

Rare compound heterozygous missense *SPATA7* variations and risk of schizophrenia; whole-exome sequencing in a consanguineous family with affected siblings, follow-up sequencing and a case-control study

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Purpose: Whole-exome sequencing (WES) of multiplex families is a promising strategy for identifying causative variations for common diseases. To identify rare recessive risk variations for schizophrenia, we performed a WES study in a consanguineous family with affected siblings. We then performed follow-up sequencing of *SPATA7* in schizophrenia-affected families. In addition, we performed a case-control study to investigate association between *SPATA7* variations and schizophrenia.

Patients and methods: WES was performed on two affected siblings and their unaffected parents, who were second cousins, of a multiplex schizophrenia family. Subsequently, we sequenced the coding region of *SPATA7*, a potential risk gene identified by the WES analysis, in 142 affected offspring from 137 families for whom parental DNA samples were available. We further tested rare recessive *SPATA7* variations, identified by WES and sequencing, for associations with schizophrenia in 2,756 patients and 2,646 controls.

Results: Our WES analysis identified rare compound heterozygous missense *SPATA7* variations, p.Asp134Gly and p.Ile332Thr, in both affected siblings. Sequencing *SPATA7* coding regions from 137 families identified no rare recessive variations in affected offspring. In the case-control study, we did not detect the rare compound heterozygous *SPATA7* missense variations in patients or controls.

Conclusion: Our data does not support the role of the rare compound heterozygous *SPATA7* missense variations p.Asp134Gly and p.Ile332Thr in conferring a substantial risk of schizophrenia.

Keywords: Japanese, multiplex schizophrenia family, next-generation sequencing, recessive variations

Introduction

Schizophrenia is a complex disorder with heritability of approximately 80%.¹ Understanding the genetic architecture of schizophrenia has progressed steadily.²⁻⁴ Genome-wide association studies (GWASs) have discovered common loci associated with schizophrenia.⁵⁻⁷ Intriguingly, association of the major histocompatibility complex locus with schizophrenia involves structurally distinct alleles of *C4* that affect the expression of *C4A* and *C4B* in the brain.⁸ However, the heritability of schizophrenia is not fully explained by common variations, suggesting that rare variations

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also contribute to schizophrenia liability.⁹ Indeed, rare copy number variations are associated with schizophrenia.^{10–12} Whole-exome sequencing (WES) studies have demonstrated that rare sequence variations play a substantial role in the genetic etiology of schizophrenia.^{13–15} Of note, *SETD1A* was identified as a risk gene for schizophrenia with a large effect.^{16,17}

WES and whole-genome sequencing (WGS) of multiplex families is a promising strategy for identifying causative variations for common diseases.^{18,19} The number of WES and WGS studies that have examined multiplex schizophrenia families is still limited, but they have detected highly penetrant variations in *GRM5*,²⁰ *UNC13B*,²¹ *SHANK2* and *SMARCA1*,²² *RELN*,²³ *TAAR1*,²⁴ *RBM12*,²⁵ *CSPG4*,²⁶ *PTPRA*,²⁷ *ITGB4*,²⁸ *TIMP2*,²⁹ and *TENM4*.³⁰

Two recent studies suggested that a combined strategy including identity-by-descent (IBD) mapping and WES may be useful in identifying rare risk variations for schizophrenia inherited from common ancestors.^{31,32} Harold et al performed IBD mapping using Irish schizophrenia GWAS data and identified potential risk haplotypes.³¹ Subsequently, they conducted WES and identified *PCNT* p.Gly1452Arg as a potential risk haplotype, although this missense variation was not associated with schizophrenia in replication samples. In the other study, Salvoro et al performed IBD mapping and WES in multiplex families with schizophrenia, bipolar disorder, and schizoaffective disorder from Chioggia, Italy.³² Among potential risk haplotypes, they found significant enrichment of non-synonymous variations of genes involved in extracellular matrix biology and axon guidance processes.

