abstracts by the primary reviewer (BAJ) and secondary reviewer (GW) using the following inclusion criteria:

1. Primary studies of kindreds that provide information about familial clustering.
2. Primary epidemiological studies that provide evidence of heritability including ethnic differences.
3. Relevant systematic reviews that provide information about genetics or heredity for cholesteatoma.
4. Case reports that refer to familial clustering of the cholesteatoma phenotype (>1 family member affected).
5. Case reports or epidemiological studies that provide evidence of association between cholesteatoma and syndromes

Studies were excluded if they were general narrative reviews or opinion pieces, about non-human or experimental disease models, or described pathologies other than cholesteatoma.

## 2.5 | Study selection & data extraction

Full reports of potentially relevant articles were retrieved, and data were extracted by the primary reviewer (BAJ). The study design, patient characteristics and nature of the outcomes were collated and coded red for exclusion, green for inclusion and amber to indicate uncertainty (RAG review). When there were uncertainties about inclusion or data interpretation, the articles were discussed by the reviewers (GW, MB and BAJ) to reach consensus. All studies that met the inclusion criteria were included regardless of quality, which was subsequently appraised (see Risk of bias and quality assessment below).

## 2.6 | Data synthesis

A narrative synthesis was conducted to explore the review questions about heritability and genetic associations reported for the cholesteatoma phenotype. We tabulated the date of the study, first author, study design, number of subjects, subtype of cholesteatoma, genetic investigations (including family history), associated congenital

syndromes, gene nomenclature and direct quotations from discussion or conclusions.

## 2.7 | Risk of bias and quality assessment

We appraised quality of epidemiological studies by reference to the Strengthening Reporting of Observational Studies (STROBE) guidelines<sup>17</sup> and the Strengthening Reporting of Genetic association Studies (STREGA) guidelines.<sup>18</sup> We mapped the evidence for each study to the five levels of evidence described by the Oxford Centre for Evidence Based Medicine.<sup>19</sup>

## 3 | RESULTS

### 3.1 | Study selection & data extraction

Our search identified 449 unique studies, of which 36 met the initial inclusion criteria. Most studies were excluded at the abstract or primary manuscript review stage, but six manuscripts were excluded at the data extraction stage because there were no relevant primary data identified about cholesteatoma or genetic phenomena,<sup>20-23</sup> or because the study described external auditory canal cholesteatoma.<sup>24</sup> The studies identified in the initial search were supplemented by five additional reports identified by hand-searching citation lists.<sup>25-29</sup> Thirty-five studies were finally included in this narrative synthesis (see Figure 1 for a flow chart which summarises these steps).

### 3.2 | Familial clustering

Nine studies (classified as case reports, case series and epidemiological studies) present evidence for familial clustering of cholesteatoma.<sup>25,27,28,30-35</sup> The extracted study characteristics are described in Table 1.

