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Cerebrospinal fluid monoamine metabolite concentrations as intermediate phenotypes between glutamate-related genes and psychosis

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ABSTRACT

Glutamate-related genes have been associated with schizophrenia, but the results have been ambiguous and difficult to replicate. Homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) are the major degradation products of the monoamines dopamine, serotonin and noradrenaline, respectively, and their concentrations in the cerebrospinal fluid (CSF), mainly HVA, have been associated with schizophrenia. In the present study, we hypothesized that CSF HVA, 5-HIAA and MHPG concentrations represent intermediate phenotypes in the association between glutamate-related genes and psychosis. To test this hypothesis, we searched for association between 238 single nucleotide polymorphisms (SNPs) in ten genes shown to be directly or indirectly implicated in glutamate transmission and CSF HVA, 5-HIAA and MHPG concentrations in 74 patients with psychotic disease. Thirty-eight nominally significant associations were found. Further analyses in 111 healthy controls showed that 87% of the nominal associations were restricted to the patients with psychosis. Some of the psychosis-only-associated SNPs found in the D-amino acid oxidase activator (*DAOA*) and the kynurenine 3-monooxygenase (*KMO*) genes have previously been reported to be associated with schizophrenia. The present results suggest that CSF monoamine metabolite concentrations may represent intermediate phenotypes in the association between glutamate-related genes and psychosis.

1. Introduction

Glutamate is the major excitatory neurotransmitter in the central nervous system (CNS) and several lines of evidence suggest association between the glutamatergic system and schizophrenia (Cherlyn et al., 2010). Several glutamate-related genes have been associated with schizophrenia, however the results have been difficult to replicate in subsequent studies until recently, when a genome wide association study of more than 100,000 individuals found association between genes related to glutamatergic neurotransmission and the disorder (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014).

Homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) are the major degradation products of the monoamines dopamine, serotonin and noradrenaline, respectively, and their concentrations in the cerebrospinal fluid (CSF) are considered to reflect the turnover rates of the monoamines in the CNS (Stanley et al., 1985; Wester et al., 1990). Several lines of evidence suggest connections and interactions in CNS between glutamate and the monoamine systems, mainly dopamine. Dopamine regulates the activity of glutamatergic neurons in cortex, where as glutamatergic neurons innervate dopamine cells in ventral tagmental area (Sesack et al., 2003). Moreover, dopamine and glutamate modulate common target neurons in various brain regions, including prefrontal cortex and basal ganglia (Sesack et al., 2003). Glutamate is co-released

Abbreviations: HVA, Homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid; MHPG, 3-methoxy-4-hydroxyphenylglycol; CSF, Cerebrospinal fluid; CNS, Central nervous system; SNP, Single nucleotide polymorphism; SPIR, Swedish psychiatric inpatient register; HWE, Hardy–Weinberg equilibrium; NMDARs, N-methyl-D-aspartate receptors; GRIN1, Glutamate receptor, ionotropic, N-methyl-D-aspartate, subunit 1 gene; GRIN2B, Glutamate receptor, ionotropic, N-methyl-D-aspartate, subunit 2B gene; DAOA, D-amino acid oxidase activator; DAO, D-amino acid oxidase; DISC1, Disrupted in schizophrenia 1; BDNF, Brain-derived neurotrophic factor; IDO, Indoleamine 2,3-dioxygenase; TDO, Tryptophan 2,3-dioxygenase; KMO, Kynurenine 3-monooxygenase; BDNF, Brain-derived neurotrophic factor

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from the majority of serotonergic neurons in raphe nuclei (Fischer et al., 2014) and serotonin reuptake inhibitors modulate glutamate synapses onto serotonergic neurons of the dorsal raphe nucleus (Geddes et al., 2015). A synergistic regulation of glutamatergic neurotransmission in cortex by the serotonin and norepinephrine systems has also been reported (Yuen et al., 2014). Finally, the CSF concentration of kynurenic acid, a glutamate receptor antagonist (Schwarcz et al., 2012), has been reported to have positive intercorrelations with both CSF HVA and 5-HIAA (Nilsson et al., 2007).

