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Preliminary evidence that polymorphisms in dopamine-related transcription factors *LMX1A*, *LMX1B* and *PITX3* are associated with schizophrenia

Olle Bergman [a,*], Lars Westberg [a], Lars-Göran Nilsson [b], Rolf Adolfsson [c], Elias Eriksson [a]

[a] Department of Pharmacology, The Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden
[b] Department of Psychology, Stockholm University, Stockholm, Sweden
[c] Division of Psychiatry, Department of Clinical Sciences, Umeå University, Umeå, Sweden

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ABSTRACT

The early development of dopaminergic pathways has been attributed importance for the aetiology of schizophrenia. Several transcription factors are involved in the survival and maturation of dopamine neurons, including *LMX1A*, *LMX1B* and *PITX3*. The possibility that polymorphisms in these genes may influence the development and/or the maintenance of dopaminergic neurons prompted us to investigate if five single nucleotide polymorphisms (SNPs) previously linked to Parkinson's disease are associated with this disorder. Preliminary evidence that genetic variation in *LMX1A* (rs6668493, rs4657411), *LMX1B* (rs10987386) and *PITX3* (rs4919621) may increase the risk of developing schizophrenia is presented.

1. Introduction

Schizophrenia is a severe and heterogeneous mental disorder hosting a wide spectrum of symptoms, including psychosis, impairment in cognitive function and social withdrawal. A meta-analysis of several twin studies estimated the heritability of schizophrenia to 80% (Sullivan et al., 2003), and this result is supported by family studies, first-degree family members of schizophrenic patients displaying a nine-fold increase in the risk of developing the disorder (from 1% to 9%) (Tsuang, 2000). However, as yet few specific genes of importance in this context, and exerting a major effect, have been identified and confirmed beyond doubt.

The hypothesis that dopamine plays an important role for the pathophysiology of schizophrenia is based largely on pharmacological evidence (Carlsson, 1988), but has been confirmed also by means of positron emission tomography (PET) studies showing enhanced dopamine release in the striatum of patients with this disorder (Laruelle et al., 1996; Breier et al., 1997). Moreover, functional magnetic resonance imaging (fMRI) endophenotype studies investigating the effect of dopamine regulating genes on brain activation during working memory and verbal fluency tasks also support the case of dopaminergic imbalance in schizophrenia (Weinberger et al., 2001; Prata et al., 2009a,b,c). Needless to say, the emerging notion that schizophrenia is to be regarded as a neurodevelopmental disorder is not incompatible with the dopamine hypothesis; several authors thus have suggested that a dysfunction in the early development of the dopaminergic pathways could cause an imbalance in the dopamine neurotransmission that leads to manifest schizophrenia at a later stage of brain maturation (Murray and Lewis, 1987; Sesack and Carr, 2002).

Several transcription factors are involved in the survival and maturation of postmitotic dopaminergic neurons. Induction of LIM homeobox transcription factor 1 alpha (*LMX1A*) thus initiates a regulatory cascade involving several other transcription factors, which results in a subsequent differentiation and maturation of mesencephalic dopamine neurons (Friling et al., 2009). Silencing the expression of *Lmx1a* using small interfering RNA in chick embryos hence resulted in loss of these neurons (Andersson et al., 2006), and Dreher mice carrying a hypomorphic mutation in the *Lmx1a* gene exhibit neurogenesis defects in mesencephalic dopamine neurons (Ono et al., 2007). Furthermore, forced expression of *LMX1A* generates mesencephalic dopamine neurons from human and mouse embryonic stem cells (Friling et al., 2009).

When expression of LIM homeobox transcription factor 1 beta (*LMX1B*) is initiated in precursor cells of mesencephalic dopamine neurons, it is co-expressed with *LMX1A* (Alavian et al., 2008). Studies

Abbreviations: LMX1A, LIM homeobox transcription factor 1 alpha; LMX1B, LIM homeobox transcription factor 1 beta; PITX3, Paired-like homeodomain 3; BDNF, Brain-derived neurotrophic factor; COMT, Catechol-O-methyltransferase; DRD1/2/4, Dopamine receptor D1/2/4; DAT1, Dopamine transporter gene; PET, Positron emission tomography; fMRI, Functional magnetic resonance imaging; SNP, Single nucleotide polymorphism.

* Corresponding author. Department of Pharmacology, Institute of Neuroscience and Physiology, Sahlgrenska Academy at the University of Gothenburg, P.O. Box 431, SE-405 30 Gothenburg, Sweden. Tel.: +46 31 786 3228; fax: +46 31 786 3064.
E-mail address: olle.bergman@pharm.gu.se (O. Bergman).

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of Lmx1b$^{-/-}$ knock-out mice have demonstrated that, while this gene is not essential for early differentiation of embryonic dopamine neurons, it is necessary for survival of these neurons, and it also appears to regulate an independent pathway necessary for expression of *Paired-like homeodomain transcription factor 3* (*Pitx3*) (Smidt et al., 2000) which is required for the development and survival of mesencephalic dopamine neurons (Nunes et al., 2003; van den Munckhof et al., 2003). Although *Pitx3* is expressed in all mesencephalic dopamine neurons, only cells of the substantia nigra are lost in *Pitx3* deficient mice (Semina et al., 2000; Hwang et al., 2003). *In vitro* studies of *Pitx3*-deficient embryonic stem cells have shown that these generate 50% fewer mature dopamine neurons and that dopamine release was dysfunctional in these neurons (Papanikolaou et al., 2009).

