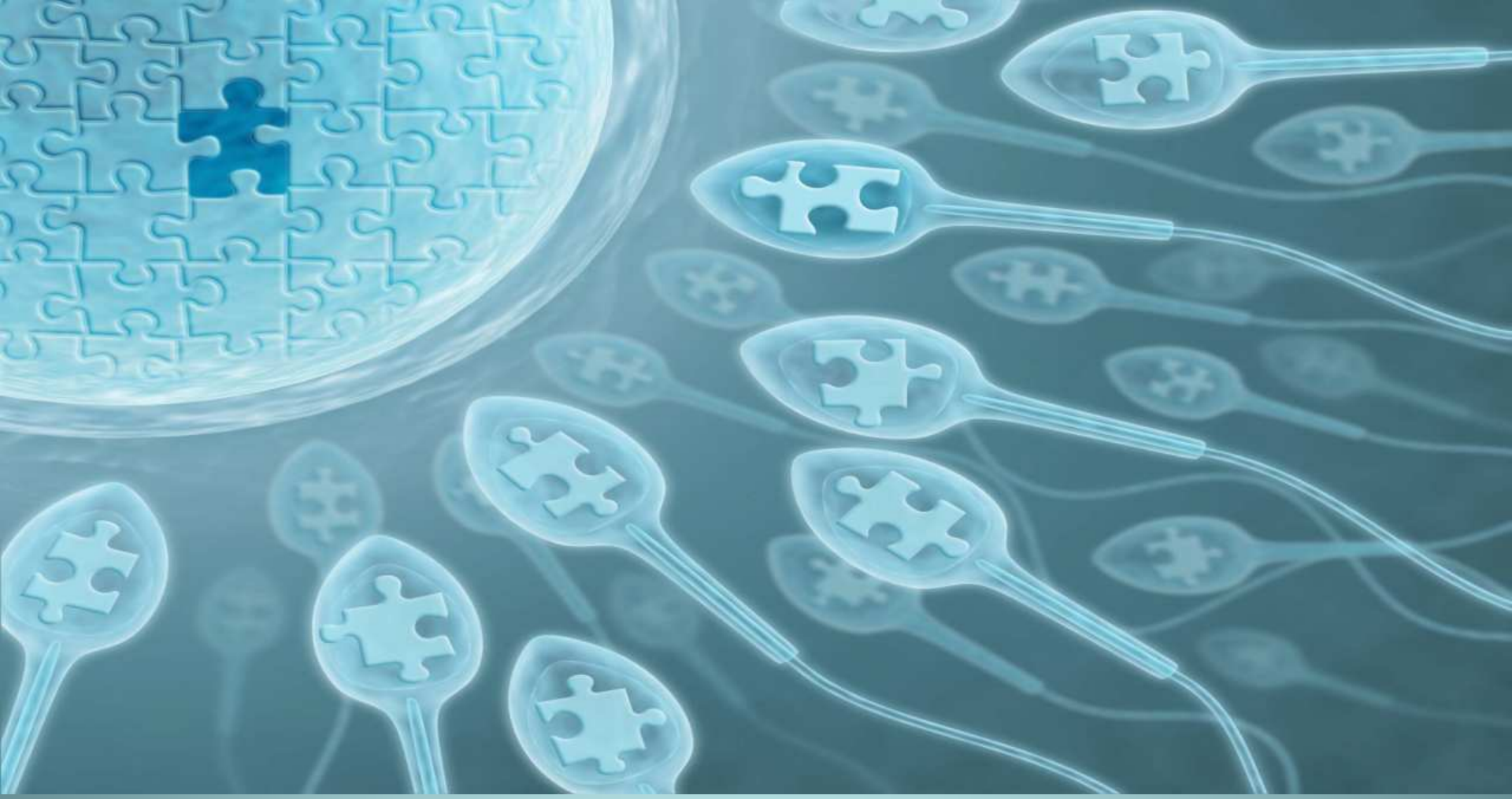
XIV Annual Meeting of the Mediterranean Society for Reproductive Medicine (MSRM)
April 21-24 2016, Izmir, Turkey

"Evaluation of in Vitro Fertilization Outcomes Using the FMR1 CGG Repeat Level and Genotypes as a Criterion"

Eleni Theochari, MSc Biologist

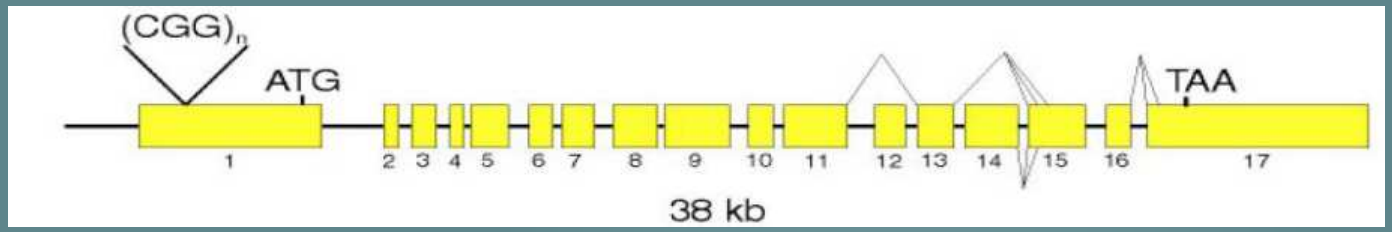
Master of Reproductive – Regenerative Medicine
School of Medicine, Athens University, Greece



