Breakthrough Research in Fragile X Carrier Testing:
AGG Interruptions and Modification of Expansion Risk

Elizabeth Berry-Kravis MD, PhD
Professor of Pediatrics, Dept. Neurological Sciences
and Biochemistry
Rush University Medical Center

National Society of Genetic Counselors

www.nsgc.org

Introduction:

Fragile X-Associated Disorders (FXD)

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Fragile X-Associated Disorders (FXD) Prevalence

- **Premutation "carriers" - risk for FXTAS/FXPOI**
  - **1:100-1:250 females, 1:250-1:800 males**
  - **RUSH/UCD/UNC 10,000 newborn screening samples (Tassone) ~ 1:160 F, 1:500 M**
- **FXPOI 25% female carriers, ~ 1:650 females**
- **FXTAS 50% male carriers, ~ 1:1000 males**
- **Full mutation fragile X syndrome - FXS**
  - **1:4000 males and females**
- **All ethnic groups worldwide**
- **Affect families in multiple generations**
- **FXS is:**

THE MOST COMMON KNOWN INHERITED FORM OF COGNITIVE DISABILITY

THE MOST COMMON KNOWN GENETIC CAUSE OF AUTISM

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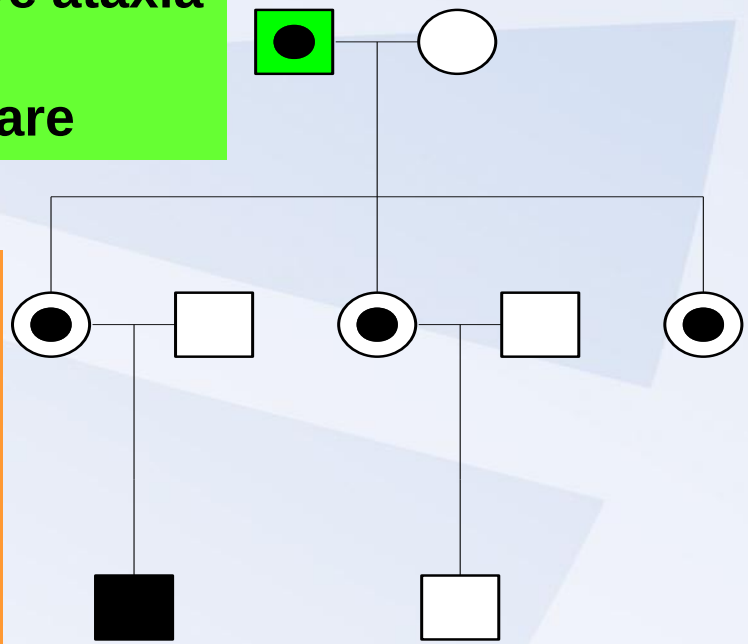
FXTAS, FXS and FXPOI Affect Multiple Generations in a Family

Progressive ataxia
Dementia
Full time care



Full time caregiver
Stress/Anxiety
The future: Will I get it? What will I get? If I do who will take care of my son?

Anxiety
Hyperactivity
Autistic behavior
Poor verbal skills
Full time care

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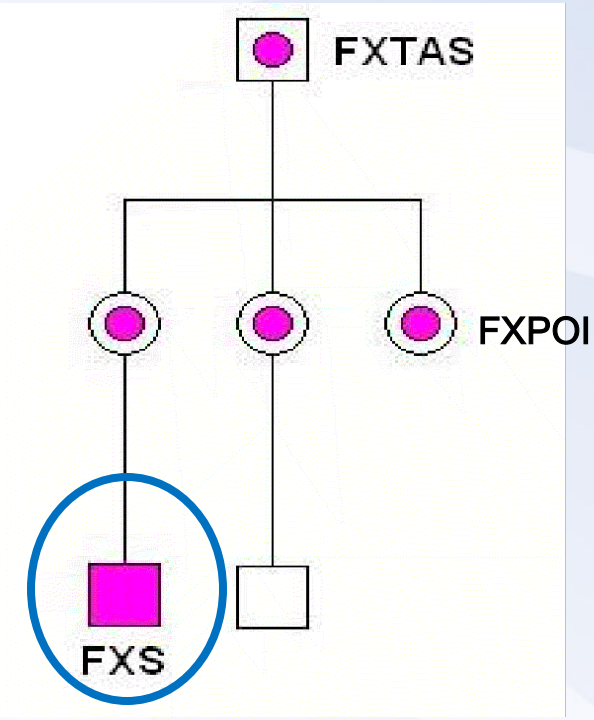
Fragile X Syndrome (FXS)



