Are we Close to Solve the Mystery of Fragile X Associated Premature Ovarian Insufficiency (FXPOI) in FMR1 Premutation Carriers?

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אוניברסיטת תל-אביב
TEL AVIV UNIVERSITY

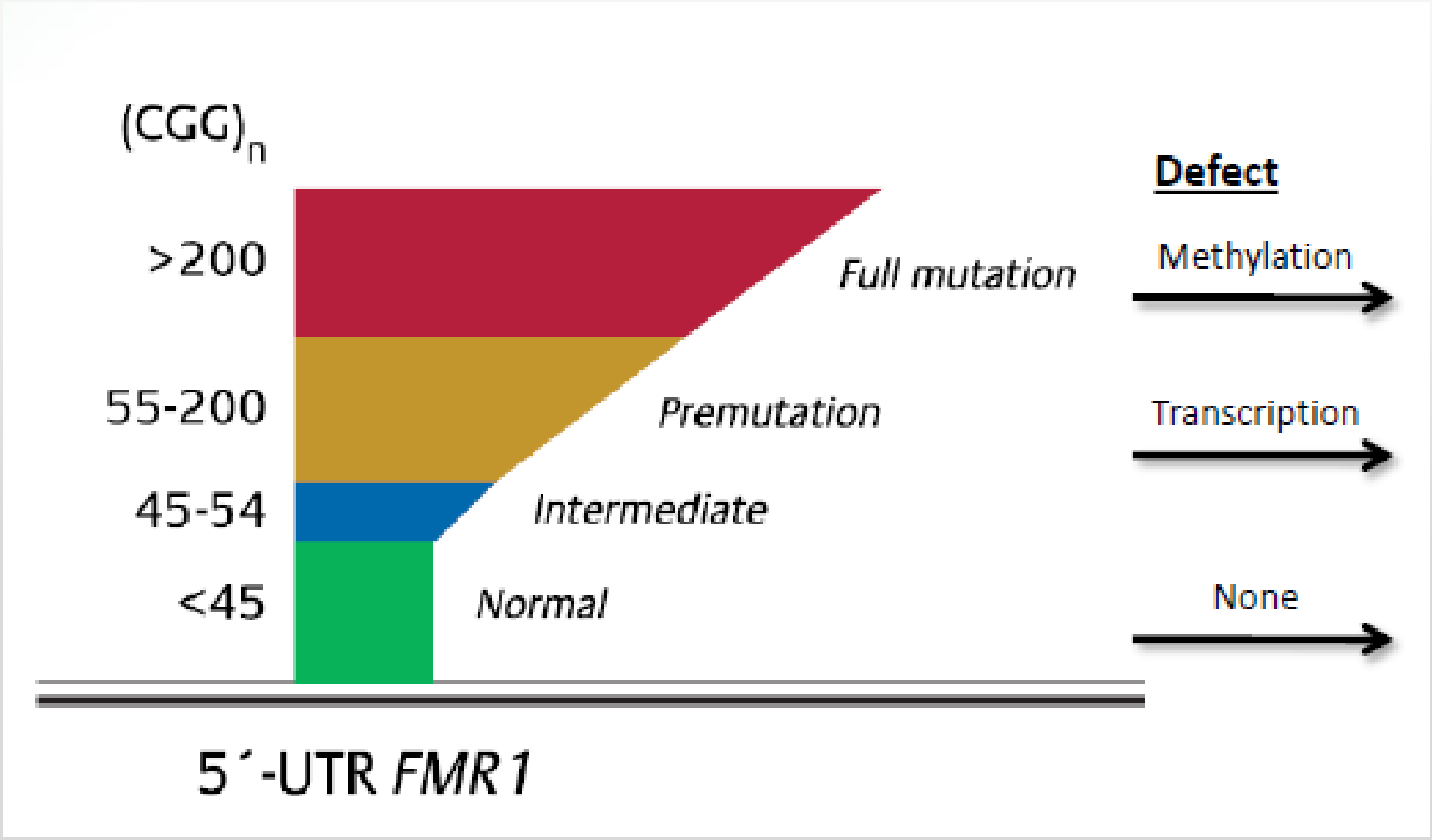
Fragile X Syndrome

- CGG repeat expansions in the 5’ untranslated region of the Fragile X Mental Retardation-1 (FMR1) gene
- Prevalence- 1:4000 males, 1:8000 females
- The most common single cause of inherited mental retardation
-
- The most common known genetic cause of autism

Fragile X Syndrome

- Patients with mental retardation had more than 200 CGG repeats
- The expanded sequence of CGG is hypermethylated in affected individuals
- Methylation of the expanded CGG repeat lead to FMR1 transcriptional silencing

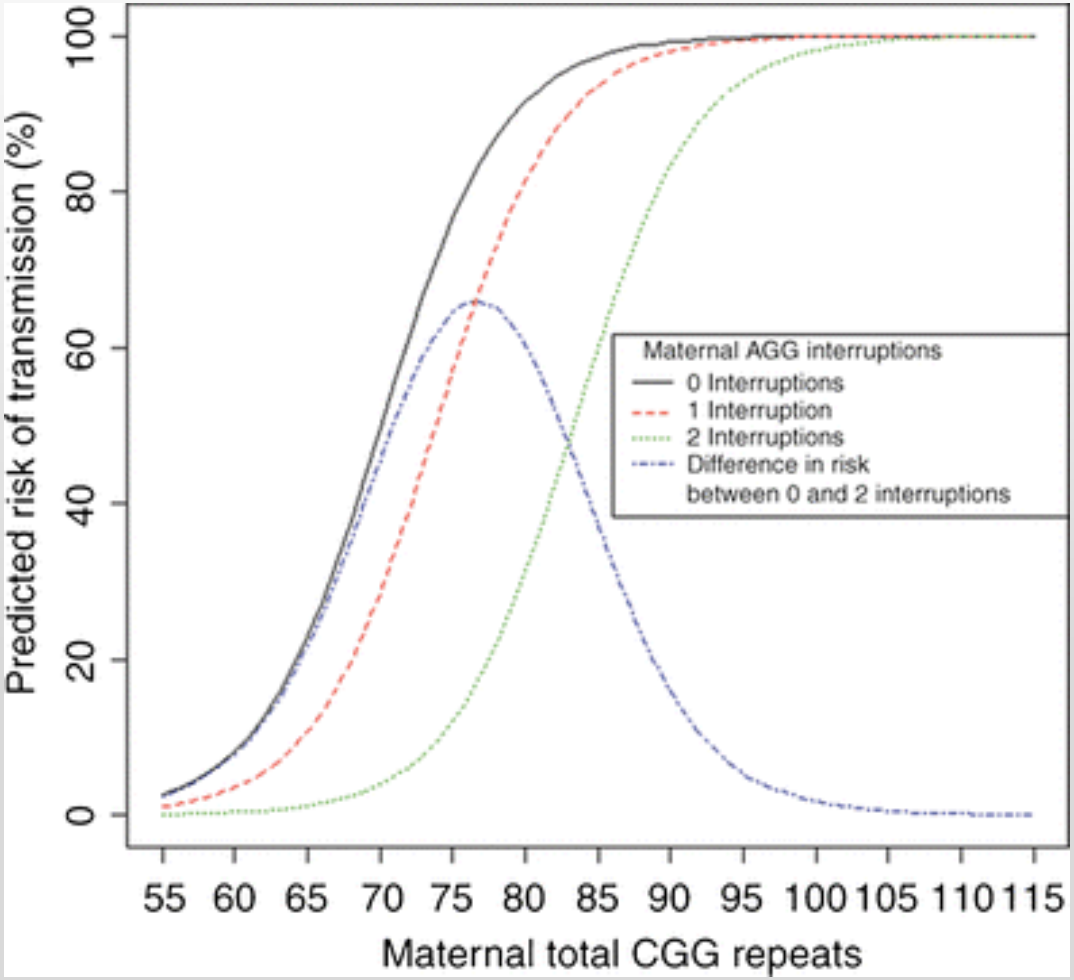
CGG repeats are unstable



AGG interruptions

- AGG interruptions are commonly seen within FMR1 alleles
- AGG triplet are associated with a reduced risk of expansion
- AGG interruptions within the CGG repeat tract do not influence FMR1 mRNA levels
- Significant differences in AGG interruption patterns in various populations
- Genetic counselling- taking into account the AGG interruptions

AGG interruptions and stability of the CGG tract



Yrigollen CM, et al. (2012) Genet Med

FMR1 Premutation

- Premutation: 55-200 CGG repeats
- Prevalence of 1:800 in males and 1:150 in females
- Fragile X tremor ataxia syndrome (FXTAS)
- Fragile X associated premature ovarian insufficiency (FXPOI)
- Others: emotional problems, ADHD, and autism

Fragile X associated tremor ataxia syndrome (FXTAS)

Clinical features:

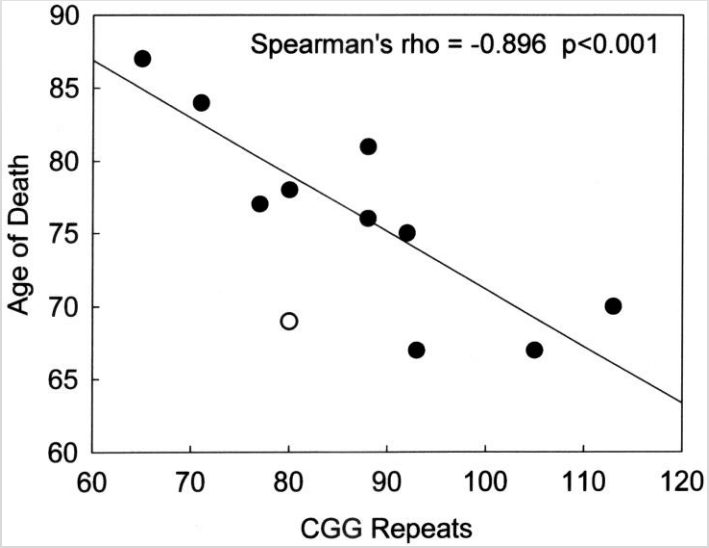
- Intention tremor
- Cerebellar ataxia
- Parkinsonism
- Memory/cognitive function deficits

Signs of neurodegenaration:

- Brain atrophy
- Middel cerebellar peduncle lesions (MCP sign)
- Neuropathy
- Intranuclear inclusions

Fragile X associated tremor ataxia syndrome (FXTAS)

- 45% of the male >50y
- 8% of female >50y
- Age-dependent penetrance
- Larger CGG repeat correlates with earlier age of onset and with earlier age of death



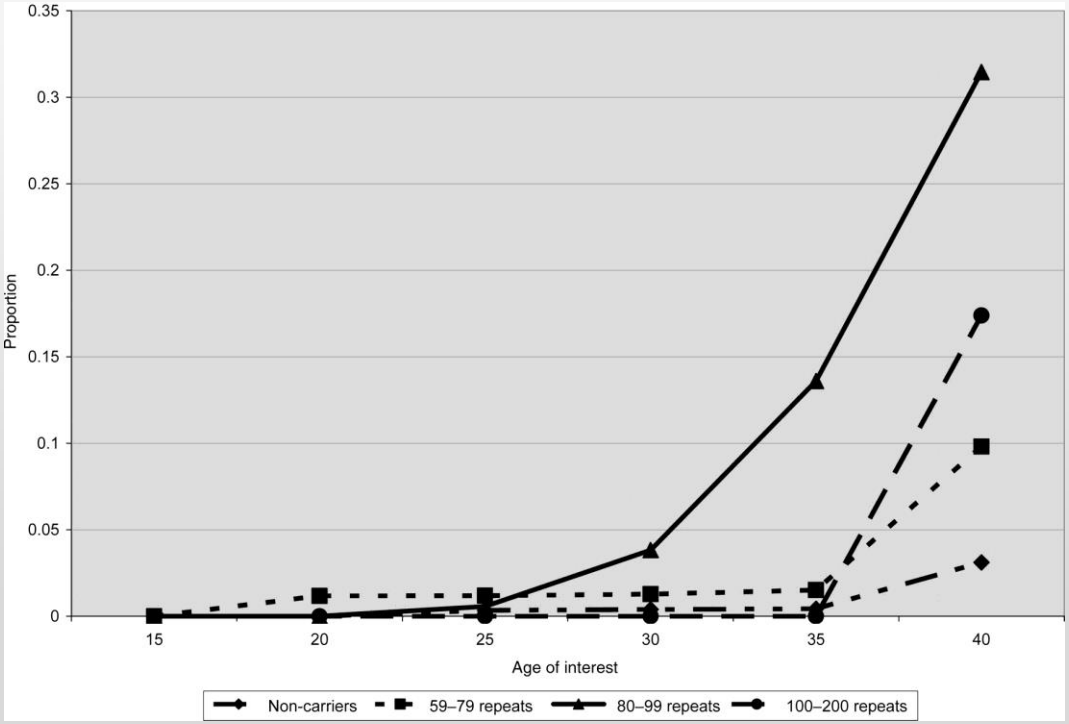
Tassone F, et al. (2005) Human Genetics

Fragile X associated premature ovarian insufficiency (FXPOI)

