Genetic Advances in the Understanding of Microtia

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Abstract

Microtia is a genetic condition affecting the external ears and presents clinically along a wide spectrum: minimally affected ears are small with minor shape abnormalities; extremely affected ears lack all identifiable structures, with the most extreme being absence of the entire external ear. Multiple genetic causes have been linked to microtia in both animal models and humans, which are improving our understanding of the condition and may lead to the identification of a unified cause for the condition. Microtia is also a prominent feature of several genetic syndromes, the study of which has provided further insight into the possible causes and genetic mechanisms of the condition. This article reviews our current understanding of microtia including epidemiological characteristics, classification systems, environmental and genetic causative factors leading to microtia. Despite our increased understanding of the genetics of microtia, we do not have a means of preventing the condition and still rely on complex staged, surgical correction.

Keywords
- microtia
- genetic causes
- gene
- anotia
- embryology

Introduction

Microtia encompasses a spectrum of congenital anomalies of the auricle that range in severity from mild partial structural abnormalities to complete absence of the ear (anotia).[1] Currently, there is no consensus on the terminology that should be used to describe and classify this condition. Some authors prefer to use the term "microtia"[2–5] while others use "microtia–anotia" or "microtia/anotia."[6–10] The term "microtia" includes anotia (complete absence of the ear) as the most severe end of the microtia spectrum for the purpose of this review.

The condition presents with aesthetic concerns for the child and parents and can lead to severe psychological sequelae secondary to the condition itself and the complexity of surgical treatments to correct associated anomalies as well as the ear deformity. Because microtia is associated with absence of the external ear canal, the condition may present with hearing problems, and if the clinical presentation is bilateral, may be associated with significant language delays.

Embryology and Anatomy

The external ear is composed of several important landmarks that may be affected to varying degrees in microtia and are depicted in ►**Fig. 1**. The external ear begins to develop during the 5th week of gestation from the first and second pharyngeal arches on the ventral surface of the embryo.[5,11] Mesenchymal cells of mesodermal and cranial neural crest origin are the primary cell type in the pharyngeal arches. Reciprocal signaling between neural crest cells (NCC) and the craniofacial ectoderm plays an important role in driving facial development, including that of the external ear.[12,13] The historically accepted embryological pattern for ear development has been that the pharyngeal arches give rise to six hillocks that form the major anatomic structures of the ear. Hillocks one to three arise from the first arch and form the tragus, helical root, and helix. The second arch gives rise to hillocks four to six which comprise the antihelix, concha, and antitragus (►**Fig. 2**). During the 7th week of gestation, the

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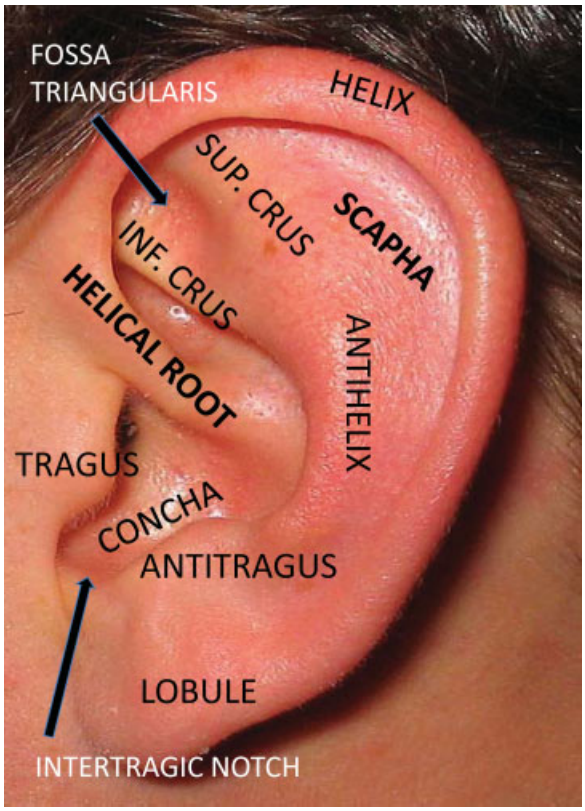

