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HTR2A gene polymorphisms and Inward and Outward Personal Meaning Organisations

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Objective: Caregiver behaviours and emotional expressions may induce development of two basic categories of constructing identity and of regulating cognitive and emotional processes: an Inward or an Outward Personal Meaning Organisation (PMO). Inwards read environmental signals through their internal activations. Their emotions are more distinct, and reciprocity is more based on physical distance (protection, loneliness). Outwards read internal activations through the environment. Their emotions are more blurred, and reciprocity is more based on a semantic sight of relations (approval, rules).
It has recently been shown that PMO development may also have physiological and genetic bases. In a functional magnetic resonance imaging (fMRI) study, Inward and Outward subjects showed different amygdala activation patterns and an association with the SLC6A4 serotonin transporter gene 5-HTTLPR polymorphism.
Methods: In this work, 149 healthy subjects were examined with respect to Inward and Outward PMOs. We explored the association with 10 serotonin receptor 2A (HTR2A) gene single nucleotide polymorphisms (SNPs) selected by bioinformatics methods.
Results: An intronic SNP (rs55948462) was found to be significantly associated with an Inward and an Outward PMOs development. However, after statistical adjustments, these results did not remain significant.
Conclusion: We did not find associations between considered SNPs and Inward/Outward PMOs. However, the role of HTR2A polymorphisms was not considered in this study and that of the other serotonin-related genes should be valued.

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Keywords: genetic association studies; personality; serotonin

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Significant outcomes

- Ten HTR2A polymorphisms are not associated with Inward/Outward Personal Meaning Organisations.

Limitations

- False-negative results could be due to the small sample size.

Introduction

The construct of Personal Meaning Organisation (PMO) has been described as 'the specific arrangement of personal meaning processes by which each individual is provided with a sense of oneness and historical continuity in the course of his/her lifespan' (1).

Constancy and predictability of the caregiver behaviours and emotional expressions allow early decoding of his/her basic feelings (2–4). For example,

the child begins to perceive situations in which he/she feels secure or in danger, protected or lonely and reads the environmental signals through his/her internal activations (e.g. anger, fear or calmness, sadness or happiness). These conditions promote development of an internal focus with an 'Inward' reading of experience. In contrast, when caregiver behaviours and expressions are perceived as being more complex, and to change as a function of external situations, thus being less predictable and more difficult to decode, the child needs to store, and update, a greater amount of information. His/her emotional activations are connected to a self-evaluating cognitive schema (guilt, shame or serenity, sense of self-adequacy or inadequacy, dubiousness or certainty). In these conditions, the self develops beginning from a preliminary evaluation of the environment, which orients the recognition of internal activations and self-perception, leading to the development of an external focus with an 'Outward' reading of experience (5–7). Inward/Outward patterns are more or less evident in different subjects, but one prevails in each individual, at least within specific categories of experience (8).

However, the PMO model, whose value in clinical practice is well established (9–12), and attachment theories, which generally refer to learned behaviours, tend not to be investigated from the genetic viewpoint (2–4). In the last few years, advances in neuroscience have suggested possible physiological and adaptive bases for PMO (8). A recent functional magnetic resonance imaging (fMRI) study has documented different amygdala activation patterns in relation to personality style (13). Inward subjects asked to look at facial expressions of fear, showed a greater activation of amygdala, hippocampus and medial prefrontal cortex compared with Outward subjects, who exhibited greater activation at the level of the fusiform gyrus, dorsal–lateral frontal cortex and occipital cortex. Another investigation by the same group showed greater activation of the medial prefrontal cortex in Inwards exposed to threatening stimuli, very probably due to greater neuronal recruitment occurring during cognitive evaluation of primary emotions (14). In an fMRI study by our group, where we investigated the activation induced by standardised emotional stimuli, Inward subjects were seen to respond to anger with a more intense and univocal pattern, activation being mainly detected in the right amygdala and less in cortical areas compared with Outwards. As regards happiness, greater activation of the verbal hemisphere was observed in Outwards, in line with the clinical observation that they construct relations on semantic reciprocity, while Inwards construct relations on physical reciprocity. Activation in both groups was greater when shown the facial expressions of an unknown person (third person experience) with respect to their own (first person experience), probably as a result of a 'surprise effect' and to a keener attention to unfamiliar aesthetic details (15).

