Cardiopharyngeal mesoderm origins of musculoskeletal and connective tissues in the mammalian pharynx

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DOI: 10.1242/dev.185256

Editor: Patrick Tam

Review timeline
Original submission: 2 October 2019
Editorial decision: 5 November 2019
First revision received: 27 November 2019
Accepted: 16 December 2019

Original submission

First decision letter

MS ID#: DEVELOP/2019/185256

MS TITLE: Cardiopharyngeal mesoderm origins of musculoskeletal and connective tissues in the mammalian pharynx

AUTHORS: Noritaka Adachi, Marchesa Bilio, Antonio Baldini, and Robert G. Kelly

I have now received all the referees' reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPressand click on the 'Manuscripts with Decisions' queue in the Author Area.

As you will see, the referees express interest in your work, but have some criticisms and recommend a revision of your manuscript before we can consider publication. If you are able to revise the manuscript along the lines suggested (please also see editor's note appended below), which may involve further experiments, I will be happy receive a revised version of the manuscript. Your revised paper will be re-reviewed by one or more of the original referees, and acceptance of your manuscript will depend on your addressing satisfactorily the reviewers' concerns. Please also note that Development will normally permit only one round of revision.

Please attend to all of the reviewers' comments and ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion. I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

"Editor’s note:

• Highlight the novelty of the present study and the overlap with the published works identified by Reviewer 1.
• Provide a discourse of the disparity of the finding of myogenic tissues in the sixth arch reported by Poopalasundaram et al. Zoological Letters, https://doi.org/10.1186/s40851-019-0123-5
• Clarify the intrinsic requirement of Tbx1 activity in the connective tissues for the patterning of the pharyngeal tissues."

Reviewer 1

Advance summary and potential significance to field

In the paper "Cardiopharyngeal mesoderm origins of musculoskeletal and connective tissues in the mammalian pharynx", Adachi et al. perform lineage analyses to identify Cardio-Pharyngeal Mesoderm (CPM) derivatives during mouse development. They show that CPM gives rise to several pharyngeal structures and to different cell types including musculo-skeletal and connective tissues. They describe the fact that CPM and neural crest cells contribute differently to mediolateral pharyngeal structures. They further claim that Tbx1 is required for muscle patterning with CPM-derived connective tissue. They conclude that CPM participates to muscle patterning during development in a way reminiscent to the role of cranial neural crest cells in cephalic regions.

Comments for the author

Unfortunately I find that, despite the good quality of this extensive experimental work, the manuscript from Adachi et al. is not suitable for publication in Development as it does not provide sufficiently novel findings and some conclusions are questionable:

Indeed, the major claims of the paper are not novel as:
- the neural crest cells and mesodermal contributions to larynx skeleton and muscle connective tissue (CT) have been previously reported (Heude et al. 2018 eLife, Tabler et al. 2017 eLife)
- the neck muscle phenotype of Tbx1 mutants has already been described (Heude et al. 2018 eLife)
- the authors do not consider that Tbx1 CT derivatives might be of NCC origin (see Funato et al. 2015 HMG)
- and that, given that Tbx1 is expressed in 1) CPM-derived muscles, 2) CT, 3) pharyngeal endoderm and 4) ectoderm, the results obtained in Tbx1 mutants do not support that CPM-derived CT is directly implicated in neck muscle patterning.

Reviewer 2

Advance summary and potential significance to field

In this paper, Adachi et al uncovered a novel contribution of cardiopharyngeal mesoderm (CPM) to the development of musculoskeletal and connective cell types in the pharyngeal region. The authors show complementary contribution of CPM, somite and neural crest-derived cells to cartilages, muscles, and connective tissue in the pharynx. Intriguingly, this work identified a novel contribution of CPM to pharyngeal skeletons, connective tissue of the somite-derived muscles, and showed that the majority if not all branchiomeric muscles are derived from the CPM. Importantly, Adachi et al demonstrated that two copies of the transcription factor Tbx1 mutated in DiGeorge patients are required for the proper patterning of neck muscles. The majority of DiGeorge patients have one functional copy of Tbx1, and often exhibit previously unexplained defects in feeding and speech associated with defective movement of the hyoid bone. Adachi et al show that haploinsufficiency of Tbx1 in mice results in mispatterning and aberrant attachments of muscles pharyngeal muscles explaining these defects in human patients.

