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Reduced expression of glyoxalase-1 mRNA in mood disorder patients

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ABSTRACT

Glyoxalase-1 (Glo1) is an antioxidant enzyme which detoxifies α-ketoaldehydes to prevent the accumulation of pro-oxidant compounds, such as methylglyoxal, in all cell types. Glo1 has been suggested to be involved in anxiety disorders, autism, and Alzheimer's disease. Mood disorders have a high rate of comorbidity with anxiety disorders although, to date, little is known of the involvement of Glo1 in the pathophysiology of these conditions. In the present study, we examined the expression levels of Glo1 mRNA in peripheral white blood cells of mood disorder patients to understand the role of Glo1 in mood disorders. Quantitative real-time polymerase chain reaction experiments revealed that reduced expression of Glo1 mRNA was observed in major depressive and bipolar disorder patients in a current depressive state, as compared with healthy control subjects. In contrast, the expression of Glo1 mRNA in major depressive and bipolar patients, in a remissive state, showed no significant alteration when compared with healthy control subjects. These results suggest that the aberrant expression of Glo1 might be involved in the pathophysiology of mood disorders.

A growing body of evidence has implicated a role of chronic or moderate oxidative stress in the pathogenesis of anxiety in humans [2]. Previous clinical investigations have reported an imbalance of antioxidant enzyme activities in patients with social phobia and obsessive-compulsive disorder [17]. Glyoxalase-1 (Glo1) is an antioxidant enzyme that, together with the cofactor glutathione, is involved in the detoxification of α-ketoaldehydes, thereby preventing the accumulation of pro-oxidant compounds such as methylglyoxal [27,28]. The association between altered Glo1 expression levels and anxiety disorders in mice supports the hypothesis that Glo1 is involved in the pathogenesis of these conditions [11,15].

The manifestation of anxiety in a number of psychiatric disorders such as generalized anxiety disorder, depressive disorder, panic disorder, phobia, obsessive-compulsive disorder and post-traumatic stress disorder [6] highlights the importance of gaining a better understanding of common biomarkers for these disorders. The significant association between anxiety and depression in behavioral studies [15] resembles the clinical situation of a high comorbidity between anxiety disorders and major depressive disorder [18]. Although Glo1 has been reported to be associated with anxiety [22], little is known about the involvement of Glo1 in the pathophysiology of mood disorders. To investigate the role of Glo1 in the pathophysiology of mood disorders, we examined the expression levels of Glo1 mRNA in the peripheral white blood cells of major depressive and bipolar disorder patients in a depressive, as well as a remissive, state.

Major depressive and bipolar disorder patients were diagnosed according to the criteria in the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) [1]. These included both outpatients and inpatients of the Division of Neuropsychiatry of the Yamaguchi University Hospital. The extent of the depressive state was assessed by a 21-item "Hamilton depression rating scale" (HDRS). Subjects were classified as being under a current depressive state when they showed a score of more than 18 on HDRS and met the DSM-IV criteria for a major depressive episode. Subjects were classified as being in remission when they showed a score of less than six on HDRS and did not show any symptoms of a major depressive episode in the DSM-IV criteria for more than 2 months. Individuals were excluded from the present study if they had abnormal physical examinations or abnormal results for routine medical laboratory tests such as a complete blood count and renal, liver or thyroid function. Female subjects who were pregnant or took oral contraceptives were also excluded. All healthy control subjects were screened to exclude significant current or past medical or neurological illnesses, significant alcohol or drug abuse and past or current axis I psychiatric illnesses. This protocol was approved by the Institutional Review Board of Yamaguchi University Hospital. Informed written consent was obtained for all subjects.