Clinical features:

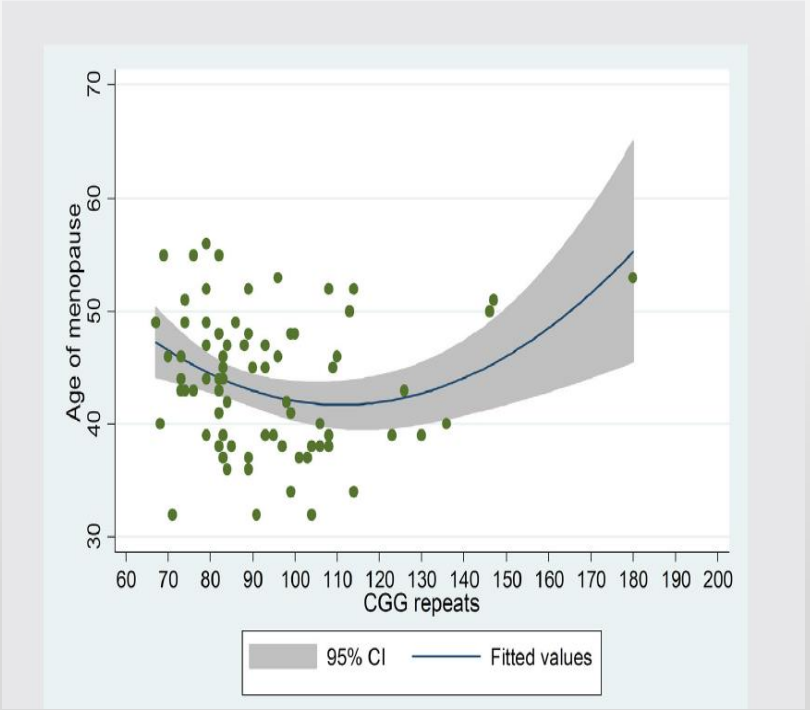
- High frequency of POF (20% vs 1%)
- Full mutation have the same risk as non carriers- 1%
- Lower fertility (visit a doctor, time to 1st preg)
- High frequency of POI (>25%)
  - Skipped cycles, Irregular cycles, Short cycle lengths
  - Increased levels of day 3 Follicle-Stimulating Hormone (FSH)
  - Decreased levels of Anti -Müllerian hormone (AMH)
- Experience menopause 5-7 years earlier