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Features of FXS

- **Physical: large prominent ears, long face, large head, prominent jaw and forehead, midfacial hypoplasia, hyperflexible joints, large testis**
- **Intellectual Disability or LD**
- **Behavior problems: hyperactivity, distractibility, anxiety, perseveration**
- **Autism: 18-36% AD, 43-67% ASD**
- **Seizures: 15%**
- **Strabismus: 30%**
- **Medical: otitis, sinus, MVP, reflux, sleep apnea, loose stools, allergies**

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FXS – Affected Females

- More mildly involved
- Average IQ 80
- NVLD, VIQ>PIQ, poor math,
  very impaired executive function, distractibiity
- Same cognitive pattern as males
- Physical features/medical problems variably present
- Social/psychiatric disability common – anxiety/shyness, oddness
- Decreased education, job stability, socioeconomic status

FXS Mosiac Males

- Mildly affected, IQ > 70
- Mosaic = Partially or fully unmethylated mutation
- Methylation mosaic - unmethylated full mutation
- Size mosaic – premutation and full mutation
- Long repeats - decreased translation even if unmethylated
- Cognitive abilities similar to females
- Variable physical features

Patient with learning disability, brother has ID

Unmethylated full mutation

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FXS Diagnostic Testing Guidelines

Clinician should test for *FMR1* mutation if the patient has any of the following:

- **Intellectual Disability of unknown etiology**
- **Autism or Autism Spectrum Disorder of unknown etiology (including PDD-NOS or Aspergers)**

Clinician should test for *FMR1* mutation if the patient has any of the following AND additional cognitive or physical features of FXS OR family history of FXS or FXTAS:

- **Learning Disability, especially Nonverbal Learning Disabilities or math disability**
- **Behavioral issues, including poor eye contact, anxiety, attention problems, hyperactivity**
- **Seizures**

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from Berry-Kravis et al. 2007

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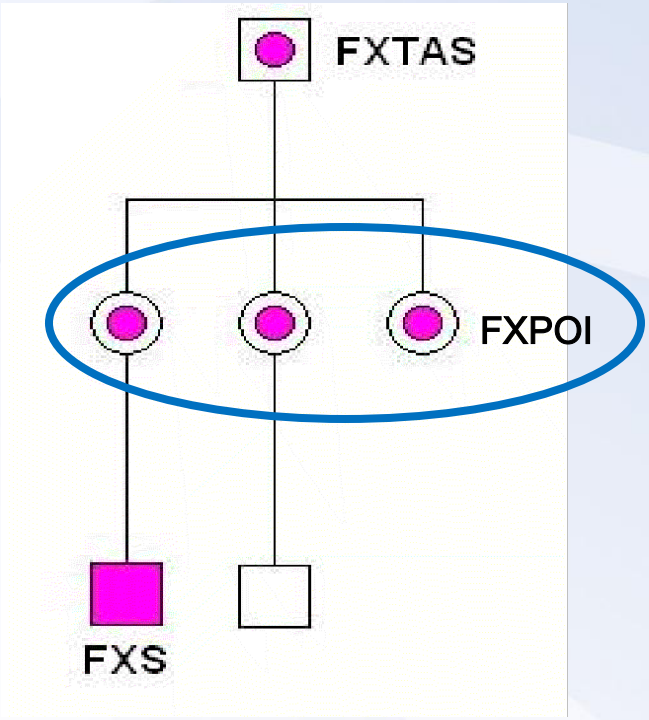
Fragile X Associated Primary Ovarian Insufficiency (FXPOI)



