ている．非麦角系のタリペキソール，プラミペキソール，ropinirol は腎排泄型のため，高齢者や腎障害のある患者では投与量に注意する必要がある．一方，麦角系ではシトクローム P-450 で代謝されるために，他の薬剤との併用に影響を受けることが予想される．しかしながら，薬剤の併用で臨床的に問題となることは少ない．

先に触れたように ELLDOPA STUDY により，DA で治療開始に関する考えは弱まっている感があるが，神経保護や運動症状以外の付加効果も認められることより DA の長所を十分に理解することが必要である．効果に関しては，エビデンスに基づき評価することが重要になっているが，新しい薬ほどレベルの高いエビデンスが存在する．エビデンスには，DA の単独療法，Levodopa との併用療法，wearing off や dyskinesia を総称とした motor complication に対する治療効果や予防効果についてレベルの高い評価をされている[12)14)15)17)]．**Table 1** にわが国で利用可能な DA のエビデンスに基づく治療評価を示す[4)]．ここで重要なのは，対照薬が Levodopa であり，DA 間の治療効果の違いについてはエビデンスがないということである．わが国で行われたプラミペキソールの試験はブロモクリプチンとプラセボを対照としているが，プラミペキソールとブロモクリプチンの両者には有意差はない[9)]．パーキンソニズムに対する効果は DA 間において大きな違いがないことを示すのか，あるいは十分なスタディが存在していていないためと思われるが，今後は DA 間における効果の違いを証明することが課題となろう．注目すべきは，個々の患者において DA に対する効果や耐用性が異なることである．つまりエビデンスを参考し，そのうえで医師の判断に基づき患者ごとの治療を行うことが重要となってくる．

DA 間における違いは検討されているスタディに依存されるので，ドパミン受容体の親和性に依存した効果や臨床現場の実践的な要素も十分に考慮したうえで使用することが望ましいが，詳細な検討は存在しない．また半減期の長さを考慮した処方も臨床現場では十分に考慮すべきだが，エビデンスは存在しない．さらに DA の併用療法についても PD の予後が改善され，治療歴が長期に及ぶことを考えると検討の余地がある．Stocchi ら[18)]は，プラミペキソールないし ropinirol と Levodopa 併用 35 例についてカベルゴリンの併用の検討をしているが，作用時間の長さを反映してか，オフ時間の減少，オフ時の Unified Parkinson's Disease Rating Scale（UPDRS）の改善に差を見出している．運動合併症状に抑制には continuous dopaminergic stimulation（CDS）が重要であり，カベルゴリンのように作用時間の長い DA は，理論的にも運動合併症状の出現を抑制することが予想される．プラミペキソールには D3 作用が存在することが指摘されており，PD 患者の anhednia に対する効果が期待されている．Anhednia とは気分障害で，本来喜びが得られるような行為においても快楽を感じえないことをいい，ドパミン欠乏により食欲，性欲に対しても快感が得られなくなることが多くなる．この，実際に Snaith-Hamilton Pleasure Scale（SHAPS-D）を用いた検討では，振戦を含むパーキンソニズムの改善だけでなく，うつに対しても効果を示すことがわかっている[16)]．ペルマックスに関しては D1 レセプター刺激作用があり，神経因性膀胱に効果があるとされている[7)]．またペルマックスには，D3 レセプター刺激作用もあり，中枢性疲労に対し効果を示すとされている[1)]．さらに PET スタディを用いた検討でも，非麦角系のみならず麦角系 DA にも神経保護作用の可能性が指摘され，DA の共通機構に進行阻止作用の存在がクローズアップされている．今後，わが国で使用可能となる ropinirol は，ドパミンと構造が類似していることより，他の DA に比べて精神症状などの副作用が少ないことが予想されている．個々の DA にはそれぞれ臨床経験に基づく治療効果の違いが存在するが，十分なエビデンスが存在するわけではない．したがって DA にはそれぞれ特徴があり，絶対的かつ普遍的効果を持つ DA は存在せず，個々の患者に合わせて処方することが大事になる．

先に触れた DA の神経保護作用については十分なエビデンスが存在しないが，プラミペキソール，ropinirol での SPECT，PET を用いた検討では，その可能性を示している．2006 年に報告された『Neurology』[20)]では，エビデンスに基づいたレビューで証明された神経保護作用を持つ治療方法はないと結論づいている．

DA のリスク

運動合併症状のリスクは Levodopa より DA で少ないが，一方，副作用の点では圧倒的に DA 群で頻度が高い．便秘，悪心，嘔吐などの消化器症状，下肢の浮腫，睡眠発作，病的賭博などの薬剤誘発性精神症状，心臓弁の線維化による弁膜症などが挙げられる（**Table 2**）[6)]．特に最近では心臓弁膜症の問題が大きく取り上げられているが，この副作用については弁置換術が必要となったケースは少なく，定期的に心臓超音波検査をすることで予防可能と考えている．もともと麦角系エルゴタミンによる線維化の報告が発端になり，Van Camp ら[21)]の『Lancet』誌のペルゴリドの心臓弁膜症の報告となっている．論文に

Table 2 Dopamine agonist therapy in early Parkinson's disease: A systematic review of randomised controlled trials (ref. 6)

	Odds ratio	95% Confidence Interval	*P* values
Mortality rates	1.03	0.84～1.26	$P=0.8$
Dyskinesia*	0.48	0.40～0.57	$P<0.00001$
Motor fluctuation*	0.74	0.62～0.89	$P=0.002$
Dystonia*	0.64	0.49～0.84	$P=0.001$

Side effects	Odds ratio	95% Confidence Interval	*P* values
Edema	2.90	2.01～4.21	$P<0.00001$
Somnolence	2.73	2.12～3.51	$P<0.00001$
Hallucinations	2.21	1.50～3.27	$P=0.00007$
Nausea	1.89	1.53～2.35	$P<0.00001$
Constipation	1.81	1.35～2.41	$P=0.00006$
Dizziness	1.58	1.23～2.01	$P=0.0003$
Insomnia	1.41	1.07～1.87	$P=0.01$
Drop-out by side effects	2.77	2.26～3.40	$P<0.00001$

Twenty-eight randomiased trials of DA therapy involving 5,000 patients with early PD were identified. The trials were identified using the Cochrane Library, MEDLINE, Embase, PubMed and Web of Science for the years 1966～2003. Major journals in the field, abstract books and meeting proceedings were also hand-searched. Eligible trials were randomsed trials of a DA (with or without Levodopa) versus a placebo or Levodopa* in patients with early stage PD. Results were combined using the methods of Mantel and Haenszel. This study confirmed that patients treated with Das are less likely to develop motor complications than LD-treated patients. In contrast, other side effects which may be more important for patients and their caregivers are substantially increased.

よれば，高用量の平均投与量が 9.3 mg/日，低用量群でも 3.5 mg/日であり，わが国の投与量と比較すれば大きな差が存在する．わが国では保険診療での最高投与量が 1.25 mg であることを考えれば，最近の Yamamoto らの報告[22]で，海外の投与量の違いもあり，ペルゴリドでの心臓弁膜症の頻度は少ないとしている．むしろカベルゴリンでの頻度の高さを指摘している．ドイツでは高用量カベルゴリン（6～12 mg）での安全性が確認されており，拘束性心臓弁膜症の問題は，過度に神経質になることも患者のメリットを奪いかねないので，個々の症例について投与を健闘すべきと著者は考えている．線維化については，麦角系 DA に共通しているわけではなく，5-HT2b の親和性がその原因と推定されている．事実，Lisuride は 5-HT2b に対しむしろ拮抗作用を示すとされており[5]，1968 年に発売されているものの，線維化の頻度はきわめて少ない．このことからも，5-HT2b への作用が問題となることが，最近のデータからは強く推定される．またこの線維化については可逆的変化といわれており，早期発見早期中止により線維化現象を正常化に戻すことも可能となる．そのような意味でも心臓超音波検査の定期的検査を行うことは重要になる．

眠気と病的睡眠発作についても非麦角系で頻度が高いことが報告されているが，これも DA に共通しているものの，特定の DA に観察されるものではないと結論づけられている．ただ運転中の事故など重篤な事故の報告があり問題となっているので，運転などの機会が多い患者については，少しでも眠気，睡眠発作が存在するようであれば，他の薬剤に変更することが望ましい．DA の副作用については，モニタリングが可能であれば副作用は避けられるが，病的睡眠発作だけは昼間の眠気がなく急激に出現することよりモニタリングの困難さが推定される．

薬剤誘発性精神症状は抗 PD 剤共通の副作用であり，抗コリン剤，塩酸アマンタジン，DA，Levodopa に共通している．最近，dopamine dysregulation syndrome (DDS) の考え方が注目されているが，これは末期まで残存する ventral tegmental area (VTA) のドパミン神経細胞が残ることと関連があるとされている[2]．VTA から側坐核への投射が DDS の中核にあり，このために末期になっても PD ではプラセボ効果が生まれるとされている．この側坐核への影響が大きく，さまざまな症状が出現する (**Table 3**)．ここで注目すべき点は，PD のオフには non-motor off も存在することである．運動障害だけでなく精神症状を含めた neuropsychiatric disorders であることの所以である．Anhednia の考え方はドパミン欠乏に基づいており (**Fig. 2**)，一般的なうつ的症状とは異なる．この

Table 3 Dopamine Dysregulation Syndrome (DDS) (ref. 2)

- Craving
- Euphoria/hypomania
- Dysphoria/non-motor offs
- Appetitive behaviours
- Aggression
- Psychosis
- Motor stereotypies (Punding)

Sensitization of the ventral striatal circuits to dopamine replacement therapy and appetitive behaviour may be analogous to the neurpalstic changes in the dorsal striatum thought to contribute to the motor complications of DRT such as dyskinesia and repetitive motor acts.