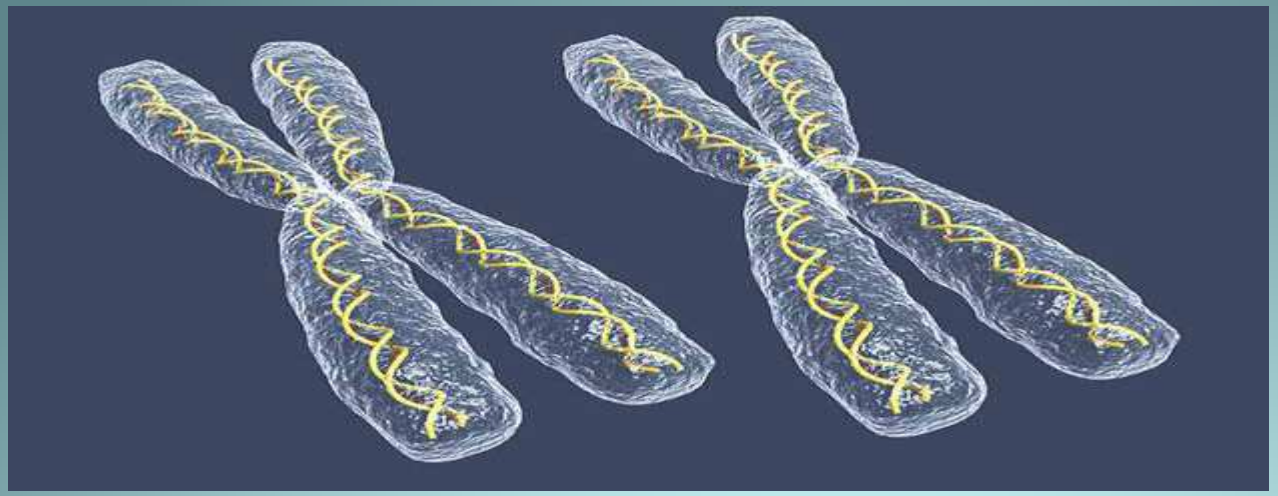
Variability in the subfertile patient population excludes the possibility of a single approach to controlled ovarian stimulation (COS) covering all the requirements of a patient. In the future, genetic screening may allow an individual patient's response to COS to be predicted based on genotype.

(*Alviggi et al. Reproductive Biology and Endocrinology 2012,10:9)*

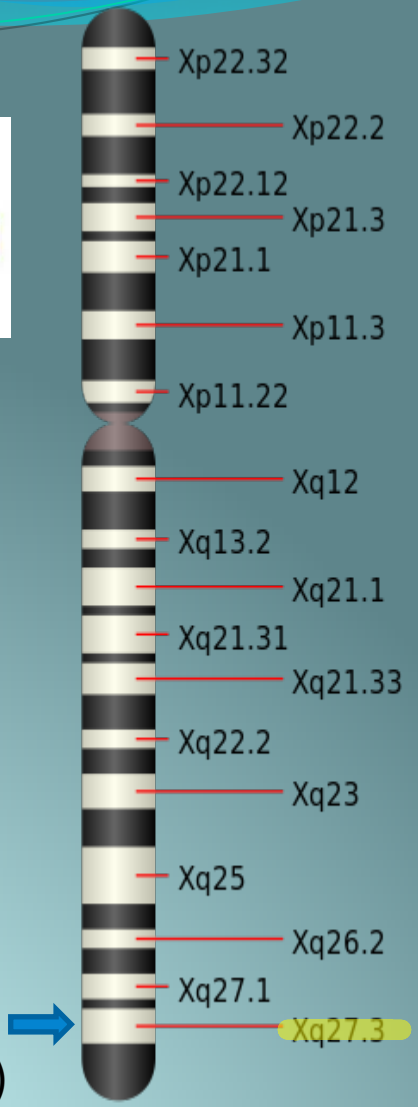
The FMR1 gene



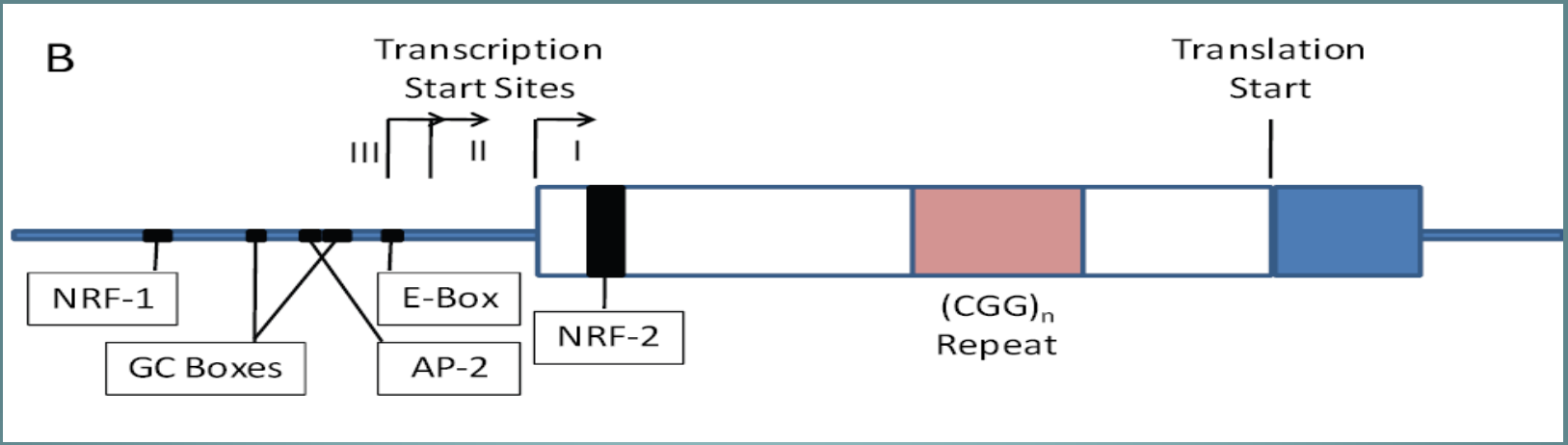
- Location : Xq27.3 17 exons – 16 introns Alternative Splicing