Comments for the author

The paper by Adachi et al entitled "Cardiopharyngeal mesoderm origins of musculoskeletal and connective tissues in the mammalian pharynx" reports novel major contribution of CPM to pharyngeal muscles and skeletons. This paper is well written, the data showing the contribution of the CPM and the complimentary contribution by the NCC are immaculately done, and conclusions of this manuscript are well-supported by the data. The carefully controlled experiments leave no doubt of the extensive contribution of the CPM to cartialges muscles, and connective tissues in the posterior pharynx. The major importance of this paper lies in the identification of an important role of Tbx1 in patterning of the neck muscles and explains pathologies seen in DiGeorge patients. Importantly, the authors show that these defects arise even in the presence of one functional copy of Tbx1, and this is highly medically relevant.

This reviewer has only a few minor comments that would aid clarity of the presentation:
1) It would be helpful to have additional cartoons depicting in different colors the contribution of CPM, NCC (and when relevant) somitic mesoderm to cartilages and muscles in each figure.
2) The summary Fig. 9B is confusing. The green color on the middle and left panels are hard to see. Explain the structures that are not green, does CPM also contribute to these?
3) The structure labeled clrary in the supplemental figure S5 panel B, should be labeled clary.
4) While having the list of abbreviations is very helpful, it would be easier to examine supplemental figures, if each abbreviation was included into the legend, like in the main paper figures.

Reviewer 3

Advance summary and potential significance to field

The study shows the contribution of the mesoderm versus neural crest to formation of the laryngeal cartilages and to the cranial muscles plus their connective tissues in the developing pharynx of mice. Assuming point 1 (below) can be answered which should be straight forward, the manuscript clearly demonstrates the mesodermal versus neural crest derivatives of pharyngeal arches without potential caveats of previous studies (e.g. the Mesp1Cre line which marks both somitic and unsegmented cranial mesoderm derivatives and transplantation studies which risk contamination). The study therefore, answers outstanding questions in the field and makes an important contribution to our understanding of the development of mammalian posterior pharyngeal arches. This ultimately will have implications to our understanding of developmental disorders as illustrated for Tbx1 within the manuscript. Overall, the figures are very clear, well set out and well labelled enabling the reader to fully appreciate the data that this is being shown. The manuscript is also fully referenced and presents a balanced summary of the field.

Comments for the author

Key point to be addressed:
1. The Mef2c-AHF-Cre marks the mesodermal (and endodermal/ectodermal?) tissues at E10.5. Can the authors add some further clarification about what stages have previously been examined i.e. to confirm that this Cre line is never expressed in somitic or neural crest lineages and to reassure the reader that the structures labelled with this Cre have a cranial mesodermal origin.

Minor comments/revisions
2. The supplementary data was harder to follow/took longer as all the abbreviations are listed on one page.
Can the authors please add the appropriate abbreviations to the figure legends as for the main text.
3. The discussion is too long – I think the discussion about Tbx1 could be reduced considerably.
4. Change the second heading within the main text. E.g. CPM gives rise to skeletal structures and mesenchyme in the pharynx and shoulder. There is no need to mention the perichondrium as this is naturally part of the skeleton.
5. The discussion of the perichondrium on page 8 is slightly misleading. One would expect higher density of labelling as cell density within the perichondrium is higher than the density of chondrocytes.
6. Page 9- where is the pharyngeal endoderm in Fig. 1I,J?
7. The discussion of innervation on page 11 can be moved to the main discussion.
8. Fig. S2 Is the B,E label in (A) at the correct position?
9. A summary table would be very helpful in the supplementary data

First revision

Author response to reviewers' comments

Response to the Editor and Reviewer's comments

Thank you very much for reviewing our manuscript, "Cardiopharyngeal mesoderm origins of musculoskeletal and connective tissues in the mammalian pharynx". We appreciate all the Editor's and Reviewers' comments and suggestions, and we are very glad to know that they are interested in our work. In response to all comments, we revised our manuscript as follows (we also uploaded a PDF of the Response).

In response to the Editor

• Highlight the novelty of the present study and the overlap with the published works identified by Reviewer 1.

AU: We highlighted the following major novelties of our study.

