Association of NRH:Quinone Oxidoreductase 2 Gene Promoter Polymorphism With Higher Gene Expression and Increased Susceptibility to Parkinson’s Disease

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The N-ribosylhydronicotinamide (NRH):quinone oxidoreductase 2 (NQO2) gene encodes an enzyme that catalyzes activation of quinones. Blood DNA from 80 control individuals and 118 age-matched Parkinson’s disease patients were analyzed for NQO2 gene promoter polymorphisms. The results revealed three allelic variants, designated I-29, I-16, and D. These results were confirmed in fibroblast cell lines. In patients with Parkinson’s disease, there was a significant increase in the frequency of the D allele, but there was no difference in the frequency of the alleles in familial compared to sporadic Parkinson’s disease. The D and I-16 promoters direct higher NQO2 gene expression that results in higher enzyme activity. Overexpression of NQO2 in the catecholaminergic neuroblastoma SH-SY5Y cells resulted in increased production of reactive oxygen species when exposed to exogenous dopamine. The results suggest that the association of the D promoter with Parkinson’s disease may be due to an increase in expression of the NQO2 gene.

Key Words: NRH:quinone oxidoreductase 2—Promoter polymorphism—Parkinson’s disease.
**Genomic DNA Preparation**

Human peripheral blood (6 mL) was withdrawn from the cubital vein and collected in a heparinized tube. The white blood cells were pelleted down and washed two times with normal saline. The cells were resuspended in 5 mL of STE buffer (10 mM Tris base, 10 mM sodium chloride, 1 mM EDTA) containing 0.8% sodium dodecyl sulfate (SDS) and proteinase K at 0.4 mg/mL, and then was incubated at 37°C overnight. The genomic DNA was extracted with phenol-chloroform and precipitated with ethanol. The DNA was washed with 70% ethanol and resuspended in TE buffer (0.01 M Tris, 0.001 M EDTA, pH 8.0).

**Methods**

**Patients and Controls**

This study was approved by the Institutional Review Board for Human Subject Research for Baylor College of Medicine and Affiliated Hospitals. The clinical diagnosis of Parkinson’s disease was made in the Baylor College of Medicine Parkinson’s Disease Center and Movement Disorders Clinic by Dr. Joseph Jankovic according to established diagnostic criteria: the presence of at least two of the three cardinal signs (tremor at rest, bradykinesia, and rigidity), the absence of signs of atypical parkinsonism or evidence of secondary parkinsonism caused by other neurological diseases, known drugs or toxins, and improvement in symptoms with L-dopa therapy. The Parkinson’s Disease Center and Movement Disorders Clinic maintains a database of patients (with DNA samples from these patients). In the database, about 10% of the Parkinson’s disease patients have a family history of Parkinson’s disease, at least, in first-degree relatives. To determine whether NQO2 is associated with familial Parkinson’s disease, roughly 40% of the samples used in the current study were chosen from patients with a family history. A total of 118 patients and 80 healthy control participants were included in this study. Among the patients with Parkinson’s disease, 70 were diagnosed as sporadic and 48 as familial. The Parkinson’s disease group was composed of 70 men and 48 women with an average age (± standard deviation (SD)) of 59 ± 12.7 years, whereas the control group contained 50 men and 52 women with the average age (± SD) of 55.6 ± 16.7 years. In the control group, there were samples from 62 Caucasian, 16 Hispanic, and 2 Asian persons. In the Parkinson’s disease group, there were samples from 105 Caucasian (62 sporadic and 42 familial), 9 Hispanic (5 sporadic and 4 familial), 2 Asian (1 in each group), and 1 African-American (in the familial group) person.

**Cell Culture**

Skin fibroblast cells from 15 humans were obtained from the Coriell Cell Repository (Camden, NJ). The fibroblast cells were cultured in Minimum Essential Medium supplemented with 15% (vol/vol) heat-inactivated fetal bovine serum and 2 mM L-glutamine. Hep-G2 cells were grown in α MEM supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum and 2 mM L-glutamine. All media were also supplemented with penicillin at 100 U/mL and streptomycin at 100 μg/mL. The cultures were grown at 37°C in a humidified atmosphere containing 5% (vol/vol) CO2 in air. The media and the reagents for cell culture were obtained from Invitrogen Life Technologies (Carlsbad, CA). SH-SY5Y cells were grown in Dulbecco’s modified Eagle’s medium (GIBCO, Gaithersburg, MD) supplemented with 10% heat-inactivated fetal bovine serum (GIBCO) and cultured at 37°C in a humidified 5% CO2 atmosphere.