- Normal Female Karyotype : 46, XX → 2 FMR1 alleles (genotype)



5′UTR-(CGG)n: Size Matters

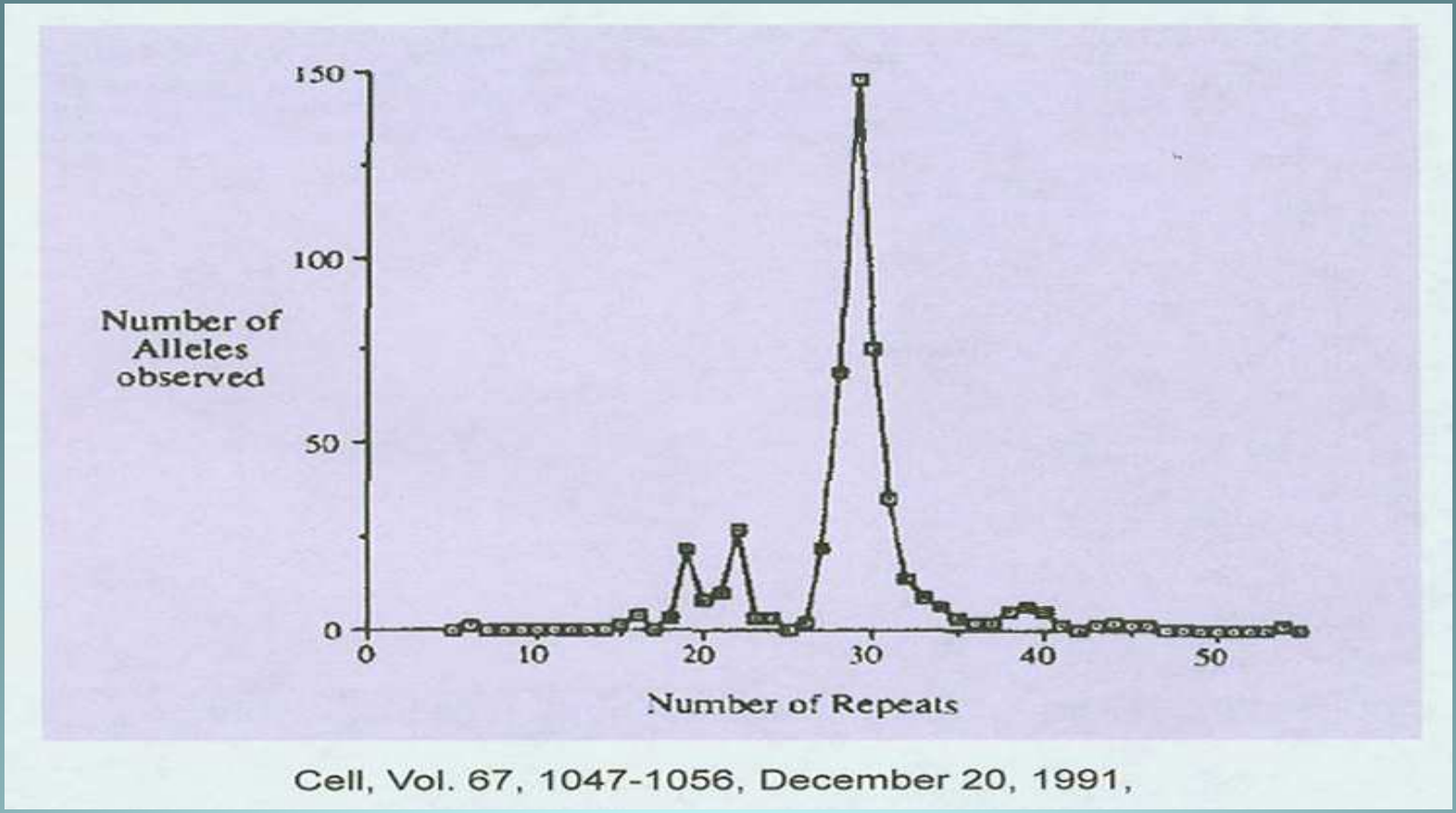


5′UTR Role : Post-transcriptional regulation of gene expression
(transport of mRNAs, translation efficiency, subcelular localization, stability)

(CGG)n : numerically polymorphic (dynamic mutation)
Can exert both positive (n<30) and negative (n>30) effect on translation.
Optimal translation near the modal repeat number within the general population.

