

A Novel Approach to Link Genetics and Human MRI Identifies AKAP7-Dependent Subicular/Prefrontal Functional Connectivity as Altered in Suicidality

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Guillermo Poblete^{1,2}, Tien Nguyen³, Savannah Gosnell^{4,5},
Olutayo Sofela⁶, Michelle Patriquin^{1,4}, Sanjay J. Mathew^{1,4,7} ,
Alan Swann^{4,7}, David A. Nielsen^{4,7}, Thomas R. Kosten^{4,5,7}
and Ramiro Salas^{1,4,5,7,8} 

Abstract

Background: Brain imaging and genetics are fields acquiring data at increasing speed, but more information does not always result in a better understanding of the underlying biology. We developed the ProcessGeneLists (PGL) approach to use genetics and mRNA gene expression data to generate regions of interest for imaging studies.

Methods: We applied PGL to past suicide attempt (ATT): We averaged the mRNA expression levels of genes ($n = 130$) possibly associated with ATT ($p \leq 10^{-3}$ in a published genome-wide association study, GWAS) in each brain region studied in the Human Allen Brain Atlas (6 ex-vivo brains, 158 to 946 regions/brain have mRNA expression data) and compared that to the averaged mRNA expression levels of all other genes in each region in each brain in the atlas.

Results: PGL revealed 8 regions where “attempt-related genes” were differentially expressed (Wilcoxon test with Bonferroni correction $8.88^{-11} = <p < 0.046$). Using resting state functional connectivity (RSFC), we studied those regions in psychiatric inpatients (male/female, $n = 132$ with [ATT], $n = 291$ without [NAT] past attempt, unrelated to those in the GWAS).

Among the 8 PGL-identified regions, the subiculum showed higher RSFC with habenula ($p < 10^{-6}$) and dorsolateral prefrontal cortex (dlPFC $p_{FWE} < 0.05$) in ATT. We genotyped one single nucleotide polymorphism (SNP) in each of the five genes (within 130 from GWAS) with most important subicular expression. AKAP7 (A-Kinase Anchoring Protein 7, important in hippocampal memory processes) showed an interaction between genotype, ATT, and subiculum/dlPFC RSFC.

Conclusion: PGL uncovered a brain function/genotype interaction in ATT by using published GWAS data to inform imaging studies. This could inform individualized therapies in the future.

Keywords

AKAP-7, dorsolateral prefrontal cortex, habenula, subiculum, suicide, resting state functional connectivity

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Introduction

In 2019 suicide was the tenth leading cause of death for 47,500 people and the second cause among individuals between the ages of 10 and 34 in the United States.¹ We believe that a reason why suicide, suicide attempt, and suicide ideation are so prevalent is that we don't really understand the etiology. Thus, the study of the mechanisms of suicide attempt is the key to fight against one of the main factors responsible for mortality. Suicidal behaviors are known to have a genetic component² and the function of some brain regions, such as raphe nucleus, have been associated with suicide.³ However, as typical in psychiatry

¹The Menninger Clinic, Houston, TX, USA

²Universidad de Buenos Aires, Buenos Aires, Argentina

³Rice University, Houston, TX, USA

⁴Baylor College of Medicine, Houston, TX, USA

⁵Baylor College of Medicine, Houston, TX, USA

⁶Baylor College of Medicine, Houston, TX, USA

⁷Michael E DeBakey VA Medical Center, Houston, TX, USA

⁸Center for Translational Research on Inflammatory Diseases, Michael E DeBakey VA Medical Center, Houston, TX, USA

Corresponding Author:

Ramiro Salas, PhD, Baylor College of Medicine, One Baylor Plaza – BCM 350, Houston, TX 77030, USA.
Email: rsalas@bcm.edu



research, there is little to no integration between the fields of genetics and brain function in suicidality, resulting in a highly fragmented pathophysiological understanding.

Genetic studies often result in large databases that must be mined to extract useful data - an example being genome-wide association studies (GWAS). GWAS typically use participants with and without a condition (eg, suicide attempters [ATT] and non-attempters [NAT]). Sample sizes are usually in the low thousands (however, larger samples are becoming more common) and typically ~ 1 million genetic variants are collected. Therefore, a very stringent multiple comparison correction is necessary (p values $\sim 10^{-8}$ are commonly necessary), filtering out false positives but creating results with many false negatives.⁴ To identify precise hypotheses, the genes must be appropriately analyzed, and sometimes there is no genetic variant that survives multiple comparisons, leaving us with little new information despite having spent millions of dollars and thousands of hours (both investigators and participants) on the study. This has led some to argue whether genome-wide association studies (GWAS) have truly advanced our understanding of disease.⁵

In human brain imaging new data is also collected at high speed.⁶ However, brain imaging also suffers from a major multiple comparison problem. Most brain imaging manuscripts use dozens of participants, and the brain is composed of thousands of voxels. Since the statistical corrections in human brain imaging are commonly less stringent than in genetics, it is possible that many false positives are published (for a perhaps exaggerated view of this, see⁷).

