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Association of serotonin and dopamine gene pathways with behavioral subphenotypes in dementia

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Abstract

Genetic association studies investigating the association between genes of serotonergic and dopaminergic systems and behavioral and psychological symptoms in dementia (BPSD) are contradictory. We have utilized 1008 probable Alzheimer's disease (AD) patients from the UK and used the 12-item Neuropsychiatric Inventory. We applied a multiple indicators-multiple causes (MIMIC) approach to investigate the effect of 11 polymorphisms on the 4 behavioral subphenotypes "psychosis", "moods", "agitation", and "behavioural dyscontrol". Significant associations were observed between the serotonin transporter gene (SERT) polymorphism STin2 and "psychosis"; the dopamine transporter gene (DAT) 3′ variable number tandem repeats (VNTR) and "agitation"; and the dopamine receptor 4 (DRD4) VNTR and "moods" factors. Direct associations were identified between the dopamine receptor 3 (DRD3) BalI polymorphism and depression; the dopamine receptor 1 (DRD1) and dopamine transporter gene 3′ VNTR polymorphisms and aberrant motor behavior; the DRD4 VNTR and sleep disturbances; and the SERT gene VNTR 5HTTLPR and apathy items. Significant interactions observed between polymorphisms suggested epistatic effects and interactions between polymorphisms and medications highlighted potential treatment response. This multiple indicators multiple causes (MIMIC) model efficiently captured the complexity of the interrelations between genetic variation, behavioral symptoms, and clinical variables.
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Keywords: Alzheimer's disease (AD); BPSD; Multiple indicators multiple causes (MIMIC) model; Genes; Dopamine; Serotonin; Medication; Interactions; Covariates; SNP; NPI

1. Introduction

Behavioral and psychological symptoms, such as hallucinations, agitation, or depression occur in the majority of people with Alzheimer's disease and are associated with considerable morbidity to patients and distress to care-givers (Donaldson et al., 1998; Lyketsos et al., 2000; Steele et al., 1990). Family, linkage, and genetic association studies (Bacanu et al., 2005; Hollingworth et al., 2007; Sweet et al., 2002; Tunstall et al., 2000) suggest a genetic component to these behavioral and psychological symptoms in dementia (BPSD). Studies investigating BPSD have focused on dopaminergic and serotonergic neurotransmission, as both systems have been implicated in many aspects of human and animal behavior and are potential targets for treatment

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of BPSD and psychiatric disorders. A number of genetic association studies have examined genes from these systems, including the serotonin receptor genes 5HT2A and 5HT2C, the serotonin transporter gene (SERT), the dopamine receptors DRD1-4 genes, the dopamine transporter gene (DAT) the catechol-O-methyl transferase gene (COMT) and the monoamine oxidase A gene (MAOA) in an effort to define the genetic basis of BPSD, but conflicting results have been reported (Assal et al., 2004; Borroni et al., 2004, 2006b; Craig et al., 2004a, 2006, 2007; Holmes et al., 1998, 2001; Lam et al., 2004; Nacmias et al., 2001; Pritchard et al., 2007b, 2008a, 2008b, 2009; Rocchi et al., 2003; Sweet et al., 1998, 2001, 2005). Inconsistent findings may reflect the small number of patients examined, which in general do not exceed 500, the various measures to define BPSD, and differences in clinical population studies, particularly in relation to disease stage and use of psychotropic medication. BPSD are complex and interrelated and the effects of allelic variants are likely to be individually small, highlighting the need for larger and more systematic approaches and more consistent definitions of abnormal behavior.

This study aimed to investigate associations between genetic variation and the presence of behavioral symptoms using data on 11 polymorphisms from 10 genes, in a large cohort ($n = 1008$) of patients with probable Alzheimer's disease (AD). In addition to associations between genes and BPSD, potential interactions between polymorphisms which may affect the expression of these behavioral symptoms were investigated. Interactions were also investigated between polymorphisms and psychotropic medication to identify potential treatment response. Finally, interactions were sought between the X-linked genes and gender to capture sex-specific effects. The polymorphisms examined have been previously associated with neuropsychiatric conditions, such as depression or schizophrenia, and all of them bar 1 (DRD2 TaqI) have been previously associated with behavioral symptoms in AD.

The co-occurrence of behavioral symptoms in AD has led to the suggestion that distinct behavioral subphenotypes exist. We have previously proposed a multiple indicators multiple causes (MIMIC) model to capture the complexity of the interrelations between behavioral symptoms, subphenotypes, and clinical variables, in the same dataset (Proitsi et al., 2009). Four behavioral subphenotypes, namely "psychosis", "moods", "agitation", and "behavioural dyscontrol" were identified and their associations with each other, as well as with covariates, such as cognitive impairment, gender, age of onset, and disease duration and each other were modeled. MIMIC models have been successfully applied in geriatric research (Gallo et al., 1994; Mast, 2004, 2005), psychiatric studies (Agrawal et al., 2007; Chung and Martin, 2005), and gene × environment studies (Gatt et al., 2009). Here, we aimed to use this model as a platform to test the association between genetic variation and these behavioral symptoms in the presence of covariates. This is a powerful approach which allows the simultaneous analysis of the entire system of variables, by forming specific hypotheses. Such systematic analysis will help shed light on the biological nature of these common and disabling symptoms in AD.

2. Methods

2.1. Subject cohorts

We have used a UK cohort of 1008 participants from the Medical Research Council Genetic Resource for Late-onset AD. AD patients were ascertained by 4 collaborating centers, comprising the Institute of Psychiatry in London, Cardiff University School of Medicine in Cardiff, Trinity College in Dublin, and Cambridge University in Cambridge. All individuals were unrelated white European, recruited through secondary care services, and diagnosed with probable AD in accordance with the National Institute of Neurological and Communication Disorders and Stroke/Alzheimer's Disease and Related Disorders Association clinical diagnostic criteria (McKhann et al., 1984). The 12-item Neuropsychiatric Inventory (NPI) (Cummings, 1997) was used to assess prevalence and severity of BPSD in participants. Frequency and severity scores are multiplied to give an overall domain score for each symptom ranging from 0 to 12. Details on the NPI and the assessment of patients can be found in (Proitsi et al., 2009). Ethical permission was obtained from the relevant Research Ethics Committees.

