Serotonin 5-HT$_{2A}$ receptor polymorphisms are associated with irritability and aggression in conduct disorder

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Abstract

In childhood and adolescence, overt antisocial and aggressive manifestations are typically diagnosed as conduct disorder (CD). Given that the emerging research has ponted to the influence of 5-HT_{2A} receptors in the ontogeny of aggression, we aimed to analyze the association of its genetic polymorphisms with CD.

The study included 228 (120 with and 108 without CD) male adolescent subjects. CD was diagnosed according to Structured Clinical Interview for DSM-IV criteria, while evaluations of aggressive/dissociative behaviors were performed using the psychometric questionares: PCL-YV, OAS-M, KADS and CBCL. Platelet 5-HT concentration was determined by spectrophotofluorometry, and genotyping of 5-HT_{2A} receptor polymorphisms rs2070040, rs9534511, rs4142900, rs9534512 was done using TaqMan SNP Genotyping Assasys.

Subjective irritability, physical aggression towards others and antisocial behavior were strongly associated with the G allele of rs2070040 and rs4142900, and the C allele of rs9534511 and rs9534512. A significantly increased platelet 5-HT concentration in CD subjects, when compared to controls, was lost after the correction according to the smoking status.

Our result indicate an association of the studied *HTR2A* polymorphisms, as well as their haplotypes with irritability and impulsivity traits, which may contribute to the aggressive and antisocial behavior in male adolescents with CD.

Key words: Serotonin, 5-HT_{2A} receptor polymorphisms, conduct disorder, irritability, aggression

1. Introduction

Antisocial behavior is characterized by aggressive and non-aggressive rule-breaking, violation of societal norms, disregard for the rights and properties of others, and violence (Buitelaar et al., 2013). In childhood and adolescence, overt antisocial manifestations are typically diagnosed as conduct disorder (CD), a condition affecting 5-9% of adolescents with a marked male predominance (Moffitt et al., 2001). Youths with CD frequently engage in cruelty to animals and people, destructive acts, lying, stealing, delinquency, and perpetration of violent crimes (including assault, rape, robbery, and murder (Spencer et al., 2007). Accordingly, about 40% of youths arrested for delinquent acts meet psychiatric criteria for CD (Harrington et al., 1991), and more than half of juvenile subjects in correctional facilities meet CD diagnostic criteria (Fazel et al., 2008). Given the robust association between delinquency and CD, this disorder is a critical public issue imposing a heavy burden on society (Kazdin, 1995). Making matters worse, preventive and therapeutic interventions for CD are highly challenging, and no drug has been approved for its treatment.

Several studies have investigated the biological factors underlying CD pathophysiology. Scarce number of genome wide association studies have focused on CD. A study performed (Dick et al., 2011) on childhood CD in an American sample detected 3 genome-wide significant loci and emphasized the association of C1QTNF7 (C1q and tumor necrosis factor-related protein 7) gene with the CD symptom count. Building on the well-documented role of serotonin (5-HT) in aggression, violence, and impulsivity (Bortolato et al., 2013; Brown and Linnoila, 1990; Ketcherside et al., 2013; Klasen et al., 2019; Mann et al., 2009; Nomura et al., 2015; Pattij and Vanderschuren, 2008; Pavlov et al., 2012; Stanley et al., 2000; Virkkunen et al., 1995), several studies have pointed to the involvement of this neurotransmitter in CD (Cadoret et al., 2003; Chang et al., 2017; Golubchik et al., 2009; Unis et al., 1997) However, aggression is a complex trait, and its relationship with 5-HT levels is bidirectional due to the influence of psychological and social factors (Krakowski, 2003). Thus, while most studies suggest that aggressive behavior is inversely correlated with circulating levels of 5-HT or its metabolites (Blumensohn et al., 1995; Duke et al., 2013; Kruesi et al., 1990; Tuinier et al., 1995), whole-blood 5-HT concentrations were found to be more elevated in CD-affected individuals (Unis et al., 1997). The pleiotropic impact of 5-HT on antisocial behavior is likely due to the different functions of its various receptors. In particular, several studies suggest a crucial role of 5-HT_{2A} in pathological aggression and antisocial behavior. 5-HT_{2A} receptor agonists and antagonists have been shown to increase and

reduce aggression in animal models, respectively (Sakaue et al., 2002). Furthermore, it was recently documented that early-life activation of 5-HT_{2A} receptors mediates gene x environment interactions in antisocial behavior in a mouse model of pathological aggression (Godar et al., 2019). More importantly, the implication of 5-HT_{2A} receptors in aggression and antisocial behavior is also supported by human data. For example, a selective 5-HT_{2A} receptor upregulation was documented in the orbitofrontal cortex of pathologically aggressive individuals (Rosell et al., 2010). Previous evidence has also shown that the role of 5-HT_{2A} receptors in pathological aggression is also shaped by genetic polymorphisms (Banlaki et al., 2015; Giegling et al., 2006). For example, a study on adult Caucasian individuals found a significant association between aggression and the *HTR2A* single-nucleotide polymorphisms (SNPs) rs7322347 in males and females (Banlaki et al., 2015). Notably, the T allele of the *HTR2A* rs7322347 was significantly related to hostility, anger, and physical aggression, suggesting that this polymorphism predicted aggressive traits (Banlaki et al., 2015). Another SNP, *HTR2A* rs6311, and its *G* allele were associated with higher total aggression levels in Hadza, Datoga, and Russian populations (Butovskaya et al., 2018). However, this association was not confirmed in further studies in Pakistani prisoners (Qadeer et al., 2021).

Based on these premises, the present study aimed to evaluate the role of 5-HT_{2A} receptor polymorphisms in CD. We assessed whether selected *HTR2A* polymorphisms, such as *HTR2A* rs2070040, HTR2A rs9534511, *HTR2A* rs4142900, *HTR2A* rs9534512, were associated with CD diagnosis or with relevant behavioral dimensions, such as aggression, irritability, and other facets of psychopathology. Furthermore, we measured platelet 5-HT in CD-affected and control individuals since this parameter can serve as a reliable operational index of central 5-HT functions (Mammadova-Bach et al., 2017; Zhuang et al., 2018), given that platelets share the same 5-HT synthetic and metabolic machinery as brain synaptosomes (Camacho and Dimsdale, 2000; Goveas et al., 2004; Zhuang et al., 2018). We conducted our studies in a cohort of male youths detained in a correctional facility, given the high prevalence of CD in this population (Fazel et al., 2008).

2. Materials and Methods

2.1. Participants

The study was performed on a cohort of 228 young male drug-naive Croatian subjects, described in a previous study (Podobnik et al., 2020). Briefly, 185 participants were detained in the Juvenile Correctional Facility Ivanec, Zagreb County, Croatia (out of which 120 with and 65 without a CD

diagnosis), while 43 healthy control adolescents were out of detention and recruited during regular medical examinations at the Family Medicine Unit in Osijek, Croatia, or the Department of Child and Adolescent Psychiatry in the Clinical Hospital Centre, Osijek.

CD diagnoses were made by child psychiatrists and psychologists using the Structured Clinical Interview for DSM-IV criteria (SCID-IV) (APA, 1994) since participants were recruited before DSM-5 criteria had been translated into Croatian. Determination of smoking status revealed that 155 adolescents smoked, while 73 did not. Exclusion criteria were the use of psychoactive compounds or alcohol, a diagnosis of autism spectrum disorder, intellectual disability, or ADHD, and treatment history of cognitive-behavioral therapy or electroconvulsive therapy. The group comprised of control youths with no psychiatric diagnoses. All participants were medication-free, and all gave voluntary informed consent to be a part of the study. The study was approved by the Ethics Committees and was fully compliant with the ethical standards laid down in the 1975 Declaration of Helsinki.

2.2. Questionnaires.

Evaluations of psychopathology and different facets of aggressive/dissociative behaviors were performed using the Hare Psychopathy Checklist: Youth Version (PCL-YV) (Forth et al., 2003), Overt Aggression Scale-Modified (OAS-M) (Coccaro, 2020; Yudofsky et al., 1986), Kutcher Adolescent Depression Scale (KADS) (LeBlanc et al., 2002) and Child Behavior Checklist (CBCL) (Achenbach, 1991).

2.3. Determination of platelet 5-HT concentrations

Sampling was performed in the morning around 8 a.m., after overnight fasting. Blood samples taken for usual laboratory routine tests were used for platelet 5-HT determination and genotyping. Whole-blood samples were collected in 8.5 mL yellow-top Vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) with 1.5 mL of acid citrate dextrose anticoagulant. With a series of centrifugation steps, platelet-rich plasma (PRP) was obtained. Platelet 5-HT concentration was determined by spectrophotofluorometry, using ortho-phthalaldehyde (OPA). Fluorescence was measured in duplicates on a Varian spectrophotofluorometer Cary Eclipse, with the excitation wavelength of 345 nm and emission wavelength of 485 nm. Platelet 5-HT concentrations were corrected for the different platelet number by the total platelet protein levels, measured using the method of Lowry (Lowry et al., 1951). The detection limit of the method was 10.0 ng/sample, with intra- and inter-assay coefficients of variation 3.66 and 8.69%, respectively.

2.4. Genotyping of 5-HT_{2A} receptor polymorphisms

Extraction of genomic DNA was carried out with the salting-out method (Miller et al., 1988). Genotyping of the *HTR2A* rs2070040, HTR2A rs9534511, *HTR2A* rs4142900, and *HTR2A* rs9534512 was performed by the real-time PCR according to the procedures described by Applied Biosystems, using the ABI Prism 7300 Real-time PCR System apparatus (Applied Biosystems, Foster City, CA, USA), with primers and probes from Applied Biosystems (Foster City, CA, USA) as TaqMan® SNP Genotyping Assays. Genotyping of all SNPs was done by researchers blinded to the clinical data, while 10% of the samples were re-genotyped for quality control.

