The role of genetic factors in microtia

by Indri Lakhsmi Putri

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SYSTEMATIC REVIEW

REVISED The role of genetic factors in microtia: A systematic review [version 3; peer review: 2 approved with reservations, 1 not approved]

Indri Lakhsmi Putri[1], Alexandria Stephanie[2], Rachmaniar Pramanasari[2], Moshe Kon[3], Citrawati Dyah Kencono Wungu[4]

[1]Department of Plastic Reconstructive and Aesthetic Surgery, Faculty of Medicine, Airlangga University, Surabaya, East Java, 60132, Indonesia
[2]Plastic Reconstructive and Aesthetic Surgery Unit, Airlangga University Hospital, Surabaya, East Java, 60115, Indonesia
[3]International Society of Auricular Reconstruction (President); Department of Plastic and Reconstructive Surgery, Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, Netherlands, 3508 GA, Netherlands Antilles
[4]Department of Physiology and Medical Biochemistry, Faculty of Medicine, Airlangga University, Surabaya, East Java, 60132, Indonesia

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Abstract

Background: Microtia is a congenital malformation of the outer ears caused by improper embryonic development. The origin of microtia and causes of its variations remain unknown. Because of the lack of clarity regarding the role of genetic variables in microtia, we conducted a systematic review to qualitatively identify the genes most important in the development of microtia to provide an up-to-date review.

Methods: Using six search engines, we searched all published studies related to the genetic factors of isolated microtia and syndromic microtia. The identified publications were screened and selected based on inclusion and exclusion criteria by the authors and assessed for methodological quality using the Joanna Briggs Institute (JBI) critical appraisal tools. We found 40 studies, including 22 studies on syndromic microtia and 18 studies on isolated microtia. Data extraction of each study was arranged in tabulation for syndromic and isolated microtia. The extracted data were: first author's surname, year of publication, country of origin, study design, sample characteristic and gene assessed.

Results: After the data were extracted, analyzed, and reviewed, the most common gene suspected to be involved in isolated microtia was *Homeobox A2* (*HOXA2*, 12.1%). Conversely, in syndromic microtia, the two most common genes supposed to play a role were *Fibroblast Growth Factor 3* (*FGF3*, 47.2%) and *Treacher–Collins–Franceschetti*

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1. **Walid D. Fakhouri**, University of Texas Health Science Center at Houston, Houston, USA
2. **Isabella Monlleó**, Universidade Federal de Alagoas, Maceió, Brazil
3. **Miriam Erandi Reyna-Fabian**, National Institute of Pediatrics, Mexico City, Mexico

Any reports and responses or comments on the

syndrome 1 (*TCOF1*, 30.2%). From the studies, the three most prevalent genes associated with microtia were *HOXA2* (10%), *FGF3* (8.4%), and *TCOF1* (5.4%). In syndromic microtia, the most common mutation types were deletion in *TCOF1* (46.9%) and missense and deletion in *FGF3* (both 38%), and in isolated microtia, the most common mutation type was silent in *HOXA2* (54.2%).
Conclusions: In summary, genetic factors are involved in microtia; thus, molecular analysis is strongly advised.
PROSPERO registration: CRD42021287294 (25/10/21).

Keywords
Microtia, Genetic Mutation, isolated, syndromic, Genetic Diversity, FGF3, HOXA2, TCOF1

article can be found at the end of the article.

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Corresponding authors: Indri Lakhsmi Putri (indrilakhsmiputri@fk.unair.ac.id), Citrawati Dyah Kencono Wungu (citrawati.dyah@fk.unair.ac.id)

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REVISED **Amendments from Version 2**

We have updated, revised, and rearrange the sentences and references as suggested by reviewers. We have also updated the percentage of genetic involvement and added the limitation of the study.

Any further responses from the reviewers can be found at the end of the article

Introduction

Microtia is a congenital malformation of the outer ears caused by improper embryonic development.[1,2] It is distinguished by small, irregularly shaped external ears, and it can occur unilaterally or bilaterally.[1] The prevalence of microtia varies among ethnic groups (0.83–17.4 per 10,000 births).[2–4] Microtia occurs unilaterally in 80%–90% of cases and bilaterally in 10%–20% of cases.[2,4] Boys are approximately twofold more likely than girls to have microtia, and the right–left bilateral ratio is typically 6:3:1.[2,3] Microtia may occur as an isolated condition, or as part of a spectrum of anomalies or syndrome and approximately 20–60% of children with microtia have associated anomalies or an identifiable syndrome.[2,4] Microtia is easily misdiagnosed during pregnancy.[5,6] If pregnancy ultrasonography suggests microtia, the diagnosis is easily confirmed and diagnosed following birth based on physical examination.[5,6]

The etiology of microtia and the causes of its variations remain unknown although there is compelling evidence that environmental factors such as maternal sociodemographic variables, multiple gestation, diseases (gestational diabetes, cold-like syndrome), and related drug treatments such as isotretinoin use during pregnancy play roles, genetic factors are also believed to influence the embryonic development of microtia.[7–9] Estimates of the prevalence of inherited microtia vary greatly, ranging from 3 to 34%.[10] Although certain studies discovered various candidate genetic disorders for microtia[1,9,11–104] there is no single specific genetic disorder that is certain and always be found in every patient with microtia.

Research on animals with isolated microtia as a prominent feature revealed mutations in *homeobox A2 (HOXA2), sine oculis homeobox (SIX), eyes absent transcriptional coactivator* and *phosphatase (EYA), TBX1, IRF6,* and *CHUK.*[105] The most common genes that identified to be involved in microtia related syndromic were *PLCB4* and *GNAI3* for *auriculo-condylar syndrome*; *TFAP2A* for *branchio-oculo-facial (BOF) syndrome*; *EYA*[106–108] *SIX1* and *SIX5* for *branchio-oto-renal (BOR) syndrome; CHD7 (SEMA3E)* for *CHARGE syndrome*; *FRAS1, FREM2*, and *GRIP1* for *Fraser syndrome*; *MLL2* and *KDM6A* for *Kabuki syndrome*; *GDF6* for *Klippel–Feil syndrome*; *fibroblast growth factor 3 (FGF3)* for *labyrinthine aplasia, microtia* and *microdontia (LAMM) syndrome*; *FGFR2, FGFR3,* and *FGF10* for *lacrimo-auriculo-dento-digital syndrome*; *EFTUD2* for *mandibulofacial dysostosis* with *microcephaly*; *ORC1, ORC4, ORC6, CDT1*, and *CDC6* for Meier–Gorlin syndrome; *HOXA2* for microtia, hearing impairment, and cleft palate; *DHODH* for *Miller syndrome*; *SF3B4* for *Nager syndrome*; *H6 family homeobox 1 transcription factor gene (HMX1)* for *oculo-auricular syndrome (OVAS)*; *SALL1* for *Townes–Brocks syndrome*; and *Treacher–Collins–Franceschetti syndrome 1 (TCOF1), POL1RC, and POL1RDT* for *Treacher–Collins syndrome (TCS).*[2]

FGF3 mutations are commonly found in *LAMM* syndrome.[11,12] The *FGF3* protein regulates a cascade of chemical processes inside cells by binding to its receptor, thereby signaling cells to undergo particular changes, such as proliferating or maturing to perform specialized activities.[109] *TCOF1* mutations can cause TCS in up to 78% of patients.[110] *HOXA2* encodes key developmental transcription factors of the second branchial arch, which contributes significantly to the development of the external and middle ear in embryonic development, and it was previously linked to autosomal recessive bilateral microtia.[10,13]

To determine what genetic factors play a role in microtia, we conducted a first systematic review to identify the most common genes in microtia development qualitatively. Hopefully, this will help improve our understanding of microtia, highlight the importance of genetic screening as a diagnostic technique for preparation of further management.

Methods

Protocol and registration

We have registered our protocol with the International Prospective Register of Systematic Reviews (PROSPERO, CRD42021287294 (25/10/21)). We have also screened PROSPERO for similar systematic reviews. No registered protocol reviewing the genetic factors of microtia was identified. The report of this systematic review was formulated according to the recommendations of the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statement.[111]

Eligibility criteria

We performed an extensive and systematic search of all published studies related to genetic factors implicated in the development or outcome of microtia. Rather than focusing on a single disease, we aimed to provide systematic evidence on all types of microtia, including isolated microtia and syndromic microtia. First, the identified publications were assessed for relevance to the topic of interest using their titles and abstracts. The identified articles were then examined for any duplication using Mendeley. Then, the complete text of all screened papers was reviewed for the inclusion criteria, which were observational studies and case reports/series in the English language that assessed genetic factors in microtia. The exclusion criteria were duplications, reviews, non-English articles, animal studies, and articles in which sufficient details on genetic factors of microtia were not provided.

Search strategy

Three of the four authors (A.S., I.L.P., and R.P.) performed the search and study selection, which was supervised by the fourth author (C.D.K.W). We used six electronic bibliographic databases, PubMed, Web of Science, Science Direct, Proquest, Springerlink, and Clinicaltrials.gov, to conduct systematic searches from 1 to 31 October 2021. We checked Medline (PubMed) to identify controlled vocabulary Medical Subject Headings terms related to genetics and microtia. Searching strategies for PubMed are presented in Supplementary Table 1 (see *Extended data*[112]) and modified for other electronic databases.

Data extraction

Data extraction was conducted independently by three reviewers (A.S., I.L.P., and R.P.) through a standardized form. The methodological quality of studies in this systematic review was assessed using Joanna Briggs Institute (JBI) critical appraisal tools.[113] We extracted data once all of the screening and selection steps had been completed. For both syndromic and isolated microtia, two different extraction forms were produced. The following data were extracted: first author's surname, year of publication, country of origin, study design, sample population, sex, age, type of microtia, analysis method, affected genes, and mutations. Disagreements between the three reviewers were settled by discussion with the fourth reviewer (C.D.K.W.).

Results

All supplementary files can be found in the *Extended data.*[112]

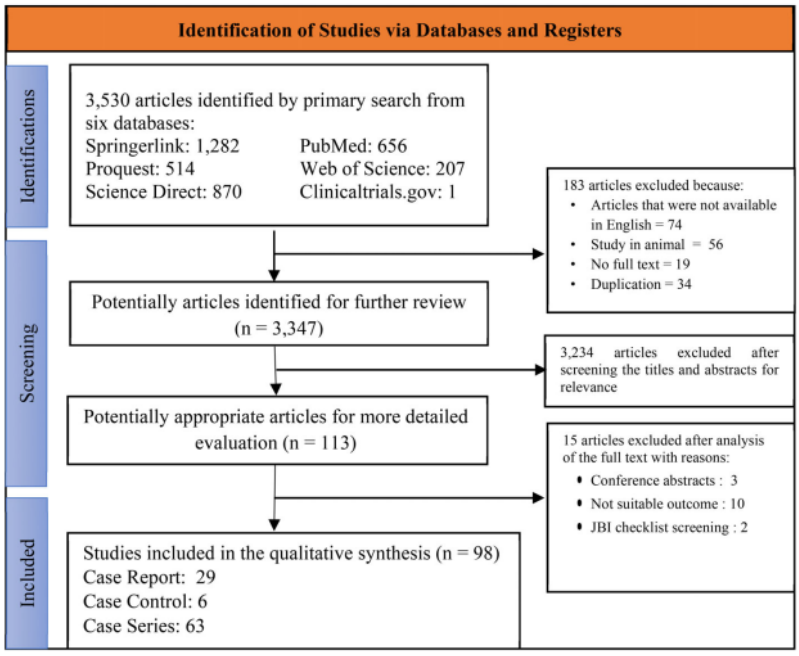

Figure 1. Systematic review flow diagram of included studies.

Table 1. Characteristic of the studies of syndromic microtia.[11,12,15–24,30–40,50–95,98–104,114,115]

No	First author's surname/ country of origin/year of publication	Study design	Family history	Sex	Sample size	Affected ear	Grade	Disorder level	Gene assessed
1	Al Yassin/UK/2019	CS	Yes	5F+M	6	Bilateral: 6	Grade 2: 12	DNA *FGF3*	
2	Alsmadi/Saudi Arabia/2009	CS	Yes	2M+2M	4	Bilateral: 4	Grade 2: 8	DNA *FGF3*	
3	Bacino/USA/1994	CS	No	M+F	2	Bilateral: 2	Grade 2: 2 Grade 3: 2	Chromosome	*Chromosome 22*
4	Balikova/Belgium/2008	CS	Yes	4M+2F	6	Right: 6	Grade 2: 6	DNA	*Gene on Chromosome 4*
5	Bardakjian/USA/2009	CR	-	M	1	Bilateral	Grade 2: 2	DNA *SALL1*	
6	Basdemirci/Turkey/2019	CS	Yes	2F	2	Bilateral: 2	Grade 2: 4	DNA *FGF3*	
7	Boudewys/Belgium/2012	CS	Yes	2M+3F	5	Bilateral: 5	Grade 2: 10	Chromosome	*Chromosome 22*
8	Bragagnolo/Brazil/2016	CR	Yes	F	1	Left	Grade 1	DNA	*Gene on Chromosome 4*
9	Burrage/USA/2015	CS	No	M+2F	3	Bilateral: 3	Grade 1: 6	DNA *GMNN*	
10	Chen/China/2018	CS	No	M+F	2	Left: 2	Grade 3: 2	DNA *MYO5C*	
11	Chen/China/2018	CS	Yes	11M+8F	19	-	Grade 1: 8 Grade 2: 6 Grade 3: 4	DNA *TCOF1*	
12	Conte/Italy/2011	CS	Yes: 3 No: 43	-	46	-	-	DNA *TCOF1*	
13	De Munnik/Canada/2012	CS	-	3M+7F	10	Bilateral: 10	Grade 2: 20	DNA *ORC1, ORC4, ORC6, CDT1, CDC6*	
14	Derbent/Turkey/2003	CS	-	2M	1	Left	Grade 3	Chromosome	*Chromosome 22*
15	Digilio/Italy/2009	CS	No	3M	3	Right: 1 Left: 2	Grade 3: 3	Chromosome	*Chromosome 22*
16	Enomoto/Japan/2021	CR	No	M	1	Right	Grade 3	DNA *POLR1B*	
17	Eser Cavdartepe/ Turkey/2019	CR	No	F	1	Left	Grade 2: 1	DNA *TCOF1*	
18	Fan/China/2019	CS	Yes: 2 No: 1	M+2F	3	-	-	DNA *TCOF1*	
19	Fenwick/China/2016	CS	Yes: 5 No: 8	6M+7F	13	Bilateral: 13	Grade 2: 26	DNA *CDC45*	

Table 1. *Continued*

No	First author's surname/ country of origin/year of publication	Study design	Family history	Sex	Sample size	Affected ear	Grade	Disorder level	Gene assessed
20	Gimelli/Switzerland/2013	CR	Yes	F	1	Left	Grade 3	Chromosome	*Chromosome 14*
21	Gonzalez-Dominiques/ Mexico/2020	CR	No	M	1	Bilateral	Grade 2	DNA *PMM2*	
22	Gripp/USA/2014	CS	No	2M+4F	6	Bilateral: 6	Grade 2: 12	DNA *TSR2, RPS26, RPS28*	
23	Guleray/Turkey/2021	CS	Yes: 10 No: 6	12M+4F	16	Right: 6 Left: 5 Bilateral: 5	-	DNA *EFTUD2*	
24	Han/China/2021	CS	Yes	2M+3F	5	Right: 5	Grade 2: 5	DNA *EYA1*	
25	Hao/China/2016	CS	No	2M+F	3	Bilateral: 3	Grade 2: 4 Grade 3: 2	DNA *TCOF1, HOXA2, GSC*	
26	Huang/China/2013	CR	No	M	1	Left	Grade 4: 1	DNA	*DNA CNV*
27	Irving/UK/2016	CS	-	-	4	-	-	DNA *SF3B4*	
28	Kim/Korea/2015	CR	Yes	F	1	Bilateral	Grade 2: 2	DNA *EYA1*	
29	Kim/Korea/2016	CR	-	F	1	Right	Grade 2: 1	Chromosome	*Chromosome 11q13*
30	Knapp/India/2021	CS	No	2M	2	Bilateral: 2	Grade 1: 4	DNA *CDC45*	
31	Knapp/USA/2020	CS	-	M	1	Right	Grade 2: 1	DNA *DONSON*	
32	Koprulu/Turkey/2020	CR	No	M	1	Bilateral	Grade 1: 2	DNA *GRIP1*	
33	Lamonica/Brazil/2010	CR	-	F	1	Left	Grade 3	DNA *TWIST1*	
34	Lee/Korea/2006	CS	Yes	M+F	2	Bilateral: 2	Grade 1: 4	DNA *EYA1*	
35	Li/China/2021	CR	No	M	1	Bilateral	Grade 3: 2	DNA CDC45	
36	Liberalesso/Brazil/2017	CR	No	M	1	Left	Grade 2	DNA *SALL1*	
37	Lines/Canada/2012	CS	Yes	6M+5F	11	Bilateral	Grade 2: 22	DNA EFTUD2	
38	Liu/China/2020	CR	No	M	1	Bilateral	Grade 3: 2	DNA TCOF1	
39	Liu/China/2021	CR	No	M	1	Bilateral	Grade 2: 2	DNA *TCOF1*	
40	Luquetti/USA/2020	CS	No	2M+F	3	Right: 2 Bilateral	Grade 2: 2 Grade 3: 3	DNA *MYT1*	

