C9orf72 hexanucleotide repeat expansion analysis in Chinese spastic paraplegia patients

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Abstract

Background: Recently, a hexanucleotide repeat expansion in the C9orf72 gene has been identified to cause frontotemporal dementia, amyotrophic lateral sclerosis families and many other neurodegenerative diseases. Owing to the overlapping phenotypes among HSP, frontotemporal dementia and amyotrophic lateral sclerosis we hypothesized that C9orf72 expansions might be a genetic risk factor or modifier of HSP.

Objectives: The aim of this study was to find out whether C9orf72 expansions also confer risk to spastic paraplegia (SPG).

Methods: We recruited 112 genetically unidentifiable SPG patients, 68 SPG4 patients and 313 controls in mainland China to determine if hexanucleotide repeat of C9orf72 plays a role in spastic paraplegia.

Results: No large expansion was detected in all subjects. C9orf72 repeat expansions were not associated with onset of HSP.

Conclusion: Our results support the notion that repeat expansions in C9orf72 may not be associated with HSP in China.

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1. Introduction

Hereditary spastic paraplegias (HSPs) are a group of clinically and genetically heterogeneous neurodegenerative disorders, which are of monogenic inheritance and primarily impair the pyramidal tracts [1]. Although progressive gait disturbances, lower limb spasticity and extensor plantar responses are hallmarks of HSP, these characteristics are also found in other neurodegenerative disorders, e.g. amyotrophic lateral sclerosis (ALS) [2]. Both neurodegenerative diseases show progressive impairment in motor functions. HSPs have been linked to ALS and frontotemporal degeneration with motor neuron disease (FTD-MND), since TDP-43 positive inclusions have recently been found in an HSP subtype, and TDP-43 are found in abundance in pathological inclusions of both ALS and FTD-MND [3]. Despite clinical and genetic heterogeneity, a number of molecular commonalities have long been recognized for disorders as amyotrophic lateral sclerosis (ALS), hereditary spastic paraplegias (HSPs) and spinocerebellar ataxia (SCA) [4–6]. Recently, a hexanucleotide repeat expansion in the C9orf72 gene has been identified to account for a significant portion of frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) patients [7,8]. The function of C9orf72 and the GGGGCC in the first intron was largely unknown [9]. Non-ATG-initiated translation of the intronic GGGGCC-repeat expansion in FTD/ALS patients leads to accumulation of insoluble dipeptide repeat (DRP) aggregates [10]. In addition to DPR and TDP-43 pathology, the C9orf72 expansion may lead to haploinsufficiency and trigger sequestration of GGGGCC-binding proteins. Drosophila and mammalian models of this expanded hexanucleotide repeat and showed that expression of the expanded GGGGCC repeat RNA (rGGGGCC) is sufficient to cause neurodegeneration [11]. Because of the overlap clinical phenotype and pathology of neurodegenerative diseases, researchers have studied the C9orf72 expansion among many neurodegenerative diseases, including AD, PD, essential tremor, restless legs syndrome, sporadic cerebellar ataxia and schizophrenia patients [12–17]. C9orf72 repeats within the normal range have ever been observed as genetic modifiers or risk factors for Alzheimer’s disease and Parkinson’s disease [18,19]. In addition, the presence of clinical features may differ greatly among SPG4 patients in a different pedigree and even among patients in the same pedigree, suggesting it might be modulated by other yet unknown parameters [20,21]. Furthermore, a majority of HSPs are still of unknown etiology, and might contribute to novel causative genes or modifiers. Considering
2. Methods

2.1. Study samples

We analyzed 180 spastic paraplegia patients, including 83 sporadic spastic paraplegia patients, 29 AD-HSP probands and 68 SPG4 patients from 26 families, and 308 normal controls from unrelated Chinese Han families. All the spastic paraplegia patients were clinically diagnosed by two experienced neurological professors according to Harding’s criteria [1]. Eighty-three sporadic spastic paraplegia patients had been genetically excluded from SPG3A, SPG4, SPG6, SPG31, SPG11, SPG15, SPG5 and SPG7, and 29 AD-HSP probands had been genetically excluded from SPG3A, SPG4, SPG6, SPG31 and SPG42. SPG4 patients were genetically diagnosed by DNA sequencing and MLPA. Age at onset was estimated as date of first onset of symptoms reported by the patient, their informant, or from the patient’s medical records. Control subjects had been excluded from any neurological diseases. All participants or their legal guardian gave informed consent for participation in the clinical and genetic studies. This study also got prior approval by the institutional review board and the Ethics Committee of Xiangya Hospital, Central South University.

