DNA methylation of SHATI/NAT8L promotor sites in the blood of unmedicated and medicated patients with depression

Hajime MIyanishi[1], Kyosuke Uno[1], Mina Iwata[1], Yuu Kikuchi[1], Hidenaga Nagamori[2], Yuka Yasuda[2], Kazutaka Ohi[2], Ryota Hashimoto[2,3], Tomiki Sumiyoshi[4,5], Atsumi Nitta[1]

[1]University of Toyama, Toyama, JAPAN, [2]Department of Psychiatry, Osaka University Graduate School of Medicine, Osaka, Japan, [3]Department of Pathology of Mental Diseases, National Institute of Mental Health, National Center of Neurology and Psychiatry, Tokyo, Japan, [4]Department of Preventive Intervention, National Institute of Mental Health, National Center of Neurology and Psychiatry, Tokyo, Japan, [5] Bio Bank of National Center of Neurology and Psychiatry, Tokyo, Japan

Introduction

The prevalence of psychiatric diseases, such as depression, schizophrenia, and bipolar disorder, is increasing worldwide. Of these conditions, depression is one of the most common ones that are often resistant to treatment (Trivedi et al., 2006). For example, antidepressants are associated with a relatively low response rate in 70%–80% of patients (Jiang et al., 2018).

The reliability of diagnosis of psychiatric diseases is an issue of concern. One of the main reasons is the lack of an objective examination or biomarker, and physicians make diagnosis on the basis of the International Classification of Disease-10 by the World Health Organization or the Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5) by the American Psychological Association. Elucidation of biomarker for psychiatric diseases, like depression, would be expected to lead to earlier intervention and treatment (Halfin, 2007), and consequently, improvement of QOL of patients, families, and society as a whole.

The SHATI/NAT8L gene shows N-acetyl transfer activity, encodes a protein synthesizing N-acetylaspartate (NAA) from aspartate and acetyl-CoA. NAA is biosynthesized to N-acetylaspartylglutamate (NAAG) by condensation with glutamic acid by NAAG synthetase (Becker et al., 2010). Levels of both NAA and NAAG have been reported to be changed in postmortem brains of patients with psychiatric diseases (Reynolds & Reynolds, 2011). Given this set of evidence, SHATI/NAT8L concentrations are thought to help diagnose psychiatric diseases.

From the perspective of epigenetics deeply involved in psychiatric diseases (Petronis, 2004), attempts to find biomarkers using SHATI/NAT8L are worthwhile. It has been reported that methylation of SHATI/NAT8L is altered at the promoter site in a mouse model of schizophrenia, possibly providing a biomarker (Uno et al., 2016). Methylation of DNA is a chemical reaction carried out by DNA methyltransferase (DNMT), which adds a carbon atom to the fifth position of the pyrimidine ring of cytosine. Methylation mainly occurs at CpG sites, a nucleotide sequence in which guanine appears next to cytosine. Regions containing a high amount of CpG are called a CpG island (Bird, 1986). Methylation of CpG islands is thought to be involved in the regulation of gene expression in many diseases (Bird, 1984). However, gene expression can be influenced by epigenetic changes caused by medication (Jin et al., 2017). Thus, when searching for peripheral biomarkers, the effect of medication should be carefully considered (Kageyama et al., 2017). Specifically, a history of medication should be controlled for in the comparison of data between patients and healthy control subjects.

In the present study, we collected DNA samples in the blood from unmedicated patients from all over Japan. The extent of methylation of SHATI/NAT8L DNA was examined. To our knowledge, this is the first attempt to measure methylation of SHATI/NAT8L in drug-naive patients with psychiatric diseases.

