Online Supplementary Detailed Materials and Methods

Subjects

The study population has been previously described in both its level of trauma exposure and candidate gene-association studies of PTSD and depression (Binder et al., 2008; Bradley et al., 2008; Gillespie et al., 2009). Samples were collected from patients receiving services in the primary care or obstetrical-gynecological clinics at Grady Memorial Hospital (Atlanta, GA), an inner-city hospital that offers health care to a predominantly African American patient population. Individuals at the hospital were approached while waiting for appointments and asked to participate in a study that would examine trauma exposure during childhood and adulthood (as previously described, Gillespie, et al, 2009). Those who agreed to participate in the first phase of the study were asked to provide a saliva sample for DNA collection. Only those patients who agreed to continue participation to the second, more in-depth phase of the study were asked to donate blood.

Phenotype Measures

Demographics Form. The Demographics Form is locally developed and assesses subject age, self-identified race, marital status, education, income, employment, disability status, and questions about lifetime and current substance abuse.

The Traumatic Events Inventory. The Traumatic Events Inventory (TEI (Schwartz et al., 2005, 2006; Binder et al., 2008) is a 14-item screening instrument for lifetime history of traumatic events. For each traumatic event, the TEI assesses experiencing and witnessing of events separately. The total number or types of trauma exposure variable was created and used in our data analysis because in our prior work (Bradley et al., 2008; Binder et al., 2008) and in other research on the impact of trauma exposure (Anda et al,. 2006) it relates in a predictable and consistent manner with a number of measures of adaptive functioning and trauma related functioning.

PTSD Symptom Scale. The PTSD Symptom Scale (PSS) is a psychometrically valid 17-item self-report scale assessing PTSD symptomatology (Binder et al., 2008; Coffey et al., 1998; Falsetti et al., 1993; Foa and Tolin, 2000; Schwartz et al., 2005,2006) over the prior two weeks Consistent with prior literature, we summed the PSS frequency items (“0: not at all" to ”3: >5 times a week") to obtain a continuous measure of PTSD symptom severity ranging from 0-51. For this sample, previously described in Gillespie et al. (2009) the PSS frequency items had a standardized alpha coefficient of .93 (M = 13.46, SD = 12.18). Each subscale (Intrusive, Avoidant, Hyperarousal) may also be summed to obtain a symptom subscale score, as in Figures 1C-1E.

The categorical diagnosis of PTSD was determined based on DSM-IV A-E criterion responses to the PSS questionnaire (A, presence of trauma; B, presence of at least 1 re-experiencing symptom; C, presence of

at least 3 avoidance / numbing symptoms; D, presence of at least 2 hyper-arousal symptoms; and E, present for at least 1 month).

Beck Depression Inventory: Depressed mood was assessed with the 21-item Beck Depression Inventory (BDI(Beck et al., 1961)), a commonly used continuous measure of level of depressive symptoms (Beck et al., 1988). For this sample, previously described in Gillespie et al. (2009), the BDI had a standardized alpha coefficient of .92 (M = 10.86, SD = 11.71).

Sample Collection and Handling

DNA came from saliva collected in Oragene vials (DNA Genotek Inc., Ontario Canada) or from whole blood collected in EDTA tubes. For genotyping, 500ul of whole blood and 200ul of oragene saliva were used for extraction. DNA from saliva was extracted using the DNAdvance extraction kit and DNA from blood was extracted using the Genfind v2 kit (Beckman Coulter Genomics, Danvers MA). Both extraction methods utilized automation methods on the Biomek NX (Beckman Coulter Inc., Brea, CA) For methylation analysis, 700ul of whole blood was used for DNA extraction using the MagAttract DNA Blood M48 kit (Qiagen, Valencia, CA). All DNA for methylation was extracted at the same time and used immediately for bisulfate treatment (refer to Methylation Analyses). All DNA for genotyping was quantified using PicoGreen (Invitrogen Corp., Carlsbad, CA) and normalized to a concentration of 5-10 ng/μl. DNAs that fell below 5 ng/μl were not used in downstream applications. DNA was plated into 384 plates at 10ng or 20ng for Taqman or Sequenom genotyping, respectively. All DNAs were dried down prior to performing the reactions.