Table 1. *Continued*

No	First author's surname/ country of origin/year of publication	Study design	Family history	Sex	Sample size	Affected ear	Grade	Disorder level	Gene assessed
41	Luquetti/USA/2012	CS	No	3M	3	Bilateral: 3	Grade 1: 2 Grade 2: 2 Grade 3: 2	DNA *EFTUD2*	
42	Marchegiani/ Netherland/2015	CS	Yes	7M+11F	18	-	Grade 1: 18	DNA *TWIST2*	
43	Marques/USA/2016	CS	No	-	3	Bilateral	-	DNA *SF3B4*	
44	Marszatek-kruk/Poland/2021	CS	-	-	1	-	-	DNA *TCOF1*	
45	Marszatek-kruk/Poland/2012	CS	Yes	2F	2	Bilateral	Grade 2: 4	DNA *TCOF1*	
46	Marszatek-kruk/Poland/2014	CR	No	M	1	Bilateral	Grade 3: 2	DNA *TCOF1*	
47	Mullegama/USA/2016	CR	No	F	1	Right	Grade 1	DNA *STAG2*	
48	Neuhann/German/2012	CR	No	M	1	Bilateral	Grade 2: 2	DNA *CNV*	
49	Pan/China/2020	CS	Yes	3M+2F	5	Left Bilateral: 4	Grade 2: 4 Grade 3: 5	DNA *TCOF1*	
50	Patil/India/2018	CS	Yes	M+F	2	Bilateral: 2	Grade 1: 1 Grade 3: 3	DNA *PAX1*	
51	Petek/Austria/2000	CR	No	M	1	Right	Grade 3	Chromosome	*Chromosome 4*
52	Ramsebner/Austria/2010	CS	Yes	2M+F	3	Left: 2 Bilateral	Grade 4: 4	DNA *FGF3*	
53	Riazuddin/Pakistan/2011	CS	Yes	17M + 10F	27	Bilateral: 27	Grade 1: 54	DNA FGF3	
54	Royer-Bertrand/ Switzerland/2015	CS	No: 1 Yes: 2	3F	3	Bilateral: 3	Grade 3: 6	DNA *HSPA9*	
55	Sanchez/France/2020	CS	Yes	4F	4	Left Bilateral: 3	-	DNA *POLR1B*	
56	Schlump/German/2012	CS	Yes	2M+F	3	Bilateral: 3	Grade 3: 6	DNA *TCOF1*	
57	Sensi/Italy/2011	CS	Yes	M+2F	3	Bilateral: 3	Grade 2: 6	DNA *FGF3*	
58	Smigiel/Poland/2014	CS	No	2F	2	Right: 1 Left: 1	Grade 2: 2	DNA *EFTUD2*	
59	Stevenson/USA/2007	CR	No	F	1	Bilateral	Grade 2: 2	DNA *HOXD*	
60	Su/Taiwan/2005	CR	No	M	1	Bilateral	Grade 2: 2	DNA *TCOF1*	

Table 1. *Continued*

No	First author's surname/ country of origin/year of publication	Study design	Family history	Sex	Sample size	Affected ear	Grade	Disorder level	Gene assessed
61	Su/Taiwan/2007	CS	No	2M+3F	5	Right: 1 Left: 3 Bilateral: 1	Grade 3: 3 Not Mentioned: 3	DNA *TCOF1*	
62	Tassano/Switzerland/2015	CR	Yes	F	1	Left	Grade 3	DNA *FOXI3*	
63	Tekin/Turkey/2008	CS	Yes	10M+3F	13	Bilateral: 4 Not Mentioned: 9	Grade 1: 8 Not Mention: 9	DNA *FGF3*	
64	Thiel/German/2005	CS	Yes	3M	3	Right: 1 Bilateral: 2	Grade 3: 5	DNA *TCOF1*	
65	Timberlake/USA/2021	CS	Yes	M+2F	3	Bilateral: 3	Grade 1: 6	DNA *SF3B2*	
66	Tingaud/France/2020	CR	No	M	1	Left	Grade 3	DNA *ZYG11B*	
67	Tingaud/France/2021	CS	Yes	2M	2	Bilateral	Grade 3: 3 Grade 4: 1	DNA *EYA3*	
68	Urel-Demir/Turkey/2021	CS	Yes	M+F	2	Bilateral	Grade 1: 4	DNA *UBE3B*	
69	Valdes/Mexico/2014	CR	No	F	1	Bilateral	Grade 1: 2	Chromosome	*Chromosome 113q*
70	Venditti/USA/2004	CR	No	M	1	Bilateral	Grade 3: 2	Chromosome	*Chromosome 20*
71	Vetro/Italy/2017	CR	No	M	1	Bilateral	Grade 1: 2	DNA *MCM5*	
72	Voigt/German/2013	CS	Yes: 2 No:5	4M+3F	7	Bilateral: 7	Grade 2: 14	DNA *EFTUD2*	
73	Wang/China/2018	CS	Yes	2F	2	Bilateral	Grade 2: 4	DNA *EYA1*	
74	Weaver/USA/2015	CS	Yes	2M	2	Left Bilateral: 1	Grade 2: 1 Grade 4: 2	DNA *POLR1A*	
75	Xing/China/2020	CS	Yes	2M+F	3	Right Bilateral: 2	-	DNA *EYA1*	
76	Zarate/USA/2012	CR	No	F	1	Right	Grade 3: 1	DNA *MLL2*	Case Report
77	Zeng/China/2021	CR	No	M	1	Bilateral	Grade 3: 2	DNA *TCOF1*	Case Report
78	Zhang/China/2020	CS	No	2M+4F	6	Bilateral: 6	Grade 2: 12	DNA *TCOF1*	Case Series
79	Zhang/China/2013	CS	Yes	3M+2F	5	Not Mentioned	Not Mentioned	DNA *TCOF1*	Case Series

Abbreviations: CNV, copy number variation; CS, case series; CR, case report; F, female; M, male; FGF3, fibroblast growth factor 3; FOXI3, forkhead box I3; PAX1, paired box 1; TCOF, Treacher–Collins syndrome; EFTUD, Elongation Factor Tu GTP Binding Domain Containing; HOXD, Homeobox D Cluster; MYT1, Myelin Transcription Factor; TWITST, Twist-related protein; STAG, Stromal Antigen; CNV, copy number variation; PMM, phosphomannomutase; SALL, Spalt Like Transcription Factor; GMNN, Geminin DNA Replication Inhibitor; MYO5C, Myosin VC; EYA, eyes absent transcriptional coactivator and phosphatase; SF3B2, splicing factor 3b subunit 2; SF3B4, Splicing factor 3B subunit 4; GSC, Goosecoid Homeobox; DONSON, DNA Replication Fork Stabilization Factor DONSON; GRIP1, Glutamate Receptor Interacting Protein 1; HSPA9, Heat Shock Protein Family A Member 9; POLR1A, RNA Polymerase I Subunit A; POLR1B, RNA Polymerase I Subunit B; RPS26, Ribosomal Protein S26; RPS28, Ribosomal Protein S28; TSR2, TSR2 Ribosome Maturation Factor; UBE3B, Ubiquitin Protein Ligase E3B; ZYG11B, Zyg-11 Family Member B; CDC6, Cell Division Cycle 6; CDC45, Cell Division Cycle 45; MCM5, Minichromosome Maintenance Complex Component 5; CDT1, Chromatin licensing and DNA replication factor 6; ORC1, Origin recognition complex subunit 1; ORC4, Origin recognition complex subunit 4; ORC6, Origin recognition complex subunit 6.

Systematic review outline

We discovered 3,530 articles after searching six electronic bibliographic databases. In total, 183 publications were removed because they were not available in English, they were animal studies, their full text was not available, or they were duplicated studies. Only 113 articles were eligible for more extensive evaluation after title and abstract screening. Only 98 papers were included in this study after a comprehensive full text analysis (Figure 1). The included studies were reviewed, utilizing a checklist questions form provided by JBI based on the studies' methodology. Based on the JBI Tools for case reports, case series, and case controls, all publications involved were assessed as low-risk bias (Supplementary Tables 2–4).

Study characteristics

Following the screening, selection, and data extraction of all included studies, we discovered 79 articles of syndromic microtia involving 338 subjects (Table 1 and Supplementary Table 5) and 19 articles of isolated microtia involving 585 subjects (Table 2 and Supplementary Table 6). China had the most cases of syndromic microtia (58 subjects [17.1%] in 15 studies), followed by Italy (53 subjects [15.6%] in four studies); Turkey (36 subjects [10.6%] in seven studies); USA (31 subjects [9.2%] in 14 studies); Pakistan (27 subjects [8%] in one study); UK (23 subjects [6.8%] in three studies); Canada (21 subjects [6.2%] in two studies); Netherland (18 subjects [5.3%] in one study); German (14 subjects [4.1%] in four studies); Belgium (11 subjects [3.2%] in two studies); France (7 subjects [2%] in three studies); Taiwan and Poland (6 subjects [1.7%] in four studies each); Switzerland (5 subjects [1.4%] in three studies); Arab (4 subjects [1.2%] in one study) and India, Austria, Korea (4 subjects [1.2%] in two studies each]. China had the most cases of isolated microtia (520 subjects [88.8%] in 11 studies), followed by Turkey (38 subjects [6.5%] in two studies), the USA (21 subjects [3.5%] in four studies), Iran and Italy (3 subjects [0.5%] in one study each). China had the most cases of microtia among all investigations, being the site of 578 of 923 cases (62.6%). Concerning the study design, there were 29 case reports, 63 case series, and 6 case control studies included in this analysis.

Regarding studies of syndromic microtia (Table 1), the family history was known for 299 of 338 subjects (88.5%), whereas the family history was not discussed in 39 subjects (11.5%). Of the 299 subjects, 190 (63.5%) had family histories of microtia. We also discovered that, of the 338 subjects, the gender was known for 284 subjects (84%), which included 156 males (54.9%). The severity of microtia was known for 403 of the 514 ears (78.4%) involved in this analysis. Of the 403 ears, 123 (30.5%) had grade I microtia, 202 (50.1%) had grade II microtia, 70 (17.3%) had grade III microtia, and 8 (1.9%) had grade IV microtia. The type of microtia was known in 233 of 338 subjects (68.9%); of these subjects, 31 (13.3%), 26 (11.1%), and 176 (75.5%) had right unilateral, left unilateral, and bilateral microtia, respectively. Based on the gene disorder levels in 79 studies including 338 subjects, 322 subjects (95.3%) in 70 studies had DNA-level disorders, and 16 subject (4.7 %) in 9 study had a chromosomal disorder. We discovered that of the 338 subjects, 107 subjects (31.6%) in 18 studies had *TCOF1* gene mutations, whereas 55 subjects (16.7%) in 7 studies had *FGF3* gene mutations.

According to studies of isolated microtia (Table 2), 573 of 585 subjects (97.9%) had a family history of microtia. The genders of the 585 subjects were known for 445 subjects (76%), which included 263 males (59.1%). The severity of microtia was known for 171 ears of the 639 ears (26.7%) involved in this analysis, among whom 11 (6.4%), 72 (42.1%), 88 (51.5%) had grades I, I, and III microtia, respectively. The type of microtia was specified for 87 of 585 subjects (14.8%), being right unilateral, left unilateral, and bilateral in 21 (24.1%), 12 (13.8%), and 54 subjects (62.1%), respectively. Regarding the genetic disorder level, of 585 subjects in 19 studies, 584 subjects (99.8%) in 18 articles had DNA-level disorders, and one subject (0.2%) had a chromosomal disorder. We discovered that 140 subjects (23.9%) in seven studies had mutations in the *HOXA2* gene.

We discovered that of 923 subjects, the family history was known in 872 subjects (94.5%). In total, 463 of the 872 subjects (51.9%) had family histories of microtia. The genders of 729 of the 923 total subjects (78.9%) were known. Among the 729 subjects, 419 (57.4%) were male, and 310 (42.5%) were female. The severity of microtia was known in 574 of the 1.153 ears (49.7%) analyzed in this study. Among these ears, 134 (23.34%) had grade I microtia, 274 (47.7%) had grade II microtia, 158 (27.5%) had grade III microtia, and 8 (1.4%) had grade IV microtia. Based on the type of microtia, the type of microtia was known for 320 of 923 subjects (34.6%). Among these 320 subjects, 52 (16.25%) had right unilateral microtia, 38 (11.8%) had left unilateral microtia, and 230 (71.8%) had bilateral microtia. Based on the gene disorder levels described in all 98 studies, 909 subjects (98.5%) in 89 studies had DNA-level disorders, whereas 14 subjects (1.5%) in 9 studies had chromosomal disorders. We discovered that *TCOF1* was the most common gene involved in syndromic microtia (66 subjects [19.52%]), followed by *FGF3* (51 subjects [15.1%)], and *HOXA2* was the most common gene involved in isolated microtia (22 subjects [40.2%]).

Based on the type of mutation (Table 3), we discovered that in syndromic microtia, the most common type of mutation in *TCOF1* was deletion, being detected in 26 of 66 subjects (39.4%). In *FGF3*, the most common mutation types were

Table 2. Characteristics of the studies of isolated microtia.[1,9,13,14,25–29,41–48,96,97]

No	First author's surname/ country of origin/year of publication	Study design	Family history	Sex	Sample size	Affected ear	Grade	Disorder level	Gene assessed
1	Alasti/Iran/2008	CS	Yes	3F	3	Bilateral	Grade 3: 6	DNA *HOXA2*	
2	Brown/USA/2013	CS	Yes	3M+2F	5	Left: 3 Bilateral: 2	Grade 2: 7	DNA *HOXA2*	
3	Buyukgural/Turkey/2016	CC	No	11M+8F	19	-	-	DNA *eNOS*	
4	Fan/China/2020	CS	No	3M+3F	6	Right: 4 Left: 2	Grade 3: 6	DNA *HOXA4*	
5	Guo/China/2020	CS	Yes	M+2F	3	Right: 3	Grade 3: 3	DNA *CYP26A1*	
6	Hao/China/2017	CC	No	73M+33F	106	-	-	DNA *GSC, HOXA2, PKPR*	
7	Li/China/2014	CS	Yes	3M+7F	10	Bilateral: 10	Grade 2: 20	DNA *CNV*	
8	Meddaugh/USA/2020	CS	Yes	2M+F	3	Bilateral: 3	Grade 2: 6	DNA *HOXA2*	
9	Monks/USA/2010	CC	-	7M+5F	12	Right: 8 Left: 3 Bilateral	Grade 1: 7 Grade 2: 3 Grade 3: 3	DNA *HOXA2, SIX2*	
10	Nursal/Turkey/2017	CC	No	-	19	-	-	DNA *IL-6, IL-10, IFN-γ, TGF-β1, TNF-α*	
11	Piceci/Italy/2017	CS	Yes	2M+F	3	Bilateral: 3	Grade 1: 4 Grade 2: 2	DNA *HOXA2*	
12	Si/China/2020	CS	Yes	3M+2F	5	Bilateral: 5	Grade 2: 10	DNA *HOXA2*	
13	Si/China/2020	CS	Yes	6M+6F	12	Bilateral: 12	Grade 2: 24	DNA *HMX1-ECR CNV*	
14	Wang/China/2019	CS	No	28M+12F	40	-	Grade 3: 40	DNA	131 genes associated with external/middle or inner ear deformity
15	Yang/China/2021	CS	No	6M+4F	10	Right: 6 Left: 4	-	DNA *CYP26A1*	
16	Yu/USA/2005	CR	No	F	1	Bilateral	Grade 3: 2	Chromosome	*Chromosome 6*
17	Zhang/China/2009	CC	Yes: 12 No: 109	-	121	-	-	DNA *GSC, BMP5s*	
18	Zhang/China/2010	CS	Yes	10M+4F	14	Bilateral: 14	Grade 3: 28	DNA *GSC, FGF3*	
19	Zhao/China/2019	CC	Yes	105M+88F	193	-	-	DNA *FGFR2*	

Abbreviations: CC, case control; CS, case series; CR, case report; M, male; F, female; CNV, copy number variation; CYP, cytochrome p450; eNOS, endothelial nitric oxide synthase; FGF3, fibroblast growth factor 3; GSC, goosecoid homeobox; HOXA, homeobox A; HMX, H6 family homeobox; IFN, interferon; IL, interleukin; PRKR, protein kinase R; SIX2, sine oculis homeobox 2; TGF, transforming growth factor; TNF, tumor necrosis factor; BMP5s, Bone morphogenetic proteins 5s; FGFR2, Fibroblast Growth Factor Receptor 2.

Table 3. Types of mutations.

Manifestation	Mutated gene	Type of mutation (%)
Syndromic microtia	*TCOF1*(N = 66)	Deletion: 26 (39.4%) Microdeletion: 10 (15.1%) Nonsense: 11 (16.6%) Insertion: 8 (12.1%) Missense: 6 (9.1%) Complex: 2 (3%) Splicing: 2 (3%)
	FGF3(N = 51)	Deletion: 7 (13.7%) Missense: 28 (54.9%) Nonsense: 16 (31.3%)
Isolated microtia	*HOXA2*(N = 22)	Missense: 3 (13.6%) Nonsense: 16 (72.7%) UTR: 3 (13.6%)

Abbreviation: UTR, untranslated region.

missense, being present in 28 of 51 subjects (54.9%) each, and in isolated microtia, the most common mutation type in *HOXA2* was nonsense mutation, being present in 16 of 22 subjects (72.7%).

Discussion

Microtia is a congenital external ear deformity that can range in severity from minor anatomical problems to full ears absence (anotia). Microtia can be a single birth abnormality or part of a broader set of defects or syndrome.[8] This systematic review attempted to describe the genes that play important roles in the development of syndromic and non-syndromic microtia. Only 98 studies on genetically linked microtia met our selection criteria. In this study, China had the highest number of microtia cases. The reported prevalence of microtia/anotia varies between 1 in 3000 to 1 in 20 000 births.[1,2] The prevalence might vary by country and race/ethnicity but this is likely dependent on what forms of microtia are included in studies.[116,117]

We discovered that most patients with microtia had a family history of the disease, including 97.7% of patients with isolated microtia and 63.5% of patients with syndromic microtia. This is consistent with the existing literature, which describes Mendelian hereditary variants of microtia with an *autosomal* dominant or recessive mode of inheritance.[10] The rates of familial microtia ranged from 3 to 34%.[10,118]

Based on the gender classification of each study, we discovered nearly 60% of all patients were male, including 54.9% of patients with syndromic microtia and 59.1% of patients with isolated microtia. In prior research, microtia was more common in male patients than in female patients, with a sex ratio of 1.5:1 and an estimated 20–40% greater risk in males than in females.[1,4]

Although microtia can arise bilaterally, 77–93% of patients have unilateral involvement.[4] The most common form of syndromic microtia is bilateral microtia.[1] Although bilateral microtia was more common in this study, the type of microtia was only known for 34.6% of subjects. Because of the inadequate data, this could represent a biased outcome for the most prevalent type of microtia.

