Psychiatr. Pol. 2024; 58(5): 801–814
PL ISSN 0033-2674 (PRINT), ISSN 2391-5854 (ONLINE)
www.psychiatriapolska.pl
DOI: https://doi.org/10.12740/PP/OnlineFirst/166125

The role of selected polymorphisms in regulation of gene *CD38* expression and their effect on the clinical picture of autism spectrum disorders – preliminary study

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Summary

Aim. Clinical effects observed in cases of oxytocin deficiency can also manifest themselves in disorders of mechanisms responsible, for example, for its secretion. For oxytocin, this function is played by – among others – the cluster of differentiation antigen 38 (CD38). Existing literature along with the correlation between protein CD38 and oxytocin secretion raise interest in the context of their possible relation to the clinical picture and development of the autism spectrum disorders (ASD). The aim of the study was to analyze the correlations between polymorphisms *rs3796863* and *rs6449197* in gene *CD38*, the level of gene expression and the clinical picture and the risk of ASD diagnosis.

Method. The study included 59 individuals with the mean age of 15.05 years with IQ > 90. The participants were divided into two groups: the studied group consisting of 37 persons with confirmed ASD diagnoses and the control group including 22 neurotypical individuals. Diagnosis verification was carried out via the ADOS-2 protocol.

Results. The comparative analysis with the standardized population based on the 1000Genomes database with the presence of clinically significant intensification of ASD traits showed the correlation of alleles "T" of polymorphisms *rs3796863* and *rs6449197*, which are more frequent in the general population and are treated as "wild". In the inter-group analysis, this type of dependency was weaker, and the genotype of the control group was somehow intermediate between the studied group and the standardized population. In the $\Delta\Delta Ct$ analysis, the normalized value of the relative expression level of gene *CD38* showed that in the studied group the expression level was around 1.1–1.2 times higher than in the control group.

Conclusions. The obtained results show that a significant correlation with the severity of autism spectrum disorder traits is mainly observed in the carriers of wild variants of the

studied polymorphisms, in which the related increase in the expression level of gene *CD38* is also observed.

Key words: ASD, oxytocin, *CD38*

Introduction

In recent years, disorders within the oxytocinergic system have raised increased interest in the context of studies of social competence deficits, both in neurotypical populations as well as individuals suffering from autism spectrum disorder (ASD) [1, 2]. Available literary sources provide numerous analyses devoted to the potential share of oxytocin in ASD development, both in terms of its blood concentration [3] and the polymorphisms within genes responsible for its receptors [4]; however, a great majority of these publications present unanimous results, and frequently – contradictory ones.

The clinical effect observed in oxytocin deficiency may be also present in disorders within their receptors or mechanisms responsible, for example, for their secretion. For oxytocin, this is – among others – the cluster of differentiation 38 (CD38) [5]. CD38 is a transmembrane glycoprotein with enzymatic and transporting functions, and its function includes – among others – the cyclization of nicotinamide adenine dinucleotide (NAD+) to cyclic ADP-ribose (cADPR) which plays an important role in calcium metabolism within, for example, neurons. This is of key importance for processes related to neuroplasticity and the development of the brain, as well as the secretion of some neurotransmitters, including oxytocin [5, 6]. Current literature focuses mainly on animal studies. For instance, in mice with the removed *CD38* gene disturbances were observed within social memory and maternal behaviors [7, 8]. Studies on social competence and relations depending on the *CD38* genotype were also carried out on the human population. For instance, in 2021 Makhanova et al. [9] published a study suggesting a significant correlation between the genotype of *rs3796863* polymorphism and satisfaction and success in creating marriage bonds. Also in 2021, Krol et al. [10] linked the "CC" *rs3796863* genotype with social motivation in infants. However, in the study of Hutter et al. [11] of 2020 no correlation was observed between genotype *rs3796863* and empathy levels in neurotypical individuals. Obviously, the above enlisted studies are only examples focusing on the analysis of various aspects of social competences in relation to the *CD38* genotype. Diversity of the subject matter makes the results non-homogenous; hence, it is difficult to draw clear-cut conclusions on their basis. Nevertheless, these literature data, as well as the relation of the CD38 protein with oxytocin secretion arise interest in the context of their possible link to the clinical picture and development of ASD. At present, there are only two publications devoted to this subject in the PubMed database. The first one is the original study of Munesue et al. [12] of 2010. It describes a study based on genetic material available within the Autism Genetic Resource Exchange (AGRE). A significant correlation was found between the "C" allele of polymorphism *rs3796863* and autism development in the American population; however, no such observation was made in the Japanese population. The association test based on family examinations in the second cohort of the American population revealed a statistical significance for polymorphisms *rs3796863*

and *rs6449197*. The remaining 8 polymorphisms within gene *CD38* did not reveal any statistically significant correlations with the risk of autism in any of the studied cohorts.

