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Association Analysis of Rare *CNTN5* Variants With Autism Spectrum Disorder in a Japanese Population

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ABSTRACT

Background: Contactin-5 (CNTN5), a neural adhesion molecule involved in synaptogenesis and synaptic maturation in the auditory pathway, has been associated with the pathophysiology of autism spectrum disorder (ASD), particularly hyperacusis. To investigate the role of rare *CNTN5* variants in ASD susceptibility, we performed resequencing and association analysis in a Japanese population.

Methods: We resequenced the *CNTN5* coding regions in 302 patients with ASD and prioritized rare putatively damaging variants. The prioritized variants were then genotyped in 313 patients with ASD and 1065 controls. Subsequently, we conducted an association study of selected variants with ASD in 614 patients with ASD and 61 057 controls. Clinical data were reviewed for patients carrying prioritized variants.

Results: Through resequencing, we prioritized three rare putatively damaging missense variants (W69G, I227L, and L1000S) in patients with ASD. Although we found a nominally significant association between the I227L variant and ASD, it did not remain significant after post hoc correction. Hyperacusis was found in three out of nine patients carrying prioritized variants.

Conclusion: This study does not provide evidence for the contribution of rare *CNTN5* variants to the genetic etiology of ASD in the Japanese population.

1 | Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder distinguished by impaired social communication and interaction, as well as restricted, repetitive behaviors or interests [1]. Over 90% of individuals with ASD exhibit sensory abnormalities as part of their restricted, repetitive behaviors [2]. In particular, hyperacusis, or auditory hypersensitivity, has an estimated current prevalence of 37%–45% and a lifetime prevalence of 50%–70% in the ASD population, according to a recent meta-analysis [3]. Additionally, the occurrence of auditory dysfunction in ASD is supported by findings of prolonged wave

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latencies or delayed responses in auditory brainstem response tests in infants and children with autism, suggesting immaturity in the auditory brainstem circuits [4–6].

Genetic analysis is crucial for elucidating the underlying mechanisms of auditory dysfunction in the ASD population, which is primarily explored through knock-out studies of ASD-related genes in animal models [7–10]. Among these genes, *contactin-5* (*CNTN5*) encodes CNTN5 that facilitates neurite outgrowth [11, 12] and synaptogenesis [13], critical functional pathways linked with ASD phenotypes [14, 15]. CNTN5 is a cell adhesion molecule anchored to the cell membrane by a glycosylphosphatidyl inositol (GPI) anchor, comprising six immunoglobulin-like domains and four fibronectin type III homologous repeats [16]. In mice, *Cntn5* is prominently expressed in brain regions involved in the auditory pathway [17], and its deficiency disrupts synapse formation and triggers neuronal apoptosis during postnatal development of the auditory brainstem, consequently increasing auditory brainstem response wave latencies in the adult stage [18].

In European populations, inconsistent results for an association of rare *CNTN5* variants with ASD have been reported. Mercati et al. [19] resequenced *CNTN5* in 212 patients with ASD and 217 control individuals. They found a significant association between rare *CNTN5* variants and ASD in 501 patients and 33 075 controls. In contrast, Murdoch et al. [20] failed to observe a significant association in 1030 patients and 942 controls. A two-stage analysis study aggregating exome and genome sequencing data from 42 607 ASD cases and 236 000 controls did not list *CNTN5* as an ASD-associated gene despite identifying two rare *de novo* mutations in this gene [21]. These discrepancies highlight the need for further investigation into the role of rare *CNTN5* variants in ASD susceptibility.

In a Japanese population, a whole-exome sequencing (WES) study failed to find an association between rare *CNTN5* variants in 309 ASD cases and 299 controls [22]. Here, we performed a four-stage study to investigate the association between rare putatively damaging *CNTN5* variants and ASD in a Japanese population. In the first stage, we resequenced coding regions of *CNTN5* in 302 patients with ASD. In the second stage, we genotyped rare putatively damaging *CNTN5* variants in 313 patients with ASD and 1065 controls. In the third stage, we performed an association study between rare putatively damaging *CNTN5* variants and ASD in 614 patients and 61 057 controls. In the fourth stage, we conducted a chart review to obtain clinical information from the patients carrying rare putatively damaging *CNTN5* variants.

