

Original Article

Open Access



Pharmacogenetic influences on the response to pharmacological treatment in autism spectrum disorders

Amaia Hervas¹, Alexandre Serra-Llovich², Isabel Rueda¹, Irene Targa¹, Silvina Guijarro¹, Aitana Bigorra¹, Martha Cancino¹, Valentin Bote¹, Maria Cárcel², Nare Amasi-Hartoonian², Marta Hernandez³, Maria J. Arranz²

¹Department of Child and Adolescent Psychiatry, Hospital Universitari Mútua Terrassa, Terrassa 08221, Spain.

²Research Laboratory Unit, Fundació Docència i Recerca Mútua Terrassa, Terrassa 08221, Spain.

³Facultat Blanquerna, Universitat Ramon LLull, Barcelona 08025, Spain.

Correspondence to: Dr. Maria J. Arranz, Research Laboratory Unit, Fundació Docència i Recerca Mútua Terrassa, c/Sant Antoni, 19, Terrassa 08221, Spain. E-mail: mjarranz@mutuaterrassa.es

How to cite this article: Hervas A, Serra-Llovich A, Rueda I, Targa I, Guijarro S, Bigorra A, Cancino M, Bote V, Cárcel M, Amasi-Hartoonian N, Hernandez M, Arranz MJ. Pharmacogenetic influences on the response to pharmacological treatment in autism spectrum disorders. *J Transl Genet Genom* 2021;5:278-87. <https://dx.doi.org/10.20517/jtgg.2021.25>

Received: 7 May 2021 **First Decision:** 16 Jun 2021 **Revised:** 30 Jun 2021 **Accepted:** 5 Jul 2021 **Available online:** 12 Jul 2021

Academic Editor: Richard E. Frye **Copy Editor:** Yue-Yue Zhang **Production Editor:** Yue-Yue Zhang

Abstract

Aim: About a third of patients with autism spectrum disorder (ASD) receive pharmacological treatment for comorbid symptoms. However, 30%-50% do not respond adequately and/or present severe and long-lasting side effects. Previous studies have reported the influence of variants in genes coding for drug targets on the efficacy and safety of pharmacological treatments, including genetic polymorphisms in dopaminergic and serotonergic systems. However, most studies have focused on the adult population, with relatively few studies in children and adolescents, and no clear biomarkers of response have been reported in these populations. The aim of our study was to identify genetic predictors of drug response in patients with ASD. This information may be used to personalise pharmacological treatment and improve the efficacy and safety of psychotropic drugs in patients with ASD.

Methods: Genetic variants in dopaminergic and serotonergic drug targets (*SLC6A3*, *DRD2*, *DRDRD3*, *DRD4*, *HTR2A*, and *HTR2C*) and in other genes previously associated with treatment efficacy and/or induced side effects (*ANKK1*, *BDNF*, *COMT*, and *HTR1A*) were investigated in 176 children and adolescents diagnosed with ASD and undergoing pharmacological treatment.



© The Author(s) 2021. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



Results: A *SLC6A3* genetic variant was associated with response to methylphenidate in our ASD cohort, whereas *HTR2A* and *HTR2C* allele and haplotype distributions were associated with adverse reactions such as somnolence, mood alterations, and BMI. *ANKK1*, *COMT*, and *BDNF* genetic variants were mainly associated with treatment side effects.

Conclusion: If confirmed, these genetic variants may be used as predictors of clinical outcome and help to personalise pharmacological treatments in patients with ASD.

Keywords: Autism, pharmacogenetics, methylphenidate, antipsychotic, antidepressant, dopamine, serotonin

INTRODUCTION

Autistic spectrum disorders (ASD) are severe neurodevelopmental alterations characterised by deficits in social communications and repetitive and restricted behaviours. Although there is no specific pharmacological treatment for ASD, about a third of patients receive pharmacological treatment for comorbid symptoms. Stimulant, antipsychotic, and antidepressant drugs are used for the treatment of conduct, anxiety, and mood disorders observed in patients with ASD. Pharmacotherapy with methylphenidate is the preferred treatment for attention deficit hyperactivity disorder (ADHD) comorbid symptoms, as well as antipsychotics and selective serotonin reuptake inhibitors antidepressants for the treatment of aggression and mood disorders. However, there is significant individual variability in the response to pharmacological treatment. Not all ASD subjects respond to treatment, with 30%-50% not responding and/or presenting with severe and long-lasting side effects, including increased irritability, aggressiveness, and somnolence^[1]. Furthermore, children and adolescents are more susceptible to drug-induced side effects than adults^[2]. Treatment failure and side effects have a negative effect on patients with ASD, and predictors of response for the personalisation of pharmacological treatment are required.

There is strong evidence of the influence of genetic factors on the clinical outcome of pharmacological treatments. Previous studies have reported the influence of variants in genes coding for targets of psychotropic drugs on the efficacy and safety of pharmacological treatments. The dopaminergic and serotonergic systems, both major targets for psychotropic drugs, have been implicated in the modulation of treatment outcome^[1,3,4]. Enzymes involved in the metabolism of catecholamines and proteins involved in stress and mood alterations have also been implicated in the modulation of treatment response^[3,5,6]. However, relatively few pharmacogenetic studies have been performed on drug treated ASD subjects.

