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Genes of the serotonergic and dopaminergic pathways and their interaction affect the expression of Behavioural and Psychological Symptoms in Dementia (BPSD): A Multiple Indicators Multiple Causes (MIMIC) approach.

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ABSTRACT

Although there is evidence for the involvement of genes of serotonergic and dopaminergic systems in the manifestation of the Behavioural and Psychological Symptoms in Dementia (BPSD), genetic association studies are contradictory. We used 1008 probable AD patients from the UK and applied a Multiple Indicators Multiple Causes (MIMIC) approach to investigate the effect of 11 polymorphisms in the serotonergic and dopaminergic systems, on four behavioural sub-phenotypes, namely "psychosis"," moods", "agitation" and "behavioural dyscontrol", as well as on 12 NPI items. Significant findings included the association of DRD1 A48G with "psychosis" (p=0.037), the association of DAT1 VNTR with "agitation" (p=0.006) and the association of DRD4 with "moods" sub-phenotype (p=0.008). In addition, associations were identified between DRD1 A48G and DAT1 VNTR with aberrant motor behaviour (AMB) symptoms (p=0.001 and p=0.015 respectively), between DRD4 and sleep disturbances (p=0.018) and between 5HTTLPR and apathy (p=0.033). Finally, significant interactions were observed between COMT Val158Met and 5HTTLPR with "psychosis" (p=0.026), between HTTLPR and STin2 with "psychosis" (p=0.005), between DAT1 3'UTR VNTR and COMT Val158Met with "agitation" (p=0.0001) and between DAT1 3'UTR VNTR and 5HTTLPR with the "moods" factor (p=0.0027). The complexity of the interrelations between genetic variation, behavioural symptoms and clinical variables was efficiently captured by this MIMIC model.

KEYWORDS

Alzheimer's disease (AD), BPSD, Multiple Indicators Multiple Causes (MIMIC) model, genes, neurotransmitters

INTRODUCTION

Behavioural 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 carers (1-3). Family, linkage and genetic association studies (4-7) suggest a genetic component to those Behavioural and Psychological Symptoms in Dementia (BPSD). Studies investigating BPSD have focused on the dopaminergic and serotonergic neurotransmission, as both systems have been implicated in many aspects of human and animal behaviour and are potential targets for treatment 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 1 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 (8-25). Inconsistent findings may reflect the small number of patients examined, which in general do not exceed 500, as well as 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 inter-related 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 behaviour.

This study aimed to investigate associations between risk alleles/genotypes and the presence of behavioural symptoms and sub-phenotypes, using data on eleven

polymorphisms from ten genes, in a large cohort (n = 1008) of patients with probable AD. In addition to associations between genes and BPSD, potential interactions between polymorphisms which may affect the expression of these behavioural symptoms were investigated. Interactions were also investigated between the X-linked genes and gender to capture sex-specific effects. All of the polymorphisms were chosen because they have been previously associated with neuropsychiatric conditions such as depression or schizophrenia and all of them bar one (DRD2 Taq1) have been previously associated with behavioural symptoms in AD.

The fact that behavioural symptoms in AD tend to co-occur has led to the suggestion that distinct behavioural sub-phenotypes exist. We have previously proposed a Multiple Indicators Multiple Causes (MIMIC) model to capture the complexity of the interrelations between behavioural symptoms, sub-phenotypes and clinical variables, in the same dataset (26). Four behavioural sub-phenotypes, 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 modelled. MIMIC models have been successfully applied in geriatric research (27-29), psychiatric studies (30;31) as well gene X environment studies (32). In the current study we aimed to use this model as a platform to test the association between risk alleles/genotypes with these behavioural symptoms and sub-phenotypes in the presence of covariates. This is a powerful approach which allows us to perform a simultaneous analysis of the entire system of variables, by forming specific hypotheses. Such systematic analysis will help shed light into the biological nature of these common and disabling symptoms in AD.

METHODS

Subject cohorts

We have used a UK cohort comprising of more than 1000 participants from the Medical Research Council Genetic Resource for Late-onset AD. AD patients were ascertained by four collaborating centres, 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 (33). The 12-item Neuropsychiatric Inventory (NPI) (34) was used to assess prevalence and severity of BPSD in participants. Details on the NPI and the assessment of patients can be found in (26). Ethical permission was obtained from the relevant Research Ethics Committees.

Genotyping analyses

DNA was available for all 1008 patients.

Genotyping of Single Nucleotide Polymorphisms (SNPs)

The genotypes of the 5HT2A C102T (rs6313), 5HT2C Cys23Ser (rs6318) , DRD1 -48A/G (rs4532), DRD2 A1 allele (rs1800479), DRD3 Gly9Ser (rs6280) and COMT Val158Met (rs4680) polymorphisms were determined by allelic discrimination assays based on fluorogenic 5' nuclease activity: TaqMan Single Nucleotide Polymorphism (SNP) Genotyping Assay (Applied Biosystems,). SNP-specific primers and probes were designed and assays were performed according to the manufacturer's instructions.

