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FGF20 and Parkinson's disease: No evidence of association or pathogenicity via α-synuclein expression

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Abstract

Genetic variation in *fibroblast growth factor 20* (*FGF20*) has been associated with risk of Parkinson's disease (PD). Functional evidence suggested the T allele of one SNP, rs12720208 C/T, altered PD risk by increasing FGF20 and α-synuclein protein levels. Herein we report our association study of *FGF20* and PD risk in four patient-control series (total: 1,262 patients and 1,881 controls), and measurements of FGF20 and α-synuclein protein levels in brain samples (nine patients). We found no evidence of association between *FGF20* variability and PD risk, and no relationship between the rs12720208 genotype, FGF20 and α-synuclein protein levels.

Keywords

Parkinson's disease; *FGF20*; α-synuclein; association study; genetics

Introduction

Elucidating the genetic factors involved in complex disorders such as Parkinson's disease (PD) is crucial as we move into the realm of individualized medicine. Recently, genetic association between variants in *fibroblast growth factor 20* (*FGF20* [MIM*605558]) and PD has been reported,[1-4] however further results have been conflicting.[5-7] In one of the studies that showed a positive association between *FGF20* and PD in a series of 1089 patients and 1165 controls

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from 729 families, functional evidence was presented in support of the association findings.[4] Namely, the T allele of one single-nucleotide polymorphism (SNP) (rs12720208 C/T) associated with increased risk of PD was shown to disrupt a micro-RNA (miRNA-433) binding site. Results from *in vitro* (*renilla* luciferase assay in fibroblasts) experiments showed the rs12720208 T allele reduced binding of miRNA-433 and increased *FGF20* expression. In addition, FGF20 was reported to increase α-synuclein protein levels in dopaminergic cells. *In vivo* studies using brain tissue from three PD patients suggested the T allele of rs12720208 is associated with increased protein levels of FGF20 and α-synuclein. The study proposed that the T allele of rs12720208 confers significant PD risk by promoting *FGF20* gene expression, increased FGF20 protein levels and concomitantly α-synuclein levels.

Herein we describe our association study of *FGF20* variants in four series of unrelated PD patients and controls (total: 1,262 patients, 1,881 controls), and our assessment of *FGF20* rs12720208 genotype and human brain levels of both FGF20 and α-synuclein proteins.

Methods

A U.S. (n=840) and an Irish (n=348) patient-control series, matched for age and gender, an unmatched Norwegian (n=1653) patient-control series and an unmatched North-American pathological brain (n=302) patient-control series were examined for *FGF20* association with PD (Table 1). PD diagnosis was established according to published criteria with each living patient examined by a movement disorders neurologist and the post-mortem cases by experienced neuropathologists.[8] Controls were free of neurological disease or a family history of parkinsonism. The ethical committees of each institution approved the study and each living subject signed an informed consent. Brains were collected under IRB approved protocols.

DNA was extracted from blood and brain tissue using standard protocols. We genotyped four SNPs across *FGF20*, including the three SNPs associated with PD in the initial report by van der Walt et al.2 (rs12720208, rs1721100 which was independently confirmed,[5] and rs1989754), and one SNP which yielded borderline results in the only positive replication study (rs12718379) (Figure 1).[5] Genotyping was performed using MALDI-TOF on a Sequenom platform (>95% genotype calls). SNP genotypes were in Hardy-Weinberg equilibrium for each control population as determined using chi-square goodness of fit tests (all $p>0.05$).[9] Linkage disequilibrium between SNPs was measured in controls by pair-wise r^2 values (Supplementary Table 1).10 For the matched U.S. and Irish series, association between PD and each marker was measured by odds ratios (OR's) with 95% confidence intervals (CI's) obtained from single variable conditional logistic regression models. For the unmatched North-American pathological brain series, Norwegian patient-control series, and combined series, association between PD and each marker was measured by OR's with 95% CI's obtained from logistic regression models adjusted for age, sex, and series (combined series only). Haplotype analysis was performed using S-Plus score tests for association,[11] adjusted for age, sex, and series (combined series only); *p*-values were obtained from the asymptotic distribution of the score statistic (haplotypes <1% were not considered). Statistical significance was set at the 5% level and multiple testing was adjusted for using the Bonferroni method for each family of statistical tests.