2 | Methods

2.1 | Participants

We enrolled 302 patients with ASD to resequence *CNTN5* coding regions for variant screening (Table 1). Then, we genotyped the variants prioritized via resequencing in 313 patients with ASD and 1065 control individuals. At this genotyping stage, 191 ASD samples overlapped with those previously included in the study by Kimura et al. [22]. There was no overlap between the individuals with ASD in the resequencing and genotyping processes. Child psychiatrists established the ASD diagnosis based on the criteria for ASD in the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5). Control individuals did not have any personal or familial history of psychiatric disorders among their first-degree relatives. All subjects recruited in this study were of Japanese descent.

To increase statistical power, we used additional controls by obtaining the genome or exome data of 60 000 Japanese individuals from the Tohoku Medical Megabank Organization (ToMMo) 60KJPN allele frequency panel (https://jmorp.megabank.tohoku.ac.jp/) [23].

2.2 | *CNTN5* Coding Region Resequencing

We examined 302 patients with ASD using the Sanger sequencing method, as described previously [24], to identify variations in the *CNTN5* coding region, spanning from exon 3 to exon 25 (RefSeq accession number NM_014361). We designed custom amplification primers (Table S1) to cover this region.

The variations identified from resequencing were prioritized if they were predicted using *in silico* tools as deleterious variants that had a Phred score of ≥20 from Combined Annotation Dependent Depletion (CADD) v1.7 (https://cadd.gs.washington.edu/) [25] and categorized as possibly or probably damaging by Polymorphism Phenotyping v2 (PolyPhen-2; http://genetics.bwh.harvard.edu/pph2/) [26]. We included rare variants with a mutant allele frequency (MAF) < 0.01 in ToMMo 60KJPN. Variants with MAF < 0.01 are more likely to be deleterious and have larger effect sizes that contribute to disease risk, as these rare variants are often under negative selection, including weak and strong selection [27–29]. This cutoff enriches for variants more likely to impact complex diseases, particularly when using a combination of predictor tools. However, not all variants will show statistically significant associations because association

TABLE 1 | Characteristics of participants.

	ASD		Control
Characteristic	**Resequencing**	**Genotyping**	**Genotyping**
Number of samples	302	313	1065
Male (%)	234 (77.5%)	236 (75.4%)	460 (43.2%)
Age (year)[a]	19.4 ± 9.5	19.6 ± 10.2	39.0 ± 12.1

Abbreviation: ASD, autism spectrum disorder.
[a]Mean ± standard deviation.

strength depends on factors such as effect size, selection pressure, and sample size [29]. Next, the prioritized variants were investigated through a conservation analysis of human CNTN5 orthologs across different vertebrae and were compared against other human CNTN family members. This evolutionary conservation was analyzed using the Constraint-based Multiple Alignment Tool (COBALT; https://www.ncbi.nlm.nih.gov/tools/cobalt/cobalt.cgi#) [30].

2.3 | Case–Control Study

We genotyped the selected variants in 313 patients with ASD and 1065 control individuals using the TaqMan 5′-exonuclease assay (Thermo Fisher Scientific, Waltham, MA, USA; Table S2), as previously described [31]. For the case–control analysis, we combined samples from resequencing, genotyping, and acquired data from public databases, resulting in 614 ASD cases and 61 057 controls.

