原著

香川小児病院重症心身障害児(者)病棟における重症化と高齢化の現状 -過去22年間の死亡症例の検討-

福田邦明† 中川義信*

IRYO Vol. 65 No. 7 (371-376) 2011

要旨 香川小児病院重症心身障害児(者)[重症児(者)]病棟(200床)における過去22年間の死亡症例の基礎疾患,合併症,死亡病名を調査し,経年的に進行した重症化の実態と高齢化の現状について報告した.前期(1986-1996年)の死亡症例数は50名であったが,後期(1997-2007年)は34名に減少した.基礎疾患では脳性麻痺が27名から16名と著明に減少し,変性・代謝性疾患も7名から4名と減少した.基礎疾患別の死亡時年齢は,奇形・染色体異常症が他の疾患に比べて有意($p \leq 0.01$)に若年であった.合併症については前・後期で胃食道逆流症は変化がなかったが,気道狭窄は前期16%から後期38%と著明に増加した.死亡病名は肺炎が37名と最も多く,急性心呼吸不全(いわゆる突然死)が11名,心不全9名,多臓器不全6名,敗血症と失血死が4名であった.前期から後期にかけて感染症が29名から12名と著明に減少し,急性心呼吸不全も9名から2名と減少した.一方,現代医療の最終病態といえる多臓器不全が1名から5名に,対応が困難な動脈性の失血死が1名から3名と増加し,後期に2名が成人病で死亡した.前期末の入院患者の大島分類Iの占める割合は39%であったが,後期末には44%と軽度増加した.入院患者の年齢構成の推移は,1996年から2007年にかけて30歳未満は減少し,40歳以上が増加し,60歳以上が6名となった.Post-NICU児の受け入れには,現在入院中の重症児(者)の重症化と高齢化対策を並行して行う必要がある.

キーワード 重症心身障害児(者),死亡原因,重症化,高齢化,Post-NICU

はじめに

近年,重症心身障害児(者)[重症児(者)]の病棟で高齢化が進行し,高齢化とともに医療の高度化に基づく重症化が課題となっている[1].そして,社会的にはPost-NICU児の受け入れ施設としての重症児(者)病棟の役割が強く求められている[2].この,Post-NICU児の受け入れを実行可能にするために

国立病院機構香川小児病院 神経内科,*脳神経外科 †医師
別刷請求先:福田邦明 現所属 麻田総合病院神経小児科 〒763-8507 香川県丸亀市津森町219番地
(平成22年7月9日受付,平成22年12月10日受理)
The Present Situation of Advancing of Diseases and Aging in the Wards for Children and Persons with Severe Motor and Intellectual Disabilities (SMID) : A Study of the Cases of Death in the Wards for Children and Persons with SMID during the 22 Years from 1986 to 2007
Kuniaki Fukuda and Yoshinobu Nakagawa*. Department of Neurology and *Neurosurgery, NHO Kagawa Children's Hospital. Zentsuji
Key Words: children and persons with SMID, cause of death, advanced disease, aging, Post-NICU

は，現在の重症児（者）病棟が抱えている課題を明らかにする必要がある．重症化にともない必要となる医療の課題を明らかにするために，死亡症例の合併症と死亡原因を検討することは最も有用な研究と考える．今回われわれは，香川小児病院重症児(者)病棟における過去22年間の死亡症例を対象に，基礎疾患，重症度（大島分類→377p を参照），合併症，死亡病名について調査するとともに，前期・後期に分けて比較し，重症児（者）病棟における医療課題の変化を明らかにし，さらに入院患者の年齢構成の推移を検討し高齢化の実態についても報告する．

対象と方法

当院の重症児（者）病棟は５病棟（200床）で超重症児（者）[3]から強度行動障害児（者）を療育している．今回，われわれは当院重症児（者）病棟における1986年１月から2007年12月までの22年間の死亡症例を対象に死亡時年齢・大島分類・基礎疾患・合併症・死亡病名について調査するとともに，1986年から1996年の11年間を前期，1997年から2007年までの11年間を後期として死亡時年齢・大島分類・基礎疾患・合併症・死亡病名の変化と入院患者の年齢推移について検討した．

結　　果

１）死亡時年齢：22年間に死亡退院した症例数は84名で，前期が50名で，後期は34名に減少した．年齢分布では10歳未満が22名，10歳以上20歳未満が22名，20歳以上30歳未満が15名，30歳以上40歳未満が20名で40歳以上が５名であった．前期から後期にかけて40歳未満（とくに10歳未満）の死亡症例が減少し，40歳以上は軽度増加した（表１）．

表１　死亡症例の年齢構成の変化（人数）

年　齢	前 期(1986-1996年)	後 期(1997-2007年)	合 計
10 歳未満	16	6	22
10～19 歳	13	9	22
20～29 歳	9	6	15
30～39 歳	11	9	20
40～49 歳	0	2	2
50～59 歳	1	2	3
60 歳以上	0	0	0
計	50	34	84

２）重症度（大島分類）：前期末（1996年）の入院患者の大島分類Ⅰの割合は39％で，狭義の重症児（者）[3]（大島分類Ⅰ～Ⅳ）の占める割合は54％であった．一方，死亡症例における大島分類Ⅰの割合は72％で，狭義の重症児（者）の占める割合も88％と，入院患者に比べて高く，重度の症例が多かった．後期末（2007年）の入院患者における大島分類Ⅰの割合も44％で，狭義の重症児（者）の占める割合も63％といずれも前期に比べ軽度増加した．死亡症例における狭義の重症児（者）の占める割合は76％と軽度減少したが，大島分類Ⅰは71％で前期とほぼ同じ割合であった（表２）．

表２　大島分類による重症度の比較（％）

	前期入院患者	前期死亡症例	後期入院患者	後期死亡症例
大島分類Ⅰ	39	72	44	71
狭義の重症児（者）	54	88	63	76
周辺群も含む重症児（者）	65	92	70	85

３）基礎疾患別の死亡症例：死亡症例の基礎疾患は脳性麻痺が43名，奇形・染色体異常症が13名，変性・代謝性疾患が11名，精神遅滞が11名，筋ジストロフィー症が４名，その他が２名であった．前期から後期にかけて脳性麻痺が27名から16名と著明に減少し，変性・代謝性疾患も７名から４名に減少した（表３）．

表３　基礎疾患別の死亡症例の推移と死亡時年齢

基礎疾患	前期	後期	死亡時年齢	危険率（対：奇形・染色体異常症）
脳性麻痺(43)	27	16	23.5±12.5	p＝0.0004
奇形・染色体異常症(13)	7	6	9.2± 8.7	—
変性・代謝性疾患(11)	7	4	20.5± 9.7	p＝0.0065
精神遅滞(11)	6	5	27.5±13.6	p＝0.0006
筋ジストロフィー症(4)	2	2	22.8± 4.6	p＝0.0104
その他(2)	1	1	5，3	—
計	50	34		

基礎疾患別の死亡時平均年齢は，脳性麻痺が23.5歳，奇形・染色体異常症が9.2歳，変性・代謝性疾患が20.5歳，精神遅滞が27.5歳，筋ジストロフィー症が22.8歳で，奇形・染色体異常症の死亡年齢は他の疾患に比べて有意（$p \leqq 0.01$）に若年であった（表３）．

４）主な合併症：主な合併症は気道狭窄が21名と最も多く，次が胃食道逆流症の13名で，ほかに腎不全を４名，糖尿病を３名に認めた．前期と後期の比較では気道狭窄が，16％から38％と著明に増加した．胃食道逆流症は変化がなかった（表４）．

表４　主な合併症［人数（　）内は％］

	前期	後期	合計
気道狭窄	8(16.0)	13(38.2)	21(25.0)
胃食道逆流症	8(16.0)	5(14.7)	13(15.5)
腎不全	3(6.0)	1(2.9)	4(4.8)
糖尿病	2(4.0)	1(2.9)	3(3.6)

5）死亡病名：死亡病名は肺炎が37名と最も多く，次いで急性心呼吸不全（いわゆる突然死）が11名，心不全が９名，多臓器不全が６名，敗血症と失血死が４名であった．前期から後期にかけて肺炎・敗血症・腹膜炎といった感染症が29名から12名と著明に減少し，急性心呼吸不全も９名から２名に減少した．一方，多臓器不全が１名から５名，失血死が１名から３名に増加し，後期に膀胱癌と脳梗塞による死亡を各１名認めた（表５）．

表５　死亡病名（人数）

死亡病名	前期	後期	計
肺炎	25	12	37
急性心呼吸不全	9	2	11
心不全	5	4	9
敗血症	3	0	3
腹膜炎	1	0	1
多臓器不全	1	5	6
横紋筋融解症	1	0	1
肝不全	0	1	1
けいれん重積	1	1	2
腸閉塞	1	0	1
悪性腫瘍	0	1	1
脳梗塞	0	1	1
失血死	1	3	4
窒息	0	1	1
脳幹死	0	2	2
肺水腫	0	1	1
その他	2	0	2
合計	50	34	84

基礎疾患別の死亡病名の検討では，各疾患とも肺炎が第１位で脳性麻痺は49％，奇形・染色体異常症は46％，変性・代謝性疾患は45％，筋ジストロフィー症は75％と多くを占めた．精神遅滞は心不全と同率で18％と低かった．第２位以下は基礎疾患により異なり，精神遅滞はその他が最も多く症例ごとに原因が異なった（表６）．

発症後24時間内の死亡を22名認め，原因は急性心呼吸不全11名，失血死３名，敗血症性ショック２名，その他６名であった．前期から後期にかけて急性心呼吸不全と敗血症性ショックが減少した（表７）．　死亡時間帯の検討では，急性心呼吸不全以外の11名は日勤帯３名，準夜帯４名，深夜帯４名と均等であったが，急性心呼吸不全は11名中５名が深夜帯で，ほかの６名は日勤帯で内５名が午前中であった．

6）入院患者の推移：1996年，2001年，2007年の入院患者の年齢構成の推移をみると，30歳未満は減少し，とくに10歳未満は階段状に減少した．30歳以上40歳未満は変化がなかったが，40歳以上は階段状に増加し，2007年には60歳以上が６名になった（表８）．

7）2007年以後の超重症児（者）[3]の推移：2007年の超重症児者は28名であったが，2009年には40名に増加した．年齢構成では20代，30代の増加が顕著であった（図１）．

考　察

重症心身障害児（者）病棟では高齢化に加えて，超重症児（者）が増加し，重症化が指摘されている[1]．今回の当院の入院患者における大島分類の検討でも，大島分類Ⅰの占める割合は前期末（1996年）が39％に比べ，後期末（2007年）には44％と軽度増加した．死亡症例は運動障害が強いほど多いことが指摘[4]されているが，われわれの検討においても入院患者における大島分類Ⅰの占める割合は39–44％であったが，死亡症例の大島分類Ⅰは前期72％，後期71％と著明に高く，寝たきりの症例に対する医療は非常に

