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Fragile X Gray Zone Alleles Are Associated With Signs of Parkinsonism and Earlier Death

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Abstract

Background: Premutation size (55–199 CGG repeats) in the fragile X mental retardation 1 (*FMR1*) gene cause fragile X-associated tremor/ataxia syndrome, but it is unclear whether smaller "gray" zone expansions of 41–54 repeats are also associated with movement disorders. The objectives of this study were to determine the association between the *FMR1* gene gray zone expansions, AGG interspersions, and the presence of parkinsonism and motor and cognitive function in an elderly community-based population.

Methods: Automated *FMR1* polymerase chain reaction was performed on existing samples from 2 longitudinal aging studies whose subjects agreed to brain donation. A detailed clinical evaluation including a modified Unified Parkinson's Disease Rating Scale score, a composite score of global motor function, 17 cognitive tests summarized as a global measure of cognition, and neuropathological examination were obtained for genotyped participants.

Results: The average age of the population (n = 2362) was 85.9 ± 7.3 years, and average age at death was 88.6 ± 6.4 years (n = 1326), with 72% women. The prevalence of *FMR1* gray zone alleles was 5.2% (122 of 2362). There was no difference between participants with gray zone expansions or those lacking AGG interspersions compared with normal participants in global cognition, global motor function, clinical diagnosis, or pathological changes. Gray zone alleles were associated with signs of parkinsonism in men ($P = 0.01$), and gray zone carrier men weremore likely to die (hazard ratio, 2.34; 95%confidence interval, 1.31–4.16).

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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

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Conclusion: This is the largest study to investigate gray zone alleles in a community population. The key findings are that in men, the gray zone allele is associated with signs of parkinsonism and higher risk of death, but not with intranuclear neuronal inclusions.

Keywords

atypical parkinsonism; fragile X; FXTAS

More than 22.5 million people in the United States are carriers of smaller "gray zone" expansions in the trinucleotide repeat of the fragile X mental retardation 1 (*FMR1*) gene. Fragile X–associated disorders are caused by a CGG repeat expansion in the 5′ untranslated region of *FMR1*, located on the X chromosome, and include fragile X–associated tremor/ataxia syndrome (premutation range, 55–199 CGG repeats) and fragile X syndrome (full mutation range, >200 CGG).[1] However, there has been less focus on disorders that occur in *FMR1* "gray zone" (41–54 CGG) expansion carriers or in those who lack AGG interspersions within the *FMR1* CGG repeat.

Prior studies by our group screening consecutive patients with parkinsonism at a tertiary movement disorder clinic showed that 11% of women with Parkinson's disease (PD, n = 98) were gray zone carriers compared with a control rate of 4.4% (n = 135; odds ratio, 3.23; confidence interval, 1.2–8.6).[2] Hedrich et al found similar results in women with parkinsonism (n = 208), who had a rate of 7.2% gray zone alleles compared with a control rate of 4.6%.[3] Screens conducted in Australia and Tasmania showed that both women and men with parkinsonism had higher rates of gray zone alleles at 7.5% (n = 228) compared with a control rate (n = 578) of 3.3%.[4,5] However, screening studies focused in PD cohorts have not yielded many gray zone carriers.[6] The results of these studies cause a lack of clarity on the role of this genetic factor in the development of parkinsonism.

It has been reported that individuals with *FMR1* gray zone expansions have elevated and potentially toxic mRNA levels that are similar to premutation carriers.[7,8] Pathologically, premutation carriers have neuronal intranuclear inclusions that contain *FMR1* mRNA throughout the central nervous system.[9,10] A second genetic feature of the *FMR1* CGG repeat is the presence of typically 2–3 AGG interspersions within the repeat, which stabilize the repeat on transmission. A lack of AGG interspersions within the *FMR1* CGG repeat destabilizes the trinucleotide repeat, resulting in a CGG that expands when transmitted from parent to child.[11] Pilot data from our group suggest that a loss of AGG interspersions may be associated with the presence of movement disorders or cognitive deficits in adult populations.