We excluded the variant with a genotyping call rate of less than 95% and that also deviated from Hardy–Weinberg equilibrium using the chi-square test for goodness-of-fit. To investigate the contribution of each prioritized *CNTN5* variant identified through resequencing to ASD susceptibility in the Japanese population, we conducted an association analysis using Fisher's exact test. The α level was adjusted using Bonferroni correction for multiple testing. To test the joint effects of all prioritized variants in *CNTN5*, our combined resequencing and genotyping data (614 cases and 1057 controls) without additional controls from ToMMo 60KJPN were converted into PLINK-formatted files (http://pngu.mgh.harvard.edu/purcell/plink/) [32] and then analyzed using the sequence kernel association test (SKAT; https://rdrr.io/cran/SKAT/src/R/Function.R) [33]. To estimate the statistical power of our results, we used the Genetic Power Calculator (http://zzz.bwh.harvard.edu/gpc/cc2.html) [34] with an α level of 0.05 and assuming a disease prevalence of 0.01.

2.4 | Chart Review of Patients Carrying Rare Putatively Damaging *CNTN5* Variants

We conducted a chart review to obtain clinical information from the medical records of patients with ASD carrying prioritized *CNTN5* mutations. The information entailed the presence of hyperacusis, full-scale intellectual quotient scores, neuropsychiatric comorbidities, and family history with ASD diagnosis.

3 | Results

The variant screening by resequencing the *CNTN5* coding region in 302 patients with ASD identified 12 variants (Table 2). We prioritized three rare missense variants (W69G, I227L, L1000S) that

TABLE 2 | *CNTN5* variants identified via resequencing.

				Genotype[c]	*In silico* analysis		Mutant allele frequency (MAF)
Position[a]	Allele[b]	dbSNP ID	Amino acid	ASD	PolyPhen-2	CADD	ToMMo 60KJPN
99 819 555	T/G	rs10790978	S23A	215/86/1	Benign	14.76	0.405[d]
99 819 693	**T/G**	**rs778655565**	**W69G**	**300/2/0**	**Probably damaging**	**21.9**	**0.0006**
99 844 969	G/A	rs577549789	S132N	301/1/0	Benign	7.808	0.003
99 845 151	A/T	rs201145645	T156S	300/2/0	Benign	16.42	0.002
99 956 811	**A/C**	**rs771277271**	**I227L**	**298/4/0**	**Possibly damaging**	**20.5**	**0.003**
100 061 278	A/T	rs186615197	K349N	293/9/0	Probably damaging	22.0	0.011
100 061 356	T/A	rs201982881	R375=	301/1/0	—	4.886	0.002
100 191 133	A/G	rs11223168	I530V	266/33/3	Benign	15.78	0.068
100 341 174	**T/C**	**rs757538847**	**L1000S**	**301/1/0**	**Possibly damaging**	**23.7**	**0.003**
100 350 849	A/C	rs140703637	I1060L	299/3/0	Benign	22.6	0.002
100 356 152	T/A	rs1216183	S1079T	129/138/35	Benign	0.328	0.388
100 356 197	A/C	—	M1094L	301/1/0	Benign	1.007	—

Note: Mutations with bolded fonts fulfilled the criteria of prioritized variants: MAF < 0.01 and predicted damaging by PolyPhen-2 and CADD score > 20.
Abbreviations: ASD, autism spectrum disorder; CADD, Combined Annotation Dependent Depletion; PolyPhen-2, Polymorphism Phenotyping v2; ToMMo 60KJPN, Tohoku Medical Megabank Organization 60KJPN allele frequency panel.
[a]Position according to GRCh38.
[b]Reference/alternative allele.
[c]Homozygous for reference allele/heterozygous/homozygous for mutant allele.
[d]Reported as fail during filtering implying the low-quality mutation.

were predicted to be probably damaging or possibly damaging using PolyPhen-2 and had CADD scores over 20. We did not include I1060L, which was predicted as benign using PolyPhen-2, or K349N, as its MAF was 0.011 in ToMMo 60KJPN. We mapped the prioritized variants onto CNTN5 protein domains (Figure 1). I227L and L1000S were located in the Immunoglobulin-like 2 (Ig2) and Fibronectin type III 4 (FN4) domains, respectively, while W69G was not localized within any known domains. All residues corresponding with our prioritized variants were conserved among distinct vertebrates (Table S3).