2.2. Genotyping analyses

DNA was available for all 1008 patients.

2.3. Genotyping of SNPs

The genotypes of the 5HT2A C102T (rs6313), 5HT2C Cys23Ser (rs6318), DRD1 48 A/G (rs4532), DRD2 A1 allele (rs1800479), DRD3 Gly9Ser (rs6280) and COMT Val158Met (rs4680) SNPs were determined by allelic discrimination assays based on fluorogenic 5′ nuclease activity: TaqMan single nucleotide polymorphism (SNP) genotyping assays were performed the ABI Prism 7900HT and analyzed with SDS software according to the manufacturer's instructions (Applied Biosystems, Warrington, UK).

2.4. Genotyping of VNTRs

Genotyping of SERT 5HTTLPR and STin2 VNTRs, MAOA and DAT 3′ UTR promoter variable number tandem repeats (VNTRs) and DRD4 exon 348 bp VNTR were performed using protocols described elsewhere with few modifications (Assal et al., 2004; Edenberg and Reynolds, 1998; Jonsson et al., 2000; Sabol et al., 1999) (Supplementary methods 1).

2.5. Statistical analyses

All polymorphisms were investigated for significant departure from the Hardy-Weinberg Equilibrium (HWE) using PLINK (Purcell et al., 2007). Associations between risk alleles/genotypes for each SNP were examined using the same MIMIC model method described in Proitsi et al. (2009). Structural equation modeling (SEM) analyses were conducted in Mplus version 5.1 (Muthen and Muthen, 2006) using the robust maximum likelihood (MLR) estimator. MLR estimates the parameters by maximum likelihood and the standard errors by asymptotically robust methods using the asymptotic covariance matrix, which successfully addresses issues of nonindependence of observations and nonnormality. Disease duration, cognitive impairment measured by the Mini Mental State Examination (MMSE) (Folstein et al., 1975) (1 = MMSE scores 0–10, 2 = MMSE scores 11–20, 3 = MMSE scores 21–28), current age or age of onset (due to colinearity), gender, Apolipoprotein (APOE) ε4 status, and use of psychotropic medication, such as antipsychotics, antidepressants, and sedatives were used as covariates. To avoid issues of multiple testing, 1 genetic model was tested for each SNP by adding in the MIMIC model the risk or protective allele implicated in previous studies. For the SERT 5HTTLPR polymorphism we investigated for the presence of short allele or genotype, whereas for the SERT STin2 we examined for the presence of 12R repeats. For MAOA we sought for associations with the high activity (4 repeats) alleles of the promoter VNTR. For DAT we sought for associations with either 9 or 10 repeats (9R or 10R) and finally for DRD4, associations were sought with 7 repeats (7R), 4 repeats (4R) or 2 repeats (2R).

Analysis took place in 2 stages. An initial model was developed without polymorphisms (covariates only). A final model was constructed where all polymorphisms, their interactions, and interactions between polymorphisms, medication, and gender were modeled. This revealed the amount of variation on each subphenotype/symptom attributable to the polymorphisms and/or interactions. Models were built using stepwise backward regression. In each step the fit of the simpler model was compared with that of the more complex using the Satorra-Bentler scaled χ^2 test as described in http://www.statmodel.com/chidiff.shtml and the scaling correction factor (MLR), supplied by Mplus, for each model. The test of χ^2 difference continued until the final model was no longer significant using an alpha level of 0.05. Satorra-Bentler scaled χ^2 test was also used to test which of the 2 models had the best fit.

Direct paths between polymorphisms or covariates and NPI items which indicated direct differences in NPI items attributed to each polymorphism/covariate after controlling for the factor, (differential item functioning, DIF), were estimated as described before (Proitsi et al., 2009). After this, a significant effect of the polymorphism on the factor would imply differences on the latent mean score. To simplify interpretation, associations were performed assuming no directionality between the factors but measuring their correlations.

As described in Proitsi et al. (2009), the χ^2 test relative to the degrees of freedom was used to assess the model. The root mean squared error of approximation (RMSEA) and the comparative fit index (CFI) were used to evaluate fit of each model tested. Modification indices (MI) were included if they were >8 (modification indices >3.84 for 1 degree of freedom are indicative of significant drop in the χ^2 if the path is freed) and whether they were accepted from a theoretical standpoint.

2.6. Power calculations

Power calculations were performed using QUANTO (Gauderman, 2002).

3. Results

The key demographic characteristics of the 1008 patients are presented in Table 1 and the frequencies of the alleles examined for each polymorphism are presented in Table 2. Power calculations were made using the allele frequencies in Table 2. Assuming a type I error rate of 0.05 and using a 2-sided test, this study gave us >75% power to detect the effect a gene with a minor allele frequency of 0.1 explaining a 1% proportion of variance of a trait and >75% power to detect a significant interaction between 2 genes with minor allele frequencies of 0.1 which explains 1% proportion of variance, assuming a recessive mode of inheritance.

3.1. Multiple indicators multiple causes (MIMIC) model using covariates only (simple model)

An initial model assessed the effect of covariates on the factor structure as described in Proitsi et al. (2009). This model consisted of 4 behavioral subphenotypes: "psychosis", "agitation", "moods", and "behavioral dyscontrol". The model controlled for gender, age of onset, disease duration, MMSE score, and APOE ε4 status. Some differences were observed to the previously published model because the present cohort utilized the 12-item NPI (instead of the 10-item NPI used in Proitsi et al., 2009), did not use

Table 1
Basic characteristics of the sample (n = 1008)

	Mean (SD), range
Age (years)	81.6 (6.5), 63–100
Age at onset (years)	76.1 (6.6), 60–95
Disease duration (months)	66.2 (39.3), 0–192
MMSE score	12.8 (8.8), 0–28
Females/males (%)	726/282 (72/28)
Antipsychotics	158 (17%)[a]
Antidepressants	229 (24%)[a]
Sedatives	115 (12%)[a]

Key: MMSE, Mini-mental State Examination.
[a] n = 946.

