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鈴木, 智陽

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Short communication

Auditory function analysis in immunodeficient STAT1 knock-out mice: Considerations for viral infection models

Tomoharu Suzuki [a], Junki Maruyama [b], Rebecca Cook [a], Shinji Urata [a], Slobodan Paessler [b], Tomoko Makishima [a,*]

[a] Department of Otolaryngology, University of Texas Medical Branch, USA
[b] Department of Pathology, University of Texas Medical Branch, USA

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ABSTRACT

The STAT1 knock-out (KO) mouse is a frequently used transgenic immunodeficient strain to model human viral and bacterial diseases. The Lassa fever model was established in the STAT1 KO mice mimicking phenotypes seen in human patients including deafness in survivors. This model develops hearing loss at high prevalence and is a valuable tool to investigate viral infection-induced hearing loss. However, Lassa virus is a highly contagious and regulated agent requiring the unique logistics of the biosafety level 4 posing limitations for experimental work. Therefore, we did a detailed auditory analysis of the STAT1 KO mice to assess baseline auditory function in preparation for further auditory behavioral studies. Auditory brainstem response and distortion product otoacoustic emission tests were performed on males and females of the STAT1 KO mice and was compared to 129S6/SvEv wild type (WT) mice. The male WT mice had the best auditory performance and the female WT mice had the worst hearing performance. The male and female STAT1 KO mice had similar auditory performance to each other, which was intermediate between WT males and females. We conclude that both male and female STAT1 KO mice are suitable for studying viral infection-induced hearing loss.

1. Introduction

Viral infections have been an ongoing threat to human health, and there is a constant effort to overcome this threat through research. Viral infections that cause hemorrhagic fever in particular continue to have high mortality rates to this day. Lassa fever (LF), an acute hemorrhagic fever, and zoonotic infection caused by Lassa virus (LASV), has a fatality rate of 1–15 % [1–3] while most of humans infected with the LASV likely develop only mild disease or no disease at all. One unique feature of LF is that approximately one-third of LASV infected patients develop sudden onset sensorineural hearing loss either after surviving the acute phase of the disease or later in the convalescence phase of the disease [4]. However, the mechanism of inner ear or CNS injury (or both) caused by LASV infection is unknown.

Mice are frequently used as model animals for human infectious diseases, but many wild type mouse strains are resistant to infection with human viruses. Therefore, studying human viral diseases in mouse models has been often achieved by using immunocompromised mouse strains. For example, strains such as SCID [5,6], STAT1 deficient (STAT1 KO) [7,8] and IFN receptor deficient mice have been used successfully in viral infection models [7,9]. Previously, we successfully developed a LF mouse model that had similar disease phenotypes as in human LF patients, including hearing loss in survivors [7,8]. Given this high similarity, our LF model mice, which are STAT1 KO mice infected with the human LASV isolates, most likely share molecular mechanisms leading to hearing loss in humans.

The mouse is a well-established model animal for studying the inner ear auditory system due to ease of genetic manipulations and for the similarities of the auditory system in humans. In order to study hearing loss induced by a viral infection using the mouse model, it is important to fully assess the baseline auditory functional behavior in each mouse background strain. Differences in auditory function in lab mice have been demonstrated depending on the background strain [10,11]. Genetically engineered mice are frequently bred on a mixed C57BL6 and 129 background strain. The STAT1 KO mice used in this study were kept on a 129S6 background strain. It is important to note that the WT 129S6 strain has mild to moderate hearing loss in the low and high frequencies at baseline [12,13]. STAT1 mediates cisplatin-induced ototoxicity

* Corresponding author at: Department of Otolaryngology, University of Texas Medical Branch, 301 University Blvd, Galveston, TX 77555-0521, USA.
E-mail address: tomakish@utmb.edu (T. Makishima).

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affecting the inner ear hair cells [14], but its role in auditory function is unknown. Thus, we investigated whether STAT1 deficiency itself can affect auditory function or not.

The focus of this study was to review the baseline auditory behavior in a mouse strain used for various viral infections. Specifically, we assessed whether the auditory function was affected by background strain, gender, or repeated testing over time. In order for these mice to become a reliable model for viral infection-induced human auditory disease models, these results are essential in designing future experiments and interpreting their results.

