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ORIGINAL ARTICLE

Frequency of *C9orf72*, *GRN*, and *MAPT* pathogenic variants in patients recruited at the Belgrade Memory Center

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Abstract
Most of the heritability in frontotemporal dementia (FTD) is accounted for by autosomal dominant hexanucleotide expansion in the chromosome 9 open reading frame 72 (*C9orf72*), pathogenic/likely pathogenic variants in progranulin (*GRN*), and microtubule-associated protein tau (*MAPT*) genes. Until now, there has been no systematic analysis of these genes in the Serbian population. Herein, we assessed the frequency of the *C9orf72* expansion, pathogenic/likely pathogenic variants in *GRN* and *MAPT* in a well-characterized group of 472 subjects (FTD, Alzheimer's disease - AD, mild cognitive impairment - MCI, and unspecified dementia - UnD), recruited in the Memory Center, Neurology Clinic, University Clinical Center of Serbia. The *C9orf72* repeat expansion was detected in 6.98% of FTD cases (13.46% familial; 2.6% sporadic). In the UnD subgroup, *C9orf72* repeat expansions were detected in 4.08% (8% familial) individuals. Pathogenic variants in the *GRN* were found in 2.85% of familial FTD cases. Interestingly, no *MAPT* pathogenic/likely pathogenic variants were detected, suggesting possible geographical specificity. Our findings highlight the importance of wider implementation of genetic testing in neurological and psychiatric practice managing patients with cognitive-behavioral and motor symptoms.

Keywords Genetics · Heritability · Frontotemporal dementia · Alzheimer's dementia · Mild cognitive impairment · Unspecified dementia

Introduction

Frontotemporal dementia (FTD) is associated with frontal and temporal lobe degeneration that results in progressive personality/behavior changes and impairment of language functions [1]. FTD is a highly heritable group of neurodegenerative disorders, with around 40% of patients with a strong family history [2].

The most common pathogenic/likely pathogenic variants with autosomal dominant inheritance are found in the chromosome 9 open reading frame 72 (*C9orf72*), progranulin (*GRN*), and microtubule-associated protein tau (*MAPT*) genes each causing between ~5 and 10% of all FTD [3], with geographical variability presented in some case series like the predominance of *GRN* pathogenic/likely pathogenic variants in Northern Italy and the Basque country [4, 5]. *C9orf72* hexanucleotide expansion seems to be the most common global cause of genetic FTD, followed by *GRN* and *MAPT* genes. These pathogenic/likely pathogenic variants account for 20–30% of the familial and 5–10% of sporadic FTD cases [3]. In Caucasian/Western populations, pathogenic/likely pathogenic variants in *C9orf72*, *GRN*, and *MAPT* could be responsible for 8–25%, 5–22%, and 5–15%, cases respectively [6], whereas in other genes are found in less than 5% patients with FTD [7].

Alzheimer's disease (AD) and FTD may share clinical features in early stages, making it difficult to differentiate between these two diseases. Hexanucleotide repeat expansion in *C9orf72* was reported either within clinically diagnosed AD patients or pathologically confirmed AD cases [8, 9]. In the study that included asymptomatic and symptomatic individuals with a family history of FTD, the

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pathogenic/likely pathogenic variants in one of the genes (*C9orf72*, *GRN*, or *MAPT*) were reported, with the bvFTD as the most common finding. Further, in *C9orf72* expansion carriers, the behavioral ($n=3$) and cognitive ($n=6$) mild cognitive impairment (MCI) variants are found. In *MAPT* carriers, the bvFTD showed high frequency, followed by the MCI behavior variant, while *GRN* carriers had more diverse phenotypes in FTD and AD dementia spectrum phenotypes [10].

Until now, no comprehensive analyses of pathogenic/likely pathogenic variants in the major three genes have been conducted in the Serbian population. In the present study, we screened *C9orf72*, *GRN*, and *MAPT* in the cohort with cognitive-behavioral disorders (FTD, MCI, AD, and unspecified dementia -UnD) recruited at the Memory Center of the Neurology Clinic in Belgrade, University Clinical Centre of Serbia.