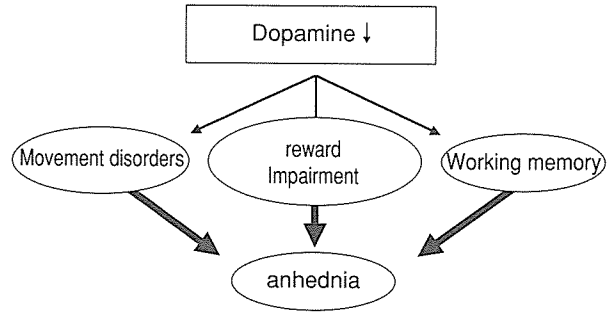

Fig. 2 Deficiency of dopamine induces anhednia
Dopamine deficiency induces movement disorders such as parkinsonism, reward impairment, working memory. All of these factors can also trigger anhednia.

anhednia に対し，D3 が有効であることが報告されているが，反面この D3 が病的賭博などの DDS との関連が指摘されているのも事実である．対象となる患者によって，D3 刺激は両刃の剣となることが予想される．

ELLDOPA STUDY のもたらしたもの

2006 年に発表されたエビデンスに基づいたレビュー[20]によれば，Levodopa の毒性は否定された．また ELLDOPA STUDY で途中 washout してプラセボ群との比較で，UPDRS で差を認めたことより，むしろ神経保護作用が存在している可能性を見出している．一方，β-CIT 取り込み低下がプラセボ群と比較して高くなっており，PD の進行を促進しているかのような矛盾する結果が得られている．この画像診断での解釈については，その後いくつかの問題点が指摘されており議論の余地がある．ただ Levodopa を用いたスタディ結果は，washout 期間の問題や評価時期の問題などいくつか問題はあるものの，早期 PD においても Levodopa の使用の可能性を示したものであり，Levodopa の開始時期については再検討されると思われる．今回の検討で Levodopa の開始時期については，早期は DA で開始し，もし日常生活レベルが低下すれば早期に併用することも積極的選択肢として考慮すべきと考える．今後，日本神経学会でのガイドラインの早期改訂が必要となろう．今日，情報が患者にも容易に得られることより，患者自身 Levodopa の副作用に対し過敏に反応している傾向が臨床の現場でも経験される．日常生活レベルが十分に上がっていないにもかかわらず Levodopa の使用時期が遅れることは，QOL を考えた場合，患者自身の大きな損失になる．日本では副作用を恐れるがあまり十分な投与量が得られないことが多い．皮肉にも先の拘束性心臓弁膜症がわが国で少ないのは投与量が少ないからである．もちろん，むやみに投与量を増やすことはないが，十分量投与することの重要性を強調したい．

他の抗 PD 剤の特徴と問題点

1 セレギリン

MAO 阻害剤であるセレギリンに関しては，わが国では進行期 PD に用いることが推奨されている．しかしながら，最近のセレギリンの治療効果は，むしろ早期に用いることのメリットが報告されている．Levodopa の併用により運動合併症状の出現の抑制や，わが国では単独使用の保険適応がないが，Levodopa との併用は認可されているので，今後はセレギリンの効果を活かすためにも Levodopa 開始時期から併用することも視野に入れて使うべきと考える．欧米では早期から使用することで運動症状の改善が期待されている．また一時，英国でセレギリンによる死亡率の増加が報告されたが，メタアナリシスでの検討で否定されている[13]．ただチーズ効果（チーズ効果：MAO-A と MAO-B の両酵素を阻害するとノルアドレナリンの分解も抑制され，高血圧が生じる）による急激な血圧上昇には留意が必要である．

2 塩酸アマンタジン

A 型インフルエンザの治療薬として近年にわかに注目されているが，インフルエンザに罹患している患者が服用し治療効果を示したことに始まる．作用機序については不明な点が多く，NMDA 受容体拮抗薬として注目されている[19]．グルタミン酸をニューロトランスミッターで持つ視床下核の興奮性を抑制することで，抗 PD 作用を示すことが示唆されている．最近では，進行期の dyskinesia に対し効果を示すことが期待されている．一方で，

小動物視などの視覚性幻覚の問題があり，進行期に使用できないケースも経験する．dyskinesia が出現しているケースでは積極的に用いるべきと考える．

③ 抗コリン剤

塩酸トリヘキシフェニジルが，わが国でも抗 PD 作用を期待されて使用されている．古い薬剤のためエビデンスの高いレベルの試験は少ないが，PD の運動症状に対し塩酸アマンタジンと同様の効果を示す．振戦に対し効果を示すことが経験的に指摘されていたが，Levodopa と比して効果が優れていることはない．むしろ副作用として認知症の問題があり，近時記憶障害が問題となっている．この近時記憶障害は可逆的であるので，中止により回復することが報告されている[11]．

④ ドロキシドパ

すくみ足の現象に青斑核の関与が推定され，その神経伝達物質がノルアドレナリンであることから，その前駆物質であるドロキシドパが開発された．ドロキシドパは，進行期 PD を対象としている．有効率は低いものの，Levodopa 抵抗性の症状に対し，ある程度有効を示す．特に，すくみ足，構音障害，姿勢反射障害に有効であったとしている．起立性低血圧に対しても効果が期待されている．臨床データはわが国に集中しており，レベルの高いスタディがない．

⑤ ゾニサミド

日本で開発された抗てんかん薬で，10 年以上の使用経験がある．したがって安全性については十分な確証が存在する．この薬剤が臨床的に PD に有効であることが，Murata ら[10]により報告された．作用機序については，MAO-B 阻害作用，チロシン水酸化酵素の mRNA の発現レベルの増加，その蛋白の増加を介したドパミン合成促進作用が推定されている．臨床的には wearing off の改善が主体となっており，しかも効果については通常用いられる投与量よりはるかに少ない量（てんかんでは，200～600 mg/日，抗 PD 作用としては 25，50，100 mg）で効果を示すことがわかっている．このゾニサミドの抗 PD 効果については，臨床現場における詳細な観察に基づいており，臨床現場の重要性が再認識された．今後ゾニサミドは，ランダム比較試験での有効性からわが国から発信された薬剤として注目されるであろう．

おわりに

現在 PD に使用可能な薬剤について解説した．最近のエビデンスに基づいた効果判定により，科学的証明に基づいた治療選択が可能となった．一方，PD は多様性に富んだ疾患群の集団であり，個々の患者によって治療効果は異なることも忘れてはならない．さらに最近では，基底核の生理学的アプローチから脳深部刺激療法の開発が行われ，有効性が報告されている．機能外科手術も選択肢に加わり，PD 治療は多岐にわたってきているといえる．選択肢は増えているものの，終生薬物療法は欠かすことができず，生活レベルは満足のいくものとは言い難い．また薬物療法と機能外科手術の時期的な問題など，解決しなければばらない問題が残っている．本稿では薬物療法の概略について解説したが，薬物療法で生命予後は劇的に改善されているものの，依然未解決な問題があり，本質的原因の解明が望まれる．今後分子生物学的アプローチから一次的原因が明らかにされるのもそう遠くない未来にくるものと信じている．

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要　旨

パーキンソン病の薬物療法：最近の動向と問題点

服部　信孝

近年，わが国でも多数のドパミン作動薬の使用が可能になっている．一方，ELLDOPA STUDY の結果は，再度 Levodopa の作用が見直されることになった．今年『Neurology』に発表されたエビデンスに基づくレビューでは，はっきりと Levodopa は細胞毒性がないと肯定している．しかしながら，運動合併症状などを考慮すればドパミン作動薬から使用されることが推奨されるが，ELLDOPA STUDY の結果を受けて，Levodopa の開始時期については，従来より躊躇せずに早期の投与できることが想定される．もちろん，十分なエビデンスの検証が必要であることはいうまでもない．さらに機能的外科手術の登場で，薬物療法の限界と機能的外科手術の時期的問題など新たな課題を抱えている．

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Prognosis of Parkinson's Disease: Time to Stage III, IV, V, and to Motor Fluctuations

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Abstract: We report a long-term outcome on a large cohort of Japanese patients with Parkinson's disease (PD). A total of 1,768 (793 men, 975 women) consecutive patients visited our clinic from 1 January 1989 to 31 December 2002. Among them, 1,183 patients (531 men, 652 women) came to our clinic within 5 years from the onset of disease and at the Hoehn & Yahr Stage III or less at the first visit. Long-term outcome was evaluated in this subcohort of the patients. We examined the duration to reach Stage III, IV, and V, and the duration to develop wearing off and dyskinesia. Time to reach Stage III was slightly but significantly shorter in women, in that 23.8% of men and 35.3% of women reached Stage III by the end of the 5th year; 49.7% of men and 63.3% of women reached Stage III by the end of the 10th year, and 88.9% of men and 79.9% of women by the end of the 15th year ($P < 0.001$). Also, durations to develop wearing off and dyskinesia were shorter in women compared to men. These data suggest that the disease progression may be slightly faster for women. Young-onset patients showed significantly longer duration to reach Stage III, IV, and V but shorter duration to develop wearing off and dyskinesia. Not many studies are available in the literature on the long-term outcome of PD, and our data would be useful as a reference.

Key words: Parkinson's disease; treatment; prognosis; Hoehn & Yahr stage; mortality

Long-term prognosis of patients with Parkinson's disease (PD) has greatly improved since the introduction of levodopa and other anti-PD drugs to the treatment. But not many studies are available on the long-term outcome of PD under the optimum treatment. There are many studies on the mortality of PD; however, it is hard to find studies addressing how long it would take to reach Hoehn & Yahr Stage III, IV, and V under the usual clinical practice setting. This situation prompted us to investigate the long-term outcome of PD patients on a large cohort. Our Medical Center (Juntendo University School of Medicine) is located in a central part of Tokyo and is one of the referral centers for movement disorders. Therefore, our data could be considered as a representative on the long-term outcome of PD in Japan.

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PATIENTS AND METHODS

Patients

We retrospectively reviewed all the hospital charts on patients who had visited our clinic and were diagnosed as PD from 1 January 1989 to 31 December 2002. The diagnosis of PD was made according to the criteria of Calne and colleagues,[1] and those patients who fulfilled the criteria for clinically probable PD or clinically definite PD were enrolled (total number = 1,768). Patients with secondary and symptomatic Parkinsonism were excluded. Hospital charts were reviewed systematically by board-certified or board-eligible neurologists of our department. Items to be checked are defined, and each reviewer was given a check sheet. Items checked included name, sex, date of birth, date of first visit., onset date and initial symptoms, order of medication and approximate date of start of each medication, date they reached Hoehn & Yahr Stage III, IV, and V, date and the

causes of death, if applicable, date of onset of wearing off and drug-induce dyskinesia. End-of-dose deterioration, off-period dystonia, and off-period freezing were taken as the evidence for wearing off. Drug-induced dyskinesias were defined abnormal involuntary movements that appeared either at peak dose or at onset and end of dose. We did not include off-period dystonia in the drug-induced dyskinesia. Missing information was obtained by the telephone or letters to patients or their family members. The final neurological evaluation was made at the hospital visit closest to 31 December 2002. When the evaluation was made before 31 December 2002, absence of significant change in the neurological condition was confirmed at the next visit after 31 December. When significant changes occurred during that period, the data of the final evaluation were revised according to the information obtained from the patients and their caregivers. When patients did not visit our clinic on a regular base until 31 December 2002, we sent questionnaires to those patients so that we could evaluate the final condition during the study period. When they failed to respond to our questionnaires, neurological findings at their last visits were used and the disease durations were counted from the onset of the disease to the date of the last examination. We sent 825 inquiry letters and obtained response from 411. Of the 825 letters, 464 were to the subjects who fulfilled the entry criteria for long-term outcome analysis (n = 1,183) and we received responses from 213.