**Genotyping of NQO2 Gene Promoter Polymorphism**

The polymorphic alleles of the human NQO2 promoter were genotyped using polymerase chain reaction (PCR). The lengths of the PCR amplicons from the alleles were 323, 339, and 352 bp for D, I-16, and I-29 alleles, respectively. The primer sequences used were as the following: forward, 5′-CTGCTCTGAAGTTCAAGGGTC-3′; reverse, 5′-GACCCAGGCGGTGGGACCCCG-3′. One microgram of genomic DNA was used as the template. PCR conditions were programmed as follows: denature DNA at 95°C for 15 minutes, 35 cycles of denaturing at 94°C for 30 seconds followed by annealing at 62°C for 30 seconds, and amplification at 72°C for 30 seconds. HotStar Taq enzyme and Q-solution from Qiagen ( Valencia, CA) were used to amplify the highly GC-rich region. PCR products were separated on 2% agarose–ethidium bromide gel and photographed.

**Cloning and Plasmid Construction**

The polymorphic human NQO2 promoters (~1.3 kb) were cloned from the genomic DNA samples of patients or controls by using PCR. The primers used for the PCR were: forward, 5′-GGAAGTACCCGGATCTGGACCTCAAGA CAAG-3′; reverse, 5′-GAAGATCTTTCGCGGTCCAGT CCGGGAA-3′. The KpnI and BglII restriction sites (underlined sequences) were introduced in the forward and reverse primers, respectively. The PCR products were digested with KpnI and BglII and then subcloned into pGL2 Basic vector. All constructs were confirmed by sequencing the entire 1.3-kb NQO2 promoter DNA from the three polymorphic alleles.

The pcDNA-NQO2-V5 plasmid was constructed as follows. The mouse NQO2 complementary DNA (cDNA) was amplified from mouse liver total RNA using the following primers: forward primer: 5′-CAGAGATCTATCTCCTCTT CCAACATGGCAGG-3′ and reverse primers without the stop codon: 5′-CTCTTGGGTAAGTCAACAGGGG-3′. The stop codon was removed to clone NQO2 in frame with V5 peptide tag. The reverse transcription–PCR (RT–PCR) product was subcloned into the pcDNA3.1D/V5-His-TOPO vector (Invitrogen Life Technologies), and the construct was designated as pcDNA-NQO2-V5. The construct was...
confirmed by sequencing. This plasmid encodes V5-tagged NQO2 in transfected cells. V5-tag is used for easy detection of NQO2-V5 protein with anti-V5 antibody (21). The addition of V5 to NQO2 had no effect on NQO2 protein stability and activity (Wang W, Jaiswal AK, 2005, unpublished data).

**Transient Transfection and Luciferase Assay**

Hep-G2 cells were plated in six-well plates at a density of 3 × 10^5 cells/well 1 day prior to transfection. The cells were transfected with 0.5 μg of NQO2 promoter–luciferase plasmids. To normalize the transfection efficiency, 0.05 μg of pRL-TK plasmid was used as a control and was included in each transfection. Effectene Transfection Reagent (Qiagen) was used for transfection of cells. Transfection was carried out as described previously (20). Briefly, the DNA and 4 μL of Enhancer were dissolved in EC buffer to a total volume of 100 μL. The DNA–Enhancer mixture was incubated at room temperature for 5 minutes. After incubation, 5 μL of Effectene Transfection Reagent was added to the mixture, mixed, and incubated at room temperature for 10 minutes to allow transfection–complex formation. Medium (200 μL) was added to the mixture and mixed. The mixture was then immediately added to the well containing the cells and 1.5 mL of fresh medium. The cells were harvested 48 hours after the transfection, and luciferase assay was conducted using a Dual-Luciferase Reporter Assay System (Promega, Madison, WI). The data presented are the results from 3 independent experiments.