Fig. 1 Normal anatomy of the external ear.

auricular hillocks differentiate, enlarge, and eventually fuse. This morphogenesis determines the final auricular shape, size, and position. The ears gradually move from their initial position low on the neck to their final more cranial position.[11]

Recent reports, however, raise doubts as to the actual origin of the major anatomic subunits of the ear including which tissues actually give rise to the external auditory canal (EAC). Minoux et al found that the mouse pinna derives from the *HOXA2*-expressing neural crest–derived mesenchyme of the second pharyngeal arch, and not from a composite of first and second arch mesenchyme as previously proposed using genetic fate mapping.[14] These authors also demonstrated that the mouse EAC is entirely lined by *HOXA2*-negative first arch mesenchyme and does not develop at the first pharyngeal cleft, as previously assumed. Given these new findings, a reorganization of our understanding of the embryonic origin of the anatomical structures of the ear is underway.

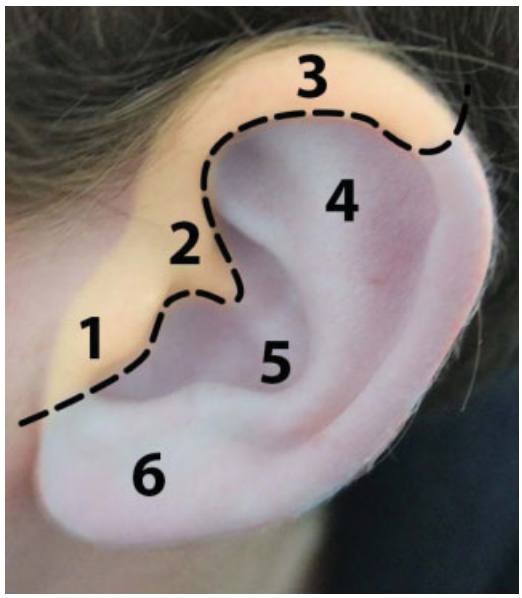

Fig. 2 Yellow shading indicates embryological origin from branchial arch 1: 1, tragus; 2, helical root; 3, helix. Light purple indicates a branchial arch 2 origin: 4, antihelix; 5, antitragus; 6, lobule.

Incidence

Population-based studies on the incidence of microtia in Italy, France, Sweden, Finland, and the United States range between 0.83 and 4.34 per 10,000 births.[3,7–10,13] Studies conducted using nonpopulation-based data reported higher rates for Ecuadorians,[2,15] Chileans, and among Native Americans in the United States.[16–18] Among U.S. Native Americans, the overall incidence is between 1/4,000 and 1/6,500 live births. This is even higher in the Navajo population. In a study of 15,890 Navajo Indians, 1:935 were found to have microtia, specifically clustered to one quarter of the Western reservation.[18] Differing rates between studies may be due to under- or overreporting in hospital records because there is no standard definition or classification system used for what constitutes microtia—not at the extreme end (anotia), which is easy to identify clinically, but at the less extreme end, where the auricle is small with more limited structural abnormalities.

Classification Systems

Many classification systems have been proposed for ear abnormalities on the microtia spectrum, the first of which was proposed by Hermann Marx in 1926. In 1978, Tanzer attempted to classify the anomalies according to their surgical correction.[19] Finally, Weerda modified and combined these classifications and added embryologic development as part of the metric for organization.[13,20] Hunter et al published a standardized terminology article in the *American Journal of Genetics* in an attempt to improve reporting on microtia and chose the Weerda classification system as the basis for their proposed classification system (►**Table 1**; ►**Fig. 3**).

Despite the difficulties associated with accurate classification of the range of presentations in microtia, it is imperative to adopt a clear classification system to assist with data collection, practitioner communication, and our understanding of the genetic links to variations in presentation of microtia.

