Fragile X Testing in 2012: Case Studies for How New Technologies Improve Detection of Methylation Mosaicism and Risk of Expansion

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Panel Presentation: Clinical Practice and/or Research

Disclosures

Jennifer Skeen – employed by and stock holder of Asuragen, Inc.

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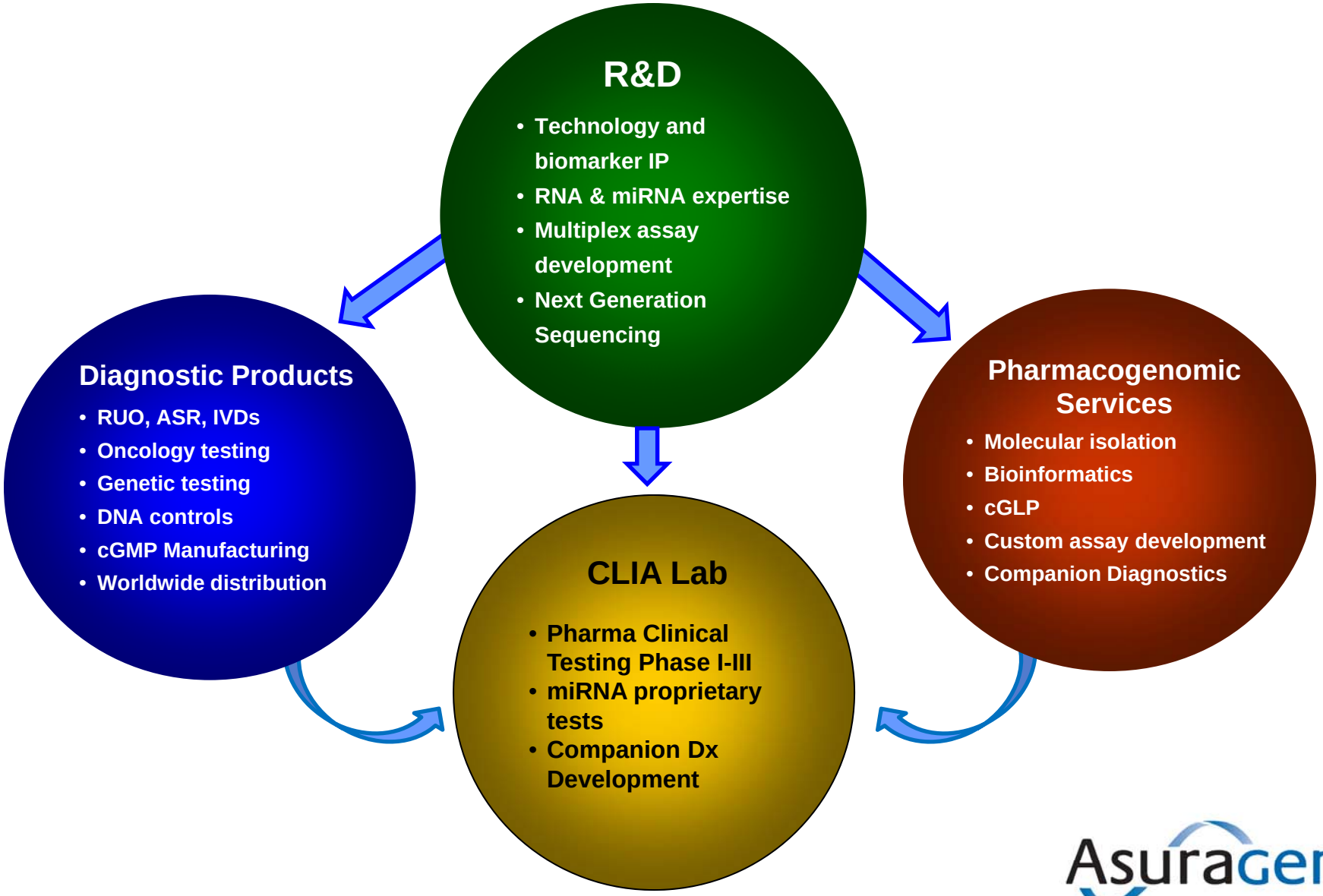