Cerebellar brain tissue from nine PD patients was selected based on rs12720208 genotype; one homozygote TT; four heterozygotes TC; and four homozygotes CC. Total tissue lysates were prepared using RIPA extraction buffer. Protein levels were measured by Western blot using rat monoclonal anti-FGF20 antibody (R&D Systems, Minneapolis, MN) and mouse monoclonal anti-α-synuclein antibody (Invitrogen, Carlsbad, CA), and normalized to GAPDH controls.

Results

Examination of the individual *FGF20* SNPs including rs12720208 (Table 2) and subsequent haplotype analysis of all four SNPs simultaneously ($p>0.25$ in each series) revealed no significant association with PD in four separate series. Allele and genotype frequencies are given in Supplemental Table 2. Minor allele frequencies were consistent across the four series; therefore they were suitable for the combined analysis, which showed no association between *FGF20* variability and PD. Examination of FGF20 protein levels in nine brain samples showed no association with the rs12720208 genotype (Figure 2). In addition, α-synuclein protein levels were not associated with FGF20 protein levels (Figure 2).

Discussion

Wang *et al*. propose a pathomechanism for *FGF20* genetic association with PD via the over-expression of FGF20 and α-synuclein.[4] They postulate a 3′UTR SNP (rs12720208) effects miRNA binding and results in the differential allele-specific expression of *FGF20*, thereby altering FGF20 protein levels and consequently α-synuclein. This group first proposed *FGF20* as a candidate PD gene as it was located under a linkage peak they identified in a study of small singleton and multiplex U.S. families.[1-4] Subsequent studies have shown inconsistent results, positive only in one Japanese population,[5] and negative in three Caucasian populations.[6, 7] Likewise, the present study did not find any association between *FGF20* genetic variability and risk of PD. In contrast to the original *FGF20* study which used a pedigree-based analysis of familial and sporadic patients and controls,[2, 4] replication studies, including ours, used an unrelated patient-control design; however, recent evidence suggests the genetic risks in sporadic PD may overlap with familial forms of the disease.[12, 13] While the pedigree-based statistical method avoids bias from population stratification,[2, 4, 14] cryptic relatedness may be negligible in outbred populations.[15] Furthermore, in such populations, the classical patient-control association method may require similar or even smaller sample sizes than the pedigree-based test to assess genetic risk factors;[16] therefore, differences in statistical approach are unlikely to account for our discrepant results.

Although we can not rule out an effect of SNP rs12720208 on miRNA binding, our functional study neither showed any association between the rs12720208 genotype and levels of FGF20 or α-synuclein, nor a relationship between the levels of FGF20 and α-synuclein. The considerable random variability between our nine post-mortem cases in both FGF20 and α-synuclein protein expression may help explain the differences observed in brain samples from only one patient of each rs12720208 genotype (n=3 in the original study).[4]

Our study does not support a significant role of *FGF20* variability in PD risk, or a pathomechanism involving co-dependent FGF20 and α-synuclein protein levels. This study highlights the need for caution in interpreting association studies, even with functional data, before genetic findings have been adequately replicated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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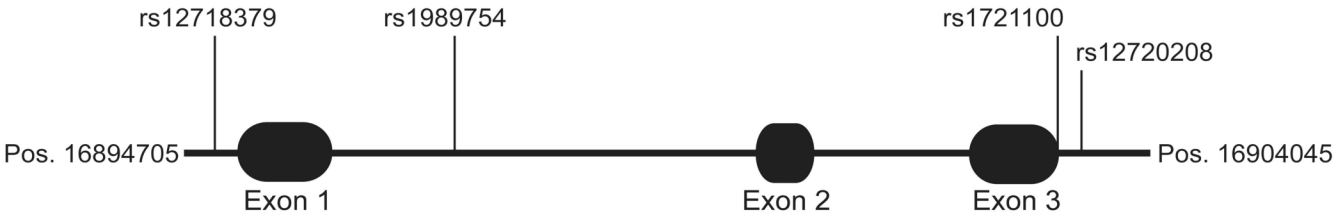

