The involvement of serotonin polymorphisms in autistic spectrum symptomatology

Amaia Hervás[a], Claudio Toma[c,e], Patricia Romarís[a], Marta Ribasés[f], Marta Salgado[a], Mònica Bayes[g], Noemí Balmaña[a], Bru Cormand[b,d,e], Marta Maristany[h], Silvina Guijarro[a] and María J. Arranz[b,i,j]

Background **Autism spectrum disorders (ASD) are highly inherited developmental syndromes, resulting from a complex interaction between environmental and genetic factors. To date, only a limited number of genetic variants have been discovered with respect to autism, and their contribution to the development of the disorder has not been clearly determined. Investigation of specific autistic symptomatology may improve the chances of identifying related genes and may help to better understand these disorders.**

Materials and methods **We investigated the contribution of 80 genetic variants in 15 serotonin genes to ASD phenotypes [intelligence quotation (IQ), intellectual disability (ID) and language onset delay (LD)] in a cohort of 141 children and young adults (121 male patients and 20 female patients, average age 14.5 ± 5.1 years).**

Results **Two polymorphisms in the *HTR2B* gene, rs10194776 and rs16827801, were associated with IQ ($P = 0.0004$ and 0.003, respectively), ID ($P = 0.02$ and 0.03) and LD ($P = 0.04$ and 0.004). Nominal associations were also detected between the ASD phenotypes investigated and 5-HT2A, 5-HT4 and 5-HT6 genetic variants.**

Conclusion **Our study provides evidence of the contribution of serotonergic variants to IQ, ID and LD in ASD patients. *Psychiatr Genet* 24:158–163 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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[a]Department of Child Psychiatry, [b]Fundació Docència i Recerca Mútua Terrassa, Hospital Universitari Mútua Terrassa, [c]Department of Genetics, University of Barcelona, [d]Institut de Biomedicina de la Universitat de Barcelona (IBUB), [e]Biomedical Network Research Centre on Rare Diseases (CIBERER), [f]Psychiatric Genetics Unit, Vall d"Hebron Research Institute (VHIR), [g]National Center for Genome Analysis (CNAG), [h]Department of Psychiatry, Hospital Sant Joan de Deu, [i]Department of Psychiatry, Hospital Santa Creu i Sant Pau, Barcelona, Spain and [j]Department of Neuroscience, Institute of Psychiatry, King's College London, London, UK

Correspondence to Maria J. Arranz, PhD, Fundació Docència i Recerca Mútua Terrassa, Hospital Universitari Mútua Terrassa, University of Barcelona, c/Sant Antoni, 19, 08221 Terrassa, Barcelona, Spain
Tel: +34 937 365 050 x1177; fax: +34 937 367 011;
e-mail: maria.arranz@kcl.ac.uk, mjarranz@mutuaterrassa.es

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Brief report

Autism spectrum disorders (ASD) are highly inherited developmental syndromes, resulting from a complex interaction between environmental and genetic factors. To date, only a limited number of genetic variants have been discovered with respect to autism. Mutations of the fragile X mental retardation protein (*FXMRP*) account for 5% of ASD cases. Genes such as *Neuroligin X*, *SHANK2*, *SHANK3*, *CNTNAP2*, *EN2* and *MET* among others have also been reported in association with ASD (State, 2010), although their contribution to the development of the disorder has not been clearly determined. Investigation of specific autistic symptomatology may improve the chances of identifying related genes and may help to better understand these disorders.