Here, we performed a three-stage study to identify rare recessive variations that play a substantial role in conferring schizophrenia risk. First, we undertook a WES study in a multiplex family with two siblings with schizophrenia whose unaffected parents were second cousins. Second, we sequenced the coding region of *SPATA7*, a potential risk gene identified by the WES study, in 142 affected offspring from 137 families for whom parental DNA samples were available. Third, we conducted a case-control study to examine association of rare recessive *SPATA7* variations, identified by WES and sequencing, with schizophrenia in 2,756 patients and 2,646 controls.

Materials and methods

Participants

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics

Committee of each participating institute. All participants gave written informed consent, and all were of Japanese descent.

We included two siblings with schizophrenia (#4 and #5) and their unaffected parents (#1 and #2) in a WES study (Figure 1). In this family, the female proband (#4) and her younger sister (#5) were diagnosed with schizophrenia. Their older sister (#3) was suspected of having postpartum depression. Their younger brother (#6) died one day after a Caesarean section delivery. Their younger brother (#7) was not diagnosed with any psychiatric disorder. Their unaffected father (#1) and mother (#2) were second cousins. Diagnoses of each family member were made using Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) criteria, as previously described.³³

For sequencing *SPATA7* coding regions, we included 142 affected offspring (79 men and 63 women; mean age, 29.4±9.0 years) from 137 families for whom parental DNA samples were available for genotyping. These affected offspring were diagnosed with schizophrenia according to DSM-IV or DSM-5 criteria and were not included in the case-control study.

The case-control study population comprised 2,756 patients with schizophrenia and 2,646 controls, who were recruited from Fujita Health University,⁶ Kobe University,³⁴ and Niigata University³⁵ (Table 1). The patients were diagnosed according to DSM-IV or DSM-5 criteria. Controls had no personal or family history (first-degree relatives) of psychiatric disorders.

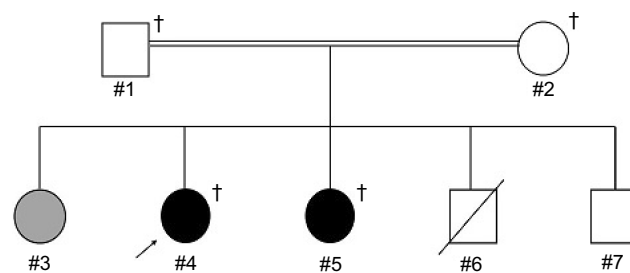


Figure 1 Pedigree of a consanguineous family with two schizophrenia siblings. The female proband (#4), indicated by an arrow, and her younger sister (#5) were diagnosed with schizophrenia, indicated by black shaded symbols. Their older sister (#3) was suspected of having postpartum depression, indicated by a gray shaded symbol. Their parents (#1 and #2) and younger brothers (#6 and #7) were not diagnosed with any psychiatric disorder, indicated by unshaded symbols. Their younger brother (#6) died one day after a Caesarean section delivery, indicated by a diagonal line through the symbol. Their parents (#1 and #2) were second cousins, indicated by a double line between individuals. Squares and circles represent males and females, respectively. Crosses represent individuals from whom genomic DNA samples were available.

Table 1 Characteristics of case-control study participants

Institute	Patients			Controls		
	n	Men (%)	Mean age \pm SD	n	Men (%)	Mean age \pm SD
Fujita Health University	1,111	569 (51.2%)	46.2 \pm 14.8	1,124	486 (43.2%)	40.5 \pm 12.8
Kobe University	939	489 (52.1%)	54.3 \pm 15.0	851	400 (47.0%)	52.3 \pm 18.6
Niigata University	706	377 (53.2%)	43.1 \pm 13.5	671	341 (50.8%)	38.3 \pm 10.9
Total	2,756	1,435 (52.1%)	48.1 \pm 15.2	2,646	1,227 (46.4%)	43.8 \pm 15.8