The clinical outcome of psychotropic drugs varies between children and adults^[7]. Most studies have focused on the adult population, with relatively few studies in children and adolescents. Several studies have associated genetic variants in the gene coding for the dopamine transporter (*SLC6A3*), a direct target of methylphenidate in children and adolescents affected by ADHD^[7,8]. Variants in genes coding for dopaminergic receptors type 2, 3, and 4 (*DRD2*, *DRDRD3*, and *DRD4*, respectively) have also been associated with response to methylphenidate in young ADHD subjects^[6,9,10]. Findings of association between polymorphisms in genes coding for the adrenergic receptor 2A (*ADRA2A*), brain derived neurotrophic factor (*BDNF*), catechol-O-methyltransferase (*COMT*), serotonin receptor 2A (*HTR2A*), serotonin transporter (*SLC6A4*), norepinephrine transporter (*SLC6A2*), and methylphenidate clinical outcome in young ADHD subjects have been reported in independent studies^[7,11,12]. However, these findings have not been universally replicated or showed inconsistent results^[7]. A meta-analysis by Bonvicini *et al.*^[13] did not support an association of a polymorphism in the 3'-untranslated region (UTR) in the dopamine transporter (*SLC6A3*) with response to methylphenidate. Significant associations between variants in dopamine receptors 1, 3, and 4 (*DRD1*, *DRD3*, and *DRD4*), *ADRA2A*, *COMT*, *SLC6A3*, and *SLCA4* genes and response

to methylphenidate have been detected in a sample of 64 children with ASD^[14]. A study by Correia *et al.*^[15] described the influence of genetic variants in the multidrug resistance 1 (*MDR1* or *ABCB1*) gene on clinical improvement with risperidone therapy in $N = 45$ ASD patients. Furthermore, associations between treatment response and polymorphisms in *BDNF*, *HTR2A*, serotonin receptor 2C (*HTR2C*), serotonin receptor 6 (*HTR6*), and cytochrome P450 2D6 (*CYP2D6*) genes were reported in the same study. However, these findings were not conclusive. Considering the limited number of pharmacogenetic studies in ASD and the moderate sample sizes, further investigation is required to identify predictors of response that could improve the efficacy and safety of pharmacological treatments in this population group.

The aim of our study was to identify genetic predictors of drug response in a population group who are particularly susceptible to adverse reactions. This information may help to improve the efficacy and safety of pharmacological treatments in children and adolescents with ASD.

METHODS

Study samples

A total of $N = 176$ children (86% boys and 14% girls, average age = 11.77 ± 4.64 SD) diagnosed with ASD according to DSM-5 criteria and undergoing pharmacological treatment ($N = 146$ with methylphenidate and $N = 30$ with antipsychotic, antidepressant, anxiolytics, and mood stabilizers) for at least 8 weeks were included in the study. Treatment response was assessed using the Aberrant Behaviour checklist, (ABC-CV, Aman *et al.*, 1985), Autism Treatment Evaluation Checklist (Rimland & Edelson, 1999), Clinical Global Impression-Severity (CGI-S) for autism symptoms, Conners Rating Scale-Revised for parents and teachers for the assessment of ADHD symptoms (Conners, 1997), Child Behaviour Check list for parents, and Teacher's Report Form for teachers to assess general child psychopathology symptoms. Response to pharmacological treatment was assessed retrospectively from the parents' CGI categorical scores (0 = poor response, 1 = some response, 2 = good response, 3 = very good response). Global side effects were assessed with a score between 0 and 3 (0 = no side effects, 1 = mild side effects lasting less than two weeks, 2 = moderate side effects lasting more than 2 weeks, 3 = bad side effects with long lasting side effects of more than a month of duration or intolerable side effects resulting in suppression of medication). Specific information on the presence or absence of aggression, shutdowns, irritability, mood alterations, and somnolence were obtained via parents' interviews. This sample has a statistical power $\geq 85\%$ to detect moderate effect sizes ($f \geq 0.25$, $\alpha = 0.05$). This project was approved by the Ethics committee of the Hospital Universitari Mutua Terrassa. Informed consent was obtained from all participants or their legal carers prior to introduction in the study.

Genetic characterisation

Selected candidates included genes coding for dopaminergic and serotonergic drug targets (*SLC6A3*, *DRD2*, *DRD3*, *DRD4*, *HTR2A*, and *HTR2C*) and other genes previously associated with treatment efficacy and/or induced side effects (*ANKK1*, *BDNF*, *COMT*, and *HTR1A*). DNA was extracted from whole blood samples using a commercial kit (EZNA SQ Blood DNA Kit II, Omega Bio-Tech, USA) and following manufacturers' instructions. Sixteen single nucleotide polymorphisms (SNPs) and variable number tandem repeats (VNTRs) within the 10 selected genes were genotyped using iPlex® Gold chemistry and the MassARRAY platform (CEGEN-PRB2-ISCI, University of Santiago de Compostela, Spain) for the SNPs and agarose gel genotyping methods for the VNTRs. Table 1 contains a complete list of the genotyped polymorphisms. Polymorphisms were selected based on previously reported associations with response to pharmacological treatment.

