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Rapid Communication

Preservation of cochlear function in *Fabp3* (H-Fabp) knockout mice

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ABSTRACT

Fatty acid-binding protein 3 (Fabp3) is an intracellular lipid trafficking protein that mediates energy metabolism and long-chain fatty acid-related signaling. Fabp3 is expressed in the spiral ganglion neurons and supporting cells of the organ of Corti. However, it is unclear what role Fabp3 plays in the cochlea. Here, we demonstrated that the ABR thresholds of young and aged *Fabp3* knockout mice were unchanged compared with those of wild-type mice. Compared with the wild-type mice, the adult mutant mice demonstrated no differences in their vulnerability to acoustic overexposure. These results suggest that Fabp3 deficiency alone does not adversely affect hearing function.

1. Rapid communication

Fatty acid-binding proteins (Fabps) are intracellular lipid trafficking proteins that bind to fatty acids (FAs) and other lipophilic substances and regulate metabolic pathways. Fabps also modulate gene expressions (Furuhashi and Hotamisligil, 2008; Owada, 2008; Storch and Thumser, 2010). The Fabp family has at least 10 members in mammals (Liu et al., 2008); two members, Fabp3 (heart-type, H-Fabp) and Fabp7 (brain-type, B-Fabp), have been identified in the mouse cochlea (Saino-Saito et al., 2010).

Fabp3 is required for long-chain FA transport to maintain efficient mitochondrial beta-oxidation, and it interacts with the nuclear receptors (e.g., PPAR-alpha) in the heart (Tan et al., 2002; Storch and Thumser, 2010). *Fabp3* knockout (KO) mice exhibit major alterations in peripheral free long-chain FA utilization, acute exercise intolerance in young age groups, and cardiac hypertrophy in old age groups (Binas et al., 1999). Fabp3 is also expressed in the adult brain, and it is necessary to maintain the n-6/n-3-polyunsaturated fatty acid (PUFA) balance, particularly for arachidonic acid (ARA) uptake and metabolism in neurons (Murphy et al., 2005; Storch and Thumser, 2010). An imbalance in the n-6/n-3-PUFA ratio may be a pathological factor in several neuropsychiatric disorders (Sakayori and Osumi, 2013). FABP3 levels are decreased in the brains of patients with Down syndrome and Alzheimer's disease (Cheon et al., 2003), which provides indirect evidence of a relationship between FABP3 and neurological functions and diseases (Storch and Thumser, 2010). These studies indicate that Fabp3 may have an important role in maintaining normal brain functions.

The cochlea is a main pathological region for sensorineural hearing loss (e.g., age-related hearing loss [AHL] and noise-induced hearing loss [NIHL]) (Ohlemiller, 2008; Kidd Iii and Bao, 2012). There are no effective treatments for sensorineural hearing loss; therefore, it is important to recognize the molecular mechanisms at work in the cochlea. Fabp3 is expressed in supporting cells (e.g., the inner and outer pillar cells and outer phalangeal cells) in the organ of Corti (OC) and spiral ganglion (SG) neurons (Saino-Saito et al., 2010), which suggests its role in regulating the hearing function. However, no studies of the Fabp3 function in the cochlea have been reported. Fabp3 is involved in apoptosis (Zhu et al., 2011; Song et al., 2012), which is critical in various pathophysiological processes. Apoptosis is also critical for AHL (Yamasoba et al., 2013) and NIHL (Op de Beeck et al., 2011). Therefore, we evaluated the cochlear function of *Fabp3* KO mice and focused on AHL and NIHL.

