C9ORF72 expansions, parkinsonism, and Parkinson disease

A clinicopathologic study

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ABSTRACT

Objective: To determine the histopathologic bases for the observed incidence of parkinsonism in families with *C9ORF72* expansions, which typically cause amyotrophic lateral sclerosis (ALS) and/or frontotemporal dementia.

Methods: DNA was extracted from 377 brains with the histopathologic diagnosis of idiopathic Parkinson disease or related disorders and analyzed for *C9ORF72* expansions. α-Synuclein and p62 immunohistochemistry of the substantia nigra (SN) was undertaken in brains of 17 ALS cases with (*C9ORF72+*) and 51 without (*C9ORF72−*) the *C9ORF72* expansion.

Results: Only 1 of 338 cases with pathologically confirmed idiopathic Parkinson disease had a *C9ORF72* expansion. Similarly, only 1 of 17 *C9ORF72+* brains displayed features suggestive of α-synucleinopathy. In contrast, p62-positive, TDP-43–negative neuronal cytoplasmic inclusions within the SN were considerably more frequent in *C9ORF72+* brain tissue than in the *C9ORF72−* brains ($p = 0.005$). Furthermore, there was a more marked loss of dopaminergic neurons in the SN of *C9ORF72+* ALS brains than *C9ORF72−* ALS brains ($p = 0.029$).

Conclusions: SN involvement is common in *C9ORF72+* ALS but can be clearly distinguished from Parkinson disease–related mechanisms by the presence of p62-positive inclusions and the absence of α-synuclein–positive Lewy bodies or Lewy neurites. ***Neurology*® 2013;81:808–811**

GLOSSARY

ALS = amyotrophic lateral sclerosis; ***C9ORF72*** = chromosome 9 open reading frame 72; **FTD** = frontotemporal dementia; **FTLD** = frontotemporal lobar degeneration; **iPD** = idiopathic Parkinson disease; **PD** = Parkinson disease; **SN** = substantia nigra; **TDP-43** = TAR DNA-binding protein 43.

Substantia nigra (SN) involvement in amyotrophic lateral sclerosis (ALS) has previously been noted clinically[1] and neuropathologically.[2,3] Expansions of *C9ORF72* with >30 repeats (*C9ORF72+*) are the most common identifiable genetic cause of ALS and frontotemporal dementia (FTD).[3,4] We and others have reported parkinsonian phenotypes at a greater frequency within *C9ORF72+* families[3,5] and sporadic cases,[3,6,7] than in those with ALS/FTD who did not have a *C9ORF72* expansion (*C9ORF72−*). Thus, it seems likely that intronic expansions of *C9ORF72* explain at least in part the observed association between ALS and parkinsonism. However, it is currently unclear whether *C9ORF72+* mutation carriers develop parkinsonism due to *C9ORF72+* causing an α-synucleinopathy—as observed in idiopathic Parkinson disease (iPD)—or whether the underlying pathology in these patients is more in keeping with typical *C9ORF72+* extramotor pathology with p62-positive, TDP-43–negative, ubiquitylated neuronal and glial cytoplasmic inclusions.[3] Of note, the 9p21 locus has not been implicated in genetic association studies of iPD.[8]

To further clarify these crucial issues, we have genotyped a large number of brain tissue samples with the histopathologically confirmed diagnosis of iPD or related disorders for *C9ORF72* expansions. We also hypothesized that subclinical involvement of the SN may be more common

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in *C9ORF72*+ than in *C9ORF72*− brains of patients who clinically presented only with features in keeping with ALS/FTD.

METHODS **Standard protocol approvals, registrations, and patient consents.** Ethical approval was obtained from the respective local and national ethics committees. Postmortem histology reports were provided by the Parkinson's UK Brain Bank and the Queen Square Brain Bank for Neurological Disorders.

Subjects. A total of 377 cases with a clinical diagnosis of PD were included; Lewy-body–positive, α-synucleinopathy was pathologically confirmed in 338 (90%) of cases (see table). Thus, a sufficient number of cases was obtained to allow comparison with the known frequency of expanded *C9ORF72* in controls.[3]

