The *USH2A* Gene: An Analysis of Ultrasonic Vocalizations in a Mouse Model of Usher Syndrome Type 2

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INTRODUCTION

Usher syndrome type 2 is a complex autosomal recessive genetic disorder that is characterized by moderate to severe congenital sensorineural hearing loss, onset of retinitis pigmentosa in the second decade of life, and in some cases, vestibular dysfunction. Mutations in the *USH2A* gene account for 85% of cases of type 2 [1]. The gene is responsible for encoding the protein usherin, which has an important role in the development and function of inner ear hair cells (stereocilia) and retinal photoreceptors.

Until recently, it has been believed that carriers of the *USH2A* mutation were phenotype free. However, recent data has suggested that carriers may exhibit certain deficits in sensorineural hearing [2].

Homozygous *Ush2a* knockout mice mimic the visual and hearing deficits seen in *USH2A* human patients. Little attention has been paid to the heterozygous preparation, but given recent evidence of subtle language deficits in these human carriers, we included heterozygous KO mice in the current study. Since ultrasonic mouse vocalizations (USV's) are abundant in male mice when in the presence of a female, and can be used to assess defects in vocal communication, we measured and analyzed USV's from homozygous and heterozygous *Ush2a* KO mice.

AIMS AND HYPOTHESIS

- Our goal was to analyze the structures of ultrasonic vocalizations of a transgenic mouse model with a mutation of the *USH2A* mouse homolog. We also wanted to analyze the total time spent vocalizing.
- We hypothesized that a mutation in *Ush2a* would lead to significant differences in call type structures, and total time spent vocalizing, indicating vocal production deficits.

SUBJECTS

The *USH2A* knockout mice were purchased outside of our lab, and re-derived at UCHC's GTTF. HT x HT breedings were conducted at the University of Connecticut to generate the testing subjects. All of the subjects were genotyped and weaned, and then housed in standard cages with food and water. All procedures were conducted in compliance with the National Institutes of Health and approved by the University of Connecticut's IACUC.

There were 12 litter-matched wildtype (WT) male controls, 12 heterozygous *Ush2a* male knockouts (HT) and 11 homozygous *Ush2a* male knockouts (KO).

METHODS

All subjects were recorded individually for 5 minutes while interacting with an unfamiliar female mouse in oestrus. The USV's were detected using a well-established vocalization recording paradigm. An analysis was conducted of the recorded audio files to determine time spent vocalizing, and the structures of the USV's. Using previously established call types, the vocal recording of each mouse was individually and manually analyzed for shape and duration. Call types were defined and adapted from a study of mouse USV's published in previous years [3].

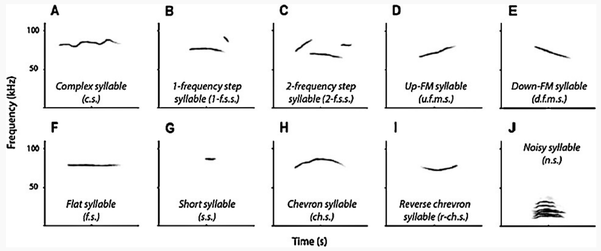
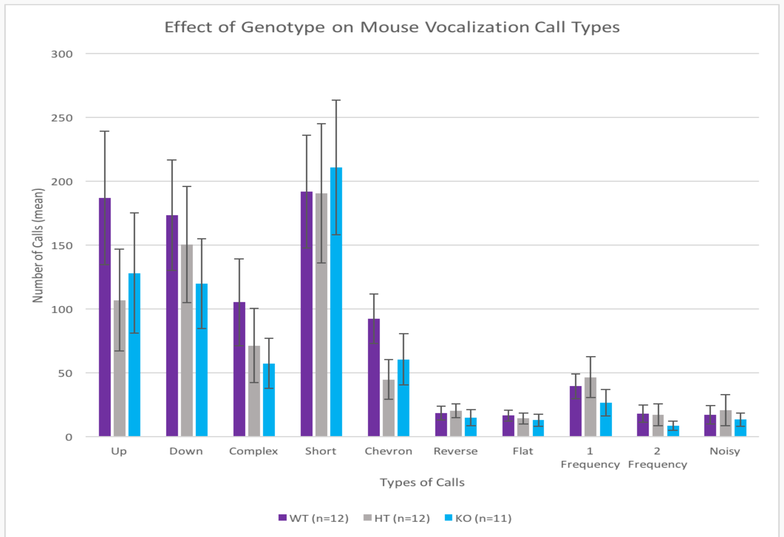


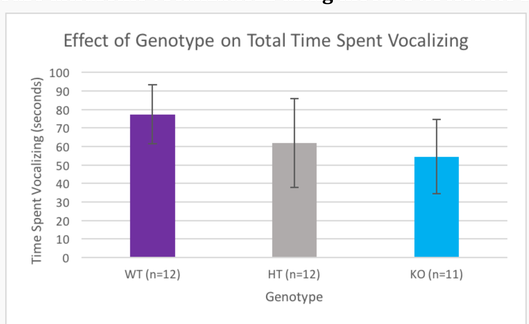
Figure 1. Presentation of the various call types, with spectrograms of examples of vocalizations within each call type. Images taken from Ey et al., 2013 [3].

RESULTS

A test of between-subject effects found no significant effect of genotype on the structure of call types (Graph 1), and no significant effect of genotype on total time spent vocalizing (Graph 2).



Graph 1. Effect of genotype on mouse ultrasonic vocalizations using the ANOVA test. No significant effect of genotype on call type was observed.



Graph 2. Effect of genotype on total time spent vocalizing by the subject. No significant effect of genotype on time spent vocalizing was observed.

CONCLUSIONS

Overall, genotype had no significant effect on the results. The total time spent vocalizing did not differ significantly between genotypes, and the structures of the calls were not significantly different between genotypes. This insignificance is something to take note of – the lack of findings may indicate that there are no vocal production deficits associated with the *USH2A* gene. Receptive differences have been noted, therefore lack of vocal production differences is something that may be worth taking another look at.

However, it is important to acknowledge the limitations of this collected data. Each subject was only recorded for a single 5 minute trial. Furthermore, there were only 35 subjects total. The subjects were all male, which does not allow for a comprehensive phenotyping of the gene since an entire portion of the population is not included in the testing. Furthermore, only one experimenter went through and analyzed each structure individually, and determined how much time total was spent vocalizing.

FUTURE DIRECTIONS

Given emerging evidence of language anomalies associated with Usher Syndrome type 2, as well as more recent evidence of subtle impairments in carriers, it may be useful to conduct additional research on USV call structures in this mouse model. This future research should include generating a larger n, using both sexes as subjects, and adding additional social conditions.

Ultimately, this research is meant to help improve health outcomes in those diagnosed with Usher syndrome.

ACKNOWLEDGEMENTS

This project was supported by the Murine Behavioral Neurogenetics Facility (MBNF) at the University of Connecticut by NSF IGERT grant DGE-1144399, and by the Connecticut Institute for Brain and Cognition (IBACS).

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