To integrate genetics and human brain imaging and help to partially solve these multiple comparison problems, we developed the ProcessGenesList (PGL) approach, a two-step procedure. The first step is intended to have genetics data inform brain imaging studies, and the second step is designed to have brain imaging results inform a genotyping step. Using a large and diagnostically heterogeneous sample of psychiatric inpatients that included both ATT and NAT, we applied PGL to ATT. We started with a set of genes extracted from a GWAS that compared ATT versus NAT, with the hypothesis that PGL (by using the mean mRNA expression of genes likely associated with suicide and comparing it with the average of all other genes in each region studied by the Human Allen Atlas) would uncover brain regions that are altered in ATT. This is because genes truly associated with suicide attempt must have an expression pattern across brain regions associated with ATT, and their averaged expression is likely to “point” to such regions. On the other hand, false positives will likely have a random expression pattern and will not be considered relevant to determine brain regions related to suicidality (each false positive gene will likely “point” to different, non-relevant regions, and their weight in the final average will be negligible). In the second step, we hypothesized that the genes from the list that are highly expressed

in regions of interest (ROIs) found to be altered in suicidal patients may show differential genotype/brain function interactions in ATT inpatients.

Methods and Materials

Participants and Clinical Measures

This study was approved by the Baylor College of Medicine Institutional Review Board. Participants provided informed consent. Inpatients were recruited at The Menninger Clinic, in Houston, TX USA between October 2012 and January 2016. Of 423 psychiatric in-patients included, 132 patients presented with past suicide attempt (ATT) and 291 patients presented without past suicide attempt (NAT). Participants with any psychiatric comorbidity were included, to investigate an ecologically valid sample, as has been argued is needed in neuroimaging studies.⁸ Past ATT was assessed using the Columbia-Suicide Severity Rating Scale (C-SSRS, any attempt was considered regardless of severity).⁹ The SCID-I and SCID-II manuals were used to diagnose psychiatric comorbidities. See Supplementary methods for additional information.

ProcessGenesList Pipeline

Figure 1 shows a general pipeline, that includes the suicide-related list of genes creation, the use of PGL¹⁰ to get the most important regions and genes associated with that phenotype, and the imaging and genetic studies that complete the whole process. Other ways of creating a list of genes associated with a phenotype or disease would work similarly.^{11,12} Below, we describe each step of the pipeline. The code for PGL can be freely accessed (<https://github.com/GuillermoPoblete/PGL>).

1. Creating a suicide-related gene list from a GWAS. We used a published GWAS that compared ATT versus NAT (all bipolar disorder patients),¹³ and set up different p-values (10^{-3} , 10^{-4} and 10^{-5}) with the aim of obtaining a list of genes large enough to get a representative average of mRNA expression in each brain region, while minimizing false positives. Using 10^{-4} threshold resulted in a number of genes that allow PGL to find ROIs in just 2 out of 6 brains, deteriorating the quality of its final result, while 10^{-5} resulted in no ROIs at all from PGL. On the other hand, 10^{-3} was the smallest p-value that allowed us to get the shortest list of genes that let PGL to find ROIs in the six brains. Since the GWAS was only on bipolar disorder patients, any brain regions uncovered by PGL could be related to ATT in general, or ATT in the setting of bipolar disorder. This possible limitation was dealt with by studying a second GWAS using only patients with major depressive disorder (MDD) (see below). Next, we used the software

