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LETTER TO THE EDITOR

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On the conflicting reports of imprinting status of mouse *ATP10a* in the adult brain: strain-background-dependent imprinting?

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To the Editor:

We read with interest a report by Kashiwagi et al. (2003) in the April issue of the journal, in which the authors detailed their results on imprinting studies in the mouse *Atp10a* gene, previously called as *Atp10c*/*pfatp*, the mouse ortholog of the human *ATP10C* gene. They analyzed the imprinting status using a nucleotide polymorphism in 30-week-old adult F1 hybrid mice of crosses between two different strains (C57BL/6J x JF1) of *Mus musculus*. They demonstrated that *Atp10a* is imprinted in a tissue-specific manner, with predominant expression of the maternal allele in the hippocampus and olfactory bulb.

Contrary to their results, we have recently reported that the mouse *Atp10a* gene escapes genomic imprinting in all fetal (embryonic day 16) and adult (6 weeks) tissues examined, including the hippocampus (Kayashima et al. 2003). In our imprinting study, the PWK strain of *Mus musculus* was mated with C57BL/6 J strain to make F1 hybrid mice, and a polymorphic site, different from that reported by Kashiwagi et al. (2003), was used for intron-spanning RT-PCR assay. Differences in materials and methods between the two studies were mouse strains, ages of adult mice, and polymorphic sites used for the imprinting analysis.

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To verify the discrepancy in the imprinting status of *Atp10a* in adult mice, three F1 hybrid mice at the age of 30 weeks were investigated. The allelic expression of *Atp10a* was analyzed in RT-PCR assay using two polymorphic sites; one in *Atp10a* ORF (Kayashima et al. 2003) and the other in the 3'UTR reported in JF1 strain (Kashiwagi et al. 2003), which was also polymorphic in PWK strain. The experiments were independently performed in each of three F1 hybrids and revealed bi-allelic expression of the *Atp10a* gene in both the hippocampus and olfactory bulb (Fig. 1). On the other hand, an imprinted gene, *Ube3a,* showed predominant expression from the maternal allele in the identical cDNAs from the frontal cortex, olfactory bulb, and hippocampus (Fig. 1). We also analyzed the imprinting status in the primary brain-cell culture system and found that *Atp10a* was not imprinted in cultured neurons (data not shown), where *Ube3a* was completely imprinted (Yamasaki et al. 2003). These results confirmed that *Atp10a* escapes genomic imprinting whereas *Ube3a* shows neuron-specific imprinting in the brain in our F1 hybrid (PWK x C57BL/6 J) mice system.

How can we explain such conflicting results in the imprinting status of *Atp10a* between two studies? The most plausible explanation may be strain background-dependent imprinting. A good example is the mouse *Kvlqt1* gene, of which imprinted expression is detected in fetal tissues from female CAST/Ei (CS) mice x male 129/SvEv (129) mice, whereas *Kvlqt1* exhibits partial loss of imprinting (LOI) in fetal tissues from female 129 x male CS mice (Jiang et al. 1998).

We have revealed bi-allelic expression of *Atp10a* in C57BL/6 J and PWK strains, but our result does not absolutely rule out its imprinted expression in other strains, including JF1. However, strain background-dependent imprinting cannot easily explain the results, because C57BL/6 J strain was used for a common parent of F1 hybrid mice in both studies. Other factors, such as the strain-dependent total expression level of *Atp10a*, may also affect its allele expression in C57BL/6 J and JF1. The fact that imprinting of the human

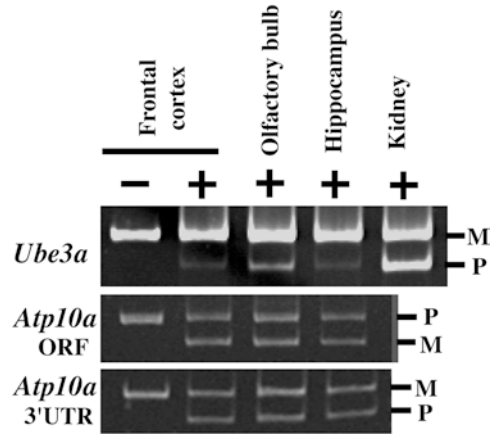

Fig. 1 Imprinting analysis of *Ube3a* and *Atp10a* in 30-week-old adult F1 hybrid mice from female PWK x male C57BL/6 J mice. Predominant *Ube3a* expression from the maternal allele was demonstrated in the identical cDNAs from the brain samples (*upper panel*) using polymorphism (Yamasaki et al. 2003; Chamberlain and Brannan 2001). Bi-allelic expression of *Atp10a* was detected using a polymorphism (*middle and lower panels*) reported in the *Atp10a* ORF (Kayashima et al. 2003) and 3′UTR (Kashiwagi et al. 2003), respectively. All experiments were performed under the same PCR conditions as described previously (Yamasaki et al. 2003; Kayashima et al. 2003; Kashiwagi et al. 2003). *Plus* and *minus* signs mean with and without *Tsp*509I, *Hpy*Ch4IV, and *Msp*I digestions (*upper, middle, and lower panels*), respectively. *M* and *P* mean RT-PCR products originated from the maternal and paternal alleles, respectively

ATP10C gene was not seen in the brain from one normal individual (Meguro et al. 2001) may support strain-background-dependent imprinting of mouse *Atp10a*.

In addition to repeated experiments in the F1 hybrid mice, further investigations on the imprinted expression of *Atp10a* in their F1 hybrid mice (C57BL/6 J x JF1) will elucidate the molecular mechanism of brain-specific imprinting. It also remains to be investigated when *Atp10a* imprinting becomes prominent in the brain regions and whether it correlates with imprinted *Ube3a* expression under control of an imprinting center.

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