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The association between repeat number in *C9orf72* and phenotypic variability in Turkish patients with frontotemporal lobar degeneration

Ebru Erzurumluoglu [a,*], Oguz Cilingir [a], Belgin Demet Ozbabalik Adapinar [b], Basar Bilgic [c], Sinem Kocagil [a], Hulya Ozen [d], Beyhan Durak Aras [a], Cinar Yenilmez [e], Sevilhan Artan [a]

[a] Department of Medical Genetics, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey
[b] Acibadem Hospital Neurology Clinic, Eskisehir, Turkey
[c] Department of Neurology, Istanbul University, Istanbul Faculty of Medicine, Behavioural Neurology and Movement Disorders Unit, Istanbul, Turkey
[d] Department of Biostatistics, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey
[e] Department of Psychiatry, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey

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ABSTRACT

Frontotemporal lobar degeneration (FTLD) describes a group of progressive brain disorders. The expansion of a noncoding GGGGCC (G_4C_2) hexanucleotide repeat in the *C9orf72* gene is a major cause of both familial FTLD and amyotrophic lateral sclerosis. The aim of this study was to determine the prevalence of *C9orf72* G_4C_2-repeat expansion in a Turkish population with FTLD and to determine its effects on the phenotype. The G_4C_2 expansion in the *C9orf72* gene was analyzed in 100 cases of FTLD without mutations of the *MAPT, PGRN, CHMP2B, VCP, TARDBP,* and *FUS* genes and 100 age-matched healthy controls by using repeat-primed polymerase chain reaction and fragment length analysis techniques. A possible pathogenic repeat ($\geq$30) was found in one of the familial cases (1/33), but none of the sporadic cases. The difference in the allele length between the cases and controls was statistically significant ($p < 0.01$). Intermediate (20–30) repeats were detected in 4% of our cases. Patients with psychotic symptoms appear to be enriched for intermediate and possibly pathogenic repeats. To determine whether the intermediate and $\geq$30-repeat allele carriers shared the *C9orf72* risk haplotype, we examined rs4879515 and rs3849942 in all samples and family members of patients with possibly pathogenic alleles. We identified at least one risk allele for each single-nucleotide polymorphism in all intermediate and possibly pathogenic repeat carriers. We observed that $\geq$8 unit repeats were strongly correlated with the tagging risk alleles for both single-nucleotide polymorphisms ($p < 0.001$). To our knowledge, this is the first study to evaluate *C9orf72* G_4C_2 repeats in Turkish patients with FTLD. The present findings suggest that pathogenic expansions of the *C9orf72* repeat are uncommon in Turkish patients with FTLD, but intermediate repeats may be a risk factor for FTLD and act as a genetic modifying factor for psychotic symptoms.

1. Introduction

Frontotemporal lobar degeneration (FTLD) describes a group of clinically, pathologically, and genetically heterogeneous progressive disorders featuring predominant neuronal death of the temporal and/or frontal lobes of the brain, clinically characterized by behavioral and/or language deficits (Mann and Snowden, 2017). It represents the second most common cause of early-onset dementia after Alzheimer's disease, is observed in patients aged less than 65 years, and accounts for approximately 5%–15% of all dementias (Neumann et al., 2009; Suhonen et al., 2017). In 2006, the genetic linkage at chromosome 9p21.3-9p21.1 was reported in familial FTLD/amyotrophic lateral sclerosis (ALS) cases (Gijselinck et al., 2017). Technological developments made in the last decade, including next-generation sequencing, have enabled a clearer understanding of the molecular pathophysiology of familial/sporadic frontotemporal dementia (FTD) and the identification of more than 13 genes that cause and/or are responsible for susceptibility to the disease (Rainero et al., 2017). Among these genes, the pathogenic

* Corresponding author at: Department of Medical Genetics, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey. Tel./fax: +90 5397946467.
E-mail address: ebruerzurumluoglu@gmail.com (E. Erzurumluoglu).

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expansion of a noncoding G_4C_2 hexanucleotide repeat in the *C9orf72* gene, mapping to chromosome 9p21, has been defined simultaneously as a major cause of both familial FTLD and ALS (DeJesus-Hernandez et al., 2011; Renton et al., 2011).