Materials and methods

Participants

The present study was conducted as a part of the project on dementia genetics from January 2011 to January 2021. Patients with various cognitive-behavioral presentations were prospectively recruited at the Memory Center, Neurology Clinic, University Clinical Center of Serbia (UCCS). The consecutive subjects ($n=472$) consisted of 129 patients with FTD, 176 with AD, 118 with MCI, and 49 with UnD. The patients were referred from specialized dementia inpatient and outpatient units, covering dementia cases from all over Serbia. The patients were unrelated, except for two sisters in the AD group, and one FTD patient had a sister with MCI. The group of healthy controls ($n=96$) without a history of dementia, parkinsonism, and motor neuron disease were tested as well for the *C9orf72* expansion.

The three major FTD genes (*C9orf72*, *GRN*, and *MAPT*) were screened in 265 (83 in FTD, 80 in AD, 22 in UnD, and 80 in MCI) patients. Sequencing analysis of *GRN* and *MAPT* genes was performed in 271 (84 in FTD, 84 in AD, 23 in UnD, and 80 in MCI) and 276 patients (88 in FTD, 82 in AD, 23 in UnD, and 83 in MCI), respectively. All recruited patients ($n=472$) were screened for the *C9orf72* expansion.

The participants underwent detailed clinical and cognitive-behavioral examination and imaging protocols: computerized tomography, magnetic resonance imaging (MRI), and FDG positron emission tomography (PET) imaging. The diagnosis of AD was made on the existing criteria [11]. The cerebrospinal fluid (CSF) AD biomarkers (amyloid-beta, tau, and phospho-tau) were determined for the patients who gave consent for lumbar puncture (~74%). The enrolled patients with FTD met the research criteria for behavioral variant FTD (bvFTD), primary progressive aphasia (PPA), Semantic dementia (SD), and progressive nonfluent aphasia (PNFA) following the criteria: (a) International Behavioural Variant FTD Criteria Consortium [12]; and (b) developed by an international group of PPA investigators [13]. The STROBE checklist for the present study was completed based on the STROBE cohort guidelines [14].

The Goldman scores were used to determine the family histories. Each patient was assigned a score from 1 to 4 with an adjustment that split Goldman category 3 into 3 and 3.5 [15]. Score 1 is assigned to autosomal dominant inheritance with at least 3 people in 2 generations affected with FTLD, ALS, CBD, or PSP, with one person being a first-degree relative of the other two. Category 2 means a family aggregation if there were at least three relatives with dementia or ALS but do not meet the criteria for autosomal dominant inheritance. A score of 3 is assigned if there was a single affected first-degree family member with dementia or ALS with the age of onset before 65y and 3.5 for the onset above 65y. Category 4 was assigned when there was no contributory family history or with an unknown family history. Medical history records were obtained for affected family members whenever possible.

Standard protocol approvals, registrations, and patient consents

The study was approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade, and Ethics from the UCCS. A neurologist took the necessary clinical information after obtaining informed written consent from the patients and their participating family members. Whenever a patient was not capable of consenting, a legally authorized representative was included. For all patients, information regarding the age of onset, disease duration, family history, and clinical features were obtained from patients and caregiver reports.

Genetic analyses

After obtaining the informed consent, blood samples were drawn from all patients, and DNA was extracted using standard protocols. The first coding exon, as well as the exons 9–13 of *MAPT* gene (transcript *NM_001377265.1*), and all exons of *GRN* gene (transcript NM_002087.4) with their surrounding regions (up to 30 base pairs), were sequenced using Sanger sequencing. All the variants were classified according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology Guidelines [16]. The variants were searched through the

ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) [17]. Minor allele frequency in the non–Finnish European population was obtained from the Genome Aggregation Database (gnomAD (v.3.1.1), http://gnomad.broadinstitute.org/) [18] and pathogenicity prediction was performed using *in silico* prediction software SIFT (https://sift.bii.a-star.edu.sg) [19], PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) [20] and CADD (https://cadd.gs.washington.edu) [21]. We applied a stringent pathogenicity cut-off of 20 for the CADD score. Sizing of hexanucleotide repeats in the *C9orf72* gene was performed by fragment analysis on ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using the two-step protocol. First, normal-size alleles were genotyped. In the second step, for the homozygous samples, we performed repeat-primed PCR (RP-PCR) with previously published primers by Renton et al., 2011 [22]. To exclude false negative results, additional RP-PCR with primers by DeJesus-Hernandez et al., 2011 [23] was performed, as recommended by Rollinson et al., 2015 [24]. Pathogenic cut-off size was 30 repeats [22]. All the expansion carriers were confirmed with Southern blot.

Statistical analyses

Statistical analyses were performed with the Statistica program (version 12). The potential differences between groups were assessed with the Pearson chi^2 test, and Fischer's exact test was used when the expected values were <5 in more than 20% of the contingency cells. A p-value of <0.05 was considered significant. In the correlation analysis of the repeat size and the age of onset in the FTD group, we have used the maximum value of the obtained repeats. The analysis was performed using the Spearman correlation coefficient.

Results

All subjects originated from the population of Serbia. Demographic and clinical data of each group (FTD, AD, MCI, and UnD) are presented in Table 1., and in the Supplementary file text (Supplementary material). In the FTD group there were 84 patients with bvFTD, 5 with FTD-ALS, 34 with PNFA, and 6 with SD. To our knowledge, parental consanguinity of either degree was not present in our cohort.

In 265 subjects tested for *C9orf72*, *GRN*, and *MAPT*, 2.64% had a pathogenic variant in *C9orf72* ($n=6$) and in *GRN* ($n=1$), whereas in the group of 472, we found almost the same frequency 2.54% ($n=12$).

C9orf72 gene

Nine patients with repeat expansions were from the FTD group (9/129; 6.98%), and two patients belonged to the UnD group (2/49; 4.08%). We did not detect any presence of the pathogenic *C9orf72* repeat expansion in MCI or AD subjects. The distribution of the *C9orf72* alleles is presented in Supplementary Fig. 1 and Supplementary file text. The most frequent alleles in patients and the control group were in the *wild-type* range with 2, 5, and 8 repeats. In addition, one FTD and one AD patient were carriers of the intermediate number of repeats (24 and 28 repeats, respectively) in *C9orf72* (Supplementary Fig. 2a&b, and Supplementary file text).

Table 1 Demographic and clinical characteristics of the cohorts tested on 3 major genes for FTD

	FTD	AD	UnD	MCI
N	129	176	49	118
Gender m: f	74:55	79:87	22:27	34:84
age mean (SD) range	60.99 (7.79) (39–81)	61.24 (6.90) (36–85)	61.41 (9.44) (32–79)	62.38 (9.00) (39–83)
Education mean (SD) Range	12.26 (2.98) (4–18)	11.31 (3.0) (4–22)	11.55 (2.84) (5–18)	13.21 (2.47) (4–20)
Age at onset mean (SD) range	57.13 (7.71) (36–78)	57.74 (6.61) (35–83)	57.55 (9.64) (30–76)	59.33 (9.64) (36–82)
Duration of the disease Mean (SD) range	3.87 (2.43) (0.5–11)	3.51 (2.18) (0.2–10)	3.85 (2.53) (0.1–10)	3.06 (2.32) (0.3–10)
MMSE mean (SD) range	17.38 (7.53) (2–30)	15.48 (6.67) (3–28)	16.10 (8.03) (3–29)	26.69 (2.96) (17–30)
Goldman modified score				
1	5 (3.87%)	7 (3.97%)	0	4 (3.39%)
2	6 (4.65%)	3 (1.70%)	5 (10.20%)	8 (6.78%)
3	24 (18.6%)	15 (8.52%)	11 (22.45%)	11 (9.32%)
3.5	17 (13.18%)	40 (22.72%)	9 (18.37%)	36 (30.51%)
4	77 (59.68%)	111 (63.07)	24 (48.98%)	59 (50.0%)

