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White matter changes in basis pontis in small expansion FMR1 allele carriers with parkinsonism

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Abstract

Examples of white matter hyperintensities (*wmh*) on magnetic resonance images in a basis pontis are presented in two male carriers, each of whom carry a small CGG expansion Fragile X Mental Retardation (FMR1) allele. One carried a premutation (PM) allele of 85 CGG repeats and the other, a grey zone (GZ) allele of 47 repeats. Both were originally diagnosed with idiopathic Parkinson's Disease (iPD). Similar changes are also shown in one PM carrier of 99 repeats affected with mild tremor and imbalance, who was ascertained through a fragile X syndrome family. These examples draw attention to the occurrence of *wmh* in a basis pontis in the carriers of small CGG expansions presenting with tremor and ataxia. Moreover, the presence of this change in GZ, as well as PM, allele carriers originally diagnosed with iPD supports our earlier suggestion that both these alleles may contribute to the neurodegenerative changes in this disorder which, in the examples presented, have been reflected by *wmh*, most prominent in the cerebellar peduncles and/or pontine area.

Keywords

MRI changes; Parkinson's Disease; Fragile X

Introduction

Expansions of CGG repeat in the Fragile X Mental Retardation (FMR1) gene within the premutation (PM) range of 55-200 [Maddalena et al., 2001] repeats cause the late onset Fragile X-Associated Tremor Ataxia syndrome (FXTAS) first described in 2001 [Hagerman et al., 2001]. Major clinical features of FXTAS, which occurs in nearly 40% of male PM carriers after the age of 65, are intention tremor, ataxia and cognitive decline [Hagerman et al., 2001; Jacquemont et al., 2003; Jacquemont et al., 2004; Loesch et al., 2005]. The other changes occurring with disease progression may include autonomic dysfunction, psychiatric problems, and peripheral neuropathy. Notably, parkinsonian manifestations are seen in 1/3 of the PM carriers diagnosed as FXTAS [Jacquemont et al., 2004], with mild resting tremor in nearly half of the patients, and bradykinesia and mild rigidity, in 57% and 71%, respectively. It is not unexpected therefore that some patients diagnosed with idiopathic Parkinson's Disease (iPD) were found to be carriers of the PM alleles [Hall et al., 2009]. The MRI changes, apart from white matter lesions of the middle cerebellar peduncles (MCP) in 50% of patients, include widespread cerebellar and cerebral white matter T2 hyperintensities (*wmh*), and cortical atrophy [Brunberg et al., 2002; Hagerman et al., 2001; Jacquemont et al., 2003; Jacquemont et al., 2004; Leehey 2009; Loesch et al., 2005; Loesch et al., 2007a]. The brain pathology reveals white matter degeneration with spongiosis distinct from that related to vascular or inflammatory changes, as well as ubiquitin-positive intranuclear inclusions abundant in both neuronal and astrocytic nuclei throughout the cerebrum, cerebellum, and brainstem [Greco et al., 2002].

The premutation (PM) is associated with elevated levels of FMR1 mRNA [Tassone et al., 2000], and the pathogenesis of FXTAS has been attributed to RNA becoming toxic through a 'gain-of-function' and thus leading to neurodegeneration [Greco et al., 2002; Hagerman et al., 2001; Jacquemont et al., 2004; Tassone et al., 2000]. Notably, an elevation of FMR1 transcript has also been reported in carriers of the grey zone (GZ) alleles [Loesch et al., 2007], which were originally defined as having 45-54 CGG repeats [Maddalena et al., 2001]. This suggested that these alleles, which occur commonly (~ 1 in 30) in the general population, might also be associated with phenotypic changes similar to those seen in the PM carriers. The GZ carriers cannot normally be ascertained, as can PM carriers, through fragile X families, because of the low rate of CGG expansion across generations. Therefore, the hypothesis that these alleles are associated with neurological involvement may realistically be tested by screening groups with the disorders reminiscent of FXTAS that is, associated with tremor and ataxia, for an excess of these alleles.

We have recently screened a sample of 228 Australian males with parkinsonism for the size of CGG repeat in the FMR1 gene [Loesch et al., 2009], and found a significant excess of both PM and GZ alleles compared with their frequencies in a population-based sample of 578 Guthrie spots from consecutive local male newborns (controls). We adopted, in the 2009 study, 40 CGG repeats as the lower boundary of GZ range, instead of an earlier recommended 45 repeats [Maddalena et al., 2001] based on the finding that transcriptional activity increases when FMR1 alleles exceed 39 repeats [Loesch et al., 2007]. The results of the screening suggested that both PM and GZ alleles might play a role in the aetiology of parkinsonism, and that modest elevation of 'toxic' RNA, especially in the GZ and lower-end

PM carriers, might present an additional risk for neurodegeneration by synergizing with other PD susceptibility genetic or environmental factors, thus contributing to the severity of this group of disorders..

