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HSP70 variations in the acute treatment with mood stabilizers in patients with bipolar disorder: results of a preliminary work

Research Article

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Abbreviations: analysis of covariance, (ANCOVA); Analysis of variance, (ANOVA); bipolar I disorder, (BID); brain derived neurotrophic factor, (BDNF); Clinical Global Impression, (CGI); DSM-IV Axis I disorders-Clinical Version, (SCID-CV); endoplasmic reticulum, (ER); heat shock protein, (HSP); HSP70 family, (HSPA1L); hystone deacetylase, (HDAC); linkage disequilibrium, (LD); small heat-shock proteins, (sHSPs); Young Mania Rating Scale, (YMRS)

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Summary

A pharmacogenetic approach was used to investigate the role of heat shock protein (HSP) 70 on the effect of mood stabilizers since a line of evidence has proposed a possible involvement of its chaperone activity in the pathophysiology of bipolar disorders. Forty five patients with bipolar I disorder were treated for an average of 36.5 (±19.9) days with mood stabilizers (lithium, valproate, or carbamazepine), were evaluated with using the Clinical Global Impression (CGI) scale and the Young Mania Rating Scale (YMRS), and were genotyped for their HSP 70 variants (rs2227956 C/T, rs2075799 A/G, rs1043618 C/G, rs562047 C/G, rs539689 C/G). Results: No association was found between the investigated variations and response to mood stabilizer treatments even considering possible stratification factors. The small number of subjects is an important limitation to the present study, nonetheless HSP 70 gene variants seem not to be involved in acute antimanic effect. Adequately-powered study would properly address the potential role of HSP 70 gene variants for the effect of mood stabilizers.

I. Introduction

Lithium, valproate and carbamazepine are the first line agents for both acute and long-term treatments of bipolar disorder (American Psychiatric Association, 2002). Given the paucity of clinical predictors of treatment response (Maj, 1992; Kleindienst et al, 2005), genetic predictors would be of a great help to clinicians. Bipolar disorder itself as well as antidepressant and antipsychotics may be influenced by genetic factors (Malhotra et al, 2004; Althoff et al, 2005; MacQueen et al, 2005; Serretti et al, 2005; Savitz and Ramesar, 2006). Moreover, a partial genetic control over the long term prophylactic effect of lithium has recently been reported (Maj, 1992; Grof et al, 1994; Alda, 1999; Serretti et al, 2002a; Serretti et al, 2002b; Serretti and Artioli, 2003). A list of pivotal genes that are probably involved in the action mechanism of mood stabilizers such lithium has been recently identified. Although such results are still inconsistent, the findings are presented in **Table 1**.

The antimanic effects of carbamazepine and valproate have been less widely investigated comparing with lithium. Nevertheless, their prophylactic action has been associated with some genes or proteins as listed in **Table 2**, giving some rational for a pharmacogenetic approach

Table 1. Pharmacogenetic studies on lithium prophylactic action

References	Gene	Gene Variant	Subjects	Length of observation	Results
(Mamdani et al, 2007)	PREP	rs3799990; rs3818281; rs1149320; rs6902415; rs12192054; rs1028792; rs9486069; rs9500087; rs720225	Caucasian 249 BID 126 controls	13.25±7.26 years	No significant association
(Michelon et al, 2006)	BDNF, INPP1, AP-2β, SERTPR	G196A, C973A, VNTR, s-l	Caucasian 134 BID	2 years	No significant association
(Szczepankiewicz et al, 2006)	GSK-3β	T-50C	Caucasian 89 BID	5 years	No significant association
(Masui et al, 2006b)	BDNF	Val66Met	Japanese 83 BPI 78 BD II	1 year	No significant association
(Masui et al, 2006a)	Xbp-1	-116C/G	Japanese 83 BPI 78 BD II	1 year	C associated with better response
(Benedetti et al, 2005)	GSK3-β	-50 T/C	Caucasians 88 BID	More than 2 years before and 2 years on lithium treatment	C associated with better response
(Rybakowski et al, 2005a)	SERTPR	s-l	Caucasians 67 BID	More than 5 years	S associated with worse response
(Rybakowski et al, 2005b)	BDNF	Val66Met and -270C/T	Caucasians 88 BID	5-27 years (mean 15 years)	Met and T associated with better response
(Dimitrova et al, 2005)	IMPA2	8 SNPs →	Caucasian 237 parents-offspring trios 174 BID 170 controls	3 years	No significant association
(Serretti et al, 2004)	SERTPR	s-l →	Caucasian 83 BID	3 years	l/l associated with poor response l/s associated with better response
(Washizuka et al, 2003)	mtDNA	10398 A/G	Japanese 54 BID	4.4 - 5.6 years	A associated with better response
(Serretti et al, 2002b)	COMT, MAO-A, Gβ3	G158A, 30-bp repeat, C825T	Caucasian 160 BID	4.9 years	No significant association
(Serretti et al, 2001)	SERTPR	s-l	Caucasian 167 BID	4.85 years	s/s associated with worse response
(Serretti et al, 2000)	HTR2A, HTR2C, HTR1A	T102C, C-1420T, Cys23Ser, C-1019G	Caucasian 102 BID	4.3 years	No significant association
(Serretti et al, 1999b)	TPH	A218C; A779C	Caucasian 90 BID	4.2 years	TPH*A/A variant showed a trend toward a worse response
(Serretti et al, 1999a)	DRD2, DRD4, GABRA1	SerCys, VNTR, VNTR	Caucasian 100 BID	4.41 years	No significant association
(Steen et al, 1998)	INPP1	C973A	Caucasian pilot bipolar sample	/	C973A transversion was present in responders
(Serretti et al, 1998)	DRD3	BalI	Caucasian 43 BID	4.08 years	No significant association