Wes

From the family, we obtained genomic DNA samples from the proband (#4), her affected younger sister (#5), and their unaffected father (#1) and mother (#2; **Figure 1**). WES was performed at Takara Bio Inc. (Shiga, Japan), using the HiSeq2500 system (Illumina, San Diego, CA, USA). We prepared exome libraries using the SureSelect Human All Exon V6 Kit (Agilent, Santa Clara, CA, USA). WES data were processed using GeneData Expressionist for Genomic Profiling v9.1.4a (Genedata, Basel, Switzerland). Adaptor sequences and low-quality reads were removed from raw sequence reads using Trimmomatic v0.1.9 (<http://www.usadellab.org/cms/?page=trimmomatic>).³⁶ Cleaned sequence reads were mapped against the reference human genome (UCSC hg19) using the Burrows–Wheeler Aligner-MEM v0.7.12 (<http://bio-bwa.sourceforge.net/>).³⁷ Variations were annotated using SnpEff v3.6c (<http://snpeff.sourceforge.net/>)³⁸ and VCFtools v0.1.9 (<https://vcftools.github.io/index.html>).³⁹ We calculated the coefficient of relationship from the WES data for each pair of individuals using peddy (<https://github.com/brentp/peddy>).⁴⁰

To prioritize variations, we applied the following filtering steps (**Table 2**). First, we included variations on autosomes. Second, we included variations covered by ≥ 10 reads. Third, we included “HIGH” or “MODERATE” Effect_Impact variations predicted using SnpEff v3.6c. Fourth, we included recessive homozygous and compound heterozygous variations identified in both affected siblings. Fifth, we included rare variations with mutant allele frequency < 0.01 in the Japanese Multi Omics Reference Panel (jMorp) 3.5KJPNv2 (<https://jmorp.megabank.tohoku.ac.jp/201808/>),⁴¹ the Human Genetic Variation Database (HGVD) v1.42 (<http://www.genome.med.kyoto-u.ac.jp/SnpDB/>),⁴² the BioBank Japan Whole-Genome Sequencing (BBJWGS) database (<http://jenger.riken.jp/>),⁴³ Japanese data from the 1000 Genomes Project (1KGP) phase 3 (<https://www.ncbi.nlm.nih.gov/variation/tools/>

1000genomes/),⁴⁴ and East Asian data from the Genome Aggregation Database (gnomAD) v2.1 (non-neuro) (<http://gnomad.broadinstitute.org/>).⁴⁵

To validate prioritized variations, we performed Sanger sequencing using a 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), as previously described.⁴⁶

Sequencing the SPATA7 coding region

The *SPATA7* coding region (RefSeq accession number, NM_018418) was sequenced in 142 affected offspring from 137 families. In 32 offspring, we screened for rare recessive *SPATA7* variations using our published^{35,47} and unpublished WES data. In the remaining 110 offspring, we performed Sanger sequencing. Primer sequences for amplification are listed in **Table S1**.⁴⁸

Case-control study

We performed an association study of rare recessive *SPATA7* variations, prioritized via WES and sequencing, with schizophrenia in 2,756 patients and 2,646 controls. We genotyped p.Asp134Gly and p.Ile332Thr in our case-control samples, using the TaqMan 5'-exonuclease assay (Thermo Fisher Scientific, Waltham, MA, USA; **Table S2**), as previously described.³³

In silico analysis

We performed in silico analysis to predict the functional effects of *SPATA7* variations identified via WES and resequencing using Polymorphism Phenotyping v2 (PolyPhen-2; <http://genetics.bwh.harvard.edu/pph2/>),⁴⁹ Protein Variation Effect Analyzer v1.1 (PROVEN; http://provean.jcvi.org/genome_submit_2.php?species=human),⁵⁰ and Combined Annotation Dependent Depletion (CADD; <http://cadd.gs.washington.edu/home>) scores.⁵¹

Results

The mean read depth varied from 48.0 \times to 67.6 \times , and 97.1–98.1% of the target regions were covered by 10 or