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Table 2
Polymorphisms investigated for significant associations with BPSD

Gene	Chromosome	Polymorphism	Rs	Type	Genetic model examined	Frequency of examined allele
5HT2A	13	102 T/C	6313	Synonymous	CC + CT versus TT	0.402
5HT2C	X	68 C/G-Cys23Ser	6318	Nonsynonymous	GG + CG versus CC	0.170
SERT	17	40-bp insertion/deletion in promoter		VNTR	SS + LS versus LL (S: short allele; L: long allele)	0.423
SERT	17	9,10, or 12 repeats of STin.2		VNTR	presence of 12 repeats (12R)	0.592
MAOA	X	3–5 repeats of VNTR in promoter		VNTR	1. Presence of 3 repeats 2. Presence of 4 repeats	0.329 0.641
DAT	5	40-bp promoter VNTR	28363170	VNTR	1. Presence of 10 repeats (10R) 2. Presence of 9 repeats (9R)	0.726 0.269
COMT	22	G/A-Val/158/Met	4680	Nonsynonymous	GG + GA versus AA	0.464
DRD1	5	A/G 48 bp 5′ of mTSS (A48G)	4532	Promoter	GG + GA versus AA	0.386
DRD2	11	A1 allele (TaqI)	1800479	3′ of the gene	A1A1 + A1A2 versus A2A2	0.186
DRD3	3	BalI biallelic polymorphism Gly9Ser	6280	Nonsynonymous	CC and CT versus TT	0.323
DRD4	11	48 bp repeat in exon 3		VNTR	1. Presence of 7 repeats 2. Presence of 2 repeats	0.189 0.107

Key: BPSD, behavioral and psychological symptoms in dementia; COMT, catechol-O-methyl transferase gene; DAT, dopamine transporter gene; DRD, ; MAOA, monoamine oxidase A gene; Rs, ; SERT, serotonin transporter gene; VNTR, variable number tandem repeats.

a disease duration cut-off point of 2.5 years, included only patients from the MRC Genetic Resource Centre, and correlations rather than directions between the factors were modeled. In addition, this study controlled for use of antipsychotics, antidepressants, and sedatives. Associations are presented in Supplementary Fig. 1 and Supplementary Tables 1–4.

The model had a good fit ($\chi^2 = 117.86$, $df = 106$, $p = 0.203$, RMSEA = 0.011, CFI = 0.993, MLR = 1.166), and the covariates explained 16.7% of the variability of "psychosis" factor, 10% of the variability of "agitation" factor, 5.7% of the variability of "moods" factor, and 36% of the variability of "behavioral dyscontrol" factor.

3.2. MIMIC model using covariates, polymorphisms and their interactions

A full MIMIC model was then built by adding the polymorphisms described in Table 2 and looking for interactions between (1) polymorphisms, which could highlight epistatic effects, (2) polymorphisms and medication, which could modify the effect of medication on behavioral symptoms, and (3) polymorphisms and gender because the MAOA and 5HTC genes are on the X chromosome (Figure 1, Table 3).

A negative association was observed between the SERT STin2 12R allele and "psychosis" ($\beta = -0.670$, SE = 0.300, $p = 0.025$). DAT 10R was associated with higher "agitation" ($\beta = 0.928$, SE = 0.310, $p = 0.003$), and the DRD4 2R allele with higher "moods" levels ($\beta = 0.653$, SE = 0.226, $p = 0.004$). Direct associations were observed between the DRD3 BalI C and lower depression ($\beta = -0.082$, SE = 0.030, $p = 0.007$), the DRD1 G allele and higher irritability as well as lower aberrant motor behavior (AMB) ($\beta = -0.547$, SE = 0.211, $p = 0.01$ and $\beta = 0.44$, SE = 0.193, $p = 0.023$, respectively), the DAT 10R allele and higher AMB ($\beta = 0.90$, SE = 0.344, $p = 0.009$), the STin2 12R allele and less apathy ($\beta = -0.544$, SE = 0.202, $p = 0.007$), and between the DRD4 2R allele and increased sleep abnormalities ($\beta = 0.637$, SE = 0.297, $p = 0.032$). No associations were observed between any genes and covariates, except for that of APOE ε4 and age of onset.

The interactions investigated were between polymorphisms reported to interact with each other in previous BPSD studies or other neuropsychiatric disorders. In more detail, we investigated whether the DAT polymorphism interacts with the DRD1, DRD4, HTTLPR, or COMT polymorphisms, whether the DRD1 polymorphism interacts with the DRD3 or DRD4 polymorphisms, whether HTTLPR interacts with the COMT, MAOA, DRD4 or STin2 polymorphisms and whether the MAOA polymorphism interacts with the COMT or DRD4 polymorphisms. An interaction was observed between the HTTLPR SS genotype and the COMT G allele. Bearers of the HTTLPR SS genotype who did not bear COMT G alleles had higher "psychosis" levels ($\beta = 1.69$, SE = 0.534, $p = 0.029$) (Fig. 2). An interaction was also observed between the DAT 10R and the COMT G allele. Although presence of DAT 10R was associated with higher "agitation", absence of both COMT G and DAT 10R was associated with lower "agitation" ($\beta = -1.349$, SE = 0.551, $p = 0.014$) (Fig. 2). Finally, an interaction was observed between the SERT HTTLPR S and the DAT 10R alleles. Patients who carried neither the SERT HTTLPR S allele nor the DAT 10R allele had lower "moods" scores ($\beta = -1.094$, SE = 0.538, $p = 0.042$) compared with carriers of both or either alleles (Fig. 2).

