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ORIGINAL ARTICLE

Hereditary spastic paraplegia is not associated with *C9ORF72* repeat expansions in a Danish cohort

TT Nielsen[1,2], K Svenstrup[1,2], M Duno[3] and JE Nielsen[1,2]

Objectives: Hereditary spastic paraplegia (HSP) is a heterogeneous group of neurodegenerative disorders characterized by a progressive gait disorder, lower limb spasticity, hyper-reflexia, weakness and extensor plantar responses. Recently, large intronic hexanucleotide repeat expansions (GGGGCC) in *C9ORF72* have been found to cause frontotemporal dementia (FTD), amyotrophic lateral sclerosis and FTD with motor neuron disease. Owing to the overlapping phenotypes among HSP, amyotrophic lateral sclerosis and FTD with motor neuron disease along with shared pathological findings, we hypothesized that *C9ORF72* expansions might be a genetic risk factor or modifier of HSP.
Methods: Clinically characterized HSP patients were investigated for elongations in the hexanucleotide repeat of *C9ORF72*.
Results: Upon analyses of the repeat lengths in the *C9ORF72* gene in a Danish cohort of HSP patients, we found no expansions.
Conclusion: We conclude that HSP is most likely not associated with repeat expansions in *C9ORF72*.
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Keywords: *C9ORF72*; HSP; hereditary spastic paraplegia; modifier

INTRODUCTION

Hereditary spastic paraplegia (HSP) is a heterogeneous group of neurodegenerative disorders characterized by a progressive gait disorder, lower limb spasticity, hyper-reflexia, weakness and extensor plantar responses.[1] More than 50 loci have been identified in HSP, which can be inherited in an autosomal dominant, autosomal recessive or X-linked manner.[2] However, a large proportion of the HSPs are isolated cases with no known genetic cause of the disease.[3] Similarly, the age of onset is highly variable even within families with known mutations, suggesting that yet unknown genetic or environmental factors influence disease development and severity.

Recently, large intronic hexanucleotide repeat expansions (more than 30 GGGGCC repeats) in *C9ORF72* have been found to cause frontotemporal dementia (FTD), amyotrophic lateral sclerosis (ALS)[4,5] and FTD with motor neuron disease.[6] ALS, FTD and FTD with motor neuron disease are part of the same spectrum of diseases with overlapping phenotypes, as the motor neuron disease is found in FTD and a cognitive decline in ALS.[7] In addition, they share pathological hallmarks in terms of TDP-43 (transactive response DNA-binding protein 43-kDa)-positive inclusions in neurons. Moreover, HSP can be regarded as the outermost part of this spectrum, as it clinically shows involvement of the corticospinal motor neurons and as TDP-43 pathology has also been found in the HSP subtype SPG6 (caused by mutations in *NIPA1*).[8,9] Furthermore, significantly decreased regional cerebral blood flow in the frontotemporal cortex has previously been reported in SPG4-HSP.[10]

Owing to these similarities, and because *C9ORF72* repeat expansions have also been suggested to be associated with other neurodegenerative disorders e.g. Alzheimer's disease,[11,12] we speculated that such expansions might also associate with HSP or serve as a modulator of the disease in HSP patients. However, on screening a cohort of HSP patients for repeat expansions in the *C9ORF72* gene, we found no association between the expansions in the *C9ORF72* gene and HSP.

MATERIALS AND METHODS

Patients

A total of 182 HSP patients, referred to the Section of Neurogenetics, Department of Cellular and Molecular Medicine, University of Copenhagen, were included in the study. The ethics committee of Copenhagen approved the study (KF 01–142/94), and all participating individuals gave informed consent. The patients fulfilled the criteria of definite or probable HSP[13] and were tested for alleles with a pathogenic hexanucleotide expansion in *C9ORF72*. Ninety-three patients were familial cases with known mutations, 25 patients were suspected of a genetic background with an affected first-degree relative, but without a known mutation, whereas 64 patients were isolated cases (Table 1). All individuals were of Danish origin and representative of the general Danish population, which is considered to be homogeneous.

Molecular analyses

DNA was isolated from EDTA blood samples by standard methods. A repeat primed-PCR assay was used to assess the GGGGCC repeat of the *C9ORF72* gene essentially as described by Lindquist *et al.*[14] In short, the primers 5′- FAM-CCC AAA CAG CCA CCC GCC AGG ATG C-3′, 5′-TAC GCA TCC CAG TTT GAG ACG GGG GCCG GGG CCG GGG CCG GGG-3′ and 5′-TAC GCA TCC CAG TTT GAG ACG-3′ were combined, and repeat primed-PCR products were generated via a 35-cycle two-step PCR reaction (96 °C for 20 s;

[1]Danish Dementia Research Centre, Neurogenetics Clinic, Department of Neurology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [2]Department of Cellular and Molecular Medicine, Section of Neurogenetics, The Panum Institute, University of Copenhagen, Copenhagen, Denmark and [3]Department of Clinical Genetics, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark
Correspondence: Dr TT Nielsen, Danish Dementia Research Centre, Neurogenetics Clinic, Department of Neurology, Section 6702, Rigshospitalet, Copenhagen University Hospital, Blegdamsvej 9, Copenhagen DK-2100, Denmark.
E-mail: troelsn@sund.ku.dk
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71 °C for 120 s) using the Tempase PCR system (Amplic in combination with a GC-rich solution (Roche). Products were resolved on an ABI3130Xl. Samples were categorized into two groups with normal or expanded *C9ORF72* repeats (<20 or >30 repeats, respectively).

