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Biological tests for major depressive disorder that involve leukocyte gene expression assays

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ABSTRACT

Background: Development of easy-to-use biological diagnostic tests for major depressive disorder (MDD) may facilitate MDD diagnosis and delivery of optimal treatment. Here, we examined leukocyte gene expression to develop a biological diagnostic test for MDD.
Methods: 25 drug-naive MDD patients (MDDs) and 25 age- and sex-matched healthy subjects (Controls) participated in a pilot study. A subsequent replication study involved 20 MDDs and 18 Controls. We used custom-made PCR array plates to examine mRNA levels of 40 candidate genes in leukocyte samples to assess whether any combination of these genes could be used to differentiate MDDs from Controls based on expression profiles.
Results: Among 40 candidate genes, we identified a set of seven genes (*PDGFC, SLC6A4, PDLIM5, ARHGAP24, PRNP, HDAC5, and IL1R2*), each of which had expression levels that differed significantly between MDD and Control samples in the pilot study. To identify genes whose expression best differentiated between MDDs and Controls, a linear discriminant function was developed to discriminate between MDDs and Controls based on the standardized values of gene expression after Z-score transformation. Ultimately, five genes (*PDGFC*, *SLC6A4*, *ARHGAP24*, *PRNP*, and *HDAC5*) were selected for a multi-assay diagnostic test. In the pilot study, this diagnostic test demonstrated sensitivity and specificity of 80% and 92%, respectively. The replication study yielded nearly identical results, sensitivity of 85% and specificity of 89%.
Conclusions: Using leukocyte gene expression profiles, we could differentiate MDDs from Controls with adequate sensitivity and specificity. Additional markers not yet identified might further improve the performance of this test.

1. Introduction

Major depressive disorder (MDD) is a highly prevalent and moderately heritable psychiatric disorder that causes major psychological, physical, and social impairments. Lifetime prevalence for MDD is estimated at about 16% or 17% and is definitely over 10%; women are affected twice as often as men (Kessler et al., 2003; Faravelli et al., 2013). Biological (Nestler et al., 2002), genetic (Levinson, 2006), and environmental factors (Caspi et al., 2003) can affect the onset of MDD, but MDD pathogenesis is largely unknown. Clusters of symptoms are currently used to diagnose MDD, and most care is delivered by general practitioners. Recent evidence indicates that diagnostic accuracy is highly variable in practice (Cepoiu et al., 2008). Undoubtedly, the development of easy-to-use biological diagnostic tests for MDD can radically improve diagnostic accuracy.

Quantitative profiling of leukocyte mRNA expression is an emerging and promising approach for assessing mental conditions. Psychological changes associated with depression clearly affect the hypothalamus-pituitary-adrenal (HPA) axis and the neuroendocrine, autonomic nervous, and immune systems (Connor and Leonard, 1998; Raison and Miller, 2003). Importantly, receptors

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for stress mediators are expressed in leukocytes; for example, neurotransmitter, hormone, growth factor, and cytokine receptors are found in leukocytes; additionally these cells produce various cytokines, including pro-inflammatory cytokines that stimulate the HPA axis directly (Arzt, 2001; Ohmori et al., 2005).

Recently, changes in leukocyte gene expression have been linked to MDD (Iga et al., 2008; Hepgul et al., 2013). However, expression of any one gene explains only a small proportion of the variance associated with depression. Combining measurements from individual markers into a single measurement often results in superior diagnostic test performance. The goal of present work was to develop and test the performance of a composite, multi-assay diagnostic test for MDD based on leukocyte gene expression profiles.

2. Methods and materials

2.1. Subjects

The protocol was approved and monitored by the Institutional Review Boards at each participating center. Written informed consent was obtained from each participant before any study procedures were performed.

For the pilot study, we enrolled 25 MDDs from four psychiatric hospitals in the Tokushima Prefecture of Japan: each MDD participant was experiencing a single or recurrent major depressive episode; 25 non-depressed healthy individuals were recruited from Tokushima University Hospital to serve as Controls. The replication study included 20 MDDs (14 drug-naïve patients and 6 medicated patients) from psychiatric hospitals in Tokushima and Kochi Prefectures and 18 Controls recruited from Tokushima University Hospital. Each of the six medicated patients was being treated with antidepressants, but none reached remission (HAM-D $\leq$ 7). The diagnosis of MDD was established according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria by at least two trained psychiatrists. None of the patients or controls in our study had any other medical disorder or any concomitant medication. Antidepressant-treated patients (n = 6) in the replication study had the antidepressants listed in Table 2. Exclusion criteria for MDDs included not having used non-steroidal anti-inflammatory agents, steroids or anticonvulsants within at least the 2 months before study initiation. Demographic data for participants in each study are shown in Tables 1 and 2.