In this constructivistic framework, a recent work examined the correlation between genotype and Inward/Outward PMO, showing that the 5-HTTLPR polymorphism of the serotonin transporter gene is associated with Inward/Outward personality styles (13). These findings are in line with the hypothesis that the Inward/Outward PMO also has genetic bases and is correlated to serotonergic system. This correlation was expected because serotonin regulates activity of the amygdala that interprets the environmental stimulations and therefore, it has a role in construction of personality and of the sense of self (13).

In this work, we chose another serotonin-related gene, *HTR2A*. It is associated with personality and behaviour (16–18) as well as with psychiatric conditions, e.g. schizophrenia, mood disorders, attention deficit hyperactivity disorder (ADHD), suicidal behaviour, anxiety, obsessive-compulsive behaviour, eating disorders and Alzheimer's disease (AD) (19–21). The aim of this study was to investigate whether 10 *HTR2A* polymorphisms are associated with an Inward or an Outward PMO.

Material and methods

Subjects

Subjects were assessed for PMO using a Post-rationalist clinical interview and two questionnaires: Personal Meaning Questionnaire (PMQ) and Mini Questionnaire of Personal Organisation (MQPO), the validated self-rating questionnaires for PMO. In the clinical interview, the Inward/Outward PMO was blindly diagnosed by two trained psychotherapists from the Psychiatric Unit of the Neuroscience Department with at least 10-year experience in cognitive post-rationalist psychotherapy (BN, EA). The interview was conducted in line with a recent work (13), through three steps: (a) a detailed account of two meaningful episodes involving fear or anger, (b) a detailed description of emotional experience of fear or anger to assess the personal style of emotional activation and regulation and (c) a reconstruction of the onset, expression and extinction of the emotional experience. The PMQ questionnaire (22) includes 68 items addressing two Inward (phobic-prone and depressive-prone) and two Outward (eating disorders-prone and obsessive-prone) personality styles. The MQPO questionnaire (23) has 20 items and addresses two Inward (controller-prone and

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detached-prone) and two Outward (contextualised-prone and principles oriented-prone) personality styles. A diagnosis of PMO was made when the score in one PMO scale was at least 10% higher than the other scores, according to author's guidelines (22,23). Subjects were enrolled if the separate clinical evaluations by the two psychiatrists were also concordant.

In total, 149 healthy unrelated volunteers (65 males and 84 females) were recruited by call. All worked or studied at the School of Medicine of the Polytechnic University of Marche and at the Health Public Services of Ancona. All subjects were Italian, to minimise genetic heterogeneity. There were 58 Inwards and 91 Outwards (mean age 42.1 ± 10.3 and 37.6 ± 8.7, respectively, Student's *t*-test *p*-value = 0.005). Males were 60.3% of Inwards and 32.9% of Outwards (chi-squared *p*-value = 0.001). All subjects having any psychiatric diagnosis (assessed with the Structured Clinical Interview for DSM-IV, Axis I and II) were excluded. The study was approved by the Ethics Committee of Azienda Universitaria-Ospedaliera Ospedali Riuniti, Ancona. All subjects gave their written informed consent to participate.

Markers selection

The NCBI dbSNP (release 131) and HapMap (www.hapmap.org) databases were searched for data regarding HTR2A single nucleotide polymorphism (SNPs). Potentially phenotype-affecting polymorphisms were selected using the most accurate bioinformatics tools according to the literature (24), or the most recent ones in case of lack of literature data.