**NQO2 Enzyme Activity**

Cytosolic extract was prepared from the fibroblast cells using a Nuclear Extract Kit from Active Motif (Carlsbad, CA). The Bradford protein assay (Bio-Rad, Richmond, CA) was used to determine the protein concentration of the cytosolic extract. NQO2 activity was determined as described before (20). Briefly, NRH was synthesized by adding 1,000 units of calf intestinal alkaline phosphatase (Sigma, St. Louis, MO) to 500 μL of 10 mM nicotinamide mononucleotide (Sigma) in phosphate-buffered saline (PBS). The reaction was allowed to proceed for 15 minutes at room temperature. Ten microliters of the NRH was added to 50 mM Tris, pH 7.4, 100 μM dichlorophenolindophenol, and cytosolic extract in a 1-mL standard cuvette. The decrease in absorbance was followed at 600 nm for 1 minute with a Beckman DU640 spectrophotometer (Beckman Coulter, Fullerton, CA). Cytosolic extract concentrations were used that produced a 0.08–0.15 absorbance change per minute. The specific activity of NQO2 was calculated from the change in absorbance per microgram of protein.

**Semiquantitative RT–PCR**

TRIZOL reagent (Invitrogen Life Technologies) was used to extract total RNA from the 15 primary fibroblast cultures. Semiquantitative RT–PCR was performed using the one-step RT–PCR kit from Qiagen. In each reaction, 0.5 μg of total RNA was used. The primers used for the RT–PCR reactions are as follows: NQO2 forward 5’-CATGGCACATTACACTTCTTGG-3’; NQO2 reverse 5’-CTCTTTGCTTGCGCTTGG-3’; GAPDH forward 5’-ACCACAGTCATGCCACATCAC-3’; GAPDH reverse 5’-TCCACCACCCATGGCACATTACACTTCTTGG-3’; RT–PCR conditions used for the various primer sets are as follows: denaturation of DNA at 50°C for 30 minutes followed by 95°C for 15 minutes, followed by 25 cycles of denaturing at 94°C for 30 seconds, annealing at 55°C for 30 seconds, amplification at 72°C for 1 minute, and a final extension at 72°C for 10 minutes. The RT–PCR products were resolved on 1% ethidium bromide agarose gel, and the band densities were quantitated using an Eagle Eye System (Stratagene, La Jolla, CA). The same 15 samples were run twice. The NQO2 to GAPDH ratio was calculated for each sample and averaged.

**Western Blot Analysis**

The SH-SY5Y cells were transiently transfected with pcDNA vector or pcDNA-NQO2-V5 at 0.5 μg/well or 1.0 μg/well in six-well plates. Twenty-four hours after the transfection, the cells were washed 3 times with ice-cold PBS before being scraped off the plates. The cells were spun down, and cytosolic proteins were prepared using RIPA buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholate, 0.1% SDS, supplemented with 1× proteinase inhibitor cocktail; Roche Applied Sciences, Mannheim, Germany). Western blot analyses were performed to determine the expression of NQO2-V5. Ten micrograms of total protein were separated on a 10% SDS polyacrylamide gel and transferred onto nitrocellulose membranes. Membranes were incubated overnight with anti-V5 horseradish peroxidase–conjugated antibody (Invitrogen Life Technologies) to detect NQO2-V5 fusion protein. Bands were revealed using enhanced chemiluminescence (ECL) reagents (Amersham Biosciences, Piscataway, NJ).

**Transient Transfection, Dopamine Treatment, and Measurement of ROS**

The SH-SY5Y cells were transiently transfected with pcDNA vector or pcDNA-NQO2-V5 using Lipofectamine 2000 (Invitrogen Life Technologies) in 96-well plates. Twenty-four hours later, the cells were treated with 100 μM NRH and 50 μM dopamine (Sigma) for an additional 24 hours. Dopamine was freshly dissolved in distilled water with 0.25% ascorbic acid and further diluted immediately before use. The cells were analyzed for ROS measurement by procedures as previously described (22). To determine the intracellular amount of ROS, cells were loaded with 2’,7’-dichlorodihydrofluorescein diacetate (DCF-DA), which freely enters the cells. DCF-DA is a nonfluorescent compound and is deacetylated by viable cells to the highly fluorescent 2’,7’-dichlorofluorescein (DCF) by intracellular ROS. The fluorescence of DCF was assessed as described before with modifications (22). Briefly, after treatment with dopamine, the transfected cells were rinsed twice with ice-cold PBS, and 1 mL of 0.05% trypsin/0.02% EDTA was added to each well. Cells were collected, rinsed three times with a PBS solution, and incubated with 10 μM of DCF-DA (dissolved in dimethyl sulfoxide) in PBS for 30 minutes at 37°C. Cells were then rinsed twice with PBS on ice, and the fluorescence was quantified using a fluorometer (Cytofluor II; PerSeptive Biosystems, Framingham, MA) at excitation/emission wavelengths of 485/535 nm.
**Results**

Genotyping of human NQO2 gene promoter from 80 normal and 118 age-matched Parkinson’s disease patients revealed the presence of three polymorphic alleles. The alignment of the nucleotide sequences of the alleles (Figure 1) shows that the deletion or insertion of sequences in the same region of the promoter generated the three alleles. Thus they are designated as I-29, I-16, and D alleles (Figure 2A, representative alleles shown). Two of these polymorphic alleles (I-29 and D) have been previously reported (19).

