SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES

FORMAT BY SYRCLE (WWW.SYRCLE.NL)
VERSION 2.0 (DECEMBER 2014)

Item #	Section/Subsection/Item	Description	Check for approval
	A. General		
1.	Title of the review	Cognitive deficits in targeted knock-in APOE4 mice: A systemic review	x
2.	Authors (names, affiliations, contributions)	P. Roemers[1], T. Metz[1], M.J. van Heuvelen[2],P.P. DeDeyn[3] C.R. Hooijmans[4], E.A. van der Zee[1] [1] Molecular Neurobiology, Groningen Institute for Evolutionary Life Sciences (GELIFES), University of Groningen, Groningen, The Netherlands [2] Center for Human Movement Sciences, University Medical Center Groningen, Groningen, The Netherlands [3] Department of Neurology and Memory Clinic, Hospital Network Antwerp, Middelheim and Hoge Beuken, Belgium 14 Reference Centre for Biological Markers of Dementia (BIODEM), Laboratory of Neurochemistry and Behavior, Department of Biomedical Sciences, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium [4] Department of SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE), Radboud University Medical Centre, 6500 HB, Nijmegen, the Netherlands	x
3.	Other contributors (names, affiliations, contributions)		x
4.	Contact person + e-mail address	P.Roemers, p.roemers@rug.nl	x
5.	Funding sources/sponsors	ZonMW, Deltaplan Dementie	x
6.	Conflicts of interest	None	x
7.	Date and location of protocol registration	Groningen, 03-04-2017	x
8.	Registration number (if applicable)		x
9.	Stage of review at time of registration	Conducting pre-screening	x
	B. Objectives		
	Background		
10.	What is already known about this disease/model/intervention? Why is it important to do this review?	A number of studies have found that targeted replacement of the murine APOE gene with the human APOE4 gene impairs cognition in various behavioural tasks. There is a strong suspicion that these effects are age-related and sex-specific, but the available studies cannot conclude this with certainty. Similarly, it is unknown what cognitive task is most suitable to reveal cognitive deficits in APOE4 mice. A systematic review of available literature could clear up these issues, or clearly point out gaps in current knowledge. Moreover, since APOE4 is the only universal risk factor for sporadic Alzheimer's Disease but little is known about its	x

		effects on the brain, the targeted knock-in APOE4 mouse model could prove a powerful tool to investigate the pathology of sporadic Alzheimer's Disease. The current review will not investigate possible neuro-molecular mechanisms, but could help target mechanisms related to certain cognitive domains or brain areas by investigating cognition. Finally, this review will support proper study design for researchers that utilize this mouse-model.	
	Research question		
11.	Specify the disease/health problem of interest	The effects of APOE4 on various cognitive domains. This is relevant in the context of sporadic Alzheimer's disease.	x
12.	Specify the population/species studied	Mouse, rat	x
13.	Specify the intervention/exposure	Targeted knock-in of the APOE4 gene	x
14.	Specify the control population	Targeted APOE3 knock-in mice	x
15.	Specify the outcome measures	Cognitive output parameters related to learning and memory and anxiety in any standardized behavioural test	x
16.	State your research question (based on items 11-15)	What are the effects of APOE4 genotype on learning and memory and anxiety in targeted replacement mice?	x
	C. Methods		
	Search and study identification		
17.	Identify literature databases to search (*e.g.* Pubmed, Embase, Web of science)	X MEDLINE via PubMed X Web of Science ☐ SCOPUS ☐ EMBASE ☐ Other, namely: ☐ Specific journal(s), namely:	x
18.	Define electronic search strategies (*e.g.* use the step by step search guide[15] and animal search filters[20, 21])	When available, please add a supplementary file containing your search strategy: [Search strategy APOE4 review.pdf]	x
19.	Identify other sources for study identification	X Reference lists of included studies ☐ Books ☐ Reference lists of relevant reviews ☐ Conference proceedings, namely: ☐ Contacting authors/ organisations, namely: ☐ Other, namely:	x
20.	Define search strategy for these other sources	If an additional paper is encountered in one of the included studies, and this paper meets the same criteria, the paper will be included.	x
	Study selection		
21.	Define screening phases (*e.g.* pre-screening based on title/abstract, full text screening, both)	Pre-screening based on title /abstract. Full text screening for all pre-screening selected studies.	x
22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved	a) Two reviewers per phase. b) Pre-screening discrepancies: Study which is subject of discussion will be included for full-text screening. b) Full text screening discrepancies:	x

