The role of the potassium channel gene KCNK2 in major depressive disorder

The role of the potassium channel gene KCNK2 in major depressive disorder.

Congiu C, Minelli A, Bonvicini C, Bortolomasi M, Sartori R, Maj C, Scassellati C, Maina G, Trabucchi L, Segala M, Gennarelli M.

Abstract

Six single nucleotide polymorphisms (SNPs) of the KCNK2 gene were investigated for their association with major depressive disorder(MDD) and treatment efficacy in 590 MDD patients and 441 controls. The A homozygotes of rs10779646 were significantly more frequent in patients than controls whereas G allele of rs7549184 was associated with the presence of psychotic symptoms and the severity of disease. Evaluating the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) dataset, we confirmed our findings.

1. Introduction

Ion channels are important mediators of intrinsic neuronal excitability, widely distributed in the central nervous system. Their relevant importance as modulators of action potential duration modulators and neurotransmitter release regulators make them interesting to study due to their putative role in susceptibility to psychiatric illness and relative pharmacological treatments (Imbrici et al., 2013).

Several studies have identified associations between voltage gated potassium channelsand bipolar disorder and schizophrenia (Borsotto *et al*., 2007; Heide *et al*., 2012; Judy and Zandi, 2013 ; Smolin *et al*., 2012) whereas few data are available in association with major depressive disorder (MDD). Studies have focused in particular on the two-pore domain potassium channel, and noteworthy Heurteaux et al. (2006) showed that *KCNK2*$^{-/-}$ knockout mice have an antidepressant-like phenotype. In their study, KCNK2-deficient mice showed an increased efficacy in serotonin neurotransmission, resistance to depression, and a substantially reduced level of corticosterone under stress conditions.

In humans, genetic variations in the KCNK2 (also called TREK-1) gene have been linked to susceptibility to depression as well as to antidepressant response (Liou et al., 2009). The Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study reported an association between genetic variants in KCNK2 locus and differential responses to next-step treatment in individuals who did not remit with citalopram treatment (Perlis et al., 2008). These results suggested that certain KCNK2 genotypes could be involved in resistance to antidepressants, in line with the hypothesis raised in the Heurteaux animal model (Heurteaux et al., 2006).

However, these literature findings have not been confirmed by the integrated analyses of the most recent GWASs (Genome-Wide Association Studies) both for MDD vulnerability (Ripke et al., 2013) and for antidepressant efficacy (Uher et al., 2013).

In order to better understand the role of KCNK2 in MDD and antidepressant response, we carried out: 1) an association study in a representative cohort with a well-delineated phenotype characterization evaluating six SNPs previously resulted associated with response in STAR*D cohort (Perlis et al., 2008); 2) a single gene reanalysis on available case-control and pharmacogenetic GWAS datasets.

2. Materials and methods

2.1. Sample

Five hundred ninty DSM-IV MDD patients (of whom 372 treatment resistant depression(TRD) and 218 no-TRD) with at least moderately severe depression were voluntarily enrolled along with 441

unrelated healthy volunteers. Inclusion criteria and all sample characteristics are shown in Supplementary Data A file.

2.2. Genotyping and statistical analyses

The KCNK2 polymorphisms rs2841608, rs2841616, rs12136349, rs10779646, rs7549184 and rs10494996 were genotyped using the BeadXpress System and the VeraCode Assay according to the manufacturer′s protocols (www.illumina.com).
Chi-square (χ^2) tests and logistic regression analyses were conducted to evaluate the association between groups and categorical variables.
All data about genotyping method and statistical analyses performed are reported in Supplementary Data B file.

2.3. GWAS datasets replication studies

We analyzed KCNK2 data on two different GWAS datasets (Ripke *et al*., 2013 ; Uher *et al*., 2013) through *ricopili* interrogation tool from the Broad Institute.
Moreover, we had access to clinical and genetic data of the dataset 2 of STAR*D study. All details about the dataset evaluated and the analyses performed are shown in Supplementary Data B file.

3. Results

3.1. Our sample results

We first carried out a multiple comparison permutation test including all KCNK2genotyped SNPs and the principal clinical features such as pathology, response to treatment, symptoms.
The results of association analyses showed a significant difference in allelic distribution between patients and controls for the rs10779646 (p=0.01) and a trend of association (p=0.06) between rs7549184 and the severity of depression. All further analyses were performed only on these two SNPs.
Analysis results on rs10779646 (see Table 1A) demonstrated differences in genotype frequencies in case-control comparison (p=0.002). In particular, the AA homozygotes were more frequent in patients than controls (F=9.10, p=0.003, OR=1.42, 95% CI:1.10–1.85).
Regarding the rs7549184, significant effects were found for the presence of psychotic symptoms and the severity of depression (see Table 1B and C). Assuming a recessive model, homozygous GG patients presented an OR=2.37 (95% CI:1.22–4.63; p=0.009) of developing psychotic symptomatology during a depression episode compared to the A allele carriers (Table 1B).
Furthermore, this SNP was associated with the severity of the depressive episode. In particular, G allele carriers showed an increased risk of developing a more severe MDD symptomatology than AA (p=0.03; OR=1.68; 95% CI=1.14–2.48; Table 1C).
We performed LD analysis on all the five SNPs genotyped in the case-control population. Between the SNPs rs10779646 and rs7549184 no LD block was present and the haplotype analysis by sliding windows approach did not detect any association in haplotype frequencies.