Next, three prioritized variants were genotyped in 313 patients with ASD and 1065 controls. The genotyping rate of the W69G variant was less than 95%, and we excluded the variant from a subsequent association analysis. There was no significant deviation from Hardy–Weinberg equilibrium in the genotype distributions of all variants (Table S4). We also excluded one ASD patient and eight controls from further analysis due to missing genotyping data for all prioritized variants.

By incorporating additional controls from ToMMo 60KJPN, we expanded our case–control analysis to include 614 cases and 61 057 controls (Table 3). A nominally significant association was detected between the I227L and ASD ($p = 0.04$), although this did not withstand the Bonferroni correction with an adjusted α of 0.025. Similarly, no significant association was observed between the set of prioritized *CNTN5* variants and ASD using the SKAT method in 614 cases and 1057 controls (PSKAT = 0.111).

Clinically, most patients with ASD carrying the prioritized variants were males with sporadic ASD in the resequencing and genotyping phases (Table 4). Of the three prioritized variants, only two (I227L and L1000S) had sufficient genotyping call rates. We were able to obtain clinical information from five of seven patients carrying I227L, and hyperacusis developed in one of these patients. Similarly, one out of two patients carrying I1000L had hyperacusis. Although W69G had a low genotyping call rate, one in two patients carrying this variant complained of hyperacusis. Tabulating all available data, three out of nine (33.3%) patients with prioritized variants had hyperacusis. Regarding comorbidity, attention deficit hyperactivity disorder was observed in one patient with W69G and three patients with I227L. A patient with the L1000S mutation also had an intellectual disability as a comorbidity.

4 | Discussion

In this study, we screened the coding region of *CNTN5* in 302 Japanese patients with ASD and identified three rare putatively damaging variants. Of these, I227L was nominally associated with ASD in the analysis involving 614 ASD cases and 61 057 controls, although this association did not pass the Bonferroni correction threshold. Overall, our analysis did not provide substantial evidence for the involvement of rare putatively damaging *CNTN5* variants in the genetic etiology of ASD.

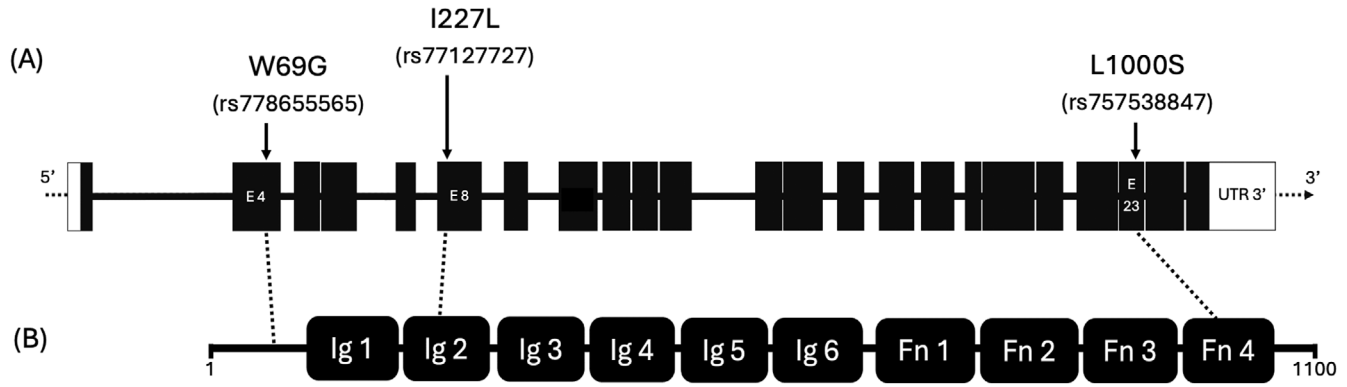

FIGURE 1 | Locations of rare putatively damaging *CNTN5* variants. (A) The genomic structure of *CNTN5* is based on RefSeq accession number, NM_014361. Coding and untranslated regions are shown as black and white rectangles, respectively. (B) The CNTN5 protein structure follows the UniProt protein databases for CNTN5 (UniProt ID O94779). Black arrows and dotted lines indicate the location of each putatively damaging variant found through resequencing in both structure.