Plasma was collected in EDTA blood collection tubes, put on ice immediately, centrifuged at 3000rpm for 15min, then aliquoted into 1ml samples and placed at -80^{o}C. Plasma samples used for the radioimmunoassay (described below) were thawed one or more times prior to analysis.

Genotyping

Pairwise tagging ($R^2 > 0.8$, MAF > 0.1) was used to choose tag-SNPs for both ADCYAP1 and ADCYAP1R1 (P.I.W. de Bakker et al., 2005). The coordinates were chr18:885000-906000 and chr7:31048667-31117836 for ADCYAP1 and ADCYAP1R1, respectively (NCBI B36) which includes approximately 10kb upstream and 5kb downstream of the coding regions for both genes. To capture tag-SNPs in the African American population, we included tag-SNPs for both the YRI (Yoruba in Ibadan, Nigeria) and CEU (Utah residents with Northern and Western European ancestry from the CEPH collection populations) populations. SNPs were generated from HapMap Data Phase III/Rel#2, Feb09, dbSNP b126. Coding SNPs were also included in the genotyping panel.

Genotypes for the tag-SNPs were generated using Sequenom iPlex. Further genotyping for rs2267735 (ADCYAP1R1) was done using Taqman. Sequenom genotypes were collected using the iPLEX

chemistries and the MassARRAY system (Sequenom, Inc., San Diego, CA). SNP assays that had a < 90% call rate for any of the chips were not analyzed (8/42 for ADCYAP1R1; 5/22 for ADCYAP1). SNP assays that were non-polymorphic or failed HWE at p<.02 were also removed and not analyzed (4/34 for ADCYAP1R1; 3/17 for ADCYAP1). The averaged call rate ranged from 93-98%. For Taqman, SNP assays with a call rate less than 95% were excluded. Taqman reactions were performed using Taqman SNP Genotyping Assays along with Taqman Genotyping Master Mix (Applied Biosystems Inc., Foster City, CA). Alleles were discerned using the 7900HT Fast Real-Time PCR system. In final, 14 ADCYAP1 and 30 ADCYAP1R1 SNPs were used in the analysis.

Negative controls and within- and across-plate duplicates were used for quality control. Discordant samples were removed prior to analysis For all Sequenom genotypes there was a 11.9% duplication rate and .005% discordance rate. The duplication rate for Taqman genotypes was 6.8%. There were no within method discordants. rs2267735 was genotyped using both Taqman and Sequenom with an across method duplication rate of 6.8% and a discordance rate of 0.1%. All negative controls passed QC.

Sequence Analysis for ER binding sites

A 65kb region (chr7:31084099-31149140; GRCh37/hg19) spanning ADCYAP1R1 was used in MatInspector (Matrix Family Library Version 8.2; Genomatix Software GmbH, Munich, Germany) to detect predicted estrogen response elements (EREs). Core similarity and matrix similarity scores are reported for each predicted ERE. In addition, we note whether or not a SNP is located within the sequence (underlined in *Supplemental Table 3*). Core similarity refers to the degree of similarity within the core sequence. The core sequence consists of the highest conserved, consecutive positions within the whole sequence. In Supplemental Table 3 the core sequence is represented by capitalized bases. The matrix similarity score takes into account the conservation across the entire sequence such that mismatches in highly conserved positions of the matrix decrease the matrix similarity more than mismatches in less conserved regions. The maximum core similarity of 1.0 is only reached when the highest conserved bases of a matrix match exactly in the sequence. Matrix similarity scores above 0.80 are considered “good” matches. (Cartharius K, et al., 2005)