Genotyping of Variable Number Tandem Repeats (VNTRs)

Genotyping of the 5HTTLPR, STin2, MAOA and DAT1 VNTRs was performed using protocols described elsewhere with few modifications (8;35-37) (Supplement Methods 1).

Statistical analyses

All polymorphisms were investigated for significant departure from the Hardy-Weinberg Equilibrium (HWE) using the program PLINK (38). Associations between risk alleles/genotypes for each SNP were examined using the same MIMIC model method described in (26). Structural Equation Modelling (SEM) analyses were conducted in Mplus Version 5.1 (39) 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 is appropriate when using non- normally distributed indicator variables such as the NPI items. Disease duration, cognitive impairment as measured by the Mini Mental State Examination (MMSE) (40) (1= MMSE scores 0-10, 2= MMSE scores 11-20, 3= MMSE scores 21-28), current age or age of onset (due to co-linearity between age) gender and psychotropic medication were used as covariates in the MIMIC model. Patients were classified as drug-naïve if they were not receiving any antipsychotic, antidepressant, sedative or anxiolytic medication. In order to avoid issues of multiple testing, one genetic model was tested for each SNP by adding in the MIMIC model the risk or protective allele implicated in previous studies. For the 5HTTLPR polymorphism we investigated for the presence of short allele or genotype (S or SS & LS), whereas for STin2 we examined the association between BPSD and

presence of (12R) repeats. For MAOA we sought for associations between BPSD and the presence of high activity (4 repeats) alleles of the promoter VNTR. For the DAT1 40bp VNTR we sought for associations between BPSD and the presence of either 9 or 10 repeats (9R or 10R) and finally for the 48bp repeat in exon 3 of DAT1, associations were sought between BPSD and the presence of 7 repeats (7R), 4 repeats (4R) or 2 repeats (2R).

Analysis took place in three stages. An initial model was developed without polymorphisms (investigating the presence of covariates). A second model was then constructed where all polymorphisms were added simultaneously in the presence of covariates followed by stepwise backward regression. This would highlight any potential changes in the association of covariates with behavioural sub-phenotypes/symptoms in the presence of genetic variation and would also reveal the amount of variation on each sub-phenotype/symptom attributable to the polymorphisms. Finally, a complete model was built which included interactions between polymorphisms and between polymorphisms and gender. All three models were built using stepwise backward regression. In each step the fit of the simpler model was compared to that of the more complex model by using Satorra-Bentler scaled chi-square test between the two models as described in http://www.statmodel.com/chidiff.shtml. To calculate this test the scaling correction factor, supplied by Mplus, for each model is used. The test of chi-square difference continued until the final fitted model was no longer significant using an alpha level of 0.05. Satorra-Bentler scaled chi-square test was also used to test which of the three models (simple model without genetic information, model with genetic information, and final model with genetic interactions) had the best fit.

Direct paths between polymorphisms or covariates and the factor indicators (i.e. NPI items) which indicated differences in the factor indicators (e.g. delusions) that can be attributed to each polymorphism/covariate after controlling for the factor also know as Differential Item Functioning (DIF), were also estimated as described before (26). This process was used in order to identify direct associations between a polymorphisms and symptoms. After this, a significant effect of the polymorphism on the latent variable would imply differences on the latent mean score. To simplify interpretation, associations were performed assuming no directionality between the factors but measuring their correlations after adjusting for the rest of the variables instead.

As described in Proitsi et al., (2009) (26), the χ^2 test relative to the degrees of freedom, the root mean squared error of approximation (RMSEA), and Comparative Fit Index (CFI) were used to evaluate fit of each model tested. Modification indices (MI) were included if they were >8 (MI>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.

Power Calculations

Power calculations were performed using the program QUANTO (41).

RESULTS

The key demographic characteristics of the 1008 patients are presented on 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. For a quantitative trait design and unrelated individuals, assuming a type I error rate of 0.05 and using a two-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 two genes with a minor allele frequencies of 0.1 which explains 1% proportion of variance. These power values were obtained assuming a recessive mode of inheritance, and are therefore greater when dominant or additive models were used.

Multiple Indicators Multiple Causes (MIMIC) models

A. MIMIC model using covariates only

An initial model to assess the effect of covariates on the factor structure was created using the method described in Proitsi et al., (2009) (26). This cohort utilised the 12 item NPI (instead of the 10 item NPI used in (26)), controlled for use of psychotropic medication, did not use a disease duration cut-off point of 2.5 years and included only patients from the MRC Genetic Resource Centre for Late-Onset AD. In addition correlations rather than directions between the four factors were modelled and therefore some small differences to the previously published model were observed (Supplementary Table 1).