(Chen et al. Hum Mol. Gen,2003,Vol.12, N°.23, 3067-3074)

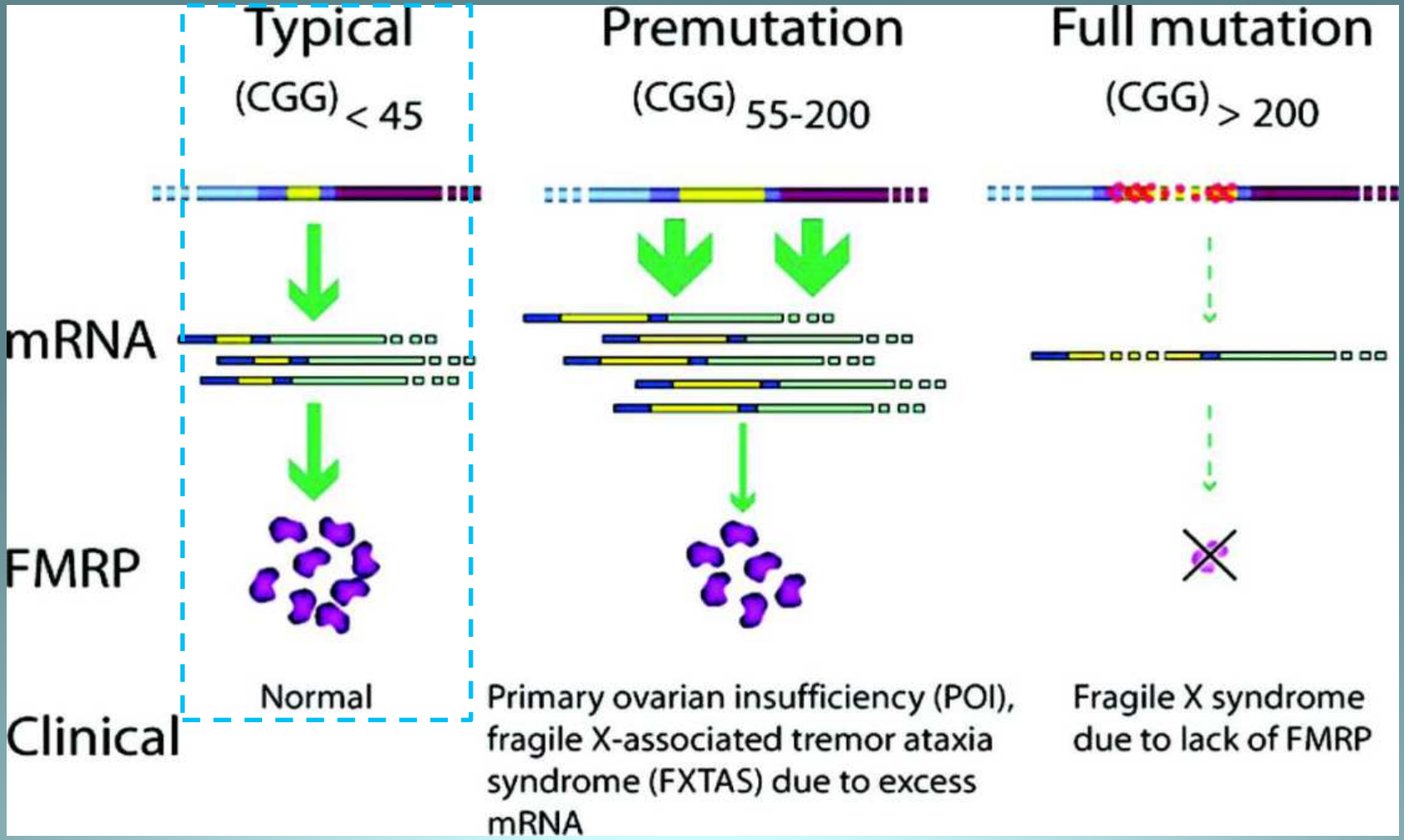
Distribution of CGG lengths in the general population