AP-2 = activator protein-2; **BDNF** = brain-derived neurotrophic factor; **COMT** = catechol-O-methyltransferase; **DRD2** = dopamine receptor D2; **DRD3** = dopamine receptor D3; **DRD4** = dopamine receptor D4; **GABRA1** = GABA(A) receptor α1 subunit; **GSK3-β** = glycogen synthase kinase β3; **HTR1A** = serotonin receptor 1A; **HTR2A** = serotonin receptor 2a; **HTR2C** = serotonin receptor 2C; **IMPA2** = Inositol Monophosphatase 2; **INPP1** = inositol polyphosphate 1-phosphatase **INPP1** = inositol polyphosphate 1-phosphatase; **MAO-A** = monoamine oxidase A; **mtDNA** = mitochondrial DNA; **PREP** = prolyl endopeptidase; **SERTPR** = promoter of serotonin transporter gene; **TPH** = tryptophan hydroxylase; **Xbp-1** = X-box binding protein 1

Table 2. Potential candidate targets for the action mechanism of mood stabilizers.

References	Drugs	Targets
(Kazuno et al, 2007)	Valproate	Mitochondrial DNA
(Rao et al, 2007)	Carbamazepine	AP-2 DNA-binding activity; AP-2 α protein expression
(Montezinho et al, 2006)	Carbamazepine; Valproate	dopamine D2-like and β-adrenergic receptors
(Phiel et al, 2001)	Valproate	HDACs
(Sharma et al, 2006)	Valproate	acH3; acH4
(Gurvich et al, 2005)	Valproate	HDACs
(Dokucu et al, 2005)	Valproate; Lithium	GSK-3β
(Zhou et al, 2005)	Valproate; Lithium	BAG-1
(Chen et al, 2005)	Valproate	B56γ regulatory subunits; transcriptional coactivator p300
(Shaltiel et al, 2004)	Valproate	inositol biosynthesis
(Chetcuti et al, 2006)	Valproate	ZIC1; SFMBT2; SCM4L1; PAR-4
(Ju and Greenberg, 2003)	Valproate	Inositol; phospholipid biosynthesis
(Bown et al, 2002)	Valproate	ER stress proteins
(Shao et al, 2006)	Valproate; Lithium	ER stress proteins
(Okada et al, 2004)	Valproate	Pc-G
(Lagace et al, 2004)	Valproate	leptin secretion; leptin messenger ribonucleic acid
(Sullivan et al, 2004)	Valproate	HTR2A
(Du et al, 2004)	Valproate; Lithium	AMPA glutamate receptor
(Nelson-DeGrave et al, 2004)	Valproate	androgen
(Yildirim et al, 2003)	Valproate	acH; 5-lipoxygenase
(Sands et al, 2000)	Valproate	TH mRNA
(Chen et al, 1999b)	Valproate	AP1 family of transcription factors