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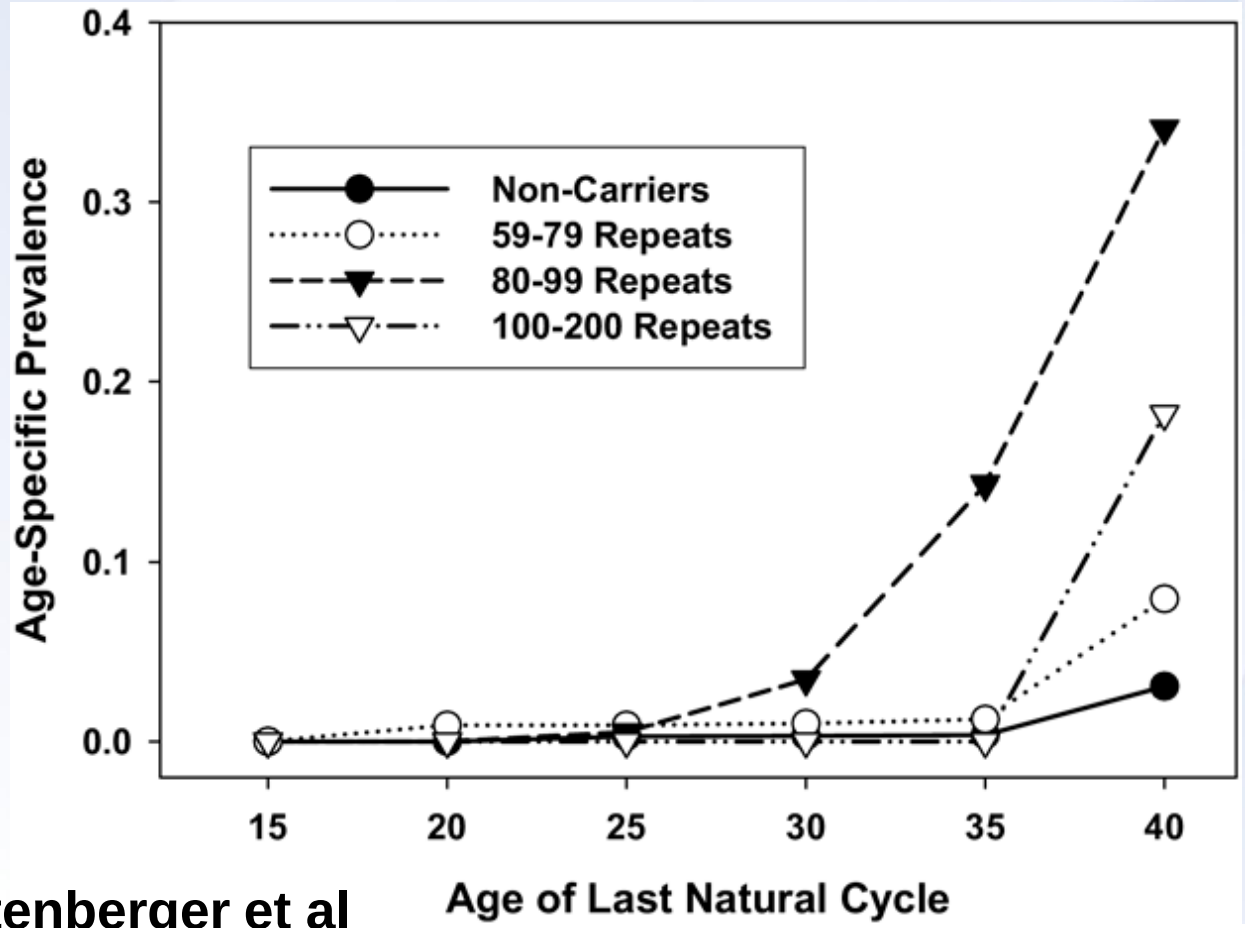
Features of FXPOI

- **15-22% of female premutation carriers have POF (early menopause)**
- **0.8-7.5% of women with POF have *FMR1* premutation, 13% if FHx of POF**
- **Now called POI because many have ovarian dysfunction early but don't fully stop menses by 40 years**
- **Premutation carriers have increased FSH across early, mid, late follicular phase**
- **Carrier females enter menopause average of 5 years earlier than non-carrier family members**

POF/POI Risk and CGG Repeats

Risk for POF/POI increases gradually for CGG<80, rapidly 80-100 and then levels off or