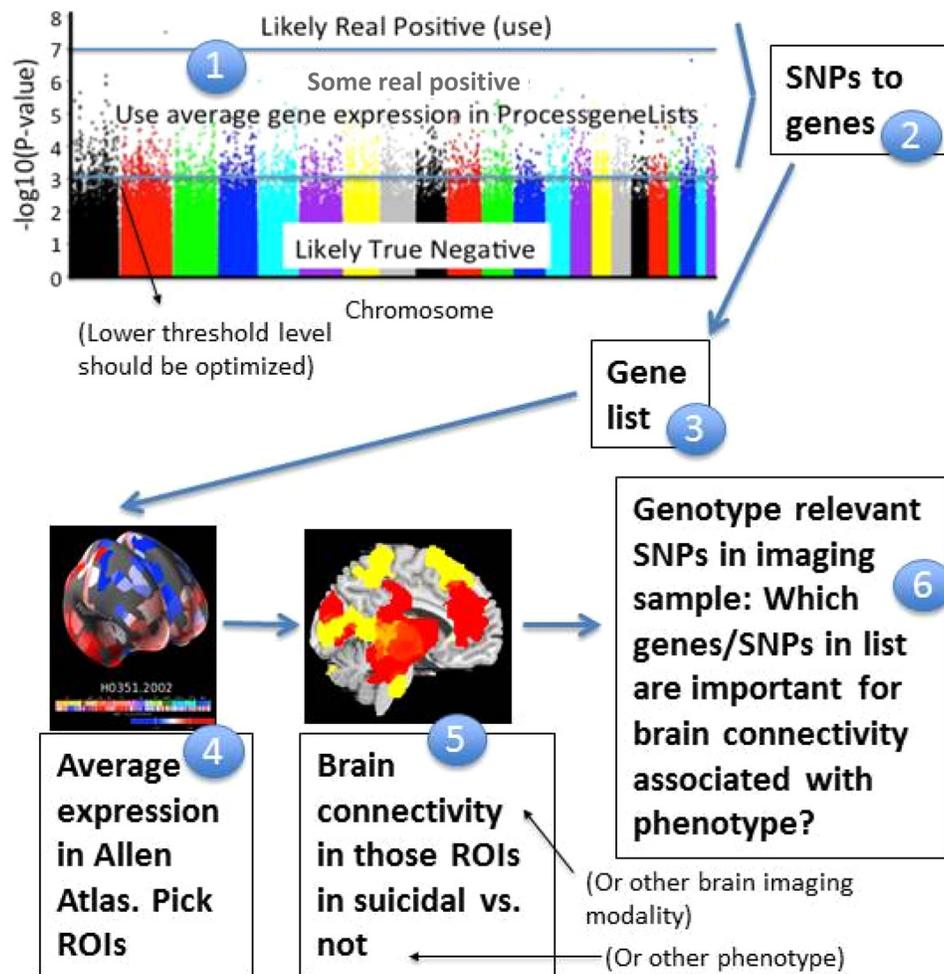


Figure 1. The PGL approach. (1) A generic Manhattan plot from GWAS. SNPs with $p \leq 10^{-3}$ are used (2) to generate a list of associated genes (3). The mRNA expression of those genes in each brain region is normalized and averaged (4) to obtain a series of ROIs in which the expression levels of the genes in the list are significantly different from the expression of all other genes present on the Allen Atlas. The brain connectivity (other brain imaging modalities can be used, such as volumetry or diffusion tensor imaging) of those ROIs is studied in a sample that includes patients with and without the genotype studied in the GWAS (5). Finally, the genes can be genotyped (6) in the patients from the imaging sample and imaging data re-analyzed based on those genotypes. This addresses the question: Which gene variants, via gene expression in which areas of the brain, are important for the studied phenotype (in our case, suicide attempt).

scandb (Univ of Chicago) to create a list of genes associated with the SNPs isolated in the first step (termed “ATT-associated genes” for simplicity).

- Using PGL to find regions where suicide-associated genes are significantly differentially expressed. Using the human Allen Brain Atlas¹⁴ and the PGL GetROIs function,¹⁰ we performed a Wilcoxon test (with Bonferroni correction for number of regions) for each brain region in each of 6 brains included in the atlas, to find regions in which the difference between the mean mRNA expression levels of suicide-associated genes was significantly different from the average of “all other genes”. We chose areas that were significant in at least 5 brains for further study, for two reasons: First, using regions that appeared significant in one or two of the brains would probably increase false positives and

second, because using only those that appeared in 6 brains was likely to give very few results (given the variability of sampling in the Allen Atlas). As expected, PGL uncovered a small list of regions (8 in this case) of possible interest. Not all brains contain the same sampled regions (range: 158-946 regions per brain), creating a possible bias in that not all brain regions are equally represented, but treating them individually gave us the possibility to create an ensemble of the results coming from each brain. While taking just the top brain regions can seem too restrictive, the main idea of PGL in this context was to provide the most relevant regions to be used as seeds in resting state functional connectivity studies over the whole brain. It is important to notice that they are by PGL considered probably the most important regions but not the only ones.

3. Studying functional connectivity of the relevant brain regions. Given a list of gene expression-determined ROIs, we studied the RSFC between each of those 8 regions and every other region in the brain ($n=125$, see below for parcellation description), to find ATT-specific RSFC features. We used t-tests with Bonferroni corrections for the total number of pairs of regions in each comparison. In addition, we used the 8 regions in seed-to-voxel RSFC studies (see supplementary methods).
4. Using PGL Get Genes function for finding genes/SNPs likely to be associated with the alterations in RSFC in ATT patients. Once we had found at least one of the eight regions uncovered by PGL as altered in suicide, we used the PGL GetGenes function to find the top 5 genes associated with that region (higher relative expression) using a random forest classification.
5. Genotyping. Then, from those 5 genes, one SNP per gene was chosen to be genotyped, using a combination of largest possible minor allele frequency (to increase the chances of having a sample well balanced for the genotypes), and lowest possible p value in the original GWAS (to increase the chances that the SNP was actually associated with ATT in the GWAS). We studied only the top 5 genes to avoid an additional multiple comparison problem.
6. Finding SNP/RSFC interactions. Patients (ATT, NAT) were further subdivided into genotypes (for each of the 5 SNPs studied, dominant model). The RSFC between the relevant brain region (that showed differences in RSFC in ATT vs. NAT patients) and the rest of the brain was studied for those groups.

As part of a verification process, we used a separate GWAS on ATT on mood disorders¹⁵ to verify that any regions that appeared as important in PGL and showed differences between ATT and NAT were replicated in an independent sample.

Neuroimaging Data Acquisition Analysis

Brain imaging data was collected on a 3 T Siemens Trio MRI. RSFC analysis was performed in Conn.¹⁶ For details, see Supplementary methods.

