CACNA1C polymorphism and brain cortical structure in bipolar disorder

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Background: The *CACNA1C* gene encodes the 1C subunit of L-type voltage-gated calcium channels and has been associated with several psychiatric syndromes — including bipolar disorder — in several genome-wide association studies. Experimental and clinical studies have reported changes with respect to behaviour and biomarkers in risk allele carriers, corroborating the essential role of the *CACNA1C* gene in neurons, during development and in the mature brain. However, the association of this gene with regional cortical thickness has not been evaluated in patients with bipolar disorder. **Methods:** Using magnetic resonance imaging, we measured the average cortical thickness of 68 brain regions in 87 patients genotyped for the single-nucleotide polymorphism rs1006737 in *CACNA1C*. **Results:** We found associations with the mean thickness of several cortical areas: the left lateral orbitofrontal and rostral anterior cingulate cortices, as well as other parts of the frontal and parietal cortices. **Limitations:** This cross-sectional cohort study could not fully differentiate correlation from causation. **Conclusion:** The *CACNA1C* polymorphism rs1006737 is associated with the mean thickness of cortical brain areas that have been shown to be altered in bipolar disorder.

Introduction

Bipolar disorder is a serious mental disorder that affects approximately 1% of the population;[1] people with bipolar disorder experience both decreased mood (depression) and increased mood (mania).[2] The etiopathogenesis of bipolar disorder is not completely understood, but twin and family studies estimate a heritability of 60% to 80%,[3,4] prompting major efforts to identify risk alleles. A consistent finding from genome-wide association studies has been an association with commonly occurring single nucleotide polymorphisms (SNPs) in the *CACNA1C* gene.[5–7] Such SNPs in this gene have also been associated with schizophrenia[8,9] and major depression.[9]

The *CACNA1C* gene encodes the 1C subunit of L-type voltage-gated calcium (Ca^{2+}) channels that enable the influx of extracellular Ca^{2+} upon depolarization. The Ca^{2+} channels are expressed in excitable cells, including neurons, where they play an essential role during development and in the mature brain.[10,11]

The *CACNA1C* SNP rs1006737 (adenine instead of guanine) is located in an intronic region, and it affects only the level of expression, not the protein structure itself (either on its own or by some SNP in close proximity). Whether it leads to increased or decreased expression is dependent on cis-regulatory elements such as enhancers.[12] For example, in postmortem samples of patients with schizophrenia, the risk allele was associated with higher *CACNA1C* expression in the dorsolateral prefrontal cortex.[13] In contrast, the risk allele was associated with lower *CACNA1C* expression in the cerebellum of patients with bipolar disorder.[14] In neurons derived from induced pluripotent stem cells of risk allele carriers, *CACNA1C* expression has been reported to be both lower[12] and higher[15] than in cells with wild genotypes.

To shed light on the mechanisms by which genetic variation confers vulnerability to psychiatric disorders, allelic variation needs to be studied in relation to phenotypes and endophenotypes. Studies using MRI have described altered brain structure in bipolar disorder,[16–18] but little is known about whether allelic variation in the *CACNA1C* gene is associated with such changes. In healthy participants, the rs1006737 risk allele has been associated with variations in cerebral grey matter volume,[19] amygdala volume[20] and limbic activity.[21] One study demonstrated increased grey matter volume and reduced functional connectivity in the corticolimbic frontotemporal neural system in healthy carriers of *CACNA1C* risk alleles.[22]

The aim of this study was to investigate whether the risk variant of *CACNA1C* SNP rs1006737 was associated with regional brain cortical thickness, determined from MRI scans of patients with bipolar disorder.

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Methods

Participants

Our sample included 87 patients with bipolar disorder who underwent both genotyping and MRI. We did not compare these with controls, because we were primarily interested in disease-specific differences and we had only 14 genotyped controls with MRI scans. Patients were recruited from the St. Göran Bipolar Project, which enrols patients from the bipolar unit at the Northern Stockholm Psychiatric Clinic in Stockholm, Sweden.