Numerous studies have demonstrated that *C9orf72* repeat expansion is the most common mutation compared with other known genes, accounting for approximately 25% of familial FTD, 37% of familial ALS, and 88% of both familial FTD and ALS cases (Van Mossevelde et al., 2017). Pathogenic G_4C_2 expansion has also been reported in nearly 5% and 10% of patients with sporadic FTD and ALS, respectively. Numerous *C9orf72* repeat expansion studies conducted worldwide have shown that the frequency of *C9orf72* G_4C_2 expansion greatly depends on geographical region and the ethnic structure of the population. In European populations, particularly in Scandinavian countries, the carrier rate is markedly high, with the highest frequency reported in a Finnish population (29%) (Couratier et al., 2017; Van der Zee et al., 2013). The repeat expansion is believed to be originated from a single founder haplotype in the chromosome 9p21 locus. This haplotype is associated with ALS, FTD, and combined ALS-FTD phenotypes (Laaksovirta et al., 2010; Mok et al., 2012). Most cases of *C9orf72* expansion have the same 20 single-nucleotide polymorphisms (SNPs) including rs4879515 and rs3849942 in a 140-kb long region surrounding *C9orf72,* corroborating the Scandinavian founder effect (Byrne et al., 2012; Mok et al., 2012; Ratti et al., 2012). However, these expansions are rarer in Asian populations than in Caucasian Europeans (Kim et al., 2014; Ogaki et al., 2013). Considering Turkey's geographical location and history, we expected to see a correlation between these populations.

Although Drosophila and zebrafish models have indicated that the expansion level of >30 units is sufficient for neurodegeneration (Swinnen et al., 2018; Xu et al., 2013) and patients carry from hundreds up to 1600 repeats (Jiao et al., 2014; Renton et al., 2011), the size of expansion is highly variable. Although the pathogenic cutoff value is not yet established, most studies conducted to date have considered expansions of >30 repeat units as possibly pathogenic. Normal repeat lengths of up to 24 units have been identified in most healthy cohorts (Van Mossevelde et al., 2017), but patients with FTD and their affected siblings with 20–22 repeat alleles (Gómez-Tortosa et al., 2013) or unaffected individuals with 32–34 repeats and even larger expansions have also been reported (McGoldrick et al., 2017; Xiao et al., 2015). Even the pathogenic cutoff value is not yet established; most of the studies carried out to date have considered >30 units as possibly pathogenic expansion (DeJesus-Hernandez et al., 2011; Guven et al., 2016; Mahoney et al., 2012; Renton et al., 2011). Information is limited as to whether the magnitude of the expansion of intermediate alleles increases over generations, as seen in other repeat expansion diseases, and whether intermediate allele sizes are associated with specific clinical features. Previously, wild-type *C9orf72* alleles (<20 repeats) have been shown to be stable between generations, but intermediate alleles (20–30 repeats) have been reported to be susceptible to unfaithful inheritance or somatic instability (Benussi et al., 2014; Ng and Tan, 2017; van Blitterswijk et al., 2013). Recently, Ng and Tan (2017) reviewed 49 studies on the role of intermediate alleles and reported that individuals with intermediate repeat lengths may have a predisposition to neuropsychiatric sequelae. During genetic counseling in individuals with intermediate repeat lengths, challenges are encountered as to whether future generations are faced with the risk of recurrence and whether specific clinical traits will appear at older ages.

The aims of this study were to determine the prevalence of *C9orf72* G_4C_2-repeat expansions in Turkish patients with FTLD and to investigate the genotype-phenotype correlations.

2. Methods

2.1. Participants

We studied 100 patients diagnosed with FTLD with or without a family history of the disease by neurologists specializing in neurodegenerative diseases at the Neurology department of Eskisehir Osmangazi University. Mutations in the FTLD-related genes (*MAPT, PGRN, CHMP2B, VCP, TARDBP, FUS*) analyzed using next-generation sequencing had been previously excluded in all patients. A hundred individuals in the control cohort were evaluated by neurologists to ensure that they did not exhibit any clinical manifestations of neurodegenerative diseases. The Turkish individuals in both the FTLD and control cohorts originated from Central Anatolia.

This study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Clinical Practice Ethics Committee of the Faculty of Medicine at Eskisehir Osmangazi University. Each patient provided informed consent.

2.2. C9orf72 G_4C_2-repeat genotyping

Genomic DNA was isolated from peripheral lymphocytes using Magna Pure Compact LC (Roche Applied Science, Basel, Switzerland), according to the manufacturer's recommendations. A total of 100 patients with FTLD and all control individuals were screened for the *C9orf72* G_4C_2 repeat by fragment length analysis (DeJesus-Hernandez et al., 2011) and repeat-primed polymerase chain reaction (Renton et al., 2011) on an automated ABI 3130 genetic analyzer, as described previously. Allele identification was performed using GeneMapper software (Applied Biosystems, CA, USA).