The second publication is the study of Lerer et al. [13] of 2010, in which polymorphisms and expression levels for gene *CD38* were studied in individuals with low-functioning autism (IQ <70) and in their neurotypical parents. The authors of this paper simultaneously analyzed haplotypes (including polymorphism *rs3796863*) as well as *CD38* expression levels. Allele "C" rs3796863 was significantly correlated with the autism diagnosis in four out of five studied haplotypes. Apart from this, allele "C" was linked with a reduced *CD38* expression level, and a correlation was shown between a reduced *CD38* expression level and autism risk in individuals with intellectual disorders.

In addition, there was another publication in the PubMed database which analyzed transcription levels of chosen genes linked in the past with the risk of ASD, including gene *CD38*. Thanseem et al. [14] in their study carried out on brain tissues collected *post mortem* from 8 patients with ASD and 13 neurotypical individuals observed increased levels of gene *CD38* expression in patients with ASD. They linked expression changes within the analyzed genes with expression changes for gene *Transcription Factor Specificity Protein 1* (Sp1). The Sp1 factor regulates expressions of a wide range of genes, including many which are linked with the autism spectrum disorders.

Therefore, the available literature which focuses on the relationship between autism diagnosis and genotype of polymorphisms within *CD38* is extremely limited, and the existing results – ambiguous. Due to this fact, undertaking a detailed analysis of the relationship between ASD symptoms and polymorphisms and expression of gene *CD38* is an interesting challenge. The present paper is a summary of preliminary results of a project aiming at the assessment of the impact of genotypes of polymorphism within gene *CD38*, as well as the levels of its expression in peripheral blood on the clinical picture and risk of ASD.

Materials and methods

Participants

The study was carried out at the Department of Psychiatry and Psychotherapy of Developmental Age of the Medical University of Silesia at the John Paul II Pediatric Centre in Sosnowiec, Poland in cooperation with the Department of Molecular Biology, Faculty of Medical Sciences of the Medical University of Silesia in Katowice, Poland. Participants were recruited among the patients of the John Paul II Pediatric Centre in Sosnowiec from the Department of Psychiatry and Psychotherapy of Developmental Age, the outpatient clinic of mental health, and among pupils from Silesian schools. Informed consent to participate in the study was obtained upon providing information both from parents and participants themselves.

Inclusion criteria for the studied group included confirmed autism spectrum disorders in the ADOS-2 protocol carried out earlier by a child and adolescent psychiatrist. Exclusion criteria for the studied and control groups included the following: 1) a co-

existing diagnosis of another mental disorder; 2) age below 12 and above 19 years; 3) a coexisting intellectual disability; 4) epilepsy; 5) confirmed genetic, neurometabolic and similar grounds of the observed symptoms (e.g., fragile X syndrome) – "autism plus"; 6) poor somatic condition; 7) a serious liver, kidney or heart dysfunction diagnosis; 8) hypothyreosis. Additionally, for the control group exclusion criteria also included: 1) suspected or previously diagnosed autism spectrum disorders; 2) a first- or second-degree relative diagnosed with autism spectrum disorder.

Data collected in the study were pseudonymized. The study covered 59 individuals of a mean age of 15.05 years (95% CI: 14.24-15.86; min/max: 8/20) with IQ >90. Participants were divided into two groups based on a confirmed or excluded diagnosis of autism spectrum disorders on the basis of DSM-5 and ICD-10 criteria and then the ADOS-2 protocol. The studied group with a confirmed ASD diagnosis comprised of 37 individuals of a mean age of 14.1 years (95% CI: 13.23-15.15), out of whom 32.4% (n = 12) were females. The control group contained 22 individuals with a mean age of 16.3 years (95% CI: 15.01-17.65), out of whom 18.1% were females (n = 4). The observed age difference was of statistical significance ($p < 0.05$), while in the combined-group analysis of the correlation between genotypes of the studied polymorphism and the gene expression level no significant differences were observed in terms of age.