2.5. Statistical analyses

Statistical analyses were performed using Sigma Stat 3.5 (Jandel Scientific Corp., San Jose, CA, USA). Since the Kolmogorov–Smirnov test documented a significant deviation from normality, results were reported as median and interquartile range. Non-parametric Mann–Whitney U test and Kruskal–Wallis ANOVA by ranks were used to analyze the differences in 5-HT levels and scores on different psychiatric scales between two or more group of subjects. Spearman correlation was used to investigate the relationship between these scores and platelet 5-HT levels, and two-way ANOVA was conducted to examine the effect of multiple factors on platelet 5-HT. Genotype, allele, and haplotype frequencies between groups were compared using χ^2-test. G*Power 3.1 Software (Faul et al., 2009) was used to determine the required sample size and actual statistical power. For p = 0.05, medium effect size, power (1 – β) = 0.800, the required sample size was 134 for Mann-Whitney test, 200 for Kruskal-Wallis, 84 for bivariate correlation and 108 for χ^2-test with df=2. Since the study included 228 participants in total, but psychometric data was avaliable only for 185 (predominantely in CD group), it had adequate sample size and statistical power for majority, but not for all statistical analyses. Hardy-Weinberg (HW) equilibrium for tested SNPs was determined using Haploview software v. 4.2 (Barrett et al., 2005). Haploview was also used to perform haplotype analysis and to determine LD pairwise values between rs2070040, rs9534511, rs4142900, and rs9534512 polymorphisms. Two haplotype blocks were identified based on the strongest link between rs2070040 and rs9534511 polymorphism (D'=0.95; LOD>=2) and rs4142900 and rs9534512 SNP (D'=0.98; LOD>=2) determined by the confidence interval (CI) method by Gabriel et al. (Gabriel et al., 2002), and best-estimated haplotypes were attributed to each subject using PLINK 1.7 software (Purcell et al., 2007).

3. Results

3.1. Demographic data

The study included 228 male adolescents, including 120 subjects with CD (61 with moderate symptoms and 59 with severe symptoms, respectively) and 108 controls (43 from the general population and 65 detained in a correctional facility but without a CD diagnosis). Forty control subjects from the correctional facility (61.5%) were social cases (i.e., with no history of convictions). In contrast, 25 participants (38.5%) had records of delinquency adjudications. They were referred to as court cases similar to subjects with CD, where 58 of them (48.3%) were social cases, and 62 (51.7%) were court cases (p=0.086, Table 1). Subjects with CD were slightly older than healthy control subjects (p=0.023), had greater prevalence of smoking (p<0.001) and alcohol abuse (p<0.001), as well as a much more frequent history of severe damages infliction (p<0.001) (Table 1).

Table 1. Demographic data of control subjects and subjects with conduct disorder. The data are represented as median and interquartile range or as total number and frequency. P values denoted in bold represent statistical significance.

		Control subjects (N=108)	**Subjects with CD (N=120)**	**Statistics**
Age		16 (15; 18)	17 (16; 18)	U=4491.0; **p=0.023**
Delinquency adjudications	Yes	25 (38.5%)	62 (51.7%)	χ^2=2.951; df=1; p=0.086
	No	40 (61.5%)	58 (48.3%)	
Infliction of severe damage	Yes	5 (7.7%)	60 (50.0%)	χ^2=33.114; df=1; **p<0.001**
	No	60 (92.3%)	60 (50.0%)	
Smoking	Yes	49 (45.4%)	106 (88.3%)	χ^2=48.203; df=1; **p<0.001**
	No	59 (54.6%)	14 (11.7%)	
Alcohol abuse	Yes	5 (9.4%)	11 (55.0%)	χ^2=17.616; df=1; **p<0.001**
	No	48 (90.6%)	9 (45.0%)	

CD – conduct disorder

All subjects were genotyped for 4 *HTR2A* polymorphisms: rs2070040, rs9534511, rs4142900 and rs9534512 with minor/alternate allele frequency (MAF) for each polymorphism 0.412 (A allele), 0.475 (T allele), 0.437 (T allele) and 0.604 (C allele), respectively, which is mostly in line with estimated MAF in European population for given polymorphisms: MAF (rs2070040, A allele)=0.466, MAF (rs9534511, T allele)=0.482, MAF (rs4142900, T allele)=0.479, MAF (rs9534512, C allele)=0.486 (ALFA project). All polymorphisms were in HW equilibrium (p=0.381, p=0.460, p=0.684, p=0.577 for rs2070040, rs9534511, rs4142900 and rs9534512, respectively).

Haplotype analysis identified two haplotype blocks based on the strongest link between rs2070040 and rs9534511 polymorphism (haplotype block 1; D'=0.95; LOD>=2) and rs4142900

and rs9534512 SNP (haplotype block 2; D'=0.98; LOD>=2) determined by confidence interval (CI) method by Gabriel et al. (2002) (Figure 1). The haplotypes that were present in ≤ of 1% (AC for haplotype block 1 and GA for haplotype block 2) of subjects were excluded from further analyses.

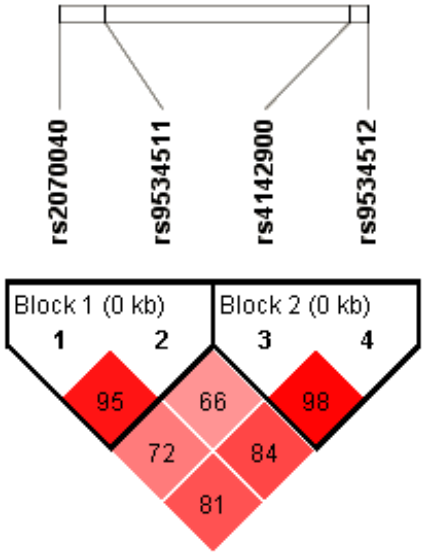

Figure 1. Linkage disequilibrium (LD) plot for 4 HTR2A polymorphisms (rs2070040, rs9534511, rs4142900 and rs9534512) in subjects with CD and control subjects. Pairwise LD value (D' x 100) was calculated for each SNP combination and is denoted in red rectangles, where more intense shading represents stronger link between loci. Determined D' values indicated the strongest link between rs2070040 and rs9534511 SNP identified as haplotype block 1, and rs4142900 and rs9534512 SNP, identified as haplotype block 2.

There were no significant differences in the distribution of genotypes or alleles of the *HTR2A* polymorphism or haplotype blocks (rs2070040-rs9534511 and rs4142900-rs9534512) between control subjects and subjects with CD (Table 2). However, significant changes in the distribution of rs2070040 genotypes (p=0.019) and alleles (p=0.032) were observed between court and social cases, which included subjects with and without CD from the correctional facility (Table 2). Subjects facing court charges were mostly GG homozygotes (47.1%), while this genotype was present in 27.6% of social cases, who were most frequently GA heterozygotes for rs2070040 polymorphism. Other polymorphisms and haplotypes were not associated with the history of delinquency adjudications.

Table 2. The distribution of genotypes, alleles and haplotypes in control subjects and subjects with CD and in subjects with or without delinquency adjudications, depending on *HTR2A* polymorphisms. The data are represented as total number and frequency, while significant p values are denoted in bold.

***HTR2A* SNP**		**Control subjects (N=108)**	**Subjects with CD (N=120)**	**Statistics**	**Social cases (N=98)**	**Court cases (N=87)**	**Statistics**
rs2070040	AA	17 (15.7%)	24 (20.0%)	χ^2=1.291; df=2; p=0.524	18 (18.4%)	14 (16.1%)	χ^2=7.945; df=2; **p=0.019**
	AG	48 (44.4%)	56 (46.7%)		53 (54.1%)	32 (36.8%)	
	GG	43 (39.8%)	40 (33.3%)		27 (27.6%)	41 (47.1%)	
	A	82 (38.0%)	104 (43.3%)	χ^2=1.358; df=1; p=0.244	89 (45.4%)	60 (34.5%)	χ^2=4.574; df=1; **p=0.032**
	G	134 (62.0%)	136 (56.7%)		107 (54.6%)	114 (65.5%)	
rs9534511	CC	34 (31.8%)	33 (27.5%)	χ^2=1.277; df=2; p=0.528	21 (21.4%)	31 (36.0%)	χ^2=4.843; df=2; p=0.089
	CT	51 (47.7%)	55 (45.8%)		51 (52.0%)	37 (43.0%)	
	TT	22 (20.6%)	32 (26.7%)		26 (26.5%)	18 (20.9%)	
	C	119 (55.6%)	121 (50.4%)	χ^2=1.223; df=1; p=0.269	93 (47.4%)	99 (57.6%)	χ^2=3.752; df=1; p=0.053
	T	95 (44.4%)	119 (49.6%)		103 (52.6%)	73 (42.4%)	
rs4142900	GG	38 (36.2%)	35 (29.9%)	χ^2=1.067; df=2; p=0.586	29 (29.9%)	30 (35.7%)	χ^2=0.716; df=2; p=0.699
	GT	48 (45.7%)	57 (48.7%)		47 (48.5%)	38 (45.2%)	
	TT	19 (18.1%)	25 (21.4%)		21 (21.6%)	16 (19.0%)	
	G	124 (59.0%)	127 (54.3%)	χ^2=1.027; df=1; p=0.311	105 (54.1%)	98 (58.3%)	χ^2=0.648; df=1; p=0.421
	T	86 (41.0%)	107 (45.7%)		89 (45.9%)	70 (41.7%)	
rs9534512	AA	15 (13.9%)	22 (18.3%)	χ^2=2.635; df=2; p=0.268	19 (19.4%)	12 (13.8%)	χ^2=2.641; df=2; p=0.267
	AC	47 (43.5%)	59 (49.2%)		47 (48.0%)	37 (42.5%)	
	CC	46 (42.6%)	39 (32.5%)		32 (32.7%)	38 (43.7%)	
	A	77 (35.6%)	103 (42.9%)	χ^2=2.514; df=1; p=0.113	85 (43.4%)	61 (35.1%)	χ^2=2.664; df=1; p=0.103
	C	139 (64.4%)	137 (57.1%)		111 (56.6%)	113 (64.9%)	
Haplotype block 1 rs2070040-rs9534511	GC	113 (54.3%)	115 (49.1%)	χ^2=1.226; df=2; p=0.542	88 (46.1%)	97 (58.4%)	χ^2=5.435; df=2; p=0.066
	AT	79 (38.0%)	99 (42.3%)		85 (44.5%)	57 (34.3%)	
	GT	14 (6.7%)	18 (7.7%)		18 (9.4%)	12 (7.2%)	

Haplotype block 2 rs4142900- rs9534512	GC TA TC	121 (58.2%) 74 (35.6%) 12 (5.8%)	127 (54.3%) 100 (42.7%) 7 (3.0%)	χ^2=3.707; df=2; p=0.157	105 (54.1%) 84 (43.3%) 5 (2.6%)	96 (57.8%) 58 (34.9%) 12 (7.2%)	χ^2=5.904; df=2; p=0.052

3.2. Association of platelet 5-HT concentrations with demographic factors and *HTR2A* polymorphisms and psychometric scales

Subjects with CD had significantly higher platelet 5-HT concentration than control subjects (U=5029.5; p=0.008). A two-way ANOVA was conducted to examine the effect of diagnosis and smoking, as well as diagnosis and alcohol abuse on platelet 5-HT concentration. There was no significant interaction between the effects of diagnosis and smoking (F(1, 221)=0.213; p=0.645) or significant main effect of diagnosis (F (1, 221)=0.875; p=0.350) on platelet 5-HT concentration. However, a significant main effect was observed in relation to smoking (F=5.413; p=0.021), which was revealed to depend on significantly higher 5-HT concentrations in smokers. Platelet 5-HT concentrations were not significantly associated with the interaction of CD diagnosis and alcohol use (F(1, 69)=2.627; p=0.110). Likewise, no main effects of diagnosis (F (1, 69)=0.869; p=0.355) and alcohol consumption (F (1, 69)=1.538; p=0.052) were found. Additionally, platelet 5-HT concentrations did not differ in relation to the history of court convictions (U=3639.0; p=0.168); however, control subjects in the correctional facility had significantly higher platelet 5-HT concentrations than non-detained controls (U=893.0; p=0.002; data not shown).