Genotyping

Once the RSFC of a region or regions were shown to be associated with differences in ATT versus NAT, we genotyped, in our patient sample, one SNP per gene in the five genes that had highest relative expression in the affected region(s). For details, see Supplementary methods.

Results

Table 1 shows demographic and clinical data. The two groups did not differ on age. We assessed the current and past presence of any psychiatric comorbidity to decide which covariates may be of interest. The ATT and NAT groups differed on sex and the presence of depression current (but not MDD), substance dependency, eating disorders, obsessive compulsive disorder, post-traumatic stress disorder current, obsessive compulsive personality disorder, borderline personality disorder, and avoidant personality disorder. We used those as covariates in a *post-hoc* analysis. No additional differences were found between the two patient groups (Table 1).

Eight brain regions were highlighted by PGL-suicide

We used all SNPs with a p value of 10^{-3} or less (2507 SNPs) from the GWAS described in¹³ and studied which genes those SNPs were associated with. This resulted in a list of 130 genes according to the ScanDB Tool (www.scandb.org, University of Chicago). Of those 130 genes, 118 were found in the Human Allen Brain Atlas, and therefore were used in subsequent analyses (Supplemental table 1). Next, we compared the distribution of the mRNA expression levels of those 118 genes with the distribution of the expression levels of the rest of the genes, for each region sampled on each brain in the atlas. For these comparisons we used the Wilcoxon test, and we considered ROIs to all the regions which the Wilcoxon's null hypothesis was rejected (H_0 : There was no difference between the distribution in the expression's levels of the 118 genes under study vs. the distribution on the rest of the genes present in the Atlas) being the corrected p-value resulted less than 0.05 (using the Bonferroni method for multiple comparisons according to number of regions sampled on each brain). For subsequent analysis, we used all regions that showed higher co-expression of the list of 118 genes in at least 5 of the 6 brains available in the atlas. This resulted in eight brain regions being highlighted by PGL as related to suicide.

ROI-to-ROI Analysis. Subiculum/Habenula RSFC was Higher in ATT Than in NAT

Using the 8 brain regions identified in Table 2 (except corpus callosum because it contains only white matter), we performed ROI-to-ROI RSFC between those regions and all other regions in the brain (see methods for ROI-to-ROI regions used). Since we did many comparisons for each of the 7 (eight minus callosum) *a priori* hypotheses, we used a Bonferroni correction for the P value for each ATT versus NAT comparison. The only ROI-to-ROI RSFC difference that survived Bonferroni correction was left subiculum to left habenula (Figure 2A). This comparison was significant when using each of all the possible covariates defined in table

Table 1. Demographic and Clinical Characteristics of the Study Groups^a.

Characteristic	NAT (N = 291)		ATT (N = 132)		Statistical Analysis	
	Mean	SD	Mean	SD	t-statistic	P
Age	30.98	12.1	30.53	12	0.3569	0.7213
Sex (male/female) ^c	N 175/116	% 60.14/39.86	N 60/72	% 45.45/ 55.45	χ^2 7.9288	P 0.005
Major Depressive disorder ^b	147	50.52	79	59.85	3.1789	0.075
Bipolar disorder	54	18.56	27	20.45	0.2113	0.646
Depressive disorder ^c	46	15.81	10	7.58	5.3573	0.021
Anxiety disorders	134	46.05	50	37.88	2.4658	0.116
Substance abuse	106	36.43	43	32.58	0.5900	0.442
Substance dependency ^c	128	43.99	41	31.06	6.3240	0.012
Social phobia	38	13.06	13	9.85	0.8824	0.348
Eating disorder ^c	40	13.75	29	21.97	4.4990	0.034
Obsessive compulsive Disorder ^c	8	2.75	11	8.33	6.6007	0.010
PTSD ^c	29	9.97	24	18.18	5.5933	0.018
Obsessive compulsive PD ^c	25	8.59	28	21.21	13.2702	0.000
Borderline PD ^c	50	17.18	39	29.55	8.4323	0.004
Avoidant PD ^c	45	15.46	35	26.52	7.2955	0.007
	Mean	SD	Mean	SD	t-statistic	P
Columbia Scale for Rating of Suicide, Severity, lifetime ideation subscale	8.51	6.53	14.44	3.61	9.7780	0.000

^aAbbreviations: NAT: no past suicide attempt; ATT: past suicide attempt; PD: Personality Disorder;

^bCovariates with n > 50 are shown in order to characterize the sample: Major Depressive Disorders (“MDDSingle”, “MDDRecur”); Bipolar Disorders (“bipolarI”, “bipolarII”, “bipolarOther”); Anxiety (“GAD”, “AnxNOS”); Depressive Disorder [“depress”]; Substance Abuse [“alcohol abuse”, “subabuse”]; Substance Dependency [“alcoholdep”, “subdep”]; Social phobia [“socialphobia”];

^cCovariates with significant association to past suicide attempts are shown: Eating disorder [“bulimia”, “anorexia”, “binged”, “ENOS”]; OCD [“OCD”]; PTSD [“PTSD”]; Obsessive Compulsive Personality Disorder [“SCID2PDOCDX”]; Borderline Personality Disorder [“SCID2PDBPDDX”]; Avoidant Personality Disorder [“SCID2PDAVOIDDX”].

