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Integrated profiling of phenotype and blood transcriptome for stress vulnerability and depression

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ABSTRACT

Etiology of depression and its vulnerability remains elusive. Using a latent profile analysis on dimensional personality traits, we previously identified 3 different phenotypes in the general population, namely stress-resilient, -vulnerable, and -resistant groups. Here we performed microarray-based blood gene expression profiling of these 3 groups (n = 20 for each group) in order to identify genes involved in stress vulnerability as it relates to the risk of depression. Identified differentially expressed genes among the groups were most markedly enriched in ribosome-related pathways. These ribosomal genes, which included ribosomal protein L17 (*RPL17*) and ribosomal protein L34 (*RPL34*), were upregulated in relation to the stress vulnerability. Protein-protein interaction and correlational co-expression analyses of the differentially expressed genes/non-coding RNAs consistently showed that functional networks involving ribosomes were affected. The significant upregulation of *RPL17* and *RPL34* was also observed in depressed patients compared to healthy controls, as confirmed in 2 independent case-control datasets by using pooled microarray data and qPCR experiments (total number of subjects was 122 and 166, respectively). Moreover, the upregulation of *RPL17* and *RPL34* was most marked in DSM-IV major depressive disorder, followed by in bipolar disorder, and then in schizophrenia, suggesting some diagnostic specificity of these markers as well as their general roles in stress vulnerability. These results suggest that ribosomal genes, particularly *RPL17* and *RPL34*, can play integral roles in stress vulnerability and depression across nonclinical and clinical conditions. This study presents an opportunity to understand how multiple psychological traits and underlying molecular mechanisms interact to render individuals vulnerable to depression.

1. Introduction

Depression is a highly prevalent psychiatric disorder characterized by persistently low mood and/or diminished interest in once pleasurable activities. It is now widely recognized that the development of depression is affected by complex interactions between an individual's vulnerability and a broad range of environmental factors (Belsky and Pluess, 2009). Despite extensive research, however, the pathophysiology of depression is largely unknown and candidate biomarkers remain impractical for clinical practice. This difficulty in elucidating pathology of depression and establishing biomarkers for its diagnosis and prognosis will, at least partly, be explained by the heterogeneity of the disorder.

Our understanding of vulnerability to stress can be described via phenotypical and biological perspectives. At the phenotypic level, stress vulnerability manifests as a failure to adapt to stress, probably because of poor coping styles associated with maladaptive personality traits. These psychological constructs of coping and personality are multidimensional and encompass several different dimensions (or aspects) pertaining to the construct. Moreover, it is postulated that such different dimensions are not independent of each other; rather, they can dynamically interact, thereby establishing a psychological profile of the

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individual (Markon et al., 2005). This also implies that the relationship of these psychological traits with mental health can be complicated, such that any single dimension can have both advantages and disadvantages depending on the context (Ferguson et al., 2014) (Supplementary Fig. S1 top). In line with this notion, we previously applied an individual-based profile analysis on dimensional personality traits of nonclinical adults and identified 3 different phenotypes in accordance with different degrees of (qualitative and quantitative) stress vulnerability, namely "resilient", "vulnerable", and "resistant" groups (Hori et al., 2014).

At the biological level, it is recognized that nervous system homeostasis and stress response are regulated by a complex network of genes and transcripts (Qureshi and Mehler, 2012). Here, depression can be understood as a breakdown of homeostatic mechanisms due to dysregulated crosstalk between genes and molecules which would include –but not limited to– those involved in the regulation of hypothalamic-pituitary-adrenal (HPA) axis, immune system, and neurotransmission (e.g., Network and Pathway Analysis Subgroup of Psychiatric Genomics Consortium, 2015). Analyses of pathways and networks that mirror interactions between multiple genes and transcripts may therefore help to gain perspective on intricate phenomena like stress vulnerability and depression. Gene expression profiling is considered advantageous in this regard, as it captures a dynamic picture of genomic responses to environmental stimuli at a particular time point, thereby providing a snapshot of the transcriptomic landscape (Gibson, 2008). Using microarrays and RNA-seq, several studies have examined blood-based gene expression profiles in depression, although their findings have not been consistent (Hori et al., 2016; Jansen et al., 2016; Leday et al., 2018; Liu et al., 2014; Mostafavi et al., 2014; Segman et al., 2010).

Another notable aspect of stress vulnerability is that it is thought to exist on a continuum ranging from nonclinical subjects to clinically depressed patients. Many individuals sometimes experience distress related to subclinical levels of depressive symptoms, but do not meet the diagnostic criteria of depression, or major depressive disorder (MDD). These symptoms are usually qualitatively similar to those observed in patients with MDD, as reflected, for example, in the fact that the formal diagnosis of MDD is made quantitatively in accordance with the number of symptoms (5 or more) and their duration (2 weeks or longer) (APA, 2013). This symptomatic overlap between nonclinical/subclinical distress and clinical depression suggests that these behavioral states could also represent a pathophysiological continuum (Supplementary Fig. S1 bottom).