We then analyzed the association of platelet 5-HT concentrations with *HTR2A* polymorphisms and haplotypes. These analyses were run in the total sample as well as separately in smokers and non-smokers, given the significant difference in platelet 5-HT concentrations between these two groups. None of the tested *HTR2A* polymorphisms was significantly associated with platelet 5-HT concentration (genetic, allelic, haplotype analysis) even after subdividing the subjects into smokers and non-smokers (Supplementary Table 1).

Spearman's correlation was used to analyze the association of platelet 5-HT concentration with severity of depressive, psychopathic, aggressive, and problematic behavior symptoms in control subjects from the correctional facility and subjects with CD. Platelet 5-HT concentrations did not significantly correlate with KADS (ρ=-0.131; p=0.298 in healthy subjects, ρ=-0.079; p=0.398 in patients with CD), PCL-YV (ρ=-0.227; p=0.069 in healthy subjects ρ=-0.051; p=0.584 in patients with CD), OAS-M (ρ=-0.069; p=0.584 in healthy subjects, ρ=-0.039; p=0.679 in patients with CD) or CBCL total scores (ρ=-0.148; p=0.238 in healthy subjects, ρ=-0.028; p=0.768 in patients with CD).

3.3. Association of *HTR2A* polymorphisms and psychometric scales

We next studied the association of the severity of depressive symptoms, psychopathology, open aggression and other emotional and behavioral difficulties (measured with the KADS, PCL-YV,

OAS-M and CBCL scales, respectively) and *HTR2A* polymorphisms and haplotypes. Spearman analysis showed significant positive interscale correlation between KADS, PCL-YV, OAS-M and CBCL in all subjects, with the exception of KADS, related to depressive symptoms, and PCL-YV total scores, reflecting psychopathology in youth, when subjects were subdivided by diagnosis (Table 3). The strongest correlation was detected between scales estimating aggressive behavior (OAS-M) and behavioral difficulties including delinquency (CBCL). The association analyses of these scores and *HTR2A* polymorphisms were performed separately in CD-affected and control subjects detained in the correctional facility, since we found that subjects with CD had significantly higher total KADS (U=3168.5; p=0.034), PCL-YV (U=769.0; p<0.001), OAS-M (U=1383.0; p<0.001) and CBCL (U=1143.0; p<0.001) scores than non-affected controls (Table 4).

Table 3. Interscale correlations between KADS, PCL-YV, OAS-M and CBCL in all subjects, control subjects and subjects with CD from correctional facility. The data are represented as Spearman's correlation coefficient and p value. Significant p values are denoted in bold.

	PCL-YV total scores	**OAS-M total scores**	**CBCL total scores**
All subjects			
KADS total scores	ρ=0.184; **p=0.012**	ρ=0.315; **p<0.001**	ρ=0.390; **p<0.001**
PCL-YV total score		ρ=0.640; **p<0.001**	ρ=0.667; **p<0.001**
OASM total scores			ρ=0.716; **p<0.001**
Healthy subjects			
KADS total scores	ρ=0.084; p=0.506	ρ=0.305; **p=0.013**	ρ=0.374; **p=0.002**
PCL-YV total score		ρ=0.397; **p=0.001**	ρ=0.515; **p<0.001**
OASM total scores			ρ=0.665; **p<0.001**
Subjects with CD			
KADS total scores	ρ=0.079; p=0.394	ρ=0.276; **p=0.002**	ρ=0.390; **p<0.001**
PCL-YV total score		ρ=0.490; **p<0.001**	ρ=0.495; **p<0.001**
OASM total scores			ρ=0.586; **p<0.001**

CBCL - Child Behavior Checklist; CD – conduct disorder; KADS - Kutcher Adolescent Depression Scale; OAS-M - The Modified Overt Aggression Scale; PCL-YV - The Hare Psychopathy Checklist: Youth Version

ρ None of the tested *HTR2A* polymorphisms were significantly associated with the KADS or PCL-YV total scores in control or CD subjects (genetic, allelic or haplotype analysis) (Table 4). However, the rs2070040 SNP was significantly associated with the OAS-M and CBCL total scores in patients with CD, but not in controls. Carriers of the GG genotype had the highest score on OAS-M (p=0.018), while G allele carriers had higher total scores than A carriers on both OAS-M (p=0.018) and CBCL (p=0.031) scale (Table 4). The SNP rs4142900 was significantly associated with the CBCL total scores in allelic model (p=0.043) where G allele carriers had higher scores than T allele carriers (Table 4). Other polymorphisms (rs9534511 and rs9534512) and haplotype blocks were not significantly associated with these scales in either CD patients or controls (Table

4). To further evaluate specific aggressive, behavioral and emotional domains, scores on KADS, PCL-YV, OAS-M and CBCL subscales were also analyzed in relation to *HTR2A* polymorphisms.

Table 4. KADS, PCL-YV, OAS-M and CBCL scores in control subjects and subjects with CD from correctional facility depending on the *HTR2A* polymorphisms and haplotypes. The data are represented as median and interquartile range, while significant p values are denoted in bold.

***HTR2A* SNP**		**KADS total scores**		**PCL-YV total scores**		**OAS-M total scores**		**CBCL total scores**	
		Control subjects	Subjects with CD	Control subjects	Subjects with CD	Control subjects	Subjects with CD	Control subjects	Subjects with CD
rs2070040	AA	4 (2; 10)	3 (2; 5)	10 (8; 14)	22 (18; 30)	17 (8; 28)	32 (18; 39)	60 (25; 70)	67 (54; 77)
	AG	3 (0; 6)	4 (2; 7)	11 (7; 15)	27 (18; 32)	9 (6; 18)	30 (19; 41)	44 (30; 55)	72 (66; 85)
	GG	3 (2; 4)	4 (2; 7)	12 (9; 16)	27 (23; 33)	15 (5; 21)	37 (31; 49)	48 (33; 62)	78 (67; 96)
		H=1.921; df=2; p=0.383	H=1.481; df=2; p=0.477	H=0.626; df=2; p=0.731	H=2.775; df=2; p=0.250	H=1.360; df=2; p=0.507	H=8.012; df=2; **p=0.018**	H=1.859; df=2; p=0.395	H=4.489; df=2; p=0.106
	A	3 (2; 7)	4 (2; 6)	10 (7; 15)	26 (18; 32)	14 (6; 20)	32 (18; 39)	45 (30; 65)	71 (57; 83)
	G	3 (2; 4)	4 (2; 7)	11 (8; 15)	27 (21; 32)	13 (5; 20)	35 (25; 47)	46 (31; 60)	75 (66; 91)
		U=1902.5; p=0.960	U=6766.0; p=0.563	U=1749.5; p=0.424	U=6165.0; p=0.088	U=1902.5; p=0.961	U=5809.0; **p=0.018**	U=1884.5; p=0.891	U=5925.0; **p=0.031**
rs9534511	CC	3 (2; 4)	4 (2; 5)	11 (8; 14)	27 (23; 32)	18 (5; 25)	33 (27; 40)	45 (28; 60)	75 (64; 91)
	CT	3 (1; 4)	4 (2; 7)	11 (7; 15)	27 (19; 34)	13 (6; 18)	34 (21; 50)	46 (31; 57)	75 (68; 89)
	TT	5 (2; 10)	3 (2; 6)	12 (9; 19)	23 (17; 30)	13 (6; 23)	27 (13; 38)	48 (37; 67)	67 (54; 77)
		H=2.614; df=2; p=0.271	H=2.208; df=2; p=0.332	H=0.500; df=2; p=0.779	H=4.299; df=2; p=0.117	H=0.326; df=2; p=0.850	H=4.731; df=2; p=0.094	H=0.304; df=2; p=0.859	H=5.922; df=2; p=0.052
	C	3 (2; 4)	4 (2; 6)	11 (7; 15)	27 (22; 32)	14 (5; 22)	33 (25; 46)	45 (30; 60)	75 (66; 91)
	T	3 (2; 7)	4 (2; 6)	11 (7; 15)	26 (18; 32)	13 (6; 20)	32 (18; 41)	46 (32; 63)	72 (57; 85)
		U=1798.5; p=0.274	U=6866.5; p=0.533	U=1914.5; p=0.600	U=6305.5; p=0.096	U=1980.5; p=0.836	U=6254.5; p=0.079	U=1940.5; p=0.690	U=6278.5; p=0.087
rs4142900	GG	3 (2; 5)	4 (2; 6)	11 (7; 14)	27 (23; 33)	13 (5; 19)	33 (26; 40)	44 (30; 62)	77 (66; 96)
	GT	3 (1; 6)	4 (3; 7)	13 (8; 20)	27 (19; 32)	11 (6; 22)	35 (20; 50)	46 (36; 56)	72 (64; 85)
	TT	3 (2; 4)	3 (2; 5)	10 (7; 12)	20 (18; 30)	17 (9; 25)	32 (17; 38)	52 (25; 67)	68 (54; 77)