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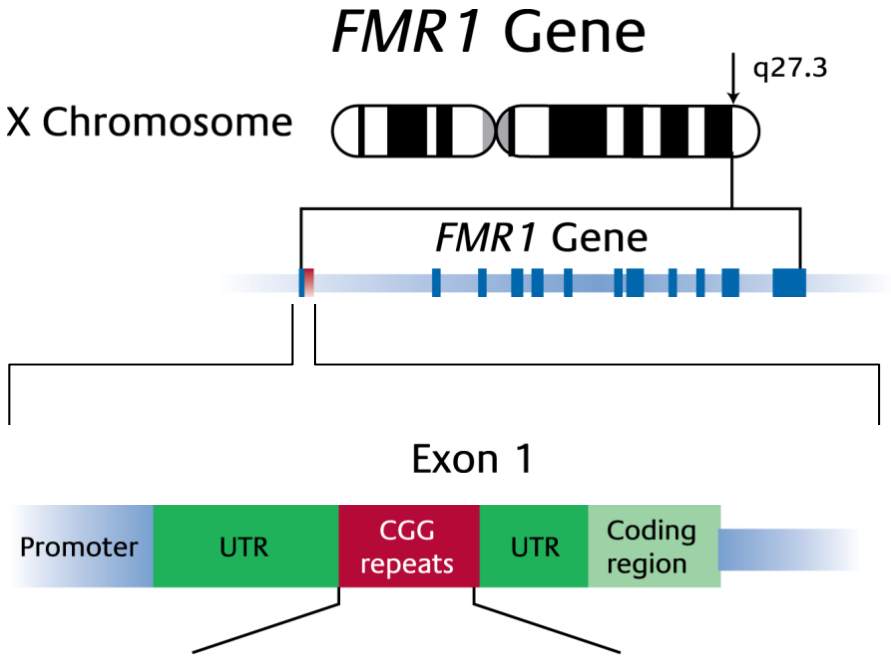
Fragile X Syndrome (FXS) and associated disorders FXPOI and FXTAS impact a broad range of individuals

- Associated with CGG repeat expansion and methylation in the *FMR1* Gene.
- Most common form of inherited mental retardation and most common known genetic cause of autism
- Premutation alleles are associated with ovarian insufficiency (FXPOI) and tremor and ataxia disorders (FXTAS)
- Approximately 1.5 million Americans are at risk for a premutation disorder and expansion to a full mutation in their child.



Normal	<45 CGG
Intermediate	45-54 CGG
Premutation	**55-200 CGG**
Full mutation	>200 CGG

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AGG Mapping provides an important new model for fragile X screening

"…we conclude that failure to account for AGG interruptions can result in profound errors in predicted risk for fragile X syndrome"

Carolyn Yrigollen et al,
Genetics in Medicine, 2012

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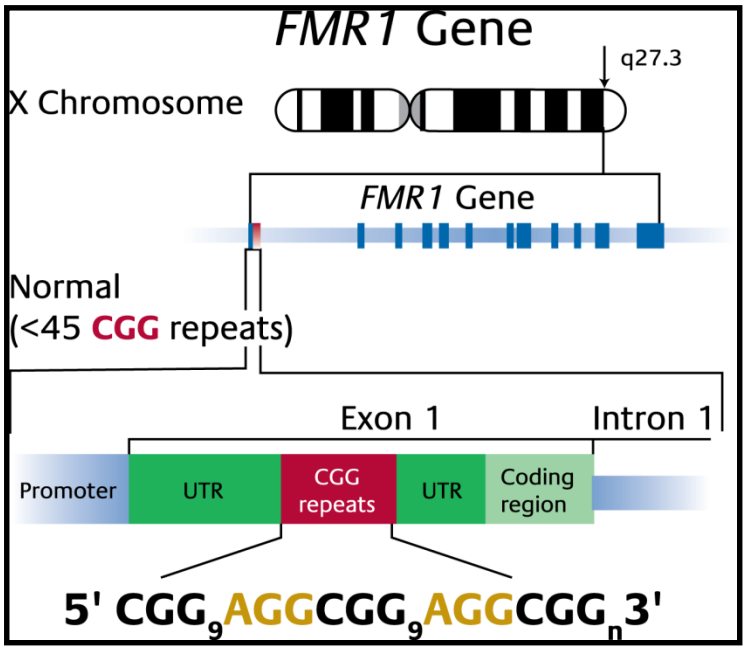
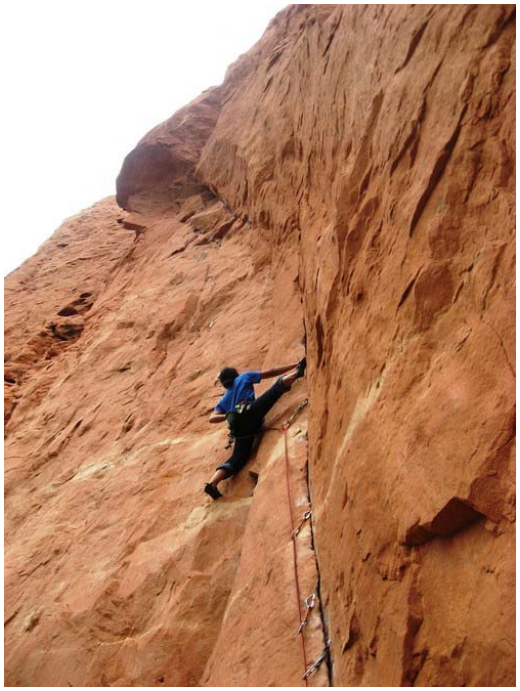
What are AGG interruptions?