Figure 1.
Gene structure of *FGF20* on chromosome 8p22 showing the four SNPs analyzed in the present study; one in the 5′UTR (rs12718379); one in the first intron (rs1989754); and two in the 3′ UTR regulatory region (rs1721100, rs12720208).

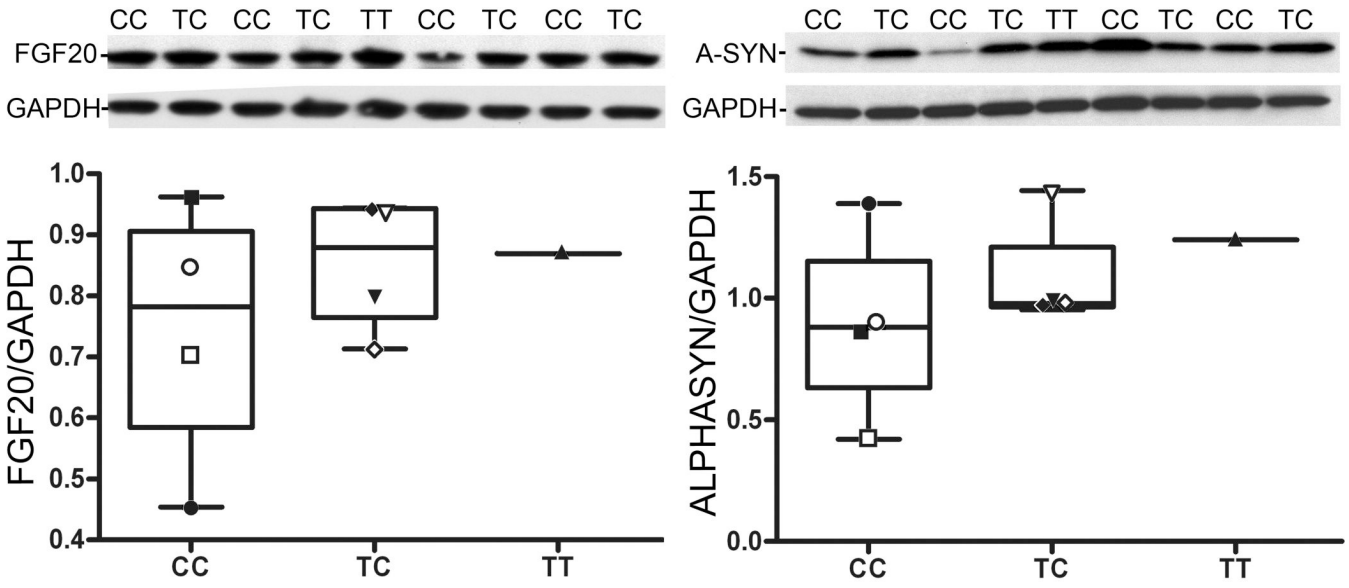

Figure 2.

Top, expression of FGF20 (*left*) and α-synuclein (*right*) in nine PD patients. The rs12720208 genotype (CC, TC, TT) is indicated. *Bottom*, relative expression levels of FGF20 and α-synuclein (normalized against GAPDH; imaging software, ImageJ/NIH) with median values, 25^{th}-75^{th}-tiles (boxes) and confidence intervals. Each symbol (squares, circles, and triangles) indicates normalized FGF20 (*left*) and α-synuclein (*right*) levels for one patient. There is no association between the rs12720208 genotype (CC, TC or TT) and levels of FGF20 and α-synuclein. Comparison in individual patients shows no relationship between FGF20 and α-synuclein levels (for example, the patient with a filled square has relatively high FGF20 levels but average α-synuclein levels, the reverse being true for the patient with a filled circle).