Analysis of the genotype and allele frequency in the 80 controls and 118 Parkinson’s disease patients led to several interesting observations (Table 1). The frequency of the I-29 allele was highest, followed by the frequency of the D allele in both the control and Parkinson’s disease groups. The only heterozygote detected was the I-29/D. Neither I-29/I-16 nor the D/I-16 heterozygotes were detected. This finding suggests that the distributions of alleles are not in Hardy–Weinberg equilibrium. Whether this is due to sample size and chance or due to a biological issue with the I-16 allele is not known. Because no heterozygote with the I-16 allele was observed, perhaps the I-16 allele is only stable when paired with another I-16 allele.

The distribution of genotypes and alleles was significantly different in the Parkinson’s disease patients compared to the control group (\( p < .005; 2 \times 4 \) contingency table, chi-square test) with an increase in frequency of D/D and I-29/D genotypes. Among the 118 patients with Parkinson’s disease, 48 were classified as familial and 70 as sporadic Parkinson’s disease. The genotype distribution for these subgroups was significantly different from the distribution in the control group (\( p < .05 \)), but not different from each other. The influence of gender on the risk for Parkinson’s disease was split into familial and sporadic disease groups, the \( p \) value was adjusted using a Bonferroni correction. Student’s \( t \) test was used to evaluate mean (± standard error of the mean [SEM]) differences where appropriate. Differences were considered significant if the \( p \) value was <.05.

### Table 1. Genotypes and Allele Frequencies of Human NQO2 Promoter Polymorphism in Control and PD Patients

<table>
<thead>
<tr>
<th>Participants (No.)</th>
<th>Genotypes</th>
<th>Allele Frequencies</th>
<th>( p ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I-29/I-29 (%)</td>
<td>I-16/I-16 (%)</td>
<td>D/D (%)</td>
</tr>
<tr>
<td>Controls (80)</td>
<td>68 (85.0%)</td>
<td>3 (3.8%)</td>
<td>8 (10.0%)</td>
</tr>
<tr>
<td></td>
<td>D: 0.1062</td>
<td>I-29: 0.6525</td>
<td>I-16: 0.0339</td>
</tr>
<tr>
<td>PD (118)</td>
<td>66 (55.9%)</td>
<td>4 (3.4%)</td>
<td>26 (22.0%)</td>
</tr>
<tr>
<td></td>
<td>1-29: 0.08562</td>
<td>I-16: 0.0339</td>
<td>D: 0.3136</td>
</tr>
<tr>
<td></td>
<td>I-29: 0.6875</td>
<td>I-16: 0.0417</td>
<td>D: 0.2708</td>
</tr>
<tr>
<td>PD-familial (48)</td>
<td>28 (58.3%)</td>
<td>2 (4.2%)</td>
<td>8 (16.7%)</td>
</tr>
<tr>
<td></td>
<td>1-29: 0.6875</td>
<td>I-16: 0.0417</td>
<td>D: 0.2708</td>
</tr>
<tr>
<td>PD-sporadic (70)</td>
<td>38 (54.3%)</td>
<td>2 (2.9%)</td>
<td>18 (25.7%)</td>
</tr>
</tbody>
</table>

**Notes**: *Genotype comparison.

\(^1\)Allelic frequency comparison. Each group was compared to the control group. A Bonferroni correction was done for the familial and sporadic groups.

NQO2: \( N \)-ribosylhydroxycinnanikamide:quinone oxidoreductase 2; PD = Parkinson’s disease.
disease was specifically addressed by asking whether there was a difference in the distribution of the D/D and I-29/D genotypes between male and female patients in the Parkinson’s disease group. There was no effect of gender.

