行政院國家科學委員會專題研究計畫　期中進度報告

Parkin 基因多型性與巴金森氏病致病感受性的關聯性、以及 DJ-1 基因變異在巴金森氏病扮演之角色(1/2)

計畫類別：個別型計畫
計畫編號：NSC93-2314-B-002-241-
執行期間：93 年 08 月 01 日至 94 年 07 月 31 日
執行單位：國立臺灣大學醫學院神經科

計畫主持人：吳瑞美
共同主持人：李銘仁

報告類型：精簡報告
報告附件：出席國際會議研究心得報告及發表論文
處理方式：本計畫可公開查詢

中　華　民　國 94 年 3 月 28 日

Comprehensive analysis of DJ-1 in a cohort of Taiwanese ethnic Chinese: Implications for the genetic etiology of early-onset Parkinsonism.

Abstract

Recently, mutations in DJ-1 (PARK7) were described as a novel cause of early-onset parkinsonism. We have performed a comprehensive analysis of the DJ-1 gene in a cohort of early-onset patients with Parkinson's disease, originating from Taiwan; 41 subjects were clinically and genetically examined. These patients have previously been evaluated for the presence of parkin mutations (PARK2) and found to be negative. The entire *DJ-1* open reading frame was amplified from cDNA and sequenced to identify coding variants. In addition, we developed quantitative PCR assays to examine the genomic copy number of *DJ-1* exons. No sequence alterations or exon deletion/duplications were detected. We conclude that in ethnic Chinese, Taiwanese patients alterations to *DJ-1* are not a common cause of disease. Given the low frequency of parkin mutations in this early-onset cohort, our findings suggest other causes of parkinsonism, genetic and/or environmental, remain to be identified.

Keywords: PARK7, Parkinson's disease, genetics, Taiwanese

Introduction

Parkinson's disease (PD; MIM 168600) is a common neurodegenerative disorder that is characterized by bradykinesia, resting tremor, muscular rigidity and postural instability. The pathological features include loss of dopaminergic neurons, in particular within the substantia nigra pars compacta, and eosinophilic inclusions termed Lewy bodies (1). Treatment with L-dopa and dopaminergic agonists provides good symptomatic benefit, however therapy fails to halt disease progression and may provoke undesirable effects (2).

Recent studies have identified causal mutations in familial parkinsonism and highlighted the importance of genetic susceptibility in sporadic PD (3, 4). A molecular genetic approach is powerful as it facilitates the design of novel therapeutics, which may ameliorate symptoms of disease by targeting the underlying cause(s) (5). In the past six years, ten genetic loci have been linked with parkinsonism and five of the underlying mutant genes have been identified (6, 7). Three loci have been associated with early-onset, autosomal recessive parkinsonism (EO-PD); *Parkin* (8) (PARK 2, MIM 602544), the as yet unidentified PARK 6 (9) (PARK 6, MIM 605909) and *DJ-1* (10) (PARK 7, MIM 606324). Loss of Parkin function is the predominant genetic cause of EO-PD in Japanese, Northern European and North African populations. *Parkin-proven* disease accounts for approximately 49% of familial and 19% of sporadic EO-PD (with onset prior to 45 years) (11-16). The phenotype is consistent with typical, late-onset PD, with a good response to levodopa. The disease is slowly progressive although an earlier-onset is often associated with dystonia and marked benefit from sleep (17). Parkin encodes a 465

amino acid protein that functions as an E3 ubiquitin-protein ligase (18), suggesting dysfunction of the proteasomal system can result in disruption of the dopaminergic system. Recent studies have demonstrated that Parkin can protect dopaminergic neurons from a variety of toxic insults (19, 20).

Very recently, a second gene for EO-PD (PARK7/*DJ-1*) was identified (7). In one Dutch kindred, a homozygous deletion of exons 1 to 5 of the *DJ-1* gene was identified in affected individuals. In a second Italian family, a homozygous T to C transition was found that resulted in the substitution of a conserved leucine to proline at amino acid 166 of the protein. Recently, we and others (21, 22) demonstrated 166P DJ-1 to be rapidly and efficiently degraded by the ubiquitin proteasomal system (UPS). Additionally, both mutations show complete segregation with disease in homozygous individuals, while heterozygous carriers are unaffected, suggesting complete loss of function of *DJ-1* is pathogenic. The phenotype of affected individuals is similar to *Parkin*-proven EO-PD, although behavioral disturbances were noted early in the disease course (10, 23). The mechanism by which loss of DJ-1 causes parkinsonism is unclear. The protein appears to have multiple functions, having been implicated in hydroperoxide response, infertility and mRNA expression and stability (reviewed in(24)). To date there is no information regarding the frequency of *DJ-1* mutations in parkinsonism, nor in different populations.

