Association between cardiac denervation and parkinsonism caused by α-synuclein gene triplication

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Summary
Parkinson’s disease patients frequently have symptoms and signs of autonomic nervous dysfunction that are the source of considerable disability. Recent studies have revealed that most patients with Parkinson’s disease, and all with Parkinson’s disease-associated orthostatic hypotension, have a loss of cardiac sympathetic innervation. Familial Parkinson’s disease, caused by mutation of the gene encoding α-synuclein, also features orthostatic hypotension, sympathetic neurocirculatory failure and cardiac sympathetic denervation. We have recently described a whole-gene triplication of α-synuclein causing Lewy body parkinsonism in a large, well characterized family called the ‘Iowa kindred’. Here we report the results of cardiac PET scanning using the sympathetic nerve imaging agent, 6-[18F]fluorodopamine in affected and unaffected members of this kindred. Four family members were studied, two with parkinsonism, one clinically normal and one with benign essential tremor alone. Both affected members had obvious loss of cardiac sympathetic innervation; the unaffected member had normal innervation, as did the member with isolated essential tremor. The results indicate that, in this family, where disease is caused by overexpression of normal α-synuclein, cardiac sympathetic denervation cosegregates with parkinsonism. Post-mortem studies have demonstrated synuclein-positive Lewy body formation in the brains of individuals with parkinsonism who were also in the family described here and who also carry this triplication. These results indicate that both parkinsonism and cardiac sympathetic denervation can result from an excess of normal synuclein.

Keywords: fluorodopamine; norepinephrine; orthostatic hypotension; alpha-synuclein; baroreflex


Introduction
Parkinson’s disease is characterized by at least two of the following: rigidity, bradykinesia, gait difficulties, and a characteristic ‘pill roll’ resting tremor. Additional signs and symptoms, include those of autonomic dysfunction, are common and can be disabling, including chronic constipation, urinary urgency and incontinence, erectile failure in men, and orthostatic or post-prandial light-headedness (Turkka, 1987; Martignoni et al., 1995; Niimi et al., 1999; Chaudhuri, 2001).

Until recently, Parkinson’s disease was viewed as a non-genetic disease; however, increasing evidence suggests the disease often has a genetic component (Gwinn-Hardy and Farrer, 2002). Genetic insights have included those gained from the study of affected members of the ‘Iowa kindred’, who share autosomal dominantly inherited parkinsonism caused by a recently identified triplication of the α-synuclein gene (Singleton et al., 2003). In this family, parkinsonism begins at a relatively early adult age, with an average age at onset of 34 years and range of 20–48 years. Disease course is fulminant, with survival approximately 10 years after diagnosis. Another well-described phenotypic trait in the Iowa kindred is a form of progressive essential tremor, which does not affect lifespan and does not segregate with the pathogenic α-synuclein multiplication.

The clinical and pathological findings in this family resemble those observed in families carrying missense
mutations in α-synuclein, especially the Contursi kindred (Gwinn-Hardy et al., 2000). Familial Parkinson’s disease caused by α-synuclein gene mutation in the Contursi kindred has been previously described to include orthostatic hypotension, sympathetic neurocirculatory failure and cardiac sympathetic denervation (Polymeropoulos et al., 1997). Whether other forms of familial Parkinson’s disease involve such features remains unknown. In this study we sought to determine whether an analogous dysautonomia existed in the Iowa kindred.

**Methods**

This study was performed under a protocol approved by the National Institutes of Health institutional review board. Written informed consent was obtained from all participants. Following informed consent, subjects underwent a neurological history and physical examination, which included the Unified Parkinson’s Disease rating scale (UPDRS) by a fellowship-trained movement disorders specialist (K. G.-H.). To determine the frequency of symptoms or signs of dysautonomia in the Iowa kindred, medical records were reviewed for members in four generations, including 21 affected individuals.

Molecular genetic analysis of the kindred was performed as previously described in an unpublished manuscript (Singleton et al., 2003) to determine which individuals were carriers and non-carriers of the pathogenic triplication mutation.

Caffeine-containing beverages, cigarettes and alcohol were not allowed for at least 24 h before testing. Subjects were studied while taking their usual medications, which did not include drugs known to inhibit neuronal uptake of catecholamines (but did include carbidopa/levodopa).

Orthostatic hypotension was defined by a decrease in systolic blood pressure of at least 20 mmHg and a decrease in diastolic blood pressure of at least 5 mmHg between 15 min of lying supine and 5 min of standing upright (Goldstein et al., 2002). Beat-to-beat blood pressure and pulse rate responses to the Valsalva manoeuvre were recorded, with attention to whether there was a progressive decline in blood pressure during Phase II and absence of pressure overshoot in Phase IV (Goldstein and Tack, 2000). Antecubital venous blood was drawn through an indwelling catheter, after at least 15 min of supine rest and then after 5 min of standing. Plasma norepinephrine was assayed by high-pressure liquid chromatography with electro-