Association between CGG repeat length and ovarian dysfunction



Age-specific prevalence of POF by repeat size group

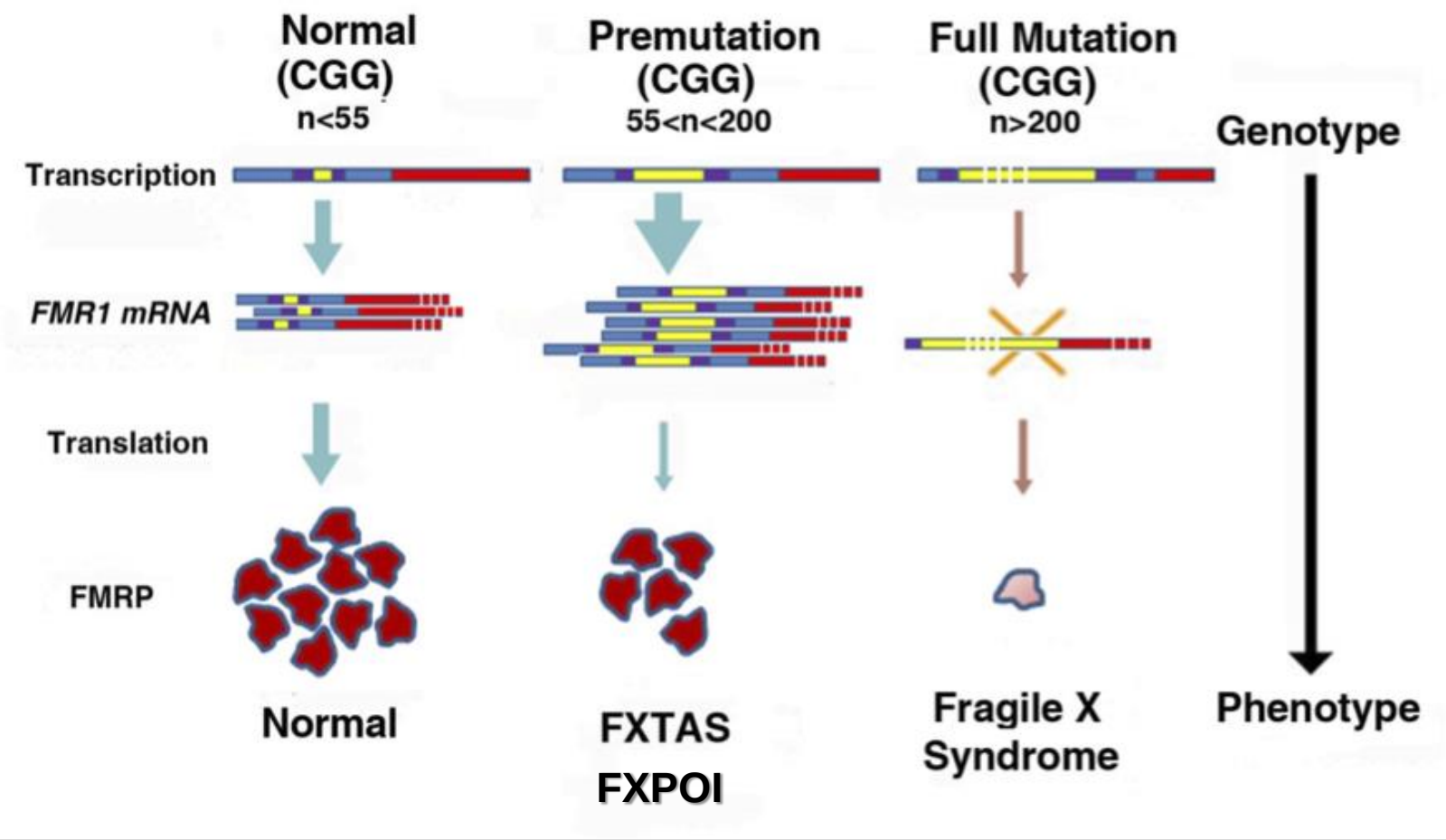
Allen et al (2007) Hum. Reprod



Age-specific prevalence of menupause

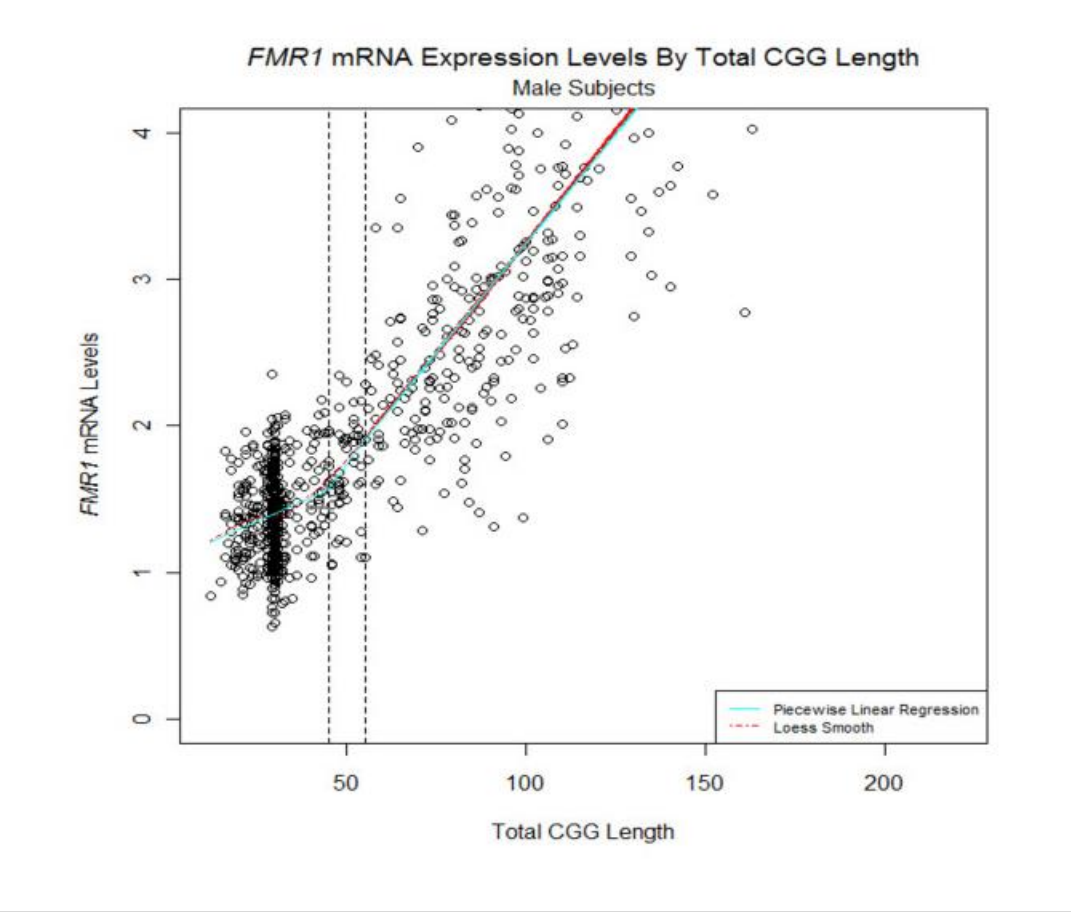
Mailick et al (2014) Am J Med Genet

One gene, Three Major Disorders
The Molecular Basis



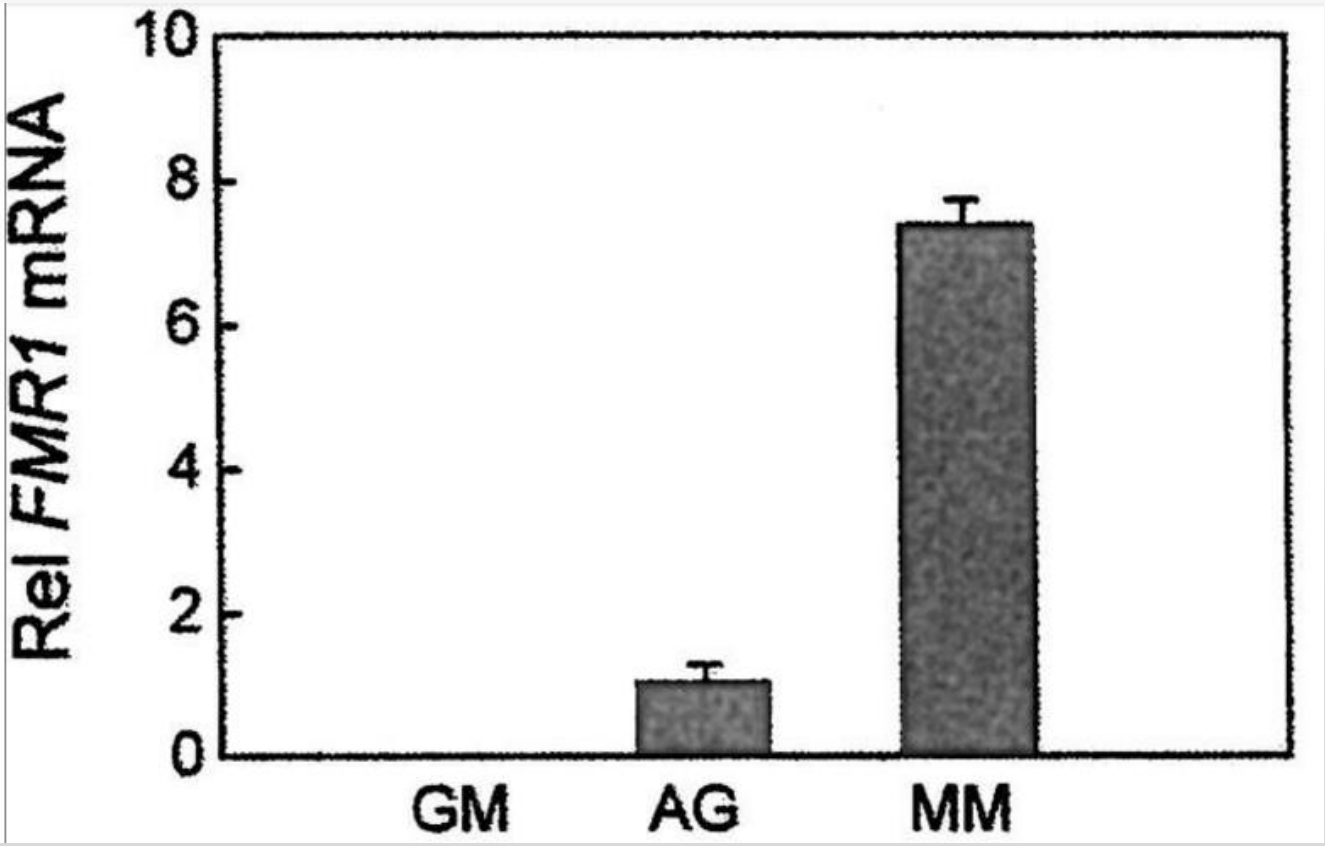
adapted from Hagerman and Hagerman 2002

Expression of FMR1 mRNA is increased in premutation carriers



Sellier C, et al. (2014) *J Neurodev Disord*

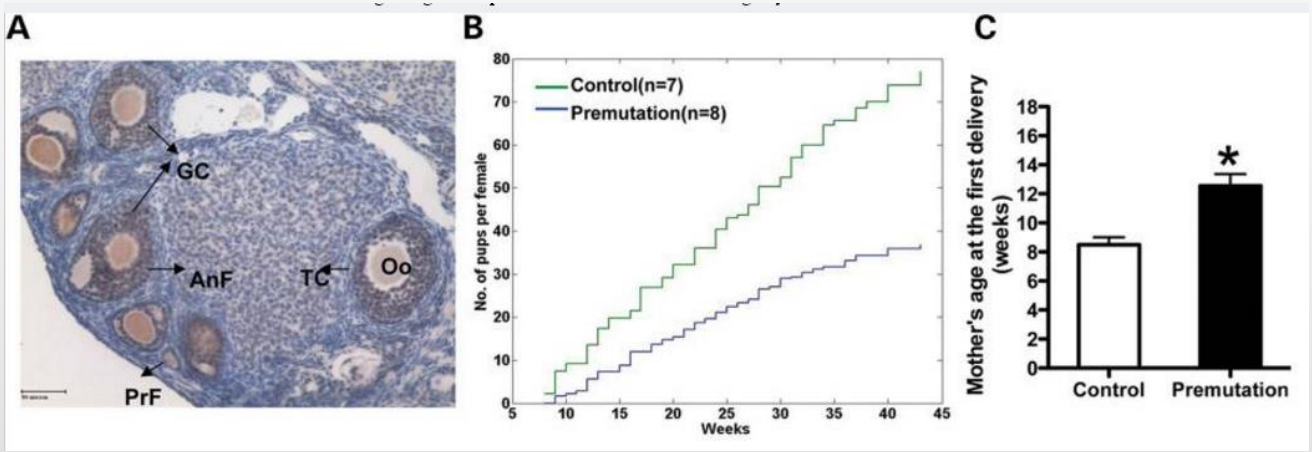
Elevated level of FMR1 mRNA in premutation carriers is caused by increased transcription efficiency

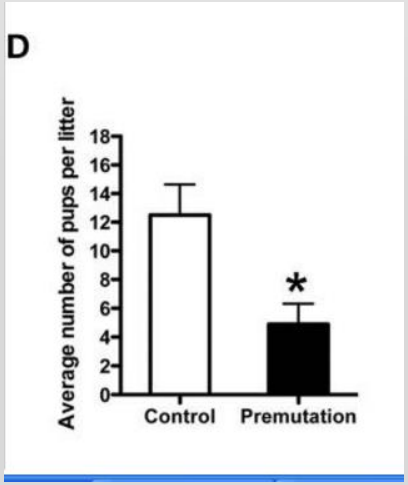


- normal female (AG) 16/29 repeats
- premutation male (MM) 160 repeats
- full mutation male (GM) with ~600 repeats

Tassone F, et al. (2007) RNA

Fragile X premutation RNA is sufficient to cause primary ovarian insufficiency in mice





Transgenic mouse - premutation of 90 CGG repeats

- Fmr1 mRNA and Fmrp in granulose cells and oocytes
- Increased mRNA level
- Smaller cumulative number of pups
- PM females delivered their first litter 1 month later than WT
- Average number of pups was significantly reduced

Lu C et al. (2012) Hum Mol Genet.

Fragile X premutation RNA is sufficient to cause primary ovarian insufficiency in mice

- **Reduced number of growing follicles**
  - At PD8 and 25 the dimensions of the ovaries were similar to those of WT
  - The numbers of follicles in adult females were less than in WT
- **Altered serum hormone levels**
  - From 9 to 22 weeks, the levels of serum FSH were significantly higher
  - The serum LH level were significantly lower
- **LH receptor (Lhr) was significantly downregulated**
- **Reduced phosphorylation of Akt and mTOR**
  - Significant reduction of phosphorylated Akt
  - Dramatic reduction of phosphorylated mTOR in FMR1 premutation ovaries

Lu C et al. (2012) Hum Mol Genet.

Results

OPEN ACCESS Freely available online

PLOS ONE

Elevated Levels of FMR1 mRNA in Granulosa Cells Are Associated with Low Ovarian Reserve in FMR1 Premutation Carriers

Shai E. Elizur[¶], Oshrit Lebovitz[¶], Sanaz Derech-Haim, Olga Dratviman-Storobinsky, Baruch Feldman, Jehoshua Dor, Raoul Orvieto, Yoram Cohen*

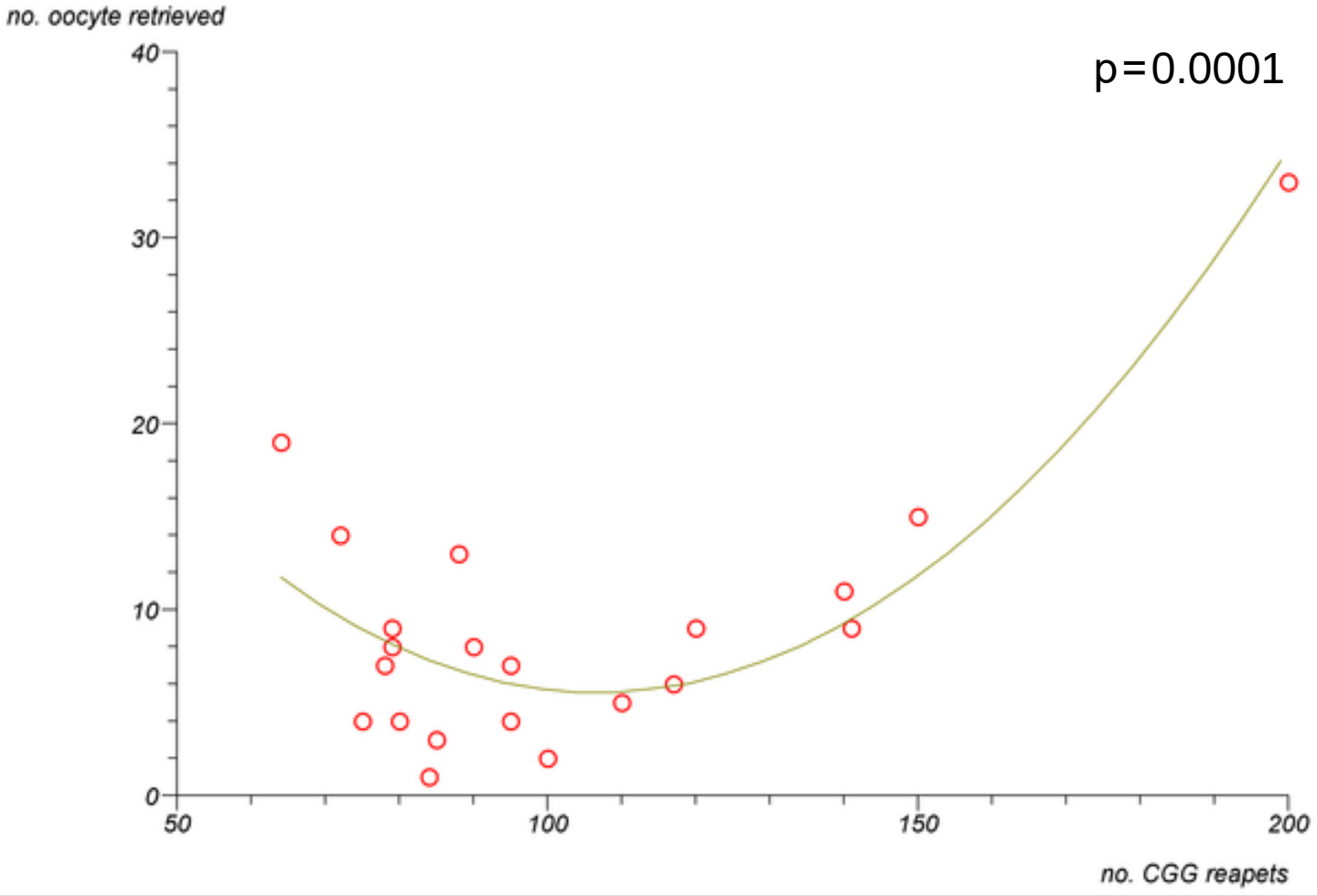
Department of Obstetrics and Gynecology, Chaim Sheba Medical Center, (Tel Hashomer), Ramat Gan, Israel, and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Clinical and laboratory characteristics of the study

	FMR1 Premutation	Control	
	N = 21	N = 15	P value
Age (mean) (SD)	31.5 (3.4)	30.8 (4.3)	ns
Parity (median)	0	0	ns
Mean FSH (IU) (basal) (SD)	8.2 (2.0)	7.0 (1.7)	0.08
Mean LH (IU) (basal) (SD)	3.7 (1.7)	4.9 (1.9)	ns
Mean Basal FSH/LH ratio (SD)	2.4 (1.3)	1.4 (0.7)	0.01
Mean Estradiol (basal) (pmol/L) (SD)	152 (58)	156 (87)	ns
Mean Total Gonadotropins used in stimulation (IU) (SD)	2588 (1198)	1865 (990)	0.04
Mean duration of stimulation (days) (SD)	10.8 (2.8)	10 (1.9)	ns
Mean peak estradiol (pmol/L) (SD)	6399 (3347)	8470 (2508)	0.06
Mean no. oocyte retrieved (SD)	9 (7.1)	13.1 (5.7)	0.02
Mean no. embryo transferred (SD)	1.4 (1.2)	1.7 (0.7)	ns
No. of pregnancies	4	2	ns
Mean FMR1 repeats (range)	102 (64–200)	<55	