Most published research on microtia reported the existence or absence of microtia and/or anotia with no further details on severity. Marx (1926), Weerda (1988), Roger (1977), Tanzer (1978), and Hunter (2009) all provided classifications of microtia.[5] Their classification system was nearly identical, consisting of four grading levels.[4,119] Most of the listed paper used in our study had same definition to grade microtia, Hunter classification. To homogenize the definition to grade microtia. In our study we use Hunter classification system for microtia when extracting data from 98 studies and data provided data in Tables 1 and 2. The most prevalent severity of microtia in our study was grade II, accounting for 47.7% of all cases, owing to the higher number of syndromic microtia cases than isolated microtia cases in our study that are commonly with a grade II microtia presentation.[1,4]

From all examined studies involved, the most common genes associated with microtia were *HOXA2, FGF3*, and*TCOF1*. We discovered that in syndromic microtia, the most common types of mutation were *TCOF1* deletion (39.4%), and nonsense in *FGF3* (54.9%), whereas in isolated microtia, the most common type of mutation in *HOXA2* was nonsense

mutation (72.7%). This was consistent with the literature, which indicated that the most common genes involved in microtia were *HOXA2, FGF3, HOXD, ORC1, ORC4, ORC6, CDT1, CDC6, DHODH, HMX1, EYA1,* and. *TCOF1.*[105]

FGF3 encodes a protein member of the FGF family.[109] The FGF family has extensive mitogenic and cell survival activities and is involved in various biological processes such as embryonic development, morphogenesis, tissue repair, cell growth and inner ear formation in mice and chicken.[109] *FGF3* activates a cascade of chemical reactions inside cells that activate certain changes, such as dividing or maturing to take on specialized functions, by attaching to another protein known as a receptor.[109] *FGF3* haploinsufficiency could also be related with dental and hearing problems. *FGF* signaling is required for the appropriate development of the otic placode, a thickening of the ectoderm on the outer surface of a developing embryo from which the ear develops.[120] *FGF3* mutation is often found in syndromic microtia, mainly in *LAMM* syndrome. Congenital deafness with *LAMM* syndrome is an autosomal recessive disorder characterized by significant bilateral congenital sensorineural deafness coupled with inner ear defects, grade I bilateral microtia, and microdontia (small teeth) as its major phenotypic features.[10,15] The finding of biallelic pathogenic mutations in *FGF3* on molecular genetic testing confirms the diagnosis of *LAMM* syndrome in the proband.[11,12,16,17]

The *TCOF1* gene, which encodes a suspected nucleolar phosphoprotein known as treacle, has been identified as the cause of TCS,[10,121] an autosomal dominant craniofacial development disorder, in up to 78% of patients.[18,110,122] Inhibition of mature RNA ribosomal (rRNA) production and gene transcription in neural folds prefusion during the early stage of embryogenesis may cause abnormal development due to treacle haploinsufficiency, caused by mutation in the *TCOF1* gene, thus affecting proliferation and proper differentiation of these embryonic cells.[123] To date, more than 50 mutations have been identified in the *TCOF1* gene, most of which are insertions or deletions. TCS is characterized by cleft palate, hypoplasia of facial bones (particularly the mandible and zygomatic complex), downward slanting of palpebral fissures with colobomas of lower eyelids, external ear deformity, conductive hearing loss, and defects in brain development such as microcephaly and mental retardation.[19–24] *TCOF1* was the most prevalent gene found in this systematic review. This was because *TCOF1* is a causal gene for TCS, which features microtia as one of its clinical symptoms.[20] In addition to clinical findings, TCS diagnosis is also confirmed by detection of pathogenic variants of *TCOF1, POLR1D, or POLR1B* using molecular genetic testing, mainly inherited in an autosomal dominant pattern.[124] In accordance with our result, *TCOF1* gene deletions were reported to range from a single exon to a whole gene. Despite the fact that >97% of reported cases contained a pathogenic mutation identifiable by sequencing, Bowman *et al.* (2012) reported 5% of cases (5/92) with a big deletion, suggesting that the rate of large deletions may be higher than current data suggest.[110]

In Vertebrate, Hox genes are a subset of homeobox genes of which there are four cluster groups (A–D).[1] Depending on the animal, there are four to 48 per genome of Hox genes.[1] In human, there are 39 homeobox genes of the HOX family at four loci, HOXA, HOXB, HOXC, and HOXD on chromosomes 7, 17, 12, and 2, respectively.[1] All Hox genes encode proteins that share a 60 amino acid domain called the homeodomain.[1] In a variety of organisms, this structural motif is found in many different transcription regulators for a wide range of genes and plays important roles in cell differentiation.[1] Homeobox genes mutation during development result in the transformation of different parts of the body.[1] Homeodomain is a helix-turn-helix motif which is consist of three alpha-helices and an N-terminal arm.[1] Site-specific DNA binding is achieved by interaction of the third helix with the major DNA groove.[1] The N-terminal arm residues normally mediate contacts with the minor groove of DNA.[1] Different pathogenic changes in the sequence of a homeodomain can affect stability and/or DNA-binding activity.[1]

Homeobox genes participate in the formation of the pharyngeal arches.[4,25] They encode transcription factors that determine cell positional identity and morphogenesis during development, as well as switch on cascades of other genes that shared a 180 bp segment of DNA.[1,4,25,125,126] The *Hox* gene family was discovered to be grouped inside the genome and ordered on the chromosome in the order of expression during development. This ordered pattern of gene expression could be part of a mechanism that generates morphogenetic specification.[4,25] HoxA2, a member of the HOXA cluster, encodes a protein with a molecular weight of 41kD and is important in the regulation of development and morphogenesis in patterning the antero-posterior axis of the embryo of almost all metazoans such as in patterning and morphogenesis of the neural-crest-derived mesenchyme.

HOXA2 was discovered to be highly expressed in the second brachial arches (BA2), to express critical developmental transcription factors BA2, to play an important role in the development of the external and middle ear during embryonic development, and to be associated with autosomal recessive bilateral microtia as a member of the *HOX* gene family.[10,13,25] In humans, abnormal or lost *HOXA2* function, as well as early and late *HOXA2* inactivation, results in auditory system malformations, primarily in the external and middle ear, such as a duplicated or absent auricle[10,25] Consequently, *HOXA2* has been proposed as a key transcriptional regulator of auricle morphogenesis.[125] Individuals with a homozygous *HOXA2* mutation have far more severe clinical symptoms than those with a heterozygous mutation. In a mouse model,

inactivation of *Hox2* early in development results in the absence of the pinna, whereas late inactivation results in a hypomorphic auricle.[127]

From the study of mice, we know that the HoxA2 protein is important for the development of the auditory system, mainly the outer and middle ear.[1] The embryological origin of the inner ear is different than that of the middle ear and outer ear, which share a common origin.[1] The inner ear is derived from an epidermal otic placode at the level of the hindbrain, whereas the middle and outer ears originate from the mesenchyme at the first and second pharyngeal or branchial arches.[1] The formation of many craniofacial tissues is influenced by Hox genes.[1] Hoxa2 is also a key gene for the facial somatosensory map.[1] A significant percentage of microtic patients also present deficient facial components that originate from the same embryological structures.[1]

Aside from *HOXA2, FGF3,* and *TCOF1*, other genes linked to microtia include *HMX1*, *POL1RC*, *POLR1D*, *GSC*, *SIX1*, *EYA1*, *SALL1*, *EFTUD2*, *SF384*, *FGFR2*, *GRIP1*.[105] Among these, *HMX1* located on chromosome 4p16.1 is prominent. It is involved in the differentiation of the lateral facial mesenchyme downstream of embryonic patterning genes.[9,128] In humans, duplication in the intergenic region downstream of HMX1 have been linked to *OVAS*, which is characterized by external ear and eye deformity.[9] In a five-generation Chinese family with isolated bilateral microtia, a 10-Mb linkage locus covering 4p16 was discovered.[9,26,128]

We hypothesized that a link existed between the types of genes involved and the grade of microtia. For example, *FGF3* deletions in *LAMM* syndrome have been clinically identified as grade I microtia.[11,12,16,17] According to the MARX classification,[10] the *HOXA2* gene was common in the form of microtia type II and was exclusive to isolated microtia,[1,13,14,26–29,125,127,128] and no specific type of microtia has been linked to the deletion of *TCOF1*.[10,29] However this requires further studies to confirm these data.

The field of genetic variables in microtia research is still relatively extensive. More research on genetic variables that contribute to microtia is required, particularly using the next generation sequencing (NGS) and DNA microarray approach. NGS, massively parallel sequencing, or deep sequencing are all terms that refer to DNA sequencing technology that has transformed genomic research.[29,129] In comparison to other technologies, NGS can sequence the entire human genome in a single day. Genomes may be examined without prejudice, allowing mosaic mutations to be detected.[28] On the other hand, DNA microarray is a revolutionary technique for gene expression profiling.[130] Microarrays were created as a method for mapping and sequencing vast amounts of DNA. DNA microarrays offer a far higher throughput and are less time consuming than previous approaches. By applying NGS and microarray for screening of genetic risk factors in microtia, the diagnosis of microtia could be made earlier and the patients could get a more comprehensive treatment.

Strengths and limitations of the study

This systematic review used recent available evidence and is the first systematic review to describe genetic factors in microtia. All studies included in this review were assessed as being of high quality. However, the limitations of this study included the heterogeneity of studies on the genetic evaluation of microtia in online databases, as well as the absence of information on the details of the subjects; thus, we could not perform a meta-analysis in this study. Gender information was unknown in 21% of patients, severity was unknown in 50.2% of subjects, and the type of microtia was unclear in 65.3% of patients. Case reports and case series were the most common study types in this systematic review. More observational research on genetic microtia is required to perform a more comprehensive systematic review and even meta-analysis. By applying NGS and microarrays to screen for genetic risk factors in microtia, it is possible to diagnose microtia earlier whether it belongs to isolated microtia or syndromic microtia. So that experts can provide education to parents regarding the diagnosis of microtia and further management plans that can be taken to treat these patients can be more thorough because they have been well prepared beforehand.

Conclusions

According to this study, most cases of microtia (62.6%) occurred in China, 51.9% of subjects had a family history of microtia, 57.4% of cases occurred in males, 71.8% of cases were bilateral, and 47.7% of cases were grade IImicrotia. From the studies involved, the three most common genes associated with the development of microtia were *HOXA2* (40.2%), *FGF3* (15.1%), and *TCOF1* (19.52%). The most prevalent syndromes related to microtia were TCS and *LAMM* syndrome. Deletion mutations in *TCOF1* were found in 26 patients (39.4%), missense were present in *FGF3* in 28 patients (54.9%), and nonsense were present in *HOXA2* in 16 patients (72.7%).

More research on genetic variables in microtia is required, particularly the use of NGS, massively parallel sequencing, or deep sequencing. We recommend that investigations on genetically associated microtia be conducted using observational studies, and the features of patients involved should be described more clearly and comprehensively in the future for better systematic reviews or even meta-analysis.

By understanding the three most dominant genes associated with microtia *(HOXA2, FGF3,* and *TCOF1)*, we could promote the early screening and detection of microtia in the next generation, allowing us to provide better education and genetic counseling to patients with microtia regarding the possibility of microtia development in their children, and we hope that this systematic review will serve as a reference for the establishment of a global database of patients with microtia.

Data availability

Underlying data

All data underlying the results are available as part of the article and no additional source data are required.

Extended data

Harvard Dataverse: The Role of Genetic Factors in Microtia: A Systematic Review. https://doi.org/10.7910/DVN/4RRHH0.[112]

This project contains the following extended data:

- Supplementary Files.docx (Table 1. Medline (Pubmed) search strategy to identify published literature, Tables 2-4 Risk of bias evaluation of studies involved in this systematic review using JBI Checklist, Tables 5-6 additional characteristics of studies involved in this systematic review)
- Table Manuscript.docx (Tables 1-2 main characteristics of studies involved in this systematic review, Table 3 type of mutation found on analysis)

Reporting guidelines

Harvard Dataverse: PRISMA checklist and flowchart for 'The role of genetic factors in microtia: A systematic review'. https://doi.org/10.7910/DVN/4RRHH0.[112]

References

1. Alasti F, Sadeghi A, Sanati MH, *et al.*: **A Mutation in HOXA2 Is Responsible for Autosomal-Recessive Microtia in an Iranian Family.** *Am. J. Hum. Genet.* 2008; **82**(4): 982–991.
PubMed Abstract | Publisher Full Text
2. Luquetti DV, Leoncini E, Mastroiacovo P: **Microtia-Anotia: A Global Review of Prevalence Rates.** *Birth Defects Res. A Clin. Mol. Teratol.* 2011; **91**(9): 813–822.
PubMed Abstract | Publisher Full Text
3. Klockars T, Rautio J: **Embryology and epidemiology of microtia.** *Facial Plast. Surg.* 2009; **25**(3): 145–148.
Publisher Full Text
4. Luquetti DV, Heike CL, Hing AV, *et al.*: **Microtia: Epidemiology & Genetics.** *Am. J. Med. Genet. A.* 2012; **158A**(1): 124.
Publisher Full Text
5. Calzolari F, Garani G, Sensi A, *et al.*: **Clinical and radiological evaluation in children with microtia.** *Br. J. Audiol.* 1999; **33**(5): 303–312.
PubMed Abstract | Publisher Full Text
6. Wei J, Ran S, Yang Z, *et al.*: **Prenatal ultrasound screening for external ear abnormality in the fetuses.** *Biomed. Res. Int.* 2014; **2014**: 1–5.
PubMed Abstract | Publisher Full Text
7. Paput L, Czeizel AE, Bánhidy F: **Possible multifactorial etiology of isolated microtia/anotia—A population-based study.** *Int. J. Pediatr. Otorhinolaryngol.* 2012; **76**(3): 374–378.
PubMed Abstract | Publisher Full Text
8. Tripathee S, Zhang J, Xiong M: **Risk factors of microtia: A systematic review and meta-analysis.** *Eur. J. Plast. Surg.* 2016; **39**(5): 335–344.
Publisher Full Text
9. Si N, Si N, Meng X, *et al.*: **Duplications involving the long range HMX1 enhancer are associated with human isolated bilateral concha-type microtia.** *J. Transl. Med.* 2020; **18**(1): 244.
PubMed Abstract | Publisher Full Text
10. Alasti F, van Camp G: **Genetics of Microtia and Associated Syndromes.** *J. Med. Genet.* 2009; **46**(6): 361–369.
Publisher Full Text
11. Riazuddin SS, Ahmed ZM, Hegde RS, *et al.*: **Variable expressivity of FGF3 mutations associated with deafness and LAMM syndrome.** *BMC Med. Genet.* 2011; **12**.
PubMed Abstract | Publisher Full Text
12. Sensi A, Ceruti S, Trevisi P, *et al.*: **LAMM syndrome with middle ear dysplasia associated with compound heterozygosity for FGF3 mutations.** *Am. J. Med. Genet. A.* 2011; **155**(5): 1096–1101.
PubMed Abstract | Publisher Full Text
13. Brown KK, Viana LM, Helwig CC, *et al.*: **HOXA2 haploinsufficiency in dominant bilateral microtia and hearing loss.** *Hum. Mutat.* 2013; **34**(10): 1347–1351.
Publisher Full Text
14. Piceci F, Morlino S, Castori M, *et al.*: **Identification of a second HOXA2 nonsense mutation in a family with autosomal dominant non-syndromic microtia and distinctive ear morphology.** *Clin. Genet.* 2017; **91**(5): 774–779.
Publisher Full Text
15. Al Yassin A, D'arco F, Morín M, *et al.*: **Three New Mutations and Mild, Asymmetrical Phenotype in the Highly Distinctive LAMM Syndrome: A Report of Eight Further Cases.** *Genes (Basel).* 2019; **10**(7).
PubMed Abstract | Publisher Full Text
16. Ramsebner R, Ludwig M, Parzefall T, *et al.*: **A FGF3 mutation associated with differential inner ear malformation, microtia, and microdontia.** *Laryngoscope.* 2010; **120**(2): 359–364.
PubMed Abstract | Publisher Full Text

17. Tekin M, Öztürkmen Akay H, Fitoz S, *et al.*: **Homozygous FGF3 mutations result in congenital deafness with inner ear agenesis, microtia, and microdontia.** *Clin. Genet.* 2008; **73**(6): 554–565.
Publisher Full Text

18. Liu J, Dong J, Li P, *et al.*: **De novo TCOF1 mutation in Treacher Collins syndrome.** *Int. J. Pediatr. Otorhinolaryngol.* 2021; **147**: 110765.
PubMed Abstract | Publisher Full Text

19. Marszałek-Kruk BA, Wójcicki P: **Identification of three novel TCOF1 mutations in patients with Treacher Collins Syndrome.** *Hum. Genome Var.* 2021; **8**(1): 36.
PubMed Abstract | Publisher Full Text

20. Chen Y, Guo L, Long LC, *et al.*: **Mutation screening of Chinese Treacher Collins syndrome patients identified novel TCOF1 mutations.** *Mol. Gen. Genomics.* 2018; **293**(2): 569–577.
PubMed Abstract | Publisher Full Text

21. Zhang X, Fan Y, Zhang Y, *et al.*: **A novel mutation in the TCOF1 gene found in two Chinese cases of treacher collins syndrome.** *Int. J. Pediatr. Otorhinolaryngol.* 2013; **77**(9): 1410–1415.
PubMed Abstract | Publisher Full Text