Here we present the first examples of T2 hyperintensity in basis pontis occurring amongst male carriers of small expansions of CGG repeat identified in our earlier screening study [Loesch et al., 2009]. Although this trait has not been listed among the typical MRI changes in FXTAS, attention to this feature has been drawn by the recent presentation of this change in a female carrying a PM allele of 85 CGG repeats who, like our patients, was originally diagnosed with iPD [Morís et al., 2010]. These examples show, firstly, that the white matter changes in basis pontis may not be rare amongst the carriers of small expansions affected with tremor ataxia. Secondly, further investigation of this abnormality in context with parkinsonian and/or cerebellar manifestations in these carriers may have important implications in both clinical diagnosis, and provide a better understanding of the mechanisms involved in the contribution of these expansions to the severity of neurodegenerative changes in this group of disorders.

Materials and Methods

In the sample of 228 Australian (Caucasian) males with parkinsonism screened for the CGG repeat size in our earlier study [Loesch et al., 2009], 226 patients met the UK Brain Bank Criteria for iPD [Hughes et al., 1992], with 28 manifesting atypical features, and 2 patients diagnosed with MSA. All patients (except one presented here as *Case 1*) were responsive to levodopa treatment. In this sample, 4 patients were identified as PM carriers and 19, as GZ carriers (within a broader range of 40-54 repeats). The patients with initial diagnoses of atypical iPD or MSA were largely over-represented in the sample of identified carriers, with more than 30-fold increase of atypical iPD, and nearly 90-fold increase of MSA, relative to these proportions in the sample screened. Three PM carriers (55, 56, and 85 CGG repeats, respectively) and 10 GZ carriers (40-47 CGGs) aged 54-82 years signed the informed consent to participate in our further in-depth study comprising clinical neurological, neuropsychological, and molecular investigations (Loesch et al., in press; Loesch et al., paper in preparation).

MR images were available in 2 PM carriers of 56 and 85 repeats, and in 7 GZ carriers of 41-47 CGGs. These images were obtained using a 3-D coronal gradient echo volume acquisition sequence as well as routine axial and coronal T2 and proton density weighted images. Axial and coronal TSE T2 and PD, axial and coronal MPRS, and coronal T1 MPR volume whole brain imaging were acquired on a Siemens Avanto 1.5T MRI.

CGG repeat sizing was based on DNA extracted from saliva samples. Processing of saliva samples and the assessment of the size of CGG repeat from the extracted DNA (with precision of +/- one repeat) was conducted using a fully validated PCR amplification assay as detailed in our previous studies [Khaniani et al., 2008; Loesch et al., 2009].

Case presentation

The T2 hyperintensity in basis pontis occurred in one out of 2 PM carriers (*Case 1*), and one out of 7 GZ carriers (*Case 2*) identified in our 2009 screening study [Loesch et al., 2009]. This abnormality was more prominent in the PM carrier of 85 CGG repeats, in whom there was also the MCP sign, than in the GZ carrier of 47 repeats, who did not manifest the MCP sign, but had a few *wmh* foci in corona radiate of frontal and parietal lobes, as well as in the basis pontis. For comparison, we also present similar changes in a male carrying a PM allele of 99 repeats ascertained, unlike *Case 1* and *Case 2*, through a fragile X family, and presenting with a mild form of tremor/ataxia meeting the criteria of FXTAS (detailed clinical description of this individual has been given in: Loesch et al., 2007 [Loesch et al., 2007a]).

Case 1

A 70 year old male was diagnosed with iPD at the age of 60. At that time he started having resting and action tremor in his right arm, which gradually spread to his left arm, trunk, head and lower limbs. He received Sinemet treatment with poor response, and his tremor deteriorated to the degree that he could not perform any manual work, hold a glass of water, and had severe difficulty with eating. He had no history of hypertension. Neurological examination revealed striking wing-beating tremor especially on the right, becoming more severe with posture or action. There was also moderate resting and postural tremor of the head, face and tongue, and resting, postural and action tremor of the lower limbs. He had moderate upper limb incoordination on finger-nose testing, mild limb ataxia and dysarthria, and gait ataxia with inability to tandem walk. He had mild rigidity of neck, slight to mild rigidity of the upper arms (l>r) and legs (l>r). The results of neuropsychological testing revealed mild dementia with slowing of processing speed and significant impairment of learning and long term memory. MRI (see Fig 1A and B) showed intense T2 hyperintensity in basis pontis and the MCP sign bilaterally. The other changes highlighted included T2 *wmh* band around body/anterior horns/left atria, and involving corpus callosum, few small to medium *wmh* in the corona radiate, and generalized cerebral and cerebellar atrophy.