2. Material and methods

2.1. Mice

STAT1 constitutive knockout (KO) mice (5 males and 5 females) and 129S6/SvEv wild type (WT) mice (5 males and 5 females) at ages 6–14 weeks were used. STAT1 KO (cat #2045-M and 2045-F) and its background WT mice (cat #129SVE-M and 129SVE -F) were obtained from Taconic Biosciences, Inc., USA. Mouse study protocols were approved by the Institutional Animal Care and Use Committee at University of Texas Medical Branch, and compliant with AAALAS and NIH guidelines.

2.2. Auditory tests

Mice were anesthetized by intraperitoneal (IP) injection of ketamine 100 mg/kg + xylazine 10 mg/kg. Auditory tests were done in mice weekly from age 6 weeks to 14 weeks. The mice were kept in a soundproof box while auditory tests were performed.

Auditory Brainstem Response (ABR) was performed using the Intelligent Hearing System SmartEP software with the high frequency transducer. Averages of 256–512 responses elicited by 8-, 16-, 24- and 32-kHz tone pip or click stimulus was recorded in descending 5 dB steps [15]. Threshold was determined by identifying the smallest stimulus level with recognizable wave II or wave III.

Distortion Product Otoacoustic Emission (DPOAE) was detected using the Starkey DP2000 system (Starkey Laboratories, USA). Ear inserts were modified to fit the mouse external auditory canal. Distortion product 2F1 -F2 was obtained at high frequency setting, with F2 value between 8 kHz–16 kHz. F1/F2 = 1.22, F1 = 65 dB, F2 = 55 dB [16].

2.3. Statistical analysis

Auditory test results were compared between different genotypes, background strains, gender, and timepoints. Statistical difference ($P < 0.05$) was assessed using ANOVA with posthoc Tukey test (StatistiXL 2.0 software) (https://www.statistixl.com/).

3. Results

Characterization of the auditory phenotype typically includes determining the degree, age at onset, and progression of hearing loss. In our previously reported LF model pilot study, we used mice at ages 6 weeks to 14 weeks and assessed the gross auditory function by startle reflex (7). Within this age range, mice with early onset hearing loss would have already developed noticeable hearing loss, whereas the mice with age related hearing loss would not have developed hearing loss yet. First, we determined whether the STAT1 KO mice had hearing loss at 6 weeks of age by ABR and DPOAE. We compared WT vs KO, as well as males vs females.

3.1. ABR of STAT1 KO mice at baseline

At 6 weeks of age, we compared ABR thresholds of the better-hearing ear of STAT1 KO and WT mice. The ABR thresholds of STAT1 KO male mice (n = 5), STAT1 KO female mice (n = 5), WT male mice (n = 5), and WT female mice (n = 5) were overall similar when tested with click-, 8 kHz-, 16 kHz- and 24 kHz tone pip stimuli. However, with 32 kHz tone pip stimulus, the WT male mice had better hearing than wild type female mice, with ABR threshold of 59 ± 4 dB and 72 ± 8 dB respectively, with approximately 13 dB difference (p = 0.048). Overall, there was a trend of male WT mice having the best auditory performance in all tested frequencies. Also, there was a trend of male WT mice having better auditory performance compared to male STAT1 KO mice, whereas female WT mice had similar auditory performance with female STAT1 KO mice (Fig. 1A).

3.2. DPOAE of STAT1 KO mice at baseline

Next, we performed DPOAE to assess outer hair cell and cochlear function. At 6 weeks of age, we observed a trend of WT males having the largest distortion product (DP) value in the higher frequencies, whereas the WT female mice had the smallest DP value. The STAT1 KO males and females showed roughly intermediate DP values, which is a similar trend with the ABR results. This difference was prominent at F2 frequencies 11 kHz – 14 kHz range (Fig. 2A).

To evaluate the auditory function change over time, we compared the sum of the DP across F2 ranges 8 kHz – 16 kHz of the better performing ear weekly from 6–14 weeks of age (Fig. 2B). The results showed a similar trend to the ABR results. The DP sum was significantly larger in WT male mice compared to WT female mice only at 6 weeks of age (p = 0.04).

3.3. The effect of repeated weekly auditory testing

Next, we analyzed whether weekly testing with ABR and DPOAE, and repeated anesthesia under ketamine/xylazine had any effect on auditory performance. We compared results of the weekly auditory testing from 6–14 weeks of age.