We used the Swedish version of the Affective Disorder Evaluation for clinical assessment. Bipolar diagnoses were made according to DSM-IV criteria as per the Structured Clinical Interview for DSM-IV (SCID)[23] and categorized as either type 1, type 2 or not otherwise specified. To screen for other psychiatric diagnoses, we tested participants using the Mini International Neuropsychiatric Interview. Details on exclusion and inclusion criteria, diagnostic tools and methods can be found elsewhere.[24] The study was approved by the Regional Ethics Committee in Stockholm and conducted in accordance with the Helsinki Protocol. After a complete description of the study, all enrolled patients consented to participate in the study, both orally and in writing.

Magnetic resonance imaging

All patients underwent MRI brain scans[18] at the MR Research Center, Karolinska University Hospital, Stockholm, Sweden. We acquired coronal 3-dimensional T_1-weighted images using a spoiled gradient echo recall sequence (3D-SPGR) and the following parameters: repetition time 21.0 ms, echo time 6 ms, field of view 18 cm, flip angle 30°, acquisition matrix $256 \times 256 \times 128$, and voxel size $0.7 \times 0.7 \times 1.8$ mm^3. We used a 1.5 T Sigma Excite MRI medical scanner (General Electric) equipped with an 8-channel head coil. We acquired additional axial fluid attenuation inversion recovery T_2-weighted scans for examination by senior radiologists to ensure that the investigated sample was free of clinically significant anatomic abnormalities and neuropathology.

Image processing

We obtained measures of cortical thickness using the semi-automated segmentation and cortical surface reconstruction methods provided by FreeSurfer (version 5.1; https://surfer.nmr.mgh.harvard.edu), as described elsewhere.[25–30] In brief, the procedure includes intensity normalization; removal of non-brain tissue; segmentation of cortical grey, subcortical white and deep grey matter volumetric structures; and triangular tessellation of the grey matter–white matter interface and the grey matter–cerebrospinal fluid boundary (pial surface). Cortical thickness was defined as the closest distance from the pial surface to the boundary of the grey matter–white matter surface. All surface reconstructions were visually inspected and, where necessary, corrected manually using editing tools provided by FreeSurfer, including correc-

tions of erroneous skull stripping and white matter and grey matter segmentations. FreeSurfer also allows for automated parcellation of the cortical surfaces into 34 anatomic regions of interest as defined by the Desikan atlas.[28,31] We measured 68 regions in total. For each region of interest, we obtained average thickness measures from participants' native space. We performed analyses using these regional average thickness measures.

Genotyping

We determined genotypes for the SNP rs1006737 using Kompetitive allele specific polymerase chain reaction (PCR) technology (LGC Genomics). We used standard PCR cycling conditions according to the manufacturer's instructions. The samples were analyzed on a 7900HT Fast Real-Time PCR System (Applied Biosystems). All cluster plots were manually inspected, and ambiguous results were excluded.

Statistical analysis

Most analyses were performed using custom-made scripts in MATLAB (R2017b; MathWorks). We conducted hypothesis testing using the unpaired Student t test for numerical data and Fisher exact test or the χ^2 test for dichotomous data. We performed χ^2 tests using SPSS (version 25; IBM). We presented numeric values as means and standard deviations. For each cortical region, we compared the value for those who had the risk allele with the value for those who did not. We addressed the problem of multiple comparisons by controlling the false discovery rate (FDR) using a threshold FDR value of 0.10. We used Bonferroni correction to control for multiple hypotheses testing for clinical characteristics. We constructed confidence intervals adjusted for multiple comparisons using the p value (0.0017) corresponding to an FDR of 0.1 as base. Thus, as in Bonferroni correction, 99.83% confidence intervals were constructed.