With this background, we have performed a comprehensive mutation analysis of *DJ-1* in a cohort of 41 EO-PD probands of Taiwanese/Ethnic Chinese origin. All cases had previously be assessed for mutations in the parkin gene, and found to be negative (25). To detect point mutations and small deletions or insertions, the complete *DJ-1* open

reading frame (ORF) was amplified by RT-PCR and examined by gel electrophoresis and direct sequencing. In addition, to test for gene dosage alterations, we developed semi-quantitative multiplex PCR assays for all genomic exons.

Results

All subjects presented with early-onset parkinsonism (mean age at onset 41.3 years ± 5.6 S.D, range 29-49, male:female ratio =25:16, n=41) without atypical features. Patients were previously evaluated for *Parkin* mutations by direct sequencing and gene dosage studies (25). For all patient samples, PCR amplification of the *DJ-1* cDNA resulted in two products of 819 and 761 base pairs respectively (Figure 1). The different product sizes arise via alternate splicing of the non-coding exon 1^{B}, as reported previously (7). No PCR products of a different size were observed for any subject, suggesting there were no mutations affecting consensus splice motifs. Similarly, no sequence alterations were detected in the untranslated or coding portion of the *DJ-1* cDNA in any of the patients examined. All samples encoded the A allele at dbSNP:3203837, suggesting this SNP is rare in the ethnic Chinese, Taiwanese population. There was one difference in comparison to the Genbank reference sequence (Accession NM_007262) a G to T transversion at position 376, resulting in a C119F amino acid substitution. This coding change was present in all subjects in the Taiwanese cohort and over sixty other control and PD affected individuals from a variety of populations (data not shown). Additionally, a G at position 376 was not detected in any of the human ESTs in the public database (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi).

To exclude the possibility of large genomic deletion/duplication of the DJ-1 locus, we performed a comprehensive analysis of all seven *DJ-1* exons for genomic copy

number. A semi-quantitative multiplex PCR assay utilizing fluorescent primers and an ABI3100 capillary array was developed (Table 1). No alterations in exon copy number (duplication and/or deletion) were detected in any patient sample. Our methodology was sensitive and quantitative, as demonstrated by analysis of appropriate haploid genomic control samples (Figure 2). For all exons (1 through to 5) where a haploid control sample was available, we could accurately detect ~50% reduction in peak area, indicative of exon deletion.

Discussion

The frequency of *DJ-1* (PARK7) mutations in parkinsonism, in different ethnic populations, has yet to be fully evaluated (7). Although pathogenic mutations were reported in two consanguineous European kindreds, analysis of nine additional EO-PD families and 22 sporadic EO-PD cases failed to identify any additional *DJ-1* mutations (7). However, not all of these cases were evaluated for *Parkin* mutations, and surprisingly nor was *DJ-1* gene dosage analysis performed.

We report the first comprehensive analysis of the DJ-1 gene in a cohort of ethnic Chinese, Taiwanese patients with EO-PD. Patients were formerly clinically and genetically evaluated and found to be negative for mutations in the *Parkin* gene (25). In the present study, we have assessed all possible variants within the *DJ-1* gene by RT-PCR, direct sequencing of cDNA and exon dosage methods. However, no pathogenic sequence alterations were identified, in any affected individual. Most likely the G376 base change represents a sequencing error in the *DJ-1* reference sequence, or a rare

polymorphism. Similarly, we did not detect any EO-PD cases with exon dosage alterations.

The pathogenic deletion of exons 1 to 5 of *DJ-1*, reported with a frequency of 0.9% in an isolated Dutch community and consistent with a founder effect, was not observed in our study (7). However, *DJ-1* maps to a fragile region of 1p36 analogous to the FRA6E/*Parkin* locus, suggesting the *DJ-1* locus also might be prone to deletion and loss of heterozygosity (29, 30). In *Parkin*, *de novo* exonic deletions/duplications are the most common mutations (14). Hence, despite our results, gene dosage studies must be considered an important component of future studies of *DJ-1* and Parkinsonism in different populations.

Recent reports have functionally associated variation in the *Parkin* promoter and coding sequence with risk of idiopathic PD (31, 32) and suggested *Parkin* haploinsufficiency may confer susceptibility to deficiency in the dopaminergic system (15, 33, 34). Further studies will be required to address whether variability in *DJ-1* sequence or expression might similarly contribute to late-onset, typical PD, in the community.

