

What we know or need to know about fragile X premutation

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The effect of small expansions on phenotypes is an important problem considering their high population prevalence. The PM (using 55 repeats as the lower bound) is relatively common in the general population affecting 1 in 113 to 259 females and 1 in 260 to 813 males.

Although the carriers of FMR1 premutation allele which have between 55 and 200 CGG repeats were originally thought by both researchers and clinical geneticists to show no clinical effects, some problems related to the carrier status had been reported two decades ago. In 1991 an elevated rate of premature ovarian failure (POF) was documented in carriers compared to controls, and confirmed by many other research groups. This term, POF, has later been renamed the fragile X- associated primary ovarian insufficiency (FXPOI) to emphasize the association with the premutation, and also the fact that some women may have fertility and hormonal problems but can still reproduce. Further research is required to determine if EXPO is a late onset condition, or the changes, such as diminished ovarian reserve, start early in life. Another major issue to be resolved is what causes these problems. In 2001 our US collaborators led by Prof Randi Hagerman discovered that premutation FMR1 alleles are overactive, resulting in the elevated levels of 'messenger RNA' (mRNA), which normally translate the DNA sequence into the relevant protein structure. These researchers suggested that the elevated levels of this mRNA may have a toxic effect on some tissues causing damage and premature loss of cells. It remains to be seen if the ovarian changes can be attributed to this RNA toxicity, or to some other, as yet unknown, changes occurring in, or interacting with, the premutation.

Soon after the association between premutation and fertility problems had been identified, we reported a few minor yet significant physical features such as hyperextensible joints, as well as mild cognitive deficits in some proportion of males and females carrying the premutation allele; and later, other research groups used magnetic resonance imaging (MRI) to demonstrate some changes in the volumes of certain brain areas, reminiscent of those changes seen in the fragile X syndrome (FXS). We subsequently showed that these changes were associated with a small deficit of the FMR1 gene protein product, FMRP. This protein is grossly depleted in the individuals affected with FXS, where the full mutation leads to severe physical and cognitive changes. It is well known that this depletion results from an inactivation of the FMR1 gene's promoter, which normally regulates the rate of production of FMRP, but is silenced if the size of CGG repeat exceeds 200. However, it still remains

uncertain if, to some degree, the promoter's inactivation also applies to premutation alleles, and our team at the Murdoch Institute laboratory led by Dr David Godler is presently studying this issue using a novel methodology.

In 2001, the fragile X-associated tremor ataxia syndrome (FXTAS) was discovered in aging male carriers amongst the grandfathers of children with FXS by our collaborating US team from the M.I.N.D. Institute led by Prof Randi Hagerman. This syndrome included mainly tremor and imbalance (ataxia), but also nerve damage, autonomic dysfunction (sudden falls in blood pressure), neuropsychiatric problems and cognitive decline sometimes leading to dementia. Since then a large number of studies of this, and related disorders, supported by a collaborative National Institutes of Health (US) grant, have been conducted by the US and our Australian teams. In both centres we found a similar overall prevalence of FXTAS in males that ranged between ~35-40%, with the frequency of ~17% at the age of 50 gradually increasing to 75% at the age of 85 years. We described the associated MRI changes occurring in a majority of affected carriers, with a typical degeneration of white matter in cerebellar peduncles seen in approximately 60% of FXTAS patients. Both centres also noted that some proportion of FXTAS patients have been initially (and mistakenly) diagnosed with Parkinson's Disease or some other related syndromes.

Apart from the typical manifestations of FXTAS, we have observed some other forms of neurological involvement in male premutation carriers, such as isolated mild tremor or imbalance on clinical examination or with the use of more sensitive computerized tools, such as 'CATSYS', or MRI changes without, or with only minimal clinical manifestations, or cognitive changes, particularly executive function deficits. It is yet to be established if these isolated or minor changes develop into the full FXTAS with progressing age, or they merely represent a mild form of neurological involvements in some individuals. Answering this question would require a large scale longitudinal study to be supported for at least 5 years, in order to follow up aging premutation carriers manifesting the wide range of clinical involvements. Unfortunately, it is extremely difficult to obtain this type of research grant, or ensure such a long term commitment of the participants. FXTAS has also been reported in 8% to 16.5% of female carriers, and occurs at a later age than in men, which is largely because of the protective role of the second X chromosome. Our own study in a sample of Australian female carriers showed a much lower prevalence (~2%), and the reason for this discrepancy is still not clear and requires further studies in larger samples.