Investigating the association between gene variants and intermediate phenotypes in psychotic individuals is a powerful approach that can result in more robust results as well as a deeper understanding of the genotype-phenotype associations (Freimer and Sabatti, 2003; Bilder et al., 2009). An intermediate phenotype can be defined as a mechanism-related manifestation of a complex phenotype, in our case the psychotic disorder (Goldman and Ducci, 2007). An endophenotype should meet specific criteria, i.e. heritability, disease association, state-independence and co-segregation within families (Gottesman and Gould, 2003). Moreover, for complex diseases, such as schizophrenia, it has been proposed that an endophenotype should be found at a higher rate in non-affected family members relative to general population (Gottesman and Gould, 2003).

The term intermediate phenotype is used by many authors for traits that have not been formally shown to meet the criteria for endophenotypes (Goldman and Ducci, 2007). In the present study, we have used the term intermediate phenotypes to characterize the monoamine metabolite concentrations relative to psychosis, as they have been reported to be heritable and to some extend psychosis-related, but do not formally fulfill all the endophenotype-related criteria required.

Regarding heritability, a study in human twins has shown that CSF MHPG is under major genetic influence, whereas CSF 5-HIAA and HVA are under both genetic and environmental influence (Oxenstierna et al., 1986). Studies in other primates also indicate that monoamine metabolite CSF concentrations are partially under genetic influence (Higley et al., 1993; Rogers et al., 2004). Regarding disease association, schizophrenia has been associated with monoamine metabolite concentrations, mainly HVA. HVA concentrations have been reported to be significantly lower in drug-free patients with schizophrenia compared to controls (Bjerkenstedt et al., 1985; Lindström, 1985; Wieselgren and Lindstrom, 1998). Increased CSF MHPG concentrations have also been associated with psychosis (Hsiao et al., 1993).

N-methyl-D-aspartate receptors (NMDARs), one of the main glutamate receptor classes, play a critical role in neurodevelopment, learning and memory (Hirasawa et al., 2003; Riedel et al., 2003). It has been proposed that a hypofunction of the NMDARs is implicated in the pathophysiology of schizophrenia, generating cognitive, negative and positive symptoms (Javitt and Zukin, 1991; Krystal et al., 1994; Javitt, 2008; Labrie and Roder, 2010). In the present study, we chose two genes encoding NMDAR subunits, i.e. glutamate receptor, ionotropic, N-methyl-D-aspartate, subunit 1 (*GRIN*1) encoding the NR1 subunit and glutamate receptor, ionotropic, N-methyl-D-aspartate, subunit 2B (*GRIN2B*) encoding the NR2B subunit, being the most studied NMDAR subunits-related genes in schizophrenia. (www.szgene.org). *GRIN*1 and *GRIN2B* are located on chromosomes 9q34.3 and 12p12, respectively.

The D-amino acid oxidase activator (DAOA) protein is located in various regions of CNS, mainly amygdala and nucleus caudatus (Chumakov et al., 2002). DAOA regulates the function of D-amino acid oxidase (DAO), a flavoprotein catalyzing the oxidative deamination of D-amino acids, including D-DOPA and D-serine, a co-agonist of the NMDARs (Wu et al., 2006; Kawazoe et al., 2007). The *DAO* gene is located on chromosome 12q24, whereas the *DAOA* gene is located on chromosome 13q34.

Disrupted in schizophrenia 1 (DISC1) is a protein, involved in neurodevelopment, plasticity and migration of neurons (Thomson et al., 2013). It affects glutamate neurotransmission in several ways, mainly by modulating serine racemase, an enzyme that generates D-serine, altering the NMDAR neurotransmission (Snyder and Gao, 2013). The *DISC*1 gene is located on chromosome 1q42.1.