Wittenberger et al
***Fertil Steril* 2006**

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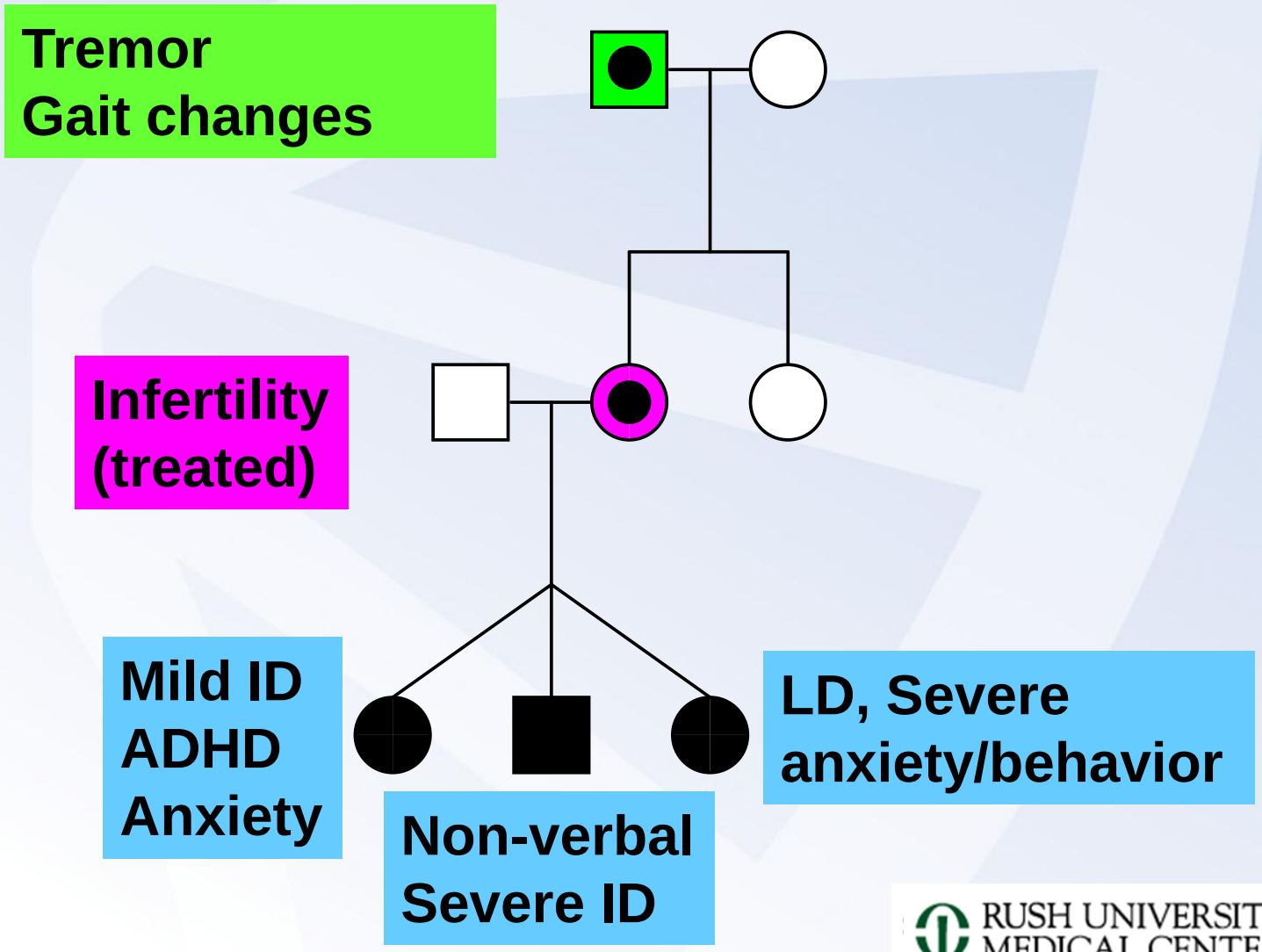
FXPOI, Infertility and FXS
The Trouble with Triplets