The disruption of the serotonin (5-HT) system is one of the most consistent and well-replicated findings in ASD (Zafeiriou *et al.*, 2009). Increase in platelet serotonin levels was the first finding to relate 5-HT to ASD (Zafeiriou *et al.*, 2009; Hammock *et al.*, 2012), with hyperserotonemia observed in 25–50% of patients with ASD (Zafeiriou *et al.*, 2009). Sequence variants in the serotonin genes may contribute to this disruption, and several studies have investigated their involvement in the development of ASD. The serotonin transporter (5-HTT) gene is a strong autism candidate because of its association with anxiety, aggression, attention and the effectiveness of selective serotonin reuptake inhibitors in treating certain behavioural symptoms (Brune *et al.*, 2006), and it is located in an area linked to autism, 17q11. A polymorphism in the promoter region of this gene (*SLC6A4* LPR) was found associated with the disorder (Brune *et al.*, 2006; Calahorro *et al.*, 2009), level of language impairment (Kistner-Griffin *et al.*, 2011) and childhood aggression (Beitchman *et al.*, 2006). Genetic variants in 5-HT1B, 5-HT2A, 5-HT3A, 5-HT3C and 5-HT4A receptor genes have also been linked to the predisposition to ASD (Coutinho *et al.*, 2004; Anderson *et al.*, 2009; Orabona *et al.*, 2009; Hranilovic *et al.*, 2010). However, most of these findings have not been replicated in independent studies, and the reported genetic

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Table 1 List of serotonin polymorphisms genotyped in the sample

Genes	Symbol	Polymorphisms
5-HT1A	*HTR1A*	rs878567
5-HT1B	*HTR1B*	rs130058, rs6296
5-HT1D	*HTR1D*	rs676643, rs604030
5-HT1E	*HTR1E*	rs828358, rs1581774, rs1408449, rs2209639, rs9344666
5-HT1F	*HTR1F*	rs1503433
5-HT2A	*HTR2A*	rs2070040, rs9534511, rs2296973, rs2770304, rs1410657, rs2770296, rs4941570, rs2770293, rs6561335, rs2224721, rs7984966, rs9534495, rs2770300, rs1923887, rs6561333, rs6561332, rs7997012, rs7322347
5-HT2B	*HTR2B*	rs10194776, rs16827801, rs4973377
5-HT3A	*HTR3A*	rs1150222, rs1176717, rs10160548
5-HT3B	*HTR3B*	rs1985242, rs11214763, rs12270070, rs1176744, rs11214775, rs1672717, rs7129190
5-HT4	*HTR4*	rs6865654, rs9686886, rs7721747, rs10223307, rs7711800, rs2910098, rs6873382, rs13166761, rs1368384, rs2005953, rs4597955, rs3995090, rs13156542, rs4274967
5-HT5A	*HTR5A*	rs6320, rs2241859, rs2581841, rs6597455, rs1657268
5-H-T6	*HTR6*	rs4912138, rs9659997
5-HT7	*HTR7*	rs1298056, rs11817364, rs1274446, rs7904560, rs7916403, rs11186320, rs12261011, rs10785973, rs2226116, rs7084468, rs12259062
5-HTT	*SLC6A4*	rs2020942, rs140701
TPH	*TPH1*	rs10488683, rs172423, rs1800532, rs211102

effects are relatively small. We propose to investigate the genetic contribution to specific phenotypes within ASD, such as intelligence quotation (IQ) and its components, intellectual disability (ID) and language onset delay (LD). This may facilitate the identification of related genes and help to better understand the pathophysiology of the disease.

A total of 141 children and young adults (121 male patients and 20 female patients, average age 14.5 ± 5.1 years) were recruited at the Child Psychiatric Unit of the Hospital Universitari Mútua Terrassa (HUMT, Barcelona, Spain). All patients were unrelated and fulfilled diagnosis of autistic spectrum disorders (ASD) according to DSM-IV-TR, Autism-Diagnostic Interview-Revised and Autism Diagnostic Observation Schedule (Lord *et al.*, 2000a, 2000b). Intellectual capacity was measured by the Wechsler intelligence scale for children [Wechsler Adult Intelligence Scale Revised. (WAIS-IV)] or by the Wechsler adult intelligence scale-Revised. Full scale IQ was the principal IQ measure. If differences among Wechsler subscales were 23 points or more, the General Ability Index was calculated and taken as principal IQ measure. Participants had an average IQ of 88.41 (SD = 20.28). ID was defined by a full scale IQ of 70 or less. Language delay was defined as onset of first meaning words later than 24 months or onset of two word phrases later than 33 months. ID and LD were observed in 37 and 36% of the participants, respectively. Karyotype analyses and Fragile X testing was conducted in all patients. All study participants and their tutors gave written consent to participate in this study, which has been approved by the HUMT Ethics Committee. This sample size has a statistical power of 80–85% to detect genetic effects, with an odds ratio of 2.5 for polymorphisms with a minimum allele frequency of 0.15 or higher (considering 240 chromosomes, calculated with EpiInfo v7.0.8.3; Centers for Disease Control and Prevention, Atlanta, Georgia, USA).

