Crossing the Border: A novel mouse line using Fgf10-Cre to drive Gata3 overexpression in nonsensory transcriptional context

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Crossing the Border

A novel mouse line using Fgf10-cre<sup>ERT2</sup> to drive Gata3 overexpression in nonsensory transcriptional context of the organ of Corti.

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🌟 Abstract:

Hearing loss can occur in a variety of ways, and in every case, they have equally devastating effects. While hearing loss and the people who suffer from it shall not be discriminated against, attempting to offer options for treatments nevertheless should be of priority. As such, there has been much progress in the field of genetically based forms of treatment for sensorineural hearing loss. A gene that has been almost always implicated in the conversation of regeneration, Hair Cell and Spiral Ganglia development, and inner ear development, in general, is the Zinc Finger transcription factors Gata3. Furthermore, since the Organ of Corti is a complex organization of cells, we found a perfect opportunity to document the effect of Gata3 in cells of the GER that do not usually express high levels of Gata3. Thus, we were interested in documenting the effects of Gata3 overexpression on the genetic profile of these cells. To do this we decided to overexpress gata3 in the cells of the GER. We utilized the tamoxifen-inducible Cre recombinase transgenic mouse line Fgf10-cre<sup>ERT2</sup> to characterize Fgf10 expression in the GER (Fgf10-cre<sup>ERT2</sup>:tdTomato), to then have certainty in our method of inducing overexpression of Gata3 (Fgf10-cre<sup>ERT2</sup>:Gata3<sup>OE</sup>) in the cells of the GER. In doing this, we found no significant evidence that Gata3 overexpression causes any drastic morphologic or cell fate changes in the OC. This grants us some confidence. That if Gata3 ever be used as a treatment option in the future; for either sensorineural hearing loss treatment, HDR (Gata3 haploinsufficiency), or preemptive protection against expected otic trauma, it should not create any abnormal changes in the OC.

🌟 Introduction:

- **Hearing loss and the layout.**

Sound perception is for many people, an extremely intricate piece of a subject’s experience of the world around them. Hearing is associated with the pinnae, the ear canal, and the eardrum as a regular visit to the ear, nose, and throat doctor might have already revealed; however, the full story behind hearing is far more complex than any little look in the mirror should reveal. Loss of hearing, of any kind, is an extremely common affliction in the world, however, due to the complexity and the variety of different ways hearing loss can occur, in combination with the familiarity bred ignorance we have over this sense, hearing loss and hearing disorders have long been overlooked. In the United States, 15% of the population over eighteen years of age indicate some form of hearing impairment, while 13% percent of the population indicates hearing loss in both ears (Lin et al., 2011; Blackwell & Lucas, 2012). Sources of hearing loss include congenital hearing loss, which is a result of a genetic disorder, ototoxic drugs, often due to the overuse of antibiotics or use of chemotherapeutic drugs, such as cisplatin, noise-induced trauma, and age-related hearing loss (Prell et al., 2019; Hodge et al., 2021). As a result of the multitude of ways one can lose their hearing, treatment option...
development has been slow, and insufficient in many cases. Apparatuses, such as hearing aids and cochlear implants are some of the most recognizable. Reasons for the inefficiency of these methods can range from the necessity of the presence of the same sensory cells which are already damaged, or because they are simply unpleasant to wear and use.

Sound transduction is a multifaceted process, with various checkpoints which allow for a variety of complications to occur. Hearing begins once sound waves are funneled into, essentially captured, by the auricle. The ear canal then allows the sound waves to travel and vibrate the tympanic membrane. Once the tympanic membrane is struck, it causes the vibration of the attached ossicles (from malleus to incus, to stapes), in the middle ear, which faithfully vibrates at the frequency of the sound waves that entered the ear canal. Transduction continues once these sound waves are transduced from the ossicles to the inner ear, by vibrating the oval window, which touches the sound transducing organ, the cochlea.

The inner ear and its labyrinth anatomy consist of six sensory organs that should be familiar to anyone investigating the functional anatomy of the organ. The detection of gravity, balance, linear and angular acceleration, and of course, sound, rely on the relationships between populations of ectoderm-derived cells that interact within the inner ear. There are six organs responsible for the perception of the five senses stated above and are distributed amongst two sensory domains, cochlear and vestibular. The first three vestibular organs are the three semicircular canals and their respective cristae organized in an orthogonal axis to each other, allowing for the perception of angular acceleration and balance. Next, are the other two vestibular organs responsible for the perception of gravity and linear acceleration, the saccule, and utricle. Finally, the cochlea exerts its role as the complex organ responsible for auditory function.