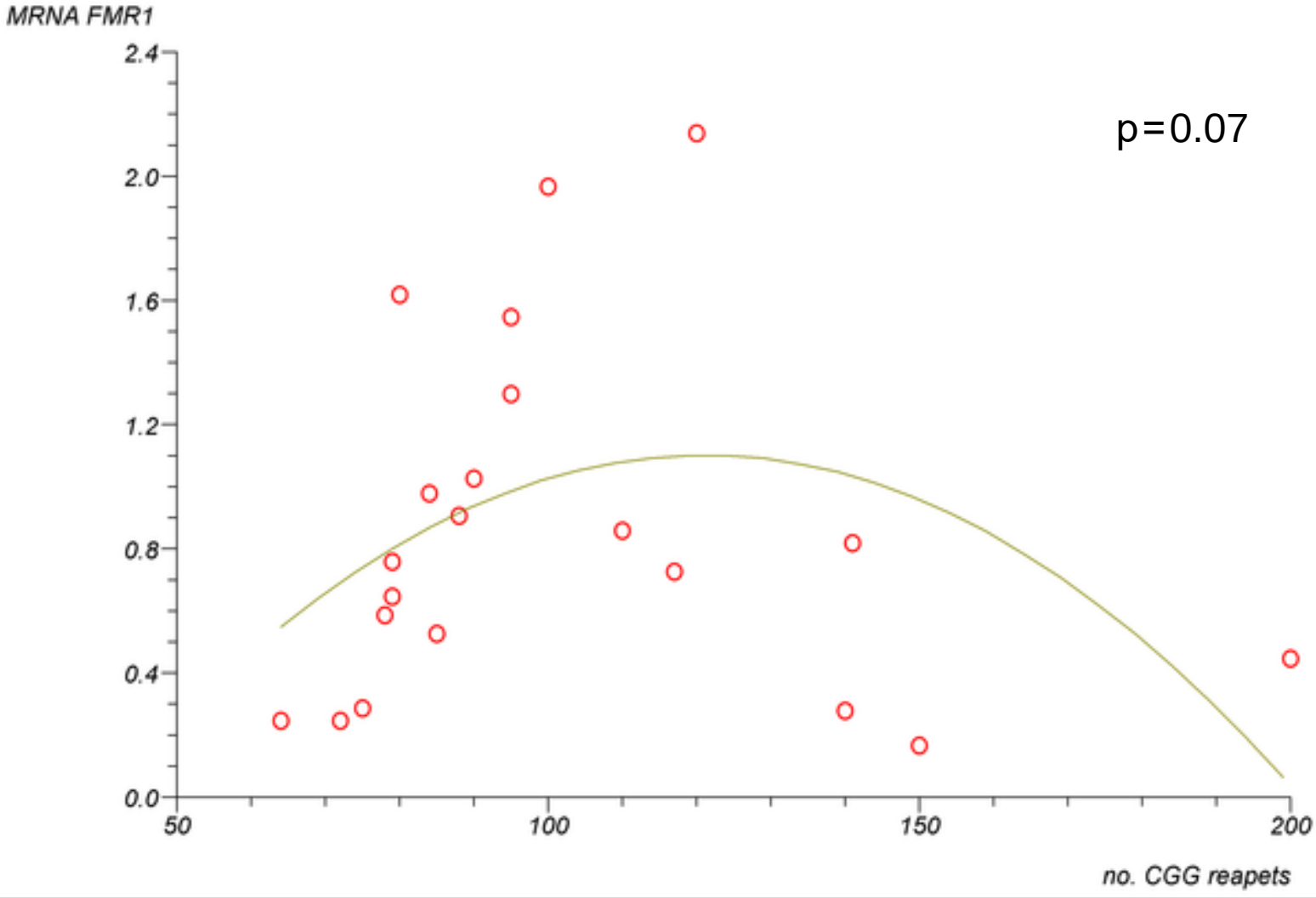
Elizur SE, et al. (2014) PLoS ONE

A non-linear association between the number of retrieved oocytes during IVF cycle and the number of CGG repeats in FMR1 premutation



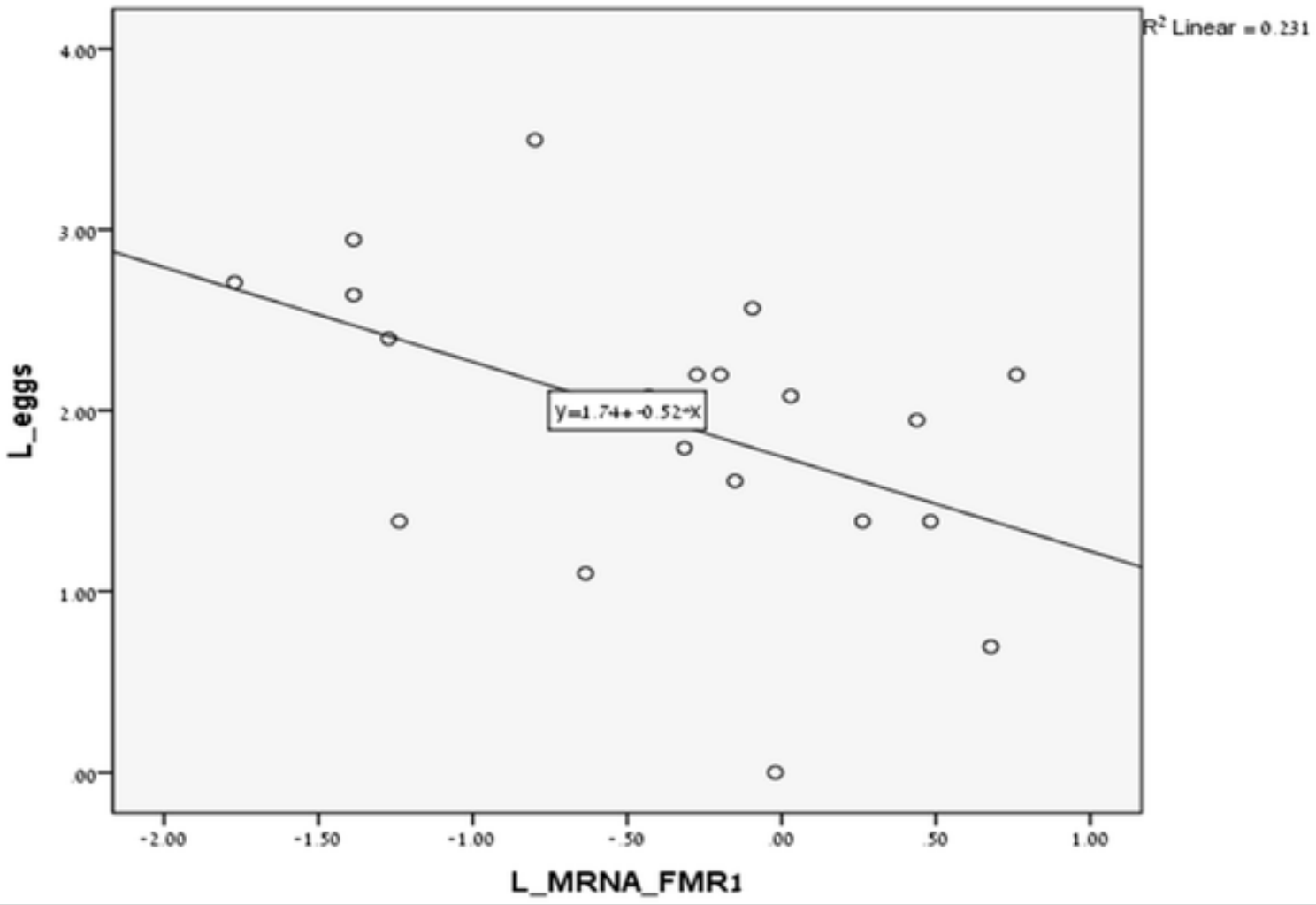
Elizur SE, et al. (2014) PLoS ONE

A trend for a non-linear association between FMR1 mRNA levels in FMR1 premutation carriers and the number of CGG repeats



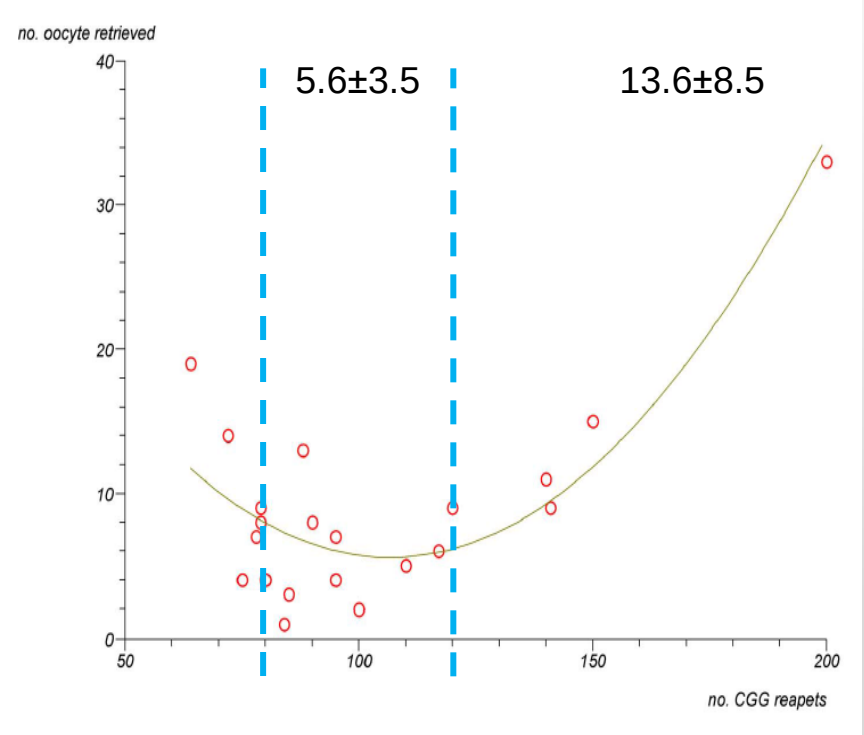
Elizur SE, et al. (2014) PLoS ONE

The number of retrieved oocyte according to mRNA levels of FMR1 in FMR1 premutation carriers

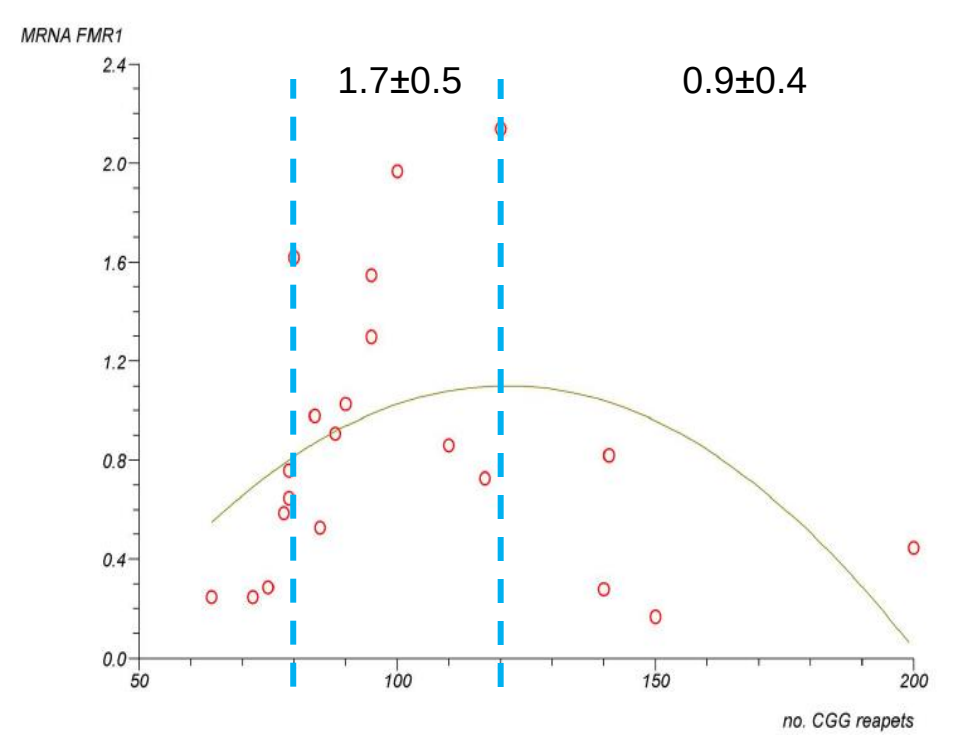


Elizur SE, et al. (2014) PLoS ONE

The effect of CGG repeat number on ovarian response among fragile X premutation carriers



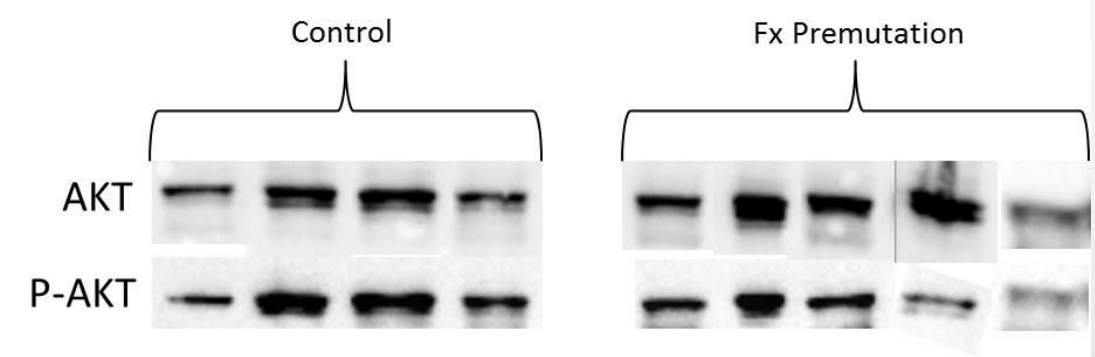
80-120 repeats associated with reduced number of retrieved oocytes



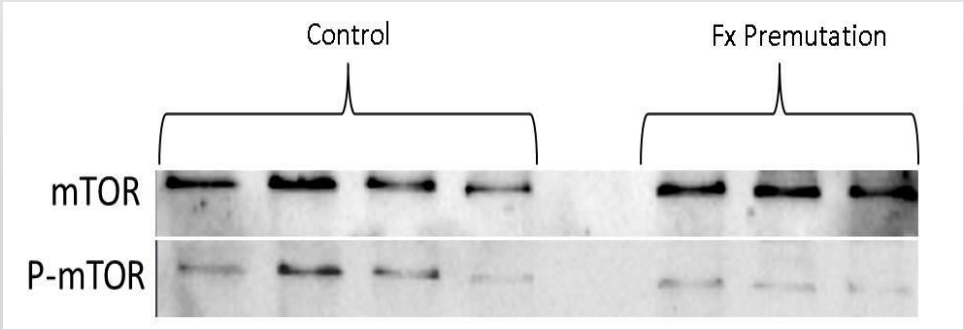
80-120 repeats associated with highest FMR1 mRNA levels