Our results indicate that in the ethnic Chinese, Taiwanese population, mutations in *DJ-1* are not a common cause of EO-PD. Similarly, we failed to detect any *DJ-1* mutations in a cohort of 50 Caucasian EO-PD cases (21). These observations suggest that mutations in *DJ-1* appear to be a rare cause of recessively inherited EO-PD, accounting for <1% of cases with onset prior to 50 years. Interestingly, the frequency of *Parkin* mutations in this ethnic Chinese, Taiwanese cohort also was considerably lower, approximately 3% (25) compared with 19-50% of EO-PD cases reported in European,

Caucasian populations (11-16). As twin studies suggest the genetic contribution to EO-PD is considerable (35), other causal genes may be responsible for disease in this population. PARK6 is a potential candidate, with 8 of 28 European *Parkin*-negative EO-PD families suggestive of linkage to the 1p35-36 (9, 36, 37).

In conclusion, the nosology of Parkinson's disease and EO-PD is complex. It is evident that a clinical diagnosis of Parkinson's disease may fit a number of genetic causes (4-7). Furthermore, ethnic differences may have a profound effect on the expression and classification of different neurodegenerative disorders as clinical Parkinson's disease (38, 39). Our analysis of the contribution of *Parkin* and *DJ-1* mutations to EO-PD, in ethnic Chinese, Taiwanese cases suggests that genetic cause(s) once identified are likely to be population specific. This background may confound the notion of consensus criteria for disease diagnosis, typically developed in Caucasian cohorts, as well as cross-cultural epidemiological studies. However, unlike hereditary ataxias where polyglutamine inclusions explain the majority of disease, and the profile of gene expression rather than its function is important (40), functional analysis of proteins identified in familial parkinsonism and EO-PD implicates a common pathway affected in idiopathic Parkinson's disease (5). Finding additional genes for parkinsonism, and in different populations, will undoubtedly facilitate an accurate diagnosis and provide a rationale for novel drug design and more specific treatments.

References

1. Fearnley JM, Lees AJ. Ageing and Parkinson's disease: substantia nigra regional selectivity. Brain 1991;114 (Pt 5):2283-2301.

2. Melamed E, Offen D, Shirvan A, Ziv I. Levodopa--an exotoxin or a therapeutic drug? J Neurol 2000;247 Suppl 2:II135-139.

3. Hicks AA, Petursson H, Jonsson T, et al. A susceptibility gene for late-onset idiopathic Parkinson's disease. Ann Neurol 2002;52(5):549-555.

4. Gasser T, Bressman S, Durr A, Higgins J, Klockgether T, Myers RH. State of the art review: Molecular diagnosis of inherited movement disorders. Movement Disorders Society task force on molecular diagnosis. Mov Disord 2003;18(1):3-18.

5. Skipper L, Farrer M. Parkinson's genetics: molecular insights for the new millennium. Neurotoxicology 2002;23(4-5):503-514.

6. Gwinn-Hardy K. Genetics of parkinsonism. Mov Disord 2002;17(4):645-656.

7. Bonifati V, Rizzu P, van Baren MJ, et al. Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. Science 2003;299(5604):256-259.

8. Kitada T, Asakawa S, Hattori N, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature 1998;392(6676):605-608.

9. Valente EM, Bentivoglio AR, Dixon PH, et al. Localization of a novel locus for autosomal recessive early-onset parkinsonism, PARK6, on human chromosome 1p35-p36. Am J Hum Genet 2001;68(4):895-900.

10. van Duijn CM, Dekker MC, Bonifati V, et al. Park7, a novel locus for autosomal recessive early-onset parkinsonism, on chromosome 1p36. Am J Hum Genet 2001;69(3):629-634.

11. Lucking CB, Durr A, Bonifati V, et al. Association between early-onset Parkinson's disease and mutations in the parkin gene. French Parkinson's Disease Genetics Study Group. N Engl J Med 2000;342(21):1560-1567.

12. Abbas N, Lucking CB, Ricard S, et al. A wide variety of mutations in the parkin gene are responsible for autosomal recessive parkinsonism in Europe. French Parkinson's Disease Genetics Study Group and the European Consortium on Genetic Susceptibility in Parkinson's Disease. Hum Mol Genet 1999;8(4):567-574.

13. Kann M, Jacobs H, Mohrmann K, et al. Role of parkin mutations in 111 community-based patients with early-onset parkinsonism. Ann Neurol 2002;51(5):621-625.