2.2. Tissue processing, RNA purification, and sample preparation for real-time PCR analysis

PAX gene blood RNA tubes (Qiagen, Tokyo, Japan) and PAX gene Blood RNA kits (Qiagen, Tokyo, Japan) were used according to the manufacturer's recommendations to extract total RNA from peripheral leukocytes taken from whole blood samples. More specifically, PAX gene Blood RNA kits were used to purify total RNA from 2.5-ml samples of human whole blood collected in PAX gene Blood RNA tubes. RNA concentration and RNA integrity were analyzed with an Agilent 2100 Caliper LabChip Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). The RNA Integrity Number (RIN) values calculated using Agilent 2100 Caliper LabChip Bioanalyzer were sufficient for the samples to be used for real-time PCR analysis; mean RIN for all leukocyte samples was 7.12 ± 0.84. After assessing RNA quality and quantity, individual total RNA samples (2 μm each), random (N6) primers, and Quantiscript Reverse Transcriptase (Qiagen, Tokyo, Japan) were used to synthesize cDNAs.

Table 1
Demographic data of participants in the pilot study.

	MDD	Healthy subjects	p Value
N	25	25	
Male	7	9	
Female	18	16	0.54
Age	43.0 ± 14.0	40.4 ± 11.9	0.26
HAM-D score	22.4 ± 7.1		

Table 2
Demographic data of participants in the replication study.

	MDD (naïve)	Healthy subjects	p Value
N	20 (14)	18	
Male	6	5	
Female	14	13	0.60
Age	47.0 ± 11.9	44.8 ± 11.2	0.56
HAM-D score	20.8 ± 6.5		

Concomitant antidepressants: sertraline 50 mg/day (n = 1), sertraline 25 mg/day (n = 1), fluvoxamine 75 mg/day (n = 1), sertraline 25 mg/day (n = 1), sulpiride 50 mg/day (n = 1), mirtazapine 7.5 mg/day (n = 1).

2.3. PCR array procedure

A customized PCR array plate was used to examine gene expression levels (Fig. 1). This plate had 96 wells and could house a single unique genetic probe in each well. We could use this plate to simultaneously assess the expression level of multiple genes. The following criteria were used to select 40 candidate genes for the pilot study: 1) expression must be altered in the leukocytes of patients with MDD (Hobara et al., 2010; Iga et al., 2005, 2006, 2007a; Numata et al., 2009), 2) expression must be altered by lithium administration (Watanabe et al., 2014), and 3) the gene must be previously associated with the neurobiology of MDD and

Gene Symbol	1	2	3	4	5	6
A		NTF3	ARTN	PDLIM5	GLO1	REST
B	ARRB1	NR3C1	EMP1	NMUR1	HSPH1	PRNP
C	FASLG	IL1B	IL6	IFNG	TNF	HDAC2
D	HDAC5	PDE4B	TNFSF12	CREB1	SLC6A4	VEGFA
E	NSUN7	ARHGAP24	UBE2B	ANK3	IL1R2	PDGFC
F	IL12A	IL15	IL18	CXCL1	MDK	SOCS3
G	MPO	FOS	JUN	ATF2	STAT3	RN18S1
H	ABL1	GAPDH	HPRT1	ACTB	B2M	18S

Fig. 1. Specific criteria were used to select 40 candidate genes; mRNA levels of these candidates were examined simultaneously with custom-made PCR array plates. Expression of genes indicated in blue was lower in patients than in controls. Expression of genes indicated in red was higher in patients than in controls. Genes indicated in green were highly expressed in leukocytes. Based on previous findings, expression of genes indicated in yellow changed following lithium treatment. Genes indicated in gray are housekeeping genes. Abbreviations: NTF3: neurotrophin3, ARTN: artemin, PDLIM5: PDZ and LIM domain5, GLO1: glyoxalase1, REST: RE1-silencing transcription factor, ARRB1: arrestin beta1, NR3C1: nuclear receptor subfamily3 group C member1, EMP1: epithelial membrane protein1, NMUR1: neuromedin U receptor1, HSPH1: heat shock 105 kDa/110 kDa protein 1, PRNP: prion protein, FASLG: Fas ligand, IL1B: interleukin1 beta, IL6: interleukin6, IFNG: interferon gamma, TNF: tumor necrosis factor, HDAC2: histone deacetylase2, HDAC5: histone deacetylase5, PDE4B: phosphodiesterase4B cAMP-specific, TNFSF12: tumor necrosis factor superfamily menber12, CREB1: cAMP responsive element binding protein1, SLC6A4: solute carrier family6 member4, VEGFA: vascular endothelial growth factor A, NSUN7: NOP2/Sun domain family member7, ARHGAP24: Rho GTPase activating protein 24, UBE2B: ubiquitin-conjugating enzyme E2B, ANK3: ankyrin3 node of Ranvier, IL1R2 interleukin1 receptor type II, platelet derived growth factor C, IL12A: interleukin 12A, IL15: interleukin15, IL18: interleukin18, CXCL1: chemokine ligand1, MDK: midkine, SOCS3: suppressor of cytokine signaling3, MPO: myeloperoxidase, FOS: FBJ murine osteosarcoma viral oncogene homolog, JUN: jun proto-oncogene, ATF2: activating transcription factor2, STAT3: signal transducer and activator of transcription3, RN18S1: 18S ribosomal RNA, ABL1: c-abl oncogene1 non-receptor tyrosine kinase, GAPDH: glyceraldehydes-3-phosphate dehydrogenase, HPRT1: hypoxanthine phosphoribosyltransferase1, ACTB: actin beta, B2M beta-2-microglobulin, 18S: 18S ribosomal RNA. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)