In two previously published studies we reported that single nucleotide polymorphisms (SNPs) in *PITX3* (Bergman et al., 2010), *LMX1A* and *LMX1B* (Bergman et al., 2009) were associated with another disorder known to be closely related to aberrations in dopaminergic neurotransmission, *i.e.* Parkinson's disease. The possibility raised by these findings, that polymorphisms in these genes may influence the development and/or the maintenance of dopaminergic neurons in a significant way, have now prompted us to investigate if the same SNPs are also associated with schizophrenia.

2. Methods

2.1. Subjects

Patients with schizophrenia ($n=213$) were recruited from care centres in Umeå in Northern Sweden. All patients fulfilled the DSM-IV criteria for schizophrenia (DSM-IV. 1994). At inclusion all patients had at least two discharge diagnoses of schizophrenia and a life-time diagnosis of schizophrenia was validated by information obtained from medical records up to this date. Notably, in a previous study we showed that information from medical records was highly reliable and valid in patients with a diagnoses of schizophrenia when compared to diagnoses based on semi-structured interviews (Ekholm, Ekholm et al., 2005). To complete diagnostic work-up, research psychiatrists and nurses used semi-structure interviews based on parts of the Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger et al., 1994). Final diagnosis was determined by the consensus of two research psychiatrists, and only patients for whom full consensus was reached were included in the study. All of the patients were Caucasian, and none were of Finnish, Norwegian or Lappish descent. Mean age of patients at the time of DNA sampling was 49.3 years, and mean age at onset was 25.3 years. Control subjects were recruited via the Betula project ($n=2718$), a random population prospective longitudinal study in Umeå, described in detail elsewhere (Nilsson et al., 1997, 2004). A prerequisite for inclusion in the population study was the ability to comply with a test battery consisting of a health examination, interviews and self-rating scales about somatic and mental health, and ability to comply with an extensive cognitive test battery. Follow-up studies after 10–20 years revealed that none of the controls had a life-time diagnoses of schizophrenia or any other psychotic disorder. Mean age of controls was 58.6 years. The sex ratio was similar in the two samples: the schizophrenic patients consisted of 110 men (51.6%) and 103 women (48.4%) and the control subjects 1222 men (45.0%) and 1496 women (55.0%). All subjects participated after giving written informed consent and the regional Medical Ethical Committee at the University of Umeå approved the study.

2.2. Genotyping

In order to avoid the risk of chance significances following analyses of many different polymorphisms, the only SNPs addressed in this study are those that we have previously found to be associated with Parkinson's disease, i.e. rs6668493, rs4657411 and rs4657412 in *LMX1A*, rs10987386 in *LMX1B* and rs4919621 in *PITX3*. Genomic DNA was isolated from blood samples collected from each subject and diluted to 2.5–5.0 ng/μl. Genotyping was performed at Sequenom Inc. in Hamburg, Germany and at Region Skåne Competence Centre (RSKC Malmö), Malmö University Hospital, Malmö, Sweden, using the Sequenom iPLEX® Gold assay and MassARRAY® MALDI-TOF mass spectrometry platform in accordance with the manufacturer's instructions (Sequenom Inc., San Diego, CA). Primers for PCR amplification and sequencing were designed using the Sequenom MassARRAY® System Designer software.

2.3. Statistical analysis

The five SNPs were tested individually for association using SPSS (version 17.0 for Macintosh; SPSS Inc., Chicago, IL). Pearson chi-square test was used to analyse group differences in genotype frequencies. In order to increase statistical power of analyses of polymorphisms with one low frequency genotype (<1%), dichotomized variables were analysed, using Fisher's exact test. Chi-square tests of allele frequency were performed with Haploview 4.1 as were analyses of possible associations between LMX1A haplotypes and schizophrenia. Differences were considered significant when P was less than 0.05.

3. Results

Frequencies for the five SNPs did not deviate from Hardy–Weinberg equilibrium. The power to detect a genotype relative risk of 2 for carriers of the minor allele varied from 81% to 94% for the investigated SNPs. Genotype-wise analyses showed that four of the five SNPs (rs6668493 and rs4657411 in *LMX1A*, rs10987386 in *LMX1B* and rs4919621 in *PITX3*) were associated with schizophrenia (Table 1). In contrast, allele-wise analyses of individual SNPs and an analysis of LMX1A haplotypes did not reveal any significant associations with schizophrenia.

4. Discussion

In the present study we have studied the possible association between schizophrenia and five SNPs in three dopamine-related transcription factors, *LMX1A*, *LMX1B* and *PITX3*. Of the studied SNPs, all of which have previously been linked to Parkinson's disease (Fuchs et al., 2009; Bergman et al., 2009, 2010; Haubenberger et al., in press; Le et al., in press), four were significantly associated also with schizophrenia. The alleles that were found to be more common in subjects with schizophrenia were the same as those previously shown to be overrepresented in subjects with Parkinson's disease.

As discussed in the Introduction, the three studied genes are all involved in the development and/or survival of mesencephalic dopaminergic neurons. Certain alleles in these genes being associated with enhanced risk of developing Parkinson's disease (and/or of developing Parkinson's disease at an early age) has led to the suggestion that carriers of these alleles may be characterised by reduced formation of dopaminergic neurons during development, leading to lower density of such neurons in the adult brain and hence to a higher risk of displaying motor symptoms when the degeneration of dopaminergic neurons starts, the reserve capacity being lower than in non-carriers. It is however also possible that these genes may partly counteract the loss of dopaminergic neurons in patients with Parkinson's disease, and that this protective influence is reduced in subjects carrying the studied alleles (Fuchs et al., 2009; Bergman et al., 2009, 2010; Haubenberger et al., in press; Le et al., in press). If any of these hypotheses were correct, the implication of the present finding would be that also schizophrenia, like Parkinson's disease, might be associated with reduced density of dopaminergic neurons.

The dopamine hypothesis of schizophrenia originally emerged from the finding that drugs counteracting dopamine transmission by