Risk Factors for Developing Microtia

Vascular Disruption

Though vascular disruption as an explanation for the development of microtia is losing ground, there continue to be adherents of this theory.[21] Localized ischemia and tissue

Table 1 Summary of Weerda and Hunter et al classifications for microtia

Weerda classification	Hunter et al classification
First degree dysplasia	Microtia, first degree
Most structures of a normal auricle are recognizable (minor deformities): (A) macrotia, (B) protruding ears, (C) cryptoptia, (D) absence of upper helix, (E) Small deformities, (F) colobomata, (G) lobule deformities, (H) cup ear deformities	Presence of all the normal ear components and the median longitudinal length more than 2 SD below the mean
Second degree dysplasia	Microtia, second degree
Some structures of a normal auricle are recognizable: (A) cup ear deformity type III and (B) miniear	Median longitudinal length of the ear more than 2 SD below the mean in the presence of some, but not all, parts of the normal ear
Third degree dysplasia	Microtia, third degree
None of the structures of a normal auricle are recognizable: (A) unilateral, (B) bilateral, and (C) anotia (peanut ears are included in this group)	Presence of some auricular structures, but none of these structures conforms to recognized ear components
	Anotia
	Complete absence of the ear

Abbreviation: SD, standard deviation.

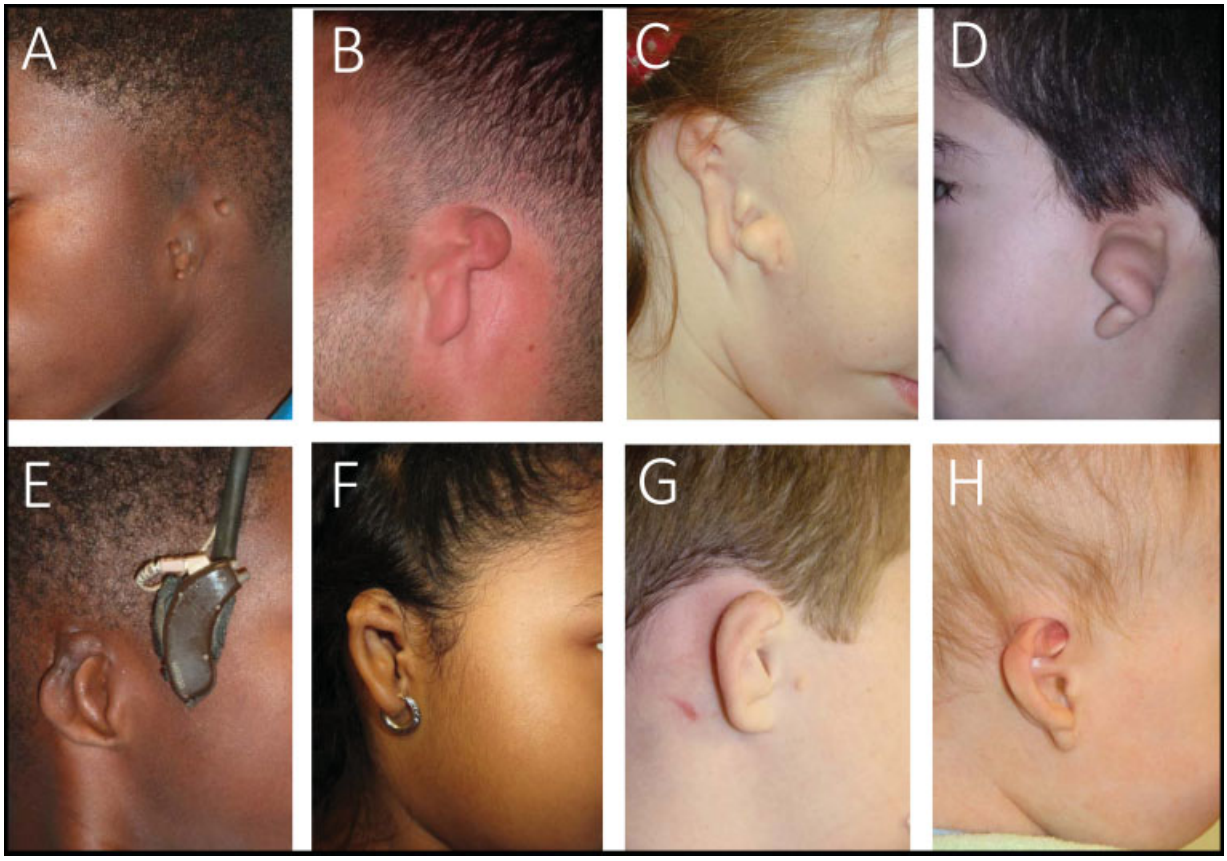

Fig. 3 Degrees of dysplasia associated with microtia: Anotia (**A**), with variations of third degree dysplasia (**B** and **C**); second degree dysplasia (**D**–**F**), and first degree dysplasia, including cup ear (**G**) and cryptotia (**H**).

necrosis may develop with disruption in the vascular supply leading to malformation of the auricular tissues. This may arise by several mechanisms including underdevelopment of the arterial system to specific tissues, occlusion of the vessels secondary to compression or hemorrhage, or by means of vasoconstriction secondary to a pharmacological or genetic cause.

Poswillo conducted the key study suggesting vascular disruption as a cause for craniofacial deformities in monkeys and mice exposed to thalidomide and triazine, respectively.