Interactions between polymorphisms and medication showed that use of sedatives was associated with higher "agitation" only in the presence of COMT G allele ($\beta = 1.925$, SE = 0.606, $p = 0.001$). Antipsychotics users had higher "agitation" levels only in the presence of APOE ε4 allele ($\beta = 1.667$, SE = 0.649, $p = 0.010$) and higher

irritability (β = 0.172, SE = 0.068, p = 0.012) in the absence of the SERT HTTLPR S compared with the APOE ε4 noncarriers and SERT HTTLPR S carriers. Finally, bearers of HTTLPR S allele who were treated with sedatives had more eating problems (β = 0.167, SE = 0.053, p = 0.002) (Fig. 3).

No interactions were observed between either the MAOA or the 5HT2C genes and gender. The final full model (Fig. 1, Table 3) explained 19.3% of "psychosis", 13.3% of "agitation", 8.9% of "moods", and 36% of "behavioral dyscontrol" factors (χ^2 = 260.82, df(272), p = 0.676, RMSEA = 0.0, CFI = 1.0, MLR = 1.104) and had a significantly better fit compared with the previous model likelihood ration test (LRT) (χ^2 = 141.54, df(166), p = 0.92) Correlations between covariates, between factors and amount of variance explained for each factor/NPI item are displayed in Supplementary Tables 2–4.

4. Discussion

A number of studies have examined the association of polymorphisms in the serotonergic and dopaminergic system with BPSD but with conflicting results. This may partly be a consequence of small sample sizes and differences in approaches employed. This study has utilized the largest AD cohort so far and has employed a very systematic MIMIC approach to investigate simultaneously the association of 11 common polymorphisms of the serotonergic and dopaminergic pathways and their interactions with both the behavioral subphenotypes and the individual NPI symptoms in AD patients. This study had a minimum of 75% power to

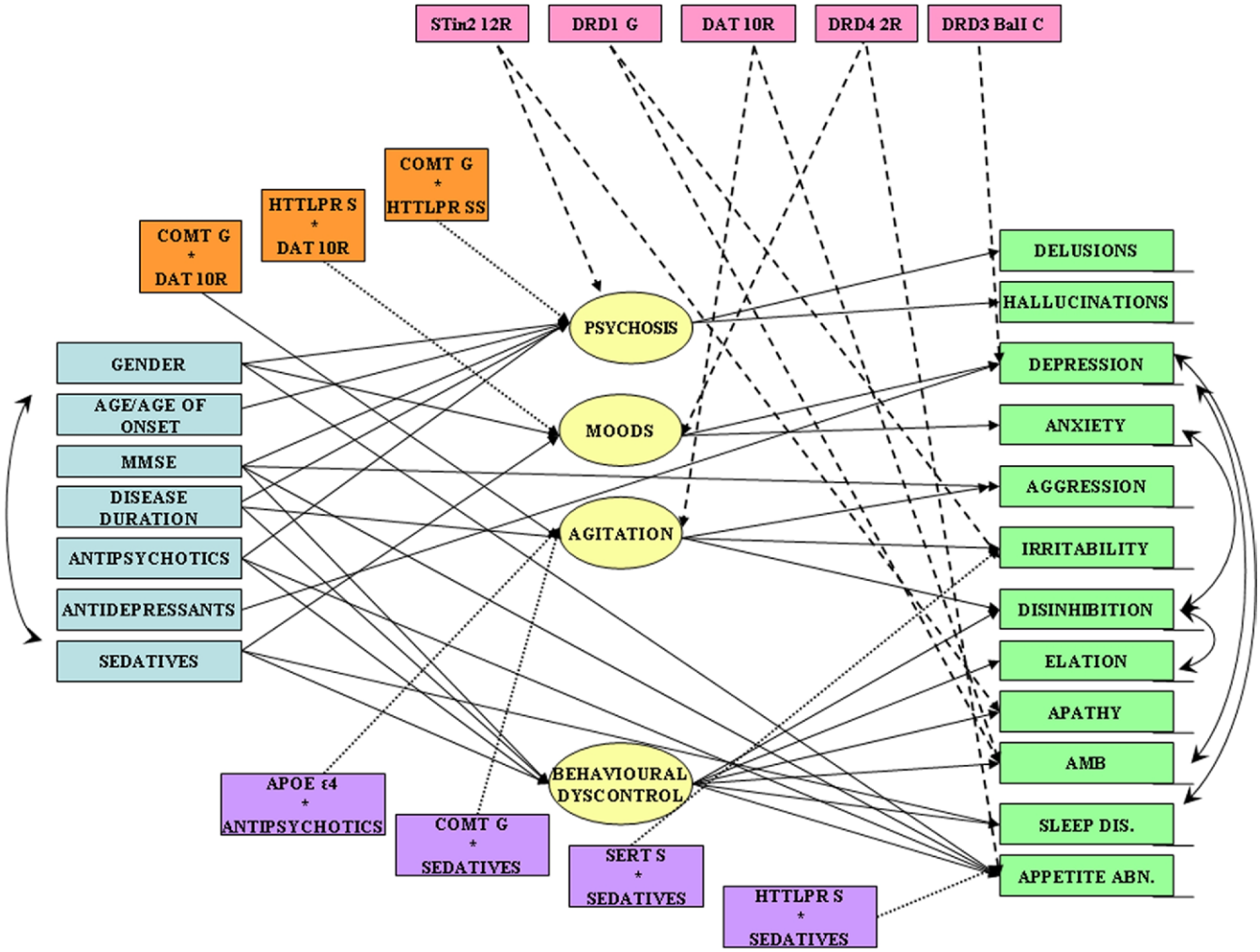

Fig. 1. Multiple indicators multiple causes graphical model of the impact of polymorphisms, covariates, and their interactions on the 4 factors. Measured variables are represented by a box and latent variables are represented by circles. Dashed lines highlight associations between polymorphisms and behavioral symptoms/Neuropsychiatric Inventory (NPI) items. Dotted lines indicate interaction effects. Arrows to individual NPI symptoms indicate a direct effect after keeping the relevant factor constant. Bidirectional arrows on the right of the NPI items show error covariances. Proportion of variance explained for each NPI item and correlations between factors and between covariates are presented in the Supplement. All associations presented are significant at the 0.05 level (except for elation p = 0.143).

