SHORT COMMUNICATION

Neurologia i Neurochirurgia Polska
Polish Journal of Neurology and Neurosurgery
2022, Volume 56, no. 3, pages: 276–280
DOI: 10.5603/PJNNS.a2022.0039

ISSN: 0028-3843, e-ISSN: 1897-4260

C9orf72 hexanucleotide repeat expansion found in suspected spinobulbar muscular atrophy (SBMA)

Wiktoria Radziwonik[1], Ewelina Elert-Dobkowska[1], Filip Tomczuk[1], Aleksandra Wozniak[1], Anna Sobanska[1], Iwona Stepniak[1], Dariusz Koziorowski[2], Jacek Zaremba[1], Anna Sulek[1]

[1]Institute of Psychiatry and Neurology, Warsaw, Poland
[2]Department of Neurology, Medical University of Warsaw, Poland

ABSTRACT

Introduction. The expansion of a hexanucleotide GGGGCC repeat (G_4C_2) in the *C9orf72* locus is the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). In addition, *C9orf72* expansion has also been detected in patients with a clinical manifestation of Parkinson's Disease (PD), Alzheimer's Disease (AD), Huntington's Disease (HD), and ataxic disorders.

Material and methods. A total of 1,387 patients with clinically suspected ALS, HD or spinal and bulbar muscular atrophy (SBMA) were enrolled, and the prevalence of *C9orf72* expansions was estimated.

Results. The hexanucleotide expansion accounted for 3.7% of the ALS patients, 0.2% of the HD suspected patients with excluded *HTT* mutation, and 1.3% of the suspected SBMA patients with excluded mutation in *AR* gene.

Conclusions: This is the first report revealing the presence of *C9orf72* expansion in patients with a suspected SBMA diagnosis. Consequently, we advise testing for *C9orf72* expansion in patients presenting with the SBMA phenotype and a genetically unsolved diagnosis.

Key words: ALS, C9orf72 locus, dynamic mutation, FTD, microsatellite repeats expansion, SBMA

(*Neurol Neurochir Pol 2022; 56 (3): 276–280*)

Introduction

Hexanucleotide expansion (GGGGCC) in the *C9orf72* locus is thought to be the most frequent genetic cause for autosomal dominant ALS, FTD or ALS-FTD [1, 2]. The normal number of G_4C_2 repeats ranges from 2 to 24, and in most studies alleles with > 60 G_4C_2 repeats are considered to be highly pathogenic [2].

A recently published meta-analysis revealed that the *C9orf72* mutation occurs in 22.5% of patients with familial ALS (fALS), and in 3.1% of patients with sporadic ALS (sALS). The frequency can range between 3.4% and 54.5% in fALS and 0.3–5.1% in sALS [3, 4]. The incidence is estimated from 0.0 to 61.5% depending on the origin of the analysed cohorts and the neurological conditions [5]. The specific phenotypic features in patients with the *C9orf72* expansion were first observed by Millecamps et al. [6] who reported that they presented more frequent bulbar onset of ALS, and older age at disease onset, compared to *SOD1* or *FUS* mutation carriers. Reports of an association between *C9orf72* expansion and parkinsonism, AD, HD phenotype and ataxia also exist, as well as an association between *C9orf72* intermediate alleles with neuropsychiatric phenotypes [7–9]. European studies on HD-like patients negative for *HTT* gene mutations revealed *C9orf72* expansion in 2–5% of cases [10, 11].

In this study, we report the prevalence of *C9orf72* pathogenic expansion in patients presenting with ALS, HD and, for the first time, SBMA phenotype.

Address for correspondence: Anna Sulek, Institute of Psychiatry and Neurology, 9 Sobieskiego St, 02-957 Warsaw, Poland; e-mail: suleka@ipin.edu.pl

Received: 30.11.2021; Accepted: 7.04.2022; Early publication date: 3.06.2022

Material and methods

Patients

The studied group retrospectively analysed comprised:

1. 108 patients (43 female and 65 male) with clinical symptoms of ALS-FTD according to the revised El Escorial criteria; 101 presented with ALS (18 fALS and 83 sALS), 4 with FTD (one familial FTD), and 3 with ALS-FTD phenotype. The mean age at onset was 46 years in familial and 48 in sporadic cases. Mean age at examination was 53.6.
2. 1,054 patients (588 female and 466 male) with suspected HD and negative for *HTT* mutations (233 familial and 821 sporadic cases). The mean age at onset was 37.2 for familial and 43 for sporadic cases.
3. 225 male patients with the bulbar phenotype, suspected of SBMA and negative for mutation in the androgen receptor (*AR*) gene (18 familial cases), with a mean age at onset of 58.3.
4. 106 healthy controls, aged 18–98 years, with neither neurological nor muscular complaints and
5. 401 cases with genetic confirmation of HD (200 individuals) or SCA (201 individuals).