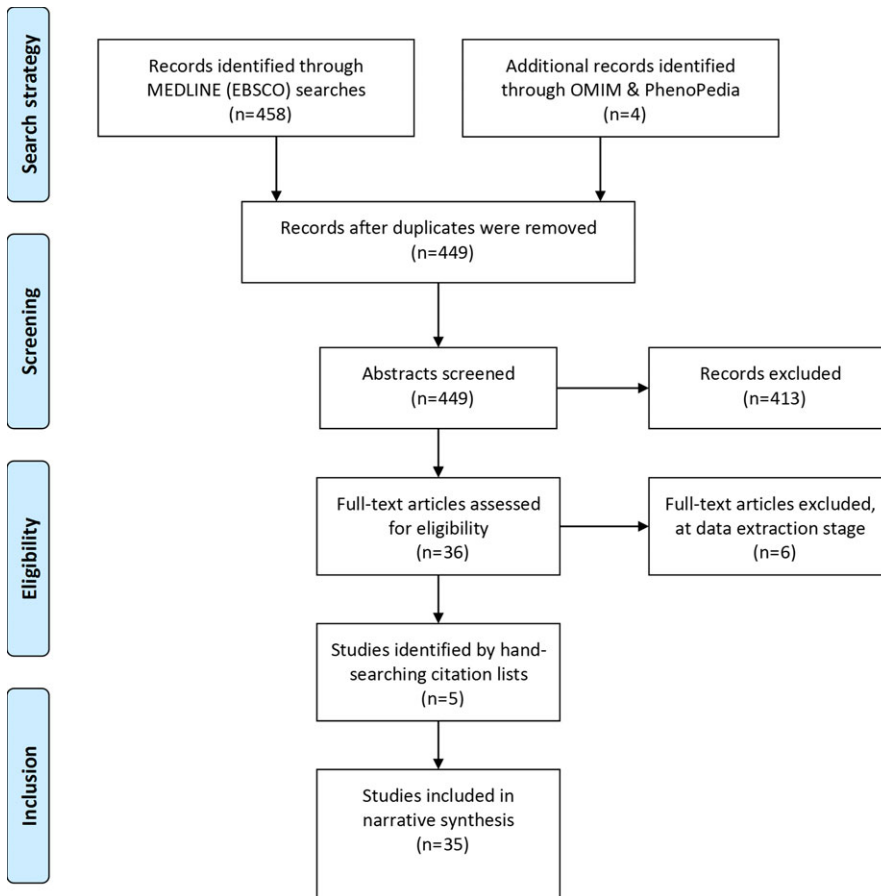
### 3.3 | Congenital syndromes and cholesteatoma

Twenty-two case reports and epidemiological studies describe the occurrence of cholesteatoma in patients affected by congenital and malformation syndromes,<sup>13,26,30,35-52</sup> several of which have a known underlying genetic aetiology. These are summarised in Table 2.

Some of these reports are of cholesteatoma occurrence in a single case of a particular syndrome, for example Beckwith-Wiedemann syndrome, granulomatosis with polyangiitis, Nager syndrome, primary ciliary dyskinesia, Tolosa-Hunt syndrome, Treacher Collins syndrome and Wolf-Hirschhorn syndrome. Single occurrences of a disease, whether associated with a syndrome or not, are susceptible to publication bias and so do not add to understanding of disease risk in isolation.

### 3.4 | Candidate genes and gene variants

We identified just two published studies of DNA-based laboratory investigations of particular gene sequences considered in association



**FIGURE 1** The identification and screening of studies for inclusion in the narrative synthesis is illustrated in this PRISMA flow diagram. OMIM: Online Mendelian Inheritance in Man.<sup>12</sup> PhenoPedia is an information database curated by the US Center for Disease Control (CDC)

with the cholessteatoma phenotype. One is a case report of a 6-year-old boy with a congenital cholessteatoma who was shown to have a deletion in the *APC* tumour suppressor gene.<sup>53</sup> The other is a candidate gene association study of polymorphisms of the *GJB2* and *GJB6* loci that encode connexins<sup>54</sup> in a cohort of 98 children undergoing surgery for cholessteatoma. These studies are also described in Table 3.

### 3.5 | Risk of bias and quality assessment

We identified only a small body of literature that was relevant to our questions about a heritable component for cholessteatoma aetiology. Many of the studies provide some indirect evidence only, given that the authors' objectives were to describe cholessteatoma management or associated environmental factors.

Most of the studies identified in the literature search, and described here, are case reports and so represent the lowest level of evidence. Case reports were automatically categorised as level 5 (see Tables 1, 2 and 3). The remaining observational studies include case series, cross-sectional surveys, case-control studies and cohort studies; each of these manuscripts was reviewed by BAJ and GW to define the level of evidence presented; STROBE and STREGA guidelines were referred to in classifying the quality of the methodology used in the case-control and cohort studies. The level of evidence ranged from 4 (for low-quality case-control studies, surveys and case

series) and 2b for a high-quality cohort study<sup>35,49</sup> (see Tables 1, 2 and 3).