1.1. As we state clearly in the introduction, the two prior studies referred to by Reviewer 1 show a mesodermal contribution to the pharyngeal skeleton; however in these studies the origin of this mesoderm was unknown. A major novelty of our study is the identification of cardiopharyngeal mesoderm (CPM), defined by the Mef2c-AHF-Cre driver, as the source of mesoderm contributing to the pharyngeal skeleton. We consider that the novel finding that cartilage and connective tissue are CPM-derivatives, in addition to the established CPM derivatives of cardiac and branchiomeric muscles and endothelial cells, is an important advance in understanding the biology of this progenitor cell population in the early embryo.

1.2. The loss of neck muscles in homozygous mutant Tbx1 embryos was first described by us in 2004 and later analysed in studies by Theis and Heude (Kelly et al., 2004; Theis et al., 2010; Heude et al., 2018). In contrast, the neck muscle phenotype of Tbx1 heterozygous mutant embryos, the focus of the last part of our study, has not been previously described. We highlight the potential importance of these observations are for understanding craniofacial defects in 22q11.2 deletion syndrome patients who are haploinsufficient for TBX1.

1.3. We discuss the study of Funato et al., 2015 in the light of potential Tbx1 expression in neural crest cells. Mef2c-AHF-Cre, however, has not been reported to be expressed in neural crest cells. In our study we confirm this and indeed observe a strikingly complementary contribution of CPM and NCC to the connective tissue of certain neck muscles.

1.4. In our revised manuscript we have included new data obtained in CPM conditional Tbx1 heterozygous mutant embryos showing that neck muscle patterning defects are also observed when one copy of Tbx1 is deleted only in the Mef2c-AHF-Cre lineage (new Fig. 9). This finding strongly supports a role for CPM-derived CT in neck muscle patterning.

• Provide a discourse of the disparity of the finding of myogenic tissues in the sixth arch reported by Poopalasundaram et al. Zoological Letters, https://doi.org/10.1186/s40851-019-0123-5

AU: We thank the Editor for letting us know about this paper. Poopalasundaram et al., 2019 used RNA probe and whole-mount in situ hybridization to detect MyoD transcripts. In our manuscript, we used MYOD antibody and immunostaining on the paraffin sections with TSA amplification method. It is likely that these methodological differences may bring the distinct outcomes. Thus, while our findings that the pharyngeal arch 6 develops very differently to anterior arches agree with the conclusion of Poopalasundaram et al., we have clearly shown that myogenesis takes place in the 6th arch. This is also visible in the panels below from Kelly et al., 2004, showing MyoD expression in pharyngeal arches 4-6 at E10.5 at sites of more superficial neck muscle development (Reviewer Figure 1). This latter site appears to be incorrectly labelled by a white arrowhead as hypoglossal cord in panel E of Figure 3 in Poopalasundaram et al's paper.

In our revised manuscript, we discuss this paper on page 24, line 540, as follows:

Our finding that arch 6 develops differently to anterior arches is consistent with a recent study by Poopalasundaram et al. (2019) showing that posterior PA development in amniotes is markedly distinct from that in other vertebrate clades. However, while Poopalasundaram et al. suggest that myogenesis is suppressed in posterior PAs, we observed MYOD protein accumulation in posterior CPM in the anlagen of branchiomeric muscles derived from arches 4-6 from early developmental stages.

Reviewer Figure 1, showing MyoD in situ hybridisation at E10.5 in wildtype and Tbx1 null embryos (right) identifying branchiomeric muscle anlagen of arches 1-6 (if the figure is not visible here, please check the PDF of the Response, or Kelly et al., 2004 Figure 5):

• Clarify the intrinsic requirement of Tbx1 activity in the connective tissues for the patterning of the pharyngeal tissues.

AU: In our revised manuscript, we have included new data obtained in CPM conditional Tbx1 heterozygous mutant embryos showing that neck muscle patterning defects are also observed when one copy of Tbx1 is deleted only in the Mef2c-AHF-Cre lineage (new Fig. 9). This finding strongly supports a role for CPM-derived CT in neck muscle patterning. We added new sentences as well as an additional figure to show the conditional Tbx1 KO experiment in the following pages:

Page 17, line 380.
Page 21, line 461.
Page 40, line 965.