![Fig. 1 Blood pressure and heart rate associated with the Valsalva manoeuvre in a control subject, a patient with sporadic Parkinson’s disease and orthostatic hypotension, a patient with familial Parkinson’s disease in the Contursi kindred, and a patient with familial Parkinson’s disease in the Iowa kindred. Note the progressive decline in blood pressure in Phase II and the absence of pressure overshoot in Phase IV of the Valsalva manoeuvre in the patients with sporadic or familial Parkinson’s disease.](http://brain.oxfordjournals.org/)

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**Fig. 1** Blood pressure and heart rate associated with the Valsalva manoeuvre in a control subject, a patient with sporadic Parkinson’s disease and orthostatic hypotension, a patient with familial Parkinson’s disease in the Contursi kindred, and a patient with familial Parkinson’s disease in the Iowa kindred. Note the progressive decline in blood pressure in Phase II and the absence of pressure overshoot in Phase IV of the Valsalva manoeuvre in the patients with sporadic or familial Parkinson’s disease.
Table 1 Autonomic function indices in Iowa kindred family members with and without Parkinson’s disease

<table>
<thead>
<tr>
<th>Subject</th>
<th>Fx ΔMAP</th>
<th>Val BP</th>
<th>BRS (ms/mmHg)</th>
<th>NE Sup. (nmol/l)</th>
<th>Fx ΔNE</th>
<th>EPI (nmol/l)</th>
<th>18F Sept. (nCi-kg/ml-mCi)</th>
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<tr>
<td>Parkinson’s disease</td>
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<td></td>
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<tr>
<td>1023</td>
<td>−0.06</td>
<td>II, IV</td>
<td>5.77</td>
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<td>1011</td>
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<td>II, IV</td>
<td>0.37</td>
<td>0.70</td>
<td>0.96</td>
<td>0.02</td>
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<tr>
<td>No Parkinson’s disease</td>
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<tr>
<td>2002</td>
<td>0.03</td>
<td>II, IV</td>
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<td>1.15</td>
<td>2.40</td>
<td>0.04</td>
<td>10736</td>
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<td>II, IV</td>
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<td>II, IV</td>
<td>6.08</td>
<td>1.28</td>
<td>1.11</td>
<td>0.08</td>
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Values in bold face are abnormal (for quantitative data, more than 2 SD below the normal mean). Fx ΔMAP = fractional change in mean arterial pressure after standing for 5 min; Val BP = Valsalva blood pressure response pattern; BRS = baroreflex-cardiovagal gain; NE Sup. = plasma norepinephrine level during supine rest; Fx ΔMAP = fractional change in plasma norepinephrine level after standing for 5 min; EPI = plasma epinephrine level; 18F Sept. = septal myocardial concentration of 6-[18F]fluorodopamine 5 min after completion of a 3-min i.v. injection of 6-[18F]fluorodopamine; II = Phase II of the Valsalva manoeuvre; IV = Phase IV of the Valsalva manoeuvre.

Results
The presence or absence of autonomic symptoms or signs per chart review revealed that, of 21 records from affected members, a total of 11 had documented symptoms. Of these, six had complaints and findings of orthostatic hypotension explicitly documented. An additional five other affected family members had complaints of symptoms consistent with dysautonomia, including urinary or fecal incontinence, constipation, or light-headedness upon standing. One member suffered from micturition syncope as well. In order to prevent unmasking of genetic data to participants and others, gender is not given and ages are given in a 10-year range only.

Subject 1023
This individual, in the fifth decade of life, was diagnosed with Parkinson’s disease in the fourth decade, 10 years prior to evaluation. This subject carries the pathogenic triplication of α-synuclein. Review of systems revealed orthostatic dizziness. Physical examination showed masked facies, hypophonic speech, severe bilateral resting tremor (more on the left than on the right), bradykinesia and cogwheel rigidity. Orthostatic tachycardia was present, heart rate increasing from 72 to 104 b.p.m. after 5 min of standing, but did not have orthostatic hypotension. Gait was moderately impaired due to shuffling, postural instability and significant slowing. The parkinsonian symptoms all improved with levodopa/carbidopa, but the patient experienced moderate to marked ‘on’ dyskinesia. Other medications at the time of evaluation included pergolide and amantadine.

Beat-to-beat blood pressure failed to increase from the nadir in Phase II of the Valsalva manoeuvre, and there was no blood pressure overshoot in Phase IV (Fig. 1). Baroreflex-cardiovagal gain was within the normal range for a person of the patient’s age. The plasma norepinephrine level was normal during supine rest and increased normally during standing (Table 1). Levels of other catechols were as expected in a patient taking levodopa/carbidopa.

Thoracic PET scanning after i.v. 6-[18F]fluorodopamine demonstrated virtually absent myocardial radioactivity (Fig. 2).