Results

Participant characteristics

Participant characteristics are shown in Table 1. We genotyped the *CACNA1C* SNP rs1006737, and 56 of 87 (64%) patients were carrying 1 or 2 risk alleles (GA or AA), compared to 30% in the 1000 Genomes project.[32] The frequency for the A allele was 0.42, and the genotypes were distributed according to Hardy–Weinberg equilibrium ($\chi^2 = 0.30$; $p = 0.86$). We found no significant difference in clinical characteristics (including subtype, comorbidity or treatment) between patients with risk alleles and those without.

Associations with brain cortical thickness

Table 2 shows the comparisons of average regional cortical thickness between people with or without risk alleles after correcting for multiple testing. Significant associations are shown in Figure 1. Patients who had the rs1006737 risk allele showed

Table 1: Characteristics of patients with the risk allele or wild type for rs1006737

Characteristic	Risk allele	Wild type	p value*
Patients, *n*	56	31	—
Age, yr, mean ± SD	41.1 ± 14	37.6 ± 12.8	0.25‡
Females, %	57.1	54.8	1§
Illicit drugs, %	12.5	19.4	0.53§
Postsecondary education, %	55.4	38.7	0.18§
Bipolar disorder, %			0.43¶
Type 1	51.8	54.8	
Type 2	33.9	22.6	
NOS	14.3	22.6	
Personality disorder, %	5.4	0	0.55§
MADRS score, mean ± SD	4.2 ± 6.2	3.6 ± 4.5	0.67‡
YMRS score, mean ± SD	1.5 ± 2.5	1.7 ± 3.2	0.81‡
Duration of illness, y, mean ± SD	22.3 ± 14	18.8 ± 11.1	0.23‡
Suicide attempt, %	44.6	38.7	0.65§
Pharmacotherapy, %†			
Mood stabilizers	83.4	80.7	0.77§
Lithium	48.2	67.7	0.11§
Antipsychotics	26.8	12.9	0.18§
Antidepressants	39.2	32.3	0.64§
Anxiolytics	19.6	0	0.007§

MADRAS = Montgomery–Åsberg Depression Rating Scale; NOS = not otherwise specified; SD = standard deviation; YMRS = Young Mania Rating Scale.
*Bonferroni correction with *n* = 15 yields $\alpha_{Bonferroni}$ = 0.05/15 = 0.0033.
†Values for pharmacotherapy mean ongoing treatment.
‡Unpaired Student *t* test.
§Fisher exact test.
¶χ^2 test.

Table 2: Significant differences in brain region thickness (mm) dependent on genotype

Region	Confidence interval*		p value†	FDR‡	Fold change
	Risk allele	Wild type			
Left paracentral	2.30–2.44	2.42–2.60	< 0.001	0.019	1.0021
Left caudal middle frontal	2.49–2.64	2.59–2.73	0.011	0.072	0.98
Left inferior parietal	2.45–2.59	2.55–2.68	0.005	0.072	0.98
Left superior frontal	2.79–2.94	2.90–3.03	0.005	0.072	0.99
Left superior parietal	2.13–2.25	2.21–2.32	0.011	0.072	0.98
Left supramarginal	2.49–2.61	2.55–2.73	0.010	0.072	1.0006
Right inferior parietal	2.50–2.62	2.59–2.72	0.002	0.072	0.98
Right inferior temporal	2.90–3.03	2.97–3.13	0.011	0.072	0.998
Right paracentral	2.38–2.49	2.43–2.58	0.010	0.072	0.995
Right superior frontal	2.79–2.94	2.89–3.03	0.010	0.072	0.99
Right superior parietal	2.12–2.24	2.21–2.32	0.005	0.072	0.98
Left lateral orbitofrontal cortex	2.66–2.79	2.73–2.89	0.017	0.074	0.99
Left rostral anterior cingulate cortex	2.89–3.08	2.97–3.25	0.015	0.074	0.98
Right precentral	2.37–2.51	2.46–2.59	0.015	0.074	0.97
Right precuneus	2.33–2.45	2.41–2.51	0.016	0.074	0.98
Right thickness	2.51–2.61	2.58–2.67	0.013	0.074	0.99
Left thickness	2.49–2.59	2.55–2.65	0.019	0.08	0.99

