Research paper

Associations between a locus downstream DRD1 gene and cerebrospinal fluid dopamine metabolite concentrations in psychosis

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HIGHLIGHTS

• Dopamine receptor D1 gene variation is significantly associated with cerebrospinal fluid homovanillic acid.
• The significant associations are restricted to psychotic patients.
• The results support the dopamine hypothesis of schizophrenia.

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ABSTRACT

Dopamine activity, mediated by the catecholaminergic neurotransmitter dopamine, is prominent in the human brain and has been implicated in schizophrenia. Dopamine targets five different receptors and is then degraded to its major metabolite homovanillic acid (HVA). We hypothesized that genes encoding dopamine receptors may be associated with cerebrospinal fluid (CSF) HVA concentrations in patients with psychotic disorder.

We searched for association between 67 single nucleotide polymorphisms (SNPs) in the five dopamine receptor genes i.e., DRD1, DRD2, DRD3, DRD4 and DRD5, and the CSF HVA concentrations in 74 patients with psychotic disorder. Nominally associated SNPs were also tested in 111 healthy controls.

We identified a locus, located downstream DRD1 gene, where four SNPs, rs11747728, rs11742274, rs265974 and rs11747886, showed association with CSF HVA concentrations in psychotic patients. The associations between rs11747728, which is a regulatory region variant, and rs11742274 with HVA remained significant after correction for multiple testing. These associations were restricted to psychotic patients and were absent in healthy controls. The results suggest that the DRD1 gene is implicated in the pathophysiology of psychosis and support the dopamine hypothesis of schizophrenia.

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1. Introduction

Dopaminergic innervation is prominent in the central nervous system and is critically implicated in many central and peripheral functions [5]. The catecholaminergic neurotransmitter dopamine is released from the presynaptic terminals of dopaminergic neurons and exerts its action by targeting five different G-protein-coupled receptors located both pre- and postsynaptically [5]. Dopamine is then degraded to its major metabolite homovanillic acid (HVA) by catechol-O-methyltransferase and monoamine oxidase.

Dopaminergic dysfunction is considered to be implicated in various mental disorders, mainly schizophrenia [5,9,22]. Schizophrenia affects approximately 1% of the world’s population with a heritability up to 80% [37]. Many dopaminergic gene variants have been associated with the disorder, however the results have been ambiguous and difficult to replicate until recently, when a genome-wide significant association was found between the dopamine receptor D2 (DRD2) gene and schizophrenia [1]. The
association between dopaminergic gene variants and measurable biological markers may be more robust and consistent than the associations between gene variants and the disorder itself. Moreover, this approach can shed further light to the understanding of genotype-phenotype associations.

We consider the cerebrospinal fluid (CSF) HVA to be a relevant measurable marker as it reflects the dopamine turnover rate in the central nervous system (CNS), it is partially genetically determined and it has been associated with schizophrenia. Concentrations of HVA in ventricular, cisternal, and lumbar CSF show a cranio-caudal gradient [25,28]. Studies in postmortem human brains have shown that CSF HVA reflects brain HVA concentrations [36,42]. Studies in human twins and other primates have shown that HVA concentrations are partially under genetic influence [30,32]. HVA concentrations are significantly lower in drug-free patients with schizophrenia relative to controls [6,43]. Moreover, quetiapine and olanzapine administration was found to be associated with a significant increase in CSF HVA [26,33].

Dopamine receptors, i.e., D1, D2, D3, D4 and D5, are classified as D1-class dopamine receptors, including D1 and D5 [39], and D2-class dopamine receptors, including D2, D3 and D4 [5,40]. D1 and D2 receptors have the highest level of expression in multiple brain regions, whereas D4 receptors have the lowest level of expression in the brain [5]. Dopamine receptors activation, mainly D1 and D2, affects among others locomotion, reward mechanisms, psychotic symptomatology, sleep and attention [5,24].

The dopamine receptors D1, D2, D3, D4 and D5 are encoded by the dopamine receptor D1 gene (DRD1), the dopamine receptor D2 gene (DRD2), the dopamine receptor D3 gene (DRD3), the dopamine receptor D4 gene (DRD4) and the dopamine receptor D5 gene (DRD5), respectively. DRD1 and DRD5 have no introns, whereas DRD2, DRD3 and DRD4 contain six, five and three introns in their coding regions, respectively. Providing splice variants [16], DRD1 is located on chromosome 5q35.1, DRD2 is located on chromosome 11q23.1, DRD3 is located on chromosome 3q13.3, DRD4 is located on chromosome 11p15.5 and DRD5 is located on chromosome 4p16.1.