Table 2 Filtering steps applied to variations identified by WES

Filtering step	Number of remaining variations
Called	213,038
On autosomes	209,389
Covered with 10 or more reads	102,293
HIGH or MODERATE Effect_Impact	11,210
Recessive	156
Homozygous	60
Compound heterozygous	96
With mutant allele frequency <0.01	2

Abbreviation: WES, whole-exome sequencing.

more reads (Table S3). We identified a total of 213,038 variations via WES (Table 2). The coefficient of relationship observed for the parents (#1 and #2) was 0.038, which was similar to 0.031, the value expected for second cousins (Table S4). The coefficients of relationship observed for the other pairs of individuals ranged from 0.430 to 0.522, which were similar to 0.5, the value expected for parent-offspring or siblings. After the filtering steps (Table 2), we prioritized rare compound heterozygous missense variations in *SPATA7* (Table 3). One was previously unidentified: an A to G transition (g.88892604A>G) at codon 134 resulting in an aspartic acid to glycine substitution (p.Asp134Gly). The other, a T to C transition (g.88895774T>C) at codon 332 resulting in an isoleucine to threonine substitution (p.Ile332Thr), had been previously reported (rs534658921). Unaffected father (#1) and mother (#2) transmitted the mutant p.Ile332Thr and p.Asp134Gly alleles, respectively, to both affected siblings (#4 and #5). In silico analysis predicted these variations to be “benign” and “neutral” using PolyPhen-2 and PROVEN, respectively (Table 3). CADD scores for p.Asp134Gly and p.Ile332Thr were 3.243 and 8.805, respectively, indicating that these variations were not deleterious.

Sequencing *SPATA7* coding regions identified eight variations in 142 affected offspring (Table S5). However, there were no rare recessive variations. In the case-control study, p.Asp134Gly was not found in 2,732 patients or 2,627 controls, while heterozygous p.Ile332Thr was observed in five patients and one control (Table 4). In these individuals, we did not detect other rare variations by sequencing *SPATA7* coding regions. The frequency of mutant alleles (0.0002) of p.Ile332Thr in our control group was similar to that in large databases including jMorp

Table 3 Rare compound heterozygous missense *SPATA7* variations prioritized by WES

dbSNP ID	Position ^a	Allele ^b	Exon	Protein	Inheritance	In silico analysis		CADD	MAF					
						PolyPhen-2	PROVEN		jMorp	HGVD	BBJWGS	IKGP	gnomAD	
–	88892604	A/G	6	Asp134Gly	Maternal	Benign	Neutral	3.243	–	–	–	–	–	–
rs534658921	88895774	T/C	8	Ile332Thr	Paternal	Benign	Neutral	8.805	0.0001	0.0004	0.000974659	0	0.0005220	

Notes: ^aPosition according to GRCh37. ^bReference/mutant allele.

Abbreviations: IKGP, 1000 Genomes Project; BBJWGS, BioBank Japan Whole-Genome Sequencing; CADD, Combined Annotation Dependent Depletion; gnomAD, the Genome Aggregation Database; HGVD, the Human Genetic Variation Database; jMorp, Japanese Multi Omics Reference Panel; MAF, mutant allele frequency; PolyPhen-2, Polymorphism Phenotyping v2; PROVEN, Protein Variation Effect Analyzer; WES, whole-exome sequencing.

Table 4 Genotyping of two missense *SPATA7* variations in the case-control study

Variation	Sample	Patient			Control		
		1/1 ^a	1/2 ^a	2/2 ^a	1/1 ^a	1/2 ^a	2/2 ^a
p.Asp134Gly	Fujita	1,108	0	0	1,120	0	0
	Kobe	918	0	0	837	0	0
	Niigata	706	0	0	670	0	0
	Combined	2,732	0	0	2,627	0	0
p.Ile332Thr	Fujita	1,109	0	0	1,120	0	0
	Kobe	913	4	0	836	1	0
	Niigata	705	1	0	670	0	0
	Combined	2,727	5	0	2,626	1	0

Note: ^aGenotypes: reference and mutant alleles are denoted by 1 and 2, respectively.