FDR = false discovery rate.
*Adjusted for multiple comparisons.
†Unpaired Student *t* test.
‡FDR 0.1 corresponds to *p* = 0.0017, and a 99.83% confidence interval.

bilateral lower average thickness of the inferior parietal, superior frontal and superior parietal cortices than patients without the risk allele. As well, the complete cortices were bilaterally thinner. The left lateral orbitofrontal and rostral anterior cingulate cortices, as well as the right precentral and precuneus cortices, were also thinner in patients with the risk allele. For the
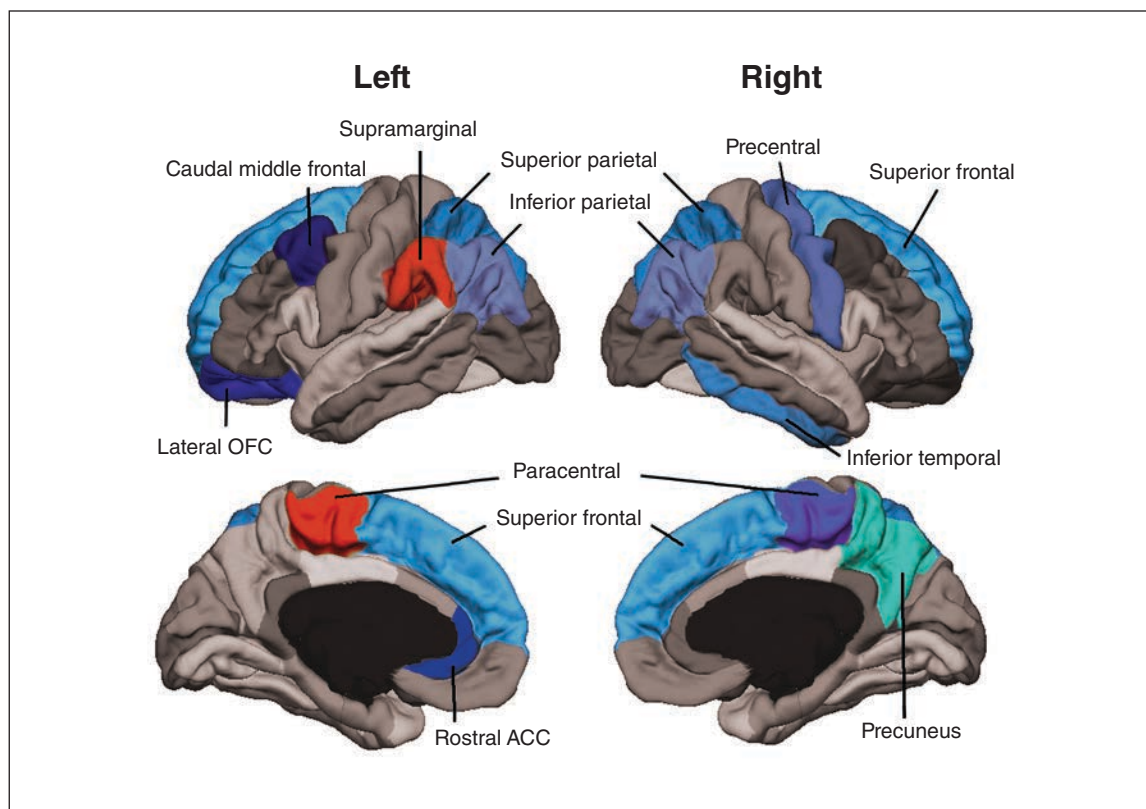
Fig. 1: Significant differences in cortical brain regions for patients marked in colours. Warm colours indicate that the mean cortical thickness was higher in patients with the risk allele, and cold colours indicate that the mean cortical thickness was lower. ACC = anterior cingulate cortex; OFC = orbitofrontal cortex.

paracentral cortical region, the cortex was thicker on the left side and thinner on the right side in patients with the risk allele. See Appendix 1, available at jpn.ca/190029-a1, for complete results.

