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Research article

A part of patients with autism spectrum disorder has haploidy of HPC-1/syntaxin1A gene that possibly causes behavioral disturbance as in experimentally gene ablated mice

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HIGHLIGHTS

- STX1A gene ablated mice exhibit abnormal behavioral profiles, similar to human autistic symptoms.
- Some ASD patients were haploid for the STX1A gene similar to STX1A heterozygote mutant mice.
- In ASD patients with STX1A gene haploidy, STX1A mRNA expression was reduced to about half of controls.
- A part of ASD patients had haploidy of STX1A gene and lower STX1A gene expression.

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ABSTRACT

Autism spectrum disorder (ASD) is highly heritable and encompasses a various set of neuropsychiatric disorders with a wide-ranging presentation. HPC-1/syntaxin1A (STX1A) encodes a neuronal plasma membrane protein that regulates the secretion of neurotransmitters and neuromodulators. STX1A gene ablated mice (null and heterozygote mutant) exhibit abnormal behavioral profiles similar to human autistic symptoms, accompanied by reduction of monoamine secretion. To determine whether copy number variation of STX1A gene and the change of its expression correlate with ASD as in STX1A gene ablated mice, we performed copy number assay and real-time quantitative RT-PCR using blood or saliva samples from ASD patients. We found that some ASD patients were haploid for the STX1A gene similar to STX1A heterozygote mutant mice. However, copy number of STX1A gene was normal in the parents and siblings of ASD patients with STX1A gene haploidy. In ASD patients with gene haploidy, STX1A mRNA expression was reduced to about half of their parents. Thus, a part of ASD patients had haploidy of STX1A gene and lower STX1A gene expression.

1. Introduction

According to the *Diagnostic and Statistical Manual of Mental Disorders*, 4th Edition (*DSM-IV*), autism spectrum disorder (ASD) is characterized by three core symptoms: impaired social interactions, communication difficulties, and repetitive and restricted behavior and interests. ASD affects more than 1% of the general population [1]. Both family and twin studies indicate that ASD is a highly heritable neuropsychiatric disorder [2,3], with a heritability of approximately 50% [4–6], but the identity and number of genes responsible for ASD are not yet known. However, recently, tradi-

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; ASD, autism spectrum disorder; CNS, central nervous system; CNV, copy number variation; *DSM-IV*, *Diagnostic and Statistical Manual of Mental Disorders-IV*; OXT, oxytocin; OXTR, oxytocin receptor; qRT-PCR, real-time quantitative reverse transcription polymerase chain reaction; SNAP-25, 25 kDa synaptosomal-associated protein; SNARE, soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein receptor; SSRI, selective serotonin reuptake inhibitor; STX1A, HPC-1/syntaxin1A; VAMP-2, vesicle-associated membrane ptotein-2; WS, Williams syndrome.

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tional genetic and genomic studies have identified over 100 genes, and about 50 genomic loci (some including these genes) have been implicated by recurrent chromosomal abnormalities or copy number variations (CNVs) [7,8]. It is generally assumed that the genes involved in ASD pathogenesis are associated with neuronal signal transmission, such as synaptic scaffolding molecules, cell adhesion molecules, secreted proteins, and receptors and transporters with synaptic functions [9–11]. In contrast, epigenetic and environmental factors also contribute to ASD [12–14].

HPC-1/syntaxin1A (STX1A) is abundantly expressed in most neurons and localizes to the neuronal synaptic plasma membrane [15,16]. STX1A is one of neuronal soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein receptors (SNAREs), which form the molecular machinery driving vesicle exocytosis [17,18], and regulates the secretion of neurotransmitters and neuromodulators, including monoamines and neuropeptides. In addition, STX1A modulates the activity of various plasma membrane-bound monoamine (*e.g.*, noradrenaline, serotonin, and dopamine) transporters [19–22]. STX1A is also closely related to synaptic plasticity, which is strongly influenced by neuromodulators [23–25]. Previously, we generated STX1A gene ablated mice (null and heterozygote mutant) [26]. STX1A gene ablated mice showed normal, fast synaptic transmission of glutamate and GABA [26], while monoamine release was reduced in those mice [27]. Furthermore, STX1A gene ablated mice exhibited abnormal recognition profiles, which recovered with selective serotonin reuptake inhibitor (SSRI) treatment [27], and hypersensitivity in pain and auditory sensations [27] and [28]. These unusual properties resemble the symptoms observed in ASD patients. Furthermore, STX1A gene has been implicated in neuropsychiatric alterations, for example, schizophrenia [29], attention-deficit/hyperactivity disorder (ADHD) [30,31], and ASD [32–35]. In particular, STX1A gene expression was reported to be disturbed in ASD patients [32,33], and we also showed that there was a wide variation of STX1A gene expression in ASD patients [36,37].