In ABR, WT males had the most consistent weekly auditory threshold throughout the time points tested at 6- to 14-weeks at all frequencies. WT females showed an 8 dB difference (range 58–66 dB, p = 0.025) only at 8 kHz, STAT1 KO males showed an 11 dB difference in both 8 kHz (range 48–59 dB, p = 0.002)) and 24 kHz (range 44–55 dB, p = 0.044), and STAT1 KO females showed differences of 11 dB with click (range 41–52 dB, p = 0.025) and 9 dB (range 44–53 dB, p = 0.04) with 24 kHz. These differences were observed spontaneously at different time points, and none of the genotypes had a trend for worsening auditory thresholds at later time points (Fig. 1B).

WT male mice had significantly better auditory thresholds than WT female mice at various auditory stimulus frequencies. WT males had smaller thresholds compared to WT females by 6 dB with click stimulus at 10 weeks (p = 0.027), 8 dB at 8 weeks (p = 0.001) and 9 dB at 10 weeks (p = 0.014) with 8 kHz stimulus, 10 dB with 16 kHz stimulus at 12 weeks (p = 0.047) and 13 dB with 32 kHz stimulus at 6 weeks (p = 0.048).

There was no significant difference in DP sum over time for all genotypes, although there was a trend of DP sum being slightly smaller at 14 weeks. There was a consistent trend for WT males having larger DP sum compared to WT females at all tested timepoints, which were significantly different at 6 weeks (p = 0.044) and 9 weeks (p = 0.045). The STAT1 KO male and STAT1 KO female mice had intermediate auditory performances compared to WT males and WT females, but were similar to each other (Fig. 2B).

In summary, there was no significant difference in auditory function between male and female STAT1 KO mice. The only significant difference observed repeatedly with different auditory stimulus conditions was between male and female WT mice, with male mice having better auditory function compared to female mice. However, there was a trend for male STAT1 KO mice having worse auditory performance than male WT mice and female STAT1 KO mice having better auditory performance than the female WT mice. Importantly, while the male WT mice

