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Investigation of an Epistastic Effect Between a Set of TAAR6 and HSP-70 Genes Variations and Major Mood Disorders

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Epistasis, the interaction between genes, is a topic of current interest in molecular and quantitative genetics. We have further studied a previously investigated sample of 187 major depressive disorder (MDD) patients, 171 bipolar disorder (BD) patients, and 288 controls, and tried to analyze the interaction between a set of variations of independent genes: the trace amine receptor 6 (rs4305745, rs8192625, rs7452939, rs6903874, and rs6937506) and the heat shock protein 70 (rs562047, rs1061581, rs2227956). The multifactor dimensionality reduction (MDR) method was applied and the covariates associated with diagnosis were also controlled. A significant predictive value of specific interactions between genotypes located in the investigated genes was found. We then report preliminary evidence that the epistasis between trace amine receptor 6 and heat shock protein 70 variations may compose a risk profile for major mood disorders. The level of statistical significance ($P < 0.001$) and the testing balancing accuracy over 0.62 suggest a cautious optimism toward this result, although the possibility of false positivity warrants further analyses in independent samples.

Key words: epistasis; major depressive disorder; bipolar disorder; trace amines receptor 6; heat shock proteins

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Major depressive disorder (MDD) is associated with high rates of psychiatric and non-psychiatric medical comorbidities [Angst et al., 1999; Aina and Susman, 2006; Fabrazzo and De Santo, 2006; Delville and McDougall, 2008] and bipolar disorder (BP) imposes both impressive personal sufferance to the affected individuals as well as high socioeconomic and public health costs [Keck et al., 2008] since it is associated with high rates of unemployment [Laxman et al., 2008], suicide behavior [McIntyre et al., 2008], car accidents, violent acts, and loss of productivity [Fieve, 1999]. Prevention is a mandatory first step in a rational medical approach to these conditions: a timely diagnosis along with the administration of the most proper treatment for the individual patient will hopefully change the clinical prognosis, allowing both a more favorable life style for patients and a consistent economic benefit for the community. There has been emerging evidence that occurrence of and treatment response to such mood disorders are in part driven by genetic factors [Manji et al., 2001; Danese, 2008; Escamilla and Zavala, 2008; Jabbi et al., 2008; Porteous, 2008; Serretti et al., 2009]. However, underpowered study samples, incomplete coverage of genetic variations, and inadequate molecular paths have been common weaknesses in this research field, although high complexity of molecular understanding for mood disorders may also attribute to poor replication of previous positive genetic studies. In fact, there is evidence that the investigation of genetic variations alone, which is the standard nowadays, both considering candidate gene approach and genome-wide investigations, does not permit a sufficiently detailed view on the field and most part of key molecular actors are eventually lost within this kind of perspective. For example, the investigation of a variation which enhances the expression of a certain protein involved in the metabolism of key

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cellular components of the central nervous system may be considered as rational way to start the disentanglement of the molecular orchestrations whose disrupted equilibrium leads to psychiatric disorders. Nevertheless, whenever the association analysis omits other events such as mRNA editing, RNA interfering, molecular path adaptations, the presence of copy number variations and the methylation rates may suffer from a sufficient stratification bias to shadow the real effect of the investigated variation or set of variations. The reactions between the genetic and non-genetic components of the cell functions and neuronal nets activities may be considered as a more valuable target for research: it goes under the name of reactome [Joshi-Tope et al., 2005] and it can be at least partially investigated by the means of epistasis [Phillips, 2008]. Epistasis is referred to as event of functional interaction between genes in its most widely accepted meaning. In particular, the statistical epistasis may play a strategic role in sorting out specific interactions between mutations located in different genes, which are the mirror of what is happening at the enzymatic level in the cells and neuronal nets consequently. This may provide new insights into the complexity of the molecular aspects of mood disorders and cast some light in the field, indicating some molecular crossing points that could help unravel the matter. This is the background that stays behind the present investigation in which we re-analyzed a set of variations located in relevant candidate genes (namely TAAR6: rs4305745, rs8192625, rs7452939, rs6903874, and rs6937506; HSP-70: rs562047, rs1061581, and rs2227956) under the perspective of epistasis. Our group yet separately analyzed these variations in previously published articles [Pae et al., 2007, 2008] challenging the hypothesis that these proteins mutated may be associated with a higher risk of experiencing a mood disorder or a poor response to treatment through the analysis of genotypes and haplotypes in each gene.

Sample characteristics, inclusion and exclusion criteria, battery test, and the timing of the study have been described elsewhere along with the details related to the genotyping and the rational of the selection of the investigated variations (187 MDD patients, 171 BD patients, and 288 controls) [Pae et al., 2007, 2008]. The diagnosis was based on a strict consensus between two board-certified psychiatrists and based on the DSM-IV criteria for MDD and BD. Patients with any additional axis I disorders were excluded. Psychiatrists who conducted the study and performed the tests were blind to the genotypes. All patients were interviewed with the structured Clinical Interview for DSM-IV Axis I Disorders—Clinician Version [First et al., 1997].