2.3. Single-nucleotide polymorphisms genotyping

We genotyped all FTLD cases for 2 SNPs, rs4879515 and rs3849942, to determine whether the intermediate and >30-repeat allele carriers shared the *C9orf72* risk haplotype. The primer sequences designed using Primer3 software flanking rs4879515 were F: 5′TGCTAAAACGCCACTGTGATCC3′, R: 5′ TTCCTTTCTCTTCTTCCTCCTCTCC 3′ and rs3849942 F:5′ CTTCTGCTTAATGGCTCTCCAAC 3′, 5′ACCATGCTAGGCACTGAGAC 3′. These 2 SNPs were analyzed through direct DNA sequencing with the BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI 3130 genetic analyzer (Applied Biosystems, Life Technologies, CA, USA), and sequencing data were analyzed using Sequencing Analysis Software (V. 5.4., Applied Biosystems, Life Technologies).

2.4. Statistical analysis

Statistical analysis was performed using the Statistical Package of the Social Sciences 21.0 (IBM, NY, USA). Qualitative variables are presented as frequencies and percentages, whereas quantitative variables are presented as means ±standard deviation or medians (Q1–Q3). The Student *t*-test was used to compare the age values of the FTLD and control groups. In addition, sex distribution in the FTLD and control cohorts was evaluated using χ^2 analysis. The Mann–Whitney *U* test was used to compare non-normally distributed allele length values in the FTLD and control groups. The relationship between psychotic symptoms and the number of G_4C_2-repeat groups was determined using χ^2 analysis. Linear regression analysis was used to test the correlation between the number of repeats and age at onset. χ^2 Test was used in the comparisons of ≥8 unit repeats and <8 unit

Table 1
Clinical and demographic features of the FTLD cases and control individuals

Sex/age		FTLD cohort		Control cohort	*p*-value
N		100		100	
Sex (female/male)		45/55		60/40	0.567
Age		60.64 ± 9.6		58.30 ± 9.1	0.078
Age at onset		55.73 ± 9.7		–	
Clinical diagnosis subtype	n	Family history + (%)	Psychotic symptoms + (%)	Intermediate repeats	Possibly pathogenic repeats
bvFTD	71	23	15	2	1
SD	6	1	–	–	–
PNFA	10	4	–	–	–
FTD-MND	6	3	2	1	–
CBD	4	1	–	–	–
PSP	2	–	–	–	–
FTD-Park	1	1	–	1	–
Familial		33 (33)	13 (76.4)	4	1
Sporadic		67 (67)	4 (23.6)	–	–

Key: bvFTD, behavioral variant frontotemporal dementia; CBD, corticobasal degeneration; FTD-Park, frontotemporal dementia with Parkinsonism; FTLD, frontotemporal lobar degeneration; FTD-MND, frontotemporal dementia with motor neuron disease; PNFA, progressive nonfluent aphasia; PSP, progressive supranuclear palsy; SD, semantic dementia.

repeats in the patient group with the risk allele. Statistically significant evidence of association was determined by *p*-values of 0.05 or below.

3. Results

3.1. Demographic and clinical features of the study groups

The patients included in this study consisted of 45 women and 55 men (100 probands) with FTLD (71 with behavioral variant FTD, 6 with semantic dementia, 10 with progressive nonfluent aphasia, 6 with FTD-motor neuron disease, 4 with corticobasal degeneration, 2 with progressive supranuclear palsy, and 1 with FTD with Parkinsonism). The mean age and age of disease onset in the case group were 60.64 ± 9.6 and 55.73 ± 9.7 years, respectively. The mean age of the 60 women and 40 men in the control group was 58.30 ± 9.1 years. Sex and age distributions were similar in both the FTLD and control cohorts. A history of dementia in first-degree relatives was found in 33% of the FTLD cohort and psychotic symptoms were observed in 17% of cases. The characteristics of the study groups are presented in Table 1.

3.2. Results of C9orf72 G_4C_2-repeat analysis

C9orf72 G_4C_2 hexanucleotide repeats were classified according to the data obtained in previous studies (Cannas et al., 2015; Gómez-Tortosa et al., 2013; Nuytemans et al., 2013): wild-type alleles (<20 repeats), intermediate repeats (20–29 repeats), and possibly pathogenic repeat expansion (≥30 repeats). Among the non-expanded alleles, the most frequent numbers of repeats were 2, 8, and 5 in all groups. In the FTLD cohort, intermediate and possibly pathogenic repeats were detected in 4% and 1%, respectively whereas remaining patients had wild type alleles. Neither intermediate nor pathogenic repeats were detected in the sporadic cases. The difference in allele length between the cases and controls was statistically significant ($p = 0.005$) (Table 2, Fig. 1).

