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RS2710102 polymorphism of the *CNTNAP2* gene is related to autism susceptibility in a Kazakhstani population

Abstract. Autism spectrum disorders (ASDs) represent serious mental development disorders characterized by deficits in verbal and non-verbal communication, reciprocal social interactions and stereotypical behaviors. Genetically determined pathologies of neurodevelopment and synaptic functioning are increasingly considered to be a cause of ASDs. Contactin associated protein-like 2 (*CNTNAP2*) gene encodes a protein, which plays an essential role in brain development. Genetic variations in the *CNTNAP2* gene can perturb its functions, contributing to the genetic predisposition to ASDs. The study aimed to investigate an association of the *CNTNAP2* rs2710102 with ASDs in a Kazakhstani population. The study involved patients diagnosed with ASDs and healthy controls of Kazakhstani origin. PCR-RFLP assay was used for the genotyping rs2710102 *CNTNAP2* SNP. The distribution of the rs2710102 genotype was under the Hardy-Weinberg equilibrium in both cases and controls. C allele and *CC genotype were associated with a significantly increased risk of ASDs (OR* = 3.04*, 95% CI* = 1.96-4.72*, p<0.001 and OR* = 6.41*, 95% CI* = 2.47-16.63*, p<0.001, respectively), which was also confirmed for males (OR* = 2.25*, 95% CI* = 1.23-4.10*, p=0.007 and OR* = 2.95*, 95% CI* = 1.06-8.18*, p=0.029, respectively) and females (OR* = 4.75*, 95% CI* = 1.91-11.77*, p<0.001 and OR* = 7.20*, 95% CI* = 0.89-58.53*, p=0.002, respectively). In contrast,* there was no statistically significant association of the rs2710102 with deficits of verbal communication in ASD patients. The obtained results provide the first significant link between rs2710102 *CNTNAP2* and autism susceptibility in Asian populations.

Key words: Autism spectrum disorders (ASDs), *CNTNAP2* gene, single-nucleotide polymorphism, genetic susceptibility, Kazakhstani population, neurodevelopment.

Introduction

Autism spectrum disorders (ASDs) are a group of heterogeneous neurodevelopmental diseases, which are characterized by deficits in verbal and non-verbal communication, reciprocal social interactions and stereotypical behavior. In Kazakhstan, as everywhere in the world, the number of children with ASDs has been increasing annually. In Kazakhstan, according to the Service of psychological, medical and pedagogical consultation, autism was diagnosed in 3820 children (in the 2020 year), however, according to the international experts, the real number of children with ASD significantly exceeds this figure. For, according to the Institute of Autism at Oregon State University (USA), in our country 59 thousand children have autism spectrum disorders.

Genetic factors are one of the main components of ASDs. Literature shows hundreds of candidate genes for ASDs, which may be a subject of closer attention and deserve a separate study. Here, we focused on the contactin associated protein-like 2, which has been proposed to be a candidate gene for ASDs [1].

CNTNAP2 gene is located in 7q35–36 and encodes the transmembrane protein Caspr2, which is a member of the neurexin family. This protein is localized at the juxtaparanodes of myelinated axons providing interactions between neurons and glia during nervous system development. It is also involved in clustering K+ channels in myelinated axons [2].

Several genetic, neurobiological and mouse model studies have supported the role of CNTNAP2 in ASDs and other related neurodevelopmental disorders. In the Old Order Amish community, the mutations of *CNTNAP2* have been identified as causes of symptomatic childhood-onset epilepsy, characterized by the presence of neuronal migration

abnormalities, intellectual disability, language regression, seizures, hyperactivity, impulsive/ aggressive behavior, as well as ASDs [3].