In response to Reviewer 1

- the neural crest cells and mesodermal contributions to larynx skeleton and muscle connective tissue (CT) have been previously reported (Heude et al. 2018 eLife, Tabler et al. 2017 eLife)

AU: Two papers, Heude et al., 2018 and Tabler et al., 2017, used Mesp1-Cre line to genetically trace the cell fate of larynx skeletons and CT. However, Mesp1-Cre line labels a broad range of mesoderm, including the head, somitic and lateral plate mesoderm, so the precise mesodermal origins of the skeletons and CT remain elusive. Moreover, the early distribution of NCCs and mesoderm remains unknown and the biological significance of the cellular origins of the pharynx has not been well discussed in the clinical and evolutionary contexts.
In our paper, we employed the Mef2c-AHF-Cre line that specifically labels a subset of the head mesoderm, cardiopharyngeal mesoderm (CPM), and investigated the contribution of Mef2c-AHF-Cre derivatives to musculoskeletal and connective tissues in the pharyngeal and neck regions in detail. As a result, we revealed the more precise embryonic origins of the pharyngeal and laryngeal components. We also discussed implications of CPM derived CT in the patterning of the pharynx and the phenotype of 22q11.2 deletion syndrome patients. Our finding of the unique cellular distribution in the pharynx and posterior pharyngeal arches provides interesting insights into the evolution of the vertebrate pharynx. Therefore, we consider that our manuscript has multiple novelties that advance the research field.
We thank Reviewer 1 for pointing this out, since our sentence in the Introduction was not so specific and straightforward regarding this. We thus changed the sentence on page 5, line 94, as follows:

The view that NCCs contribute to skeletal and connective tissues and CPM gives rise to musculature during pharyngeal development has been challenged by evidence for a mesodermal origin of components of the pharyngeal skeleton and CT in amniotes (Noden, 1988; Tabler et al., 2017; Heude et al., 2018). In these studies, however, the lateral mesoderm (mesoderm adjacent to the otic vesicle and the 1st somite) was traced in chicken-quail chimera analysis, and Mesp1-Cre mice, labeling anterior somites, lateral plate mesoderm (LPM) and head mesoderm, were employed in genetic lineage analysis. Therefore, the precise mesodermal origins of the pharyngeal skeleton and CT remain elusive.

We also highlighted our novel findings concerning this point in the discussion, page 24, line 523, as follows:

Our results using two different Cre lines now identify CPM as the source of medial skeletons, CT and mesenchymal cells in the mammalian pharynx.

- the neck muscle phenotype of Tbx1 mutants has already been described (Heude et al. 2018 eLife)

AU: The phenotype described in Heude et al. 2018 concerns Tbx1 homozygous mutant mice, and not Tbx1 heterozygous mutant mice. Actually, the trapezius muscle phenotype in Tbx1 null mutant embryos has been already described before (Kelly et al. 2004; Theis et al, 2010, Lescroart et al., 2015). Moreover, while Heude et al., 2018 described severely affected or missing non-branchiomeric infrahyoid muscles in Tbx1 homozygous mutant, we now show that infrahyoid muscles are in fact present but mispatterned in the same mutant. The altered attachment site of these muscles in Tbx1-/- mice, as well as hypoplasia of the hypoglossal cord in the absence of Tbx1, likely explains why these muscles were overlooked. We interpreted this phenotype as a muscle patterning defect, because muscles are formed and located in a position comparable to the wildtype, but are ectopically connected to the clavicle. In addition to this, and a major novel point of our study, is the first demonstration of muscle patterning defects in Tbx1 heterozygous mutant mice, affecting the sternocleidomastoid and omohyoid muscles. While these phenotypes have not been reported previously, they are highly relevant for human 22q11.2 deletion syndrome patients haploinsufficient for TBX1. Therefore, we found totally new phenotypes both in Tbx1 homozygous and heterozygous mutant embryos.
However, we admit that the way these results were presented may have been unclear, as pointed out by Reviewer 1. To clarify this point, we have changed the subheading of the Results on page 15, line 331, as follows:

Muscles with CPM-derived CT are mispatterned in Tbx1 heterozygous mutant embryos

- the authors do not consider that Tbx1 CT derivatives might be of NCC origin (see Funato et al. 2015 HMG)