- First reports on AGGs published in 1994 (Eichler & Nelson et al)
- “Protective sequences” are reported in other repeat disorders:

Disorder	Repetitive Sequence	Interruption
Fragile X (FRAX)	CGG	AGG
SCA1	CAG	CAT
SCA2	CAG	CAA
Freidreich’s ataxia	GAA	GAGGAA
SCA10	ATTCT	ATGCT

http://katerawlings.com/2011/01/24/another-bump-in-the-road/





http://www.summitpost.org/images/medium/334984.jpg

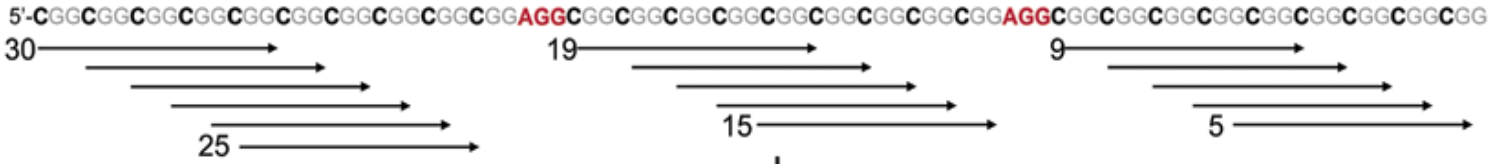
AGGs are like molecular “anchors” for replication and meiotic integrity

New PCR methods can map the CGG repeat region and identify AGG elements

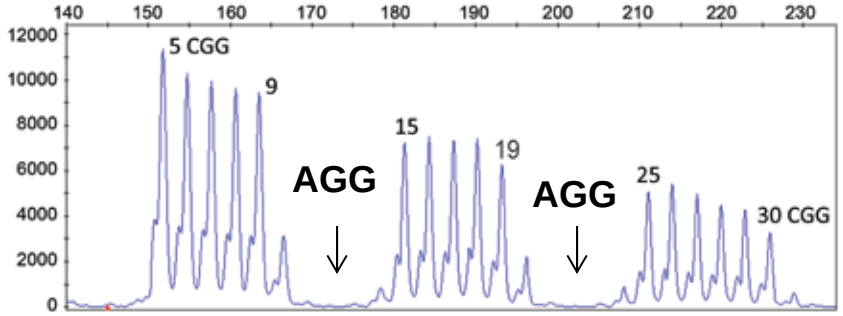
These methods overcome the historical difficulties of mapping AGG

3-primer design used to size and map the repeat region

CGG primer annealing is specific to CGG repeats



Capillary electrophoresis detection reveals AGG



Chen et al (2010). "An information-rich CGG repeat primed PCR that detects the full range of fragile X expanded alleles and minimizes the need for southern blot analysis." J Mol Diagn, 12:589-600.

AGG mapping in a large-scale maternal transmissions study

- *FMR1* genotyping of over 1000 DNA samples from families with and without a history of FXS

- Focused on 456 mother-to-child transmissions from maternal alleles with 45-69 CGG repeats

New York State Institute for Basic Research

RUSH UNIVERSITY MEDICAL CENTER

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Publication submitted.

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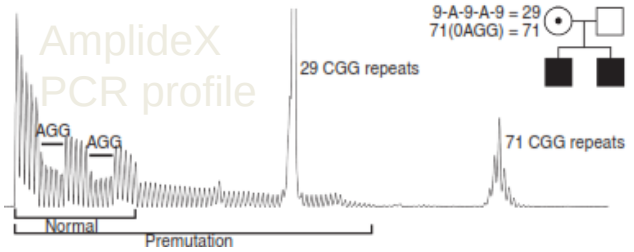
AGG interruptions refine the risk of expansion to full mutations

ORIGINAL RESEARCH ARTICLE | Genetics inMedicine

AGG interruptions within the maternal *FMR1* gene reduce the risk of offspring with fragile X syndrome