They showed that these exposures led to ipsilateral hematomas at the junction of the pharyngeal and hyoid arteries with associated unilateral ear and mandibular defects.[22,23] Further supporting this concept, a transgenic mouse line carrying a nonexpressed transgene was noted to develop a phenotype similar to hemifacial microsomia.[24,25] These authors reported rupture of the vasculature of the second pharyngeal arch with histologically confirmed hemorrhage and subsequent phagocytosis. However, a causative gene has not been identified.

Several authors refute the vascular disruption theory. A reassessment of Poswillo's work by Johnston and Bronsky led them to conclude that the hematomas occurred too late in relation to drug delivery, at a time after which the underdevelopment of tissues was already present.[26] Furthermore, they argue, the vascular disruption of a single vessel in the head and neck region could not adequately explain cases of bilateral microtia and the occurrence of microtia with other noncraniofacial malformations affecting the hands, kidneys, and heart.

Environmental Factors

Rick factors for developing microtia include maternal illness and anemia during pregnancy, diabetes, and maternal race[7,8]; high maternal or paternal age[2,7,9]; and multiple births.[8,9] A Japanese study analyzing 592 patients with microtia found 28% of the patients' mothers had a cold, imminent spontaneous abortion, or anemia during pregnancy.[27] A study conducted in Latin America found acute maternal illness during the first trimester to be a major risk factor in the development of microtia in that patient population.[28] Mothers with chronic type I diabetes are at significantly higher risk for having a child with microtia. In patients with microtia, low birth weight is more common than in healthy children.[4,6,29]

There is strong evidence for association between microtia and exposure to the teratogens retinoic acid (RA), thalidomide,[1] and mycophenolate mofetil.[30,31] In mothers exposed to isotretinoin, 83% of pregnancies result in infants with serious birth defects including microtia.[32] Exposure to high altitude,[2,16,33] and being of Hispanic, Asian, or Native American ethnicity[2,8–10,15,34] are also established risk factors.

Table 2 Genes identified in animal studies with microtia as a major feature

Animal model	Gene(s) identified
Mouse	*HOXA1*, *HOXA2*, *HOXB1*, *SIX1–4*, *TBX1*, *IRF6*, *CHUK*, *EYA*, *SALL1*, *Prx1*, *Prx 2*, *TCOF1*, *GSC*, *HMX1*, *BMP5*, *FGFR1*, *FGF8*, *FGF10*, *Wnt5a*
Chick	*HMX1*, *SIX1*
Frog	*SIX1*
Rat	*HMX1*
Cow	*HMX1*

Note: The underlined genes are also present in human studies.