Methylation Analyses

DNA concentration was determined by PicoGreen quantitation using the Quant-iT dsDNA Assay Kit (Invitrogen, Carlsbad, CA) on a SpectraMax Gemini XPS plate reader (Molecular Devices, Sunnyvale, CA). Samples were resolved on a 1% agarose gel to verify that the DNA was of high molecular weight (at least 2kb). One µg DNA was bisulfite-treated for cytosine to thymine conversion using the EZ DNA Methylation-Gold kit (Zymo Research, Orange, CA). The DNA was whole-genome amplified, fragmented, and hybridized

to the HumanMethylation27 BeadChip (Illumina, San Diego, CA). The XStain was performed on a Tecan Evo 150 liquid handling robot. Individual samples were stratified to BeadChips according to PTSD status to limit bias. The BeadChips were scanned using a BeadStation 500GX, and the methylation level (beta value) was calculated using the Methylation Module of the BeadStudio software.

Samples with probe detection call rates < 90% were excluded from further analysis as were those with an average intensity value of either <50% of the experiment-wide sample mean or < 2000 arbitrary units (AU). One sample of pooled female DNA was included on each BeadChip as a control throughout the experiment and assessed for reproducibility using a Pearson R^2 coefficient. For individual sample I, and CpG site j, the signals from methylated (M) and unmethylated (U) bead types are used to calculate a b value: $\beta_{ij} = \frac{M}{U + M + 100}$, which can be treated as an approximation of the proportion of CpG dinucleotides methylated at a particular site.

To identify CpG sites for which methylation varied significantly with each outcome, we fit a separate linear mixed effects model for each CpG site. To ensure that our dependent variable was roughly normally distributed, we worked with the log ratio of β-values, $\log\left(\frac{\beta}{1-\beta}\right)$. For each CpG site, we regressed $\log\left(\frac{\beta}{1-\beta}\right)$ on the variable of interest, as well as sex and age. We included chip-specific random effects to allow for chip-to-chip differences in measurement of the proportion of DNA methylated. We fit the above model for all 27,578 CpG sites.

<u>Radioimmunoassay to determine PACAP38 levels</u>

PACAP38, denoting the 38-amino acid peptide form of PACAP, in human plasma samples was concentrated on C18 Sep-Pak solid-phase cartridge minicolumns (Waters, Milford, MA) as described previously (Brandenburg et al., 1997). Briefly, the samples were recycled 3 times onto the minicolumns, washed with 0.1% trifluoroacetic acid (TFA) and eluted with 80%/0.1% TFA. The eluates were dried under reduced pressure and resuspended in 0.1 M sodium phosphate buffer (pH 7.4) containing 0.5% bovine serum albumin and 0.3 mg/ml phenylmethylsulfonylfluoride, immediately before assay. Peptide recovery from the Sep-Pak cartridges was 85%. PACAP38 radioimmunoassay (1:30,000, Peninsula Laboratories, Belmont, CA) was performed using double antibody immunoprecipitation as previously described (Girard et al., 2006). Each sample was assayed over a dilution range to ensure data interpolation within the linear range of the standard curve; assay midpoint was 3.5 fmol. With the initial sample, the median level was 20pM, such that 'low' PACAP were those $\leq$20pM, and 'high' PACAP were those with levels >20pM.

An additional 93 subjects were analyzed from the same initial population for a replication of the PACAP – PTSD association analysis. For the replication sample, the same above RIA methods were used. Due to increased variability in the overall second assay, the extreme tails of the samples (the lowest 10% and highest 10%) of the PACAPpM levels in the replication cohort (blinded to patient status) were removed to avoid spurious high or low readings. These data were analyzed in the same way, with the identification of subjects with high vs. low PACAP levels to predict level of PTSD symptoms. The median PACAP level sample, the median was 17.2pM, such that 'low' PACAP were those $\leq$17.2pM, and 'high' PACAP were those with levels >17.2pM.