TABLE 3 | Association analysis of rare putatively damaging *CNTN5* variants.

	Allele count[a]							
	ASD			Control				
Variant	Resequencing	Genotyping	Combined	Genotyping	ToMMo 60KJPN	Combined	*p* value	Odd ratio
I227L	600/4	611/3	1211/7	2062/4	119 560/316	121 622/320	0.046	2.2
L1000S	603/1	623/1	1226/2	2107/7	119 566/306	121 673/313	0.775	0.6

Abbreviations: ASD, autism spectrum disorder; ToMMo 60KJPN, Tohoku Medical Megabank Organization 60KJPN allele frequency panel.
[a]Reference allele/alternative allele.

TABLE 4 | Clinical characteristics ASD patients carrying rare putatively damaging *CNTN5* variants.

ID	Variant	Sex	Age	Hyperacusis	Full scale IQ	Comorbid	Family history
1	W69G	Male	14	Yes	107	No	No
2		Female	26	No	66	Suspected ADHD	No
3	I227L	Male	9	No	94	ADHD	No
4		Male	8	No	89	Suspected ADHD	No
5		Male	22	No	87	No	No
6		Male	44	No	72	ADHD	No
7		Male	10	Yes	NA	No	No
8		Male	24	NA	NA	NA	NA
9		Female	42	NA	NA	NA	NA
10	L1000S	Male	24	Yes	98	No	No
11		Female	20	No	50	Intellectual disability	NA

Abbreviations: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; IQ, intelligence quotient; NA, not available.

CNTN5 plays a crucial role as a neuronal cell adhesion molecule during neurodevelopment by regulating neurite outgrowth [11, 12], neuronal migration [35], synaptic formation, and maturation [18]—the processes associated with ASD [36]. A study using induced pluripotent stem cells derived from patients with ASD demonstrated that the heterozygous loss of *CNTN5* increases neuron excitability, indicating impaired synaptic function [37]. Although the knock-down of the *Cntn5* gene in rodents did not result in behavioral abnormalities [35], it was associated with synaptic immaturity leading to auditory impairment [17, 18].

Furthermore, Mercati et al. found a significant increase in the prevalence of hyperacusis (reaching 87.5%), evaluated using objective audiometry assessments, among 24 patients with ASD carrying 12 rare missense *CNTN5* variants [19]. Collectively, these findings suggest that disruptions in *CNTN5* neurobiological functions may contribute to the development of hyperacusis in ASD. In the present study, the prevalence of hyperacusis, assessed by child psychiatrists using unstructured interviews, was 33.3% among nine patients with ASD carrying rare putatively damaging missense variants (W69G, I227L, and L1000S). We then performed a chart review and obtained information of hyperacusis in six of seven patients carrying the other non-prioritized rare missense variants (S132N, T156S, I1060L, and M1094L) of *CNTN5*. Four of those patients had hyperacusis, and thus, the prevalence of hyperacusis was 44.7% among 15 patients with ASD carrying rare missense *CNTN5* variants. Moreover, subjectively reported hyperacusis observed by parents ranged from 5%–38% in Japanese patients with ASD using subjective measurement tools [38–40]. Taken together, unlike the Mercati et al. study [19] that used objective audiometry assessments that could assess latent hyperacusis in patients with ASD [41], we hypothesized that our lower hyperacusis prevalence might be due to reliance on subjective reports from unstructured interviews documented in medical records.