Elizur et al, 2014

AKT/mTOR pathway in granulosa cells



	p-AKT/AKT
Control	0.88 (±0.2)
FMR1 Premutation	0.5 (±0.14)
	p<0.05



	p-mTOR/mTOR
Control	0.48 (±0.13)
FMR1 Premutation	0.15 (±0.08)
	p<0.05

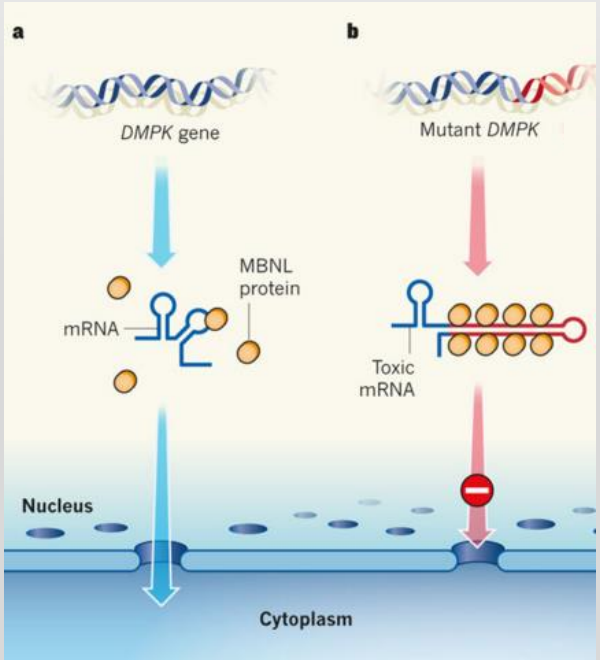
Is pathology the result of an RNA gain-of-function mechanism

- RNA gain-of-function- myotonic dystrophy type 1

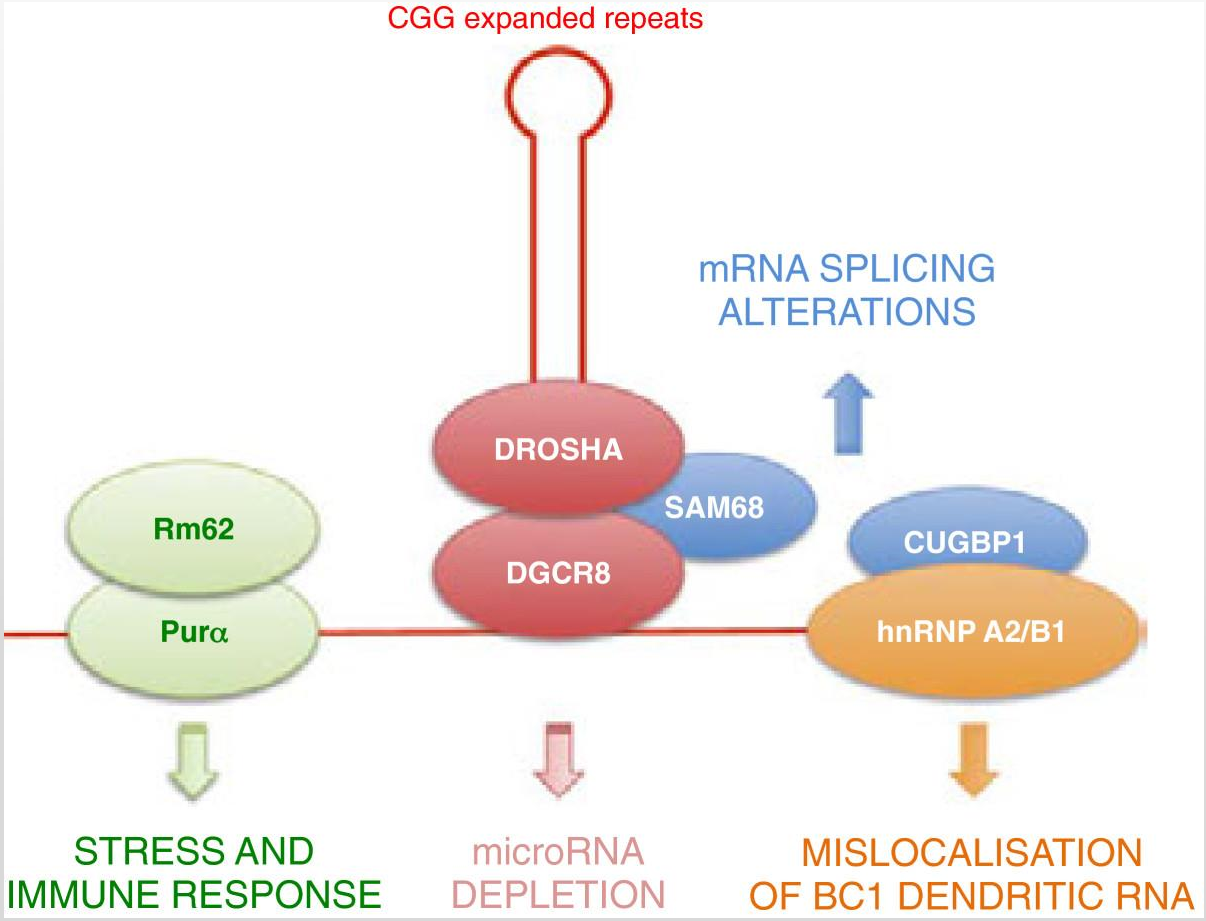
- CUG (DM1) accumulate in nuclear RNA aggregates that sequester the Muscleblind-like (MBNL) splicing factors and retaining them in the nucleus

- Depletion of the free pool of MBNL1 leads to specific alternative splicing changes and symptoms of DM

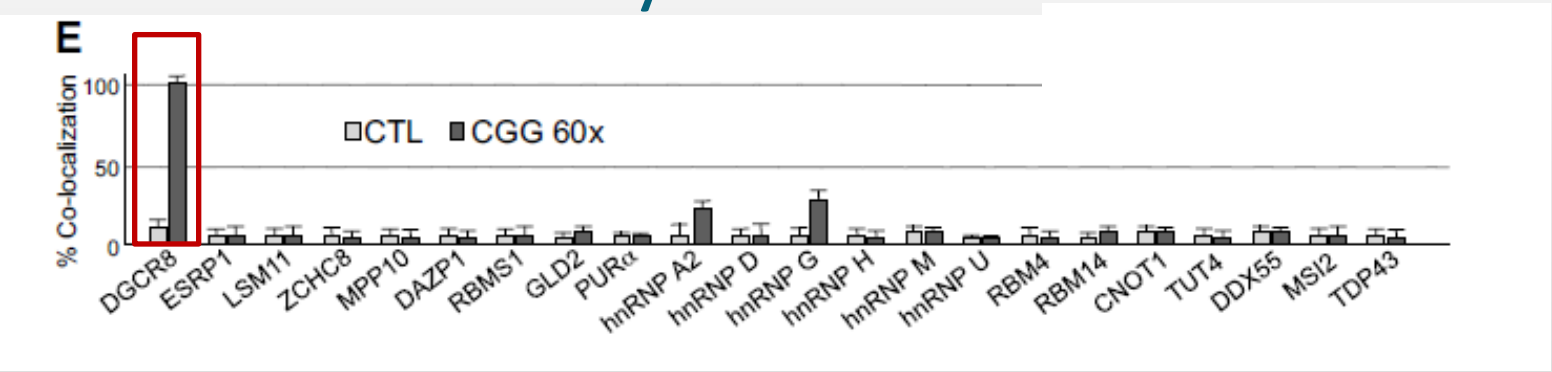
- Symptoms of DM can be reversed by supplying additional MBNL in animal models of the disease

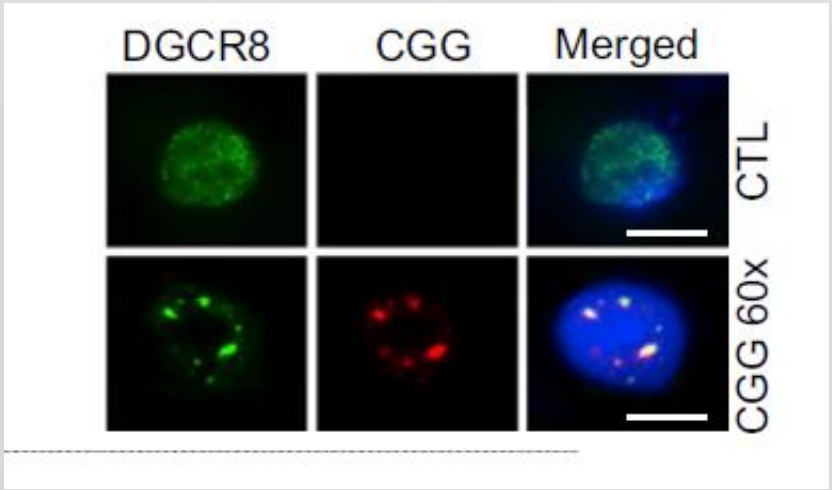


RNA gain-of-function mechanism in FXPOI and FXTAS



Sequestration of DROSHA and DGCR8 by Expanded CGG RNA Repeats Alters MicroRNA Processing in Fragile X-Associated Tremor/Ataxia Syndrome