Startle Response Measurement

The human startle response data were acquired at a 1000 Hz sampling frequency using the electromyography (EMG) module of the Biopac MP150 for Windows (Biopac Systems, Inc., Aero Camino, CA). The acquired data were filtered, rectified, and smoothed using MindWare software (MindWare Technologies, Ltd., Gahanna, OH) and exported for statistical analyses. The EMG signal was filtered with low- and high- frequency cutoffs at 28 and 500 Hz, respectively. The maximum amplitude of the eyeblink muscle contraction 20 – 200 ms after presentation of the startle probe was used as a measure of the acoustic startle response.

As previously described (Jovanovic et al, 2005; Jovanovic et al, 2006) the eyeblink component of the acoustic startle response was measured by EMG recordings of the right orbicularis oculi muscle with two 5-mm Ag/AgCl electrodes filled with electrolyte gel. One electrode was positioned 1cm below the pupil of the right eye and the other was 1cm below the lateral canthus. Impedance levels were less than 6 kilo-ohms for each participant. The startle probe was a 108-dB (A) SPL, 40ms burst of broadband noise with near instantaneous rise time, delivered binaurally through headphones.

The fear-potentiated startle task included two phases: habituation and conditioning. The habituation phase consisted of six startle probes presented alone (noise-alone trials, NA). Immediately following habituation, participants underwent the conditioning phase, which consisted of three blocks, each of which included four trials of each CS type and four NA trials for a total of 12 trials per block. All CS+ (i.e., danger cue) trials were reinforced with the unconditioned stimulus (US), while the CS- (i.e. safety cue) trials were not reinforced. Both conditioned stimuli were different colored shapes presented on a computer monitor and were six seconds in duration. The US was a 250 ms air blast with an intensity of 140 psi directed to the larynx. The air blast was emitted by a compressed air tank attached to the polyethylene tubing and controlled by a solenoid switch. This US has been used in our studies previously (Jovanovic et al, 2005;

Norrholm et al, 2006) and produces robust fear-potentiated startle. In all phases of the experiment, inter-trial intervals were of randomized duration ranging from 9 to 22 seconds.

The dark-enhanced startle task included five phases: habituation, light phase 1, dark phase 1, light phase 2, and dark phase 2. The habituation phase consisted of 8 startle probes presented alone at 108 dB (noise-alone trials, NA). Immediately following habituation, participants underwent alternative exposure to the light and dark phases with counterbalanced order of presentation of phases. During each 1-min light and dark phase, participants were exposed to 4 trials of the 108-dB noise probe for a total of 24 trials in the experimental session. Transition from light and dark phases was controlled by a digital timer and there was no perceptible ambient light in the startle booth during the dark phase. Participants were monitored throughout the experiment with the use of a closed circuit camera. In all phases of the experiment, inter-trial intervals were of randomized duration ranging from 9 to 22 seconds.

Mice Fear Conditioning and Amygdala / PFC qPCR

Animals. Adult male 7-9 week old C57BL/6J mice (Jackson Labs) were used. Mice were housed four per cage in a temperature-controlled (24 ^{o}C) animal colony, with *ad libitum* access to food and water, on a 12-h light-dark cycle, with all behavioral procedures done during the light cycle. All procedures used were approved by the Institutional Animal Care and Use Committee of Emory University and in compliance with National Institutes of Health (NIH) guidelines for the care and use of laboratory animals.

Fear Conditioning Apparatus. Mice were fear conditioned in eight identical startle response systems (SR-LAB, San Diego Instruments). Each system consisted of a nonrestrictive Plexiglas cylinder (5.5 cm in diameter and 13 cm long) mounted on a Plexiglas platform and located in a ventilated, sound-attenuated chamber. Cylinder movements were sampled each millisecond by a piezoelectric accelerometer mounted under each platform. The footshock unconditioned stimulus (US) was generated by a programmable animal shocker (San Diego Instruments) located outside the isolation chambers and was delivered through the cage floor bars. The conditioned stimulus (CS) was a tone delivered by a speaker located about 15 cm above the chambers. Sound intensities were measured by an audiometer (Radio Shack). Stimuli presentation and data acquisition were controlled, digitized, and stored by a Dell computer using SR-LAB software.