Eighty tagged single nucleotide polymorphisms (SNPs) in 15 serotonin genes were chosen using the HapMap programme (*http://www.hapmap.org*) and the parameters r2 less than 0.85 and minimum allele frequency greater than 0.15 (see Table 1 for list of polymorphisms and Table 2 for linkage disequilibrium figures in our sample). DNA was extracted from whole blood samples using the standard methods. Genotyping was performed using SNPlex technology (Applied Biosystems, Foster City, California, USA). Linear regression analyses, including age and sex as covariates, and χ^2 analyses were calculated to investigate the associations with linear (IQ) and bimo dal variables (ID and LD). Phased (using the E–M algo rithm) haplotype analyses including all SNPs within a gene were performed (data available on request). The statistical packages SPSS (v2.0; IBM, Armonk, New York, USA), PLINK (v1.07) (Purcell *et al.*, 2007) and EpiInfo (v7.0.8.3; IBM) were used.

Table 3 summarizes the results of the analyses. In general, the allelic frequencies agree with those observed in previous studies on unaffected Spanish controls, although there are no data for some of the polymorphisms investigated in our study. Several serotonin polymorphisms were associated with the level of IQ in our cohort. The most significant finding was the association with the 5-HT2B receptor gene (*HTR2B*) rs10194776 polymorphism ($P = 0.0004$), a finding still significant after corrections for multiple analyses (correction factor: 15 genes $\times$ 3 = 45). This same polymorphism was also found associated with ID ($P = 0.02$) and LD ($P = 0.04$), although these marginal associations did not survive multiple analyses corrections. A second *HTR2B* polymorphism, rs16827801, was also significantly associated with IQ, ID and LD, respectively, confirming the relevance of this gene. Haplotype analyses revealed association between allelic combinations of *HTR2B* SNPs and IQ, ID and LD. No information on the functional effect of these polymorphisms exists, and no significant influence on

Table 2 Linkage disequilibrium values (r^2) calculated for the genotyped polymorphisms

Genes	r^2
5-HT1A	
rs6296	0.898
rs130058	[a]
5-HT1D	
rs604030	0.383
rs676643	[a]
5-HT1E	
rs828358	[a]
rs1581774	0.48
rs1408449	0.04
rs2209639	0.5
rs9344666	0.157
5-HT2A	
rs7322347	0.019
rs7997012	0.013
rs6561332	0.033
rs6561333	0.017
rs1923887	0.015
rs2770300	0.017
rs9534495	0.005
rs7984966	0.048
rs2224721	[a]
rs6561335	0.007
rs2770293	0.031
rs4941570	0.043
rs2770296	0.011
rs1410657	0.108
rs2770304	0.14
rs2296973	0.043
rs2070040	0.004
rs9534511	0.015
5-HT2B	
rs10194776	[a]
rs4973377	0.14
5-HT3A	
rs1150222	[a]
rs1176717	0.34
rs10160548	0.652
5-HT3B	
rs11214763	0.068
rs12270070	0.53
rs1176744	[a]
rs11214775	0.25
rs1672717	0.33
rs7129190	0.08
rs1985242	0.001
5-HT4	
rs4274967	7.46e-005
rs13156542	0.0001
rs3995090	0.001
rs4597955	0.006
rs1368384	0.214
rs13166761	0.536
rs6873382	[a]
rs2910098	0.393
rs7711800	0.458
rs10223307	0.387
rs7721747	0.351
rs9686886	0.356
rs6865654	0.283
5-HT5A	
rs6320	0.222
rs2241859	0.876
rs2581841	[a]
rs6597455	0.853
rs1657268	0.674
5-HT6	
rs4912138	0.394
rs9659997	[a]
5-HT7	
rs1298056	0.037
rs11817364	0.060
rs1274446	0.164
rs7904560	0.055
rs7916403	
1.02e−005	
rs11186320	0.413
rs12261011	[a]
rs10785973	0.060
rs2226116	0.029
rs7084468	0.053
rs12259062	0.316