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Table 3
Multiple indicators multiple causes model results of the impact of polymorphisms, covariates and their interactions on the 4 factors[a]

Factor (% variance explained)	Variable	β	Standard Error (SE)	p
Psychosis (19.3%)	Gender[b]	0.742	0.192	< 0.001
	Age/age of onset	0.042	0.015	0.005
	MMSE	−0.048	0.013	< 0.001
	Disease duration	0.012	0.003	< 0.001
	Antipsychotics	1.336	0.370	< 0.001
	SERT STin2 12R	−0.671	0.299	0.025
	SERT HTTLPR SS genotype and COMT G allele	−1.529	0.598	0.011
	SERT HTTLPR SS genotype (no COMT G)	1.169	0.534	0.029
	COMT G allele (no SERT SS)	0.110	0.237	0.643
Agitation (13.3%)[c,d,e]	Disease duration	0.009	0.003	0.001
	DAT 10R allele and COMT G allele	−1.349	0.551	0.014
	DAT 10R repeats (no COMT G)	1.973	0.403	< 0.001
	COMT G allele (no DAT 10 R or sedatives)	1.261	0.513	0.014
	Sedatives and COMT G allele	1.925	0.606	0.001
	Antipsychotics and APOE ε4 allele	1.667	0.649	0.010
	Antipsychotics (no APOE ε4)	0.574	0.516	0.265
	Sedatives (no COMT G)	−0.629	0.454	0.166
	APOE ε4 allele (no sedative use)	−0.056	0.186	0.761
Moods (8.9%)[f]	Gender[b]	0.451	0.156	0.004
	Sedatives	0.654	0.243	0.007
	DRD4 2R allele	0.659	0.228	0.004
	SERT HTTLPR S allele and DAT 10R allele	−1.094	0.538	0.042
	SERT HTTLPR S allele (no DAT 10 R)	0.949	0.495	0.055
	DAT 10R allele (no SERT S)	0.893	0.381	0.019
Behavioral dyscontrol (36%)[c,d,e,f]	MMSE	−0.119	0.013	< 0.001
	Disease duration	0.010	0.002	< 0.001
	Antipsychotics	0.671	0.272	0.014
	Sedatives	0.826	0.334	0.013

Key: APOE, apolipoprotein E; COMPT, catechol-O-methyl transferase gene; CRI, ; DAT, dopamine transporter gene; HTTLPR, ; MMSE, Mini Mental State Examination; NPI, Neuropsychiatric Inventory; RMSEA, root mean squared error of approximation; SE, standard error; SERT, serotonin transporter gene; SS, .

[a] $\chi^2 = 260.82$, $df(272)$, $p = 0.676$, RMSEA = 0.0, CFI = 1.0; estimated direct effects of genetic variation and covariates on individual NPI items are noted on the bottom of the table.

[b] Gender coded 0 for males and 1 for females.

[c] Low MMSE was associated with aggression ($\beta = -0.078$, SE = 0.012, $p < 0.001$) and higher MMSE was associated with appetite abnormalities ($\beta = 0.061$, SE = 0.040, $p = 0.001$). Female gender was associated with appetite abnormalities ($\beta = 1.048$, SE = 0.261, $p < 0.001$).

[d] DRD3 allele 2 was negatively associated with depression ($\beta = -0.551$, SE = 0.202, $p = 0.006$), DRD1 G allele was negatively associated with AMB and positive associated with irritability ($\beta = -0.558$ SE = 0.211, $p = 0.008$ and $\beta = 0.486$, SE = 0.191, $p = 0.011$ respectively), DAT 10R had a direct positive association with AMB ($\beta = 0.898$, SE = 0.344, $p = 0.009$), STin2 12R was negatively associated with apathy ($\beta = -0.871$, SE = 0.306, $p = 0.004$), and DRD4 2R was positive associated with sleep disturbances ($\beta = 0.635$, SE = 0.199, $p = 0.033$).

[e] An interaction between sedatives and SERT S allele was associated with higher appetite abnormalities ($\beta = 0.167$, SE = 0.053, $p = 0.002$ for the interaction term). An interaction between antipsychotics and SERT S allele was associated with less irritability ($\beta = -0.158$, SE = 0.067, $p = 0.018$).

[f] Antidepressant use was associated with higher depression levels ($\beta = 0.189$, SE = 0.034, $p < 0.001$), sedative use was associated with higher sleeping disturbances levels ($\beta = 0.183$, SE = 0.040, $p < 0.001$) and antipsychotic use was associated with less appetite abnormalities ($\beta = -0.113$, SE = 0.035, $p = 0.001$).

detect significant associations and interactions that explain at least 1% of the variance of each trait (R^2) for common alleles (Minor allele frequency [MAF] = 0.1).

Some novel associations and interactions between polymorphisms have been identified. In addition, we investigated for associations between medication with behavioral problems and interactions between medication and polymorphisms. It has to be highlighted that the presence of BPSD was recorded at any time over the disease course and that medication use was assessed at baseline. It is not therefore possible to draw conclusions about the response of patients using drugs depending on their genotype because such a question could only be addressed in randomized drug trials. Nevertheless, the interactions between medication and polymorphisms are of great interest because they highlight associations that could be explored in more detail in a different clinical setting. Interestingly, differences between polymorphisms and behavioral subphenotypes were observed only in treated patients, supporting the case that the observed associations may reflect polymorphism-dependent response.

A discussion of the most interesting findings for each subphenotype is given below.