Cell, Vol. 67, 1047-1056, December 20, 1991,

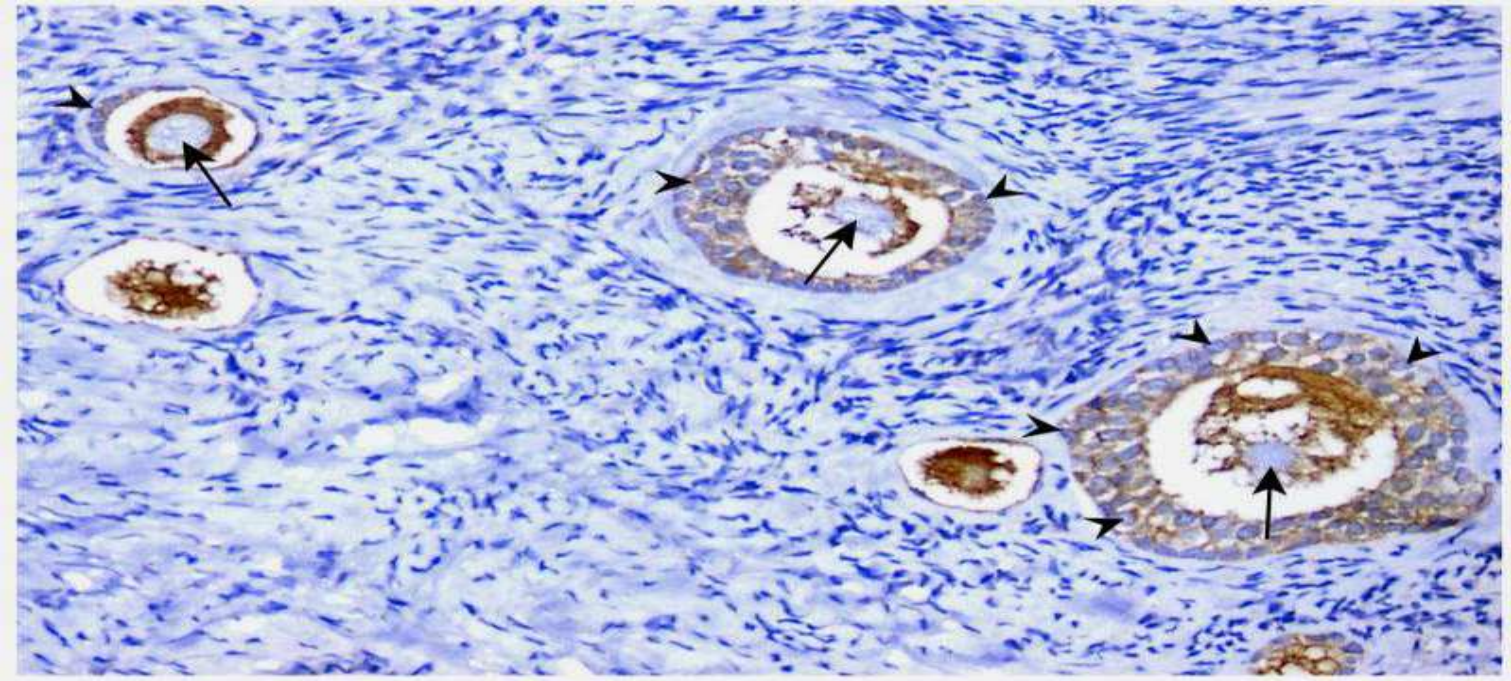
- Fu's distribution curve: $(CGG)_{29}$ the most frequent allele
- Greek general population : $(CGG)_{28}$ the most frequent allele *(Sofokleous et al. 2008)*

FMR1 : A gene with three faces



(Pediatrics, 2009 January ; 123(1): 378–390)

FMRP in the ovary



(Clin Genet. 2011 September; 80(3):214-225)

- Major cellular translation repressor protein – Controls the level of translation of multiple transcripts (including its own) – Interacts with components of RISC and miRNAs *(Garber et al. 2006)*

- FMRP is expressed after birth in female germline, predominantly in granulosa cells (human) *(J. Schuettler et al. 2011)*
- Increasing FMRP expression with advancing follicle development (rat model) *(Ferder et al. 2013)*

- Women with $(CGG)_n$ outside the range associated with normal folliculogenesis (26<n>34)
  → relaxed FMR1 transcription control → altered FMRP levels→ affect folliculogenesis *(J. Schuettler et al. 2011)*

FMR1 and Reproductive Medicine

- 29-30 CGG repeats reflective of normal ovarian reserve. Higher and Lower counts denote risk for Premature Ovarian Senescence

(Gleicher et al. 2009e)

- Definition of new genotypes *(within the typical range)* for the ovarian function of FMR1 gene

Normal (norm) : Both alleles (CGG)n= **26-34**
Heterozygous (het) : One allele outside normal range
Homozygous (hom) : Both alleles outside normal range

(Gleicher et al. 2010c,d)

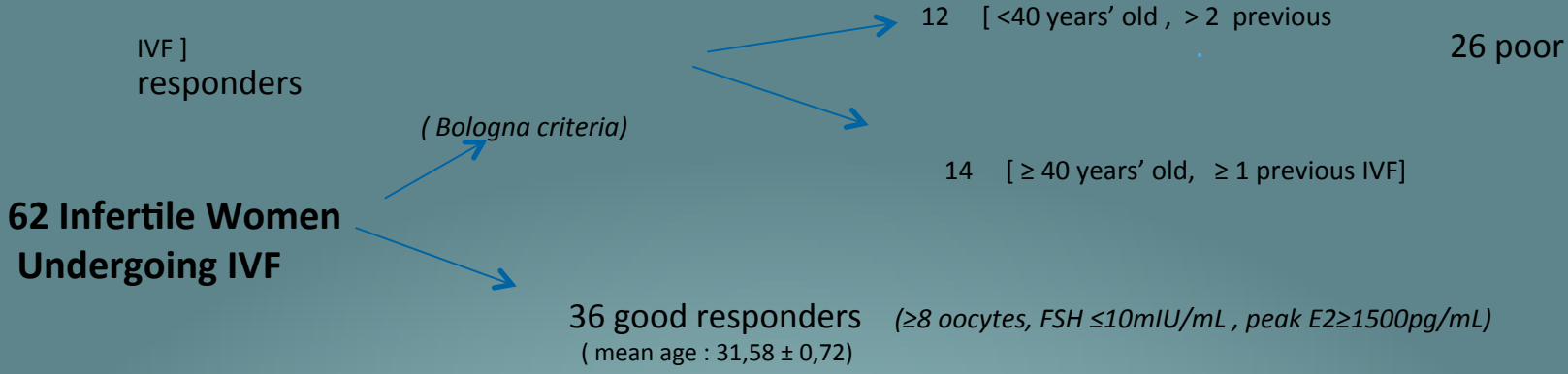
- FMR1 genotypes predictive of pregnancy, het-norm/low most significantly and with decreasing chance in comparison to norm genotypes