Genotyping and immunohistochemistry. DNA was extracted from brain tissue using standard methods and analyzed for *C9ORF72* expansions as previously described.[3] α-Synuclein and p62 immunohistochemistry was performed on 17 *C9ORF72*+ cases of ALS, including one case known to have autopsy-confirmed iPD as well as ALS.[3] Immunohistochemistry for p62 was also performed on an additional 51 *C9ORF72*− ALS cases. The SN was examined on one side of each brain. Seven-micron-thick tissue sections from selected blocks were subjected to immunohistochemistry using antibodies to α-synuclein (Novocastra, Milton Keynes, UK) and p62 (BD Transduction Laboratories, Oxford, UK). α-Synuclein pathology was assessed as present or absent in *C9ORF72*+ cases. The number of p62-positive inclusions was classified as high (≥10 positive neuronal cytoplasmic inclusions), intermediate (5–9 inclusions), or low (≤4 inclusions). The number of cases with and without the *C9ORF72* mutation with high, intermediate, and low numbers of p62-positive neuronal cytoplasmic was compared by χ^2. To determine whether the ubiquitylated neuronal cytoplasmic inclusion pathology of the SN was associated with neuronal loss, both *C9ORF72*+ and *C9ORF72*− brains were semiquantitatively assessed as having no, mild, or severe neuronal loss. The extent of the neuronal cell loss was compared by χ^2.

RESULTS Only one of the brain bank iPD cases had a *C9ORF72* expansion containing >30 repeats (1/377 = 0.2% of the total number of cases screened, and 1/338 = 0.3% of the Lewy-body–positive cases). This *C9ORF72*+ patient presented with clinically typical PD at the age of 67 years. His father had died of ALS. Neuropathologic assessment revealed features of 1) classic PD with Braak stage 6, diffuse neocortical Lewy-body pathology; 2) classic TDP-43 pathology with frontotemporal lobar degeneration (FTLD)-TDP type-A features[9]; and 3) *C9ORF72*-ALS/FTLD pathology with numerous p62-positive, TDP-43–negative neuronal cytoplasmic inclusions of star-shaped morphology in the hippocampus, and smaller cytoplasmic inclusions in cerebellar granule cells. Unfortunately, his spinal cord was not available.

All but one of the 17 *C9ORF72*+ ALS brains were devoid of α-synuclein–positive neuronal cytoplasmic inclusions in the SN. The single case with α-synuclein pathology was known to have coincident PD-ALS and has been discussed elsewhere.[3]

The 17 *C9ORF72*+ ALS brains had a considerably higher number of p62-positive, TDP-43–negative neuronal cytoplasmic inclusions in the SN (7 cases with >10, 2 cases with 5–9, and 8 cases with ≤4 p62-positive inclusions) than the 51 *C9ORF72*− ALS cases (4 cases >10, 6 cases with 5–9, and 41 cases with ≤4 p62-positive inclusions; $\chi^2 = 10.724$, $df = 2$, $p = 0.005$). No/moderate/severe neuronal cell loss was observed in 3/9/5 cases with *C9ORF72* mutations and 22/17/4 cases without this mutation ($\chi^2 = 7.074$, $df = 2$, $p = 0.029$). Thus, the burden of p62-positive disease is much greater than that seen on α-synuclein immunohistochemistry and was associated with a variable degree of neuronal loss in the SN (figure).

DISCUSSION Previous studies investigating a possible association among *C9ORF72* expansions, parkinsonism, and iPD have concentrated on patients with the clinical rather than the pathologic diagnosis of iPD.[e1–e7] These studies have all concluded that *C9ORF72* expansions are not a common cause of iPD; however, given that clinical diagnosis has a higher false-positive rate, we chose to conduct a study of pathologically confirmed iPD. Furthermore, by focusing on neuropathology, we also investigated the pathologic basis of parkinsonian presentations in *C9ORF72*+ ALS patients.

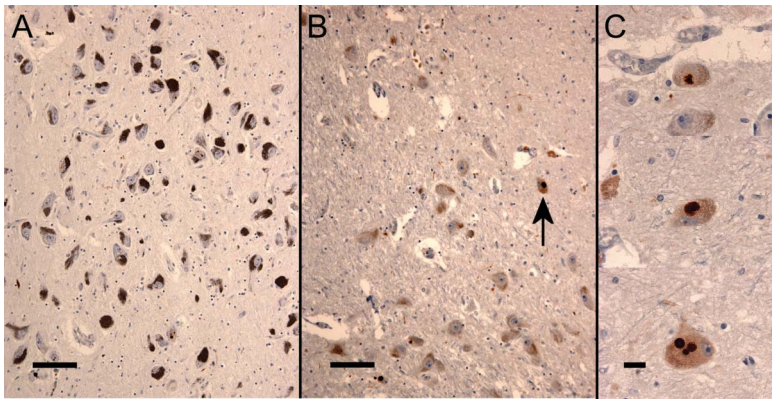
We identified a single *C9ORF72*+ patient with clinically typical iPD of 377 tested; this frequency is similar to that in controls[3] and thus we conclude that *C9ORF72* expansions are not a major cause of iPD. Notably, this patient had a family history of ALS and neuropathology consistent with *C9ORF72*+ disease; although it was not possible to investigate motor neuron loss in the spinal cord, we suspect this patient had subclinical FTD. The presence of type-A FTLD