		H=1.722; df=2; p=0.423	H=4.996; df=2; p=0.082	H=4.417; df=2; p=0.110	H=4.025; df=2; p=0.134	H=0.893; df=2; p=0.640	H=2.248; df=2; p=0.325	H=0.090; df=2; p=0.956	H=4.076; df=2; p=0.130
	G	3 (2; 5)	4 (2; 7)	11 (8; 15)	27 (21; 32)	12 (5; 19)	33 (24; 47)	45 (31; 60)	75 (66; 91)
	T	3 (1; 4)	3 (2; 5)	11 (7; 15)	25 (18; 31)	15 (6; 23)	32 (18; 42)	46 (31; 66)	71 (57; 83)
		U=1744.0; p=0.253	U=6349.5; p=0.385	U=1974.0; p=0.992	U=5783.5; **p=0.050**	U=1796.0; p=0.382	U=6075.5; p=0.163	U=1915.0; p=0.767	U=5752.5; **p=0.043**
rs9534512	AA	3 (2; 3)	3 (2; 5)	10 (7; 10)	21 (17; 30)	16 (12; 20)	31 (17; 38)	38 (31; 66)	67 (54; 77)
	AC	3 (1; 7)	4 (2; 7)	12 (7; 21)	27 (18; 32)	9 (3; 20)	32 (19; 46)	46 (30; 56)	72 (66; 88)
	CC	3 (2; 4)	4 (2; 6)	11 (8; 15)	27 (23; 32)	13 (5; 20)	33 (27; 47)	46 (34; 60)	76 (65; 92)
		H=0.301; df=2; p=0.860	H=1.965; df=2; p=0.374	H=3.405; df=2; p=0.182	H=3.630; df=2; p=0.163	H=0.925; df=2; p=0.630	H=2.746; df=2; p=0.253	H=0.256; df=2; p=0.880	H=3.574; df=2; p=0.167
	A	3 (1; 6)	3 (2; 6)	10 (7; 15)	25 (18; 32)	14 (5; 20)	32 (18; 41)	44 (30; 65)	72 (57; 83)
	C	3 (2; 4)	4 (2; 6)	11 (8; 15)	27 (22; 32)	12 (5; 20)	33 (24; 46)	46 (31; 60)	75 (66; 91)
		U=1782.5; p=0.659	U=6725.5; p=0.532	U=1641.5; p=0.256	U=6125.5; p=0.080	U=1857.5; p=0.949	U=6177.5; p=0.099	U=1802.5; p=0.736	U=6111.5; p=0.076
Haplotype block 1 rs2070040-rs9534511	GC	3 (2; 4)	4 (2; 6)	11 (7; 15)	27 (21; 32)	15 (5; 22)	35 (25; 47)	46 (30; 60)	75 (66; 91)
	AT	3 (1; 7)	4 (2; 6)	10 (7; 15)	25 (18; 31)	14 (5; 23)	32 (18; 40)	45 (30; 65)	71 (57; 80)
	GT	5 (3; 9)	7 (3; 9)	12 (10; 18)	28 (17; 34)	13 (12; 18)	38 (21; 55)	48 (43; 59)	75 (67; 96)
		H=3.862; df=2; p=0.145	H=4.866; df=2; p=0.088	H=1.305; df=2; p=0.521	H=3.394; df=2; p=0.183	H=0.058; df=2; p=0.971	H=5.556; df=2; p=0.062	H=1.013; df=2; p=0.603	H=5.190; df=2; p=0.075
Haplotype block 2 rs4142900-rs9534512	GC	3 (2; 5)	4 (2; 7)	11 (7; 15)	27 (21; 32)	13 (5; 19)	33 (24; 47)	46 (31; 60)	75 (66; 91)
	TA	3 (1; 4)	3 (2; 6)	10 (7; 13)	25 (18; 31)	14 (5; 20)	32 (18; 42)	44 (30; 65)	71 (57; 83)
	TC	2 (1; 3)	3 (2; 5)	15 (11; 21)	25 (18; 30)	23 (10; 32)	35 (34; 54)	54 (45; 66)	74 (62; 79)
		H=1.915; df=2; p=0.384	H=1.055; df=2; p=0.590	H=5.740; df=2; p=0.057	H=3.856; df=2; p=0.145	H=2.707; df=2; p=0.258	H=2.899; df=2; p=0.235	H=1.243; df=2; p=0.537	H=4.124; df=2; p=0.127

CBCL - Child Behavior Checklist; CD – conduct disorder; KADS - Kutcher Adolescent Depression Scale; OAS-M - The Modified Overt Aggression Scale; PCL-YV - The Hare Psychopathy Checklist: Youth Version

3.4. Association of *HTR2A* polymorphisms and PCL-YV subscales score

The PCL-YV scale was used to evaluate the risk of developing antisocial personality behavior and psychopathic tendencies in young people. The four factors: F1 - interpersonal dimension (impression management, arrogance, manipulative behavior, pathological lying), F2 - affective dimension (emotional numbing, lack of remorse and/or empathy), F3 - behavioral dimension (antisocial tendencies, irritability, impulsivity, lack of goals) and F4 - criminal behavior (poor anger control, criminal behavior) were assessed. Although PCL-YV total scores were not associated with any of the tested SNPs, F3 domain of PCL-YV scale, which evaluates behavioral problems in youth, was associated with rs2070040, rs4142900 and rs9534512 polymorphism in subjects with CD, but not in the control subjects (Table 5). In particular, the G allele of rs2070040 ($U=5887.0$; $p=0.025$), the GG genotype ($H=6.126$; $df=2$; $p=0.047$) and the G allele of rs4142900 ($U=5577.5$; $p=0.017$), as well as the CC genotype ($H=6.202$; $df=2$; $p=0.045$) and the C allele of rs9534512 ($U=5858.5$; $p=0.023$) were associated with the higher PCL-YV F3 subscale scores (Table 5). Other PCL-YV subscale scores were not associated with any of the tested polymorphisms or haplotypes.

Table 5. PCL-YV interpersonal, affective, behavioral and criminal domain in control subjects and subjects with CD from correctional facility depending on the *HTR2A* polymorphisms and haplotypes. The data are represented as median and interquartile range, while significant p values are denoted in bold.

***HTR2A* SNP**		**PCL-YV F1 scores**		**PCL-YV F2 scores**		**PCL-YV F3 scores**		**PCL-YV F4 scores**	
		Control subjects	Subjects with CD	Control subjects	Subjects with CD	Control subjects	Subjects with CD	Control subjects	Subjects with CD
rs2070040	AA	1 (0; 3)	6 (4; 7)	3 (1; 4)	7 (5; 9)	4 (3; 5)	5 (3; 7)	2 (2; 4)	7 (5; 8)
	AG	1 (1; 3)	5 (3; 8)	4 (2; 4)	7 (6; 10)	2 (0; 4)	6 (4; 8)	4 (2; 5)	7 (5; 9)
	GG	2 (1; 4)	6 (4; 8)	3 (2; 6)	8 (5; 10)	2 (2; 5)	7 (5; 9)	3 (2; 4)	8 (6; 10)
		H=0.908; df=2; p=0.635	H=1.565; df=2; p=0.457	H=0.862; df=2; p=0.650	H=1.463; df=2; p=0.481	H=1.426; df=2; p=0.490	H=4.915; df=2; p=0.086	H=3.276; df=2; p=0.194	H=3.485; df=2; p=0.175
	A	1 (0; 3)	5 (4; 7)	4 (2; 4)	7 (5; 9)	3 (1; 5)	5 (4; 8)	3 (2; 4)	7 (5; 8)
	G	2 (1; 3)	6 (4; 8)	3 (2; 5)	7 (6; 10)	2 (2; 4)	7 (5; 8)	3 (2; 4)	7 (6; 9)
		U=1759.5; p=0.445	U=6694.0; p=0.475	U=1728.5; p=0.362	U=6465.0; p=0.249	U=1831.5; p=0.687	U=5887.0; **p=0.025**	U=1757.5; p=0.440	U=6077.0; p=0.059
rs9534511	CC	1 (0; 4)	6 (5; 7)	3 (1; 5)	7 (5; 10)	2 (2; 5)	6 (5; 7)	3 (2; 4)	8 (6; 8)
	CT	1 (1; 3)	6 (4; 8)	4 (2; 5)	8 (6; 10)	2 (0; 4)	7 (4; 9)	4 (2; 5)	7 (6; 10)
	TT	3 (0; 4)	5 (3; 7)	4 (2; 6)	7 (4; 9)	5 (3; 6)	5 (4; 7)	3 (2; 4)	6 (5; 8)
		H=0.178; df=2; p=0.915	H=1.552; df=2; p=0.460	H=0.581; df=2; p=0.748	H=2.327; df=2; p=0.312	H=4.637; df=2; p=0.098	H=3.872; df=2; p=0.144	H=2.090; df=2; p=0.352	H=5.852; df=2; p=0.054
	C	1 (1; 3)	6 (4; 8)	3 (2; 5)	7 (6; 10)	2 (1; 4)	6 (5; 8)	3 (2; 4)	8 (6; 9)
	T	2 (1; 3)	5 (3; 7)	4 (2; 5)	7 (5; 9)	3 (1; 5)	6 (4; 8)	3 (2; 4)	7 (5; 8)
		U=1948.5; p=0.714	U=6536.5; p=0.214	U=1876.5; p=0.476	U=6554.5; p=0.225	U=1773.5; p=0.223	U=6258.5; p=0.078	U=1.875.5; p=0.470	U=6297.5; p=0.090
rs4142900	GG	1 (0; 3)	6 (4; 8)	3 (2; 5)	7 (6; 10)	2 (2; 4)	7 (5; 8)	3 (2; 4)	8 (6; 9)
	GT	2 (1; 5)	5 (4; 7)	4 (3; 6)	7 (6; 9)	4 (1; 6)	6 (4; 9)	4 (2; 5)	7 (5; 9)
	TT	2 (0; 3)	5 (4; 7)	3 (2; 4)	6 (3; 9)	3 (1; 3)	5 (3; 6)	3 (2; 4)	6 (5; 8)