22. Marszałłek-Kruk BA, Smigiel R, Sąsiadek MM: **Novel mutation in the TCOF1 gene in a patient with Treacher Collins syndrome.** *Pediatr. Pol.* 2014; **89**(6): 462–465.
Publisher Full Text

23. Marszałek-Kruk BA, Wójcicki P, Śmigiel R, *et al.*: **Novel insertion in exon 5 of the TCOF1 gene in twin sisters with Treacher Collins syndrome.** *J. Appl. Genet.* 2012; **53**(3): 279–282.
PubMed Abstract | Publisher Full Text

24. Liu J, Lin P, Pang J, *et al.*: **Identification of a novel gross deletion of TCOF1 in a Chinese prenatal case with Treacher Collins syndrome.** *Mol. Genet. Genomic Med.* 2020; **8**(8): 1–8.
PubMed Abstract | Publisher Full Text

25. Shi N, Meng X, Lu X, *et al.*: **Identification of loss-of-function HOXA2 mutations in Chinese families with dominant bilateral microtia.** *Gene.* 2020; **757**: 144945.
PubMed Abstract | Publisher Full Text

26. Li X, Hu J, Zhang J, *et al.*: **Genome-wide linkage study suggests a susceptibility locus for isolated bilateral microtia on 4p15.32-4p16.2.** *PLoS One.* 2014; **9**(7).
PubMed Abstract | Publisher Full Text

27. Hao S, Jin L, Li C, *et al.*: **Mutational analysis of GSC, HOXA2 and PRKRA in 106 Chinese patients with microtia.** *Int. J. Pediatr. Otorhinolaryngol.* 2017; **93**: 78–82.
PubMed Abstract | Publisher Full Text

28. Monks DC, Jahangir A, Shanske AL, *et al.*: **Mutational analysis of HOXA2 and SIX2 in a Bronx population with isolated microtia.** *Int. J. Pediatr. Otorhinolaryngol.* 2010; **74**(8): 878–882.
PubMed Abstract | Publisher Full Text

29. Meddaugh HR, Zambrano RM: **Novel HOXA2 variant presenting with microtia and variable hearing impairment in four-generation pedigree.** *Clin. Dysmorphol.* 2020; **29**: 104–106.
Publisher Full Text

30. Liberalesso PBN, Cordeiro ML, Karuta SCV, *et al.*: **Phenotypic and genotypic aspects of Townes-Brock syndrome: Case report of patient in southern Brazil with a new SALL1 hotspot region nonsense mutation.** *BMC Med. Genet.* 2017; **18**(1): 125.
PubMed Abstract | Publisher Full Text

31. Kim YS, Kim GH, Byeon JH, *et al.*: **Chromosome 11q13 deletion syndrome.** *Korean J. Pediatr.* 2016; **59**: S10–S13.
PubMed Abstract | Publisher Full Text

32. Tassano E, Jagannathan V, Drögemüller C, *et al.*: **Congenital aural atresia associated with agenesis of internal carotid artery in a girl with a FOXI3 deletion.** *Am. J. Med. Genet. A.* 2015; **167**(3): 537–544.
PubMed Abstract | Publisher Full Text

33. Luquetti D v, Heike CL, Zarante I, *et al.*: **MYT1 role in the microtia-craniofacial microsomia spectrum.** *Mol. Genet. Genomic Med.* 2020; **8**(10): e1401.
PubMed Abstract | Publisher Full Text

34. Thiel CT, Rosanowski F, Kohlhase J, *et al.*: **Exclusion of TCOF1 mutations in a case of bilateral Goldenhar syndrome and one familial case of microtia with meatal atresia.** *Clin. Dysmorphol.* 2005; **14**(2): 67–71.
PubMed Abstract | Publisher Full Text

35. Patil SJ, das Bhowmik A, Bhat V, *et al.*: **Autosomal recessive otofaciocervical syndrome type 2 with novel homozygous small insertion in PAX1 gene.** *Am. J. Med. Genet. A.* 2018; **176**(5): 1200–1206.
PubMed Abstract | Publisher Full Text

36. Han R, Xia Y, Liu Z, *et al.*: **A mutation of EYA1 gene in a Chinese Han family with Branchio-Oto syndrome.** *Medicine.* 2021; **100**(25): e24691.
PubMed Abstract | Publisher Full Text

37. Balikova I, Martens K, Melotte C, *et al.*: **Autosomal-Dominant Microtia Linked to Five Tandem Copies of a Copy-Number-Variable Region at Chromosome 4p16.** *Am. J. Hum. Genet.* 2008; **82**(1): 181–187.
PubMed Abstract | Publisher Full Text

38. Timberlake AT, Griffin C, Heike CL, *et al.*: **Haploinsufficiency of SF3B2 causes craniofacial microsomia.** *Nat. Commun.* 2021; **12**(1): 4680.
PubMed Abstract | Publisher Full Text

39. Alsmadi O, Meyer BF, Alkuraya F, *et al.*: **Syndromic congenital sensorineural deafness, microtia and microdontia resulting from a novel homoallelic mutation in fibroblast growth factor 3 (FGF3).** *Eur. J. Hum. Genet.* 2009; **17**(1): 14–21.
Publisher Full Text

40. Knapp KM, Sullivan R, Murray J, *et al.*: **Linked-read genome sequencing identifies biallelic pathogenic variants in DONSON as a novel cause of Meier-Gorlin syndrome.** *J. Med. Genet.* 2020; **57**(3): 195–202.
Publisher Full Text

41. Zhang Q, Zhang J, Yin W: **Pedigree and genetic study of a bilateral congenital microtia family.** *Plast. Reconstr. Surg.* 2010; **125**(3): 979–987.
PubMed Abstract | Publisher Full Text

42. Nursal AF, Bekerecioğlu M, Pehlivan S, *et al.*: **Cytokine gene variants/expressions and non-syndromic microtia — is there a link?** *ENT Updates.* 2017; **7**(2): 62–67.
Publisher Full Text

43. Zhao R, Du P, Sun H, *et al.*: **Fetal microtia and FGFR2 polymorphism.** *Exp. Ther. Med.* May 10, 2019; **18**: 384–388.
PubMed Abstract | Publisher Full Text

44. Guo P, Ji Z, Jiang H, *et al.*: **Identification of a novel CYP26A1 mutation in a Chinese family with congenital microtia.** *Int. J. Pediatr. Otorhinolaryngol.* 2020; **139**: 110488.
PubMed Abstract | Publisher Full Text

45. Wang P, Wang Y, Fan X, *et al.*: **Identification of sequence variants associated with severe microtia-astresia by targeted sequencing.** *BMC Med. Genet.* 2019; **12**(1): 28.
PubMed Abstract | Publisher Full Text

46. Büyükgüral B, Pehlivan S, Nursal AF, *et al.*: **The roles of endothelial nitric oxide synthase (eNOS) and myeloperoxidase (MPO) genes in microtia.** *ENT Updates.* 2016; **6**(3): 121–125.
Publisher Full Text

47. Yang M, Lu X, Zhang Y, *et al.*: **Whole-exome sequencing analysis in 10 families of sporadic microtia with thoracic deformities.** *Mol. Genet. Genomic Med.* 2021; **9**(5): e1657.
PubMed Abstract | Publisher Full Text

48. Fan X, Ping L, Sun H, *et al.*: **Whole-Exome Sequencing of Discordant Monozygotic Twin Families for Identification of Candidate Genes for Microtia-Atresia.** *Front. Genet.* 2020; **11**.
PubMed Abstract | Publisher Full Text

49. Boudewyns A, van den Ende J, Boiy T, *et al.*: **22q11.2 microduplication syndrome with congenital aural atresia: A family report.** *Otol. Neurotol.* 2012; **33**(4): 674–680.
PubMed Abstract | Publisher Full Text

50. Lines MA, Huang L, Schwartzentruber J, *et al.*: **Haploinsufficiency of a spliceosomal GTPase encoded by EFTUD2 causes mandibulofacial dysostosis with microcephaly.** *Am. J. Hum. Genet.* 2012; **90**(2): 369–377.
PubMed Abstract | Publisher Full Text

51. Sanchez E, Laplace-Builhé B, Mau-Them FT, *et al.*: **POLR1B and neural crest cell anomalies in Treacher Collins syndrome type 4.** *Genet. Med.* 2020; **22**(3): 547–556.
PubMed Abstract | Publisher Full Text

52. Royer-Bertrand B, Castillo-Taucher S, Moreno-Salinas R, *et al.*: **Mutations in the heat-shock protein A9 (HSPA9) gene cause the EVEN-PLUS syndrome of congenital malformations and skeletal dysplasia.** *Sci. Rep.* 2015; **5**(1): 1–8.
PubMed Abstract | Publisher Full Text

53. Marchegiani S, Davis T, Tessadori F, *et al.*: **Recurrent Mutations in the Basic Domain of TWIST2 Cause Ablepharon Macrostomia and Barber-Say Syndromes.** *Am. J. Hum. Genet.* 2015; **97**(1): 99–110.
PubMed Abstract | Publisher Full Text | Free Full Text

54. Bacino CA, Schreck R, Fischel-Ghodsian N, *et al.*: **Clinical and molecular studies in full trisomy 22: Further delineation of the phenotype and review of the literature.** *Am. J. Med. Genet.* 1995; **56**(4): 359–365.
PubMed Abstract | Publisher Full Text

55. Bardakjian TM, Schneider AS, Ng D, *et al.*: **Association of a de novo 16q copy number variant with a phenotype that overlaps with Lenz microphthalmia and Townes-Brocks syndromes.** *BMC Med. Genet.* 2009; **10**(1): 1–7.
Publisher Full Text

56. Bragagnolo S, Colovati MES, Guilherme RS, *et al.*: **Wolf-Hirschhorn Syndrome with Epibulbar Dermoid: An Unusual Association in a**

Patient with 4p Deletion and Functional Xp Disomy. *Cytogenet. Genome Res.* 2016; **150**(1): 17–22.
Publisher Full Text

57. Basdemirci M, Zamani AG, Sener S, *et al.*: **LAMM syndrome: two new patients with a novel mutation in FGF3 gene and additional clinical findings.** *Clin. Dysmorphol.* 2019; **28**(2): 81–85.
PubMed Abstract | Publisher Full Text

58. Burrage LC, Charng WL, Eldomery MK, *et al.*: **De Novo GMNN Mutations Cause Autosomal-Dominant Primordial Dwarfism Associated with Meier-Gorlin Syndrome.** *Am. J. Hum. Genet.* 2015; **97**(6): 904–913.
PubMed Abstract | Publisher Full Text | Free Full Text

59. Conte C, D'Apice MR, Rinaldi F, *et al.*: **Novel mutations of TCOF1 gene in European patients with Treacher Collins syndrome.** *BMC Med. Genet.* 2011; **12**.
PubMed Abstract | Publisher Full Text | Free Full Text

60. de Munnik SA, Bicknell LS, Aftimos S, *et al.*: **Meier-Gorlin syndrome genotype-phenotype studies: 35 individuals with pre-replication complex gene mutations and 10 without molecular diagnosis.** *Eur. J. Hum. Genet.* 2012; **20**(6): 598–606.
PubMed Abstract | Publisher Full Text

61. Derbent M, Yilmaz Z, Baltaci V, *et al.*: **Chromosome 22q11.2 deletion and phenotypic features in 30 patients with conotruncal heart defects.** *Am. J. Med. Genet. A.* 2003; **116A**(2): 129–135.
PubMed Abstract | Publisher Full Text

62. Digilio MC, McDonald-McGinn DM, Heike C, *et al.*: **Three patients with oculo-auriculo-vertebral spectrum and microdeletion 22q11.2.** *Am. J. Med. Genet. A.* 2009; **149A**(12): 2860–2864.
PubMed Abstract | Publisher Full Text

63. Enomoto Y, Tsurusaki Y, Tominaga M, *et al.*: **A Recurrent Variant in POLR1B, c.3007C>T; p.Arg1003Cys, Associated with Atresia of the External Canal and Microtia in Treacher Collins Syndrome Type 4.** *Mol. Syndromol.* 2021; **12**(2): 127–132.
PubMed Abstract | Publisher Full Text

64. Eser Çavdartepe B: **Treacher Collins syndrome with a novel deletion in the TCOF1 gene.** *Erciyes Med. J.* 2019.
Publisher Full Text

65. Fan X, Wang Y, Fan Y, *et al.*: **TCOF1 pathogenic variants identified by Whole-exome sequencing in Chinese Treacher Collins syndrome families and hearing rehabilitation effect.** *Orphanet J. Rare Dis.* 2019; **14**(1): 1–9.
Publisher Full Text

66. Fenwick AL, Kliszczak M, Cooper F, *et al.*: **Mutations in CDC45, Encoding an Essential Component of the Pre-initiation Complex, Cause Meier-Gorlin Syndrome and Craniosynostosis.** *Am. J. Hum. Genet.* 2016; **99**(1): 125–138.
PubMed Abstract | Publisher Full Text | Free Full Text

67. Gimelli S, Cuoco C, Ronchetto P, *et al.*: **Interstitial deletion 14q31.1q31.3 transmitted from a mother to her daughter, both with features of hemifacial microsomia.** *J. Appl. Genet.* 2013; **54**(3): 361–365.
PubMed Abstract | Publisher Full Text

68. González-Domínguez CA, Raya-Trigueros A, Manrique-Hernández S, *et al.*: **Identification through exome sequencing of the first PMM2-CDG individual of Mexican mestizo origin.** *Mol. Genet. Metab. Rep.* 2020; **25**.
Publisher Full Text

69. Gripp KW, Curry C, Olney AH, *et al.*: **Diamond-Blackfan anemia with mandibulofacial dystostosis is heterogeneous, including the novel DBA genes TSR2 and RPS28.** *Am. J. Med. Genet. A.* 2014; **164**(9): 2240–2249.
PubMed Abstract | Publisher Full Text

70. Huang XS, Zhu B, Jiang HO, *et al.*: **A de novo 1.38Mb duplication of 1q31.1 in a boy with hemifacial microsomia, anophthalmia, anotia, macrostomia, and cleft lip and palate.** *Int. J. Pediatr. Otorhinolaryngol.* 2013; **77**(4): 560–564.
Publisher Full Text | PubMed Abstract

71. Irving MD, Dimitrov BI, Wessels M, *et al.*: **Rodriguez acrofacial dysostosis is caused by apparently de novo heterozygous mutations in the SF3B4 gene.** *Am. J. Med. Genet. A.* 2016; **170**(12): 3133–3137.
PubMed Abstract | Publisher Full Text

72. Knapp KM, Fellows B, Aggarwal S, *et al.*: **A synonymous variant in a non-canonical exon of CDC45 disrupts splicing in two affected sibs with Meier-Gorlin syndrome with craniosynostosis.** *Eur. J. Med. Genet.* 2021; **64**(4): 104182.
PubMed Abstract | Publisher Full Text

73. Koprulu M, Kumare A, Bibi A, *et al.*: **The first adolescent case of Fraser syndrome 3, with a novel nonsense variant in GRIP1.** *Am. J. Med. Genet. A.* 2021; **185**(6): 1858–1863.
PubMed Abstract | Publisher Full Text

74. Lamônica DAC, Maximino LP, Feniman MR, *et al.*: **Saethre-Chotzen Syndrome, Pro136His TWIST Mutation, Hearing Loss, and External and Middle Ear Structural Anomalies: Report on a Brazilian Family.** *Cleft Palate Craniofac. J.* 2010; **47**(5): 548–552.
Publisher Full Text

75. Lee KY, Kim S, Kim UK, *et al.*: **Novel EYA1 mutation in a Korean branchio-oto-renal syndrome family.** *Int. J. Pediatr. Otorhinolaryngol.* 2007; **71**(1): 169–174.
PubMed Abstract | Publisher Full Text

76. Li X, Zhang LZ, Yu L, *et al.*: **Prenatal diagnosis of Meier-Gorlin syndrome 7: a case presentation.** *BMC Pregnancy Childb.* 2021; **21**(1): 381–388.
PubMed Abstract | Publisher Full Text

77. Luquetti D, Hing A, Rieder MJ, *et al.*: **"Mandibulofacial dysostosis with microcephaly" caused by EFTUD2 mutations: expanding the phenotype.** *Am. J. Med. Genet. A.* 2013; **161A**(1): 108–113.
PubMed Abstract | Publisher Full Text | Free Full Text

78. Marques F, Tenney J, Duran I, *et al.*: **Altered mRNA Splicing, Chondrocyte Gene Expression and Abnormal Skeletal Development due to SF3B4 Mutations in Rodriguez Acrofacial Dysostosis.** *PLoS Genetics.* 2016; **12**(9): e1006307.
PubMed Abstract | Publisher Full Text

79. Mullegama Sv, Klein SD, Mulatinho Mv, *et al.*: **De novo loss-of-function variants in STAG2 are associated with developmental delay, microcephaly, and congenital anomalies.** *Am. J. Med. Genet. A.* 2017; **173**(5): 1319–1327.
PubMed Abstract | Publisher Full Text

80. Neuhann TM, Müller D, Hackmann K, *et al.*: **A further patient with van Maldergem syndrome.** *Eur. J. Med. Genet.* 2012; **55**(6-7): 423–428.
PubMed Abstract | Publisher Full Text

81. Pan Z, Xu H, Chen B, *et al.*: **Treacher Collins syndrome: Clinical report and retrospective analysis of Chinese patients.** *Mol. Genet. Genomic Med.* 2021; **9**(2): e1573.
PubMed Abstract | Publisher Full Text

82. Petek E, Wagner K, Steiner H, *et al.*: **Prenatal diagnosis of partial trisomy 4q26-qter and monosomy for the Wolf-Hirschhorn critical region in a fetus with split hand malformation.** *Prenat. Diagn.* 2000; **20**(4): 349–352.
PubMed Abstract | Publisher Full Text