Durations to Stage III, IV, V, and to the onset of motor fluctuation were analyzed on a subcohort of the patients who visited our clinic within 5 years from the onset and at the Hoehn & Yahr Stage III or less so that we could obtain reliable information as to the time they had reached Stage III and the time they had developed motor fluctuations, if present at the first visit to our clinic. Hoehn & Yahr stage was evaluated at the best *on*, when they had wearing off. This study was approved by the Institutional Review Committee for the Ethics of Clinical Investigations.

Statistical Analyses

Long-term outcomes on the subcohort of patients who visited our clinic within 5 years from the onset and at Hoehn & Yahr Stage III or less were initially analyzed by Kaplan–Meier plots. The comparisons between men and women, between young-onset (50 or before) and late-onset patients (after 50), among initial symptoms, and among initial treatment were made with the log rank test. Kaplan–Meier plots and log rank tests were calculated using StatMate III (ATMS Corp., Tokyo, Japan). The comparative analysis of the age at death was performed on all PD patients. The correlation diagram was drawn to use the age and the year at death. A multivariate analysis by conditional logistic regression was used to study the individual role of each factor, including the sex, onset of age, the starting drug, and the initial symptom. A multivariate analysis was calculated using StatView.

RESULTS

A total of 1,768 patients (793 men, 975 women) were diagnosed as PD during the study period, i.e., from 1 January 1989 to 31 December 2002. Demographic data are summarized in Table 1. The mean age (±SD) of onset was 57.2 ± 11.2, and its distribution is shown in Figure 1. The peak age of onset was 60 to 64 years. The mean age (±SD) at the final evaluation was 66.1 ± 10.1. The mean disease duration (±SD) was 9.41 ± 6.28 years, and the mean Hoehn & Yahr stage at the final evaluation (±SD) was 2.50 ± 1.02. The number of the patients who received L-dopa as the initial treatment was 930 (65.2%), and the total number of patients who had ever received L-dopa was 1578 (89.2%). The maintenance doses of anti-PD drugs at the final evaluation are summarized in Table 2. Mean L-dopa dose at the final examination was 471.5 ± 198.6 mg.

Figure 2 shows distribution of Hoehn & Yahr stage according to the disease duration. When the disease duration was 5 years or less, percentages of patients at Stage I or less, II, III, IV, and V were 14.8%, 12.3%, 49.0%, 28.6%, 3.0%, and 0.7%, respectively. When the disease duration was 6 to 10 years, these percentages were 7.1%, 41.9%, 32.1%, 7.8%, and 1.8%, respectively; in the similar way, for 11 to 15 years, percentages were 2.4%, 28.7%, 33.9%, 13.3%, and 3.5%, respectively; for 16 years and longer 3.3%, 28.7%, 33.9%, 13.3%, and 3.5%, respectively. Percentages of deceased patients were 0.6% for the fist 5 years, 5.0% for the next 5 years, 12.6% for the third 5 years, and 27.0% for the last five years.

Durations to reach Hoehn & Yahr Stage III, IV, V, and to the onset of motor fluctuations were evaluated in a sub-cohort of the patients who visited our clinic within 5 years from the onset and the Hoehn & Yahr Stage III or less. A total of 1,183 patients (531 men, 652 women) fulfilled these criteria. Demographic data on this subcohort are shown in Table 3. The duration to Stage III is shown in Figure 3. Percentages of patients who reached Hoehn & Yahr Stage III by the end of the 5th, 10th, and the 15th year after the onset were 30.2%, 57.2%, and 83.5%, respectively. A slight but significant difference was noted between men and women, in that women reached Stage III slightly but significantly earlier than men; i.e., 23.8% of men and 35.3% of women reached

TABLE 1. *Demographic data on the total cohort*

	Men	Women	Total
No. of patients	793	975	1768
Age of onset (yr)	56.5 ± 11.4	57.8 ± 11.1	57.2 ± 11.2
Age at the first visit (yr)	60.3 ± 10.8	61.8 ± 10.1	61.1 ± 10.5
Age at the last examination (yr)	65.3 ± 10.5	66.8 ± 9.8	66.1 ± 10.1
Disease duration (yr)	9.31 ± 6.14	9.50 ± 6.39	9.41 ± 6.28
Hoehn & Yahr stage at the first visit	2.13 ± 0.84	2.16 ± 0.91	2.15 ± 0.88
Hoehn & Yahr stage at the final evaluation	2.40 ± 0.93	2.58 ± 1.08	2.50 ± 1.02
Years to the use of L-dopa	2.95 ± 2.62	2.81 ± 2.64	2.87 ± 2.63
Years to the use of an agonist	4.73 ± 3.81	4.87 ± 3.98	4.81 ± 3.90
No. of patients who received L-dopa first	426 (53.7%)	504 (51.7%)	930 (52.6%)
No. of total patients who received L-dopa	709 (89.4%)	869 (89.1%)	1578 (89.2%)
No. of patients who received an agonist first 68 (8.6%)	103 (10.6%)	171 (9.7%)	
No. of total patients who received agonists	534 (67.3%)	662 (67.9%)	1196 (67.7%)
No. of patients who received other anti-PD 159 (20.1%) med	241 (24.7%)	400 (22.7%)	
Initial symptoms			
Tremor	365 (47.5%)	522 (54.7%)	887 (51.5%)
Gait disturbance	225 (29.3%)	255 (26.7%)	480 (27.8%)
Bradykinesia	155 (20.2%)	146 (15.3%)	301 (17.5%)
Others	24 (3.1%)	32 (3.4%)	56 (3.2%)
Side of initial symptom			
Right	409	457	866
Left	286	392	678

Mean ± SD.

Stage III by the end of the 5th year and 49.7% of men and 63.3% of women reached Stage III by the end of the 10th year; however, at the end of the 15th year, 88.9% of men and 79.9% of women reached Stage III. The overall difference was statistically significant ($P < 0.001$ by the log rank test). The multivariate analysis also confirmed the above male–female difference (data not shown).

The duration to reach Stage IV is shown in Figure 4. Percentages of patients who reached Hoehn & Yahr Stage IV by the end of the 5th, 10th, and the 15th year after the onset were 6.5%, 27.9%, and 41.2%, respectively. There was no significant difference between men and women. The duration to Stage V is shown in Figure 5. Percentages of patients who reached Stage V by the end of the 5th, 10th, and the 15th year after the onset were 2.1%, 16.5%, and 29.4%, respectively. Again there was no statistical difference between men and women.

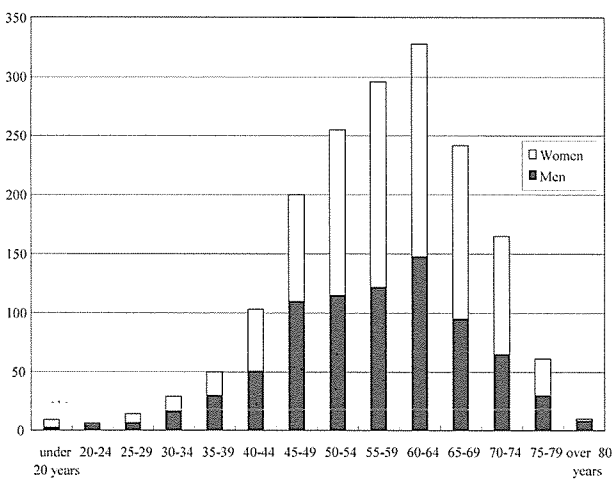

FIG. 1. The distribution of the age of onset. The peak age of onset was 60 to 64 years. The mean (± SD) age of onset was 57.2 ± 11.2. There was no difference in the age of onset between men and women.

The duration to develop wearing off fluctuations is shown in Figure 6. Percentages of patients who developed wearing off fluctuations by the end of the 5th, 10th, and the 15th year after the onset were 21.3%, 59.4%, 73.2%, respectively. Women developed wearing off slightly but significantly earlier than men ($P = 0.0064$). The duration to develop dyskinesia is shown in Figure 7. Percentages of patients who developed dyskinesia by the end of the 5th, 10th, and the 15th year after the onset were 8.4%, 35.1%, and 62.8%, respectively. Women developed dyskinesia slightly but significantly earlier than men ($P = 0.014$). Multivariate analysis also confirmed the above male–female difference (data not shown).

Then we examined influence of age of onset on the duration to reach Hoehn & Yahr Stage III, IV, and V and to wearing off and dyskinesia. Early-onset patients (50 years of age or younger) showed significantly longer duration to reach Stage III, IV and V, in that 14.7% of early-onset and 34.1% of late-onset patients (after 50) reached Stage III by the end of the 5th year from the

TABLE 2. *Maintenance dose of anti-Parkinson drugs at the final evaluation*

Drug	No. (men, women)	Men	Women	Total
L-Dopa (with DCI)	1304 (579, 725)	492.5 ± 196.7	454.7 ± 198.5	471.5 ± 198.6
Bromocriptine	139 (65, 74)	12.56 ± 8.10	10.33 ± 6.14	11.36 ± 7.17
Pergolide	514 (252, 262)	1.32 ± 0.74	1.11 ± 0.61	1.21 ± 0.68
Cabergoline	337 (135, 202)	2.68 ± 1.27	2.56 ± 1.27	2.61 ± 1.26
Talipexole	62 (24, 38)	1.48 ± 1.00	1.27 ± 0.93	1.35 ± 0.96
THP	467 (223, 244)	3.82 ± 1.72	3.99 ± 1.82	3.68 ± 1.61
Amantadine	484 (213, 271)	163.5 ± 62.6	167.0 ± 70.5	165.4 ± 67.1
Selegiline	285 (134, 151)	5.63 ± 2.17	5.01 ± 2.00	5.30 ± 2.10
L-Dopas	169 (80, 89)	466.7 ± 239.0	422.4 ± 175.2	477.9 ± 215.8

Mean ± SD.

onset, these percentages for early-onset and late-onset patients were 30.7% and 64.2% by the end of the 10th year, and 67.4 and 88.6% by the end of 15th year, respectively ($P < 0.001$ in log rank test). Regarding the duration to Stage IV, 3.5% of young-onset patients and 7.3% of late-onset patients reached Stage IV by the end of the 5th year; these percentages for young-onset and late-onset patients were 14.4% and 32.1% by the end of the 10th year and 25.4% and 42.0% by the end of the 15th year, respectively ($P < 0.001$). These percentages to reach Stage V were 1.9% and 2.1% by the end of 5th year from the onset, 7.4% and 19.4% by the end of the 10th year, and 12.9% and 35.7% by the end of the 15th year, respectively ($P < 0.001$).

