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The transcription factor PITX3 is associated with sporadic Parkinson's disease

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Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disease with typical motor symptoms due to the preferential loss of midbrain dopaminergic (mDA) neurons in the *Substantia nigra pars compacta*. Several proteins of the homeodomain family are crucial for the development of mDA neurons. These proteins remain expressed into adulthood with largely unknown functions, but potentially influence mDA neuronal survival. To determine whether genetic variation in these genes plays a role in sporadic PD, we performed a genetic association study in a screening sample of 340 PD patients and 680 controls and a large replication sample of 669 PD patients and 669 controls using 54 single nucleotide polymorphisms in and around the *Engrailed 1/2*, *PITX3*, *LMX1B* and *OTX2* genes. We provide evidence for a novel, strong and reproducible association of the *PITX3* promoter SNP rs3758549: C > T ($p = 0.004$) with PD. The C-allele appears to be a recessive risk allele with an estimated population frequency of 83%. An allele-dependent dysregulation of PITX3 expression might contribute to the susceptibility to PD.

Keywords: Parkinson's disease; PITX3; Homeoproteins; SNPs; Midbrain dopaminergic neurons; Association study; Neuronal development

1. Introduction

Parkinson's disease is a progressive neurodegenerative disease with an age-dependent prevalence. At the age of 65 approximately 1% of the population is affected, increasing to 4–5% at the age of 85 (Fahn, 2003). The preferential loss of ≈50% midbrain dopaminergic (mDA) neurons in the *substantia nigra pars compacta* (SNpc) (Fearnley and Lees, 1991) determines the onset of the characteristic motor symptoms bradykinesia, rigidity, tremor and loss of postural reflexes (Fahn, 2003).

A series of proteins with a tightly regulated spatial and temporal expression pattern, including several proteins of the homeodomain family, contribute to the differentiation and

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survival of mDA neurons during development (for review see (Prakash and Wurst, 2006; Simeone, 2005)). Factors responsible for the development of post-mitotic, mature mDA neurons and their maintenance are the homeodomain transcription factors Lmx1b (Smidt et al., 2000), *Engrailed 1/2* (Alberi et al., 2004; Simon et al., 2001; Sonnier et al., 2007), Pitx3 (Nunes et al., 2003; Smidt et al., 2004; van den Munckhof et al., 2003) and the orphan nuclear receptor Nurr1 (Smits et al., 2003; Wallen et al., 1999). Interestingly, these transcription factors remain expressed in the adult though with widely unknown function. In fact, several of these proteins may be important for mDA neuronal survival beyond development and potentially play a role in PD. For example, mutations in Nurr-1 have been implicated in a rare familial form of PD (Le et al., 2003), and polymorphisms in the gene may be associated with sporadic PD in some populations (Xu et al., 2002; Zheng et al., 2003) though not in others (Hering et al., 2004; Tan et al., 2003). Further, the presence of only one En1 allele in mice leads to a preferential and progressive loss of mDA neurons of the SNpc, reminiscent of the pathology associated with PD in humans (Sonnier et al., 2007) and Pitx3-deficient *aphakia* mice display motor deficits reversible by L-DOPA, a major drug used in the treatment of PD (Hwang et al., 2005).

In this study, we therefore investigated the genetic contribution of the homeodomain transcription factors *PITX3*, *LMX1B*, *EN1* and *EN2* and *OTX2* to the risk to develop sporadic PD in a screening sample of 340 PD patients and 680 controls and a large replication sample of 669 PD patients and 669 controls using 54 SNPs. We chose En1/En2 (generation and survival of post-mitotic mDA neurons into adulthood (Alberi et al., 2004; Simon et al., 2001)), Pitx3 (survival and tyrosine-hydroxylase induction in a subset of post-mitotic mDA neurons (Nunes et al., 2003; Smidt et al., 2004; van den Munckhof et al., 2003)) and Lmx1b (maintenance of post-mitotic mDA neurons (Smidt et al., 2000)) due to their importance for mDA neuronal development and survival and their continuous expression in the adult. Otx2 is required for the regional and neuronal specification of mDA progenitors earlier in the development (Puelles et al., 2003) and remains expressed into adulthood selectively in the VTA, but not the SNpc (Frantz et al., 1994). We hypothesise that the genetic control of the expression of these transcription factors might be a susceptibility factor in the pathogenesis of PD.

2. Materials and methods

2.1. Patients and controls

Sporadic PD patients of German origin came from three independent recruitments, referred to as sample set I (clinical centres in Munich and Tuebingen), sample set II and sample set III (clinical centres in Bonn, Rostock, Munich and Tuebingen). Sample set II and III were pooled to form the replication sample. The first set of 340 PD patients (sample set I) had a sex ratio of 1.38 (m/f) and a median age at onset of 52.5 years; the second and third replicating set of 329 (sample set II) + 340 (sample set III) PD patients had a sex ratio of 1.35 and a median age at onset of 59 years. Sample sets I and II were already previously characterized (Mueller et al., 2005a,b). For the first sample set, 680 healthy, age and sex matched individuals from the KORA (Cooperative Health Research in the Region of Augsburg) Survey 4, which studied a large population-based sample**,** were used as controls (Illig et al., 2003); for the second and third sample set 329 and 340 additional healthy matched control individuals of KORA were used. Steffens et al. compared 210 SNPs in three German populations (including 730 participants of KORA S4) and detected no major population stratification between KORA and the other populations (Steffens et al., 2006). All PD patients were examined by neurologists specialized in movement disorders. Cases had at least two of the four cardinal signs of Parkinsonism (rest tremor, rigidity, bradykinesia and/or postural instability). Patients with atypical symptoms or secondary causes of PD were excluded. After obtaining informed consent, blood samples were drawn for DNA extraction. The project was approved by the local Ethics Committees for Research.

2.2. Genotyping

Nine SNPs covering a genomic region of 41 kb including the *PITX3* gene (RefSeq NM_ NM_005029.3), 29 SNPs covering a genomic region of 88 kb including the *LMX1B* gene (RefSeq NM_002316.1), 3 SNPs covering a genomic region of 11 kb including the *EN1* gene (RefSeq NM_001426.3), 9 SNPs covering a genomic region of 15 kb including the *EN2* gene (RefSeq NM_001427.3) and 4 SNPs covering a genomic region of 12 kb including the *OTX2* gene (RefSeq isoform a NM_021728.2/isoform b NM_172337.1) were selected for genotyping. It has been shown previously, that tagSNPs selected from the HapMap data sufficiently tag the genetic variation in the German population (Mueller et al., 2005a,b). Consequently, SNPs were identified using the HapMap database (http://www.hapmap.org/cgi-perl/gbrowse/gbrowse/hapmap/) with the following selection regimen: (1) all tag SNPs with an r^2 threshold of 0.8 according to (de Bakker et al., 2005) (2) all coding SNPs and (3) all SNPs in the 5′ and 3′UTR (4) at least one promoter SNP and (5) SNPs with potential function as predicted by PupaSuite (http://www.pupasnp.org/; (Conde et al., 2004, 2005)). If inter-SNP distance exceeded 5 kb, additional SNPs were selected to ensure an overall SNP spacing of <5 kb. In sample set I and age-matched controls, all selected SNPs were genotyped, whereas only significant association signals or neighbouring SNPs were replicated in sample set II and III. Genotyping was performed using the MALDI-TOF mass spectrometry method (Sequenom) in the Genome Analysis Center, GSF, Munich. All SNPs showed high genotyping quality and Hardy–Weinberg equilibrium in all samples.