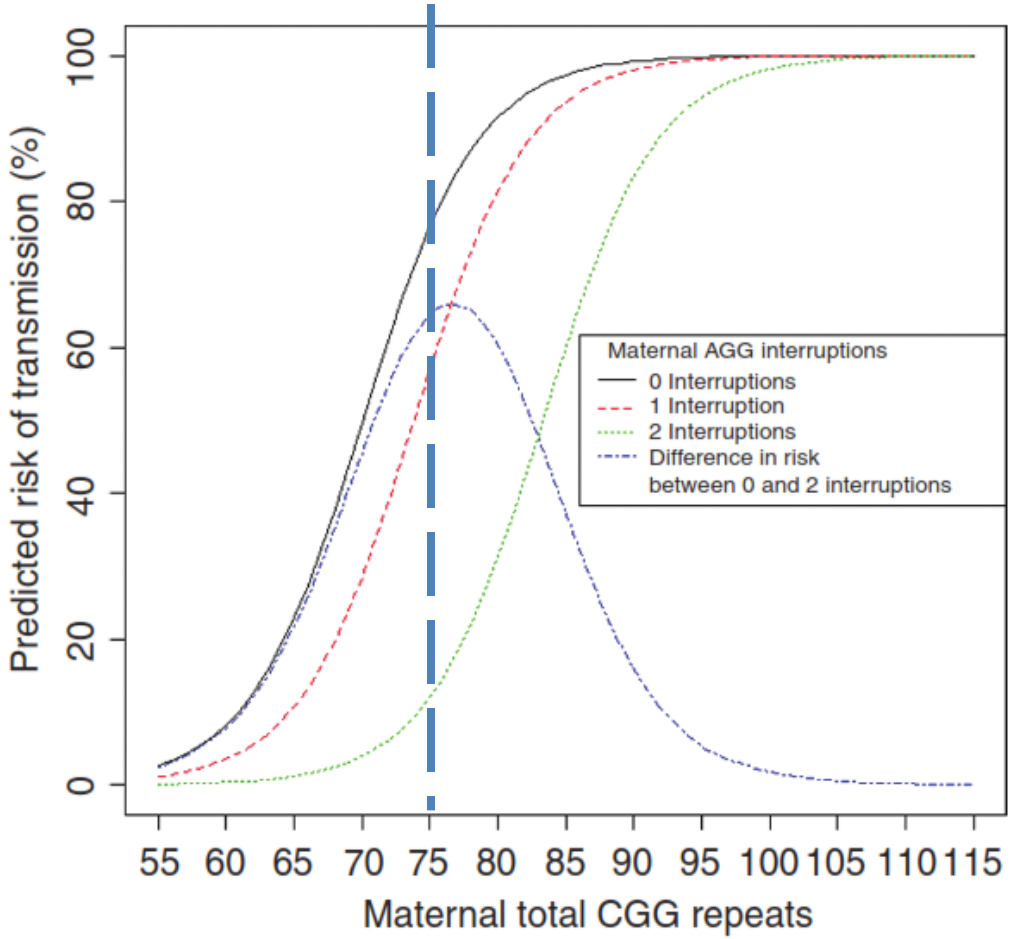
Carolyn M. Yrigollen BSc[1], Blythe Durbin-Johnson PhD[2], Louise Gane MS[3], David L. Nelson PhD[4], Randi Hagerman MD[3,5], Paul J. Hagerman PhD, MD[1,3] and Flora Tassone PhD[1,3]



- AGG mapping using AmplideX technology
  (enabled by Chen et al., 2010)

- 267 mothers (55-175 CGG)
- 373 transmissions
- 296 expansions to full mutations

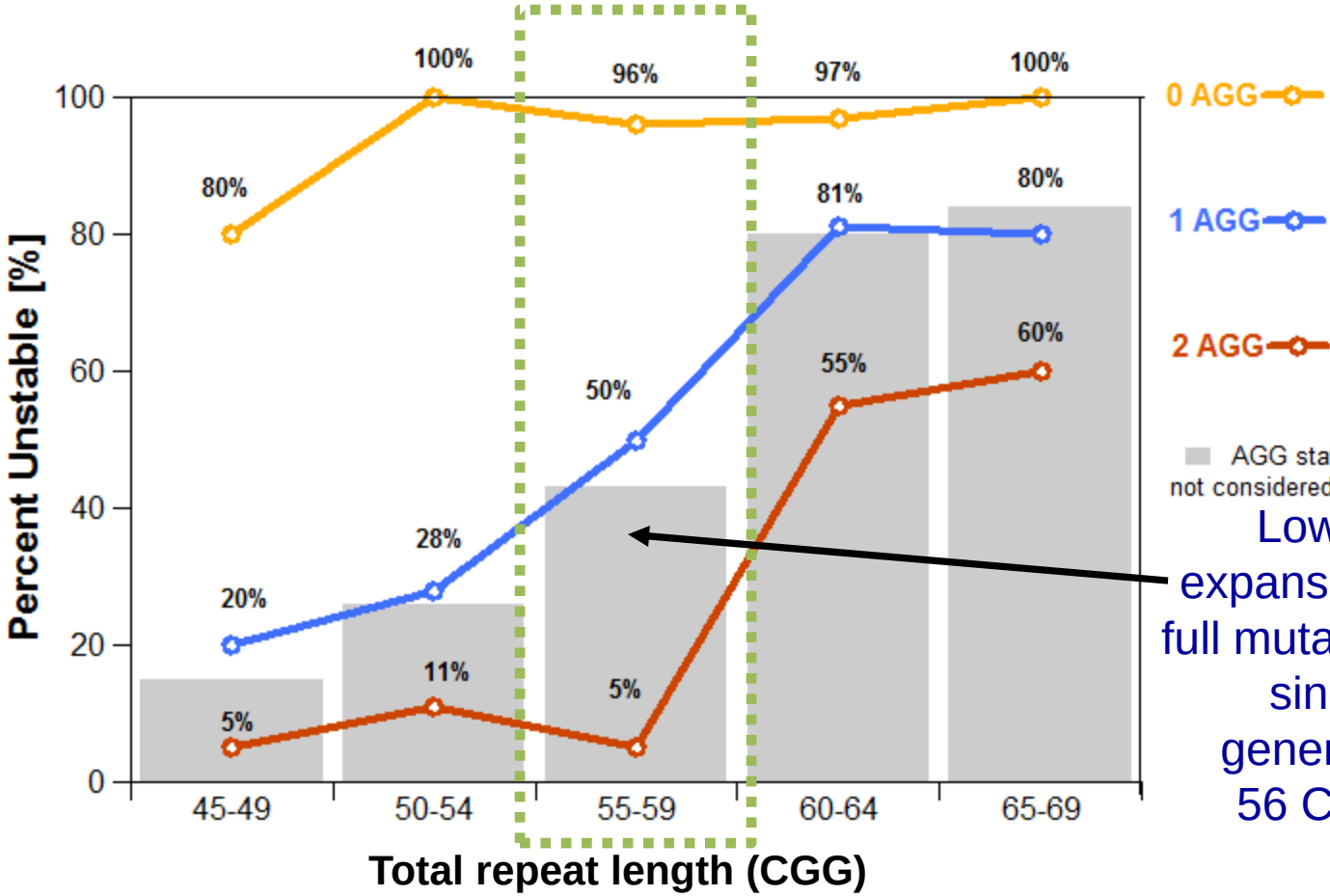
- Risk of expansion to full mutation reclassified
  - o 75 CGG, **0 AGG=77%**
  - o 75 CGG, **2 AGG=12%**



