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Journal

Movement Disorders, 33(7)

ISSN

0885-3185

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Publication Date

2018-07-01

DOI

10.1002/mds.27420

Peer reviewed

HHS Public Access

Author manuscript

Mov Disord. Author manuscript; available in PMC 2019 July 01.

Published in final edited form as:

Mov Disord. 2018 July ; 33(7): 1178–1181. doi:10.1002/mds.27420.

Evidence for the role of *FMR1* grey zone alleles as a risk factor for parkinsonism in females

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Abstract

Objective—There is convincing evidence that small CGG expansion (41–54 repeats)- *FMR1* 'grey zone' alleles (GZ) contribute to the risk of parkinsonism in males, but there is insufficient corresponding data in females. This study intends to fill this gap.

Methods—We screened whole blood-derived DNA from a cohort of 601 females diagnosed with idiopathic Parkinson's disease (iPD), and from dry Guthrie blood spots from a local sample of 1005 female newborns (population controls), for the size of the *FMR1* CGG repeat using a PCR technique.

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DZL: Conception, organization and partial execution of research project; review of statistical analysis; writing of the first draft of a manuscript.

FT: Organization and execution of major aspects of research project; review and critique of manuscript.

GDM: Organization and execution of certain aspects of research project; review of manuscript.

MH: Organization and execution of certain aspects of research project; review of manuscript.

JPR: Organization and execution of certain aspects of research project.

MQB: Design and execution of statistical analysis.

DF: Organization and execution of certain aspects of research project.

ES: Conception and organization of research project; review and contribution to writing the final version of a manuscript.

Results—We found a significant excess (8.2%) of GZ carriers compared with 5.2% in the control sample, with a p-value of 0.009 for the difference in proportions.

Conclusion—*FMR1* grey zone alleles are a significant risk factor for parkinsonism in females. These population data and occasional reports of FXTAS-like or parkinsonian manifestations in carriers suggest possible mechanisms whereby the effects of these alleles synergize with the existing pathologies underpinning parkinsonism.

Keywords

FMR1 gene; grey zone alleles; Parkinson's disease; carrier screening; increased risk

Introduction

The multiple causes of Parkinson's disease (PD) result in a spectrum of phenotypes within the broad clinical picture of bradykinesia, rigidity and/or resting tremor, and postural instability, with or without Lewy body formation. A number of rare genetic causes have been established (notably LRRK2 and α-synuclein mutations), although not all (*e.g. Parkin* mutations) faithfully recapitulate the α-synuclein deposits in cytoplasmic Lewy bodies, which are the pathological hallmark of idiopathic Parkinson's disease (iPD)[1]. It was recently discovered that heterozygotes for mutations in the glucocerebrosidase gene have a 20- to 30-fold increased risk of developing PD, with an earlier age of onset and greater cognitive impairment than in the idiopathic disease[2].

In 2009 we reported that intermediate range (GZ) alleles of the fragile X mental retardation 1 (*FMR1*) gene, containing small (41–54) CGG repeat expansions, were significantly over-represented in a cohort of 228 male iPD patients from two Australian states[3]. Comparison with the frequency of GZ alleles in a sample of 578 Guthrie spots from consecutive local male newborns showed an increase in GZ carriers in the iPD patient cohorts (6.8%, *vs* 3.3% in controls), with an odds ratio (OR) of 2.36 (95% confidence intervals 1.20–4.63). A more recent screening of a larger independent cohort of 817 males from the Queensland Parkinson's Project (QPP) also showed a 2.5-fold excess of GZ carriers[4] compared with the same sample of newborn controls. In both studies the patients had been diagnosed using the UK Brain Bank criteria[5]. We discussed the inconsistent results from some other, earlier studies of males with iPD, which were attributed to inadequate sample sizes, falling well below the frequency stabilization threshold[4], and to other major problems inherent in this type of screening, such as the choice of control population or ascertainment issues.

Moreover, comparison of the prevalence rates with those early surveys is not straightforward because the definitions of the GZ alleles, especially of the lower threshold, varied between 35 and 45 CGG repeats[6]. Our own population studies, however, as well as more recent surveys, have considered that the lower repeat number should be 40/41. This is based on the threshold for elevation of *FMR1* mRNA blood levels[7] that were originally linked to neurotoxicity in carriers of *FMR1* premutations in the 55–200 CGG range.[8–11]. The lower end of this particular range was determined by the potential for expansion into the full mutation (>200) over a single generation[12].