Table 2. Brain Regions That Appeared in ≥5 of the Brains Available in the Human Allen Brain Atlas That Showed Significant Differences in mRNA Expression of the Suicide Genes vs all Other Studied Genes.

Region of Interest	Number of appearances in Donors	AAL nomenclature
VIIb, left, lateral hemisphere	6	Cerebellum 7b
medial geniculate complex, left	5	Medial geniculate nucleus
subiculum, left	5	Parahippocampus
superior temporal gyrus, left, inferior bank of gyrus	5	Temporal superior
putamen, left	5	Putamen
Crus II, left, lateral hemisphere	5	Cerebellum crus 2
globus pallidus, internal segment, left	5	Globus Pallidus
corpus callosum	5	Corpus callosum

1 as nominally significantly different between the ATT and NAT groups. Other pairs of regions that may be worth exploring in the future but did not survive Bonferroni corrections were Superior Temporal Pole/Cerebellum VIIb

(p = 0.0024), Superior Temporal gyrus/Cuneus (p = 0.0026), and Pallidum/Medial Temporal Pole (p = 0.0026).

Seed-to Voxel Analysis. Subiculum to Medial Prefrontal cortex RSFC Connectivity was Higher in ATT Than in NAT

For seed-to-voxel analysis we used 3 mm radius seeds centered in the MNI coordinates according to the Atlas, of the eight PGL-highlighted regions (except callosum). Seed-to-voxel RSFC was performed in Conn for all 8 regions using $P_{uncorrected} = 0.001$ for peak voxel and $P_{FWE} = 0.05$ for cluster. In addition, since we performed seven parallel analyses, the final P value was Bonferroni-corrected for seven comparisons. Again, only the seed in the left subiculum showed significant results: The RSFC between the left subiculum seed and the right and left dorsolateral prefrontal cortex (dlPFC) was higher in ATT than in NAT, surviving FWE and Bonferroni corrections (Figure 2B and C). Note that the habenula was unlikely to appear in this seed-to-voxel analysis because of small size. This analysis included sex as covariate.