The clinical findings of FTLD cases with intermediate (21–29) and possibly pathogenic G_4C_2 expansion (≥30) repeats are presented in Table 3. Linear regression analysis of the FTLD group revealed no significant association between repeat number and age at disease onset ($\beta = -0.065$, $t = -0.372$, $p = 0.711$). Of the 17 FTD cases with psychotic symptoms, 3 had intermediate repeats and one had ≥30 repeat carriers, and a statistically significant association was observed between repeat number groups and psychotic symptoms ($p = 0.003$) (Table 4).

The pedigree of the carrier with the 33 repeats is presented in Fig. 2. The symptoms in the proband started at the age of 45 years with severe anxiety and the gradual involvement of stereotyped movements, apathy, episodic memory impairment, and visual and auditory hallucinations. Children of the proband described similar symptoms in the mother of the proband; however, the mother could not be evaluated because she had died and the siblings, aged >60 years, did not approve genetic analyses. The repeat lengths of the proband's children were within normal ranges.

3.3. Genotyping of the risk SNPs

To determine whether the intermediate and ≥30-repeat allele carriers shared the *C9orf72* risk haplotype, we examined rs4879515 and rs3849942 in all intermediate allele carriers and family members of the patient with possibly pathogenic alleles (Table 5). The analysis of the known markers of risk SNPs rs4879515 and rs3849942 showed the presence of the risk allele in intermediate and possibly pathogenic repeat carriers. All intermediate and possibly pathogenic repeat carriers carried the T allele of SNP rs4879515. The proband with the 33 unit repeat was homozygous for the T allele, whereas the children and intermediate repeat carriers were heterozygous.

All intermediate repeat carriers, possibly pathogenic expansion-positive cases, and their children carried the A allele of SNP rs3849942, which tags the haplotype as the least frequent allele on the haplotype.

We examined the 2 SNPs in the FTLD and control groups. As shown in Table 6, the frequencies of both the tagging T allele for rs4879515 and the A allele for rs3849942 were significantly higher in the FTLD cohort compared to the controls.

We classified the number of repeats into 2 groups (≥8 and <8 repeat carriers) to investigate the relationship between the repeat

Table 2
Distribution of allele lengths between the frontotemporal lobar degeneration and control cohorts

Allele length	Cases median (Q1–Q3)	Controls median (Q1–Q3)	
Number of repeats	5.5 (2–8)	5 (2–7.5)	$p = 0.005$

p: Level of significance according to the Mann–Whitney *U* test.