Abbreviations: **acH** = acetylated Histone protein; **AP-2** = phospholipase A2; **BAG-1** = glucocorticoid receptor cochaperone protein; **ER** = endoplasmic reticulum; **GSK-3β**= glycogen synthase kinase β3; **HDACs** = histone deacetylases; **HTR2A** = serotonin receptor 2A; **PAR-4** = prostate apoptosis response-4; **Pc-G** = Polycomb group; **SCM4L1** = structural maintenance of chromosome 4-like 1; **SFMBT2** = Scm-related gene containing four mbt domains; **TH** = tyrosine hydroxylase; **ZIC1** = zinc finger protein of the cerebellum 1

onto their antimanic effects. This field of research is not only difficult but also intriguing since the specific action mechanisms leading to mood stabilization still need to be identified (Chen et al, 1999a; Brunello and Tascedda, 2003; Harwood and Agam, 2003; Einat and Manji, 2006; Kazuno et al, 2007). Generally, the inheritance of acute antimanic response remains still unclear (Dooley and Andermann, 1989; Skarpa et al, 1994; Hwang et al, 1998). Even though the prophylactic action of mood stabilizers has been widely investigated, pharmacogenetic studies investigating the acute antimanic effect are lacking till today.

The first step in this research is to identify which molecular mechanisms are relevant to the antimanic effects of mood stabilizers. Within the variety of theories dealing with mood stabilizers pharmacodynamic aspects (Harwood and Agam, 2003), the neuroprotective pathway seems to play a relevant role (Jope, 1999). Consistently, early effects of lithium and valproate over cell–life promoting mechanisms have been reported as follows: influence on apoptotic or antiapoptotic mechanisms, action on glutamate induced reactions, induction of brain derived neurotrophic factor (BDNF) cascade (i.e., BDNF, TrkB), stimulation of neuroblasts proliferation, stabilization of lysosomal membrane, hystone deacetylase (HDAC) inhibition, as well as stimulation of heat shock factor 1, and inhibition of glycogen synthase kinase 3. Chaperones associated with endoplasmic reticulum (ER) including GRP78, GRP94, PDI, calreticulin, caspase 3 together with the cytosolic chaperones belonging to the heat shock protein (HSP) family, may be involved in the pathways where are related to the effects of mood stabilizers (Bown et al, 2000, 2002; Chen et al, 2000; Ren et al, 2003, 2004; Chuang, 2004, 2005; Hiroi et al, 2005; Kim et al, 2005; Pan et al, 2005; Sinn et al, 2007). Moreover, neuroprotective action of lithium and valproate has been also demonstrated *in vivo* (Ren et al, 2003; Chuang, 2004; Chuang, 2005; Xu et al, 2006; Sinn et al, 2007).

All the above mentioned proteins could represent good targets for pharmacogenetic research on the acute antimanic effect of mood stabilizers. In fact, even not a classical neurodegenerative disease, bipolar disorder has been recently found to be associated with a neurodegenerative pathophysiology (Savitz et al, 2005). Moreover, cell–life promoting events are at reasonably responsible for the neuronal plasticity and resilience, which have been also recognized to be relevant with bipolar disorder (Chen et al, 1999a; Ikonomov and Manji, 1999; Manji et al, 1999, 2000). In this direction, HSPs are essential neuroprotective proteins, they have been proven to be influenced by lithium and valproate and they were recently proposed as possible state markers of acute manic episode (Shen et al, 2006). Moreover, there is some evidence that HSPs are associated with other psychiatric disorders as well (Shimizu et al, 1996; Pae et al, 2005; Pae et al, 2007).

Taken together above mentioned, we employed a pharmacogenetic approach to investigate the hypothesis of an involvement of HSPs in acute antimanic effects of mood stabilizers. A set of genetic variations (rs2227956 C/T, rs2075799 A/G, rs1043618 C/G, rs562047 C/G, rs539689 C/G) was studied on the basis of previous results (Pae et al, 2005).

II. Materials and Methods

A. Subjects

The sample was composed by 45 patients (20 males) suffering from bipolar I disorder (BID, diagnosed with a consensus between C.U.P. and J.J.K.), and scoring at least 13 in YMRS at baseline. The patients were assessed using a Structured Clinical Interview, DSM-IV Axis I disorders-Clinical Version (SCID-CV) and patients with comorbid Axis I disorder other than BID were excluded. Subjects with neurological and current medico-surgical illness were also excluded. Patients were administered the Clinical Global Impression (CGI) (Guy, 1976) scale and the Young Mania Rating Scale (YMRS) (Young et al, 1978) for evaluation of the effectiveness of mood stabilizers. Patients were assessed with such effectiveness measures at the time of admission and discharge. All subjects were biologically unrelated, native Korean descendants residing in Korea. Written informed consent was provided by the subjects after being explained the purpose and method of the study. The institutional review board of Kangnam St. Mary's Hospital approved the study that was conducted in accordance with the Declaration of Helsinki.