Supplementary Table 1 and Supplementary Figure 1 show the associations between covariates and the four factors. Stepwise backwards regression was used as described to generate the model with the best fit. Overall the model had a good fit (χ^2=95.459, df=85, p=0.225, RMSEA=0.011, CFI=0.993), and the five covariates (Gender,

age/age of onset, MMSE, disease duration and psychotropic medication) explained 14.3% of the variability of "psychosis" factor, 8.6% of the variability of "agitation" factor, 13.3% of the variability of "moods" factor and 32% of the variability of "behavioural dyscontrol" factor.

B. MIMIC model using covariates and polymorphisms.

A MIMIC model was then built by adding simultaneously all the polymorphisms described in Table 2. For the DAT1, HTTLPR and DRD4 polymorphisms individual models were first tested and only the alleles of each polymorphism showing significant associations or trends were added in the final model. Stepwise backward regression was used as before.

Adding the polymorphisms in the model indicated a significant association between DRD1 A48G G allele and lower "psychosis" levels (β=-0.093, SE=0.251, p=0.026). A significant association was also observed between DAT1 10R allele and higher "agitation" levels ((β=0.106, SE=0.276, 0.0062). Finally a direct association was observed between DRD4 2R allele and increased sleep abnormalities (β=0.092, SE=0.360, p=0.0114) (Supplementary Figure 2 and Supplementary Table 2). Direct associations were also observed between the DAT1 10R allele and the DRD1 G allele and increased aberrant motor behaviour (AMB) (β=0.077, SE=0.430, p=0.0154 and β=-0.107, SE=0.254, p=0.0015 respectively). (Supplementary Figure 2 and Supplementary Table 2). The inclusion of the polymorphisms in the final model predicted an additional ~1%,~2% and ~1% of the variation of the "psychosis" "agitation" and "moods" factors respectively but it failed to predict any additional variation of the "behavioural dyscontrol" factor. The model had a good fit (χ^2=169.912, df=224, p=0.997, RMSEA=0.011, CFI=0.993) and was a significant

improvement to the model where no genetic variation was added (Supplementary Table 3).

C. MIMIC model using covariates, polymorphisms and interactions between polymorphisms.

The final model allowed for the investigation of specific interactions between different polymorphisms. We looked for interactions between the polymorphisms which showed significant associations with BPSD in the previous model and between polymorphisms that have been reported to interact with each other in previous studies examining BPSD or other neuropsychiatric disorders. In more detail, we investigated whether the DAT polymorphism interacts with DRD1 or DRD4 polymorphisms, whether the DRD1 polymorphism interacts with DRD3 or DRD4 polymorphisms, whether the COMT polymorphism interacts with HTTLPR, MAOA, or DAT polymorphisms, whether HTTLPR interacts with DAT, MAOA, DRD4 or STin2 polymorphisms and finally whether the MAOA polymorphism interacts with DRD4 polymorphism. All of the interactions were added in the previous model where only direct associations with the polymorphisms were included and the final model was produced by using stepwise backwards regression and the Satorra-Bentler scaled chi-square test as before. In addition to the hypothesised interactions between polymorphisms, we also hypothesised that the effect of the MAOA and 5HT2C polymorphisms may be modified by gender, since both genes are on the X chromosome and gender specific association have been reported for both.

As seen in Table 1, all of the previously observed associations between polymorphisms and sub-phenotypes or NPI items remained significant. A very significant interaction was observed between the DAT1 10R and the COMT G

alleles. Patients carrying the 10R allele but no COMT G allele (AA carriers) had significantly lower "agitation" levels (β=-0.395, SE=0.563, p=0.0001) (Figure 2). As seen above, DAT1 10R was significantly associated with higher "agitation" levels and this association was independent of COMT G allele. However, absence of DAT1 10R allele was significantly associated with lower levels of "agitation" only in the absence of COMT G allele.

We also observed an interaction between the HTTLPR SS genotype the COMT G allele. Bearers of the HTTLPR SS genotype who did not bear COMT G allele had significantly higher "psychosis" levels (β=-0.261, SE=0.657, p=0.0255) (Figure 2).

Another significant interaction was observed between the HTTLPR SS genotype and the STin2 12R allele. Patients bearing the HTTLPR SS genotype who did not carry a STin2 12R allele had significantly higher "psychosis" levels (β=-0.481, SE=1.103, p=0.0008) (Figure 2).