		Objective and clear inclusion criteria should eliminate discussion about which studies are to be included in the systemic review. If issues do arise, we will ask an uninvolved staff-member from the research group to resolve it.	
	Define all inclusion and exclusion criteria based on:		
23.	Type of study (design)	Inclusion criteria: Primary research Exclusion criteria: Reviews	x
24.	Type of animals/population (*e.g.* age, gender, disease model)	Inclusion criteria: Targeted replacement APOE3 and APOE4 mice Exclusion criteria: All other species, non-targeted replacement APOE3 or 4 knock-in mice	x
25.	Type of intervention (*e.g.* dosage, timing, frequency)	Inclusion criteria: Targeted replacement of the murine APOE gene with human APOE3 and 4 polymorphisms. Targeted replacement is a knock-in method that results in substitution of the murine APOE gene with the human APOE3 or 4 gene so that all murine regulatory sequences remain intact. Thus APOE3 and 4 expression is regulated in the same manner that murine APOE expression is regulated. Exclusion criteria: Studies based on a pharmaceutic, behavioural or dietary intervention. Any intervention other than the targeted replacement of the murine APOE gene with human APOE3 and 4 polymorphisms.	x
26.	Outcome measures	Inclusion criteria: Any behavioural test that measures learning and memory or anxiety. Exclusion criteria:	x
27.	Language restrictions	Inclusion criteria: Exclusion criteria: None	x
28.	Publication date restrictions	Inclusion criteria: Exclusion criteria: None	x
29.	Other	Inclusion criteria: Exclusion criteria:	x
30.	Sort and prioritize your **exclusion** criteria per selection phase	Selection phase: Pre-screening 1. Not a study conducted in mice or rats 2. Not a primary study 3. No APOE4 group Selection phase: Full text screening Same as above + 1. No targeted replacement APOE3 and APOE4 mice or rats 2. Does not include behavioural testing for cognitive function or anxiety 3. Co-intervention: Does not include a control group (in case of any intervention other than the targeted replacement of the murine APOE (e.g. pharmaceutical, dietary or other transgenes knocked-in)	x

	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
31.	Study ID (*e.g.* authors, year)	Authors, year, journal	x
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	Experimental groups, number of animals	x
33.	Animal model characteristics (*e.g.* species, gender, disease induction)	Strain, gender, age, genotype	x
34.	Intervention characteristics (*e.g.* intervention, timing, duration)	Mouseline or ratline	x
35.	Outcome measures	Type behavioural test; Name test; output parameter (unit of measurement) E.g. Spatial learning; Morris Water Maze; Escape latency (s) Spatial learning; Morris Water Maze; Distance to platform (cm) Spatial memory; Morris Water Maze; Time spent in target quadrant (% total time)	x
36.	Other (*e.g.* drop-outs)		x
	Assessment risk of bias (internal validity) or study quality		
37.	Specify (a) the number of reviewers assessing the risk of bias/study quality in each study and (b) how discrepancies will be resolved	a) Two b) If issues do arise, we will ask an uninvolved staff-member from the research group to resolve it.	x
38.	Define criteria to assess (a) the internal validity of included studies (*e.g.* selection, performance, detection and attrition bias) and/or (b) other study quality measures (*e.g.* reporting quality, power)	☐ By use of SYRCLE's Risk of Bias tool[4] **X**By use of SYRCLE's Risk of Bias tool, adapted as follows: to be extended with some reporting quality items (any measure used for randomization or blinding the outcome assessment ☐By use of CAMARADES' study quality checklist, e.g [22] ☐By use of CAMARADES' study quality checklist, adapted as follows: ☐Other criteria, namely:	x
	Collection of outcome data		
39.	For each outcome measure, define the type of data to be extracted (*e.g.* continuous/dichotomous, unit of measurement)	For each outcome measure: Mean, SD, ~~SEM~~, n per group. Effect sizes will be calculated per outcome measure (APOE E4 vs E3) If the ES cannot be calculated due to a lack of information in the article, the level of significance (p) is provided.	x
40.	Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting authors)	1. Direct extraction of data from tables or text 2. Extraction from graphs using digital screen ruler 3. Contacting the authors. A maximum of two attempts will be made. After the second attempt, we will wait 2 weeks for an answer.	x

41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	a) Two b) If issues do arise, we will ask an uninvolved staff-member from the research group to resolve it.	x
	Data analysis/synthesis		
42.	Specify (per outcome measure) how you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	If possible, a meta-analysis will be performed for all outcome measures. If a meta-analysis is not possible (too few or too heterogeneous datasets) the data will be reported by descriptive summary.	x
43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	A meta-analysis will be performed if there are at least 3 studies reporting on a specific outcome measure. Subgroup analyses are only conducted in case of minimal 5 independent comparisons	x
	If a meta-analysis seems feasible/sensible, specify (for each outcome measure):		
44.	The effect measure to be used (*e.g.* mean difference, standardized mean difference, risk ratio, odds ratio)	Mean difference if applicable (if units of measurement are the same), standardized mean difference if outcome measures are presented in different units of measurements. To be determined for every included behavioural test.	x
45.	The statistical model of analysis (*e.g.* random or fixed effects model)	Random effects model	x
46.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	I^2	x
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	Sex, age, type of cognitive test used	x
48.	Any sensitivity analyses you propose to perform		x
49.	Other details meta-analysis (*e.g.* correction for multiple testing, correction for multiple use of control group)	Correction of p-value for the number of subgroup analyses by Bonferroni-Holmes correction for multiple comparisons with the same control group by dividing the number of control animals by the number of comparisons with the control group	x
50.	The method for assessment of publication bias	Funnel plot	x
Final approval by (names, affiliations):		P. Roemers[1] T. Metz[1] M.J. van Heuvelen[2] E.A. van der Zee[1] [1] Molecular Neurobiology, Groningen Institute for Evolutionary Life Sciences (GELIFES), University of Groningen, Groningen, The Netherlands [2] Center for Human Movement Sciences, University Medical Center Groningen, Groningen, The Netherlands	Date: 016-02-2017