FTD = Frontotemporal dementia; AD = Alzheimer's disease; UnD = unspecified dementia; MCI = mild cognitive impairment; MMSE = Mini Mental Status Examination

C9orf72 repeats expansion in FTD

Demographic and clinical characteristics of *C9orf72* carriers in the FTD group In the FTD group ($n=129$), among 52 positive family history and 77 sporadic, there was no statistically significant difference in the age of onset ($p=0.55$). Nine patients (6.98%; 5 females, 4 males) were *C9orf72* repeat expansion heterozygous carriers with increasing frequency (13.46%) within the group with a positive family history, and in 2.6% of sporadic cases. A significant difference was shown for the age of onset between the *C9orf72* carriers and non-carriers ($p=0.007$), indicating that heterozygous carriers of the *C9orf72* expansion in the FTD group were about 7 years younger at the onset than FTD patients without the expansion (mean age 50.56 ± 8.25 vs. 57.63 ± 7.47). There was no difference in the age of onset ($p=0.756$) nor in disease duration ($p=0.379$) between the female and male carriers. Details of demographic and clinical characteristics and imaging findings are presented in Table 2. The typical clinical phenotypes varied between *C9orf72* repeat expansion carriers; 7 patients with bvFTD and 2 with FTD-ALS overlapping presentation. Detailed descriptions of patients' neurological signs and behavioral symptoms are given in Table 2.

Positive family history ($n=7$) was present significantly more in patients harboring *C9orf72* repeat expansion than in patients without ($p=0.018$). FTD-ALS overlapping phenotype was present in five individuals (3.88%) within the FTD group. Overlapping FTD-ALS clinical presentation was more common in expansion carriers relative to patients without (22.22% vs. 2.5%, respectively).

The maximum number of *C9orf72* repeats obtained among all FTD patients was ~742. The correlation analysis of the number of expanded repeats and patients' age of onset did not show significance ($p>0.05$).

C9orf72 carriers within the UnD group

In the group with UnD ($n=49$; 25 positive family history, 24 sporadic), 2 female patients (4.08%) carried the *C9orf72* repeat expansion. Within the positive family history, both expansion carriers (8%) had a Goldman score category 3. One patient had a family member (aunt) with ALS at an age before 65y, and the other with a score 3.5 had a mother with dementia (aged over 65y). The clinical picture is described in Table 2 with various cognitive-motor problems that included stuttering, agrammatism, apraxia, walking and postural stability disorders, dysphagia, dysarthria, and cerebellar ataxia. The maximum number of the obtained repeats with Southern blot, for these two patients, was ~590 repeats.

GRN variants

Pathogenic *GRN* variant (c.1252 C>T; p.R418*) was identified in one out of 271 screened patients (Table 2). Based on the *in silico* prediction software CADD, it has a score of 27.5. The variant is also present in the ClinVar database and characterized as pathogenic by multiple sources. The patient was a female aged 67y, with first clinical symptoms in the language domain that appeared several years before, with difficulties finding the right word when speaking or starting a conversation. Also, she exhibited hesitant speech, and preferred listening. The symptoms worsened as time passed, with pauses in speech, speaking in shorter sentences, and grammatical errors. She was diagnosed with a PNFA variant of FTD. The patient's mother was diagnosed with dementia at the age of 64y, while the patient's sister had a more severe clinical picture diagnosed at the age of 52y.

MAPT variants

Screening of selected *MAPT* exons in 276 patients did not reveal proven pathogenic variants in our cohort. We identified only 1 benign variant in one patient (Excel file in Supplementary material).