## 4 | DISCUSSION

This is the first systematic review to explore the constitutional genetics of individuals affected by cholessteatoma. We have synthesised data from 35 published studies about familial aggregation of disease. The association of cholessteatoma with congenital syndromes and genes that were directly analysed in patients with cholessteatoma were also considered.

### 4.1 | Heritability

We have summarised the published evidence about the heritability of acquired and congenital cholessteatomas. We only identified a few case reports and case series that show two or more affected first-degree relatives; therefore, there is insufficient evidence to describe cholessteatoma as a heritable trait.

However, there are some compelling individual observations to consider, including affected monozygotic<sup>27</sup> and dizygotic twins,<sup>28,33</sup> families with two or more affected generations,<sup>30,32,33</sup> and high rates of bilateral disease in affected families.<sup>28,33</sup> Such observations suggest rare genetic variants underlie the disease in some families.

**TABLE 1** Familial clustering of the cholesteatoma phenotype

Year of publication	First author	Study design	Number of subjects	Phenotypes CC = congenital cholesteatoma AC = acquired cholesteatoma	Author conclusions extracted from manuscript (original language in parentheses)	Level of evidence
1973	Ray, J.	Case reports	2 siblings	Intramastoid CC in two brothers aged 3 years, and 8.5 months.	"First, and above all, the cholesteatomas are considered to be congenital.. (D'abord et surtout parce qu'elles sont a inscrire au repertoire des cholestéatomes congénitaux..)"	5
1986	Lipkin, A.F.	Case reports	2	A mother and her daughter had CC treated at age 5.	"These two cases may represent a unique variant of branchio-oto dysplasia."	5
1986	Podoshin, L.	Prospective cross-sectional survey of Kibbutz population	3056 individuals; 12 (0.4%) had a cholesteatoma.	Unilateral cholesteatoma in 11 cases; bilateral disease in one case. No data presented on congenital/acquired subtypes but histological examination of surgical tissue was carried out to confirm diagnoses.	"Among cholesteatoma patients a family history was found in 64%. . . One or more of their close family members had chronic otitis media or cholesteatoma."	4
1986	Naito, Y.	Case reports	2 siblings	Bilateral cholesteatoma in one dizygotic twin and unilateral cholesteatoma in other. Developed on a background of long-standing otitis media with effusion.	"More dizygotic and monozygotic twins with cholesteatoma should be studied to decide whether hereditary factors have any significant influence on the occurrence of this disease."	5a
2007	Homoe, P.	Case series	Family; two parents and seven siblings.	Mother and three siblings have AC; all surgically treated. All five family members seen had a dolicocephal appearance.	"to our knowledge this is the first report in the world literature of family clustering of AC."	4
2009	Prinsley, P.	Case series	15 families with $\geq 2$ family members affected by cholesteatoma.	"no distinction is made but the majority had drum abnormalities and would not therefore normally be considered congenital."	"this observation of family clustering in East Anglia is remarkable." "It supports the suggestion that a genetic predisposition exists for cholesteatoma."	4
2013	Al Balushi, T. 2013	Case reports	2	CC	"we report congenital cholesteatoma in identical twins, a previously unreported occurrence."	5

(Continues)

TABLE 1 (Continued)

Year of publication	First author	Study design	Number of subjects	Phenotypes CC = congenital cholesteatoma AC = acquired cholesteatoma	Author conclusions extracted from manuscript (original language in parentheses)	Level of evidence
2013	Landegger, L.D.	Case report	2.	CC The index case was diagnosed at 34 months, his older brother was treated for CC at 2 years of age.	"This case joins a single, previous report describing congenital cholesteatoma in multiple family members, suggesting that in some cases, hereditary factors may play a role in the formation of the disease."	5
2015	Djurhuus, B.D.	Historic Cohort Study (Health Outcomes and demographic data extracted from Danish Cleft Lip and Palate database and the Danish National Patient Register)	441 014 individuals were included in the study, comprising 8593 cases of orofacial cleft and 6989 siblings and a random control group from the Danish population.	201 and 21 Surgically treated cholesteatomas in a population affected by orofacial cleft and their siblings, respectively. The authors note that "in Denmark, cholesteatomas are rarely histologically examined."	"We found a twofold increased risk of cholesteatoma in siblings of cleft palate cases." "A possible increased risk of cholesteatoma in siblings of CP cases may be explained by familial aggregation of subclinical muscular defect."	2b

Studies are presented in chronological order by year of publication.