		H=4.171; df=2; p=0.124	H=1.462; df=2; p=0.481	H=4.702; df=2; p=0.095	H=3.145; df=2; p=0.208	H=2.059; df=2; p=0.357	H=6.126; df=2; **p=0.047**	H=0.822; df=2; p=0.663	H=3.054; df=2; p=0.217
	G	1 (1; 3)	6 (4; 8)	3 (2; 5)	7 (6; 10)	2 (2; 4)	7 (4; 8)	3 (2; 4)	7 (6; 9)
	T	2 (1; 3)	5 (4; 7)	3 (2; 4)	7 (5; 9)	3 (1; 4)	5 (3; 7)	3 (2; 4)	7 (5; 8)
		U=1834.0; p=0.482	U=6280.5; p=0.315	U=1906.0; p=0.731	U=6002.5; p=0.120	U=1952.0; p=0.906	U=5577.5; **p=0.017**	U=1974.0; p=0.992	U=5944.5; p=0.096
rs9534512	AA	1 (0; 2)	6 (3; 7)	3 (2; 4)	7 (4; 9)	3 (1; 3)	4 (3; 7)	2 (2; 4)	6 (4; 8)
	AC	1 (1; 5)	5 (4; 8)	4 (3; 5)	8 (6; 10)	3 (0; 7)	6 (4; 9)	4 (2; 5)	7 (6; 9)
	CC	2 (0; 3)	6 (5; 8)	3 (2; 5)	7 (6; 10)	2 (2; 4)	7 (5; 8)	3 (2; 4)	8 (6; 9)
		H=3.610; df=2; p=0.164	H=1.162; df=2; p=0.559	H=2.336; df=2; p=0.311	H=2.565; df=2; p=0.277	H=0.842; df=2; p=0.656	H=6.202; df=2; **p=0.045**	H=1.783; df=2; p=0.410	H=4.190; df=2; p=0.123
	A	1 (0; 3)	5 (4; 7)	3 (2; 4)	7 (5; 9)	3 (0; 4)	5 (3; 8)	3 (2; 4)	7 (5; 8)
	C	2 (1; 3)	6 (4; 8)	3 (2; 5)	7 (6; 10)	2 (2; 5)	7 (5; 8)	3 (2; 5)	7 (6; 9)
		U=1773.5; p=0.624	U=6610.5; p=0.400	U=1708.5; p=0.418	U=6329.5; p=0.167	U=1771.5; p=0.618	U=5858.5; **p=0.023**	U=1741.5; p=0.516	U=6186.5; p=0.099
Haplotype block 1 rs2070040-rs9534511	GC	1 (1; 3)	6 (4; 7)	3 (2; 5)	7 (6; 9)	2 (1; 4)	6 (5; 8)	3 (2; 4)	7 (6; 9)
	AT	1 (0; 3)	5 (3; 7)	4 (2; 4)	7 (5; 9)	3 (1; 5)	5 (3; 7)	3 (2; 4)	7 (5; 8)
	GT	3 (1; 4)	5 (3; 8)	5 (3; 6)	8 (5; 10)	4 (2; 5)	7 (4; 9)	3 (2; 4)	7 (6; 10)
		H=1.226; df=2; p=0.542	H=1.241; df=2; p=0.538	H=2.478; df=2; p=0.290	H=1.637; df=2; p=0.441	H=0.922; df=2; p=0.631	H=5.773; df=2; p=0.056	H=1.367; df=2; p=0.505	H=3.999; df=2; p=0.135
Haplotype block 2 rs4142900-rs9534512	GC	1 (0; 3)	6 (4; 8)	3 (2; 5)	7 (6; 10)	2 (2; 4)	7 (4; 8)	3 (2; 4)	7 (6; 9)
	TA	1 (0; 3)	5 (4; 7)	3 (2; 4)	7 (5; 9)	3 (0; 4)	5 (3; 7)	3 (2; 4)	7 (5; 8)
	TC	3 (2; 6)	6 (4; 6)	4 (3; 6)	7 (3; 10)	4 (2; 7)	5 (5; 7)	4 (2; 5)	7 (5; 9)
		H=5.579; df=2; p=0.061	H=1.014; df=2; p=0.602	H=1.387; df=2; p=0.500	H=2.416; df=2; p=0.299	H=1.923; df=2; p=0.382	H=5.952; df=2; p=0.051	H=1.847; df=2; p=0.397	H=2.937; df=2; p=0.230

CD – conduct disorder; PCL-YV - The Hare Psychopathy Checklist - Youth Version; PCL-YV F1 - interpersonal dimension; PCL-YV F2 – affective dimension, PCL-YV F3 - behavioral dimension; PCL-YV F4 - criminal behavior

3.5. Association of *HTR2A* polymorphisms and OAS-M subscales scores

The OAS-M scale was used to assess the aggressive behavior in young male subjects in the correctional facility. The OAS-M contains three domains describing different phenotypic features of aggression: aggressive behavior, irritability, and suicidality. In turn, the aggressive domain can be further divided into four subdomains: verbal aggression, aggression toward objects, auto-aggression, and physical aggression toward other people. The irritability domain can be divided into open and subjective irritability subdomains, while suicidality is further dissected into suicidal tendencies, suicidal attempt, and severity of suicidal attempt.

HTR2A rs2070040 SNP was associated with the OAS-M aggressive subscale scores (H=6.475; df=2; p=0.039, codominant model; U=5971.0; p=0.039, allelic model) where the GG homozygotes, or the G allele carriers had higher scores than carriers of the AA and AG genotype, or the A allele, respectively (Table 6). Other polymorphisms were not associated with the OAS-M aggressive subscale scores (Table 6). However, significant associations were found between the OAS-M subdomain of physical aggression toward others and the GG genotype, as well as the G allele of rs2070040 (H=9.120; df=2; p=0.010, codominant model; U=5851.0; p=0.017, allelic model). Furthermore, CC and CT carriers, and the C allele carriers of rs9534511 (H=7.118; df=2; p=0.028, codominant model; U=5992.5; p=0.019, allelic model), as well as their GC haplotype (H=6.482; df=2; p=0.039) had higher scores on the OAS-M subscale for physical aggression toward other people (Table 7). Other types of aggressive behaviors measured with the OAS-M verbal aggression, aggression toward objects, and auto-aggression subscales were not associated with any polymorphism (Table 7).

Table 6. OAS-M aggression, suicidality and irritability subscale scores in control subjects and subjects with CD from correctional facility depending on the *HTR2A* polymorphisms and haplotypes. The data are represented as median and interquartile range, while significant p values are denoted in bold.

***HTR2A* SNP**		**OAS-M aggression subscale scores**		**OAS-M suicidality subscale scores**		**OAS-M irritability subscale scores**	
		Control subjects	Subjects with CD	Control subjects	Subjects with CD	Control subjects	Subjects with CD
rs2070040	AA	10 (5; 20)	23 (12; 32)	0 (0; 1)	1 (0; 1)	4 (2; 7)	6 (4; 7)
	AG	5 (2; 12)	23 (12; 34)	0 (0; 1)	1 (0; 1)	4 (2; 5)	6 (5; 7)
	GG	10 (1; 15)	29 (21; 40)	1 (0; 2)	1 (0; 2)	4 (3; 6)	7 (6; 8)
		H=0.740; df=2; p=0.691	H=6.475; df=2; **p=0.039**	H=0.937; df=2; p=0.626	H=2.056; df=2; p=0.358	H=0.614; df=2; p=0.736	H=6.726; df=2; **p=0.035**

	A	9 (2; 13)	23 (12; 32)	0 (0; 1)	1 (0; 1)	4 (2; 5)	6 (5; 7)
	G	9 (1; 12)	27 (18; 38)	0 (0; 1)	1 (0; 2)	4 (2; 6)	6 (5; 8)
		U=1895.5 ; p=0.933	U=5971.0; **p=0.039**	U=1737.5 ; p=0.346	U=6337.0 ; p=0.141	U=1797.5 ; p=0.569	U=5828.0 ; **p=0.018**
rs9534511	CC	10 (0; 20)	27 (21; 31)	1 (0; 2)	1 (0; 1)	5 (2; 6)	6 (6; 8)
	CT	8 (4; 12)	27 (15; 40)	0 (0; 1)	1 (0; 2)	4 (2; 5)	6 (5; 8)
	TT	9 (3; 15)	21 (8; 31)	0 (0; 1)	1 (0; 1)	4 (2; 6)	5 (4; 7)
		H=0.049; df=2; p=0.976	H=3.602; df=2; p=0.165	H=3.934; df=2; p=0.140	H=1.968; df=2; p=0.374	H=1.113; df=2; p=0.573	H=9.839; df=2; **p=0.007**
	C	9 (1; 17)	27 (19; 35)	1 (0; 2)	1 (0; 1)	4 (2; 6)	6 (5; 8)
	T	9 (4; 12)	24 (12; 34)	0 (0; 1)	1 (0; 1)	4 (2; 5)	6 (5; 7)
		U=1978.5 ; p=0.829	U=6447.5; p=0.162	U=1668.5 ; p=0.062	U=7027.5 ; p=0.733	U=1858.5 ; p=0.424	U=5778.5 ; **p=0.007**
rs4142900	GG	9 (1; 12)	27 (20; 31)	1 (0; 2)	1 (0; 1)	4 (3; 6)	6 (5; 8)
	GT	7 (3; 13)	27 (15; 40)	0 (0; 1)	1 (0; 2)	4 (2; 6)	6 (5; 8)
	TT	11 (5; 21)	22 (12; 31)	1 (0; 1)	0 (0; 1)	4 (2; 6)	6 (4; 7)
		H=1.571; df=2; p=0.456	H=1.721; df=2; p=0.423	H=3.252; df=2; p=0.197	H=2.127; df=2; p=0.345	H=0.128; df=2; p=0.938	H=3.948; df=2; p=0.139
	G	9 (1; 12)	27 (17; 37)	0 (0; 1)	1 (0; 2)	4 (2; 6)	6 (5; 8)
	T	9 (3; 19)	24 (12; 34)	1 (0; 1)	1 (0; 1)	4 (2; 6)	6 (5; 7)
		U=1738.0 ; p=0.246	U=6170.5; p=0.226	U=1957.0 ; p=0.919	U=6383.5 ; p=0.395	U=1920.0 ; p=0.784	U=5794.5 ; **p=0.049**
rs9534512	AA	10 (8; 18)	22 (12; 31)	1 (0; 1)	0 (0; 1)	3 (2; 5)	6 (4; 7)
	AC	5 (1; 12)	26 (14; 35)	0 (0; 1)	1 (0; 2)	4 (2; 5)	6 (5; 8)
	CC	9 (1; 12)	27 (21; 37)	0 (0; 1)	1 (0; 1)	4 (3; 6)	6 (5; 8)
		H=1.397; df=2; p=0.497	H=2.083; df=2; p=0.353	H=0.009; df=2; p=0.995	H=1.948; df=2; p=0.377	H=2.011; df=2; p=0.366	H=3.621; df=2; p=0.164
	A	9 (2; 17)	24 (12; 34)	1 (0; 1)	1 (0; 1)	4 (2; 5)	6 (5; 7)
	C	9 (1; 12)	27 (17; 35)	0 (0; 1)	1 (0; 1)	4 (2; 6)	6 (5; 8)
		U=1795.5 ; p=0.710	U=6295.5; p=0.153	U=1853.5 ; p=0.926	U=6651.5 ; p=0.418	U=1611.5 ; p=0.195	U=6065.5 ; p=0.059
Haplotype block 1 rs2070040 - rs9534511	GC	9 (1; 17)	27 (19; 37)	1 (0; 1)	1 (0; 1)	4 (2; 6)	6 (5; 8)
	AT	9 (2; 17)	22 (12; 33)	0 (0; 1)	1 (0; 1)	4 (2; 5)	6 (5; 7)
	GT	9 (8; 11)	30 (16; 42)	0 (0; 1)	2 (0; 3)	4 (3; 6)	5 (4; 9)
		H=0.031; df=2; p=0.985	H=4.268; df=2; p=0.118	H=2.567; df=2; p=0.277	H=3.912; df=2; p=0.141	H=0.589; df=2; p=0.745	H=6.693; df=2; **p=0.035**
Haplotype block 2	GC	9 (1; 12)	27 (17; 37)	0 (0; 1)	1 (0; 2)	4 (2; 6)	6 (5; 8)
	TA	9 (2; 17)	24 (12; 34)	1 (0; 1)	1 (0; 1)	4 (2; 5)	6 (5; 7)
	TC	17 (6; 24)	28 (24; 47)	0 (0; 2)	1 (0; 1)	6 (4; 6)	6 (5; 8)

rs4142900 - rs9534512	H=2.213; df=2; p=0.331	H=2.147; df=2; p=0.342	H=0.064; df=2; p=0.968	H=0.723; df=2; p=0.697	H=4.019; df=2; p=0.134	H=3.911; df=2; p=0.141

CD – conduct disorder; OAS-M - Modified Overt Aggression Scale

Table 7. OAS-M aggression subscales in control subjects and subjects with CD from correctional facility depending on the *HTR2A* polymorphisms and haplotypes. The data are represented as median and interquartile range, while significant p values are denoted in bold.