DNA was extracted from patients' peripheral blood leukocytes. All individuals were of Polish ethnicity. They were referred from all over Poland for genetic testing and counselling to the Institute of Psychiatry and Neurology between 2000 and 2020. All provided informed consent for genetic testing, and our study was approved by the Bioethics Committee of the Institute of Psychiatry and Neurology in Warsaw.

Genetic analysis

Two-step *C9orf72* genotyping protocol was implemented to detect G_4C_2 expansion. Firstly, a polymerase chain reaction (PCR) across the repeats was conducted in all tested individuals. To detect G_4C_2 expansion, repeat-primed PCR (RP-PCR) was carried out in cases with only one normal allele observed. The PCR and RP-PCR products were separated by capillary electrophoresis and analysed by GeneMapper software (Thermo Fisher Scientific).

Results

The ranges of *C9orf72* alleles and expansions detected in our groups are presented in Figure 1 and Table 1. Within five studied groups, the number of normal alleles detected ranged between 2 and 21 G_4C_2 repeats.

The first case with *C9orf72* expansion in ALS-FTD group was a 50-year-old male with a 6-month history of muscle fasciculations of the upper limbs, similarly to his father who had subsequent progression to weakness of all extremities, dysphagia and dysarthria, and who died 5 years later due to respiratory failure. No sensory deficits were noted. Nerve conduction studies and electromyography (EMG) revealed a generalised disorder of the motor neuron. Similar symptoms were observed in 4 other relatives of his.

The second patient was a 66-year-old male with a 6-month history of muscle cramps and progressive lower extremity weakness. He also reported postural instability, slurred speech and swallowing difficulties without sensory deficits. Neurological examination showed pseudobulbar syndrome, hypophonic dysarthria, and muscle atrophy. EMG revealed an active generalised disorder of the motor neurons with the presence of fasciculations in all the sampled muscles. Brain MRI showed mild cortico-subcortical cerebral and cerebellar atrophy. Irritability during the last 3–4 years was observed. Rapidly progressing generalised weakness and muscle wasting up to loss of gait, speaking and swallowing difficulties were characteristic for the proband and his 6 relatives with a disease onset between 50 and 65 years of age. Additionally, one of the patient's brothers experienced hyperhydrosis, hypomimia, progressive bradykinesia and global muscle rigidity, but with domination of symptoms related to a lower motor neuron disorder. An EMG revealed an active generalised disorder of the motor neurons. No dementia was recognised. Autonomic system examination showed slightly reduced activity of the parasympathetic nervous system.

The third patient was a 61-year-old female with a 3-year history of dementia. Psychological assessment revealed significant cognitive disturbances with dominant executive function deterioration. Speech disturbances and language deficits reflected motor aphasia. MRI examination showed considerable atrophy of the frontal and temporal lobes, supporting the diagnosis of frontotemporal dementia. The patient's father had been diagnosed with dementia at the age of 65, and her brother died in the course of ALS.

In the fourth, female patient presented with FTD symptoms, in addition to expansion in the *C9orf72* locus, a p.Arg307Ser variant in the *PSEN1* gene was detected. According to the ACMG guidelines, *PSEN1* variant (NM_0000221.3: c.921G > C, rs554783173) has been classified as variant of unknown significance not detected in the European population to date. Her brother presented with ALS and dementia symptoms, but he has not been evaluated genetically yet.

Within a cohort of 1,054 HD-like patients, G_4C_2 expansion was confirmed in two individuals, accounting for approximately 0.2%. The first case was a 46-year-old male with affected movement coordination since the age of 31. (*HTT* — 17/19 CAG, *C9orf72* — 2/EXP G_4C_2). The second was a 61-year-old male with an age at onset of 50 years (*HTT* — 17/22 CAG, *C9orf72* — 5/EXP G_4C_2) manifesting as lower limb spasticity, dysarthria and dementia. His MRI revealed generalised cerebral atrophy. Both cases were sporadic.