To determine the ability of the different alleles to promote gene expression, 1.3 kilobase pairs of the gene for the NQO2 promoters containing the allelic variants were separately cloned in pGL2 Basic vector, transfected into Hep-G2 cells, and analyzed for luciferase gene expression. Luciferase activity for each allele was averaged and compared with a grouped \( t \) test. The results showed a significant increase in expression of luciferase from the D and I-16 promoters compared to the I-29 promoter (Figure 2B). The D and I-16 alleles expressed similar amounts of luciferase activity. These experiments demonstrate that the sequence in this region of the promoter has a significant effect on gene expression.

Next, we extended the genotyping studies to 15 established skin fibroblast cell lines from normal human individuals (Figure 3A). Among the 15 cell lines, 12 were I-29 homozygote, 2 were I-16 homozygote (numbers 2 and 3), and 1 was D homozygote (number 9). To confirm the relative strengths of the polymorphic promoters determined in the luciferase assay, the fibroblast cells were analyzed for NQO2 messenger RNA (mRNA) levels, using GAPDH as the control (Figure 3B). The normalized band density from one experiment is shown in Figure 3C. The results were confirmed by a repeat experiment. The average density for the bands representing NQO2 mRNA from I-16 cell lines (samples 2 and 3) and the D cell line (sample 9) was significantly higher than the average density for the bands representing NQO2 mRNA from the I-29 cell lines (0.46 \( \pm \) 0.11, \( n = 12 \), mean \( \pm \) SEM, \( p < .001 \), grouped \( t \) test). Finally, to confirm that this change in mRNA level results in a change in the activity of the enzyme, the activity of NQO2 was determined in the 15 fibroblast cell lines. Consistent with the mRNA level, the average NQO2 activity in the I-16 and D fibroblasts was significantly higher than that in the I-29 (1.6 \( \pm \) 0.2 nmol of 2,6-dichlorophenolindophenol reduced/min/mg cytosolic protein for the I-16/I-16 and D/D \( [n = 3 \) total]; 1.2 \( \pm \) 0.1 for the I-29/I-29 \( [n = 12] \), \( p < .05 \); grouped
Thus the enzyme activity for NQO2 in the fibroblast cell lines correlated with the mRNA levels. Cells with I-16 or D alleles had higher relative levels of mRNA for NQO2 and higher NQO2 enzyme activity compared to cells with the I-29/I-29 genotype.

To begin to address the question of the connection between higher NQO2 enzyme activity and Parkinson’s disease, we chose to use the catecholaminergic cell line SH-SY5Y. These cells have the capacity to take up, store, and metabolize dopamine. Application of dopamine to cultures of SH-SY5Y cells will result in cytotoxicity due to the generation of oxidative stress from dopamine that is not properly stored in vesicles (23). We tested whether overexpression of NQO2 can alter this dopamine-induced generation of oxidative stress. Levels of ROS were measured in SH-SY5Y cells that had been transfected with pcDNA-NQO2-V5 so that they would overexpress NQO2. Western analysis of transfected SH-SY5Y cells showed plasmid concentration-dependent overexpression of NQO2-V5 (Figure 4A). Transfection of the control vector (pcDNA) did not result in expression of NQO2-V5. Addition of dopamine and NRH to cells transfected with the control vector did not result in a significant change in levels of ROS (Figure 4B, compare columns 1 and 2). However, treatment of cells overexpressing NQO2 led to a 30% increase in ROS generation \( (p < .005, \text{grouped } t \text{ test comparing column 2 to columns 3 and 4 in Figure 4B}). \) NRH was included in the culture medium to ensure that the cells had sufficient NRH (enzyme cofactor) when NQO2 is overexpressed (16). The effect of overexpression of NQO2 in the absence of NRH and dopamine gave inconsistent results (data not shown). It is noteworthy that increasing the plasmid concentration from 0.5 \( \mu \text{g} \) to 1.0 \( \mu \text{g} \) for transfection increased NQO2 protein but did not further increase the ROS generation (Figure 4, data from columns 3 and 4 in Figure 4B were not different). This presumably is due to limited NRH availability inside the cells.