Evidence from female cohorts is still limited. An excess (12%) of GZ carriers in 41–54 range was found in a small sample of 98 females from the USA diagnosed with iPD[13]. Similar results were reported in a Chinese sample of 147 females[14], with the rate of 6.5% compared with 0.0% in a small sample of population controls. Most recently an estimate of 11% of GZ allele carriers amongst females with iPD has been reported from the Movement Disorders Clinic in Denver, USA[15]. Here we present the data from a much larger sample, which provides statistical evidence for the significant role of *FMR1* GZ alleles as a risk factor for parkinsonism in females, and stimulates consideration of possible mechanisms of synergistic effect of these alleles with other iPD–predisposing factors.

Sample and Methods

We included 601 adult females clinically diagnosed with iPD by movement disorders experts, using the UK Brain Bank criteria[5]. This sample included 430 individuals from a larger cohort of iPD patients recruited since 2005 to participate in the Queensland Parkinson's Project as described previously[4]; and 171 subjects from the Australian Parkinson's Research Register encompassing three other states: Victoria, New South Wales and Western Australia. The population-based control data were obtained from newborn Guthrie blood spots collected, in an anonymous fashion, from an unselected sample of 1005 consecutive Australian females born in the State of Victoria in the years 2007/2008. A great majority of patients and controls were Caucasians of European origin residing in Australia. Since the screening of both groups was anonymous, we assumed, based on government data on ethnic composition of the general population, that both samples had a small admixture (<5%) of individuals of East and South-East Asian origin, which is the largest other ethnic group residing in this country.

Data collection and analysis from PD patients were approved by the Human Ethics Committees of Griffith University in Brisbane, and Monash and La Trobe Universities in Melbourne; screening of a control sample of the Victorian newborns was approved by the Human Ethics Committee at the Royal Children's Hospital in Melbourne. All PD participants gave informed consent for their involvement in the study.

CGG repeat size was measured on DNA extracted from whole blood or dry Guthrie blood spots. Testing of the major proportion of the iPD sample and of the Victorian newborn sample (for cross-checking and validation of dual samples) was performed at the MIND Institute, UC Davis, California, where genomic DNA was amplified using either a standard method[13] or a recently developed *FMR1* PCR method[16,17] utilizing primers flanking the CGG repeat sequence, in addition to internal repeat primers (Asuragen, Inc., Austin, TX). PCR products were subjected to capillary electrophoresis to determine allele size relative to known standards. Testing at the Victorian Clinical Genetics Services, Royal Children's Hospital in Melbourne, was conducted using PCR[18], with all assays fully validated by internal and external quality assessment to provide a precision of +/− one repeat.

Results

We identified 49 GZ allele carriers (range 41–54 CGGs) amongst 601 iPD females, compared with 52 in the sample of 1005 Victorian female newborns, with the expansion in heterozygous state in all identified carriers. Using a two-sample test for proportions, this represents a significantly greater percentage of GZ carriers in the iPD cohort (8.2%) than in the population-based cohort (5.2%), with the p-value for the difference in proportions = 0.009. Fisher's exact test generated a (two-sided) p-value of 0.019 for this difference. The odds ratio for the likelihood of being in the PD/GZ group was 1.62 with a 95% confidence interval of 1.06–2.49.

Discussion

The present results provide evidence for a significant role of *FMR1* GZ alleles as a risk factor for parkinsonism in females, and are consistent with the findings from both Australian studies in iPD males[3,4], and also with the earlier findings in smaller female samples[13–15]. The population-based data is supported by clinical reports of the occurrence of a mild version of the Fragile X-Associated Tremor Ataxia Syndrome (FXTAS) in several male and female GZ carriers[19–21]. This syndrome was originally linked to *FMR1* premutation alleles of 55–200 repeats[9] and, although its core features are cerebellar ataxia and action tremor, about a third of those affected manifest parkinsonian features[22,23]. The neurodegenerative changes in FXTAS have been attributed to a toxic gain-of-function effect of the elevated *FMR1* mRNA resulting from CGG expansions in the premutation range[8–11]. However, this mRNA elevation, though at a lower level, has also been reported in the carriers of GZ alleles with a threshold of 40/41 repeats[7], which implies that the neurotoxic effect of this transcript may also apply to the GZ range, leading to neurodegenerative changes typical or reminiscent of FXTAS[22,23], or aggravating any existing pathologies underpinning parkinsonian manifestations. Indeed, neurological manifestations in a series of 31 adult carriers of GZ alleles in the 40–54 CGG range seen in movement disorders clinics in Denver and Chicago featured parkinsonism and/or ataxia and tremor[15]. However, the evidence based on the observed occurrence of these changes in individual GZ carriers is limited, because a causal relationship between the carrier status and neurological manifestations could not yet be confirmed by neuropathological data, or statistically verified. The most recent changes in the 'required' molecular diagnostic criteria for FXTAS, which include GZ as well as PM carrier status, have been an important step forward[24]. However, the uncertainty of this association, if not yet fully supported by evidence, suggests that the term 'not excluding' rather than 'including' (in Fig 1.4c) might be considered.