3.2. GWAS datasets replication results

Considering both case-control and pharmacogenetic meta-analyses, no SNPs reached significant *p*-Value after Bonferroni correction for the number of the SNPs considered in KCNK2 region. The rs10779646 and the rs7549184 have not been analyzed in both GWAS. Moreover the two SNPs are not in LD with any polymorphisms in the datasets, thus making impossible an association analysis through a genotype imputation.
Evaluating only the STAR*D dataset, the comparison between no-TRD and TRD subjects showed 12 significant polymorphisms associated with treatment resistance (Supplementary Data C file).

Moreover, two SNPs resulted associated with the severity of the depressive episode that resulted a negative predictor of response (p<0.001) confirming the wide literature.

4. Discussion

Our study showed that KCNK2 variants were significantly associated with MDDsusceptibility and negative predictor of response to treatment as severity of disease and the presence of psychotic symptoms. Moreover, analyses on STAR*D data supported our results associating KCNK2 SNPs both with treatment response and severity of MDD.

We observed that one intronic SNP (rs10779646) of the KCNK2 gene was associated with the risk of developing MDD since AA homozygotes were more frequent in patients. These data are in line with the literature regarding the involvement of KCNK2 and its genetic variations with the pathophysiology of the disease (Heurteaux *et al*., 2006 ; Liou *et al*., 2009) supported by its biological role as a modulator of the excitability of neurons by affecting neural firing and action potential generation and duration and by regulating neurotransmitter release.

In our sample, we found also a significant association of rs7549184 both with the presence of psychotic symptoms and the severity of the depressive episode. G allele carriers correlated with a more severe pathology and GG homozygotes showed twice the risk of developing psychotic symptoms during a depression episode compared to the A allele carriers. This is of particular importance since the presence of psychotic symptoms is one of the strongest negative predictor factors of resistance to treatment in MDD (Perlis *et al*., 2013 ; Schlaepfer *et al*., 2012). To support our finding, several studies showed a significant association of KCNK2 with inter-individual variations in treatment response in MDD both on data from animal models and humans (Heurteaux *et al*., 2006; Liou *et al*., 2009 ; Perlis *et al*., 2008).

Preclinical studies on animal models have proved that KCNK2 is a downstream target of SSRIs (Heurteaux et al., 2006). In Heurteaux et al.′s study, KCNK2-deficient mice behavior was nearly exactly the same as wild-type mice that were treated with fluoxetineor paroxetine. In addition, knockout mice were insensitive to fluoxetine or paroxetine.

In humans, Perlis and collaborators found an association between KCNK2 genetic variants and the differential response to next-step treatment among individuals who did not remit with citalopram (Perlis et al., 2008). Furthermore, one study showed that KCNK2 may be involved in depression resistant phenotype (Dillon et al., 2010).

Nonetheless, performing a single-gene study in the most recent GWAS meta-analyses (Ripke *et al*., 2013 ; Uher *et al*., 2013), we did not find KCNK2 as a candidate gene both for MDD susceptibility and response. Nevertheless, the two SNPs resulted implicated in our study in susceptibility to MDD and resistance to antidepressant mechanisms have not been included in both GWASs.

This heterogeneity of data could be the result of the contrasts between strength and limitations of a candidate gene approach and GWAS technique (Fabbri et al., 2013). In particular, the need to obtain large sample sizes entails as consequence a high heterogeneity in clinical and socio-demographic features that negatively affect the increase in power obtained in these studies.

Since the literature has reported that a substantial proportion of responses to an initial antidepressant trial may be placebo-like or nonspecific (Perlis *et al*., 2008 ; Walsh *et al*., 2002), we excluded Level 1 responders in no-TRD group of STAR*D dataset. Comparing them with TRD ones, it was confirmed the involvement of KCNK2 in treatment resistance showing 12 SNPs associated with the outcome. Moreover, two SNPs were correlated with the severity of the MDD episode confirming our results regarding a role of KCNK2 in more severe form of depression.

Unfortunately, since the presence of psychotic symptom was an exclusion criterion for the enrollment of STAR*D project, it has not been possible to perform any replication analysis.