Fear Conditioning. After pre-exposure to the conditioning chambers, mice were placed in the chamber, and after 5 min presented with ten tone-shock pairings at an inter-trial interval (ITI) of 3-5 min. Each pairing consisted of a 30-s tone (6 kHz, 85 db, CS) that terminated with a 0.5 s footshock (1.0mA, US). Freezing in startle-reflex chambers during fear acquisition was assessed as described previously (Choi et al., 2010; Maguschak & Ressler, 2008). Two hours after training, mice were sacrificed, brains rapidly dissected

and placed in ice cold phosphate buffered saline, and amygdala or prefrontal cortex tissue removed using a micropunch form 2mm coronal sections.

RNA Preparation. Total RNA was prepared from frozen amygdala and prefrontal cortex dissections in mice. Briefly, tissue samples were homogenized and centrifuged at 13,000g for 3 minutes. RNA was washed with 70% ETOH and purified using RNeasy columns (Qiagen). RNA amount and quality were determined using a nanodrop spectrophotometer.

Quantitative RT-PCR. 140 micrograms of total RNA were reverse transcribed using the RT2-First Strand Kit (C-03, SA Biosciences). Quantitative PCR was performed using the Applied Biosystems 7500 Fast Real-Time PCR System. Online detection of reaction products was carried out using the TaqMan Gene Assay for mouse *ADCYAP1R1* (Applied Biosystems, Assay ID: Mm01326453_m1) and for *GADPH* (Applied Biosystems) and the TaqMan Standard Universal Master mix (Applied Biosystems) according to manufacturer's instructions. Calculated values are presented as mean +/- SEM to indicate accuracy of measurement. *ADCYAP1R1* values were normalized for measurements of GADPH. PCR conditions were 2 min at 50 deg C, 10 min at 95 deg C and 40 cycles with 15s 95 deg C, 60 s 60 deg C.

Correlation between *ADCYAP1R1* levels and Fear. The 4th trial of the fear conditioning session demonstrated the most variance with regards to freezing across animals, thus that trial was chosen to examine the rate of acquisition compared to the levels of amygdala and mPFC *ADCYAP1R1* gene expression levels. Bivariate correlations were used to demonstrate a significant relationship between level of freezing at trial 4 and level of *ADCYAP1R1* gene expression post-training. Note that a significant relationship was also found of the trial 4 – trial 1 freezing levels were used (essentially the slope of rate of acquisition).

Expression of ADCYAP1R1 in rats exposed to estrogen

Quantitative PCR was performed exactly as described previously (Hammack et al., 2009; Girard et al. 2006; Braas et al., 2007). Rats were implanted with 21-day continuous release pellets for delivery of estrogen. After rat euthanasia, the different brain regions were quickly dissected and frozen on dry ice. The tissues were homogenized in Stat-60 total RNA/mRNA isolation reagent (Tel-Test "B", Friendswood, TX). The RNA (2μg) was used to synthesize first strand cDNA using SuperScript II reverse transcriptase and random hexamer primers with the SuperScript II Preamplification System (Invitrogen, Carlsbad, CA) in a 20 μl final reaction volume. The same tissue regions from all rats were reverse transcribed simultaneously to obviate variability. Following the reverse transcriptase reaction, the cDNA samples were treated with RNase H to remove residual RNA. Real-time quantitative PCR methods and oligonucleotide primers were exactly as described previously. The melting profiles for amplified DNA fragments were performed to verify unique

product amplification in the quantitative PCR assays. For each target sequence, all samples from the same brain region were amplified together in the same assay to minimize variability. All data were normalized to 18S RNA levels; all assays were repeated 2 - 3 times to verify data reproducibility.