AU: We agree that Tbx1-Cre line may label a small portion of NCCs and NCC derivatives. However, we observed that Tbx1-Cre positive CT derivatives, such as perichondrial cells in medial pharyngeal and shoulder skeletons and muscle CTs of the medial branchiomeric and infrahyoid muscles, were also Mef2c-AHF-Cre lineage positive, but Wnt1-Cre lineage negative (please see Figs. 1, 2, 3, 6, S4, S5 and S6). Also, we detected TBX1 expression in the cells surrounding the hypoglossal cord at E10.5 (Fig. 6I-J''). These cells are Mef2c-AHF-Cre lineage positive, but Wnt1-Cre lineage negative (please see Figs. 4 and 5). In addition, Funato et al. 2015 showed the immunostaining of TBX1 and observed TBX1 positives in cartilage cells, but not CT cells (Funato et al. 2015 Figure 5). These observations strongly support the conclusion that Tbx1-Cre positive CT is derived from CPM. However, the consideration of this point is very important and we really appreciate the comment form Reviewer 1. We mention this point on page 14, line 312, as follows:

In contrast to Mef2c-AHF-Cre, Tbx1 has been reported to be expressed in a subset of NCC derivatives (Funato et al., 2015). However, the Tbx1 and Mef2c-AHF-Cre lineages displayed highly similar labelling of Wnt1-Cre lineage negative CT, and moreover, we did not observe significant GFP signal in the hyoid bone, lateral thyroid cartilage or the lateral CT of branchiomeric muscles where Wnt1-Cre derivatives were detected (Figs. 1, 6 and S5).

- and that, given that Tbx1 is expressed in 1) CPM-derived muscles, 2) CT, 3) pharyngeal endoderm and 4) ectoderm, the results obtained in Tbx1 mutants do not support that CPM-derived CT is directly implicated in neck muscle patterning.

AU: In our revised manuscript, we have included new data obtained in CPM conditional Tbx1 heterozygous mutant embryos showing that neck muscle patterning defects are also observed when one copy of Tbx1 is deleted only in the Mef2c-AHF-Cre lineage (new Fig. 9). Since the omohyoid muscle is surrounded by CPM-derived CT and the Mef2c-AHF-Cre line does not label somitic muscle fibers, pharyngeal endoderm or ectoderm, this finding strongly supports a role for CPM-derived CT in neck muscle patterning. We added new sentences as well as an additional figure to show the conditional Tbx1 KO experiment in the following pages:

Page 17, line 380.
Page 21, line 461.
Page 40, line 965.

In response to Reviewer 2

1) It would be helpful to have additional cartoons depicting in different colors the contribution of CPM, NCC (and when relevant) somitic mesoderm to cartilages and muscles in each figure.

AU: We have added a color cartoon to the revised manuscript showing the contribution of CPM, NCC and somite to pharyngeal structures. Instead of adding this to the cartoons already included in each figure, to avoid introducing a bias in interpretation for the reader, we have added this cartoon in Fig. 10.

2) The summary Fig. 9B is confusing. The green color on the middle and left panels are hard to see. Explain the structures that are not green, does CPM also contribute to these?

AU: We agree with this point. In the revised Fig. 10B we have made the panels bigger and also added labels on both CPM and non-CPM components. In addition, we have made the text more visible.

3) The structure labeled clrary in the supplemental figure S5 panel B, should be labeled clary.

AU: Thank you for pointing out this typo. The lateral cricoarytenoid muscle is correctly labelled in the supplemental figure S5.

4) While having the list of abbreviations is very helpful, it would be easier to examine supplemental figures, if each abbreviation was included into the legend, like in the main paper figures.

AU: As suggested by the Reviewer, we have added abbreviations in the supplemental figure legend.

In response to Reviewer 3

1. The Mef2c-AHF-Cre marks the mesodermal (and endodermal/ectodermal?) tissues at E10.5. Can the authors add some further clarification about what stages have previously been examined i.e. to confirm that this Cre line is never expressed in somitic or neural crest lineages and to reassure the reader that the structures labelled with this Cre have a cranial mesodermal origin.

AU: We agree that the clarification of this point is crucial to our study. We cite two papers that used Mef2c-AHF-Cre line and showed no obvious expression of Cre in somite, neural crest cells, lateral plate mesoderm, ectoderm and endoderm, and mentioned this in the Result section, page 7, line 134, as follows:

In contrast, NCC, LPM and somitic derivatives as well as ectodermal and endodermal structures were YFP negative (see details in Figs. S1, S2) (Köntges and Lumsden, 1996; Chai et al., 2000; Jiang et al., 2000; Huang et al., 2000; Le Douarin et al., 2004; Noden and Trainor, 2005; Matsuoka et al., 2005; Heude et al., 2018). Consistent with these results, activation of Cre or Mef2c-AHF-Cre derivatives has not been observed in NCCs, LPM, somite, ectoderm and endoderm for this transgenic line during early development (from E7.5 to E12.5; Verzi et al., 2005; Lescroart et al., 2015) (also see Figs. 4 and 5).

