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Novel missense *SETD1A* variants in Japanese patients with schizophrenia: Resequencing and association analysis

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ABSTRACT

SETD1A has been identified as a substantial risk gene for schizophrenia. To further investigate the role of *SETD1A* in the genetic etiology of schizophrenia in the Japanese population, we performed resequencing and association analyses. First, we resequenced the *SETD1A* coding regions of 974 patients with schizophrenia. Then, we genotyped variants, prioritized via resequencing, in 2,027 patients with schizophrenia and 2,664 controls. Next, we examined the association between *SETD1A* and schizophrenia in 3,001 patients with schizophrenia and 2,664 controls. Finally, we performed a retrospective chart review of patients with prioritized *SETD1A* variants. We identified two novel missense variants (p.Ser575Pro and p.Glu857Gln) via resequencing. We did not detect these variants in 4,691 individuals via genotyping. These variants were not significantly associated with schizophrenia in the association analysis. Additionally, we found that a schizophrenia patient with the p.Glu857Gln variant had developmental delays. In conclusion, novel *SETD1A* missense variants were exclusively identified in Japanese patients with schizophrenia. However, our study does not provide evidence for the contribution of these variants to the genetic etiology of schizophrenia in the Japanese population.

1. Introduction

The genetic architecture of schizophrenia has not been fully elucidated but has been progressively uncovered (Legge et al., 2021 Smeland et al., 2020; Sullivan and Geschwind, 2019;). Genome-wide association studies have identified 270 loci associated with schizophrenia in European and East Asian populations (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2020), and several rare copy number variants have also been associated with schizophrenia in these populations (CNV and Schizophrenia Working Groups of the Psychiatric Genomics Consortium; Psychosis Endophenotypes International Consortium, 2017 Kushima et al., 2017; Li et al., 2016;). Whole-exome sequencing studies have been performed mainly in European populations and have revealed that rare variants contribute substantially to the genetic risk for schizophrenia (Singh et al., 2020).

The *SETD1A* gene has been identified as a schizophrenia risk gene with a large effect size (Singh et al., 2016 Takata et al., 2016;). The Schizophrenia Exome Sequencing Meta-analysis Consortium confirmed that ultra-rare loss-of-function (nonsense, frameshift, and splice site) variants and predicted damaging missense variants of *SETD1A* conferred risk for schizophrenia in 24,248 patients, 97,322 controls, and 3402 parent-proband trios, mainly from European populations (https://schema.broadinstitute.org/results Singh et al., 2020;). Five novel singleton loss-of-function and missense *SETD1A* variants were found in 786 Ashkenazi Jewish patients with schizophrenia but not in 463 controls (Lencz et al., 2021). Furthermore, a whole-genome sequencing study of 251 families with a proband with schizophrenia and related disorders found two rare *de novo* loss-of-function and missense *SETD1A* variants in Ashkenazi Jewish patients with schizophrenia (Alkelai et al., 2021). However, no *de novo SETD1A* variants were identified in 1695 Taiwanese trios included in the Schizophrenia Exome Sequencing Meta-analysis (Howrigan et al., 2020). Further studies in non-European populations are warranted to firmly establish the association between *SETD1A* and schizophrenia.

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A novel missense variant was identified via resequencing of the *SETD1A* coding regions of 390 Japanese patients with schizophrenia (Kimura et al., 2016). However, this variant was not associated with schizophrenia in 1783 patients with schizophrenia and 2213 controls. In this previous study, ultra-rare variants may not have been sufficiently identified because of the sample size used for resequencing ($n = 390$). By increasing the sample size, we attempted to further investigate the role of ultra-rare *SETD1A* variants in the genetic etiology of schizophrenia in the Japanese population. First, we resequenced the *SETD1A* coding regions in 974 patients with schizophrenia. Then, we genotyped variants, prioritized via resequencing, in 2027 patients with schizophrenia and 2664 controls. Next, we examined the association between *SETD1A* and schizophrenia in 3001 patients with schizophrenia and 2664 controls. Finally, we performed a retrospective chart review to obtain the clinical characteristics of patients with prioritized *SETD1A* variants.

2. Methods

2.1. Participants

This study was approved by the Ethics Committee of each participating institute, and written informed consent was obtained from all participants.

All participants were unrelated and of Japanese descent. Resequencing of the *SETD1A* coding regions was conducted on 974 patients with schizophrenia (Table 1). Genotyping of variants prioritized via resequencing was performed on 2027 schizophrenia patients and 2664 controls. The population of patients who underwent resequencing did not overlap with the population of patients who underwent genotyping.