The levels of evidence were mapped to those described by the Oxford Centre for Evidence Based Medicine.<sup>19</sup>

<sup>a</sup>Data from Naito et al. extracted from abstract only.

If a disease is inherited as a monogenic or oligogenic trait, next generation sequencing (NGS) studies<sup>55</sup> can now be used to identify DNA variants that are co-inherited with that trait. NGS studies of affected family members may reveal mutations that are unique to a single kindred (these are known as *private* mutations). But such findings can also be generalisable if they identify the genes and biological pathways that are altered in other cholesteatoma patients with more complex aetiologies.

Observations about the familial aggregation of phenotypes are often followed by more discriminating epidemiological methods to distinguish the influence of heritability from shared environments. But because cholesteatoma is rare, a classical twin study has not been conducted and is not feasible. However, a study or register to collect data about bilateral disease might provide information about a genetic component to the aetiology of cholesteatoma. The incidence of bilateral disease for individuals could be compared to the coincidence of disease in dizygotic twins, in a manner analogous to studies of monozygotic vs dizygotic twins.

## 4.2 | Congenital syndromes

Several lines of evidence suggest that variants in genes regulating ear embryogenesis and tissue architecture also increase the risk of

cholesteatoma. For example, congenital cholesteatoma is more common in the malformed ears of people with branchio-oto-renal syndrome.

The association of acquired cholesteatoma with Down's syndrome, Turner syndrome and cleft palate is more difficult to disentangle, as these syndromes also place individuals at increased risk of COME, which often precedes development of cholesteatoma. Whether these syndromes are in themselves associated with increased risk of cholesteatoma is difficult to say. In contrast, Djurhuus et al.<sup>35</sup> showed a doubling of risk of cholesteatoma in siblings of patients with cleft palate. This finding should nevertheless be taken with some caution, the associated *P*-value was .026, which would be considered insignificant if it had been subject to Bonferroni adjustment due to the multiple hypothesis testing present in this study.

## 4.3 | Gene associations

Little evidence is presented in our results about the role of particular genes in cholesteatoma biology because only two studies reported the analysis of gene sequences: a case report and a small candidate gene association study. The case report describes a 6-year-old boy affected by familial adenomatous polyposis (FAP) who had cholesteatoma, and an inherited deletion in the tumour suppressor gene

**TABLE 2** Reports of cholesteatoma and congenital syndromes

Name of syndrome	First author and year of publication	Study design	Number of subjects	Subtype CC = congenital cholesteatoma AC = acquired cholesteatoma (original language in parentheses)	Genetic investigations and genetic loci noted	Author conclusions extracted from manuscript (original language in parentheses)	Level of Evidence
Beckwith-Wiedemann syndrome OMIM #130650	Daugbjerg, P. 1984	Case Report	7-year-old female	A small primary CC between the long process of incus and the handle of the malleus.	None	"The primary cholesteatoma might represent a fetal detachment of epidermis."	5
Branchio-oto-renal syndrome OMIM #113650	Graham, G.E. 1999	Case Report	14-month-old female	Unilateral CC. Also, bilateral middle ear anomalies and abnormal inner ear morphology.	None	"Our index patient represents the third individual reported to have congenital cholesteatoma in association with BOR syndrome."	5
	Lipkin, A.F. 1986 (also presented in Table 1)	Case Report	Mother and daughter. Both treated for cholesteatoma at 5 years.	CC	None	"These two cases may represent a unique variant of branchio-oto dysplasia."	5
	Worley, G.A. 1999	Case Report	8-year-old female	Bilateral CC	None	"An association between Branchio-oto-renal syndrome and congenital cholesteatoma has never been documented."	5
Cleft palate OMIM #119530 1988	Djurhuus, B.D. 2015 (also presented in Table 1)	Historic Cohort Study	8593 cases of orofacial cleft; 201 of whom were treated for cholesteatoma.	Surgically treated cholesteatoma.	None	"a 20-fold increase in risk of cholesteatoma was found for individuals with cleft palate."	2b
	Lau, C.C. 1988	Cross-sectional study.	83 patients with cleft palate; two (6%) patients had cholesteatoma.	34 (23%) had middle ear disease including cholesteatoma.	None	"There is a need for early otological assessment of all patients with cleft palate at an early age."	4