Case 2

A 71 year old male was diagnosed with iPD at the age 62, but the tremor started 2 years prior to the diagnosis. Since the diagnosis he received Sinemet treatment with adequate response. He had no history of hypertension. On examination he presented with moderately severe asymmetric akinetic rigid parkinsonism, mild postural instability, subtle limb and mild gait ataxia, and cognitive decline. Neuropsychological examination revealed borderline dementia with slowing of processing speed and inefficient memory. His MRI revealed T2 hyperintensity in the basis pontis (Fig 1C), and a few T2 hyperintense foci in the corona radiate.

Discussion

The reporting of clinical and neuropathological findings in FXTAS has mainly focused on changes in the cerebellar and cerebral white matter, with MCP sign considered the most

characteristic MRI feature. However, it was also suggested that the MCP sign arose from degenerative changes in the cerebellar peduncles that were a consequence of damage to pontine neurones or their afferents [Greco et al., 2002]. Although the main emphasis in reporting MRI changes in FXTAS has been on the MCP sign, the pontine changes reflecting this damage were noted (although not specifically reported) on neuropathological examination of a series of FXTAS patients (C. Greco, unpublished data). These changes comprised white matter degeneration and spongiosis distinct from that associated with hypertensive vascular disease.

Although there has been only one recent report of T2 hyperintensity in the basis pontis in a female carrying 85 CGG repeats, with manifestations consistent with the diagnosis of iPD [Morís et al., 2010], our observations suggest that this particular change may be not uncommon in carriers of the small CGG expansion FMR1 alleles with neurological involvement. Furthermore, it is possible that this change is more prevalent in carriers presenting with parkinsonism than in those with predominant cerebellar signs typical of FXTAS. Clearly a much larger study is required to test this hypothesis, and also to confirm that indeed, T2 hyperintensities in basis pontis, as distinct from vascular pathology, are significantly associated with the PM and GZ alleles.

In our small sample, one out of 2 PM carriers, and one out of 2 GZ carriers with CGG repeats within the commonly accepted range of 45-54 [Maddalena et al., 2001] manifested this change; whereas it was not apparent amongst the remaining GZ carriers with lower number of repeats within the 40-44 range, where the synergizing effect of an expansion with other PD susceptibility factors may be less discernible. Thus, the larger PM allele of 85 CGG repeats (*Case 1*) may induce a more significant effect of expansion resulting in the presence of T2 hyperintensity in basis pontis, as well as MCP sign (such as observed in another example of PM carrier with FXTAS), and clinically, prominent resting/action tremor and gait ataxia, and dementia. The carrier of the smaller grey zone allele of 47 repeats (*Case 2*) showed mild gait and limb ataxia, his clinical manifestations and cognitive decline were milder and responsive to levodopa, and his *wmh* in basis pontis, though not associated with MCP sign, was similar to this change in both PM carriers presented (Fig 1A&D). On the other hand, *Case 2* showed a more severe parkinsonian manifestation than case 1, such as bradykinesia and muscle rigidity, suggesting that the smaller alleles, i.e. GZ alleles, might synergise in some way with other PD susceptibility factor(s) to exacerbate neurological changes.

In conclusion, our data combined with the earlier neuropathological findings of Greco et al. (2002) [Greco et al., 2002] suggest that *wmh* in basis pontis may represent white matter changes in carriers of small CGG expansions, identifiable on MR images, which precede those involving the cerebellar peduncles. The fact that this change occurs in GZ, as well as PM carriers, and in patients with the original diagnosis of either iPD or FXTAS, supports our suggestion that, while the major effect of larger expansions within the PM range results in FXTAS with typical neurological and MRI manifestations, the smaller expansion GZ alleles may contribute to severity of neurological involvement in Parkinson's spectrum disorders. The occurrence of *wmh* in basis pontis in these conditions, unless obviously related to vascular pathology, may thus represent an early specific marker of this

contribution. If our limited data is supported in a large comprehensive study, this will suggest that when hyperintensities in the basis pontis are found in iPD patients, testing for these alleles is indicated, which may create opportunities for targeted therapies as soon as they become available.