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Blood sample preparation, total RNA isolation and cDNA synthesis were performed as previously described [19]. In brief, blood was obtained by vein puncture between 10:00 a.m. and 11:00 a.m. and total RNA was isolated using the QIAamp RNA blood mini kit (Qiagen, Chatsworth, CA) according to the manufacturer's manual. One microgram of total RNA was used for cDNA synthesis using random hexamer primers and omniscript reverse transcriptase (Qiagen). The cDNA was stored at −80 °C until use. Quantitative real-time polymerase chain reaction (PCR) was performed in an Applied Biosystems 7300 fast real-time PCR system with SYBR green PCR master mix (Applied Biosystems, Foster City, CA), as previously reported [19]. PCR conditions were 15 min at 95 °C, 45 cycles of 15 s at 95 °C and 30 s at 60 °C. Amplification of the single PCR product was confirmed by monitoring the dissociation curve and electrophoresis on 1% agarose gels stained with ethidium bromide. The expression level of GAPDH mRNA was used for normalization and the expression value was normalized by dividing the mean of the value for control subjects. All measurements were performed in duplicate and two-independent experiments were conducted. The following PCR primers were used: Glo1 forward, 5′-CGAGGATTCGGTCATATTGG-3′; Glo1 reverse, 5′-CCAGGCCTTTC-ATTTTACCA-3′; GAPDH forward, 5′-CAGCCTCAAGATCATCAGCA-3′; GAPDH reverse, 5′-TGTGGTCATGAGTCCTTCCA-3′. A subgroup of subjects in a current depressive state underwent the dexamethasone (Dex)/corticotropin-releasing hormone (CRH) test as previously reported [19]. All data are expressed as means ± standard error of the mean (SEM). Statistical analysis was performed with commercial software (SPSS version 16.0; Chicago, IL). Multivariable analysis was conducted using Glo1 mRNA level as a dependent variable and with age, gender, state (depressive and remissive states) and type of drugs used (antidepressants and mood stabilizers) as independent variables. Gender distribution was analyzed by the χ^2-test. The data of Glo1 mRNA levels were subjected to a factorial analysis of variance (ANOVA) followed by *post hoc* comparison (Dunnett test). The Spearman rank correlation was calculated to assess the correlation between data. Two group comparisons, such as suppressors and non-suppressors of the Dex/CRH test on Glo1 mRNA expression, were performed using the Student's *t*-test. In all cases, *p*-values were two-tailed, and comparisons were considered to be statistically significant for $p < 0.05$.

Table 1 shows the demographic and clinical characteristics of the subjects used in this study. The mean ages were not significantly different among major depressive disorder patients, bipolar disorder patients and healthy control subjects ($F_{(2, 104)} = 1.84$, $p = 0.16$). Regarding the gender distribution, bipolar disorder patients showed a significantly larger ratio of females to males ($\chi^2 = 11.77$, $p = 0.001$). Multivariable analyses demonstrated that the variable "state (depressive and remissive states)" was solely and significantly associated with the expression level of Glo1 mRNA ($p = 0.004$), when analyzed together with the control variables: age, gender, and type of drugs used (antidepressants and mood stabilizers). Quantitative real-time PCR experiments revealed that reduced expression of Glo1 mRNA was observed in major depressive disorder patients ($F_{(2, 58)} = 5.70$, $p < 0.01$) and bipolar disorder patients in a current depressive state ($F_{(2, 58)} = 5.70$, $p < 0.05$), compared with healthy control subjects (Fig. 1). In a remissive state, by contrast, there was no significant difference in the expression levels of Glo1 mRNA in major depressive disorder patients ($F_{(2, 98)} = 0.19$, $p = 0.82$) or bipolar disorder patients ($F_{(2, 98)} = 0.19$, $p = 1.00$), compared with healthy control subjects (Fig. 2). There was a significant correlation between Glo1 mRNA levels and HDRS scores in major depressive disorder patients ($r = -0.358$, $p = 0.005$) (Fig. 3), but not in bipolar disorder patients ($r = -0.198$, $p = 0.187$).