Considering the complexity of the resistance/response phenotype, the literature suggests that pharmacogenetic study designs should take into account the manifold biological, clinical, socio-demographical and environmental variables that impact on treatment response. This would enable putative associations to be identified with clinical and environmental factors that influence the

outcome in MDD. This would then suggests that some genetic variants may be indirectly implicated in the response through an involvement in negative or positive predictors of response.

The present study replicates and extends the findings about the involvement of KCNK2 gene in the aetiopathogenesis of MDD and in treatment resistance mechanisms. Variations in potassium-channel genes seem to have pleiotropic effects on a broader psychopathology and consequently they play important biological role in psychiatric disorders.

Contributors

CC conceived of the study, participated in its design and its coordination, carried out all genetic analyses, performed the statistical analyses, and co-wrote the manuscript; AM participated in study design and its coordination, screened patients and controls, performed the statistical analyses, and co-wrote the manuscript; CB and CS participated in the design of the study and carried out all genetic analyses; RS supervised the statistical analyses; CM performed the GWAS dataset analyses; MB, GM, LT, MS enrolled and screened patients; MG conceived of the study, participated in its design and coordination, and helped draft the manuscript and critically reviewed it for intellectual content. All the authors read and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This research was supported by grants from the Italian Ministry of Health (RC and RF2007 Conv. 42) and Regione Lombardia (ID: 17387 SAL-13). We thank Federica Centin and Barbara Bertasi for laboratory support and Elisabetta Tessari, Maria Abate and Giulio Gainelli for the recruitment of the patients. Furthermore, the authors would like to express sincere gratitude to all volunteers that participated in the study.
Acknowledgment for Depression Sample Biomaterials and Clinical Data from STAR*D study: Study 18-Data and biomaterials were obtained from the limited access datasets distributed from the NIH-supported “Sequenced Treatment Alternatives to Relieve Depression” (STAR*D). STAR*D focused on non-psychotic major depressive disorder in adults seen in outpatient settings. The primary purpose of this research study was to determine which treatments work best if the first treatment with medication does not produce an acceptable response. The study was supported by NIMH Contract # N01MH90003 to the University of Texas Southwestern Medical Center. The ClinicalTrials.gov identifier is NCT00021528.

References

1. Borsotto et al., 2007
M. Borsotto, L. Cavarec, M. Bouillot, G. Romey, F. Macciardi, A. Delaye, M. Nasroune, M. Bastucci, J.L. Sambucy, J.J. Luan, A. Charpagne, V. Jouet, R. Leger, M. Lazdunski, D. Cohen, I. Chumakov **PP2A-Bgamma subunit and KCNQ2 K+ channels in bipolar disorder** The Pharmacogenomics Journal, 7 (2007), pp. 123–132
2. Dillon et al., 2010
D.G. Dillon, R. Bogdan, J. Fagerness, A.J. Holmes, R.H. Perlis, D.A. Pizzagalli **Variation in TREK1 gene linked to depression-resistant phenotype is associated with potentiated neural responses to rewards in human.** Human Brain Mapping, 31 (2010), pp. 210–221
3. Fabbri et al., 2013
C. Fabbri, G. Di Girolamo, A. Serretti **Pharmacogenetics of antidepressant drugs: an update after almost 20 years of research** American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 162B (2013), pp. 487–520
4. Heide et al., 2012
J. Heide, S.A. Mann, J.I. Vandenberg **The schizophrenia-associated Kv11.1-3.1 isoform results in reduced current accumulation during repetitive brief depolarizations** PLoS One, 7 (2012), p. e45624
5. Heurteaux et al., 2006

C. Heurteaux, G. Lucas, N. Guy, M. El Yacoubi, S. Thummler, X.D. Peng, F. Noble, N. Blondeau, C. Widmann, M. Borsotto, G. Gobbi, J.M. Vaugeois, G. Debonnel, M. Lazdunski **Deletion of the background potassium channel TREK-1 results in a depression-resistant phenotype** Nature Neuroscience, 9 (2006), pp. 1134–1141

6. Imbrici et al., 2013
P. Imbrici, D.C. Camerino, D. Tricarico. **Major channels involved in neuropsychiatric disorders and therapeutic perspectives**. Frontiers in Genetics, 4 (2013), p. 76

7. Judy and Zandi, 2013
J.T. Judy, P.P. Zandi. **A review of potassium channels in bipolar disorder**. Frontiers in Genetics, 4 (2013), p. 105

8. Liou et al., 2009
Y.J. Liou, T.J. Chen, S.J. Tsai, Y.W. Yu, C.Y. Cheng, C.J. Hong. **Support for the involvement of the KCNK2 gene in major depressive disorder and response to antidepressant treatment**. Pharmacogenetics and Genomics, 19 (2009), pp. 735–741

9. Perlis et al., 2013
R.H. Perlis, B. Fijal, S. Dharia, J.P. Houston. **Pharmacogenetic investigation of response to duloxetine treatment in generalized anxiety disorder**. The Pharmacogenomics Journal, 13 (2013), pp. 280–285