表６　基礎疾患別の死亡原因（人数）

基礎疾患	症例数	肺炎	急性心呼吸不全	心不全	多臓器不全	失血死	その他
脳性麻痺	43	21	6	2	4	3	7
奇形・染色体異常症	13	6	2	3	0	0	2
変性・代謝性疾患	11	5	2	0	2	1	1
精神遅滞	11	2	1	2	0	0	6
筋ジストロフィー症	4	3	0	1	0	0	0

表７　24時間内の死亡症例数

	人数	男女比	急性心呼吸不全	失血死	敗血症性ショック	その他
前期	14	7:7	9	1	2	2
後期	8	4:4	2	2	0	4
計	22	11:11	11	3	2	6

表８　入院患者年齢分布の推移（人数）

年　齢	1996年	2001年	2007年
10歳未満	15	9	3
10～19歳	38	18	18
20～29歳	57	57	40
30～39歳	55	59	54
40～49歳	29	40	48
50～59歳	4	16	30
60歳以上	0	1	6
計	198	200	199

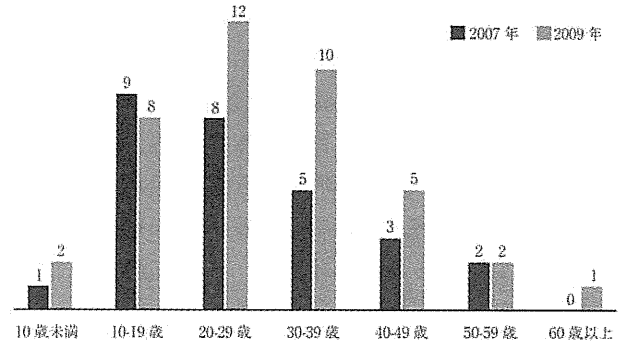

図１　超重症児・者の推移

重要と考えられる．

近年，重症児（者）の死亡調査により合併症，死亡原因が少しずつ明らかになり，予防，治療が進歩し，死亡症例の減少が指摘されている[4)5)]．今回のわれわれの検討においても前期の死亡症例数が50名に対し，後期は34名に減少した．基礎疾患では脳性麻痺の27名が16名に減少し，死亡病名では肺炎・敗血症等の感染症と急性心呼吸不全（いわゆる突然死）の減少が顕著であった．折口[1)]は重症児（者）の死亡の減少について，1982年より行っている死亡原因の調査で常に第１である呼吸器系感染症の減少が主要因と指摘している．呼吸器系感染症の減少は抗生物質の進歩によるところもあるが，嚥下障害，気道狭窄による呼吸障害に対する外科的医療の進歩[6)]に負うところが大きい．今回の検討においても，主要な合併症である胃食道逆流症は変化がなかったが，気道狭窄の頻度は16％から38％と２倍以上に増加し，気道狭窄に基づく呼吸障害に対応する医療は非常に重要である．気道狭窄の原因としては，舌根沈下が最も多いが，これは気道確保で対応が可能である．これ以外には胸郭の扁平化による主気管支の扁平狭窄[7)]と気管軟化症[8)]がある．気管軟化症に関しては一定の治療方針がなく，対応を模索中である．胸郭の扁平化にともなう上気道狭窄に対し，江川ら[7)]は腕頭動脈バイパスと前胸骨部分切除術を行い良好な結果を得ている．腕頭動脈バイパスと前胸骨部分切除術は，気管狭窄による呼吸障害の改善とともに，呼吸管理時の最も重篤な合併症である気管腕頭動脈瘻の予防を兼ねたすぐれた方法といえる．今回のわれわれの死亡原因の検討でも感染症による死亡は減少したが，現代医学の最終病態といえる多臓器不全とともに突然の動脈性の失血死は増加しており，気管腕頭動脈瘻に対する予防は治療[9)]に勝るものと考える．

重症児（者）に多い予知困難な死因に急性心呼吸不全（いわゆる突然死）がある[4)10)11)]．この病態・病理は不明[11)]であるが，死亡時間帯の特徴[10)]が指摘されている．今回，われわれの24時間内に死亡した22名の検討においても，死因が明らかな11名は日勤，準夜，深夜帯に均等に発生したが，いわゆる突然死は11名中10名が深夜を含む午前中に発生しており，病態にサーカディアンリズムが関わっていると考えられた．そして，呼吸と心拍のモニタリングおよび積極的呼吸管理を行うことにより前期９名あった突然死が２名に減少したことは，重症児（者）へのきめ細かい医療の重要性と必要性を指摘している．

生きるために最も重要な呼吸器系に進行性の重篤な障害が発生する重症児（者）をケアするためには緻密な看護体制と複数科にまたがる専門的な医療体制が必要である．

重症児（者）病棟で，確実に進んでいる高齢化[12)]に向けては成人病対策が不可欠である．われわれの検討においても少数ではあるが前期にはなかった成人病による死亡を後期は２名認めた．現在，重症児（者）の成人病の特徴はいまだ検診制度もなく不明

のままである．また，医療体制も多くを小児科医が診ている現状[12]を政策的に見直す時期になっている．

重症児（者）病棟の重症化に対しては呼吸・消化器系を中心とした外科的医療の需要が高く，高齢化に対しては婦人科を含め内科的医療の需要が高まっている．これらの分野をより専門的に行えるシステムを構築すれば，多くを診ている小児科医が，Post-NICU児を受ける体制づくりにより積極的に関われると考える．

結　　語

前期に比べ後期は脳性麻痺の感染症・急性心呼吸不全による死亡が減少した．一方，現代医療の最終病態ともいえる多臓器不全と予期せぬ死につながる動脈性の失血死が軽度増加した．また，後期には2名が成人病により死亡した．合併症では気道狭窄の頻度が倍増し，予防と外科的治療が今後の重要な課題である．入院患者の年齢分布では30歳未満が減少し，40歳以上が著明に増加し，60歳以上も徐々に増加した．今後，重症化・成人病・高齢化対策が必要である．

〈本論文の要旨は第63回国立病院総合医学会（仙台市）のシンポジウム「Post-NICUの現状と今後の展望-何を求められ，何が必要か-」において「重症心身障害児（者）医療施設の現状と問題点」の一部として発表した〉

[文献]

1）佐々木征行，宮野前健，山本重則ほか．SMIDデータベースから見た重症心身障害児（者）の重症化．医療 2009；63：708-13.

2）宮野前健．重症心身障害児（者）の重症化．-ポストNICU児等受け入れ施設としての重症心身障害児病棟の役割と課題-．医療 2009；63：715-9.

3）平山義人．重症心身障害児（者）の総論と実態．In：栗原まな編．発達障害医学の進歩．東京：診断と治療社；2007：p1-6.

4）折口美弘．重症心身障害児（者）の死亡原因．In：栗原まな編．発達障害医学の進歩．東京：診断と治療社；2007：p85-90.

5）三上史哲，三田勝己，花岡知之ほか．公法人立重症心身障害児施設入所児（者）の実態調査の分析-施設入所児（者）の死亡-．日重障誌 2009；34：171-80.

6）岩中督．重症心身障害児のQOLの向上を目指して-外科医の役割-．脳と発達 2007；39：126-30.

7）江川善康，川人智久，菅野幹雄ほか．重症心身障害児(者)における胸郭変形にともなう気管狭窄の治療-腕頭動脈バイパスと前胸骨部分切除術-．医療 2010；64：40-5.

8）加藤晋，木部哲也，横地健治．重症心身障害児(者)の気管軟化症5例の管理経験．日児誌 2007；111：692-9.

9）吉田誉，江川善康，川人智久．気管切開術後の気管腕頭動脈瘻の2救命例-予防，止血，術式について検討-．日心外会誌 2007；36：265-8.

10）石崎朝世，篠崎昌子，吉田玲子ほか．重症心身障害児（者）における死亡症例の検討．脳と発達 1990；22：357-63.

11）吉田玲子，石崎朝世，福山幸夫ほか．重症心身障害児（者）の突然死例の臨床的検討．脳と発達 1995；27：466-72.

12）倉田清子．高齢期を迎える重症心身障害児の諸問題-加齢を重ねる重症児（者）の臨床的特徴-合併症と死亡原因の検討-．脳と発達 2007；39：121-5.

The Present Situation of Advancing of Diseases and Aging in the Wards for children and persons with Severe Motor and Intellectual Disabilities (SMID) : A Study of the Cases of Death in the Wards for children and persons with SMID during the 22 Years from 1986 to 2007

Kuniaki Fukuda and Yoshinobu Nakagawa*

Department of Neurology and Department of Neurosurgery*, NHO Kagawa Children's Hospital

Abstract We studied the causes and severe complications of patients, who died in the wards for children and persons with severe motor and intellectual disabilities (SMID), during the 22 years from 1988 to 2007. Of eighty-four patients who died during the 22 years, fifty patients died in the early period (1988-1996) and thirty-four patients died in the latter period (1997-2007). As the causes of death, infections (pneumonia and sepsis) and sudden death decreased in the latter period conspicuously. On the other hand, multiple organ failure and arterial bleeding increased and two patients died due to lifestyle diseases.

The ages of death in the anomaly group were significantly ($p \leqq 0.01$) younger than those in other groups.

The main complications were upper airway constriction (25.0%), gastroesophageal reflux disease (15.5%), renal failure (4.8%), and diabetes (3.6%). The frequency of upper airway constriction in the latter period was more than twice compared with that of the early period.

The percentage of patients who were hospitalized and classified as Ohshima's I in the early period was 39% and that in the latter period became 44%. The percentage of patients who died and were classified as Ohshima's I in the early period and in the latter period were 72% and 71%, respectively.

During these 22 years, the aging of patients hospitalized in the wards for SMID advanced remarkably.

J Epidemiol 2011;21(3):211-216
doi:10.2188/jea.JE20100149

Short Communication

How Can the National Burden of Parkinson's Disease Comorbidity and Mortality Be Estimated for the Japanese Population?

Yuriko Doi[1], Tetsuji Yokoyama[2], Yoshikazu Nakamura[3], Masaki Nagai[4], Kenichi Fujimoto[5], and Imaharu Nakano[5]

[1]Department of Education and Training Technology, National Institute of Public Health, Saitama, Japan
[2]Department of Human Resources Development, National Institute of Public Health, Saitama, Japan
[3]Department of Public Health, Jichi Medical University School of Medicine, Tochigi, Japan
[4]Department of Public Health, Saitama Medical University, Saitama, Japan
[5]Division of Neurology, Department of Medicine, Jichi Medical University School of Medicine, Tochigi, Japan

Received October 8, 2010; accepted December 22, 2010; released online March 12, 2011

ABSTRACT

Background: Good medical care results in long survival for patients with Parkinson's disease (PD). However, little is known about the burden of PD comorbidity and mortality in Japan. This is the first study to examine comorbid diseases of PD decedents and extrapolate PD death rates from multiple-cause coding mortality data for the total population of Japan.