Genetic Factors

There are multiple studies involving animal models suggesting that particular genetic pathways cause microtia (►**Table 2**). In an excellent review on the genetics of microtia, Luquetti et al[34] propose that a genetic cause for microtia is suggested by five observations: (1) higher concordance in monozygotic twins (38.5%) than in dizygotic twins (4.5%),[35] (2) estimates of familial cases ranging from 3 to 34%,[2,6,27,36] (3) reports of familial cases with autosomal recessive or dominant modes of inheritance with variable expression and incomplete penetrance,[37–49] (4) more than 18 distinct microtia-associated syndromes for which single-gene defects or chromosomal aberrations have been reported, and (5) mouse models demonstrating that mutations in specific genes *HOXA2*, *SIX* and eyes absent (*EYA*), *TBX1*, *IRF6*, and *CHUK* result in microtia.

Genetic Studies in Animals

Murine models have elucidated the mechanisms of aberrations in craniofacial development, including microtia. Auricular defects in these mice range from mild deformities to complete anotia (similar to the spectrum in humans). The *Hox* genes are a large group of homeobox genes, which express critical transcription factors in embryonic development and are strongly linked to microtia. NCC in the second branchial arch express *HOXA2* over a prolonged period of time.[4,50] *HOXA2* knockout mice present with microtia.[51–53] In addition, inactivation of *HOXA1* in a mouse model results in hypoplastic external ears, and a combination of *HOXA1* and *HOXB1* knockout mice present with complete anotia.[54]

Members of the *SIX* homeobox gene family (*SIX1–6*) have been implicated in external ear development.[55] *SIX* functions appear conserved across evolutionary development evidenced by the fact that knockout of *SIX1* in frogs, chicks, and mice, all result in craniofacial abnormalities.[56–58] *SIX1* and *SIX4* exert their effect via *PAX3* gene expression, which controls the early steps of myogenic cell delamination and migration from the somite.[59] Heterogeneous *SIX1* to *SIX4* mice present with microtia as well as severe rib anomalies.

EYA forms a complex with *SIX* (*EYA–SIX*) to regulate the development of several tissues and organs. Natural target genes of the *EYA–SIX* complex include *SIX2* and (sal-like-1) *SALL1*. Studies on *EYA1* expression have shown a major role in pinna development, apparently related to cartilage formation, while knockout mice for *EYA1* present with anotia.

TBX1 is a member of the T-box gene family of transcription factors. Mutations in *TBX1* result in failure of middle and outer ear development in a mouse model. Inactivation of *TBX1* in the pharyngeal arch endoderm causes similar defects in outer ear formation indicating a primary role for this gene in pharyngeal arch morphogenesis.[60]

Mice that are homozygous null for *IRF6* lack external ears in addition to exhibiting abnormal skin, limbs, and short snouts and jaws. A similar phenotype was observed in mice deficient for *CHUK* (also known as *IKK-α*). Abnormalities in the *IRF6* and *CHUK* mice are secondary to defects in epidermal differentiation and cell proliferation.[61,62] In the double mutant mice model for *Prx1*|*Prx2* homeobox genes, defects in the

external, middle, and inner ear, a lack of tympanic rings, cleft mandible, and polydactyly are noted.[63] Treacher Collins–Franceschetti 1 (*TCOF1*) encodes a protein named treacle. Expression studies of *TCOF1* in the mouse embryo support a role for treacle in the development of the craniofacial complex. Creation of a knockout mouse model for *TCOF1* results in heterozygous mice with severe craniofacial malformations.[4,64] Human goosecoid (*GSC*) is a homeodomain transcription factor and a downstream target of endothelin. Mice with a homozygous disruption of *GSC* have multiple developmental defects including the ear.[65]

HMX1 is a homeodomain transcription factor found in the developing mouse and chick nervous system and eye.[66] HMX1 expression appears in the branchial arches in the mouse model.[67] Mutations in the HMX1 locus have also been identified in rats and cows, leading to what appear to be isolated ear malformations. These isolated auricular malformations have been attributed to disruption of a conserved noncoding element downstream.