Among 225 males with a primary clinical diagnosis of SBMA, expansion of G_4C_2 was found in 3 individuals, accounting for 1.3% of this group. Two patients with suggested SBMA phenotype presented slowly progressive pure lower motor neuron disease. EMG examination confirmed chronic

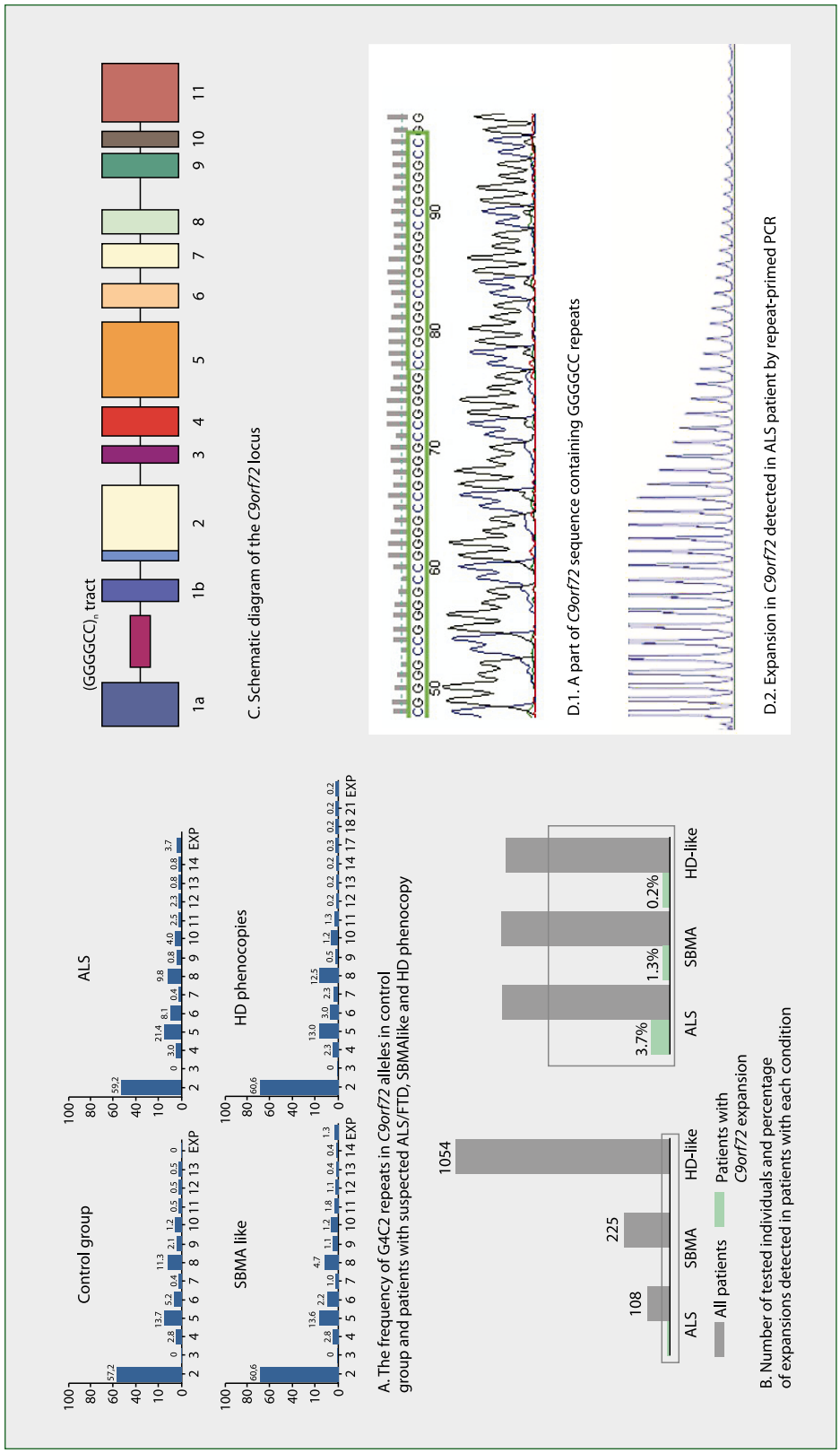

Figure 1. Results of molecular analysis of G_4C_2 repeats in *C9orf72* locus in all studied groups

Table 1. Results of *C9orf72* genetic analysis in different groups of patients and controls