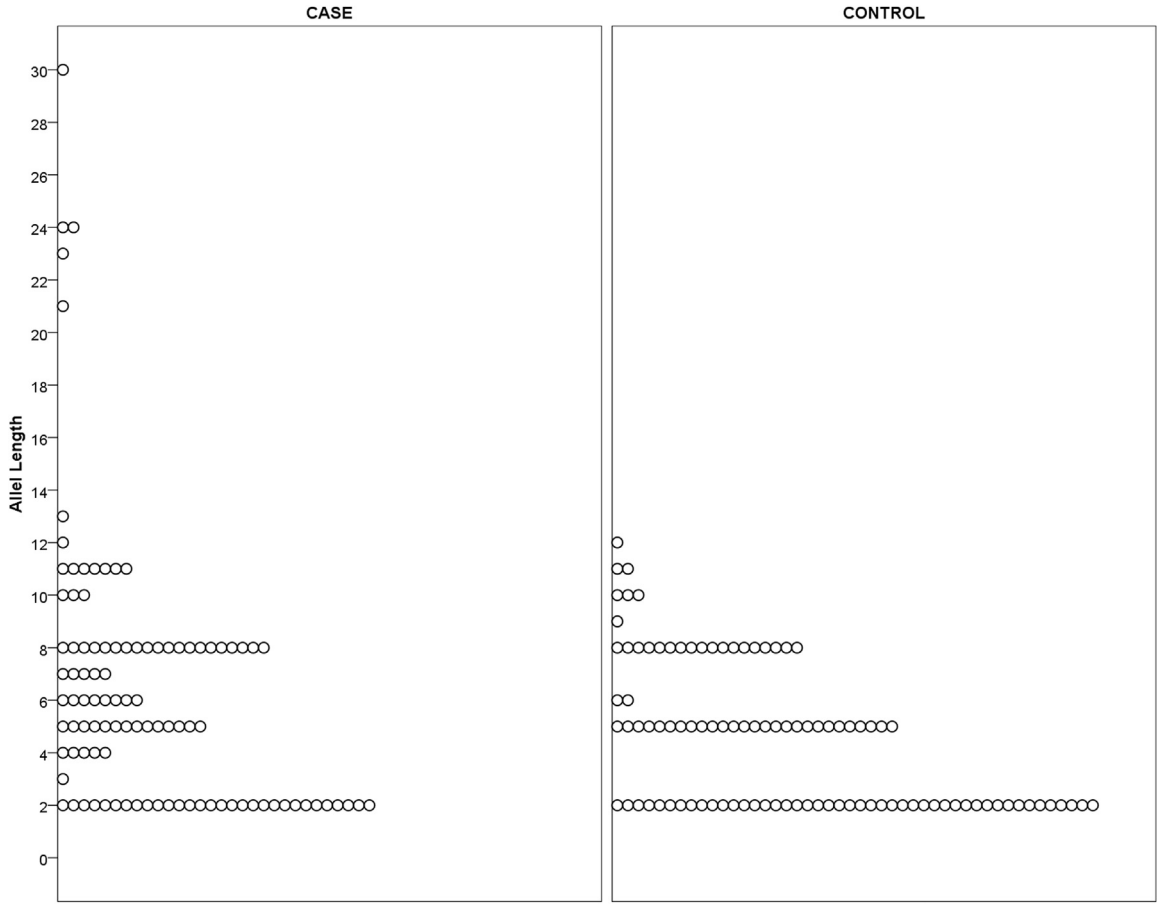

Fig. 1. Scatter plot of the distribution of allele lengths in the frontotemporal lobar degeneration and control cohorts.

number and risk alleles, according to a previous study (Nuytemans et al., 2013; Van der Zee et al., 2013). The rs4879515 T allele was found in 37/37 patients with ≥8 units compared to 41/63 patients (65%) with <8 unit repeat carriers ($p < 0.001$; Table 7). Similarly, a statistically significant difference was observed for rs3849942, with all patients with ≥8 unit repeats and 46% of patients with <8 unit repeats (29/63) exhibiting the tagging A allele. The patients carrying at least one rs3849942 A allele had a greater repeat length than those without the A allele ($p < 0.001$) (Table 7, Fig. 3).

4. Discussion

4.1. Frequency of C9orf72 repeats

Although several studies have cited the presence of abnormal G_4C_2 expansion in the *C9orf72* gene as a cause of FTD and as evidence of the association between FTD and ALS, data on the prevalence of *C9orf72* expansion in Turkish patients with FTD are highly limited. To our knowledge, only 2 studies have evaluated *C9orf72* G_4C_2 repeats in Turkey; one study determined the frequency of this expansion among ALS and dementia groups and another reported the frequencies of *MAPT*, *GRN*, and *C9orf72*, which are major FTD genes, in 95 dementia cases. Therefore, as far as we know, this is the first study reporting the frequency of G_4C_2 expansion in Turkish patients with FTLD but without FTLD-related gene mutations. Özoğuz et al., 2015 reported G_4C_2 expansion frequencies of 3% in sporadic and 18% in familial ALS cases. However, among 4 cases found to have expansion, 3 cases had FTD-ALS and 1 had dementia. In another study, *C9orf72* G_4C_2 expansion was reported in 2 progressive nonfluent aphasia cases and 1 behavioral variant FTD case; the expansion frequency was reported as 3.2%, but the frequency was higher (10.7%) if only the patients with FTD were considered. Of these cases, 2 were Turkish and 1 was Macedonian in origin (Guven et al., 2016).

Table 3
Clinical findings of frontotemporal lobar degeneration cases with intermediate and possibly pathogenic repeats

Case	Age at onset	Subtype	Clinical findings	MRI results	Repeat number
I1	53	bvFTD	Decrease in visuospatial ability and loss of insight, difficulties in abstract thinking, apathy, apraxia, visual hallucination	Bilateral frontotemporal atrophy	6–21
I2	58	FTD-MND	Memory deficit, depression, apathy, muscle weakness, hallucination	Frontotemporal atrophy	4–24
I3	59	bvFTD	Apathy, loss of insight, difficulties in abstract thinking, visual hallucination	Frontal lobe atrophy	2–23
I4	48	FTD-Park	Executive dysfunction and attention deficit, rigidity, bradykinesia	Frontotemporal and parietal lobe atrophy	2–24
E1	45	bvFTD	Stereotyped movements, anxiety, apathy, obsession, impairment of episodic memory, visual and auditory hallucinations	Severe frontotemporal atrophy	2–>30

Key: bvFTD, behavioral variant frontotemporal dementia; FTLD, frontotemporal lobar degeneration; FTD-MND, frontotemporal dementia with motor neuron disease; FTD-Park, frontotemporal dementia with Parkinsonism; MRI, magnetic resonance imaging.

