OPEN PEER REVIEW REPORT 1

Name of journal: Neural Regeneration Research
Manuscript NO: NRR-D-21-00394
Title: Crry Silencing Alleviates Tau Pathology by Regulating the Expression of Neuroinflammatory Cytokines and the Complement System
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COMMENTS TO AUTHORS

Crry alleviated tau phosphorylation and improved cognitive function by regulating neuroinflammation and the complement system in the AD mouse model. Thus, CR1 is a potential therapeutic target for AD treatment.

This is an interesting and important study addressing the role of a key rodent complement regulator namely CRRY. The experiments are carried out well and are mostly well described however the authors appear unable to suggest a viable reason for their rather surprising data showing that knocking down the key complement regulator actually improves the inflammatory state, cognitions and phosphorylation of TAU. I address the discussion in more detail at the end.

There are a number of other issues:
Materials and Methods
Lentiviral particles (3 μL) were injected into the cerebral cortex (anteroposterior:-0.3, mediolateral: 2, dorsoventral: -1.5 mm and anteroposterior: -2, mediolateral: 1.2, dorsoventral:-1.2 mm for the cerebral cortex) and the hippocampus (anteroposterior:-2; mediolateral: 1.2; dorsoventral: -2 mm for the hippocampus) of the brain (6 months in P301S mice) Did each mouse receive all the region dosings? The authors need to make this clear.
P9 Western Blot : Mouse brain tissues were lysed with 100 μL/50 mL protein lysate RIPA. Please check: should this be 100 uL/50 mg?
All % should be annotated correctly ie w/v; v/v as appropriate.
P10 QRT-PCR: quantity of RNA used for Reverse transcription should be in ug NOT ul
Double Immunofluorescence staining: thickness of sections: should be uM NOT mm
Results
Statistics: materials and methods say SEM's are presented but Figure legends 2-10 say "SD" which is correct?
Fig 2 Crry expression goes up with age and in the tau tg mice
Need to insert a bar to show which groups are being compared statistically.
Fig 4 why does tau get phosphorylated? How does complement influence this? Why does ko CRRY improve this? Little discussion on this. Needs to be expanded.
Fig 5
The actin blots are not loading controls. To be loading controls there would have to be one actin blot for each test blot (or one for the groups of blots if stripping of membranes was carried out).
Fig 6 number of neurons decreased in TG mice. Crry KO ameliorates this; cleaved caspase down in Crry KO;
"Moreover, cleaved caspase-3 expression was lower in P301S mice with Crry shRNA compared with P301S mice with control shRNA (Figure 6D, E), suggesting possible apoptosis"
Do the authors mean that there is less apoptosis in the those mice receiving the Crry shRNA?

Fig 9 CRRY SILENCING MODULATED NEUROINFLAMMATION AND COMPLEMENT

SYSTEM
Downregulation of Crry significantly suppressed
the expression of IL-1β (by 47.3%, n=6, P<0.05, Figure 9A), TNF-α (by 53.4%, n=6, P<0.05, Figure 9B) and IL-6 (by 35.4%, n=6, P<0.05, Figure 9C

Why qPCR? Why not do westerns or ELISA on the homogenates?

Levels of C3 and C3b proteins decreased by 48.6% and increased by 1.52-fold, respectively, (Figures 10C and 10D) compared with P301S mice with control shRNA (Figure 10E). Both should be expressed as % OR fold change.

Discussion
1) Regarding the levels of C3 and C3b this looks like consumption of C3 together with increase in activation products. SO we have more complement activation in the shCrry treated animals. How does this mediate the interesting data shown regarding the beneficial effects of CRRY knockdown? The section discussing the role of crry and complement is not very informative and needs to be completely re-written, please see: J Exp Med. 1995 Jan 1;181(1):151-9. doi: 10.1084/jem.181.1.151. Mouse complement regulatory protein Crry/p65 uses the specific mechanisms of both human decay-accelerating factor and membrane cofactor protein Y U Kim et al
2) There is a clear difference between complete knockout of Crry which was shown by Ruseva et al (Mol Immunol. 2009 Feb;46(5):803-11. doi: 10.1016/j.molimm.2008.09.003. Epub 2008 Oct 22.) and the partial knockdown achieved by the authors in this manuscript. This needs careful thought and to be added to the discussion.
3) How is the increase in C3b beneficial? What is this C3b doing? Normally one might expect that this would go to form C3 convertases and also be involved in opsonisation. Indeed the limited complement data shown (C3 and C3b) appear to show a fall in intact C3 levels probably due to increased activation in the crry knockdown group and a concomitant rise in the activation product C3b. This later probably accumulates because the fI mediated cleavage to iC3b that would normally be catalysed by CRRY is slowed in the knockdown animal group.
4) It would be very informative if the authors had focussed more experiments on determining the levels of other complement activation products: C3a, C5a and the terminal complement complex (TCC/MAC).
5) There is some evidence that CRRY can induce signalling in cells: J Leukoc Biol. 2005 Dec;78(6):1386-96. doi: 10.1189/jlb.1104642. Complement regulatory protein Crry/p65-mediated signalling in T lymphocytes: role of its cytoplasmic domain and partitioning into lipid rafts and Crry/p65, a Membrane Complement Regulatory Protein, Has Costimulatory Properties on Mouse T Cells Elena Fernández-Centeno et al, J Immunol May 1, 2000, 164 (9) 4533-4542; DOI: https://doi.org/10.4049/jimmunol.164.9.4533

How does knocking down CRRY in this context affect the biology/immunobiology in the brains of the experimental mice used?

Given all the questions regarding the possible mechanisms underlying the presented data, it seems a little premature at this point to describe CR1 as a good target for therapy.