Skeletal muscles are necessary for normal development of vertebrate bone, cartilage, and other connective tissue structures.1 The effects that skeletal muscles have on the surrounding structures during embryonic development are generally viewed as mechanical.1 The micromovements and tone imparted by skeletal musculature are thought to serve a crucial role for normal embryogenesis of adjacent structures. This concept is supported by studies that have suggested a muscular cause to various congenital auricular deformities, and middle and inner ear development was previously reported using the same mouse model. Wild-type mouse embryos were used as controls to compare the histomorphometric outcomes.

**Background:** Mechanical stimuli imparted by skeletal muscles play an important role during embryonic development in vertebrates. Little is known whether skeletal muscles are required for normal external ear development.

**Methods:** We used Myf5−/−:MyoD−/− (double-mutant) mouse embryos that completely lack skeletal musculature and analyzed the development of the external ear. We concentrated on the external ear because several studies have suggested a muscular cause to various congenital auricular deformities, and middle and inner ear development was previously reported using the same mouse model. Wild-type mouse embryos were used as controls to compare the histomorphometric outcomes.

**Results:** Our findings demonstrated an absence of the external auditory meatus, along with an abnormal auricular appearance, in the double-mutant mouse embryos. Specifically, the auricle did not protrude laterally as noted in the wild-type mouse ears. However, histomorphometric measurements were not significantly different between the wild-type and double-mutant mouse ears.

**Conclusion:** Overall, our study showed that the development of the mouse external ear is dependent on the presence of skeletal muscles. (Plast Reconstr Surg Glob Open 2015;3:e382; doi: 10.1097/GOX.0000000000000352; Published online 1 May 2015.)

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auricular anomalies. Specifically, intrinsic auricular muscle irregularity has been associated with underdeveloped antihelical fold, and abnormal placement and function of both extrinsic and intrinsic auricular muscles have been suggested to cause a range of external ear anomalies.

Regulatory transcription factors, Myf5 and MyoD, serve essential roles in skeletal muscle formation. Subsequently, compound-mutant mouse embryos lacking both Myf5 and MyoD give rise to skeletal muscle agenesis. These double-mutant (Myf5−/−:MyoD−/−) fetuses are typically born alive, but die soon after birth due to respiratory arrest. Yet, they can still be valuable in assessing the role of skeletal muscle in the development of various structures during embryogenesis.

Middle and inner ear development with the Myf5−/−:MyoD−/− mouse model was previously reported by our group. However, the influence of skeletal muscles on external ear development has not been reported.

The objective of this study was to determine the influence of skeletal muscles on embryonic ear development using an animal model. Specifically, the ear development in double-mutant mouse model lacking skeletal musculature was compared with that in wild-type mouse model.

**METHODS**

**Interbreeding and Collection of Embryos**

Ethics approval was obtained from Dalhousie University Committee on Laboratory Animals. Mouse embryos lacking both Myf5 and MyoD were attained by a 2-generation breeding scheme, as described earlier. Briefly, MyoD−/− mice were first bred with Myf5+/− mice to generate Myf5+/−:MyoD+/− mice. Second, Myf5+/−:MyoD+/− mice were interbred to obtain embryos of 9 different genotypes including Myf5−/−:MyoD−/− (double-mutant) that appeared with an incidence of 1:16.

**Tissue Processing and Staining**

Mouse embryos were collected by Cesarean section on embryonic day 18.5 (E18.5). Mouse embryonic development lasts 18–19 days; by E18.5, all major organs and vital structures are developed and can be analyzed. The embryonic tissues were prepared for whole-mount hematoxylin and eosin staining and transverse sectioning.

**Photography and Morphometry**

Digital photographs were acquired using a stereomicroscope (Nikon Eclipse TE2000-S, Amstelveen, The Netherlands). Ear sections of wild-type (n = 3, 6 sides) and double-mutant (n = 3, 6 sides) mice were examined under 10× magnification.

**RESULTS**

The mouse auricles, at embryonic stage E18.5, did not contain any noticeable intrinsic skeletal muscles in both genotypes. However, there were some skeletal muscles on the medial most aspect of the auricle in all wild-type ears (Fig. 1). Presumably, this is analogous to the auricularis muscles in humans, which anchor the auricle to the craniofacial skeleton. There were no similar skeletal muscles observed in any of the double-mutant ears (Fig. 2). The distal external auditory meatus was present in wild-type mouse ears, which was located medial to the auricular fold (Fig. 1). This was a consistent finding in all wild-type phenotypes. The external auditory meatus was not observed in any of the double-mutant ears. As well, the auricle of the double-mutant mouse did not protrude laterally as observed in the wild-type samples. There was a continuation of the epithelium covering the auricle and the lateral aspect of the craniofacial region in all double-mutant ears (Fig. 2). This was in contrast to the clear separation of the auricle from the lateral head observed in the wild-type ears.

Histomorphometric measurements were made along the longest and widest dimensions of the auricle on all samples. Overall, there was no significant difference between the histomorphometric measurements of the wild-type (length, 1044.74 μm; SD = 93.72; width, 225.05 μm; SD = 18.13) and Myf5−/−:MyoD−/− (length, 1033.33 μm; SD = 72.15; width, 236.25 μm; SD = 11.79) embryos (length P = 0.85 and width P = 0.34; unpaired t test).

**Fig. 1.** Hematoxylin and eosin–stained transverse section of wild-type E18.5 embryo external ear. EAC indicates external auditory canal/meatus; Ext Ear, external ear; Fd, auricular fold; M, auricularis muscle. Bar, 100 μm.
DISCUSSION

Using the same double-mutant mouse model, we previously reported various developmental abnormalities affecting other anatomic regions. Specifically, disordered morphogenesis of the mandible and palate was demonstrated in the complete absence of skeletal musculature.9,11,12

Skeletal muscle abnormalities have been suggested to play a role in some congenital ear anomalies.5 Some have proposed that normal auricular development is dependent on the presence of normally functioning auricular muscles.5 For instance, abnormal antitragus muscle has been suggested to be an etiological factor of protruding ears in humans.6 Animal studies have supported this concept as well. Chiu et al13 performed cranial nerve VII neurectomy in a rat model and assessed auricular and craniofacial morphology. They noted paralysis of the operated auricle with flattening of the cartilaginous fold. The conclusion was that the functional activity of normally innervated auricular muscles significantly affects auricular shape in the rat model.13

In our double-mutant mouse model, the external auditory meatus was absent, which indicates malformation or complete collapse of the distal ear canal. Although the pathogenetic mechanism of this anomaly is unclear, it is not uncommon to observe deformities involving both the auricle and the ear canal in humans. An example of this dual congenital anomaly would be microtia and external auditory canal atresia (aural atresia). Both are conditions that arise typically from disruptions in branchial arch development,14 and the absence of skeletal muscles associated with the relevant branchial apparatus may be the underlying cause of these anomalies.

The gross form and size of the double-mutant auricle were similar to the wild-type ears. However, the auricle was found buried under the epithelium, which may be analogous to cryptotia seen in humans. Interestingly, the middle and inner ear (cochlea) were found essentially unaffected in the double-mutant mouse model, as reported previously.10

CONCLUSION

Although the exact nature and significance of our findings is unclear, they do support the premise that skeletal muscle abnormalities may affect external ear development.

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