In mouse models, CNTNAP2 deficiency has shown extraordinary similarity to the main deficits of behavioral and cognitive functions of ASD patients. Neurophysiological features, such as cortical neuronal migration abnormalities, observed in CNTNAP2-/- mice, further supported the involvement of the *CNTNAP2* gene in ASD pathologies [4]. The migration of neurons in the developing cerebral cortex plays a key role in the development of brain and brain networks [5]. A number of neuroimaging studies demonstrated abnormal connectivity and altered brain networks in individuals with ASDs [6–8]. Moreover, mouse model studies have demonstrated a capacity of anti-Caspr2 auto-ABS to generate ASD-like behavior [9, 10].

Besides, the *CNTNAP2* gene is expressed in language-related cortical areas [11]. Hence, given the speech impairment in autism, CNTNAP2 becomes a strong candidate gene for autism.

Genetic variations in the *CNTNAP2* gene can affect its functions contributing to the genetic predisposition to ASDs. It has been shown that the loss of even one CNTNAP2 allele leads to elicit axonal growth alterations [12]. Several common and rare variants of CNTNAP2 have been reported to be associated with autism as well as related to phenotypes such as intellectual deficiency, impaired language, abnormal social behavior, epilepsy and schizophrenia [13–17].

The single-nucleotide polymorphism (SNP) rs2710102 is located at intron 13. There are still insufficient data on the relation of this common variant with ASDs in Asian populations. Therefore, in the present study, we carried out a case–control association study between rs2710102 and ASDs in a Kazakhstani population.

Materials and methods

The study involved patients diagnosed with ASDs and healthy controls of Kazakhstani origin. A total of 51 healthy individuals and 280 ASD patients had been recruited in rehabilitation centers of Kazakhstan starting from March 2018 to December 2020.

Collection of the clinical material in families with children with ASD was carried out in the "Autism Pobedim" Foundation based on the Memorandum of Cooperation, as well as in public funds, working with ASD children in Almaty, Astana, Zhezkazgan, Karaganda, Kokshetau, Kyzyl-Orda, Pavlodar, Petropavlovsk, Temirtau, Ust-Kamenogorsk, Shymkent, Ekibastuz and South Kazakhstan region.

Buccal epithelium samples were collected from patients with ASDs, as well as from healthy individuals using sterile cotton-tipped applicators. The collected material was transported to the Institute of General Genetics and Cytology in a portable refrigerated container and frozen at -80° C for further molecular-genetic studies.

The collection of biomaterial was conducted exclusively voluntarily after signing an informed consent by at least one of the parents. The protocol of the study was approved by the Ethics Committee of S. Asfendiyarov Kazakh National Medical University (Protocol #57 from 05.09.2017).

The clinical diagnosis of autism was established by senior psychiatrists and assessed by the CARS for children over 3 years and the M-Chat-R for children under three years old. CARS and M-Chat-R have been used as standardized, investigator-based instruments for the detection of ASDs [19, 20]. ASD patients with M-CHAT or CARS scores in the low-risk range were excluded from the study. Additional exclusion criteria were a recognizable neurological or genetic disorder (Rett syndrome, Fragile X syndrome and others), noncitizens of Kazakhstan.

For the control group, the exclusion criteria were the presence of autism or other mental disorders in personal and family history, M-CHAT or CARS scores in the medium/high-risk range, noncitizens of Kazakhstan.

Verbal communication in the ASD group was assessed by interviewing parents of ASD children and classified as without (verbal communication is appropriate for age and situation) or with speech disorders (delayed speech, echolalia, meaningless speech, etc.).

DNA from buccal swabs was isolated using a DNA extraction kit (AmpliSens). DNA samples were stored at -20°C and -80°C. PCR-RFLP assay was used for the genotyping rs2710102 CNTNAP2 SNP as described earlier [18].

Statistical analysis was performed using "Case-Control Study Estimating Calculator" by TAPOTILI company (Laboratory of Molecular Diagnostics and Genomic Dactyloscopy of "GosNIIGenetika" State Scientific Centre of Russian Federation). Hardy-Weinberg equilibrium (HWE) test was used to compare the observed and expected genotype frequencies. Relative risks were estimated by odds ratios (OR) with a logistic regression 95% confidence interval. Statistical analysis considering the models of inheritance– the multiplicative, dominant, and recessive – was conducted for the examined SNP. $P < 0.05$ was considered statistically significant.