Methods: Data for 4589 certified deaths due to PD as the underlying cause of death (ICD-10 code: G20) were obtained from the 2008 Japanese vital statistics. Of those, comorbidities listed in the death certificates of 477 randomly selected decedents were analyzed. All diseases or conditions mentioned on death certificates were counted and ranked in descending order of frequency. The death rates (per 100 000 population) from PD were calculated using Japanese National Vital Statistics. The estimated rate of deaths with PD was extrapolated using US death data from a multiple-cause coding system, as no such system is available in Japan, with adjustment for the difference in disease structure between countries.

Results: Average age at death was 80.9 years. The top 5 comorbid diseases ranked as contributory causes of death were cerebrovascular diseases (4.0%), dementia (3.8%), diabetes mellitus (3.6%), malignant neoplasm (2.5%), and heart diseases (2.3%). Overall, the death rates from and with PD were 3.6 and 5.8, respectively.

Conclusions: Analysis restricted to data from the underlying-cause coding system underestimated the national burden of PD comorbidity and mortality. Use of death certificates and multiple-cause mortality data complement the existing system.

Key words: Parkinson's disease; comorbidity; mortality; causes of death; Japan

INTRODUCTION

Under the Statistics Act of Japan, the Japanese Ministry of Health, Labour and Welfare is charged with overseeing the annual collection of vital statistics surveys to analyze vital events and obtain a basic population data source for policy making on health, labor, and welfare.[1] The procedures adhere to international standards for mortality statistics regarding underlying cause of death, which is defined by the World Health Organization as the disease or injury that initiated the train of morbid events leading directly to death or the circumstances of the accident or violence that produced the fatal injury.[2] Coders select underlying causes of deaths in accordance with the rules and guidelines on coding for deaths and diseases.[3] Overall, underlying cause of death data are reported to capture approximately 90% of deaths mentioned in the death certificates for malignant neoplasms.[4–8] Statistics on underlying cause of death can be valuable in describing types of death for which a single primary cause is clinically considered to contribute.

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the 4 cardinal motor signs of tremor at rest, bradykinesia, rigidity, and postural instability, and by other non-motor clinical manifestations. Average age at onset is approximately 55 years. With the development of various kinds of treatments, the average age at

Address for correspondence. Yuriko Doi, Department of Education and Training Technology, National Institute of Public Health, 2-3-6 Minamai, Wako, Saitama 351-0197, Japan (e-mail: yuriko@niph.go.jp).

death of patients with PD is now close to that of the general population. Because the disease is incurable, patients survive for a long period, often decades, under medical supervision.[9]

As with other chronic diseases, the causes of PD death are more likely to represent a number of co-existing conditions among which there may be no direct etiologic chain to facilitate the identification of a single underlying cause. To complement statistics on underlying cause of death, statistics encompassing multiple causes of death have been introduced as standard practice in a number of Western countries. Analysis of multiple cause of death revealed that data on underlying cause of death represent only 30% to 50% of deaths with PD mentioned in death certificates.[4,8,10] How can we estimate the national burden of PD comorbidity and mortality without a multiple-cause coding system for mortality statistics in Japan? The present study is the first to examine comorbid diseases of decedents from PD using their death certificates and to extrapolate PD death rates from multiple-cause coding mortality data.

METHODS

Data

Death certificates

There were 4589 Japanese decedents for whom PD was the underlying cause of death in the 2008 vital statistics. To analyze these death certificates, a random sample of decedents was selected, with a 10% probability for each prefecture. There were no significant differences in demographic characteristics between the vital statistics dataset and the sampled death certificates (Table 1).

Two of the authors (YD and TY) are medical epidemiologists and transcribed all mentioned causes of death from the copied death certificates of the sampled decedents, after obtaining permission to do so from the Statistics and Information Department, Minister's Secretariat, Ministry of Health, Labour and Welfare, Japan. The transcribed information did not contain any personally identifiable information. Based on standard clinical practice, one author (YD), who has more than 10 years of experience in clinical medicine, classified all mentioned causes of death other than PD into several categories of diseases and medical conditions. The present study was approved in March 2010 by the Institutional Review Board of the National Institute of Public Health, Japan.

Vital statistics

Data for 4589 decedents from PD as the underlying cause of death were extracted from the national mortality database of vital statistics, after obtaining permission from the Statistics and Information Department, Minister's Secretariat, Ministry of Health, Labour and Welfare, Japan. The International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) uses the code G20 for PD.[2,11] The data used in the present study did not contain any personally identifiable information.

Table 1. Demographic characteristics of decedents for whom Parkinson's disease was listed as the underlying cause of death on death certificates (Japan, 2008)

Characteristics	All decedents (n = 4589)		Sampled decedents[a] (n = 477)	
	Number or Mean	% or SD	Number or Mean	% or SD
Sex				
Men	2124	46.3	208	43.6
Women	2465	53.7	269	56.4
Age[b]				
Men	79.5	7.2	80.1	7.2
Women	82.0	7.3	82.7	6.9
Both	80.9	7.4	81.6	7.1
Marital status				
Married	2380	51.9	239	50.1
Single	184	4.0	21	4.4
Bereaved	1865	40.6	200	41.9
Separated	158	3.4	17	3.6
Unknown	2	0.0	0	0.0
Occupation				
Farming	349	7.6	48	10.1
Self-employed	242	5.3	31	6.5
Employed	570	12.5	60	12.5
Other	302	6.6	29	6.1
Unemployed	2718	59.2	266	55.8
Unknown	408	8.9	43	9.0
Place of death				
Hospital	3496	76.2	363	76.1
Clinic	156	3.4	11	2.3
Healthcare facility for the aged	103	2.2	15	3.1
Nursing home	325	7.1	33	6.9
Home	466	10.2	48	10.1
Other	43	0.9	7	1.5

[a]Sampled decedents were randomly selected with a 10% probability from all deaths attributed to Parkinson's disease, by prefecture, in 2008.
[b]Values are means and standard deviations (SDs).

Statistical analysis

Analysis of death certificates

All categorized causes or conditions mentioned, other than PD, in death certificates of the sampled decedents were counted and ranked in descending order of frequency as 1 of 3 types of causes: immediate, intermediate, or contributory. An immediate cause is a final disease or condition resulting in death and is described on the top line in Part (I) of the death certificate. Intermediate causes are diseases, injuries, or complications (other than immediate cause) in the chain of events that directly caused the death and are described in Part (I) of the death certificate. Contributory causes are other significant conditions that contributed to the causes described in Part (I), but did not directly result in those causes. Contributory causes are described in Part (II) of the death certificate.[12]

Analysis of vital statistics

The number and rate of deaths in individuals for whom PD was listed as the underlying cause of death were calculated according to 5-year age intervals using the Japanese national

vital statistics and data on the Japanese population for the year 2008. Currently, there is no multiple-cause coding system in Japan; however, the US Centers for Disease Control and Prevention (CDC) publically releases data on multiple cause-of-death. Therefore, we calculated the number and rate of PD as a multiple cause of death according to sex and age group by weighting the underlying cause of death data from the Japanese national vital statistics in 2008 with multiple cause of death data from publically available US national vital statistics in 2006 (see Appendix).[13] The equations, (1)–(3), are as follows: $P_{JAPAN,j}(G20|D_i)$ is the proportion of death certificates mentioned with PD to those for D_i as the underlying cause of death from disease i in the j-th sex-age-group in Japan; D_i is ischemic heart disease (ICD-10: I20–I25), malignant neoplasm (C00–C75), cerebrovascular disease (I60–I69), pneumonia (J10–J18), PD (G20), or other (all other codes). The same is true for $P_{US,j}(G20|D_i)$. $P_{JAPAN,j}(G20|D_i) = 1$ when D_i is G20. Otherwise, equation (1) can be used: (1) $P_{JAPAN,j}(G20|D_i) = P_{US,j}(G20|D_i) \times \frac{R_{JAPAN,j}}{R_{US,j}}$, where $R_{JAPAN,j}$ and $R_{US,j}$ are the death rates from PD as the underlying cause of death in the j-th sex-age-group in Japan and the United States, respectively. Equation (1) is used to adjust for the difference in disease structure between the 2 countries.

Then, the number of multiple-cause deaths from PD in the death certificate with D_i as the underlying cause of death in the j-th sex-age-group, $M_{i,j}$, is estimated as: (2) $M_{i,j} = N_{i,j} \times P_{JAPAN,j}(G20|D_i)$, where $N_{i,j}$ is the number of the underlying cause of death from D_i in the j-th sex-age-group. The number of the multiple cause of death from PD in the j-th sex-age-group, M_j, is obtained by summing $M_{i,j}$ for all underlying causes of death as: (3) $M_j = \sum_i M_{i,j}$. The sex- and age-specific rates of multiple-cause of deaths from PD were calculated by dividing M_j by the corresponding sex- and age-specific Japanese population for the year 2008. The multiple cause of death rates, by sex and in total, were calculated from the corresponding numbers of summed multiple-cause deaths and populations.

RESULTS

Mean age at death among PD decedents was 79.5 years for men, which exceeded the average life expectancy of men in the general population (79.3 years), and 82.0 years for women, which was lower than that of women in the general population (86.1 years; Table 1).

Table 2 shows all causes or conditions mentioned, other than PD, in the death certificates of 477 decedents. The 5 most frequent comorbid diseases listed as contributory causes of death were cerebrovascular diseases, dementia, diabetes mellitus, malignant neoplasm, and heart diseases. The most common immediate or intermediate cause of death was aspiration or suffocation that caused pneumonia, respiratory failure, or multiple organ failure leading to death.

Table 3 shows the death rates per 100 000 population according to underlying and multiple cause of death. Both rates increased with age, from 0.5 and 0.7, respectively, in those aged 55 to 64 years to 40.8 and 71.3 in those aged 85 years or older. The overall death rate estimated from extrapolation of US data on multiple-cause deaths was approximately 1.6 times that obtained from Japanese data on underlying cause of death: 5.8 versus 3.6, respectively.

DISCUSSION

Death certificate analysis

Our most noteworthy finding was that dementia and diabetes mellitus appeared together with the 3 leading causes of death—cerebrovascular disease, malignant neoplasm and heart disease—as comorbid diseases in a representative sample of the Japanese PD population. Because our analysis was able to detect chronic diseases and conditions that, while not fatal by themselves, could contribute to causing death, diabetes mellitus was highly ranked in our death certificate analysis, even though it is not among the 10 leading causes of death in the underlying-cause coding system of Japanese vital statistics. Much the same was true for dementia. Dementia is a major long-term cause of disability in people with PD and is reported in 30% to 80% of individuals with PD.[14] The value observed in the present study, 4.8%, was quite low. This difference between studies may be due to differences in methods. The high prevalence of dementia in PD patients was mostly reported in clinical studies of PD patients who had undergone comprehensive neuropsychological assessment. Oral health, which is important in PD,[15] was not mentioned anywhere in the death certificates of the present study.

