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Genetic analysis of the glyoxalase system in schizophrenia

Fabian N. Bangel [a,b], Kazuo Yamada [b], Makoto Arai [c], Yoshimi Iwayama [b], Shabeesh Balan [b], Tomoko Toyota [b], Yasuhide Iwata [d], Katsuaki Suzuki [d], Mitsuru Kikuchi [e], Tasuku Hashimoto [f], Nobuhisa Kanahara [f], Norio Mori [d], Masanari Itokawa [c], Oliver Stork [a], Takeo Yoshikawa [b,*]

[a] Department of Genetics and Molecular Neurobiology, Institute of Biology, Otto-von-Guericke University Magdeburg, Magdeburg, Germany
[b] Laboratory for Molecular Psychiatry, RIKEN Brain Science Institute, Saitama, Japan
[c] Department of Psychiatry and Behavioral Sciences, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan
[d] Department of Psychiatry, Hamamatsu University School of Medicine, Shizuoka, Japan
[e] Department of Psychiatry and Neurobiology, Kanazawa University Graduate School of Medicine, Kanazawa, Japan
[f] Department of Psychiatry, Graduate School of Medicine, Chiba University, Chiba, Japan

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ABSTRACT

Recent reports suggest that carbonyl stress might affect a subset of schizophrenia patients suffering from severe symptoms. Carbonyl stress protection is achieved by the glyoxalase system consisting of two enzymes, glyoxalase 1 and 2, which in humans are encoded by the genes *GLO1* and *HAGH*, respectively. Glyoxalase 1 and 2 catalyze the detoxification of reactive alpha-oxoaldehydes such as glyoxal and methylglyoxal, which are particularly damaging components of carbonyl stress. Here, we investigated the role of the glyoxalase system in schizophrenia by performing association analyses of common genetic variants ($n = 12$) in *GLO1* and *HAGH* in a Japanese sample consisting of 2012 schizophrenia patients and 2170 healthy controls. We detected a nominally significant association with schizophrenia ($p = 0.020$) of rs11859266, a SNP in the intronic region of *HAGH*. However, rs11859266 did not survive multiple testing (empirical $p = 0.091$). The variants in *HAGH*, rs11859266 and rs3743852, showed significant associations with schizophrenia in males at allelic and genotype levels, which remained persistent after multiple testing with the exception of rs3743852 for the genotype model. We further measured the mRNA expression of both genes in postmortem brain, but did not detect any changes in transcript expression levels between case and control samples or in sex-specific comparisons. Therefore, our findings suggest that an explanation of elevated carbonyl stress in a substantial part (reported as ~20%) of patients with schizophrenia will require the examination of a much larger cohort to detect risk alleles with weak effect size and/or other risk factors.

1. Introduction

Despite extensive research, the pathological mechanisms of schizophrenia are still poorly understood. Recently, several studies reported that a subset of schizophrenia is strongly associated with biomarkers for carbonyl stress in the Japanese and French populations (Arai et al., 2010; Katsuta et al., 2014; Kouidrat et al., 2013; Miyashita et al., 2014a,b). Carbonyl stress is the accumulation of toxic reactive carbonyl compounds such as glyoxal and methylglyoxal, which promote the formation of advanced glycation end products (AGEs) (Fig. 1). AGE accumulation has been linked to aging and a variety of diseases, including diabetes, renal insufficiency, atherosclerosis and neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease (Bierhaus et al., 1998; Grillo and Colombatto, 2008; Jaisson and Gillery, 2010; Takeuchi and Yamagishi, 2009). In 2009, it was discovered that alterations of AGE-mediated signaling are associated with schizophrenia (Steiner et al., 2009). In 2010, Arai et al. reported enhanced AGE levels in schizophrenia patients (Arai et al., 2010), which has been confirmed by the following studies (Arai et al., 2010; Katsuta et al., 2014; Kouidrat et al., 2013; Miyashita et al., 2014a,b).