(Continues)

TABLE 2 (Continued)

Name of syndrome	First author and year of publication	Study design	Number of subjects	Subtype CC = congenital cholesteatoma AC = acquired cholesteatoma (original language in parentheses)	Genetic investigations and genetic loci noted	Author conclusions extracted from manuscript (original language in parentheses)	Level of Evidence
Down Syndrome OMIM #190685	Bacciu, A. 2005	Retrospective observational study.	Nine children with Down's syndrome who had been surgically treated for cholesteatoma	AC: two had bilateral disease.	Trisomy 21	"cholesteatoma is found to be more severe and extensive (in children with Down syndrome), probably related to a delay in diagnosis."	4
	Saito, R. 1983	Case Report	7-day-old girl	CC and congenital aural atresia	"Trisomy of G-group chromosome" (either +21 or +22) n.b. authors included this case report in the Down's syndrome subclassification because of cytogenetic findings but no confirmation in the manuscript.	"The formation of cholesteatoma was observed through the inter-mingling of the stratified squamous epithelium in the mucous epithelium of the hypotympanum."	5
	Suzuki, C 2004	Case Reports	45-year-old female with Down's syndrome and 77-year-old male	Rupture of cholesteatoma.	None	"infection of cholesteatoma matrix, rupture in particular, might have produced the easy transfer of endogenous substances from the cholesteatoma sac and epithelial debris that provoked the bone destruction in cholesteatoma."	5
Focal Dermal Hypoplasia (FDH)/Goltz syndrome. OMIM #305600	Reber, T. 1987	Case Report	21-year-old male.	"... partial cholesteatoma development in the upper part of the left tympanum ... (linkes Trommelfell subtotale randständige, im oberen anteil polyose Cholesteatombildung) ..."	Cytogenetic analysis revealed a normal male karyotype, 46,XY.	"The detected cholesteatoma present in our patient is probably linked to GGS (Möglicherweise steht das bei unserem Patienten festgestellte Cholesteatom ebenfalls in Zusammenhang mit dem GGS.)"	5
	Buchner, S.A. 1992	Case Report	68-year-old male diagnosed with FDH; treated for cholesteatoma at 56 years.	Bilateral cholesteatoma	No genetic report but syndrome associated with X-linked dominant pattern of inheritance	"Of interest in this case is the development of bilateral cholesteatoma. To date, only one case of cholesteatoma associated with FDH has been reported in the literature."	5
Granulomatosis Polyangiitis [Wegener's Granulomatosis] OMIM #608710	Kornblut, A.D. 1982	Case series	60 patients with Granulomatosis Polyangiitis	Four case reports of aural disease; one case of cholesteatoma in a 77-year-old male.	None	"Only one patient was found to have a cholesteatoma, and required radical mastoidectomy for management."	4

(Continues)



