GWAS-identified risk variants for major depressive disorder: Preliminary support for an association with late-life depressive symptoms and brain structural alterations

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Abstract

A number of genome-wide association studies (GWAS) have investigated risk factors for major depressive disorder (MDD), however there has been little attempt to replicate these findings in population-based studies of depressive symptoms. Variants within three genes, *BICC1, PCLO* and *GRM7* were selected for replication in our study based on the following criteria: they were identified in a prior MDD GWAS study; a subsequent study found evidence that they influenced depression risk; and there is a solid biological basis for a role in depression. We firstly investigated whether these variants were associated with depressive symptoms in our population-based cohort of 929 elderly (238 with clinical depressive symptoms and 691 controls), and secondly to investigate associations with structural brain alterations. A number of nominally significant associations were identified, but none reached Bonferroni-corrected significance levels. Common SNPs in *BICC1* and *PCLO* were associated with a 50% and 30% decreased risk of depression, respectively. *PCLO rs2522833* was also associated with the volume of grey matter ($p=1.6 \times 10^{-3}$), and to a lesser extent with hippocampal volume and white matter lesions. Among depressed individuals *rs9870680* (*GRM7*) was associated with the volume of grey and white matter ($p=10^{-4}$ and 8.3×10^{-3}, respectively). Our results provide some support for the involvement of *BICC1* and *PCLO* in late-life depressive disorders and preliminary evidence that these genetic variants may also influence brain structural volumes. However effect sizes remain modest and associations did not reach corrected significance levels. Further large imaging studies are needed to confirm our findings.

Keywords: Depression; elderly; imaging; grey matter; hippocampus volume; GWAS

1. Introduction

Depression is a highly prevalent psychiatric disorder, affecting up to a fifth of people in the general population and similar numbers are diagnosed with a major depressive disorder across their lifetime (Alonso et al., 2004). Depression is considered one of the leading causes of disability, being highly comorbid with other health problems, and in the elderly with daily activity limitations and mortality (Ryan et al., 2008).

Family studies provide evidence that there is a genetic component to depressive disorders, with estimates that heredity may account for around 40% of depression cases (Kendler et al., 2006). Uncovering the genetic basis of susceptibility to depression has thus become an important field of research, and one that is highly feasible since publication of the complete human genome. Candidate-gene studies of depression have logically focused, most predominantly, on genes involved in neurotransmitter signaling (Levinson, 2006), due to their involvement in the aetiology of depression and because they are the principal targets of the most widely-used antidepressants. However, despite the exponential increase in studies over the last decade, only a few candidate genes have consistently been implicated (Licinio et al., 2009; Wray et al., 2009). Genome-wide association studies (GWAS) offer a unique opportunity to discover new genes, pathways or systems implicated in the disease, by examining thousands of common gene variants across the entire genome. A number of novel genes have been identified through this work (Cross-Disorder Group of the Psychiatric Genomics, 2013; Levinson et al., 2014; Major Depressive Disorder Working Group of the Psychiatric et al., 2013), although very few have reached the stringent significance levels currently accepted for GWAS. Importantly, the majority of genes have only been identified in individual GWAS studies and most use broad measures of depression phenotype without detailed information on participant characteristics. Replication remains the only way to validate GWAS hits and help determine their utility as markers in the wider community. Furthermore,

the vast majority of GWAS have focused on clinically diagnosed major depressive disorder (MDD). Whether identified variants are also associated with other forms of the disease, such as depressive symptoms, which are much more prevalent in the community, especially in the elderly, remains to be determined.

We selected three genes which were identified as a 'strong hit' in at least one GWAS study, where there was good biological evidence for their involvement in MDD, and they were subsequently identified as a likely risk factor for depression in an additional study (GWAS or candidate-gene). This included the bicaudal C homologue 1 gene (*BICC1*) (Bermingham et al., 2012; Lewis et al., 2010); the piccolo presynaptic cytomatrix protein (*PCLO*) (Hek et al., 2010; Sullivan et al., 2009); and the gene encoding the glutamate receptor metabotropic 7 (*GRM7*) (Muglia et al., 2010; Shyn et al., 2011). *BICC1* codes for the BicC family RNA binding protein 1, which forms complex interactions with other proteins including RNA. It is expressed as various isoforms throughout the brain, and one of the isoforms contains a sterile alpha motif (SAM) domain, an important postsynaptic targeting signal (Grace et al., 1995). Expression of *BICC1* is upregulated in the post-mortem brains of individuals with MDD, and in rat models of chronic stress, while treatment with antidepressants reduces expression. In the latter models, knockdown of this gene in the hippocampus also prevents anhedonia, a key feature of depression (Ota et al., 2014). *PCLO* is localised in the brain and part of the presynaptic cytoskeletal matrix. It is involved in establishing active synaptic zones and in synaptic vesicle trafficking, and plays an important role in monoaminergic neurotransmission (Leal-Ortiz et al., 2008). It was thought to play a role in behavioral plasticity even prior to its identification in GWAS studies of depression (Cen et al., 2008). The *GRM7* gene is expressed throughout the brain and codes for a highly conserved presynaptic receptor (mGluR7) which regulates neurotransmission. *A*nimal models have shown that *GRM7* plays a role in stabilizing mood and could be a target for antidepressant treatment (Palucha et al., 2007; Wieronska et al., 2007). In knockout mice

models, deletion of *GRM7* was associated with upregulation of glucocorticoid receptor feedback on the hypothalamic–pituitary–adrenal axis and increased levels of brain derived neurotrophic factor in the hippocampus (Mitsukawa et al., 2006), as well as decreased depression-like behaviors (Cryan et al., 2003).

The study will use data collected from a large well-characterized prospective elderly cohort recruited from the community and assessed for MDD and depressive symptoms. A subset of these participants also underwent neuroimaging. The first aim of this study was to examine whether GWAS identified genes for MDD are associated more broadly with clinically significant depressive symptoms in our elderly cohort. Secondly, we sought to determine whether these gene variants were associated with structural brain alterations, as intermediate phenotypes of depression (Hornung and Heim, 2014). Depressive patients often display brain structural abnormalities (Drevets et al., 2008) which could potentially proceed the onset of symptoms and genetic variants have been associated with brain imaging markers (Scharinger et al., 2010).