Finally, a significant interaction was observed between the HTTLPR S and the DAT1 10R alleles. Patients who carried neither the HTTLPR S allele nor the DAT1 10R allele had significantly lower "moods" factor level (β=-0.597, SE=0.634, p=0.0027) compared to those carrying both or either (Figure 2). In addition, bearers of an S allele who did not carry an 10R allele had significantly higher "moods" levels compared to carriers of an S allele who did carry a 10R allele.

No significant interactions were observed between the rest of the hypothesised interactions except for a marginal interaction between DRD1 and DRD3. It was however not included in the final model since it did not result in significant fit improvement, in contrast to the rest of the interactions.

No interactions were observed between either the MAOA or the 5HT2C genes and gender except for a weak trend between the absence of high activity MAOA 4R and male gender with agitation (p=0.07; not included in the final model).

In the presence of these interactions a significant association was also observed between the presence of the HTTLPR genotype and higher apathy (β=0.074, SE=0.372, p=0.033).

The final model is presented in Table 3 and Figure 1 and had a very good fit (χ^2= 209.72 df=274, p=0.998, RMSEA=0, CFI=1) and had a significantly better fit than the models which included covariates only or genetic associations with no interactions (Supplementary Table 3). This model explained 19.9% of "psychosis", 11.5% of "agitation", 17% of "moods" and 32% of "behavioural dyscontrol" factor, showing that the interactions predicted an additional 1-3% of the variation of the sub-phenotypes. Associations between the covariates are depicted in Figure 1 (Left side). Lower MMSE was significantly associated with longer disease duration, male gender and lack of psychotropic medication use (all p<0.0001). In addition, older age/age of onset is associated with female gender (p=0.0078) and with shorter disease duration and lack of drug use (both p<0.0001). Finally, long disease duration was associated with use of psychotropic medication (p= 0.0017).

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 due of small sample sizes and differences in approaches employed. This study has utilised the largest AD cohort so far to investigate the association of polymorphic variation in the dopaminergic and serotenergic systems with BPSD. It is also the first study to employ a systematic MIMIC approach to investigating simultaneously the association of 11 common polymorphisms and their interactions with both the behavioural sub-phenotypes and the individual NPI symptoms in AD patients, in the presence of covariates. This study had a minimum of 75% power to detect significant associations and interactions that explain at least 1% of the variance of each trait (R^2) for common alleles (MAF=0.1). Increasing allele frequencies resulted in power estimates of almost 99%.

This study has replicated some previously reported associations between BPSD and polymorphisms of the serotonergic and dopaminergic pathways but has also identified some novel associations. In addition, this study reports novel interactions between polymorphisms which may highlight epistatic effects.

A significant association was identified between DRD1 A48G G allele and "psychosis" sub-phenotype. Allele G corresponds to the B1 allele in the studies published by Sweet et al., and Holmes et al., (15;23). It is interesting that both these studies identified an association between DRD1 A48G and psychotic symptoms, although the reported allele/genotype frequencies were not in full agreement. Here, we replicate the finding of Sweet and partly that of Holmes and report an association between the presence of allele G (GG and GA) and lower level of "psychosis" factor. Of note, a similar association between DRD1 A48G and psychosis has been reported in young adults with schizophrenia (42).

This is the first study to report an association of DRD1 A48G with AMB. As with the association with "psychosis", allele G was associated with lower AMB symptoms. Although the most recent to date investigating the association of DRD1 A48G and BPSD (21) did not identify any significant associations, the present study employs a much larger cohort and in addition, the cohort of Pritchard et al (52) had moderate cognitive impairment (mean MMSE =18.6) compared to the present cohort (Table 1). Of note, our study does not report an association between "psychosis" and the 5HT2A C102T SNP, which reported in a recent meta-analysis (43) to be significantly associated with psychotic symptoms in AD.

Our findings of a significant association between DAT1 3'UTR VNTR and AMB are consistent with previous data (19). In addition, we identified a significant association between the DAT1 10R allele and higher scores of the "agitation" sub-phenotype. The finding between the DAT1 VNTR and "agitation" is an intriguing one, since the polymorphism has been implicated in violent and anti-social behaviour in adolescents (44;45) and further investigation into the role of DAT1 in aggressive symptoms is warranted.

The finding of an association between presence of the DRD4 2R allele and higher 'moods' scores is a novel one. Previous BPSD studies have focussed upon the 4 or 7 alleles as risk factors (21;23), although the 2R allele has also been implicated in depressive symptomatology (46) and in sleep disturbance following smoking cessation (47). Pritchard et al., (2009) (57) reported an association between depression and the decrease of 7R allele/increase of 4R allele. They also reported an association between the presence of aggression/agitation and a decrease in 4R allele/increase in 7R allele. Here, we did not identify any significant associations with the 7R or 4R alleles and any symptoms/factors.