In the present study, we have searched for association between DRD1, DRD2, DRD3, DRD4 and DRD5 single nucleotide polymorphisms (SNPs) and dopamine turnover rate in the CNS, as reflected by the CSF concentrations of the major dopamine metabolite HVA, in patients with psychosis.

2. Material and methods

2.1. Subjects

The subjects of the present study have been investigated as previously described [4]. Patients with psychotic disorder were recruited among inpatients at four psychiatric university clinics in Stockholm County between 1973 and 1987 and were asked to participate in pharmacological and/or biological research projects [8]. The participants were observed for at least 48 h without any antipsychotic medication and CSF samples (12.5 ml) were drawn by lumbar puncture.

Three to 34 years after the first investigation, patients were asked to participate in genetic research studies and blood was drawn for genotyping. Patients were then asked to participate in a diagnostic structured interview [35] and permit the researchers to retrieve their medical records. Available records were scrutinized by researchers to obtain a life-time diagnosis according to DSM-III-R and DSM-IV. In 2010, hospital discharge diagnoses were also obtained from the Swedish psychiatric inpatient register, a register covering all inpatient hospitalizations in Sweden since 1973. For each hospitalization the diagnosis was recorded according to the International Classification of Diseases, 8th, 9th or 10th revisions. The majority of the participants had experienced several hospitalizations, but only one diagnosis was given per participant, following a diagnostic hierarchy [14,41]. The final diagnoses, used in the present study, were based on the Swedish psychiatric inpatient register, as it was not possible to retrieve all medical records and several of the patients were not willing to participate in a diagnostic interview.

Analyses in healthy controls were conducted for SNPs that were nominally associated with HVA concentrations in psychotic patients, in order to evaluate whether the effects of the associated SNPs were restricted to patients with psychosis. CSF samples were drawn by lumbar puncture from unrelated healthy Caucasians between 1973 and 1987. Eight to 20 years after the first investigation, the subjects were interviewed to re-assess the absence of psychiatric morbidity [18] and whole blood was drawn for genotyping.

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Karolinska University Hospital. Written informed consent was obtained from all the participating subjects.

2.2. CSF monoamine metabolite concentrations

CSF samples (12.5 ml) were drawn by lumbar puncture with the patients and controls in a sitting or recumbent position between 8 and 9 a.m., after at least 8 h of bed-rest and absence of food intake or smoking. HVA concentrations were measured by mass fragmentography with deuterium-labeled internal standards [38].

2.3. DNA analysis

Genomic DNA was extracted from whole blood. Totally, 67 SNPs were genotyped in DRD1 (n = 17), DRD2 (n = 23), DRD3 (n = 18), DRD4 (n = 7) and DRD5 (n = 2). These SNPs were either candidate SNPs reported to be associated with mental disorders, mainly schizophrenia, enzyme function or monoamine metabolite concentrations, or tag-SNPs selected using HapMap to cover the genes of interest with an r2 threshold of 0.8. The genotyping was performed using the Illumina BeadStation 500GX and the 768-plex Illumina Golden Gate assay (Illumina Inc., San Diego, CA, USA) [15].

2.4. Statistical analysis

The associations between SNPs and HVA CSF monoamine metabolite concentrations were tested with multiple linear regression (STATA 12.1), where concentration was modeled as a linear function of the allele count and three to five covariates. In the analyses of psychotic patients, back-length, gender, age at lumbar puncture, diagnosis (i.e., schizophrenia spectrum psychosis or other psychosis) and use of antipsychotics were included as covariates. Back-length was defined as the distance between the external occipital protuberance and the point of needle insertion. Antipsychotic treatment was considered as present if the patient had taken antipsychotics during a three-week period prior to the lumbar puncture. In the analyses of healthy controls, back-length, gender and age at lumbar puncture were included as covariates. Hardy–Weinberg (HW) equilibrium was tested using exact significance as implemented in STATA 12.1. Normality of residuals was checked graphically with STATA 12.1. Adjustments for multiple testing were performed using Bonferroni correction taking into account the number of tests conducted (α = 0.05/67 = 7.5 × 10−4).

3. Results

Psychotic patients (n = 74, 45 men and 29 women) participated in the present study. The mean age of disease onset ± standard
deviation was 27.6 ± 7.8 years, whereas their mean age ± standard deviation was 30.4 ± 7.2 years at lumbar puncture. Twenty-six of the patients were treated with antipsychotics at the time of lumbar puncture, whereas thirty-six patients were free from antipsychotic medication since three weeks or longer. Sixty-four patients were diagnosed with schizophrenia spectrum disorder (schizophrenia n = 60, schizoaffective disorder n = 4) and ten with other psychosis (psychosis not otherwise specified n = 7, delusional disorder n = 1, bipolar disorder n = 1, alcohol induced psychotic disorder n = 1).