The presence of AGG reclassifies risk of repeat change across 45-69 repeats

Carrier rate for premutation allele is as high as 1:178. 75% of female premutation carriers have less than 70 CGG with ambiguous risk profiles for expansion.

Risk for any change in repeat length[a]



Lowest expansion to a full mutation in a single generation 56 CGG[b]

[a]Nolin et al (2011) Prenat Diagn. 10:925-31 and estimates from Asuragen Validation study
[b]Fernandez-Carajval et al (2009) J. Molec Diag. 11:306-10

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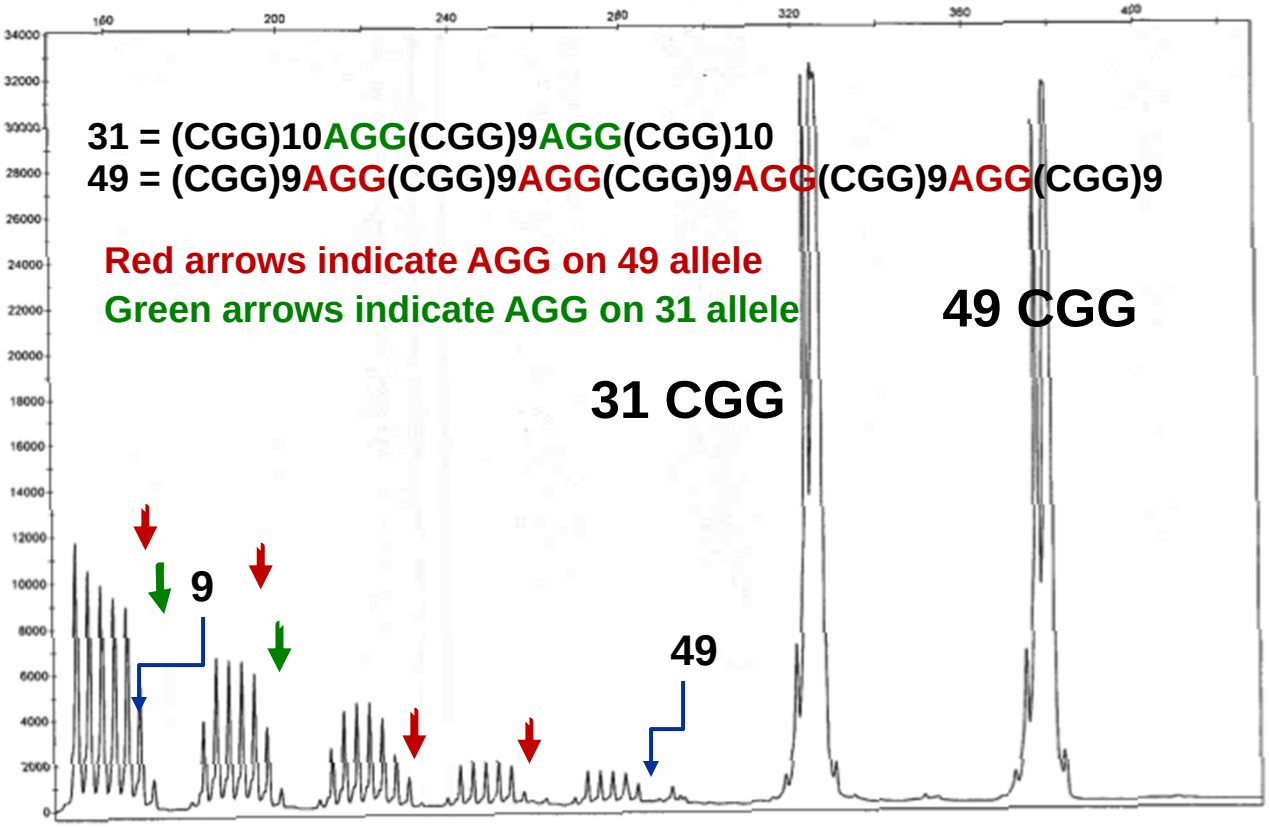
Case Study