Prediction of the effects of the SNPs lying on the promoter region was accomplished using the rVISTA 2.0 software (25), which is based on the recent 10.2 version of the TRANSFAC database (26). The software can predict the gain or loss of transcription factor binding sites related to polymorphisms. The extension of the window straddling the transcription start site (TSS) to be analysed is debated. Some researchers use sequences of 5000 bp upstream the TSS (27), others consider a region encompassing −1750 to +250 bp (28), and others still a region from −500 to +500 bp (29). We used an intermediate stretch of 2000 bp upstream the TSS. Promoter sequences were extracted using the UCSC Genome Browser (version hg18).

rBLOSUM64 (30), the recently revised BLOSUM matrix, and the SIFT (31) and PolyPhen (32) tools were applied to determine whether SNPs negatively affected protein function. NNSPLICE (33) was used to predict the 5′ and 3′ splice sites, and SpliceAid (www.introni.it/splicing.html) (34) to predict the binding sites of splicing regulatory proteins. Because SpliceAid uses a database collecting all experimentally assessed target RNA sequences in humans, it avoids the false positives that are obtained with tools using score matrices.

Genotyping

Genomic DNA was extracted from buccal swabs using the Maxwell™ 16 Blood DNA Purification Kit on an automated Maxwell™ 16 system (Promega Corporation, Madison, Wisconsin, USA).

Selected SNPs were genotyped using a minisequencing assay with the SnaPshot™ Multiplex Kit [Applied Biosystems Inc., Carlsbad, CA, USA (ABI)] and the capillary electrophoresis discrimination assay in an automated ABI 3130 machine (ABI).

The minisequencing method involved PCR amplification and extension. Multiplex PCR primers and extension primers were designed using PrimerExpress™ v2.0 (ABI); secondary structure and primer–dimer interactions were tested with the Autodimer software (www.cstl.nist.gov/biotech/strbase/AutoDimerHomepage/AutoDimerProgramHomepage.htm). Given the large number of primers, their sequences are available on request. Multiplex PCR amplification was performed using the QIAGEN Multiplex PCR kit following the manufacturer's recommendations. The PCR product was purified by enzymatic digestion with ExoI and CIP (Calf Intestinal Alkaline Phosphatase, New England BioLabs Inc., Ipswich, MA, USA) to inactivate unincorporated dNTPs and remove excess primer.

The purified PCR product was subjected to single-base extension of unlabelled oligonucleotide primers using the SnaPshot™ Multiplex Kit (ABI) under the thermal cycling conditions specified by the manufacturer. A further purification step with 0.5 U of CIP enzyme was run to inactivate unincorporated ddNTPs, by removing 5′ phosphoryl groups. Then, 0.5 µl of purified minisequencing product was mixed with 0.25 µl of the internal size standard LIZ120 (ABI) and 9.25 µl Hi-Di Formamide (ABI), and injected in an automated ABI 3130 5-colour sequencer (ABI). Raw data were processed using the GeneMapper ID software, v3.2.1 (ABI), which automatically assigned the allelic state to all SNPs.

Statistical analysis

The pairwise linkage disequilibrium (LD) between any two markers and the haplotype block structure was evaluated using the Haploview software (35). Haplotye blocks (segments with strong LD) were defined according to validated criteria (36).

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Hardy-Weinberg equilibrium (HWE) was tested in the Inward and Outward groups, and within the whole population. For each marker, HWE was tested using the goodness-of-fit implemented in the PowerMarker software (37).

Associations between alleles, genotype and the two PMO phenotypes were tested by comparing allele and genotype frequency distributions in Inwards and Outwards with the chi-squared test and an exact test using PowerMarker. The software automatically selects the suitable exact test depending on sample numerousness. In particular it can choose between the Markov chain Monte Carlo (MCMC) approach (38,39), the permutation approach and the asymptotic chi-squared test. Odds ratios (ORs) and 95% confidence intervals (CI 95%) were calculated to assess the relative risk conferred by a given allele or genotype. To assess the strength of the combined evidence from multiple tests for the association between the HTR2A gene and the PMOs, the significance level α of the tests was corrected for multiple comparisons. The software SNPSpD was used to generate the experiment-wide significance threshold required to keep Type I error below 5%. This approach consists in a simple correction for multiple testing of SNPs in LD with each other, on the basis of the spectral decomposition (SpD) of matrices of pairwise LD between SNPs (40).