Results and discussion

General Characteristics of Patients. Characteristics of the ASD patients and healthy controls are summarized in Table 1. The ethnic heterogeneity of both groups was Kazakh, Russian and other Europeans and Asians. The mean age for the ASD group and control subjects was 7.41±8.39 years (range 2-34 years) and 7.08±4.42 years (range 1-39 years), respectively. In the ASD group, 79.3% were males and 20.7% were females. In the control group, 47.1% were males and 52.9% were females. 173 ASD patients had speech disorders and 68 without.

Table 1 – Characteristics of ASD and control groups

Characteristic		ASD N	(%)	Controls N	(%)
Sample size		280		51	
Ethnicity	Kazakh	180	64.3	39	76.5
	Russian	68	24.3	5	9.8
	Other Europeans	3.2	9.0	4	7.8
	Other Asians	8.2	23.0	3	5.9
Age (years)	Median	7.41±8.39		7.08±4.42	
Gender	Male	222	79.3	24	47.1
	Female	58	20.7	27	52.9

Analysis of the Association of the rs2710102 *CNTNAP2 Polymorphism with the Risk of ASD in a Kazakhstani Population.* The genotype distributions of the rs2710102 polymorphism were under Hardy-Weinberg equilibrium (HWE) for both control (p=0.585) and ASD cases (p=0.818). The distribution of rs2710102 CNTNAP2 genotypes is presented in Table 2.

As shown in Table 2, the CNTNAP2 C allele and CC genotype were associated with a significantly increased risk of ASD (OR = 3.04, 95% CI = 1.96-4.72, p<0.001 and OR = 6.41, 95% CI = 2.47-16.63, p<0.001, respectively). Furthermore, a significantly increased risk of ASD was found for CC+CT genotypes versus the TT genotype in the dominant model (OR = 3.56, 95% CI = 1.84-6.89, p<0.001) and for CC genotype versus the combined variant of CT+TT genotypes in the recessive model (OR = 6.41, 95% CI = 2.47-16.63, p<0.001).

Due to a gender difference in rates of ASDs we further calculated the association of the rs2710102 CNTNAP2 polymorphism with ASD risk in the sub-groups stratified into genders (Table 2). C allele and CC genotype showed a significant association with ASD in both males (OR = 2.25, 95% CI = 1.23-4.10, *p=0.007* and OR = 2.95, 95% CI = 1.06-8.18, *p=0.029*, respectively) and females (OR = 4.75, 95% CI = 1.91-11.77, *p<0.001* and OR = 7.20, 95% CI = 0.89-58.53, *p=0.002*, respectively). Furthermore, the C allelotype (CC+CT genotypes) had a high risk for ASD development in both male and female patients in the dominant model (OR = 2.85, 95% CI = 1.09-7.49, *p=0.027* and OR = 7.03, 95% CI = 2.16-22.88, *p=0.001*, respectively).

Table 2 – Genotype and allele distributions of *CNTNAP2* in ASD patients and controls

rs2710102 *CNTNAP2*	ASD Patients 280	Controls 51	OR	95% CI	p-Value
CC	115	5	6.41	2.47-16.63	*<0.001*
CT	125	27	0.72	0.39-1.30	
TT	40	19	0.28	0.15-0.54	
Dominant model					
CC+CT	240	32	3.56	1.84-6.89	*<0.001*
TT	40	19	0.28	0.15-0.54	