2.2. Genotyping assays

DNA samples of patients and controls were isolated from peripheral blood leukocytes using a QIAGEN kit. DNA samples of one ALS patient with large C9orf72 repeat expansions (> 30 repeats) were served as positive controls. The presence of the GGGGCC hexanucleotide expansion was screened using a 2-step polymerase chain reaction protocol. In the first step, we used a previously reported repeat-primed polymerase chain reaction assay to detect the size of the longest expanded allele. Briefly, DNA samples (100 ng/PCR, 50 ng/μl) were amplified using three primers (MRX-F: 5′-FAM-ACAGTACTCCGTAGGGTGAAA; MRX-R1: 5′-CAGGAAAAACGCTATGACCGGCCCCGCACCGCAGGCCCCGCCCCGCGG; and MRX-M13R: 5′-CAGAAGGACGGTATGACC), and the primers ratio was modified to improve PCR efficiency. The total process was performed using a touchdown thermonuclease program. In the second step, we performed a classical FAM-fluorescent labeled PCR assay to detect the accurate genotype in the non-pathogenic mutation carriers. The fragment length analysis was performed on ABI 3730X1 DNA analyzer and visualized by Genemapper software version 3.2 (Applied Biosystems).

2.3. Statistical methods

A cut-off value of 30 repeats was used to define pathogenic threshold. Descriptive statistics of sample characteristics (eg, sex, age at onset, and familial history between cases and controls) were analyzed and measurement data were described as mean ± standard deviation; differences in the distributions of longest repeats allele between cases and controls were tested using Mann–Whitney U test or Kruskal–Wallis H test, a 2-tailed significance was set at P < 0.05. Considering the role of 7–27 units which were strongly correlated with the risk haplotype on diseases, we converted a continuous variable of repeat units in an individual’s longest repeat allele into a binary categorical variable, including short alleles (<7 repeat units) and intermediate alleles (7–27 units). To account for the fact that non-mutation carriers have 2 alleles in the normal range, the number of repeats corresponding to the longer of the 2 normal alleles was used to evaluate a dominant effect. We considered the number of GGGGCC repeats as both a continuous variable and a categorical variable. The association of the number of repeats with spastic paraplegia disease status was evaluated using logistic regression models adjusted for gender and age at onset. The associations of the number of repeats with age at onset of spastic paraplegia or SPG4 was examined using linear regression models adjusted for gender. Statistical analysis was performed using SPSS (version 20.0; IBM SPSS). In order to adjust for multiple testing, we performed a Bonferroni adjustment in logistic regression and linear regression, after which p-values < 0.0166 were defined as statistically significant.

3. Results

A total of 112 genetically un-identified spastic paraplegia cases, 68 SPG4 patients and 308 healthy control individuals have been successfully subjected to repeat-primed PCR and genotyping PCR. However, no pathological repeat expansion was detected in any patients group or controls.
controls. Details of the characteristics information were presented in Table 1. The wide range of repeat expansions in all cases and control individuals was 2–14 units. In spastic paraplegia patients and controls the most frequent was 2-repeat allele, while the frequency of the 6-repeat allele was marginally increased in SPG4 patients. For a complete overview of the allele distribution in patients and controls, a graphical representation was provided in Fig. 1. But C9orf72 repeat expansions were not associated with onset of HSP (supplementary materials).

4. Discussion

Pathological C9orf72 expansions underlie a spectrum of neurodegenerative phenotypes, including ALS/FTD and Alzheimer’s disease. The pathogenic mechanism underlying C9orf72 expansion is largely unknown, and the formation of toxic RNA inclusions may contribute to disease [10,11]. However a number of questions remain to be answered including the diversity in clinical phenotype and the pathogenic mechanism associated with C9orf72 expansion-related diseases [9,22]. Our study did not find any pathological C9orf72 expansions in 112 genetically un-identified spastic paraplegia patients, 68 SPG4 patients and 308 healthy control individuals. Although it is possible that our cohort was underpowered to detect the C9orf72 expansion, this study at least demonstrated that such expansions were likely to be a rare occurrence in HSP, certainly rarer than that seen in Alzheimer’s disease, where approximately 1% of clinically diagnosed patients harbor the mutation [12,23]. The current study had a power >0.95 to detect a genetic risk factor with a prevalence of 1%. As 83 sporadic spastic paraplegia patients had been genetically excluded from SPG3A, SPG4, SPG6, SPG31, SPG15, SPG5 and SPG7, and 29 AD-HSP probands had been genetically excluded from SPG3A, SPG4, SPG6, SPG31 and SPG42, it is possible that C9orf72 repeat expansions are very rarely associated with spastic paraplegia. Although limited phenotypic data were available in our cohort, this study does not support genetic testing for C9orf72 expansion in sporadic HSP and AD-HSP.

In conclusion, our spastic paraplegia patients and control study cohorts did not support the hypothesis that the length of the normal allele of the GGGGCC hexanucleotide repeat in C9orf72 has an effect on spastic paraplegia patients. However, larger cohorts are needed to draw conclusions in general.

Potential conflict of interest

The authors have reported no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jns.2014.09.028.

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