Published in final edited form as:

Parkinsonism Relat Disord. 2007 October ; 13(7): 386–388. doi:10.1016/j.parkreldis.2007.01.010.

Lack of evidence for association of *Parkin* promoter polymorphism (*PRKN*-258) with increased risk of Parkinson's disease

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Abstract

Parkin (*PRKN*) mutations are a common cause of early-onset parkinsonism, however the role of this gene in typical late-onset Parkinson's disease (PD) remains unresolved. A single nucleotide polymorphism in the promoter region (*PRKN* -258; rs9347683) has been observed to associate with PD, affect age-at-onset of symptoms, and to functionally effect differential expression of the *PRKN* transcript. In the present study *PRKN* -258 did not associate with PD, and no evidence for an age-at-onset effect was observed in three age- and gender-matched Caucasian patient-control series from Norway, Ireland and the US. These data do not support a role for this common variant in PD etiology.

Keywords

Parkin; promoter SNP; Parkinson's disease

Introduction

Parkin is functionally recognized as an E3 ubiquitin ligase that plays a critical role in the development of autosomal recessive juvenile parkinsonism (ARJP) [1-3]. *Parkin* (*PRKN*)

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mutations are a frequent cause of early-onset Parkinson's disease (EOPD) and are reported to account for up to 49% of early-onset parkinsonism and 18% of sporadic EOPD, defined as symptomatic onset <45 years [4-6]. Over one hundred genetic variants have been described in the *PRKN* locus since the identification of the first large *PRKN*-positive parkinsonism kindred [1,2]. However, the role of *PRKN* variants in the more common late-onset form of PD remains unresolved [7].

A limited number of studies have investigated the frequency of coding single nucleotide polymorphisms (SNPs) situated within the *PRKN* locus which may influence risk of disease [8-12]. A SNP at position -258 in the promoter of *PRKN* has been identified to associate with disease and to functionally effect differential expression of the gene mRNA [12]. It has also been suggested that *PRKN* -258 affects transcriptional activity which appears to be up-regulated when neuronal cells are placed under oxidative stress conditions [13]. However, this association could not be replicated in a family-based study of primarily late-onset PD patients [10], and no association with the promoter region was identified in a Spanish patient-control series [9].

To investigate the influence of the *PRKN* -258 SNP in PD we examined the frequency of this variant in three age- and gender-matched Caucasian patient-control series. Given the role of *PRKN* in EOPD this SNP was also examined as a possible age-at-onset (AAO) disease modifier.

Methods

The Norwegian series included 338 patients with a mean age ($\pm$ SD) of 70 ± 11 years (AAO 58 ± 11 SD years; range 25-88 years), the Irish series included 186 patients with a mean age of 61 ± 13 years (AAO 50 ± 11 SD years; range 18-77 years), and the US series included 257 patients with a mean age of 71 ± 10 years (AAO 62 ± 12 SD years; range 23-85 years). All patients were examined and observed longitudinally by a movement disorders neurologist (JOA, JMG, TL, ZKW & RJU) and diagnosed with PD according to published criteria [14]. Each patient was individually matched based on age (± 4 years), gender, and ethnicity to an unrelated control without evidence of neurological disease. The ethical review board at each institution involved approved the study and all participants provided informed consent.

Genotyping of SNP *PRKN* -258 (A>C rs9347683; T>G in the reading frame of *PRKN* as the gene is encoded centromeric to telomeric) was performed using TaqMan chemistry on an ABI7900 genetic analyzer (PCR product 125bp; primer and probe sequences available on request). In cases where genotype data was only available for one sample of a matched pair, the other subject was retained in the analysis. For the controls in each population, χ^2 tests of Hardy-Weinberg equilibrium (HWE) were implemented using Haploview [15]. Associations between susceptibility to PD and *PRKN* -258 were measured by odds ratios (ORs) and corresponding 95% confidence intervals (CIs) obtained from logistic regression models adjusted for age and sex. In PD cases, linear regression models adjusted for sex were used to examine individual associations between AAO and *PRKN* -258. Model stratification separately by age (only in models involving susceptibility to PD) and sex was also

considered. Models involving patients from all three sample locations were additionally adjusted for sample location. Genotyping call rates were >95% for rs9347683. Statistical significance was determined at the 5% level.

Results and Discussion

The present study demonstrated no evidence of association between *PRKN* -258 and PD (Table 1). This lack of association between *PRKN* -258 and PD was consistent across age and gender for all sample groups (data not shown) except Norwegian females, for whom there was marginal evidence of a negative association with PD (OR: 0.62, 95% CI: 0.38 – 0.99, $P=0.050$). There was no statistically significant correlation with age-at-onset of symptoms (Table 2), which also was consistent for males and females (data not shown).