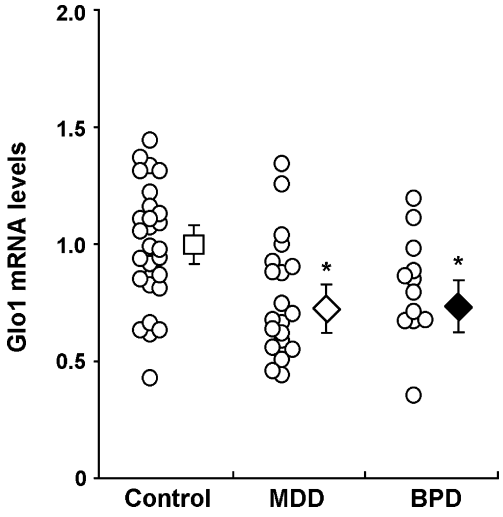

Fig. 1. Expression levels of Glo1 mRNA for mood disorder patients in a current depressive state. Quantitative real-time PCR experiments revealed reduced expression levels of Glo1 mRNA (open circles) for major depressive disorder patients in a current depressive state (MDD, $n = 20$) and bipolar disorder patients (BPD, $n = 13$), as compared to normal control subjects ($n = 28$). Data is represented as means ± S.E.M. (control, open square; MDD, open diamond; BPD, closed diamond). Asterisk represents statistically significant difference at $p < 0.05$.

Dysfunction of the hypothalamic–pituitary–adrenal (HPA) system is the most characteristic biological alteration found in the majority of depressed patients. Accumulating evidence suggests

Table 1
Demographic and clinical characteristics of subjects

	Controls	Patients			
		MDD		BPD	
		Depressive	Remissive	Depressive	Remissive
Number of subjects	28	20	40	13	33
Mean age (years)	50.0 ± 1.8	52.3 ± 3.5	57.2 ± 2.2	55.5 ± 3.5	52.7 ± 2.6
Gender (male/female)	15/13	10/10	15/25	2/11	7/26
HDRS		25.9 ± 1.9	3.3 ± 0.2	24.6 ± 1.0	2.8 ± 0.2
Medication					
No medication	28	3	4	1	0
SSRI/SNRI	0	10	38	9	9
TCA/other antidepressants	0	23	28	6	14
Li	0	0	2	4	17
VPA	0	0	0	7	15
CBZ	0	0	0	2	8

MDD, major depressive disorder; BPD, bipolar disorder; HDRS, Hamilton depression rating scale; SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin–noradrenaline reuptake inhibitor; TCA, tricyclic antidepressant; Li, lithium; VPA, valproic acid; CBZ, carbamazepine.

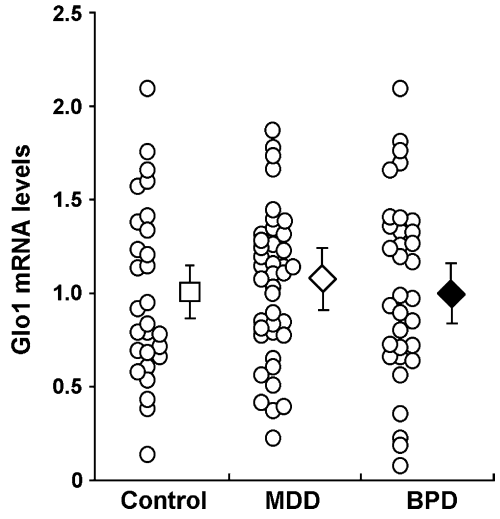

Fig. 2. Expression levels of Glo1 mRNA for mood disorder patients in a remissive state. Quantitative real-time PCR experiments revealed that expression of Glo1 mRNA (open circles) for major depressive disorder patients in a remissive state (MDD, $n = 40$) and bipolar disorder patients (BPD, $n = 33$) were not significantly different to that of normal control subjects ($n = 28$). Data is represented as means ± S.E.M. (control, open square; MDD, open diamond; BPD, closed diamond).

that the combined Dex/CRH test is highly sensitive and is able to detect HPA system abnormalities [10]. ACTH and cortisol responses to this test are exaggerated in depressed patients [7,8]. To examine the association between Glo1 mRNA levels and HPA axis activity, the mRNA levels for Glo1 of mood disorder patients in a current depressive state were compared between suppressors ($n = 11$; 8 major depressive disorder patients and three bipolar disorder patients) and non-suppressors ($n = 15$; 8 major depressive disorder patients and seven bipolar disorder patients) of the Dex/CRH test. There was no significant difference in the expression levels of Glo1 mRNA between suppressors and non-suppressors ($F_{(1,24)} = 3.68$, $p = 0.67$). In addition, there was no significant correlation between Glo1 mRNA levels and the plasma cortisol concentration in healthy control subjects ($r = -0.09$, $p = 0.72$), major depressive disorder patients ($r = 0.42$, $p = 0.27$) or bipolar disorder patients ($r = -0.50$, $p = 0.39$).