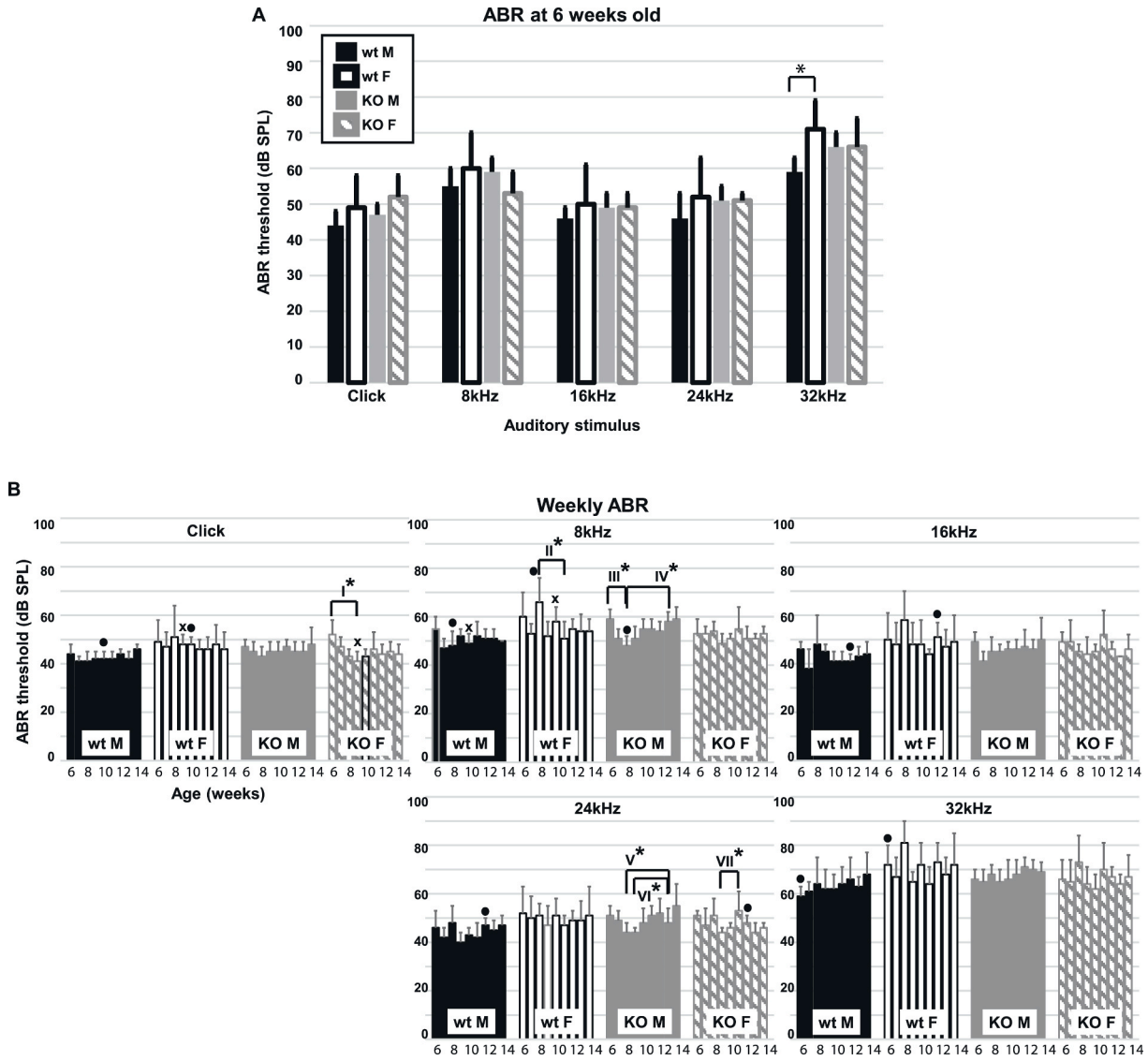

Fig. 1. Auditory brainstem response (ABR). (A) ABR threshold at 6 weeks old. Average threshold ± SD is shown for each group (n = 5 each). wt M, 129S6/SvEv wild type male; wt F, 129S6/SvEv wild type female; KO M, STAT1 knockout male; KO F, STAT1 knockout female. * represents significant difference between wt M and wt F at 32 kHz (p = 0.048). **(B)** Weekly ABR results. ABR threshold ± SD by genotype and by auditory stimulus condition is shown for each group (n = 5 each). * represents statistically significant timepoint differences within the same group: I* (p = 0.025), II* (p = 0.029), III* (p = 0.002), IV* (p = 0.002), V* (p = 0.044), VI* (p = 0.044), VII* (p = 0.040). Other symbols represent significant differences between groups: closed circle in "Click" panel, wt M vs wt F (p = 0.027). X in "Click panel, wtF vs STAT1 KO F (p = 0.041). Closed circle in "8 kHz" panel, wt M vs wt F and wt F vs STAT1 KO F (p = 0.001). X in "8 kHz" panel, wt M vs wt F (p = 0.014). Closed circle in "16 kHz" panel, wt M vs wt F (p = 0.047). Closed circle in "24 kHz" panel, wt M vs KO F (p = 0.034). Closed circle in "32 kHz" panel, wt M vs wt F (p = 0.048).

had the best auditory performance and the female WT mice had significantly worse auditory performance, the auditory performances of both the male and female STAT1 KO mice were in-between and similar to each other.

While the ABR thresholds did not trend towards worse auditory performance over time, the DPOAE sum had a trend towards worse auditory performance at later weeks.

4. Discussion

4.1. Considerations for mice gender differences

Auditory brainstem response (ABR), otoacoustic emission (OAE), or acoustic startle reflex (ASR) are frequently used methods to determine the auditory function in mice. Most studies have not taken into account gender differences, which in recent years have been identified as a major factor affecting outcome especially in behavioral studies, including auditory studies in mice [17]. Our goal was to determine whether there were gender differences in ABR and/or DPOAE results in mouse strains used as infectious disease models. Based on our pilot study, we observed a slight gender difference in auditory function in WT 129S6 strain, but this difference likely diminishes when the STAT1 gene is deficient. Therefore, when using the STAT1 KO mice, both the male and female mice can be used in the same cohort. However, it is still unknown whether the reaction to a stimulus such as a viral infection will be different between the two genders. Thus, the results of auditory tests in these viral infection model mice must be interpreted with caution and needs to account for gender differences at the planning stage of experiments in anticipation of this effect.