(0.0001), HGVD (0.0004), and gnomAD (0.0005; Table 3).

Discussion

In the first-stage of this study, we did not identify rare recessive homozygous variations, but rare compound heterozygous missense *SPATA7* variations, p.Asp134Gly and p.Ile332Thr, via WES in a family with two affected siblings whose unaffected parents were second cousins. Even in a consanguineous pedigree, a disease trait may be caused by compound heterozygous variations.⁵² For example, rare compound heterozygous missense *AACS* variations were identified in a consanguineous Pakistani family with autosomal recessive intellectual disability.⁵³ In the second-stage of our study, sequencing *SPATA7* coding regions did not detect rare recessive variations in 142 affected offspring for whom parental DNA samples were available for genotyping. In the third-stage of the study, we did not provide statistical evidence for the associations of *SPATA7* p.Asp134Gly and p.Ile332Thr with schizophrenia in 2,756 patients and 2,646 controls.

There is no converging evidence that rare recessive variations play an important role in the genetic etiology of schizophrenia. In a WES study of seven Italian schizophrenia patients with a high number of large runs of homozygosity (ROH), Giacomuzzi et al identified 119 low frequency, homozygous, recessive, non-synonymous and splice-site variations in 107 genes within ROH regions.⁵⁴ These genes significantly overlapped with the composite set of 1,796 genes of a Swedish case-control sample.¹⁴ Using WES data of the Swedish case-control sample, Magri et al found that rare homozygous variations in genes of the gamma-aminobutyric acid system were more frequent in patients (6/4,225) compared with controls

(0/5,834).⁵⁵ However, Ruderfer et al observed no significant difference in rare recessive gene-disrupting variations between Swedish patients (229 of 2,477) and controls (233 of 2,481).⁵⁶ WES of 604 Bulgarian parent-affected offspring trios did not find an increased burden of rare recessive non-synonymous variations.⁵⁷ To draw any conclusion on the effect of rare recessive variations on schizophrenia, further studies should be performed using sufficiently large sample sizes.

SPATA7 encodes spermatogenesis-associated protein 7 (SPATA7). *Spata7* was identified in rat testis, and *SPATA7* was isolated by screening a human testis library.⁵⁸ *SPATA7* mRNA levels are high in retina, brain and testis.⁵⁹ Recessive loss-of-function *SPATA7* variations cause Leber congenital amaurosis and juvenile retinitis pigmentosa.^{48,59,60} In mouse retina, SPATA7 plays a critical role in the proper localization of proteins at the distal connecting cilium.^{61,62} However, the functions of SPATA7 in the brain remain unclear. In silico analysis predicted the *SPATA7* variations, p.Asp134Gly and p.Ile332Thr, to be not damaging. Nevertheless, functional analyses are required to assess the functional implications of these variations. Earlier WES studies also reported no significant association between rare *SPATA7* variations and schizophrenia¹⁴ and no de novo *SPATA7* variations in schizophrenia.¹³ *SPATA7* expression in the dorsolateral prefrontal cortex was not altered in schizophrenia patients.⁶³ There were no available data regarding the methylation of *SPATA7* in the three postmortem brain studies that are registered in the schizophrenia database (SZDB) v2 (<http://www.szdb.org/index.html>).⁶⁴ Taken together, these findings do not support the role of *SPATA7* in the development of schizophrenia.