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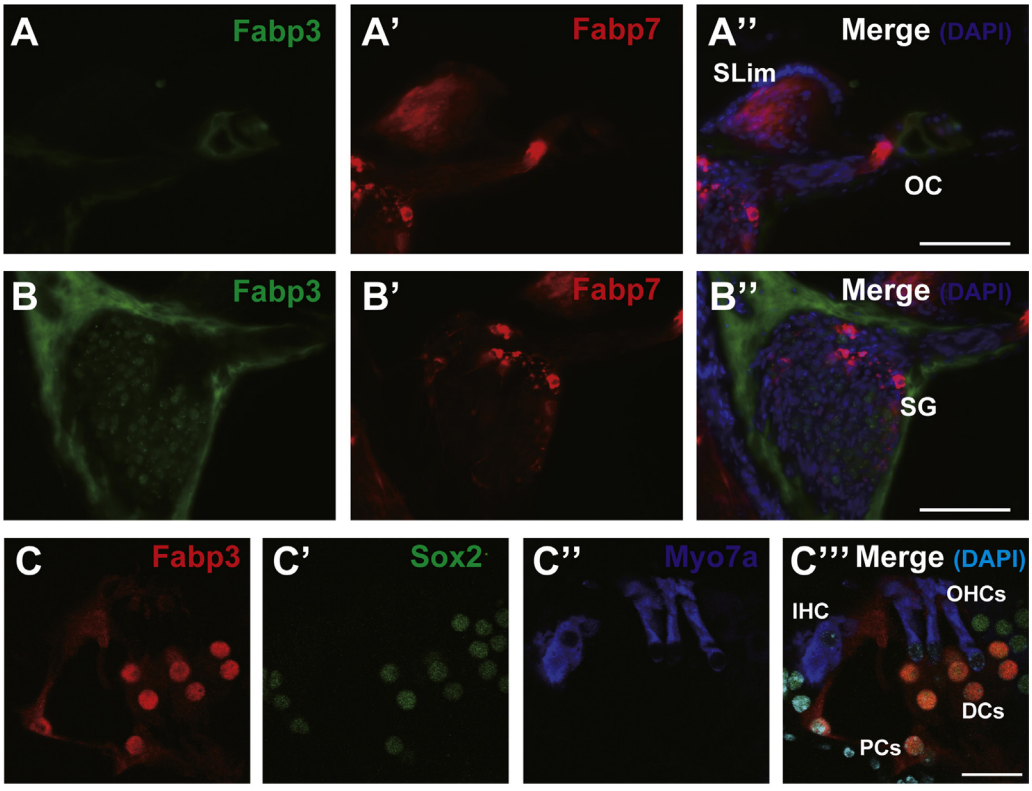
Table 1A
A list of antibodies used in this study.

Antibodies and dye	Dilution	Suppliers	Note
Anti-Fabp3	1:50	Hycult Biotechnology, HM2016	Mouse monoclonal IgG1
Anti-Fabp7	1:1000	A gift from Dr. Owada	Rabbit polyclonal Igs
Anti-Sox2	1:500	R&D systems, AF2018	Goat polyclonal Igs
Anti-Myosin 7a	1:500	Abcam, ab3481,	Rabbit polyclonal Igs
Alexa Fluor 488 Donkey Anti-Mouse IgG (H + L)	1:400	Invitrogen, A-21202	For anti-Fabp3
Alexa Fluor 488 Donkey Anti-Goat IgG (H + L)	1:400	Invitrogen, A-11055	For anti-Sox2
Cy3 Donkey Anti-Mouse IgG (H + L)	1:400	Jackson ImmunoResearch, 715-165-150	For anti-Fabp3
Cy3 Donkey Anti-Rabbit IgG (H + L)	1:400	Jackson ImmunoResearch, 711-165-152	For anti-Fabp7
DyLight 649 Donkey Anti-Rabbit IgG (H + L)	1:500	Jackson ImmunoResearch, 711-495-152	For anti-Myosin 7a
Biotin.SP. Donkey Anti-Mouse IgG (H + L)	1:1000	Jackson ImmunoResearch, 715-065-150	For anti-Fabp3

Table 1B
A list of primers used in this study.

Gene Name	Reference sequence	Forward primer (5′–3′)	Reverse primer (5′–3′)	Product size (bps)
Fabp3	NM_010174.1	GAA TAG AGT TCG ACG AGG TGA	CCT CCT TCT CAT AAG TCC GAG T	195
Fabp7	NM_021272	TGG ATG GAG ACA AGC TCA TTC	AAC AGC GAA CAG CAA CGA TA	126



D **A summary of the Fabp3 expression**

cochlear region	Fabp3-expressing cells
Organ of Corti (OC)	Deiters' cells (DCs)
	Inner pillar cells (PCs)
	Outer pillar cells (PCs)
Spiral ganglion (SG)	Neurons

Fig. 1. Fabp3 expression in the cochlea of an adult mouse (age 3–4 months). (A and A″) Fabp3 and Fabp7 expression patterns in the organ of Corti (OC) and the spiral limbus (SLim). Fabp3 is localized only in the OC (A and A″). (B and B″) Fabp3 and Fabp7 expression patterns in the spiral ganglion (SG). Fabp3 is detected in the SG neurons (B and B″), and Fabp7 is detected in the satellite cells (B′ and B″). (C and C‴) Confocal images of the OC. Fabp3 is localized in the nuclei and cytoplasms of Sox2-positive and Myo7a-negative supporting cells: Deiters cells (DCs) and inner and outer pillar cells (PCs) (C and C‴). A summary of the Fabp3 expression is shown in (D). Abbreviations: inner hair cell (IHC) and outer hair cells (OHCs). Scale bars represent (A″ and B″) 100 μm and (C‴) 20 μm.

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