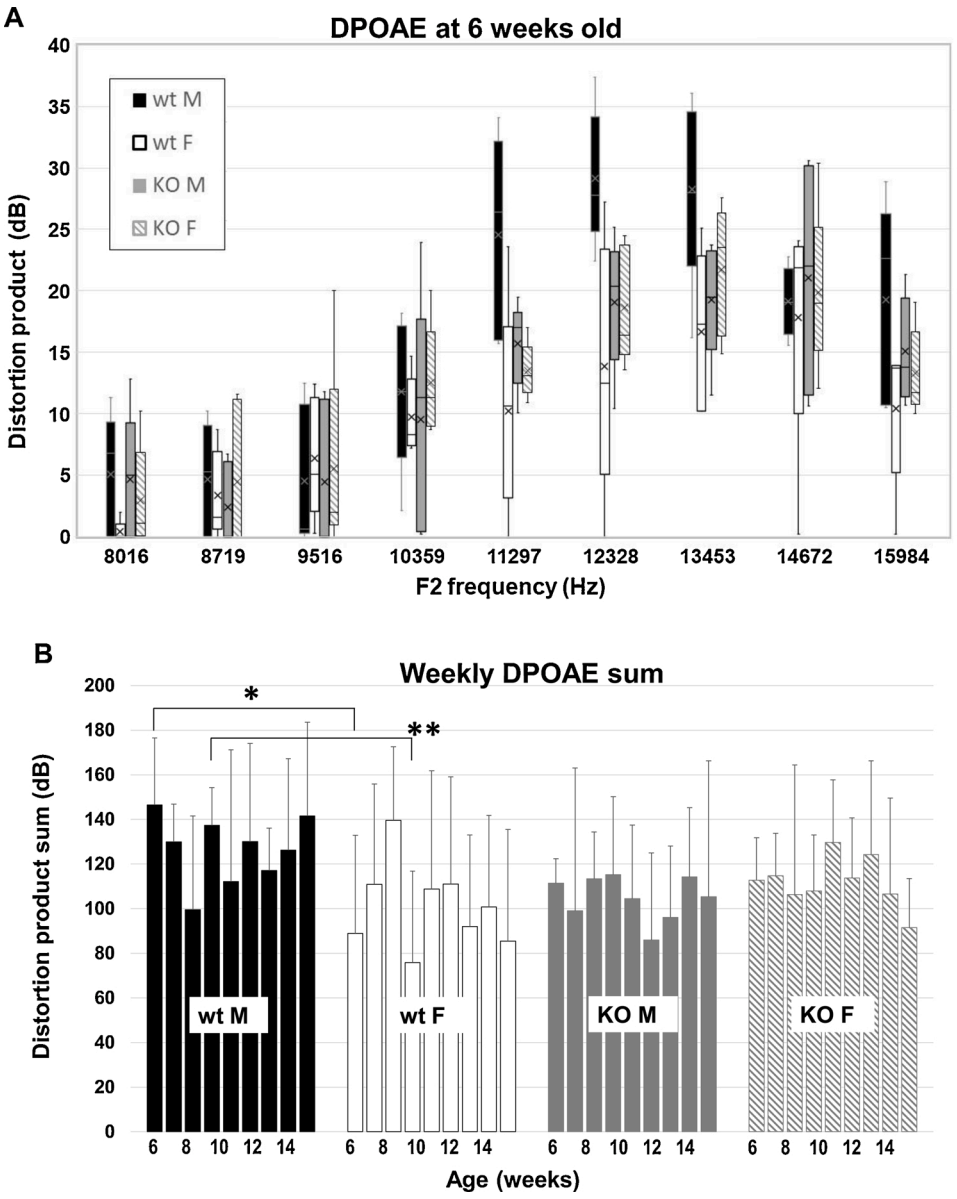

Fig. 2. Distortion product otoacoustic emission (DPOAE). (A) DPOAE at 6 weeks old. Distortion product (DP) 2F1-F2 at each F2 frequency for each group (n = 5 each) was compared. Box and whisker graph; X, mean; middle horizontal line, median; box top line, 3rd quartile; box bottom line, 1st quartile; whiskers, maximum and minimum range. **(B)** Weekly DPOAE results. The sum of DP across all frequencies was compared weekly between 6- and 14-weeks of age in each group. The average ± SD is shown in the bar graph. Black bar, wild type male; white bar, wild type female; gray bar, STAT1 knockout male; striped bar, STAT1 knockout female. * represents significant difference between wt M and wt F at 6 weeks of age ($p = 0.044$). ** represents significant difference between wt M and wt F at 9 weeks of age ($p = 0.045$).