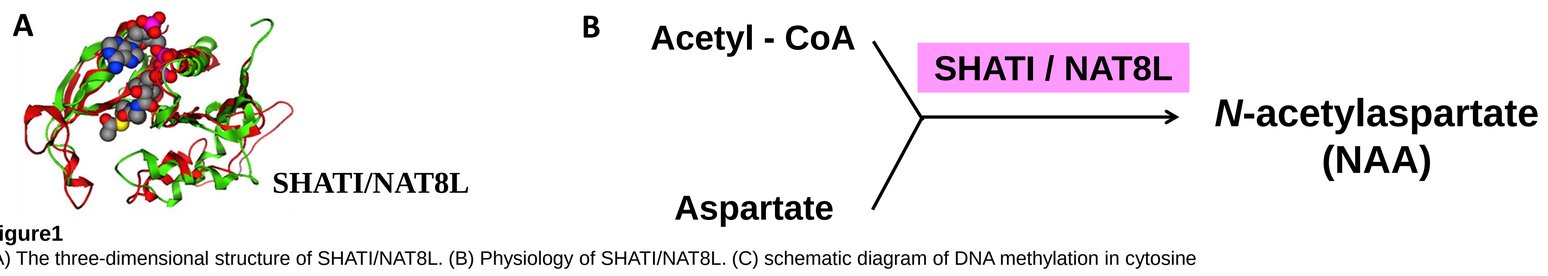


Figure1
(A) The three-dimensional structure of SHATI/NAT8L. (B) Physiology of SHATI/NAT8L. (C) schematic diagram of DNA methylation in cytosine

Methods

Study design and subjects

This study was performed in accordance with the Declaration of Helsinki and was approved by the institutional review board at the University of Toyama (permit number 24-6). Approved samples from the NCNP Biobank (the only high-quality biobank in Japan) and the COCORO consortium at the University of Osaka (permit number 423) were used. All subjects provided written informed consent. DNA samples were obtained from 81 patients diagnosed of schizophrenia, bipolar disorder, or depression. Each patient had been diagnosed and assessed by at least two trained psychiatrists according to DSM-IV, criteria based on a structured clinical interview for DSM-IV axis I disorders (SCID-I). Of these, 41 patients from NCNP Biobank were untreated. They have never taken psychotropic drugs including typical antipsychotics and atypical antipsychotic. Whereas 40 patients from the University of Osaka were treated. Patients ranged in age from 12 to 60 years old. For controls, 69 subjects aged 12 years and older were recruited from the NCNP Biobank and the University of Osaka. Detailed subject characteristics are shown in Table 1. Patients with a history of drug or alcohol dependence, head injury, or epilepsy were excluded. Patients with an IQ below 75 and pregnant women were also excluded. The small sample size reflects the difficulty in recruiting unmedicated patients with depression.

Table1. Participant characteristics.

Sample 1		n	Age (years)	Sex (male/female)
Control		29	36.43 ± 2.48	15/14
Patient (no-treatment)	Mood disorder	20	40.75 ± 2.58	12/8
	Schizophrenia	19	33.00 ± 3.26	8/11
	Bipolar disorder	2	39.75 ± 8.12	2/2

Sample 2		n	Age(years)	Sex(male/female)
Control		40	54.65 ± 0.91	15/25
Patient (treatment)	Mood disorder	40	54.90 ± 2.11	14/26

Data are expressed as mean ± SEM

Bisulfite conversion

Genomic DNA was used in bisulfite reactions, in which unmethylated cytosine residues were converted to uracil residues, using an EpiTect Bisulfite Kit (Qiagen, Hilden, Germany) according to the manufacturer's guidelines. DNA Protect Buffer, bisulfite solution, and DNA (200 ng) were mixed and incubated under the cycle conditions recommended by the manufacturer using a TaKaRa PCR Thermal Cycler Dice® Gradient (TaKaRa, Shiga, Japan). The bisulfite conversion thermal cycler conditions were as follows: 1 cycle at 95 ° C for 5 min, 1 cycle at 60 ° C for 10 min, 1 cycle at 95 ° C for 5 min, and 1 cycle at 60 ° C for 10 min. Converted DNA was then purified and eluted with elution buffer.