Table 1. Summary of statistical analyses in study sample. Regression coefficient and P value (within brackets) provided

Gene	Polymorphism	Response	Side effects	Aggress	Shutd	Irritab	Mood	Somnol	BMI
<i>ANKK1</i>	rs1800497	0.51 (0.61)	2.18 (0.03)	-1.72 (0.08)	1.21 (0.23)	-0.19 (0.85)	-1.22 (0.22)	-0.28 (0.77)	0.62 (0.54)
<i>BDNF</i>	rs6265	0.46 (0.65)	-2.16 (0.03)	1.53 (0.13)	0.91 (0.36)	1.32 (0.19)	0.58 (0.56)	2.13 (0.03)	-1.39 (0.17)
<i>COMT</i>	rs4680	0.37 (0.72)	-2.29 (0.02)	0.06 (0.95)	0.56 (0.58)	-1.34 (0.18)	-1.76 (0.07)	0.71 (0.48)	-2.05 (0.04)
<i>DAT1</i>	3'UTR-VNTR	1.82 (0.07)	-0.51 (0.61)	0.30 (0.76)	-0.85 (0.39)	-0.59 (0.55)	0.01 (0.99)	2.29 (0.02)	-1.41 (0.16)
<i>DRD2</i>	rs1801028	0.65 (0.51)	-0.66 (0.51)	-1.33 (0.18)	-1.27 (0.20)	-0.57 (0.57)	-0.79 (0.43)	-1.36 (0.18)	-0.77 (0.44)
<i>DRD3</i>	rs167771	0.44 (0.66)	-0.66 (0.51)	0.80 (0.42)	-0.06 (0.95)	-0.50 (0.62)	0.68 (0.49)	-0.32 (0.75)	-0.99 (0.32)
	rs6280	0.28 (0.78)	-0.84 (0.41)	-0.26 (0.79)	0.51 (0.61)	0.69 (0.50)	-0.84 (0.40)	-1.53 (0.12)	-1.11 (0.27)
<i>DRD4</i>	48bp VNTR	-0.01 (0.99)	0.67 (0.50)	-0.83 (0.41)	0.98 (0.33)	-0.19 (0.85)	-0.33 (0.74)	-1.00 (0.31)	0.55 (0.58)
<i>HTR1A</i>	rs6295	0.55 (0.58)	0.63 (0.53)	-0.10 (0.92)	0.58 (0.56)	0.04 (0.97)	1.10 (0.27)	0.65 (0.52)	0.10 (0.92)
	rs878567	0.35 (0.73)	0.41 (0.68)	-0.04 (0.97)	0.62 (0.53)	-0.21 (0.84)	1.21 (0.22)	0.74 (0.46)	0.22 (0.83)
<i>HTR2A</i>	rs6311	-0.74 (0.46)	-0.15 (0.88)	0.55 (0.58)	1.47 (0.14)	0.13 (0.89)	0.86 (0.39)	1.71 (0.09)	0.42 (0.67)
	rs6313	-0.35 (0.72)	-0.18 (0.85)	0.61 (0.54)	1.10 (0.28)	-0.13 (0.89)	0.95 (0.34)	1.55 (0.12)	0.38 (0.70)
	rs6314	0.07 (0.94)	1.16 (0.25)	-0.65 (0.51)	-0.95 (0.34)	0.15 (0.88)	-2.17 (0.03)	-1.73 (0.08)	-1.96 (0.05)
<i>HTR2C</i>	rs1414334	-1.41 (0.16)	0.03 (0.97)	0.005 (0.99)	0.13 (0.89)	1.05 (0.29)	0.75 (0.45)	1.00 (0.13)	1.85 (0.07)
	rs3813929	0.16 (0.86)	0.77 (0.44)	-0.36 (0.71)	-0.63 (0.53)	-0.37 (0.71)	-0.78 (0.44)	-0.55 (0.58)	-0.08 (0.94)
	rs6318	0.65 (0.52)	0.20 (0.84)	-0.002 (0.99)	-0.002 (0.99)	0.004 (0.99)	0.002 (0.99)	0.04 (0.97)	0.51 (0.61)

Statistical analyses

Multivariate analyses, including gender, age, drug type, and dose as covariables, were conducted for each single polymorphism analysed. Haplotype analyses were also conducted within those genes with more than one polymorphism genotyped. Separate analyses were also conducted for the subgroup of patients treated with methylphenidate. Statistical analyses were performed using the statistical package PLINK (version 1.07.2)^[16].

RESULTS

All SNPs and individuals investigated showed genotyping success rates over 95%. Additionally, all genotyped SNPs were in Hardy-Weinberg equilibrium and were included in the analyses. [Table 1](#) summarises the results of the multivariate analyses in the study sample. Single marker analyses including gender, age, drug type (i.e., methylphenidate, antipsychotics, antidepressants, or others), and dose as covariates did not reveal any significant association with the level of response to pharmacological treatment in patients with ASD. The *ANKK1* rs1800497 polymorphism was associated with presence of side effects ($P = 0.03$) as were SNPs in *BDNF* (rs6265, $P = 0.03$) and *COMT* (rs4680, $P = 0.02$). Analyses of specific side effects revealed association between the *HTR2A* rs6314 polymorphism and mood alterations ($P = 0.03$). The level of somnolence was associated with *BDNF* (rs6265, $P = 0.03$) and *SLC6A3* (3' UTR VNTR, $P = 0.02$) variants. Finally, *COMT* and *HTR2A* variants (rs4680 and rs6314, respectively) were nominally associated with BMI ($P = 0.04$ and $P = 0.05$, respectively). Haplotype analyses (data facilitated on request) revealed association between *HTR2A* allelic combinations and mood alterations, presence of somnolence, and BMI ($P = 0.02$, $P = 0.01$, and $P = 0.04$, respectively). A *HTR2C* haplotype was significantly associated with BMI ($P = 0.005$). Finally, a *DRD3* haplotype was nominally associated with the presence of shutdowns ($P = 0.04$). No other statistically significant associations were observed.