Death certificate analysis has limitations. First, there is no multiple-cause coding system in Japan. Therefore, it was impossible to obtain death certificates in which PD was mentioned, but was not the underlying cause of death, from the 1.14 million death certificates filed in 2008. It may also be that parts of the analyzed death certificates were incomplete or inaccurate because of the possibility that (1) contributory causes are disregarded in data on underlying cause of death in national mortality statistics, (2) detailed medical records are sometimes unavailable at death, especially for decedents with long periods of morbidity, and (3) physicians sometimes have difficulty in reporting detailed medical information on the death certificates of such decedents.

Analysis of vital statistics

The present study showed that analysis restricted to data on underlying cause of death underestimates PD mortality in Japan. Our estimated number of PD decedents was nearly 60% higher than the number of decedents due to PD as the underlying cause of death.

Table 2. All causes or conditions mentioned, other than Parkinson's disease, in death certificates of 477 decedents randomly selected from 4589 deaths due to Parkinson's disease as the underlying cause of death (Japan, 2008)

Immediate causes[a]	Number	%	Intermediate causes[b]	Number	%	Contributory causes[c]	Number	%
Aspiration or suffocation	106	22.2	Aspiration or suffocation	64	13.4	Cerebrovascular diseases	19	4.0
Pneumonia	70	14.7	Senile deterioration	8	1.7	Dementia	18	3.8
Respiratory failure	61	12.8	Pneumonia	8	1.7	Diabetes mellitus	17	3.6
Senile deterioration	52	10.9	Dementia	5	1.0	Malignant neoplasms	12	2.5
Heart diseases	30	6.3	Cerebrovascular diseases	4	0.8	Heart diseases	11	2.3
Multiple organ failure	12	2.5	Respiratory failure	4	0.8	Lung diseases	10	2.1
CO_2 narcosis or hypoxemia	9	1.9	Lung diseases	3	0.6	Infection, sepsis or DIC	8	1.7
Infection, sepsis or DIC	5	1.0	Heart diseases	3	0.6	Hypertension or hypotension	8	1.7
Renal diseases	3	0.6	Neuroleptic malignant syndrome	2	0.4	Diseases of the gastrointestinal tract	8	1.7
Lung diseases	2	0.4	Mental disorders	2	0.4	Fracture	6	1.3
Neuroleptic malignant syndrome	2	0.4	Infection, sepsis or DIC	2	0.4	Connective tissue diseases	6	1.3
Cerebrovascular diseases	2	0.4	Disuse syndrome	2	0.4	Diseases of arteries or arterioles	4	0.8
Diseases of the gastrointestinal tract	1	0.2	Diseases of the gastrointestinal tract	2	0.4	Senile deterioration	4	0.8
Disuse syndrome	1	0.2	CO_2 narcosis or hypoxemia	2	0.4	Pneumonia	4	0.8
Unknown	1	0.2	Renal diseases	1	0.2	Mental disorders	4	0.8
			Hypertension or hypotension	1	0.2	Lung diseases	4	0.8
			Diabetes mellitus	1	0.2	Disuse syndrome	4	0.8
			Decubitus ulcer	1	0.2	Aspiration or suffocation	3	0.6
			Anemia or hypoalbuminemia	1	0.2	Liver diseases	3	0.6
						Decubitus ulcer	3	0.6
						Respiratory failure	1	0.2
						Anemia or hypoalbuminemia	1	0.2

Abbreviation: DIC, disseminated intravascular coagulation.
[a]An immediate cause is a final disease or condition resulting in death, described on the top line in Part (I) of the death certificate.
[b]Intermediate causes are diseases, injuries, or complications, other than immediate causes, in the chain of events that directly cause death, as described in Part (I) of the death certificate.
[c]Contributory causes are other significant conditions that contribute to the causes cited in Part (I), but do not directly result in those causes; they are described in Part (II) of the death certificate.

Table 3. Number and rate of Parkinson's disease deaths, based on national vital statistics, by sex and age group (Japan, 2008): Underlying cause of death versus multiple cause of death

Age (years)	Underlying cause of death						Multiple cause of death[a]					
	Number of deaths			Rate per 100 000 population			Estimated number of deaths			Estimated rate per 100 000 population		
	Men	Women	Total	Men	Women	Total	Men	Women	Total	Men	Women	Total
0–14	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0
15–24	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0
25–34	0	0	0	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0
35–44	2	1	3	0.0	0.0	0.0	2	2	4	0.0	0.0	0.0
45–54	6	7	13	0.1	0.1	0.1	9	10	19	0.1	0.1	0.1
55–64	53	32	85	0.6	0.3	0.5	83	45	128	0.9	0.5	0.7
65–74	381	297	678	5.4	3.7	4.5	623	407	1030	8.8	5.1	6.9
75–84	1187	1214	2401	29.4	21.2	24.6	2023	1769	3792	50.1	30.9	38.8
85+	495	914	1409	52.4	36.4	40.8	911	1549	2461	96.5	61.8	71.3
Total	2124	2465	4589	3.4	3.8	3.6	3652	3782	7434	5.9	5.8	5.8

[a]The estimated number and rate of multiple-cause PD deaths were calculated according to sex and age group by weighting data on underlying cause of death from 2008 Japanese national vital statistics with multiple cause of death data from publically available 2006 US national vital statistics (see reference 13).

In the equation used in the present study, the estimates were adjusted for differences in disease structure between the United States and Japan. However, this adjustment could not fully account for racial differences between populations in vulnerabilities and comorbidities regarding PD. Age-standardized PD prevalence and incidence are lower in Japanese studies than in US studies.[16] The underlying cause of death as a percentage of multiple cause of death reports was 49% in the US population in 2000–2001 and 56% in the UK population in 2001–2006.[4,10] It remains to be seen

Appendix. Number and rate of deaths with Parkinson's disease mentioned on death certificates, by sex and age group (United States, 2006): Underlying cause of death versus multiple cause of death

Age (years)	Underlying cause of death						Multiple cause of death					
	Number of deaths			Rate per 100 000 population			Number of deaths			Rate per 100 000 population		
	Men	Women	Total	Men	Women	Total	Men	Women	Total	Men	Women	Total
0–14	0	1	1	0.0	0.0	0.0	0	2	2	0.0	0.0	0.0
15–24	0	1	1	0.0	0.0	0.0	0	0	0	0.0	0.0	0.0
25–34	0	0	0	0.0	0.0	0.0	3	0	0	0.0	0.0	0.0
35–44	4	1	5	0.0	0.0	0.0	4	4	0	0.0	0.0	0.0
45–54	36	16	52	0.2	0.1	0.1	67	30	97	0.3	0.1	0.2
55–64	251	130	381	1.7	0.8	1.2	450	239	689	3.0	1.5	2.2
65–74	1512	783	2295	17.4	7.6	12.1	2677	1359	4036	30.9	13.3	21.3
75–84	5517	3544	9061	104.1	45.7	69.5	9665	6147	15812	182.4	79.3	121.2
85+	3894	3741	7635	230.7	103.7	144.1	6860	6809	13669	406.3	188.7	258.1
Total	11214	8217	19431	7.6	5.4	6.5	19726	14590	34316	13.4	9.6	11.5

Note: The data source is multiple cause of death data from publically available US national vital statistics (see reference 13).

whether these percentages are accurate for the Japanese population.

Epidemiological implications

To estimate more accurately the national burden of PD comorbidity and mortality, a multidimensional approach is also required for the Japanese population. This approach is not a substitute for, but rather an extension of, existing data on underlying cause of death,[5,17,18] and has already been adopted in the United States, the United Kingdom, Sweden, Spain, and France.[17–21] The addition of this multiple-cause coding system to the current Japanese system would be an ideal long-term solution. It should also be recognized that, in a multiple-cause coding system, certain epidemiological catchment areas should be designated for the regular collection and release of necessary reference data on comorbidity and mortality statistics. Most importantly, it is essential to maintain the quality of death certificates by enhancing understanding of their importance in the fields of medicine, public health, and health policy.[5–7]

Conclusion

The present study showed that analysis using only data from the underlying-cause coding system underestimated the national burden of PD comorbidity and mortality. Use of death certificates and multiple-cause mortality data are thus desirable complements to the existing system.

ACKNOWLEDGMENTS

This study was supported by grants from Health and Labour Sciences Research of the Ministry of Health, Labour and Welfare, Japan: Research Committee on Epidemiology of Intractable Diseases and Research Committee on CNS Degenerative Diseases of Intractable Diseases.

Conflicts of interest: None declared.

REFERENCES

1. Statistics and Information Department, Minister's Secretariat, Ministry of Health, Labour and Welfare, Japan. Vital statistics of Japan 2008 volume 1. Tokyo: Statistics and Information Department, Ministry of Health, Labour and Welfare, Japan; 2010 (in Japanese).
2. Geneva: World Health Organization [cited 2010 Aug 25]. Available from http://www.who.int/healthinfo/statistics/mortdata/en/.
3. Statistics and Information Department, Minister's Secretariat, Ministry of Health, Labour and Welfare, Japan. Rules and guidelines on coding the causes of deaths and diseases. In: International statistical classification of diseases and related health problems, tenth revision: ICD-10. Tokyo: Health and Welfare Statistics Association; 2004. p. 50–151 (in Japanese).
4. Redelings MD, Sorvillo F, Simon P. A comparison of underlying cause and multiple causes of death: US vital statistics, 2000–2001. Epidemiology. 2006;17:100–3.
5. Israel RA, Rosenberg HM, Curtin LR. Analytical potential for multiple cause-of-death data. Am J Epidemiol. 1986; 124:161–79.
6. Chamblee RF, Evans MC. New dimensions in cause of death statistics. Am J Public Health. 1982;72:1265–70.
7. Dorn HF, Moriyama IM. Uses and significance of multiple cause tabulations for mortality statistics. Am J Public Health Nations Health. 1964;54:400–6.
8. Goldacre MJ, Duncan ME, Cook-Mozaffari P, Griffith M. Trends in mortality rates comparing underlying-cause and multiple-cause coding in an English population 1979–1998. J Public Health Med. 2003;25:249–53.
9. Disease Prevention Study Group. Parkinson's disease. In: Disease Prevention Study Group, editor. Guidelines of diagnoses and treatments for intractable diseases. 3rd ed. Tokyo: Tokyo Roppo Publishing; 2005. p. 249–69 (in Japanese).
10. Goldacre MJ, Duncan M, Griffith M, Turner MR. Trends in death certification for multiple sclerosis, motor neuron disease, Parkinson's disease and epilepsy in English populations 1979–2006. J Neurol. 2010;257:706–15.