***Phenotype*: Female sample 31 and 49 CGG. Referred from fertility clinic prior to IVF due to gray zone allele and concerns about expansion.**

CGG RP



***Outcomes*: CGG PCR shows location of AGGs at 10, 20, 30, and 40, only 9 consecutive CGGs, suggest no risk of size change, completely stable allele, thus assist with risk prediction for patient in IVF decision.**

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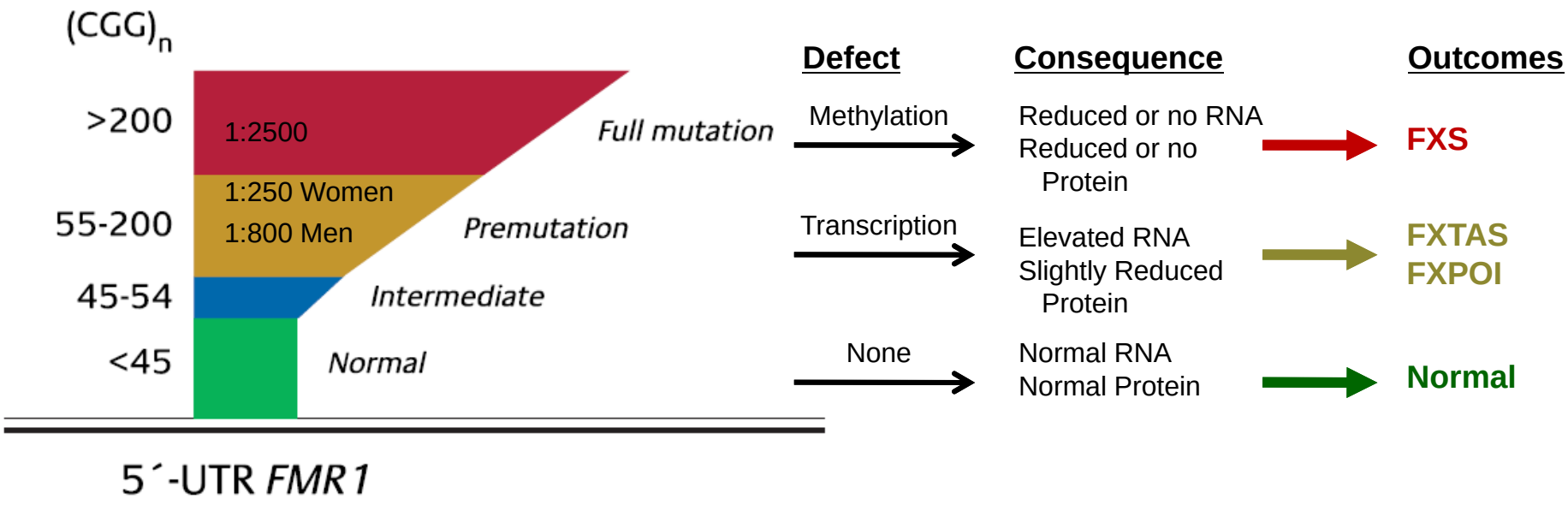
The challenge of fragile X molecular analyses

Expansion of CGG repeats and methylation status of the *FMR1* gene are used to study Fragile X Syndrome and related disorders.



Problem: The CG-rich nature of the triplet repeat and neighboring region means that some full mutation alleles may represent up to 99% GC content and are prone to methylation.

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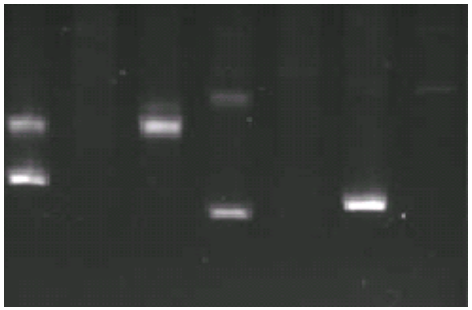
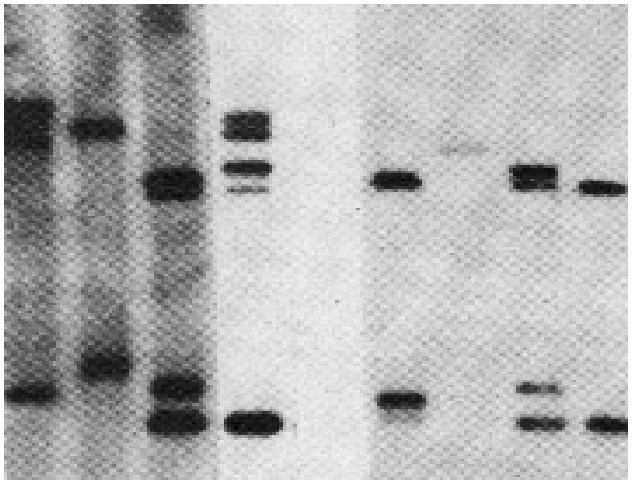
Current Fragile X testing: a combination of PCR and Southern blot analyses