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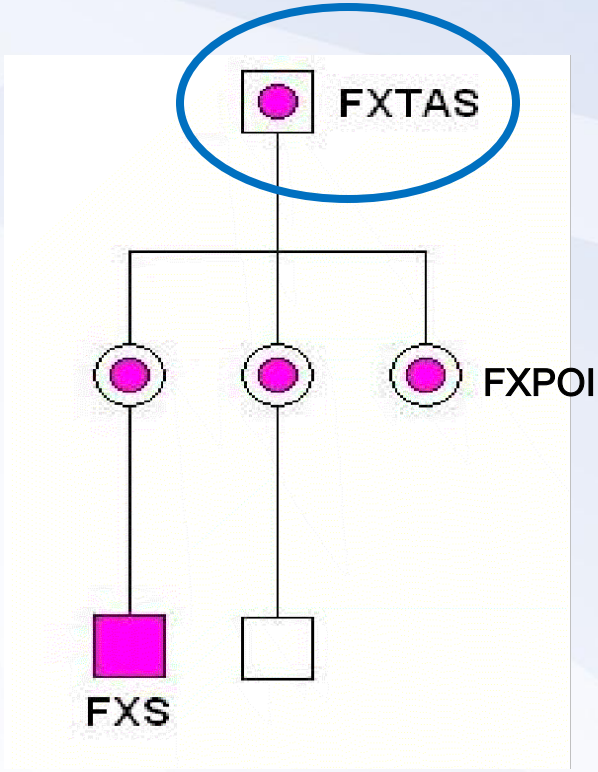
Recommendations for *FMR1* Testing in Reproductive Clinics

- All women with ovarian failure (cessation of menstrual cycles), particularly if FSH elevated
- Egg and sperm donors
- All women with personal or FHx of ID, DD, autism
- Women with fertility concerns but normal or erratic cycles if:
  - Elevated FSH
  - FHx of POF, FXS, or FXTAS, or undiagnosed ID/DD/autism or movement disorder
  - Especially if doing fertility tx – want to avoid multiples with FXS

Fragile X Tremor/Ataxia Syndrome (FXTAS)



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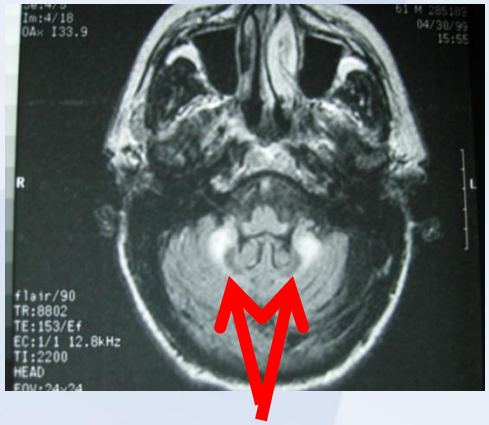
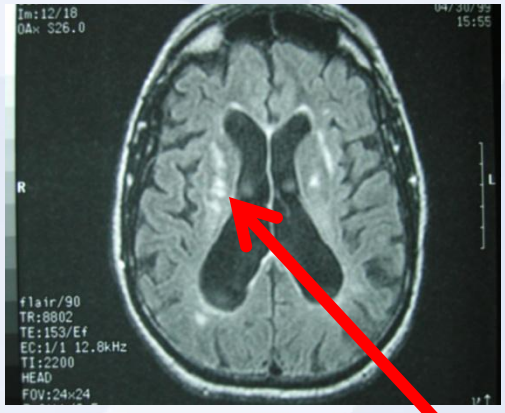
Features of FXTAS

- Multidimensional tremor
- Ataxia
- Parkinsonian symptoms
- Neuropathy
- Executive function problems and cognitive deterioration (frontal subcortical dementia)
- Characteristic MRI with white matter changes and MCP sign
- Neuronal inclusions



MCP sign



Abnormal white matter signal
Gray and white matter atrophy

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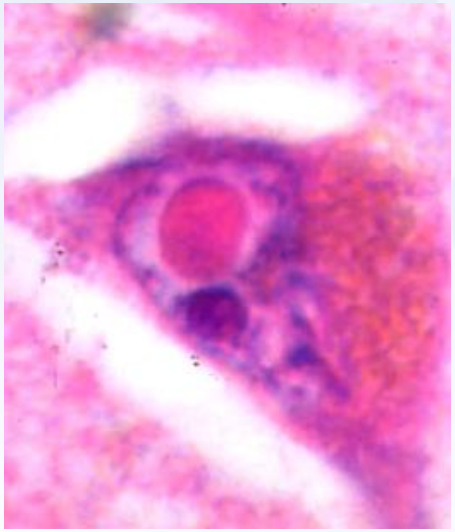
Many Features of Disease in FXTAS are Dependent on CGG Repeat Length in Males