14. Periquet M, Lucking C, Vaughan J, et al. Origin of the mutations in the parkin gene in Europe: exon rearrangements are independent recurrent events, whereas point mutations may result from Founder effects. Am J Hum Genet 2001;68(3):617-626.

15. West A, Periquet M, Lincoln S, et al. Complex relationship between Parkin mutations and Parkinson disease. Am J Med Genet 2002;114(5):584-591.

16. Hedrich K, Marder K, Harris J, et al. Evaluation of 50 probands with early-onset Parkinson's disease for Parkin mutations. Neurology 2002;58(8):1239-1246.

17. Bonifati V, De Michele G, Lucking CB, et al. The parkin gene and its phenotype. Italian PD Genetics Study Group, French PD Genetics Study Group and the European Consortium on Genetic Susceptibility in Parkinson's Disease. Neurol Sci 2001;22(1):51-52.

18. Shimura H, Hattori N, Kubo S, et al. Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. Nat Genet 2000;25(3):302-305.

19. Petrucelli L, O'Farrell C, Lockhart PJ, et al. Parkin protects against the toxicity associated with mutant alpha-synuclein: proteasome dysfunction selectively affects catecholaminergic neurons. Neuron 2002;36(6):1007-1019.

20. Staropoli JF, McDermott C, Martinat C, Schulman B, Demireva E, Abeliovich A. Parkin Is a Component of an SCF-like Ubiquitin Ligase Complex and Protects Postmitotic Neurons from Kainate Excitotoxicity. Neuron 2003;37:735-749.

21. Lockhart PJ, Lincoln S, Hulihan M, et al. DJ-1 mutations are a rare cause of recessively inherited early-onset Parkinsonism mediated by loss of protein function. J Med Genet 2003;In Press.

22. Miller DW, Ahmad R, Hague S, et al. L166P mutant DJ-1, causative for recessive Parkinson's disease, is degraded through the ubiquitin-proteasome system. J Biol Chem 2003;Jul 8 [Epub ahead of print].

23. Bonifati V, Breedveld GJ, Squitieri F, et al. Localization of autosomal recessive early-onset parkinsonism to chromosome 1p36 (PARK7) in an independent dataset. Ann Neurol 2002;51(2):253-256.

24. Cookson MR. Pathways to parkinsonism. Neuron 2003;37(1):7-10.

25. Wu R-M, Bounds R, Lincoln S, et al. Parkin mutations are not prevalent in early-onset Parkinsonism in Taiwanese ethnic Chinese. Arch Neurol 2003;In Press.

26. Gelb DJ, Oliver E, Gilman S. Diagnostic criteria for Parkinson disease. Arch Neurol 1999;56(1):33-39.

27. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12(3):189-198.

28. Fahn S, Elton RL, Committee. aMotUD. Unified PD rating scale. In: Fahn S, Marsden CD, Goldstein M, Calne DB, eds. Recent Developments in PD. New York: Macmillan, 1987:153-163.

29. Smith DI, Huang H, Wang L. Common fragile sites and cancer (review). Int J Oncol 1998;12(1):187-196.

30. Smedley D, Sidhar S, Birdsall S, et al. Characterization of chromosome 1 abnormalities in malignant melanomas. Genes Chromosomes Cancer 2000;28(1):121-125.

31. West AB, Maraganore D, Crook J, et al. Functional association of the parkin gene promoter with idiopathic Parkinson's disease. Hum Mol Genet 2002;11(22):2787-2792.

32. Foroud T, Uniacke SK, Liu L, et al. Heterozygosity for a mutation in the parkin gene leads to later onset Parkinson disease. Neurology 2003;60:796-801.

33. Hilker R, Klein C, Hedrich K, et al. The striatal dopaminergic deficit is dependent on the number of mutant alleles in a family with mutations in the parkin gene: evidence for enzymatic parkin function in humans. Neurosci Lett 2002;323(1):50-54.

34. Hilker R, Klein C, Ghaemi M, et al. Positron emission tomographic analysis of the nigrostriatal dopaminergic system in familial parkinsonism associated with mutations in the parkin gene. Ann Neurol 2001;49(3):367-376.

35. Tanner CM. Is the cause of Parkinson's disease environmental or hereditary? Evidence from twin studies. Adv Neurol 2003;91:133-142.

36. Valente EM, Brancati F, Caputo V, et al. PARK6 is a common cause of familial parkinsonism. Neurol Sci 2002;23 Suppl 2:S117-118.