Potential confounding effects due to differences in demographic characteristics (age and sex) between the PMO phenotypes may occur. Therefore, logistic regression analysis was performed to estimate the effect of HTR2A markers on PMO development adjusted for demographic covariates. A backward stepwise logistic regression method was conducted using the program PASW 17.0 (SPSS Inc., Chicago, IL, USA), with the PMO phenotypes as the dependent variable, and the age, sex and alleles as the independent variables. For allele data, only the information of the minor allele from each SNP was entered in the regression model. Minor alleles were coded as 1, 0.5 or 0, respectively, for the minor allele homozygote, the heterozygote or the major allele homozygote.

Results

Ten HTR2A SNPs were genotyped to assess their association with the Inward/Outward PMO. Among these, nine SNPs were selected because they could severely affect one or more biological functions, i.e. transcription, protein structure and RNA splicing (Table 1). rs6312 is included, although it was not predicted to influence any biological process, because it has been reported to decrease promoter activity in combination with rs6311 (41).

Table 1. Polymorphisms predicted to be capable of affecting important biological processes

Marker	Region	Potentially affected processes
rs1058576	Exon 3	Protein, Splicing
rs1928040	Intron 2	Splicing
rs3742278	Intron 2	Splicing
rs55948462	Intron 2	Splicing
rs594242	Intron 2	Splicing
rs6311 (A-1438G)	Promoter	Transcription
rs6312 *	Promoter	Transcription
rs6313 (T102C)	Exon 1	Splicing
rs643627	Intron 2	Splicing
rs7997977	Promoter	Transcription

Transcription: SNP may enhance or undermine transcription, giving rise to a greater or smaller amount of pre-mRNA, respectively; Splicing: SNP may increase or decrease the amount of correctly processed mRNA; Protein: SNP may damage protein folding.
*rs6312 was included, although it was not predicted to influence any biological process, because it has been reported to decrease promoter activity in combination with rs6311.

In general, an SNP lying in the gene promoter region can strengthen, weaken or even create or destroy a DNA binding site for a transcription factor, therefore up or downregulate the transcription level of a gene, thus altering in this case, the amount of synthesised receptor. In Table 1, the three selected SNPs predicted to affect transcription process, according to rVISTA tool, are reported.

Generally, an SNP lying in a gene-coding region and causing an amino acid substitution could affect the protein structure by enhancing or reducing its ligand affinity. We selected one SNP using rBLOSUM64 matrix, the SIFT and PolyPhen, software able to predict possible protein structure changes.

Both exonic and intronic SNPs could affect the splicing process by altering 5′ and 3′ splice sites or regulative elements such as exonic splicing enhancers (ESEs), exonic splicing silencers (ESSs), intronic splicing enhancers (ISEs) or intronic splicing silencers (ISSs). We selected seven SNPs using NNSPLICE and SpliceAid, tools able to predict the alteration of splicing regulatory motifs.

Pairwise LD analysis in the 149 subjects showed that some markers lay in a haplotype block (Fig. 1). Markers rs1058576 and rs7997977 were not polymorphic in our sample and were excluded by LD calculation. rs6311, rs6312 and rs6313 were in complete LD ($D' = 1$) and belong to the same haplotype block (Fig. 1).

The genotype frequency distribution of the markers was in HWE, except for rs594242, which significantly departed from it in the whole sample and in the Outward group ($p < 0.01$), but was in HWE in the Inward group. The HWE test results are reported in Table 2.