***HTR2A* SNP**		**OAS-M verbal aggression scores**		**OAS-M physical aggression toward objects scores**		**OAS-M auto-aggression scores**		**OAS-M physical aggression toward other people scores**	
		Control subjects	Subjects with CD	Control subjects	Subjects with CD	Control subjects	Subjects with CD	Control subjects	Subjects with CD
rs2070040	AA	5 (2; 8)	9 (4; 11)	0 (0; 3)	4 (2; 11)	0 (0; 0)	0 (0; 6)	3 (1; 9)	9 (3; 9)
	AG	3 (1; 6)	7 (5; 10)	2 (0; 2)	5 (2; 6)	0 (0; 0)	0 (0; 6)	3 (0; 3)	6 (3; 9)
	GG	4 (1; 7)	10 (6; 10)	2 (0; 4)	6 (2; 12)	0 (0; 3)	5 (0; 6)	3 (0; 3)	9 (9; 11)
		H=0.499; df=2; p=0.779	H=2.548; df=2; p=0.280	H=2.833; df=2; p=0.243	H=3.357; df=2; p=0.187	H=5.343; df=2; p=0.069	H=2.826; df=2; p=0.243	H=1.797; df=2; p=0.407	H=9.120; df=2; **p=0.010**
	A	4 (1; 6)	7 (5; 10)	0 (0; 2)	4 (2; 8)	0 (0; 0)	0 (0; 6)	3 (0; 3)	8 (3; 9)
	G	4 (1; 7)	10 (6; 10)	2 (0; 2)	6 (2; 10)	0 (0; 0)	0 (0; 6)	3 (0; 3)	9 (3; 9)
		U=1856.5; p=0.781	U=6518.0; p=0.289	U=1595.5; p=0.098	U=6402.0; p=0.200	U=1657.5; p=0.065	U=6295.5; p=0.170	U=1705.5; p=0.280	U=5851.0; **p=0.017**
rs9534511	CC	4 (0; 7)	7 (6; 10)	2 (0; 2)	6 (2; 10)	0 (0; 6)	3 (0; 6)	2 (0; 3)	9 (6; 9)
	CT	3 (1; 7)	10 (6; 11)	2 (0; 2)	6 (2; 10)	0 (0; 0)	0 (0; 6)	3 (0; 3)	9 (3; 9)
	TT	5 (2; 6)	7 (4; 10)	0 (0; 2)	2 (2; 7)	0 (0; 0)	0 (0; 6)	3 (0; 6)	4 (3; 9)
		H=0.327; df=2; p=0.849	H=2.080; df=2; p=0.354	H=1.673; df=2; p=0.433	H=2.130; df=2; p=0.345	H=3.136; df=2; p=0.208	H=0.286; df=2; p=0.867	H=0.878; df=2; p=0.645	H=7.118; df=2; **p=0.028**
	C	4 (0; 7)	8 (6; 10)	2 (0; 2)	6 (2; 10)	0 (0; 0)	0 (0; 6)	3 (0; 3)	9 (3; 9)
	T	4 (1; 6)	8 (5; 10)	2 (0; 2)	4 (2; 8)	0 (0; 0)	0 (0; 6)	3 (0; 3)	9 (3; 9)
		U=1920.5; p=0.616	U=7138.5; p=0.908	U=1835.5; p=0.337	U=6587.5; p=0.246	U=1791.5; p=0.102	U=6810.0; p=0.576	U=1843.5; p=0.358	U=5992.5; **p=0.019**
rs4142900	GG	2 (1; 7)	8 (6; 10)	2 (0; 4)	6 (2; 12)	0 (0; 2)	0 (0; 6)	1 (0; 3)	9 (3; 9)
	GT	4 (1; 7)	10 (5; 10)	1 (0; 2)	6 (2; 8)	0 (0; 0)	0 (0; 6)	3 (0; 3)	9 (3; 9)
	TT	5 (2; 9)	7 (3; 10)	2 (0; 2)	4 (2; 8)	0 (0; 3)	0 (0; 6)	3 (1; 8)	9 (3; 9)

		H=1.239; df=2; p=0.538	H=1.841; df=2; p=0.398	H=1.020; df=2; p=0.601	H=3.532; df=2; p=0.171	H=4.536; df=2; p=0.104	H=0.133; df=2; p=0.936	H=3.445; df=2; p=0.179	H=1.576; df=2; p=0.455
	G	3 (1; 7)	10 (6; 10)	2 (0; 3)	6 (2; 10)	0 (0; 0)	0 (0; 6)	3 (0; 3)	9 (3; 9)
	T	4 (1; 7)	7 (5; 10)	2 (0; 2)	4 (2; 8)	0 (0; 0)	0 (0; 6)	3 (0; 3)	9 (3; 9)
		U=1739.0; p=0.243	U=6289.5; p=0.318	U=1879.0; p=0.615	U=5969.5; p=0.103	U=1892.0; p=0.549	U=6525.0; p=0.741	U=1605.0; p=0.055	U=6202.5; p=0.230
rs9534512	AA	4 (2; 6)	7 (3; 10)	2 (0; 2)	4 (2; 10)	0 (0; 0)	0 (0; 6)	3 (2; 3)	8 (3; 9)
	AC	3 (1; 6)	8 (5; 10)	0 (0; 2)	4 (2; 6)	0 (0; 0)	0 (0; 6)	3 (0; 3)	9 (3; 9)
	CC	4 (1; 7)	10 (6; 10)	2 (0; 4)	6 (2; 12)	0 (0; 3)	3 (0; 6)	2 (0; 3)	9 (3; 9)
		H=0.314; df=2; p=0.855	H=0.823; df=2; p=0.663	H=4.224; df=2; p=0.121	H=4.513; df=2; p=0.105	H=5.488; df=2; p=0.064	H=0.505; df=2; p=0.777	H=1.195; df=2; p=0.550	H=2.641; df=2; p=0.267
	A	4 (1; 6)	7 (4; 10)	2 (0; 2)	4 (2; 8)	0 (0; 0)	0 (0; 6)	3 (0; 3)	9 (3; 9)
	C	4 (1; 7)	10 (6; 10)	2 (0; 2)	6 (2; 10)	0 (0; 0)	0 (0; 6)	3 (0; 3)	9 (3; 9)
		U=1832.5; p=0.849	U=6584.5; p=0.367	U=1647.5; p=0.239	U=6295.5; p=0.146	U=1712.5; p=0.248	U=6595.0; p=0.475	U=1649.5; p=0.243	U=6254.5; p=0.115
Haplotype block 1 rs2070040 - rs9534511	GC	4 (1; 7)	10 (6; 10)	2 (0; 2)	6 (2; 10)	0 (0; 0)	2 (0; 6)	3 (0; 3)	9 (3; 9)
	AT	4 (1; 7)	7 (4; 10)	0 (0; 2)	4 (2; 8)	0 (0; 0)	0 (0; 6)	3 (0; 3)	9 (3; 9)
	GT	5 (1; 6)	10 (6; 15)	2 (0; 4)	5 (2; 12)	0 (0; 2)	3 (0; 9)	3 (0; 3)	9 (3; 9)
		H=0.187; df=2; p=0.911	H=3.672; df=2; p=0.159	H=3.160; df=2; p=0.206	H=1.938; df=2; p=0.379	H=3.350; df=2; p=0.187	H=1.818; df=2; p=0.403	H=1.421; df=2; p=0.491	H=6.482; df=2; **p=0.039**
Haplotype block 2 rs4142900 - rs9534512	GC	4 (1; 7)	10 (6; 10)	2 (0; 3)	6 (2; 10)	0 (0; 0)	0 (0; 6)	3 (0; 3)	9 (3; 9)
	TA	4 (1; 6)	8 (5; 10)	2 (0; 2)	4 (2; 8)	0 (0; 0)	0 (0; 6)	3 (0; 3)	9 (3; 9)
	TC	9 (3; 10)	7 (5; 10)	2 (2; 2)	4 (0; 14)	0 (0; 3)	6 (0; 9)	3 (0; 6)	9 (9; 18)
		H=3.349; df=2; p=0.187	H=1.034; df=2; p=0.596	H=2.589; df=2; p=0.274	H=2.694; df=2; p=0.260	H=1.765; df=2; p=0.414	H=1.452; df=2; p=0.484	H=3.623; df=2; p=0.163	H=3.075; df=2; p=0.215

CD – conduct disorder; OAS-M - Modified Overt Aggression Scale

Higher scores on the OAS-M irritability subscale (Table 6), and more specifically the OAS-M subjective irritability subdomain scores (Table 8) were associated with: 1) the GG genotype (H=6.726; df=2; p=0.035) and the G allele of rs2070040 (U=5828.0; p=0.018); 2) the CC and CT genotypes (H=9.839; df=2; p=0.007) and the C allele of rs9534511 (U=5778.5; p=0.007); and 3) the GC haplotype (H=6.693; df=2; p=0.035) and the G allele of rs4142900 (U=5794.5; p=0.049).