This study also identified a trend between the DRD3 BalI polymorphism and the "moods" factor. Only one study (21) has investigated the association between DRD3 BalI and depression, failing however to report any significant associations. DRD3 BalI has been previously implicated in depressive disorders, although meta-analyses have shown a weak association (48).

A number of significant interactions between different polymorphisms were also identified. Epistatic interactions have been rarely investigated in BPSD and only two study to date (23;49) has reported additive effects between COMT and HTTLPR, as well as between DRD1 and DRD3 on psychosis. Specific hypotheses on the effect of epistatic interactions on the behavioural sub-phenotypes and the individual NPI items were tested based on the results from the initial models and previously published associations.

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 DAT1 10R allele was significantly associated with lower levels of "agitation", whereas in the presence of COMT G there was no significant difference in "agitation" levels between DAT1 alleles (Figure 2c). DAT and COMT regulate synaptic levels of dopamine in the brain, and thus, modulate central dopaminergic function. DAT is a presynaptic protein and removes dopamine from the synaptic cleft especially in subcortical regions where it is abundant. In cortical regions however, where DAT is less abundant regulation of dopamine is achieved though intracellular degradation by COMT (50;51). Interactions therefore between COMT and DAT genes are of interest and have been reported in cortical regions in relation to schizophrenia (52) as well as on reward processing and cognition (53-55).

An interaction was also observed between the HTTLPR SS genotype and the absence of COMT G allele (presence of the low activity AA genotype) which was associated with higher "psychosis" levels. Borroni et al., (2006) (49) reported a cumulative effect of COMT and HTTLPR (presence of COMT G and HTTLPR S allele) on "psychosis" although we failed to observe any additive effects and here "psychosis" is 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 allele (56-60). Both SERT and COMT are responsible for the inactivation of serotonin and dopamine respectively and the effect of such an interaction here could be interpreted as the results of both genes producing an excess of monoamines in the synaptic cleft.

Another interesting finding was that the HTTLPR SS genotype seemed to have a significant effect on "psychosis" only in the absence of the STin2 12R allele (a weak trend was observed between STin 12R and "psychosis" prior to the addition of the interactions in the model; data not presented). Such a significant association is of interest since it highlights potential haplotypic effects. Variable patterns of LD have been observed between the HTTLPR and STin2 polymorphisms, although in this study LD was modest (D'=0.5). This relationship is of interest because of the involvement of both polymorphisms with "psychosis" in BPSD (18) as well as schizophrenia. It is also very interesting that the recent meta-analysis on psychosis in AD (43) failed to support the involvement of HTTLPR with "psychotic" symptoms; a main contributor to this could be its potential interaction with other SERT polymorphisms.

Finally an interaction was also identified between the absence of both the HTTLPR S and the DAT1 10R alleles which resulted in significantly lower "moods" levels (or

between HTTLPR S carriers carrying no DAT1 10R allele which had significantly higher "moods" levels). Both HTTLPR and DAT1 are responsible for the clearance of serotonin and dopamine respectively from the synaptic cleft and are both implicated in depressive disorders and response to anti-depressant treatment, and interactions between the two polymorphisms have been associated with harm avoidance and reward dependence traits (61-66).

The significant interactions identified in this study highlight 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 (67). Interactions therefore between genes involved in the two systems, which may modulate behaviour, are very interesting. However, it has to be highlighted that although the present study is well powered to detect these interactions, the combination of allele responsible for the significant effects observed were present in 2-7% of the patients which highlights the need for even larger cohorts. The significant genetic associations and interactions reported here were weak and individually only explained an addition 1-5% of each trait investigated, as expected when investigating behavioural phenotypes. Overall the presence of covariates and genetic variation explained ~ 20%, 12% 17% and 32% of the variation of "psychosis", "agitation", "moods" and "behavioural dyscontrol" factors respectively highlighting that there is a large proportion of unexplained variation.

This is the largest study to date investigating the association between BPSD and genetic variation in the serotonergic and dopaminergic systems. Single chi-square type analyses on the tested polymorphisms and the individual NPI symptoms have 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, in the MIMIC model the DRD1 G allele was significantly associated with "psychosis" factor after controlling for the individual NPI items, although regression analysis (controlling for the same covariates) indicated an association only with delusions. More interestingly, none of the individual NPI items of aggression, irritability or disinhibition was significantly associated with DAT 10R allele in simple regression analysis which showed non significant trends ($p=0.103$, $p=0.204$, $p=0.256$), but the association of the 10R allele with the "agitation" factor in the MIMIC after controlling for these variables was very significant ($p<0.001$). In addition, separate regression analyses showed that the associations between DRD4 2R allele with depression and anxiety ($p=0.043$ and $p=0.022$) were strengthened when using the latent "moods" construct in the MIMIC model ($p=0.008$) and that association of DRD4 2R with sleep disturbances also became slightly stronger ($p=0.0175$ in the MIMIC model compared to $p=0.030$). Finally the direct associations between DRD1 G and DAT 10R alleles with AMB, after controlling for the presence of the factors, was again strengthened ($p=0.0014$ and $p=0.0149$) compared to simple regression analysis ($p=0.012$ and $p=0.092$ respectively).