10. Perlis et al., 2008
R.H. Perlis, P. Moorjani, J. Fagerness, S. Purcell, M.H. Trivedi, M. Fava, A.J. Rush, J.W. Smoller. **Pharmacogenetic analysis of genes implicated in rodent models of antidepressant response: association of TREK1 and treatment resistance in the STAR*D study.** Neuropsychopharmacology, 33 (2008), pp. 2810–2819

11. Ripke et al., 2013
S. Ripke, N.R. Wray, C.M. Lewis, S.P. Hamilton, M.M. Weissman, G. Breen, E.M. Byrne, D.H. Blackwood, D.I. Boomsma, S. Cichon, A.C. Heath, F. Holsboer, S. Lucae, P.A. Madden, N.G. Martin, P. McGuffin, P. Muglia, M.M. Noethen, B.P. Penninx, M.L. Pergadia, J.B. Potash, M. Rietschel, D. Lin, B. Muller-Myhsok, J. Shi, S. Steinberg, H.J. Grabe, P. Lichtenstein, P. Magnusson, R.H. Perlis, M. Preisig, J.W. Smoller, K. Stefansson, R. Uher, Z. Kutalik, K.E. Tansey, A. Teumer, A. Viktorin, M.R. Barnes, T. Bettecken, E.B. Binder, R. Breuer, V.M. Castro, S.E. Churchill, W.H. Coryell, N. Craddock, I.W. Craig, D. Czamara, E.J. De Geus, F. Degenhardt, A.E. Farmer, M. Fava, J. Frank, V.S. Gainer, P.J. Gallagher, S.D. Gordon, S. Goryachev, M. Gross, M. Guipponi, A.K. Henders, S. Herms, I.B. Hickie, S. Hoefels, W. Hoogendijk, J.J. Hottenga, D.V. Iosifescu, M. Ising, I. Jones, L. Jones, T. Jung-Ying, J.A. Knowles, I.S. Kohane, M.A. Kohli, A. Korszun, M. Landen, W.B. Lawson, G. Lewis, D. Macintyre, W. Maier, M. Mattheisen, P.J. McGrath, A. McIntosh, A. McLean, C.M. Middeldorp, L. Middleton, G.M. Montgomery, S.N. Murphy, M. Nauck, W.A. Nolen, D.R. Nyholt, M. O'Donovan, H. Oskarsson, N. Pedersen, W.A. Scheftner, A. Schulz, T.G. Schulze, S.I. Shyn, E. Sigurdsson, S.L. Slager, J.H. Smit, H. Stefansson, M. Steffens, T. Thorgeirsson, F. Tozzi, J. Treutlein, M. Uhr, E.J. van den Oord, G. Van Grootheest, H. Volzke, J.B. Weilburg, G. Willemsen, F.G. Zitman, B. Neale, M. Daly, D.F. Levinson, P.F. Sullivan. **A mega-analysis of genome-wide association studies for major depressive disorder**. Molecular Psychiatry, 18 (2013), pp. 497–511

12. Schlaepfer et al., 2012
T.E. Schlaepfer, H. Agren, P. Monteleone, C. Gasto, W. Pitchot, F. Rouillon, D.J. Nutt, S. Kasper. **The hidden third: improving outcome in treatment-resistant depression**. Journal of Psychopharmacology, 26 (2012), pp. 587–602

13. Smolin et al., 2012
B. Smolin, R. Karry, S. Gal-Ben-Ari, D. Ben-Shachar. **Differential expression of genes encoding neuronal ion-channel subunits in major depression, bipolar disorder and schizophrenia: implications for pathophysiology**. The International Journal of Neuropsychopharmacology, 15 (2012), pp. 869–882

14. Uher et al., 2013
R. Uher, K.E. Tansey, M. Rietschel, N. Henigsberg, W. Maier, O. Mors, J. Hauser, A. Placentino, D. Souery, A. Farmer, K.J. Aitchison, I. Craig, P. McGuffin, C.M. Lewis, M. Ising, S. Lucae, E. Binder, S. Kloiber, F. Holsboer, B. Müller-Myhsok, S. Ripke, S.P. Hamilton, J. Soundy, G. Laje, F.J. McMahon, M. Fava, A.J. Rush, R.H. Perlis. **Common genetic variation and antidepressant efficacy in major depressive disorder: a meta-analysis of three genome-wide pharmacogenetic studies**. The American Journal of Psychiatry, 170 (2013), pp. 207–217

15. Walsh et al., 2002
B.T. Walsh, S.N. Seidman, R. Sysko, M. Gould. **Placebo response in studies of major depression: variable, substantial, and growing.** The Journal of the American Medical Association, 287 (2002), pp. 1840–1847