**TABLE 2** (Continued)

Name of syndrome	First author and year of publication	Study design	Number of subjects	Subtype CC = congenital cholesteatoma AC = acquired cholesteatoma (original language in parentheses)	Genetic investigations and genetic loci noted	Author conclusions extracted from manuscript (original language in parentheses)	Level of Evidence
Nager Syndrome [Nager acrofacial dystosis] OMIM #154400	Herrmann, B.W. 2005	Retrospective case series	10 patients with Nager acrofacial dystosis.	Cholesteatoma in one patient.	None	"pediatric patients with Nager acrofacial dystosis exhibit conductive hearing loss due to middle and external ear pathology."	4
Microtia OMIM #610706 & #612290	Jin, L. 2010	Retrospective cross-sectional study	208 microtia patients (21 of 208 had a family history of microtia)	15 (7.2%) cases had middle ear cholesteatoma	None	15 cases (7.2%) had middle ear cholesteatoma. "Therefore we suggest that every patient with microtia undergo a temporal CT scan examination at an early age."	4
Primary Ciliary Dyskinesia	el-Sayed, Y. 1997	Case series	16 electron microscope-confirmed cases observed between 1991 and 95.	One adult case had bilateral cholesteatoma treated with bilateral mastoidectomy.	None	"Otitis media is a prominent part of the primary ciliary dyskinesia syndrome."	4
Tolosa-Hunt Syndrome	Colnaghi, S. 2006	Case Report	40-year-old male	40-year-old male surgically treated for a relapsed right middle ear cholesteatoma.	None	"There are no previous reports about a relationship between Tolosa-Hunt syndrome and cholesteatoma and with the present case we only wish to point out an association between the two diseases, without any causal implications."	5
Treacher Collins Syndrome OMIM #154500 & #606847	Mann, W. 2014	Case Report	16-year-old male.	Hypotympanic cholesteatoma.	None	"50% of TC patients have a congenital ...hearing loss resulting from major or minor ear anomaly."	5

(Continues)

TABLE 2 (Continued)

Name of syndrome	First author and year of publication	Study design	Number of subjects	Subtype CC = congenital cholesteatoma AC = acquired cholesteatoma (original language in parentheses)	Genetic investigations and genetic loci noted	Author conclusions extracted from manuscript (original language in parentheses)	Level of Evidence
Turner Syndrome OMIM references #312865 (X-linked loci)	Bergamaschi, R. 2008	Observational Case Series	173	10 cases of cholesteatoma (5.7%); and 9 of 10 had bilateral disease.	45,XO	"high prevalence of middle ear infections and CHL in TS probably due to growth disturbances of the structures from the 1st and 2nd branchial arches" "the incidence of cholesteatoma is higher in these children than in the general population."	4
	Hall, J.E. 2009	Retrospective case series	178	Six patients (3.4%) were found to have cholesteatoma	None	"recurrent and chronic otitis media is common in patients with Turner syndrome."	4
	Lim, D.B. 2014	Retrospective Cohort Study	179	Seven (3.9%) had cholesteatoma; two had bilateral disease	"risk factors include 45,X and 46i(Xq)"	"This study has confirmed a cholesteatoma prevalence . . . . . approximately 1000 times higher than in the general population." "Our data support the observation that loss of one of the short arm pairs, for example through 45X monosomy or duplication of the q arm of X (iXq) is a risk factor for serious middle ear disease."	2b
Wolf-Hirschhorn Syndrome	Iino, Y. 1987	Case Report	7-month-old female	"two types of cholesteatoma in the middle ear cleft of the right temporal bone" "Cholesteatoma, which might be congenital in origin"	46,XX,4p- (deletion of the short arm of chromosome 4).	"the most interesting finding was the presence of two types of cholesteatoma in the middle ear cleft of the right temporal bone." It "might have been derived from developmental migration of keratinised epithelia in the middle ear cleft."	5
Unclassified Congenital Syndromes	Vaglio, A. 2008	Case Report	9-year-old female with dysmorphism and clinical features that included the absence of speech and poor mobility	Treated with surgery for cholesteatoma in her right ear.	46,XX,add(22)(q13.3) (pseudodiscentric bisatellited chromosome 22 associated with partial trisomy of chromosome 22). Duplication was paternal in origin.	"The serious clinical picture reported suggests that the proximal 22q region contains dosage-sensitive genes involved in development."	5

OMIM #: the numerical identifier used to catalogue entries about genes and traits in the Online Mendelian Inheritance in Man.<sup>12</sup>  
The levels of evidence were mapped to those described by the Oxford Centre for Evidence Based Medicine.<sup>19</sup>