Each participant was subjected to a psychiatric assessment, as previously described (Igeta et al., 2019). In brief, patients were diagnosed in accordance with the Diagnostic and Statistical Manual of Mental Disorders, Fourth or Fifth Edition criteria by at least two experienced psychiatrists. The controls included mentally healthy individuals, with no personal or family history (within first-degree relatives) of psychiatric disorders.

We performed a retrospective chart review to obtain the clinical characteristics of patients with *SETD1A* variants that were prioritized via resequencing.

2.2. Resequencing of the SETD1A coding region

Genomic DNA was extracted from blood leucocytes using a QIAamp DNA Blood Maxi Kit (QIAGEN, Germany).

SETD1A coding regions (RefSeq NM_014712) were resequenced in 974 patients with schizophrenia using Sanger sequencing (Supplementary Table 1), as previously described (Nunokawa et al., 2010).

We prioritized ultra-rare loss-of-function variants and missense variants with a Missense badness, PolyPhen-2, and Constraint (MPC) score ≥ 2 because these *SETD1A* variants have been significantly associated with schizophrenia (Singh et al., 2020). The MPC score is a deleteriousness metric for missense variants (Samocha et al., 2017). An ultra-rare variant was defined as a variant with five or fewer alternative allele counts in the Tohoku Medical Megabank Organization 8.3KJPN allele frequency panel from the Japanese Multi Omics Reference Panel (https://jmorp.megabank.tohoku.ac.jp/202,102/variants Tadaka et al., 2021;), the Genome Aggregation Database v2.1.1 (non-neuro) (http://gnomad.broadinstitute.org/ Karczewski et al., 2020;), and the Trans-Omics for Precision Medicine freeze 8 database (Taliun et al., 2021) from BRAVO (https://bravo.sph.umich.edu/freeze8/hg38/).

We also prioritized novel singleton missense variants that were not registered in these three databases because Lencz et al. (2021) reported that novel singleton loss-of-function and missense *SETD1A* variants were identified only in patients with schizophrenia and not in controls.

Table 1
Characteristics of study participants.

Characteristic	Resequencing Schizophrenia	Genotyping Schizophrenia	Control
N	974	2027	2664
Men (%)	504 (51.7%)	1050 (51.8%)	1243 (46.7%)
Mean age (SD)	42.1 (14.6)	50.3 (15.3)	43.7 (15.8)

2.3. In silico analysis

We predicted the functional impact of the missense variants prioritized via resequencing using the MPC score and the Polymorphism Phenotyping v2 (PolyPhen-2; http://genetics.bwh.harvard.edu/pph2/ Adzhubei et al., 2010;), Sorting Intolerant From Tolerant (SIFT; https://sift.bii.a-star.edu.sg/ Sim et al., 2012;), and Combined Annotation Dependent Depletion (CADD) v1.6 (https://cadd.gs.washington.edu/ Rentzsch et al., 2021;) tools.

2.4. Genotyping

We genotyped variants prioritized via resequencing in 2027 patients with schizophrenia and 2664 controls using the TaqMan 5′-exonuclease assay (Thermo Fisher Scientific, Waltham, MA, USA; Supplementary Table 2), as previously described (Watanabe et al., 2006).

2.5. Statistical analysis

To determine whether the *SETD1A* variants prioritized via resequencing contribute to the genetic etiology of schizophrenia in the Japanese population, we performed a gene-based association analysis of 3001 patients and 2664 controls, including 974 patients who underwent resequencing and 2027 patients and 2664 controls who underwent genotyping, using the cohort allelic sums test (https://rdrr.io/cran/AssotesteR/src/R/CAST.R Morgenthaler and Thilly, 2007;). A probability level of $p < 0.05$ was considered to indicate statistical significance.

3. Results

We identified 40 common and ultra-rare variants via resequencing of the *SETD1A* coding region of 974 patients with schizophrenia (Supplementary Table 3). There were no common or ultra-rare loss-of-function variants, and one common missense variant was found, with an MPC score of 2.42: p.Gln90His (g.30972611G>C; rs976305904). The alternative allele count of p.Gln90His was 35 of 16,760 in the Tohoku Medical Megabank Organization 8.3KJPN database, whereas this variant was not registered in the Genome Aggregation Database v2.1.1 (non-neuro) or the Trans-Omics for Precision Medicine freeze 8 database. Therefore, p.Gln90His was not ultra-rare and was not prioritized.