Psychometric analysis

All participants were examined with the ADOS-2 protocol with modules corresponding to their age and communication level. The ADOS-2 protocol is a tool which includes a set of tests provoking participants to defined social behaviors. Based on observation, participants are assessed in the following five categories: 1) Language and communication, 2) Social reciprocity, 3) Play/Imagination, Stereotypical behaviors and Restricted interests, and 5) Other abnormal behaviors. The outcomes of this test are a quantitative score on two scales (social affect and restricted and repetitive behaviors) and a final score to define the degree of severity of autism spectrum symptoms. Further analyses focus mainly on the comparative outcome (ADOS: CO); social affect domain (ADOS: SA) and Restricted and Repetitive Behaviors (ADOS: RRB). Apart from the above, the following were applied within the study:

- "Reading the Mind in the Eyes" Test (RMET) – a tool designed by Baron-Cohen [15, 16]. In the version applied in this study participants look at 28 pictures presenting human eyes and need to select one of four terms describing the mental state that best describes the picture. There are no time limitations for this test.
- "Autism Quotient" Test (AQ) [17] – a questionnaire constructed in 2001 to assess the probability level of autism spectrum disorder diagnosis. It consists of 50 questions with multiple-choice answers based on the 4-point Likert scale.
- "Empathy Quotient" Test (EQ) – a tool designed by Baron-Cohen (translated by Jankowiak-Siuda et al. [18] in 2017) to define individual differences in

terms of the ability to empathize. The Wakabayashi et al. [19] version, which consists of 22 testing items, was applied in this study.

Bioethics Committee consent and source of financing

The study was carried out with the consent granted by the Bioethics Committee of the Medical University of Silesia in the resolution no. KNW/0022/KB1/123/18/19 of January 8, 2019.

The study was funded by the Medical University of Silesia within contracts of statutory work no. KNW-2-K18/D/9/N and KNW-1-178/N/9/K.

Molecular analysis

Analysis of DNA polymorphisms

DNA was isolated from 200 µl of peripheral blood collected to EDTA (name of tubes used to draw blood) with the GeneMatrix Quick Blood DNA Purification Kit (EuRX, Poland) in line with the manufacturer's instructions. The qualitative and quantitative assessments of DNA were performed spectrophotometrically on the NanoDrop 2000 (Thermo Fisher Scientific, USA) tool. Next, double gene detection was performed (twice) on the thermocycler LightCycler480 II (Roche, Germany) with the probes TaqMan SNP Genotyping Assay (for *rs3796863*: c_1216944_10; for *rs6449197*: c_31096525_20) (Applied Biosystems, USA) and TaqPath ProAmp Master Mix (Thermo Fisher Scientific, USA), as per the manufacturer's instructions.

Analysis of RNA expression

Total mRNA was isolated from the peripheral blood collected to PaxTube in order to maintain RNA integrity with the PaxGene Blood RNA Kit (Qiagen, Germany), as per the manufacturer's instructions. The qualitative and quantitative assessments of RNA were performed spectrophotometrically on the NanoDrop 2000 (Thermo Fisher Scientific, USA) tool. For the reverse transcription with High Capacity cDNA Reverse Transcription Kit (Roche, Germany), 500 ng of total RNA was collected. Expression analysis was carried out with the TaqMan Gene Expression Assay – probe Hs01120071_m1 for gene *CD38* and probe Hs03929097_g1 for the reference gene, *GAPDH* (Applied Biosystems, USA) and TaqMan Gene Expression Master Mix (Applied Biosystems, USA). The reaction for 50 ng of cDNA was carried out two times in a thermocycler of real time LightCycler480 II (Roche, Germany). Expression outcomes were analyzed with the GenEx ver6 (MultiD Analyses AB, Sweden). The analysis of raw data was conducted with the ΔCt method, i.e., the relative expression in the following manner: raw data were normalized to technical repetitions, to cDNA amount and finally, to the reference gene *GAPDH*. In the case of the $\Delta\Delta Ct$ method, i.e., the comparative method, the normalization to calibrator was an additional step.

Statistical analysis

Statistical analysis was carried out with the StatSoft Statisctica software, ver. 13. The assumed level of statistical significance was $\alpha = 0.05$. The analysis of allele frequency of selected polymorphisms was carried out with the frequency formula resulting from the Hardy-Weinberg equilibrium defining the relationship between allele and genotype frequencies in a population.