Furthermore, among CD-affected subjects, higher scores on the OAS-M subjective irritability subscale were found in: 1) GG homozygotes and G allele carriers of the rs2070040 polymorphism (H=9.550; df=2; p=0.008, codominant model; U=5462.0; p=0.002, allelic model); 2) CC homozygotes and C allele carriers of rs9534511 (H=13.272; df=2; p=0.001, codominant model; U=5628.5; p=0.002, allelic model); 3) GG homozygotes and G allele carriers of rs4142900 (H=10.854; df=2; p=0.004, codominant model; U=5174.5; p=0.001, allelic model); 4) CC homozygotes and C allele carriers of rs9534512 (H=10.024; df=2; p=0.007, codominant model; U=5.474.5; p=0.002, allelic model); and 5) the GC haplotype from both haplotype blocks (H=10.762; df=2; p=0.005 for haplotype block 1; H=10.947; df=2; p=0.004, for haplotype block) (Table 8).

Table 8. OAS-M irritability subscale scores in control subjects and subjects with CD from correctional facility depending on the *HTR2A* polymorphisms and haplotypes. The data are represented as median and interquartile range, while significant p values are denoted in bold.

***HTR2A* SNP**		**OAS-M open irritability subscale scores**		**OAS-M subjective irritability subscale scores**	
		Control subjects	Subjects with CD	Control subjects	Subjects with CD
rs2070040	AA	2 (1; 3)	3 (2; 4)	2 (1; 4)	3 (2; 3)
	AG	1 (1; 2)	3 (2; 3)	3 (1; 3)	3 (3; 4)
	GG	2 (1; 3)	3 (2; 4)	3 (2; 3)	4 (3; 4)
		H=4.305; df=2; p=0.116	H=4.610; df=2; p=0.100	H=0.236; df=2; p=0.889	H=9.550; df=2; **p=0.008**
	A	1 (1; 2)	3 (2; 4)	3 (1; 3)	3 (2; 4)
	G	1 (1; 3)	3 (2; 4)	3 (1; 3)	3 (3; 4)
		U=1685.5; p=0.237	U=6489.0; p=0.258	U=1869.5; p=0.828	U=5462.0; **p=0.002**
rs9534511	CC	2 (1; 3)	3 (2; 4)	3 (1; 3)	4 (3; 4)
	CT	1 (1; 2)	3 (2; 4)	3 (1; 3)	3 (3; 4)
	TT	1 (1; 3)	3 (2; 4)	3 (1; 4)	3 (2; 3)
		H=3.769; df=2; p=0.152	H=3.441; df=2; p=0.179	H=0.077; df=2; p=0.962	H=13.272; df=2; **p=0.001**
	C	1 (1; 3)	3 (2; 4)	3 (1; 3)	3 (3; 4)
	T	1 (1; 2)	3 (2; 4)	3 (1; 3)	3 (2; 4)

		U=1714.5; p=0.116	U=6404.5; p=0.126	U=1977.5; p=0.819	U=5628.5; **p=0.002**
rs4142900	GG	1 (1; 3)	3 (2; 4)	3 (2; 3)	4 (3; 4)
	GT	1 (1; 3)	3 (2; 4)	3 (1; 3)	3 (3; 4)
	TT	1 (1; 3)	3 (2; 4)	3 (1; 4)	3 (2; 3)
		H=0.050; df=2; p=0.975	H=0.410; df=2; p=0.815	H=0.109; df=2; p=0.947	H=10.854; df=2; **p=0.004**
	G	1 (1; 3)	3 (2; 4)	3 (1; 3)	3 (3; 4)
	T	1 (1; 3)	3 (2; 4)	3 (1; 3)	3 (2; 4)
		U=1947.0; p=0.881	U=6496.5; p=0.550	U=1967.0; p=0.964	U=5174.5; **p=0.001**
rs9534512	AA	1 (1; 2)	3 (2; 4)	2 (1; 3)	3 (2; 3)
	AC	1 (1; 2)	3 (2; 4)	3 (1; 3)	3 (3; 4)
	CC	2 (1; 3)	3 (2; 4)	3 (2; 3)	4 (3; 4)
		H=4.409; df=2; p=0.110	H=0.291; df=2; p=0.865	H=0.097; df=2; p=0.953	H=10.024; df=2; **p=0.007**
	A	1 (1; 2)	3 (2; 4)	3 (1; 3)	3 (2; 4)
	C	1 (1; 3)	3 (2; 4)	3 (2; 3)	3 (3; 4)
		U=1.554.5; p=0.096	U=6.783.5; p=0.597	U=1.813.5; p=0.770	U=5.474.5; **p=0.002**
Haplotype block 1 rs2070040-rs9534511	GC	1 (1; 3)	3 (2; 4)	3 (1; 3)	3 (3; 4)
	AT	1 (1; 2)	3 (2; 4)	2 (1; 3)	3 (2; 4)
	GT	2 (0; 3)	3 (2; 5)	3 (2; 3)	4 (2; 5)
		H=2.260; df=2; p=0.323	H=1.833; df=2; p=0.400	H=0.280; df=2; p=0.869	H=10.762; df=2; **p=0.005**
Haplotype block 2 rs4142900-rs9534512	GC	1 (1; 3)	3 (2; 4)	3 (1; 3)	3 (3; 4)
	TA	1 (1; 2)	3 (2; 4)	3 (1; 3)	3 (2; 4)
	TC	3 (2; 3)	3 (2; 4)	3 (2; 3)	3 (3; 4)
		H=7.899; df=2; **p=0.019**	H=0.358; df=2; p=0.836	H=0.462; df=2; p=0.794	H=10.947; df=2; **p=0.004**

CD – conduct disorder; OAS-M - Modified Overt Aggression Scale

3.6. Association of *HTR2A* polymorphisms and CBCL subscales scores

Behavioral and emotional functioning, and in particular delinquent and aggressive behavior, was assessed using the CBCL aggression and delinquent subscales. The rs2070040 SNP was significantly associated with the CBCL total scores in patients with CD (Table 4) and in particular, its aggression domain. The GG homozygotes of the rs2070040 (H=7.396; df=2; p=0.025) and the G allele carriers (U=5693.0; p=0.010), the C allele carriers of rs9534511 (U=6099.5; p=0.041), and the GT haplotype of haplotype block 1 (H=6.852; df=2; p=0.033) were significantly associated with the higher scores in the CBCL aggression subscale in patients with CD, while the TC haplotype of haplotype block 2 (H=7.813; df=2; p=0.020), but no other SNPs, were associated with this subscale in control subjects. However, no significant association between SNPs and haplotypes was detected regarding the CBCL delinquent subscale scores (Table 9).

Table 9. CBCL aggression and delinquent subscales in control subjects and subjects with CD from correctional facility depending on the *HTR2A* polymorphisms and haplotypes. The data are represented as median and interquartile range, while significant p values are denoted in bold.

***HTR2A* SNP**		**CBCL aggression subscale scores**		**CBCL delinquent subscale scores**	
		Control subjects	Subjects with CD	Control subjects	Subjects with CD
rs2070040	AA	13 (2; 18)	21 (15; 31)	4 (2; 9)	15 (10; 18)
	AG	8 (5; 13)	24 (19; 32)	5 (3; 7)	13 (10; 17)
	GG	13 (5; 20)	29 (24; 33)	7 (5; 10)	16 (13; 19)
		H=2.485; df=2; p=0.289	H=7.396; df=2; **p=0.025**	H=4.197; df=2; p=0.123	H=4.546; df=2; p=0.103
	A	10 (4; 14)	23 (17; 31)	5 (2; 8)	14 (10; 17)
	G	12 (5; 16)	26 (20; 32)	6 (4; 9)	15 (12; 18)
		U=1680.5; p=0.256	U=5693.0; **p=0.010**	U=1542.5; p=0.069	U=6408.0; p=0.212
rs9534511	CC	12 (4; 21)	26 (23; 32)	6 (2; 9)	15 (12; 17)
	CT	10 (6; 16)	25 (19; 32)	6 (3; 8)	14 (11; 18)
	TT	12 (4; 16)	22 (14; 30)	7 (2; 9)	14 (9; 18)
		H=0.544; df=2; p=0.762	H=4.366; df=2; p=0.113	H=0.071; df=2; p=0.965	H=0.313; df=2; p=0.855
	C	12 (4; 19)	26 (20; 32)	6 (3; 9)	15 (12; 17)
	T	11 (5; 16)	24 (17; 32)	6 (3; 8)	14 (10; 18)
		U=1891.5; p=0.526	U=6099.5; **p=0.041**	U=1974.5; p=0.814	U=6887.5; p=0.561
rs4142900	GG	9 (4; 16)	29 (22; 33)	6 (3; 10)	15 (12; 19)
	GT	12 (7; 19)	25 (19; 32)	6 (3; 7)	13 (11; 17)
	TT	12 (3; 16)	21 (15; 30)	6 (2; 10)	15 (10; 18)
		H=1.406; df=2; p=0.495	H=3.928; df=2; p=0.140	H=0.358; df=2; p=0.836	H=0.964; df=2; p=0.617
	G	10 (5; 16)	26 (20; 32)	6 (3; 9)	14 (11; 18)
	T	12 (5; 17)	24 (17; 31)	6 (2; 8)	14 (10; 18)
		U=1917.0; p=0.774	U=5762.5; **p=0.045**	U=1853.0; p=0.549	U=6600.5; p=0.706
rs9534512	AA	11 (4; 13)	21 (15; 30)	5 (2; 10)	15 (9; 18)
	AC	10 (5; 19)	25 (19; 32)	6 (2; 7)	14 (11; 18)
	CC	12 (5; 17)	29 (22; 32)	6 (3; 10)	15 (12; 18)
		H=1.337; df=2; p=0.513	H=3.183; df=2; p=0.204	H=1.977; df=2; p=0.372	H=0.305; df=2; p=0.858
	A	11 (4; 14)	25 (17; 32)	5 (2; 8)	14 (10; 18)
	C	12 (5; 17)	26 (20; 32)	6 (3; 9)	14 (12; 18)
		U=1625.5; p=0.225	U=6106.5; p=0.074	U=1641.5; p=0.255	U=6894.5; p=0.762

Haplotype block 1 (rs2070040-rs9534511)	GC	12 (5; 19)	26 (20; 32)	6 (3; 9)	15 (12; 18)
	AT	10 (4; 17)	22 (17; 31)	5 (2; 8)	14 (10; 17)
	GT	13 (5; 16)	30 (22; 33)	7 (7; 10)	16 (10; 19)
		H=1.361; df=2; p=0.506	H=6.852; df=2; **p=0.033**	H=5.579; df=2; p=0.061	H=1.542; df=2; p=0.463
Haplotype block 2 rs4142900-rs9534512	GC	10 (5; 16)	26 (20; 32)	6 (3; 9)	14 (11; 18)
	TA	11 (4; 14)	25 (17; 32)	5 (2; 7)	14 (10; 18)
	TC	19 (16; 21)	23 (19; 31)	7 (5; 9)	15 (12; 17)
		H=7.813; df=2; **p=0.020**	H=4.038; df=2; p=0.133	H=2.145; df=2; p=0.342	H=0.151; df=2; p=0.927