Durations to the onset of wearing off and dyskinesia were significantly shorter for young-onset patients. By the end of the 5th year from the onset, 33.3% of young-onset and 18.1% of late-onset patients developed wearing off; these percentages for young-onset and late-onset patients were 69.4% and 56.5% by the end of the 10th year and 85.1% and 68.2% by the end of the 15th year, respectively ($P < 0.001$). Regarding dyskinesia, 14.3% of young-onset patients and 6.8% of late-onset patients developed dyskinesia by the end of the 5th year from the onset; these percentages for young onset and late-onset patients were 39.2% and 34.0% by the end of the 10th year and 72.4% and 57.9% by the end of the 15th year, respectively ($P < 0.001$).

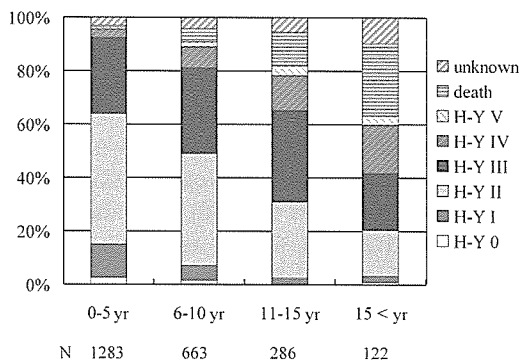

FIG. 2. Distribution of Hoehn & Yahr stages according to the disease duration. When the disease duration was 5 years or less, percentages of patients at Stage I or less, II, III, IV, and V were 14.8%, 49.0%, 28.6%, 3.0%, and 0.7% respectively. When the disease duration was 6 to 10 years, these percentages were 7.1%, 41.9%, 32.1%, 7.8%, and 1.8%, respectively; in the similar way, for 11 to 15 years, percentages were 2.4%, 28.7%, 33.9%, 13.3%, and 3.5%, respectively; for 16 years and longer 3.3%, 28.7%, 33.9%, 13.3%, and 3.5%, respectively. Percentages of deceased patients were 0.6% for the fist 5 years, 5.0% for the next 5 years, 12.6% for the third 5 years, and 27.0% for the last five years.

Then we analyzed the influence of initial symptoms on the duration to reach Hoehn & Yahr Stage III, IV, and V. The tremor-onset group and the bradykinesia-onset group showed significantly longer duration to reach Stage III compared to the gait disturbance-onset group ($P < 0.01$, data not shown). There was no significant difference between the tremor-onset and the bradykinesia-onset group. Initial symptoms in the bradykinesia group were usually disturbances of hand dexterity such as hand writing. Initial symptoms had no effect on the duration to reach Stage IV or Stage V.

In the same way we analyzed the influence of initial symptoms on the duration to develop wearing off or dyskinesia. Tremor-onset group showed significantly longer duration to develop wearing off compared with bradykinesia-onset and gait disturbance-onset groups ($P < 0.001$, data not shown). But initial symptoms were of no effect on the duration to develop dyskinesia.

Then we analyzed the effects of initial treatment on the duration to reach Hoehn & Yahr Stage III, IV, and V. The initial L-dopa group showed slightly but significantly shorter duration to reach Stage III. By the end of the 5th year, 32.7% of the L-dopa group and 23.1% of the dopamine agonist group reached Stage III; these percentages for the L-dopa group and the dopamine agonist group were 61.1% and 41.1% by the end of the 10th year and 82.1% and 76.3% by the end of the 15th year, respectively ($P < 0.05$). The group which was started with other drugs, mainly an anticholinergic or amanta-

TABLE 3. *Demographic data on the subcohort in which long-term outcome was evaluated*

	Men	Women	Total
No. of patients	531	652	1183
Age of onset (yr)	58.6 ± 10.7	59.9 ± 9.7	59.3 ± 10.1
Age at the first visit (yr)	60.8 ± 10.9	62.2 ± 9.7	61.6 ± 10.3
Age at the last examination (yr)	64.9 ± 10.6	66.4 ± 9.5	65.7 ± 10.1
Disease duration (yr)	6.35 ± 3.75	6.50 ± 3.69	6.43 ± 3.72
Hoehn & Yahr stage at the first visit	1.99 ± 0.77	2.01 ± 0.84	2.00 ± 0.81
Hoehn & Yahr stage at the final evaluation	2.38 ± 1.05	2.48 ± 1.13	2.43 ± 1.10
Years to the use of L-dopa (yr)	2.37 ± 1.49	2.44 ± 1.48	2.41 ± 1.49
Years to the use of a agonist (yr)	3.57 ± 2.51	3.46 ± 2.24	3.51 ± 2.36
No. of patients received L-dopa first	271 (51.0%)	300 (46.0%)	571 (48.3%)
No. of total patients received L-dopa	476 (89.6%)	583 (89.4%)	1059 (89.5%)
No. of patients received an agonist first	56 (10.5%)	93 (14.3%)	149 (12.6%)
No. of total patients who received agonists	337 (63.5%)	433 (66.4%)	770 (65.1%)
No. of patients received other anti-PD med	115 (21.7%)	175 (26.8%)	290 (24.5%)
Initial symptoms			
Tremor	242 (45.6%)	354 (54.3%)	596 (50.4%)
Gait disturbance	148 (27.9%)	168 (25.8%)	316 (26.7%)
Bradykinesia	111 (20.9%)	97 (14.9%)	208 (17.6%)
Others	15 (2.8%)	22 (3.4%)	37 (3.1%)
Side of initial symptom			
Right	268	307	575
Left	198	271	469

Mean ± SD.
PD, Parkinson's disease.

dine HCl, also showed similar time course as the dopamine agonist group (data not shown). Similar difference was also noted in the time to reach Stage IV ($P < 0.05$, data not shown). Initial treatment was of no effect on the time to reach Stage V or to develop wearing off or dyskinesia (data not shown).

Duration to death is shown in Figure 8. Percentages of patients who died by the end of the 5th, 10th, and 15th year from the onset were 0.7%, 10.2%, and 18.7%, respectively. No significant difference was noted between men and women. But age of onset had a significant effect on the duration to death. Duration to death was

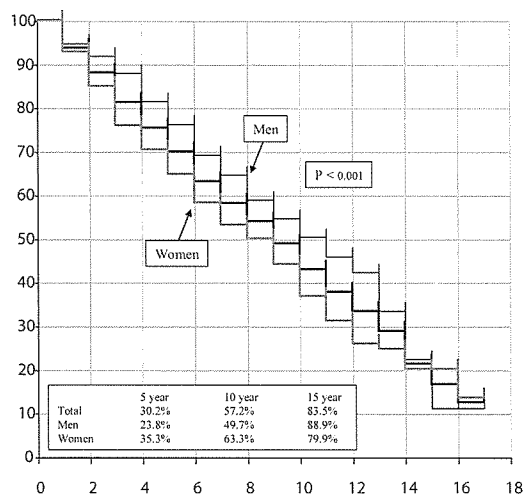

FIG. 3. The duration to reach Stage III. The ordinate indicates the proportion (percentage) of the patients remaining at Stage II or less. The abscissa indicates the years from the onset. Total number of the patients analyzed was 1,178 (men, 530; women, 648). The fine solid black line indicates men, the gray line women, and the heavy black line men and women combined. Women reached Stage III slightly but significantly earlier than men (P < 0.001).

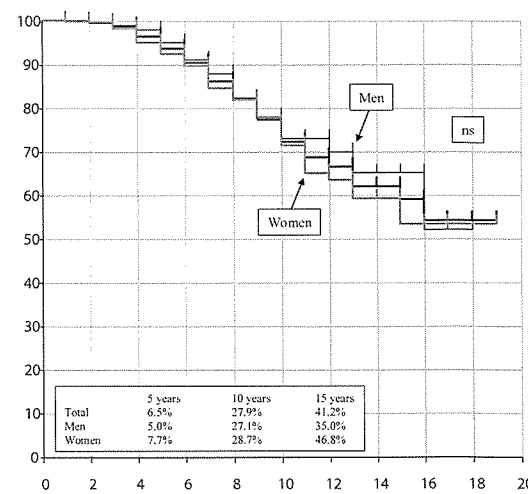

FIG. 4. The duration to reach Stage IV. The ordinate indicates the proportion (%) of the patients remaining at Stage III or less. The abscissa indicates the years from the onset. Total number of the patients analyzed was 1,181 (531 men, 650 women). The fine black line indicates men, the gray line women, and the heavy black line men and women combined. No significant difference was noted between men and women ($P = 0.212$).

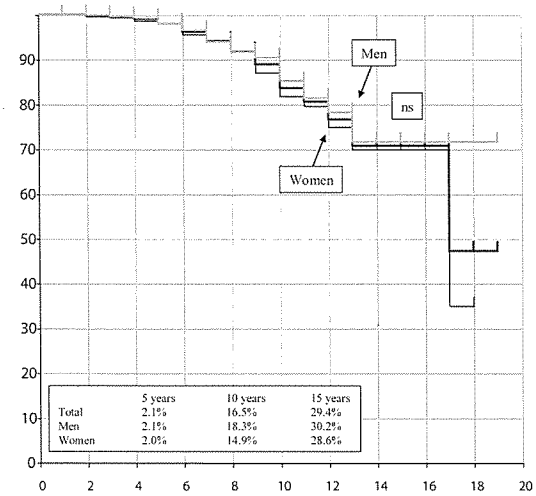

FIG. 5. The duration to reach Stage V. The ordinate indicates the proportion (%) of the patients remaining at Stage IV or less. The abscissa indicates the years from the onset. Total number of the patients analyzed was 1,181 (531 men, 650 women). The fine black line indicates men, the gray line women, and the heavy black line men and women combined. No significant difference was noted between men and women ($P = 0.444$).