RESULTS

In order to determine whether hexanucleotide expansions in the *C9ORF72* gene associate with HSP, we screened a total number of 182 HSP patients. Upon analyses, we found no HSP patients with expansions in the GGGGCC hexanucleotide repeat of the *C9ORF72* gene (Figure 1).

Table 1 Characterization of the HSP cohort

Type	*Gene/locus (encoded protein)*	*Known gene mutation*	*Unknown gene mutation*	
			Familial cases	*Isolated cases*
SPG3A	ATL1 (Atlastin-1)	7 (1–2)		
SPG4	SPAST (Spastin)	70 (0–55)		
SPG5	CYP7B1 (Cytochrome P450 polypeptide)	2 (14–18)		
SPG6	NIPA1 (Non-imprinted in Prader-Willi/Angelman syndrome region protein 1)	1 (10)		
SPG7	SPG7 (Paraplegin)	3 (30–43)		
SPG11	KIAA1840 (Spatacsin)	2 (5–6)		
SPG13	HSPD1 (Heat shock 60 kDa protein/chaperonin 60)	2 (50–52)		
SPG31	REEP1 (Receptor-expression-enhancing protein 1)	6 (1–35)		
Total		93 (0–55)	25 (1–53)	64 (1–66)

Overview of the distribution of HSP patients with known mutations, familial cases with probable autosomal dominant or autosomal recessive inheritance without known identified mutation and isolated cases. Numbers in parentheses denote age of onset.

DISCUSSION

Among the motor neuron diseases, two share the clinical features of prominent upper motor neuron signs, namely ALS and HSP. Although genetic testing can assist in the identification of several variants, in the remaining cases, including those in which spasticity may be associated with amyotrophy, clinical differentiation of the disorders may prove to be difficult. The recent reports showing association between ALS and repeat expansions in two different genes have raised an interest in the involvement of repeat expansions in neurodegenerative disorders in general. CAG repeat expansions in *ATXN2*, the causative gene of spinocerebellar ataxia type 2, have shown to be a risk factor of ALS and progressive supranuclear palsy,[4,5,15] and recently we have shown that the age of onset in HSP is likely to be modulated by CAG repeat expansions in *ATXN2*, although expansions in *ATXN2* do not seem to be a direct risk factor of HSP.[16] The studies reporting that the hexanucleotide repeat expansions in the *C9ORF72* gene cause FTD and account for 46% of familial ALS cases and 21% of sporadic ALS cases in a Finish population[4,5] prompted us to investigate the involvement of this type of hexanucleotide expansion in HSP, given the shared pathological findings and the phenotypic overlap of ALS, FTD with motor neuron disease and HSP. Moreover, it has been suggested that expanded *C9ORF72* repeats compromise the basic RNA metabolism of the cell, and the gene has further been shown to be expressed throughout the brain implying that pathogenic mutations in this gene could have a more general role in neurodegeneration, although a recent report does not find an association between C9ORF72 repeat expansions and Parkinson's disease.[11,17,18]

Here, we analyzed the *C9ORF72* repeat length in a Danish cohort of HSP patients and found no expansions, and we therefore conclude that an expansion in *C9ORF72* is not causative of HSP in our cohort and that it is most likely not a modifier of HSP either. However, larger cohorts and new methods for more exact repeat size determination are needed to draw conclusions in general.

DATA ARCHIVING

There were no data to deposit.

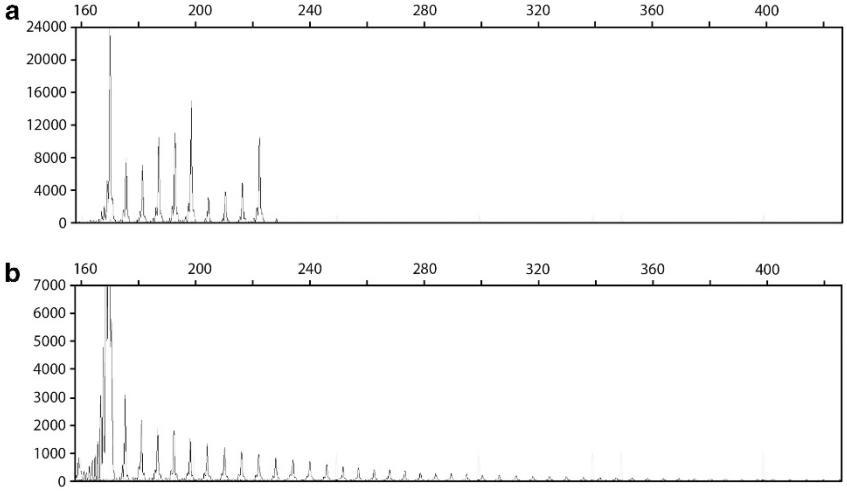

Figure 1 Repeat primed-PCR products resolved on an ABI3130XL. Panel **a** depicts a normal heterozygous sample from an HSP patient, whereas panel **b** shows a pathogenic expanded allele in a sample from a positive control[14] not related to HSP. Numbers on the X-axis denote size (bp) of the product and numbers on the Y-axis denote arbitrary units of fluorescence.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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