CBCL - Child Behavior Checklist; CD – conduct disorder

4. Discussion

The present study investigated the association of *HTR2A* SNPs and platelet 5-HT levels with CD and several behavioral dimensions related to this disorder, including aggression and irritability. One of our main results was that several *HTR2A* polymorphisms were associated with irritability and aggressive behavior in CD-affected, but not control, subjects. The strongest association of all four tested polymorphisms (*HTR2A* rs2070040, rs9534511, rs4142900, and rs9534512, and corresponding haplotype blocks) was detected with subjective irritability. Specifically, we showed that higher irritability and aggression towards others, and aggressive behavior as a part of externalizing domain within CBCL scale) were associated with the GG genotype (G allele) of *HTR2A* rs2070040 and CC and CT genotype (C allele) of rs9534511, and GT and GC haplotype (block 1: rs2070040-rs9534511) carriers. These results were expected, since OAS-M and CBCL scales are in strong positive correlation, and are estimating similar phenotypes. Additionally, higher antisocial behavior and impulsivity in the F3 domain of PCL-YV scale was seen in G allele carriers of rs4142900. These results are in substantial alignment with previous findings in a cohort of Mexican-American youths, substantiating that the C allele of the rs9534511 was associated with an increased risk of social disinhibition manifested as impolite behavior, extreme emotional outbursts, and defiance of rules or laws (Archer et al., 2014). More in general, our findings are in keeping with other evidence on different *HTR2A* polymorphisms in traits related to CD, such as anger and aggression (Giegling et al., 2006) and impulsivity (Nomura et al., 2006; Tomson et al., 2016). Our results showed the lack of association between rs2070040, rs9534511, rs4142900 and rs9534512 polymorphisms and rs2070040-rs9534511 and rs4142900-rs9534512 haplotype blocks with smoking and alcohol consumption, a characteristic often tied to sensation-seeking behavior and disinhibition in adolescent age (Wilkinson et al., 2012).

In partial contrast with our findings, a recent study (Langevin et al., 2019) also reported the association of the rs2070040, rs9534511, rs4142900, and rs9534512 polymorphisms and rs2070040-rs9534511 and rs4142900-rs9534512 haplotype blocks with general delinquency, antisocial personality disorder symptoms and self-reported physical partner violence in early adulthood. However, in that study, the AT (block 1: rs2070040-rs9534511) and the TA (block 2: rs4142900-rs9534512) haplotypes were associated with the more exaggerated characteristics of deviant behavior, while our findings showed that subjects carrying these haplotypes (haplotype AT, block 1: rs2070040-rs9534511, and TA, block 2: rs4142900-rs9534512) had milder symptoms of aggression and irritability. We did not observe the association of rs2070040, rs9534511, rs4142900, and rs9534512 polymorphisms and rs2070040-rs9534511 and rs4142900-rs9534512 haplotype blocks with delinquency domain within CBCL scales, however subjects with history of delinquency adjudications (court cases) were more often rs2070040 GG homozygotes, compared to subjects without record (social cases). Many factors may account for this apparent discrepancy, including potential epistatic interactions with other genetic polymorphisms, age differences in clinical samples, as well as the use of different psychometric scales (self-reported vs. clinician-administered). At any rate, it is worth noting that we found the highest levels of aggressive symptoms and irritability in relation to the GT haplotype of block 1 in CD subjects and the TC haplotype of block 2 in controls, while both of these haplotypes were excluded from analyses in the Langevin's study (Langevin et al., 2019) due to their low frequency in their samples.

Of all 5-HT receptors, 5-HT_{2A} are an ideal candidate for the regulation of aggressive traits and behaviors since they are highly expressed in the prefrontal cortex (PFC), the critical brain region that orchestrates impulse control, socio-affective responses, aggressive reactivity, inhibitory function, and reinforcement-based decision making after acute threat stimuli (Blair, 2016). In addition to the role of 5-HT_{2A} receptors in aggression and antisociality, emerging evidence has pointed to the association of these molecules with several psychopathological states connected with emotional dysregulations, including major depression (Hrdina et al., 1995; Hrdina et al., 1993; Mintun et al., 2004), suicidal behavior (Pandey et al., 1995; Turecki et al., 1999), and schizophrenia (Rasmussen et al., 2010).

The most investigated *HTR2A* SNPs, located in promotor and exon regions of the *HTR2A* gene, such as rs6311, rs6313 and rs6314, have been regarded as critical for the modulation of 5-HT_{2A} receptor expression and intracellular signaling, even though most results have proven inconsistent (Blasi et al., 2013; Bray et al., 2004; Khait et al., 2005; Nomura et al., 2015; Ozaki et

al., 1997; Parsons et al., 2004; Spies et al., 2020), likely reflecting the importance of other genetic and environmental factors in the general responsiveness of the PFC (and, likely, different brain regions) and psychopathology. Nevertheless, the association of these SNPs with several mental disorders and traits related to impulsive behavior, such as anorexia nervosa (Collier et al., 1997; Nishiguchi et al., 2001; Ricca et al., 2004), schizophrenia (Abdolmaleky et al., 2004; Joober et al., 1999; Serretti et al., 2008), depression (Lin et al., 2015; Petit et al., 2014; Tan et al., 2014; Zhao et al., 2014) and crime commission in males (Berggard et al., 2003), as well as with tobacco use (do Prado-Lima et al., 2004) and development of alcohol dependence and alcoholism-associated impulsivity (Nakamura et al., 1999; Preuss et al., 2001), supports the idea that genetic factors play a role in influencing the implication of 5-HT_{2A} receptors in neuropsychiatric disorders.

The four polymorphisms investigated in our study are intron and upstream *HTR2A* variants and their role in expression and binding kinetics of platelet or CNS 5-HT2A receptors is not known. In our study, none of the polymorphisms or haplotypes were associated with platelet 5-HT concentration, suggesting the unchanged platelet HT_{2A} receptor functions. However, many intrinsic and external factors moderate platelet 5-HT concentrations, so functional and mechanistic studies are necessary to elucidate the potential role of these polymorphisms on 5-HT_{2A} properties. In our study, higher platelet 5-HT concentrations were associated with smoking, which is in line with previous studies that reported increased 5-HT concentration in platelets of male alcoholic smokers compared to alcoholic non-smokers (Nedic Erjavec et al., 2021; Nenadic-Sviglin et al., 2011; Svob Strac et al., 2019). Several studies showed the association of platelet 5-HT concentrations with alcohol dependence, different alcohol-related phenotypes, and withdrawal symptoms in patients with chronic alcohol abuse and alcohol dependence (Nedic Erjavec et al., 2021; Pivac et al., 2004). However, in our study, alcohol abuse was not related to differences in 5-HT concentrations, probably due to the young age of participants and relatively short alcohol exposure.

Modifications in platelet 5-HT concentrations have been documented in many neuropsychiatric disorders. For example, higher 5-HT levels were associated with violence in male adolescent offenders (Unis et al., 1997) and impulsivity in children with ADHD (Hercigonja Novkovic et al., 2009), but also with impulsive verbal and physical aggression in veterans with PTSD (Ljubin-Golub et al., 2021), psychosis (Muck-Seler et al., 1996) and schizophrenia with predominantly positive symptoms (Muck-Seler et al., 2004; Pivac et al., 1997), while lower platelet 5-HT was related to suicidality in various mental disorders: depression (Muck-Seler et al., 1996; Roggenbach et al., 2007), first episode of psychosis (Marcinko et al., 2007), PTSD (Kovacic et

al., 2008) and schizophrenia with present depressive symptoms (Peitl et al., 2016), but also with more severe alcohol dependence, withdrawal symptoms and late onset of alcohol abuse in patients with alcohol dependence (Nedic Erjavec et al., 2021). In this study, platelet 5-HT did not correlate with severity of aggression, depression, or psychopathic tendencies and did not differ between subjects with or without CD or between social and court cases. Subjects with CD had higher platelet 5-HT than control subjects. Still, this association was lost when smoking was taken into account, since, unlike control subjects, most CD-affected participants were smokers. However, it is essential to mention that, because the majority of participants were detainees in a correctional facility, problematic behavior was observed also in control subjects from the correctional facility. Indeed, youths living in correctional facilities have a higher risk for various behavioral problems and psychiatric disorders (Goncalves et al., 2016). Moreover, the detention itself could be a strong environmental factor associated with biological changes and poorer global and mental health in youth (Barnert et al., 2019; Podobnik et al., 2020), with possible repercussions on the regulation of platelet 5-HT concentrations, as seen in our study where control subjects from correctional facility had higher platelet 5-HT than control subjects outside the detention system.

5. Conclusion

This study indicates a weak, yet significant, role of the *HTR2A* rs2070040, rs9534511, rs4142900, and rs9534512 SNPs, as well as their haplotypes, in moderating the irritability and impulsivity traits, possibly resulting in aggressive and antisocial behavior in male, Caucasian adolescents with CD. The strength of this study is a relatively uniform group of subjects that excluded sex-, and ethnicity-based differences in the distribution of the genotypes and possible interaction of the serotonergic system with hormonal and developmental changes in male and female sex, and different age groups, resulting in a distinctive impact of 5-HT on aggression in children, adolescents and adults (Dadds and Rhodes, 2008). Also we tried to distinguish between different phenotypic manifestations of aggression such as reactive aggression, mostly related to impulsivity and proactive aggression, commonly linked with psychopathic tendencies (Blair et al., 2005; Blair, 2016), antisocial behavior, and related personality traits such as irritability, impulsivity, lack of empathy and remorse, using several clinician-assessed psychiatric and psychological measures.

However, the limited number of participants and the lack of functional evidence associated with these findings represent significant limitations of our study. Although we performed multiple testing, the study was designed as exploratory, and no p value correction was needed. Thus, we

advocate caution in the interpretation of these findings and emphasize the importance of future replication studies in larger cohorts to validate the present results.

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Disclosure of interest

The authors declare no conflict of interest.

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Author Contributions

Conceptualization, NP and MB.; Methodology, GNE, JB, KDC, MC and MC; Formal Analysis, GNE and MNP; Investigation, GNE and LT; Data Curation, LT; Writing – Original Draft Preparation, NP and LT; Writing – Review & Editing NP, MB, GNE, LT and DSS; Supervision, NP and MB.

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