All these highlight the usefulness of using such a model which allows the modelling and testing of complex patterns of relationships between genes, environmental factors and behavioural constructs simultaneously in a joint model. In addition, using other methods of analysis would require multiple separate analyses. By using such a statistical approach, power is gained and the multiple comparison problem of standard regression analysis is avoided. Multiple testing was limited here in the examination of different alleles of the VNTRs and in the examination of the interactions. Applying

correction for multiple testing, such as Bonferroni correction would result in very few of the significant associations passing the threshold of significance even in the case of the MIMIC model since the p values observed were moderate. However, when examining behavioural traits, moderate p values are expected and application of multiple testing criteria could potentially mask variants of small effect size and overlook significant associations. Results should however be replicated in larger cohorts and this may be easily achieved using the large scale genetic collaborations on AD. Another advantage of structural equation modelling approaches such as MIMIC compared to other commonly used statistical methods is that these modelling methods allows the estimation and partitioning of measurement error. When measurement error exists, estimated parameters can be biased (68).

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

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DISCLOSURE STATEMENT

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Table 1. Basic characteristics of the sample (n=1008). [1]n=951

	Mean,(SD), range (n=1008)
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)
Psychotropic medication use (%)	398 (39.5%)[1]

Table 2. Polymorphisms investigated for significant associations with psychosis in AD

Gene	Chromosome	Polymorphism	Rs	Type	Genetic Model examined	Frequency of examined allele
5HT2A	13	102 T/C	6313	Synonymous	CC+CT vs TT	0.402
5HT2C	X	68C/G- Cys23Ser	6318	Non-synonymous	GG + CG vs CC	0.170
SERT	17	40 bp insertion/deletion in promoter		VNTR	SS + LS vs LL	0.423
SERT	17	9,10 or 12 repeats of STin.2		VNTR	Presence of 12 repeats (12R)	0.592
MAOA	X	3 to 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	Non-synonymous	GG+GA vs AA	0.464
DRD1	5	A/G 48 bp 5' of mTSS (A48G)	4532	Promoter	GG + GA vs AA	0.386
DRD2	11	A1 allele (Taq 1)	1800479	3' of the gene	A1A1 + A1A2 vs A2A2	0.186
DRD3	3	Bal I biallelic polymorphism Gly9Ser	6280	Non-synonymous	CC and CT vs TT	0.323
DRD4	11	48bp repeat in exon 3		VNTR	1. Presence of 7 repeats 2. Presence of 2 repeats	0.189 0.107

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Table 3. MIMIC model results: impact of genetic variation, genetic interactions, gender, age/age of onset, MMSE, disease duration, and psychotropic mediation use on the "psychosis", "agitation", "moods" and "behavioural dyscontrol" factors.

Estimated direct effects of genetic variation and covariates on individual NPI items are noted on the bottom of the table.

Factor (% variance explained)	Significant covariates and polymorphisms	β	SE	P
Psychosis (19.9%)	Gender	0.125	0.227	0.0006
	Age/Age of Onset	0.165	0.018	0.0001
	MMSE	-0.121	0.014	0.0059
	Disease duration	0.246	0.004	<0.0001
	Psychotropic Medication	0.097	0.258	0.0351
	DRD1 A48G G	-0.09	0.248	0.0372
	HTTLPR SS *COMT G	-0.261	0.657	0.0255
	HTTLPR SS * STin2 12R	-0.584	1.227	0.0053
Agitation (11.5%)[1]	Disease duration	0.197	0.003	<0.0001
	Psychotropic Medication	0.216	0.254	<0.0001
	DAT1 10R repeats	0.284	0.379	<0.0001
	DAT 10R * COMT G	-0.397	0.574	0.0001
Moods (17%)	Gender	0.176	0.201	0.0025
	Psychotropic Medication	0.236	0.237	0.0018
	DRD4 2R	0.189	0.275	0.008
	SERT S * DAT 10R	-0.601	0.632	0.0027
Behavioural Dyscontrol (32%)[1234]	MMSE	-0.354	0.014	<0.0001
	Disease duration	0.213	0.003	<0.0001
	Psychotropic Medication	0.207	0.236	<0.0001