4.1. "Psychosis" subphenotype

A negative association was identified between the presence of the SERT STin2 12R allele and "psychosis". STin2

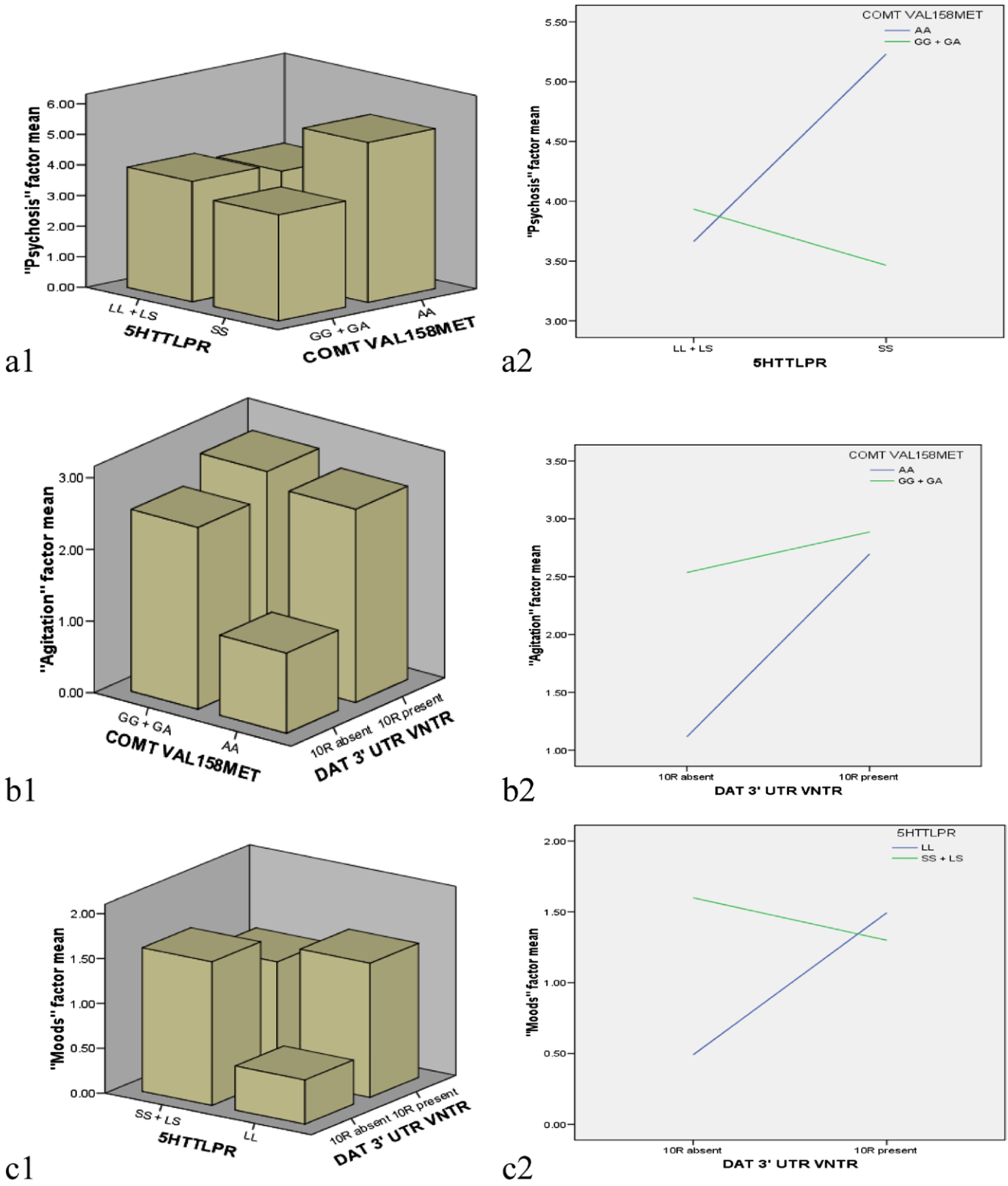

Fig. 2. a1-c1: Boxplots displaying the means of “psychosis”, “agitation” and “moods” factors in the presence of different allelic combinations; a2-c2: Lines highlight the interaction effects between different polymorphisms on the four factors. Differences in the directions of the factor slopes indicate an interaction between the polymorphisms. The y axis represents mean factor scores for the different genotypes on the x axis.