[Table 2](#) summarises the results in the subgroup of ASD subjects treated with methylphenidate. The 3' UTR VNTR variant in *SLC6A3* was associated with response to methylphenidate ($P = 0.03$) and the *BDNF* rs6265 polymorphisms was associated with the presence of side effects ($P = 0.03$). No other single marker association was detected. Haplotype analyses within this subgroup revealed association between *HTR2C*

Table 2. Summary of statistical analyses in group of ASD subjects treated with methylphenidate. Regression coefficient and P value (within brackets) provided

Gene	Polymorphism	Response	Side effects	Aggress	Shutd	Irritab	Mood	Somnol	BMI
<i>ANKK1</i>	rs1800497	0.67 (0.50)	1.83 (0.07)	-0.93 (0.35)	1.29 (0.20)	0.33 (0.74)	-1.46 (0.14)	NA	0.52 (0.61)
<i>BDNF</i>	rs6265	-0.01 (0.99)	-2.25 (0.03)	1.51 (0.13)	1.14 (0.25)	1.48 (0.14)	1.42 (0.15)	NA	-0.78 (0.43)
<i>COMT</i>	rs4680	1.53 (0.13)	1.42 (0.16)	-1.65 (0.10)	-1.90 (0.06)	-0.48 (0.63)	-0.52 (0.61)	NA	-0.05 (0.96)
<i>DAT1</i>	3'UTR-VNTR	2.21 (0.03)	-0.24 (0.81)	0.71 (0.48)	-1.10 (0.27)	-0.35 (0.73)	0.33 (0.74)	NA	-1.52 (0.13)
<i>DRD2</i>	rs1801028	0.66 (0.51)	-0.68 (0.50)	-1.41 (0.16)	-1.24 (0.22)	-0.51 (0.61)	-0.81 (0.42)	NA	-0.77 (0.44)
<i>DRD3</i>	rs167771	0.51 (0.61)	-1.00 (0.32)	0.41 (0.68)	0.53 (0.60)	-0.66 (.51)	0.71 (0.48)	NA	0.74 (0.46)
	rs6280	0.20 (0.84)	-1.15 (0.25)	-0.71 (0.48)	0.22 (0.83)	-1.02 (0.30)	-0.46 (0.64)	NA	-0.33 (0.74)
<i>DRD4</i>	48bp VNTR	0.06 (0.95)	0.46 (0.65)	-0.42 (0.67)	0.84 (0.40)	-0.05 (0.96)	0.13 (0.89)	NA	0.77 (0.45)
<i>HTR1A</i>	rs6295	0.76 (0.45)	1.43 (0.16)	0.53 (0.60)	0.29 (0.77)	0.44 (0.66)	0.86 (0.39)	NA	-0.12 (0.91)
	rs878567	0.54 (0.59)	1.21 (0.23)	0.58 (0.56)	0.33 (0.74)	0.17 (0.86)	0.98 (0.33)	NA	0.002 (0.99)
<i>HTR2A</i>	rs6311	-1.22 (0.22)	0.38 (0.71)	1.06 (0.29)	0.65 (0.51)	0.21 (0.84)	0.49 (0.62)	NA	0.06 (0.96)
	rs6313	-0.83 (0.41)	0.29 (0.77)	1.19 (0.23)	0.20 (0.84)	-0.04 (.97)	0.56 (0.58)	NA	0.01 (0.99)
	rs6314	0.14 (0.89)	0.83 (0.41)	-0.57 (0.57)	-0.80 (0.42)	0.18 (0.85)	-1.91 (0.06)	NA	-1.57 (0.12)
<i>HTR2C</i>	rs1414334	-1.90 (0.06)	-0.40 (0.69)	0.004 (0.99)	0.21 (0.84)	0.60 (0.55)	0.84 (0.40)	NA	1.79 (0.08)
	rs3813929	0.35 (0.73)	0.47 (0.64)	-0.64 (0.52)	-0.64 (0.52)	-0.65 (0.52)	-0.93 (0.35)	NA	-0.12 (0.90)
	rs6318	0.97 (0.34)	-0.23 (0.82)	-0.002 (0.99)	0.002 (0.99)	0.001 (0.99)	0.002 (0.99)	NA	0.61 (0.55)

NA: Not available.

allelic combinations and response to methylphenidate treatment ($P = 0.02$) and BMI ($P = 0.02$). Association was also observed between a *HTR2A* haplotype and mood alterations ($P = 0.04$). Finally, a *DRD3* allelic combination was associated with presence of side effects ($P = 0.05$).

DISCUSSION

We aimed to identify genetic predictors of response to pharmacological treatment by investigating 16 SNPs and VNTRs within 10 candidate genes and their influence on clinical outcome in a cohort of $N = 176$ children and adolescents with ASD. Several significant associations were observed that may help to identify patients with ASD likely to show poor response and/or develop side effects.

Previous evidence indicates that variants in dopaminergic genes are associated with emotional dysregulation and ADHD symptoms in patients with ASD^[17] and with methylphenidate response in children with ADHD^[6,9,18-21], although these findings have not been universally replicated^[22-24]. We investigated polymorphisms in several dopaminergic genes including *SLC6A3*, *DRD2* (and the *ANKK1* Taq I), *DRD3*, and *DRD4*, and their possible relation to symptom improvement after pharmacological treatment in ASD children.