83. Stevenson DA, Bleyl SB, Maxwell T, *et al.*: **Mandibulofacial dysostosis in a patient with a de novo 2;17 translocation that disrupts the HOXD gene cluster.** *Am. J. Med. Genet. A.* 2007; **143A**(10): 1053–1059.
PubMed Abstract | Publisher Full Text

84. Su PH, Chen JY, Chen SJ, *et al.*: **Treacher Collins syndrome with a de Novo 5-bp deletion in the TCOF1 gene.** *J. Formos. Med. Assoc.* 2006; **105**(6): 518–521.
PubMed Abstract | Publisher Full Text

85. Tingaud-Sequeira A, Trimouille A, Salaria M, *et al.*: **A recurrent missense variant in EYA3 gene is associated with oculo-auriculo-vertebral spectrum.** *Hum. Genet.* 2021; **140**(6): 933–944.
PubMed Abstract | Publisher Full Text

86. Ürel-Demir G, Aydln B, Karaosmanoğlu B, *et al.*: **Two Siblings with Kaufman Oculocerebrofacial Syndrome Resembling Oculoauriculovertebral Spectrum.** *Mol. Syndromol.* 2021; **12**(2): 106–111.
PubMed Abstract | Publisher Full Text

87. Valdes-Miranda JM, Soto-Alvarez JR, Toral-Lopez J, *et al.*: **A novel microdeletion involving the 13q31.3–q32.1 region in a patient with normal intelligence.** *Eur. J. Med. Genet.* 2014; **57**(2-3): 60–64.
PubMed Abstract | Publisher Full Text

88. Venditti CP, Hunt P, Donnenfeld A, *et al.*: **Mosaic paternal uniparental (iso) disomy for chromosome 20 associated with multiple anomalies.** *Am. J. Med. Genet.* 2004; **124A**(3): 274–279.
PubMed Abstract | Publisher Full Text

89. Vetro A, Savasta S, Russo Raucci A, *et al.*: **MCM5: a new actor in the link between DNA replication and Meier-Gorlin syndrome.** *Eur. J. Hum. Genet.* 2017; **25**(5): 646–650.
PubMed Abstract | Publisher Full Text

90. Voigt C, Mégarbané A, Neveling K, *et al.*: **Oto-facial syndrome and esophageal atresia, intellectual disability and zygomatic anomalies - Expanding the phenotypes associated with EFTUD2 mutations.** *Orphanet J. Rare Dis.* 2013; **8**(1): 1–12.
PubMed Abstract | Publisher Full Text

91. Weaver KN, Watt KEN, Hufnagel RB, *et al.*: **Acrofacial Dysostosis, Cincinnati Type, a Mandibulofacial Dysostosis Syndrome with Limb Anomalies, Is Caused by POLR1A Dysfunction.** *Am. J. Hum. Genet.* 2015; **96**(5): 765–774.
PubMed Abstract | Publisher Full Text

92. Wang Y-G, Sun S-P, Qiu Y-L, *et al.*: **A novel mutation in EYA1 in a Chinese family with Branchio-oto-renal syndrome.** *BMC Med. Genet.* 2018; **19**(1): 139.
PubMed Abstract | Publisher Full Text

93. Xing ZK, Wang SY, Xia X, *et al.*: **Targeted next-generation sequencing identifies a novel frameshift EYA1 variant causing**

branchio-otic syndrome in a Chinese family. *Int. J. Pediatr. Otorhinolaryngol.* 2020; **138**: 110202.
PubMed Abstract | Publisher Full Text

94. Zarate YA, Zhan H, Jones JR: **Infrequent Manifestations of Kabuki Syndrome in a Patient with Novel MLL2 Mutation.** *Mol. Syndromol.* 2012; **3**(4): 180–184.
PubMed Abstract | Publisher Full Text

95. Zeng H, Xie M, Li J, *et al.*: **A novel nonsense mutation in the TCOF1 gene in one Chinese newborn with Treacher Collins syndrome.** *Int. J. Pediatr. Otorhinolaryngol.* 2021; **141**: 110561.
PubMed Abstract | Publisher Full Text

96. Zhang Q-G, Zhang J, Yu P, *et al.*: **Environmental and Genetic Factors Associated with Congenital Microtia: A Case-Control Study in Jiangsu, China, 2004 to 2007.** *Plast. Reconstr. Surg.* 2009; **124**(4): 1157–1164.
PubMed Abstract | Publisher Full Text

97. Yu M, Obringer AC, Fowler MH, *et al.*: **Prenatal detection of deletion 6q13q15 in a complex karyotype.** *Prenat. Diagn.* 2005; **25**(12): 1084–1087.
PubMed Abstract | Publisher Full Text

98. Chen X, Xu F, Liu F, *et al.*: **Whole-exome sequencing for monozygotic twins discordant for hemifacial microsomia.** *J. Craniomaxillofac. Surg.* 2018; **46**(5): 802–807.
PubMed Abstract | Publisher Full Text

99. Kim SH, Shin JH, Yeo CK, *et al.*: **Identification of a novel mutation in the EYA1 gene in a Korean family with branchio-oto-renal (BOR) syndrome.** *Int. J. Pediatr. Otorhinolaryngol.* 2005; **69**(8): 1123–1128.
PubMed Abstract | Publisher Full Text

100. Schlump JU, Stein A, Hehr U, *et al.*: **Treacher Collins syndrome: Clinical implications for the paediatrician - A new mutation in a severely affected newborn and comparison with three further patients with the same mutation, and review of the literature.** *Eur. J. Pediatr.* 2012; **171**(11): 1611–1618.
PubMed Abstract | Publisher Full Text

101. Smigiel R, Bezniakow N, Jakubiak A, *et al.*: **Phenotype analysis of Polish patients with mandibulofacial dysostosis type Guion-Almeida associated with esophageal atresia and choanal atresia caused by EFTUD2 gene mutations.** *J. Appl. Genet.* 2015; **56**(2): 199–204.
PubMed Abstract | Publisher Full Text

102. Su PH, Yu JS, Chen JY, *et al.*: **Mutations and new polymorphic changes in the TCOF1 gene of patients with oculo-auriculo-vertebral spectrum and Treacher-Collins syndrome.** *Clin. Dysmorphol.* 2007; **16**(4): 261–267.
PubMed Abstract | Publisher Full Text

103. Tingaud-Sequeira A, Trimouille A, Marlin S, *et al.*: **Functional and genetic analyses of ZYG11B provide evidences for its involvement in OAVS.** *Mol. Genet. Genomic Med.* 2020; **8**(10): e1375.
PubMed Abstract | Publisher Full Text | Free Full Text

104. Zhang C, An L, Xue H, *et al.*: **Mutation analysis of TCOF1 gene in Chinese Treacher Collins syndrome patients.** *J. Clin. Lab. Anal.* 2021; **35**(1): e23567.
PubMed Abstract | Publisher Full Text | Free Full Text

105. Gendron C, Schwentker A, van Aalst JA: **Genetic Advances in Craniofacial Malformations: Genetic Advances in the Understanding of Microtia.** *J. Pediatr. Genet.* 2016; **5**(4): 189–197.
PubMed Abstract | Publisher Full Text

106. Abdelhak S, Kalatzis V, Heilig R, *et al.*: **Clustering of mutations responsible for branchio-oto-renal (BOR) syndrome in the eyes absent homologous region (eyaHR) of EYA1.** *Hum. Mol. Genet.* 1997; **6**(13): 2247–2255.
PubMed Abstract | Publisher Full Text

107. Rodríguez Soriano J: **Branchio-oto-renal syndrome.** *J. Nephrol.* 2003 Jul-Aug; **16**(4): 603–605. Accessed April 18, 2023.
PubMed Abstract

108. Kumar S, Deffenbacher K, Cremers CWRJ, *et al.*: **Branchio-Oto-Renal Syndrome: Identification of Novel Mutations, Molecular Characterization, Mutation Distribution, and Prospects for Genetic Testing.** *Genet. Test.* 2009; **1**(4): 243–251.
PubMed Abstract | Publisher Full Text

109. Smith LB, Belanger JM, Oberbauer AM, *et al.*: **Fibroblast growth factor receptor 3 effects on proliferation and telomerase activity in sheep growth plate chondrocytes.** *J. Anim. Sci. Biotechnol.* 2012; **3**(1): 1–8.
Publisher Full Text

110. Bowman M, Oldridge M, Archer C, *et al.*: **Gross deletions in TCOF1 are a cause of Treacher–Collins–Franceschetti syndrome.** *Eur. J. Hum. Genet.* 2012; **20**: 769–777.
Publisher Full Text

111. Hutton B, Salanti G, Caldwell DM, *et al.*: **The PRISMA extension statement for reporting of systematic reviews incorporating network meta-analyses of health care interventions: checklist and explanations.** *Ann. Intern. Med.* 2015; **162**(11): 777–784.
PubMed Abstract | Publisher Full Text

112. The Role of Genetic Factors in Microtia: **A Systematic Review – Harvard Dataverse.** 2023. Accessed April 18, 2023.
Publisher Full Text

113. **Checklist for Systematic Reviews and Research Syntheses Critical Appraisal Checklist for Systematic Reviews and Research Syntheses 2.** 2017. Accessed April 20, 2022.
Reference Source

114. Güleray N, Koşukcu C, Oğuz S, *et al.*: **Investigation of Genetic Causes in a Developmental Disorder: Oculoauriculovertebral Spectrum.** *Cleft Palate Craniofac J.* 2021; **59**(9): 1114–1124.
PubMed Abstract | Publisher Full Text

115. Hao S, Jin L, Wang H, *et al.*: **Mutational Analysis of TCOF1, GSC, and HOXA2 in Patients With Treacher Collins Syndrome.** *J. Craniofac. Surg.* 2016; **27**(6): e583–e586.
PubMed Abstract | Publisher Full Text | Free Full Text

116. *BIRTH DEFECTS SURVEILLANCE A MANUAL FOR PROGRAMME MANAGERS SECOND EDITION.* CDC.
Reference Source

117. 4.4 Congenital Malformations of the Ear | Birth Defects Surveillance Toolkit | CDC. Accessed July 8, 2022.
Reference Source

118. Suutarla S, Rautio J, Ritvanen A, *et al.*: **Microtia in Finland: comparison of characteristics in different populations.** *Int. J. Pediatr. Otorhinolaryngol.* 2007; **71**(8): 1211–1217.
Publisher Full Text

119. Campo AA, Victor-Baldin A, Contreras-Mérida SM: **Surgical-based classification for microtia.** *J. Craniofac. Surg.* 2018; **29**(6): 1651–1654.
Publisher Full Text

120. Gregory-Evans CY, Moosajee M, Hodges MD, *et al.*: **SNP genome scanning localizes oto-dental syndrome to chromosome 11q13 and microdeletions at this locus implicate FGF3 in dental and inner-ear disease and FADD in ocular coloboma.** *Hum. Mol. Genet.* 2007; **16**(20): 2482–2493.
PubMed Abstract | Publisher Full Text

121. Grzanka M, Piekiełko-Witkowska A: **The Role of TCOF1 Gene in Health and Disease: Beyond Treacher Collins Syndrome.** *Int. J. Mol. Sci.* 2021; **22**(5): 1–19.
PubMed Abstract | Publisher Full Text

122. Sakai D, Trainor PA: **Treacher Collins syndrome: unmasking the role of Tcof1/treacle.** *Int. J. Biochem. Cell Biol.* 2009; **41**(6): 1229–1232.
PubMed Abstract | Publisher Full Text

123. Gonzales B, Henning D, So RB, *et al.*: **The Treacher Collins syndrome (TCOF1) gene product is involved in pre-rRNA methylation.** *Hum. Mol. Genet.* 2005; **14**(14): 2035–2043.
Publisher Full Text

124. Trainor PA, Dixon J, Dixon MJ: **Treacher Collins syndrome: etiology, pathogenesis and prevention.** *Eur. J. Hum. Genet.* 2009; **17**(3): 275–283.
PubMed Abstract | Publisher Full Text | Free Full Text

125. Alexander T, Nolte C, Krumlauf R: **Hox genes and segmentation of the hindbrain and axial skeleton.** *Annu. Rev. Cell Dev. Biol.* 2009; **25**: 431–456.
Publisher Full Text

126. Mallo M, Wellik DM, Deschamps J: **Hox genes and regional patterning of the vertebrate body plan.** *Dev. Biol.* 2010; **344**(1): 7–15.
PubMed Abstract | Publisher Full Text

127. Minoux M, Kratochwil CF, Ducret S, *et al.*: **Mouse Hoxa2 mutations provide a model for microtia and auricle duplication.** *Development.* 2013; **140**(21): 4386–4397.
Publisher Full Text

128. Si N, Zhang Z, Meng X, *et al.*: **Generation of an induced pluripotent stem cell line from a congenital microtia patient with 4p16.1 microduplication involving the long-range enhancer of HMX1.** *Stem Cell Res.* 2021; **53**: 102357.
PubMed Abstract | Publisher Full Text

129. Wright TJ, Mansour SL: **Fgf3 and Fgf10 are required for mouse otic placode induction.** *Development.* 2003; **130**(15): 3379–3390.
PubMed Abstract | Publisher Full Text

130. Yee AJ, Ramaswamy S: **DNA Microarrays in Biological Discovery and Patient Care.** *Essentials of Genomic and Personalized Medicine.* January 1, 2010; pp. 73–88.
Publisher Full Text

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Miriam Erandi Reyna-Fabian
[1] Molecular Biology Laboratory, Medical Research Sudirectorate, National Institute of Pediatrics, Mexico City, Mexico
[2] Molecular Biology Laboratory, Medical Research Sudirectorate, National Institute of Pediatrics, Mexico City, Mexico

Microtia and anotia are a spectrum of congenital anomalies with variable phenotypic expression, heterogeneous etiology and differences in its prevalence across ethnicities: Hispanic (1.12/10,000), U.S.-born Hispanic (0.83/10,000), Asian (0.54/10,000), Pacific Island native (4.61/10,000), and Philippine (4.77/10,000)[1]. It may occur as an isolated condition, or as part of chromosomal and monogenic syndromes.

Since genetic and non-genetic (prenatal exposure to alcohol, retinoids, maternal diabetes)[2-4] factors have been associated to this condition, a multifactorial origin has been proposed. Even though, the genetic contribution to the development of microtia has been addressed from various perspectives such as: identification of families segregating as an autosomal dominant (AD), autosomal recessive (AR), or multifactorial trait[5]; studies in monozygotic and dizygotic twins[6]; knockout murine models developing microtia due to pathogenic variants in orthologous genes[7], only in few cases the responsible genotype has been characterized[8-11].

Recently, other genetic studies using DNA chromosomal microarray analysis[12,13], transcriptomic and proteomic profiles[14] and next-generation sequencing studies[15-19] have also been performed in patients with this entity, but few candidate genes have been identified so far. Therefore, more studies should be addressed in order to dilucidated the genetic etiology underlying of microtia which remains unknown in most patients.

In this review, Putri *et al.*, make a great effort to investigate the genetic etiology of isolated and syndromic microtia according to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) recommendations with systematic searchers from 1 to 31 October 2021. The objectives of the article are clearly stated, and the detailed methods provided by the authors allow replication of the study.

Comment 1:
The summary is not updated as it states, "We found 40 studies, including 22 studies on syndromic microtia and 18 studies on isolated microtia", and it should include the 98 studies that were added to Version 2.

Comment 2:
In introduction, third paragraph, it is stated “SIX1 and SIX5 for branchio-oto-renal (BOR) syndrome”, however, EYA1 has also been identified as a gene underlying this syndrome[20-22].

Comment 3:
In the discussion section, last paragraph, the authors write “By applying NGS and microarray for screening of genetic risk factors in microtia, the diagnosis of microtia could be made earlier and the patients could get a more comprehensive treatment”. This proposal seems very ambitious since these molecular studies have identified the genetic cause only in a few cases, mainly in isolated and sporadic microtia, so proposing these molecular studies as routine tests should be taken with caution, mainly because of the incidental findings. In this sense: how do you consider that this genetic screening can help patients with a more comprehensive treatment? Do you consider that the molecular results can impact on the patient’s treatment?

In addition, if the authors consider that the diagnosis of microtia could be made earlier, please read the publication by Chen *et al.*, (2022)[23], where they propose an early diagnosis of congenital microtia patients based on metabolomic characterization, as they showed a panel of metabolic biomarkers that has a high sensitivity and specificity to separate patients with microtia from controls. This is indeed a direct test that could have an impact on the early diagnosis of microtia. Therefore, please consider rephrasing this sentence or be more cautious in the proposals.

Besides, in the introduction section, last paragraph, the authors stated “This may help to improve our understanding of microtia, underline the necessity of gene screening and even make prevention of microtia in the near future possible with the help of gene modification”. Please consider rephrasing this sentence as the prevention of microtia by genetic modification lacks clear arguments and proposals.

Comment 4:
Microtia could be considered a minimal expression of the oculo-auriculo-vertebral spectrum (OAVS, MIM #164210) and there is no agreement on the minimum diagnostic criteria for OAVS. Does this review consider the published studies of the OAVS genetic causes?

Comment 5:
This review performed searches until October 31, 2021. Do the authors have consider expanding the search to, at least January 2023 as new findings have contributed to the knowledge of microtia genetics etiology.[12, 24-31].