Patient and control groups	Number of individuals	Female/Male %	Range of G_4C_2 repeats	Number of expansions detected	%
Control group (healthy individuals)	106	52/48	2–13	0	0
HD clinical diagnosis (mutation in *HTT* confirmed)	200	56/44	2–17	0	0
SCA1/2 clinical diagnosis (mutation in *ATXN1/2* confirmed)	201	51/49	2–13	0	0
ALS/FTD clinical diagnosis	108	40/60	2–14 + EXP	4	3.7
HD clinical diagnosis (mutation in *HTT* excluded)	1054	56/44	2–21 + EXP	2	0.2
SBMA clinical diagnosis (mutation in *AR* excluded)	225	0/100	2–14 + EXP	3	1.3

lower motor neuron involvement and mild sensory axonal neuropathy. The third individual had a more complicated medical and family history. His 2 brothers died tragically at a young age, and his mother had a history of Alzheimer's Disease. The patient was aged 65 at the onset of dementia and had diabetes mellitus type 2. Shortly thereafter, quickly progressing asymmetrical muscle atrophy was observed without significant sensory deficits. Eight years earlier, the patient underwent surgery and chemotherapy due to a large intestine carcinoma with full remission. Neuropsychological tests confirmed mild dementia. EMG revealed motor-sensory axonal-demyelinating polyneuropathy. EEG was normal. MRI of the brain showed cortico-subcortical cerebral atrophy.

Discussion

Our study on G_4C_2 repeat analysis in the *C9orf72* locus in a total of 1,894 individuals with diverse clinical statuses revealed the presence of expansion in nine individuals. As we expected, *C9orf72* expansion was not observed in individuals with other confirmed mutations (HD/SCA1/SCA2). The results in the Polish control group revealed that the range of G_4C_2 repeats (2–13) might be similar to, or even lower than, other populations [1, 12, 13]. This fact might have an impact on the low frequency of ALS-FTD due to G_4C_2 expansion in Poland, as the mutation is known to arise from the upper normal range alleles.

Though as many as 1,054 patients with suspected HD but excluded mutation in the *HTT* gene (HD phenocopy) were analysed for *C9orf72* expansion, our results were lower than those reported in other studies. Ranging from 1% to even 5% [10, 11, 14, 15], such frequencies account for a significant number of ultimate diagnosis confirmations in patients with excluded *HTT* mutations. The very low frequency of G_4C_2 expansion detected in our cohort — 0.2% — does not endorse the inclusion of the *C9orf72* gene into genetic testing algorithms in Polish HD-like patients.

Considering the clinical phenotype of SBMA and ALS-FTD overlapping i.e. progressive lower neuron syndrome, including bulbar region, presenting with muscle weakness and atrophy, dysarthria, dysphagia, fasciculations, muscle cramps and cognitive alterations or mood disorders, we retrospectively tested a group of clinically suspected SBMA individuals with no *AR* gene mutation.

In contrast to SBMA cases, the clinical characteristics of ALS patients are rapidly progressing upper and lower limb muscular weakness, as well as rapidly developing generalised muscular atrophy. Moreover, respiratory muscle involvement is observed in ALS. ALS patients may also present with pseudobulbar or pyramidal signs, which are not observed in SBMA. Extrapyramidal syndrome is a rarely observed ALS symptom that also does not occur in Kennedy's Disease. As the onset of both diseases often slightly differs, with SBMA occurring in the 4^{th} or 5^{th} decades and ALS in the 6^{th} or 7^{th} decades, patients primarily suspected of SBMA could develop upper motor neuron symptoms later in the disease course. In the case of our patients, a clinical diagnosis of SBMA was justified prior to referring the patients for genetic testing. Only retrospective analysis and identification of *C9orf72* expansions responsible for a more severe phenotype revealed that the suspicion of ALS should have been considered as well.

Clinical implications

To the best of our knowledge, this is the first report presenting a 1.3% frequency of G_4C_2 repeat expansion in patients clinically suspected of SBMA. Our results show that *C9orf72* testing is a valuable strategy and an important element of differential diagnosis in diseases progressing with motor neurons involvement. We believe that it is crucial to analyse the clinical differences between individuals with *C9orf72* expansions in order to enable the precise selection of patients for *C9orf72* testing.

