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Research article

# Mutation of the key residue for extraribosomal function of ribosomal protein S19 cause increased grooming behaviors in mice

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## HIGHLIGHTS

- Gln137Glu-RP S19 knock-in mice show increased grooming behavior.
- Gln137Glu-RP S19 knock-in mice show enhanced anxiety-like behavior.
- Gln137Glu-RP S19 knock-in mice show enhanced fear memory.

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## ABSTRACT

Ribosomal protein S19 (RP S19) possesses ribosomal function as RP S19 monomer and extraribosomal function as cross-linked RP S19 oligomers which function as a ligand of the complement 5a (C5a) receptor (CD88). We have generated a Gln137Glu-RP S19 knock-in (KI) mouse, which is shown to possess the weakened extraribosomal function of RP S19. Because whether the extraribosomal function of RP S19 has a role in brain function had been unclear, we performed behavioral analysis on these mice and demonstrated that KI mice displayed an increased grooming behavior during open-field test and elevated plus maze test and an enhanced freezing behavior in contextual fear conditioning test. These results suggest an involvement of RP S19 oligomers in some anxiety-like behavior, especially grooming behavior.


## 1. Introduction

Ribosomal protein S19 (RP S19) is a component of the small ribosomal subunit, playing a role in ribosome biogenesis [1]. RP S19 is also present in blood plasma forming a complex with prothrombin [2]. RP S19 is oligomerized *via* the intermolecular crosslinking between Lys122 and Gln137 by a transglutaminase-catalyzed reaction and gains a ligand capacity to the complement 5a (C5a) receptor (CD88) as an extraribosomal function [2,3]. The RP S19 oligomer-C5a receptor system plays roles in apoptotic cell clearance, in blood coagulum resorption and in erythrocyte maturation with enucleation [4].

We recently generated the Gln137Glu-RP S19 knock-in (KI) mice to reveal further the extraribosomal roles of RP S19 oligomers *in vivo*. This mutation did not apparently affect the ribosomal function of RP S19 because homozygote mice with Gln137Glu-RP S19 were born alive and fertile [5]. Our previous *in vitro* study of a Gln137 mutation suggested at least a 70% reduction in the extraribosomal function of RP S19 in these KI mice [6]. Indeed, a significant delay of clearance of KI mouse-derived blood coagulum was observed in a hematoma resorption model [5].

In the central nervous system (CNS), several evidences gave a role of complement system in development, neuropretection, neurogenesis and synaptic plasticity [7]. Expression of C5a receptor is revealed in astrocytes and microglia of the CNS [8] and most neurons in the cerebral cortex, hippocampus and cerebellum [9].

*Abbreviations:* ANOVA, analysis of variance; CNS, central nervous system; RP S19, ribosomal protein S19; KI, knock-in; WT, wild-type; Gln, glutamine; Glu, glutamic acid; C5a, complement 5a.