The cochlea is composed of three major delineated spaces: the Scala vestibuli, Scala tympani, and the cochlear duct. The oval window, once vibrated, moves the fluid termed perilymph in the Scala vestibuli back and forth, maintaining the initial frequency. This fluid is then able to vibrate a crucial structure of the cochlear duct called the basilar membrane. This structure is of great importance because it is able to selectively vibrate in response to different frequencies, which is termed mechanical tuning. This then creates a frequency gradient along the length of the cochlea. Higher frequencies, perceived as higher pitch, travel the smallest distance, due to the impedance of the fluid in the cochlea, and the basilar membrane vibrates to these frequencies at the basal region (closest to the oval window). Lower frequencies, perceived as lower pitch, have longer wavelengths that travel further, and as such the basilar membrane vibrates with selectivity to these frequencies in the apex of the cochlea. This allows for the concept of a spatial-frequency colocalization along the length of the cochlea, which allows for selective sensorineural transduction towards the central nervous system (CNS), otherwise known as the tonotopic map.

The perception of sound is attributed to the function of two crucial sensory cell populations, hair cells (HC) and spiral ganglion neurons (SGN). Within the cochlear duct (CD) between the greater epithelial ridge (GER) and the lesser epithelial ridge (LER), we can find a patch of tissue where the mechanosensory cells are found (Fig. 1) (Ohyama et al., 2010). The organ of Corti (OC), is home to several types of specific cells, which house two main populations: supporting cells (SC) and HCs (Kelly et al., 2009, Groves and Fekete 2012). The OC is the specialized epithelia in the CD and contains three rows of outer hair cells (OHC) and one row of inner hair cells (IHC) (Fig. 1) (Dabdoub et al., 2008; Basch et al., 2016). The IHCs are the cells that get directly stimulated by the moving fluid in the CD caused by the vibrating tectorial membrane, which is connected to the basilar membrane. On the other hand, OHCs offer a modulatory and tuning function by contracting and increasing IHC stimulation or protecting the same from excessively loud sounds (Puligilla et al., 2010).

This population is followed by its opposing neuronal population, which innervates and allows for the final step in auditory transduction for central processing. The specific pattern of innervation in the OC is of utmost importance. The IHCs and OHCs respectively have their own specific innervating populations of SGNs. The two main types of SGNs are: Type I SGNs, which account for 95% of SGNs, and Type II SGNs, which account for 5% of SGNs (Benoudiba et al., 2013; Nishimura et al., 2016). Extending SGNs obey the border exertion set by the OC and the two nonsensory regions. Type I SGNs solely innervate IHCs along the more medial region of the OC, presenting multiple points of synapsing on these cells. Type IIs, on the other hand, innervate OHCs and extend past the tunnel of Corti, making a right “base oriented” turn to innervate and synapse with multiple OHCs along the three rows (Fig. 1). The multiple synapsing abilities of Type II SGNs further their role as a modulatory pathway for sound perception, and “loudness” (Fritzsch et al., 2010; Weisz et al., 2009). The SGNs innervate the cochlea (Liu et al., 2000; Ma et al., 1998), and along with the vestibular neurons, will coalesce into the VIIIth cranial nerve, which then extends to the hindbrain (Bouchard et al., 2012). Upon reaching the hindbrain, the vestibular afferents project to the vestibular nucleus in the hindbrain, while cochlear afferents project towards the cochlear nucleus. Within the cochlear nucleus, the tonotopic organization, base to apex pattern, is preserved in multiple subdivisions, which offers our perception of frequency discrimination (Pickels 2015; Koundakjian et al., 2007). These physiological variations between HCs and SGNs, their organization, and the particular molecular interactions they have, are the microscopic tools that enable the auditory abilities of the inner ear.

- **Early otic development and crucial genes and signaling cascades.**

FGF signaling in general can work one of two ways, it can operate through the general receptor tyrosine kinase (RTK), or through the JAK-STAT pathway. FGF10, which is part of the keratinocyte growth factor superfamily or the FGF7
The hindbrain region, a precursor area of competency for otic development, is crucial for the proper development of the inner ear, and at different times it performs different functions throughout otic development. Gata3, along with Sox2, is one of the earliest sensory epithelia specifying genes, and they are both present at the earliest stages of invagination. While Sox2 seems to have, a role in early stages of proliferative, Gata3 is not so simple. From an early stage, Gata3 has patterns of expression that appear to prime future sensory epithelia regions and has restricted expression just two days after invagination E10.5 (Karis et al., 2001).

