Dermal Lef1 Expression Regulates Skin and Whisker Development, Maturation, and Aging in the Face of Mice

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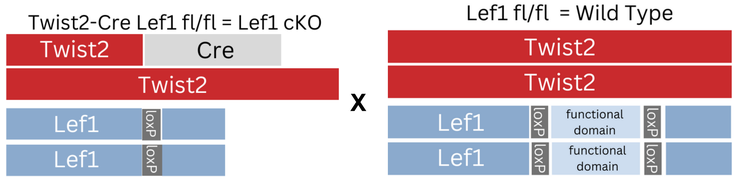
Background

Today, there are no effective treatments for hair loss caused by aging, scarring, or disease, with 85% of men and 50% of women affected[1]. Hair is important to normal skin function, as human hair is highly innervated and vascularized. However, mice are the most common model for humans, yet their fur is distinct from human hair. Human hair is larger, has a distinct molecular signature, and is more vascularized than mouse fur[3,4]. For hair regeneration and wound repair technology to improve, a more analogous model for human hair must be identified. Expression of the Wnt transcription factor *Lef1* in dermal cells in mouse skin has been shown to impact fur development and regeneration[2]. Mouse whiskers more closely resemble human hair, suggesting they may be a model for studying human-like hair. However, the role of dermal Lef1 in face and whisker development, maturation, and aging is unknown. In this study we investigated the structure and layering within the face and cheekpads of mice to establish the effect of Lef1 knockout on whisker and dermal development, maturation, and aging.

Methods

Cre/lox Transgenic Mouse System



Histology

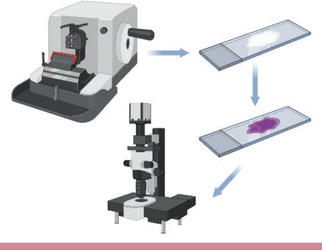


Fig 1. Process of collecting, staining and quantifying data. Cheek pad samples were embedded in paraffin blocks and cut on the microtome, then stained by H&E or Herovici [6]. Samples were then analyzed with a brightfield microscope.

Whisker Collection and Imaging System

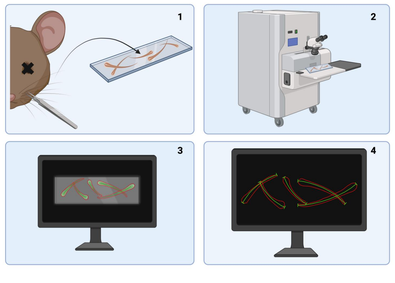
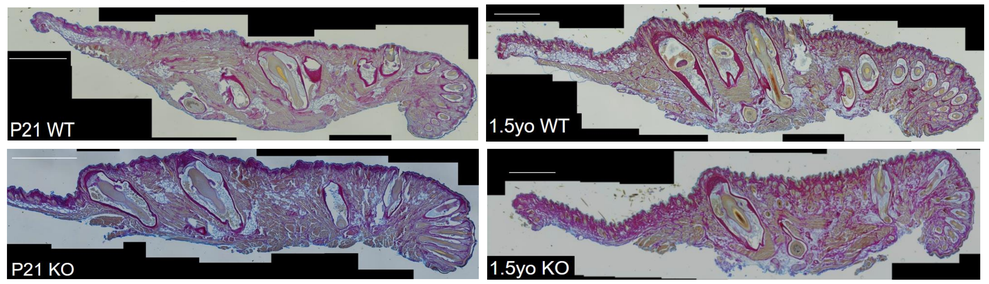


Fig 2. Process of collecting, imaging, and analyzing whiskers. Whiskers were collected from 21-day old (P21) mice and placed on microscope slides. Slides were imaged, and whiskers were digitally traced so their dimensions could be analyzed using a computer vision tool.

Results

1). Dermal Lef1 KO Alters Face Shape Without Altering Craniofacial Development

Histological Comparison of Cheekpad Maturation and Aging



"Cone-face" is caused by reduced cheekpad thickness

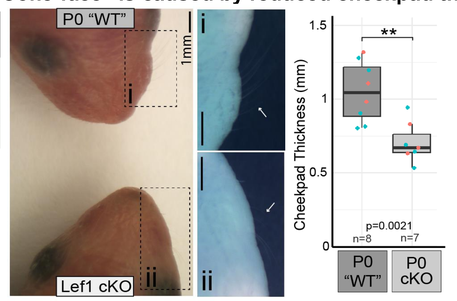
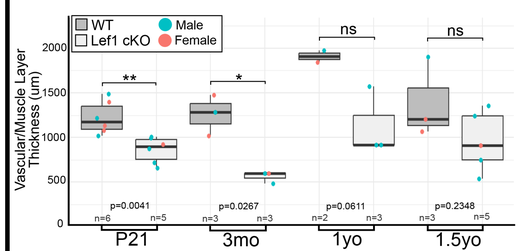
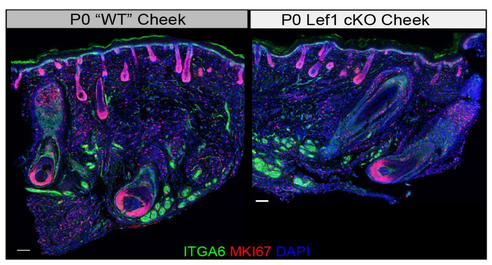


Fig 3. Analysis of Face Phenotypes. (left) Representative Herovici stains for juvenile (P21) and aged (1.5 year old) cheekpads. (mid) Close-up of faces of WT and KO newborn (P0) littermates. Inlays are zoomed in and color inverted to emphasize whiskers. (right) Quantification of cheekpad thickness in P0 mice. Statistics were performed using a t-test with p-values and samples sizes indicated on the plots. Scale bars indicate 1mm

Lef1 KO reduces dermal muscle layer thickness



Lef1 KO alters dermal cell proliferation?