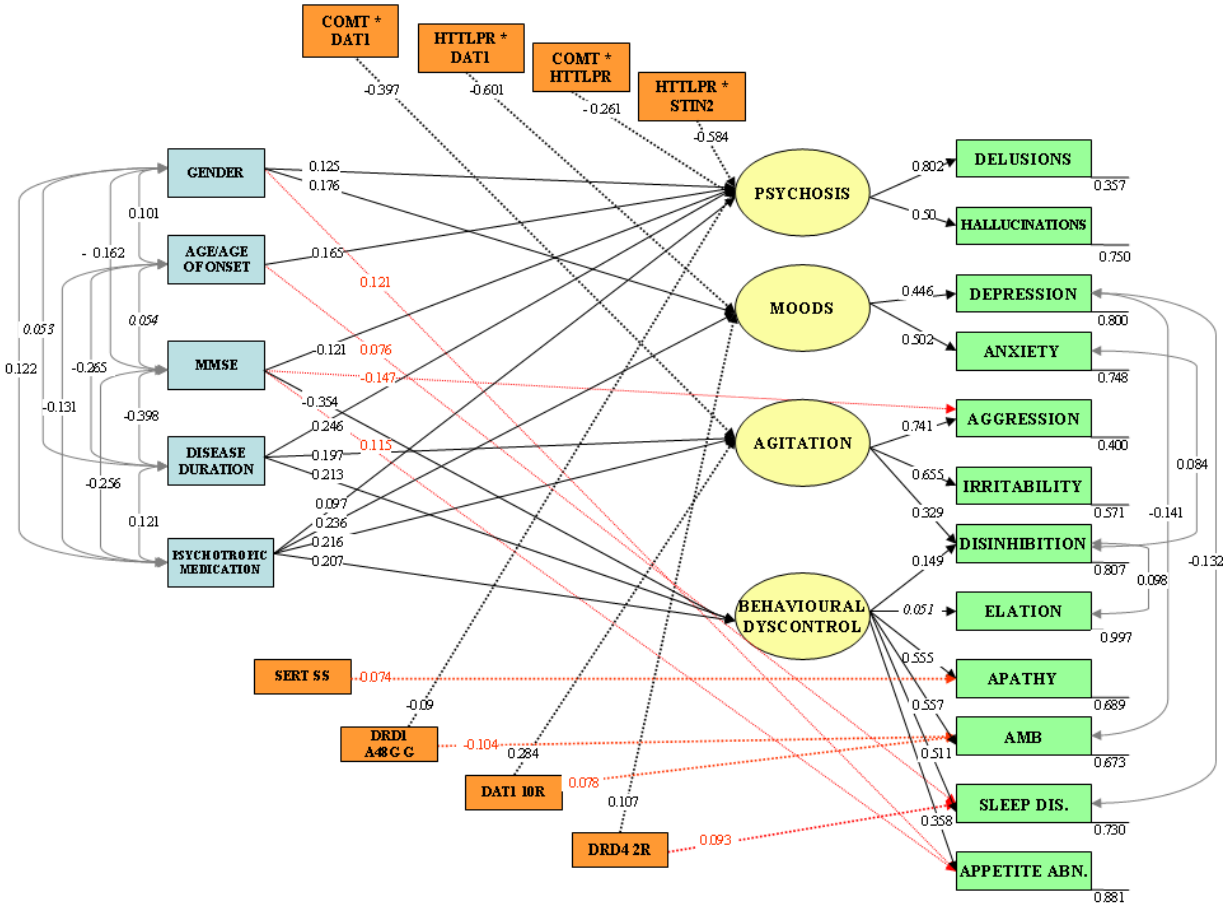
[1]Low MMSE had a significant effect on aggression variable after controlling for "agitation" factor (β =-0.146, SE=0.013, p<0.001) and higher MMSE had a significant effect on appetite abnormalities variable after controlling for "behavioural dyscontrol" factor (β =0.113, SE=0.021, p=0.0123).

[2]Female gender was significantly associated with appetite abnormalities after controlling for "behavioural dyscontrol" factor (β =0.121, SE=0.328, P=0.0007) and sleeping abnormalities were associated with older age/age of onset (β =0.080, SE=0.360, p=0.0343).

[3] DRD1 G allele had a direct negative association with AMB (β =-0.108, SE=0.254, 0.0014), DAT 10R had a direct positive association with AMB (β =0.078, SE=0.431, 0.0149) and DRD4 2R had a direct positive association with sleep abnormalities (β =0.088, SE=0.363, p= 0.0175).