A more effective approach enabling statistical analysis of the association between a particular set of clinical manifestations or other relevant findings and the GZ carrier status is to observe any differences in these changes between GZ carriers and non-carriers with a primary syndromic diagnosis of iPD. Our earlier results using this approach[25] suggested that, at the cellular (blood lymphoblasts) level, the toxic effect of the elevated *FMR1* transcript in GZ carriers might contribute to cellular stress and the mitochondrial dysfunction long known to be associated with iPD[26], and thus to the severity of parkinsonian manifestations. Indeed, in the same small sample, we found an elevation of the UPDRS

(motor) score in GZ carriers compared with iPD controls matched for age and disease duration.

On the other hand, the effects of pathological processes linked to GZ alleles and to other iPD-predisposing factors, respectively, might synergize at brain level. A remarkable study using dopamine transporter (^{123}I) beta-CIT single photon emission tomography (SPECT) imaging in two female, and one male GZ carriers clinically diagnosed with iPD showed well- preserved dopamine transporter density in the putamen, implying an absence of dopaminergic deficits typically associated with this disorder[27]. These results suggest that the contribution of GZ alleles to the risk of parkinsonism may be related to mechanisms other than pre-synaptic dopaminergic insufficiency. The nature of these mechanisms has been suggested by MRI scanning, by which we have shown that total brain white matter changes in deep frontal and parieto-occipital regions are significantly more severe in GZ male carriers with PD than in age-matched non-carriers with PD[28]. These results imply that the additional damage inflicted by the toxic gain-of-function RNA from the GZ alleles along the relevant non-dopaminergic neural pathways may limit (presynaptic) compensatory responses at the earlier stages of the disorder.

Considering that only a small proportion of iPD cases has so far been linked to a particular major gene, the potential importance of GZ-alleles as a substantial risk factor in iPD is emphasized by their high population frequency, reaching 5% in females[4,6,21,22,29,30]. Hence, the paucity of relevant large- scale studies is disconcerting, and may be partly accounted for by the requirement for relatively large sample sizes to obtain reliable estimates of the risk figures associated with GZ alleles from iPD cohorts. This was demonstrated by modelling the cumulative GZ allele frequency recorded in consecutive batches of 80–90 male newborn genotypes[4]. The results showed that this frequency fully stabilizes to the actual population estimate at a sample size of ~2000 alleles. Here we have applied the same modelling to a sample of female newborn genotypes used as population controls, with the stabilization occurring at a sample size of ~1600 alleles.

In summary, our data, based on screening of a large cohort of female patients, consolidates the evidence for the role of GZ alleles in the risk of Parkinson’s spectrum disorders, and suggests pathological mechanisms involved in this allele’s effect. An ongoing study being conducted by the authors in a large sample of GZ carriers with PD intends to establish whether these carriers represent a new, clinically and/or radiologically separate subtype within the iPD spectrum, or if it is a more severe form within the typical iPD phenotype. This will ultimately determine the criteria for standard testing for these alleles in clinical patients, and will allow for further exploration of specific targeted treatment.

Acknowledgments

Financial support to authors related to present study: DZL- The National Institutes of Child Health and Human Development Grant HD 36071; Brain Foundation Research Grant; Bethlehem Griffiths Foundation Research Grant. FT- The National Institutes of Child Health and Human Development Grant HD 36071 to DZL; GDM- Brain Foundation Research Grant and Bethlehem Griffiths Research Foundation Grant. MH- CRC for Mental Health, University of Melbourne; Brain Foundation Research Grant and Bethlehem Griffiths Research Foundation Grant to DZL, ES & GDM; MQB- Brain Foundation Research Grant and Bethlehem Griffiths Research Foundation Grant to DZL, ES & GHM; DF- Victorian Clinical Genetic Services special grant for Newborn Screening; ES- Brain Foundation Research Grant and Bethlehem Griffiths Research Foundation Grant.

Funding sources for study: The National Institutes of Child Health and Human Development Grant HD 36071 to DZL; Brain Foundation Research Grant; Bethlehem Griffiths Research Foundation Grant; Victorian Clinical Genetic Services special grant for Newborn Screening to DF.

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