References
1. Forrester MB, Merz RD: Descriptive epidemiology of anotia and microtia, Hawaii, 1986-2002. *Congenit Anom (Kyoto)*. 2005; **45** (4): 119-24 PubMed Abstract | Publisher Full Text
2. Foroud T, Wetherill L, Vinci-Booher S, Moore ES, et al.: Relation over time between facial measurements and cognitive outcomes in fetal alcohol-exposed children.*Alcohol Clin Exp Res*. 2012;

36 (9): 1634-46 PubMed Abstract | Publisher Full Text
3. Berenguer M, Darnaudery M, Claverol S, Bonneu M, et al.: Prenatal retinoic acid exposure reveals candidate genes for craniofacial disorders.*Sci Rep*. 2018; **8** (1): 17492 PubMed Abstract | Publisher Full Text
4. Gendron C, Schwentker A, van Aalst JA: Genetic Advances in the Understanding of Microtia.*J Pediatr Genet*. 2016; **5** (4): 189-197 PubMed Abstract | Publisher Full Text
5. Llano-Rivas I, Ariadna, del Castillo V, Reyes R, et al.: Microtia. *Archives of Medical Research*. 1999; **30** (2): 120-124 Publisher Full Text
6. Artunduaga M, Quintanilla-Dieck M, Greenway S, Betensky R, et al.: A Classic Twin Study of External Ear Malformations, Including Microtia. *New England Journal of Medicine*. 2009; **361** (12): 1216-1218 Publisher Full Text
7. Luquetti DV, Heike CL, Hing AV, Cunningham ML, et al.: Microtia: epidemiology and genetics.*Am J Med Genet A*. 2012; **158A** (1): 124-39 PubMed Abstract | Publisher Full Text
8. Piceci F, Morlino S, Castori M, Buffone E, et al.: Identification of a second HOXA2 nonsense mutation in a family with autosomal dominant non-syndromic microtia and distinctive ear morphology.*Clin Genet*. 2017; **91** (5): 774-779 PubMed Abstract | Publisher Full Text
9. Brown KK, Viana LM, Helwig CC, Artunduaga MA, et al.: HOXA2 haploinsufficiency in dominant bilateral microtia and hearing loss.*Hum Mutat*. 2013; **34** (10): 1347-51 PubMed Abstract | Publisher Full Text
10. Si N, Meng X, Lu X, Zhao X, et al.: Identification of loss-of-function HOXA2 mutations in Chinese families with dominant bilateral microtia.*Gene*. 2020; **757**: 144945 PubMed Abstract | Publisher Full Text
11. Meddaugh HR, Zambrano RM: Novel HOXA2 variant presenting with microtia and variable hearing impairment in four-generation pedigree.*Clin Dysmorphol*. 2020; **29** (2): 104-106 PubMed Abstract | Publisher Full Text
12. Celse T, Tingaud-Sequeira A, Dieterich K, Siegfried G, et al.: OTX2 duplications: a recurrent cause of oculo-auriculo-vertebral spectrum.*J Med Genet*. 2022. PubMed Abstract | Publisher Full Text
13. Glaeser AB, Diniz BL, Deconte D, Santos AS, et al.: Microarray-Based Comparative Genomic Hybridization, Multiplex Ligation-Dependent Probe Amplification, and High-Resolution Karyotype for Differential Diagnosis Oculoauriculovertebral Spectrum: A Systematic Review.*J Pediatr Genet*. 2020; **9** (3): 149-157 PubMed Abstract | Publisher Full Text
14. Chen X, Xu Y, Li C, Lu X, et al.: Key Genes Identified in Nonsyndromic Microtia by the Analysis of Transcriptomics and Proteomics.*ACS Omega*. 2022; **7** (20): 16917-16927 PubMed Abstract | Publisher Full Text
15. Lopez E, Berenguer M, Tingaud-Sequeira A, Marlin S, et al.: Mutations in MYT1, encoding the myelin transcription factor 1, are a rare cause of OAVS.*J Med Genet*. 2016; **53** (11): 752-760 PubMed Abstract | Publisher Full Text
16. Güleray N, Koşukcu C, Oğuz S, Ürel Demir G, et al.: Investigation of Genetic Causes in a Developmental Disorder: Oculoauriculovertebral Spectrum.*Cleft Palate Craniofac J*. 2022; **59** (9): 1114-1124 PubMed Abstract | Publisher Full Text
17. Rengasamy Venugopalan S, Farrow E, Sanchez-Lara PA, Yen S, et al.: A novel nonsense substitution identified in the AMIGO2 gene in an Occulo-Auriculo-Vertebral spectrum patient. *Orthod Craniofac Res*. 2019; **22 Suppl 1**: 163-167 PubMed Abstract | Publisher Full Text
18. Tingaud-Sequeira A, Trimouille A, Marlin S, Lopez E, et al.: Functional and genetic analyses of ZYG11B provide evidences for its involvement in OAVS.*Mol Genet Genomic Med*. 2020; **8** (10): e1375 PubMed Abstract | Publisher Full Text
19. Luquetti DV, Heike CL, Zarante I, Timms AE, et al.: MYT1 role in the microtia-craniofacial

microsomia spectrum.*Mol Genet Genomic Med*. 2020; **8** (10): e1401 PubMed Abstract | Publisher Full Text
20. Abdelhak S, Kalatzis V, Heilig R, Compain S, et al.: Clustering of mutations responsible for branchio-oto-renal (BOR) syndrome in the eyes absent homologous region (eyaHR) of EYA1.*Hum Mol Genet*. 1997; **6** (13): 2247-55 PubMed Abstract | Publisher Full Text
21. Kumar S, Deffenbacher K, Cremers CW, Van Camp G, et al.: Branchio-oto-renal syndrome: identification of novel mutations, molecular characterization, mutation distribution, and prospects for genetic testing.*Genet Test*. **1** (4): 243-51 PubMed Abstract | Publisher Full Text
22. Rodríguez Soriano J: Branchio-oto-renal syndrome.*J Nephrol*. 2003; **16** (4): 603-5 PubMed Abstract
23. Chen SJ, Zhang HS, Huang XP, Li WH, et al.: Metabolomic characterization of congenital microtia: a possible analysis for early diagnosis.*Ann Transl Med*. 2022; **10** (24): 1330 PubMed Abstract | Publisher Full Text
24. Si N, Zhang Z, Huang X, Wang C, et al.: De novo 22q11.2 deletions and auricular findings in two Chinese patients with microtia.*Mol Genet Genomic Med*. 2022; **10** (1): e1862 PubMed Abstract | Publisher Full Text
25. Lin K, You DY, Zhang LH, Zhou LJ, et al.: The genes for sensory perception of sound should be considered in gene diagnosis of congenital sensorineural hearing loss and microtia.*J Appl Genet*. 2022; **63** (2): 327-337 PubMed Abstract | Publisher Full Text
26. Chou W, Chen J, Shiao Y, Tsauer J, et al.: Prenatally diagnosed microdeletion in the TCOF1 gene in fetal congenital primary Treacher Collins Syndrome. *Taiwanese Journal of Obstetrics and Gynecology*. 2022; **61** (3): 514-516 Publisher Full Text
27. Jamshidi F, Shokouhian E, Mohseni M, Kahrizi K, et al.: Identification of a homozygous frameshift mutation in the FGF3 gene in a consanguineous Iranian family: First report of labyrinthine aplasia, microtia, and microdontia syndrome in Iran and literature review.*Mol Genet Genomic Med*. 2023. e2168 PubMed Abstract | Publisher Full Text
28. Quinn S, Kavanagh K, McArdle L, Betts D, et al.: Unilateral microtia found in association with a de-novo 20q13.33 deletion, is there a causal link?. *Clin Dysmorphol*. 2023; **32** (1): 39-42 PubMed Abstract | Publisher Full Text
29. Quiat D, Timberlake AT, Curran JJ, Cunningham ML, et al.: Damaging variants in FOXI3 cause microtia and craniofacial microsomia.*Genet Med*. 2023; **25** (1): 143-150 PubMed Abstract | Publisher Full Text
30. Liu W, Wang Q, Guo Y, Lin L, et al.: Whole-Genome Sequencing Identifies Two Novel Rare Mutations in BMP5 and BMP2 in Monozygotic Twins With Microtia.*J Craniofac Surg*. **33** (2): e212-e217 PubMed Abstract | Publisher Full Text
31. García-Castro M, Martinez-Merino T, Puente N, Riancho J: Expanding the Etiology of Oculo–Auriculo–Vertebral Spectrum: A Novel Interstitial Microdeletion at 1p36. *International Journal of Molecular Sciences*. 2022; **24** (1). Publisher Full Text

Are the rationale for, and objectives of, the Systematic Review clearly stated?

Yes

Are sufficient details of the methods and analysis provided to allow replication by others?

Yes

Is the statistical analysis and its interpretation appropriate?

Yes

Are the conclusions drawn adequately supported by the results presented in the review?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Medical genetics, molecular biology, monogenic and multifactorial diseases.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 04 Apr 2023

Citrawati Wungu

Comment 1:
The summary is not updated as it states, "We found 40 studies, including 22 studies on syndromic microtia and 18 studies on isolated microtia", and it should include the 98 studies that were added to Version 2.

Comment Response 1:
Thank you for this excellent input and comment. We have checked, revised and updated the summary to Version 2. (Page 1, line 14-15)

Comment 2:
In introduction, third paragraph, it is stated "SIX1 and SIX5 for branchio-oto-renal (BOR) syndrome", however, EYA1 has also been identified as a gene underlying this syndrome.[20-22]

Comment Response 2:
We thank the reviewer for giving us a chance to improve our manuscript. We have now added EYA1 as a gene underlying BOR syndrome (in introduction, 3rd paragraph) and cited it in reference number 13-15.

Comment 3

- In the discussion section, last paragraph, the authors write "By applying NGS and microarray for screening of genetic risk factors in microtia, the diagnosis of microtia could be made earlier and the patients could get a more comprehensive treatment". This proposal seems very ambitious since these molecular studies have identified the genetic cause only in a few cases, mainly in isolated and sporadic microtia, so proposing these molecular studies as routine tests should be taken with caution, mainly because of the incidental findings. In this sense: how do you consider that this genetic screening can help patients with a more comprehensive treatment? Do you consider that the molecular results can impact on the patient's treatment?

- In addition, if the authors consider that the diagnosis of microtia could be made earlier, please read the publication by Chen *et al.*, (2022)23, where they propose an early diagnosis of congenital microtia patients based on metabolomic

characterization, as they showed a panel of metabolic biomarkers that has a high sensitivity and specificity to separate patients with microtia from controls. This is indeed a direct test that could have an impact on the early diagnosis of microtia. Therefore, please consider rephrasing this sentence or be more cautious in the proposals.

- Besides, in the introduction section, last paragraph, the authors stated "This may help to improve our understanding of microtia, underline the necessity of gene screening and even make prevention of microtia in the near future possible with the help of gene modification". Please consider rephrasing this sentence as the prevention of microtia by genetic modification lacks clear arguments and proposals.

Comment Response 3:

- Thank you for this excellent input and comment, which have helped us to improve our manuscript. We have revised and added our explanation related to the statement about 'NGS and microarray for screening of genetic risk factors in microtia, the diagnosis microtia could be made earlier and the patients could get more comprehensive treatment' in last sentence of last paragraph of discussion.

- We thank the reviewer for giving us a chance to improve our manuscript. We have read the publication by Chen *et al.*, (2022)[23], a panel of metabolic biomarkers that has a high sensitivity and specificity to separate patients with microtia from controls. This is a very good study, but in their study, they also stated the limitations of their study in that the field of metabolomics is still immature, and easily disrupted by upstream organisms. And in addition, this metabolic biomarker does not discriminate whether it belongs to isolated or syndromic microtia, in which we all know that syndromic microtia requires further treatment compared to isolated microtia.

- Thank you for this comment. In the last paragraph of our introduction section, we have rephrased the sentences as you suggested.

Comment 4:

Microtia could be considered a minimal expression of the oculo-auriculo-vertebral spectrum (OAVS, MIM #164210) and there is no agreement on the minimum diagnostic criteria for OAVS. Does this review consider the published studies of the OAVS genetic causes?

Comment Response 4:

Thank you for this excellent input and comment. We agree that Microtia could be considered a minimal expression of the oculo-auriculo-vertebral spectrum (OAVS, MIM #164210). However, regarding there is no agreement on the minimum diagnostic criteria for OVAS, we disagree, referring to the study of Devriendt, et al (2023)[1], Luquetti, et al (2012)[2] where they propose the minimum diagnostic criteria for OVAS from several researchers. And in our review, we do not propose what the exact genetic cause of OVAS is, but here we only review and conclude data from various genetic studies related to microtia, some of which state that genetic disorders belong to isolated microtia which can be categorized as OVAS from their clinical condition.

Comment 5 :

This review performed searches until October 31, 2021. Do the authors have consider

expanding the search to, at least January 2023 as new findings have contributed to the knowledge of microtia genetics etiology.[12, 24-31]

Comment Response 5 :
We thank the reviewer for excellent input and suggestion. We did the search up to October 31, 2021 due to the time when we first submitted the manuscript. However, the review process itself in the journal was pretty slow thus it took for about one year and a half. If we update and re-do the searching, it would take a longer time to rewrite the manuscript and change the overall data. It would be nice if further research was carried out from our systematic review later.

Competing Interests: We declare that we have no competing interest

Reviewer Report 07 March 2023

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Isabella Monlleó
[1] Faculty of Medicine, Clinical Genetics Service, University Hospital, Universidade Federal de Alagoas, Maceió, State of Alagoas, Brazil
[2] Faculty of Medicine, Clinical Genetics Service, University Hospital, Universidade Federal de Alagoas, Maceió, State of Alagoas, Brazil

INTRODUCTION

1. **Paragraph** "Roughly one-third to one-half of patients with microtia have craniofacial microsomia[2,3].":

   Comment: References 2 and 3 do not provide this information. About 40% of patients present syndromic microtia. Craniofacial microsomia is an example of syndromic microtia (Luquetti DV, *et al*., 2013[1];Meng Lu *et al*., 2020[2]).

2. **Paragraph** "Although certain studies discovered candidate genetic variations for microtia, no causal genetic mutation has been identified.[11]"

   and

   Paragraph "FGF3 mutations are commonly found in LAMM syndrome.[13,14] (...) TCOF1 mutations can cause TCS in up to 78% of patients. (...) HOXA2.... was previously linked to autosomal recessive bilateral microtia.[10,17]"

   Comment: Reference 11 brings results on a truncating mutation in *HOXA1* gene that causes

a monogenic disorder of microtia in pigs. References 4, 7, 9, 10, 12, 13, 14, 16, 17, 69, 72... list genes and pathogenic variants associated with non-syndromic and syndromic microtia.

3. **Paragraph** "Genes identified to be involved in the development of major syndromic microtia"

   Comment: What does it mean? Major genes involved in syndromic microtia? Genes involved in the major syndromes associated with microtia? Genes involved in syndromes with microtia as a major feature? Or genes involved in the development of the ears?

The above excerpts do not make clear the existing etiological models and clinical groups of microtia. According to their excellent references 4, 7, 10, 12, and 130, we have:

1. **1-ETIOLOGY OF NON-SYNDROMIC (ISOLATED) MICROTIA**

   1.1.Sporadic microtia: it corresponds to the majority of cases and follows the multifactorial model, i.e. genetic liability (polygenes) plus environmental triggered factors ("maternal sociodemographic variables, multiple gestation, gestational diabetes", etc). This model explains the mild male predominance and ethnic-geographic variation of the phenotype;

   1.2. Familial microtia (3-34% of the cases): it includes the expected precurrence and recurrence of isolated microtia related to the multifactorial model, and rare cases of single gene/Mendelian disorders segregating as a dominant, recessive, or X-linked trait in families.

   1.3. Unrecognized

2. **2-ETIOLOGY OF SYNDROMIC MICROTIA:**

   2.1. Single gene (Mendelian) disorders, for example, Treacher-Collins syndrome;

   2.2. Cytogenetic and cytogenomic rearrangements, for example, trisomy, translocation, deletion syndromes;

   2.3. Teratogenic, for example, retinoic acid embryopathy;

   2.4. Unrecognized: non-random pattern of multiple congenital malformations.

AIM, METHODS, RESULTS, AND DISCUSSION

To "conducted the first systematic review to qualitatively identify the most important genes in the development of microtia" authors have selected 98 articles consisting of 29 case reports, 6 case-control, and 63 case series studies.

I agree that this strategy is suitable for the above aim. However, the selected studies do not allow the following statements:

1. **Paragraph "**(...) In this study, China had the highest number of microtia cases. The reported prevalence of microtia/anotia varies between 1 in 3000 to 1 in 20 000 births.1,2 The prevalence might vary by country and race/ethnicity but this is likely dependent on what forms of microtia are included in studies.20,21"

   and

2. **Paragraph** "According to this study, most cases of microtia (62.6%) occurred in China, 51.9% of subjects had a family history of microtia, 57.4% of cases occurred in males, 71.8% of cases were bilateral, and 47.7% of cases were grade II microtia."

To do so, authors should systematically review population-based, hospital-based, multicenter-based, international, or national-wide studies.

In the following paragraphs, authors put together sporadic and familial cases and non-syndromic and syndromic cases which are etiologically and epidemiologically distinct:

1. **Paragraph** "We discovered that most patients with microtia had a family history of the disease, including 97.7% of patients with isolated microtia and 63.5% of patients with syndromic microtia. This is consistent with the existing literature, which describes Mendelian hereditary variants of microtia with an autosomal dominant or recessive mode of inheritance.[1,4,22] The rates of familial microtia ranged from 3 to 34%.[10]".

   Comment: No, this is not consistent with the existing literature. This is an ascertainment bias.

2. **Paragraph** "Based on the gender classification of each study, we discovered nearly 60% of all patients were male, including 54.9% of patients with syndromic microtia and 59.1% of patients with isolated microtia. In prior research, microtia was more common in male patients than in female patients, with a sex ratio of 1.5:1 and an estimated 20–40% greater risk in males than in females.[4,23]"

   Comment: This is an ascertainment bias.

3. **Paragraph** "Although microtia can arise bilaterally, 77–93% of patients have unilateral involvement.[4,23] The most common form of syndromic microtia is bilateral microtia.[1] Although bilateral microtia was more common in this study, the type of microtia was only known for 34.6% of subjects. Because of the inadequate data, this could represent a biased outcome for the most prevalent type of microtia".

   Comment: This is an ascertainment bias.

Concerning the aim of the study in itself:

1. **Paragraph** "in isolated microtia, the most common type of mutation in HOXA2 was silent mutation (54.2%)".