2. EXPERIMENTAL PROCEDURES

2.1 Study population

The prospective ESPRIT study is a general population-based study of psychiatric disorders among older adults in France (Ritchie et al., 2004). Eligible participants who were aged 65 years or older and not living in an institution were randomly selected from electoral rolls in the Montpellier region between 1999

and 2001. The study protocol was approved by the Ethical Committee of the University Hospital of Kremlin-Bicêtre and all procedures were undertaken with verbal and written consent of the participants. Of the 2189 participants without dementia who agreed to participate in the ESPRIT Study, 1076 provided buccal samples for genotyping analysis, and 944 (87.7%) had complete and validated genotyping data for all single nucleotide polymorphisms (SNPs). Twelve of these participants did not undergo assessment for depression and an additional three were missing data concerning at least one of the important potential confounding factors. The analysis of depression associations was thus performed on 929 participants, while the imaging sub-study (described further below) involved 336 of these participants. Compared to the participants included in the analysis, those excluded were significantly older ($t = 1.95$, $p=0.05$), and were also more likely to have physical activity limitations ($\chi 2 =11.65$, $p=0.0006$) and smaller hippocampal volumes ($t = -3.74$, $p=0.0002$). Included and excluded participants however, were not significantly different in terms of depression prevalence, genotypes or other brain measures.

2.2 Depression diagnosis

MDD was diagnosed based on the diagnostic and statistical manual of mental disorders (DSM-IV) criteria using the Mini-International Neuropsychiatry Interview (MINI) (Sheehan et al., 1998). This standardized psychiatric examination has been validated in France. The MINI provides an extensive examination according to DSM-IV criteria, with diagnostic algorithms applied to assess 'caseness', and positive cases were further reviewed by a panel of psychiatrists to validate the initial diagnosis (Ritchie et al., 2004). The Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff, 1977), validated for use in the elderly, was used for the assessment of current depressive symptoms. A clinically significant level of depression was examined (Ancelin et al., 2013)), defined as a current diagnosis of MDD or high levels of

depressive symptomatology based on the CES-D (CESD≥16) (Radloff and Locke, 1986). Current use of antidepressants was validated by presentation of the prescription or the medication itself.

2.3 Genotyping

Participants provided buccal samples and DNA was extracted as detailed previously (Ancelin et al., 2013) and stored at -80°C. Genotyping was performed by LGC Genomics (Hoddesdon Herts, UK) using their KASPar genotyping system, a competitive allele-specific PCR incorporating a FRET quencher cassette to identify Single-Nucleotide Polymorphism (SNP). The amplified PCR products were analysed by fluorescence scanning in a BMG labtech Pherastar scanner. Results were interpreted using KlusterCaller 1.1 software. The error rate for the KASPar assay system is less than 0.3%.

We selected three genes for analysis in this study based on those which had been reported in at least one GWAS study of depression (i.e. mentioned as "top hits" in the study, even if they did not reach genome-wide significant levels), and which were identified as being implicated in depression in at least one additional study. For the three genes selected, there was also a good biologically basis for their involvement in depression. For each gene we chose three SNPs for analysis, focusing on the variants reported in previous studies.

BICC1 is located on chromosome 10 *(10q21.1)* with 22 exons. The SNPs investigated in this study included *rs9416742* and *rs999845,* which were the top-ranked hits in the original GWAS study of major recurrent depression (Bermingham et al., 2012; Lewis et al., 2010), and *rs7903712,* which showed the strongest association after genotype imputation (Lewis et al., 2010). *BICC1 rs9416742* and *rs999845* were also associated with age at onset of MDD in a similar cohort (Power et al., 2012) and the latter with structural brain changes in depression (Bermingham et al., 2012).

PCLO is located on chromosome 7 (7q21.11) and has 26 exons. The three SNPs chosen for analysis were *rs2715148* and *rs2522833*, as the top-ranked variants for this gene (Hek et al., 2010; Sullivan et al., 2009) and *rs2107828*, which was the fourth ranked variant. Further studies supported a role for *PCLO* and *rs2522833* in particular, in depression (Hek et al., 2010).

The *GRM7* gene is found on chromosome 3 (3p26.11) and has 14 exons. SNPs chosen for analysis included *rs9870680* (Muglia et al., 2010; Shyn et al., 2011) and *rs162209* (Muglia et al., 2010), identified from GWAS studies of MDD, and *rs1485171,* which was the most significant risk variant for bipolar disorder in the large Wellcome Trust Case Control Consortium where depression was not investigated specifically (WTCCC, 2007).

2.4 Brain structures

Of the participants recruited to the ESPRIT study, those aged 80 years or less were randomly selected to take part in the imaging study. Magnetic Resonance Imaging (MRI) data was obtained as detailed below. Transversal fast multi-slice double echo T2-weighted 2D axial data, (TR=4400ms, TE1 and TE2= 16ms and 98ms, slice thickness = 4mm, gap= 0.4mm, matrix = 256x256, in-plane resolution = 0.98 x 0.98mm^2), covering the whole brain, was acquired using a 1.5T GE Signa Imaging System (General Electric Medical Systems, Wisconsin, USA). T1-weighted volumetric MRI was also obtained by using the spoiled gradient recalled (SPGR) sequence (TR = 97ms, TE = 4ms), consisting of a 124 set of adjacent transverse sections parallel to the anterior commissure-posterior commissure line with a section thickness of 1.5mm (without a gap).

Brain volumes (cm^3) were calculated by segmenting each T1-weighted SPGR image into its component tissue classes (grey and white matter; cerebrospinal fluid) with SPM5 (Wellcome Department of Cognitive Neurology, London, UK) and using a specific segment.m script (developed by Jon Jackson; http://www.fil.ion.ucl.ac.uk/spm/ext/#spm_segment). Included in the total grey matter and white matter volumes were subcortical /deep grey / white matter structures. Other details of the script included separable smoothing kernel = 3x3x3, no lesion masks and default threshold (0). Total intracranial volume (ICV) was calculated as the sum of grey and white matter plus cerebrospinal fluid volumes (Acosta-Cabronero et al., 2010). All outputs were manually inspected to ensure accurate segmentation and valid data. Brain atrophy was estimated as the cerebrospinal fluid to ICV ratio.

Hippocampal volumes (mm^3). Using consecutive coronal slices, ROIs were manually outlined and verified from axial and sagittal orientations (Maller et al., 2007). The hippocampus head and body were measured as the anterior tip of the hippocampus until the slice before the opening of the crus of the fornix (CF) and included the subiculum, CA1–(4) areas, and dentate gyrus (DG). Given the inherent difficulties and frequent inconsistencies in hippocampal measurement, a well-validated standardized procedure has been adopted which has been previously described elsewhere (Ryan et al., 2014).