The multifactor dimensionality reduction (MDR) method was employed to investigate the gene–gene interactions [Lou et al., 2007]. The model computes the data reducing the n-dimensional space formed by a given set to a single dimension to analyze n-way interactions. Each class is then classified as either high risk or low risk according to the relative proportion of cases to controls in that class. This is performed for all possible combinations of SNPs, and the combination with the best predictive capacity is identified. According to the settings of the software, we tested all the possible interactions using 10-fold cross-validation in an exhaustive search, thus considering all possible SNP combinations. As outcome parameters, we considered the cross-validation consistency, the testing balanced accuracy, and the significance test. The cross-validation (CV) consistency score measures the degree of consistency through which the identification of the selected variation takes place, on the basis of the best model applied to the computation. The testing balanced accuracy measures the degree to which the interaction accurately predicts case–control status (label 1 indicating good prediction of the model, label 0.50 suggesting that the model is no better than chance in selecting cases from controls). The significance test gives the *P*-value of the calculation.

The sample was composed by 187 MDD patients, 171 BD patients, and 288 controls. The analysis of the epistasis between the selected variations successfully distinguished between cases and controls and showed the following genetic associations: the homozygosis at rs4305745 turned out to be a risk for suffering a BP only if co-occurrent with the homozygosis for the G allele at the rs452939, this being independent from the genotype expressed in rs562047. On the other hand, heterozygosis at rs4305745 was at risk of BP only if concurrent with heterozygosis at rs452939. Under this model, the presence of both alleles G at rs562047 counteracted the risk of association with BP. Finally, homozygosis of the T allele at the rs4305745 was at risk of BP only when co-occurring with the homozygosis for the C allele at rs562047 and A allele at rs452939. Results are presented in Figure 1. CV consistency was set at 09/10; *P*-level was inferior to 0.0010 and the testing balancing accuracy was found to be as high as 0.65. With regard to the association with the diagnosis of depressive disorders, we found that homozygosis for the C allele at rs4305745 was at risk of depressive disorder only when associated with the presence of the C allele at the rs562047 (either homozygosis or heterozygosis) and of the homozygosis for the G allele at the rs452939. Conversely, homozygosis at the rs4305745 was not found to be at risk of depressive disorder when differently combined with the other variations. Instead, heterozygosis or homozygosis for the T allele at rs4305745 turned out to be at risk of depressive disorder in the co-occurrence of the C allele at rs562047 and heterozygosis at rs452939. Results are shown in Figure 2. CV consistency was set at 9/10; *P*-level was inferior to 0.0010 and the testing balancing accuracy was found to be as high as 0.62 for this association.

Trace amine receptors are endogenous amine compounds that can be found in the mammalian nervous system in trace amounts (0.1–10 nM). Trace amines have been associated with depression [Sandler et al., 1979; Davis and Boulton, 1994] and psychosis [Potkin et al., 1979; Jacob and Presti, 2005]. Consistently, high concentrations of trace amines have been proven to influence the catecholamine and indolamine release, reuptake and biosynthesis, with a final presynaptic "amphetamine-like" effect, resulting in increased alertness, euphoria, irritability, decreased appetite, insomnia, and tremor. On the other hand, a low trace amines concentration has been associated with enhanced dopamine and serotonin neurotransmission [Burchett and Hicks, 2006]. Heat shock proteins (HSPs) represent a protein family dedicated to house keeping and stress-induced cellular activities; they can bind and stabilize otherwise unstable protein, facilitating its correct fate in vivo, be it folding, oligomeric assembly, transport to a particular subcellular compartment, or disposal to degradation [see review Hartl, 1996]. Moreover, HSPs are involved in the uncoating of clathrin-coated vesicles, in the mitochondrial activity and in the control of regulatory proteins [Lindquist and Craig, 1988;

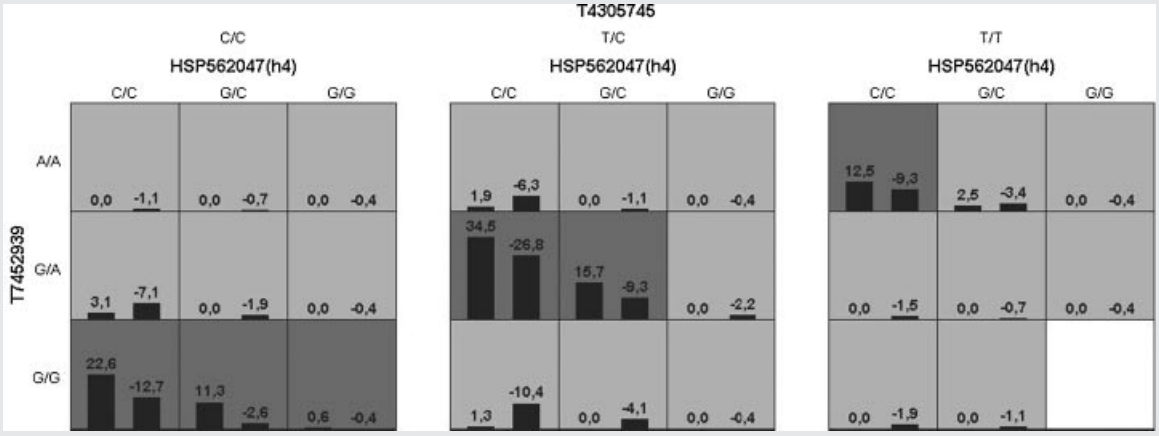

FIG. 1. Epistasis between TAAr6 and HSP-70 variations distinguishes BD patients from controls. CV consistency = 9/10; $P < 0.0010$; testing balancing accuracy = 0.65. Number upon columns are the ratios of the number of cases to the number of controls. "High-risk" cells are indicated by dark shading, "low-risk" cells by light shading, and "empty" cells by no shading [Lou et al., 2007].