[a]Reference polymorphism.

regulatory factors was predicted using the FuncPred programme (*http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm*). However, a nearby stop codon polymorphism, Q20*, had previously been related to antisocial personality and impulsivity, and studies in knockout mice related this polymorphism to impulsivity (Bevilacqua *et al.*, 2010). Our findings suggest that *HTR2B* polymorphisms may contribute to specific phenotypes within ASD, and the relationship of these polymorphisms with impulsivity should be investigated.

ID was significantly associated with several 5-HT2A receptor gene (*HTR2A*) polymorphisms and haplotypes ($P=0.03$). Interestingly, this significant effect was not observed when analysing LD and IQ, suggesting that *HTR2A* variant affects specifically ID. Several lines of evidence suggest that 5-HT2A may play a role in ASD. Higher level of 5-HT2A receptor expression has been observed in ASD patients (Kazek *et al.*, 2010). Significant reduction in cortical 5-HT2A receptor binding has been observed in patients with Asperger's syndrome and has been related to abnormal social communication (Murphy *et al.*, 2006). In addition, numerous studies have reported associations between *HTR2A* polymorphisms and cognitive impairment and memory (Uçok *et al.*, 2007; Hasselbalch *et al.*, 2008; Sigmund *et al.*, 2008; Wagner *et al.*, 2008; Alfimova *et al.*, 2010). Our results contribute evidence supporting the involvement of 5-HT2A receptor alterations on cognitive impairment in ASD patients.

Two 5-HT6 receptor gene (*HTR6*) SNPs and a haplotype were significantly associated with IQ. Although no report has directly associated 5-HT6 receptors with ASD, these receptors are involved in learning, memory processes and cognitive impairment (Da Silva Costa-Aze *et al.*, 2012). Our data would suggest that *HTR6* genetic variants contribute to cognitive impairment in ASD patients.

In addition to *HTR2B* variants, LD was significantly associated to 5-HT4 receptor gene (*HTR4*) polymorphisms and haplotypes. Previous studies have implicated 5-HT4 receptors with learning processes (King *et al.*, 2008; Marchetti *et al.*, 2008, Levallet *et al.*, 2009). However, this is the first report associating *HTR4* variants with LD or with ASD.