The general trend that is observed in the earlier days of otocyst invagination and general domain specification is mainly spatiotemporal. Probably because of the severe redundancy that FGF signaling has in early development, and the incredible number of genes that are controlled by GATA3 it is hard to pin what is the specific connection between these two, most likely because there is not just one. Because of this parallel expression, the relationship between Gata3 and Fgf10, and their respective phenotypes in null models have revealed extensive thinking as to why look further into this relationship at later and more specific stages.

- FGF10 and GATA3 role in Sensory epithelia and organ of Corti development.

A crucial characteristic of FGF signaling in the OC is in its specification of sensory and nonsensory cells in the prosensory region, a precursor area of competency for OC development. Specifically, FGF signaling is heavily involved in OC patterning by being presented in gradients. Fgf20 is seen to be

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**Figure (2)** series of morphogen gradients in the developing organ of Corti form E 11.5 to E 18.5. As can be seen in E 13.5 the previous Fgf10 expression begins to specify the pro-sensory region and future organ of Corti region, where Sox2 and Gata3 expression dominate (Taken from: Groves & Fekete, 2012).
can become HCs and will mature into functional ones. There has been a significant investigation in the very tight regulation and specification between IHCs, phalangeal cells, pillar cells, and GER border cells, all of which elucidate the complexity of border specification. Specifically, it has been shown how this is regulated by Hes/Hey factors being active or not, of which themselves are regulated by Gata3, which has generated suspicion of this being a direct role of this gene (Basch et al., 2015; Luo et al., 2013).

In two studies, which eliminated Gata3 at two different times, it was observed that by deleting Gata3 at E8, during initial inner ear development, its deletion had great morphologic effects which either directly or by proxy caused the absence of any sensory tissue in the cochlea. The cochlea was severely atrophied and demonstrated little to no development at all, and logically, there was also no present sensory epithelia (Duncan et al., 2011). Deleting Gata3 just a day later at E9.5, after some sensory specification has already taken place, Gata3 conditional knockouts (CKO) also demonstrated severe morphologic defects, however, these were less prominent than the earlier null mutants. Most specifically, however, there was a very sparse development of sensory epithelia and some HCs. The OC presented almost no organization, HCs were few, and also had significant development malfunctions, primarily observed by maldeveloped stereocilia.

The role of FGF signaling and most specifically of Fgf10 expression in the inner ear has been in large part due to the data collected from null models. One null model, which eliminated Fgf10 prior to any otic development shows a number of developmental problems. Most notable, is the presumable role FGF signaling has on the morphologic development of the vestibular system, as Fgf10−/− mice demonstrated complete loss of the posterior semicircular canal, as well as the atrophied cochlea. Furthermore, OC development demonstrated great defects as well. Null Fgf10 models demonstrated a dose-dependence since Fgf10+/− mice demonstrated graded effects, such as the loss of a proper Reissner’s membrane, shortened cochlear ducts, and small OC. However, there was the presence of HCs (Urness et al., 2015). It is interesting to note that Fgf10 has a transient expression around the OC, as it begins to be expressed in the LER, supposedly priming future BMP4, and then progresses counterclockwise through Reissner’s membrane and finally to the GER where it ends in the GER (Urness et al., 2015; Groves and Fekete, 2012). Thus, Fgf10 seems to have a great morphologic impact by specifying nonsensory regions of the OC, which ultimately leads to interest in what is its role within nonsensory cells of the OC that are just adjacent to the OC, where Gata3 is presently active, also taking into consideration that Gata3 regulates Fgf10 (Luo et al., 2013; Lillevåli et al., 2007).

The interaction of Gata3 and Fgf10 in the development of the sensory epithelia and neurons in the inner ear has been long suspected. Studies have shown that Gata3 does indeed directly regulate Fgf10 levels of mRNA expression.
The interest of investigation and plan of approach.