We did not find association between the *SLC6A3* 3' UTR VNTR variant investigated and treatment response in the study sample that included subjects treated with a variety of psychotropics. Nevertheless, the *SLC6A3* 3' UTR VNTR variant was associated with somnolence ($P = 0.02$) in the total cohort and with response in the subgroup of methylphenidate treated patients. Interestingly, previous studies have reported association between this variant and response to methylphenidate in children with ADHD^[14,20]. The dopamine transporter is a direct target of methylphenidate, a drug widely used in the ASD population for the treatment of ADHD co-morbid symptoms. Although suggestive, these results require further investigation. An association was observed between the *ANKK1* rs1800497 polymorphism (alternative nomenclature: *DRD2* Taq I) and presence of side effects in the study cohort ($P = 0.03$). Interestingly, a

previous study reported this polymorphism associated with insulin-resistance in patients with ASD treated with risperidone^[25]. We did not find any significant association with the other dopaminergic variants investigated (*DRD2* rs18012028, *DRD3* rs167771 & rs6280, and a 48bp repeat in *DRD4*), although *DRD3* haplotype combinations were found nominally associated with shutdowns in the total sample ($P = 0.04$) and with side effects in the methylphenidate subgroup ($P = 0.05$). Previous studies reported association between the *DRD3* rs6280 polymorphism and methylphenidate response in a group of 64 children with ASD^[14] and risperidone response in a sample of 45 patients with ASD^[15]. These findings require further investigation in larger samples to confirm the possible contribution of *DRD3* variants to treatment response variability in ASD.

Abnormalities in the serotonergic system have been implicated in several psychiatric disorders. A significant reduction of serotonin type 1A and 2A (5-HT_{1A} and 5-HT_{2A}) receptor binding densities was observed in brain regions of patients with ASD^[26]. *HTR1A* variants, including rs878567, have been associated with ADHD risk^[27]. *HTR2A* polymorphisms have also been associated with depression, gastrointestinal disorders, and risk in patients with ASD^[28-32]. Several studies have associated *HTR2A* and *HTR2C* polymorphisms with response to antipsychotic and antidepressant drugs as well as weight gain or increased BMI during antipsychotic treatment^[3,33,34]. *HTR1A* variants have also been shown to associate with antipsychotic response^[35] but not with antidepressant outcomes^[36]. In our study, we found significant associations between the *HTR2A* rs6314 (His452Tyr) polymorphism and BMI and mood alterations. Carriers of the Tyr452 variant, with reduced functionality^[37], were more likely to experience mood alterations and somnolence during treatment but showed less BMI. Haplotype analyses of *HTR2A* allele combinations showed significant findings with mood alterations ($P = 0.02$), somnolence ($P = 0.01$), and BMI ($P = 0.04$) in the total cohort and with mood alterations in the methylphenidate subgroup ($P = 0.04$). These findings seem to agree with previous studies that linked *HTR2A* variants with BMI during pharmacological interventions^[33,38] and with major depression^[39].

The serotonin 2C (5-HT_{2C}) receptor modulates eating behaviour and has been reported to influence antipsychotic-induced weight gain and BMI^[3,16,40]. Although we did not observe single marker associations, we found significant associations between *HTR2C* haplotype combinations and BMI in the study sample ($P = 0.005$) and between overall response ($P = 0.02$), as well as between BMI ($P = 0.01$) in the subgroup of methylphenidate patients. Previous studies had also reported association between *HTR2C* genetic variants and response to psychotropic treatments^[3]. Finally, we did not find any significant association between the two *HTR1A* polymorphisms genotyped, rs6295 and rs878567, and the phenotypes investigated.

BDNF is a protein that modulates stress and mood alterations and several studies link BDNF altered levels with ASD^[41]. It has been reported that methylphenidate treatment increases BDNF serum levels in children with ADHD^[20,42]. *BDNF* genetic variants may contribute to ASD pathogenesis^[43] and methylphenidate response in children with ASD^[44]. Our own results showed an association between the *BDNF* rs6265 variant and presence of side effects ($P = 0.03$ for both the study cohort and the methylphenidate subgroup) during pharmacological treatment in children with ASD. Additionally, patients carrying the Met66 allele showed higher levels of somnolence ($P = 0.03$ in total cohort). However, we were not able to find association between the rs6265 Val66 allele and response to methylphenidate ($P = 0.26$) as previously reported in Korean children with ADHD^[44]. Reports of association between the rs6265 polymorphism and aggression in patients with schizophrenia were not confirmed by us and other investigators^[45]. Correia *et al.*^[15] found association between the Met66 allele and higher prolactin levels during risperidone treatment of children in ASD, although no direct association with risperidone response was detected. Insulin resistance during risperidone treatment was associated with this polymorphism in adolescents with ASD^[25]. These results,

taken together, suggest that genetic variation in *BDNF* contributes to adverse reactions rather than to the efficacy of pharmacological treatment in ASD subjects. However, the possible role of these genetic variants on *BDNF* plasma levels and their contribution to treatment side effects need further investigation.

COMT is one of the main enzymes involved in the degradation of catecholamines including dopamine, epinephrine, and norepinephrine, whose pathways are targeted by methylphenidate and other psychotropics. The *COMT* rs4680(Val158Met) polymorphism has been associated with methylphenidate response in children and adolescents with ADHD^[19,48-50] and children with ASD^[14]. Furthermore, the level of irritability was predicted by *COMT* variants in children with ADHD treated with methylphenidate^[48]. The *COMT* rs4680 variant was not associated with treatment response in our sample, but was marginally associated with presence of side effects, with rs4680-G/G (Val/Val) individuals presenting more lasting side effects ($P = 0.02$) and BMI ($P = 0.04$) in the study cohort.

In summary, we observed several associations between the candidate genes analysed and clinical outcome in patients with ASD treated with a variety of psychotropics. A *SLC6A3* genetic variant predicted response to methylphenidate in our ASD cohort, whereas *HTR2A* and *HTR2C* allele and haplotype distributions were mainly associated with adverse reactions such as somnolence, mood alterations, and BMI. *ANKK1*, *COMT*, and *BDNF* genetic variants were mainly associated with treatment side effects. These associations resembled those observed in other pathologies, suggesting a similar mechanism of action in ASD and/or confirming the common origin of the symptoms treated.