PCR



Southern Blot



- Direct detection of very large expansions
- Assesses both allele size and methylation status
- Can reveal the X activation ratio (AR) and mosaicism
- Well validated across large multicenter studies for phenotype-genotype associations

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Genotype-Phenotype correlates in fragile X

Mutation Type	Number of CGG Repeats	Methylation Status of *FMR1*	Clinical Status	
			Male	**Female**
Premutation	55-200	Unmethylated	At risk for FXTAS	At risk for POI and FXTAS
Full mutation	>200	Completely methylated	100% with ID	~50% with ID, ~50% normal intellect
Repeat size mosaicism	Varies across the triplet repeat range	Partial: Commonly unmethylated in PM, methylated in FM	**May be higher functioning than males with full mutation only**	Highly variable: Ranges from normal intellect to affected
Methylation mosaicism	>200	Partial: Mixture of methylated and unmethylated FM		
Unmethylated full mutation	>200	Unmethylated	**Often have high-functioning ID to low-normal intellect**	

Adapted from GeneReviews (Saul & Tarleton, 2011):
http://www.ncbi.nlm.nih.gov/books/NBK1384/

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Challenges of *FMR1* Southern blot analyses

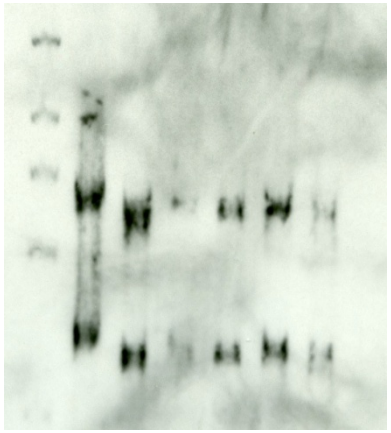
- Laborious
- ~1 week TAT
- Low throughput
- 5-10 ug DNA input (a lot!)
- Inexact CGG quantification
- Sometimes difficult to interpret

Southern Blot

Steps	*Time*
1. Genomic DNA digestion	16 h
2. Electrophoresis	16 h
3. DNA transfer	16 h
4. DNA fixing	1 h
5. Hybridization	16 h
6. Detection	3 h
7. Result analysis	15 min
Total	~83 h