Previous reports have suggested the involvement of Glo1 in neuropsychiatric disorders, including anxiety disorders and autism. A significant association of the Glo1 Ala111Glu polymorphism has been observed in a subgroup of patients with panic disorder without agoraphobia [22] and patients with autism [12]. Reduced Glo1 enzyme activity has also been observed in the brains of patients

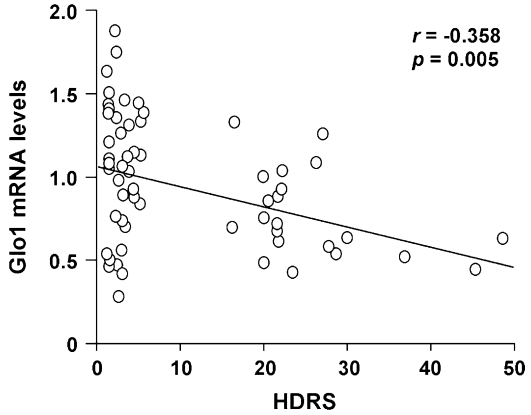

Fig. 3. Significant inverse correlation between HDRS scores and Glo1 mRNA levels was found in the major depressive disorder patients ($n = 60$). HDRS, Hamilton depression rating scale.

with autism [12]. Moreover, a possible association between Glo1 and mood disorders has been found in a linkage study of families with mood disorders [26]. There is a wealth of data demonstrating the comorbidity of mood disorders with anxiety disorders [3,31,24], including panic disorder [5,13,25]. Genetic data with regard to panic disorder and major depressive disorder have been inconsistent, although there is some evidence for a shared diathesis for anxiety and depression [29]. These data suggest an important role for Glo1 in the pathophysiology of many neuropsychiatric disorders, especially with regard to the anxiety symptoms of these conditions.

Krömer et al. [15] have reported an association between reduced Glo1 expression and high anxiety-like behaviors in mice. Importantly, the reduced expression of Glo1 was observed not only in the amygdala, but also in peripheral red blood cells [15], suggesting that the expression levels of Glo1 in the brain is well correlated with that in peripheral blood cells. These data and our present study raise the possibility that the expression levels of Glo1 in mood disorder patients may be reduced in multiple systems. However, a recent study has shown that local overexpression of Glo1 in various brain regions, e.g. cingulate cortex, resulted in increased anxiety-like behavior [11]. This finding is discordant with that of Krömer et al. [15] and thus, it is still unclear how Glo1 is involved in the pathophysiology of anxiety and depression.

A previous report has shown that the number of Glo1 immunopositive neurons and astroglia increase up to, approximately, 55 years of age and decrease progressively thereafter in humans [16]. Glo1 mRNA levels also showed a biphasic course similar to those observed with protein determination [16], suggesting that the expression of Glo1 is primarily regulated at the transcriptional level. The promoter region of the human Glo1 gene contains several consensus sequences for known transcriptional regulatory elements, including: insulin responsive element, metal responsive element and glucocorticoid responsive element [23]. The existence of the glucocorticoid responsive element in the human Glo1 promoter is particularly interesting, because the glucocorticoid receptor (GR) has been shown to be associated with mood disorders and in the adaptation to stress [4,9,20]. Reduced expression of GRα has been observed in the cerebral cortex, hippocampus and amygdala in mood disorder patients [30,14,21]. In addition, we have previously reported that the expression of GRα mRNA is also reduced in the peripheral white blood cells of mood disorder patients [19]. This raises the possibility that dysfunction of GR plays a causal role in the aberrant Glo1 expression observed in mood disorder patients.

Considering our results from multivariable analysis and the significant correlation between Glo1 mRNA levels and HDRS scores in major depressive disorders, it could be interpreted that the reduced expression of Glo1 mRNA is "state-dependent" at least in major depressive disorders. However, our study has the limitation that all the patients were on medication; therefore, we cannot exclude completely the influence of medication on the expression levels of Glo1 mRNA. To our knowledge, however, there is no evidence showing altered levels of Glo1 expression by treatment with antidepressants or mood stabilizers *in vitro* or *in vivo*. Further study conducted in medication-free subjects is needed to elucidate this issue.

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