Our study has several limitations. None of the findings reported in this study survived multiple analyses corrections, considering the number of polymorphisms and phenotypes analysed. Our findings require confirmation in independent studies. The study sample size is moderate, which may have affected the statistical significance of the findings and produced false positives or negatives. However, it is one of the largest cohorts collated for ASD pharmacogenetic studies. Furthermore, most of our findings coincide with the pharmacogenetic results observed in other pathologies, suggesting they are true findings. Another limitation is that we did not investigate functional variants in drug metabolising hepatic enzymes. Although there is extensive evidence on the influence on functional variants in cytochrome P450 (CYP) metabolic enzymes on treatment response, the main drug used in our study cohort, methylphenidate, is metabolised mainly by *CES1*. Inconsistent results on the genetic influence of *CES1* variants on treatment response have been reported^[51,52]. However, reports of associations between genetic variants in CYP enzymes and response to psychotropic treatment in children with schizophrenia or ASD merit further investigation in independent studies^[2,53].

In conclusion, our study showed that genetic variation in dopamine (*SLC6A3*) and serotonin (*HTR2A* and *HTR2C*) may influence response to psychotropic treatment in patients with ASD and side effects, whereas *ANKK1*, *COMT*, and *BDNF* polymorphisms may contribute to adverse reactions. Associations between the *SLC6A3* and methylphenidate response have been reported in other pathologies and may constitute a useful biomarker for the selection of adequate treatment. The genetic associations with adverse reactions may help to predict or prevent the development of side effects, although their value to discriminate between treatments is unclear. Nevertheless, if confirmed these genetic variants may be used as predictors of clinical outcome and help to personalise pharmacological treatments in patients with ASD.

DECLARATIONS

Authors' contributions

Study design, sample recruitment, data analyses, results interpretation, and paper writing: Hervas A

Genotyping, data analyses, and results interpretation: Serra-Llovich A, Cárcel M, Amasi-Hartoonian N, Hernandez M

Sample recruitment and results interpretation: Rueda I, Targa I, Guijarro S, Bigorra A, Cancino M, Bote V

Study design, data analyses, results interpretation, and paper writing: Arranz MJ

Availability of data and materials

Clinical and genotyping data were collected by our team and can be provided on request.

Financial support and sponsorship

Fondos Investigación Sanitaria, Instituto de Salud Carlos III, PI11/0260.

Conflicts of interest

Dr. Amaia Hervas has consulted for Exeltis and given seminars sponsored by Shire. Dr. Hervas participates in several clinical trials. None of these activities have influenced the present study. No other interests were declared by the rest of the co-authors.

Ethical approval and consent to participate

This study complies with the Declaration of Helsinki and has been approved by our Hospital Ethics Committee, as mentioned in the methods section.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2021.

REFERENCES

1. Brown JT, Eum S, Cook EH, Bishop JR. Pharmacogenomics of autism spectrum disorder. *Pharmacogenomics* 2017;18:403-14. [DOI](#) [PubMed](#)
2. Dodsworth T, Kim DD, Procyshyn RM, Ross CJ, Honer WG, Barr AM. A systematic review of the effects of CYP2D6 phenotypes on risperidone treatment in children and adolescents. *Child Adolesc Psychiatry Ment Health* 2018;12:37. [DOI](#) [PubMed](#) [PMC](#)
3. Arranz MJ, Blanco JP, Samperiz BA. Pharmacogenetics of the efficacy of antipsychotic drugs in schizophrenia. In: Rybakowski JK, Serretti A, editors. Genetic influences on response to drug treatment for major psychiatric disorders. Cham: Springer International Publishing; 2016. p. 1-20. [DOI](#)
4. Fabbri C, Serretti A. Pharmacogenetics of the efficacy and side effects of antidepressant drugs. In: Rybakowski JK, Serretti A, editors. Genetic influences on response to drug treatment for major psychiatric disorders. Cham: Springer International Publishing; 2016. p. 39-54. [DOI](#)
5. Fabbri C, Hosak L, Mössner R, et al. Consensus paper of the WFSBP Task Force on Genetics: Genetics, epigenetics and gene expression markers of major depressive disorder and antidepressant response. *World J Biol Psychiatry* 2017;18:5-28. [DOI](#) [PubMed](#)
6. Pagerols M, Richarte V, Sánchez-Mora C, et al. Pharmacogenetics of methylphenidate response and tolerability in attention-deficit/hyperactivity disorder. *Pharmacogenomics J* 2017;17:98-104. [DOI](#) [PubMed](#)
7. Joensen B, Meyer M, Aagaard L. Specific genes associated with adverse events of methylphenidate use in the pediatric population: a systematic literature review. *J Res Pharm Pract* 2017;6:65-72. [DOI](#) [PubMed](#) [PMC](#)
8. Purper-Ouakil D. [Use of psychotropic drugs in children]. *Arch Pediatr* 2008;15:1834-6. [DOI](#) [PubMed](#)
9. Gomez-Sanchez CI, Carballo JJ, Riveiro-Alvarez R, et al. Pharmacogenetics of methylphenidate in childhood attention-deficit/hyperactivity disorder: long-term effects. *Sci Rep* 2017;7:10391. [DOI](#) [PubMed](#) [PMC](#)
10. Naumova D, Grizenko N, Sengupta SM, Joobar R. DRD4 exon 3 genotype and ADHD: Randomised pharmacodynamic investigation of treatment response to methylphenidate. *World J Biol Psychiatry* 2019;20:486-95. [DOI](#) [PubMed](#)
11. Myer NM, Boland JR, Faraone SV. Pharmacogenetics predictors of methylphenidate efficacy in childhood ADHD. *Mol Psychiatry* 2018;23:1929-36. [DOI](#) [PubMed](#) [PMC](#)
12. Fageera W, Chaumette B, Fortier MÉ, et al. Association between COMT methylation and response to treatment in children with ADHD. *J Psychiatr Res* 2021;135:86-93. [DOI](#) [PubMed](#)
13. Bonvicini C, Faraone SV, Scassellati C. Attention-deficit hyperactivity disorder in adults: A systematic review and meta-analysis of genetic, pharmacogenetic and biochemical studies. *Mol Psychiatry* 2016;21:872-84. [DOI](#) [PubMed](#) [PMC](#)
14. McCracken JT, Badashova KK, Posey DJ, et al. Positive effects of methylphenidate on hyperactivity are moderated by monoaminergic