(Gleicher et al. 2011)

- A low FMR1 allele ($CGG_{<26}$) is associated with significant poorer morphologic embryo quality and pregnancy chance

(Gleicher et al. 2014)

Our Study



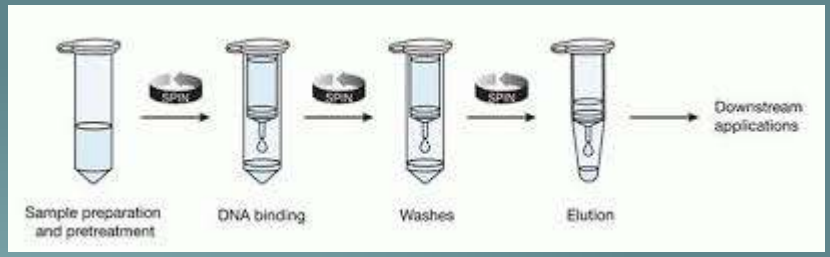
Infertility Reason: tubal, male, unexplained

Data 1. Age, weight, BMI, years of infertility, previous IVF
2. Hormonal profile (FSH, LH, PRL, TSH)
3. Parameters of ovarian stimulation/induction (days of stimulation, total FSH dose, peak E2)
   - < 40 years: long agonist protocol
   - ≥ 40 years: short agonist protocol
4. IVF Outcome (№ follicles, № oocytes, maturation rate, fertilization rate, embryo quality, pregnancy)

