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# Study of the genetic association between selected 3q29 region genes and schizophrenia and autism spectrum disorder in the Japanese population

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## ABSTRACT

Psychiatric disorders are highly inheritable, and most psychiatric disorders exhibit genetic overlap. Recent studies associated the 3q29 recurrent deletion with schizophrenia (SCZ) and autism spectrum disorder (ASD). In this study, we investigated the association of genes in the 3q29 region with SCZ and ASD. *TM4SF19* and *PAK2* were chosen as candidate genes for this study based on evidence from previous research. We sequenced *TM4SF19* and *PAK2* in 437 SCZ cases, 187 ASD cases and 524 controls in the Japanese population. Through targeted sequencing, we identified 6 missense variants among the cases (ASD & SCZ), 3 missense variants among controls, and 1 variant common to both cases and controls; however, no loss-of-function variants were identified. Fisher's exact test showed a significant association of variants in *TM4SF19* among cases (p=0.0160). These results suggest *TM4SF19* variants affect the etiology of SCZ and ASD in the Japanese population. Further research examining 3q29 region genes and their association with SCZ and ASD is thus needed.

Keywords: 3q29, *TM4SF19, PAK2,* schizophrenia, autism spectrum disorder

Abbreviations:
ASD: autism spectrum disorder
SCZ: schizophrenia



## INTRODUCTION

Schizophrenia (SCZ), a neuropsychiatric disorder that affects approximately 1% of the worldwide population,[1] exhibits positive symptoms (delusions and hallucinations; so-called psychotic symptoms in which contact with reality is lost), negative symptoms (particularly impaired motivation, reduction in spontaneous speech, and social withdrawal) and cognitive impairment.[2]

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SCZ is highly heritable, with rates ranging up to 80%.[3]

Autism spectrum disorder (ASD) is a term used to describe a constellation of early appearing social communication deficits and repetitive sensory-motor behaviors associated with a strong genetic component as well as other causes.[4] The heritability of ASD is estimated to range from 64% to 91%.[5]

Even though SCZ and ASD are different disorders, they exhibit phenotypic similarities and genetic overlap. A particularly compelling example of overlapping genetic vulnerability is the high rates of ASD and SCZ seen in individuals with 22q11.2 deletion syndrome[6] and 3q29 deletion syndrome.[7,8]

Recent studies have reported that individuals with 3q29 deletion syndrome have a high risk of developing SCZ and ASD.[9] The typical 3q29 recurrent deletion is 1.6 Mb in size and contains 22 protein-coding genes, including *PAK2*, *TFRC*, *TNK2*, *APOD*, *HES1*, *OPA1*, *TM4SF19*, *MUC4*, and *DLG1*.[10] The 3q29 recurrent deletion is characterized by neurodevelopmental and/or psychiatric manifestations, including mild-to-moderate intellectual disability, ASD, anxiety disorders, attention deficit/hyperactivity disorder, executive function deficits, graphomotor weakness, and psychosis/SCZ.[11]

The 3q29 deletion confers a 41.1-fold increased risk for developing SCZ.[8] Several SCZ and ASD candidate genes have been implicated in this region, including *DLG1*, *PAK2,*[12] and *FBXO45*.[8,13] In addition, *TM4SF19*[14,15] have been reported as possible ASD candidate genes. As 3q29 deletion plays a significant role in SCZ and ASD patients, we wanted to examine genes in this region may also affect the risk for developing SCZ and ASD. In previous studies, we examined *DLG1*[16] and *FBXO45*.[13] Therefore, in this study, we examined *TM4SF19* and *PAK2* as candidate genes.

The aim of this study was to investigate the relationship between variants in 3q29 region genes and SCZ and ASD. We conducted targeted sequencing of *TM4SF19* and *PAK2* and performed association analyses on rare missense variants.

## MATERIALS AND METHODS

### *Samples*

The sample set included 437 SCZ cases, 187 ASD cases, and 524 healthy controls. All participants in our study were Japanese recruited by Nagoya University Hospital and its co-institutes and co-hospitals. Cases were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, fifth edition criteria for SCZ and ASD. Controls were evaluated using an unstructured interview to confirm they had neither personal nor family history of psychiatric disorders. We obtained written informed consent from all participants and/or their guardians. This study was approved by the Ethics Committees of Nagoya University Graduate School of Medicine and co-institutes and co-hospitals.

### *Sequencing and data collection*

Genomic DNA was extracted from whole peripheral blood or saliva via standard protocols. We included only coding regions. The Ion AmpliSeq Library Preparation protocol was used to prepare a DNA library for the selected genes, and the Ion Xpress barcode adapter was used for each DNA library. Sequencing was performed on an Ion PGM Sequencer using Ion 318 Chip v2. Up to 48 barcoded libraries were loaded onto a single ion chip. All procedures followed the Ion Personal Genome Machine System– Reference Guide (revision: 2018.09). Sequencing data were analyzed using Ion Reporter software (plugins: coverage analysis, variant caller, file

exporter). Fisher's exact test (two-tailed) was used for calculating associations between samples and selected genes, with the threshold of significance set at $p<0.05$.