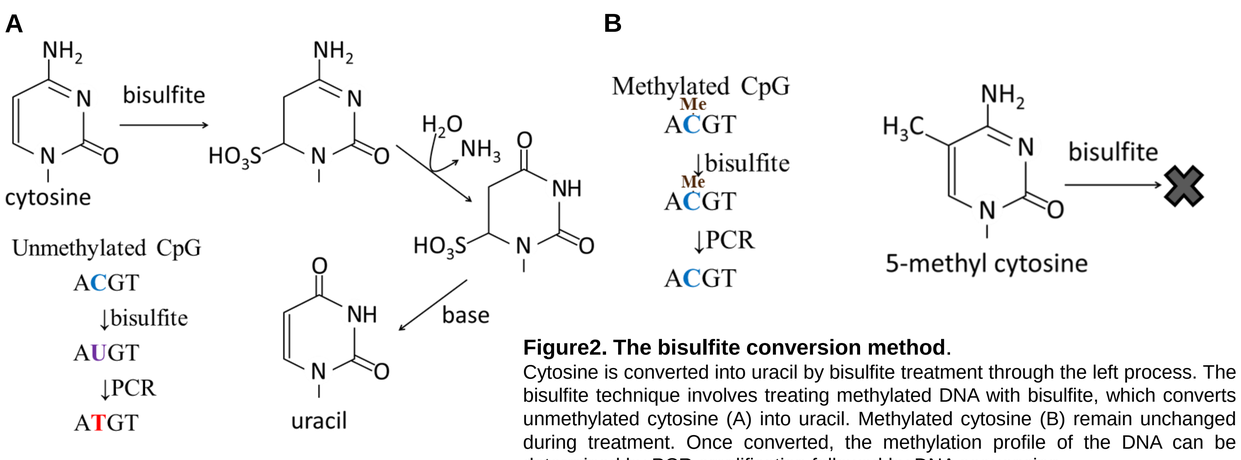


Figure2. The bisulfite conversion method.
Cytosine is converted into uracil by bisulfite treatment through the left process. The bisulfite technique involves treating methylated DNA with bisulfite, which converts unmethylated cytosine (A) into uracil. Methylated cytosine (B) remain unchanged during treatment. Once converted, the methylation profile of the DNA can be determined by PCR amplification followed by DNA sequencing.

PyroMark PCR

To prepare a single-stranded PCR product for use in the subsequent pyrosequencing procedure, one PCR primer must be labeled with biotin at its 5' mark. PCR primers were designed by PyroMark Assay Design Software 2.0 (www.qiagen.com). PyroMark PCR was performed using the PyroMark PCR Kit (Qiagen) according to the manufacturer's guidelines. PCR Master Mix, CoralLoad Concentrate, primers, RNase-free water, and converted DNA (20 ng) were briefly mixed. The reaction was performed under the recommended cycle conditions with a TaKaRa PCR Thermal Cycler Dice® Gradient (TaKaRa): 1 cycle at 95 ° C for 15 min; 45 cycles at 94 ° C for 30 s, 56 ° C for 30 s, and 72 ° C for 30 s; and 1 cycle at 72 ° C for 10 min. The PCR primer sequences used were as follows: 5'-GGAGTTATGTGGGATTTTTAAAGATTA-3' and 5'-AAAAAAACAAAATACCTTCCAACAAATTACC-3' as primers for the CpG sites from the transcription start site (TSS); -1714, -1700, -1696 bp (up and down), 5'-GGGTAATTGTTGGAAGGTATT-3' and 5'-CCCCCTCAATATC TAAAACC-3' as primers for the CpG sites from TSS; -1532, -1509, -1492, -1482, and 1480 bp (up and down).

Pyrosequencing

Pyrosequencing was performed according to the PyroMark Q24 User Manual. Streptavidin beads (GE Healthcare, Buckinghamshire, UK), PyroMark binding buffer (Qiagen), PCR product, and water were mixed and then agitated for 10 min using a mixer at 1400 rpm. The sequencing primer was diluted to 0.3 μM in annealing buffer (Qiagen), and the solution was added to each PyroMark Q24 Plate (Qiagen). PCR products were separated, denatured, washed, and added to the sequencing primer in annealing buffer using the PyroMark Q24 Vacuum Workstation (Qiagen). The primer was annealed by heating to 80 ° C for 2 min and then cooling to room temperature (25 ° C). PyroMark Gold Q96 reagents (Qiagen), which were used for the reaction and the PCR product plate, were set and analyzed by the PyroMark Q24 device (Qiagen). The following primers were designed by PyroMark Assay Design Software 2.0 (www.qiagen.com): 5'-AGAGATATTTGAGTATAGGGTTTTAGT-3' as the primer for CpG sites from TSS: -1714, -1700, -1696, and 5'-ATTTTTGGTGAATTTAGGAG-3' as the primer for CpG sites from TSS: -1532, -1509, -1492, -1482, and -1480.

Statistical analysis

All data are shown as the mean ± SEM. Student's t-test was used to compare differences in the means between the two groups. The level of statistical significance was set at $p < 0.05$.