4.2. Considerations for anesthetic agents

Agents frequently used for anesthesia in mice behavioral studies have been investigated regarding their effects on auditory function. Isoflurane and halothane, a volatile inhaled anesthetic, are popular agents due to its fast induction and recovery and consistency in the depth of anesthesia. However, inhaled anesthesia have been reported to protect mice against noise induced-hearing loss [18]. With isoflurane anesthesia, ABR threshold was elevated compared to ketamine/xylazine/acepromazine anesthesia, while DPOAE threshold was similar [19]. Ketamine-based anesthetics and pentobarbital *via* IP route are also widely used. We chose ketamine/xylazine combination IP injection for general anesthesia during auditory testing. The advantage of ketamine/xylazine IP injection is its stable anesthetic property and minimal impact on auditory testing. Indeed, even after testing from 6 weeks to 14 weeks of age, resulting in eight consecutive weekly tests in this study, the auditory function in mice remained relatively stable both in ABR and in DPOAE. We conclude ketamine/xylazine IP injection is suitable for repeated weekly administration for extended time course auditory function analysis in mice.

4.3. Considerations for study design using viral infection induced hearing loss mouse model

The main objective of this study was to determine which tests were most likely to show differences in the STAT1KO immunodeficient mice. As studying human viral infectious disease models generally takes place in restricted biosafety level environments, time and resource constraints require a minimalist approach to the study design. The results from this study is directly useful information as we plan to perform these auditory tests in the Biosafety level 4 (BSL4) laboratory to study LASV-induced hearing loss in mice. To date, ABR and DPOAE tests have never been performed in mice within the BSL4 environment.

To minimize testing time in ABR, we will most likely choose the two conditions with click and 32 kHz stimulus to detect differences. The click stimulus resulted in lower threshold, but less variability among groups. Thus, the click is suitable for a rough detection of outliers. On the other hand, the 32 kHz stimulus resulted in more variability. Thus, we speculate that this highest frequency will help detect more subtle differences. For the DPOAE results, we used the distortion product (DP) sum of all frequencies tested. Instead of comparing single frequency DP results, this will likely eliminate any artifact from the background environment.

Either male or female STAT1 KO mice are suitable to carry out the studies as the baseline auditory performance are similar. This is favorable for minimizing resources, as we may need to test only one gender or combine with smaller numbers of each for future experiments.

In summary, we were able to determine the baseline auditory function in the STAT1 KO mice, which had minimal change in hearing from ages 6–14 weeks and similar auditory function in males and females. This is the first report characterizing the auditory function in STAT1 KO mice, and we conclude this strain is suitable as a mouse model for viral infection induced hearing loss studies.

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CRediT authorship contribution statement

Tomoharu Suzuki: Formal analysis, Investigation, Data curation, Writing - original draft, Visualization, Project administration. **Junki Maruyama:** Formal analysis, Investigation, Data curation, Writing - review & editing, Visualization, Project administration. **Rebecca Cook:** Investigation. **Shinji Urata:** Formal analysis, Writing - review & editing, Visualization. **Slobodan Paessler:** Writing - review & editing, Supervision, Funding acquisition. **Tomoko Makishima:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - original draft, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

None.

References

[1] G.O. Akpede, D.A. Asogun, S.A. Okogbenin, S.O. Dawodu, M.O. Momoh, A. E. Dongo, et al., Caseload and case fatality of lassa fever in Nigeria, 2001-2018: a specialist center's experience and its implications, Front. Public Health Serv. Syst. Res. 7 (June) (2019) 170.

[2] O.O. Bello, O.R. Akinajo, K.H. Odubamowo, T.A.O. Oluwasola, Lassa fever in pregnancy: report of 2 cases seen at the university college hospital, Ibadan. Case Rep Obstet Gynecol. 2016 (2016) 1–3.

[3] N.F. Takah, P. Brangel, P. Shrestha, R. Peeling, Sensitivity and specificity of diagnostic tests for Lassa fever: a systematic review, BMC Infect. Dis. 19 (1) (2019) 1–11.

[4] L.P. Rybak, Deafness associated with lassa fever, JAMA 264 (October (16)) (1990) 2119.