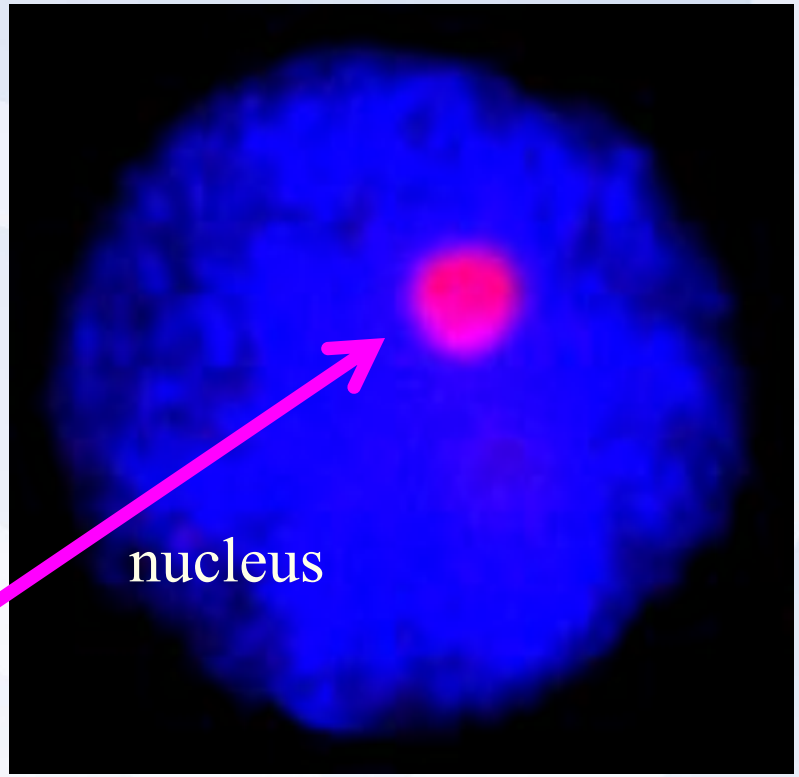
- **FXTAS Rating Scale Score (combined tremor, ataxia, PDism severity)** ***Leehey et al. 2007***
- **Risk of developing disease** ***Jacquemont et. al. 2004***
- **Age of onset** ***Tassone et al. 2006***
- **Neuropathic signs** ***Berry-Kravis et al. 2007***
- **Brain atrophy** ***Cohen et al. 2006***
- **Inclusions** ***Greco et al. 2005***

Mechanism for FXTAS - RNA Gain-of-Function/Toxicity

- predicts that the *FMR1* mRNA should be in the intranuclear inclusions found in FXTAS patients

Tassone et al. 2004





Fluorescent staining of the FXTAS inclusions with a probe that is specific for the fragile X (*FMR1*) mRNA

Inclusions do contain FMR1 mRNA
Contain key nuclear proteins eg. lamin

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FXTAS Involvement in Females

- Clearly occurs although symptoms “patchy”
- Normal X protection - related to activation ratio
- Less frequent (5-10%), less severe than males
- Longer CGG – increases risk for neuropathy and ataxia when corrected for activation ratio
- Increased thyroid disease, HTN, seizures, fibromyalgia symptoms in females with FXTAS symptoms
- Increased thyroid, parasthesias, muscle pain in non-FXTAS female carriers
- May be particular families at-risk – see family clustering

Testing Guidelines for FXTAS* :
test for *FMR1* mutation if the patient has any of the following:

- Unexplained cerebellar gait ataxia, onset > 50 yr
- Unexplained action tremor in person with parkinsonism or dementia, onset >50 yr
- Diagnosis of multiple system atrophy, cerebellar subtype
- MCP sign on MRI, family history of *FMR1* mutation, or infertility/POF in self or family if have signs consistent with FXTAS**

from Berry-Kravis et al. 2007

*FXTAS is less common in females.