Signaling Pathways

Growth factors involved in signaling pathways of outer ear development include the bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs), RA, and wingless/INT (Wnts). Dysregulation of these signaling pathways can lead to malformations of the auricle. BMPs, especially BMP5, have been considered as candidate genes for microtia in humans; however, studies in mice have shown that BMP5 is more related to ear growth than the actual formation of the external ear.[13] FGFR1–3 play various roles in pinna development.[68,69] FGF8 and -10 mutant mice present with small outer ears.[68] Mice homozygous for a hypomorphic FGFR1 allele present with very small ears and abnormal EACs.[70] Retinoid embryopathy results in apoptosis of NCC before migration into the pharyngeal arches has been observed and may interfere with cell survival once present in the pharyngeal arches.[71] Members of the Wnt family have been implicated in NCC formation and development. It has been shown that Wnt5a is expressed in the mesenchyme of the developing outer ear and Wnt5a knockout mice present with small ears.[72]

Human Studies in Microtia

A wide range of patients with microtia (15–60%) present with additional abnormalities.[2,4,8,10,13,15] Among 5 million live and still births, 818 cases were identified as having at least one major associated congenital anomaly; these findings form the basis for defining specific syndromes. The most frequent simultaneous dysmorphic features associated with microtia are cleft palate (12.8%), cleft lip and palate (11.5%), anophthalmia/microphthalmia (11.5%), facial asymmetry (10.6%), macrostomia (6.4%), preaxial polydactyly (2.2%), holoprosencephaly (2.2%), and epibulbar dermoids (1.7%).[34]

The most common syndromes associated with microtia are oculoauriculovertebral spectrum (OAVS), Goldenhar syndrome (GS)/hemifacial microsomia/craniofacial microsomia (CFM), Treacher Collins, Nager, DiGeorge, or 22qdeletion syndrome, Townes–Brock syndrome (TBS), and branchio-oto-renal (BOR) syndrome.[4,13]

Oculoauriculovertebral Spectrum

OAVS is the most extensively studied syndrome associated with microtia. The reader is referred to the article recently published by Beleza-Meireles et al for a comprehensive review of all available genetic literature on OAVS.[73] OAVS is a complex heterogeneous disorder involving the first and second branchial arch derivatives. The OAVS is broad, with anomalies including facial asymmetry resulting from maxillary and/or mandibular hypoplasia; preauricular or facial tags; ear malformations such as microtia, anotia, or aural atresia; and hearing loss. In a Turkish population with GS, microtia was present in 52% of patients.[4,74]

Forty percent of patients with OAVS show a strong allelic expression of *BAPX1*, a gene that belongs to the NK-2 family of transcription factors and plays an essential role in craniofacial development.[75] BAPX1 anomalies are present in patient fibroblasts, suggesting that epigenetic dysregulation of BAPX1 plays an important role in this syndrome.[76]

Goldenhar Syndrome/Hemifacial Microsomia/Craniofacial Microsomia

CFM is a congenital condition characterized by asymmetric hypoplasia of the craniofacial structures, most commonly including the mandible and ear.[77] Other clinical features for diagnosis include cranial nerve palsies, epibulbar dermoids, maxillary hypoplasia, soft tissue deficiency, orbital asymmetry, and extracranial malformations.[78,79] Heterozygous mutations in the *EFTUD2* gene have been identified in a subgroup of patients with mandibulofacial dysostosis with microcephaly that overlaps with CFM.