Brain-derived neurotrophic factor (BDNF) is the most expressed neurotrophic factor in the brain and is considered to play an important role in the development, survival and regeneration of neurons (Balaratnasingam and Janca, 2012; Nurjono et al., 2012). BDNF is implicated in the glutamatergic as well as dopamine and serotonergic neurotransmitter systems (Nurjono et al., 2012). The *BDNF* gene is located on chromosome 11p13.

Dysbindin is a protein implicated in synaptic structure and signaling, as well as in neurodevelopment (Benson et al., 2001; Ghiani et al., 2010). It is involved in both dopamine and glutamate neurotransmission in CNS (Talbot et al., 2004; Weickert et al., 2004). Dysbindin is encoded by the dystrobrevin-binding protein 1 (*DTNBP*1) gene, located on chromosome 6p22.3.

Indoleamine 2,3-dioxygenase (IDO), tryptophan 2,3-dioxygenase (TDO) and kynurenine 3-monooxygenase (KMO) are important enzymes implicated in the kynurenine pathway of tryptophan degradation (Schwarcz et al., 2012). Kynurenic acid and other neuroactive metabolites of this pathway are implicated in glutamatergic, dopaminergic and noradrenergic neurotransmissions (Myint and Kim, 2014). Dysregulation of the kynurenine pathway has also been associated with mental disorders, mainly schizophrenia (Schwarcz et al., 2012). IDO, TDO and KMO are encoded by the *IDO*1, *TDO*2 and *KMO* genes, located on chromosomes 8p12-p11, 4q31-q32 and 1q42-q44, respectively.

In the present study, we considered the major metabolites of dopamine, serotonin, and noradrenaline as intermediate steps between glutamate-related genes and psychosis. We hypothesized that single nucleotide polymorphisms (SNPs) in these genes affect the CSF concentrations of HVA, 5-HIAA, and MHPG in psychotic patients.

2. Methods

2.1. Subjects

Patients with psychosis, recruited among inpatients from four psychiatric university clinics in Stockholm County between the years 1973 and 1987, were asked to participate in pharmacological and/or biological research projects (Bjerkenstedt et al., 1977; Wode-Helgodt et al., 1977; Härnryd et al., 1984; Oxenstierna et al., 1996). All participants were observed for at least 48 hours without any antipsychotic medication and CSF samples were drawn by a lumbar puncture.

Seventy-four psychotic patients (45 men and 29 women) participated in the present study. The mean age (standard deviation) at lumbar puncture was 30.4 (7.2) years, whereas the mean age of disease onset (standard deviation) was 27.6 (7.8) years. Thirty-five percent of the patients ($N=26$) were treated with antipsychotic medication, whereas 49% ($N=36$) were free from antipsychotics since three weeks or more. Sixty-four patients were diagnosed with schizophrenia spectrum disorder (schizophrenia $n=60$ and schizoaffective disorder $n=4$), whereas ten patients were diagnosed with other psychosis (psychosis not otherwise specified (NOS) $n=7$, delusional disorder $n=1$, bipolar disorder $n=1$, alcohol induced psychotic disorder $n=1$).

Three to 34 years after the first investigation, the psychotic patients were asked to participate in genetic research studies and whole blood was drawn for genotyping. The participants were asked to undergo a structured interview (Spitzer et al., 1988) and permit the researchers to retrieve their medical records. Available records were scrutinized by researchers in order to obtain a life-time diagnosis according to DSM-III-R and DSM-IV. In 2010, hospital discharge diagnoses were obtained from the Swedish psychiatric inpatient register (SPIR), a register covering all inpatient hospitalizations in Sweden since 1973. Psychiatric diagnoses were recorded for each hospitalization according to the International Classification of Diseases, 8th, 9th or 10th revisions. Most patients had experienced several hospitalizations. However, each patient obtained one diagnosis, following a diagnostic hierarchy (Ekholm et al., 2005; Vares et al., 2006). The final diagnoses were based