Hippocampus outlines were blindly traced by two trained researchers that were unaware of the participants' identity, study hypotheses or group assignment. The intra- and inter-class correlations (intra- hippocampus and inter- hippocampus) were calculated using a formula that presumes random selection of raters (Shrout and Fleiss, 1979), and these values provided a measure of the reliability of the hippocampus measurements. Two of the researchers (JM, CM) each retraced five MRI images, which were randomly selected among the images previously traced, and five images which belonged to the

group previously traced by the other researcher. Intra- hippocampus was 0.942 for JM and 0.970 for CM. Inter- hippocampus was 0.939. These values are well within acceptable limits.

White matter lesion (WML) volume (cm^3) was estimated using a semiautomatic method (Brickman et al., 2009; Gurol et al., 2006). This involved the T2-weighted sequence segmentation of areas of supratentorial WML appearing as hyperintensities, using MRIcro software (Rorden and Brett, 2000). Using a semi-automated technique based on intensity thresholding, a first layer of ROIs corresponding to WML was created, and a second ROI layer was then manually outlined on each slide by roughly contouring of all WML. The intersection of the first and second layer was then manually inspected and the total WML volume was automatically obtained, irrespective of underlying cause (although people with extensive stroke-related damage were excluded). All scans were examined by an experienced reader and an experienced neurologist examined a random selection of 80 scans to assess inter-rater reliability. Inter-rater and intra-rater intra-class correlation coefficients showed good-to-excellent agreement (0.79 and 0.95, respectively).

2.5 Socio-demographic, clinical and health factors

Detailed information gathered on each of the participant's included their age, level of education, alcohol consumption, and smoking status. High alcohol consumption was defined as at least 24 grams of alcohol each day and regular smoking as having at least 10 packs year. Body mass index (BMI) was calculated as weight (kg) divided by height (m^2). The health of the participants was assessed through measurements made on 12-hour fasting blood samples, a complete inventory of drug use in the preceding month, detailed medical questionnaires and additional information from general practitioners. The questionnaires included information on cardiovascular disease history (angina pectoris, myocardial infarction, stroke, cardiovascular surgery, and arteritis), or other chronic illnesses including

hypertension, defined as resting blood pressure ≥160/95mm Hg or treatment; diabetes (fasting glucose ≥7.0mmol/l or reported treatment); and high cholesterol levels (total cholesterol ≥ 7.2 mmol/l or treatment). Blood pressure was measured twice in a sitting position using a digital electronic tensiometer OMRON M4, and the average value was used in the analyses. The accuracy of current medication reporting was verified by presentation of the prescription or the medication itself.

Participants were assessed for activity limitations using the Rosow and Breslau mobility scale (Rosow and Breslau, 1966) and the Instrumental Activities of Daily Living (IADL) scales (Lawton and Brody, 1969). The presence of limitations was defined as the inability to complete one or more activities from both scales. The Mini-Mental State Examination (MMSE) was used to measure global cognitive function, and cognitive impairment was defined as scoring 24 or less on this scale. Dementia diagnosis was made according to DSM-IV criteria and validated by independent neurologists. Participants diagnosed with dementia were excluded from this analysis.

2.6 Statistical analyses

Chi-squared tests were used to compare the distribution of *BICC1, PCLO,* and *GRM7* genotypes with those predicted under the Hardy-Weinberg equilibrium. Pair-wise linkage disequilibrium was estimated using Haploview version 4.2 (Barrett et al., 2005) to determine the correlation between individual SNPs. Chi-squared tests and t-tests were used to examine the characteristics of participants with and without depression. Age and gender adjusted logistic regression models were used to examine the association between gene variants and clinical depression. Additional models included covariates that were independently associated with depression (Table 1), to ensure other factors did not mediate the associations observed. Gender interactions were considered by including a SNP*gender interaction term in the models.

Linear regression models were then used to examine the independent association between gene variants and brain volumes. In addition to grey and white matter and WML, we examined hippocampal volume given that it is one of the key brain regions thought to be involved in the pathogenesis of depression (Vu and Aizenstein, 2013). Based on the findings of some previous studies, a first-order interaction between these SNPs and gender or depression status was also examined by including a product term in the models. When significant, subsequent analysis was stratified to determine independent group effects. Grey matter, white matter and total hippocampus volume were expressed as a ratio of ICV, thus controlling/normalising for differences in overall brain size. WML values were transformed by a $\log_{10}(x+0.01)$ function given their highly asymmetric distribution and possible null values. All models with WML as the dependent variable were adjusted for total white matter volume. SAS version 9.4 (SAS Institute, Inc., Cary, North Carolina) was used for all of the statistical analysis. For the analysis of the association between 9 SNPs and depression, the Bonferroni corrected p-value was <0.0055, while for the analysis of SNPs and brain volumes it was $p<0.0014$ (9 SNPs x 4 brain volumes). Based on the smallest genotype group with a frequency of 25%, and the prevalence of depressive symptoms in this population, our study would have over 80% power (at 5% significance level) to detect an odds ratio of 1.6 for depression associated with a given genotype.

3. RESULTS

3.1 Population characteristics

Of the 929 participants (368 men and 561 women) included in this study, 238 (25.6%) had clinically significant levels of depression at baseline (Table 1). Participants with depression were more likely to be female, to have fewer years of education, lower cognitive function, and to take a higher number of current medications, compared to non-depressed participants.

The genotype frequencies of SNPs examined in this study were as follows: for *BICC1 rs7903712* GG=582, GC=309, CC=38; *rs9416742* GG=604, AG=282, AA=43; *rs999845* GG=596, AG=291, AA=42; for *PCLO rs2107828* AA=234, TA=483, TT=212; *rs2522833* AA=359, AC=435, CC=135; *rs2715418* AA=269, CA=468, CC=192; and for *GRM7 rs1485171*: GG=673, TG= 234, TT=22; *rs162209* CC=372, CT=422, TT=135, *rs9870680* CC=277, CT=430, TT=222. All of the SNPs were in Hardy-Weinberg Equilibrium (p>0.20), with the exception of *rs9870680 GRM7* which was not in equilibrium overall (χ^2=4.69, p=0.03), but was when depressed participants were excluded (χ^2=0.73, p=0.39). The *BICC1* SNPs were in strong linkage disequilibrium (|D'|>0.90 for all pairwise comparisons), as were the three *GRM7* SNPs (|D'|=0.98). The *PCLO* SNPs were not in high disequilibrium with one another (|D'|≤0.80). To maximize the power of subsequent analyses, homozygotes for the variant minor allele were combined with the heterozygotes.