Continuation of the table

rs2710102 *CNTNAP2*	ASD Patients 280	Controls 51	OR	95% CI	p-Value
Recessive model					
CC	115	5	6.41	2.47-16.63	*<0.001*
CT+TT	165	46	0.16	0.06-0.40	
Allele					
C	355	37	3.04	1.96-4.72	*<0.001*
T	205	65	0.33	0.21-0.51	
Male					
CC	97	5	2.95	1.06-8.18	*0.029*
CT	97	12	0.78	0.33-1.80	
TT	28	7	0.35	0.13-0.92	
Dominant model					
CC+CT	194	17	2.85	1.09-7.49	*0.027*
TT	28	7	0.35	0.13-0.92	
Recessive model					
CC	97	5	2.95	1.06-8.18	*0.031*
CT+TT	125	19	0.34	0.12-0.94	
Allele					
C	291	22	2.25	1.23-4.10	*0.007*
T	153	26	0.44	0.24-0.81	
Female					
CC	18	1	7.20	0.89-58.53	*0.002*
CT	28	5	2.24	0.70-7.17	
TT	12	11	0.14	0.04-0.46	
Dominant model					
CC+CT	46	5	7.03	2.16-22.88	*0.001*
TT	12	12	0.14	0.04-0.46	
Recessive model					
CC	18	0	7.20	0.89-58.53	*0.036*
CT+TT	40	17	0.14	0.02-1.13	
Allele					
C	64	7	4.75	1.91-11.77	*<0.001*
T	52	27	0.21	0.08-0.52	

OR analysis was performed to evaluate the effect of rs2710102 CNTNAP2 on verbal communication in ASD subjects. As shown in Table 3, there was no statistically significant association of the polymorphism with speech impairments in patients with ASD in the general group, as well as in males or females.

We carried out a case-control association study of the rs2710102 CNTNAP2 in 280 patients and 51 controls to assess the genetic contribution of this genetic variant to ASDs in a Kazakhstani population. The results of the study showed significant associations between rs2710102 CNTNAP2 and autism both in males and females. Further analysis found no significant association between the rs2710102 CNTNAP2 and speech impediments in ASD patients.

Discordant results have been reported by previous studies. No association was reported between the rs2710102 CNTNAP2 and autism in

640 trios of Han Chinese descent [19]. No significant differences between the frequencies of the CC risk genotype were demonstrated in the 210 autistic patients and 200 controls representing a Brazilian population [18]. A study of 67 autism cases and 100 controls did not find significant associations between the rs2710102 *CNTNAP2* gene polymorphism and autism in an Iranian population [20]. A case–control association study of 322 Spanish autistic patients and 524 controls found no association of this polymorphism with autism [21]. Finally, the rs2710102 variant was not significantly associated with autistic-like traits in a Swedish study of 12,319 subjects [22]. Moreover, an updated meta-analysis found no association between the rs2710102 CNTNAP2 and autism [19].

Table 3 – The relation between rs2710102 *CNTNAP2* and verbal communication in ASD subjects

rs2710102 *CNTNAP2*	ASD Patients with speech disorders	ASD Patients without speech disorders	OR	95% CI	p-Value
	173	68			
CC	77	25	1.38	0.77-2.46	*0.298*
CT	70	35	0.64	0.36-1.13	
TT	26	8	1.33	0.57-3.10	
Dominant model					
CC+CT	147	60	0.75	0.32-1.76	*0.512*
TT	26	8	1.33	0.57-3.10	
Recessive model					
CC	77	25	1.38	0.77-2.46	*0.273*
CT+TT	96	43	0.72	0.41-1.29	
Allele					
C	224	85	1.10	0.73-1.66	*0.645*
T	122	51	0.91	0.60-1.37	
Male					
CC	65	21	1.66	0.88-3.13	*0.115*
CT	50	31	0.52	0.28-0.97	
TT	19	6	1.43	0.54-3.79	
Dominant model					
CC+CT	115	52	0.70	0.26-1.85	*0.469*
TT	19	6	1.43	0.54-3.79	
Recessive model					
CC	65	21	1.66	0.88-3.13	*0.116*
CT+TT	69	37	0.60	0.32-1.14	
Allele					
C	180	73	1.20	0.76-1.90	*0.422*
T	88	43	0.83	0.53-1.31	
Female					
CC	12	4	0.67	0.16-2.80	*0.805*
CT	20	4	1.58	0.38-6.48	
TT	7	2	0.88	0.15-5.05	
Dominant model					
CC+CT	32	8	1.14	0.20-6.59	*0.881*
TT	7	2	0.88	0.15-5.05	

