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Supplementary Data

LRRTM3 is Dispensable for Amyloid-β Production in Mice

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LACK OF LRRTM3 DOES NOT CAUSE COMPENSATORY UPREGULATION OF LRRTM4 MRNA EXPRESSION

Mouse *Lrrtm4* gene has most of its coding sequence (including the stop codon) in exon-3 and is predicted to have two alternative start codons, either in exon-1 or in exon-2 [1]. We analyzed the expression of the alternative transcripts using two different primer pairs spanning exon-1 to exon-3 (e1–3) or exon-2 to exon-3 (e2-3). Total RNA was isolated with the Nucleospin RNA L kit (Macherey-Nagel) from the forebrain (excluding the olfactory bulb). One μg of total RNA was reverse transcribed using the iScript cDNA synthesis Kit (Bio-Rad) according to the manufacturer's protocol. The cDNA samples were amplified in triplicate using the Maxima SYBR Green qPCR Master Mix (Thermo Scientific) and detected via the CFX96 Real Time Detection System (Bio-Rad). Primers for *Lrrtm4* and *Gapdh* (used for normalization) were designed with the PrimerQuest software (eu.idtdna.com) and were validated by gel electrophoresis to produce a single band of expected size:

Product	Forward	Reverse
Lrrtm4(e1–3)	5′-GCACAATA AGGAATGCC AGGTTTC-3′	5′-TAACCAGCA GCAGGGTG GGAAATA-3′
Lrrtm4(e2-3)	5′-TTTGGATG ACAAAGGA TGGGTTTC-3′	5′-TAACCAGCA GCAGGGTG GGAAATA-3′
Gapdh	5′-TGCACCAC CAACTG CTTAGC-3′	5′-TGGCAT GGACTGTG GTCATG-3′

Real-time PCR analysis showed that the relative expression of both *Lrrtm4* mRNA transcripts in the brain was similar between 6-month-old *Lrrtm3*-KO and WT littermates (*Lrrtm4*(e1–3): KO/WT = 1.1 ± 0.3, $p = 0.8$; *Lrrtm4*(e2-3): KO/WT = 1.1 ± 0.2, $p = 0.4$ between the genotypes using *t*-test; $n = 5$ mice in both genotypes).

REFERENCES

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