Table Characteristics of tissue samples

Biobank	No. of cases	Male: female	Mean age (range) at onset, y	Family history of parkinsonism, n (%)	Cognitive impairment, n (%)	Lewy bodies present, n (%)
UK Parkinson's Disease Society Tissue Brain Bank	141	2.4:1	65.2 (35–86)	21 (25)	98 (70)	114 (81)
Queen Square Brain Bank	236	1.5:1	63.8 (30–85)	NA	120 (51)[a]	224 (95)

Abbreviation: NA = not available.
[a] Information about cognitive impairment was not available for postmortem tissue of 62 patients in the Queen Square Brain Bank for Neurological Disorders.

Figure Substantia nigra histopathology in *C9ORF72*− and *C9ORF72*+ cases



Photomicrographs of the substantia nigra after immunohistochemistry for p62 in *C9ORF72*− brains (A) and *C9ORF72*+ brains (B, low power, and C, high power) of patients with the clinical diagnosis of amyotrophic lateral sclerosis showing neuronal loss and p62-positive cytoplasmic inclusions (arrow) in the *C9ORF72*+ brain. Bar = 100 μm (A and B), and 20 μm (C).

pathology suggests that motor neuron pathology was unlikely.

It is noteworthy that of the 2 cases that were known to have coincident PD-ALS in the Sheffield brain bank, one did[3] and one did not have the *C9ORF72* repeat expansion.

The absence of α-synuclein pathology in the SN of the vast majority of *C9ORF72*+ brains further strengthens our assumption that the intracellular mechanisms leading to neuronal cell loss in ALS/FTD and those causing α-synuclein pathology in iPD are distinct. In contrast, p62-positive, TDP-43–negative inclusions in combination with neuronal loss are considerably more common in the SN of *C9ORF72*+ ALS patients than in *C9ORF72*− ALS. This p62-positive extrapyramidal pathology is therefore the likely cause of the previously reported increased incidence of parkinsonian features in *C9ORF72*-related ALS. One could therefore consider *C9ORF72*-related neurodegeneration as a clinically and pathologically heterogeneous syndrome characterized by a combination of TDP-43 proteinopathy with superimposed extramotor p62-positive, TDP-43–negative pathology. The distribution and severity of this latter pathology is likely to govern the presence of cognitive impairment (in the presence of hippocampal and neocortical pathology) or parkinsonism (in the presence of basal ganglia pathology).

Until the pathogenesis of *C9ORF72* disease is fully understood, it remains impossible to exclude *C9ORF72* expansions as a very rare cause of α-synucleinopathy and clinical iPD. However, our observation of an alternative pathologic basis for the observed incidence of parkinsonism in *C9ORF72*+ patients, significantly strengthens the case that *C9ORF72* disease and α-synucleinopathy represent distinct pathologic entities.

Understanding that *C9ORF72* expansions are a cause of both ALS and a parkinsonian phenocopy is likely to be crucial to the counseling and management of patients with ALS presenting with parkinsonian features, particularly if they have a family history of ALS/PD. Genetic testing for expansions of *C9ORF72* will help to differentiate patients with *C9ORF72* neurodegeneration from those who have developed more typical PD; a similar suggestion has been made for the use of *C9ORF72* genotyping in the differentiation of true Alzheimer disease from FTD caused by mutation of *C9ORF72*.[10]

AUTHOR CONTRIBUTIONS

The study was conceived and designed by authors J.C.-K., J.R.H., G.C., P.J.S., N.W.W., and O.B. Data acquisition was performed by authors J.C.-K., A.F., J.R.H., G.C., J.K., A.M., J.H., S.B.W., P.G.I., C.M.D., T.L., and T.R. Data analysis and interpretation were performed by J.C.-K., A.F., J.R.H., G.C., T.L., T.R., N.W.W., and O.B. The manuscript was critically revised by J.C.-K., J.R.H., P.J.S., and O.B. The study was supervised by T.R., P.J.S., N.W.W., and O.B.

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