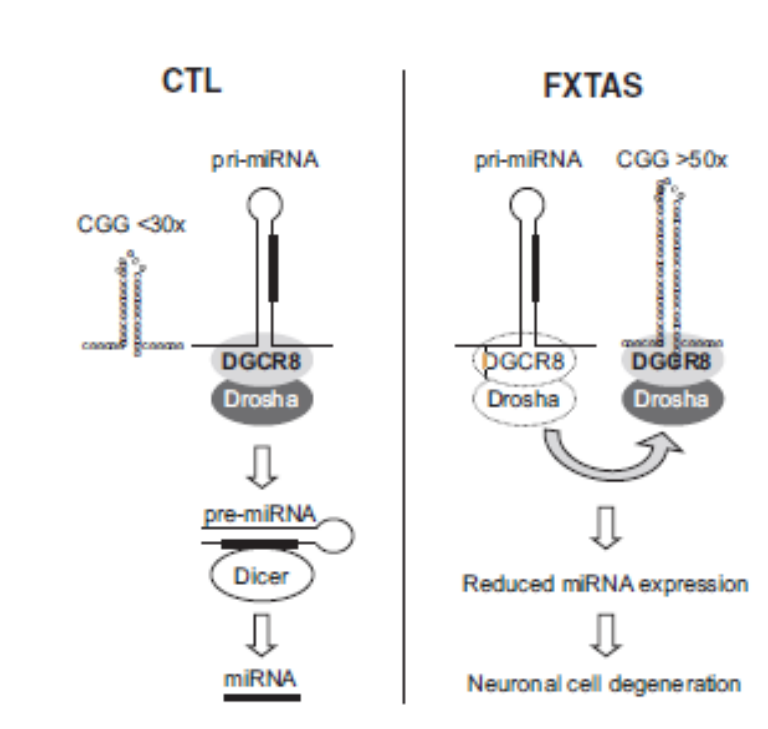


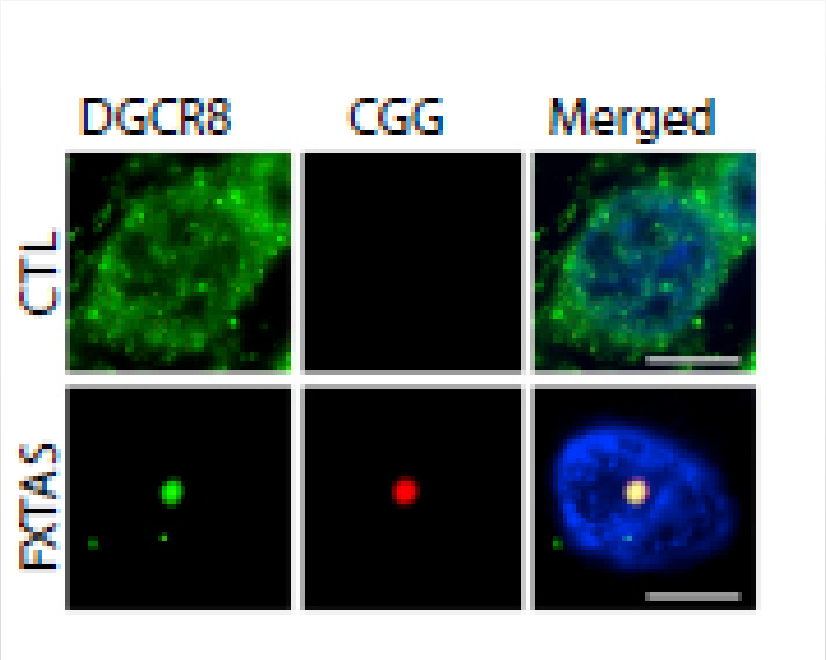


DGCR8 colocalized with CGG RNA aggregates

Sellier C, et al. (2013) Cell Reports

Sequestration of DROSHA and DGCR8 in Fragile X-Associated Tremor/Ataxia Syndrome

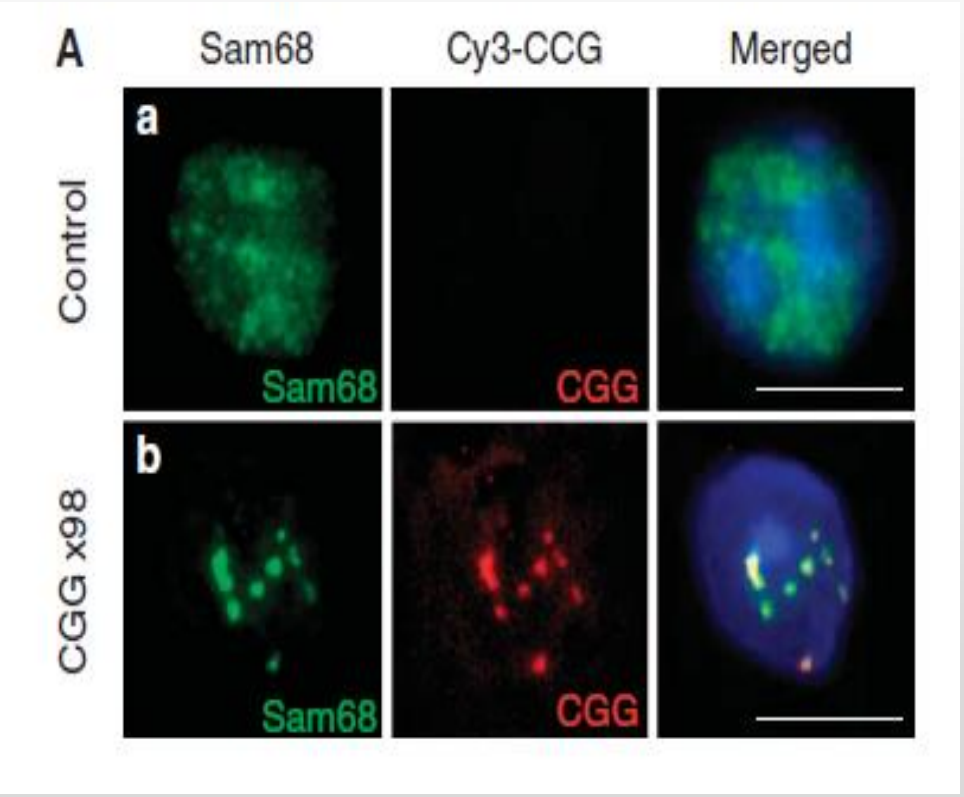


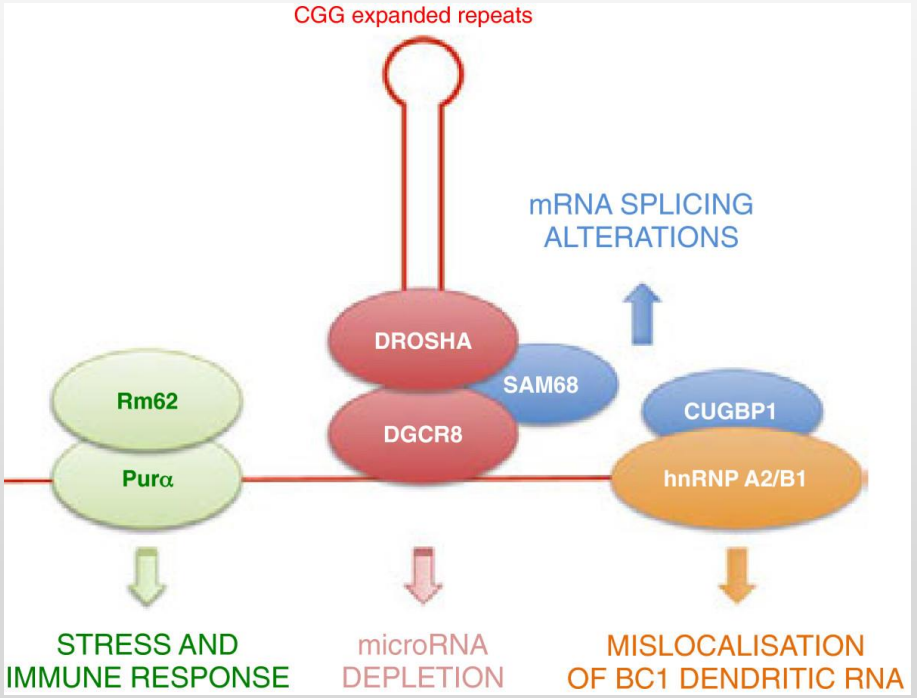


DROSHA and DGCR8 were diffusely localized in age-matched non- FXTAS controls

Sellier C, et al. (2013) Cell Reports

Sam68 co-localizes with expanded CGG RNA





CGG(98) transfected cells

Sellier C, et al. (2013) Cell Reports

Human Molecular Genetics, 2010, Vol. 19, No. 24 **4886–4894**
doi:10.1093/hmg/ddq422
Advance Access published on September 29, 2010

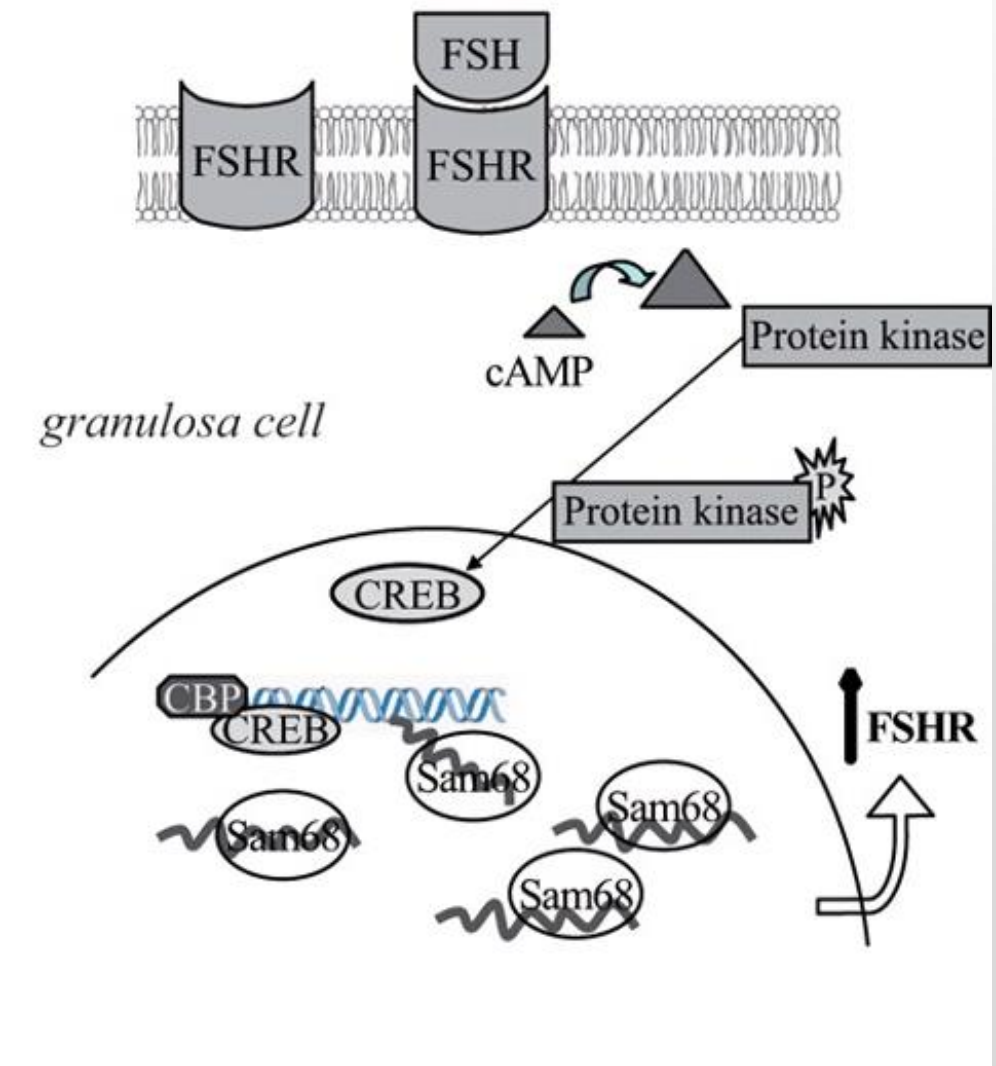
Ablation of the *Sam68* gene impairs female fertility and gonadotropin-dependent follicle development

Enrica Bianchi[1,2], Federica Barbagallo[1,2], Claudia Valeri[1], Raffaele Geremia[1], Antonietta Salustri[1], Massimo De Felici[1] and Claudio Sette[1,2,*]

[1]Department of Public Health and Cell Biology, University of Rome Tor Vergata, 00133 Rome, Italy and [2]Laboratory of Neuroembryology, Fondazione Santa Lucia, 00143 Rome, Italy

Bianchi, et al. (2010) Hum Mol Genet

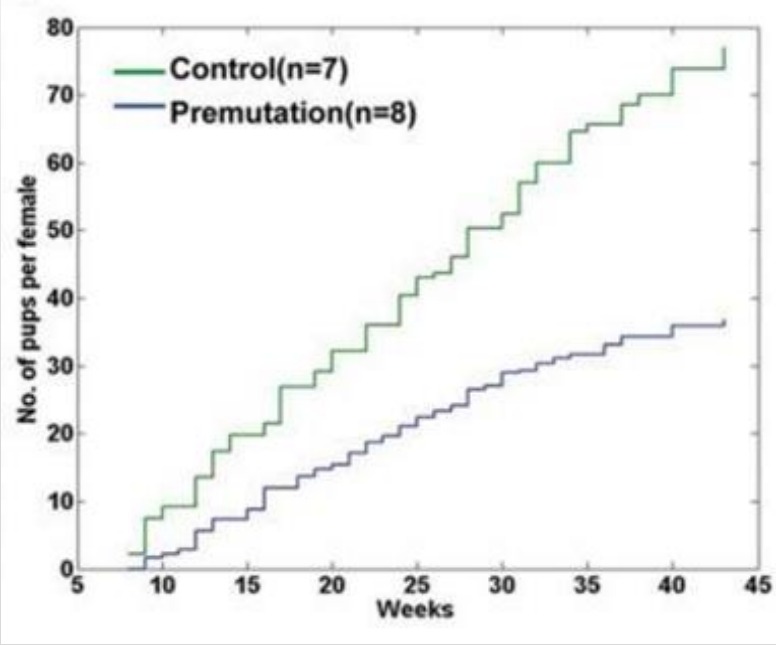
Hypothetical model of the function of Sam68 in the gonadotropin response of follicular cells



Bianchi, et al. (2010) Hum Mol Genet

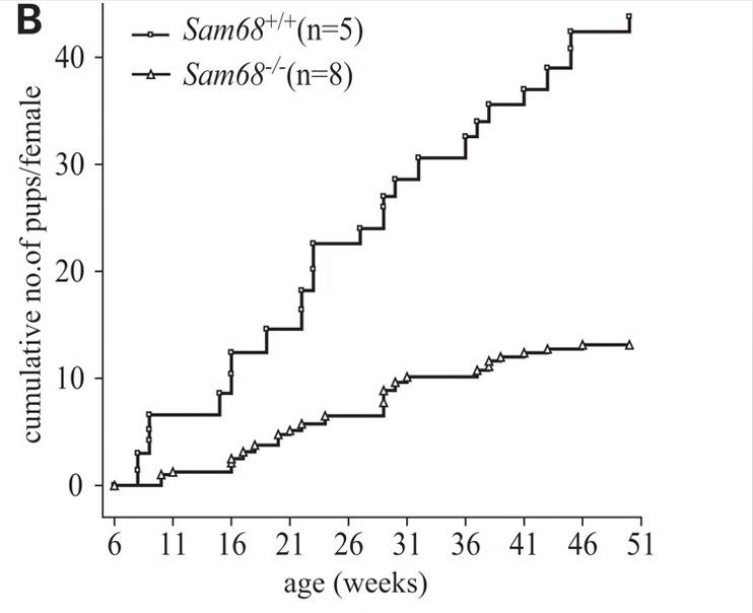
Fertility outcomes in Sam68 knockout female and FMR1 premutation mouse female

Lifespan breeding assays of randomly chosen PM mouse or control females



Lu C et al.