Discussion

This report presents the first analysis of the frequency of pathogenic variants in the three major FTD genes (*C9orf72*, *GRN*, and *MAPT)* within the Serbian population from the Memory Center. The study included individuals with neurodegenerative conditions such as AD, FTD, MCI, and UnD. Among the 472 individuals tested, pathogenic variants were found in 12 cases, accounting for a frequency of 2.64%. Specifically, pathogenic variants were identified in *C9orf72* (11 cases) and *GRN* (1 case), while in *MAPT*, there were no pathogenic variants.

In total, 7.75% of the FTD group had pathogenic variants in the *C9orf72* ($n=9$) and *GRN* ($n=1$) genes. These results are lower when compared to findings from Greece, Germany, Turkey, and Sweden [25–28]. The frequency of pathogenic variants with a positive family history was highest in our FTD cases (15.38%) and lower in sporadic cases (2.60%). A recent study in the Turkish cohort revealed a 25% rate of pathogenic variants in the selected genes among familial cases [27]. Similarly, the Greek study reported a 23.9% rate in familial cases and only one sporadic case with a likely pathogenic variant in *MAPT* [25]. Overall, pathogenic variants in *GRN*, *MAPT*, and hexanucleotide repeat expansions in *C9orf72* can be present in 60% of familial FTD cases [29].

Table 2 The demographic, clinical and imaging findings in *C9orf72* repeat expansion carriers

Patients, sex, age of onset	Variant	FH	Diagnosis	Symptoms	Disease progression	MRI/PET
1/f, 64y	Repeat expansion	3.5	bvFTD	Indifference, inertia, impulsivity, apathy, stubbornness, inattention	The disease progresses towards a greater degree of behavioral change, disorientation in time and space; died at the age of 71y	MRI - global cortical reduction supratentorial, FT region atrophy bilateral FDG PET - significantly reduced glucose metabolism in frontal and anterior temporal lobes and thalamus
2/f, 50y	Repeat expansion	3	bvFTD	Personal neglect, hoarding, disexecutive, loss of empathy, inattention, naming problems	Feeling of dizziness and falls, the episodes of forgetfulness in everyday activities, cognitive slowness, slurred speech, low extremities dyspraxia; died at the age of 61y	MRI - pronounced cortical reduction changes of the supratentorial region FDG PET - bilateral predominantly left side hypometabolism FT cortex
3/f, 42y	Repeat expansion	4	FTD-ALS	Personality changes, social withdrawal, forgetfulness, agitation, dysarthria, dysphagia, hallucinations	Expressed agitation, unintelligible speech, moderate spastic quadriparesis, progression to FTD-ALS phenotype; died at the age of 44y	MRI - discrete asymmetrical right FT cortical atrophy FDG PET - significantly reduced glucose metabolism in frontal and anterior temporal lobes, striatum, thalamus
4/f, 49y	Repeat expansion	1	bvFTD	Change in eating habits-binge eating, affective incontinence, loss of independent living, intensive appetite, face recognition problems, disorientation	Psychomotor slowness, difficulty understanding certain orders, disinhibition; died at the age of 56y	MRI - supratentorial reduction of parenchymal changes to atrophy with ex vacuo expanding the ventricular system, a pair of small micro ischemias in the right frontal subcortical area FDG PET - significantly reduced glucose metabolism in right frontotemporoparietal regions
5/m, 52y	Repeat expansion	2	FTD	Impersistance, forgetfulness, depression, social withdrawal, hoarding, seizure	Scarce speech production, emotional lability, postural instability and ataxia	MRI - bilateral atrophy of FT lobe FDG PET - significantly reduced glucose metabolism in frontal and temporal lobes
6/m, 51y	Repeat expansion	3	bvFTD-ALS	Apathy, executive deficits, eating habits changes, confabulation forgetfulness, leg weakness, bilateral foot drop,	Agitation, confusion, hallucinations disorientation in time and space, cognitive deterioration, progression to FTD-ALS phenotype; died at the age of 60y.	MRI - Supratentorial microangiopathic changes, incipient periventricular leukoencephalopathy, possible iron deposits in the globus pallidus and substantia nigra bilaterally, possibly as part of neurodegeneration with iron accumulation FDG PET - scan significantly reduced glucose metabolism in frontal and temporal lobes, striatum, thalamus and mesencephalon
7/m, 36y	Repeat expansion	4	bvFTD	Inappropriate behavior, empathy loss, eating changes, executive deficits, perseverative poor judgment, language production,	Hypomimia, forgetfulness, postural tremor on upper left extremity, occasional urinary incontinence; died at the age of 44y	MRI - FT atrophy FDG PET - significantly reduced glucose metabolism in frontal and anterior temporal lobes and thalamus
8/m, 59y	Repeat expansion	3	bvFTD	Social withdrawal, transitory alcoholism, disorganization, motor stereotypes, shaking of the right leg, smacking of the lips	Oromandibular dyskinesia, cognitive deterioration, disinhibition, sleeping problems	MRI - FT atrophy without involvement of hippocampal regions FDG PET - FT hypometabolism bilateral left predominant, PT left+, thalamus striatum
9/f, 52y	Repeat expansion	3	bvFTD	Hoarding, eating habits change, loss of empathy, forgetfulness, oromandibular dyskinesia	Apraxia, speech problems, space disorientation and motor stereotypes, loss of sphincter control, cognitive deterioration; died at the age of 56y	MRI - global cortical atrophy predominantly frontal bilateral FDG PET - reduced glucose metabolism FTP bilaterally, striatum, thalamus