With these relationships in mind, it is why the tamoxifen-inducible Fgf10-cre\textsuperscript{ERT2}: Gata3\textsuperscript{OE} line is of such interest. Although much of the developmental cues and interaction between these two genes, this study was nonetheless interested in investigating the effect that Gata3 would have on postnatal time points. In adult time points, the OC and all of its cell types have been specified, differentiated, and matured. However, Gata3 was still shown that in late embryonic time points and early postnatal time points if manipulated could allow for the presence of ectopic HC courses (Walters et al., 2017). Thus, given the various roles of Gata3 and its influence on the crucial, equally multitasking, Fgf10 gene, we set out to investigate this interplay. Our line utilizes Cre-LoxP technology to enable overexpression of Gata3 in a cell-specific and time-specific manner. We first set out to characterize Fgf10-cre\textsuperscript{ERT2} expression in the mature cochlea, using the Fgf10-cre\textsuperscript{ERT2}: tdTomato line, with an injection of pups at P14 with tamoxifen (6 mg / 40 kg). This allowed us to investigate and characterize where we would overexpress Gata3 within the over-expresser model and determine the effects of Gata3 overexpression in the tissues that correspondingly we identified with tdTomato recombination. We would like to know if the relationships outlined throughout embryonic development, and overexpression of Gata3 would have any drastic effects on an adult and differentiated OC.

**Methods:**

- **Transgenic Mouse lines.**

The Fgf10-cre\textsuperscript{ERT2} mouse line was gifted to the Duncan lab by Dr. Belluscii (Agha et al., 2021). This mouse line was characterized using the following primers: forward (ATTTGCCATCGATTACGCTC), reverse (ATCAAGCTTGTGGTTTCGGA). The Rosa26 locus TdToma transgene mouse room was acquired from Jackson Laboratory. The following were used for identification: forward (AAGGAGGCTAGTGGAATG) and reverse (CCGAAAATCTGAGGGAGTTC). The Gata3OE mouse line was gifted to the Duncan lab by Dr. Bouchard (Nguyen et al., 2013). The following primers were used: forward (AAGTGCAGCTGAGGTTGTTAT), reverse Mutant (GGAGAAGCTGTCTTCAACCC), and reverse WT (GGAGCGGGAGAAATGGATATG).

- **Mouse Collections and Preparations.**
Mice were injected with 6 mg per 40 kg tamoxifen (Sigma, cat. T5648) pup body weight to induce the modified estrogen reception on the Cre-recombinase protein at P14. Pups were then collected at P21 by euthanization using a lethal 500 mg/kg dose of Avertin (2,2,2-tribromoethanol; Sigma cat. T48402). Pups were fixed using 4% paraformaldehyde (PFA) via transcardial perfusion using a peristaltic pump. All animal breeding and euthanasia were conducted following the procedures approved by Western Michigan University’s Institutional Animal Care and Usage Committee (IACUC) #21-01-11. Before completing animal studies, researchers concluded the Collaborative Institutional Training Initiative (CITI) for animal research.

- **Immunohistochemistry**

Pups were dissected in PBS under a microscope, and the cochlea was then placed into a 96-well plate, where the cochleae remained in PBS for upwards of 1 hour. After removal, ears were prepared by being washed with 0.05% Tween20/PBS 5 times with each wash consisting of 5 minutes on a rocker. Ears were then blocked for 30 minutes on a rocker in a blocking solution, composed of 2.5 (5%) mL of donkey solution, 47.5 mL of PBS, 0.5 g of Bovine Serum (BSA), and 50 µL of TritonX-100. The ears were then washed with several exchanges of 0.05% Tween20/PBS. Ears were then placed into a combination of diluted primary antibody solutions. For Fgf10-creERT2; tdTomato as well as Fgf10-creERT2; Gata3OE samples the following primary antibodies were added; Rabbit MyoVIIa (Proteus Biosciences) were diluted in Blocking solution 1:500, Chick NF200 (Aves©) diluted in Blocking solution 1:200, DAPI (Hoechst dye) diluted 1:2000, and Phalloidin 488 diluted 1:1000 in blocking solution all were added to individual ears, and Phalloidin 488 diluted 1:1000 in blocking solution. Ears were then incubated for at least two days at 4˚C on a rotating platform. Following the incubation, period ears were then removed from the primary antibody solution and rinsed with one rapid exchange of 0.05% Tween20/PBS. Then, four washes of 30 minutes of the same 0.05% Tween20/PBS were carried out on a rocking plate. Following this, secondary antibody solutions were added in equal amounts to each ear. All secondary antibody solutions were spun in a centrifuge for 10 minutes at 14000 rpm before addition into wells containing ears. Samples were then incubated at 4°C on a rotating platform overnight. Following second incubation, samples were washed for 30 minutes three times with PBS. Following washes, samples were transferred onto glass slides arranged with hair cells facing upwards, and coverslips were added after samples were immersed in glycerol.