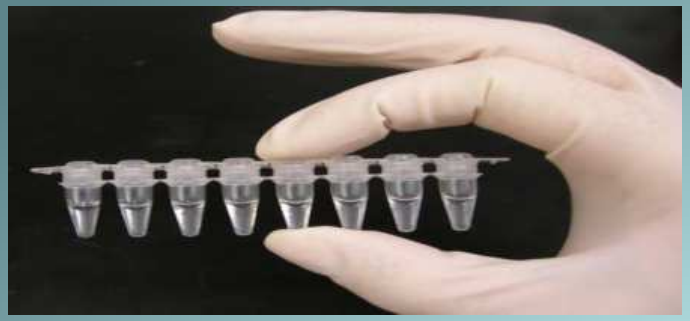
Methods

Sample/Data Collection

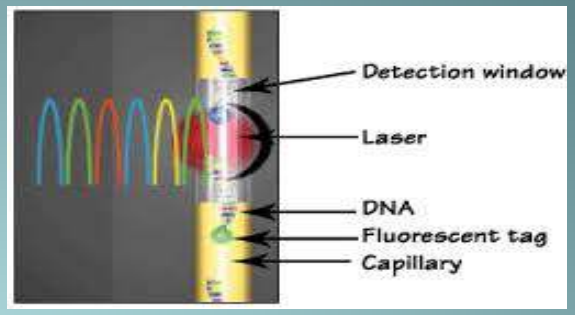
DNA extraction



PCR Amplification



Capillary Electrophoresis

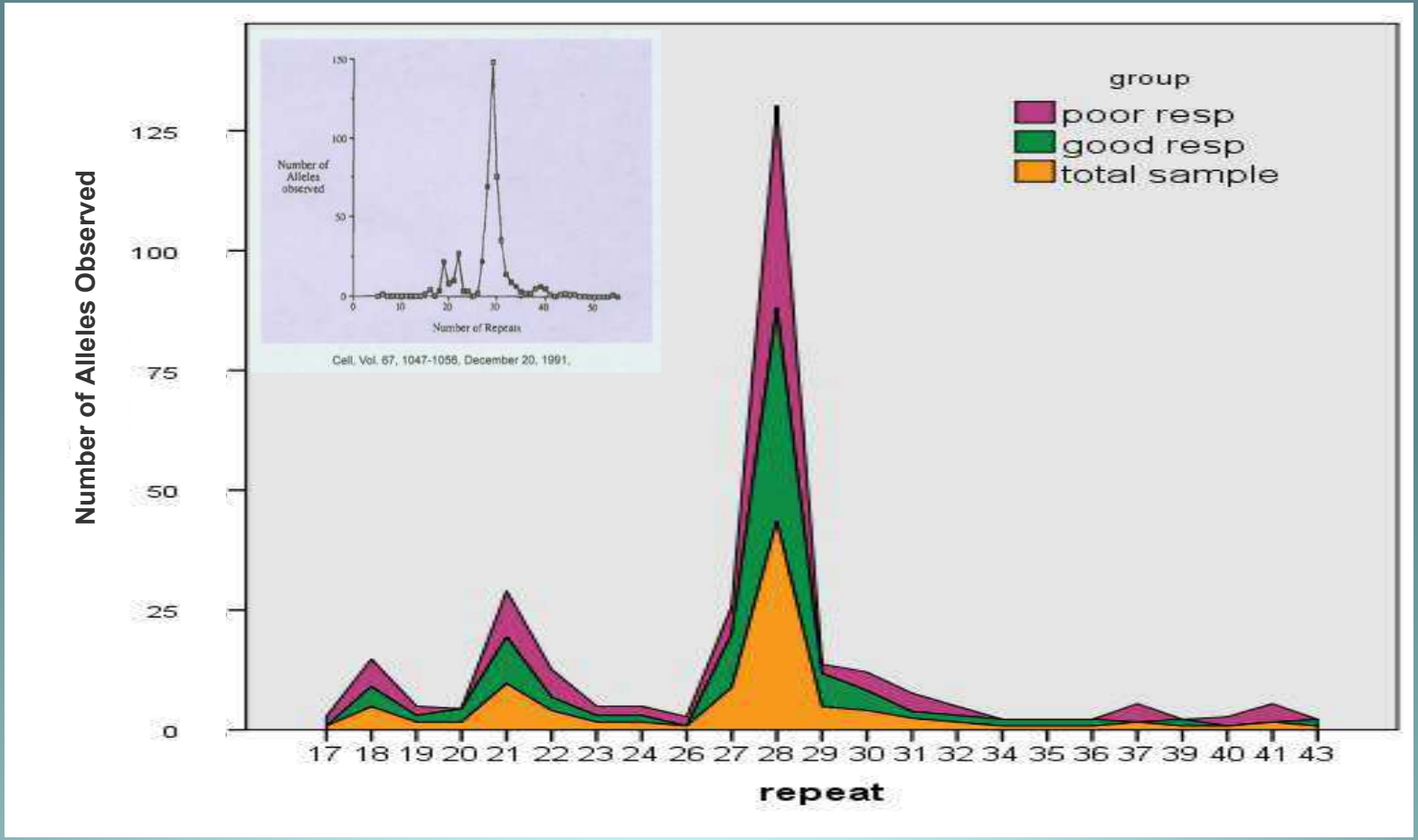


Software Analysis

Statistical Analysis

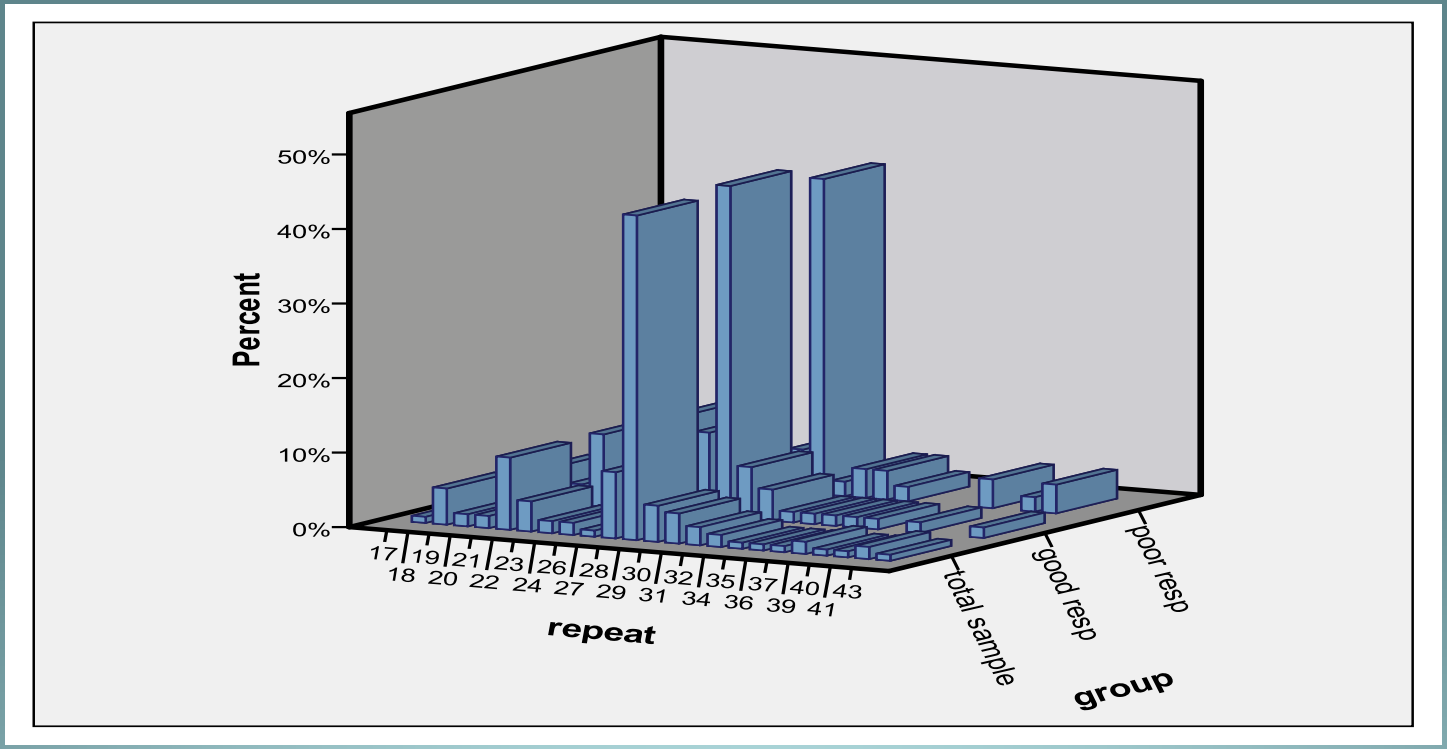
RESULTS

Distribution of FMR1 CGG triple repeat expansions in the study infertile population