Human cortex gene expression

Normalized human cortex gene expression data for ADCYAP1R1 and ADCYAP1 in 192 individuals were downloaded at http://labs.med.miami.edu/myers/LFuN/LFuN.html a dataset previously published in Myers et al,. 2007. Figure 4C, demonstrating the association between ADCYAP1R1 and ADCYAP1 mRNA levels in cortex includes data from these 192 individuals.

The rs2267735 SNP was not present on the Affymetrix SNP array used in the Myers et al., 2007 data set and was therefore imputed using genotype data of 18 SNPs within the ADCYAP1R1 locus. Impute version 2.1.0 with 10 burn-in MCMC iterations and a total of 30 MCMC iterations and the HapMap genotype data for the CEU population as a reference was used for imputation of rs2267735 genotypes. rs2267735 genotypes were imputed with an average certainty of 0.852. 99 subjects (42 female and 57 male) had both GWAS data for the imputed rs2267735 SNP and cortex mRNA data for the genotype association with ADCYAP1R1 mRNA levels (Figure 4D).

mRNA analytic approaches: mRNA was prepared as in Myers et al and Webster et al. Briefly, RNA was prepared using RNeasy Lipid Tissue Mini Kit (Qiagen, Valencia, CA, cat #: 74804) with the Qiagen RNase-Free DNase Set (cat #: 79254) in each prep to eliminate any possible cross contamination with genomic DNA. RNA samples were hybridized to Illumina ref-seq 8 chips (Illumina, San Diego, CA) using an Illumina Beadstation (Illumina, San Diego, CA) to read the chips. RNA profiles were corrected for putative age, gender, post mortem interval, and regional artifacts.

DNA Analysis Alzheimer's Disease cohort: DNA samples were genotyped using Affymetrix 500K microarrays (Affymetrix, Santa Clara, CA) including positive and negative controls on each plate (controls were 2% of cohort). DNA samples were screened (Myers et al.) to eliminate samples with gender errors, poor quality samples (as assessed by low call rates) or whose genotypes did not match reported ethnicity.

Statistical Methods

Primary and Descriptive Analysis All statistical analyses were done in SPSS version 17.0. Levels of PACAP38 were analyzed relative to PTSD total symptoms and subscales (defined in PTSD Symptom Scales section) using Univariate ANOVA. PACAP38 measures were divided into two levels, designated as high (N=33; $\geq$20.8 pM) and low (N=32; $\leq$20.4 pM), at the median. These values were analyzed by sex (Figure1B-E). The correlation, using bivariate correlation analyses, between all PACAP38 measures and the total PTSD symptoms is shown in Figure 1A (significant at $p \leq .005, r=0.497$). Additionally, the correlation analysis

between mRNA levels for ADCYAP1 and ADCYAP1R1 reveals an inverse and significant relationship (Figure 4C; p=.001, r=0.276) .

Given the relationship between PACAP38 and PTSD, our primary goal for statistical analysis was to determine if genetic variants in either ADCYAP1 or ADCYAP1R1 have an effect of PTSD symptoms Furthermore, we sought to determine if such a relationship was sex dependent. Genotypes were recoded into categorical variables representing each of the three genotypes . Missing data was designated as missing in the system so that it would be excluded from the analysis. We performed a logistic regression with PTSD coded as a dichotomous variable for either having or not having PTSD (defined in PTSD Symptom Scale) against each of the tag-SNPs. The –log (p) values were calculated and graphed in the physical order of the tag-SNPs along the chromosome (Figure 2A and Supplemental Figure 1). This analysis was repeated selecting cases by sex. SNPs that passed bonferroni correction for 44 SNPs at p<.0011 (-log (p)>3.0) were examined further.