There are some limitations to our study. First, our WES study had no power to statistically analyze the results and assess their significance. Therefore, we performed follow-up sequencing of *SPATA7* and a case-control study. However, we did not confirm the findings from the WES study. Second, we prioritized rare recessive variations because we hypothesized that these variations play a substantial role in conferring risk for schizophrenia in the consanguineous family with affected siblings. However, the inclusion of compound heterozygous variations may partially contradict the original study design. When we included low frequency, homozygous, recessive variations with mutant allele frequency <0.05, we identified two missense variations: *HEBP2* p.Arg140Gln (rs3734303) and *UPK2* p.Arg152Cys (rs137900462). Even when we genotyped rs3734303 and rs137900462 in our case-control samples, we found no significant associations between these two low frequency missense variations and schizophrenia (Table S6). It is possible that we overlooked the role of other kinds of variations, eg de novo variations^{13,17} and copy number variations.^{10–12} In our family, we identified no rare de novo variations or large homozygous deletions shared by two affected siblings. Third, genomic DNA samples from three siblings (#3, #6 and #7) were not available, and thus we were unable to assess whether they had rare compound heterozygous missense *SPATA7* variations (p.Asp134Gly and p.Ile332Thr). Therefore, it was difficult to distinguish whether the variations that were prioritized in the family were potential risk variations or were coincidentally shared by two affected siblings. Fourth, our results did not exclude the possibility that common variations are implicated in schizophrenia vulnerability in consanguineous families. Interestingly, a WGS study of eight families with monozygotic twin pairs discordant for schizophrenia revealed that polygenic risk scores were higher in probands than in unaffected parents.⁶⁵ Because we performed WES but not WGS, we were unable to calculate the polygenic risk scores.

Conclusion

Our data provide no evidence for the contribution of the rare compound heterozygous *SPATA7* missense variations p.Asp134Gly and p.Ile332Thr to the risk of schizophrenia.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Table S1 Primer sequences for sequencing *SPATA7* coding regions

Exon	Forward	Reverse
1	5'-CGCAACTGCCTCCTAGTACC-3'	5'-ACAAATTCAGGGCAAAGAAGC-3'
2	5'-TTTAATGCTGTAACCTCAGACTTCCT-3'	5'-TGAAGTTCAAATATTCGTCAAATG-3'
3	5'-AAGGTTTGAACCCAAATGGTC-3'	5'-CAAAAATGGGTATGAATTTGCT-3'
4	5'-CAAGGTCTGGAACATTTTGTGA-3'	5'-TGTTTATGTGGCACAGGAATTT-3'
5	5'-ATCTAGAGGCACATGTGAAATAAA-3'	5'-CAAAGTCAGATTGTACCACTAAAGAA-3'
6.1 ^a	5'-TTTTGTAAACCTTGAGGCTATC-3'	5'-GGAGTGAATGGCAATTGTTTGT-3'
6.2 ^a	5'-AGTCATCACAAATGGTCCTGAG-3'	5'-TTCCAATCAAAGGGCACTATC-3'
7	5'-TCTGGCAGTAGGTTTTAGTTGTTTT-3'	5'-TGATGATAAGTGCCACCAACAG-3'
8	5'-TGCTGTGTTATATTCTGCTTTTCG-3'	5'-TAGATTGGAGCATGCAATTTAAA-3'
9	5'-CATTAACTTAGTCAAATGTCTATTG-3'	5'-TGGTTTCTTTGATTCTTAATCCTTG-3'
10	5'-CCCAGTGGATTGCATTTGA-3'	5'-GGTGAACCTCCCCTAGAGTATGA-3'
11	5'-TTTTCAACCTTTGTAGTTTCAGTG-3'	5'-TTCCTTTCACTTCTCCCACCAC-3'
12.1 ^a	5'-AATCCTGTGAGATTTTCAGCAC-3'	5'-TCACAGAAGTTTCCCGATCTGT-3'
12.2 ^a	5'-GAAGTAACAATTCAGCAGGAACG-3'	5'-TGAGTTACTGGCCATTTGAGGT-3'

Note: ^aExons 6 and 12 were amplified as two overlapping fragments.