A suggestive linkage to a region on chromosome 14q32 was found by genome-wide linkage analysis in two families with features of CFM.[80] The most interesting candidate gene in the linked region was *GSC*. No disease-causing mutation in the coding region of the gene analyzed by Southern blotting could be identified in these two families or in 120 sporadic cases of CFM.[78]

Treacher Collins Syndrome

Treacher Collins syndrome is an autosomal dominant disorder that presents phenotypically with hypoplastic facial bones, microtia, micrognathia, cleft palate, and hearing abnormalities.[64,81] Mutations in the *TCOF1* gene have been identified as the cause of Treacher Collins syndrome in up to 78% of patients.[82–85] *TCOF1* encodes a protein called treacle, which plays an active role in the early embryonic development in structures that become bones and other tissues of the face.

Nager Syndrome

Nager syndrome presents with micrognathia, external ear defects, EAC stenosis, bilateral conductive hearing loss, cleft palate, down-slanting palpebral fissures, a high nasal bridge, hypoplastic or absent thumbs, and variable lower limb and toe defects.[86–88] Most cases of Nager syndrome are sporadic, although both autosomal recessive and dominant familial cases have been reported.[89,90] DNA sequencing of eight patients with Nager syndrome for a possible mutation in

either of the *PRX1* and *PRX2* genes was performed, but no pathogenic variant was found.[91]

DiGeorge Syndrome

DiGeorge syndrome is one of the most common presentations of microdeletion associated with 22.q11.2 deletion syndrome. In most cases, the deletion eliminates 3 mbp of DNA encoding for approximately 30 genes.[92] Specifically, the human *TBX1* gene, which is required for ear development and is expressed in multiple tissues during embryogenesis, is deleted.[93] Features of DiGeorge syndrome include ear defects, hearing impairment, craniofacial abnormalities, thymus and parathyroid gland hypoplasia, and heart malformations.[94] The ears are typically low-set, small and with abnormal folding of the pinna.

Townes–Brock Syndrome

TBS is a rare autosomal dominant syndrome with a combination of anal, renal, limb, and ear anomalies. TBS is caused by mutations in the *SALL1* gene on chromosome 16q.[4] TBS and GS have a significant number of overlapping features, including first and second arch defects and preaxial defects of the upper limbs. The phenotypic similarities between TBS and GS suggest that they may have a common genetic etiology.[95]

Branchio-oto-renal Syndrome

Branchio-otic syndrome (BOS) is an autosomal dominant developmental disorder characterized by branchial cleft cysts, auricular or EAC abnormalities, preauricular pits, and hearing loss. BOR syndrome is diagnosed when BOS is accompanied by additional malformations of the kidney or urinary tract.[96–98] Mutations in *SIX1* and *EYA1* have been shown to cause BOS, while mutations in *SIX5* and *EYA1* can cause BOR syndrome. Both are associated with microtia, among several other craniofacial defects.[99–103]

Four genetic loci have been mapped for BOS/BOR[4]: BOR1,[100] BOR2,[104] BOS2,[101] and BOS3.[102] Except for BOS2, the corresponding genes have been identified. *EYA1* was the first gene identified for BOR syndrome at the BOR1 locus[100] and is found in approximately 40% of cases.[98] The gene *SIX5* has been cloned for the BOR2 locus and missense mutations were identified in *SIX5* in 5.2% (5/95) of the patients with BOR.[104] *SIX1* is the responsible gene at the BOS3 locus.[97]

Genetic Studies in Humans

Microtia has been reported in individuals with trisomies 21 and 22, as well as with mosaicism of trisomies 13 and 18[105,106]; and in deletions of 4p, 5p; 18p, 18q; and 22q11.2.[13] Chromosomal translocations involving the 6p24 region have also been associated with bilateral microtia and orofacial clefting.[107] Microtia, usually anotia, occurs in approximately two-thirds of all patients who have a terminal deletion of 18q. The extent and nature of the chromosome 18 deletions has been studied by array comparative genomic hybridization and a critical region of 5 Mb was deleted in all patients with anotia on 18q22.3–18q23, making this a candidate chromosomal region for anotia.[108]