- gene variants in children with autism spectrum disorders. *Pharmacogenomics J* 2014;14:295-302. DOI PubMed PMC
15. Correia CT, Almeida JP, Santos PE, et al. Pharmacogenetics of risperidone therapy in autism: association analysis of eight candidate genes with drug efficacy and adverse drug reactions. *Pharmacogenomics J* 2010;10:418-30. DOI PubMed
 16. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007;81:559-75. DOI PubMed PMC
 17. Gadow KD, Pinsonneault JK, Perlman G, Sadee W. Association of dopamine gene variants, emotion dysregulation and ADHD in autism spectrum disorder. *Res Dev Disabil* 2014;35:1658-65. DOI PubMed PMC
 18. Froehlich TE, Epstein JN, Nick TG, et al. Pharmacogenetic predictors of methylphenidate dose-response in attention-deficit/hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry* 2011;50:1129-1139.e2. DOI PubMed PMC
 19. Akay AP, Resmi H, Güney SA, et al. Serum brain-derived neurotrophic factor levels in treatment-naïve boys with attention-deficit/hyperactivity disorder treated with methylphenidate: an 8-week, observational pretest-posttest study. *Eur Child Adolesc Psychiatry* 2018;27:127-35. DOI PubMed
 20. Winsberg BG, Comings DE. Association of the dopamine transporter gene (DAT1) with poor methylphenidate response. *J Am Acad Child Adolesc Psychiatry* 1999;38:1474-7. DOI PubMed
 21. Nuntamool N, Ngamsamut N, Vanwong N, et al. Pharmacogenomics and efficacy of risperidone long-term treatment in Thai autistic children and adolescents. *Basic Clin Pharmacol Toxicol* 2017;121:316-24. DOI PubMed
 22. Tharoor H, Lobos EA, Todd RD, Reiersen AM. Association of dopamine, serotonin, and nicotinic gene polymorphisms with methylphenidate response in ADHD. *Am J Med Genet B Neuropsychiatr Genet* 2008;147B:527-30. DOI PubMed
 23. Ji HS, Paik KC, Park WS, Lim MH. No association between the response to methylphenidate and DRD4 gene polymorphism in Korean attention deficit hyperactivity disorder: a case control study. *Clin Psychopharmacol Neurosci* 2013;11:13-7. DOI PubMed PMC
 24. Zeni CP, Guimarães AP, Polanczyk GV, et al. No significant association between response to methylphenidate and genes of the dopaminergic and serotonergic systems in a sample of Brazilian children with attention-deficit/hyperactivity disorder. *Am J Med Genet B Neuropsychiatr Genet* 2007;144B:391-4. DOI PubMed
 25. Sukasem C, Vanwong N, Srisawasdi P, et al. Pharmacogenetics of risperidone-induced insulin resistance in children and adolescents with autism spectrum disorder. *Basic Clin Pharmacol Toxicol* 2018;123:42-50. DOI PubMed
 26. Oblak A, Gibbs TT, Blatt GJ. Reduced serotonin receptor subtypes in a limbic and a neocortical region in autism. *Autism Res* 2013;6:571-83. DOI PubMed PMC
 27. Park YH, Lee KK, Kwon HJ, et al. Association between HTR1A gene polymorphisms and attention deficit hyperactivity disorder in Korean children. *Genet Test Mol Biomarkers* 2013;17:178-82. DOI PubMed
 28. Abdelrahman HM, Sherief LM, Alghobashy AA, et al. Association of 5-HT2A receptor gene polymorphisms with gastrointestinal disorders in Egyptian children with autistic disorder. *Res Dev Disabil* 2015;36C:485-90. DOI PubMed
 29. Smith RM, Banks W, Hansen E, Sadee W, Herman GE. Family-based clinical associations and functional characterization of the serotonin 2A receptor gene (HTR2A) in autism spectrum disorder. *Autism Res* 2014;7:459-67. DOI PubMed PMC
 30. Hervás A, Toma C, Romarís P, et al. The involvement of serotonin polymorphisms in autistic spectrum symptomatology. *Psychiatr Genet* 2014;24:158-63. DOI PubMed
 31. Gadow KD, Smith RM, Pinsonneault JK. Serotonin 2A receptor gene (HTR2A) regulatory variants: possible association with severity of depression symptoms in children with autism spectrum disorder. *Cogn Behav Neurol* 2014;27:107-16. DOI PubMed
 32. Valencia AV, Páez AL, Sampredo ME, et al. [Evidence for association and epistasis between the genetic markers SLC6A4 and HTR2A in autism etiology]. *Biomedica* 2012;32:585-601. DOI PubMed
 33. Al-Janabi I, Arranz MJ, Blakemore AI, et al. Association study of serotonergic gene variants with antipsychotic-induced adverse reactions. *Psychiatr Genet* 2009;19:305-11. DOI PubMed
 34. Arranz MJ, Salazar J, Hernández MH. Pharmacogenetics of antipsychotics: clinical utility and implementation. *Behav Brain Res* 2021;401:113058. DOI PubMed
 35. Takekita Y, Fabbri C, Kato M, et al. HTR1A polymorphisms and clinical efficacy of antipsychotic drug treatment in schizophrenia: a meta-analysis. *Int J Neuropsychopharmacol* 2016;19:pyv125. DOI PubMed PMC
 36. Zhao X, Huang Y, Li J, et al. Association between the 5-HT1A receptor gene polymorphism (rs6295) and antidepressants: a meta-analysis. *Int Clin Psychopharmacol* 2012;27:314-20. DOI PubMed
 37. Hazelwood LA, Sanders-Bush E. His452Tyr polymorphism in the human 5-HT2A receptor destabilizes the signaling conformation. *Mol Pharmacol*. 2004;66:1293-300. PubMed
 38. Li P, Tiwari HK, Lin WY, et al. Genetic association analysis of 30 genes related to obesity in a European American population. *Int J Obes (Lond)* 2014;38:724-9. DOI PubMed PMC
 39. Zhao X, Sun L, Sun YH, et al. Association of HTR2A T102C and A-1438G polymorphisms with susceptibility to major depressive disorder: a meta-analysis. *Neurol Sci* 2014;35:1857-66. DOI PubMed
 40. Reynolds GP, Hill MJ, Kirk SL. The 5-HT2C receptor and antipsychotic-induced weight gain - mechanisms and genetics. *J Psychopharmacol* 2006;20:15-8. DOI PubMed
 41. Saghazadeh A, Rezaei N. Brain-derived neurotrophic factor levels in autism: a systematic review and meta-analysis. *J Autism Dev Disord* 2017;47:1018-29. DOI PubMed
 42. Amiri A, Torabi Parizi G, Kousha M, et al. Changes in plasma brain-derived neurotrophic factor (BDNF) levels induced by methylphenidate in children with Attention deficit-hyperactivity disorder (ADHD). *Prog Neuropsychopharmacol Biol Psychiatry* 2013;47:20-4. DOI PubMed
 43. Yoo HJ, Yang SY, Cho IH, Park M, Kim SA. Polymorphisms of BDNF gene and autism spectrum disorders: family based association