Gray zone *FMR1* research has been scarce because gray zone carriers are difficult to ascertain. Only gray zone genes with fewer AGGs (0–1) are unstable, causing expansion of the *FMR1* repeat as it is passed down within families. It typically takes more than 3 generations for a gray zone repeat to expand into a full mutation range, which frequently triggers family testing. This study was community based with ascertainment of gray zone carriers and AGG interspersions in 2 longitudinal studies in the elderly community characterizing phenotype, genotype, and pathological findings.[12,13] The hypothesis was that *FMR1* gray zone carriers in the elderly community would be more likely than noncarriers

to have parkinsonism, neuronal and glial intranuclear inclusions on pathology, and ataxia similar to that seen in fragile X–associated tremor ataxia syndrome.

Methods

Data were ascertained from 2 large epidemiological studies being conducted at Rush University in Chicago: the Religious Orders Study (ROS) and the Rush Memory and Aging Project (MAP).[12,13] Both studies provided (1) clinical data on measures of parkinsonism and motor and cognitive function, (2) DNA for *FMR1* testing, and (3) autopsy data and brain tissue, available from a large number of subjects who agreed to organ donation at study entry. The ROS is an epidemiological study evaluating older Catholic nuns, priests, and brothers from across the United States who have had structured annual clinical evaluations since 1994.[12] At baseline, the participants had a medical history, neurological examination, and cognitive function testing. All participants signed informed consent, had an annual evaluation, had blood collected, and signed an Anatomic Gift Act for brain donation. They also signed a repository consent to allow their data and biospecimens to be repurposed. Follow-up evaluations occur at 1-year intervals and are identical to baseline. Examiners are blinded to prior data, and the autopsy rate is >90%. The MAP[13] is an epidemiological study evaluating community-dwelling persons living in >40 retirement communities or senior subsidized-housing facilities or individual homes in the Chicago area. Participants have annual structured clinical evaluations that include a medical history, cognitive testing, neurological examination, blood collection, and donation of brain, spinal cord, nerve, and muscle at time of death. All participants sign an informed consent, Anatomic Gift Act and repository consent. Data collection for both ROS and MAP are collected by the same team, with 1 trainer for research assistants and 1 trainer for nurses. The autopsy rate is >80%. Persons eligible for this project were enrolled in either ROS or MAP and had DNA available for genotyping. Each study was approved by a Rush University Medical Center Institutional Review Board.

Clinical Analyses

Demographic information collected from both cohorts included age, sex, race, ethnicity, and years of education. Individuals were classified into 3 categories as previously described: dementia and its principal causes (eg, Alzheimer’s disease, vascular dementia), mild cognitive impairment (MCI), and cognitively normal.[14,15] A board-certified clinical neuropsychologist, blinded to the person’s age, sex, and race, reviewed the results of all cognitive tests and data on educational level, occupation, motor deficits, and effort and rendered a clinical judgment regarding the presence of impairment in episodic memory and other cognitive domains. An experienced heath care professional evaluated participants and made diagnostic decisions based on a review of all available data from that year. The diagnosis of dementia and Alzheimer’s disease (AD) followed the criteria of the joint working group of the National Institute of Neurologic and Communicative Disorders and Stroke and the Alzheimer Disease (AD) and Related Disorders Association.[16] The diagnosis of MCI was based on 2 criteria: (1) the presence of cognitive impairment as determined by a neuropsychologist after a review of raw test scores and educationally adjusted impairment ratings and (2) the absence of dementia as determined by an experienced health care

professional based on a review of all available clinical data and in-person evaluation of the participant.[14] The parkinsonism and motor and cognitive function scores were determined for each subject, with data from the most recent examination used for the primary outcome measure in this project.

The parkinsonism score was the total score of a modified Unified Parkinson's Disease Rating Scale (mUPDRS).[17] To facilitate its implementation for nonphysicians, who perform some of the assessments, the mUPDRS was modified to minimize ambiguity and enhance uniformity, and the wording of some of the items was altered to make it applicable to non-PD participants[18] and it was subsequently validated for use in a community population.[19] Response options were added and others specified further. A turning item from the parkinsonian assessment in the Consortium to Establish a Registry for AD[20] was added to increase the number of items assessing postural stability. The total possible score for the modified UPDRS was 127. Logarithmic transformation of the score was used for the regression analysis.