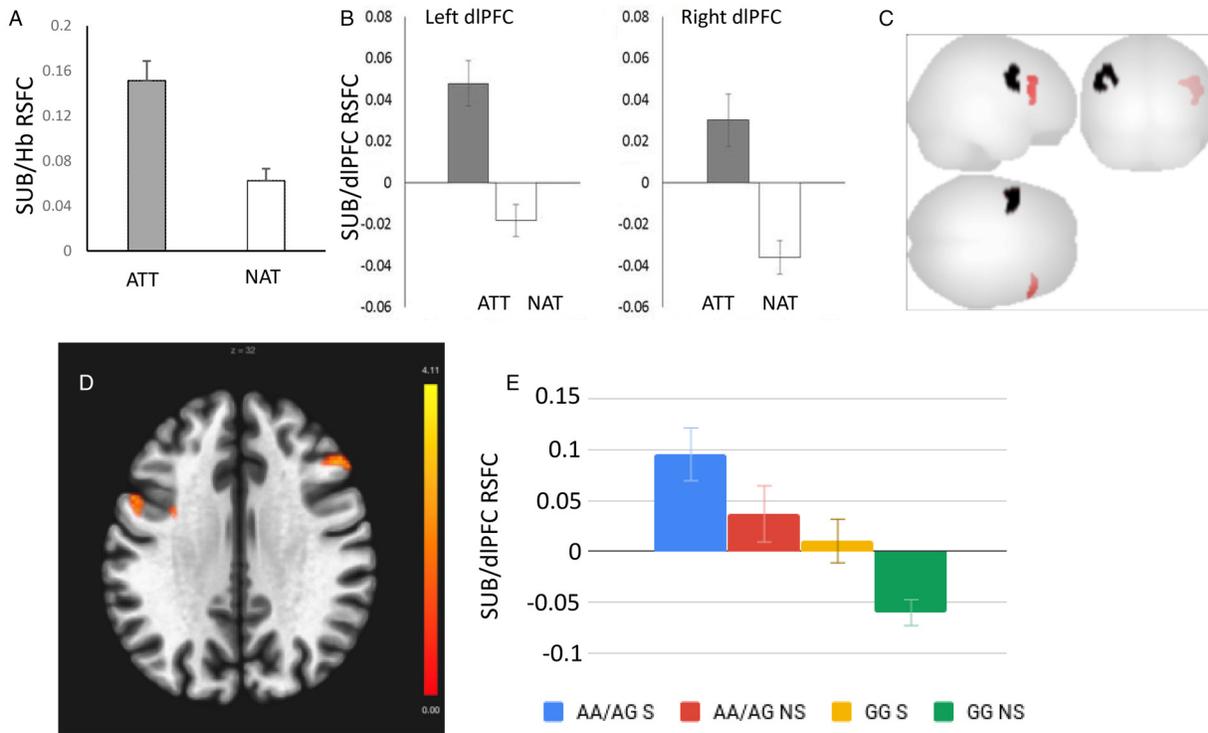


Figure 2. Subiculum resting state functional connectivity (RSFC). Left subiculum resting state functional connectivity (RSFC) in patients with past attempt (ATT, gray bars) and patients without past attempt (NAT, white bars). A) RSFC between left subiculum (SUB, whole parahippocampus region of interest) and habenula (Hb) is higher in ATT versus NAT. B) RSFC of left subiculum (3 mm radius ROI placed in coordinates from Allen Atlas) to left and right dlPFC in patients with and without past suicide attempt. C) Glass brain with right and left dlPFC clusters with higher left subiculum RSFC in ATT than NAT. D, E) RSFC between left subiculum/left dlPFC interacts with AKAP7 rs3777487 genotype in the setting of past suicide attempt. D) Clusters of interaction between genotype and group when left subiculum is used as seed in RSFC analysis. E) RSFC between left subiculum and left dlPFC cluster, separated by genotype (dominant model, AA/AG pooled vs GG) and ATT versus NAT.

Table 3. Genotyping Variants Possibly Associated with Subicular Involvement in Suicide.

Gene	# Wilcoxon	RFI	SNP	MAF	GWAS p
GRIK1	6	0.33	rs3777487	G = 0.268	4.62×10^{-4}
GRM8	5	0.64	rs2832414	C = 0.196	2.66×10^{-4}
NFIA	5	0.75	rs1120908	G = 0.254	1.89×10^{-4}
AKAP7	4	1.27	rs2474388	A = 0.101	8.26×10^{-4}
FARPI	4	0.90	rs12261	T = 0.498	2.66×10^{-4}

Wilcoxon, number of brains in the Allen Atlas that showed significant differences in Wilcoxon test between subicular expression and other expression. RFI: Random forest importance in the same comparison. SNP: Single nucleotide polymorphism chosen in each gene. MAF: minor allele frequency, 1000 Genomes project. GWAS p: p value in GWAS for each SNP in Willour et al.

Genotyping the Genes Most Responsible for Uncovering the Subiculum

We genotyped the top five genes that were significantly related to the subiculum in the original analysis. To discern which genes to genotype we implemented a tree-steps procedure.

First, we examined gene by gene in order to determine which of them had a difference in their subiculum's expression level's distribution compared to the levels distribution in the rest of the brain. In this procedure, we used the Wilcoxon test where the null hypothesis stated that there wasn't difference between both distributions. Next, we ordered the genes by its obtained p-value in ascending order and conserved the top 20 genes from each brain.

Secondly, we determined in how many brains each brain appears after performing the previously explained procedure. Trying to get results from most of the brains, we preserved those genes that survived the Wilcoxon p-value filter in at least 4 brains.

As a final step, we run the Random Forest classification method on each brain, to determine which genes were most important to distinguish the subiculum region from the rest of the regions, according to their expression levels. After obtaining the importance level of each gene on every brain, we averaged them across all the brains, getting a final list in descending order of importance. Because our goal is to reduce the number of genes as much as possible, we decided to set a selection threshold of just 5 genes among the most important ones according to PGL (table 3). These

genes were *GRIK1*, *GRM8*, *NFIA*, *AKAP7*, and *FARPI*. To decide which SNP to genotype in each gene, we used a combination of smallest p value in the Willour GWAS and largest minor allele frequency (MAF) according to the dbSNP 1000 genomes database (<https://www.ncbi.nlm.nih.gov/snp/>). This resulted in the genotyping of rs2832414 (*GRIK1*), rs1120908 (*GRM8*), rs2474388 (*NFIA*), rs3777487 (*AKAP7*), and rs12261 (*FARPI*) (table 3).

Subiculum/dIPFC RSFC is Increased in ATT Regardless of Comorbid Diagnoses

To verify whether the differences in the prevalence of some diagnoses between ATT and NAT was possibly responsible for the subiculum/dIPFC RSFC result, and because the GWAS we used to start PGL contained only bipolar disorder patients, we used as covariates each of the diagnoses that were significantly different between ATT and NAT in our inpatient sample. Only slight differences were found (see supplemental figure 1) suggesting that the subiculum/dIPFC RSFC result is not significantly associated with any of the diagnoses that differed between the two groups. Thus, the subicular association with past suicide attempt is likely to be an across-diagnostic suicide biomarker. We studied whether each one of the features nominally significantly different between the ATT and NAT groups (see table 1) had an impact on subiculum/dIPFC RSFC when used as covariates. As shown in Supplemental figure 1, the results were robust to each of those covariates.

ROI-to-ROI RSFC/Genotype Interactions

Since in ROI-to-ROI analysis we found that the subiculum/habenula RSFC was higher in ATT than in NAT patients, we studied whether the SNPs genotyped in the five genes in the suicide GWAS that were most associated with subicular expression (from Allen Atlas data), as described in supplemental table 3, would show interactions between gene variant, subiculum/habenula RSFC, and ATT versus NAT. No significant interactions were found.