The allele and genotype frequencies of the HTR2A SNPs are shown in Table 3. Allele, genotype and

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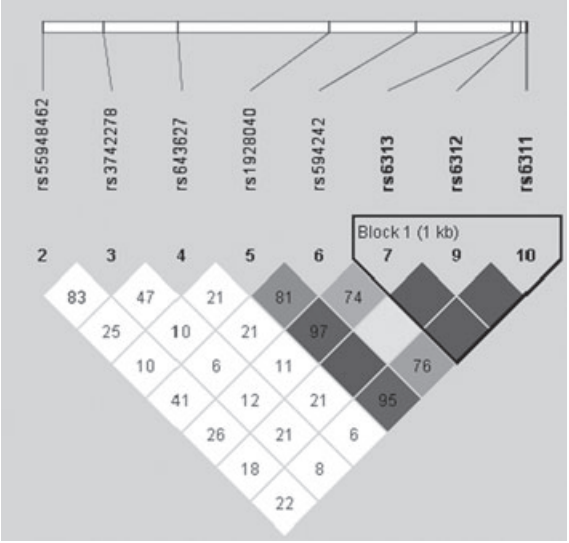

Fig. 1. Pairwise LD between HTR2A markers in unrelated Italians subjects. (D′ = 1 in blank squares; numbers inside the grey squares are D′× 100%; white squares represent low D' and low r^2 values).

Table 2. Hardy–Weinberg equilibrium test

Marker	Exact *p*-value		
	Whole sample	Inward subjects	Outward subjects
rs1928040	0.729	0.178	0.673
rs3742278	0.751	0.333	0.454
rs55948462	0.736	0.183	0.105
rs594242	**0.003**	0.228	**0.029**
rs6311	0.623	0.290	0.833
rs6312	1	0.468	0.585
rs6313	0.742	0.599	1
rs643627	0.246	1	0.204

The chi-squared test was used to assess compliance with the HWE. The SNP not in equilibrium is shown in bold.

phenotype associations were tested by comparing allele and genotype frequency distributions in Inwards and Outwards using the chi-squared and exact tests. Allele and genotype frequencies in Inwards and Outwards were not significantly different except for marker rs55948462, whose genotypes differed significantly ($p < 0.05$) between Inwards and Outwards (chi-squared and exact *p*-values 0.029 and 0.022, respectively).

Analysis of Odd ratios (ORs) (Table 4) shows that the association of rs55948462 with PMO was due to the significantly greater number (2.5 times) of heterozygotes in Inwards compared with Outward subjects (OR = 2.500; CI (95%) = 1.16–5.38).

As significant differences in sex and age between Inward and Outward subjects were observed (*p*-values < 0.05 for both variables), we tested whether the association between HTR2A variants and PMO development was attributable to differences in age- and/or sex-related penetrance. Although our data might suggest an association between HTR2A polymorphisms and PMOs, the results of logistic regression analysis, performed to detect the effect of alleles adjusted for demographic covariates, showed that none of the allele considered significantly affected the probability to develop an Inward or an Outward PMO.

A further regression analysis, performed by grouping AG and GG genotypes of rs55948462 within the same group, showed that G allele increases the probability to develop an Inward PMO ($\beta = 0.75$), although with a poor significance ($p = 0.09$).