Table 3 Genes identified in human syndromes with microtia as a major feature

Human syndrome	Gene(s) identified
Oculoauriculovertebral spectrum	*HMX1*
Treacher Collins	*TCOF1*, *POL1RC*, *POL1RD*
Craniofacial Microsomia	*GSC*
Branchio-oto	*SIX1*, *EYA1*
Branchio-oto-renal	*SIX5*, *EYA1*
Townes–Brock	*SALL1*
Nager	*SF3B4*
Mandibulofacial dysostosis with microcephaly	*EFTUD2*
Auriculocondylar	*PLCB4*, *GNAI3*
CHARGE	*CHD7*
Lacrimo-auriculo-dental-digital	*FGFR2*, *FGFR3*, *FGF10*
Kabuki	*MLL2*, *KDM6A*
Fraser	*FRAS1*, *FREM2*, *GRIP1*

Note: The underlined genes are also present in animal studies.

A missense mutation in exon 3 of *GSC* was found by sequence analysis in 2/121 patients with isolated microtia. In the same study, screening of the BMP5 locus revealed a missense mutation in four patients. None of these mutations were detected in control subjects, suggesting a potential causative role in the development of microtia (►**Table 3**).[109]

The methylation status of the *EYA1* gene promoter was analyzed in 64 individuals with microtia and 36 healthy controls. The methylation levels at this locus were significantly lower in individuals with microtia than in controls; based on this information, these authors suggest that hypomethylation may be related to the pathogenesis of microtia.[110]

A coding variant of the *HMX1* gene underlies a recessive disorder referred to as oculoauricular syndrome which is characterized by malformations of the pinna accompanied by variable eye defects.[111,112] The same *HMX1* noncoding element, when present in different species (mouse, rats, cows, and humans), yields similar phenotypes, providing some of the strongest evidence to date for noncoding, regulatory elements playing an important role in these more "isolated" disease presentations.[113] This finding also highlights that any noncoding mutations, in *HMX1* or any other gene associated with syndromic microtia, could be sufficient to cause isolated microtia phenotypes in humans.

Current Genetic Hypotheses for Microtia

The most likely underlying cause for the development of microtia is a disturbance of NCC, although the exact mechanism(s) remain unknown. However, given the clinical heterogeneity of microtia, it is possible that different pathogenic processes affecting the NCC lead to the different grades of microtia (►**Fig. 3**).[13] In addition, defects in NCC function have

been associated with numerous craniofacial syndromes.[114] In Treacher Collins syndrome, *TCOF1* mutations result in haploinsufficiency of the protein treacle, as discussed previously, leading to insufficient ribosome genesis, diminished cell proliferation, and increased neuroepithelial apoptosis. This process results in depletion of NCC precursors leading to a reduced number of cells migrating into the first and second pharyngeal arches which results in the most severe Treacher Collins phenotype, including severe, bilateral microtia.[115]

Apoptosis of NCC before migration into the pharyngeal arches has been observed in retinoid embryopathy and may interfere with cell survival once present in the pharyngeal arches. The endothelin signaling pathway, which regulates *Hox* gene expression, is also affected by retinoid exposure and may affect the positional identity of NCC within the pharyngeal arches. In diabetes, hyperglycemia causes downregulation of *PAX3*, which encodes a transcription factor critical for early NCC survival and migration.[71] Finally, the effects of thalidomide may include downregulation of FGF8[116] and BMP signaling,[116,117] though direct antiangiogenic effects and oxidative stress are also postulated as independent mechanisms,[118,119] that disrupt NCC.

Future Directions

The final common pathway in both environmental and genetic causes resulting in the development of microtia appears to involve disruption of the number, migration, and final position of NCC within the pharyngeal arches. Though many genetic causes for microtia have been identified, with the development of animal models that predictably result in either isolated microtia or a constellation of abnormalities that includes microtia, we still do not understand the abnormal processes well enough to control them. Future work must focus on treatment strategies for microtia in animal models that may eventually be translatable to humans.

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