3.2 Gene variants associated with depression

The association between gene variants and depression was investigated firstly using logistic regression models adjusted for age, education and gender, and then with further adjustment for cognitive impairment and number of medications used (Table 2), given that these factors were themselves associated with depression in this population (see Table 1). In the case of *BICC1* SNPs there was some evidence of a gender-SNP interaction (*rs7903712:* χ^2=2.75, p=0.098; *rs9416742*: χ^2=3.82, p=0.050; *rs999845* χ^2=4.27, p=0.039), and gender-stratified analysis was thus undertaken. For *BICC1,* minor alleles of *rs9416742* and *rs999845* were associated with a more than 50% lower risk of depression in men at nominal significance levels, while no association was observed among women despite a much larger sample size. Two of the three *PCLO* minor alleles were also associated with an approximately 30% lower risk of depression among all participants, but none of these associations reached the p<0.0055 threshold

for Bonferroni corrected significance. In terms of *GRM7*, no associations were found with any of the SNPs.

3.3 Gene variants associated with brain volumes

Table 3 details the characteristics of the participants involved in the imaging substudy, and Table 4 according to depression status. Except for being younger, these participants were not significantly different from the overall population. Table 5 lists the median and interquartile brain volumes of participants in this study, according to their depression status. Both the absolute volumes (grey and white matter, hippocampus) as well as the ratio of each volume according to the total ICV are given. Six depressed and 12 non-depressed participants had no detectable WML.

Linear regression models were used to investigate whether these gene variants were associated with structural brain alterations. Analysis adjusted for age and gender (Table 6), while additional models consider inclusion of other key covariates (cf. Table 3) to ensure these factors did not influence the associations (Supplementary Table 1). *BICC1* variants were not found to be significantly associated with any brain volumes, and there was no evidence of a gender interaction ($p>0.10$ for all tests). *GRM7 rs9870680* was associated with the volume of grey matter at nominal significance levels. In terms of *PCLO*, minor alleles of all three SNPs were associated with smaller grey matter relative to total ICV, and the minor C allele of *rs2522833* was also associated with a smaller hippocampus and greater WML. None of the associations would remain significant after Bonferroni correction for multiple testing ($p<0.0014$), but the association between *rs2522833* and grey matter volume was very close to this threshold level ($p=0.0016$).

3.4 Depression-stratified analysis

We next investigated whether these associations between gene variants and brain volumes differed according to depression status (n=76 with and n=260 without depression), or whether they were independent risk factors. A highly significant interaction between depression status and *GRM7 rs9870680* on both grey and white matter was identified (p=0.0003 and p=0.0094, respectively). Depression-stratified analysis suggested that the previous observed association between the T allele of *rs9870680* and smaller grey matter, with a trend for larger white matter volume, was only significant among participants with depression (age and gender adjusted models: β(SE): -2.57 (0.64), p=0.0001; β(SE): 1.53 (0.56), p=0.0083 respectively). Among participants without depression there was no such association (β (SE): -0.08 (0.33), p=0.82; β (SE): 0.08 (0.26), p=0.76 for grey and white matter volume, respectively) (Figure 1). Again, these associations were not modified after further adjustment for covariates such as education and cognitive impairment.

In the case of *BICC1*, while there were no association between gene variants and brain volumes overall, there was evidence of a depression-gene variant interaction on hippocampal volume (*rs9416742*: χ^2=1.90, p=0.058; *rs999845* χ^2=2.02, p=0.044). In stratified analysis, there was some indication that the minor alleles of these SNPs (A allele of *rs9416742* and *rs999845* with n=26 and n=27 respectively) were associated with larger hippocampal volume among depressed individuals only, compared to those homozygous for the G allele (n=50 and n=49 respectively). These associations however, were not significant and the depression stratified analysis was underpowered to detect true associations even if they existed.

4. DISCUSSION

Despite numerous GWAS and candidate-gene studies which have predominantly focused on clinically diagnosed MDD, only a few genes have been identified in more than one study, and whether these genes are also risk factors for depressive symptoms among the general population remains unclear. Our study aimed to investigate the association between three GWAS identified risk variants for MDD and depressive symptoms in an independent older population-based cohort, while also investigating their association with structural brain alterations. We provide further support for the involvement of two GWAS identified genes in late-life depression, and identified novel associations with brain volumes. In terms of *PCLO*, risk variants identified in some previous studies and those found to be associated with reduced grey matter volume in our analysis, were also associated with a decreased risk of depression. Two common SNPs in *BICC1* were associated with the risk of late-life depressive symptoms in men only. In contrast, variants in *GRM7* were not found to be associated with depressive symptoms in this older population, although they were associated with the volume of grey and white matter. Our findings thus add further evidence to suggest the involvement of these genes in depression, with the limitation that only nominally significant associations were found. Future studies must therefore use even larger sample sizes to determine the true significance of these associations.

4.1 Bicaudal C homologue 1 gene (*BICC1*)

A GWAS comparing 1638 adult depression cases and 1594 controls free of psychiatric disorder identified the *BICC1* gene as the strongest hit (Lewis et al., 2010). While none of the SNPs reached genome-wide significance levels, two SNPs in *BICC1* showed strong associations, with the A allele of both *rs9416742* and *rs999845* being protective. A subsequent GWAS investigating age at MDD onset, also identified the same two *BICC1* variants (Power et al., 2012), however their cohort was similar to the previous study. A later study of adults (aged 18-65 years) examined *rs999845* and found no differences in genotype

frequencies between the 44 cases (current clinical diagnosis of MDD) and 44 controls (balanced for age) (Bermingham et al., 2012), but lacked statistical power to observe previously reported effect sizes. The authors did find however, that the right hippocampal head was larger in individuals with the protective A allele compared to those homozygous GG, but only in the absence of early-life adversity. No other significant associations were found (overall hippocampal volume or left hemisphere, and body or tail) and no gender-specific interactions were examined.