We prioritized two novel singleton missense variants, p.Ser575Pro (g.30976925T>C) and p.Glu857Gln (g.30978268G>C Table 2;), which were confirmed by repeat PCR analysis and repeat Sanger sequencing (Supplementary Fig. 1). The MPC scores for p.Ser575Pro and p.Glu857Gln were 0.68 and 0.63, respectively. These missense variants were predicted to be probably damaging and damaging by PolyPhen-2 and SIFT, respectively. The CADD scores for p.Ser575Pro and p.Glu857Gln were 25.2 and 24.6, respectively, indicating that these variants were predicted to be among the top 1% of the most damaging variants. We also identified a novel singleton synonymous variant: p.Ala483Ala (g.30976512C>A; Supplementary Table 3). However, this variant was not a missense variant and was not prioritized.

Next, we genotyped p.Ser575Pro and p.Glu857Gln in 2027 patients with schizophrenia and 2664 controls (Table 2). These variants were not detected in any of the 4691 individuals. When we combined the samples used for resequencing and genotyping, no significant association was observed between the set of novel missense *SETD1A* variants and schizophrenia (cohort allelic sums test $p = 0.63$).

Table 2
Novel missense *SETD1A* variants.

Position[a]	Allele[b]	Amino acid	In silico analysis		Genotype[c]				
			MPC	PolyPhen-2	SIFT	CADD	Resequencing Schizophrenia	Genotyping Schizophrenia	Control
30976925	T/C	Ser575Pro	0.68	Probably damaging	Damaging	25.2	973/1/0	2026/0/0	2663/0/0
30978268	G/C	Glu857Gln	0.63	Probably damaging	Damaging	24.6	973/1/0	2027/0/0	2664/0/0

CADD, Combined Annotation Dependent Depletion; MPC, Missense badness, PolyPhen-2, and Constraint; PolyPhen-2, Polymorphism Phenotyping v2; SIFT, Sorting Intolerant From Tolerant.
[a] Position according to GRCh37.
[b] Reference/alternative.
[c] Homozygous for reference allele/heterozygous/homozygous for alternative allele.

The clinical characteristics of schizophrenia patients with novel missense *SETD1A* variants are shown in Table 3. Ser575Pro was identified in a 63-year-old female patient who developed delusions in her thirties. At age 60, she visited a psychiatrist for the first time. At age 63, her persecutory delusions and disorganized behaviors deteriorated. She was hospitalized for 6 months and was treated with perospirone (16 mg/day). Her total scores on the 16-item Brief Psychiatric Rating Scale, which ranges from 0 to 96, on admission and at discharge were 32 and 23, respectively. Her full-scale intelligence quotient was 92, as assessed using the Wechsler Adult Intelligence Scale-Revised. Brain magnetic resonance imaging showed mild ischemic lesions in the deep white matter around the lateral ventricles. She also had type 2 diabetes but did not have epilepsy or a family history of psychiatric disorders. Genomic DNA samples from her parents were not available, and we were unable to determine whether p.Ser575Pro was a *de novo* or an inherited mutation.

A Glu857Gln variant was identified in a 59-year-old male patient who was born one month preterm. He exhibited a prolonged fever at approximately 10 months of age and had developmental delays in walking and speaking. He presented persecutory delusions and psychomotor agitation at age 33, and these symptoms were successfully treated with haloperidol (1.5 mg/day) and sulpiride (100 mg/day). Although negative symptoms remained, he continued his job. His intellectual functioning was not evaluated. Brain magnetic resonance imaging at age 39 showed mild frontal lobe atrophy. He also had hypertension but did not have epilepsy. His older brother had also been diagnosed with schizophrenia. However, genomic DNA samples from his brother and parents were not available. Therefore, we were unable to determine whether this brother was heterozygous for p.Glu857Gln and if the variant was *de novo* or inherited.

4. Discussion

We identified two novel missense variants (p.Ser575Pro and p.Glu857Gln) in patients with schizophrenia via resequencing. These variants were not detected in the case-control sample used for genotyping. Therefore, Ser575Pro and Glu857Gln were exclusively identified in Japanese patients with schizophrenia, although it is possible that these patients have some loss-of-function variants of other risk genes for schizophrenia. Nevertheless, we were unable to provide statistical evidence for the association of these novel missense *SETD1A* variants with schizophrenia. Our sample sizes for resequencing (974 patients) and genotyping (2027 patients and 2664 controls) were larger than those used for resequencing (390 patients) and genotyping (1783 patients and 2213 controls) in an earlier Japanese study (Kimura et al., 2016). However, the most recent meta-analysis included 24,248 patients, 97,322 controls, and 3402 trios, indicating that ultra-rare loss-of-function and putatively damaging missense variants confer risk for schizophrenia (Singh et al., 2020). Our negative results may be attributable to an insufficient sample size.