Lifespan breeding assays of randomly chosen *Sam68*$^{+/+}$ or *Sam68*$^{-/-}$ females

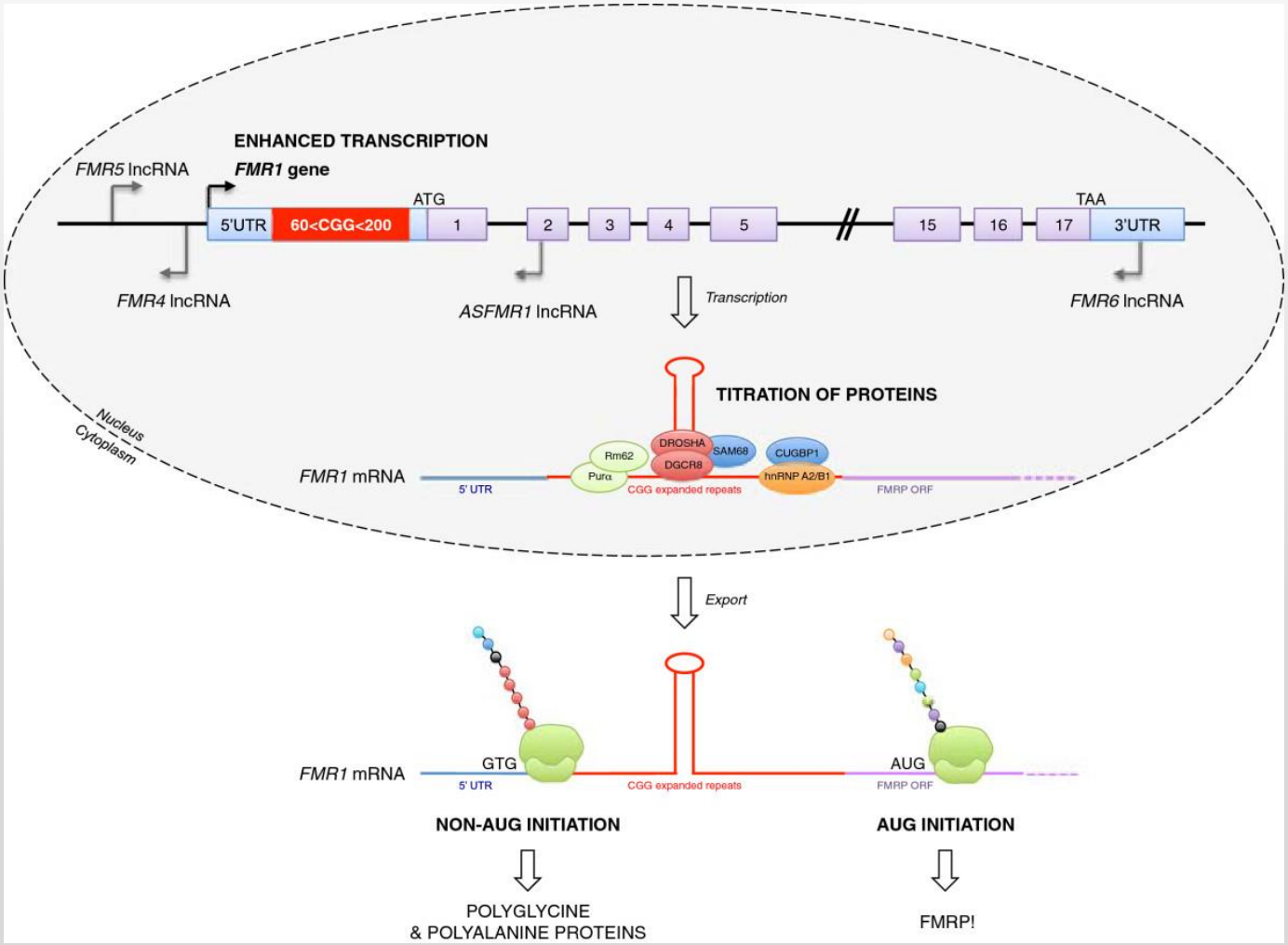


Bianchi E et al.

Fertility outcomes in Sam68 knockout female compared to FMR1 premutation mouse female

Phenotype compared to WT	**PM (98)**	**SAM68-/-**
Affect female fertility	Y	Y
Sterile	27% (3/11)	25% (2/8)
Delayed fertility (delivery of first litter)	↓	↓
Number of pups per litter	↓	↓
Cumulative number of pups	↓	↓
Uterine weight	↓	NA
Number of immature follicles (<PD25)	NS	NS
Growing follicles at 9 and 8 weeks (res)	↓	↓
Ovarian weight	↓	↓
PMSG→hCG→Ovulated oocytes	NS	↓
LH receptor	↓	↓
FSH receptor	NS	↓

Repeat associated non-ATG (RAN) translation: new starts in microsatellite expansion disorders



Results

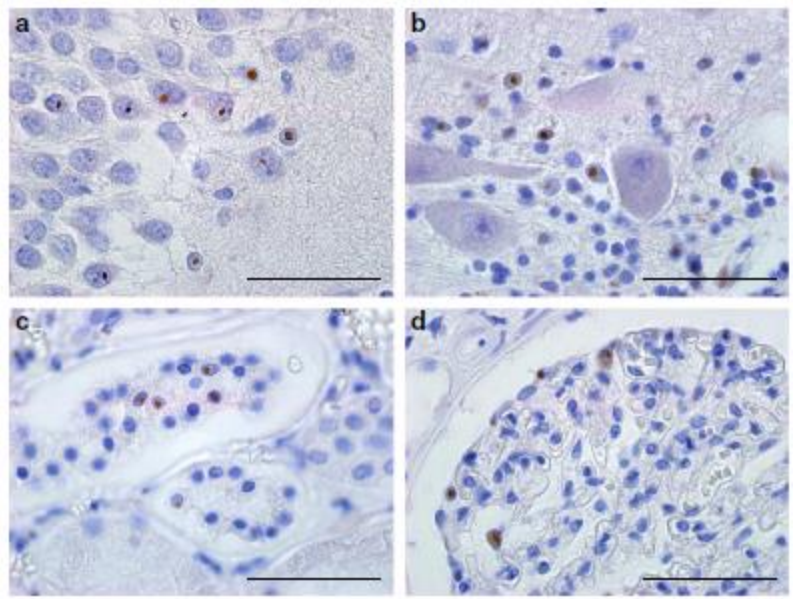
Buijsen et al. Acta Neuropathologica Communications 2014, 2:162
http://www.actaneurocomms.org/content/2/1/162

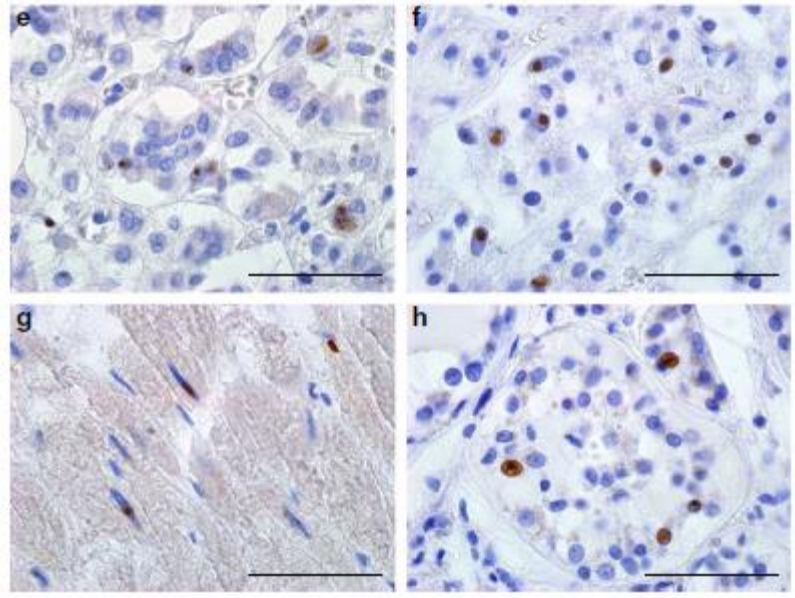
ACTA NEUROPATHOLOGICA COMMUNICATIONS

LETTER TO THE EDITOR Open Access

FMRpolyG-positive inclusions in CNS and non-CNS organs of a fragile X premutation carrier with fragile X-associated tremor/ataxia syndrome

Ronald AM Buijsen[1], Chantal Sellier[2], Lies-Anne WFM Severijnen[1], Mustapha Oulad-Abdelghani[2], Rob FM Verhagen[1], Robert F Berman[3], Nicolas Charlet-Berguerand[2], Rob Willemsen[1*] and Renate K Hukema[1*]





FMRpolyG-positive (9FM) intranuclear inclusions in a hippocampus, b cerebellum, c glomeruli and d distal tubule of the kidney, e zona glomerulosa and f zona reticularis of adrenal gland, g cardiomyocytes and h thyroid

Hum. Reprod. Advance Access published November 3, 2015
Human Reproduction, Vol.0, No.0 pp. 1–11, 2015
doi:10.1093/humrep/dev280

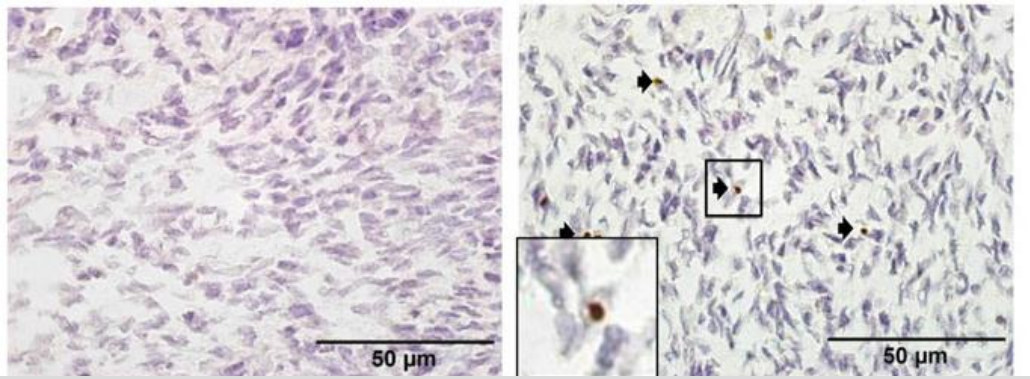
human reproduction

ORIGINAL ARTICLE *Reproductive biology*

Presence of inclusions positive for polyglycine containing protein, FMRpolyG, indicates that repeat-associated non-AUG translation plays a role in fragile X-associated primary ovarian insufficiency

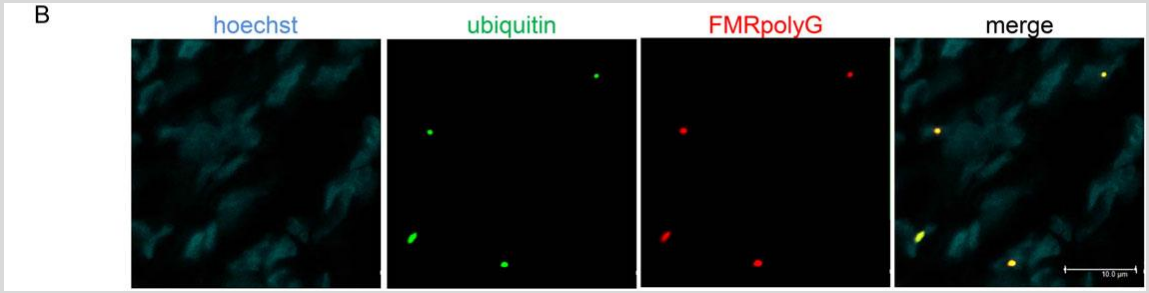
R.A.M. Buijsen[1,†], J.A. Visser[2,†], P. Kramer[2], E.A.W.F.M. Severijnen[1], M. Gearing[3], N. Charlet-Berguerand[4], S.L. Sherman[5], R.F. Berman[6], R. Willemsen[1], and R.K. Hukema[1,*]

FMRpolyG

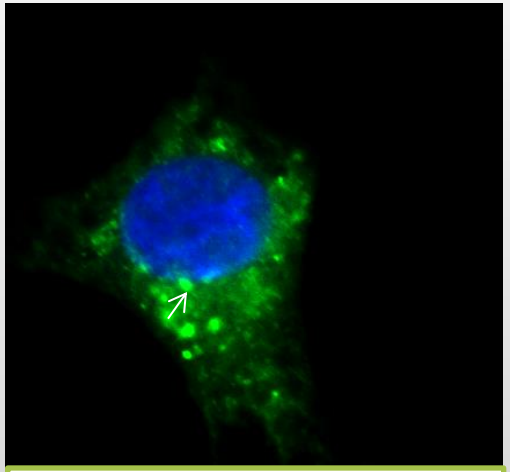


Control Fx premutation

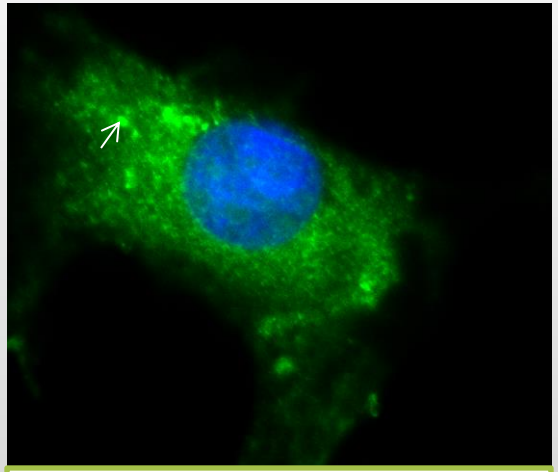
Intranuclear inclusions in ovarian stromal cells of a fragile X-associated primary ovarian insufficiency (FXPOI) patient.