This study aimed to explore the molecular basis of stress vulnerability and depression by integrating phenotypic and transcriptomic profiling, based on the assumptions of interactive relationships among psychological traits within each individual and of the continuum between nonclinical vulnerability and clinical depression. Specifically, we first investigated an association between stress vulnerability determined in accordance with the individual-oriented psychological profiles and blood transcriptome profiles in nonclinical subjects. We then tested whether the genes/molecules that were found to play an integral role in stress vulnerability in these subjects would also be associated with depression, using 2 independent sample sets of patients with clinical depression and age- and sex-matched healthy controls. In addition, the potential diagnostic specificity of these genes/molecules was examined using sample sets of patients with MDD, those with bipolar disorder, those with schizophrenia, and healthy control subjects.

2. Methods

Details of the methods are provided in Supplementary Methods.

2.1. Subjects and procedure

The overall procedure of this study is depicted in Fig. 1. This study comprised 2 main parts: Part 1) investigation of gene expression signatures of stress vulnerability, defined based on an individually-oriented profile analysis of psychological features in a nonclinical sample, and Part 2) investigation of whether the most salient findings in Part 1 can be extrapolated to clinically depressed populations.

Subjects were recruited from the outpatient clinic of the National Center of Neurology and Psychiatry Hospital, Tokyo, Japan, or through an advertisement in a free local magazine and on our website. Clinical diagnoses made by experienced psychiatrists were confirmed by a research psychiatrist using either the Structured Clinical Interview for DSM-IV Axis-I disorders (First et al., 1997) or the Mini International Neuropsychiatric Interview (M.I.N.I; Sheehan et al., 1998). Depression severity was evaluated by the total score on the Hamilton Depression Rating Scale 21-item version (HAMD; Hamilton, 1967). A total HAMD score of 15 or more was considered to indicate at least moderately severe depression, while a score of 7 or less was used for defining remission. For healthy subjects, the absence of current Axis-I psychiatric disorders was ascertained by M.I.N.I.

The present study was approved by the ethics committee of the National Center of Neurology and Psychiatry, and was conducted in accordance with the Declaration of Helsinki. After description of the study, written informed consent was obtained from every participant.

2.1.1. Part 1

In this part, we utilized a sample employed in our previous study, in which 455 nonclinical adults in the general population had been classified into the 3 homogeneous groups, namely "resilient", "vulnerable", and "resistant" groups, based on different personality profiles (Hori et al., 2014). The resilient group was characterized by a combination of essentially adaptive personality profile with some unique components, intermediate levels of distressing symptoms, and greater use of adaptive coping styles. The vulnerable group was characterized by maladaptive personality profile, highest level of distressing symptoms, and least use of social support. The resistant group showed overall adaptive personality profile and fewest distressing symptoms, without using active coping strategies frequently. Blood sampling for RNA analysis had been conducted for these subjects as part of our larger study.

In the present study we selected a total of 60 subjects from among the 455 subjects, with each of the 3 groups comprising 20 age- and sex-matched nonclinical adults. Demographic and psychological characteristics of the sample are presented in Supplementary Table S1. The microarray-based transcriptome analysis was performed for these 60 subjects.

2.1.2. Part 2

In the second part, we first utilized pooled microarray data used in our previous studies (Hori et al., 2016; Sasayama et al., 2013) for the targeted examination of key findings from Part 1. These microarray datasets comprised a total of 122 subjects, including 54 currently depressed outpatients (including 47 patients with MDD and 7 with bipolar disorder) who were at least moderately ill, 14 age- and sex-matched patients remitted from depression (including 12 patients with MDD and 2 with bipolar disorder), and 54 matched healthy controls. This dataset was independent of the sample (i.e., 60 subjects) in Part 1. Sample characteristics for these subjects are shown in Supplementary Table S2. To confirm these microarray data, a reverse transcription quantitative real-time polymerase chain reaction (qPCR) experiment was performed in a subset of the sample (i.e., 65 of the 122 subjects).

Finally, we performed a replication qPCR experiment for the key findings in an independent case-control dataset of 106 depressed patients (including 59 patients with MDD and 47 with bipolar disorder) who were at least moderately ill and 60 matched healthy controls. To examine disease specificity, 43 patients with schizophrenia were also included, and the qPCR data were compared between these 4 diagnostic groups (i.e., MDD, bipolar disorder, schizophrenia, and healthy controls). These 209 subjects (and their RNA samples) were drawn from our database, with age, sex, and ethnicity (Japanese) being matched