Only rs2267735 passed the criteria for further analysis. Genetic demographics (Supplemental Table 1) were calculated using a dataset pared-down to include only those samples with genotypes for rs2267725. Variables suspected to have an impact on PTSD symptoms are described, relative to PTSD diagnosis, in Supplementary Tables 1-2 ('Initial Genetic Sample'). The same was done for PACAP demographics paring-down to only those samples that had measurements for PACAP38 (Supplemental Tables 1-2; N=64, 'PACAP Blood Sample').

Secondary Analysis rs2267735 was coded as a dichotomous variable representing a dominant model for the more rare allele. Using Univariate ANOVA, both the three level categorical genotypes and the two level categorical genotypes were analyzed relative to PTSD and the subscales, controlling for total trauma (child and adult), age and race (data only shown for original plus replication sample set; Figure 3B-E). Univariate ANOVA was also used to determine if rs2267735 has an indirect impact on PTSD by effecting either depressive symptoms or drug abuse (Supplemental Figure 2A-D). There was no statistical difference in either BDI total or lifetime drug abuse relative to the genotype at rs2237735. These variables were subsequently removed from regression models. Similar analyses were done for PTSD by startle measures and mRNA levels (Figure 1E, Figure 3F,G, Figure 4D and Figure 5).

Correlation with a two-tailed test was used to examine the relationship between the beta values for methylation by PTSD total symptoms.(Figure 4A). The data were then spit by high and low methylation (high, N=50; low, N=45) and analyzed by a dichotomous variable, PTSD diagnosis, using Chi-square (Figure4B).

Replication A replication group representative of a second subset from the same original sample set was analyzed as above (N=555; Figure 3A; Demographics in Supplementary Tables 1-2, 'Replication Genetics Sample'). Data from the combined cohorts was used for Figures 3B-3E.

Analyses of rs2267735 genotypes with the GAIN schizophrenia and bipolar sample: To test the association of rs2267735 with schizophrenia and bipolar disorder we used the genome-wide genotyping data has been produced with the Affymetrix 6.0 platform for the Genetic Association Information Network (GAIN) schizophrenia and bipolar studies accessible through http://www.ncbi.nlm.nih.gov/projects/gap. The study accession numbers are phs000021.v3.p2 (schizophrenia) and phs000017.v3.p1 (bipolar disorder). To test the association of rs2267735 with schizophrenia, we used the available pre-computed analyses in 1378 European ancestry (EA) cases, 1351 EA controls, 954 African American (AA) cases, 1195 AA controls (analysis accession number: pha002857.1 and pha002859.1). For association analyses with bipolar disorder, 1001 EA cases, 1034 EA controls, 363 AA cases, 671 AA controls were available (analysis accession number: pha002858.1 and pha002863.1). All pre-computed p-values for associations of rs2267735 with schizophrenia or bipolar disorder in the two ethnic groups were higher than 0.01, indicating no major contribution of this variant to these disorders.

Analysis of rs2267735 association with Alzheimer's Disease samples Genomic DNA from Alzhimer's disease samples was prepared using the DNeasy Tissue Kit (Qiagen, cat #: 69504).Genotyping of rs2267735 in an additional Alzheimer's disease cohort was done using Taqman assays and a 7900 system (Applied Biosystems, Foster City, CA). DNA samples had been previously screened (Corneaveaux et al) to eliminate samples with gender errors, poor quality samples (as assessed by low call rates) or whose genotypes did not match reported ethnicity. Samples were obtained from the following sites: **Newcastle Brain Tissue Resource** (funding via the Medical Research Council, local NHS trusts and Newcastle University): C.M. Morris, MD, Ian G McKeith, Robert H Perry **MRC London Brain Bank for Neurodegenerative Diseases** (funding via the Medical Research Council): Simon Lovestone, Md PhD, Safa Al-Sarraj. MD, Claire Troakes.

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