   Comment: A silent mutation occurs when the nucleotide alteration does not affect the amino acid of the protein. As expected, these alterations do not change the phenotype. Reported causative alterations of *the HOXA2* gene are nonsense variants that segregate as autosomal dominant or recessive non-syndromic microtia (References 1, 17, 22, 50...)

2. **Paragraph** "This was consistent with the literature, which indicated that the most common genes involved in microtia were HOXA2, FGF3, HOXD, ORC1, ORC4, ORC6, CDT1, CDC6, DHODH, HMX1, EYA1, and. TCOF1.[2]"

   Comment: Reference 2 (Luquetti DV, *et al.*, 2011) does not mention genes involved with microtia.

3. **Paragraph** "FGF3 haploinsufficiency could also be related with dental and hearing problems".

   Comment: *FGF3* alterations are a well-established etiology for LAMM syndrome (MIM #610706).

4. **Paragraph** "A significant percentage of microtic patients also present deficient facial components that originate from the same embryological structures."

   Comment: Microtic patients is a pejorative term.

5. **Paragraph** "In humans, recessive loss-of-function mutations in HMX1 have been linked to OVAS, which is characterized by external ear and eye deformity.[9]"

   Comment: Reference 9 brings information on the dosage-sensitive effects of *HMX1* enhancer in patients with isolated bilateral concha-type microtia. It reports on duplications in the intergenic region downstream of *HMX1* in four families, and duplication between *HMX1* and *CPZ* in another family.

6. **Paragraph** "We hypothesized that a link existed between the types of genes involved and the grade of microtia".

   Comment: Why and how?

7. **Paragraph** "Approximately 80.2% of the genetic factors that contribute to microtia remain unknown".

   Comment: Authors have explained how they achieved this figure in their response letter: "We got this number from the calculation of the number of 100% of genetic factors involved in this birth defect minus 3 most common genes found in our systematic review: %HOXA2 (6.4%) minus %FGF3 (4.9%) and minus %TCOF1 (8.5%)". This is not an appropriate way to measure or estimate the genetic contribution to complex and etiologically heterogeneous congenital malformations such as microtia.

Tables: the authors' responses to my prior comments 8 and are not appropriate.

REFERENCES

1. There are some duplicated references.

2. Reference number 74 is the manuscript under review.

References

1. Luquetti DV, Cox TC, Lopez-Camelo J, Dutra Mda G, et al.: Preferential associated anomalies in 818 cases of microtia in South America.*Am J Med Genet A.* 2013; **161A** (5): 1051-7 PubMed Abstract | Publisher Full Text

2. Lu M, Lu X, Jiang H, Pan B: Review of Preferential Suspicious Genes in Microtia Patients Through Various Approaches.*J Craniofac Surg.* 2020; **31** (2): 538-541 PubMed Abstract | Publisher Full Text

Are the rationale for, and objectives of, the Systematic Review clearly stated?
Partly

Are sufficient details of the methods and analysis provided to allow replication by others?
Partly

Is the statistical analysis and its interpretation appropriate?
Partly

Are the conclusions drawn adequately supported by the results presented in the review?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Medical and human genetics, craniofacial anomalies, intellectual disability, disorders of sex development

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 04 Apr 2023

Citrawati Wungu

INTRODUCTION
1. Paragraph "Roughly one-third to one-half of patients with microtia have craniofacial microsomia2,3.":**Comment:** References 2 and 3 do not provide this information. About 40% of patients present syndromic microtia. Craniofacial microsomia is an example of syndromic microtia (Luquetti DV, *et al*., 20131;Meng Lu *et al*., 20202).

Comment Response 1 :
We thank the reviewer for the correction. We have revised our sentence regarding this.

2. **Paragraph** "Although certain studies discovered candidate genetic variations for microtia, no causal genetic mutation has been identified.11"
and
Paragraph "FGF3 mutations are commonly found in LAMM syndrome.13,14 (...) TCOF1 mutations can cause TCSin up to 78% of patients. (...) HOXA2.... was previously linked to autosomal recessive bilateral microtia.10,17"6
Comment: Reference 11 brings results on a truncating mutation in *HOXA1* gene that causes a monogenic disorder of microtia in pigs. References 4, 7, 9, 10, 12, 13, 14, 16, 17, 69, 72... list genes and pathogenic variants associated with non-syndromic and syndromic microtia.

Comment Response 2 :
We thank the reviewer for giving us a chance to improve our manuscript. We have revised

our sentence regarding this. What we mean here is, although there are many candidate genetic disorders associated with microtia. There is no exact single specific genetic disorder that is certain to and always be found in every patient with microtia.

3. **Paragraph** "Genes identified to be involved in the development of major syndromic microtia"
Comment: What does it mean? Major genes involved in syndromic microtia? Genes involved in the major syndromes associated with microtia? Genes involved in syndromes with microtia as a major feature? Or genes involved in the development of the ears?

Comment Response 3 :
We thank the reviewers for this comment and correction. We have corrected the sentence. What we mean here is the most common genes that identified to be involved in microtia-related syndrome.

The above excerpts do not make clear the existing etiological models and clinical groups of microtia. According to their excellent references 4, 7, 10, 12, and 130, we have:

1. ETIOLOGY OF NON-SYNDROMIC (ISOLATED) MICROTIA
    1. Sporadic microtia: it corresponds to the majority of cases and follows the multifactorial model, i.e. genetic liability (polygenes) plus environmental triggered factors ("maternal sociodemographic variables, multiple gestation, gestational diabetes", etc). This model explains the mild male predominance and ethnic-geographic variation of the phenotype;

    2. Familial microtia (3-34% of the cases): it includes the expected precurrence and recurrence of isolated microtiarelated to the multifactorial model, and rare cases of single gene/Mendelian disorders segregating as a dominant,recessive, or X-linked trait in families.

    3. Unrecognized

2. ETIOLOGY OF SYNDROMIC MICROTIA:
    1. Single gene (Mendelian) disorders, for example, Treacher-Collins syndrome;

    2. Cytogenetic and cytogenomic rearrangements, for example, trisomy, translocation, deletion syndromes;

    3. Teratogenic, for example, retinoic acid embryopathy;

    4. Unrecognized: non-random pattern of multiple congenital malformations.

Response to these comment :
We thank the reviewers for these comments and suggestions regarding the etiology of microtia whether it is an isolated or associated syndrome. Here in our review, we want to highlight the specific genetic factors associated with microtia although many studies have found various genetic disorders in microtia.

AIM, METHODS, RESULTS, AND DISCUSSION
To "conducted the first systematic review to qualitatively identify the most important genes in the development of microtia" authors have selected 98 articles consisting of 29 case reports, 6 case-control, and 63 case series studies.

I agree that this strategy is suitable for the above aim. However, the selected studies do not allow the following statements:

1. Paragraph "(...) In this study, China had the highest number of microtia cases. The reported prevalence of microtia/anotia varies between 1 in 3000 to 1 in 20.000 births.[1,2] The prevalence might vary by country and race/ethnicity but this is likely dependent on what forms of microtia are included in studies.[20,21]"

and

2. Paragraph "According to this study, most cases of microtia (62.6%) occurred in China, 51.9% of subjects had a familyhistory of microtia, 57.4% of cases occurred in males, 71.8% of cases were bilateral, and 47.7% of cases were grade IImicrotia."

To do so, authors should systematically review population-based, hospital-based, multicenter-based, international, ornational-wide studies.

Response to these comment :
We thank the reviewer for giving us a chance to improve our manuscript, although it could be a very good suggestion for the next study. We did not do a systematic review by population-based, hospital-based, multicenter-based, international, or national-wide studies due to limited data / study of each classification.

In the following paragraphs, authors put together sporadic and familial cases and non-syndromic and syndromic cases which are etiologically and epidemiologically distinct:

1. Paragraph "We discovered that most patients with microtia had a family history of the disease, including 97.7% of patients with isolated microtia and 63.5% of patients with syndromic microtia. This is consistent with the existingliterature, which describes Mendelian hereditary variants of microtia with an *autosomal* dominant or recessive mode ofinheritance.[1,4,25] The rates of familial microtia ranged from 3 to 34%.[10]
Comment: No, this is not consistent with the existing literature. This is an ascertainment bias.

Response to Comment 1 :
We thank the reviewer for giving us a chance to improve our manuscript. We have double-checked and corrected the citation and reference related to the mendelian hereditary variant of microtia and the percentage of its familial variant. (Page 11, discussion section, paragraph 1)

2. Paragraph "Based on the gender classification of each study, we discovered nearly 60% of all patients were male, including 54.9% of patients with syndromic microtia and 59.1% of

patients with isolated microtia. In prior research, microtia was more common in male patients than in female patients, with a sex ratio of 1.5:1 and an estimated 20–40%greater risk in males than in females.[4,23]"
Comment: This is an ascertainment bias.

Response to Comment 2 :
We thank the reviewer for giving us a chance to improve our manuscript. We have double-checked and corrected the citation of references that accordance with the results of our review. (Page 11, discussion section, paragraph 2)

3. Paragraph "Although microtia can arise bilaterally, 77–93% of patients have unilateral involvement.[4,23] The most common form of syndromic microtia is bilateral microtia.1 Although bilateral microtia was more common in this study, the type of microtia was only known for 34.6% of subjects. Because of the inadequate data, this could represent a biased outcome for the most prevalent type of microtia".
Comment: This is an ascertainment bias.

Response to Comment 3 :
We thank the reviewer for giving us a chance to improve our manuscript. We have double-checked and corrected the citation of references that accordance with the results of our review. (Page 11, discussion section, paragraph 3) Although microtia can occur bilaterally, 77–93% of affected individuals have unilateral involvement.[4] Syndromic forms of microtia occur in conjunction with other abnormalities and the associated malformations are mainly found in bilateral cases.[1] In our study, the most common microtia cases found was bilateral and only 34.6% of all subjects known for its type of microtia. Because of the inadequate data, this could represent a biased outcome for the most prevalent type of microtia.

Concerning the aim of the study in itself:
1. Paragraph "in isolated microtia, the most common type of mutation in HOXA2 was silent mutation (54.2%)".**Comment:** A silent mutation occurs when the nucleotide alteration does not affect the amino acid of the protein. As expected, these alterations do not change the phenotype. Reported causative alterations of *the HOXA2* gene are nonsense variants that segregate as autosomal dominant or recessive non-syndromic microtia (References 1, 17, 22,50...)

Response to this comment :
Thank you for this comment. We have double-checked the calculation of type mutation in HOXA2. We found that the most common type of mutation in HOXA2 is nonsense variant mutation 16 out of 22 (72.7%).

2. Paragraph "This was consistent with the literature, which indicated that the most common genes identifed in microtia were HOXA2, FGF3, HOXD, ORC1, ORC4, ORC6, CDT1, CDC6, DHODH, HMX1, EYA1, and.TCOF1.[2]"
Comment: Reference 2 (Luquetti DV, *et al.*, 2011) does not mention genes involved with microtia.

Response to this comment :

Thank you for this comment. We have double-checked the reference and revised it.

3. Paragraph "FGF3 haplo insufficiency could also be related with dental and hearing problems".
Comment: *FGF3* alterations are a well-established etiology for LAMM syndrome (MIM #610706).
Response to this comment :
Thank you for this comment. We have edited the sentence to avoid misunderstanding.

4. Paragraph "A significant percentage of microtic patients also present deficient facial components that originate from the same embryological structures."
Comment: Microtic patients is a pejorative term.

Response to this comment :
We thank the reviewer for this comment and for giving us a chance to improve our manuscript. We did not mean to belittle anyone here. Hence we have corrected our words with better terms.

5. Paragraph "In humans, recessive loss-of-function mutations in HMX1 have been linked to OVAS, which ischaracterized by external ear and eye deformity.[9]"
Comment: Reference 9 brings information on the dosage-sensitive effects of *HMX1* enhancer in patients with isolated bilateral concha-type microtia. It reports on duplications in the intergenic region downstream of *HMX1* in four families, and duplication between *HMX1* and *CPZ* in another family.

Response to this comment :
We thank the reviewer for giving us chance to improve our manuscript. We have re-read the reference and edited our sentence regarding this comment.

6. Paragraph "We hypothesized that a link existed between the types of genes involved and the grade of microtia".
Comment: Why and how?

Response to this comment :
Thank you for this comment. We hypothesized that a link existed between the types of genes involved and the grade of microtia. For example, *FGF3* deletions in *LAMM* syndrome have been clinically identified as grade I microtia.[16,17,30,31]According to the MARX classification,[10] the *HOXA2* gene was common in the form of microtia type II and was exclusive to isolated microtia.[1,20,25,44,50–53] And, no specific type of microtia has been linked to the deletion of*TCOF1.*[10,53] However this requires further studies to confirm these data.

7. Paragraph "Approximately 80.2% of the genetic factors that contribute to microtia remain unknown".
Comment: Authors have explained how they achieved this figure in their response letter: "We got this number from the calculation of the number of 100% of genetic factors involved

in this birth defect minus 3 most common genes found in our systematic review: %HOXA2 (6.4%) minus %FGF3 (4.9%) and minus %TCOF1 (8.5%)". This is not an appropriate way to measure or estimate the genetic contribution to complex and etiologically heterogeneous congenital malformations such as microtia.

Response to this comment :
We thank the reviewers for these comments. We have removed the line regarding this comment to set it straight.

Tables: the authors' responses to my prior comments 8 and are not appropriate.

REFERENCES
1. There are some duplicated references. :

Response: We thank the reviewer for the correction and comment. We have doubled check the references and deleted duplicated references.

2. Reference number 74 is the manuscript under review:

Response: Thank you for this comment. We have added citations here to state the supplementary files

Competing Interests: We declare that we have no competing interest

Version 1

Reviewer Report 09 August 2022

https://doi.org/10.5256/f1000research.123721.r143637

Isabella Monlleó 12
[1] Faculty of Medicine, Clinical Genetics Service, University Hospital, Universidade Federal de Alagoas, Maceió, State of Alagoas, Brazil
[2] Faculty of Medicine, Clinical Genetics Service, University Hospital, Universidade Federal de Alagoas, Maceió, State of Alagoas, Brazil
[3] Faculty of Medicine, Clinical Genetics Service, University Hospital, Universidade Federal de Alagoas, Maceió, State of Alagoas, Brazil

Microtia is a multidisciplinary and exciting subject. Although easy to recognize clinically, there is a

lack of consensus on its definition and terminology, so, over the last 80 years, several classification systems have been launched and coexist. For many dysmorphologists, surgeons, and researchers, microtia is a spectrum of congenital anomalies spanning from minor dysmorphologies of the external ear to anotia plus the absence of the auditory canal. Therefore, it can be referred to as microtia, anotia, or microtia-anotia, involving only the external or the external and middle ear (Luquetti *et al.* 2011[1]; 2012[2]). Whatever definition is employed, this congenital anomaly may be seen as an isolated defect or as a clinical sign of multiple anomalies collectively named syndromic microtia, several related to recognized syndromes (Luquetti *et al.* 2011[1]; 2012[2]). The subtle and age-dependent nature of some dysmorphologies and the above-mentioned disagreement may result in inconsistent phenotypic data that lead to misdiagnoses and research selection bias.

In the case of syndromic microtia, the etiology encompasses chromosomal abnormalities (aneuploidies, large rearrangements, and submicroscopic disequilibrium), single gene disorders, and teratogenic embryopathies. Different genetic approaches have been used as karyotyping, chromosomal microarray analysis, and DNA sequencing, to extend the list of known genes and causative variants (Gendron *et al.* 2016[3], Luquetti *et al.* 2011[1], 2012[2]). On the other hand, the etiology of isolated microtia remains poorly understood. Despite this, the higher concordance in monozygotic twins compared to dizygotic twins, the disproportional sexual distribution, the existence of multiplex families, and the prevalence variation across different geographic regions and ethnicities are strong evidence that this is a complex or multifactorial trait. Therefore, the phenotype should be looked at as the result of an imbricate gene-gene and gene-environment interaction instead of the isolated effect of each one (Chen & Zhang 2019[4], Fan *et al.* 2020[5], Gendron *et al.* 2016[3], Luquetti *et al.* 2011[1], 2012[2]).

Strategies to investigate the genetic factors underlying multifactorial phenotypes should include linkage analysis, GWAS, exome and genome sequencing, gene-editing technologies, and predictive and functional analysis, with bioinformatics support. The study of familial cases, twins, and large series of patients, and experimental studies using animal models, are equally important (Chen & Zhang 2019[4], Fan *et al.* 2020[5], Gendron *et al.* 2016[3], Luquetti *et al.* 2011[1], 2012[2]). The enormous volume of data generated by these approaches is available through many genetic databases.

Therefore, to study microtia (isolated or syndromic) is challenging and should consider the many factors involved in its etiopathogenesis and expressivity, such as genes, environment, exposition, and epigenetic alterations (Chen & Zhang 2019[4], Fan *et al.* 2020[5], Gendron *et al.* 2016[3]).

In this systematic review registered at the PROSPERO platform, the authors aimed to analyze the role of genetic factors in isolated and syndromic forms of microtia. They adhered to the PRISMA guidelines and clearly outlined their inclusion criteria, information sources, search strategies, and details of data collection.

Taking the above considerations into account and the authors' intent 'to qualitatively identify the most important genes in the development of microtia', I have some questions and suggestions:

1. Why did the authors not include human gene and human phenotype databases such as HGMD, ClinVar, MedGen, OMIM, etc., in their search strategy?

2. Why did the authors limit their review to observation studies?

3. What are the reasons for excluding 15 articles from the 55 'potentially appropriate articles´(Figure 1)?

4. Why was the authors' list of genes involved in the microtia phenotype not exhaustive?

5. Why did the authors dedicate part of the discussion to epidemiological data since this was not their research question? To do so they would change or widen their searching criteria.

6. It would be appreciated if authors make clear the existence of different definitions and classification systems for microtia. Also, they would include information in this regard in Tables 1 and 2. Did the listed papers use the same definitions to grade microtia?