Table 2 (continued)

Patients, sex, age of onset	Variant	FH	Diagnosis	Symptoms	Disease progression	MRI/PET
10/f, 30y	Repeat expansion	3	UnD	Forgetfulness, shyness, headache, scarce speech, slowness, stuttering	Agramatism, apraxia, disinhibition, loss of functionality, psychomotor slowness, spastic paraparetic gait.	MRI - arachnoid cyst in left anterior temporal lobe, cortical reduction widening of the temporal horn, atrophyof the right hippocampal volume reduction FDG PET - significantly reduced glucose metabolism in frontal and anterior temporal lobes and anterior cingulum
11/f, 59y	Repeat expansion	3	UnD	Saccadic speech, inattention, working memory deficits, acalculia, ataxia, falls, dysphagia	Broken prosody with elements of bradylalia, deterioration of gait, swallowing difficulties, emotional incontinence; died at the age of 61y	MRI - lacunar zones supratentorially in the white matter FDG PET - significantly reduced glucose metabolism in frontal and temporal lobes
12/f, 67y	Single nucleotide variant (nonsense) p.R418*	1	PNFA_FTD	Speech problems in the form of dysnomia, pauses in speaking dyslexia, dysgraphia	Progression of speech problems: agrammatism, perseverations, echolalia, preferring to listen, withdrawal, died at the age of 78y	FDG PET - reduced glucose metabolism in left frontotemporal parietal and right frontal lobes

FTD-Frontotemporal dementia; ALS-amyotrophic lateral sclerosis; FH-family history; UnD-unspecified dementia; CT-computerized tomography, MRI- Magnetic Resonance Imaging, FDG PET-Positron Emission Tomography

In this study, the *C9orf72* repeat expansion was detected in 13.46% of familial FTD cases and 2.60% of sporadic FTD cases, which is relatively lower compared to other studies. Published data on the frequency of the *C9orf72* repeat expansion in FTD cohorts from Western Europe shows a possible north-south descending gradient. The reported frequency reaches up to 28.7% in familial and up to 6% in sporadic FTD cases [30, 31]. The highest mutation rates were observed in Scandinavian familial FTD cases, such as Sweden (26.5%) [28] and Finland (29.33%) [32], while Italy and Portugal had the lower rates (6.09% and 6.62%, respectively). Germany registered the lowest frequency at 4.82% [32]. Our findings show that the frequency of *C9orf72* repeat expansion in sporadic cases was below 3%, consistent with previously published data [31]. In our study, the presence of a positive family history was significantly more frequent among expansion carriers compared to those without the expansion, aligning with previously published data [33].