In order to evaluate how the Swedish in-patient resister-based diagnoses conformed to other diagnostic tools, separate analyses were conducted. Evaluations originating from the medical records in 52 of the patients resulted in a diagnosis of a psychotic disorder in 98% of these individuals. Of 44 patients participating in a diagnostic interview 91% displayed a psychotic disorder [35]. These results are in accordance with previous reports showing that the register-based diagnoses of schizophrenia spectrum psychosis have a high validity, with 85% to 94% of the patients displaying these diagnoses when diagnostic evaluations using information from medical records and a structured clinical interview were made [41].

In the 74 psychotic patients, 67 SNPs in five genes encoding dopamine receptors were selected and genotyped. The results are illustrated in Supplementary Table S1 (see Supplementary Table S1 in the online version DOI: 10.1016/j.neulet.2016.03.005). The minor allele frequencies for the selected markers ranged from 2% to 50%. In psychotic patients, the mean (standard deviation) concentration of HVA was 178.6 (79.3) nmol/L. Six of the investigated polymorphisms were found to be nominally associated with CSF HVA concentrations and none of these SNPs showed departure from Hardy-Weinberg equilibrium (p-value < 0.05) (Table 1). The residuals of the nominal associations were approximately normally distributed.

Taking into account the total number of tests conducted, we applied a Bonferroni correction (α = 0.05/67 = 7.5 × 10^{-4}) and two of the nominal associations i.e., rs11747728 (p = 4 × 10^{-4} and rs11742274 (p = 5 × 10^{-4}) remained significant after correction for multiple testing (Table 1).

The six SNPs that were associated with HVA concentrations in psychotic patients were tested in 111 healthy Caucasians (63 men and 48 women). Their mean age ± standard deviation were 28.4 ± 7.5 years at lumbar puncture. In healthy controls, the mean (standard deviation) concentration of HVA was 167.5 (68.4) nmol/L. The minor allele frequency for the six selected markers ranged from 12% to 39%. Departure from Hardy-Weinberg equilibrium (p < 0.05) was found in one of the SNPs analyzed. The residuals were approximately normally distributed. No associations were found in healthy individuals between the selected SNPs and HVA (Table 1).

Preliminary analysis excluding the SNPs showed that HVA concentrations were not associated with antipsychotic treatment in psychotic patients. Our independent variables, i.e., the SNPs, are not expected to be associated with the presence or absence of antipsychotic treatment and moreover we have included the use of antipsychotics as a covariate in our analyses. Thus, the use of antipsychotics in a cluster of patients should not confound our analyses.

### 4. Discussion

To our knowledge, there is only one previously published study searching for associations between gene variants and CSF monoamine metabolite concentrations, including HVA, in patients with psychosis. In that study, nominal associations between genes encoding enzymes implicated in the monoamine metabolism and CSF monoamine metabolite concentrations were found [4]. No previous studies have searched for associations between dopamine receptor gene variants and CSF monoamine metabolite concentrations in psychotic patients.

#### 4.1. DRD1

Almost all independent studies and one meta-analysis failed to show evidence of association between DRD1 gene variants and schizophrenia (http://www.szgene.org). DRD1 has been associated with bipolar disorder [11,34], attention-deficit/hyperactivity disorder [7] and autism [17].

DRD1 rs4532 has been associated with treatment response to antipsychotic drugs [29] and tardive dyskinesia in patients with schizophrenia [21]. In the present study, rs4532 was not associated with CSF HVA concentrations. We identified four SNPs, i.e., rs11747728, rs11742274, rs265974 and rs11747886, located in a region 3.5–8 kbp downstream DRD1, that were associated with CSF HVA concentrations in psychotic patients. The associations between rs11747728 (p value = 0.0004) and rs11742274 (p value = 0.0005) with CSF HVA remained significant after correction for multiple testing. The SNP with the lowest p value, rs11747728, is a regulatory region variant, located 4 kbp downstream DRD1. These associations were restricted to psychotic patients and were absent in healthy controls. These SNPs have not been ascribed any association with schizophrenia or other mental disorders.

D1 receptors are expressed at a high level of density in various regions in CNS [5] and the lack of association between DRD1 and schizophrenia leads to other approaches, such as the investigation of intermediate phenotypes, in order to implicate DRD1 in the disease processes. A recent study displayed that the expression of DRD1 mRNA was decreased in dorsolateral prefrontal cortex of patients with schizophrenia compared to controls [19]. The DRD1 density in different brain regions in patients with schizophrenia and healthy controls has also been studied with positron emission tomography scan with some studies finding an increased DRD1 availability in prefrontal cortex in patients relative to controls [2], mainly in the case of drug-naive patients [3]. This increase may rep-
resent a compensatory upregulation secondary to a mesocortical dopamine function deficiency. The identification of a locus downstream \( DRD1 \) affecting the major degradation product of dopamine supports the hypothesis of the implication of \( DRD1 \) in psychosis and generally the dopamine hypothesis of schizophrenia.