Elias et al. Genet Test Mol Biomarkers. 2011 Jun;15(6):387-93

Southern blots from two labs

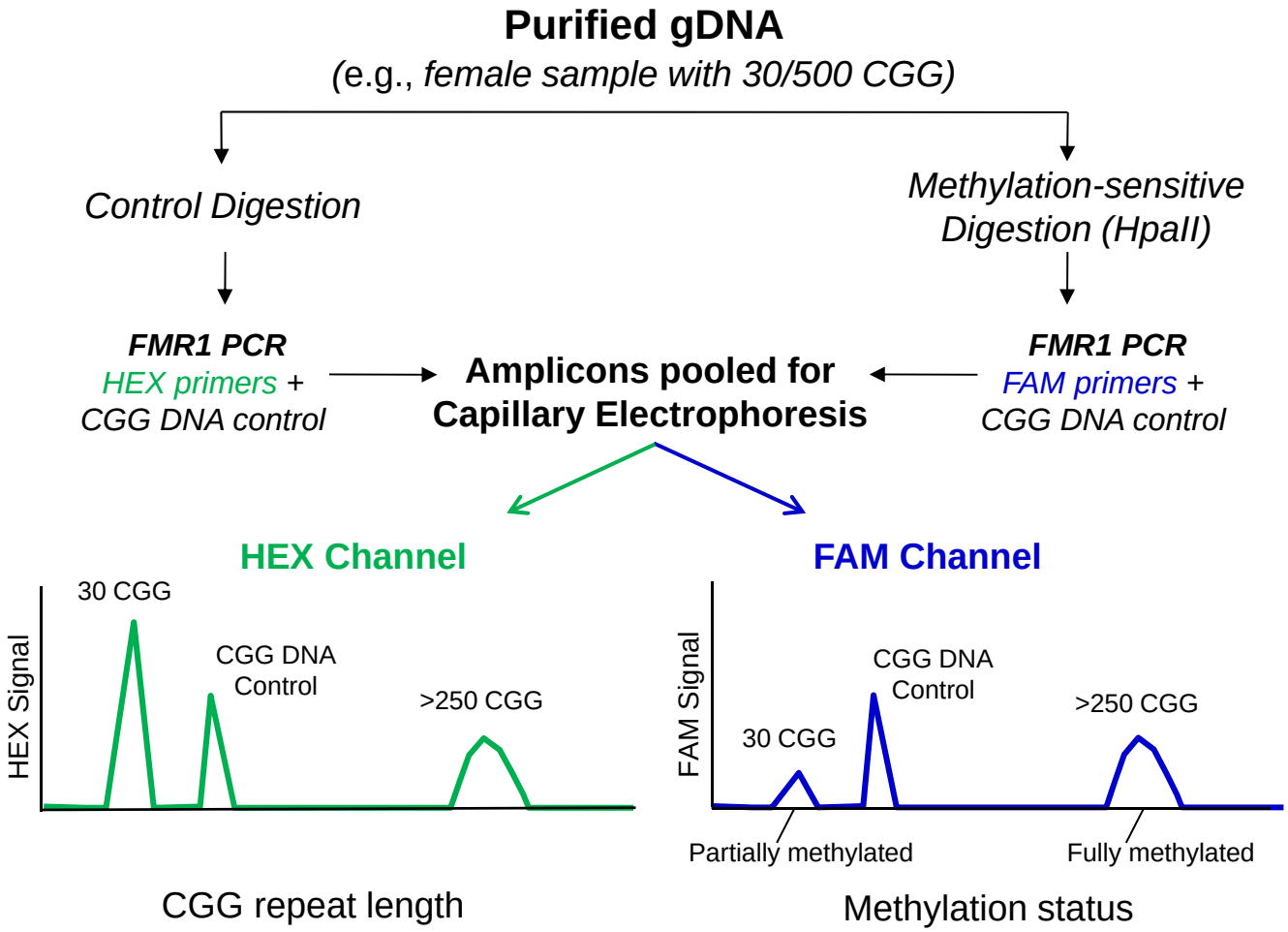


PM and 5 normal female alleles

Male FM?

Methylation PCR-CE – Chen et al. (2011)

Workflow for mPCR-CE prototype research reagents

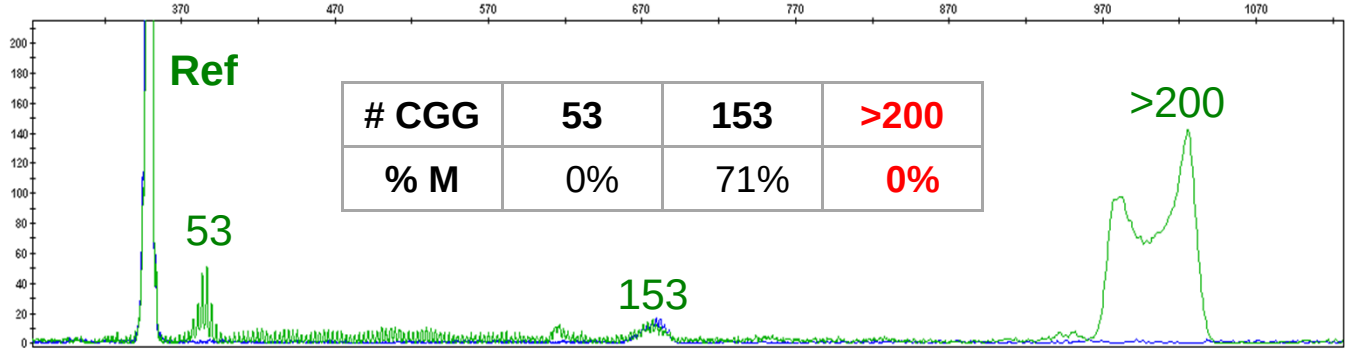


Genet Med. 2011 Jun;13(6):528-38.

mPCR-CE Case Studies: Phenotype-Genotype correlations revealed through clinical research

***Phenotype*: Male in his 50s with normal cognitive function, severe anxiety, self-referred because brother & sister have FXS. Daughter has a premutation.**

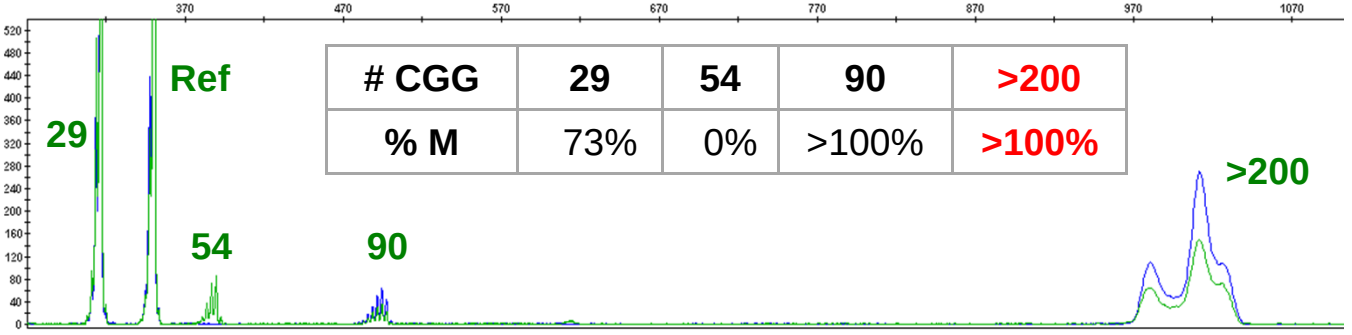
Methylation



# CGG	53	153	>200
% M	0%	71%	0%

***Phenotype*: Low functioning FXS female, 65 IQ, anxiety, mood swings, ADHD, self-referred for FXS testing because of psych diagnoses after male cousin with FXS.**

Methylation



# CGG	29	54	90	>200
% M	73%	0%	>100%	>100%

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Courtesy of Dr. Elizabeth Berry-Kravis, Rush University Medical Center

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