In the present study, we found hyperacusis in patients carrying rare putatively damaging missense variants (W69G, I227L, and L1000S), while Mercati et al. [19] observed hyperacusis in patients carrying 12 rare missense *CNTN5* variants. Notably, none of the rare variants carried by patients with hyperacusis in the European cohort of the study by Mercati et al. overlapped with those found in our Japanese cohort. W69G and I227L were specifically identified in the East Asian population (gnomad v 4.1.0, https://gnomad.broadinstitute.org) [42], while L1000S was predominantly detected in the East Asian population, with only one occurrence in a non-East Asian individual. Of 12 rare missense *CNTN5* variants carried by patients with ASD having hyperacusis in the Mercati et al. study [19], seven (D242Y, L254F, E805D, A871P, A884V, I899T, and S933F) were absent in the East Asian population, while the other five were very rare (I158M, I528T, L790I, and V935I with MAF <0.0001 in gnomAD v4.1.0 East Asian; R837Q with MAF of 0.000055 in ToMMo 60KJPN). This lack of shared rare variants may reflect the impact of demographic history and natural selection, driving population differentiation [43–45].

The human *CNTN5* gene has an alternative splicing isoform that omits 74 amino acids (Refseq accession number NM_175566.2), corresponding to the exon 4 region of the *CNTN5* canonical isoform [46]. Similarly, other CNTN members also lack this region, making CNTN5 the most extended protein [46]. A previous WES study identified a *de novo* D96G mutation in a proband with ASD [21], while we found a W69G mutation in exon 4 of the N-terminal region of CNTN5. Although the function of this region is not well understood, a copy number variant lacking this region was found in an individual with ASD who developed hyperacusis and abnormal motor coordination [19]. The W69G variant corresponds to the substitution of tryptophan, which is large, rigid, and hydrophobic with an aromatic side chain [47], for glycine, which is small, flexible, and neutral with a side chain consisting of a single hydrogen atom [48]. This variant may cause a shift toward greater disorder, potentially altering its regional structure and function [49, 50]. This assumption should be validated through in vitro and in vivo neuron culture studies.

We excluded W69G from the association analysis due to a low genotyping call rate of less than 95% observed only in the control

group, which was significantly different from that of the ASD group ($p < 0.001$). This discrepancy might have resulted from various technical issues, such as the low quantity or quality of DNA samples, the presence of PCR inhibitors, or pipetting errors, particularly in 4 out of 12 control group batches [51, 52]. However, we acknowledge the limitation of our study, as we were unable to reexamine these samples due to the insufficient quantity of reserved DNA and limited resources for new DNA extraction.

CNTN5 is tethered to the plasma membrane by a GPI anchor, implying that it needs to form coreceptor complexes with other transmembrane proteins to transmit signals inside the cell in the absence of an intracellular region [53, 54]. One key interacting protein is the protein tyrosine phosphatase receptor gamma (PTPRG), which binds to the Ig2 and Ig3 domains of CNTN5 at four specific sites comprising identical residues shared with other CNTNs [55, 56]. Our study identified I227L within the Ig2 domain. Notably, this variant does not occur at residues that interface with PTPRG. The I227 residue in human CNTN5 is not identical with its paralogs, which possess a serine at this position (Table S5). Several deleterious *CNTN5* variants in Ig2 and Ig3 domains have been identified in patients with ASD of European descent [19, 57]. *CNTN4* and *CNTN6* variants within Ig2 and Ig3 domains identified in ASD cases altered neurite outgrowth and synaptogenesis, despite not substituting PTPRG contact residues [19, 58]. Functional studies are needed to examine the impact of *CNTN5* variants within Ig2 and Ig3 domains on protein stability, structure, and interactions.