- Distribution curve of FMR1 allele frequencies based on the CGG repeat number of an infertile Greek population was found in accordance with Fu's Distribution curve.
- No shift towards higher ends neither in the total sample, nor the good or the poor responders separately.
- No intermediate or premutation alleles found

Most frequent allele in the study population



	mean ± SEM	95% CI	MAX REPEATS	PERCENT
poor resp	27,12 ± 0,76	[25,68 - 28,65]	28	42,3% (22/52)
good resp	27,06 ± 0,53	[26,00 - 28,12]	28	44,4% (32/72)
total sample	27,08 ± 0,44	[26,20 - 27,96]	28	43,5% (54/124)

$(CGG)_{28}$ the most frequent allele observed in the total sample, good or poor responders (43,5%, 42,3%, 44,4% respectively).

No different from Greek general population.

Distribution of FMR1 Genotypes among good and poor responders

	Good responders n (%)	Poor responders n (%)
norm	19 (52,8%)	12 (46,2%)
het	14 (38,9%)	8 (30,8%)
hom	3 (8,3%)	6 (23,1%)
total	36 (100%)	26 (100%)
Fisher's exact test	**P-value = 0,298**	

- The most prevalent genotype in both groups was Norm (52,8% , 46,2% respectively).
- The distribution of genotypes between good and poor responders did not statistically differ (p-value 0,256).

Impact of Genotypes on IVF Outcomes

Genotype	N	Days of Stimulation marginal Means	Std. Error	p-value
norm	31	9,877	0,278	
het	22	10,943	0,334	**0,023**
hom	9	11,118	0,514	
adjusted for age (GLM model)				

- In the whole study population, women carrying the Normal genotype had less mean number of days of stimulation compared to those carrying the Hom genotype (p-value = 0,023).

Genotype	N	Maturation Rate marginal Means	Std. Error	p-value
norm	31	0,744	0,028	
het	18	0,796	0,038	**0,026**
hom	7	0,598	0,060	
adjusted for age (GLM model)				

- In the whole study population, women carrying the Norm or Het genotype had statistically significant higher mean of oocyte maturation rates compared to those carrying the Hom genotype (p-value = 0,026).

Impact of Genotypes on IVF Outcomes

					95,0% C.I. for EXP(B)	
Binary Logistic Model	B	S.E.	p-value	OR	Lower	Upper
age	-0,081	0,059	0,168	0,922	0,822	1,0347
Geno (het)	-1,774	0,856	**0,038**	0,170	0,032	0,9081
Geno (hom)	-0,531	0,909	**0,559**	0,588	0,099	3,4935
Constant	2,067	2,055	0,315	7,900		
Binary Logistic Model						
Dependent variable - Pregnancy						
Independent variables - Genotype (Norm is reference) and Age						

- In the whole study population, women carrying a Het genotype had 83% (1-0,170) less pregnancy odds compared to those carrying a Norm genotype (95% C.I [32%-91%]), p-value = 0,038. Women carrying a Hom genotype did not have statistically significant different pregnancy odds compared to those carrying a Norm genotype, p-value = 0,559.

Impact of a Low Allele On IVF Outcomes

Low allele	N	**Days of Stimulation marginal Means**	Std. Error	p-value
Yes	26	10,952	0,309	**0,033**
No	36	10,063	0,262	
adjusted for age (GLM model)				

- In the whole study population women not carrying a Low Allele in their Genotype had less mean number of days of stimulation compared to those carrying a Low Allele (p-value = 0,033).

					95,0% C.I.for EXP(B)	
Binary Logistic Model	B	S.E.	p-value	OR	Lower	Upper
age	-0,067	0,059	0,257	0,935	0,833	1,050
low_allele (Yes)	-0,868	0,674	**0,198**	0,420	0,112	1,572
Constant	1,357	2,052	0,508	3,885		
Binary Logistic Model						
Dependent variable - Pregnancy						
Independent variables - Low allele (No is reference) and Age						

- In the whole study population the presence of a low allele ($CGG_{<26}$) was not associated with differences in pregnancy odds (p-value=0,198).

CONCLUSIONS

FMR1 genotypes seem to have both a quantitative (days of stimulation) and a qualitative (maturation rates) effect on IVF outcomes, as well as on pregnancy odds.

With regards to their FMR1 genetic background :

- Infertile women carrying a Norm genotype had less mean days of stimulation, higher mean of oocyte maturation rates and presented the best pregnancy odds.

- Infertile women carrying a Hom genotype needed the most mean days of stimulation and had the lowest mean of oocyte maturation rates. The comparative pregnancy odds in this underrepresented subgroup remains for us inconclusive as a sample effect cannot be ruled out.

- Infertile women carrying a Het genotype had 83% less pregnancy odds compared to those carrying a Norm genotype.

CONCLUSIONS

- The FMR1 could be considered as a candidate gene implicated in IVF success.

- A rationale of building up a multi-genetic, individualized profile with other genes involved in the IVF process is also an option, where FMR1 could prove informative.

Limitations of the Study

1. The relative small size of the study population allows for only preliminary results, which require confirmation in a larger study population.

2. The random X-inactivation could act as a possible modifier of the impact of FMR1 genotypes in the ovary.

Thank you for your attention