The results of our study also support an association between the minor A allele of both *rs9416742* and *rs999845* and a lower risk of depression, although this was specific to older men (gender x SNP interactions p=0.04 and 0.02 respectively). In terms of brain volumes, our results also provided some evidence that minor alleles of these *BICC1* SNPs were associated with larger hippocampal volumes in depressed elderly only, which is in the same direction as the associations reported previously in the clinical sample (Bermingham et al., 2012). Thus it appears likely that *BICC1* gene variants can influence brain volumes, although the mechanism by which this occur remains to be determined.

4.2 Presynaptic cytomatrix protein (*PCLO*)

The first epidemiological study implicating *PCLO* was a GWAS of 1738 MDD cases and 1802 controls, recruited from the Genetic Association Information Network (GAIN) study (Sullivan et al., 2009), comprising the Netherlands Study of Depression and Anxiety (NESDA) and the Twin Registry (NTR) cohorts. Cases were defined as a lifetime MDD diagnosis, while controls had no history of MDD or anxiety and reported low depressive symptomatology at baseline. All participants were aged 18-65 years (mean 44 years) and were matched with controls on age and gender. Although no SNP reached genome-wide significance levels, the strongest evidence was for *PCLO*, with 11 of the 2200 top hits located in a region overlapping the gene. The strongest variants were *rs2522833* and *rs2715148*, with the C alleles

being associated with an approximate 20% increased risk of depression. In secondary analysis, it was found that the associations were strongest for early onset recurrent MDD, and for women, although no significant gender interaction was identified (Aragam et al., 2011). The original findings could not be replicated at appropriate thresholds in a combined sample of five independent European Ancestry cohorts, totaling almost 12,000 participants. However, there was large heterogeneity in findings across cohorts, and the finding was replicated in some individual studies (discussed further below). While some additional GWAS studies did not identify this gene (Muglia et al., 2010; Shyn et al., 2011; Wray et al., 2009), a candidate gene study of 5968 older adults (≥55 years) found that *rs2522833* was associated with MDD (n=145) (Hek et al., 2010). On the other hand, they reported no association with their measure of depressive symptoms, but this was very broad and included, in addition to clinically assessed depression, a general practitioner or self-report stating they were depressed, and antidepressant use in the absence of depression. Thus it is likely that at least some of their depression 'cases' were incorrectly assigned.

While our study also provides support for *PCLO rs2522833* and *rs2715148* in depression, and suggests for the first time it may influence the risk of late-life depressive symptoms, we found that the C alleles were associated with an approximately 40% decreased risk, which is in contrast to the findings of Sullivan (Sullivan et al., 2009). Although at first this seems surprising, it is of note that a number of replication cohorts examined in the paper by Sullivan, also had odds ratios in the same direction as that found in our study, particularly those from the STAR*D (Sequenced Treatment Alternatives to Relieve Depression) study. STAR*D cases recruited from primary care or outpatient clinics, had an MDD diagnosis (DSM-IV criteria) and current depressive symptoms (Hamilton Depression Rating Scale ≥ 14), but were free of other psychiatric conditions. They were aged 18-75 years, with a mean of 43 years, 59% were female and almost 74% reported recurrent MDD. Controls were randomly recruited from the

general population and were free of psychiatric disorders (Shyn et al., 2011). Sullivan suggested that the heterogeneity across cohorts could partly be explained by case selection, with differences in studies which selected from the population versus clinical samples. However, our results do not fit with this theory, as our cohort was entirely population-based, which is similar to that of the GAIN, rather than the STAR*D study. Our study focused on late-life depression in an elderly cohort, where less than 25% reported a past MDD and the majority an age at onset after 45 years. This contrasts with the GAIN study which reported the strongest findings with early onset recurrent MDD in an adult cohort. However, the STAR*D cohort had a similar adult population to that of GAIN, and also contained a high proportion of cases with recurrent MDD.

In our elderly population, the AA homozygote of *rs2522833* which was associated with an increased risk of depression, was also associated with higher grey matter and hippocampal volume, as well as reduced WML. We have previously reported in this same ESPRIT cohort that participants reporting specific childhood traumas were at increased risk of late-life depression (Ritchie et al., 2009) but a lower risk of poor cognitive functioning and higher hippocampal volume (Ritchie et al., 2012). In the current study, only a small number of participants with both genotyping and MRI data also completed the childhood adversity questionnaire. Whether the *PCLO* gene could thus be involved in a form of cognitive adaptation in response to early adverse environment, remains to be examined in larger lifetime prospective cohorts.

Very few prior imaging studies have investigated *PCLO*. Functional MRI was used to investigate processing of emotional memory tasks and executive functioning, in a Dutch study of 159 adults (mean age 38 years, 64% female and 74% MDD) (Woudstra et al., 2012). The C allele of *rs2522833* was associated with increased activation in the left amygdala in response to specific stimuli, but had no

effect on executive functioning, and was not independently associated with MDD. Another study examined memory encoding in the subgenual cingulated cortex region and reported that carriers of the *rs2522833* C allele of *PCLO* had worse memory performance and lower encoding-related hippocampal activation (Schott et al., 2014).

4.3 Glutamate receptor metabotropic 7 gene (*GRM7*)

The chromosome region 3p26-3p25 (including *GRM7)* has been implicated in MDD, in a study of 839 families with sibling pairs affected with severe recurrent depression (Breen et al., 2011). A GWAS of 1221 MDD cases and 1636 controls from the STAR*D cohort, failed to detect significant variants at genome-wide corrected levels, but identified strong evidence for three genes and in particular *GRM7* (*rs9870680)* (Shyn et al., 2011). A subsequent study of recurrent depression in two adult German cohorts (n=1022 cases, 1000 controls; 429 cases and 1052 controls respectively) found no SNPs reaching genome-wide significance but some evidence of an association with *GRM7 rs162209* (Muglia et al., 2010). Further evidence for the involvement of GRM7 in depression has come from animal models and transcriptome studies using human postmortem brain samples (Chang et al., 2014; Palucha et al., 2007).

In our study we reported no independent association of the three *GRM7* SNPs, including *rs9870680* and *rs162209,* and late-life depression. This may be due to a lack of power, as only very small effect sizes have been reported previously (odds ratio of 1.19) (Shyn et al., 2011). We did however find that *rs9870680* was associated with grey and white matter volume, in particular among depressed individuals. These results were highly significant ($p=10^{-4}$ and $p=8.3 \times 10^{-3}$, respectively), although in opposing directions. The so-called T risk allele for *rs9870680* was associated with smaller grey matter, but a larger white matter volume in those with depression. Reductions in grey matter volume are commonly seen in individuals with depression, although these are region specific (Grieve et al., 2013).