Table 1
Demographic characteristics of the four patient-control series and the combined series

	PD patients	Controls
Irish series (174 patients, 174 controls)		
Age, y	61 ± 12 (33 – 90)	61 ± 12 (33 – 90)
Gender (Male)	68 (39%)	68 (39%)
Age of PD onset, y	49 ± 11 (18 – 77)	
Positive family history (%)	16	
U.S. series (420 patients, 420 controls)		
Age, y	72 ± 11 (29 – 91)	71 ± 11 (32 – 92)
Gender (Male)	226 (54%)	226 (54%)
Age of PD onset, y	62 ± 12 (16 – 85)	
Positive family history (%)	37	
Norwegian series (515 patients, 1138 controls)		
Age, y	72 ± 11 (30 – 99)	73 ± 11 (43 – 106)
Gender (Male)	310 (60%)	538 (47%)
Age of PD onset, y	59 ± 11 (25 – 88)	
Positive family history (%)	23	
North-American brain series (153 patients, 149 controls)		
Age, y	78 ± 7 (60 – 93)	75 ± 16 (27 – 102)
Gender (Male)	102 (67%)	77 (52%)
Age of PD onset, y	63 ± 10 (40 – 86)	
Positive family history (%)	NA	
Combined series (1262 patients, 1881 controls)		
Age, y	71 ± 12 (29 – 99)	71 ± 12 (27 – 106)
Gender (Male)	706 (56%)	909 (48%)
Age of PD onset, y	59 ± 12 (16 – 88)	
Positive family history (%)	NA	

Legend. The sample mean ± SD (minimum, maximum) is given for *age* and *age of PD onset*. NA, not available.

Table 2

Single SNP associations with PD – additive model

	Irish series (174 patients, 174 controls)		U.S. series (420 patients, 420 controls)		Norwegian series (515 patients, 1138 controls		North-American brain series (153 patients, 149 controls)		Combined series (1262 patients, 1881 controls)	
	Estimated OR (95% CI)	**p-value**	**Estimated OR (95% CI)**	**p-value**	**Estimated OR (95% CI)**	**p-value**	**Estimated OR (95% CI)**	**p-value**	**Estimated OR (95% CI)**	**p-value**
rs12718379 (C)	1.35 (0.98, 1.87)	0.067	0.92 (0.76, 1.12)	0.41	0.95 (0.82, 1.11)	0.53	0.92 (0.76, 1.11)	0.38	0.97 (0.87, 1.08)	0.58
rs1989754 (G)	0.72 (0.53, 0.99)	0.043	1.01 (0.84, 1.23)	0.88	1.10 (0.94, 1.28)	0.23	1.03 (0.84, 1.25)	0.80	1.03 (0.92, 1.14)	0.62
rs1721100 (G)	1.16 (0.81, 1.64)	0.42	0.97 (0.79, 1.19)	0.75	0.89 (0.75, 1.05)	0.16	0.96 (0.78, 1.18)	0.70	0.94 (0.84, 1.06)	0.32
rs12720208 (T)	1.08 (0.62, 1.89)	0.78	1.15 (0.83, 1.58)	0.41	0.88 (0.67, 1.16)	0.37	1.13 (0.82, 1.56)	0.46	1.03 (0.86, 1.24)	0.76

Legend. Estimated odds ratios correspond to an increase of one minor allele. The choice of an additive model is based on the hypothesis and evidence from Wang *et al*.[4] suggesting an additive effect of the rs12720208 T allele. *p*-values ≤ 0.0125 are considered statistically significant after a Bonferroni adjustment for multiple testing.