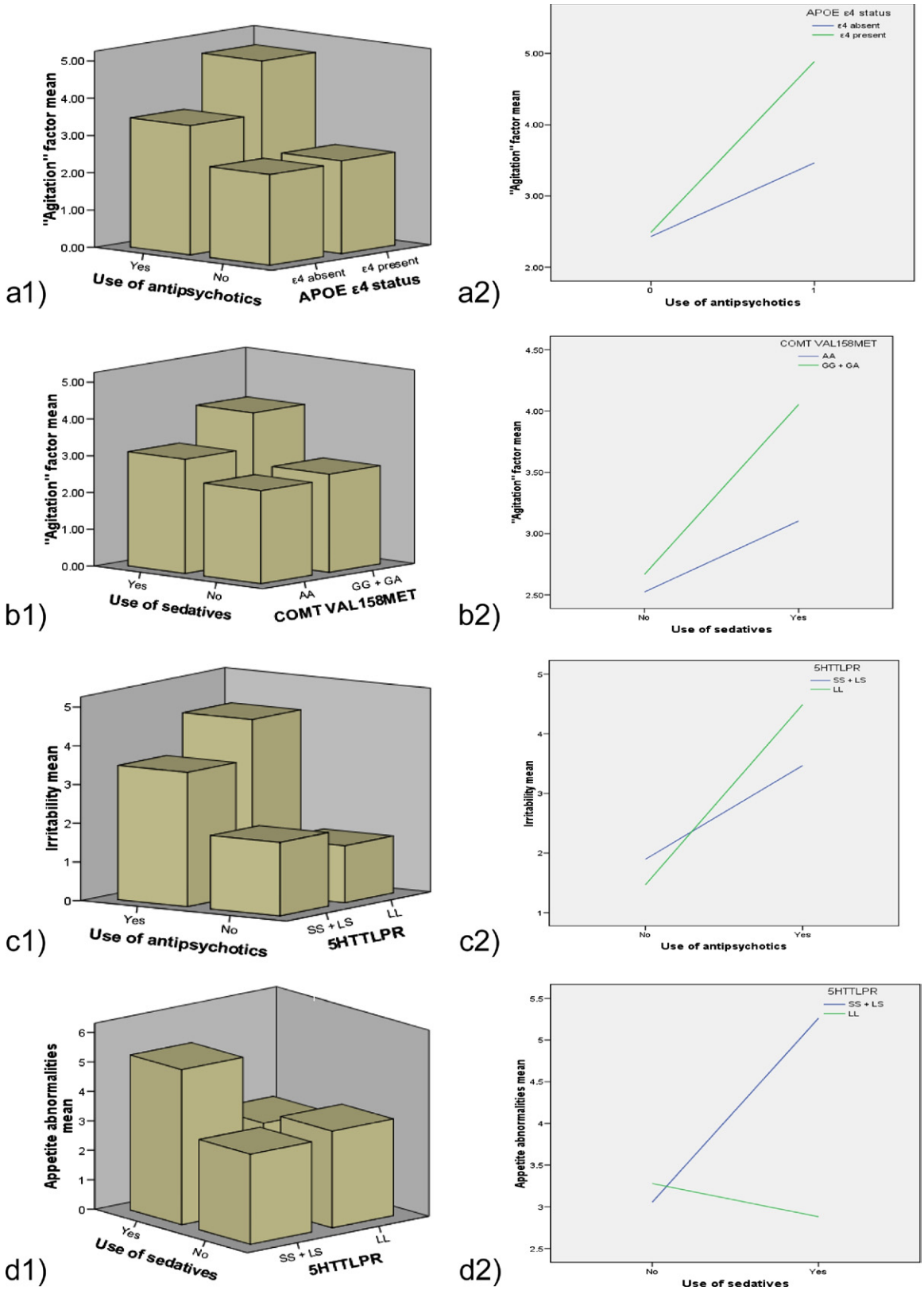

Fig. 3. a1-d1: Boxplots display differences in the means of "psychosis", "agitation" and "moods" factors for different combinations of medication and polymorphic variation; a2-d2: Lines highlight the interaction effects between medication and polymorphisms on the 4 factors. The y axis represents mean factor scores.

12R has been implicated in schizophrenia (Fan and Sklar, 2005) although associations are not consistent. However, Pritchard et al. (2007b) identified a positive association between psychosis and the 10R allele and therefore this association warrants further investigation.

Presence of the SERT HTTLPR SS genotype and absence of COMT G allele was associated with higher "psychosis". Borroni et al. (2006a) reported a cumulative effect of COMT and HTTLPR on "psychosis" but we failed to observe any additive effects and here "psychosis" was associated with the absence rather than the presence of COMT G allele. Although psychotic status has been mainly associated with the presence of the high activity G allele, studies have reported an association with the low activity A allele (Benjamin et al., 2000; Kotler et al., 1999; Lachman et al., 1998; Strous et al., 1997, 2003). Both SERT and COMT are responsible for the inactivation of serotonin and dopamine respectively and the effect of this interaction could be interpreted as the results of both genes producing an excess of monoamines in the synaptic cleft.

4.2. "Agitation" subphenotype

Associations were identified with the DAT and DRD1 polymorphisms and the "agitation" factor and irritability symptoms respectively. The DRD1 G allele corresponds to the B1 allele in the studies published by Sweet et al. (1998), and Holmes et al. (2001) although both studies identified an association with aggression and did not agree on the genetic model. In addition, Pritchard et al. (2009) did not identify any associations between DRD1 and irritability. The present study however employs a larger cohort and in addition, the cohort of Pritchard et al. had moderate cognitive impairment (mean MMSE = 18.6) compared with the present cohort (Table 1). Another intriguing finding was the association of DAT VNTR with "agitation". In addition, the effect of DAT 10R on "agitation" seemed to be modified by the COMT G allele whereby in the absence of COMT G allele (AA genotype), the DAT 10R allele was associated with less "agitation". DAT and COMT regulate synaptic levels of dopamine in the brain, and modulate central dopaminergic function. Interactions between COMT and DAT genes have been reported in cortical regions in relation to schizophrenia (Prata et al., 2009) as well as on reward processing and cognition (Bertolino et al., 2006; Caldu et al., 2007; Yacubian et al., 2007). The DAT polymorphism has been implicated in violent behavior in adolescents (Chen et al., 2003; Guo et al., 2007) and the COMT Val158Met SNP has been implicated in aggression in schizophrenia (Jones et al., 2001) and was associated with lower "frontal" subphenotype by Borroni et al. (2006b). Interestingly, COMT G allele seemed to also modify the effect of sedatives on "agitation". Patients treated with sedatives had higher "agitation" in the presence of the G allele. Interactions between the COMT Val158Met SNP and antipsychotic medication have been reported in schizophrenia (Bertolino et al., 2004; Weickert et al., 2004); however there are no studies to our knowledge investigating their interactions in relation to aggressive symptoms. Use of sedatives could also reflect patients with acute episodes of violence or combined psychotic/aggressive episodes and could underline an association of COMT with this combined phenotype. The associations and interactions of DAT, COMT, and sedatives warrant further investigation.

Another noteworthy association was that of APOE ε4 allele and "agitation". Patients treated with antipsychotics had high "agitation" scores only when they carried the APOE ε4 allele, highlighting that presence of ε4 modifies response to drugs or that APOE ε4 is associated with higher "agitation" when patients also experience "psychotic" symptoms and therefore receive antipsychotics. Studies investigating the association between APOE ε4 allele and BPSD are inconclusive (Craig et al., 2004b; Holmes et al., 1996; Pritchard et al., 2007a; Scarmeas et al., 2002). To our knowledge, no studies have investigated the association of APOE and antipsychotics in AD, although increased APOE levels have been reported in schizophrenia suggesting that APOE could be important in the therapeutic effects of antipsychotics (Dean et al., 2003).