The glyoxalase system plays a key role in carbonyl stress protection by detoxification of reactive carbonyl compounds. It consists of two enzymes, glyoxalase 1 (GLO1) and glyoxalase 2 (HAGH, also known as GLO2), which catalyze the detoxifying reaction of alpha-oxoaldehydes such as glyoxal and methylglyoxal to aldonic acids, using glutathione as a cofactor (Fig. 1). Alpha-oxoaldehydes are particularly damaging components of carbonyl stress. They promote AGE formation and

Abbreviations: AGE, advanced glycation end products; GLO1, glyoxalase 1; HAGH, hydroxyacyl glutathione hydrolase; DSM-IV, The Diagnostic and Statistical Manual of Mental Disorders-IV; SNP, single nucleotide polymorphism; LD, linkage disequilibrium.

* Corresponding author at: Laboratory for Molecular Psychiatry, RIKEN Brain Science Institute, 2-1 Hirosawa, Wako-city, Saitama 351-0198, Japan. Tel.: +81 48 467 5968; fax: +81 48 467 7462.
E-mail address: takeo@brain.riken.jp (T. Yoshikawa).

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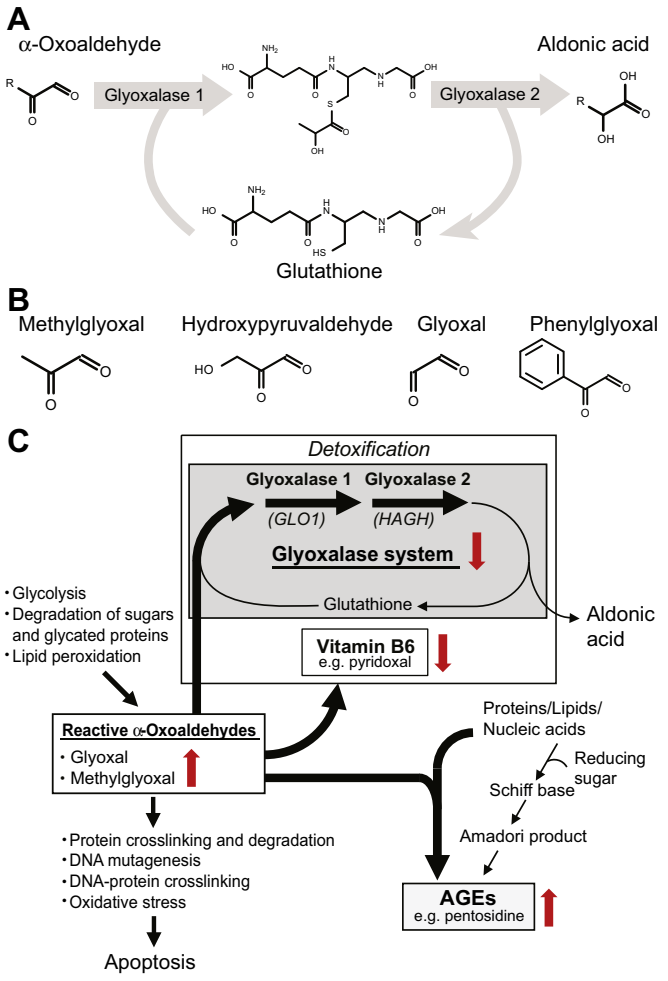

Fig. 1. The glyoxalase system and its role in eliminating carbonyl stress observed in schizophrenia. (A) The glyoxalase system consists of two enzymes, glyoxalase 1 and glyoxalase 2, which subsequently catalyze the detoxification of reactive carbonyl compounds. (B) The glyoxalase enzymes metabolize a broad spectrum of substrates including the main substrate glyoxal and other alpha-oxoaldehydes at lower turnover rates. (C) Hypothetical contribution of reduced glyoxalase function to alterations of carbonyl stress markers in schizophrenia patients (based on the observation by Arai et al., 2010).

contribute to protein, DNA and lipid membrane damage and oxidative stress aggravation. Therefore, the detoxification of alpha-oxoaldehydes is crucial for the protection against oxidative and carbonyl stress.