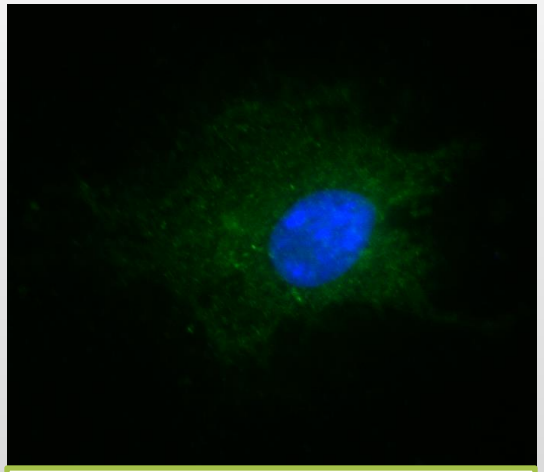
FMRpolyG-positive cytoplasmic inclusions of fragile X carriers in granulose cells



9FM-F25



9FM-F5



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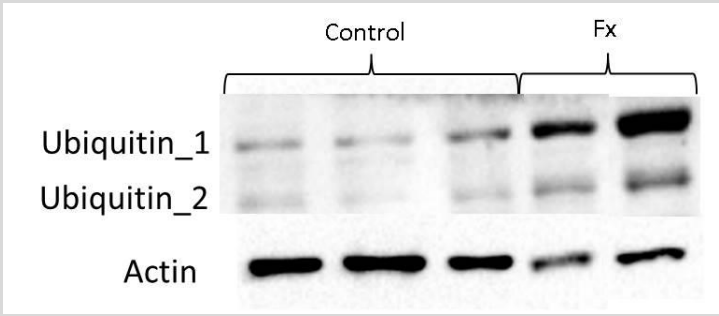
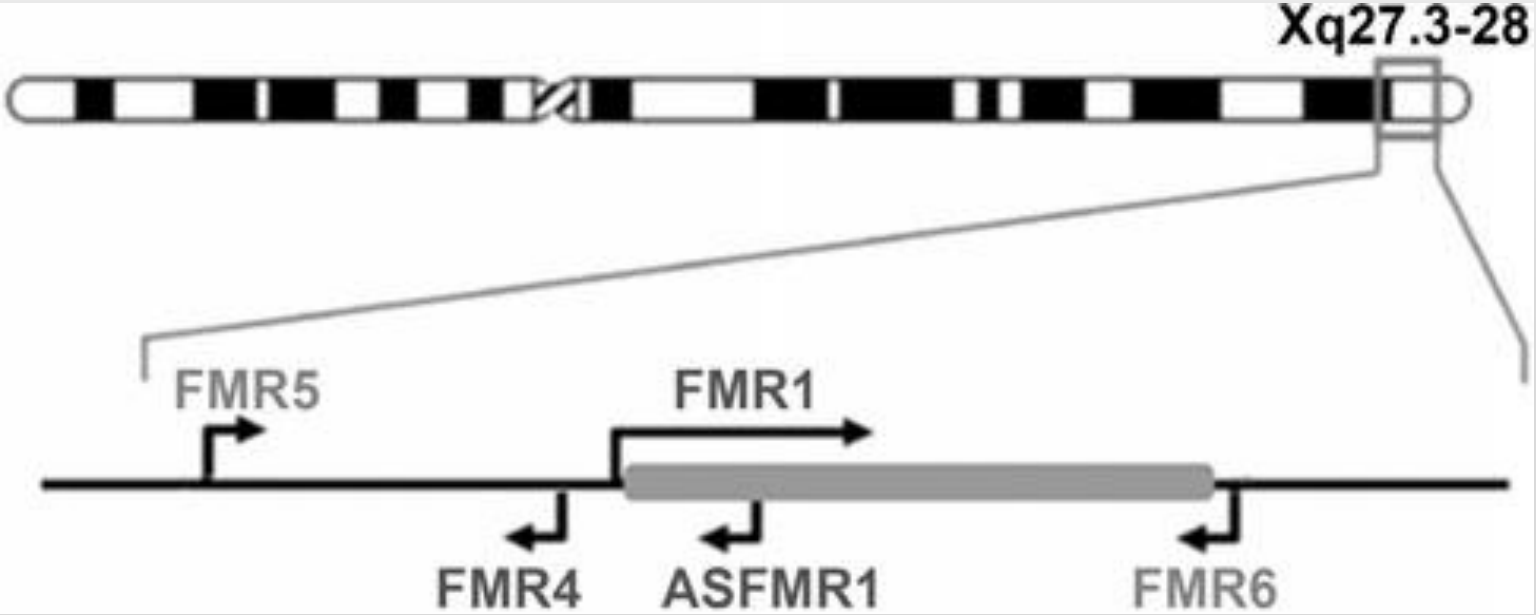


Results

Comprehensive analysis of the transcriptional landscape of the human *FMR1* gene reveals two new long noncoding RNAs differentially expressed in Fragile X syndrome and Fragile X-associated tremor/ataxia syndrome

Chiara Pastori · Veronica J. Peschansky · Deborah Barbouth · Arpit Mehta · Jose P. Silva · Claes Wahlestedt



Pastori C, et al. (2014) Hum Gent

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ORIGINAL ARTICLE

FMR6 may play a role in the pathogenesis of fragile X-associated premature ovarian insufficiency

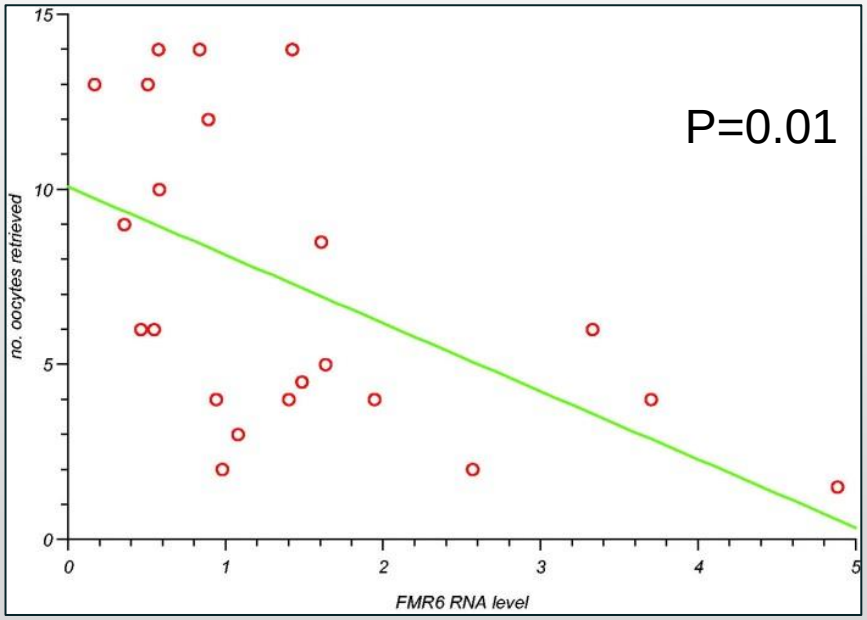
S. E. Elizur[1,2], Olya Dratviman-Storobinsky[1,2], Sanaz Derech-Haim[1,2], Oshrit Lebovitz[1,2], Jehoshua Dor[1,2], Raoul Orvieto[1,2], and Yoram Cohen[1,2]

Clinical and laboratory characteristics of control and FMR1 premutation carriers groups

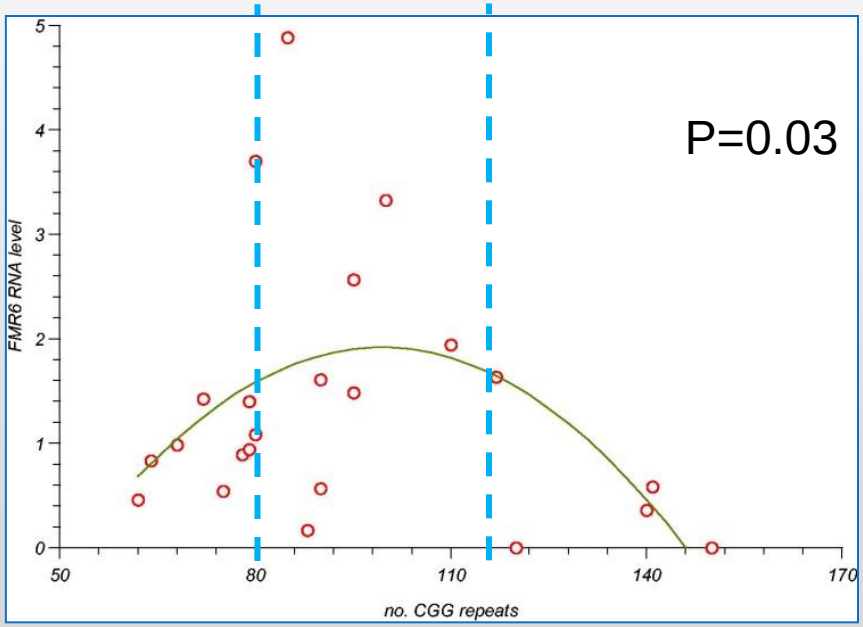
	FMR1 premutation $N=22$	Control $N=11$	p value
Age [mean (SD) years]	31.9 (3.4)	31.3 (5.4)	n.s.
Parity (median)	0	0	n.s.
Mean FSH (IU) (basal) (SD)	9.3 (3.5)	6.6 (1.2)	0.01
Mean LH (IU) (basal) (SD)	4.5 (2.2)	5.0 (1.7)	n.s.
Mean basal FSH/LH ratio (SD)	2.5 (1.3)	1.4 (0.5)	0.01
Mean estradiol (basal) (pmol/L) (SD)	153 (53)	161 (71)	n.s.
Mean total gonadotropins used in stimulation (IU) (SD)	2730 (1274)	1803 (1062)	0.06
Mean duration of stimulation (days) (SD)	11.2 (2.8)	10 (1.7)	n.s.
Mean peak estradiol (pmol/L) (SD)	6464 (3005)	7930 (2412)	0.06
Mean no. oocyte retrieved (SD)	7.9 (4.8)	13.6 (6.5)	0.008
Mean no. embryo transferred (SD)	1.4 (1.2)	1.7 (0.8)	n.s.
No. of pregnancies	4	2	n.s.
Mean FMR1 repeats (range)	93 (64–150)	<55	

Elizur et al, 2015

Elevated levels of FMR6 mRNA in carriers in peripheral blood and granulosa cells



FMR6 vs number of oocyte retrieved



FMR6 vs CGG repeats

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Thank you for listening

IVF Unit

Dr. Nicolas Charlet-Berguerand

3 rd International Conference on:

FMR1 Premutation:
Basic Mechanism and Clinical Involvement

JERUSALEM Sep 2017

Summary

- FMR1 premutation carriers are at increased risk for premature ovarian insufficiency leading to variable medical complications of early menopause and fertility loss.
- The presence of RAN translation protein product and high levels of FMR1 and FMR6 RNA in granulosa cells are associated with diminished ovarian response suggesting the role of RNA toxic gain of function and protein toxicity mechanism in FXPOI.
- Further exploration of the mechanism leading to ovarian damage in FMR1 premutation carriers will hopefully assist in developing novel medications in order to prevent ovarian failure.