Table 3 Summary of statistical analyses

			IQ			ID			LD		
SNP	Genes	Allele (frequency)	*B*	*P*	R^2	χ^2	*P*	OR (95% CI)	χ^2	*P*	OR (95% CI)
rs878567	*HTR1A*	C (0.47)	0.74	0.81	0.007	0.37	0.55	1.19 (0.67–2.10)	0.52	0.48	1.24 (0.69–2.20)
rs130058	*HTR1B*	T (0.34)	−3.5	0.25	0.01	0.09	0.75	0.91 (0.51–1.63)	0.01	0.91	0.97 (0.53–1.75)
rs6296	*HTR1B*	C (0.23)	−1.2	0.73	0.001	0.20	0.65	0.85 (0.43–1.69)	0.25	0.62	1.2 (0.59–2.45)
rs676643	*HTR1D*	A (0.18)	−1.3	0.67	0.001	0.006	0.94	1.03 (0.50–2.12)	1.13	0.29	1.51 (0.70–3.25)
rs604030	*HTR1D*	C (0.36)	−3.1	0.23	0.01	2.34	0.13	0.64 (0.36–113)	0.01	0.91	1.04 (0.58–1.87)
rs828358	*HTR1E*	A (0.21)	1.4	0.67	0.002	1.93	0.16	0.63 (0.33–1.21)	1.37	0.24	0.67 (0.35–1.30)
rs1581774	*HTR1E*	T (0.18)	1.2	0.74	0.001	0.79	0.37	0.72 (0.34–1.5)	2.02	0.15	0.58 (0.28–1.23)
rs1408449	*HTR1E*	A (0.31)	−4.5	0.10	0.03	3.33	0.07	0.58 (0.32–1.04)	1.53	0.21	0.68 (0.37–1.24)
rs2209639	*HTR1E*	T (0.17)	1.5	0.69	0.002	0.14	0.71	0.87 (0.43–1.78)	0.25	0.61	0.83 (0.40–1.71)
rs9344666	*HTR1E*	G (0.34)	−2.7	0.37	0.008	0.02	0.88	0.96 (0.53–1.72)	0.02	0.88	0.95 (0.53–1.74)
rs1503433	*HTR1F*	G (0.41)	−5.6	**0.04**	**0.04**	2.34	0.13	0.64 (0.36–1.13)	0.73	0.39	0.78 (0.44–1.38)
rs2070040	*HTR2A*	A (0.36)	−5.5	0.06	0.04	1.52	0.22	0.69 (0.39–1.24)	0.005	0.94	0.98 (0.54–1.78)
rs9534511	*HTR2A*	T (0.42)	−4.7	0.14	0.02	0.53	0.47	0.81 (0.46–1.42)	0.08	0.78	1.09 (0.61–1.93)
rs2296973	*HTR2A*	T (0.29)	2.8	0.45	0.006	2.73	0.10	1.70 (0.90–3.21)	0.31	0.58	1.19 (0.64–2.22)
rs2770304	*HTR2A*	C (0.36)	1.5	0.68	0.002	0.84	0.36	1.32 (0.73–2.42)	0.27	0.60	1.18 (0.64–2.14)
rs1410657	*HTR2A*	A (0.42)	1.3	0.67	0.002	1.61	0.20	1.45 (0.81–2.60)	1.43	0.23	1.43 (0.80–2.57)
rs2770296	*HTR2A*	C (0.32)	−0.5	0.88	<0.001	0.01	0.91	1.03 (0.57–1.87)	0.27	0.60	1.18 (0.64–2.17)
rs4941570	*HTR2A*	G (0.45)	5.2	0.12	0.03	0.34	0.56	1.19 (0.66–2.14)	0.31	0.58	0.84 (0.46–1.54)
rs2770293	*HTR2A*	T (0.38)	−5.7	0.06	0.04	3.77	**0.05**	**0.57 (0.32–1)**	<0.01	0.99	0.99 (0.55–1.79)
rs6561335	*HTR2A*	G (0.23)	−0.4	0.90	<0.001	0.12	0.73	0.89 (0.47–1.7)	0.002	0.96	1.01 (0.53–1.96)
rs2224721	*HTR2A*	A (0.24)	6.3	0.07	0.030	3.07	0.08	1.86 (0.92–3.78)	1.11	0.29	1.45 (0.72–2.9)
rs7984966	*HTR2A*	C (0.28)	3.4	0.34	0.01	0.03	0.87	1.05 (0.56–1.97)	0.31	0.58	0.84 (0.44–1.57)
rs9534495	*HTR2A*	G (0.39)	−0.7	0.81	<0.001	5.35	**0.02**	**2.03 (1.11–3.72)**	2.73	0.10	1.66 (0.91–3.02)
rs2770300	*HTR2A*	C (0.32)	−2.4	0.42	0.007	3.51	0.06	0.56 (0.31–1.03)	0.16	0.69	0.88 (0.47–1.64)