The applied formula:

$(p + q)^2 = p^2 + 2pq + q^2$ assuming that $p + q = 1$

In the presented study, p is the wild-type allele frequency and q mutant allele frequency.

In the context of levels of gene *CD38* expression, the $\Delta\Delta Ct$ method was applied in the analysis. For statistical analyses of questionnaire outcomes regarding the levels of gene *CD38* expression, the ΔCt value was used. While comparing expression levels between the studied and the control groups and individuals with the mutant allele and homozygotes in terms of the wild-type allele, the following formula of normalized value of the relative level of expression of the studied gene was used:

$R = 2^{-\Delta\Delta Ct}$

Where $R = 1$ means that expression levels between the analyzed groups are equal.

Results

Table 1. **Distribution of genotype frequencies for both analyzed polymorphisms**

	Studied group		Control group		Standarized population*	
	rs6449197 C > T	rs3796863 G > T	rs6449197 C > T	rs3796863 G > T	rs6449197 C > T	rs3796863 G > T
p-freq	0.89	0.75	0.83	0.65	0.74	0.57
q-freq	0.11	0.24	0.17	0.34	0.26	0.43
p-value vs. SP	p < 0.05	p < 0.05	p > 0.05	p < 0.05	n/a	n/a

Distribution of genotype frequencies for both analyzed polymorphisms was in line with the Hardy-Weinberg equilibrium. Distributions of polymorphism alleles in the control group, studied group and the standardized population [20] are presented in Table 1. Mean values of the applied questionnaires in the studied and control groups are presented in Table 2.

Table 2. **Mean values of the applied questionnaires in the studied and control groups**

	Studied group		Control group		
	Mean	95% CI	Mean	95% CI	p-value*
ADOS: SA	12.92	11.6-14.1	3.04	2.2-3.8	<0.05
ADOS: RRB	1.96	1.2-2.7	0.47	0.1-0.7	<0.05
ADOS: WP	7.21	6.5-7.9	1.57	1-2.1	<0.05
EQ	14.84	10.6-19	24.38	20.9-27.8	<0.05
AQ	26.3	22.4-30.2	15.75	12.8-18.7	<0.05
RMET	0.58	0.5-0.65	0.65	0.59-0.71	0.33

* Mann-Whitney U test

The analysis of differences in allele distributions between the studied and control groups and the standardized population showed some statistically significant differences in the *chi2* test. For both polymorphisms, the frequency of the mutant allele was lower in the studied group than in the standardized population. In the control group, this type of situation was present in polymorphism *rs3796863*, while there were no distribution differences observed for polymorphism *rs6449197*. Also, the difference in the distributions of polymorphism alleles between the control and studied group was analyzed; however, the observed distribution differences were not statistically significant.

In the analysis of gene *CD38* expression levels in the peripheral blood, the mean ΔCt value for the studied group was 7.14 ($SD = 0.43$) and for the control group – 7.37 ($SD = 0.39$) (a higher value signifies lower expression level). In the $\Delta\Delta Ct$ analysis, the normalized value of the relative expression level of the studied gene was $R = 1.16$ ($SD = 1.11$-1.21) between the studied and control groups, which means that in the studied group the gene *CD38* expression level was around 1.1-1.2 times higher than in the control group. At the same time, the effect of the genotype of the studied polymorphisms on the expression level both in the studied and control groups was analyzed. For the polymorphism *rs6449197*, the presence of the mutant allele led to a decrease in CD38 protein expression by 14% on average ($R = 0.86$; $SD = 0.84$-0.89). The mutant allele of polymorphism *rs3796863* also led to a drop of the expression level by around 6% ($R = 0.94$; $SD = 0.93$-0.95). Finally, logistic regression analysis was carried out to analyze the relation between decreased level of *CD38* expression and the risk of ASD, with no statistically significant correlation found. The odds ratio (OR) of affinity with the ASD group was OR = 0.28 (95% CI: 0.07-1.06; $p = 0.06$).

Another analysis focused on the OR of ASD development for both polymorphisms. For *rs6449197,* the presence of mutant variation was linked with OR = 0.92 (95% CI: 0.3-2.76), while for *rs3796863* – OR = 0.98 (95% CI: 0.36-2.62). Both parameters were statistically insignificant.