According to previous studies, while it is estimated that around 20% of the general schizophrenia population might suffer from carbonyl stress, causative variants of *GLO1* are found only in less than 1% (Arai et al., 2010; Itokawa et al., 2014). Therefore, in this study, we set out to examine the effects of common genetic variants of *GLO1* and *HAGH* in a large Japanese sample (2012 schizophrenia patients and 2170 controls). Further, we analyzed *GLO1* and *HAGH* mRNA expressions using postmortem brain samples. To our knowledge, this is the first study investigating the role of common variations and brain expression levels of the glyoxalase system genes in schizophrenia.

2. Experimental methods

2.1. Subjects

For genotyping, we examined 4182 unrelated Japanese as a case–control sample consisting of 2012 schizophrenia patients (1111 males, 901 females, mean age ± SD = 48.13 ± 14.40 years) and 2170 healthy controls (889 males, 1281 females, mean age ± SD = 42.40 ± 14.22 years) (Supplementary Table 1). Diagnosis of schizophrenia was based on Diagnosis and Statistical Manual of Mental Disorders IV (DSM-IV) criteria and was confirmed by at least two experienced psychiatrists. Exclusion of any history of psychiatric disorders in control subjects was confirmed by direct interviews by experienced psychiatrists.

All participants were recruited from Honshu (the largest Japanese island), which is part of a single genetic cluster (Yamaguchi-Kabata et al., 2008). We previously reported evidence that population stratification is negligible in our sample (Balan et al., 2014; Yamada et al., 2004, 2006). After providing information about study procedures and objections, written informed consent for participation was obtained from all case and control subjects. This study was carried out in line with the principles expressed in the Declaration of Helsinki. The study was reviewed and approved by the ethics committees of all participating institutes.

2.2. Postmortem brain tissues

Human postmortem brain tissues for expression analysis were obtained from the Maryland Brain Collection (Maryland Psychiatric Research Center, University of Maryland School of Medicine, http://www.mprc.umaryland.edu/mbc.asp), which include Brodmann area (BA) 46 (35 schizophrenia patients and 35 controls) and the hippocampal CA1 region (20 patients and 20 controls). Patients and controls were age-matched and did not differ in terms of postmortem interval and sample pH (Balan et al., 2013). No information was available on the comorbidity of diabetes mellitus and chronic kidney diseases (the two major causes of elevated AGEs).

2.3. Tag single nucleotide polymorphism (SNP) selection and genotyping

Tag SNP selection was performed by using the NIEHS LD TAG SNP Selection database (http://snpinfo.niehs.nih.gov/snpinfo/snptag.htm). Selected tag SNPs covered the full target genes including at least 5 kb of the 5′ and 3′ flanking regions. Selection was based on the following criteria: population, Japanese in Tokyo [JPT]; minor allele frequency ≥ 0.10; linkage disequilibrium (LD) threshold $r^2 \geq 0.80$. Selected SNPs are listed in Supplementary Table 2. For genotyping, we used the TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA), and for genotype calls we used the SDS (Sequence Detection Software) version 2.4 (Applied Biosystems).

2.4. Quantitative gene expression analysis

Total RNA was extracted using the miRNAeasy Mini kit (QIAGEN GmbH, Hilden, Germany). Synthesis of single-stranded cDNA was performed using the SuperScript VILO cDNA synthesis kit (Life Technologies Co., Carlsbad, CA, USA). Experimental protocols for both procedures were in accordance with the manufacturer's instructions.

We obtained TaqMan probes and primers for *GLO1* (Hs00198702_m1), *HAGH* (Hs00193422_m1) and *GAPDH* (Hs02758991_g1, used as an internal control) from TaqMan® Gene Expression Assays (Applied Biosystems). The QuantStudio 12 K Flex Real-Time PCR System (Life Technologies) was used for quantitative real-time PCR (qRT-PCR) analysis. Average values from triplicate qRT-PCR reactions were measured using the standard curve method.

2.5. Statistical analysis

Association analysis in case–control samples was performed using the PLINK software (http://pngu.mgh.harvard.edu/purcell/plink/) v1.07 (Purcell et al., 2007). We used Fisher's exact test to compare allele and genotype frequencies between patients and controls. To correct for multiple testing, permutation analysis was done (10,000 permutations) using the PLINK software. The LD pattern and haplotype structure were computed using the Haploview 4.2 software (http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-