rs1923887	*HTR2A*	G (0.37)	−2.6	0.41	0.007	4.40	**0.03**	**0.54 (0.30–0.96)**	0.52	0.47	0.80 (0.44–1.45)
rs6561333	*HTR2A*	T (0.42)	−4.5	0.15	0.02	6.91	**0.01**	**0.47 (0.26–0.83)**	0.56	0.45	0.80 (0.45–1.43)
rs6561332	*HTR2A*	T (0.47)	2.7	0.35	0.01	2.73	0.10	1.61 (0.91–2.83)	0.06	0.80	1.08 (0.61–1.9)
rs7997012	*HTR2A*	A (0.37)	−4.1	0.22	0.02	4.07	**0.04**	**0.55 (0.30–0.99)**	0.81	0.37	0.76 (0.42–1.38)
rs7322347	*HTR2A*	T (0.48)	−2.5	0.41	0.01	0.39	0.53	0.83 (0.47–1.47)	0.12	0.72	1.11 (0.62–1.98)
rs10194776	*HTR2B*	T (0.34)	10.7	**0.0004**	**0.13**	5.22	**0.02**	**2.03 (1.1–3.74)**	4.06	**0.04**	**1.88 (1.01–3.48)**
rs16827801	*HTR2B*	G (0.26)	9.9	**0.003**	**0.10**	4.55	**0.03**	**2.09 (1.05–4.14)**	8.26	**0.004**	**2.86 (1.37–5.98)**
rs4973377	*HTR2B*	A (0.21)	−6.5	0.07	0.03	4.01	0.04	0.53 (0.28–0.99)	0.92	0.34	0.73 (0.38–1.39)
rs1150222	*HTR3A*	T (0.17)	4.4	0.31	0.01	<0.01	0.99	0.99 (0.45–2.22)	0.01	0.90	0.95 (0.42–2.12)
rs1176717	*HTR3A*	T (0.23)	5.2	0.14	0.03	0.91	0.34	1.41 (0.70–2.83)	1.98	0.16	1.17 (0.81–3.51)
rs10160548	*HTR3A*	G (0.39)	4.2	0.15	0.02	0.67	0.41	1.27 (0.71–2.28)	3.62	0.06	1.78 (0.98–3.29)
rs1985242	*HTR3B*	A (0.39)	4.9	0.11	0.02	2.20	0.14	1.58 (0.86–2.78)	1.45	0.23	1.45 (0.79–2.67)
rs11214763	*HTR3B*	A (0.17)	1.5	0.73	0.001	0.21	0.65	0.84 (0.39–1.8)	0.007	0.93	1.03 (0.48–2.24)
rs12270070	*HTR3B*	C (0.28)	0.4	0.90	<0.001	0.03	0.86	1.06 (0.56–2.02)	2.42	0.12	1.71 (0.87–3.37)
rs1176744	*HTR3B*	G (0.43)	0.1	0.96	<0.001	0.36	0.55	1.19 (0.67–2.10)	0.44	0.51	121 (0.68–2.17)
rs11214775	*HTR3B*	A (0.26)	2.2	0.53	0.004	0.19	0.67	0.87 (0.47–1.64)	0.53	0.47	1.28 (0.66–2.48)
rs1672717	*HTR3B*	C (0.31)	−2.1	0.43	0.007	0.005	0.94	0.98 (0.54–1.76)	0.83	0.36	0.76 (0.41–1.38)
rs7129190	*HTR3B*	C (0.48)	0.3	0.89	0.002	0.35	0.55	1.19 (0.68–2.07)	1.24	0.26	0.72 (0.41–1.28)
rs6865654	*HTR4*	T (0.38)	2.6	0.37	0.01	1.34	0.25	1.40 (0.79–2.50)	0.54	0.46	1.25 (0.69–2.25)
rs9686886	*HTR4*	A (0.31)	2.3	0.44	0.006	0.58	0.45	1.26 (0.69–2.3)	0.002	0.96	0.98 (0.54–1.81)
rs7721747	*HTR4*	G (0.45)	0.4	0.88	<0.001	0.48	0.49	1.22 (0.70–2.14)	0.01	0.91	1.03 (0.58–1.83)
rs10223307	*HTR4*	T (0.47)	−2.2	0.43	0.007	1.28	0.26	0.72 (0.41–1.27)	0.67	0.41	0.79 (0.44–1.4)
rs7711800	*HTR4*	T (0.44)	2.2	0.42	0.007	0.90	0.34	1.31 (0.75–2.31)	<0.01	0.99	1.00 (0.57–1.78)
rs2910098	*HTR4*	T (0.34)	2.5	0.38	0.009	0.60	0.44	1.27 (0.70–2.31)	0.02	0.88	0.96 (0.52–1.75)