In the present study, we identified L1000S in the FN4 domain, which may affect protein stability due to its proximity to an N-linked glycosylation site (N1002) [59]. Notably, the Autism Sequencing Consortium (https://asc.broadinstitute.org) previously reported a stop-gained *CNTN5* mutation (S1064X) in the same domain, exclusively found in an individual with ASD [57]. Fibronectin repeats of contactins have been reported as essential regions that facilitate binding with the members of the amyloid precursor protein family [60], although a recent study suggests that the binding site is limited to a small conserved region in the FN2 domain of CNTN5 [61].

Additionally, we identified a novel missense mutation (M1094L), which was not recorded in any genetic databases, despite a different substitution (M1094V) previously registered as rs35208161 in the same position. M1094L is located in the C-terminal GPI domain of CNTN5. The lack of this domain in CNTN4 affects its function in dendritic spine formation through membrane localization [58]. However, our novel mutation was predicted to be benign.

Our data showed an interestingly consistent interpretation of pathogenic variants between PolyPhen-2 and CADD, except for one variant. I1060L was predicted to be benign by PolyPhen-2 yet had a CADD score of 22.6. CADD incorporates conservation analysis, epigenetic modifications, functional prediction, and genetic content, and it calculated these features relative to ~9 billion single nucleotide variants in the entire genome to produce Phred scores, of which a score of above 20 indicates the top 1% of the highest Phred deleterious-proxy scores [62]. However, PolyPhen-2 uses information on variants such as multiple sequence alignment and its predicting effect on a protein structure to predict the impact of rare variants [26]. Multiple sequence alignment in particular is one of the major indicators for predicting variant classification by assessing amino acid conservation and the conservation of surrounding regions. Highly conserved amino acids and conserved regions result in a "damaging" prediction as seen in our three missense variants: W69G, I227L, and L1000S (Tables 2 and S3). However, I1060L and its surrounding region are not highly conserved in a series of species; thus, they were predicted to be benign in PolyPhen-2. Because of their algorithmic analyses, these variant predictor tools occasionally differ in their outcome despite CADD and PolyPhen-2 being among the best variant prediction tools available [63].

We considered that the relatively small sample size of 614 ASD cases and 61 057 controls represented a limitation in this study. Although we observed a nominally significant association between I227L and ASD, this finding did not pass the Bonferroni correction threshold. Assuming the MAF of I227L in the control group (0.00262) as a risk allele frequency and the odds ratio from association analysis (2.2) as the genotypic relative risk in a dominant model of inheritance, our association only reached 57% power with α of 0.05. Therefore, the sample size may explain our negative results. Another limitation of our study was that our variant screening focused solely on the coding regions, potentially overlooking significant mutations in non-coding regions such as the promoter, untranslated regions, or intronic regions of *CNTN5*. Of note, a whole-genome sequencing study identified a novel *de novo* variant (g.100072400T > G) in a patient with ASD, located in the *CNTN5* intronic enhancer region [64]. Further studies with larger sample sizes are needed to examine associations between coding and non-coding *CNTN5* variants and ASD.

In conclusion, our present study does not provide evidence for the contribution of rare putatively damaging *CNTN5* variants to ASD susceptibility in the Japanese population.

Author Contributions

Jun Egawa and Yuichiro Watanabe designed the study. Abdul Fuad Hadi and Reza K. Arta performed resequencing, genotyping, and the statistical analyses. Jun Egawa and Itaru Kushima collected clinical data. Abdul Fuad Hadi, Reza K. Arta, Jun Egawa, and Yuichiro Watanabe wrote the first draft of the manuscript. Norio Ozaki and Toshiyuki Someya directed the project. All authors contributed to the revision of the manuscript and approved the final manuscript.

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Ethics Statement

The protocol for this study received approval from the Ethics Committee on Genetics of Niigata University School of Medicine (Approval No. G2018-0002) and was conducted following the Declaration of Helsinki.

Consent

Written informed consent was obtained from all participants and/or their families.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

We are not able to make the individual-level raw data available to readers because we do not have permission from the participating institutions to do so.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.