B. HSP variants investigated

HSPs are coded by different genes and are clustered by their molecular weights: the most important classes are HSP100, HSP90, HSP70, HSP60, HSP33, and the small heat-shock proteins (sHSPs). One of the best-characterized chaperones belong to the HSP70 family (Pilon and Schekman, 1999; Hartl and Hayer-Hartl, 2002). HSP70 have structural and functional properties in common, but vary in their inducibility in response to metabolic stress. In the class III region of the major histocompatibility complex on 6p21.3 was identified a duplicated HSP70 locus (Sargent et al, 1989), named HSPA1A and HSPA1B. Both encode identical 641-amino acid proteins but the 3' untranslated regions of these genes are divergent. Northern blot analysis of HeLa cell RNA detected an approximately 2.4-kb HSPA1B transcript that was expressed at elevated levels following heat shock. Sargent et al. also identified a region with similarity to HSPA1A located approximately 4 kb telomeric to HSPA1A; this homologous region has been defined as a gene of the HSP70 family (HSPA1L) (Milner and Campbell, 1992). In the present paper we investigated a set of polymorphisms concerning HSP70, within the mentioned HSPA1A, HSPA1B and HSPA1L genes.

C. Genetic analyses

Genomic DNA was extracted from venous blood by standard methods and quantified. The high-throughput genotyping method using pyrosequencer (Biotage AB, Sweden) was used for genotyping 5 HSP 70 SNPs (rs2227956 C/T, rs2075799 A/G, rs1043618 C/G, rs562047 C/G, rs539689 C/G). A set of genetic variations were selected based on public database (National Center for Biotechnology Information, dbSNP, http://www.ncbi.nlm.nih.gov/SNP/). PCR primers (Bioneer, Daejeon, Korea) and sequencing primers (Bioneer, Daejeon, Korea) used for the Pyrosequencing assay were designed by using the Pyrosequencing Assay Design Software v1 (Biotage AB, Sweden) and one primer of each primer set was biotinylated. Genotyping was confirmed by J.J.K.

D. Statistical analyses

Haploview 3.2 was used to generate a linkage disequilibrium (LD) map and to test for Hardy-Weinberg equilibrium. Single genotype associations with YMRS and CGI scores were analyzed by the Analysis of variance (ANOVA); when including covariates or other factors, the analysis of covariance (ANCOVA) and the multivariate analysis of co-/variance (MANOVA/MANCOVA) were employed. Baseline scores were included as covariates plus the clinical variables associated with genotypes. Associations with other clinical variables in the subjects were performed by the ANOVA or the Chi-square test. The "R" software ("A Programming Environment for Data Analysis and Graphics" Version 2.2.1) was employed to analyze haplotype with both discrete and continuous traits and to include covariates. Permutation (50,000 permutations) was used to estimate the global significance of the results for haplotype analyses to confirm the expectation-maximization values. Results were considered significant with an α level lower than 0.05.

With this level of significance, for single marker allele analyses, we had a power of 0.80 to detect a medium-large effect size of d=0.86, which corresponded to a difference of approximately 4.5% in the YMRS improvement between two main genotype variants and corresponded to an explained variance of about 15.6% (Cohen, 1988).

III. Results

Subjects description is presented in **Table 3**. A significant % reduction in YMRS score was reported (64.2 ± 4.6) for the whole subjects; lithium, valproate and carbamazepine treated subjects with reduction in YMRS score of 64.9±4.7, 64.2±2.8 and 60.7±5.8, respectively. The present study was not designed to investigate the different efficacy of the single drugs, so we did not perform any statistical investigation in that direction, however the effects were very similar.

Correlation analysis was performed between clinical variables and % reductions in the scores of YMRS and CGI. We observed a significant positive correlation (R Spearman = 0.63; $p < 0.0001$) between YMRS scores at baseline and % reduction in YMRS score by the end of treatment. We also observed an inverse association with age at onset and the % reduction in YMRS score (R Spearman = -0.29 p = 0.05). Otherwise were all non-significant. When considering the dichotomic variable "remitters" or "non remitters" results were also similar (data not shown).

As regard to the allelic analysis on the HSPs, all 45 subjects were successfully genotyped. All markers were in Hardy-Weinberg equilibrium (rs2227956 p=1.0, rs2075799 p=1.0, rs1043618 p=1.0, rs562047 p=1.0, rs539689 p=0.6). Comparing the allele frequencies with the international databases, all the investigated SNPs reported expected frequencies. No significant association was found between the antimanic effect or manic score at baseline and the investigated genetic variations even after covariate analyses (sex, age, age at onset, YMRS scores at baseline). A strong LD was found between SNPs rs2227956, rs2075799, rs1043618 and between SNPs rs562047 and rs539689 (>0.8). Haplotype analysis gave no

Table 3. Demographics of the subjects in the study.