Seed-to-Voxel RSFC/Genotype Interactions

We used the 5 genotypes obtained in our sample in seed-to-voxel analysis (ATT vs. NAT) using the left subiculum as seed. Of these five SNPs, only rs2474388 (*AKAP7*) showed an interaction with subicular RSFC in the setting of ATT versus NAT (Figure 2D and E).

Sensitivity Control: Another Suicide GWAS Points to Overlapping Regions

We used a suicide attempt GWAS on mood disorder patients¹⁵ for replication. Since this GWAS had overlapping

bipolar patients with the one we used originally (bipolar patients in¹³) we used only the non-overlapping cohort 2 [MDD patients; N=2922 No-Past-ATT, N=309 Past-ATT]. When we used the results of that GWAS in PGL using the same parameters as before, we found 19 regions. Importantly, the subiculum was again found in 6 of 6 brains, confirming that regardless of the GWAS used as starting point, the subiculum result would have been the same. Within the 8 regions found in suicide-PGL using Willour et al. the subiculum, medial geniculate nucleus, and superior temporal gyrus, left, inferior bank of gyrus were found in both GWAS.

Specificity Control: An Unrelated GWAS Does not Point to the Same Regions

Since PGL has not been extensively tested, it is possible that some brain regions are more likely to appear than others regardless of the gene list studied. To account for this possibility, we used an unrelated GWAS to verify that the list of regions obtained does not overlap substantially with the list we obtained from suicide-PGL using¹³ as starting point. We used an immunology-related GWAS,¹⁷ yielding 31 genes. Only hippocampus CA3 was found by PGL (in 5 brains), perhaps consistent with relationships between immunoglobulins and hippocampal gene expression.¹⁸ This suggests that our result was specific for suicide attempt.

Discussion

To obtain genes and brain regions (and their interactions) possibly associated with ATT, we attempted to balance problems in specificity/sensitivity conventionally associated with genetic and brain imaging studies by using a high p value (10^{-3}) in a GWAS, resulting in 130 genes (Supplemental Table 1). Of those, 118 genes have detailed brain mRNA expression available in the human Allen Brain Atlas,¹⁴ and according to PGL¹⁰ are co-expressed the most in eight regions (table 2). From these regions, we found that the subiculum showed higher RSFC with the habenula and the dIPFC in ATT than NAT, and that there was an interaction between the genotype of a SNP in *AKAP7* (from the 130 original genes, *AKAP7* was one of five that most “pointed” to the subiculum [table 3]) and the subicular/dIPFC RSFC in the setting of NAT versus ATT. A flowchart of the whole study is shown in Figure 3. The implications of these results are several-fold.

Using an overlapping sample of patients we had shown already that the parahippocampus (including the subiculum) and the habenula are functionally hyper-connected in patients with ATT and current ideation¹⁹ and that this connectivity appeared in machine learning as a feature that helped distinguish suicidal versus non-suicidal patients.²⁰ Given that the habenula signals negative prediction errors that occur when