DISCUSSION

Three allelic forms of human NQO2 gene promoter (I-29, I-16, and D) were detected. Among these, the I-29/I-29 allele was highly abundant both in controls and Parkinson’s disease patients. Therefore, it is likely that I-29 allele is the wild-type allele and the others are polymorphic alleles generated due to deletions in this region. I-29 alleles have a 29-bp sequence that contains transcription factor (Sp3) binding sites (20). Binding of Sp3 to this 29-bp sequence represses NQO2 gene transcription. The Sp3 binding site is deleted from the D allele, and loss of this binding site is known to de-repress NQO2 gene expression in humans (20). This explains the significant increase in the expression of luciferase gene from the D allele compared with the I-29

Figure 4. Increased reactive oxygen species (ROS) generation in human neuroblastoma SH-SY5Y cells overexpressing complementary DNA (cDNA)-derived \( \text{N}\)-ribosyldihydronicotinamide:quinone oxidoreductase 2 (NQO2). The cells were transfected with (pcDNA; control vector) and/or pcDNA-NQO2-V5. A, Western analysis. The cells were lysed in RIPA buffer, then the lysate was separated on sodium dodecyl sulfate–polyacrylamide gels, Western blotted, and probed with anti-V5 and actin antibodies. Anti-V5 detected V5-tagged NQO2 protein. B, Detection of ROS generation. The cells were rinsed three times with phosphate-buffered saline (PBS) and incubated with 10 \( \mu \text{M} \) of 2’,7’-dichlorofluorescein diacetate in PBS for 30 minutes at 37°C. The cells were again rinsed twice with PBS on ice, and the fluorescence was quantified using a fluorometer at excitation/emission wavelengths of 485/535 nm. Data are expressed as mean ± standard error of the mean \( (n = 3). \)

Figure 5. Model predicting regulation of \( \text{N}\)-ribosyldihydronicotinamide:quinone oxidoreductase 2 (NQO2) and role in Parkinson’s disease. The full-length promoter contains a binding site for the gene repressor Sp3. Loss of this binding site in the D promoter results in increased expression of NQO2 and increased enzyme activity. This increased NQO2 activity could increase the generation of toxic metabolites of dopamine, which contribute to the pathogenesis of Parkinson’s disease. ROS = reactive oxygen species.
allele. For the first time, an I-16 allele has been identified and shown to behave like the D alleles. This expression pattern is due to the deletion of 13 base pairs from the I-29 allele, which also leads to disruption of the Sp3 binding site and de-repression of gene expression.

The frequency of the D alleles was higher in Parkinson’s disease patients than in controls. Studies were further extended to determine the distribution of the NQO2 promoter genotypes in familial and sporadic Parkinson’s disease. It is interesting that both groups contained a higher frequency of D alleles compared with the control group. This finding suggested that the association between the human NQO2 promoter polymorphism and Parkinson’s disease does not contribute to the familial inheritance of Parkinson’s disease. The I-16 alleles were not sufficiently common to come to a conclusion about any coincidence with Parkinson’s disease. The present studies also showed a significantly higher expression of NQO2 from the D allele in transfected Hep-G2 cells and in human fibroblast cells, compared with I-29 alleles. Overexpression of NQO2 in neuroblastoma cells, which can take up, store, and metabolize dopamine, caused an increase in ROS generation after treatment with dopamine. Therefore, it is likely that individuals with the D allele have higher NQO2 levels, which produce increased levels of ROS in response to dopamine, leading to an increased susceptibility to Parkinson’s disease. This hypothesis will need to be confirmed with additional testing.

The role of increased NQO2 in Parkinson’s disease is also supported by indirect evidence from previously published reports. Melatonin and resveratrol are both known to protect against neuronal damage and prevent Parkinson’s disease in experimental models (17,24–33). Melatonin and resveratrol are also known to bind NQO2 and inhibit NQO2 activity (17,34). These observations, combined with the results in the present report, suggest that inhibition of NQO2 leads to protection against neuronal damage and inhibition of the development of Parkinson’s disease. However, this conclusion also needs confirmation.

Based on current and previous work, a hypothetical model is proposed (Figure 5). Sp1 and Sp3 regulate the transcription of the NQO2 gene with the I-29 promoter. Sp1 is an activator and Sp3 is a repressor of NQO2 gene transcription (20). This balance of activation and repression leads to a normal level of expression of NQO2. However, in the alleles without the full-length sequence (I-16 and D alleles), the Sp3 binding site is lost. This loss leads to de-repression and increased expression of the NQO2 gene. Up-regulation of NQO2 activates catecholamine-derived quinones leading to increased oxidative stress, progressive degeneration of dopaminergic neurons, and eventually Parkinson’s disease.

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Drs. Wang and Le contributed equally to this article.

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