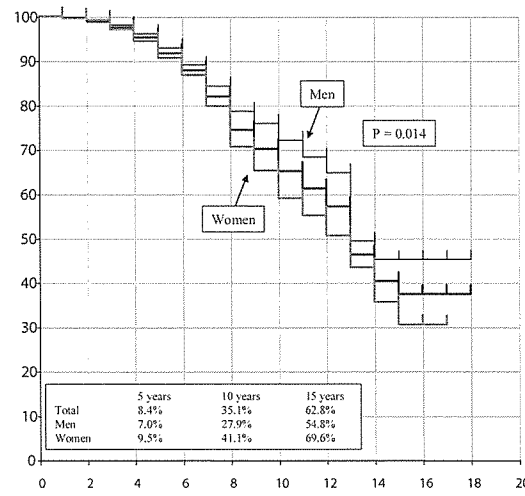

FIG. 7. The duration to develop dyskinesia. The ordinate indicates the proportion (percentage) of the patients without developing dyskinesia. The abscissa indicates the years from the onset. Total number of the patients analyzed was 1,177 (528 men, 649 women). The fine black line indicates men, the gray line women, and the heavy black line men and women combined. Women developed dyskinesia significantly earlier than men ($P = 0.014$).

significantly longer for young-onset patients in that 0.7% of young-onset patients and 0.7% of late-onset patients died by the end of the 5th year from the onset; these percentages for young-onset and late-onset patients were 1.7% and 12.8% by the end of the 10th year, and 7.6% and 22.6% by the end of the 15th year from the onset, respectively ($P < 0.01$). Initial treatment and initial symptom were of no effect on the mortality (data not shown).

As sex differences in these analyses were unexpected to us, we did multivariate conditional logistic regression analysis. Regarding sex, males had a protective effect on reaching Stage III ($P < 0.001$) and for developing wearing off ($P = 0.0002$) and dyskinesia (0.0039). But male

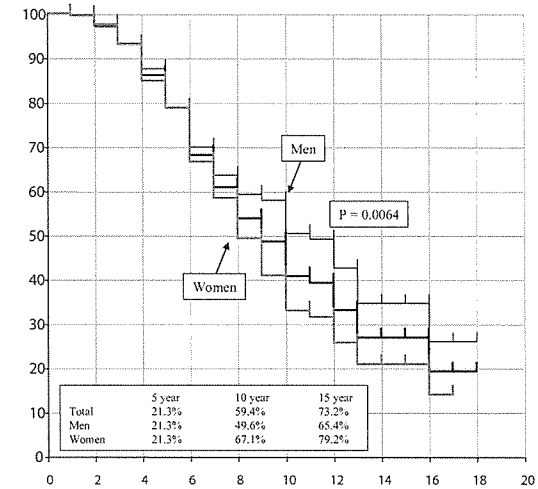

FIG. 6. The duration to develop wearing off. The ordinate indicates the proportion (percentage) of the patients without developing wearing off fluctuations. The abscissa indicates the years from the onset. Total number of the patients analyzed was 1,175 (527 men, 648 women). The fine black line indicates men, the gray line women, and the heavy black line men and women combined. Women developed wearing off significantly earlier than men ($P = 0.0064$).

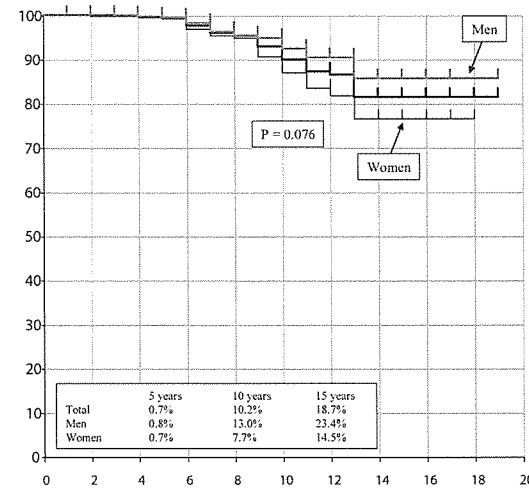

FIG. 8. The duration to death. The ordinate indicates the proportion (percentage) of the patients who remained alive. The abscissa indicates the years from the onset. Total number of the patients analyzed was 1,183 (531 men, 652 women). The fine black line indicates men, the gray line women, and the heavy black line men and women combined. No significant difference was noted between men and women ($P = 0.076$).

TABLE 4. *Demographic data on deceased patients*

	Men	Women	Total
Total no. of patients	793	975	1768
No. of patients who died	71	60	131
Age at death, yr (± SD)	71.9 ± 8.0	74.2 ± 8.7	72.9 ± 8.4
Duration to death from the onset, yr (± SD)	13.2 ± 7.9	12.4 ± 6.8	12.8 ± 7.4
Apparent mortality rate	9.0%	6.2%	7.4%

Mean ± SD.

sex was the risk factor of mortality ($P = 0.025$). Regarding the age of onset, young age of onset (50 or before) had a protective effect on reaching Stage III ($P < 0.0001$), reaching Stage IV ($P < 0.0001$), reaching Stage V ($P < 0.0001$), and mortality ($P < 0.0001$), but it was a risk factor for developing wearing off ($P < 0.0001$) and dyskinesia ($P < 0.0001$). Regarding the initial treatment, dopamine agonist was a protective factor for developing wearing off compared to L-dopa ($P = 0.020$) and with other drugs ($P = 0.034$). Regarding the initial symptom, tremor was a protective factor for developing wearing off compared to bradykinesia ($P = 0.0028$) and gait disturbance ($P = 0.0011$). No other significant correlation was noted among the factors analyzed.

Causes of the death and mortality rate were further analyzed on the total cohort (n = 1,768). During the 14 years of the study period, 131 patients (71 of 793 men and 60 of 975 women) died (Table 4). The mean age at death was 71.9 ± 8.0 years for men and 74.2 ± 8.7 years for women (not significant). The duration from the onset to the death in these patients was 13.2 ± 7.9 years for men and 12.4 ± 6.8 years for women (not significant).

Causes of death are shown in Table 5. The most common identified cause was pneumonia. Many of our patients died at home, and we were notified of the deaths of the patients several weeks after the deaths, and it was not easy to determine the exact causes of deaths. The causes of the deaths had to be concluded as unknown in 50 (38.2%) of 131 patients. In addition, details of the premorbid conditions were not known in many patients. This finding is the reason why somewhat unclear and obscure causes of the death had to be listed in the table.

Then the age and the year of death of each patient was plotted in Figure 9 (men) and Figure 10 (women) with the life expectancy curve of Japanese general population as a reference. Linear regression curve for the age at death for male PD patients gradually approached the life expectancy curve of the male general population as time went on. As of year 2003, the life expectancy of the male general population was 78.45 years. The average age at death of the male patients calculated from the curve was 76.69 years (97.76% of the general population), not significantly different from the male general population. On the contrary, the linear regression curve of the age at death of female patients had never reached that of the female general population. As of 2003, the life expectancy of the female general population was 85.63. In contrast, average age at death of the female PD patients studied was 76.23 (89.02% of the general population). The age at death for female PD patients was essentially the same as that of male PD patients. Female PD patients

TABLE 5. *Causes of the death*

Cause	No. (%)
Pneumonia	29 (22.1)
Sudden death	7 (5.3)
Cancer	5 (3.8)
Suffocation	5 (3.8)
Ileus	4 (3.1)
Death in bath tab	3 (2.3)
Trauma	3 (2.3)
Cerebral hemorrhage	3 (2.3)
Malignant syndrome	2 (1.5)
Respiratory failure	2 (1.5)
Myocardial infarction	2 (1.5)
Rupture of aortic aneurysm	2 (1.5)
Congestive heart failure	2 (1.5)
Multiple myeloma	2 (1.5)
Subarachnoid hemorrhage	2 (1.5)
Renal failure	1 (0.8)
Gastrointestinal hemorrhage	1 (0.8)
Acute cardiac failure	1 (0.8)
Hepatic failure	1 (0.8)
Septicemia	1 (0.8)
Aginge	1 (0.8)
Unknown	50 (38.2)
Total	131 (100)

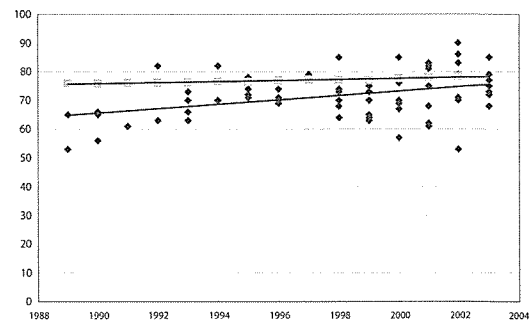

FIG. 9. The age and the year at death for the male patients. The ordinate indicates the age at death and the abscissa the year of death. The ages at death of male patients are plotted against the year at death (diamonds). The black line indicates the linear regression curve of age at death for male patients ($y = 0.006037x + 64.60$). The gray line indicates the life expectancy of the Japanese male general population (squares; $y = 0.001401x + 75.55$; the source of the data is the Vital Statistics of Japan, Ministry of Health, Labor and Welfare, Statistical Database). As of 2003, the life expectancy of the male general population was 78.36. The mean expected age of death of male Parkinson's disease (PD) patients at year 2003 was calculated as 76.69 from the linear regression curve.

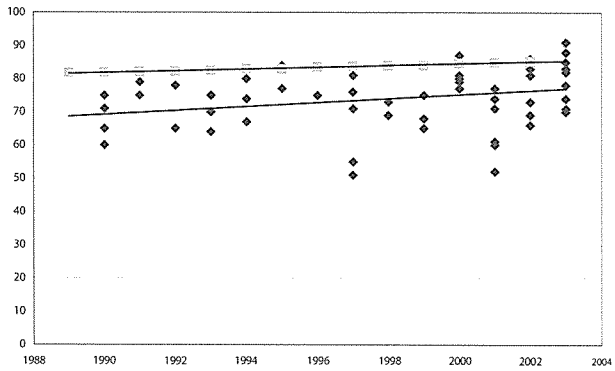

FIG. 10. The age and the year at death for the female patients. The ordinate indicates the age at death and the abscissa the year of death. The ages at death of female patients are plotted against the year at death (diamonds). The black line indicates the linear regression curve of age at death for female patients ($y = 0.003601x + 69.02$). The gray line indicates the life expectancy of the Japanese female general population (squares; $y = 0.002038x + 81.25$; the source of the data is the Vital Statistics of Japan, Ministry of Health, Labor and Welfare, Statistical Database). As of 2003, the life expectancy of the female general population was 85.33. The mean expected age of death of female PD patients at year 2003 was calculated as 76.23 from the linear regression curve.

lost approximately 8 years of longevity compared to that of the female general population in Japan.