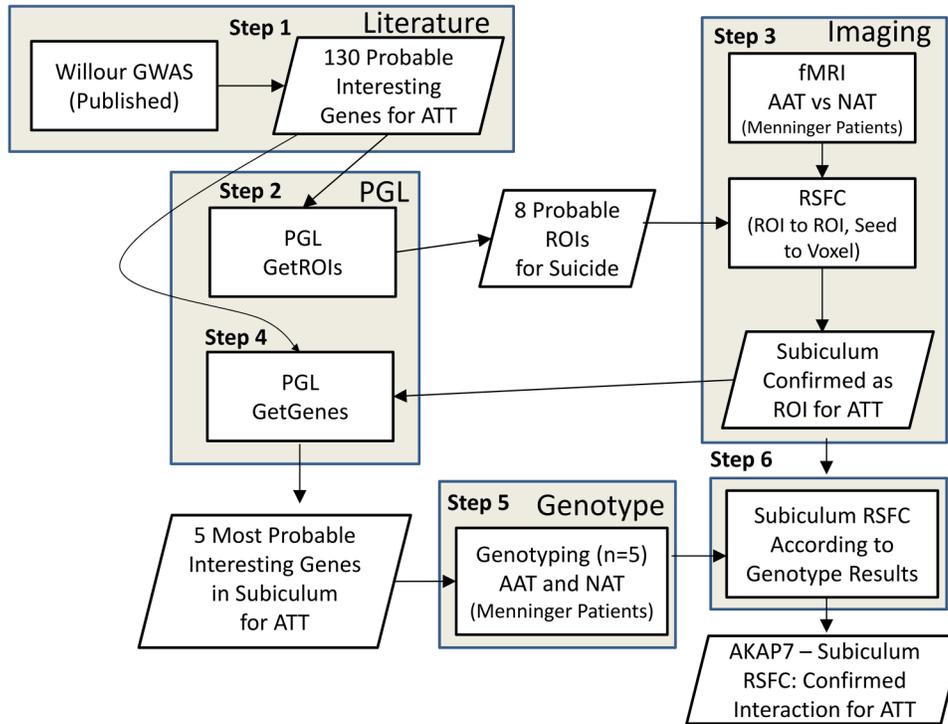


Figure 3. A general flowchart of the PGL study on suicide attempt. Steps shown are the same as in Figure 1. Starting from a published GWAS,¹³ we obtained a list of 130 genes that showed GWAS p values of 10^{-3} or less (Literature box). Using the GetROIs module of PGL software (PGL box¹⁰) we found 8 brain probable regions of interest. Then we used a group of psychiatric in-patients to perform brain imaging studies in ATT versus NAT in the 8 probable regions of interest [Imaging box, top]. Once the subiculum was found to differentiate between ATT and NAT, we used the GetGenes module of PGL to find the first 5 genes [within the 130 genes from the GWAS] that showed more importance in the subiculum [PGL box] and genotype one SNP in each [Genotype box]. Finally, we studied subicular RSFC separating patients by both genotype and ATT versus NAT [Imaging box, bottom].

reward is smaller than expected,^{21,22} and the subiculum is associated with stress-related and context-dependent memory,²³ it is tempting to speculate that the RSFC between these two regions is associated with suicidality via modulating the context in which negative prediction-associated memories are stored.

The subiculum/dIPFC RSFC was also higher in ATT than NAT. This result would have been hard to find without the PGL-generated hypothesis linking subiculum and suicidality. The dIPFC has an important role in executive functions, including planning and inhibition²⁴ which together with accessibility, have made it a prominent target in transcranial magnetic stimulation (TMS) studies. Several conditions have been studied for the possible therapeutic effects of dIPFC TMS, including addiction and depression,²⁵ and has shown promise as a therapy for suicide prevention.²⁶ Thus, the subiculum/dIPFC RSFC provides a possible mechanism and biomarker for the role of the dIPFL in suicide.

Finally, genotyping one SNP in each of the top 5 genes that implicated the subiculum revealed that AKAP7 was potentially associated with the subiculum/dIPFC RSFC. This provides a possible mechanism and biomarker for suicide risk and a possible target for suicide prevention.

AKAP7 (A-Kinase Anchoring Protein 7) has a major role in the spatiotemporal resolution of protein kinase A phosphorylation, and is important in the hippocampus: When AKAP7 was genetically removed from mouse dentate granule cells, cAMP-dependent long term potentiation was altered, and mice were deficient in pattern separation behaviors.²⁷ SNP rs2474388 is intronic and with no known clinical significance. Here we show that AKAP7 is likely involved in suicidal behaviors through modulation of subiculum/dIPFC RSFC, providing a possibly druggable target. Although therapeutic applications are not expected in the short term, malonate binds AKAP-7,²⁸ providing an important tool to explore therapeutic options.

Several limitations must be noted. First, PGL is not comprehensive: relevant brain regions may escape PGL analysis. However, other imaging techniques are not comprehensive either. Second, the 6 post-mortem brains available in the Allen Atlas have very dissimilar sampling, which restricted results to those regions that were sampled most consistently. In a newer version of PGL, we tried to mitigate this problem by using a score method that takes this into account.¹⁰ Third, we used only RSFC as the measure for brain function. We found that the corpus callosum was highly represented in

suicide-PGL, but we did not study it because it would not make much sense in RSFC. As the corpus callosum has been shown to be possibly associated with suicide,²⁹ we plan to study it under the light of PGL using volumetry and diffusion tensor imaging. Fourth, information about medical severity of suicide attempts would strengthen the proposed relationship between attempt history and risk for suicide, since most suicides occur on the first attempt. Finally, the imaged sample of inpatients at The Menninger Clinic may not be representative of the whole population and these results should be replicated in a more diverse sample.

Conclusion

In conclusion, we have shown that a) PGL is a viable strategy to mine data from already-published GWAS to find neuropsychiatry-relevant ROIs; b) the connectivity of the subiculum with both habenula and dlPFC may be a critical player in suicidality; and c) the *AKAP7* gene may have an important role in suicidality mediated by its subicular expression. Importantly, PGL allowed us to decrease the multiple comparison problem in brain imaging, and to find disorder-specific brain function alterations that are likely mediated by specific genes and genetic variants. Thus, traditional human brain imaging tries to discover those brain regions that are relevant for a phenotype, and genetic science does the same with the genes. Meanwhile, PGL determines which brain regions, through their modulations, are related to certain genes associated to a certain phenotype. In that way, we can provide both insight and possible therapeutic targets.

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Author Contributions

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. Dr. Mathew has served as a consultant to Allergan, Alkermes, Axsome Therapeutics, BioXcel Therapeutics, Clexio Biosciences, Eleusis, EMA Wellness, Engrail Therapeutics, Greenwich Biosciences, Intra-Cellular Therapies, Janssen, Levo Therapeutics,

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Ethical Approval

The Baylor College of Medicine Institutional Review Board approved the study.

Informed Consent

Informed consent was obtained from all participants.

ORCID iDs

Sanjay J. Mathew  <https://orcid.org/0000-0002-2715-6641>
Ramiro Salas  <https://orcid.org/0000-0002-1105-566X>

Trial Registration

Not applicable, because this article does not contain any clinical trials.

Supplemental material

Supplemental material for this article is available online.

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