Acknowledgments: *The authors thank the patients and their families and the referring clinicians for participation.*
Conflicts of interest: *None.*

Funding: *This study was partially supported by internal statutory grant IPIN 16202 (Polish Ministry of Education and Science).*
Data sharing and accessibility: *The data that supports the findings of this study is available from the corresponding author upon reasonable request.*

References

1. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron. 2011; 72(2): 245–256, doi: 10.1016/j.neuron.2011.09.011, indexed in Pubmed: 21944778.
2. Renton AE, Majounie E, Waite A, et al. ITALSGEN Consortium. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron. 2011; 72(2): 257–268, doi: 10.1016/j.neuron.2011.09.010, indexed in Pubmed: 21944779.
3. Chiò A, Borghero G, Pugliatti M, et al. Italian Amyotrophic Lateral Sclerosis Genetic (ITALSGEN) Consortium. Large proportion of amyotrophic lateral sclerosis cases in Sardinia due to a single founder mutation of the TARDBP gene. Arch Neurol. 2011; 68(5): 594–598, doi: 10.1001/archneurol.2010.352, indexed in Pubmed: 21220647.
4. Zou ZY, Zhou ZR, Che CH, et al. Genetic epidemiology of amyotrophic lateral sclerosis: a systematic review and meta-analysis. J Neurol Neurosurg Psychiatry. 2017; 88(7): 540–549, doi: 10.1136/jnnp-2016-315018, indexed in Pubmed: 28057713.
5. Liu Y, Yu JT, Zong Yu, et al. C9ORF72 mutations in neurodegenerative diseases. Mol Neurobiol. 2014; 49(1): 386–398, doi: 10.1007/s12035-013-8528-1, indexed in Pubmed: 23934648.
6. Millecamps S, Boillée S, Le Ber I, et al. Phenotype difference between ALS patients with expanded repeats in C9ORF72 and patients with mutations in other ALS-related genes. J Med Genet. 2012; 49(4): 258–263, doi: 10.1136/jmedgenet-2011-100699, indexed in Pubmed: 22499346.
7. Beck J, Poulter M, Hensman D, et al. Large C9orf72 hexanucleotide repeat expansions are seen in multiple neurodegenerative syndromes and are more frequent than expected in the UK population. Am J Hum Genet. 2013; 92(3): 345–353, doi: 10.1016/j.ajhg.2013.01.011, indexed in Pubmed: 23434116.
8. Burrell J, Halliday G, Kril J, et al. The frontotemporal dementia-motor neuron disease continuum. The Lancet. 2016; 388(10047): 919–931, doi: 10.1016/s0140-6736(16)00737-6.
9. Figueroa KP, Gan SR, Perlman S, et al. C9orf72 repeat expansions as genetic modifiers for depression in spinocerebellar ataxias. Mov Disord. 2018; 33(3): 497–498, doi: 10.1002/mds.27258, indexed in Pubmed: 29193335.
10. Hensman Moss DJ, Poulter M, Beck J, et al. C9orf72 expansions are the most common genetic cause of Huntington disease phenocopies. Neurology. 2014; 82(4): 292–299, doi: 10.1212/WNL.0000000000000061, indexed in Pubmed: 24363131.
11. Ida CM, Butz ML, Lundquist PA, et al. C9orf72 Repeat Expansion Frequency among Patients with Huntington Disease Genetic Testing. Neurodegener Dis. 2018; 18(5-6): 239–253, doi: 10.1159/000492499, indexed in Pubmed: 30336474.
12. Fahey C, Byrne S, McLaughlin R, et al. Analysis of the hexanucleotide repeat expansion and founder haplotype at C9ORF72 in an Irish psychosis case-control sample. Neurobiol Aging. 2014; 35(6): 1510.e1–1510.e5, doi: 10.1016/j.neurobiolaging.2013.12.003, indexed in Pubmed: 24411481.
13. Hsiao CT, Tsai PC, Liao YC, et al. C9ORF72 repeat expansion is not a significant cause of late onset cerebellar ataxia syndrome. J Neurol Sci. 2014; 347(1-2): 322–324, doi: 10.1016/j.jns.2014.10.042, indexed in Pubmed: 25467142.
14. Martins J, Damásio J, Mendes A, et al. Clinical spectrum of C9orf72 expansion in a cohort of Huntington's disease phenocopies. Neurol Sci. 2018; 39(4): 741–744, doi: 10.1007/s10072-018-3268-7, indexed in Pubmed: 29441485.
15. Alva-Diaz C, Alarcon-Ruiz CA, Pacheco-Barrios K, et al. Hexanucleotide Repeat in Huntington-Like Patients: Systematic Review and Meta-Analysis. Front Genet. 2020; 11: 551780, doi: 10.3389/fgene.2020.551780, indexed in Pubmed: 33240313.