The next step was the analysis of correlation between ΔCt values and the applied questionnaires. The increase of expression levels of gene *CD38* indicated a significant correlation with the ADOS-2 outcomes to a low degree ($r = 0.28$; $p < 0.05$) and to

a moderate degree with the social affect sub-scale ($r = 0.34$; $p < 0.05$). Similarly, within the "Reading the Mind in the Eyes" tool, the increase of *CD38* expression was related to worse outcomes ($r = – 0.38$; $p < 0.05$). The remaining tools revealed no statistically significant correlation with the level of *CD38* expression.

Furthermore, questionnaires' outcomes were compared among the carriers of at least one mutant allele of the studied polymorphisms and homozygotes in terms of the wild allele. Statistically significant differences could be observed for SNP *rs3796863* for the ADOS-2 general outcome ($p = 0.004$) and the social affect sub-scale ($p = 0.007$). For SNP *rs6449197*, no statistically significant differences were observed in the analyzed questionnaires; however, for the "Reading the Mind in the Eyes" tool, the difference was at the significance limit ($p = 0.051$). Detailed results are presented in Tables 3 and 4.

Table 3. **Average results of the surveyed questionnaires depending on the observed *rs3796863* genotype**

	Homozygote wild type		Mutant allele carrier		
	Mean	95% CI	Mean	95% CI	p-value*
ADOS: SA	12.05	10.47-13.63	8.80	6.83-10.78	0.007**
ADOS: RRB	2.20	1.72-2.69	1.29	0.74-1.83	0.01**
ADOS: WP	6.73	5.88-7.58	3	3.77-5.97	0.004**
EQ	17.21	14.04-20.39	18.68	14.39-22.96	0.55
AQ	28.1	24.93-31.27	24.84	21.16-28.52	0.15
RMET	0.61	0.57-0.66	0.66	0.6-0.72	0.27

* Mann-Whitney U test; ** statistically significant results at $p < 0.1$

Table 4. **Average results of the surveyed questionnaires depending on the observed *rs6449197* genotype**

	Homozygote wild type		Mutant allele carrier		
	Mean	95% CI	Mean	95% CI	p-value*
ADOS: SA	12.05	10.47-13.63	8.80	6.83-10.78	0.059**
ADOS: RRB	2.20	1.72-2.69	1.29	0.74-1.83	0.15
ADOS: WP	6.73	5.88-7.58	3	3.77-5.97	0.16
EQ	17.21	14.04-20.39	18.68	14.39-22.96	0.49
AQ	28.1	24.93-31.27	24.84	21.16-28.52	0.26
RMET	0.61	0.57-0.66	0.66	0.6-0.72	0.052**

* Mann-Whitney U test; ** statistically significant results at $p < 0.1$

Discussion

The analyses of the distribution of alleles within the studied polymorphisms as compared to the 1000Genomes population show similar outcomes to the observations of Munesue et al. [19] and Lerer et al. [12]. Both in the studied and the control group they differed in a statistically significant way from the distribution expected on the basis of the standardized population derived from the 1000Genomes database. Interestingly, correlation with ASD was expressed by alleles "T" of polymorphisms *rs3796863* and *rs6449197* which are manifested more frequently in the general population and are treated as "wild type". Higher results of the ADOS-2 test were also connected with them, and for the polymorphism *rs6449197* – the RMET tool as well.

However, the situation is different in the comparative analysis between the studied and control groups. Distributions of polymorphism alleles in the control group do not show significant differences against the distributions observed in the studied group. Simultaneously, for polymorphism *rs3796863* significant differences in distribution were observed in the control group as compared with the standardized population. What is interesting, while analyzing the raw distributions it could be observed that despite no statistically significant differences between the studied and control group, the genotype distribution in the control group is intermediary between the studied group and the standardized population. Similarly, results of the EQ and RMET tools in the control group are different than expected. Although for EQ the result was statistically significantly higher than in the studied group, it was also clearly lower than results found in literature, e.g., in the study of Wakabayashi et al. [19] from 2006, where the mean EQ value in the population of neurotypical males equaled 39.0 (SD = 11.56). The RMET result in the control group was also lower than expected, e.g., as in the meta-analysis of studies carried out by Penuelas-Calvo et al. [21] in 2019, where the mean result of the RMET-C test in the neurotypical group was 73.49%. At the same time, no statistically significant difference was observed in the studied and control group results. These results suggest that despite some literature reports, the percentage of correct answers in RMET – and resulting from this the ability to differentiate emotional states on the basis of face expression – does not allow to differentiate between neurotypical and ASD patients.