The global composite motor score included multiple tests of both upper and lower limb function, including the Purdue Pegboard Test, grip and pinch strength, gait, and balance.[21] This measure has been validated for use in a community-based elderly population.[22,23] The variables that were part of the summary motor measure are scaled by median value and then averaged, with larger values meaning good motor function. Scaling was sex specific given differences in measures of strength. The global composite motor score was used as interval data. In addition, an ataxia score was created that included gait speed, tandem stand, and tandem walk.[22] This score was also treated as interval data.

The global composite cognition score included measures of episodic memory, semantic memory, working memory, perceptual speed, and visuospatial ability covered by 19 cognitive tests.[14] Raw scores on each component test were converted to *z* scores using the baseline mean (SD) from all participants in both studies and averaged to form the composite global cognition measure.[24] Mean and standard deviation were used to compute *z* scores, with a negative *z* score meaning that the subject has an overall score lower than the average of the entire cohort at baseline.

Neuropathology Analyses

Brain analyses were performed by a board-certified neuropathologist blinded to all clinical data. The mean postmortem interval was a mean of 7.9 ± 5.47 hours. Brains were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer. Following an external examination of the brain to document macroscopic infarcts and grade the degree of atherosclerosis, blocks were dissected from 11 brain regions (midfrontal, midtemporal, inferior parietal, occipital, and entorhinal cortices with attached white matter, amygdala, midhippocampus, anterior thalamus, cerebellum, and midbrain at the level of the exiting third-nerve fibers). In addition, a block of the ventral pons was obtained for this study. Blocks were processed using standard techniques and embedded in paraffin.[25] Sections (6 μm) were stained with hematoxylin and eosin for detection of pathologies such as microinfarcts, arteriolosclerosis, and hippocampal sclerosis as described previously.[25] Sections of the midfrontal gyrus and ventral pons were stained with Luxol fast blue and hematoxylin and eosin to assess white-

matter pallor, which was graded as present or absent. Modified Bielschowsky silver stain was used to quantitate neuritic and diffuse plaques and neurofibrillary tangles in 5 brain regions (frontal, temporal, entorhinal, and parietal cortices and hippocampus), having the highest density of these structures as described previously.[25] The National Institute on Aging-Reagan criteria[26] were used with intermediate and high likelihood cases indicating a pathologic diagnosis of AD. The degree of nigral neuronal loss was assessed in hematoxylin and eosin–stained sections (6 μm) of the substantia nigra. Nigral neuronal loss was graded using a semiquantitative scale as none, mild, moderate, and severe as described previously.[27]

Immunohistochemistry was done using a Leica-Bond Max Autostainer (Leica Microsystems, New Buffalo, IL). Sections of the midfrontal gyrus, hippocampus, and ventral pons were immunostained with antiubiquitin antibody (1;1000 dilution; Agilent Technologies, Santa Clara, CA), to localize the intranuclear neuronal and glia inclusions characteristic of fragile x–associated tremor-ataxia syndrome (FXTAS). Immunohistochemistry for α-synuclein was used to detect Lewy bodies in 6 brain regions (entorhinal, cingulate, midfrontal, middle temporal, and inferior parietal cortices, amygdala, and substantia nigra), as described previously.[28] The transactive response DNA-binding protein 43 (TDP-43) pathology, a component of limbic-predominant age-related TDP-43 encephalopathy neuropathologic change (LATE-NC) was detected as described previously.[29] Cerebral amyloid angiopathy was assessed in meningeal and intracortical vessels in 4 cortical sections (midfrontal, midtemporal, inferior parietal, and occipital) immunostained for β-amyloid (4G8, 1:9000; Covance Labs, Madison, WI) and graded as described previously.[30]

Genetic Testing

DNA, extracted from blood or brain, was obtained from the MAP or ROS repositories, and *FMR1* polymerase chain reaction (PCR) was performed using a highly sensitive method that detects all *FMR1* expansions and allows accurate quantification of allele-specific CGG repeat length,[31] including identification of AGG interspersions,[32] using primers that flank the CGG repeat sequence in addition to internal repeat primers. PCR products were subjected to capillary electrophoresis, and CGG repeat lengths for alleles are determined by comparison of the repeat “ladder” generated by the alleles in the sample with repeat ladders from controls with known previously sized CGG repeat lengths. AGG locations are defined by areas in which the ladder is lost because of loss of annealing of the internal CGG repeat primer. AGG locations were confirmed by comparison with controls with known AGG positions. In women, allele sizes were broken down into either the larger or smaller allele for the purpose of statistical analysis. Activation ratios for women were determined using a separate commercially available kit (Asuragen, Inc., Austin, TX) by gene-specific PCR of genomic DNA that had been treated with HpaII or mock treated.