*Quality control*

For sample quality control purposes, 11 samples were excluded due to excessive/low variant calls (±3SD), and 3 samples were excluded due to low uniformity (<60%). After applying variant quality control (Variant caller QC: DP≥10; GQ≥15/20; AB>0.2 or <0.8; strand bias≤0.95), a total of 56 variants remained. Furthermore, intronic, untranslated region variants were excluded. After excluding synonymous variants, 10 candidate variants remained for further analysis.

*Filtering conditions and in silico analysis*

We determined whether the identified variants were registered in the Exome Aggregation Consortium (ExAC),[17] the Tohoku University Medical Megabank Organization (ToMMo) 8.3KJPN,[18] the Genome Medical Alliance – Japan Whole Genome Aggregation (GEM-J WGA),[19] or the Human Genetic Variation Database (HGVD).[20] The status of each variant was investigated using ClinVar.[21]

## RESULTS

We identified 10 rare variants in the *TM4SF19* (n=7) and *PAK2* (n=3) genes (Figure 1).

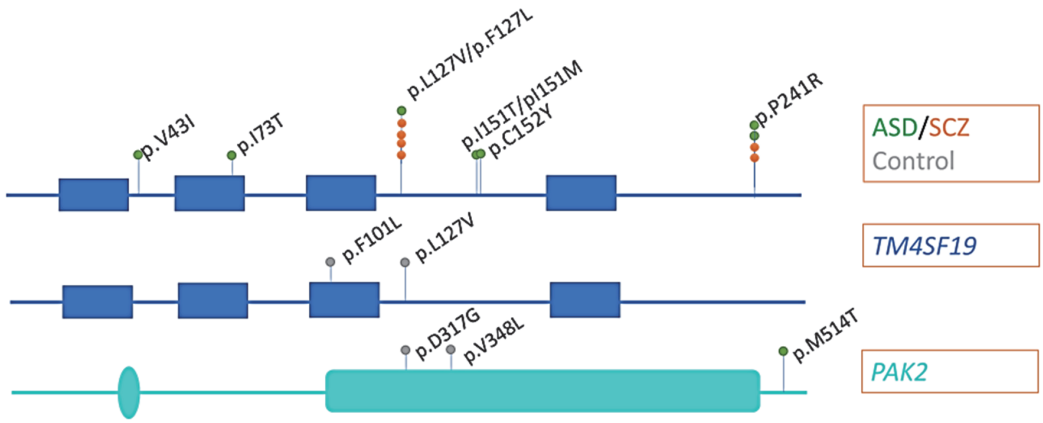


**Fig. 1** Locations of novel rare variants in *TM4SF19* and *PAK2*
*The protein structure of TM4SF19 and PAK2 is based on the Human Protein Reference Database.
**Each head indicate location at protein, amino acid change and number of patients which found in.
***Each rectangular indicates domain region of protein.
ASD: autism spectrum disorder
SCZ: schizophrenia

Fisher's exact tests showed a significant association between ASD & SCZ (n = 617) and *TM4SF19* variants ($p = 0.0160$ [$p<0.05$]; odd ratio = 5.51). Regarding *PAK2* variants, no significant association was observed between cases and the variants ($p = 0.5948$ [$p<0.05$]; odd ratio = 0.42). The effects on protein function due to amino acid changes were predicted using

the in-silico tools: Scale-invariant feature transform (SIFT),[22] PolyPhen-2,[23] and MutationTaster.[24] Three variants were predicted to have damaging effects according to all 3 tools (*TM4SF19* = 2; *PAK2* = 1; Table 1).

**Table 1** Details regarding the identified missense variants

| | Chromosome | Position | REF | ALT | Amino acid change | ASD | SCZ | Con | Gene name |
|---|---|---|---|---|---|---|---|---|---|
| 1 | chr3 | 196050592 | G | C | p.P241R | 2 | 2 | – | TM4SF19 |
| 2 | chr3 | 196050859 | C | T | p.C152Y | 1 | – | – | TM4SF19 |
| 3 | chr3 | 196050861 | G | C | p.L127V/p.I151M | 1 | 4 | 1 | TM4SF19 |
| 4 | chr3 | 196050862 | A | G | p.I151T | 1 | – | – | TM4SF19 |
| 5 | chr3 | 196051212 | A | G | p.F101L/p.F127L | – | – | 1 | TM4SF19 |
| 6 | chr3 | 196053887 | A | G | p.I73T | 1 | – | – | TM4SF19 |
| 7 | chr3 | 196054335 | C | T | p.V43I | 1 | – | – | TM4SF19 |
| 8 | chr3 | 196541336 | A | G | p.D317G | – | – | 1 | PAK2 |
| 9 | chr3 | 196541428 | G | T | p.V348L | – | – | 1 | PAK2 |
| 10 | chr3 | 196555242 | T | C | p.M514T | – | 1 | – | PAK2 |

REF: reference
ALT: alteration
ASD: autism spectrum disorder
SCZ: schizophrenia
Con: control

However, 1 of the 3 variants was found in the control group. Four variants were not registered in any general databases, including ExAC, ToMMo 8.3KJPN, GEM-J WGA, and HGVD. None of 10 variants were reported in ClinVar (Table 2).