Subject 1011
This individual, in the fifth decade of life and confirmed to have the pathogenic triplication of α-synuclein, was diagnosed with Parkinson’s disease 1 year before evaluation, after a 2-year history of fatigue, slowed movements and weight loss. Severe constipation was present and was treated for about two decades previously. The subject’s complaints on evaluation included orthostatic dizziness and light-headedness, right upper extremity resting tremor and bradykinesia. Physical examination demonstrated orthostatic hypotension (Table 1), right upper extremity resting tremor, bradykinesia, hypophonia, micrographia and shuffling gait. The parkinsonian symptoms responded to levodopa/carbidopa.
The patient had abnormal blood pressure responses to the Valsalva manoeuvre. The increment in the plasma norepinephrine level during standing was normal (Table 1). Thoracic PET scanning after i.v. 6-[18F]fluorodopamine showed markedly decreased radioactivity throughout the left ventricular myocardium, despite approximately normal myocardial perfusion based on 13N-ammonia scanning (Fig. 2, Table 1).

Subject 2002
This individual, in the eight decade, has never developed symptoms or signs of Parkinson’s disease and does not carry the pathogenic triplication of α-synuclein. Activation tremor began around age 40 years. This tremor had worsened over the past 4–5 years, causing difficulty with drinking, eating soup, holding paper and handwriting; the handwriting was large and jerky on examination. This individual does not drink alcohol and so responsiveness to that is not known. This subject was on no medications and had no medical conditions (besides tremor) other than age associated deafness. Physical examination showed supine hypertension without orthostatic hypotension (Table 1). On drawing Archimedes spirals, there was +2 tremor of the right upper extremity and +3 of the left. On finger-to-nose testing, there was +2 tremor on the right and +2.5 on the left. A +2 tremor was seen bilaterally with pouring from glass to glass and with drinking water from a spoon.

Blood pressure responses to the Valsalva manoeuvre were normal, but heart rate responses were blunted, resulted in low calculated baroreflex-cardiovascular gain (Table 1). Plasma norepinephrine increased normally with standing. Myocardial 6-[18F]fluorodopamine-derived radioactivity was normal throughout the left ventricular myocardium (Fig. 2).

Subject 1022
This individual, in the fifth decade of life, is an asymptomatic sibling of Subject 1023. Neurological examination was normal and this person does not carry the α-synuclein mutation. This subject was medication-free at the time of evaluation and had no known medical conditions. The blood pressure and heart rate responses to the Valsalva manoeuvre were normal. Plasma norepinephrine was normal during supine rest and increased normally with standing. Myocardial 6-[18F]fluorodopamine-derived radioactivity was normal throughout the left ventricular myocardium (Fig. 2).

Discussion
In the family described here, where parkinsonism is transmitted as an autosomal dominant trait, cardiac sympathetic denervation, evidenced by low myocardial concentrations of 6-[18F]fluorodopamine-derived radioactivity, cosegregated with parkinsonism and pathogenic triplication of α-synuclein. This finding indicates that, in this family, a common pathophysiological mechanism, based on overexpression of α-synuclein, causes both loss of dopamine-producing cells in the nigrostriatal system of the brain and concurrent loss of norepinephrine-producing cells in the sympathetic system of the heart.

In other patients with Parkinson disease, Lewy bodies have been identified not only in the substantia nigra, where they constitute a pathological hallmark of the disease, but also in...
sympathetic ganglia and sympathetically innervated organs (Iwanaga et al., 1999). Lewy bodies are now known to contain α-synuclein (Spillantini et al., 1997); affected members of the Iowa kindred who have come to post-mortem examination are known to have synuclein-positive Lewy bodies in the brain (Gwinn-Hardy et al., 2000). Whether, in this family, tissues outside the brain have Lewy bodies remains unknown.

The cardiac sympathetic denervation in the Iowa kindred resembles that in the Contursi kindred, where familial Parkinson’s disease results from mutation of the gene encoding α-synuclein (Goldstein and Tack, 2000; Goldstein et al., 2001). The similarities between the two kindreds point to a common feature, synucleinopathy, as a correlate and possibly pathogenic mechanism of the loss of catecholamine-producing cells. In the Iowa kindred disease has a simple cause: four copies of the normal α-synuclein gene. In terms of a mechanism of dysfunction the most parsimonious explanation is that this mutation leads to a build-up of α-synuclein and causes cellular dysfunction and/or death in vulnerable neuronal populations. There are minor differences in the cardiac denervation noted between the patients from each kindred which may be associated with stage of disease, or perhaps a result of subtle differences in the mode of action of each mutation. Assessment of additional affected patients from each kindred will allow us to examine these differences in disease in a more quantitative manner. Thus, it appears that both forms of familial parkinsonism feature cardiac sympathetic denervation from a synucleinopathy.

In both families, sympathetic denervation was most noticeable in the heart. Supine plasma norepinephrine levels were normal, consistent with normal overall release of the sympathetic neurotransmitter into the plasma; however, one must keep in mind that the norepinephrine concentration depends not only on spillover into the plasma but also clearance from it, and in autonomic failure decreased norepinephrine clearance can maintain the plasma norepinephrine level, despite decreased spillover (Meredith et al., 1992).

More than a dozen studies have agreed on the remarkable finding that patients with apparent sporadic Parkinson’s disease and orthostatic hypotension have evidence for cardiac sympathetic denervation. The present findings in the Iowa kindred support the view that cardiac sympathetic denervation can be caused not only by mutant forms of α-synuclein, but also by overproduction of normal α-synuclein protein.

**References**