- study with korean trios. *Psychiatry Investig* 2014;11:319-24. [DOI](#) [PubMed](#) [PMC](#)
44. Kim BN, Cummins TD, Kim JW, et al. Val/Val genotype of brain-derived neurotrophic factor (BDNF) Val$\square\square$Met polymorphism is associated with a better response to OROS-MPH in Korean ADHD children. *Int J Neuropsychopharmacol* 2011;14:1399-410. [DOI](#) [PubMed](#)
 45. Manchia M, Fanos V. Targeting aggression in severe mental illness: The predictive role of genetic, epigenetic, and metabolomic markers. *Prog Neuropsychopharmacol Biol Psychiatry* 2017;77:32-41. [DOI](#) [PubMed](#)
 46. Park S, Kim JW, Kim BN, Shin MS, Yoo HJ, Cho SC. Catechol-O-methyltransferase Val158-Met polymorphism and a response of hyperactive-impulsive symptoms to methylphenidate: a replication study from South Korea. *J Psychopharmacol* 2014;28:671-6. [DOI](#) [PubMed](#)
 47. Salatino-Oliveira A, Genro JP, Zeni C, et al. Catechol-O-methyltransferase valine158methionine polymorphism moderates methylphenidate effects on oppositional symptoms in boys with attention-deficit/hyperactivity disorder. *Biol Psychiatry* 2011;70:216-21. [DOI](#) [PubMed](#)
 48. McGough JJ, McCracken JT, Loo SK, et al. A candidate gene analysis of methylphenidate response in attention-deficit/hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry* 2009;48:1155-64. [DOI](#) [PubMed](#) [PMC](#)
 49. Kereszturi E, Tarnok Z, Bognar E, et al. Catechol-O-methyltransferase Val158Met polymorphism is associated with methylphenidate response in ADHD children. *Am J Med Genet B Neuropsychiatr Genet* 2008;147B:1431-5. [DOI](#) [PubMed](#)
 50. Cheon KA, Jun JY, Cho DY. Association of the catechol-O-methyltransferase polymorphism with methylphenidate response in a classroom setting in children with attention-deficit hyperactivity disorder. *Int Clin Psychopharmacol* 2008;23:291-8. [DOI](#) [PubMed](#)
 51. Bruxel EM, Salatino-Oliveira A, Genro JP, et al. Association of a carboxylesterase 1 polymorphism with appetite reduction in children and adolescents with attention-deficit/hyperactivity disorder treated with methylphenidate. *Pharmacogenomics J* 2013;13:476-80. [DOI](#) [PubMed](#)
 52. Nemoda Z, Angyal N, Tarnok Z, Gadoros J, Sasvari-Szekely M. Carboxylesterase 1 gene polymorphism and methylphenidate response in ADHD. *Neuropharmacology* 2009;57:731-3. [DOI](#) [PubMed](#)
 53. Thümmel S, Dor E, David R, et al. Pharmacoresistant severe mental health disorders in children and adolescents: functional abnormalities of cytochrome P450 2D6. *Front Psychiatry* 2018;9:2. [DOI](#) [PubMed](#) [PMC](#)