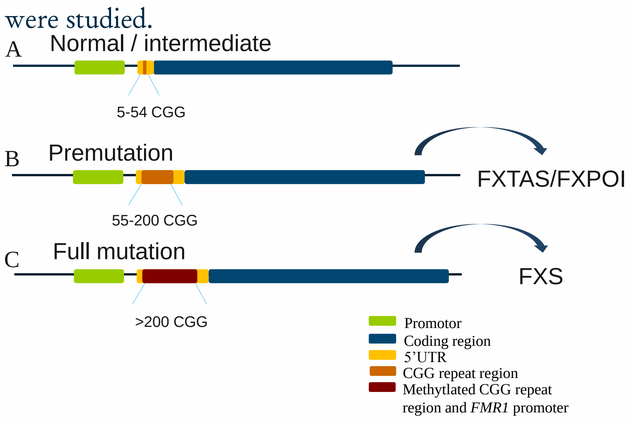
Fragile X syndrome: intergenerational allele instability and associated phenotypes in families

Joana R Loureiro[1], Paula Jorge[2], Isabel Marques[2], Rosário Santos[2], Ana I Seixas[1], Márcia Martins[3], José Vale[4], Jorge Sequeiros[1,5], Isabel Silveira[1].

[1]IBMC- Instituto de Biologia Molecular e Celular, Universidade do Porto, [2]UI&D Centro de Genética Médica Dr Jacinto Magalhães, INSA Porto, [3]Maternidade Júlio Dinis, CHP,Porto, [4]Serviço de Neurologia, Hospital de Egas Moniz, Lisboa, [5]ICBAS, Universidade do Porto.

INTRODUCTION

Fragile X syndrome (FXS) is the most common hereditary form of intellectual disability with an estimated frequency of 1/4000 males and 1/8000 females. This disease is caused by a (CGG)n expansion in the 5'UTR of the *FMR1* gene, which as a result is methylated and the gene silenced. Four classes of alleles can be found based on CGG repeat length: normal (5-44), intermediate (45-54), premutation (55-200) and full mutation (>200). Two different disorders are associated to premutation carriers, fragile X-associated tremor/ataxia syndrome (FXTAS) and Fragile X-related primary ovarian Insufficiency (FXPOI). However, recent studies suggest that other phenotypes can be associated with premutation allele carriers. To gain insights into instability of *FMR1* CGG repeats and associated phenotypes, 537 individuals from 128 FXS Portuguese families were studied.



FMR1 gene: A) Normal allele, B) Premutation allele in FXTAS and FXPOI. FXTAS is a late-onset neurodegenerative disorder with age dependent penetrance from 17% in the sixth decade to 75% after the age of 80 years, in premutation carrier males, and is rare in women. FXPOI is defined by cessation of menses before age 40 years and occurs in 20% of premutation carrier females, C) Methylated full mutation allele causing FXS.

MATERIAL & METHODS

- The sample comprised 174 premutation carriers (140 females and 34 males), detected among 537 individuals from 128 Portuguese FXS families.
- *FMR1* CGG repeat size was assessed by PCR reaction followed by automated fragment analysis and Southern blot in males where no allele was amplified and females where only a single allele was observed by PCR.

Funding sources and acknowledgements.
Funding was provided by FCT, Grant number: PIC/IC/82897/2007

15th Reunião da Sociedade Portuguesa de Genética Humana, Porto, November 22 - 24, 2012

RESULTS & DISCUSSION

A. Meiotic instability of *FMR1* allele classes

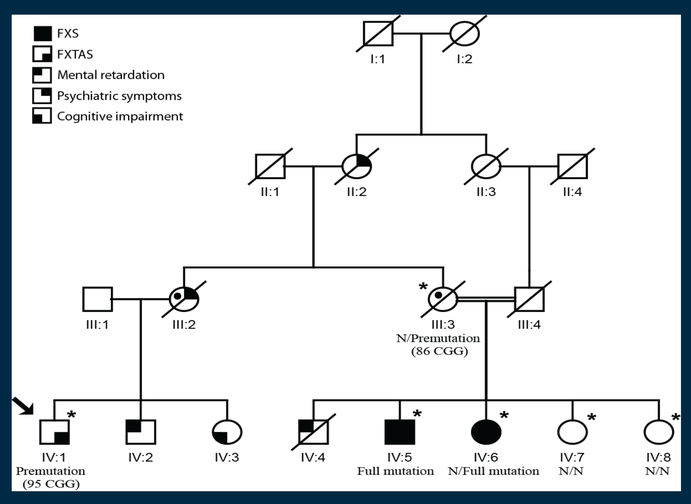
- Normal and intermediate alleles were stable upon transmission (50 normal and 5 intermediate allele transmissions).
- Only premutation alleles were inherited by daughters of premutation and full mutations males.
- The allele with 66 CGG repeats was the smallest premutation to expanded to a full mutation in a single generation.