11. Statistics and Information Department, Minister's Secretariat, Ministry of Health, Labour and Welfare, Japan. Vital statistics of Japan 2008 volume 2. Tokyo: Statistics and Information Department, Ministry of Health, Labour and Welfare, Japan; 2010 (in Japanese).
12. Statistics and Information Department, Minister's Secretariat, Ministry of Health, Labour and Welfare, Japan. Manual to fill in a death certificate. Available from http://www.mhlw.go.jp/toukei/manual/dl/manual.pdf (in Japanese).
13. Atlanta: Centers for Disease Prevention and Control, the United States of America [cited 2010 Aug 13]. Available from http://wonder.cdc.gov/.
14. Obeso JA, Rodriguez-Oroz MC, Goetz CG, Marin C, Kordower JH, Rodriguez M, et al. Missing pieces in the Parkinson's disease puzzle. Nat Med. 2010;16:653–61.
15. Nakayama Y, Washio M, Mori M. Oral health conditions in patients with Parkinson's disease. J Epidemiol. 2004;14:143–50.
16. Muangpaisan W, Hori H, Brayne C. Systematic review of the prevalence and incidence of Parkinson's disease in Asia. J Epidemiol. 2009;19:281–93.
17. Goodman RA, Manton KG, Nolan TF Jr, Bregman DJ, Hinman AR. Mortality data analysis using a multiple-cause approach. JAMA. 1982;247:793–6.
18. Weed JA. Vital statistics in the United States: preparing for the next century. Popul Index. 1995;61:527–39.
19. Lindahl BI, Johansson LA. Multiple cause-of-death data as a tool for detecting artificial trends in the underlying cause statistics: a methodological study. Scand J Soc Med. 1994;22:145–58.
20. Tardon AG, Zaplana J, Hernandez R, Cueto A. Usefulness of the codification of multiple causes of death in mortality statistics. Int J Epidemiol. 1995;24:1132–7.
21. Romon I, Jougla E, Balkau B, Fagot-Campagna A. The burden of diabetes-related mortality in France in 2002: an analysis using both underlying and multiple causes of death. Eur J Epidemiol. 2008;23:327–34.

Asari et al. BMC Neurology 2011, 11:35
http://www.biomedcentral.com/1471-2377/11/35

BMC Neurology

RESEARCH ARTICLE Open Access

Subregional 6-[^{18}F]fluoro-L-*m*-tyrosine Uptake in the Striatum in Parkinson's Disease

Sayaka Asari[1*], Ken-ichi Fujimoto[1], Akihiro Miyauchi[2], Toshihiko Sato[3], Imaharu Nakano[1] and Shin-ichi Muramatsu[1*]

Abstract

Background: In idiopathic Parkinson's disease (PD) the clinical features are heterogeneous and include different predominant symptoms. The aim of the present study was to determine the relationship between subregional aromatic l-amino acid decarboxylase (AADC) activity in the striatum and the cardinal motor symptoms of PD using high-resolution positron emission tomography (PET) with an AADC tracer, 6-[^{18}F]fluoro-L-*m*-tyrosine (FMT).

Methods: We assessed 101 patients with PD and 19 healthy volunteers. PD was diagnosed based on the UK Brain Bank criteria by two experts on movement disorders. Motor symptoms were measured with the Unified Parkinson's Disease Rating Scale (UPDRS). FMT uptake in the subregions of the striatum was analyzed using semi-automated software for region-of-interest demarcation on co-registered magnetic resonance images.

Results: In all PD patients, FMT uptake was decreased in the posterior putamen regardless of predominant motor symptoms and disease duration. Smaller uptake values were found in the putamen contralateral to the side with more affected limbs. The severity of bradykinesia, rigidity, and axial symptoms was correlated with the decrease of FMT uptake in the putamen, particularly in the anterior part. No significant correlation was observed between tremors and FMT uptake.

Conclusions: Decrease of FMT uptake in the posterior putamen appears to be most sensitive in mild PD and uptake in the anterior putamen may reflect the severity of main motor symptoms, except for tremor.

Background

Cardinal motor symptoms such as bradykinesia, rigidity, and tremor in Parkinson's Disease (PD) become apparent after a depletion of dopamine in the striatum to approximately 20% of normal levels and a reduction in aromatic L-amino acid decarboxylase (AADC) activity to 5%-20% of normal levels [1,2]. In PD, dopaminergic hypofunction in the striatum is not homogenous in association with the selective loss of ventral intermediate and lateral cell groups of the substantia nigra pars compacta that project to the posterior part of the striatum [3], although the reason for this selective vulnerability remains unknown.

Positron emission tomography (PET) is valuable for assessing altered dopamine function in PD. The first tracer used to visualize and assess the integrity of dopamine presynaptic systems was 6-[^{18}F]fluoro-L-dopa (FDOPA), a fluoro-analog of L-dopa [4]. FDOPA is taken up into the dopaminergic axon terminals and decarboxylated by AADC before being trapped and stored in synaptic vesicles. FDOPA uptake is highly correlated with viable dopaminergic cells in neurotoxin-lesioned monkeys [5] and in postmortem human PD brains [6]. A shortcoming complicating the use of this agent, however, is that metabolites of FDOPA (such as 3-*O*-methyl-[^{18}F]fluoro-L-dopa, which is formed by the action of the ubiquitous enzyme catechol-*O*-methyl-transferase (COMT)) enter the brain and diminish image contrast. An alternative agent is the non-catecholic tracer 6-[^{18}F] fluoro -L-*m*-tyrosine (FMT). FMT is also a good substrate for AADC but is not metabolized by COMT; thus, FMT uptake has approximately twice the sensitivity of FDOPA uptake and more fully represents the extent of AADC activity [7-10].

To elucidate the relationship between the main motor symptoms of PD and subregional AADC activity in the striatum, we applied a semi-automated segmentation method for extracting putaminal subregions from

* Correspondence: sasari@jichi.ac.jp; muramats@jichi.ac.jp
[1]Division of Neurology, Department of Medicine, Jichi Medical University, Tochigi, Japan
Full list of author information is available at the end of the article

high-resolution FMT PET images that were co-registered with 3.0-tesla magnetic resonance (MR) images.

Methods

Subjects and clinical evaluation

Our sample consisted of 101 patients with PD and 19 healthy individuals. PD was diagnosed clinically according to the UK PD Society Brain Bank criteria [11]. All of the patients had bradykinesia and at least one of the three features of PD: 4-6 Hz resting tremor, rigidity, and postural instability. All of the patients had asymmetric onset and showed a positive response to dopaminergic medication. None exhibited atypical symptoms such as severe gaze palsy or symptomatic dysautonomia. The control group included healthy individuals with no history of neurologic or psychiatric diseases.

Motor symptoms were evaluated using the motor examination part of the Unified Parkinson's Disease Rating Scale (UPDRS). Motor subscores were determined as follows: tremor (motor UPDRS: 20 + 21), bradykinesia (motor UPDRS: 23 + 24 + 25 + 26), rigidity (motor UPDRS: 22), and axial (motor UPDRS: 18 + 19 + 27 + 28 + 29 + 30 + 31). The mini-mental state examination (MMSE) was used to assess cognitive function.

This study was approved by the Institutional Ethics Committee of Jichi Medical University and all participants gave written informed consent.

PET imaging

All patients stopped levodopa at least 16 h before PET. To increase the availability of the tracer, all subjects took 2.5 mg/kg of carbidopa (a peripheral AADC inhibitor) orally 1 h before FMT injection. Prior to the emission scan, a 10 min transmission scan was obtained for attenuation correction. Subsequently, 0.12 mCi/kg of FMT in saline was infused into an antecubital vein and a 30-90 min static three-dimensional acquisition was started simultaneously using a PET-CT (GEMINI GXL, Philips, Amsterdam, The Netherlands). Each subject also underwent 3.0-tesla MR imaging (Achieva 3.0 T, Philips) using an inversion recovery (IR) proton density (PD)-weighted pulse sequence to enhance the contrast of anatomical structures. The PET and MR imaging data were co-registered with a fusion processing program (Syntegra, Philips) to produce fusion images. This program provided manual and point-based image registration as well as automated methods of gray-value-based image registration, including a mutual information algorithm [12]. In addition, an adaptive level set of segmentation was used for coregistration of CT and MRI imaging data [13].

Semi-automated region of interest analysis

Regions-of-interest (ROIs) in the putamen and caudate nucleus were defined in three dimensions (3-D) bilaterally on the co-registered MR images where the striatum was best visualized. The putamen and the head of caudate nucleus were delineated by manual inspection on the three to five adjacent MR planes that corresponded to those planes on the PET images. The putamen was then automatically divided into three parts in the rostrocaudal direction using dedicated software for ROI demarcation. The 3-D ROIs (volumes of interest, VOIs) were extracted automatically by connecting two-dimensional drawings on each plane using a linear interpolation algorithm for VOI outlines. For reference, cerebellar ROIs were also defined in 3-D and located bilaterally on the cerebellar cortex.

Striatal-to-cerebellum ratio (SCR) values of radioactivity counts were calculated in the 80-90-min frame for each structure, using bilaterally averaged cerebellar ROI data as the denominator. For subregional analysis of their association with major motor symptoms in the PD subjects, SCR values from the caudate nucleus and each part of the putamen were analyzed on the contralateral to the more affected side of limb.

Statistical Analysis

For comparison of more than two groups, one-way analysis of variance (ANOVA) was used. When the one-way ANOVA was significant at $p < 0.05$, post-hoc comparisons were conducted using Scheffé's test. We examined the correlation of FMT uptake in each part of the putamen with disease duration, and with the symptoms of bradykinesia, tremor, rigidity, and postural instability assessed on UPDRS motor scores. Non-linear exponential regression analysis was applied to assess the relationship between FMT uptake and disease duration (Prism, GraphPad Software, La Jolla, CA). SCR values and the UPDRS scores were compared by Spearman's rank correlation coefficient test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Characteristics of subjects

Demographic and clinical characteristics of the patients with PD and those of the control subjects are listed in Table 1 and Table 2. The mean ages of the PD patients (41 male and 60 female) and the control subjects (6 male and 13 female) were 64.0 years (SD 9.3) and 56.7

Table 1 Clinical Characteristics of the Subjects

Characteristics	PD	Normal Control	*p* value
Age, year, mean ± SD	64.0 ± 9.3	56.7 ± 11.1	0.005
Male/Female	41/60	6/13	0.542
MMSE	27 ± 2.6	29 ± 1.3	0.005

MMSE, Mini Mental State Examination.
Data are given as mean ± standard deviation (SD) values.

Table 2 Clinical Charasteristics of the PD patients

Symptom duration, year	6.0 ± 4.4
More affected side	Right 55/Left 46
Hoehn-Yahr stage, on	2.4 ± 0.9
Hoehn-Yahr stage, off	3.3 ± 1.1
UPDRS score	
Total motor	30.3 ± 16
Bradykinesia	9.86 ± 6.3
Rigidity	6.15 ± 3.8
Axial	9.54 ± 6.2
Tremor	4.80 ± 4.0

UPDRS, Unified Parkinson's Disease Rating Scale.
Data are given as mean ± standard deviation (SD) values.

years (SD 11.1), respectively. A wide range of duration and severity of symptoms was represented among the patients. The mean duration of symptoms was 6.0 years (SD 4.4) and the mean UPDRS motor score was 30.3 (SD 16.0). The right side was more affected in 55 patients.