4.2. \( DRD2 \)

\( DRD2 \) gene variations have been extensively investigated for associations with schizophrenia, with numerous positive studies, three positive meta-analyses (www.szgene.org) and a positive genome-wide association study [1]. \( DRD2 \) has also been associated with mood disorders, substance use disorders [44], Tourette's syndrome and post-traumatic stress disorder [27].

The most robust association found was between \( DRD2 \) rs6277 and schizophrenia with a positive meta-analysis with an odds ratio of 1.29 (www.szgene.org). Rs6277 has been included as a candidate SNP in the present study and has not been found to be associated with CSF HVA concentrations in psychotic patients. Another interesting result is the identification of a SNP, i.e., rs22514218, located 47 kbp downstream \( DRD2 \) found to be genome-wide significantly associated with schizophrenia [1]. Rs22514218 was not included in the present study.

In the present study, \( DRD2 \) rs2234689 was nominally associated with CSF HVA concentrations in psychotic patients. \( DRD2 \) rs2234689 is a downstream gene variant and has not been ascribed any functionality or association with mental disorders.

4.3. \( DRD3 \)

\( DRD3 \) gene variants have been associated with schizophrenia in many independent studies. However, all meta-analyses conducted failed to confirm significant associations (www.szgene.org). \( DRD3 \) SNPs have also been associated with other mental disorders such as autism [10] and unipolar depression [12].

In the present study, no \( DRD3 \) SNP was associated with CSF HVA concentrations in patients with psychotic disorder.

4.4. \( DRD4 \)

\( DRD4 \) gene variants have been associated with schizophrenia in some independent studies and two meta-analyses (www.szgene.org), as well as with other mental disorders, mainly ADHD [31].

In the present study, \( DRD4 \) rs3758653 was nominally associated with CSF HVA concentrations in patients with psychosis. Rs3758653 is an upstream gene variant previously reported to be associated with DNA methylation across the \( DRD4 \) promoter region in both lymphoblastoid cell lines and post-mortem brain tissue [13]. Rs3758653 has been also reported to be associated with Alzheimer's disease [23].

4.5. \( DRD5 \)

\( DRD5 \) gene variants have been associated with schizophrenia (www.szgene.org) and ADHD [20] in independent studies. In the present study, no \( DRD5 \) SNP was associated with CSF HVA concentrations in psychotic patients.

4.6. Limitations

The present study suffers from some limitations. First, being a genetic study the numbers of participants is small. Moreover, the assumption that a Bonferroni correction for the total number of tests conducted is a sufficient correction for a genetic study may be considered as a limitation, as there is no sufficient prior evidence to limit the analysis to the specific genes. However, as we have not tested the whole genome and have used a strong a priori hypothesis, i.e., that the selected dopamine-related genes may be regarded likely to influence dopamine metabolite concentrations in patients with psychosis, we have not applied the standard threshold for genome-wide significance but a Bonferroni correction taking into account the total number of tests conducted. The results must be seen as tentative, and there is need for independent replication. Also, several of the patients had been meditated with antipsychotics drugs. However, from a statistical point of view, the various use of antipsychotics among the patients should not confound the results as the presence of antipsychotics has not been found to be associated with HVA concentrations in the present sample and is not expected to be associated with the investigated SNPs.

5. Conclusions

In psychotic patients, we found two significant and two nominal associations between SNPs located at a locus downstream \( DRD1 \) and the CNS dopamine turnover rate, as reflected by the CSF concentration of HVA. These associations were present in patients with psychotic disorder and absent in healthy controls. The present study suggests that the \( DRD1 \) gene is implicated in the pathophysiology of schizophrenia and supports the dopamine hypothesis of schizophrenia.

Authors' contributions

DA contributed to the conception and design of the study, participated in subject assessment, subject characterization and the statistical analysis, managed the literature search and web-based database searches and drafted the article. ES performed the statistical analysis. TA was in charge of the genotyping procedures. GC made a contribution to the conception and design of the study and to the acquisition of data. JT and IA contributed to the conception and design of the study. EGJ contributed to the conception and design of the study, the acquisition and the interpretation of data. All authors revised the article critically for important intellectual content and approved the final manuscript.

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In the current study, we evaluated the association between the DRD3 receptor and a number of cognitive and neural outcomes in a sample of individuals with schizophrenia. The results suggest that the DRD3 receptor is involved in cognitive and neural processes in individuals with schizophrenia.

The findings provide preliminary evidence that the DRD3 receptor may play a role in the pathophysiology of schizophrenia.

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