Watowich and Morimoto, 1988; Hartl, 1996; Parcellier et al., 2003; Bukau et al., 2006]. HSPs may then be appropriate targets for modulating cell death pathways [Parcellier et al., 2003]. These lines of evidence suggest that both trace amine receptors and HSPs are good candidates to be investigated in the analysis of the possible disruptions that are associated with mood disorders. Our previously reported analyses along with the present epistatic investigation further suggest that this impact is likely one of the genetic imbalances, which concur to cause mood disorders. In particular, the balancing accuracy over the level of 0.50 is suggestive that a causal link really associates the combinations of the different genotypes shown here and the risk of disease. Nonetheless, these results must be treated with cautiousness: first of all, a first type mistake is a real concern to this analysis, due to the high number of tests that are performed in order to confirm or discharge the hypothesis of association. Secondly, possible stratification biases both molecular, sociodemographic and psychological in nature, have not been included: speaking about the molecular drawbacks of the study, a complete coverage of the genetic variations located in each of the candidate genes is missing along with the analysis of the principal molecular pathways that are associated with their activation. Besides, some relevant molecular events such as the presence of copy number variations, the methylation rates, and the RNA controls have not been investigated in the present study, and they are not known in much detail by the scientific community, anyway. So far, the epistasis between different genes still represents a

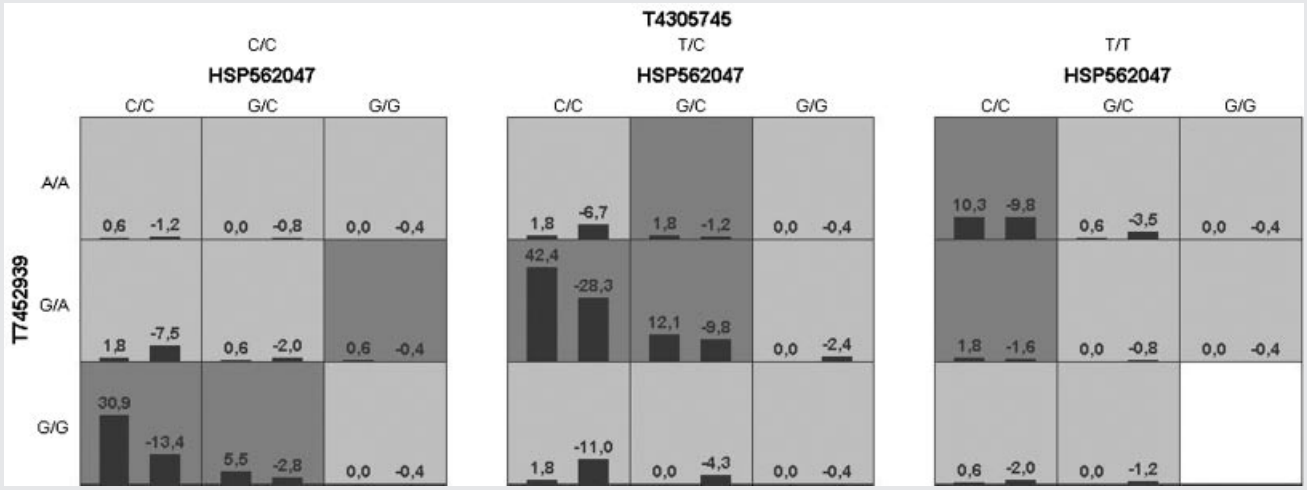

FIG. 2. Epistasis between TAAr6 and HSP-70 variations distinguishes MDD patients from controls. CV consistency = 9/10; $P < 0.0010$; testing balancing accuracy = 0.62. Numbers upon columns are the ratios of the number of cases to the number of controls. "High-risk" cells are indicated by dark shading, "low-risk" cells by light shading, and "empty" cells by no shading [Lou et al., 2007].

particularly relevant analysis to be performed in genetic association studies, being helped by the knowledge of the principal ways by which the so-called reactome displays its activities [Joshi-Tope et al., 2005]. The inclusion of the analysis of the epistasis between genes along with the analysis of the impact of single genotypes and haplotypes should be considered as a standard in the next association studies. Finally, the need of longitudinal study in investigating the epistatic interactions between candidate genes for the development of mood disorders as well as response to therapeutic agents since mood disorders have long-standing clinical course intervened by wax and wane features.

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