Discussion

We investigated for the first time, to our knowledge, whether a SNP in the *CACNA1C* gene was associated with regional brain cortical thickness as assessed by MRI in patients with bipolar disorder. We reported differences in multiple regions in patients with bipolar disorder who had the risk allele of rs1006737 compared to those who had the wild-type allele. This finding suggests that the cortical architecture of patients with bipolar disorder is related to *CACNA1C* polymorphisms.

The SNP rs1006737 in the *CACNA1C* gene has been associated with bipolar disorder.[5–7] However, genetic variation in this pleiotropic gene has also been associated with schizophrenia[8,9] and depression.[9] To gain insight into the mechanisms by which this genetic variation confers increased risk for psychiatric disorders, genotype–phenotype studies are key. For example, such studies found that risk-associated *CACNA1C* SNPs predicted higher scores on depression and anxiety questionnaires in healthy participants.[33,34] As to effects in bipolar disorder, we found no correlation between *CACNA1C* variation and cognitive test performance.[35] Nonetheless, in another study, we reported that the *CACNA1C*

SNP rs1006737 was associated with decreased hyperphosphorylated tau/total tau ratio in cerebrospinal fluid from patients with bipolar disorder.[36]

Several imaging studies have reported cortical and subcortical abnormalities in the brains of patients with bipolar disorder. The largest structural neuroimaging study to date[16] on cortical thickness in patients with bipolar disorder analyzed 6503 people. Cortical thickness was lower in the frontal, temporal and parietal regions of both brain hemispheres. In a systematic review,[37] the main differences between patients and controls was a decrease in thickness in the left anterior cingulate, left paracingulate, left superior temporal gyrus and prefrontal regions bilaterally. Although a previous study reported larger total grey matter volume in healthy carriers of *CACNA1C* risk alleles,[19] there have been no studies in patients with bipolar disorder.

By studying the relation between the *CACNA1C* SNP rs1006737 and cortical thickness in 87 patients with bipolar disorder, we found that carriers of the risk allele had bilateral lower cortical thickness in the inferior parietal, superior frontal and superior parietal cortices compared to those with the wild-type allele. For the paracentral cortical region, the cortex was thicker on the left side and thinner on the right side in patients with the risk allele than those without it. The left lateral orbitofrontal and rostral anterior cingulate cortices, as well as right precentral and precuneus cortices, were also thinner.

Although these findings suggest that the cortical architecture of patients with bipolar disorder may be related to *CACNA1C* polymorphisms, and that cortical abnormalities might be more pronounced in those who carry the risk allele, the mechanism by which *CACNA1C* genetic variants lead to changes in cortical thickness of different brain regions remains to be unravelled. One hypothesis is that risk variants of *CACNA1C* lead to changes in Ca^{2+} signalling during development and in adulthood, and therefore to altered proliferation and survival. Interestingly, Ca^{2+} signalling is known to regulate the proliferation of neural progenitors in mice.[38]

Limitations

This cross-sectional cohort study could not differentiate between correlation and causation, so it is not certain that risk alleles of *CACNA1C* actually cause changes in regional cortical thickness in patients with bipolar disorder. However, we found no significant differences between the 2 groups in terms of clinical characteristics, including pharmacological treatment with neurotropic drugs such as lithium.[39] As well, it has been suggested that education prevents age-related cortical thinning in cognitively normal people.[40] In our cohort, we found no difference in the percentage of people with at least a postsecondary education. It is not clear what clinical implications these differences in regional cortical thickness may have, because we found no significant differences with respect to clinical characteristics between the 2 groups. However, we did observe a trend for carriers of the risk allele to have less treatment with lithium and more with anxiolytics, antidepressants and antipsychotics in conjunction with more suicide attempts. Finally, we corrected our results for multiple comparisons using FDR and set the cutoff at 0.1, so 10% of the significant results listed in Table 2 might be false positives.