Table 4
Association of *C9orf72* G_4C_2-repeat expansions with psychotic symptoms in the frontotemporal lobar degeneration cohort

C9orf72 G_4C_2 expansions	Psychotic symptoms		*p*-value
	Positive (n)	Negative(n)	
Wild type	13	82	
Intermediate repeats	3	1	0.003
Possibly pathogenic repeats	1	–	

p = Level of significance according to the χ^2 test.

In our series, no pathogenic expansion was detected in sporadic FTD cases, but the percentage of possibly pathogenic G_4C_2 expansion (3.03%) found in familial FTD cases was much lower than that observed in previous studies and in European patients. G_4C_2 expansion in the noncoding region of the *C9orf72* gene was reported in 6% of sporadic FTD cases and 25% of familial FTD/FTD-ALS cases. The percentage increased to 40% in familial ALS cases (Couratier et al., 2017). The pathogenic expansion frequency has been demonstrated to differ significantly depending on the geographical origin and ethnicity of populations. The expansion frequency is higher in Caucasian European populations, particularly in Scandinavian countries but is rarer in Asian populations (Tang et al., 2016). Moreover, the finding that most of the cases with *C9orf72* expansion have the same 20 SNPs in a 140-kb long region surrounding the *C9orf72* gene corroborates the Scandinavian founder effect (Byrne et al., 2012; Mok et al., 2012; Ratti et al., 2012). The founder effect is believed to date back to the Vikings and, remarkably, the regions in which pathogenic expansion is the highest are on the migratory routes of the Vikings (Pliner et al., 2014); however, the geographic localization of Central Anatolia in Turkey is outside of these routes. Therefore, the low frequency of pathogenic expansions may have been due to the ethnic and geographical characteristics of the Turkish cohort.

We studied 2 different SNPs (rs4879515 and rs3849942) included in the previously reported risk haplotype (Mok et al., 2012). We identified at least one risk allele for each SNP in all intermediate and possibly pathogenic repeat carriers. For rs4879515, we compared the distribution of the repeat lengths for the T and C alleles and observed that ≥8 unit repeats were strongly correlated with the tagging T allele ($p < 0.001$). The only case with possibly pathogenic repeats was homozygous for this tagSNP. Similarly, for SNP rs3849942, we observed that ≥8 unit repeats alleles showed a strong correlation with the A allele ($p < 0.001$). These results support the findings of previous studies (DeJesus-Hernandez et al., 2011; Nuytemans et al., 2013; Van der Zee et al., 2013). Although the reasons for this correlation remain unknown, the repeat region of this haplotype may be unstable, thus contributing to the expansion. As suggested by Nuytemans et al. (2013), genotyping these SNPs could be used as a screening test for identifying repeat copy carriers.

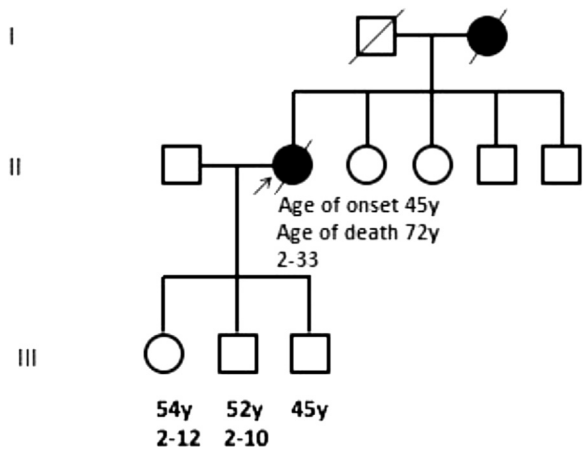

Fig. 2. Pedigree of the case with possibly pathogenic repeats.

Table 5
Genotypes in intermediate and possibly pathogenic repeat carriers and family members

SNPs	Risk allele[a]	E1	E1-1	E1-2	I-1	I-2	I-3	I-4
rs4879515	T	TT	CT	CT	CT	CT	CT	CT
rs3849942	A	GA	GA	GA	GA	GA	GA	GA
C9orf72 repeats		2–33	2–12	2–10	6–21	4–24	2–23	2–24

Key: E, expansion-positive patient; E1-1, unaffected son of an expansion-positive patient; E1-2, unaffected daughter of an expansion-positive patient; I, intermediate repeat carrier; SNP, single-nucleotide polymorphism.
[a] Reported by (Mok et al., 2012).