Less has been documented concerning white matter, although both increased and decreased volume has been described in depression. No previous study has examined *GRM7* variants and brain volumes.

4.4 Limitations and Strengths

Although the study population was randomly selected from those living in the community, individuals who agreed to participate were younger and in better health than those who did not. This limits the extent to which the findings can be generalised to the wider elderly community. Bias from population stratification also needs to be considered as French law prohibits the collection of data related to ethnicity. However, prior genotyping analysis with this data indicates that less than 1% is non-Caucasian (unpublished data and (Lambert et al., 2009)). Furthermore, all genotype frequencies were within Hardy-Weinberg Equilibrium, except *GRM7 rs9870680*, which was only in equilibrium among the non-depressed participants. Finally, as only a subsample of the participants underwent structural brain imaging, the sample size for this analysis was reduced, thus limiting the power to find significant differences if indeed they are present. In particular, this has impacted on the ability to investigate genotype x depression interactions.

This study is strengthened by its population-based design, thorough characterisation of study participants and sample size, which enabled the investigation of gender-specific interactions. Depression was assessed by trained staff using two distinct measures validated in the general population. This is the first study to investigate associations between these genetic variants and a number of structural brain measures, thus making a novel contribution to the field. Importantly, participants in the study were dementia-free and cognitive functioning was controlled for in the analysis. Furthermore, controls were free of both MDD and depressive symptoms, which may have limited findings of some previous studies

which focused on clinically diagnosed MDD, and did not consider the possibility of clinically relevant symptoms of depression among the controls.

4.5 Conclusion

These findings provide support for a role of the *PCLO* and *BICC1* genes in late-life depression, and indicate for the first time that these variants could also influence brain volumes. The common *GRM7* SNP *rs9870680* was also associated with both grey and white matter volumes in depressed individuals. Effect sizes however remain modest and none of the results reach Bonferroni-corrected significance levels. Large prospective imaging studies are needed to determine whether these variants are also associated with alterations in brain volumes over time and to help ascertain causality.

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Table 1. Characteristics of the 929[a] participants according to depression status.

Characteristic	without depression (n=691)	with depression (n=238)	Test for a difference	
	Mean (S.D)		t-value (d.f)	p
Age (years)	71.5 (4.42)	72.0 (4.48)	-1.57 (927)	0.11
Body Mass Index (kg/m^2)	24.8 (3.51)	24.8 (3.90)	0.09 (927)	0.93
	N (%)		χ^2 (d.f)	p
Gender (female)	385 (55.7)	176 (73.9)	24.60 (1)	<0.0001
≥ 12 years of education	284 (41.1)	66 (27.7)	13.48 (1)	0.0002
High alcohol consumption (≥ 24 g each day)	135 (19.9)	35 (15.2)	2.57 (1)	0.11
Smoking history (≥10 pack years)	273 (39.9)	85 (36.3)	0.94 (1)	0.33
Physical activity limitations	12 (1.74)	6 (2.52)	0.57 (1)	0.45
Low global cognitive function (MMSE≤24)	15 (5.2)	21 (12.1)	20.7 (1)	<0.0001
Ischemic-related disease	71 (10.3)	26 (10.9)	0.08 (1)	0.78
Diabetes (fasting glucose ≥ 7.0mmol/l or treatment)	50 (7.3)	16 (6.7)	0.09 (1)	0.77
Hypertension (≥ 160/95mm Hg or treatment)	311 (45.0)	104 (43.7)	0.12 (1)	0.73
Current user of ≥3 medications	243 (35.2)	124 (52.1)	21.2 (1)	<0.0001

[a]Except for alcohol consumption, smoking history and hypertension, where data were missing for 20, 11, and 5 participants, respectively.

Table 2. Logistic regression models for the adjusted[a] association between gene variants and depression.

Gene & SNP	N	All participants (n=929)			Men (n=368)			Women (n=561)		
		OR (95%CI)	χ^2 (d.f)	Nominal p	OR (95%CI)	χ^2 (d.f)	Nominal p	OR (95%CI)	χ^2 (d.f)	Nominal p
BICC1[b]										
rs7903712:GG	582	1			1			1		
GC/CC	347	0.94 (0.69-1.29)	0.14 (1)	0.71	0.57 (0.30-1.08)	1.92 (1)	0.16	1.12 (0.77-1.62)	0.33 (1)	0.57
rs9416742: GG	604	1			1			1		
AG/AA	325	0.92 (0.66-1.28)	0.23 (1)	0.63	0.48 (0.24-0.96)	4.28 (1)	**0.038**	1.14 (0.77-1.68)	0.44 (1)	0.51
rs999845: GG	596	1			1			1		
AG/AA	333	0.89 (0.65-1.23)	0.46 (1)	0.50	0.49 (0.25-0.94)	4.53 (1)	**0.033**	1.11 (0.76-1.62)	0.29 (1)	0.59
PCLO										
rs2107828: AA	234	1								
TA/TT	695	0.86 (0.61-1.22)	0.73 (1)	0.39						
rs2522833: AA	359	1								
AC/CC	570	0.65 (0.47-0.88)	7.63 (1)	**0.0058**[c]						
rs2715148: AA	269	1								
CA/CC	660	0.71 (0.51-0.99)	4.08 (1)	**0.043**						
GRM7										
rs1485171: GG	673	1								
TG/TT	256	0.99 (0.70-1.39)	0.01 (1)	0.94						
rs162209: CC	372	1								
CT/TT	557	1.04 (0.76-1.43)	0.07 (1)	0.79						
rs9870680: CC	277	1								
CT/TT	652	0.94 (0.68-1.32)	0.12 (1)	0.73						

[a] Adjusted for gender, age, education, cognitive impairment and number of medications used.

[b] Analysis was subsequently stratified by gender due to a significant gender x SNP interaction.

[c] Bonferroni-adjusted p-value is 0.052. All other adjusted p-values are >0.10.

Table 3. Characteristics of the 336[a] participants in the imaging sub-sample.