Subregional analysis of FMT uptake

Figure 1 shows representative images of FMT uptake in a normal subject and in early- and late-stage PD patients. Among the patients, FMT uptake showed the most marked decrease in the posterior putamen, regardless of disease duration, but significant decrease was seen throughout the striatum compared with the healthy controls. There were significant differences between side (ipsi- vs. contralateral to the more affected limbs), region (anterior vs. posterior putamen), and diagnosis (healthy subjects vs. PD group) ($P < 0.001$) (Figure 2a). Asymmetry between the striatum of the more and less affected sides is preserved, but shows a decrease with disease progression (Figure 2b).

Decline in FMT uptake with disease duration

Figure 3 shows scatterplots of FMT uptake against symptom duration in three regions of the putamen contralateral to the more affected limbs. Because age-related factors such as age at onset of symptoms and age-related Alzheimer-type pathology may influence disease duration, we excluded elderly-onset patients (> 70 years old; $n = 19$) in this analysis. Exponential regression curves that best fitted the data for each of the three regions analyzed are superimposed on the figure. Between 10 and 15 years of symptom duration, the FMT for all three curves leveled off to constant values that showed a statistically significant difference between the anterior and posterior putamen ($p < 0.001$). In the control group, there was no significant difference in SCR of FMT uptake between younger (< 59 years old, $n = 10$) and older (≥ 60 years old, $n = 9$) subjects (putamen, $p = 0.87$; caudate, $p = 0.81$).

Correlation of cardinal symptoms and FMT uptake

To minimize the possibility of including patients with alternative diagnoses, we analyzed patients who had cardinal motor symptoms for at least 3 years ($n = 42$). We obtained positive correlations between the severities of major motor symptoms: rigidity vs. axial symptoms ($r = 0.68$, $p < 0.001$), rigidity vs. bradykinesia ($r = 0.56$, $p < 0.001$), bradykinesia vs. postural instability ($r = 0.54$, $p < 0.001$), and tremor vs. bradykinesia ($r = 0.39$, $p = 0.014$). However, tremor did not have a significant relation with rigidity ($r = 0.20$, $p = 0.20$) or with axial symptoms ($r =$

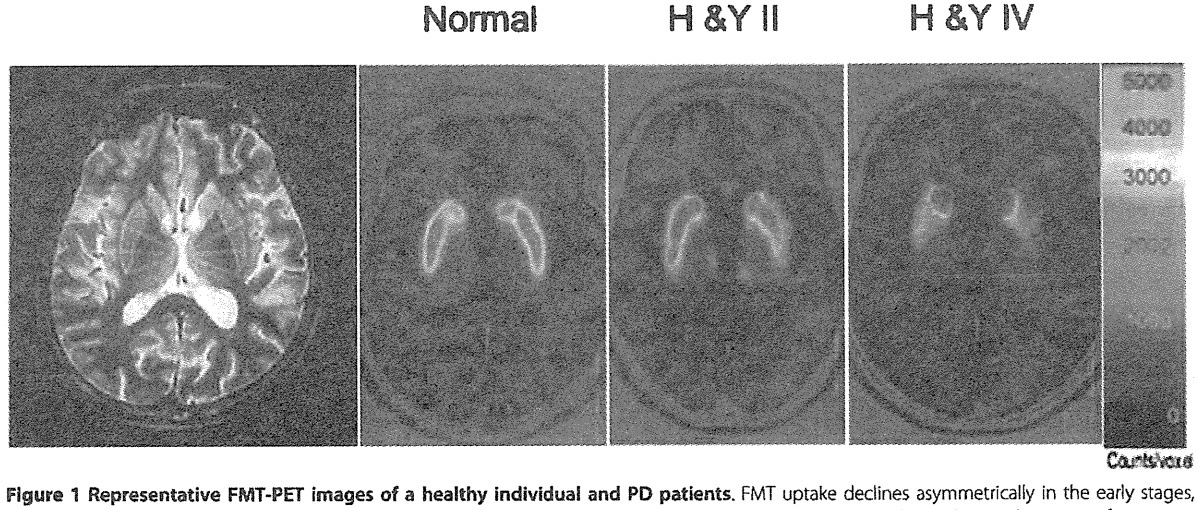

Figure 1 Representative FMT-PET images of a healthy individual and PD patients. FMT uptake declines asymmetrically in the early stages, mostly in the posterior putamen. Left: Regions-of-interest in the putamen. H &Y, Hohen and Yahr stage. The bar indicates the range of radioactive counts per voxel.

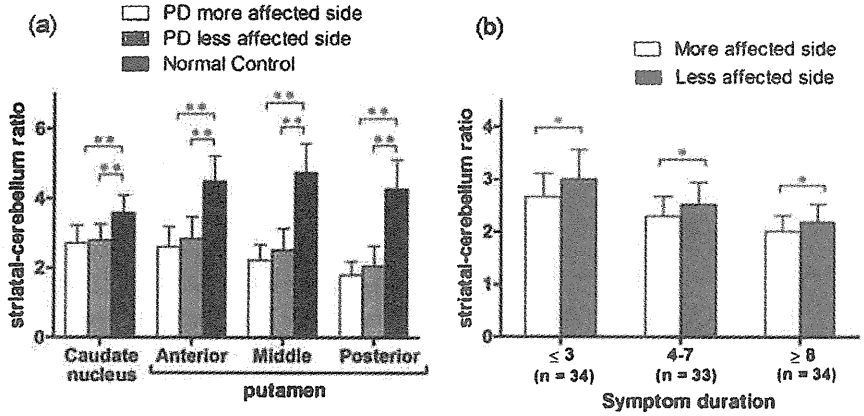

Figure 2 FMT uptake in different subregions of the striatum. Mean FMT uptake in different subregions of the striatum in normal control and PD patients (a). Comparison by side (b) shows persistant side-side asymmetry of putaminal uptake throughout the disease course. *$P < 0.05$, ** $P < 0.01$.

0.12, $p = 0.45$). Axial symptoms, rigidity, and bradykinesia scores showed a correlation with FMT uptake in the contralateral putamen, with the highest correlation in the anterior putamen, but not in the contralateral caudate (Table 3). No significant correlation was evident between unilateral tremor scores from the most severely affected limbs and any of the striatal regions. To assess the potential influence of age, we analyzed older patients (> 60 years old; $n = 25$) separately and found similar correlations between major symptoms and FMT uptake (Table 4).

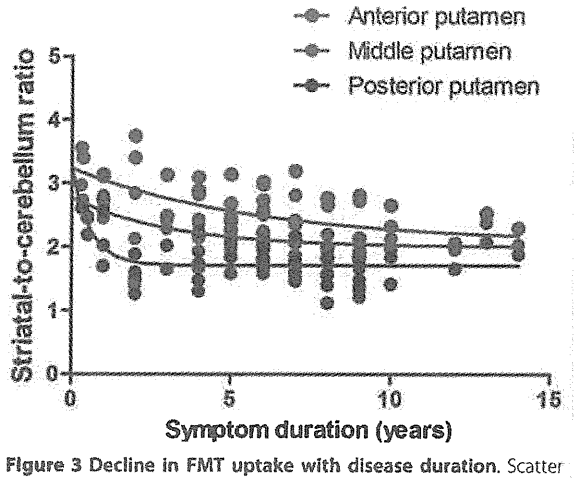

Figure 3 Decline in FMT uptake with disease duration. Scatter plots of FMT uptake against symptom duration in the putamen contralateral to the more affected limb in PD patients. Exponential decline is observed in all subregions of the putamen. Reduction of uptake is prominent at onset of the disease.

Discussion

Idiopathic PD is defined as a synucleinopathy in which Lewy bodies, pathological aggregations of the synaptic protein α-synuclein, are found in the dopaminergic neurons in the substantia nigra [14,15]. A reduction of dopamine in the striatum is a consistent finding in PD, although the clinical features are heterogeneous and include different predominant symptoms (resting tremor, bradykinesia, rigidity, or postural instability and gait disorder) with different rates of progression, and with or without dementia [16-19]. PET imaging is a valuable tool for assessing altered dopaminergic function in the striatum in PD. While FDOPA is suitable for assessing the metabolism of levodopa, FMT is superior for estimating AADC activity because it enables the production of higher-quality brain images [7,20-22]. The high resolution of FMT-PET images enables analysis of

Table 3 Correlations of UPDRS scores and FMT uptake ratio values in the each part of the putamen

Putamen	Anterior	Middle	Posterior	Whole
Symptom duration, year	-0.52 (<0.001)	-0.56 (<0.001)	-0.51 (<0.001)	-0.58 (<0.001)
Total motor score	-0.56 (<0.001)	-0.48 (0.002)	-0.41 (0.008)	-0.51 (0.001)
Bradykinesia	-0.54 (<0.001)	-0.53 (<0.001)	-0.44 (0.005)	-0.55 (<0.001)
Rigidity	-0.50 (0.001)	-0.43 (0.006)	-0.37 (0.018)	-0.44 (0.005)
Axial	-0.60 (<0.001)	-0.51 (0.001)	-0.37 (0.016)	-0.50 (0.001)
Tremor	0.069 (0.658)	0.085 (0.587)	0.015 (0.925)	0.050 (0.747)

Data are given as r (p) values. These values were calculated by Spearman's rank correlation coefficient test. UPDRS motor score in off-medication state was evaluated in 42 subjects.

Table 4 Correlations of UPDRS scores and FMT uptake ratio values in the each part of the putamen in elder patients

Putamen	Anterior	Middle	Posterior	Whole
Symptom duration, year	-0.70 (<0.001)	-0.63 (<0.005)	-0.45 (<0.05)	-0.70 (<0.001)
Total motor score	-0.56 (<0.01)	-0.50 (<0.05)	-0.37 (0.07)	-0.49 (<0.05)
Bradykinesia	-0.46 (<0.05)	-0.46 (<0.05)	-0.34(0.08)	-0.46 (<0.05)
Rigidity	-0.46 (<0.05)	-0.39 (0.05)	-0.31 (0.12)	-0.37 (0.06)
Axial	-0.69 (<0.001)	-0.59 (<0.01)	-0.45 (<0.05)	-0.58 (<0.01)
Tremor	0.26 (0.21)	0.12 (0.58)	0.06 (0.77)	0.14 (0.51)

Data are given as r (p) values. These values were calculated by Spearman's rank correlation coefficient test. UPDRS motor score in off-medication state was evaluated in 25 subjects.

dopaminergic presynaptic changes in each subregion of the striatum.

In the present study, FMT uptake in PD was reduced in the putamen, particularly in the posterior part. The anterior-to-posterior gradient of the uptake decrease in the putamen persisted to the advanced stage of PD. These results are consistent with those of previous reports that used other tracers of presynaptic dopaminergic terminals, and are considered to reflect the selective degeneration of nigrostriatal pathways that project into the posterior part of the putamen [23-25]. The lowest value of FMT uptake was observed in the posterior part of the putamen contralateral to the more affected limbs, even in the early stage of the disease. Because we analyzed regions in the posterior one-third of the putamen on high-resolution images, it is unlikely that the decreases in uptake were caused by partial volume effects, which may arise from placement of a small ROI on inaccurately co-registered images.