The ethnicity of our patients could not be directly verified, but all patients were Turkish and the pedigree analysis of both the FTLD and control cohorts revealed that the ancestors of most of the patients originated in Central Anatolia. It is well known that Turkey has a heterogeneous genetic background resulting from the large number of historical migrations. Hence, the presence of traces of Asian and European genetic identities is unsurprising. Because most cases included in the cohort of this study had origins in Central Anatolia, the low level of *C9orf72* expansion might represent a regional characteristic. The observed repeat numbers in the control group also support this notion. Although the most commonly observed number of G_4C_2 repeats is 2, followed by 5 and 8 repeats, studies have also reported >30 repeats among healthy populations (Beck et al., 2013; Ogaki et al., 2013; Renton et al., 2011; Van der Zee et al., 2013). In parallel with the literature, the range of G_4C_2 repeats detected in our control cohort was between 2 and 12 repeats, and the aforementioned repeat numbers of 2, 5, and 8 were commonly observed. In this study, neither possibly pathogenic nor intermediate *C9orf72*-expansion carriers were detected in the control group. These results showed that *C9orf72* G_4C_2 repeats among healthy Turkish patients are not as high as in the Scandinavian population. No other data on allelic expansion among a healthy Turkish population could be obtained for comparisons to determine whether regional differences exist in Turkey.

4.2. Intermediate/possibly pathogenic repeats and clinical findings

The threshold between the normal repeat alleles and pathogenic repeat expansion has not yet been fully determined. Although normal repeat lengths have been identified as up to 24 units in healthy control groups, they have also been reported as 32 to 35 repeats in healthy patients. The reduced penetrance feature of *C9orf72*-based diseases and the variable age at disease onset are considered the reasons for these different findings. Animal models

Table 6
Allele frequencies of single-nucleotide polymorphism rs4879515 and rs3849942 in patients and controls

Alleles	FTLD (%)	Control (%)	*p*-value
rs4879515			
C	80 (40.0)	130 (65.0)	<0.001
T	120 (60.0)	70 (35.0)	<0.001
rs3849942			
G	108 (54.0)	160 (80.0)	<0.001
A	92 (41.0)	40 (20.0)	<0.001

Key: FTLD, frontotemporal lobar degeneration.

Table 7
Distribution of tagging alleles in the G_4C_2-repeat groups

Repeat numbers	<8 repeat carriers (%)	≥8 repeat carriers (%)	*p*-value
rs4879515 T	41/63 (65)	37/37 (100)	<0.001
rs3849942 A	29/63 (46)	37/37 (100)	<0.001

p = Level of significance according to the χ^2 test.

have shown a minimal pathogenic expansion limit of 30 repeats, and numerous studies have taken this possible pathogenic expansion limit into consideration (DeJesus-Hernandez et al., 2011; Guven et al., 2016; Mahoney et al., 2012; Renton et al., 2011; Xu et al., 2013).

Expansion-based diseases such as Huntington disease and spinocerebellar ataxia involve a gray zone between the normal repeat range and the minimal number of pathologic repeats. In *C9orf72*-based diseases, although the number of >30 repeats is generally accepted as the possible pathogenic limit, the clinical characteristics of patients with intermediate repeats have not been reported in detail, and controversial results have been obtained for cases with intermediate repeats.

In a study of 109 FTD cases, comprising 4 mutation carriers (>30 repeats) and 5 probands involving 20–22 repeats, cases with intermediate repeats were reported as not differing clinically from cases with pathogenic expansion. Intermediate repeats were reported as possibly being related to frontotemporal deterioration (Gómez-Tortosa et al., 2013). Similarly, in our series, the clinical features of intermediate repeat cases were highly similar to cases with possibly pathogenic repeat expansion. Because the frequency of the intermediate repeats carriers was 4% and a statistically significant difference was observed between the FTLD and control cohorts in terms of repeat numbers ($p < 0.01$), we suggest that intermediate repeats should be considered a factor that might cause disease. The presence of tagging SNPs for rs4879515 and rs3849942 in all of the intermediate repeat carriers and the expansion-positive patients suggests that they share the common *C9orf72* risk haplotype.