Here, we studied human blood (leucocyte) or saliva samples to determine whether reduction of STX1A gene and the change of its expression correlate with ASD. Interestingly, STX1A gene haploidy as in STX1A heterozygote mutant mice was detected in a part of ASD patients, and those ASD patients with gene haploidy showed lower STX1A mRNA expression. Furthermore, copy number of STX1A gene was normal in the parents and siblings of ASD patients with gene haploidy. Thus, in some ASD patients, there is reduction of STX1A gene expression due to gene haploidy, which may cause unusual neuropsychological features as observed in STX1A gene ablated mice.

2. Materials and methods

2.1. Subjects

The study was approved by the Ethics Committees of the Kyorin University School of Medicine (no. 242-02) and Faculty of Health and Welfare, Prefectural University of Hiroshima (no.7 and no. 14MH047).

ASD patients were enrolled based on the *DSM-IV* criteria. Blood samples were obtained from 69 ASD patients (40 drug-naïve patients, age 6–23 years, 13.7 ± 3.3 years (mean ± SD) and 54 healthy controls (age 9–42 years, 25.7 ± 9.3 years). Saliva samples were also obtained from another 14 ASD patients (age 8–17 years, 11.4 ± 3.0 years) and 8 healthy controls (age 37–46 years, 42.6 ± 3.5 years). All the patients from whom blood samples were obtained were males. Saliva samples were obtained from one female ASD patient and two female controls. Blood or saliva samples were also prepared from the parents and siblings of ASD patients with STX1A gene haploidy. All the participants were of Japanese origin, and written informed consent was obtained from all participants including caretakers/guardians on behalf of the minors/children enrolled in this study.

2.2. Copy number assay

Peripheral blood was drawn from the cubital vein into heparin-coated plastic syringes containing RNase inhibitor. Genomic DNA was extracted from blood containing leucocytes with the Wizard Genomic DNA purification Kit (Promega, Madison, WI, USA) or extracted from saliva using the Oragene DNA kit (DNA Gentech Inc., Ottawa, Canada), according to the manufacturer's instructions. DNA was used as template for real-time quantitative RT-PCR (qRT-PCR). qRT-PCR was performed using the ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). TaqMan primer/probes for STX1A and for ribonuclease P (used as the endogenous reference) were purchased from Applied Biosystems (Taq-Man Copy Number Assays Hs00483831_cn and Hs04989378_cn for STX1A). DNA aliquots (5 ng) were amplified in 20 μl PCR reaction reagents (TaqMan Genotyping Master Mix, Applied Biosystems) containing the above TaqMan primer/probes. All reactions were performed in triplicate, according to the manufacturer's protocol. Copy number was determined using CopyCaller ver2.0 software (Applied Biosystems).

2.3. Gene expression analysis

Total RNA was extracted from blood described above using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA), and RNA quality was checked as range of A260/A280 ratio ($\geq$ 1.8). First strand complementary DNA (cDNA) was synthesized from RNA using oligo-dT primers, random primers, and reverse transcriptase (Takara Bio., Shiga, Japan). cDNA was used as template for qRT-PCR in the same system as for copy number assay. TaqMan primer/probes for STX1A and glyceraldehyde-3-phosphate dehydrogenase (used as the endogenous reference) were purchased from Applied Biosystems (TaqMan Gene Expression Assay Hs00270282_m1 for STX1A). cDNA aliquots (corresponding to 2 μg total RNA) were amplified in 50 μl of PCR reaction reagents (TaqMan Universal PCR Master Mix, Applied Biosystems) containing the above TaqMan primer/probes. All reactions were performed in triplicate, according to the manufacturer's protocol. Validation of the comparative threshold (C_T) cycle method was performed to ensure approximately equal efficiencies of target and reference amplifications (slope of log input amount vs. $\Delta C_T < 0.1$). Total RNA from human brain hippocampus (Clontech Laboratories Inc. Takara Bio group, Shiga, Japan) was used as the calibrator and amplified in each plate to correct for experimental differences between consecutive PCR runs. STX1A mRNA amount was normalized to the endogenous reference and expressed relative to the calibrator as $2^{-\Delta\Delta C_T}$ (comparative C_T method).

2.4. Statistics

All data are presented as mean ± SD. Statistical significance was determined using two-tailed Student's *t*-test or Mann–Whitney *U* test. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Some ASD patients are haploid for the STX1A gene

STX1A gene ablated mice (null and heterozygote mutant) show abnormal behavior and hypersensitivity in pain and auditory sensations, similar to human autistic behavioral and sensational profiles