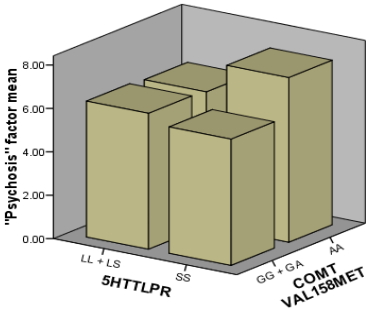
[4]HTTLPR SS had a direct negative association with apathy (β=0.074, SE=0.372, p=0.033). SE= Standard Error

Figure 1. Multiple Indicators Multiple Causes (MIMIC) model examining the impact of genetic polymorphisms and their interaction in the presence of covariates. Measured variables are represented by a box and latent variables are represented by circles. Red arrows indicate a direct effect between genes or covariates and indicators after keeping the relevant factor constant. Bidirectional arrows on the left of the figure indicate correlations between covariates, whereas on the right of the NPI items they show error covariances. The numbers on the left of each NPI item indicate residual error.



All paths drawn indicate significant associations ($p<0.05$) except for association between elation and "behavioural dyscontrol" ($p=0.20$). The four factors were significantly associated with each other at the 0.001 level (correlation of "psychosis" with "agitation", "moods" and "behavioural dyscontrol" factors was $\rho=0.425$, $\rho=0.222$, $\rho=0.313$, correlation of "agitation" with "moods" and "behavioural dyscontrol" was $\rho=0.303$ and $\rho=0.488$ and correlation between "moods" and "behavioural dyscontrol" $\rho=0.588$ respectively).

Figure 2. a1-d1: Boxplots displaying the means of "psychosis", "agitation" and "moods" factors in the presence of different allelic combinations; a2-d2: Lines highlight the interaction effects between different polymorphisms on the four factors. Differences in the directions of the factor slopes indicates an interaction between the polymorphisms. The y axis represents mean factor scores for the different genotypes on the x axis.



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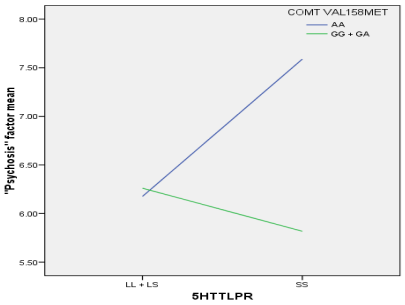

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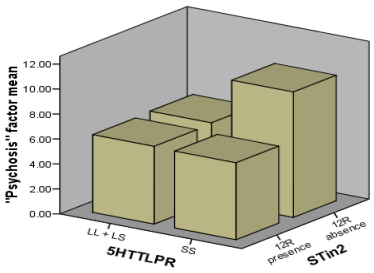

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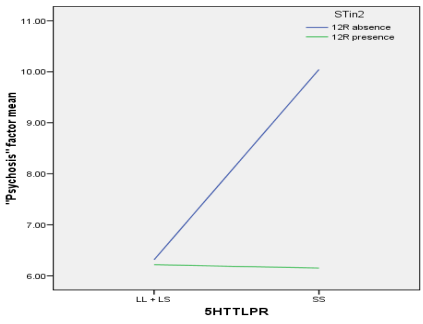

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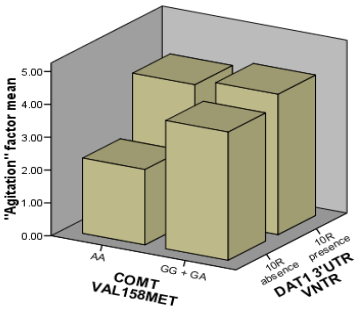
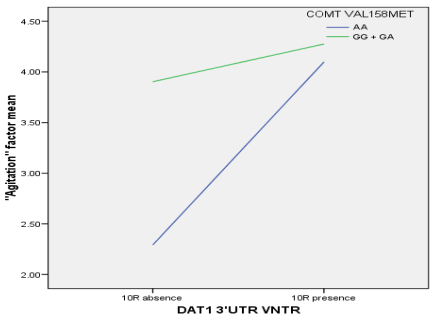


c1



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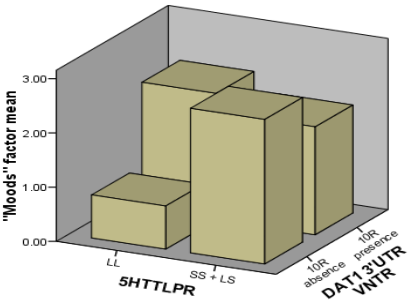

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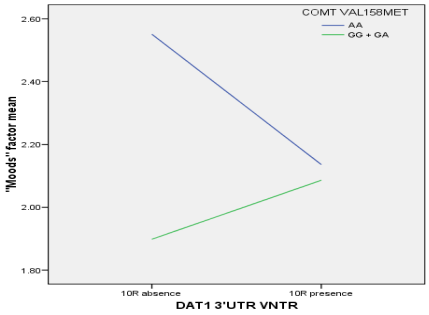

d2