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References

- Brunberg JA, Jacquemont S, Hagerman RJ, Berry-Kravis EM, Grigsby J, Leehey MA, Tassone F, Brown WT, Greco CM, Hagerman PJ. Fragile X premutation carriers: characteristic MR imaging findings of adult male patients with progressive cerebellar and cognitive dysfunction. *Am J Neuroradiol.* 2002; 23:1757–1766. [PubMed: 12427636]
- Greco CM, Hagerman RJ, Tassone F, Chudley AE, Del Bigio MR, Jacquemont S, Leehey M, Hagerman PJ. Neuronal intranuclear inclusions in a new cerebellar tremor/ataxia syndrome among fragile X carriers. *Brain.* 2002; 125:1760–1771. [PubMed: 12135967]
- Hagerman RJ, Leehey M, Heinrichs W, Tassone F, Wilson R, Hills J, Grigsby J, Gage B, Hagerman PJ. Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X. *Neurology.* 2001; 57:127–130. [PubMed: 11445641]
- Hall DA, Howard K, Hagerman R, Leehey MA. Parkinsonism in FMR1 premutation carriers may be indistinguishable from Parkinson disease. *Parkinsonism Relat Disord.* 2009; 15:156–159. [PubMed: 18565783]
- Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry.* 1992; 55:181–184. [PubMed: 1564476]
- Jacquemont S, Hagerman RJ, Leehey M, Grigsby J, Zhang L, Brunberg JA, Greco C, Des Portes V, Jardini T, Levine R, Berry-Kravis E, Brown WT, Schaeffer S, Kissel J, Tassone F, Hagerman PJ. Fragile X premutation tremor/ataxia syndrome: molecular, clinical, and neuroimaging correlates. *Am J Hum Genet.* 2003; 72:869–878. [PubMed: 12638084]
- Jacquemont S, Hagerman RJ, Leehey MA, Hall DA, Levine RA, Brunberg JA, Zhang L, Jardini T, Gane LW, Harris SW, Herman K, Grigsby J, Greco CM, Berry-Kravis E, Tassone F, Hagerman PJ. Penetrance of the fragile X-associated tremor/ataxia syndrome in a premutation carrier population. *JAMA.* 2004; 291:460–469. [PubMed: 14747503]
- Khaniani MS, Kalitsis P, Burgess T, Slater HR. An improved Diagnostic PCR Assay for identification of Cryptic Heterozygosity for CGG Triplet Repeat Alleles in the Fragile X Gene (FMR1). *Mol Cytogenet.* 2008; 1:5. [PubMed: 18471319]
- Leehey MA. Fragile X-associated tremor/ataxia syndrome: clinical phenotype, diagnosis, and treatment. *J Investig Med.* 2009; 57:830–836.
- Loesch DZ, Bui QM, Huggins RM, Mitchell RJ, Hagerman RJ, Tassone F. Transcript levels of the intermediate size or grey zone fragile X mental retardation 1 alleles are raised, and correlate with the number of CGG repeats. *J Med Genet.* 2007; 44:200–204. [PubMed: 16905681]
- Loesch DZ, Churchyard A, Brothie P, Marot M, Tassone F. Evidence for, and a spectrum of, neurological involvement in carriers of the fragile X pre-mutation: FXTAS and beyond. *Clin Genet.* 2005; 67:412–417. [PubMed: 15811008]
- Loesch DZ, Khaniani MS, Slater HR, Rubio JP, Bui QM, Kotschet K, D'Souza W, Venn A, Kalitsis P, Choo AK, Burgess T, Johnson L, Evans A, Horne M. Small CGG repeat expansion alleles of FMR1 gene are associated with parkinsonism. *Clin Genet.* 2009; 76:471–476. [PubMed: 19796183]
- Loesch DZ, Litewka L, Churchyard A, Gould E, Tassone F, Cook M. Tremor/ataxia syndrome and fragile X premutation: diagnostic caveats. *J Clin Neurosci.* 2007a; 14:245–248. [PubMed: 17194594]

- Maddalena A, Richards CS, McGinniss MJ, Brothman A, Desnick RJ, Grier RE, Hirsch B, Jacky P, McDowell GA, Popovich B, Watson M, Wolff DJ. Technical standards and guidelines for fragile X: the first of a series of disease-specific supplements to the Standards and Guidelines for Clinical Genetics Laboratories of the American College of Medical Genetics. Quality Assurance Subcommittee of the Laboratory Practice Committee. *Genet Med*. 2001; 3:200–205. [PubMed: 11388762]
- Morís G, Arias M, López MV, Álvarez V. Hyperintensity in the basis pontis: Atypical neuroradiological findings in a woman with FXTAS. *Movement Disorders*. 2010; 25:649–650. [PubMed: 20201003]
- Tassone F, Hagerman RJ, Taylor AK, Gane LW, Godfrey TE, Hagerman PJ. Elevated levels of FMR1 mRNA in carrier males: a new mechanism of involvement in the fragile-X syndrome. *Am J Hum Genet*. 2000; 66:6–15. [PubMed: 10631132]

Figure 1.

MRI changes in three carriers of the FMR1 alleles with small CGG repeat expansions.

White matter hyperintensities in the basis pontis (A), the cerebellar peduncles (MCP sign, B), in a PM carrier identified in iPD sample; white matter hyperintensities in basis pontis in a GZ carrier identified in iPD sample(C); white matter hyperintensities in the basis pontis associated with unilateral MCP sign in a PM carrier ascertained through a fragile X family (D) *.

* Modified from [Loesch et al., J Clin Neurosci 2007a, vol 14 p247, Fig 1b]: where a detailed description of this patient was also given.