"Cone-face" Is Not Due to Altered Craniofacial Bone Development

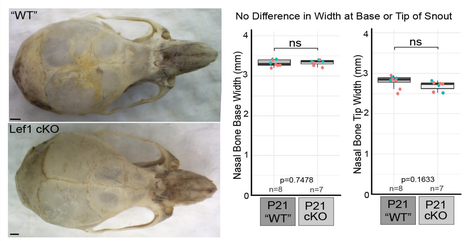


Fig 4. Validation of Cone-face Phenotype. (left) Quantification of cheekpad muscle thickness. (mid) Representative immunostains for ITGA6 and the proliferation marker MKI67 in P0 cheekpads (scale bar 100um). (right) Comparison of skulls from P21 WT and KO mice and quantification of premaxilla (snout bone). Statistics were performed using a t-test with p-values and samples sizes indicated on the plots.

2). Dermal Lef1 KO Reduces Whisker Count and Length

Fig 5. Lef1 cKO vs Wild Type whisker quantity.

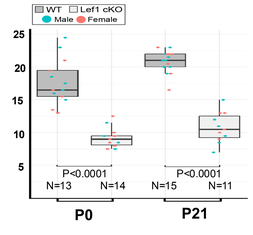


Fig 6. Leiden clustering of Whiskers represented by UMAP (swPhenome)

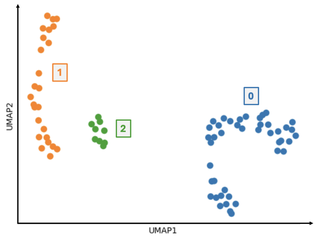


Fig 7. Area Heat Map of Whisker attribute UMAP.

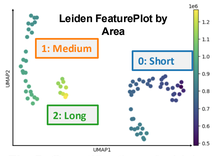


Fig 8. Perimeter Heat Map of Whisker attribute UMAP.

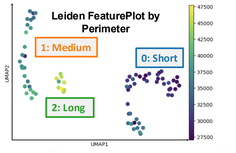


Fig 9. Whisker UMAP by mouse genotype.

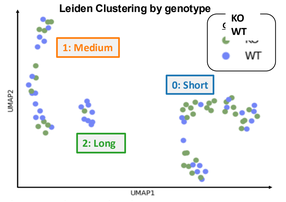


Fig 10. Lef1 KO Lacks Long Whiskers

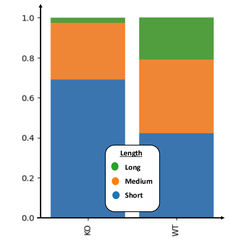


Fig 5. Quantification of whisker count in P0 and P21 mice (t-test with p-values and sample sizes on plot). Quantification of whisker structural features and subsequent visualization by UMAP with Leiden clustering. (**Fig 6**) Leiden clusters represent short, medium, and long whiskers by area (**Fig 7**) and perimeter (**Fig 8**). Visualization of genotype across the UMAP (**Fig 9**). (**Fig 10**). Proportion of total whiskers by cluster affiliation in WT and KO.

Discussion

Examining the whiskers and cheek pads of Lef1 conditional knockout mice revealed distinctions in the facial structure that were solely due to changes in the skin, suggesting that dermal regulation of Lef1 during skin and face development may influence face shape in addition to whisker formation and whisker growth. Future directions will include quantification of dermal cell proliferation in the neonatal cheekpad, inclusion of greater sample sizes to more completely define changes to cheekpads and whiskers of Lef1 KO mice across postnatal development, maturation, and aging considering sex as a biological variable, as well. Curiously, a global loss of Lef1 from all cell types in mice results in a complete absence of whiskers, fur, and narrowed snouts[5], while dermal Lef1 KO mice still have fur[2]. Based on our findings, dermal Lef1 is likely the driver of the cone face phenotype but may not be solely required for hair follicle formation. This work supports whiskers as a superior model for human hair compared to fur and lays the groundwork for understanding the molecular requirements for maintaining or regenerating human-like hair during aging or wound healing.

Acknowledgements

All animal procedures were conducted in compliance with WSU IACUC-approved protocols (#6723, 6724). WSU Franceschi Microscopy and Imaging Center (FMIC) housed and maintained the Leica SP8 used for confocal microscopy. The authors wish to thank Brietta Latham for assistance with skeletonization and dermastid beetles.
Funding for ST provided by Poncin Fellowship, JM by NIH T32GM008336, Driskell Lab by NIH NIAMS R01 AR078743

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