Variable	Results	
Sex	Male	20 (44.4)
	Female	25 (55.6)
Age (years)	32.7 (±10.9)	
Age of onset (years)	26.7 (±10.0)	
Treatment (subjects in treatment with one drug)	Lithium	30 (66)
	Valproate	9 (20)
	Carbamazepine	3 (6)
	Other	3 (6)
Duration of treatment (days)	36.5 (±19.9)	
Treatment Dose (mg/day)	Lithium	1066.7 (±174.9)
	Valproate	1166.7 (±251.2)
	Carbamazepine	600 (±200)
Suicide (suicide attempters)	3 (6.7)	
Psychotic features (subjects with psychotic features)	21 (46.6) 3 missing values	
YMRS at baseline	42.9 (±4.0)	
YMRS at retest	15.2 (±1.5)	
% reduction in YMRS	64.2 (±4.6)	
CGI at baseline	5.2 (±0.7)	
CGI at retest	3.7 (±1.2)	
% reduction in CGI	28.4 (±20.6)	

Data represent mean±standard deviation or number and frequency; Abbreviations: CGI, Clinical Global Impression scale; YMRS, Young Mania Rating Scale.

significant association results with antimanic acute effect. No correlation, even after covariation analysis, was found for YMRS at baseline or at final tests.

IV. Discussion

In the present paper we failed to find an association between the variations within the investigated HSPs coding sequence and the acute antimanic effect of lithium, valproate and carbamazepine. Minor clinical significant results were observed: a significant positive correlation between YMRS scores at baseline and % reduction in YMRS score by the end of treatment. We also observed an inverse association with age at onset and the % reduction in YMRS score. This finding is in line with some recent reports focusing on the age of onset of bipolar disorder: Lin and colleagues proposed to use it to identify more homogeneous groups of bipolar disorder families (Lin et al, 2006). This is consistent with other reports (Mick et al, 2003; Kennedy et al, 2005a,b; Leboyer et al, 2005). Age of onset has also been correlated with the severity of symptomatology (Patel et al, 2006) and poorer outcome (Carter et al, 2003).

Nevertheless, even though we reported negative association results, HSPs still represent good candidate for future research. In fact, also under physiological conditions, those proteins support folding of non-native and misfolded proteins, and prevent aggregation during proliferation and cellular differentiation. HSPs demonstrate extremely high conservation of the genetic code sequence (Karlin and Brocchieri, 1998): this is probably due to their essential role in cellular molecular homeostasis (Aufricht, 2005). This important cellular role is like-minded with the hypothesis that HSPs can represent a promising pharmacological target in mood stabilizing action, consistently, there is some evidence that bipolar disorder can be partially dependent on a neurodegenerative pathophysiology (Savitz et al, 2005).

The negative result in this study could be due to the some limitations: the small sample size, heterogeneity and different length of treatment and different doses of drugs. Drug plasma levels are an important missing variable. The lack of genomic control which is liable for stratification bias should be another limit of the study, however Korean population is considered genetically homogenous (Cavalli Sforza, 1994). We investigated only a portion of the HSPs coding sequence, on the basis of above mentioned literature evidence. In this study, five SNPs of HSP70 genes were selected. The distance between those SNPs were not evenly distributed and not enough to cover the

whole genetic variation. Therefore, it cannot be confirmed which SNPs have a critical role in the pathogenesis of bipolar disorder and possible interaction with mood stabilization by the result of this study. More SNPs on HSP70 genes in larger sample are needed in future. Epigenetic studies between possibly interconnected gene variants should be also investigated in order to cover minor gene effects. Finally, given that therapeutic agents used in bipolar disorder are heterogeneous in action mechanism, and thus unified mood stabilizer trials should be more proper for bipolar pharmacogenetic researches. In addition, to narrow down the homogeneity of subjects, study with endophenotypic diagnosis for bipolar disorder patients may be also valuable to converge the findings in this field.

The strength of this study is that it is the second one to our knowledge that focused on the pharmacogenetics of acute response of antimanic mood stabilizers treatment, and the first one that investigated a portion of the HSP coding sequence on this topic. Hence, the present paper should be considered with clear limitations as well as some potential advantages of preliminary work, highlighting an interesting but not yet deeply investigated pharmacogenetic field.

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