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Multiple system atrophy is not caused by *C9orf72* hexanucleotide repeat expansions

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Abstract

Multiple system atrophy (MSA) is a fatal neurodegenerative disorder of unknown etiology that presents with variable combinations of progressive ataxia, parkinsonism and autonomic instability. Pathologic expansion of a hexanucleotide repeat in the gene *C9orf72* gene has been demonstrated to cause neurodegeneration with diverse neurological presentations. To test the hypothesis whether pathologic expansions in *C9orf72* are a cause of MSA, we undertook genetic screening in 100 neuropathologically confirmed cases. No pathologic repeat expansions were detected suggesting that MSA is not a *C9orf72*-related neurodegenerative disease.

Keywords

multiple system atrophy; *C9orf72*

1. Introduction

Multiple system atrophy (MSA) is a progressive neurodegenerative disorder that presents with variable combinations of ataxia, parkinsonism and autonomic instability. The etiology of this fatal synucleinopathy is unknown, though genetic factors have been postulated (Mitsui and Tsuji, 2014, Scholz, et al., 2009). A hexanucleotide [GGGGCC]_n repeat expansion in *C9orf72* has been recently shown to be a major genetic cause of amyotrophic lateral sclerosis and fronto-temporal dementia (DeJesus-Hernandez, et al., 2011, Renton, et

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al., 2011). Furthermore, the pathologic repeat expansion has been identified in cases of clinically diagnosed Alzheimer's disease, in atypical parkinsonism and in subjects with psychosis (Kertesz, et al., 2013, Lindquist, et al., 2013, Majounie, et al., 2012). These data suggest that the clinical presentation of *C9orf72*-related neurodegeneration is diverse. Interestingly, patients with a pathological hexanucleotide repeat expansion presenting with clinical and neuroimaging findings suggestive of MSA have also been reported (Goldman, et al., 2014, Lindquist, et al., 2013). Neither of these cases, however, were pathologically confirmed.

The clinical diagnosis of MSA remains challenging due to frequent phenocopy and a variable clinical presentation. Consequently, the accepted criteria for this condition rely heavily on post mortem observation of synuclein-positive glial cytoplasmic inclusions in the brain tissue. To investigate whether *C9orf72* hexanucleotide repeat expansions are a true cause of MSA or a rare phenocopy of MSA, we undertook genetic screening in a cohort of pathologically proven MSA cases.

2. Methods

We studied a total of 100 pathologically confirmed MSA cases from the Queen Square Brain Bank for Neurological Disorders (University College London, London, UK, n = 89), the New York Brain Bank (Columbia University, New York, USA; n = 6), the Neurodegenerative Disease Brain Bank (University of California, San Francisco, USA; n = 2) and the McKnight Brain Institute (University of Florida, Gainesville, USA; n = 3). Appropriate institutional review boards approved the study. DNA was extracted from brain specimens using the Qiagen DNeasy extraction protocol. We performed a repeat-primed polymerase chain reaction assay of the *C9orf72* hexanucleotide repeat as described elsewhere (Renton, et al., 2011). PCR fragment analysis was carried out on an Applied Biosystems 3730xl DNA Analyzer (Life Technologies, Grand Island, NY). Positive, negative and duplicate controls were added to the PCR plate to assure accurate hexanucleotide repeat analysis.

2. Results

In our cohort the detected hexanucleotide repeat number ranged from 0 to 12 repeats. None of the patients carried a pathological repeat expansion, which for the purpose of this assay we defined as over 30 GGGGCC repeats (Renton, et al., 2011).

3. Discussion

Our data show that pathogenic *C9orf72* repeat expansions are not a common cause of MSA. Nevertheless, our cohort was relatively small (n = 100) and so it remains possible that screening of larger cohorts might lead to the identification of rare cases carrying the repeat expansion. Thus, consideration for testing of the *C9orf72* repeat expansion should be given to patients with a MSA phenotype and a family history for motor neuron disease or fronto-temporal dementia (Goldman, et al., 2014, Lindquist, et al., 2013). Ultimately, confirmation of MSA is only achieved at autopsy. Variable phenotypic presentations of *C9orf72*-related

neurodegeneration remain a challenge in the clinical setting. The MSA-like phenocopy likely represents a rare, expanded phenotype spectrum of this hereditary degenerative condition. The power of genetic testing is that it allows us to unravel overlapping clinical syndromes. Molecular characterization of neurodegenerative diseases is important as targeted therapies might become available in the near future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version.

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