**TABLE 3** Genetic association studies for cholesteatoma

First author and year of publication	Study design	Number of subjects	Subtype CC = congenital cholesteatoma AC = acquired cholesteatoma	Genetic investigations	Gene Loci & OMIM number	Author conclusions (extracted from manuscript)	Level of evidence
James, A.L. 2010	Candidate gene association study	98 children	Cholesteatoma cases include CC; AC and secondary acquired/implantation cholesteatoma.	Sequencing and MLPA analysis in candidate gene association study. But there was no control group data for comparison or HWE analysis.	GJB2 & GJB6 genes that encode connexions	"GJB2 gene variants are present in a minority of the sample of children with cholesteatoma (14%) but more commonly than in most normal populations."	4
Shaoul, R. 1999	Case report	6-year-old boy	CC	APC mutation analysis in mother and child (index case). A 5-bp deletion found in exon 15 of APC.	APC gene OMIM #175100	"This observation of a clinical association between FAP and cholesteatoma in this case, may strengthen an association of the role of APC gene mutations with abnormal control of cell growth and spatial organisation."	5

All gene symbols are congruent with Human Genome Organisation (HUGO) nomenclature and linked data about each locus can be found at The Human Gene Database GeneCards® (<http://www.genecard.org/cgi-bin/carddisp.pl?gene=GJB2>; <http://www.genecard.org/cgi-bin/carddisp.pl?gene=GJB6>; and <http://www.genecard.org/cgi-bin/carddisp.pl?gene=APC>).

OMIM #: the numerical identifier used to catalogue entries about genes and traits in the Online Mendelian Inheritance in Man;<sup>12</sup> MLPA: multiplex ligation-dependent probe amplification; HWE: Hardy-Weinberg Equilibrium.

The levels of evidence were mapped to those described by the Oxford Centre for Evidence Based Medicine.<sup>19</sup>

APC. The APC protein is expressed in many tissue types, influencing cell migration, adhesion and morphogenesis. Loss of APC expression in the colonic epithelium leads to an imbalance of cell growth over cell death,<sup>56</sup> but whether this is relevant to cholesteatoma biology is not known.<sup>53</sup> The second study was a candidate gene association study of 98 children with cholesteatoma for variants in the connexin gap-junction encoding genes, *GJB2* and *GJB6*,<sup>54</sup> some mutations of these loci are known to lead to recessive congenital deafness. Although the authors suggest a high frequency for some *GJB2* gene variants associated with cholesteatoma, no conclusions can be safely drawn from this study, because it lacked a control population and had a small sample size, placing it at risk of false discovery.

#### 4.4 | Limitations

We excluded non-English manuscripts and studies published before 1980 from our initial search (the earlier and/or non-English articles were subsequently included in the narrative synthesis because they were identified by hand-searching citation lists); it is therefore possible that we have missed relevant publications.

The over-representation of case reports, case series and historical epidemiological studies is unsurprising given that cholesteatoma is a rare disease, but such studies provide low-level evidence in the research hierarchy because they are usually retrospective with incomplete data collection or follow-up, and are subject to author bias, ascertainment bias and publication bias. In addition, such findings may not be generalisable, and should be interpreted with caution, particularly with respect to theories about the underlying aetiology of cholesteatoma.

## 5 | CONCLUSION

Cholesteatoma is a complex and heterogeneous clinical phenotype. In a handful of case reports or case series, congenital and acquired cholesteatomas have been shown to segregate within families in the pattern typical of a monogenic or oligogenic disorder with incomplete penetrance. The liability threshold for the observed cholesteatoma phenotype could therefore depend on a combination of environmental and genetic factors of variable penetrance. Evidence from syndromic cases suggests that genes controlling ear morphology may be risk factors for congenital or acquired cholesteatoma formation.

We should accommodate the hypothesis that a range of aetiological pathways exist for cholesteatoma and that these may result in disease subtypes that differ in both severity and tractability.

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#### CONFLICT OF INTERESTS

There are no other conflict of interests to declare.

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