DISCUSSION

Studies on long-term outcome of the patients with PD are few. Particularly, it was hard to find reports on the duration to reach Hoehn & Yahr Stage III, IV, and V in a large cohort of PD patients. Although the functional state of the upper extremities is not very well represented in the Hoehn & Yahr staging, it is a very useful measure to evaluate overall functions of PD patients. Generally, patients in Stage II or below were able to engage in social as well as daily activities without large difficulty. They can live an independent life, but there will be some restrictions in their activities in Stage III, and an independent life becomes difficult in Stage IV or above. Therefore, this is a good scale to estimate the overall activity state of the PD patients, and it would be nice to have some idea on how many years it would take to reach Stage III or IV for both clinicians and patients. We have to admit that there is a limitation in the accuracy of the data in retrospective studies like this one. We did Kaplan–Meier analysis instead of Cox analysis, as the former method was applicable in the presence of some missing data. We did our best to obtain missing data of patients who had been lost for follow-up by sending questionnaires and by telephoning; however, because of moves and of other reasons, follow-up information could not be obtained in 21.2% of the patients analyzed. In these subjects, we had to use the clinical data at the last visit to our clinic and the duration of the disease was calculated from the onset of the disease to the last visit to our clinic. Therefore, we believe that prognostic data in terms of the disease duration were analyzed reasonably appropriately: prognostic data were always analyzed against the duration of the disease from the onset. However, we admit that this strategy is a weak point of our analysis, including the statistical method used and in interpreting our data; readers should keep this fact in mind.

Nonetheless, we believe that our data would give us approximate information on the long-term outcome of PD patients in an usual clinical practice setting, We did not intentionally delay the use of L-dopa and we used L-dopa when it became necessary. Although our center is a referral center, according to our medical system, any patient can visit our clinic without a referral letter. Therefore, not only advanced PD patients, but also many early-stage patients come to our clinic as can be seen from Table 3, in that average and mean Hoehn & Yahr stage at the initial visit was 1.99 ± 0.77 in the cohort of the long-term outcome study, indicating that many mild cases were included. Therefore, we believe that the patients we studied represent the general PD population in our country.

Hoehn & Yahr stage was evaluated at the best *on* in each patient when wearing off was present. It was difficult to evaluate the stage at *off* when usually patients stayed at home. In addition, we thought evaluation of Hoehn & Yahr stage during *on* phase was more important to know the level of activities of PD patients. In this respect, 27.9% of our patients reached Stage IV by the end of the 10th year from onset and 41.2% by the end of the 15th year from onset. In another word, 72.1% of the patients and 58.8% of the patients remained at Stage III or less at their best *on* by the end of the 10th and the 15th year from the onset, respectively. These results appeared to be more than expected for PD patients. We wanted to compare our results with those of similar studies reported in the literature; however, we could not find a similar study.

Regarding the frequency of wearing off in relation to the duration of the disease, 21.3% of the patients developed wearing off by the end of the 5th year from the onset, 59.4% by 10 years, and 73.2% by 15 years. Time to develop wearing off in our data is somewhat longer than those reported in the literature. It has been quoted frequently that the frequency of motor fluctuations increases by approximately 10% every year from the onset of the disease.[2] This rate would mean that the frequency of motor fluctuations at 5 years from the onset would be

approximately 50%. Our data of 21.3% is considerably lower than this. As our data are from a retrospective study, we might have underestimated the frequency of wearing off. In addition, we analyzed dyskinesia separately from wearing off. These factors might in part account for the lower frequency of wearing off. However, the data comparable to ours are also reported in the literature. Koller and associates[3] reported 21% frequency of motor fluctuations with mean maintenance dose of L-dopa at 426 mg 5 years after the onset in their controlled prospective study. This frequency is comparable to ours (21.3% and 471.5 mg of L-dopa 5 years from the onset). It has frequently been claimed informally that relatively low frequency of wearing off among Japanese patients is due to lower L-dopa maintenance dose; however, according to the present study, this does not seem to hold true.

Generally, dyskinesia developed after wearing off in our study. If we compare Figures 6 and 7, frequency of dyskinesia is constantly lower than that of wearing off (21.3% vs. 8.4% at the end of the 5th year, 59.4% vs. 35.1% at the end of the 10th year, and 73.2% vs. 62.8% at the end of the 15th year, respectively). In the literature, frequencies of motor fluctuations have been reported variously. Caraceni and coworkers[4] reported the frequency of motor fluctuations (wearing off and dyskinesia combined) as 29% at 4 years and 60% at 6 years from onset; average maintenance dose of L-dopa was 449 mg at 4 years and 403 mg at 6 years. The Parkinson Study Group[5] reported the frequency of wearing off as 50% at 3 years with mean L-dopa dose of 329 mg and that of dyskinesia 30% with mean L-dopa dose of 387 mg. Thus, they also reported lower frequency of dyskinesia compared to wearing off with the same duration of the disease from the onset. Schrag and Quinn[6] also reported higher incidence of wearing off (40%) compared with dyskinesia (28%) in their community based study (total n = 124). In their study, the prevalence of motor fluctuations was best predicted by disease duration and dose of L-dopa, whereas dyskinesias could be best predicted by duration of treatment.

Contrary to our and other data shown above, McColl and colleagues[7] reported that dyskinesia appeared an average of 7 months before the onset of wearing off, which developed in 58% of the patients after a mean treatment period of 35 months. They studied the duration from the start of L-dopa treatment to the onset of motor fluctuations. We evaluated the duration from the onset of the disease to wearing off and dyskinesia, as development of wearing off depends mainly on the severity of nigral neurodegeneration. On the other hand, dyskinesia depends on both disease severity and the dose of L-dopa. Severe 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) victims developed wearing off and dyskinesia shortly after the initiation of L-dopa.[8]

Young-onset patients (50 or before) showed longer duration to reach Stage III, IV, and V suggesting the slower progression compared with late-onset patients. Diamond and associates[9] also reported a more favorable prognosis for patients with onset under 50 years of age than those whose symptoms began in later years. In addition, in their report expected mortality rate was 1.82 for those with onset under 50, 2.17 for those with onset 50 to 59 years, and 2.20 for those with onset after 60. Our data also suggest a better prognosis for young-onset patients. But young-onset patients developed wearing off and dyskinesia faster than late-onset patients. Therefore, rate of nigrostriatal degeneration may not be slower in young-onset patients compared to late-onset patients. Relatively selective nigrostriatal dopaminergic degeneration without much of nondopaminergic involvement in young-onset patients would explain longer duration to reach Stage III, despite shorter duration to develop motor fluctuations.

Tremor-dominant patients showed significantly longer duration to reach Stage III compared to gait disturbance-onset patients. Also they showed longer duration to develop wearing off. It has been said frequently that tremor-dominant patients tend to show slower progression of symptoms compared to rigid–akinetic patients.[10–13] Roos and coworkers[14] also reported that tremor-dominant patients reached Stage III significantly later than patients with rigid–hypokinetic patients. Our findings are consistent with these data.

It has been shown in recent prospective studies that starting the treatment of PD with a dopamine agonist delayed the onset of motor fluctuations.[15,16] Sixty-two percent of our patients received L-dopa as the initial treatment, 11.4% a dopamine agonist, and 26.6% other drugs such as an anticholinergic drug, amantadine, or selegiline. The number of patients who received a dopamine agonist as the initial treatment was too small to make definite conclusion whether or not starting treatment with a dopamine agonist would have delay the onset of motor fluctuations. In 1989, when this study was started, it was not a usual practice to use a dopamine agonist as the initial drug in Japan. Nonetheless, we looked at the effects of initial drug on the long-term outcome.

The initial L-dopa group showed slightly but significantly shorter duration to reach Stage III. But from the small number of the initial dopamine agonist group, we do not believe that this would mean that L-dopa was neurotoxic or other medications were neuroprotective.

The literature also does not support the idea that L-dopa is in some way neurotoxic to nigral neurons.[17–20] Types of initial treatment were of no effect on the mortality. Types of initial treatment were also of no effect on the time to develop wearing off or dyskinesia.

What was surprising to us was the significant sex difference between men and women in the time to reach Stage III, time to the onset of wearing off, time to the onset of dyskinesia, and the age at death in comparison with the life expectancy of male and female general Japanese populations. No participating neurologists expected such difference when they were treating their patients. Both time to wearing off and time to Stage III were slightly but significantly shorter for women in our study suggesting that the disease progression was slightly but significantly faster for female patients with PD. The most important contributing factor for the development of wearing off is believed to be loss of nigrostriatal dopaminergic nerve terminals. Therefore, the time to wearing off in some way may be reflecting the speed of the progression as the time to Stage III. Time to Stage IV and V did not show sex difference. Progression into Stage IV and V may be influenced not only by the natural progression of the disease but also by intervening incidents such as severe pneumonia, fractures, malignant syndrome, and other medical complications. Intervention by such events might have obscured the sex difference in advanced stages.

In the literature, Diamond and colleagues[21] reported disability and mortality as having a sex difference. They analyzed longitudinal disability scores in 47 men and 23 women with PD following them up for 6 years. They found no significant differences between the sexes in mean disability scores in any of the 6 years. But they found a difference in mortality in that the observed to expected ratio for the men was 1.7457 and for the women 2.4740, a significantly greater excess in female mortality. Hely and associates[22] reported faster progression as measured by modified Columbia Rating Scale score in female PD patients compared with male patients. Conversely, men did more poorly in other studies.[11–23]

In our total cohort, during the 14-year study period (1989–2002), 131 patients (71 of 793 men and 60 of 975 women) died. When we compared the average expected age at death in our PD patients from the linear regression curve of the age at death in PD patients (Figs. 9 and 10), there was a striking sex difference. As of year 2003, the life expectancies of the male and female general population were 78.45 years and 85.63, respectively. In contrast, average expected ages at death of the male and female PD patients calculated from the linear regression curves were 76.69 years (97.76% of the male general population) and 76.23 (89.02% of the female general population), respectively. The age at death for female PD patients was essentially the same as that of male PD patients. Female PD patients lost approximately 8 years of longevity that Japanese women in the general population were entitled to entertain.

By reviewing the literature, the relative risk for mortality of PD patients was variously reported. Among the more than 20 reports on the mortality in PD, two studies reported less than 1.5 mortality (1.17 and 1.2) compared with the general background population,[24,25] 11 studies reported mortality from 1.5 to 2.0 for PD patients,[9,26–35] and 10 studies reported higher than 2.0 mortality for PD.[34,36–44] Five studies reported sex differences: Diamond and coworkers[9] reported mortality rates of 1.75 for men and 2.47 for women compared with the respective background populations; Wermuth and colleagues[34] reported relative risks for mortality of 1.92 for men and 2.47 for women; Minami and associates[35] reported relative risks for morality of 1.74 for men and 1.97 for women; Ben-Shlomo and Marmot[40] reported a mortality rate of 2.6 without sex difference; Hely and colleagues[27] did not find sex differences in the standardized mortality ratios between men and women. On the contrary, Lilienfeld and associates[45] and Berger and coworkers[46] reported higher mortality for men compared with women. According to Berger and colleagues,[46] relative risk was 3.1 for men and 1.8 for women in European populations.