Statistical Analyses

Data from the 2 studies (MAP and ROS) were combined for the analysis. For each participant, data from the most recent visit were used. Analyses were performed separately for men and women. Demographics, genetic, and neuropathological data were compared between normal participants and gray zone allele carriers. Regression analyses were

conducted to examine the association between gray-zone expansions and the presence of parkinsonism and motor and cognitive function with adjustment for age, race, education, and ethnicity. AGG interspersions were also included in the regression models as their presence or absence to investigate their association with parkinsonism and motor and cognitive function. All statistical analyses were performed with SAS 9.3 (SAS Institute Inc., Cary NC).

Results

The study population included 1275 participants from MAP and 1087 participants from ROS. Demographics of the combined population showed that 1690 (72%) were women, and 672 (28%) were men (Table 1). Of the 2362 participants included, the prevalence of *FMR1* gray-zone allele carriers was 5.2% (122 of 2362) and that of the *FMR1* premutation carriers was 0.2% (4 of 2362). The majority of gray zone allele carriers were women (83%), and premutation carriers were 50% women. The average age of the population was 85.9 ± 7.3 years, and the average age at death was 88.6 ± 6.4 years. Ninety-seven percent of the participants were white, 2% were Hispanic, and 1% were black or Asian. There were some demographic differences between the 2 populations. Men with a gray zone expansion were younger at their last visit by approximately 4 years ($P = 0.01$) and had a lower educational level of approximately 2 years ($P = 0.03$). Gray zone carrier women were younger at death compared with normal allele carriers by approximately 3 years ($P < 0.001$). After adjusting for potential confounders, including alcohol use, smoking, hypertension, cancer, diabetes, head injury, thyroid disease, congestive heart failure, claudication, heart disease, or stroke, younger death in the gray zone carrier women remained. Regarding cognitive status, 38% of the study population had normal cognition, 27% had MCI, 33% met criteria for AD, and 2% had another cause for dementia.

The average CGG repeat size in non-gray zone carriers was 28.4 ± 4 (men) and 30.9 ± 3 (women, larger allele); see Table 2. The average CGG repeat size of the gray zone alleles was 45.5 ± 5 in men and 44.2 ± 3 in women. Five percent of men and 10% of women (both alleles combined) lacked AGG interspersions. None of the gray zone carrier men lacked AGG interspersions, and only 6% of gray zone carrier women lacked AGG interspersions. Lack of AGG interspersions between gray zone carriers and noncarriers was not significant in women (0% vs 0.38%, $P = 1$). Gray zone carrier women (89 of 101) had a mean activation ratio of 56% (SD, 23%). A premutation carrier woman with 59 and 23 *FMR1* CGG repeats was identified and had neuropathology available. She was 91 years old at death and had a modified UPDRS of 38, with scores of moderate kinetic hand tremor bilaterally, mild rest tremor, mild to moderate bradykinesia, mild rigidity, moderate gait abnormality (ataxia not specified), and severe postural instability.

In univariate analysis performed by sex, there was no significant difference between gray zone carriers and noncarriers in the measures of the parkinsonism score, global motor score, and global cognition score (Tables 3 and 4). Potential modifiers (race and ethnicity) that were not significantly related to the key measures were removed before multivariate analysis. In multivariate analysis, the parkinsonism score significantly differed between gray zone carrier men and noncarrier men ($\beta = 0.23$, SE = 0.09, $P = 0.01$), with gray zone carrier

men having a higher score. This relationship was not seen in women (β = 0.01, SE = 0.04, *P* = 0.81). Multivariate analysis was repeated to include a study population variable (ROS or MAP), and the results did not change. After adjusting for education, hazard ratios confirmed that gray zone carrier men were more likely to die (hazard ratio, 2.34; 95% CI, 1.31–4.16), but there was no significance for women.