Post-mortem investigations of PD demonstrate that the rate of decrease of nigral neurons is rapid in the initial stage of the disease: approximately 40%-50% are lost in the first decade, possibly with a slower rate of degeneration later on, to finally approach a normal age-related linear decline [26]. In the present study, loss of FMT was well fitted to symptom duration using a single exponential approximation. The exponential model provided a better fit than a linear model, indicating that the rate of decline in FMT uptake in the contralateral putamen was faster at the beginning of the disease and slowed down as the disease progressed, in agreement with the results of previous studies that used radiotracers for imaging nigrostriatal nerve terminals [23-25]. Because we performed cross-sectional analysis in the present study, and because all of the participants were on medication, the data do not provide accurate information regarding the natural course of the disease, even if PET measurements were taken in off-medication state. Even so, the present data are important for assessing the progression of dopaminergic hypofunction in the striatum under optimal medical treatment, and can provide the basis for the development of even better therapeutic strategies [27,28].

We applied striatal count ratios to analyze the relationships between subregional putaminal FMT uptake and clinical symptoms. Striatal count ratios using the cerebellum as the denominator have a strong correlation with striatal uptake constants (Ki values) [29,30]. The present FMT-PET study showed a significant correlation between cardinal motor symptoms (rigidity, bradykinesia, and axial symptoms) and uptake of the tracer in the putamen, and no significant correlation was found between tremor score and FMT uptake. These findings are consistent with the results of previous PET studies [31-33]. The clinical correlations were more significant in the anterior part of the putamen than in the posterior part, possibly reflecting a floor effect for the uptake of FMT in the posterior part of the putamen, where the decrease was severe even in the early stage of the disease.

The pathophysiological mechanism of tremor is not fully understood [34]. Tremor does not respond to L-dopa as well as do bradykinesia and rigidity. The fact that stereotactic lesion or deep brain stimulation of the ventral intermediate nucleus (Vim) of the thalamus successfully improves tremor indicates a strong association between non-dopaminergic thalamic and cerebellar systems, and tremor generation [35,36].

Conclusions

Our results indicate that FMT-PET is useful for evaluating PD patients from the early stage of the disease and for studying the relationship between AADC activity and various clinical features. Decrease of FMT uptake in the posterior putamen appears to be most sensitive in mild PD, and uptake in the anterior putamen may reflect the severity of main motor symptoms, except for tremor. These data provide an important baseline for evaluating the effects of surgical interventions, such as gene therapy for PD.

Acknowledgements

This study was supported by grants from the Japanese Government: a Grant-in-Aid from the Research Committee of CNS Degenerative Diseases via the Ministry of Health, Labour and Welfare, and grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan. The authors would like to thank Jun-ichi Saito and Seiji Nagashima for their technical assistance.

Author details

[1]Division of Neurology, Department of Medicine, Jichi Medical University, Tochigi, Japan. [2]WebNet Technology, Tochigi, Japan. [3]Utsunomiya Central Clinic, Tochigi, Japan.

Authors' contributions
SA participated in designing the study, data collection, conducted the statistical analyses, interpreted data and drafted the first manuscript.
KF participated in data collection and interpretation of data.
AM participated in data collection and interpretation of data.
TS participated in data collection and interpretation of data.
IN participated in designing the study and interpretation of data.
SM conceived the study, participated in its design, data collection, interpretation of data and drafting the manuscript.
All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Received: 6 October 2010 Accepted: 23 March 2011
Published: 23 March 2011

References

1. Hornykiewicz O: **Biochemical aspects of Parkinson's disease.** *Neurology* 1998, **51**:S2-9.
2. Nagatsu T, Ichinose H: **Molecular biology of catecholamine-related enzymes in relation to Parkinson's disease.** *Cell Mol Neurobiol* 1999, **19**:57-66.
3. Halliday G: **Substantia nigra and locus coeruleus.** In *The human nervous system.* 2 edition. Edited by: Paxinos G, Mai JK. Amsterdam; Boston: Elsevier Academic Press; 2004:pp457-458.
4. Garnett ES, Firnau G, Nahmias C: **Dopamine visualized in the basal ganglia of living man.** *Nature* 1983, **305**:137-138.
5. Pate BD, Kawamata T, Yamada T, McGeer EG, Hewitt KA, Snow BJ, Ruth TJ, Calne DB: **Correlation of striatal fluorodopa uptake in the MPTP monkey with dopaminergic indices.** *Ann Neurol* 1993, **34**:331-338.
6. Snow BJ, Tooyama I, McGeer EG, Yamada T, Calne DB, Takahashi H, Kimura H: **Human positron emission tomographic [^{18}F]fluorodopa studies correlate with dopamine cell counts and levels.** *Ann Neurol* 1993, **34**:324-330.
7. DeJesus OT, Flores LG, Murali D, Converse AK, Bartlett RM, Barnhart TE, Oakes TR, Nickles RJ: **Aromatic l-amino acid decarboxylase turnover in vivo in rhesus macaque striatum: a microPET study.** *Brain Res* 2005, **1054**:55-60.
8. DeJesus OT, Holden JE, Endres C, Murali D, Oakes TR, Shelton S, Uno H, Houser D, Freund L, Perlman SB, *et al*: **Visualization of dopamine nerve terminals by positron tomography using [^{18}F]fluoro-β-fluoromethylene-*m*-tyrosine.** *Brain Res* 1992, **597**:151-154.
9. Doudet DJ, Chan GL, Jivan S, DeJesus OT, McGeer EG, English C, Ruth TJ, Holden JE: **Evaluation of dopaminergic presynaptic integrity: 6-[^{18}F] fluoro-l-dopa versus 6-[^{18}F]fluoro-l-*m*-tyrosine.** *J Cereb Blood Flow Metab* 1999, **19**:278-287.
10. Eberling JL, Bankiewicz KS, O'Neil JP, Jagust WJ: **PET 6-[^{18}F]fluoro-l-*m*-tyrosine Studies of Dopaminergic Function in Human and Nonhuman Primates.** *Front Hum Neurosci* 2007, **1**:9.
11. Hughes AJ, Daniel SE, Lees AJ: **Improved accuracy of clinical diagnosis of Lewy body Parkinson's disease.** *Neurology* 2001, **57**:1497-1499.
12. Maes F, Collignon A, Vandermeulen D, Marchal G, Suetens P: **Multimodality image registration by maximization of mutual information.** *IEEE Trans Med Imaging* 1997, **16**:187-198.
13. Wells WM, Grimson WL, Kikinis R, Jolesz FA: **Adaptive segmentation of MRI data.** *IEEE Trans Med Imaging* 1996, **15**:429-442.
14. Dickson DW, Braak H, Duda JE, Duyckaerts C, Gasser T, Halliday GM, Hardy J, Leverenz JB, Del Tredici K, Wszolek ZK, Litvan I: **Neuropathological assessment of Parkinson's disease: refining the diagnostic criteria.** *Lancet Neurol* 2009, **8**:1150-1157.
15. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M: **Alpha-synuclein in Lewy bodies.** *Nature* 1997, **388**:839-840.
16. Halliday GM, McCann H: **The progression of pathology in Parkinson's disease.** *Ann N Y Acad Sci* 2010, **1184**:188-195.
17. Maetzler W, Liepelt I, Berg D: **Progression of Parkinson's disease in the clinical phase: potential markers.** *Lancet Neurol* 2009, **8**:1158-1171.
18. Selikhova M, Williams DR, Kempster PA, Holton JL, Revesz T, Lees AJ: **A clinico-pathological study of subtypes in Parkinson's disease.** *Brain* 2009, **132**:2947-2957.
19. van Rooden SM, Heiser WJ, Kok JN, Verbaan D, van Hilten JJ, Marinus J: **The identification of Parkinson's disease subtypes using cluster analysis: a systematic review.** *Mov Disord* 2010, **25**:969-978.
20. Brown WD, DeJesus OT, Pyzalski RW, Malischke L, Roberts AD, Shelton SE, Uno H, Houser WD, Nickles RJ, Holden JE: **Localization of trapping of 6-[^{18}F]fluoro-l-*m*-tyrosine, an aromatic l-amino acid decarboxylase tracer for PET.** *Synapse* 1999, **34**:111-123.
21. DeJesus OT, Haaparanta M, Solin O, Nickles RJ: **6-fluoroDOPA metabolism in rat striatum: time course of extracellular metabolites.** *Brain Res* 2000, **877**:31-36.
22. Elsinga PH, Hatano K, Ishiwata K: **PET tracers for imaging of the dopaminergic system.** *Curr Med Chem* 2006, **13**:2139-2153.
23. Bruck A, Aalto S, Rauhala E, Bergman J, Marttila R, Rinne JO: **A follow-up study on 6-[^{18}F]fluoro-l-dopa uptake in early Parkinson's disease shows nonlinear progression in the putamen.** *Mov Disord* 2009, **24**:1009-1015.
24. Lee CS, Schulzer M, de la Fuente-Fernandez R, Mak E, Kuramoto L, Sossi V, Ruth TJ, Calne DB, Stoessl AJ: **Lack of regional selectivity during the progression of Parkinson disease: implications for pathogenesis.** *Arch Neurol* 2004, **61**:1920-1925.
25. Nandhagopal R, Kuramoto L, Schulzer M, Mak E, Cragg J, Lee CS, McKenzie J, McCormick S, Samii A, Troiano A, *et al*: **Longitudinal progression of sporadic Parkinson's disease: a multi-tracer positron emission tomography study.** *Brain* 2009, **132**:2970-2979.
26. Fearnley JM, Lees AJ: **Ageing and Parkinson's disease: substantia nigra regional selectivity.** *Brain* 1991, **114(Pt 5)**:2283-2301.
27. Christine CW, Starr PA, Larson PS, Eberling JL, Jagust WJ, Hawkins RA, VanBrocklin HF, Wright JF, Bankiewicz KS, Aminoff MJ: **Safety and tolerability of putaminal AADC gene therapy for Parkinson disease.** *Neurology* 2009, **73**:1662-1669.
28. Muramatsu SI, Fujimoto KI, Kato S, Mizukami H, Asari S, Ikeguchi K, Kawakami T, Urabe M, Kume A, Sato T, *et al*: **A Phase I study of aromatic l-amino acid decarboxylase gene therapy for Parkinson's disease.** *Mol Ther* 2010, **18**:1731-1735.
29. Dhawan V, Ma Y, Pillai V, Spetsieris P, Chaly T, Belakhlef A, Margouleff C, Eidelberg D: **Comparative analysis of striatal FDOPA uptake in Parkinson's disease: ratio method versus graphical approach.** *J Nucl Med* 2002, **43**:1324-1330.
30. Eberling JL, Pivirotto P, Bringas J, Bankiewicz KS: **Comparison of two methods for the analysis of [^{18}F]6-fluoro-l-*m*-tyrosine PET data.** *Neuroimage* 2004, **23**:358-363.
31. Martin WR, Wieler M, Stoessl AJ, Schulzer M: **Dihydrotetrabenazine positron emission tomography imaging in early, untreated Parkinson's disease.** *Ann Neurol* 2008, **63**:388-394.
32. Otsuka M, Ichiya Y, Kuwabara Y, Hosokawa S, Sasaki M, Yoshida T, Fukumura T, Masuda K, Kato M: **Differences in the reduced ^{18}F-Dopa uptakes of the caudate and the putamen in Parkinson's disease: correlations with the three main symptoms.** *J Neurol Sci* 1996, **136**:169-173.
33. Vingerhoets FJ, Schulzer M, Calne DB, Snow BJ: **Which clinical sign of Parkinson's disease best reflects the nigrostriatal lesion?** *Ann Neurol* 1997, **41**:58-64.
34. Rodriguez-Oroz MC, Jahanshahi M, Krack P, Litvan I, Macias R, Bezard E, Obeso JA: **Initial clinical manifestations of Parkinson's disease: features and pathophysiological mechanisms.** *Lancet Neurol* 2009, **8**:1128-1139.
35. Diamond A, Shahed J, Jankovic J: **The effects of subthalamic nucleus deep brain stimulation on parkinsonian tremor.** *J Neurol Sci* 2007, **260**:199-203.
36. Terao T, Yokochi F, Taniguchi M, Kawasaki T, Okiyama R, Hamada I, Nishikawa N, Izawa N, Shin M, Kumada S, Takahashi H: **Microelectrode findings and topographic reorganisation of kinaesthetic cells after gamma knife thalamotomy.** *Acta Neurochir (Wien)* 2008, **150**:823-827, discussion 827.