Table 3
Clinical characteristics of patients with novel missense *SETD1A* variants.

Variant	Sex	Age	Age at onset	Full-scale IQ	Developmental delay	Epilepsy
Ser575Pro	Female	63	30s	92	None	None
Glu857Gln	Male	59	33	No data	Delays in walking and speaking	None

IQ, intelligence quotient.

The *SETD1A* gene (MIM 611,052) has not only been associated with schizophrenia but has also been associated with developmental disorders (Kaplanis et al., 2020 Singh et al., 2016;) Kummeling et al. (2021). reported the clinical phenotypes of 15 patients who had *de novo* loss-of-function and missense *SETD1A* variants and neurodevelopmental disorders, which were characterized by global developmental delays, intellectual disability, subtle facial dysmorphisms, and psychiatric problems (MIM 619,056) Yu et al. (2019). identified four missense *SETD1A* variants in patients with early-onset epilepsy (MIM 618,832). Moreover, delayed speech has been frequently observed in individuals with loss-of-function and missense *SETD1A* variants (Eising et al., 2019 Kummeling et al., 2021; Singh et al., 2016;). In our study, a schizophrenia patient with a p.Glu857Gln variant also had developmental delays in walking and speaking. These findings suggest that loss-of-function and missense *SETD1A* variants may cause several neuropsychiatric phenotypes.

The *SETD1A* gene encodes the histone-lysine N-methyltransferase SETD1A, which modulates mono-, di-, and trimethylation of lysine 4 at histone H3 and regulates gene transcription (Wang et al., 2021a). *SETD1A* haploinsufficient mice exhibited abnormalities in working memory (Mukai et al., 2019 Nagahama et al., 2020;), social interaction (Nagahama et al., 2020), sensorimotor gating (Nagahama et al., 2020 Bosworth et al., 2021;), and sensory processing (Hamm et al., 2020). These mice also showed reductions in spine density (Mukai et al., 2019 Nagahama et al., 2020;), deficits in short-term synaptic plasticity (Mukai et al., 2019), and attenuation of excitatory synaptic transmission (Nagahama et al., 2020). Increased morphological complexity and functional increases in bursting activity were observed in neurons from human induced pluripotent stem cells with a heterozygous frameshift *SETD1A* variant (Wang et al., 2021b). Additionally, DNA damage repair was impaired in lymphoblastoid cell lines derived from neurodevelopmental disorder patients with loss-of-function or missense *SETD1A* variants (Kummeling et al., 2021). Moreover, the dendritic spine density was decreased in cultured mouse cortical primary neurons expressing missense *SETD1A* variants that were identified in patients with early-onset epilepsy (Yu et al., 2019). Taken together, these findings suggest that loss-of-function and missense *SETD1A* variants are involved in the pathogenesis underlying schizophrenia, developmental disorders, and early-onset epilepsy. The novel *SETD1A* missense variants (p.Ser575Pro and p.Glu857Gln) exclusively identified in our patients with schizophrenia were predicted to be damaging via several *in silico* analysis metrics. These variants are located outside of the four domains of STED1A (RNA recognition motif, n-SET, SET, and post-SET domains) (Wang et al., 2021a), and the effects of these variants on the function of

SETD1A remain to be determined by functional analyses.

In conclusion, we identified novel putatively functional *SETD1A* missense variants (p.Ser575Pro and p.Glu857Gln) exclusively in Japanese patients with schizophrenia, and a patient with a p.Glu857Gln variant had developmental delays. However, our study did not provide evidence for the contribution of these variants to the genetic etiology of schizophrenia in the Japanese population.

CRediT authorship contribution statement

Ryo Morikawa: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. **Yuichiro Watanabe:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. **Hirofumi Igeta:** Investigation, Resources, Writing – review & editing. **Reza K. Arta:** Investigation, Writing – review & editing. **Masashi Ikeda:** Resources, Writing – review & editing. **Satoshi Okazaki:** Resources, Writing – review & editing. **Satoshi Hoya:** Investigation, Resources, Writing – review & editing. **Takeo Saito:** Resources, Writing – review & editing. **Ikuo Otsuka:** Resources, Writing – review & editing. **Jun Egawa:** Resources, Writing – review & editing. **Takaki Tanifuji:** Resources, Writing – review & editing. **Nakao Iwata:** Resources, Writing – review & editing. **Toshiyuki Someya:** Resources, Writing – review & editing.

Declaration of Competing Interest

All authors have no conflicts of interest to declare.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2022.114481.

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