37. Valente EM, Brancati F, Ferraris A, et al. PARK6-linked parkinsonism occurs in several European families. Ann Neurol 2002;51(1):14-18.

38. Subramony SH, Hernandez D, Adam A, et al. Ethnic differences in the expression of neurodegenerative disease: Machado-Joseph disease in Africans and Caucasians. Mov Disord 2002;17(5):1068-1071.

Figure Legends

Figure 1. Agarose gel electrophoresis of RT-PCR amplification of DJ-1.

Shown are the *DJ-1* amplification products of 4 representative patient samples (lanes 2 to 5) and a control sample. The larger 819bp fragment corresponds to the full-length DJ-1 mRNA, including all 6 coding exons and both non-coding exons (1^A and 1^B). The smaller 761bp fragment represents an alternatively spliced transcript lacking the non-coding exon 1^B.

Figure 2. Genetic analysis of *DJ-1* in EO-PD patients.

A. A representative exon dosage chromatogram demonstrating accurate detection of a heterozygous exon 5 deletion but normal exon 7 dosage in the positive control sample (heterozygous del. 1-5, (7)). B. A representative exon dosage chromatogram from an affected patient. Ratios are generated dividing the area under the DJ-1 exon peak by the area under the control peak. A ratio half of that observed for wildtype is indicative of a heterozygous exon deletion.

Table 1: Primer sets and pools used for DJ-1 gene dosage analysis.

PRIMER	SEQUENCE	PRODUCT
DJ-1 E1 F	[Hex] 5'-GCTGTCCAGCTAGAAACTCC	302bp
DJ-1 E1 R	5'- GACACCGTCCAGCACCC	
DJ-1 E2 F	[Hex] 5'-CTCTGCTTGAAAATGCTCC	392bp
DJ-1 E2 R	5'-GGCAAGACATTAACAAGCG	
DJ-1 E3 F	[Hex] 5'-TTAAAGACAGTGTTACTCTGAATT	388bp
DJ-1 E3 R	5'-CATCCAGCCACCCACTTAC	
DJ-1 E4 F	[Hex] 5'-GGCTATCTCCTGTACTTCCC	297bp
DJ-1 E4 R	5'-TCACAGCCTCCTCCCGAA	
DJ-1 E5 F	[Hex] 5'- GTGAGTGATTGGTTAGTGGC	293bp
DJ-1 E5 R	5'-ATCTCTGAAATGACACCAACTT	
DJ-1 E6 F	[Hex] 5'-CTCAAGCAATTTTTCTACCT	367bp
DJ-1 E6 R	5'-GAGGCTGAGAGAGAAGAATCG	
DJ-1 E7 F	[Hex] 5'-CGT CTTTCTCGTCACATAGC	360bp
DJ-1 E7 R	5'-TGTGACTTCCATACTTCCGC	
Control F	[Hex] 5'-ACGTTCCTGATAATGGGATC	328bp
Control R	5'-CCTCTCTCTACCAAGTGAGG	

Primers were pooled in sets of two DJ-1 exons and the control. The sets and primer concentrations were: Set 1; Exon 4 (0.6µM), Exon 2 (0.8µM) and Control (8µM), Set 2; Exon 1 (0.8µM), Exon 6 (0.6µM) and Control (0.8µM), Set 3; Exon 5 (0.8µM), Exon 7 (0.8µM) and Control (0.8µM) and Set 4; Exon 3 (1.2µM) and Control (0.6µM).

Figure 1

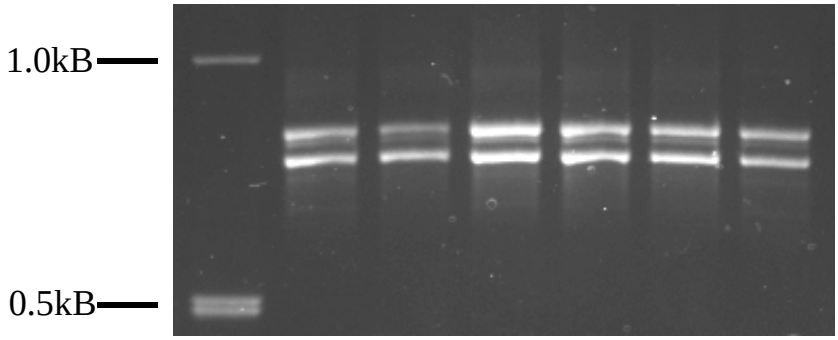

Figure 2