Conclusion

The rs1006737 risk allele of *CACNA1C* is associated with average thickness of several cortical areas in patients with bipolar disorder. This finding sheds light on possible links between genetic variants and pathophysiological mechanisms in bipolar disorder and opens up new lines of research.

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References

1. Kessler RC, Berglund P, Demler O, et al. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry* 2005;62:593-602.
2. Belmaker RH. Bipolar disorder. *N Engl J Med* 2004;351:476-86.
3. Lichtenstein P, Yip BH, Björk C, et al. Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. *Lancet* 2009;373:234-9.
4. Kieseppa T, Partonen T, Haukka J et al. High concordance of bipolar I disorder in a nationwide sample of twins. *Am J Psychiatry* 2004;161:1814-21.
5. Ferreira MA, O'Donovan MC, Meng YA, et al. Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. *Nat Genet* 2008;40:1056-8.
6. Cross-Disorder Group of the Psychiatric Genomics. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. *Lancet* 2013;381:1371-9.
7. Psychiatric GCBDWG. Large-scale genome-wide association analysis of bipolar disorder identifies a new susceptibility locus near ODZ4. *Nat Genet* 2011;43:977-83.
8. Nyegaard M, Demontis D, Foldager L, et al. *CACNA1C* (rs1006737) is associated with schizophrenia. *Mol Psychiatry* 2010;15:119-21.
9. Green EK, Grozeva D, Jones I, et al. The bipolar disorder risk allele at *CACNA1C* also confers risk of recurrent major depression and of schizophrenia. *Mol Psychiatry* 2010;15:1016-22.
10. Catterall WA. Structure and regulation of voltage-gated Ca2+ channels. *Annu Rev Cell Dev Biol* 2000;16:521-55.
11. Dolphin AC. Voltage-gated calcium channels and their auxiliary subunits: physiology and pathophysiology and pharmacology. *J Physiol* 2016;594:5369-90.
12. Roussos P, Mitchell AC, Voloudakis G, et al. A role for noncoding variation in schizophrenia. *Cell Reports* 2014;9:1417-29.
13. Bigos KL, Mattay VS, Callicott JH, et al. Genetic variation in *CACNA1C* affects brain circuitries related to mental illness. *Arch Gen Psychiatry* 2010;67:939-45.
14. Gershon ES, Grennan K, Busnello J, et al. A rare mutation of *CACNA1C* in a patient with bipolar disorder, and decreased gene expression associated with a bipolar-associated common SNP of *CACNA1C* in brain. *Mol Psychiatry* 2014;19:890-4.
15. Yoshimizu T, Pan JQ, Mungenast AE, et al. Functional implications of a psychiatric risk variant within *CACNA1C* in induced human neurons. *Mol Psychiatry* 2015;20:162-9.
16. Hibar DP, Westlye LT, Doan NT, et al. Cortical abnormalities in bipolar disorder: an MRI analysis of 6503 individuals from the ENIGMA Bipolar Disorder Working Group. *Mol Psychiatry* 2018; 23:932-42.
17. Hibar DP, Westlye LT, van Erp TG, et al. Subcortical volumetric abnormalities in bipolar disorder. *Mol Psychiatry* 2016;21:1710-6.
18. Abe C, Ekman CJ, Sellgren C, et al. Cortical thickness, volume and surface area in patients with bipolar disorder types I and II. *J Psychiatry Neurosci* 2016;41:240-50.
19. Kempton MJ, Ruberto G, Vassos E, et al. Effects of the *CACNA1C* risk allele for bipolar disorder on cerebral gray matter volume in healthy individuals. *Am J Psychiatry* 2009;166:1413-4.
20. Lancaster TM, Foley S, Tansey KE, et al. *CACNA1C* risk variant is associated with increased amygdala volume. *Eur Arch Psychiatry Clin Neurosci* 2016;266:269-75.