These results correspond to the studies of Camodeca et al. [22] and Miu et al. [23], where such phenomena constituted the fundamental traits of the so-called broad autism phenotype (BAP), i.e., the constellation of subclinical symptoms of autism spectrum which are present in the neurotypical population. Initially, this phenomenon was mainly studied in the families of ASD patients and it was treated as an abortive picture of the spectrum and as an argument for the genetic grounds of autism. However, gradually the analyses covered the general population and, for instance, in the study of Dovgan et al. [24], the percentage of BAP individuals among college students was 25.3% and was not linked with a family history of ASD. Further studies showed that the severity of various "autism spectrum traits" matches the normal distribution in the general population [21]. This in turn led to the conclusion that autism spectrum constitutes a continuum in the general population, including individuals deprived of such

traits on one extreme, and as the severity grows, qualifying them to the BAP group, and finally arriving at the full ASD diagnosis on the other extreme [24]. This type of hypothesis would explain the distribution of alleles observed in this study. Accepting currently assumed theories of multi-gene background of ASD and analyzing results of available studies [4] a mass effect hypothesis may be made. As the amount of ASD traits-conditioning alleles increases in various genes the clinical picture becomes closer to a severity which substantiates the diagnosis. Such approach would also explain the observed relations between variants linked to ASD and outcomes of the scales which analyze various diagnostic areas in non-ASD individuals.

The dependency analysis of genotype of the studied polymorphisms and the outcomes of the applied diagnostic tools showed statistically significant differences in both sub-scales and in the comparative ADOS-2 for the polymorphism *rs3796863*. For polymorphism *rs6449197*, no significant differences were observed between carriers of the mutant allele and homozygotic individuals in terms of the wild allele. However, the differences in sub-scales for the social affect and RMET were on the verge of statistical significance, which may suggest that this polymorphism may have some effect on social competences themselves, regardless of the diagnosis.

In the analysis of differences between genotype SNP distribution across various genes the issue of a real biological effect has been often ignored. The observed correlation between a polymorphism which has no structural or regulatory effects on the studied gene suggests that the observed dependency will probably be accidental. In the study of Munesue et al. [12] this fact was also underlined and the need to carry out further studies on biological effect of the studied SNPs was suggested.

Location of the studied polymorphisms in the intron areas of gene *CD38* implies that a hypothetical effect of their genotype on the risk/clinical picture of ASD will probably result from modification of their expression levels. There are no current studies of this type in the literature, which restricts the possibility to interpret the obtained outcomes. In this study, mutant alleles of both polymorphisms were connected with lower expression levels, while this effect was more than two times stronger for polymorphism *rs6449197*. It should yet be remembered that there could be a linkage disequilibrium between the studied polymorphisms and other SNPs within gene *CD38*, which could be a base for an apparent dependency between the studied genotype and the expression level. However, assuming the lack of this type of factor interfering with the interpretation of results it can be stated that both polymorphisms have a real effect on the gene expression level, and by this, the observed differences in genotype distributions may translate into actual biological effects, e.g., social cognition disorders.

Taking into account the fact that mutant variants of the analyzed polymorphisms reduced the gene *CD38* expression in this study it can be expected that in the comparative analysis of the studied and control groups *CD38* expression levels would be higher than in the control group. And indeed, in the $\Delta\Delta Ct$ analysis, the standardized value of the relative expression level of the studied gene showed that in the studied group the *CD38* expression level was around 1.1-1.2 times higher than in the control group. Moreover, in the analysis of outcomes of the applied tools, higher *CD38* expression levels were also significantly correlated with worse outcomes in ADOS-2 and RMET.

However, this dependency was not reflected in the logistic regression analysis, where (with the assumed significance level at $\alpha = 0.05$), *CD38* levels were not statistically significantly correlated with ASD risk. Yet, it should be noted that the described risk model would become statistically significant at the assumed significance level of $\alpha = 0.1$.

