PGC Secondary Analysis Proposal *(v2, revised 02-2015, pfs)*

Investigative Team. Underline PGC PI taking responsibility for all aspects of this proposal.

Date	October 2018
Title	The Heritability of Amygdala Subregions and the Relationship with Posttraumatic Stress Disorder

Investigative Team. Underline PGC PI taking responsibility for all aspects of this proposal.

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Data access requested. No permission required for published results (only pre-publication)

Group	**Individual genotypes**	**Summary results**	**Permission from group?**	**Version (e.g., MDD2, SCZ3)**

ADHD				
AN				
AUT				
BIP				
Drug/alcohol				
MDD				
OCD/TS				
PTSD		X	X	Freeze 2
SCZ				

A. Research Question, Goal, or Specific Aims

Provide a brief description (e.g., 1 paragraph) describing the aims of the proposal and the research questions to be addressed.

Decreased amygdala volume has been associated with post-traumatic stress disorder (PTSD) (Morey et al., 2012; Logue et al., 2018), but few studies have examined amygdala subregions in this disorder (Veer et al., 2015). Heritability estimates of PTSD are as high as 53%, (Isomura et al., 2015) but little is known about the genetic variants that increase risk (Duncan et al., 2018). The heritability of amygdala volume, unlike most other subcortical brain regions, remains undetermined (Hibar et al., 2015). The rationale for this work is based on a growing basic science literature that delineates the specific functions of different amygdala subregions (Swanson, 2006; Koen et al., 2016; Saygin et al., 2017). It has been suggested that the amygdala should no longer be considered as a structural and functional unit, but rather as consisting of distinct subregions/ nuclei that are distinguished from one another by their functions, cyto-architecture and connectivity and neurotransmitter profiles (SWANSON, 2006). In August 2018 FreeSurfer v6.0 released a novel ex vivo atlas allowing for segmentation of the amygdala into nine nuclei (anterior amygdaloid, corticoamygdaloid transition area; basal, lateral, accessory basal, central, cortical medial, paralaminar nuclei) (Saygin et al., 2017). This approach was able to distinguish between patients with Alzheimer's and autism from healthy controls with 84% and 51% accuracy, respectively. This is the highest predictive accuracy achieved to date (Saygin

et al., 2017). This study aims to determine the heritability of the amygdala through investigation of these subregion volumes and to refine the association between the amygdala and PTSD through investigation of case-control differences in the volumes of these subregions and determining the degree of genetic overlap between risk variants affecting these volumes and those that increase risk for PTSD. Through this investigation we may identify genetic variants that can lend insight into the pathophysiology of PTSD, determine whether amygdala subregions are genetically distict from one another and what the genetic architeture is that underlies each of these subregion volumes.

B. Analytic Plan

Provide a brief description of the analyses to be performed to address the research questions described above. Include relevant details e.g. phenotype definition, QC, analysis, plans to address population stratification and other confounders, power.

The aims of this study will be achieved via the following objectives:

1. Perform amygdala subregion parcellation (9 subregions) on healthy controls from the UK-Biobank and cases and controls from ENIGMA PTSD using the novel subregion segmentation algorithm that was released as part of FreeSurfer v6.0 (Saygin et al., 2017)
   1.1. The effects of scanning sites, sex, brain disorder diagnosis, age, and intracranial volume (ICV) from each outcome measure will be regressed out prior to analyses, using a generalized additive model (GAM)-fitting in R (v2.4.0).
   1.2. Individuals ±4 SD from the mean on any of the amygdala measures or ICV will be removed.
2. Investigate the volumetric differences between each of the amygdala subregions between PTSD cases and controls using FreeSurfer v6.0 using standardised ENIGMA protocols (Stein et al., 2012)
   2.1. Age, age of onset, gender, ethnicity, handedness, years of education, total intracranial volume, medication and the effects of different scanning sites will be included as covariates.
3. Perform a genome-wide association study (GWAS) for the volumes of the amygdala and each of its subregions using controls from the UK-Biobank using Plink (Purcell et al., 2007)

3.1. Phasing and imputation will be carried out according to the ENIGMA consortium protocols (http://enigma.ini.usc.edu)
3.2. Standard quality control parameters will be used (Weale, 2010)
3.3. Potential population stratification will be adjusted for by including the first four multidimensional scaling (MDS) measures as covariates.

4. Investigate the heritability of amygdala volume and the volumes each of its subregions.
4.1. First, the genomic data will be pruned based on linkage disequilibrium (LD)
4.2. Second, genome-wide complex Trait Analysis (GCTA) will be used to calculate the SNP-based heritability of amygdala volume and each of the subregion volumes (Yang et al., 2011).
4.3. Potential population stratification will be adjusted for by including the first four MDS measures as covariates.

5. Determine the genetic overlap between each of the amygdala subregion volumes.
5.1. Bivariate restricted maximum likelihood (REML) analysis will be applied to determine the genetic correlation between all of the regions (Lee et al., 2012).

6. Determine the genetic overlap between the amygdala and each of its subregion volumes with PTSD risk variants.
6.1. Cross-trait Linkage Disequilibrium Score regression (LDSR) (Bulik-Sullivan et al., 2015) and conditional false discovery rate (FDR) analysis (Andreassen et al., 2013) will be used to investigate the genetic overlap between PTSD and each of the amygdala subregions.
6.2. Polygenic risk scoring (Purcell et al., 2009) will also be used to investigate the predictive capacity of variants influencing amygdala subregion volumes in determining PTSD disease status

C. Analytic Personnel

Indicate who will be responsible for performing the analyses.

Ms Maryanne Mufford

D. Resources Needed

Describe the resources needed to achieve the aims of the analysis, including variables needed, analytic support, and any other issues

that may affect the feasibility of the plan.

Summary statistics from the PGC-PTSD Freeze 2 data will be required. Separate reports for the total cohort and each ancestry group studied separately (European and African etc) will be required where possible.

Analytical support will be provided by Prof Rajendra Morey

F. Timeline

Estimate time required to complete the plan and write a paper (should be ≤ 6 months).

We aim to submit a paper for publication within 6 months of receiving the PGC-PTSD Freeze 2 summary statistics

F. Collaboration

The following is the standard PGC policy about secondary analyses. Any deviation from this policy needs to be described and justified, and could negatively impact the proposal.

PGC investigators who are not named on this proposal but who wish to substantively contribute to the analysis and manuscript may contact the proposing group to discuss joining the proposal.

G. Authorship

This is an extremely important part of this proposal. Describe how authorship will be handled in the manuscript resulting from this analysis. To avoid a revision, first review the authorship policy of the group(s) whose data you wish to analyze. Points to consider:

(a) are you following the authorship policies of the groups involved?

(b) will there be a writing group and if so, who will be included?

(c) what groups or individuals will be listed as authors?

(d) will PGC members not listed as named authors be listed at the end of the manuscript?

(e) will PGC members or groups be listed as "collaborators" on the PubMed abstract page?

(f) how will funding sources be handled or acknowledged?

a) The study will include summary statistics from the PGC-PTSD Freeze 2 working group. We will follow the PGC-PTSD authorship guidelines.

b) We will be building a writing group that will allow others interested in this topic to contribute to the project.

c) All the authors listed on this proposal and the writing group will be included as authors.

d) Yes, all members of the PGC-PTSD Working Group will be listed at the end of the manuscript.

e) Yes, all PGC–PTSD members or groups will be listed as "collaborators" on the PubMed abstract page.

f) All named authors will have the opportunity to include their funding sources.

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