In our group of FTD expansion carriers, the average age of onset was around 50 years, which is lower than the previously reported 57 years [30]. The youngest carrier in our cohort was 36 years old, and the oldest was 64 years old at the age of onset, fitting within the expected range of 30 to 76.3 years [30, 31, 34].

The predominant clinical presentation in our *C9orf72* expansion-positive patients was the bvFTD phenotype (7 out of 9 cases), consistent with previous reports [30, 31, 35, 36]. In this study, the FTD-ALS phenotype strongly predicted a pathogenic *C9orf72* mutation, observed in 2 out of 5 cases.

In our UnD cases with a positive family history, we had a relatively high frequency (8%) of *C9orf72* repeat expansion carriers. This finding aligns with previous research, as *C9orf72* expansions have been detected in various neurodegenerative and psychiatric disorders [37]. Additionally, rare cases of *C9orf72* expansions associated with ataxia syndrome are reported in the literature [38], which explains the occurrence of ataxia in one of our UnD patients.

In this study, we have identified only one *GRN* pathogenic variant in the FTD group, found in a 65-year-old female patient with a PNFA phenotype and a positive family history. Previous reports indicate that clinical presentations of patients with *GRN* pathogenic variants include PNFA, progressive mixed aphasia, and social-executive disorder [39]observed in 24% of such patients [40]. The relative frequency of *GRN* variants in FTD is 4.8% overall and 12.8% in familial forms. Notably, 3.2% of apparently sporadic FTD patients carried a *GRN* pathogenic or likely pathogenic variant, suggesting the possibility of de novo mutations or incomplete penetrance [41]. Therefore, more systematic genetic testing should be considered, even in patients without an apparent family history of FTD [41].

The current study has several limitations. First, we were unable to confirm the diagnosis for many deceased family members and lacked pathohistological diagnoses. However, all subjects were evaluated using recent clinical criteria [12, 13] and utilized available CSF biomarkers and FDG PET for AD diagnosis. Challenges such as limited clinical and family history, misdiagnoses, and psychiatric disorders mimicking FTD can make it difficult to accurately estimate a disease frequency [42]. Our study focused on patients

primarily under 65 years old, but recent findings suggest FTD incidence peaks at 71 years [43].

The study confirms that *C9orf72* repeat expansion is the most common genetic cause of FTD in Serbia, although slightly lower when compared to European averages. Genetic testing for this expansion is recommended for patients with this neurodegenerative disease. It is important to consider testing patients with UnD based on the diverse clinical similarities. Further research is needed to explore geographic and ethnic implications. Our study did not find pathogenic variants in *MAPT*, possibly due to targeted analysis or the rarity of these variants in our population. Healthcare professionals should be aware of the genetic risk in patients with FTD and UnD. Genetic counseling is advised for patients and families with a detailed family history.

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Author contributions Elka Stefanova, MD, PhD: Conceptualization; Data curation; Formal analysis; Investigation; Resources; Project Administration; Visualization; Writing Drafting/revision of the manuscript. Ana Marjanović, PhD: Conceptualization; Data curation; Investigation; Methodology; Visualization; Writing Drafting/revision of the manuscript. Valerija Dobričić, PhD: Conceptualization; Investigation; Methodology; Visualization; Writing Drafting/revision of the manuscript. Gorana Mandić Stojmenović, MD, PhD: Resources. Tanja Stojković, MD, PhD: Resources. Marija Branković, PhD: Investigation; Methodology. Maksim Šarčević, MD: Resources. Ivana Novaković, MD, PhD: Conceptualization; Resources; Funding Acquisition; Writing Drafting/revision of the manuscript. Vladimir S Kostić: Conceptualization of the study, Funding Acquisition; Writing Drafting/revision of the manuscript.

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Declarations

Data sharing statement Anonymized data not published within this article will be made available by request from any qualified investigator.

Conflict of interest The authors declare no conflict of interests.

Consent statement All of the participants provided written informed consent to participate in the study.

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