Continuation of the table

rs2710102 *CNTNAP2*	ASD Patients with speech disorders	ASD Patients without speech disorders	OR	95% CI	p-Value
	173	68			
Recessive model					
CC	12	4	0.67	0.16-2.80	*0.579*
CT+TT	27	6	1.50	0.36-6.31	
Allele					
C	44	12	0.86	0.32-2.35	*0.772*
T	34	8	1.16	0.43-3.15	

Contrary, some studies suggested associations between the rs2710102 SNP and predisposition to ASDs. A relationship between frontal lobar connectivity and rs2710102 genetic variant was demonstrated by functional neuroimaging, which indirectly confirms its contribution to ASDs [23]. A positive association of the rs2710102 with ASDs has been found in a study of 152 families from the Autism Genetic Resource Exchange [24].

Besides, previous studies have demonstrated the relation of the rs2710102 with language problems, signifying its essential role in ASD pathogenicity. This SNP was associated with non-word repetition [25] and specific language impairment [26]. The risk C allele of rs2710102 was significantly associated with a delayed onset of speech, as measured by the "age at the first word", in ASD children [27]. Non-autistic homozygous for the C allele demonstrated significantly increased activation in contralateral areas of traditional left-sided language regions: the frontal operculum and middle temporal gyrus [28]. Finally, it was suggested that rs2710102, as a part of specific 4-SNP haplotypes, may influence early language development in the general population [29].

In contrast, two studies failed to replicate positive results on the association between the rs2710102 and impaired language development in ASD patients [21, 22]. Similarly, we also found no association between CNTNAP2 and speech disorders. The reason for this could be insufficient tools to assess speech impairment based on only interviewing parents.

Nevertheless, our results indicate a significant statistical association between the rs2710102 variant and autism in the general group and for both men and women. It is well known that genetic heterogeneity of different populations affects the results of case-control association studies. In this way, the analysis of genetic predisposition to ASD may require different genetic markers for different populations. The majority of case-control studies of the rs2710102 variant and autism association were performed in European and American populations. To the best of our knowledge, only two studies have been carried out in Asian populations (Chinese and Iranian) so far [19, 20]. This circumstance can explain the discrepancy between our data and the data of the above-mentioned studies.

In addition, the limitations of this study must be taken into account. The control sample is small compared to the ASD sample and not matched for gender with the ASD sample (males in ASD is 79,3% and controls is 47,1%). Subdivision of all the individuals into male and female groups, as well as into groups with and without speech disorders resulted in relatively small sample sizes, so the power of these subgroup results was < 80%, indicating that additional high-level studies are still needed. The current work investigated only one polymorphism of the *CNTNAP2* gene. However, we cannot exclude a possibility that other variants in the *CNTNAP2* gene may be involved in ASDs and language impairment. Further population-based studies that will investigate the effect of genetic variations on ASDs are needed to better understand the genetics of autism and related disorders.

Conclusion

Genetically determined pathologies of neurodevelopment and synaptic functioning are increasingly considered to be a cause of ASDs. Contactin associated protein-like 2 (CNTNAP2) gene encodes a protein, which plays an essential role in brain development. Genetic variations in the CNTNAP2 gene can perturb its functions, contributing to the genetic predisposition to ASDs.

In the current study, we provide the first significant link between rs2710102 CNTNAP2 and autism susceptibility in Asian populations. Our results suggest that the SNP rs2710102 of the CNTNAP2 gene may be associated with autism susceptibility in Kazakhstani population, but it not seems to be involved with speech disorders in the same population.

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