Riggs[47] reported an annual age-adjusted mortality rate in the United States, which was 19.15/100,000 at age 73.73 for men and 28.64/100,000 at age 78.99 for women each year from 1955 to 1986. Wermuth and associates[34] reported median ages of death at 77.29 years for men and 79.11 years for women in contrast with the median ages of death at 80.69 years for men and 84.37 years for women of the respective background populations. Elbaz and coworkers[29] reported the median survival of PD patients in Olmsted County, Minnesota, for the period of 1976 to 1995. They found 110 deaths in 196 PD cases and 79 deaths in 185 reference subjects. The median survival was 10.3 years in PD patients and 13.4 years in the reference subjects. The relative risk was 1.81 in women, and 1.49 in men. According to our data, mean disease duration from the onset to death was 13.2 years in men and 12.4 years in women (Table 5).

Imaizumi[44] studied age-specific mortality rates from PD in Japan from 1950 through 1993. The mortality rate was 2.45 per 100,000 for men and 2.12 per 100,000 for women at age 65.49 years from 1950 to 1951 and 1992 to 1993 compared with the general population. Nakashima and associates[48] studied mortality in another Japanese cohort of PD cases. They found that the mean

age at death was 75.95 ± 7.25 for men (n = 57) and 78.37 ± 6.69 (n = 57). According to our data, the mean age at death was 71.9 for men and 74.2 for women (Table 5). In both of these studies, age at death did not differ much between men and women, despite longer life expectancy by 7 to 8 years for women in Japan. Our female PD population will lose approximately 7 years of longevity over men once they get PD.

The reasons why female PD patients reached Stage III slightly but significantly earlier than men and developed wearing off and dyskinesia earlier are not known. Further studies are needed.

In summary, we reported long-term outcome on a large cohort of PD patients. Particularly we were interested in how long it would take to reach Stage III, IV, and V under a usual clinical practice setting. To our knowledge, no report has addressed this question on a large cohort with a long follow-up period.

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14-3-3η is a novel regulator of parkin ubiquitin ligase

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Mutation of the *parkin* gene, which encodes an E3 ubiquitin-protein ligase, is the major cause of autosomal recessive juvenile parkinsonism (ARJP). Although various substrates for parkin have been identified, the mechanisms that regulate the ubiquitin ligase activity of parkin are poorly understood. Here we report that 14-3-3η, a chaperone-like protein present abundantly in neurons, could bind to parkin and negatively regulate its ubiquitin ligase activity. Furthermore, 14-3-3η could bind to the linker region of parkin but not parkin with ARJP-causing R42P, K161N, and T240R mutations. Intriguingly, α-synuclein (α-SN), another familial Parkinson's disease (PD) gene product, abrogated the 14-3-3η-induced suppression of parkin activity. α-SN could bind tightly to 14-3-3η and consequently sequester it from the parkin–14-3-3η complex. PD-causing A30P and A53T mutants of α-SN could not bind 14-3-3η, and failed to activate parkin. Our findings indicate that 14-3-3η is a regulator that functionally links parkin and α-SN. The α-SN-positive and 14-3-3η-negative control of parkin activity sheds new light on the pathophysiological roles of parkin.

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Introduction

In the last decade, people working in the field of Parkinson's disease (PD) witnessed a tremendous progress in uncovering the mechanisms of PD, and several familial PD genes were discovered in succession (Vila and Przedborski, 2004). Of these hereditary PD genes, *parkin* (*PARK2*), the causative gene of autosomal recessive juvenile parkinsonism (ARJP), is of a special interest because it encodes a ubiquitin ligase, a critical component of the pathway that covalently attaches ubiquitin to specific proteins with a polymerization step to form a degradation signal (Shimura *et al*, 2000). Indeed, parkin catalyzes the addition of ubiquitin to target proteins prior to their destruction via the proteasome, suggesting that the misregulation of proteasomal degradation of parkin substrate(s) is deleterious to dopaminergic neurons (Dawson and Dawson, 2003; Bossy-Wetzel *et al*, 2004; Kahle and Haass, 2004). Consequently, impaired protein clearance can induce dopaminergic cell death, supporting the concept that defects in the ubiquitin–proteasome system may underlie nigral degeneration in ARJP and perhaps sporadic forms of PD (McNaught and Olanow, 2003). On the other hand, it was recently reported that parkin also catalyzes the formation of the K63-linked polyubiquitylation chain, independent of proteasomal destruction, in which the K48-linked polyubiquitylation chain is necessary (Doss-Pepe *et al*, 2005; Lim *et al*, 2005). Thus, it is plausible that parkin shares two roles as an E3 ligase; that is, one linking to and the other independent of the proteasome.

Among the products of major familial PD genes (Vila and Przedborski, 2004), α-synuclein (α-SN) is a product of familial PD gene (*PARK1*) identified as a presynaptic protein of unknown function. α-SN is considered in the molecular mechanisms of PD mainly because it is one of the major components of the cytoplasmic Lewy body (LB) inclusion present in the remaining nigral dopaminergic neurons of PD patients, which is the pathological hallmark of sporadic and some familial PDs (Forno, 1996). Although various studies have been conducted on α-SN (Dawson and Dawson, 2003; Bossy-Wetzel *et al*, 2004; Kahle and Haass, 2004), its pathophysiological role(s) and the interplay between α-SN and parkin are largely unknown.

To date, little is known about the role of parkin as a ubiquitin E3 ligase with respect to the underlying molecular mechanism(s) of ARJP or PD. Here we report for the first time that 14-3-3η, a member of the 14-3-3 family (β/α, γ, ε, η, ζ/δ, σ, and τ/θ) (Berg *et al*, 2003; Bridges and Moorhead, 2004; Mackintosh, 2004) identified in LB (Kawamoto *et al*, 2002; Ubl *et al*, 2002), binds primarily to the linker region of parkin and functions as a novel negative regulator of parkin. We also show that α-SN relieves parkin activity suppressed by 14-3-3η, indicating that 14-3-3η is a novel molecule handling both parkin and α-SN, and that functionally links the two familial PD gene products.

Results

Parkin specifically interacts with 14-3-3η but not with other 14-3-3 isoforms

We first examined the physical association of parkin with 14-3-3 isoforms, which are abundantly expressed in the brain (Martin *et al*, 1994; Baxter *et al*, 2002). Parkin was immunoprecipitated from mouse brain extracts, and the presence of 14-3-3 was analyzed by Western blotting (Figure 1A). 14-3-3 was clearly detected in the parkin immunoprecipitant, but not in those of control IgG or parkin antibody preabsorbed

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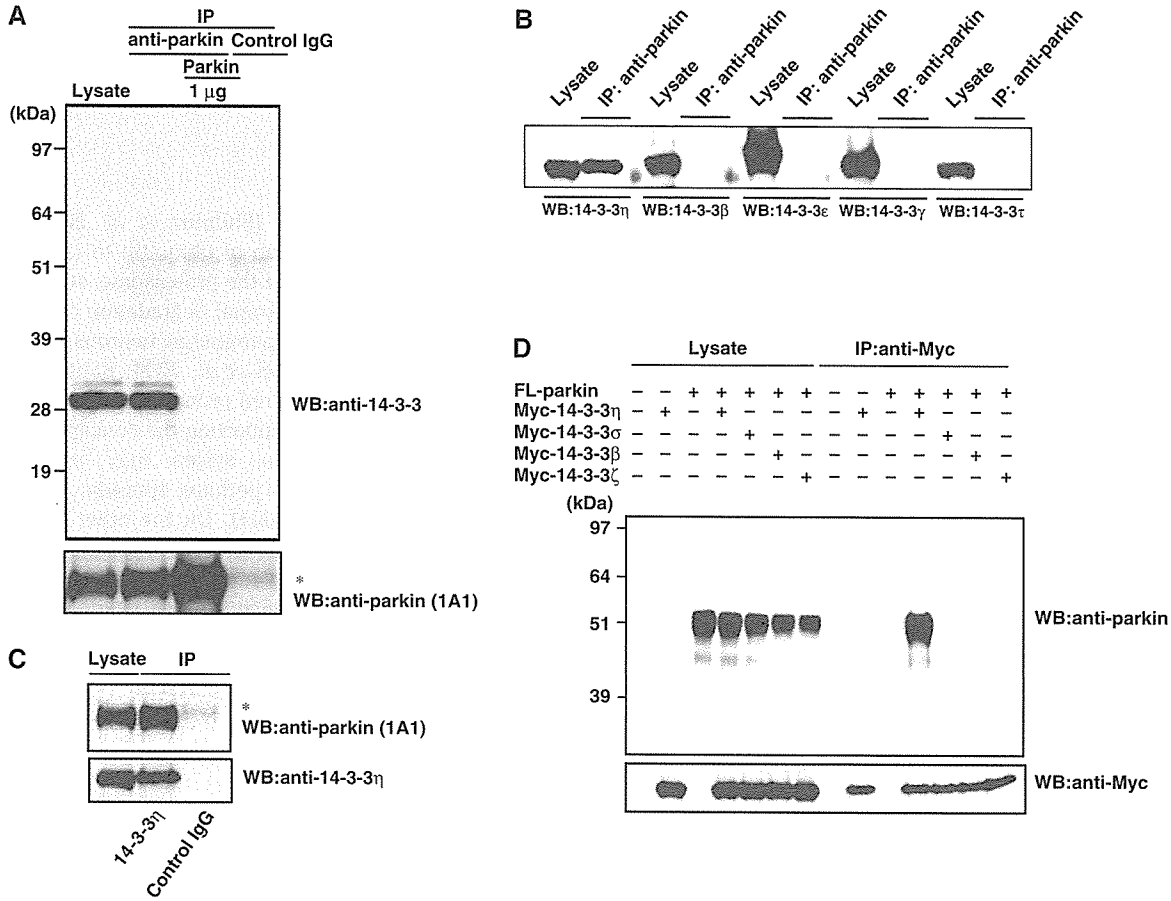