Results

Methylation rates of SHATI/NAT8L DNA in peripheral blood increased in patients without treatment.

		Subject			
		Control	Patient		
			Mood disorder	Schizophrenia	Bipolar disorder
CpG site (position from the transcription start site)	-1714	78.05 ± 0.53	78.77 ± 0.56	77.54 ± 0.88	77.79 ± 2.35
	-1700	48.90 ± 0.53	51.27 ± 0.80*	48.60 ± 0.94	48.27 ± 1.47
	-1696	31.77 ± 0.42	32.82 ± 0.50	31.28 ± 0.65	32.27 ± 1.43
	-1532	34.62 ± 0.47	36.52 ± 0.75*	34.71 ± 0.81	33.71 ± 0.49
	-1509	35.93 ± 0.26	36.63 ± 0.52	35.92 ± 0.27	34.86 ± 1.18
	-1492	42.48 ± 0.44	44.63 ± 0.53***	42.21 ± 0.61	42.18 ± 1.84
	-1482	48.88 ± 0.61	49.97 ± 0.75	48.47 ± 0.86	47.93 ± 1.06
	-1480	31.01 ± 0.39	31.78 ± 0.72	31.44 ± 0.75	30.17 ± 0.48

Data are expressed as mean ± SEM. *p < 0.05, ***p < 0.005

Table2. Methylation rates of SHATI/NAT8L DNA in peripheral blood from patients without treatment..

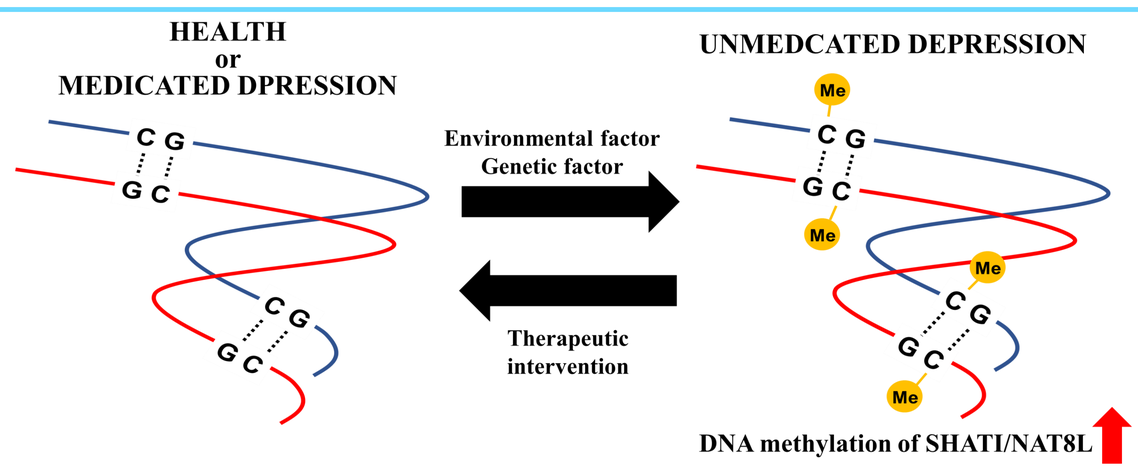
Methylation rates of SHATI/NAT8L DNA in peripheral blood decreased in patients with treatment.

		Subject	
		Control	Patient
			Mood disorder
CpG site (position from the transcription start site)	-1714	78.36 ± 0.35	78.40 ± 0.34
	-1700	51.04 ± 0.25	50.97 ± 0.30
	-1696	35.55 ± 0.40	33.81 ± 0.30***
	-1532	34.76 ± 0.39	34.08 ± 0.41
	-1509	36.62 ± 0.27	35.33 ± 0.24***
	-1492	42.98 ± 0.41	41.88 ± 0.67
	-1482	48.20 ± 0.46	47.65 ± 0.49
	-1480	31.06 ± 0.34	31.33 ± 0.47

Data are expressed as mean ± SEM. ***p < 0.005

Table3. Methylation rates of SHATI/NAT8L DNA in peripheral blood from patients with treatment.

Conclusions



DNA methylation in SHATI/NAT8l promoter sites could be new diagnostic marker for depression and evaluation marker of antidepressant therapy.