rs6873382	*HTR4*	A (0.43)	0.07	0.97	<0.001	0.23	0.63	0.87 (0.50–1.53)	4.13	**0.04**	**0.55 (0.31–0.98)**
rs13166761	*HTR4*	T (0.29)	2.04	0.53	0.004	1.27	0.26	1.44 (0.76–2.70)	0.04	0.84	1.07 (0.57–2.01)
rs1368384	*HTR4*	C (0.24)	1.4	0.68	0.002	0.32	0.57	1.22 (0.62–2.39)	0.09	0.76	1.11 (0.56–2.21)
rs2005953	*HTR4*	C (0.39)	0.8	0.77	0.001	0.005	0.94	1.02 (0.58–1.81)	2.13	0.14	0.65 (0.36–1.16)
rs4597955	*HTR4*	G (0.44)	−2.5	0.46	0.006	0.11	0.74	0.90 (0.50–1.62)	0.14	0.71	1.12 (0.62–2.03)
rs3995090	*HTR4*	C (0.39)	2.7	0.43	0.006	1.63	0.20	1.45 (0.82–2.57)	0.08	0.78	1.09 (0.61–1.93)
rs13156542	*HTR4*	C (0.41)	2.4	0.49	0.005	2.35	0.13	1.57 (0.88–2.79)	0.60	0.44	1.26 (0.71–2.24)
rs4274967	*HTR4*	C (0.23)	−1.7	0.65	0.002	0.75	0.39	1.34 (0.69–2.63)	3.84	**0.05**	**2.05 (0.99–4.21)**
rs6320	*HTR5A*	A (0.33)	3.6	0.23	0.01	0.01	0.92	1.03 (0.57–1.85)	0.81	0.37	1.32 (0.72–2.41)
rs2241859	*HTR5A*	A (0.32)	−3.8	0.21	0.02	0.05	0.82	1.07 (0.59–1.94)	0.07	0.79	0.92 (0.51–1.68)
rs2581841	*HTR5A*	G (0.34)	−4.6	0.14	0.02	0.02	0.88	0.96 (0.53–1.72)	0.42	0.52	0.82 (0.45–1.49)
rs6597455	*HTR5A*	G (0.35)	−3.3	0.30	0.01	0.37	0.54	1.2 (0.66–2.15)	0.23	0.63	0.87 (0.48–1.55)
rs1657268	*HTR5A*	C (0.41)	−3.3	0.29	0.01	0.13	0.71	1.11 (0.63–1.97)	0.22	0.64	0.87 (0.48–1.55)
rs4912138	*HTR6*	A (0.22)	10.7	**0.005**	**0.08**	2.35	0.13	0.59 (0.30–1.16)	0.01	0.92	1.04 (0.51–2.10)
rs9659997	*HTR6*	C (0.41)	−7.2	**0.02**	**0.06**	0.75	0.39	0.78 (0.44–1.37)	2.6	0.11	0.62 (0.35–1.11)
rs1298056	*HTR7*	C (0.15)	−7.2	0.08	0.03	0.77	0.38	0.70 (0.32–1.54)	1.84	0.17	1.86 (0.75–4.59)
rs11817364	*HTR7*	A (0.22)	6.8	0.06	0.03	0.56	0.46	1.29 (0.66–2.53)	0.29	0.59	0.83 (0.43–1.61)
rs1274446	*HTR7*	T (0.45)	−1.2	0.70	0.002	0.27	0.61	1.16 (0.66–2.02)	0.38	0.54	0.84 (0.47–1.47)
rs7904560	*HTR7*	A (0.22)	−2.5	0.49	0.005	0.06	0.80	0.92 (0.46–1.83)	5.24	**0.02**	**2.59 (1.12–5.96)**
rs7916403	*HTR7*	T (0.45)	2.5	0.40	0.008	0.32	0.57	1.18 (0.67–2.06)	1.42	0.23	1.42 (0.80–2.53)
rs11186320	*HTR7*	C (0.33)	3.3	0.31	0.01	0.11	0.74	0.90 (0.50–1.63)	0.58	0.21	0.68 (0.38–1.24)
rs12261011	*HTR7*	G (0.17)	7.06	0.08	0.03	0.06	0.81	0.91 (0.43–1.94)	0.25	0.62	0.82 (0.38–1.77)
rs10785973	*HTR7*	A (0.23)	6.5	0.09	0.03	2.41	0.12	1.74 (0.86–3.54)	0.07	0.79	1.10 (0.55–2.17)
rs2226116	*HTR7*	A (0.12)	4.32	0.34	0.01	0.94	0.33	1.57 (0.63–3.96)	0.54	0.46	1.41 (0.56–3.58)
rs7084468	*HTR7*	A (0.21)	0.54	0.88	<0.001	0.07	0.79	1.10 (0.55–2.17)	0.12	0.73	0.88 (0.44–1.75)