Finally, antipsychotics were associated with higher irritability when the SERT HTTLPR S allele was absent. Such an interaction could indicate that patients lacking the HTTLPR S allele did not respond to antipsychotic treatment or that HTTLPR S allele is associated with irritability when patients also exhibited psychotic symptoms. In support of the latter is the study by Sweet et al. (2001) which found that the HTTLPR S allele was associated with a combined phenotype of psychosis and aggression.

4.3. "Moods" subphenotype

The finding of an association between presence of the DRD4 2R allele and higher "moods" scores is novel. Previous BPSD studies have focused upon the 4 or 7 alleles (Pritchard et al., 2009; Sweet et al., 1998), although the 2R allele has also been implicated in depression (Lopez et al., 2005). Interestingly, Pritchard et al. (2009) reported an association between depression and the decrease of 7R allele/increase of 4R allele. Another interesting finding was between the DRD3 BalI C allele. Only Pritchard et al. (2009) investigated the association between DRD3 BalI and depression but failed to report any associations. DRD3 BalI has been previously implicated in depressive disorders and meta-analyses have shown a weak association (Lopez-Leon et al., 2008) making it a possible candidate for depression.

An interaction was identified between the absence of both the SERT HTTLPR S and DAT 10R alleles, resulting in significantly lower "moods" scores. Both SERT and DAT are responsible for the clearance of serotonin and dopamine from the synaptic cleft and are implicated in depressive disorders and response to antidepressant treatment, and interactions between the 2 polymorphisms have been associ-

ated with harm avoidance and reward dependence traits (Cervilla et al., 2006; Collier et al., 1996; Furlong et al., 1998; Greenwood et al., 2001; Kim et al., 2006; Kirchheiner et al., 2007).

4.4. "Behavioral disturbances" subphenotype

This is the first study to report an association of DRD1 A48G with AMB. Our findings of a significant association between DAT VNTR and AMB are consistent with previous data (Pritchard et al., 2008b). Dopamine is related to motor function and variation in the dopamine receptors or transporter probably reflects abnormal dopamine transmission affecting motor function. Sleep disturbances were also found to be associated with the DRD4 2R allele. Interestingly the 2R allele has been implicated in sleep disturbance following smoking cessation (Vandenbergh et al., 2007). Finally, we found that among patients who take sedatives carriers of the SERT S allele had more eating problems. SERT S allele has been implicated in eating disorders (Lee and Lin, 2009) and such an association would be an interesting one to follow up.

4.5. Conclusions

The significant interactions identified in this study highlights the complexity of the relationships between genes of the dopaminergic and serotonergic systems and BPSD. Monoaminergic systems are interconnected and serotonergic projections from the dorsal raphe nuclei project directly to the substantia nigra and inhibit the firing of dopaminergic neurons (Kapur and Remington, 1996). Interactions therefore between genes involved in the 2 systems, which may modulate behavior, are interesting. Although this study was not appropriate to evaluate drug response, the interactions observed implicate pharmacogenetic correlates which should be considered in future studies. The presence of covariates and genetic variation explained ~20%, 14% 9%, and 36% of the variation of "psychosis", "agitation", "moods", and "behavioral dyscontrol" factors respectively highlighting that there is a large proportion of unexplained variation. Single χ^2 type analyses on the polymorphisms and the individual NPI symptoms indicated that the MIMIC model has captured all the associations that conventional methods would have captured and identified additional relationships which would have been otherwise missed. For example, none of the individual NPI items of aggression, irritability, or disinhibition were significantly associated with DAT 10R in simple regression analysis showing only trends ($p = 0.103$, $p = 0.204$, $p = 0.256$), but the association of the 10R allele with the "agitation" factor in the MIMIC was highly significant ($p = 0.003$).

We have investigated the association between a number of polymorphisms and a complex intercorrelating set of behavioral domains. In this study, where complex patterns of relationships between genes, environmental factors, and behavioral constructs are tested, a MIMIC model is more appropriate than standard analyses based on multiple single polymorphism-behavioral association tests. By using a MIMIC model we have significantly minimized multiple testing and gained power. If single regression analyses were used instead of the MIMIC model, almost 2000 tests would have been performed. In the final MIMIC model there are around 200 associations tested jointly between polymorphisms and factors; if a false discovery rate (Benjamini and Hochberg, 1995) at an $\alpha = 0.05$ was applied for 200 individual tests this would result in rejection of all associations that had a $p \geq 0.007$, so that only ~30% of the significant associations between factors/NPI items and polymorphisms and their interactions would be accepted. If the same false discovery rate was applied to the single regression analysis then only associations with a $p \geq 0.0001$ would be accepted and none of the significant associations would have passed these criteria. MIMIC models are therefore a way of overcoming these issues and reduce the multiple testing penalties that would have been applied otherwise. However, investigating behavioral traits entails the risk not only of accepting false positive associations but overlooking true associations that do not pass standard multiple testing correction criteria. The observed associations should be interpreted with caution and being considered more as an indication of the involvement of the dopaminergic and serotinergic systems in BPSD rather than a definite proof which could lead to wrong inferences. Results should be replicated in larger cohorts which may be easily achieved using the large-scale AD genetic collaborations and be followed by functional approaches.

In summary, the model in Fig. 1 highlights the necessity of systematic statistical approaches, such as MIMIC modeling to be used when investigating the genetic nature of BPSD. This model can be used in future approaches to test for the association of other behavioral subphenotypes with candidates polymorphisms in a simultaneous analysis of the entire system.

Disclosure statement

There are no actual or potential conflicts of interest related to the work described in this report, either by the authors or authors' institutions.

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Appendix. Supplementary data

Supplementary data associated with this article can be found online at doi:10.1016/j.neurobiolaging.2010.06.011.

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