21. Wessa M, Linke J, Witt SH, et al. The *CACNA1C* risk variant for bipolar disorder influences limbic activity. *Mol Psychiatry* 2010; 15:1126-7.

22. Wang F, McIntosh AM, He Y, et al. The association of genetic variation in *CACNA1C* with structure and function of a frontotemporal system. *Bipolar Disord* 2011;13:696-700.

23. Kübler U. Structured Clinical Interview for DSM-IV (SCID). In: Gellman MD, Turner JR, editors. *Encyclopedia of behavioral medicine.* New York: Springer; 2013: 1919-20**.**

24. Rolstad S, Jakobsson J, Sellgren C, et al. Cognitive performance and cerebrospinal fluid biomarkers of neurodegeneration: a study of patients with bipolar disorder and healthy controls. *PLoS One* 2015;10:e0127100.

25. Dale AM, Fischl B, Sereno MI. Cortical surface-based analysis. I. Segmentation and surface reconstruction. *Neuroimage* 1999;9: 179-94.

26. Fischl B, Sereno MI, Dale AM. Cortical surface-based analysis. II. Inflation, flattening, and a surface-based coordinate system. *Neuroimage* 1999;9:195-207.

27. Fischl B, Dale AM. Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc Natl Acad Sci U S A* 2000;97:11050-5.

28. Fischl B, van der Kouwe A, Destrieux C, et al. Automatically parcellating the human cerebral cortex. *Cereb Cortex* 2004;14:11-22.

29. Reuter M, Schmansky NJ, Rosas HD, et al. Within-subject template estimation for unbiased longitudinal image analysis. *Neuroimage* 2012;61:1402-18.

30. Fischl B, Salat DH, van der Kouwe AJ, et al. Sequence-independent segmentation of magnetic resonance images. *Neuroimage* 2004; 23(Suppl 1):S69-84.

31. Desikan RS, Ségonne F, Fischl B, et al. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage* 2006;31:968-80.

32. Genomes Project, C., Auton A, Brooks LD, et al. A global reference for human genetic variation. *Nature* 2015;526:68-74.

33. Erk S, Meyer-Lindenberg A, Schnell K, et al. Brain function in carriers of a genome-wide supported bipolar disorder variant. *Arch Gen Psychiatry* 2010;67:803-11.

34. Roussos P, Giakoumaki SG, Georgakopoulos A et al. The *CACNA1C* and *ANK3* risk alleles impact on affective personality traits and startle reactivity but not on cognition or gating in healthy males. *Bipolar Disord* 2011;13:250-9.

35. Rolstad S, Sellgren Majkowitz C, Joas E, et al. Polymorphisms of *BDNF* and *CACNA1C* are not associated with cognitive functioning in bipolar disorder or healthy controls. *Cogn Neuropsychiatry* 2016;21:271-8.

36. Jakobsson J, Pålsson E, Sellgren C, et al. *CACNA1C* polymorphism and altered phosphorylation of tau in bipolar disorder. *Br J Psychiatry* 2016;208:195-6.

37. Hanford LC, Nazarov A, Hall GB, et al. Cortical thickness in bipolar disorder: a systematic review. *Bipolar Disord* 2016;18:4-18.

38. Malmersjö S1, Rebellato P, Smedler E, et al. Neural progenitors organize in small-world networks to promote cell proliferation. *Proc Natl Acad Sci U S A* 2013;110:E1524-32.

39. Giakoumatos CI, Nanda P, Mathew IT, et al. Effects of lithium on cortical thickness and hippocampal subfield volumes in psychotic bipolar disorder. *J Psychiatr Res* 2015;61:180-7.

40. Kim JP, Seo SW, Shin HY et al. Effects of education on aging-related cortical thinning among cognitively normal individuals. *Neurology* 2015;85:806-12.