2. The supplementary data was harder to follow/took longer as all the abbreviations are listed on one page. Can the authors please add the appropriate abbreviations to the figure legends as for the main text.

AU: We have added abbreviations to the supplementary figure legends.

3. The discussion is too long – I think the discussion about Tbx1 could be reduced considerably.

AU: We agree that the discussion about Tbx1 is very long, but at the same time, it is very important for us to show the significance of our results, especially as the revised manuscript includes conditional Tbx1 mutant data. For increased clarity, we separated this section of the discussion into two parts, addressing Tbx1 patterning roles and Tbx1 clinical significance, and we added an additional subheading on page 22, line 481. In addition, we deleted two sentences. While we have added the conditional data and an additional figure, as well as discussing this additional result, the manuscript remains under the word limit.

4. Change the second heading within the main text. E.g. CPM gives rise to skeletal structures and mesenchyme in the pharynx and shoulder. There is no need to mention the perichondrium as this is naturally part of the skeleton.

AU: We changed the second heading accordingly.

5. The discussion of the perichondrium on page 8 is slightly misleading. One would expect higher density of labelling as cell density within the perichondrium is higher than the density of chondrocytes.

AU: We thank Reviewer 3 for pointing this out. We removed the word ‘dense’ from page 8, line 155.

6. Page 9- where is the pharyngeal endoderm in Fig. 1I,J?

AU: The pharyngeal endoderm is the epithelial structure medial to the pharyngeal skeletons (arytenoid, cricoid and thyroid cartilages). To clarify we added the label ‘ly’ in Fig. 1I, J.

7. The discussion of innervation on page 11 can be moved to the main discussion.

AU: We agree with this point. We moved the discussion of innervation on page 11 to the discussion, page 20, line 432.

8. Fig. S2 Is the B,E label in (A) at the correct position?

AU: Yes, it is. The section level B, E is ventral to the occipital part of the skull and corresponds to the cervical region. Therefore, we observed acromiotrapezius muscle, epaxial muscles and spinal components (spinal cord, dorsal root ganglion, neural arch and vertebral body) in the sections.

9. A summary table would be very helpful in the supplementary data.

AU: We prepared a summary table of CMP and NCCs derivatives as Table S2 in the supplementary data, and referred to it on page 19, line 407.

Second decision letter

MS ID#: DEVELOP/2019/185256

MS TITLE: Cardiopharyngeal mesoderm origins of musculoskeletal and connective tissues in the mammalian pharynx

AUTHORS: Noritaka Adachi, Marchesa Bilio, Antonio Baldini, and Robert G. Kelly
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks. The referee reports and the editor's note are appended below.

Reviewer 2

Advance summary and potential significance to field

In the manuscript by Dr. Kelly's group entitled "Cardiopharyngeal mesoderm origins of musculoskeletal and connective tissues in the mammalian pharynx," the authors show novel contributions of the cardiopharyngeal mesoderm marked by the Mef2C-AHF-Cre lineage to the cartilages and connective tissues in the pharynx. The new data added to this manuscript showing the dosage requirement of Tbx1 in the Mef2C-AHF lineage for the patterning of neck muscles further strengthens this paper. Taken together, this work reports on novel functions of Tbx1 as well as on novel and unexpected derivatives and functions of the Mef2C-AHF lineage.

Comments for the author

The authors have made the clarifications requested by this reviewer, and this reviewer has no further comments

Reviewer 3

Advance summary and potential significance to field

The manuscript reports the contribution of the unsegmented cranial mesoderm versus neural crest to the posterior pharyngeal arches. The authors use a Cre line that specifically labels the unsegmented cranial mesoderm and advances the field (previous studies have used the Mesp1Cre which labels unsegmented and segmented mesoderm). The study is very detailed and will be a framework for future studies of pharyngeal arch development. Additionally, the authors identify an unexpected role of Tbx1 within the cranial mesoderm for muscle patterning and the authors identify novel muscle phenotypes in Tbx1+/- mice which are relevant to understanding defects in 22q11.2 deletion syndrome.

Comments for the author

The authors have addressed the reviewers comments.