Pre-publication history
The pre-publication history for this paper can be accessed here:
http://www.biomedcentral.com/1471-2377/11/35/prepub

doi:10.1186/1471-2377-11-35
Cite this article as: Asari *et al.*: Subregional 6-[^{18}F]fluoro-L-*m*-tyrosine Uptake in the Striatum in Parkinson's Disease. *BMC Neurology* 2011 11:35.

Hindawi Publishing Corporation
Neurology Research International
Volume 2011, Article ID 165415, 8 pages
doi:10.1155/2011/165415

Clinical Study

High-Resolution Melting (HRM) Analysis of the Cu/Zn Superoxide Dismutase (SOD1) Gene in Japanese Sporadic Amyotrophic Lateral Sclerosis (SALS) Patients

Chizuru Akimoto,[1] Mitsuya Morita,[1] Naoki Atsuta,[2] Gen Sobue,[2] and Imaharu Nakano[1]

[1] *Division of Neurology, Department of Internal Medicine, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke-shi, Tochigi 329-0498, Japan*
[2] *Department of Neurology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya-shi, Aichi 466-8550, Japan*

Correspondence should be addressed to Chizuru Akimoto, ckawamata@jichi.ac.jp

Received 19 October 2010; Accepted 29 January 2011

Academic Editor: Dirk Deleu

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder, and the majority of ALS are sporadic (SALS). Recently, several causative genes for familial ALS (FALS) were identified, but the cause of the SALS is still unknown. This time, we aimed to identify the genetic background of SALS. First, we applied the new sensitive screening methods: high-resolution melting (HRM) analysis. HRM analysis detected 18 out of 19 known SOD1 gene mutations (94.7% sensitivity). Next, we screened *SOD1*, three novel mutations (C6Y, Q22H, and S134T) were identified in our own 184 SALS cases (1.63% prevalence), and four mutations in another 255 SALS cases (1.56% prevalence) registered from all over Japan. The patients with *SOD1* mutations suggested a relatively young onset and limb involvement at onset. The HRM analysis is a sensitive and easy screening method; we will use this method for screening other ALS causative genes and revealing the genetic background of SALS.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder primarily affecting motor neurons in the spinal cord, brain stem, and cerebral cortex. Five to ten percent of ALS cases are familial; the others are believed to be sporadic [1]. Mutations in the Cu/Zn superoxide dismutase gene (*SOD1*; OMIM 147450) are the most frequent genetic defects known to underlie ALS, accounting for 20% of familial cases (FALS) and one to seven percent of apparently sporadic cases (SALS) [1–7]. Recently, other mutations like the TARDBP gene (TDP-43) [8, 9], ANG gene [10], FUS/TLS gene [11], and OPTN gene [12] were identified as causative of ALS. Despite this genetic heterogeneity, *SOD1* mutations are the most frequent cause of adult onset ALS. Here, we report the results of screening for *SOD1* mutations in the 184 SALS cases in our hospital and 265 ALS cases all over Japan by high-resolution melting (HRM) analysis.

HRM analysis is a mutation scanning technique that monitors the progressive change in fluorescence caused by the release of an intercalating DNA dye from a DNA duplex as it is denatured with marginal increases in temperature [13]. The shifts and shapes of melting curves, there are obtained as fluorescence difference plots, are used to distinguish between mutations and controls. HRM analysis of PCR products amplified in the presence of LC Green Plus can detect all heterozygous and most homozygous sequence variations through differences in shape and position of a melting curve compared with a wild-type melting profile. Although single-strand conformation polymorphism (SSCP) [2, 3, 14–20] and denaturing high-performance liquid chromatography (DHPLC) [5, 6] seem to be the main screening strategies for *SOD1* mutations, HRM analysis has its own advantages. This is the first report of HRM analysis being applied to the *SOD1* screening. In this paper, we report the high sensitivity of HRM analysis for known *SOD1*

Table 1: Reported *SOD1* mutations to determine the sensitivity of HRM analysis.

Exon1	A4V, L8V, V14G
Exon2	H43R
Exon3	D76Y
Exon4	N86S, A89V, D90A (hetero), G93S, D101G, S105L, G114A, R115G
Exon5	L126delTT, G127X, A140A, L144F type2, L144FVX

Underlined mutation could not detect the mutation by HRM analysis.

mutations, and the prevalence and clinical features of *SOD1* mutations in Japanese SALS cases.

2. Patients and Methods

2.1. Patient Group 1. A total of consecutive 184 SALS cases (109 males and 75 females) visited our Neurology Division at the Jichi Medical University Hospital in Tochigi, Japan. Ethical approval was granted by the Bioethics Committee for Human Gene Analysis of our university and informed consent was obtained from all subjects according to the Declaration of Helsinki. Every patient fulfilled the diagnostic criteria for ALS as outlined by the *El Escorial Revisited* [21] classification; 177 definite, probable or possible ALS and 7 suspected ALS. None of the cases had a family history of a neuromuscular disorder. There was no significant difference in onset age between 109 males and 75 females (males: 60.4 years on average with a range of 27–80; females: 64.3 years with a range of 34–83).

2.2. Patient Group 2. In 2006, the Japanese Consortium for Amyotrophic Lateral Sclerosis Research (JaCALS) was organized with the aim of investigating the relationships of clinical and genetic aspects of ALS in Japan. The Ethics Committee of each institution granted ethical approval. The inclusion criteria for registration with the JaCALS are: (1) adult onset, steady progressive course, (2) definite, probable or possible ALS based on the *El Escorial Revisited* [21] criteria for diagnosis of ALS, and (3) informed consent for the genetic study and clinical checking every three months. From 2006 to 2008, 265 patients (10 FALS and 255 SALS) were registered, and blood samples and clinical data having been obtained by neurologists.

2.3. Reported SOD1 Mutations. We used 19 reported *SOD1* mutations in all five exons (Table 1) to determine the sensitivity of the HRM analysis. 19 reported *SOD1* mutations were obtained from our collaborators, Dr. Andersen P. (Umeå University, Sweden) and Dr. Watanabe Y. (Tottori University, Japan), and they were already direct sequenced and confirmed they had the mutations.

2.4. HRM Analysis and Sequencing. Genomic DNA was extracted from lymphocytes using a standard procedure. We designed PCR primers for HRM analysis to screen all five exons in *SOD1*. DNA samples were amplified with double-stranded DNA-binding dye LC Green Plus (Idaho Technology). PCR was performed with a Veriti 96-Well Thermal Cycler (Applied Biosystems) in 10 μL reaction mixtures comprising 10 ng DNA, 1XPCR buffer, LC Green Plus (Idaho Technology), and 1 U Taq polymerase, with 0.25 μM each forward and reverse primers. Initial denaturation was performed at 95°C for 2 min, followed by 45 cycles of 94°C for 30 sec and 62–68°C for 30 sec, with a final cycle of 94°C for 30 sec and 25°C for 30 sec.

We performed melting acquisition with a 96-well Light Scanner (Idaho Technology). The plate was heated from 80 to 98°C at 0.1°C/sec with a 300 ms frame interval, 15 ms exposure, and 100% LED power. Light Scanner Software was used for melting curve analysis. The Light Scanner analyses of 96 samples were performed in around 10 min. Sequencing of samples indicated to include mutations was then carried out using a BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) and an ABI 310 automated sequencer (PE Applied Biosystems).

First we examined 19 reported *SOD1* mutations to determine the sensitivity of HRM analysis. Next, we applied this method to Japanese ALS patients for mutation screening of *SOD1*.

3. Results

3.1. Sensitivity of HRM Analysis. HRM analysis clearly distinguished 18 of 19 previously identified *SOD1* mutations from normal controls. The mutation detection sensitivity was 94.7% for the reported mutations. The melting curves of control samples (wild-type) were tightly grouped for all fragments, and altered difference curves were easily identified for the 18 mutations (Figure 1). The mutation that could not be detected was Gly 114 Ala.

3.2. SOD1 Mutations and the Clinical Characteristics in Group 1. We found *SOD1* mutations in three out of the 184 SALS cases (1.63%) in the group 1. The mutations identified were all novel: Cys 6 Tyr (C6Y) and Gln 22 His (Q22H) in exon 1, and Ser 134 Thr (S134T) in exon 5 (Figure 2).

In case 1, a 34-year-old woman, there was a single-base pair substitution in exon 1 at codon 6 (TGC to TAC). This change created a cysteine 6 to tyrosine missense mutation (C6Y). She awoke with painful cramping and weakness in the right leg almost every morning at the age of 33 years. The cramping resolved, but her right leg weakness progressed and become accompanied by fasciculation. One year after the onset, neurological examination showed marked muscle atrophy and prominent fasciculation in her right leg. Tendon reflexes were normal, and plantar responses were flexor. Sensations in all four modalities were intact. Nerve conduction studies revealed mild reduction of motor nerve conduction velocity without conduction block. Needle electromyographic analysis showed repetitive discharges and hyperexcitability only in the right leg. Extensive screening for causes of the motor neuropathy was negative. The muscle weakness and atrophy progressed, and spread to the other parts of her body despite treatment with intravenous