In addition, psychotic symptoms were observed in 75% of intermediate repeat carriers. The relationship between the intermediate/pathogenic expansion carriers and the presence of psychotic symptoms was statistically significant. In 17 cases with psychotic symptoms, the frequency of *C9orf72*-expansion positivity was 5.9%, whereas it was 17.7% among the intermediate repeat carriers. In previous studies, *C9orf72* expansion has been shown to increase the frequency of hallucination among FTD cases (Kertesz et al., 2013; Sharon et al., 2012; Snowden et al., 2012). Devenney et al., 2017 determined psychotic symptoms in 34% of their patients and reported that *C9orf72*-expansion carriers exhibited higher levels of psychotic features compared to noncarriers ($p = 0.006$), and that auditory, optic, or tactile hallucinations were observed in 36% of *C9orf72* carriers and 17% of noncarriers. In a study conducted by Snowden et al. (2012), psychosis was observed in 38% of cases with *C9orf72* expansion, whereas psychotic symptoms were detected in only 4% of cases with a normal number of repeats. These studies asserted that a strong relationship exists between *C9orf72* expansion and psychosis, and that repeat expansion in the *C9orf72* gene might play a critical role not only in FTD-motor neuron disease but also in the development of late-onset psychosis (Snowden et al., 2012). Similarly, the symptoms of a *C9orf72*-expansion–positive case started with severe anxiety at the age of 45 years with the gradual involvement of stereotyped movements, apathy, episodic memory impairment, and visual and auditory hallucinations. Family members described similar symptoms in the mother of the proband, but she could not be analyzed because she had died. The *C9orf72* repeats of the proband's 2 children were within the normal range; however, both children had a risk allele with ≥8 repeat units.

In conclusion, our data show that *C9orf72* G_4C_2-repeat expansion is not widespread in the Central Anatolia population. More precise results for the distribution of *C9orf72*-expansion carriers in Turkey may be obtained by analyzing cases from all regions of Turkey. In addition, we believe that intermediate repeats may be a risk factor for FTLD, particularly in cases of psychotic symptoms. Methylation, expression, and postmortem studies involving cases observed to have intermediate repeat would play an important role

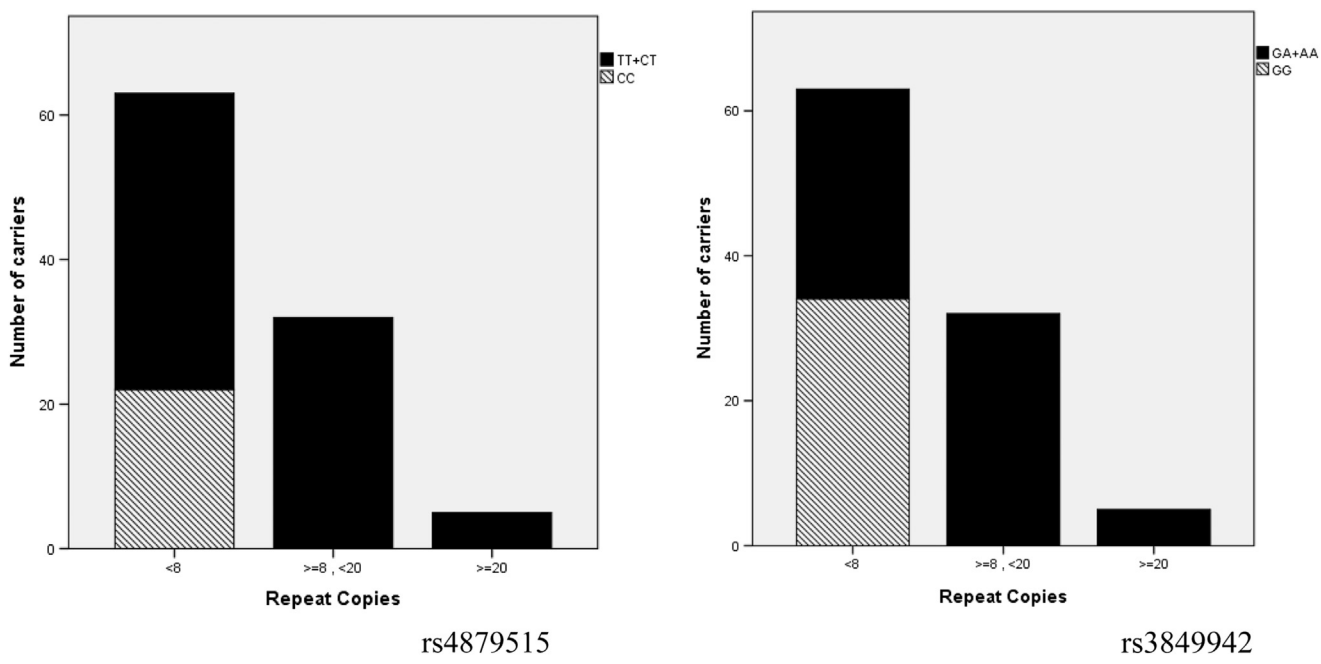

Fig. 3. Number of carriers tagging single-nucleotide polymorphism rs4879515 and rs3849942 genotype corresponding to different G_4C_2-repeat copy carriers.

in the reassessment of >30 pathogenic cutoff, which is actually accepted.

Disclosure

The authors declare no conflicts of interest. DNA samples were collected with the approval of the relevant institutional ethics boards, and informed written consent was obtained from each participant.

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