Table 3. Statistical analysis results

HTR2A marker	Genotype or allele	Inward subjects		Outward subjects		Chi-squared	Exact
		n	*F*	*n*	*F*	*p*-Value	*p*-Value
rs1928040	CC	13	0.22	21	0.23	0.137	0.137
	CT	23	0.4	48	0.53		
	TT	22	0.38	21	0.23		
	C	49	0.42	90	0.5	0.192	0.167
	T	67	0.58	90	0.5		
rs3742278	AA	41	0.71	65	0.71	0.345	0.412
	AG	17	0.29	23	0.25		
	GG	0	0	3	0.03		
	A	99	0.85	153	0.84	0.766	0.765
	G	17	0.15	29	0.16		
rs55948462	AA	37	0.65	71	0.79	**0.029**	**0.022**
	AG	20	0.35	16	0.18		
	GG	0	0	3	0.03		
	A	94	0.82	158	0.88	0.204	0.230
	G	20	0.18	22	0.12		
rs594242	CC	45	0.78	72	0.79	0.898	0.945
	CG	11	0.19	15	0.16		
	GG	2	0.03	4	0.04		
	C	101	0.87	159	0.87	0.941	1.000
	G	15	0.13	23	0.13		
rs6311	AA	17	0.29	17	0.19	0.318	0.310
	AG	26	0.45	46	0.51		
	GG	15	0.26	28	0.31		
	A	60	0.52	80	0.44	0.190	0.162
	G	56	0.48	102	0.56		
rs6312	CC	0	0	1	0.01	0.658	0.777
	CT	12	0.21	16	0.18		
	TT	46	0.79	74	0.81		
	C	12	0.1	18	0.1	0.899	1.000
	T	104	0.9	164	0.9		
rs6313	AA	16	0.28	16	0.18	0.273	0.261
	AG	28	0.48	45	0.49		
	GG	14	0.24	30	0.33		
	A	60	0.52	77	0.42	0.112	0.111
	G	56	0.48	105	0.58		
rs643627	AA	27	0.47	49	0.54	0.617	0.636
	AG	25	0.43	32	0.35		
	GG	6	0.1	10	0.11		
	A	79	0.68	130	0.71	0.541	0.508
	G	37	0.32	52	0.29		

Allele and genotype occurrences (*n*) and frequencies (*F*), chi-squared and exact *p*-values in Inwards and Outwards. Significant results are in bold and underlined.

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Table 4. OR results

HTR2A marker	Genotype	OR	CI (95%)
rs1928040	CC	0.949	0.43–2.09
	CT	0.575	0.29–1.12
	TT	2.008	0.98–4.13
rs3742278	AA	0.965	0.47–1.99
	AG	1.226	0.59–2.56
	GG	–	–
rs55948462	AA	0.495	0.24–1.04
	AG	**2.500**	**1.16–5.38**
	GG	–	–
rs594242	CC	0.913	0.41–2.03
	CG	1.186	0.5–2.8
	GG	0.777	0.14–4.38
rs6311	AA	1.805	0.83–3.91
	AG	0.795	0.41–1.54
	GG	0.785	0.38–1.64
rs6312	CC	–	–
	CT	1.223	0.53–2.81
	TT	0.881	0.39–2.01
rs6313	AA	1.786	0.81–3.93
	AG	0.954	0.49–1.84
	GG	0.647	0.31–1.36
rs643627	AA	0.746	0.39–1.44
	AG	1.397	0.71–2.74
	GG	0.935	0.32–2.73

ORs and CI 95% in Inwards and Outwards. Significant results are in bold and underlined. – : no subjects with this genotype (Table 3).

Taking into account that some of the SNPs were in LD and therefore not independent, the experiment-wide corrected significance threshold was $p_{corr} = 0.007$. Although the rs55948462 SNP was associated with development of an Inward PMO, after adjustments it followed that these results did not remain significant.

Discussion

Knowledge of the genetic bases of PMO development would permit a greater understanding of the psychotherapeutic process and its epistemological paradigms. New technologies now make it possible to observe that valuable approaches, like post-rationalist constructivism, not only have solid theoretical and epistemological bases but are also supported by specific neural patterns (e.g. biological differences in emotional activation in Inward and Outward subjects) and by polymorphism studies. This can explain how attachment processes have both learned and genetic bases.

We report the first association study of 10 serotonin receptor 2A (HTR2A) gene SNPs in Inward and Outward PMOs. The approach used to select phenotype-associated SNPs was a typical case-control test, a valuable method to identify polymorphisms involved in the development of a particular phenotype.