Characteristic	
	Mean (S.D)
Age (years)	70.54 (3.56)
Body Mass Index (kg/m^2)	24.55 (3.40)
	N (%)
Gender (female)	177 (52.7)
≥ 12 years of education	133 (39.8)
High alcohol consumption (≥ 24 g each day)	69 (21.0)
Smoking history (≥10 pack years)	140 (42.2)
Physical activity limitations	4 (1.19)
Low global cognitive function (MMSE≤24)	16 (4.8)
Ischemic-related disease	33 (9.8)
Diabetes (fasting glucose ≥ 7.0mmol/l or treatment)	29 (8.7)
Hypertension (≥ 160/95mm Hg or treatment)	144 (42.9)
Current user of ≥3 medications	112 (33.3)

[a]Except for alcohol consumption and smoking history where data were missing for 7 and 4 participants, respectively.

Table 4. Characteristics of the 336[a] participants in the imaging sub-sample according to depression status.

Characteristic	without depression (n=260)	with depression (n=76)	Test for a difference	
	Mean (S.D)		t-value (d.f)	p
Age (years)	70.5 (3.58)	70.7 (3.50)	-0.37 (334)	0.71
Body Mass Index (kg/m^2)	24.8 (3.51)	24.8 (3.90)	0.54 (334)	0.59
	N (%)		χ^2 (d.f)	p
Gender (female)	123 (47.3)	54 (71.1)	13.30 (334)	0.0003
≥ 12 years of education	105 (40.4)	28 (36.8)	0.31 (334)	0.58
High alcohol consumption (≥ 24 g each day)	58 (22.7)	11 (15.1)	1.97 (334)	0.16
Smoking history (≥10 pack years)	111 (43.2)	29 (38.7)	0.49 (334)	0.49
Physical activity limitations	3 (1.15)	1 (1.32)	0.01 (334)	0.91
Low global cognitive function (MMSE≤24)	9 (3.5)	7 (9.2)	4.29 (334)	0.04
Ischemic-related disease	24 (9.2)	9 (11.8)	0.45 (334)	0.50
Diabetes (fasting glucose ≥ 7.0mmol/l or treatment)	22 (8.5)	7 (9.2)	0.04 (334)	0.84
Hypertension (≥ 160/95mm Hg or treatment)	112 (43.1)	32 (42.1)	0.03 (334)	0.88
Current user of ≥3 medications	80 (30.8)	32 (42.1)	21.2 (334)	0.07

[a]Except for alcohol consumption and smoking history where data were missing for 7 and 4 participants, respectively.

Table 5. Brain volumes according to depression status.

Characteristic	without depression (n=260)	with current depression (n=76)	Test for difference	
	Median (IQR: 25th – 75th percentile)		**t-value (d.f)**	**p**
Volume of grey matter, cm^3	656 (618 to 705)	651 (598 to 687)	1.29 (334)	0.19
Ratio of grey matter to ICV	55.1 (53.8 to 56.9)	55.2 (53.0 to 56.7)	0.01 (334)	0.99
Volume of white matter volume, cm^3	355 (327 to 390)	347 (319 to 383)	0.81 (334)	0.42
Ratio of white matter to ICV	29.6 (28.5 to 31.0)	30.0 (28.7 to 31.4)	-0.51 (334)	0.62
Total volume of hippocampus, mm^3	5877 (5403 to 6366)	5623 (5169 to 6164)	1.36 (334)	0.17
Ratio of hippocampus to ICV	0.49 (0.45 to 0.54)	0.48 (0.44 to 0.53)	0.41 (334)	0.68
Total volume of WML, cm^3	0.7 (0.3 to 2.2)	0.6 (0.2 to 2.9)	-1.12 (334)	0.26
Volume of WML transformed (log_{10})	-0.18 (-0.51 to 0.33)	-0.25 (-0.68 to 0.47)	0.75 (334)	0.45
	N (%)	**N (%)**	**χ^2 (d.f)**	**p**
Participants with no detectable WML	12 (4.6)	6 (7.9)	1.25 (1)	0.26

Table 6. Linear regression models for the adjusted[a] association between gene variants and brain measures[b] (n=336).

Gene & SNP	N	Grey Matter		White Matter		Hippocampus		White Matter Lesions	
		β (SE)	$\chi^{2\,c}$, nominal p	β (SE)	$\chi^{2\,c}$, nominal p	β (SE)	$\chi^{2\,c}$, nominal p	β (SE)	$\chi^{2\,c}$, nominal p
BICC1									
rs7903712:GG	213								
GC/CC	123	-0.14 (0.30)	-0.48, 0.63	-0.23 (0.24)	-0.98, 0.33	-0.06 (0.08)	-0.81, 0.42	-0.04 (0.09)	-0.50, 0.62
rs9416742: GG	222								
AG/AA	114	-0.22 (0.31)	-0.69, 0.49	-0.24 (0.25)	-0.97, 0.33	-0.04 (0.08)	-0.52, 0.61	-0.01 (0.09)	-0.16, 0.88
rs999845: GG	218								
AG/AA	118	-0.16 (0.30)	-0.54, 0.59	-0.28 (0.24)	-1.18, 0.24	-0.09 (0.08)	-1.20, 0.23	-0.02 (0.09)	-0.18, 0.86
PCLO									
rs2107828: AA	83								
TA/TT	253	-0.64 (0.32)	-2.00, **0.047**	0.01 (0.26)	0.05, 0.96	-0.06 (0.08)	-0.68, 0.50	0.17 (0.10)	1.76, 0.08
rs2522833: AA	133								
AC/CC	203	-0.90 (0.28)	-3.19, **0.0016**[d]	0.14 (0.23)	0.61, 0.54	-0.16 (0.07)	-2.11, **0.036**	0.18 (0.08)	2.12, **0.035**
rs2715148: AA	95								
CA/CC	241	-0.63 (0.31)	-2.05, **0.042**	0.17 (0.25)	0.69, 0.50	-0.09 (0.08)	-1.17, 0.24	0.15 (0.09)	1.64, 0.10
GRM7									
rs1485171: GG	230								
TG/TT	106	0.26 (0.30)	0.86, 0.39	-0.41 (0.24)	-1.71, 0.09	0.05 (0.08)	0.61, 0.54	-0.03 (0.09)	-0.32, 0.75
rs162209: CC	146								
CT/TT	190	-0.17 (0.28)	-0.61, 0.54	0.02 (0.23)	0.08, 0.94	0.03 (0.07)	0.34, 0.74	0.03 (0.08)	0.33, 0.75
rs9870680: CC	105								
CT/TT	231	-0.73 (0.30)	-2.43, **0.016**	0.43 (0.24)	1.77, 0.077	0.07 (0.08)	0.90, 0.37	0.09 (0.09)	0.99, 0.32

[a]Adjusted for gender and age. [b]All brain measures are given as a percentage of total intracranial brain volume, except for the volume of white matter lesions, which were adjusted for the volume of white matter. [C]One degree of freedom. [d] Bonferroni-adjusted p-value is 0.0576. All other adjusted p-values are >0.10.