Both our Australian and US teams have also conducted numerous studies towards understanding the mechanisms leading to progressive brain damage, and why only some of the premutation carriers are affected whereas others are immune to this damage. The first step

was to explore (and find) a relationship between the relevant neurological manifestations and the size of CGG expansion, as well as the elevation of expanded FMR1mRNA. This led us and other researchers, who conducted similar studies in experimental animals, to hypothesize a toxic effect of excessive FMR1mRNA on brain tissue through deprivation of brain cells of some specific proteins essential for normal functioning of these cells, which may be sequestered by this mRNA. The most recent studies conducted by us, and also by our collaborating US team, revealed that this elevated 'toxic' mRNA may, through some mechanisms still to be explored, damage important intracellular organelles called mitochondria, which are a major source of energy for body functioning. Exploring these novel mechanisms possibly involved in the FMR1 mRNA 'toxicity' may create opportunities for specific therapies, which may also benefit people with some other neurological conditions, such as Parkinson's disease. Therefore, we need to vigorously pursue this line of research if such an opportunity arises.

Although FXPOI and FXTAS are the best known conditions associated with the premutation, a variety of additional phenotypes has been linked with this allele. They include behavioural problems in a subgroup of young male carriers including autism/autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), shyness, anxiety and seizures. In many adults with the premutation without FXTAS, including both males and females, psychopathology is common, including anxiety and depression, compared to controls. Most recently autoimmune dysfunction, including fibromyalgia and hypothyroidism, has been found to be more common in carriers compared to controls. Therefore a large spectrum of involvement associated with the premutation constitutes a newly emerging group of disorders leading to new avenues in research and clinical management.

As implied by the title, my reason for writing this article has been, firstly, to present a brief account of the problems that may arise in people carrying the premutation size FMR1 allele. Although this type of information clarifying the present status of our knowledge in this field is important for the families, the fundamental question still to be answered by further research is why some carriers are affected and some others bearing the same changes in the FMR1 gene are not. This can only be resolved through better understanding of specific mechanisms leading from expanded CGG repeats through excessive production of potentially damaging messenger RNA which, according to our preliminary findings, may interact, in some individuals, with some other yet unknown susceptibility factors, thus leading to a variety of health problems. Testing this hypothesis in larger studies would be especially important in preventing these problems and creating some novel therapies. Clearly, continuation of this research relies on grants (which become increasingly harder to obtain), large family participation, and collaboration between scientists and clinicians in these complex and

multidisciplinary studies.

I wish to thank all the families or individual family members who have participated in our past and on-going research, and we always welcome new contacts to my number at a Trobe University: 03 94791382, or e-mail me to: d.loesch@latrobe.edu.au; you are welcome to ask questions, suggest new ideas, or offer participation in our studies. I realize that some participants may expect receiving an immediate feedback describing the results of each individual study, but unfortunately most studies are just consecutive steps in search for an answer, and thus by themselves may not be conclusive. This is why the summary of overall progress and remaining questions given here seems more appropriate.

More detailed information on the present state of research in the spectrum of FMR1 mutations, with special emphasis on the clinical impact of the premutation alleles can be found in the book chapter by Loesch and Hagerman (as listed below) available through the Madame Curie Online Database, and to appear shortly in a hard book copy.

Loesch DZ, Hagerman R. Unstable Mutations in the FMR1 Gene and the Phenotypes in:

[Tandem Repeat Polymorphisms: Genetic Plasticity, Neural Diversity and Disease](#) (AJ

Hannah, ed.). Landes Bioscience, Texas, USA, 2011.

<http://www.landesbioscience.com/curie/chapter/5142/>