**Table 2** Frequency of each variant identified in this study, as shown by allele count

| | | Amino acid change | rs number | ExAC | ToMMo 8.3KJPN | GEM-J WGA | HGVD | ClinVar |
|---|---|---|---|---|---|---|---|---|
| *TM4SF19* | 1 | p.P241R | | – | 3/16,760 | 4/15,174 | – | – |
| | 2 | p.C152Y | | – | – | – | – | – |
| | 3 | p.L127V/p.I151M | rs748526139 | 6/250,462 | 16/16,760 | 16/15,148 | 8/2416 | – |
| | 4 | p.I151T | rs36016914 | 3013/281742 | – | – | – | – |
| | 5 | p.F101L/p.F127L | | – | – | – | – | – |
| | 6 | p.I73T | | – | – | – | – | – |
| | 7 | p.V43I | rs762010990 | 17/282692 | – | – | – | – |
| *PAK2* | 8 | p.D317G | | – | 16/16,760 | 15/15,188 | 4/2192 | – |
| | 9 | p.V348L | | – | – | – | – | – |
| | 10 | p.M514T | | 1/250,198 | 1/16,760 | – | – | – |

ExAC: Exome Aggregation Consortium
ToMMo 8.3KJPN: Tohoku University Medical Megabank Organization 8.3KJPN
GEM-J WGA: Genome Medical Alliance – Japan Whole Genome Aggregation
HGVD: Human Genetic Variation Database

## DISCUSSION

PAK2 is a ubiquitously expressed member of the p21-activated kinase family that plays a central role in regulating neuronal cytoskeleton dynamics. By regulating actin formation, PAK2 affects the morphology of synapses and the glutamate receptor complexes localized to synapses.[25] A previous study conducted on Han Chinese ASD probands identified a rare de novo nonsense variant and inherited damaging missense variants in PAK2.[12] Consistently, Pak2+/− mice were reported to exhibit autism-related behaviors, such as increased stereotypic behavior and reduced social interactions.[12]

The TM4SF19 gene encodes a protein that belongs to the four-transmembrane L6 superfamily. Members of this protein family are involved in several cellular processes such as proliferation, motility, and adhesion, in cooperation with integrins.[26] In humans, the *TM4SF19* is expressed at high levels in the parietal lobe, occipital lobe, hippocampus, pons, white matter, corpus callosum, and cerebellum. A de novo splice-site variant and an inherited damaging missense variant in *TM4SF19* were identified in ASD probands from the Simons Simplex Collection.[14,15] *TM4SF19* was identified as an ASD candidate gene in a study conducted in the Chinese population (ASD, n = 536; controls, n = 1457).[27]

In this study, variants in *TM4SF19* were significant among cases (ASD & SCZ) according to Fisher's exact tests. However, no significant associations were noted for *PAK2* between cases and controls. This study is the first to sequence the *TM4SF19* and *PAK2* (3q29 region) in the Japanese population. The results of this study identified some variants (p.C152Y; p.I73T) not previously reported in ExAC, ToMMo 8.3KJPN, GEM-J WGA, and HGVD, suggesting that some of the variants identified in this study may be specific to the Japanese population. Even though the function of TM4SF19 protein is unclear, a study published in Oncotarget in 2017 indicated that the C-terminus of transmembrane 4 L six family (TM4SF) proteins plays a significant role in various cellular functions, such as proliferation and migration.[28] A similar mutation within/near the C-terminus of TM4SF19 might also affect brain development (mainly expressed in the thalamus) in patients with psychiatric disorders.

We identified only three variants in *PAK2*. This may be because PAK2 is a highly conserved gene with a probability of loss-of-function intolerance score of 0.94, which suggests that deleterious mutations in this gene are likely to be associated with disease. Therefore, a larger sample would be needed to identify disease-associated missense variants in such genes.

## LIMITATIONS

The sample size (N = 1148; ASD, n = 187; SCZ, n = 437; Controls, n = 524) was too small to acquire strong evidence for any associations between variants in *TM4SF19* and *PAK2*. Due to the small sample size, we grouped SCZ and ASD case as one group (ASD/SCZ vs Control). The 3q29 deletion region contains 22 protein-coding genes. In this study, we only choose to include *TM4SF19* and *PAK2*, based on recent reports of associations with ASD and SCZ.[12,14]

## CONCLUSION

This study is the first to sequence *TM4SF19* and *PAK2* in the Japanese population. Our results suggest that *TM4SF19* variants may affect the etiology of SCZ and ASD in the Japanese population. However, further research involving larger sample sizes is needed to investigate the 3q29 region genes and their potential associations with SCZ and ASD.

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