On the other hand, the obtained results in gene *CD38* expression are in contradiction with the results presented by Lerer et al. [12]. In their study, allele "C" of polymorphism *rs3796863*, although actually related to the ASD diagnosis, was at the same time correlated with the reduction of gene *CD38* expression, and consequently – as opposed to this study – the reduced *CD38* expression was linked with the ASD diagnosis. The observed contradiction in correlations between expression level and diagnosis as well as polymorphism *rs3796863* may be a result of a fundamental difference in the studied group composition. The presented study included highly-functioning ASD individuals, i.e., within the intellectual norm. On the other hand, Lerer et al. [12] took into consideration low-functioning autism individuals (IQ < 70). Taking into account the versatility of effect of protein CD38, its participation in various processes related to neuroplasticity and development of the brain [25, 26], as well as its confirmed effect on cognitive functions (learning and memorizing) in animal models [27], the relation between this protein and intellectual disability regardless of ASD seems probable. And this could suggest that the dependence of polymorphism *rs3796863* and ASD itself obtained by Lerer et al. [12] could be an example of an ecological error and could result from a correlation with intellectual disability.

In the present study, as well as in the study by Munesue et al. [19] and Lerer et al. [12] there is the intriguing aspect of the protective role played by mutant variants of the studied polymorphisms against the risk of ASD. Assuming – as the starting point for further analyses – the hypothesis about the prevalence of autism spectrum traits in the general population and their correlation with a growing amount of mutations within various genes, it would be intriguing to reconceptualize the clinical approach to ASD as a more "primary" form of human cognitive processes, while "neurotypicality" as an evolutionary gain which is present in separate individuals to a varied degree. The gain which in the last two hundred years of technical development may have been losing its privileged role towards the advantage of ASD traits in modern society. Individuals within the spectrum manifest a wide range of abilities and talents that are a natural consequence of axial symptoms of this medical unit. In their functioning, we can observe, e.g., higher cognitive skills, mnemotechnic and musical abilities, ability to identify and analyze complex designs and systems quickly – i.e., the traits which predispose to work in the fields of science, technology, engineering and mathematics [28]. Due to this, in modern society ASD individuals commonly handle areas not only of a key role to economy and society, but also those related to prestige and good financial status. And consequently, this is favorable to good functioning and also evolutionary advantage over "neurotypical" individuals.

Both clinical and scientific implications of this hypothesis would be considerable. First, this would question the soundness of differentiating individuals with a high severity of ASD traits with rigid diagnostic criteria and would promote real needs of the examinee in the diagnostic process as well as their level of functioning in social

roles independent of symptom severity. A very important consequence of such an approach would be facilitating access to therapeutic interventions for girls with ASD. Inadequacy of diagnostic criteria to the clinical picture of ASD in females is widely discussed in the literature as well as their ability to camouflage symptoms and fundamentally high level of social functioning [29]. These traits lead to under-diagnosing, and consequently, to "a bottleneck" in the access to proper therapeutic and rehabilitation interventions. Second, in terms of future scientific studies, this would suggest the need to depart from the rigid model of differences in the studied and control groups – where it would be really impossible to univocally and objectively divide individuals into "truly neurotypical" and "truly neuroatypical" in scientific terms. A much more sound model would be to analyze the correlations among various parameters (genetic or environmental) with ASD severity in a given population, measured by means of constantly-updated diagnostic tools.

Finally, it is important to discuss the limitations of the presented study, which shed light on the assessment of the obtained outcomes. The first limitation is the risk of a misclassification of patients to the neurotypical and neuroatypical groups. This methodological difficulty is common for all scientific studies available in the literature. However, due to a two-stage classification process for this study which included a psychiatric examination with preliminary diagnosis and the ADOS-2 examination by a certified diagnostician it can be assumed that in this case the risk of such an error is low.

The next issue is the control in terms of confounding factors. In this case, the main problem is a possible effect of polymorphism not studied in this project in other locations which remain, e.g., in the linkage disequilibrium with the studied ones and generate the risk of apparent, statistically significant correlations with the analyzed parameters.

Another issue is a statistically significant difference between the studied and control groups in terms of mean age. For the applied diagnostic tools, available scientific reports point to their resistance to age differences, small ones in particular – such as in this study. And lastly, the numerical force of the studied and control groups is limited, which is also common across the literary reports. However, taking into account the obtained statistical parameters, the existing distributions of the studied polymorphisms and literary reports it can be assumed that this quantity will suffice to draw conclusions regarding the population with a satisfactory approximation.

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