Table 3 (*continued*)

			IQ			ID			LD		
SNP	Genes	Allele (frequency)	*B*	*P*	R^2	χ^2	*P*	OR (95% CI)	χ^2	*P*	OR (95% CI)
rs12259062	*HTR7*	G (0.39)	5.56	0.09	0.03	0.001	0.97	0.99 (0.56–1.76)	0.21	0.65	0.87 (0.49–1.56)
rs2020942	*SLC6A4*	A (0.39)	−0.5	0.86	<0.001	0.54	0.46	0.81 (0.46–1.42)	1.34	0.24	0.71 (0.40–1.27)
rs140701	*SLC6A4*	A (0.46)	−0.8	0.78	<0.001	1.55	0.21	1.44 (0.81–2.56)	3.99	**0.05**	**1.83 (1.01–3.3)**
rs10488683	*TPH1*	G (0.48)	1.01	0.72	0.001	0.48	0.48	1.22 (0.69–2.17)	2.51	0.11	1.60 (0.89–2.88)
rs172423	*TPH1*	C (0.36)	−0.2	0.94	<0.001	0.35	0.56	1.19 (0.66–2.15)	2.60	0.11	1.65 (0.89–3.05)
rs1800532	*TPH1*	A (0.34)	2.40	0.39	0.008	0.02	0.88	1.04 (0.58–1.89)	2.21	0.14	0.64 (0.35–1.15)
rs211102	*TPH1*	A (0.13)	1.85	0.66	0.002	<0.01	0.94	0.97 (0.42–2.24)	0.06	0.81	1.11 (0.45–2.75)

Bold indicates significant *P* values ($P < 0.05$).
ID, intellectual disability; IQ, intelligence quotation; LD, language onset delay; OR, odds ratio; SNP, single nucleotide polymorphism.

Other interesting associations were detected between *HTR1F*, *HTR2A* and *HTR7* genetic variants and IQ, ID and/or LD. However, these findings were marginal and require confirmation. We did not find clear correlations between the phenotypes investigated and *SLC6A4* polymorphisms, and we cannot confirm their involvement in the development of IQ, ID and LD phenotypes. In general, factors such as age or sex did not affect the significance of our results. However, the findings of association of this study would not survive conservative corrections for multiple analyses, with the exception of the *HTR2B* findings. Nevertheless, the replication of the association in several SNPs and haplotypes of the same genes and the existing evidence of serotonergic dysfunction in ASD patients suggest that these results may be true findings.

One of the limitations of our study is the relatively modest sample size, which is underpowered to detect small genetic effects (odds ratios <2.5). In addition, the limited number of female patients included in our cohort did not allow for separate male–female comparisons. However, reanalyses of the 121 male participants produced similar results (data not shown). Finally, the functional effect of the SNPs found in association with ASD phenotypes is so far unknown, hindering the biological plausibility of the findings.

In summary, we have identified novel associations between genetic variants in 5-HT2B, 5-HT6 and 5-HT4 receptor genes and IQ, ID and LD and have provided further evidence of the relevance of other serotonin genes in cognitive impairment in ASD patients.

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Conflicts of interest

There are no conflicts of interest.

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