This lack of association in three independent matched patient-control series or the combined sampling ($n=1570$) would suggest that this variant does not influence the parkinsonism phenotype of these patients. The *PRKN* -258G allele was first modestly associated with disease risk by West et al. ($P=0.040$) in a sample of 296 PD cases and 184 controls, where the association was driven by an increased prevalence of PD in heterozygous and *PRKN* -258GG homozygous patients. Tan et al. observed an association with PD in a sample of 386 PD cases and 367 controls that was caused by an increase in *PRKN* -258GG in patients >65 years of age ($P=0.004$).

The genotype and allele frequencies for our three populations are very similar and therefore a combined analysis is permissible (Table 3). The frequencies also match with those reported for the US controls in the initial association [6]. However the study performed in the ethnic Chinese subjects showed a greater frequency of the minor allele in their samples demonstrating population-specific variation in this SNP [13]. While it is crucial that a significant association is reproduced in independent series, it is equally important that the principle of population-specific variants is considered. A lack of association in samples of differing ethnicity may not necessarily reflect a lack of replication.

The *PRKN* SNP at position -258 (rs9347683) with relation to the first nucleotide of exon 1 is hypothesized to disrupt a consensus sequence for an NF1-like binding motif resulting in a differential profile for the *PRKN* transcript [12]. *PRKN* mutations result in a recessive form of parkinsonism indicative of loss-of-function or haploinsufficiency at this locus. This pathomechanism supports the idea that lower expression of the parkin protein may increase susceptibility to disease. Recently a family with autosomal recessive EOPD was found to harbor a homozygous deletion of the *PRKN* promoter and exon 1 ablating total *PRKN* expression [16]. Interestingly, the clinical phenotype of the affected family members is similar to that observed in other *PRKN*-associated EOPD. The *PRKN* -258G allele is observed to effect lower expression in luciferase assays and is reported to affect transcriptional activity when human neuronal cell-lines carrying either allele are placed under oxidative stress conditions [13]. Our study cannot rule out that this variant affects transcription of the *PRKN* gene, but the results indicate that the alternate expression is independent to the manifestation of disease symptoms.

Over the next decade an increasing number of polymorphic variants will be associated with complex disease. Functional studies will be necessary to elucidate the role of these variants within disease pathogenesis; however, it is essential to confirm the association in independent populations before undertaking studies of biological relevance.

Acknowledgements

Mayo Clinic Jacksonville is a Morris K. Udall Parkinson's Disease Research Center of Excellence (NINDS P50 #NS40256) and a Robert H. and Clarice Smith Fellowship (OAR). We thank Minnie Schreiber for technical assistance. We would like to thank all those who have contributed to our research, particularly the patients and their families.

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Table 1
Association between *PRKN* -258 and susceptibility to PD

Odds ratios correspond to an increase in one minor allele (G). *P*-values result from univariable tests of association in logistic regression models adjusted for age and sex. Logistic regression models involving all patients were also adjusted for sample location.

Series	Estimated OR (95% CI)	<i>P</i> -value
Norway	0.83 (0.63 – 1.08)	0.18
Ireland	0.86 (0.59 – 1.24)	0.42
US	1.08 (0.79 – 1.47)	0.63
Overall	0.91 (0.76 – 1.08)	0.29

Table 2
Association between *PRKN* -258 and age-at-onset

Estimated regression coefficients are interpreted as the increase in the mean age-at-onset with an increase of one minor allele (G). *P*-values result from univariable tests of association in linear regression models adjusted for sex. Linear regression models involving all patients were also adjusted for sample location.

Series	Estimated regression coefficient (95% CI)	<i>P</i> -value
Norway	-0.06 (-2.09 to 1.98)	0.96
Ireland	1.47 (-1.82 to 4.76)	0.38
US	0.49 (-2.21 to 3.19)	0.72
Overall	0.43 (-1.04 to 1.90)	0.56

Table 3

Genotype and allele frequencies for *PRKN* -258

Series/ <i>PRKN</i> -258	TT	TG	GG	T	G
Norwegian n=640	400 (63%)	206 (32%)	34 (5%)	1006 (79%)	274 (21%)
Controls	192	115	17	499	149
Patients	208	91	17	507	125
Irish n=363	238 (66%)	110 (30%)	15 (4%)	586 (81%)	140 (19%)
Controls	114	63	6	291	75
Patients	124	47	9	295	65
US n=489	298 (61%)	170 (35%)	21 (4%)	766 (78%)	212 (22%)
Controls	153	87	9	393	105
Patients	145	83	12	373	107
Total n=1492	936 (63%)	486 (33%)	70 (5%)	2359 (79%)	626 (21%)
Controls	459	265	32	1183	329
Patients	477	221	38	1175	297