Transmissions of premutation and full mutation alleles

Repeat size of allele	Transmitting mothers	Number of offspring with		Observed expansion to FM (%)	Transmitting fathers	Number of offspring with	
		PM	FM			PM	FM
55-59	-	-	-	-	2	3	0
60-69	6	9	1	10	2	2	0
70-79	22	16	15	48	1	1	0
80-89	17	1	24	96	2	2	0
90-99	21	1	35	95	0	0	0
100-109	6	0	8	100	1	1	0
110-200	6	0	6	100	1	1	0
>200	31	1 a)	44	-	2	4 b)	0

a) Contraction of a full mutation to an ~77CGG premutation allele.
b) Contraction of two full mutations to ~92 , ~87 , ~97 and ~103 premutation alleles.

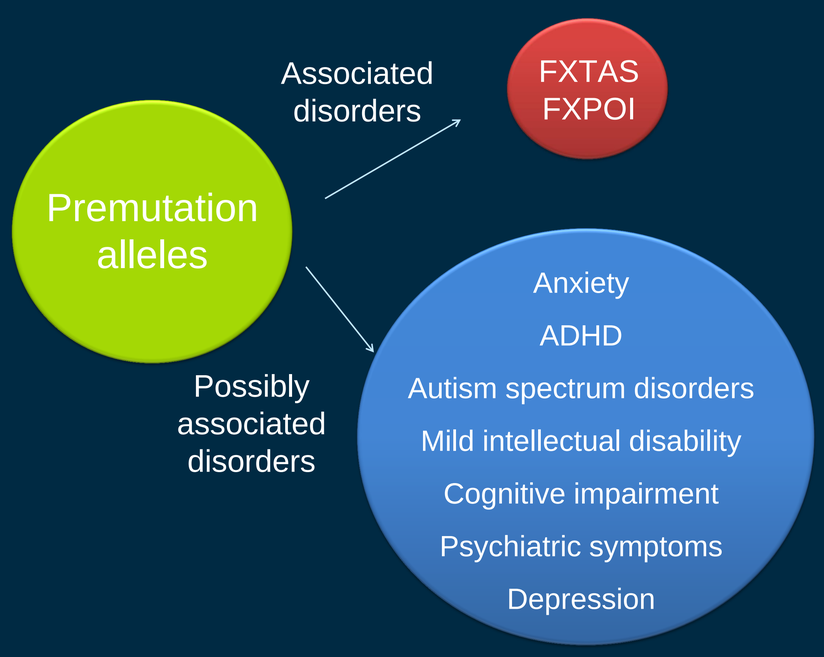
B. Intergenerational *FMR1* allele instability and associated phenotypes



Family pedigree showing FMR1 allele instability and associated phenotypes. Proband is a FXTAS patient. Individual IV:3 also presents tremor, III:2 was an obligatory carrier of an expanded allele and had a psychiatric disease. FMR1 repeat lengths are available for individuals marked with (*). Symbols with (•) are obligatory carriers of an *FMR1* expanded allele. (N – normal repeat size).

C. Phenotypes associated with premutation alleles

Disorders described in premutation allele carriers



Clinical evaluation of premutation carriers from Portuguese FXS families

Gender	Clinicaly evaluated*	Age of examination	Premutation size	FXPOI	FXTAS
Female	6	20-43	78-99	2	0
Male	1	73	95	-	1

*The cohort was not yet fully analyzed.

CONCLUSIONS

- In Portuguese FXS families, allele instability upon transmission is in agreement with previous reports:
  - ✓ Expansion and contraction of *FMR1* expanded alleles is dependent on the gender of the progenitor,
  - ✓ The observed risk of premutation to full mutation expansion increase with maternal premutation size allele.
- One FXTAS and two FXPOI cases were identified in premutation carriers among FXS Portuguese families.