- **Imaging and image editing.**

Samples were taken on a C2 Nikon confocal that had previously undergone immunohistochemistry. Images were analyzed, and Z stacks were compiled using the Fiji-ImageJ editing software. Images were finalized and edited for observation using Corel Draw; the images generated here are seen in the results sections. All images are representative of – replicates.

**Results:**

- **Characterization of the Fgf10-creERT2; tdTomato.**

Prior to overexpressing Gata3, we had to assure ourselves that the Fgf10-creERT2 method of transgenic engineering would confidently work in overexpressing Gata3 in the nonsensory cells of the GER. For this, we utilized the tamoxifen-inducible (ERT2) Cre-recombinase protein. By knowing that Cre-recombinase would only be expressed under the Fgf10 promoter. Under normal conditions, it would not be translocated to the nucleus. Upon activation by tamoxifen, which binds to the modified estrogen receptor on the Cre-
Recombinase protein, allowing for it to carry out genetic recombination. At the Rosa26 locus, a stop codon would be removed, allowing for the expression of the endogenous fluorescence of tdTomato which the mice possessed (Fig. 5).

After injection of the mice at P14, we collected mice at P21, where we prepared the samples for analysis. We observed sufficient recombination carried out by cre-Recombinase in the GER all along the length of the cochlea (Figure 6, white brackets). The border cells are specifically the most significant stained, and a decreasing gradient through the GER. Due to the observed fluorescence, we felt confident that utilizing the Fgf10-creERT2 mouse line would allow for overexpression of Gata3, only in the GER.

- **Fgf10-creERT2**: **Gata3** provokes no observable change in OC organization, morphology, or cells types.

We set out to, by the same mechanism of inducible Cre lines, overexpress Gata3 in the GER. Upon overexpression, no observable phenotypes can be seen. HCs can be seen as being stained red, and in all three figures, control, heterozygous and homozygous, the HC rows (one IHC and three OHC) can be seen as present. Gata3 overexpression at this stage also does not cause any IHC duplets, as previous data has shown to be the case in embryonic timepoints. Furthermore, overexpression of Gata3 does lead to any drastic morphological phenotypes, as seen to be the case in Gata3 null models. While null models dealt with the loss of Gata3, its role as a border maintainer gene, especially with its interactions with Fgf10 was reason to believe overexpression could cause some morphologic, or border phenotypes (Fig 6). It is interesting to note that at this age it seems like this dynamic interaction at the border has reached a point of stagnation and seems solidified. Phalloidin reveals that the pillar cells, seen in between both HC rows, maintain the proper organization as well, and taken with the observed conserved order of the OC, it seems reasonable to postulate that Gata3 overexpression does not have a significant role in border exertion, the morphology of OC, or HC fates in GER cells.

![Figure 6](image-url)

*Figure (6), Demonstrates the effects, or lack thereof, of the overexpression of Gata3 in cochleae of age P14. The cochlea was collected at age P21 and stained with MYO7A (red) and Phalloidin (Green). In all three cochleae, peripherin stains reveal the proper organ of the Corti structure by demonstrating the presence of all three rows of OHC, the presence of organized pillar cells, and single rows of IHC. MYO7A indicates that these rows of cells are indeed HCs and that no duplets were observed.*
**Conclusion:**

Most of the knowledge discussed previously has all been carried out in embryonic time points, with its focus to address the questions of congenital hearing loss, whereas postnatal studies typically focus on applying their findings to other forms of hearing loss, although not restricted to such.

It has been shown that congenital deficiencies in Gata3 lead to misexpression of Fgf10, thus causing large-scale morphology problems in inner ear development. Focusing mainly on the border of the GER and the OC, Gata3’s involved here, and subsequently its influence over Fgf10 is crucial to establish which cells become HCs and which cells become nonsensory. Clearly, there is an intriguing mechanism at play here (Basch et al., 2016). It has been shown and hypothesized by many that these cells, phalangeal cells, pillar cells, GER cells, and IHCs have a very specific way of acquiring this fate, and given that GATA3 is seen in high levels in HCs at these stages, it is not impossible to imagine that its expression pattern here is crucial for the specification of which cells become which. Previous studies have demonstrated that transdifferentiating of HCs can occur by manipulating crosstalk between GER cells, IHCs, and inner pillar cells (McGovern et al., 2019); however, no studies have shown such a relationship this late in embryonic time points. At postnatal stages, the OC has mainly been considered static in terms of cell fate after some studies have pointed out that early in the postnatal days, and late embryonic days, Gata3 seems to still play a role in the quiescence of supporting cells and GER by regulating mitotic factors p27kip1, and that if Gata3 levels are reduced, along with loss of HCs, ectopic HCs can be identified (Xu et al., 2021, Walters et al., 2017).