HWE analyses showed that the genotype frequency distributions of all HTR2A tested markers were in HWE, except for rs594242, which remained in HWD in the whole population sample and in the Outward group. In general, Hardy-Weinberg disequilibrium (HWD) data can indicate a sound disease-locus association (42,43), and in our study, it can suggest that rs594242 affects the development of Outward PMO. Deviation from HWE in a population sample could provide information on whether genotyping errors may have occurred at particular marker loci, but it also could be due to other genetic factors, e.g. protective alleles, genetic drift, population admixture, population stratification, inbreeding or deletions (44) and can be related to de novo mutations and deletions in cases, or to benign polymorphisms that are in LD with the risk locus (45). In this work, pairwise LD analysis showed a moderate LD of rs594242 with the Block 1 found in the gene region (Fig. 1). Because deviations from HWE can be detected for polymorphisms that are in LD with the risk locus (45), the HWD at rs594242 found in the whole population sample and in the Outward group could indicate a gene affecting PMOs development lying in the region. However, the low r^2 value indicates that a larger sample could be required to achieve a similar power to detect association at this locus that would be afforded by a causal locus.

We found that rs55948462 SNP alone is associated with Inward/Outward PMOs development. This SNP lies in the second intron of the HTR2A gene. According to our predictions, the rs55948462 G allele can create an ectopic 5′ splice site. This change could cause an alternative splicing event, resulting in an additional receptor form, to the detriment of the more abundant one. This modified receptor structure could affect serotonin response, i.e. receptor sensitivity and selectivity. Given that the G allele frequency is higher than about 10% in all populations according to NCBI dbSNP, a pathological effect of this allele is not expected. In this study, we only have indications that G allele could affect the PMO development. Unfortunately in our sample, there are no GG Inwards so we are not able to show an association with this genotype. However, after correction for multiple comparisons and adjustment for age and sex using logistic regression, the association between HTR2A gene region and PMOs is lost.

HTR2A gene variants have been investigated in several gene association studies in relation to various psychiatric disorders, but their findings are conflicting and generally negative. The most consistent data point to the C allele of rs6313 as a risk factor for psychosis and as affecting the antipsychotic response. rs6311 and rs6313, also analysed in this study, are among the most widely investigated SNPs

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of the HTR2A gene, but we have not found to be associated with Inward/Outward PMO. Besides, we confirm the complete LD between rs6311 and rs6313 ($D' = 1$) previously reported (46,47), and they belong to the same haplotype block (Block 1, Fig. 1). Moreover, rs55948462 belongs to no haplotype block and is not in LD with other SNPs.

However, these data do not exclude the involvement of serotonin receptor 2A (HTR2A) SNPs in Inward and Outward PMO development. In fact, it is well established that besides psychiatric disorders, also temperamental predisposition and behaviour are influenced by alterations in the serotonergic system (48,49), and, in particular, with regard to behavioural traits, serotonin receptors are known to be involved in anxiety, impulsiveness and aggression (50). Furthermore, serotonin neurotransmission interferes with the endocrine stress reaction. Indeed, increased adrenocorticotropic hormone and corticosterone secretion have been described in rat limbic cortex following HTR2A-agonist administration (51). Clinical observations predict that the reaction will be different, depending on the Inward or Outward focus. Therefore, a polymorphism that can alter receptor specificity or efficiency (i.e. by reducing the firing rate) and thus the activation of neuroendocrine systems in stress situations can play a role in an individual's coping strategies. In other words, not only attachment patterns but also upstream polymorphism arrangement can induce different behavioural skills depending on the Inward or Outward PMO.

Unfortunately, there are no literature data on rs55948462 polymorphism. A recent work found an association between avoidant attachment and the HTR2A rs6313 (T102C) SNP (52), but our data do not show its association to PMO.

In conclusion, our study has potential limitations. One concern is reduced power because of the limited sample size. Also, the possible selection bias that might have been present in the academic-based, case-control study is a relevant issue. Finally, as our study was performed on Italian subjects, these gene SNPs may have significant effects in other ethnic populations. Therefore, rs55948462 should be further investigated to assess that the Inward and Outward PMOs have a genetic basis. Also the role of HTR2A polymorphisms not considered in this study and that of the other serotonin related genes should be valued.

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