7. What evidence did the authors use to support their hypothesis of a genotype-phenotype correlation between microtia degrees and (alterations in) HOXA2 and FGF3 genes? And for the inexistent G-P correlation involving TCOF1?

8. Review the use of 'disorder level' and 'gene assessed' in Tables 1 and 2 since some studies have reported CNVs and chromosomal rearrangements.

9. Review the inclusion of the 22q11.2 syndrome as an example of isolated microtia in Table 2.

10. *Is the statistical analysis and its interpretation appropriate?* Frequency distribution was the single statistical method employed in this study that reviewed 40 case reports/series and a few case-control studies. This method and sources are not suitable to conclude the epidemiology and etiology of microtia. Authors should include national and international registries on birth defects, such as The International Clearinghouse for Birth Defects Surveillance and Research (ICBDSR), the Estudio Colaborativo Latinoamericano de Malformaciones Congénitas (ECLMAC), the European Surveillance of Congenital Anomalies (EUROCAT), South-East Asia Region's Newborn and Birth Defects Database (SEAR-NBBD), and search for genetic variants associated with microtia in human gene and phenotype databases. They should also employ robust statistics to conclude on prevalence and incidence rates worldwide. Finally, considering that microtia is a heterogenous phenotype, what is the evidence to state that 76.2% of the genetic factors involved in this birth defect remain unknown? Are the authors postulating that 23.8% are already known? I have not seen any statistics to support this inference.

References

1. Luquetti DV, Leoncini E, Mastroiacovo P: Microtia-anotia: a global review of prevalence rates. *Birth Defects Res A Clin Mol Teratol*. 2011; **91** (9): 813-22 PubMed Abstract | Publisher Full Text
2. Luquetti DV, Heike CL, Hing AV, Cunningham ML, et al.: Microtia: epidemiology and genetics.*Am J Med Genet A*. 2012; **158A** (1): 124-39 PubMed Abstract | Publisher Full Text
3. Gendron C, Schwentker A, van Aalst JA: Genetic Advances in the Understanding of Microtia.*J Pediatr Genet*. 2016; **5** (4): 189-197 PubMed Abstract | Publisher Full Text
4. Chen X, Zhang R: Microtia epigenetics: An overview of review and new viewpoint.*Medicine (Baltimore)*. 2019; **98** (41): e17468 PubMed Abstract | Publisher Full Text
5. Fan X, Ping L, Sun H, Chen Y, et al.: Whole-Exome Sequencing of Discordant Monozygotic Twin Families for Identification of Candidate Genes for Microtia-Atresia.*Front Genet*. 2020; **11**: 568052

PubMed Abstract | Publisher Full Text

Are the rationale for, and objectives of, the Systematic Review clearly stated?
Partly

Are sufficient details of the methods and analysis provided to allow replication by others?
Partly

Is the statistical analysis and its interpretation appropriate?
No

Are the conclusions drawn adequately supported by the results presented in the review?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Medical and human genetics, craniofacial anomalies, intellectual disability, disorders of sex development

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 17 Oct 2022

Citrawati Wungu

Reviewer 2

Comment 1:
Why did the authors not include human gene and human phenotype databases such as HGMD, ClinVar, MedGen, OMIM, etc., in their search strategy?

Comment Response 1:
We thank the reviewer for the excellent comments to improve our manuscript. We did not include human gene and human phenotype databases such as HGMD, ClinVar, MedGen, OMIM, etc., in our search strategy because we need certain data used in each study to be analyzed in our systematic review. The information provided on these databases is not intended for direct diagnostic use or medical decision-making without review by a genetics professional[1,2] and it only collates all known (published) gene lesions responsible for human inherited disease[3]. This statement was disclaimed on their website. In addition, the systematic review should obtain sources from primary studies, not from genetic databases.

Comment 2:
Why did the authors limit their review to observation studies?

Comment Response 2:

We thank the reviewer for giving us an excellent comment to improve our manuscript. We did limit our systematic review to observational studies/non-experimental studies of syndromic microtia and isolated microtia to be analyzed objectively in our study without any intervention from the researcher. Besides, it is impossible to take data from clinical trials/experimental studies as this type of study is not suitable for microtia.

Comment 3
What are the reasons for excluding 15 articles from the 55 'potentially appropriate articles' (Figure 1)?

Comment Response 3:
Thank you for this excellent input and comment, which have helped us to improve our manuscript. We have added the explanation of the excluded articles as requested and additional revision of the PRISMA flow in our study (page 4).

Comment 4:
Why was the authors' list of genes involved in the microtia phenotype not exhaustive?

Comment Response 4:
We thank the reviewer for giving us a chance to improve our manuscript. We have repeated the search for articles using the same keywords to get comprehensive results and added missing genes that can cause microtia in our studies (pages 5-9).

Comment 5:
Why did the authors dedicate part of the discussion to epidemiological data since this was not their research question? To do so they would change or widen their searching criteria.

Comment Response 5:
Thank you for this excellent input and comment, which have helped us to improve our manuscript. The research question in our study was what genes were most commonly associated with microtia, including syndromic microtia and isolated microtia. In the discussion section, in addition to discussing the most common gene data involved in syndromic microtia and isolated microtia, the gene examination technique used, we also add a discussion section by analyzing patient characteristics data from each study (pages 12-15).

Comment 6:
It would be appreciated if authors make clear the existence of different definitions and classification systems for microtia. Also, they would include information in this regard in Tables 1 and 2. Did the listed papers use the same definitions to grade microtia?

Comment Response 6:
We thank the reviewer for giving us excellent input and comments to improve our manuscript. Most of the listed papers used in our study had the same definition to grade microtia, Hunter classification. To homogenize the definition to grade microtia in our study, we use the Hunter classification system for microtia when extracting data from 98 studies and data provided data in Tables 1 and 2 (pages 5-9). Thank you for your suggestion, we

would like to make clear the existence of different definitions and classification systems of microtia in our discussion (page 13).

Comment 7:
What evidence did the authors use to support their hypothesis of a genotype-phenotype correlation between microtia degrees and (alterations in) HOXA2 and FGF3 genes? And for the inexistent G-P correlation involving TCOF1?

Comment Response 7:
Thank you for this excellent input and comment, which have helped us to improve our manuscript. We have discussed in our discussion about each role of HOXA2, FGF3, and TCOF1 genes in microtia (pages 13-14).

Comment 8:
Review the use of 'disorder level' and 'gene assessed' in Tables 1 and 2 since some studies have reported CNVs and chromosomal rearrangements.

Comment Response 8 :
Thank you for this comment. We use the term "disorder level" in Tables 1 and 2 to describe the types of disorders present and "gene assessed" to describe, while the term "gene assessed" refers to the genetic material being examined and whether it is found to be abnormal in genes, chromosomes, or CNV DNA.

Comment 9 :
Review the inclusion of the 22q11.2 syndrome as an example of isolated microtia in Table 2.

Comment Response 9:
We thank the reviewer for giving us a chance to improve our manuscript. We have repeated the search for articles using the same keywords to get comprehensive results and added missing genes that can cause microtia in our studies (pages 5-9).

Comment 10:
Is the statistical analysis and its interpretation appropriate? Frequency distribution was the single statistical method employed in this study that reviewed 40 case reports/series and a few case-control studies. This method and sources are not suitable to conclude the epidemiology and etiology of microtia. Authors should include national and international registries on birth defects, such as The International Clearinghouse for Birth Defects Surveillance and Research (ICBDSR), the Estudio Colaborativo Latinoamericano de Malformaciones Congénitas (ECLMAC), the European Surveillance of Congenital Anomalies (EUROCAT), South-East Asia Region's Newborn and Birth Defects Database (SEAR-NBBD), and search for genetic variants associated with microtia in human gene and phenotype databases. They should also employ robust statistics to conclude on prevalence and incidence rates worldwide.

Comment Response 10:
Thank you for this comment. As we know, A systematic review is a summary of the medical literature that uses explicit and reproducible methods to systematically search, critically

appraise, and synthesize on a specific issue. It synthesizes the results of multiple primary studies related to each other by using strategies that reduce biases and random errors[4,5]. BDSR, ECOMAC, EUROCAT, SEARCH-NBBD provide a large database as a result of a collection of various studies, we did not use the data from the ones mentioned above in our systematic review because it would be a secondary big data analysis, not the systematic review we expected from the start.

Comment 11:
Finally, considering that microtia is a heterogenous phenotype, what is the evidence to state that 76.2% of the genetic factors involved in this birth defect remain unknown? Are the authors postulating that 23.8% are already known? I have not seen any statistics to support this inference.

Comment Response 11:
Thank you for this excellent input and comment, which have helped us to improve our manuscript. Because of the additional data on missing genes in our systematic review, the percentage of genes that remain unknown increased from 76.2% to 80%. We got this number from the calculation of the number of 100% of genetic factors involved in this birth defect minus 3 most common genes found in our systematic review: %HOXA2 (6.4%) minus %FGF3 (4.9%) and minus %TCOF1 (8.5%) (Page 12).

Competing Interests: We declare that we have no competing interest.

Reviewer Report 08 July 2022

https://doi.org/10.5256/f1000research.123721.r139395

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Walid D. Fakhouri
[1] Center for Craniofacial Research, Department of Diagnostic and Biomedical Sciences, School of Dentistry, University of Texas Health Science Center at Houston, Houston, TX, USA
[2] Center for Craniofacial Research, Department of Diagnostic and Biomedical Sciences, School of Dentistry, University of Texas Health Science Center at Houston, Houston, TX, USA
[3] Center for Craniofacial Research, Department of Diagnostic and Biomedical Sciences, School of Dentistry, University of Texas Health Science Center at Houston, Houston, TX, USA

Microtia and anotia are congenital birth defects of the external ear with a frequency that ranges from 1:2000–1:10,000 live births [1,2]. Microtia and anotia can be part of syndromic disorders or isolated events. The causes of isolated and syndromic external ear malformation remain unknown in many cases [2,3,4]. However, the genetic components play a major role in causing or increasing the risk for isolated forms of external ear disorders. While microtia is defined as a small malformed external ear, anotia is a lack of external ear. The external ear malformations happen

during the first trimester between the fourth and ninth week of pregnancy. The severity can range from mild to complete missing of the external ear with possible inner ear abnormal structures [3].

In this systematic review study, Putri *et al.* analyzed the role of genetic factors in isolated and syndromic forms of microtia. They use six search engines of scientific peer-reviewed publications to screen retrieved articles and identify the relevant articles for their analysis based on a set of inclusion and exclusion criteria established by the reviewers. The authors performed and executed their analysis according to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines. The paper provides an excellent rationale for the study with clear objectives of the systematic review. The paper is also well-written and structured and includes the strength and limitations of its analysis of the retrieved articles. However, the description of the prevalence of microtia in the mentioned countries based on the included studies in their systematic review might be misleading or inaccurate. The authors cannot draw a straight line on the microtia prevalence in these countries based only on a few case reports, case-control, observational studies, or a lack of clinical studies in other countries. The authors need to validate the prevalence of microtia in these countries based on other official sources like the CDC or the authors might indicate that the incidences of the cases based on the analyzed studies included in the systematic review might infer the frequency in these countries.

Although 15 articles were relevant to the systematic review based on the titles and abstracts, the authors excluded them from the analysis after the full text was read without providing additional information or justification on why.

One point that has not been explained in the systematic review is that the altered genes in syndromic disorders of microtia did not contribute to the isolated form of microtia.

Finally, the authors also need to explain why their systematic review analysis missed several genes that mutations within their loci can cause microtia as part of syndromic disorders. The other genes that are related to microtia are described below:

- Mutations in **POLR1B** cause Treacher-Collins syndrome 4, an autosomal dominant disorder, characterized by craniofacial dysmorphisms, including downslanting palpebral fissures, malar, and mandibular hypoplasia, and microtia (Sanchez *et al.*, 2020[5]).

- Mutations within **TWIST2** cause Ablepharon-macrostomia syndrome, an autosomal dominant disorder, characterized by congenital ectodermal dysplasia characterized by absent eyelids, macrostomia, microtia, redundant skin, sparse hair, dysmorphic nose and ears, variable abnormalities of the nipples, genitalia, fingers, and hands, largely normal intellectual and motor development, and poor growth (Marchegiani *et al.*, 2015[6]).

- **HSPA9** mutations cause Even-plus syndrome (AR), which is characterized by prenatal-onset short stature, vertebral and epiphyseal changes, microtia, midface hypoplasia with a flat nose and triangular nares, cardiac malformations, and other findings including anal atresia, hypodontia, and aplasia cutis (Royer-Bertrand *et al.*, 2015[7])

- **EFTUD2** mutations cause mandibulofacial dysostosis, Guion-Almeida type (AD), a syndrome characterized by progressive microcephaly, midface and malar hypoplasia, micrognathia, microtia, dysplastic ears, preauricular skin tags, significant developmental delay, and speech delay (Lines *et al.*, 2012[8])

References
1. National Birth Defects Prevention Network: Selected birth defects data from population-based birth defects surveillance programs in the United States, 2004-2008. *Birth Defects Research (Part A): Clinical and Molecular Teratology*. 2011; **91**: 1076-1149
2. Canfield MA, Langlois PH, Nguyen LM, Scheuerle AE: Epidemiologic features and clinical subgroups of anotia/microtia in Texas.*Birth Defects Res A Clin Mol Teratol*. 2009; **85** (11): 905-13 PubMed Abstract | Publisher Full Text
3. Luquetti DV, Heike CL, Hing AV, Cunningham ML, et al.: Microtia: epidemiology and genetics.*Am J Med Genet A*. 2012; **158A** (1): 124-39 PubMed Abstract | Publisher Full Text
4. Fakhouri W, Letra A: Identification of Disease Risk DNA Variations is Shaping the Future of Precision Health. *Genes*. 2019; **10** (6). Publisher Full Text
5. Sanchez E, Laplace-Builhé B, Mau-Them FT, Richard E, et al.: POLR1B and neural crest cell anomalies in Treacher Collins syndrome type 4.*Genet Med*. **22** (3): 547-556 PubMed Abstract | Publisher Full Text
6. Marchegiani S, Davis T, Tessadori F, van Haaften G, et al.: Recurrent Mutations in the Basic Domain of TWIST2 Cause Ablepharon Macrostomia and Barber-Say Syndromes.*Am J Hum Genet*. 2015; **97** (1): 99-110 PubMed Abstract | Publisher Full Text
7. Royer-Bertrand B, Castillo-Taucher S, Moreno-Salinas R, Cho TJ, et al.: Mutations in the heat-shock protein A9 (HSPA9) gene cause the EVEN-PLUS syndrome of congenital malformations and skeletal dysplasia.*Sci Rep*. 2015; **5**: 17154 PubMed Abstract | Publisher Full Text
8. Lines MA, Huang L, Schwartzentruber J, Douglas SL, et al.: Haploinsufficiency of a spliceosomal GTPase encoded by EFTUD2 causes mandibulofacial dysostosis with microcephaly.*Am J Hum Genet*. 2012; **90** (2): 369-77 PubMed Abstract | Publisher Full Text

Are the rationale for, and objectives of, the Systematic Review clearly stated?
Yes

Are sufficient details of the methods and analysis provided to allow replication by others?
Yes

Is the statistical analysis and its interpretation appropriate?
Yes

Are the conclusions drawn adequately supported by the results presented in the review?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Genetics, Craniofacial development and disorders

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 10 Aug 2022

Citrawati Wungu

Thank you for the comments on our manuscript entitled "The Role of Genetic Factors in Microtia: A Systematic Review". We appreciate the suggested modifications and have revised the manuscript accordingly. The revised sections are shown in boldface type. The detailed responses to the reviewers' comments are presented as follows:

Reviewer 1

Comment 1:
The authors need to validate the prevalence of microtia in these countries based on other official sources like the CDC or the authors might indicate that the incidences of the cases based on the analyzed studies included in the systematic review might infer the frequency in these countries.

Comment Response 1:
We thank the reviewer for the excellent input, comments, and suggestions, which have helped us to improve our manuscript. We have checked and revised the global prevalence of microtia based on the CDC according to the suggestions. Hopefully, this revision suffices and our manuscript can be considered for indexing (Page 8, last line).

Comment 2:
Although 15 articles were relevant to the systematic review based on the titles and abstracts, the authors excluded them from the analysis after the full text was read without providing additional information or justification on why.

Comment Response 2:
Thank you for this comment. We have now added the explanation regarding why we excluded them from the analysis after the full text was read (Figure 1. Prisma Flow diagram).

Comment 3:
One point that has not been explained in the systematic review is that the altered genes in syndromic disorders of microtia did not contribute to the isolated form of microtia.

Comment Response 3:
Thank you for this excellent input and comment, which has helped us to improve our manuscript. We have added the explanation as requested (Page 14).

Comment 4:
Finally, the authors also need to explain why their systematic review analysis missed several genes that mutations within their loci can cause microtia as part of syndromic disorders. The other genes that are related to microtia are described below

Comment Response 4:
We thank the reviewer for giving us a chance to improve our manuscript. We have repeated the search for articles using the same keywords to get comprehensive results and added

missing genes that can cause microtia in our studies.

Competing Interests: We declare that we have no competing interest.

The role of genetic factors in microtia

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the BMP5 Gene Mutation", Journal of Craniofacial Surgery, 2022
Publication

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Publication

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Sanio, Andrew K. Groves, Tosso Leeb, and Giorgio Gimelli. "Congenital aural atresia associated with agenesis of internal carotid artery in a girl with a FOXI3 deletion", American Journal of Medical Genetics Part A, 2015.
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---

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Publication

---

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---

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with 4p Deletion and Functional Xp Disomy", Cytogenetic and Genome Research, 2016
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Syndrome and Review of the Current Literature", Molecular Syndromology, 2022
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76 N/A. "Current World Literature", Current Opinion in Obstetrics and Gynecology, 04/2007
Publication
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variation", European Journal of Human Genetics, 11/2004
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musculus)", Comparative Exercise Physiology, 2023
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Publication

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