Figure 1 Physical interaction between parkin and 14-3-3η. (**A**) Immunoprecipitation by anti-parkin antibody in the mouse brain. Mouse brain lysates were prepared and treated with anti-parkin or control IgG as described in Materials and methods. The resulting immunoprecipitates were subjected to SDS–PAGE, followed by Western blotting with anti-14-3-3 and parkin (1A1) antibodies. In all, 1 μg of recombinant parkin was pretreated with anti-parkin prior to immunoprecipitation. Left lane: the brain lysate (1.5% input). Asterisk denotes an IgG heavy chain. (**B**) Specificity analysis of 14-3-3 species. The immunoprecipitation with anti-parkin and subsequent SDS–PAGE were carried out as in (A). Western blotting was conducted with antibodies against various 14-3-3 isoforms as indicated for lysates and anti-parkin immunoprecipitates. (**C**) Immunoprecipitation by anti-14-3-3η antibody. After immunoprecipitation with anti-14-3-3η or control IgG of the brain lysate, the immunoprecipitates were analyzed by Western blotting with anti-parkin (1A1) and 14-3-3η antibodies, similar to (A). Left lane: the brain lysate (1.5% input). Asterisk denotes an IgG heavy chain. (**D**) Interaction between parkin and 14-3-3η in HEK293 cells. FL-parkin (5 μg), Myc-14-3-3η, σ, β, or ζ (2 μg) plasmids were transfected as indicated into HEK293 cells. After 48 h, the cell lysate was prepared and used for immunoprecipitation with anti-Myc antibody. The immunoprecipitates and the lysate (7.5% input) were analyzed by Western blotting with anti-parkin and Myc antibodies, as in (A).

with recombinant parkin protein (1 μg). Intriguingly, two 14-3-3 signals were evident: a faint band and a strongly stained band, indicating that the 14-3-3 may form homo- and/or hetero-dimers. Subsequently, we determined the type(s) of 14-3-3 species that interacts with parkin in the mouse brain in more detail. In the parkin immunoprecipitant, 14-3-3η, but not other 14-3-3 isoforms examined, that is, β, γ, ε, and τ, was detected (Figure 1B). In the next step, we examined whether parkin is coimmunoprecipitated with anti-14-3-3η antibody and found parkin in the 14-3-3η immunoprecipitant (Figure 1C). These reciprocal immunoprecipitation experiments revealed that parkin is associated with 14-3-3η in the mouse brain.

To confirm the specific interaction of parkin with 14-3-3η, Myc-tagged 14-3-3η, σ, β, or ζ was cotransfected with FLAG (FL)-parkin into HEK293 cells, and their interactions were tested. FL-parkin was detected in the immunoprecipitant of Myc-14-3-3η, but not those of Myc-14-3-3σ, β, and ζ (Figure 1D). Taken together with the results of Figure 1B, our data indicate that parkin mainly interacts with 14-3-3η.

Parkin domain interacts with 14-3-3η

We next investigated the region of parkin necessary for interaction with 14-3-3η. Structurally, parkin is characterized by the presence of the N-terminal ubiquitin-like domain (UBL) (which is highly homologous to ubiquitin), the C-terminal RING box, consisting of two RING finger motifs, RING1 and RING2, flanked by one IBR (in between RING finger) motif, and a linker region, which connects these N- and C-terminal regions (Shimura *et al*, 2000). In these experiments, various deletion mutants of FL-tagged parkin were expressed in HEK293 cells and immunoprecipitated by FL-antibody beads (Figure 2A). FL-parkin or its derivatives on the beads were further incubated with cell lysates that expressed Myc-14-3-3η, and then the amounts of Myc-14-3-3η bound to the beads were determined (Figure 2B).

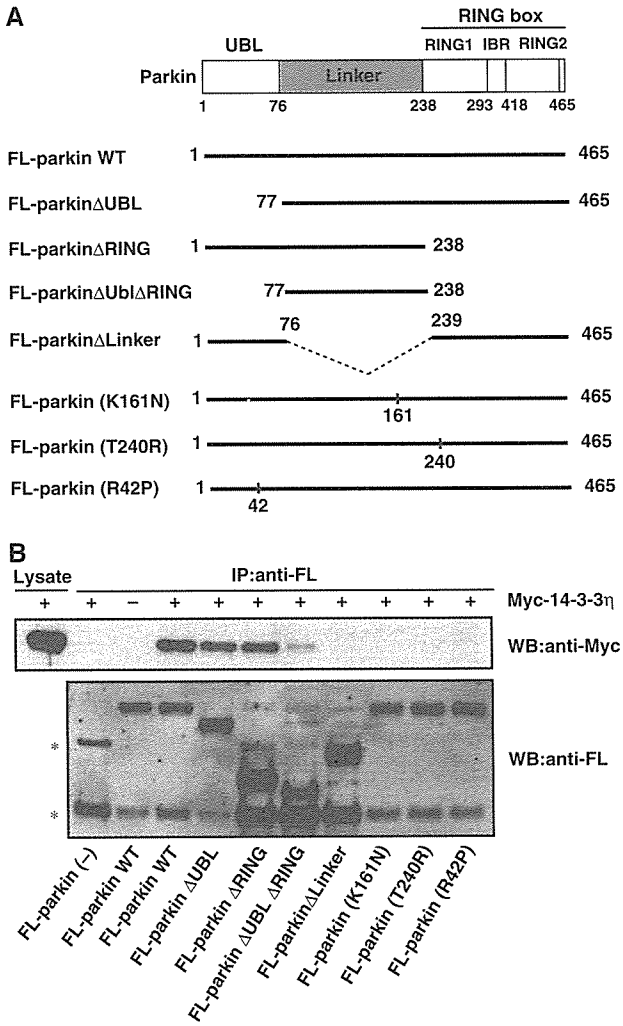

Figure 2 Domain analysis of the parkin region that interacts with 14-3-3η. (**A**) Schematic representation of WT parkin and its deletion- and disease-related missense mutants. See text for the domain structures of parkin and mutants. The dotted line denotes the deleted region. (**B**) Interaction between 14-3-3η and parkin mutants. FL-parkin (2 μg) or its mutant (10 μg) plasmids were transfected into HEK293 cells, as described in Figure 1D. The cell lysates (200–600 μl) were immunoprecipitated with anti-FL-antibody beads. Note that various amounts of the lysates were used to adjust roughly the levels of expressed parkin mutants. The resulting immunoprecipitates were mixed with other cell lysates (200 μl) prepared from cells that had been transfected with Myc-14-3-3η plasmid (2 μg) and incubated for 6 h at 4°C. Then, the extensively washed immunoprecipitates and cell lysate (7.5% input) were analyzed by Western blotting with anti-Myc and FL antibodies. Asterisks denote nonspecific bands.

The full-length parkin could bind 14-3-3η. Deletion of either UBL or RING-box domain reduced the binding compared to the full-length parkin, although these deletion mutants retained the ability to bind to 14-3-3η. Furthermore, mutants with combined deletions of the UBL and RING-box domains, that is, the linker region, could also bind 14-3-3η to a lesser extent. Conversely, deletion of the linker region resulted in the loss of ability to bind 14-3-3η. Taken together, it is concluded that the linker region is necessary for the interaction between parkin and 14-3-3η, although the UBL and RING-box domains may enhance the binding affinity.

Interestingly, the ARJP disease-causing missense mutation within the linker region, that is, parkin(K161N), in which the Lys residue at position 161 was replaced by Asn residue, showed complete loss of binding to 14-3-3η, confirming the importance of the linker region in the interaction between 14-3-3η and parkin. Unexpectedly, other disease-causing missense mutations of the UBL region, parkin(R42P), and the RING1 region, parkin(T240R), also showed complete loss of interaction with 14-3-3η (Figure 2). Thus, although the UBL and RING-box domains are not primarily required for the binding, both R42P and T240R mutations in the UBL and RING-box domains, respectively, deleteriously affect the neighboring linker domain. Alternatively, since 14-3-3 is known to form a homo- or hetero-dimer, and thus has two binding sites (Aitken *et al*, 2002), it is plausible that 14-3-3η interacts with two distinct regions of parkin, one major site of which is the linker region.

Effect of suppression of 14-3-3η on parkin E3 activity

We next investigated the role of parkin–14-3-3η binding on parkin activity. At first, we tested its effect on the ubiquitin ligase activity of parkin. We incubated recombinant His-parkin with ubiquitin, E1, and E2 (UbcH7) *in vitro*. Under this condition, His-parkin appeared as a smear band, which likely reflects self-ubiquitylation (Figure 3A). Addition of recombinant GST-14-3-3η (Figure 3A, left panel) or untagged 14-3-3η (Figure 3A, right panel) to the reaction reduced the smear of His-parkin, and such reduction was proportionate to the added amount of GST-14-3-3η or 14-3-3η and resulted in the recovery of His-parkin of intact size. In addition, we found that 14-3-3η had no effect on the ubiquitylating activity of phosphorylated IκBα by a fully *in vitro* reconstituted system, containing E1, E2 (Ubc4), and E3 (the $SCF^{\beta TrCP}$ complex; Kawakami *et al*, 2001), indicating that 14-3-3η does not interfere with ubiquitylating reactions in general (data not shown). These results strongly suggest that 14-3-3η suppresses the intrinsic self-ubiquitylation activity of parkin.

We next tested whether 14-3-3η also affects the ubiquitylation activity of parkin in HEK293 cells. First, we examined the self-ubiquitylation of parkin, whose activity was observed by cotransfections of HA-ubiquitin and FL-parkin. Myc-14-3-3η almost completely suppressed the self-ubiquitylation activity of parkin, while Myc-14-3-3σ, β, and ζ had no inhibitory effect (Figure 3B), indicating the specific role of 14-3-3η for parkin. Second, we examined the effect of 14-3-3η on the ubiquitylation of a model substrate for parkin. When V5-tagged synphilin-1, a known parkin substrate (Chung *et al*, 2001), was transfected with FL-parkin and HA-ubiquitin in the cells, V5-synphilin-1 was found in ubiquitylated form, as demonstrated by the poly-ubiquitin chain formation (detected by anti-HA antibody) in anti-V5 immunoprecipitant (Figure 3C, top panel). V5-synphilin-1 was not ubiquitylated when FL-parkin was not cotransfected, suggesting that this ubiquitylation is mediated by coexpressed FL-parkin. Indeed, FL-parkin was found to be associated with V5-synphilin-1, further supporting the above notion (Figure 3C, second panel from the top). Note that the polyubiquitylated bands observed as the smear profile were considered to include not only major synphilin-1 bands over 90-kDa size but also self-ubiquitylated bands of parkin over 52-kDa size.

In the next step, we tested the effects of 14-3-3η on the ubiquitylation and binding activities of parkin to