Sixty-six samples were included for neuropathological assessment of intranuclear inclusions (n = 31, gray; n = 35, normal). For the men, average *FMR1* CGG repeat size was 45.2 ± 4.5 for gray zone alleles and 28.3 ± 4.2 for normal alleles, with 4.6% of normal alleles and no gray zone alleles lacking AGG interspersions. For the women, average *FMR1* CGG repeat size was 43.7 ± 2.8 for gray zone alleles and 28.8 ± 4.4 for normal alleles, with 5.5% of normal alleles and 7% of gray zone alleles lacking AGG interspersions (*P* < 0.01). None of the samples from gray zone carriers contained the neuronal inclusions classically seen in FXTAS (Table 5), although inclusions were found in the premutation carrier described above who had come to autopsy. Inclusions were not seen in an additional 2 premutation carriers with premutation CGG repeats of 55 and 59. Analyses of additional age-related pathologies (Lewy bodies, neuronal loss in the substantia nigra, AD pathology, hippocampal sclerosis, LATE-NC change, amyloid angiopathy, atherosclerosis, macroscopic or microscopic infarcts) in 69 gray zone carriers compared with 1257 normal allele carriers did not reveal any differences between gray zone and noncarriers.

Discussion

This is the largest study to investigate gray zone alleles in an older community population. It is also the first to examine neuropathology in a larger set of gray zone carriers. There are several findings from this study. In examination of the demographic data in both male and female gray zone carriers, age at death was approximately 3 years earlier than in individuals who were noncarriers. Gray zone carrier men also had a lower educational level than noncarrier men by almost 2 years. In regard to phenotype, gray zone carrier men were more likely to have a higher parkinsonism score compared with noncarrier men. There were no phenotypic differences between gray zone carrier women and noncarrier women. No intranuclear inclusions were seen in the gray zone carriers. However, more than 90% of gray zone carrier men had neuronal depletion in the substantia nigra, and there were cases of both men and women gray zone carriers that had pathological Lewy body disease.

In this community-based sample, the earlier age of death for gray zone carriers is a novel finding, likely because this association has not been previously investigated, nor has there been such a large study of gray zone carriers in an aged population. Neuronal intranuclear inclusions, the hallmark of FXTAS and a sign of *FMR1*-related neurodegeneration, were not present in these individuals. Neurodegeneration from PD, suggested by higher levels of parkinsonian signs in the gray zone carrier men, was not supported by the presence of Lewy bodies or cell loss in the substantia nigra on pathology. Alzheimer’s disease or vascular disease are also not likely the explanation given the lack of differences in neuropathological findings consistent with AD, amyloid angiopathy, or other vascular abnormalities. This finding of earlier death may be explained by a synergistic effect of the gray zone allele and a

second risk factor for neurodegenerative disease or a second factor that is independent of the gray zone allele given prior cases of premutation carriers having dual pathology.[33]

Gray zone carrier men had higher parkinsonism scores compared with noncarriers, which is consistent with studies reporting parkinsonism signs in gray zone carrier men. The lack of higher parkinsonism scores in women in this study, despite prior reports from our group and others[5,34] may be explained by the methods of ascertainment. The association may be harder to identify in women in a community-based sample, unlike in women who have developed clinical parkinsonism or meet criteria for PD because of the presence of X inactivation and milder phenotypes overall.

Genetic counseling ramifications from this study for individuals with *FMR1* gray zone alleles include confirmation of the association of signs of parkinsonism later in life for gray zone carrier men. A slightly shorter life expectancy may also be associated, if the gray zone carrier lives to older age. There is no association with a lack of AGG interspersions and neurological signs in gray zone carriers and no clear neuropathological changes that can be expected. Most of the genetic counseling of gray or intermediate-zone allele carriers occurs in women of childbearing age, and changes to counseling should be considered based on these results.

In interpreting the results of this study, a few caveats should be considered. First, this elderly community-based sample does not represent an unbiased population sample or a neurologist's clinic at which these patients might present for clinical care. For this reason, it is unknown whether these findings will translate into other populations. In addition, measures directly relevant to the study hypothesis, such as scales solely focused on cerebellar signs, are not included in the data collection for the cohorts, and this is a limitation of the study. Second, this cross-sectional study cannot establish causality of a link between the genotype and phenotype. Third, although the sample size of gray zone carriers is higher than most other adult populations previously studied, careful examination by a movement disorder neurologist was not performed. Thus, under- or overestimation of signs of parkinsonism may have occurred. An outcome of this study is to provide additional information to inform clinicians and counselors of the neurological ramifications of a gray zone allele, specifically that some gray zone carriers may develop movement disorders in their elderly years. It also establishes a large database that can be used to address other questions in the fragile X field, such as concerns about "low normal" or "low-zone" allele effects (CGG ≤23). Deeper study of these gray zone allele carriers prospectively is planned in future studies in this population, which will build on the results presented here.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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TABLE 1.