Many different forms of hearing loss can occur, but they seem to target HCs, specifically noise-induced, ototoxicity, and age-related hearing loss can happen in a multitude of ways. However, typically the end result is the loss HCs or SGNs. Loss of Gata3 has also been linked with deafness, primarily through HDR syndrome. HDR syndrome involves the haploinsufficiency of Gata3. The subsequent loss of one or more alleles of Gata3 can cause congenital renal disease, sensorineural hearing loss, and hypoparathyroidism. As such Gata3 has been linked with congenital hearing loss. Given the background of HCs, their differentiation, and the role of Gata3 on border exertion, it has been postulated that Gata3 overexpression could provide some help in HC regeneration, or at least protection. Gata3, along with other transcription factors has been demonstrated to allow for the expression of HC differentiation marker Pou4f3, which has led to much excitement over Gata3 as being a new path for possible genetic therapies (Masuda et al., 2012). However, when it comes to rescuing HCs, the result has not been so promising. HCs show some form of regeneration in the vestibular system (Burns & Stone 2016), where Gata3 expression seems alike in regulating HC differentiation however in the cochlea, HCs have presented any identification of a possible regenerative function.

However, Gata3 nonetheless has been strongly linked to maintaining HCs, particularly for the data surrounding its role in upregulating Pou4f3 in GER cells, and loss of Gata3 has shown decreases in HC gene Atoh1 and a decrease in HC critical Transcription factor Eya1 (Duncan et al., 2013; Masuda et al., 2013). As such, the role of Gata3 has been more strongly linked to being one of HC fate maintenance, rather than initiating the developmental cascade, possibly improving key transcription factors and regulatory site interactions. For this reason, many therapy options have surrounding possibly granting “extra copies” of Gata3 as possible protection for expected HC trauma or for HDR syndrome patients. However, there is worry about utilizing Gata3 as such because its regulatory functions are not entirely known, specifically surrounding HC fates, OC morphology, and cochlear duct morphology. And reluctance may be held in not knowing if Gata3 requires a sort of homeostatic level for popper OC function, or if at this stage, only loss of Gata3 can affect hearing.

Our study answers this question by knowing that at least overexpression of Gata3 at this stage does cause any adverse reaction. Morphologically speaking, and most predictable the cochlea seems normal, as expected. Next, there were no observable ectopic hair cells in the GER or the OC. This is interesting because Gata3 was one of the transcription factors needed for strong Pou4f3 upregulation in the GER cells, hover it seems that at this stage, the GER cells have been specified sufficiently that they maintain the original epithelial fate. Finally, the OC and its border with the GER seems unaffected as well. It would however still be necessary to investigate what would happen if Gata3 were to be lost abruptly, at this stage in life, the early postnatal days, if this would have any effects. It is also necessary to investigate and quantify through qPCR if the Gata3 transcripts in the GER were elevated, since the possibility of these cells simply not even expressing the extra copies of Gata3 also exists.

However, our data is reassuring as it allows us to know that “too much” Gata3 is not a concern with perfectly healthy OCs. Thus, many treatments options that have been supposing transgenic methods of treating or alleviating hearing loss do not have to worry about it affecting the morphology, cell fate, or interfering with the extremely specific cell organization in the OC. Methods such as AAV injection could be used to supply patients with extra copies of Gata3 if necessary, and our data would suggest that if this dose is given to an already normal level of Gata3, targeting cells of the GER at least, the OC should remain the same, while Gata3 could offer its protective functions.

Taking all of this into account, this study demonstrates evidence that overexpression of Gata3 through Fgf10-creERT2 recombination does not cause any aberrant or observable phenotypes, however more quantitative approaches are still necessary, as well as more investigation into the role of Gata3, both embryonically as well as postnatally if it ever were to be considered as a possible avenue for hearing loss or hearing disorder therapy.
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