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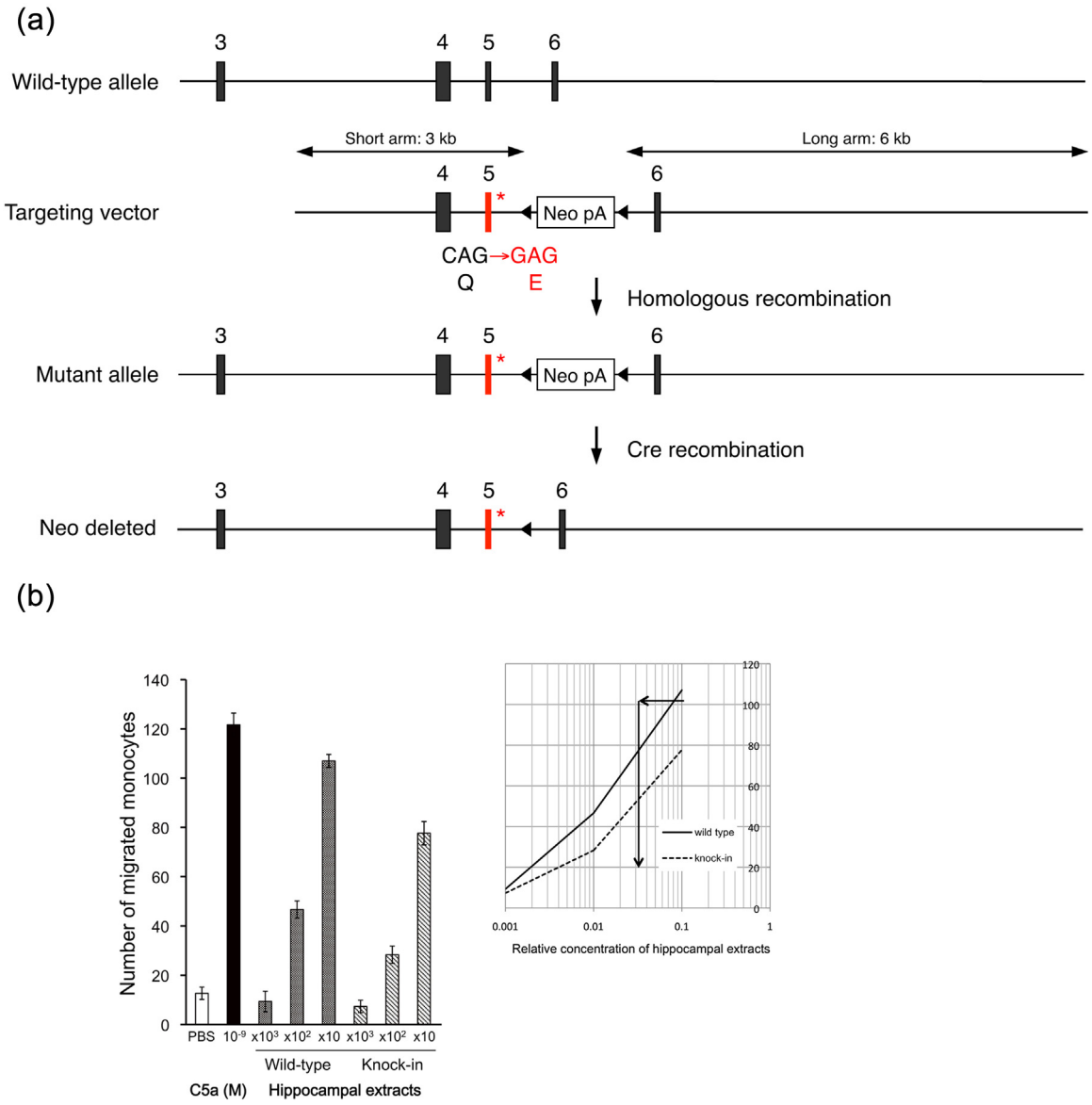


**Fig. 1.** Monocyte chemotactic activity in the hippocampus of KI mice.
(a) Schematic diagram of the endogenous *RPS19* locus and the targeting vector used to engineer embryonic stem (ES) cells by homologous recombination. This vector contains a point mutation in exon 5 that changes glutamine residue at position 137 to glutamic acid (Gln137Glu). neo, neomycin-resistant gene; pA, poly A. (b) Chemoattraction capacities of the hippocampal extracts for normal human peripheral monocytes were measured by the multiwell chamber assay at 3 logarithmic serial concentrations. The interpolated figure demonstrates concentration (logarithmic)-function (linear) relation curves to quantify the ratio of the monocyte chemotactic capacity in the KI mouse extract against that in the WT mouse extract.

Therefore, complement system *via* C5a receptor is suggested to be involved in CNS function and neurodegenerative diseases [7,10]. In that case, the RP S19 oligomers could have a role in CNS function *via* binding to C5a receptor. Moreover, RP S19 mRNA expression was revealed in pyramidal neurons of hippocampus [11]. However, relevance of C5a receptor and RP S19 extraribosomal function to *in vivo* function is largely unknown. Therefore we examined whether the Gln137Glu-RP S19 KI mice expressed some abnormalities in behavioral tests.

## 2. Materials and methods

### 2.1. Animals

We prepared Gln137Glu-RP S19 KI heterozygous mice by knock-in technology *via* homologous recombination as described previously [5]. We purchased specific pathogen-free C57BL/6J mice from Charles River (Yokohama, Japan) and the heterozygous KI mice were backcrossed with C57BL/6J mice more than six times. Then heterozygous KI mice were intercrossed to obtain homozygous mice. The homozygous KI mice and the wild-type (WT) mice were bred and maintained, respectively, in the same way at the Center for Animal Resources and Development, Kumamoto University. Genotyping is performed on tail DNA *via* polymerase chain reaction using primers (Forward, TGGGCTGTACTCATCCAGGGT; Reverse, TGCTTCTTGTTGGCAGCTGCCAC) designed across the loxP site. Mice were maintained on a 12 h light/dark cycle (7 a.m. on/7 p.m. off) with laboratory chow and water available *ad libitum*. Male mice were used for all experiments and the behavioral tests were performed at light cycle. The present animal experiments were approved by the Ethical Committee for Animal Experiment, Kumamoto University (# A27-129).