Demographics of the study subjects

Men			
Variable	**Normal (n = 651)**	**Gray zone (n = 21)**	**P**
Age at last visit, mean (SD)	85.66 (6.55)	81.86 (6.28)	0.01
Age at death, mean (SD)[a]	87.14 (6.11)	84.14 (4.71)	0.09
Race, n (%)			0.21
White	630 (96.92)	19 (90.48)	
Black	16 (2.46)	2 (9.52)	
Native American	1 (0.15)	0	
Asian or Pacific Islander	3 (0.46)	0	
Spanish, n (%)			0.57
Yes	25 (3.84)	1 (4.76)	
No	626 (96.16)	20 (95.24)	
Educational level, mean (SD)	17.07 (4.03)	15.14 (3.77)	0.03
Cognitive diagnosis, n (%)[b]			0.38
No cognitive impairment	133 (34.46)	3 (25)	
Mild cognitive impairment	95 (24.61)	3 (25)	
Mild cognitive impairment with another cause of cognitive impairment	5 (1.3)	1 (8.33)	
Alzheimer's disease	126 (32.64)	4 (33.33)	
Alzheimer's disease with another cause of cognitive impairment	19 (4.92)	1 (8.33)	
Other dementia	8 (2.07)	0	

SD, standard deviation.
[a]Missing values n = 261.
[b] **n = 274.

Women			
Variable	**Normal (n = 1589)**	**Gray zone (n = 101)**	***P***
Age at last visit, mean (SD)	86.1 (7.5)	85.45 (6.9)	0.4
Age at death, mean (SD)[902]	89.6 (6.32)	86.56 (6.32)	< 0.001
Race, n (%)[1]			0.44

Men			
Variable	**Normal (n = 651)**	**Gray zone (n = 21)**	**P**
White (1)	1499 (94.4)	93 (92.08)	
Black (2)	77 (4.85)	7 (6.93)	
Native American (3)	3 (0.19)	0	
Asian or Pacific Islander (6)	9 (0.57)	1 (0.99)	
Spanish, n (%)			0.28
Yes (1)	60 (3.78)	6 (5.94)	
No (2)	1529 (96.22)	95 (94.06)	
Educational level, mean (SD)[1]	15.77 (3.47)	16.37 (2.96)	0.06
Cognitive diagnosis, n (%)[943]			0.08
No cognitive impairment	214 (30.66)	23 (46.94)	
Mild cognitive impairment	156 (22.35)	12 (24.49)	
Mild cognitive impairment with another cause of cognitive impairment	8 (1.15)	0	
Alzheimer’s disease	274 (39.26)	10 (20.41)	
Alzheimer’s disease with another cause of cognitive impairment	32 (4.58)	3 (6.12)	
Other dementia	14 (2.01)	1 (2.04)	

Superscript indicates number of missing values.

TABLE 2.

Genetic results

Men			
Variable	**Normal (n = 651)**	**Gray zone (n = 21)**	***P***
CGG repeat size, mean (SD)	28.38 (4.34)	45.48 (4.61)	< 0.0001
AGG, n (%)[5]			
Yes	614 (95.05)	21 (100)	0.62
0	32 (4.95)	0	0.08
1	176 (27.24)	4 (19.05)	
2	429 (66.41)	15 (71.43)	
3	9 (1.39)	2 (9.52)	

Superscript indicates number of missing values.