Table S2 Probes used for the TaqMan 5'-exonuclease assay

Variation	Forward primer	Reverse primer	Reporter 1	Reporter 2
p.Asp134Gly	5'-CTCAGGCGAAC CGCAAATT -3'	5'-GACCTTGC AAAGGATGAA AATCCAT -3'	5'-VIC-CTTTTAAACAT GTCATCCTC -NFQ-3'	5'-FAM-TTAACATGCCATCCTC -NFQ-3'
p.Ile332Thr	5'-CTTTAGAAGG GCATGACTCAACA TG -3'	5'-ACCTTGGTGAGG AATGCTGAAG -3'	5'-VIC-AGCATCATCCT TAATCTCAT -NFQ-3'	5'-FAM-AGCATCATCCTTAGTCTC AT -NFQ-3'

Table S3 WES quality report summary

	Father (#1)	Mother (#2)	Proband (#4)	Affected sibling (#5)
Mean depth	58.4	55.9	67.6	48.0
Coverage at 10×	98.1	97.9	97.4	97.1

Abbreviation: WES, whole-exome sequencing.

Table S4 Coefficient of relatedness from the WES data for each pair of individuals

Pair of individuals	Coefficient of relatedness
Father (#1) and mother (#2)	0.038
Father (#1) and proband (#4)	0.522
Father (#1) and affected sibling (#5)	0.513
Mother (#2) and proband (#4)	0.494
Mother (#2) and affected sibling (#5)	0.503
Proband (#4) and affected sibling (#5)	0.430

Abbreviation: WES, whole-exome sequencing.

Table S5 SPATA7 variations identified by sequencing

dbSNP ID	Position ^a	Allele ^b	Exon	Protein	In silico analysis		MAF					
					PolyPhen-2	PROVEN	jMorp	HGVD	BBJWS	IKGP	gnomAD	
rs4904448	88852166	G/A	1	Asp2Asn	Possibly damaging	Neutral	0.0556	0.0574	0.0540936	0.0433	0.03567	
rs527236050	88857725_88857728	TCAG/del	-	Splice Acceptor Variant	-	-	0.0089	-	0.0126706	-	0.006294	
rs3179969	88862529	G/A	4	Val74Met	Benign	Neutral	0.2834	0.2731	0.280702	0.3029	0.3236	
-	88892638	C/G	6	Ser145Ser	-	Neutral	-	-	-	-	-	
rs769211713	88894018	A/T	7	Asp297Val	Possibly damaging	Deleterious	0.0038	0.0039	0.00341131	-	0.0004694	
rs375371982	88897520	A/G	9	Met345Val	Benign	Neutral	0.0046	0.0062	0.00633528	-	0.001002	
rs750676893	88904442	C/A	12	Phe492Leu	Benign	Neutral	0.0053	0.0054	0.00682261	-	0.0007465	
rs10139784	88904567	G/A	12	Arg534Gln	Benign	Neutral	0.0236	0.0211	0.0272904	0.0096	0.02946	

Note: ^aPosition according to GRCh37. ^bReference/mutant allele.

Abbreviations: IKGP, 1000 Genomes Project; BBBWGS, BioBank Japan Whole-Genome Sequencing; gnomAD, the Genome Aggregation Database; HGVD, the Human Genetic Variation Database; jMorp, Japanese Multi Omics Reference Panel; MAF, mutant allele frequency; PolyPhen-2, Polymorphism Phenotyping v2; PROVEN, Protein Variation Effect Analyzer.

Table S6 Genotyping of two uncommon missense variations in the case-control study

Variation	Sample	Patient		Control	
		1/1 ^a	2/2 ^a	1/1 ^a	2/2 ^a
rs3734303	Fujita	1,020	88	1,032	90
	Kobe	871	47	794	41
	Niigata	657	48	629	42
	Combined	2,548	183	2,455	173
rs137900462	Fujita	1,065	46	1,088	35
	Kobe	887	30	794	38
	Niigata	675	31	625	46
	Combined	2,627	107	2,507	119

Note: ^aGenotypes: reference and mutant alleles are denoted by 1 and 2, respectively.

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