Supplementary Table 1. Linear regression models for the multivariate adjusted[a] association between gene variants and brain measures[b] (n=336).

Gene & SNP	**N**	**Grey Matter**		**White Matter**		**Hippocampus**		**White Matter Lesions**	
		β (SE)	$\chi^{2\,(c)}$,p	β (SE)	$\chi^{2\,(c)}$,p	β (SE)	$\chi^{2\,(c)}$,p	β (SE)	$\chi^{2\,(c)}$,p
BICC1									
rs7903712:GG	213								
GC/CC	123	-0.17 (0.29)	-0.57, 0.57	-0.16 (0.24)	-0.67, 0.51	-0.05 (0.08)	-0.66, 0.51	-0.04 (0.09)	-0.46, 0.65
rs9416742: GG	222								
AG/AA	114	-0.24 (0.32)	-0.76, 0.45	-0.19 (0.25)	-0.74, 0.46	-0.03 (0.09)	-0.33, 0.74	-0.006 (0.09)	-0.07, 0.95
rs999845: GG	218								
AG/AA	118	-0.19 (0.30)	-0.63, 0.53	-0.21 (0.24)	-0.90, 0.37	-0.08 (0.08)	-1.08, 0.28	-0.01 (0.09)	-0.14, 0.89
PCLO									
rs2107828: AA	83								
TA/TT	253	-0.63 (0.32)	-1.97, **0.049**	0.03 (0.25)	0.10, 0.92	-0.05 (0.08)	-0.64, 0.53	0.17 (0.09)	1.74, 0.083
rs2522833: AA	133								
AC/CC	203	-0.90 (0.28)	-3.16, **0.0017**[d]	0.13 (0.23)	0.58, 0.57	-0.15 (0.07)	-2.08, **0.039**	0.17 (0.08)	2.06, **0.040**
rs2715148: AA	95								
CA/CC	241	-0.62 (0.31)	-2.00, **0.047**	0.19 (0.25)	0.79, 0.43	-0.09 (0.08)	-1.07, 0.29	0.14 (0.09)	1.58, 0.11
GRM7									
rs1485171: GG	230								
TG/TT	106	0.25 (0.30)	0.81, 0.42	-0.37 (0.24)	-1.54, 0.13	0.07 (0.08)	0.84, 0.40	-0.03 (0.09)	-0.30, 0.76
rs162209: CC	146								
CT/TT	190	-0.19 (0.29)	-0.67, 0.50	0.02 (0.23)	0.07, 0.94	0.06 (0.08)	0.71, 0.48	0.03 (0.08)	0.34, 0.73
rs9870680: CC	105								
CT/TT	231	-0.74 (0.30)	-2.47, **0.014**	0.43 (0.24)	1.78, 0.077	0.03 (0.07)	0.34, 0.74	0.10 (0.09)	1.07, 0.29

[a]Adjusted for gender, age, education, cognitive impairment and number of medications used. [b]All brain measures are given as a percentage of total intracranial brain volume, except for the volume of white matter lesions, which were adjusted for the volume of white matter. [c]One degree of freedom. [d]Bonferroni-adjusted p-value is 0.052. All other adjusted p-values are >0.10.

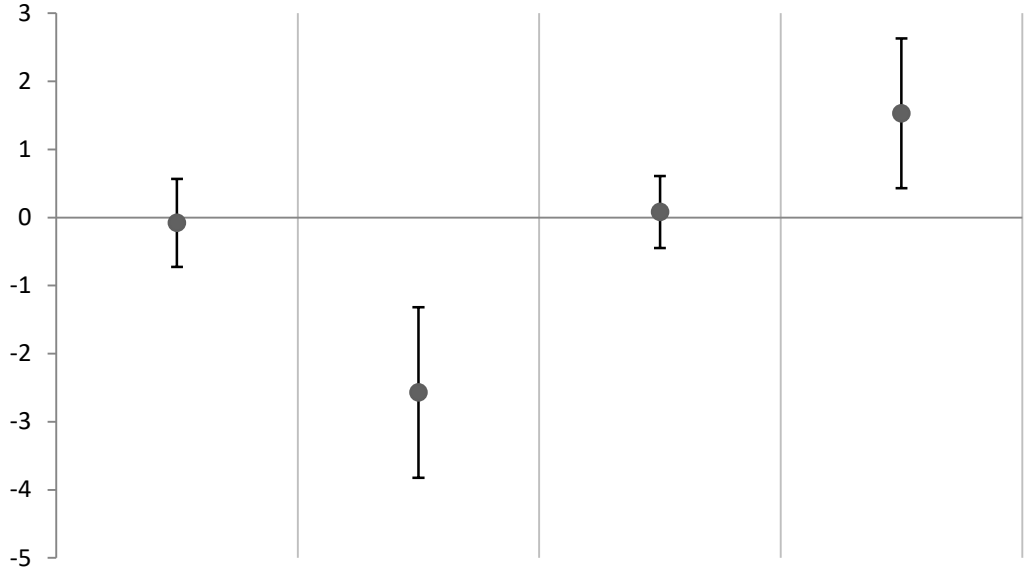

Figure 1.

Graphical representation of the β estimates and standard error from a linear regression model, of the association between *GRM7 rs9870680* and brain volumes (as a ratio of total intracranial volume), adjusted for age and gender, and stratified by depression status (n=260 non-depressed and n=76 depressed).

The circle indicates the least squares mean estimate and the bars are 1.96*SEM (standard error of the mean). The estimate corresponds to the expected change in brain volume (ratio of grey matter or white matter to intracranial volume as indicated) from *rs9870680* CC genotype to CT/TT genotype, for non-depressed (n=81 CC versus 179 CT/TT) and depressed (n=24 with CC genotype versus 52 with CT/TT) participants separately. Exact p-values are indicated.