Women						
Variable	**Normal larger allele (n = 1589)**	**Normal smaller allele (n = 1589)**	**Gray zone larger allele (n = 101)**	**Gray zone smaller allele (n = 101)**	***P* value 1[a]**	***P* value 2[b]**
CGG repeat size, mean (SD)	30.91 (3.07)	26.59 (4.27)	44.24 (3.26)	29.54 (4.37)	< 0.0001	< 0.0001
AGG, n (%)[1]						
Yes	1538 (96.85)	1464 (92.19)	95 (94.06)	95 (94.06)	1	1
0	50 (3.15)	124 (7.81)	6 (5.94)	6 (5.94)	<0.0001	<0.0001
1	245 (15.43)	570 (35.89)	20 (19.8)	14 (13.86)		
2	1242 (78.21)	894 (56.3)	65 (64.36)	80 (79.21)		
3	51 (3.21)	0	8 (7.92)	1 (0.99)		
4	0	0	2 (1.98)	0		

Superscript indicates number of missing values.

[a]Comparison between normal and gray zone on larger allele.

[b]Vomparison between normal and gray zone on smaller allele.

TABLE 3.

Outcomes in men

Univariate analysis			
Outcome variable	**Normal (n = 651)**	**Gray zone (n = 21)**	**P**
Global cognition score, mean (SD)[3]	−0.5 (1.08)	−0.37 (0.62)	0.38
Global motor score, mean (SD)[35]	0.75 (0.25)	0.76 (0.27)	0.76
Parkinsonism score, median (IQR)[32]	43 (26)	50 (29)	0.06
Superscript indicates number of missing values.			

Multivariate analysis			
	Global cognition score estimate (*P*)	**Global motor score estimate (*P*)**	**Parkinsonism score (log scale) estimate (*P*)**
Age at last visit	**−0.04 (< 0.0001)**	**−0.02 (< 0.0001)**	**0.02 (< 0.0001)**
Education	**0.04 (0.001)**	**0.01 (0.001)**	
Group (gray zone vs normal)	0.01 (0.96)	−0.05 (0.29)	**0.25 (0.01)**
Study (MAP vs ROS)	**0.25 (0.004)**	**0.17 (< 0.0001)**	**−0.1 (0.001)**
AGG (yes vs no)	−0.24 (0.21)	**0.1 (0.02)**	0.04 (0.60)

Hazard ratios

	Hazard ratio (95% CI)	*P*
Education	1.04 (1.02–1.07)	0.002
Gray zone vs normal	2.27 (1.27–4.05)	0.006

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TABLE 4.

Outcomes in women

Univariate analysis			
Outcome variable	**Normal (n = 1589)**	**Gray zone (n = 101)**	***P***
Global cognition score, mean (SD)[3]	−0.5 (1.18)	−0.33 (0.98)	0.11
Global motor score, mean (SD)[53]	0.77 (0.26)	0.82 (0.25)	0.1
Parkinsonism score, median (IQR)[33]	44 (25)	42.5 (23)	0.91
Superscript indicates number of missing values.			

Multivariate analysis			
	Global cognition score estimate (*P*)	**Global motor score estimate (*P*)**	**Parkinsonism score (log scale) estimate (*P*)**
Age at last visit	**−0.06 (< 0.0001)**	**−0.02 (< 0.0001)**	**0.02 (< 0.0001)**
Spanish (Y vs N)	**−0.34 (0.01)**		
Education	**0.07 (< 0.0001)**	**0.01 (0.0002)**	
Group (gray zone vs normal)	0.08 (0.46)	0.02 (0.35)	0.01 (0.71)
Study (MAP vs ROS)	**0.39 (< 0.0001)**	**0.18 (< 0.0001)**	**−0.12 (<0.0001)**
AGG (yes vs no)	0.16 (0.71)	0.01 (0.87)	−0.24 (0.12)

Hazard ratios		
	Hazard ratio (95% CI)	***P***
Education	1.02 (0.996–1.04)	0.11
Gray zone vs normal	1.33 (0.998–1.77)	0.052

TABLE 5.

Neuronal and astrocyte assessed for intranuclear inclusions (n = 22)

Brain regions	Area, mm^2	Counts, mean (SD)	
		Neurons	Astrocytes
Hippocampus, CA1	8.9 (3.1)	1727 (856)	2915 (1424)
Dentate gyrus	0.7 (0.2)	2560 (945)	39 (93)
Hippocampus, Hilus/CA4	4.7 (1.7)	712 (312)	1820 (852)
Midfrontal cortex	19.9 (8.8)	4498 (2537)	12,092 (6852)
Midfrontal white matter	12.1 (7.0)	—	18,304 (11,053)
Basis pontis	26.8 (3.5)	2228 (726)	18,698 (4761)