[5] N. Charlier, R. Molenkamp, P. Leyssen, J. Paeshuyse, C. Drosten, M. Panning, et al., Exchanging the yellow fever virus envelope proteins with modoc virus prM and e proteins results in a chimeric virus that is neuroinvasive in SCID mice, J. Virol. 78 (14) (2004) 7418–7426.

[6] K.L. Warfield, D.A. Alves, S.B. Bradfute, D.K. Reed, S. VanTongeren, W.V. Kalina, et al., Development of a model for marburgvirus based on severe-combined immunodeficiency mice, Virol. J. 4 (2007) 1–13.

[7] N.E. Yun, S. Ronca, A. Tamura, T. Koma, A.V. Seregin, K.T. Dineley, et al., Animal model of sensorineural hearing loss associated with lassa virus infection, J. Virol. 90 (December (6)) (2015) 2920–2927.

[8] N.E. Yun, A.V. Seregin, D.H. Walker, V.L. Popov, A.G. Walker, J.N. Smith, et al., Mice lacking functional STAT1 are highly susceptible to lethal infection with lassa virus, J. Virol. 87 (19) (2013) 10908–10911.

[9] L. Oestereich, A. Lüdtke, P. Ruibal, E. Pallasch, R. Kerber, T. Rieger, et al., Chimeric mice with competent hematopoietic immunity reproduce key features of severe lassa fever, PLoS Pathog. 12 (5) (2016) 1–22.

[10] K.R. Johnson, C. Tian, L.H. Gagnon, H. Jiang, D. Ding, R. Salvi, Effects of Cdh23 single nucleotide substitutions on age-related hearing loss in C57BL/6 and 129S1/Sv mice and comparisons with congenic strains, Sci. Rep. 7 (March) (2017) 44450.

[11] Q.Y. Zheng, K.R. Johnson, L.C. Erway, Assessment of hearing in 80 inbred strains of mice by ABR threshold analyses, Hear. Res. 130 (April (1–2)) (1999) 94–107.

[12] V.A. Street, S.G. Kujawa, A. Manichaikul, K.W. Broman, J.C. Kallman, D.J. Shilling, et al., Resistance to noise-induced hearing loss in 129S6 and MOLF mice: identification of independent, overlapping, and interacting chromosomal regions, J. Assoc. Res. Otolaryngol. 15 (October (5)) (2014) 721–738.

[13] B. Peguero, B.L. Tempel, A Chromosome 17 locus engenders frequency-specific non-progressive hearing loss that contributes to age-related hearing loss in mice, J. Assoc. Res. Otolaryngol. 16 (August (4)) (2015) 459–471.

[14] N.C. Schmitt, E.W. Rubel, N.M. Nathanson, Cisplatin-induced hair cell death requires STAT1 and is attenuated by epigallocatechin gallate, J. Neurosci. 29 (March (12)) (2009) 3843–3851.

[15] T. Makishima, C.I. Rodriguez, N.G. Robertson, C.C. Morton, C.L. Stewart, A. J. Griffith, Targeted disruption of mouse Coch provides functional evidence that DFNA9 hearing loss is not a COCH haploinsufficiency disorder, Hum. Genet. 118 (October (1)) (2005) 29–34.

[16] T. Makishima, L. Hochman, P. Armstrong, E. Rosenberger, R. Ridley, M. Woo, et al., Inner ear dysfunction in caspase-3 deficient mice, BMC Neurosci. 12 (October) (2011) 102.

[17] B. Milon, S. Mitra, Y. Song, Z. Margulies, R. Casserly, V. Drake, et al., The impact of biological sex on the response to noise and otoprotective therapies against acoustic injury in mice, Biol. Sex Differ. 9 (March (1)) (2018) 12.

[18] J.W. Chung, J.H. Ahn, J.Y. Kim, H.J. Lee, H.H. Kang, Y.K. Lee, et al., The effect of isoflurane, halothane and pentobarbital on noise-induced hearing loss in mice, Anesth. Analg. 104 (June (6)) (2007) 1404–1408, table of contents.

[19] J.M.E. Cederholm, K.E. Froud, A.C.Y. Wong, M. Ko, A.F. Ryan, G.D. Housley, Differential actions of isoflurane and ketamine-based anaesthetics on cochlear function in the mouse, Hear. Res. 292 (October (1–2)) (2012) 71–79.