**Signs consistent with FXTAS include cerebellar gait ataxia, action tremor, parkinsonism, cognitive decline, executive function deficits, neuropathy and autonomic dysfunction. Associated history consistent with FXTAS includes family history of MR, autism, ataxia, or POF

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Inheritance and Genetic Counseling in FXDs

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Inheritance Patterns for *FMR-1*

- **Normal FMR-1: does not mutate often**
- **Premutation FMR-1: mutates virtually every time it is passed on by a male or female**
  - **Does not cause FXS, just propensity to pass on FXS**
  - **Causes FXPOI and FXTAS with risk related to size**
  - **Can increase or decrease in size**
  - **Increases more often than decreases**
  - **The bigger it is, the more it increases**
  - **Eventually expands to full mutation, but only via maternal transmission**
  - **The bigger it is, the more chance of expansion to a full mutation when passed by a woman**

Inheritance Patterns for *FMR-1*

- **Full mutation FMR-1: mutates every time it is passed on reproductively and also mitotically**
  - **Causes FXS**
  - **Can mutate back to a premutation/normal, but mostly passed on as full mutation**
  - **Males with the full mutation pass a premutation to their daughters**
  - **Sperm never have a full mutation, affected males shown to have premutation in sperm while other body tissues have full mutation**

Gray Zone Allele (45-54 repeats) Inheritance

- Not clearly associated with clinical disease – may be risk factor for Parkinsonism in old age
- Variable stability
- Until recently little information has been able to be provided about stability of these
- Could look at all family members but difficult to orchestrate and get coverage

Progress in Molecular Diagnosis in FXD: Characterization of Repeat Structure with New AGG Mapping Technique

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What Are AGG Interruptions?

Normal Male 30 repeats

(((((((((A(((((((((A((((((((((

(= CGG
A = AGG

Premutation Male 80 repeats

((((((((A(((
((((

- **Most commonly 2 AGGs roughly every 10 triplets (but can be 0-4) and spacing varies**
- **Common FMR1 alleles include an AGG at repeat 9 or 10 (1^{st} interruption) and 19 or 20 (2^{nd} interruption when present)**

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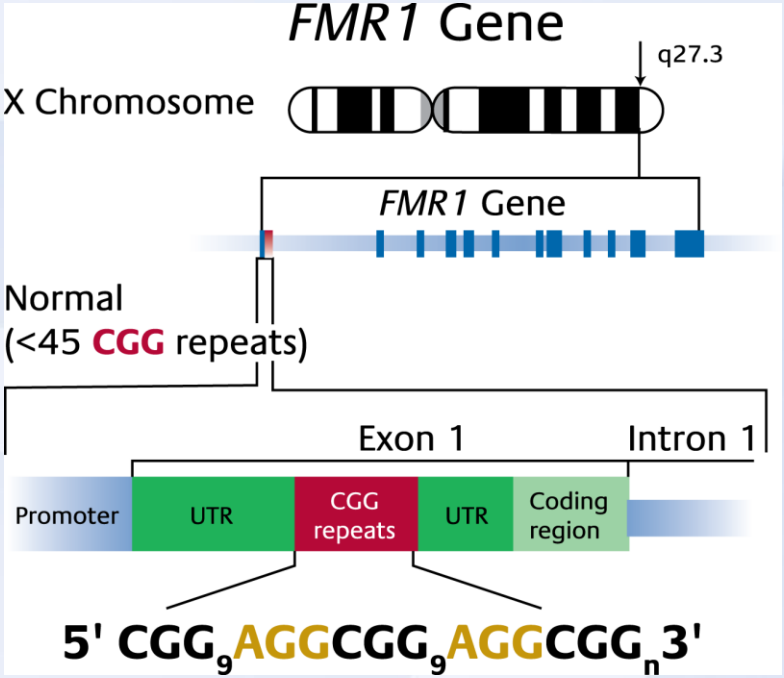
AGGs and Allele Stability

- Eichler et al. (1994) suggested AGG interruptions affect stability of *FMR1* repeat
- Big issue has been mapping AGGs
- Presence of two X chromosomes in females have made this analysis technically impossible
- New PCR assays can elucidate the AGG structure and 3' length of uninterrupted CGGs in females *(Chen et al., 2010)*



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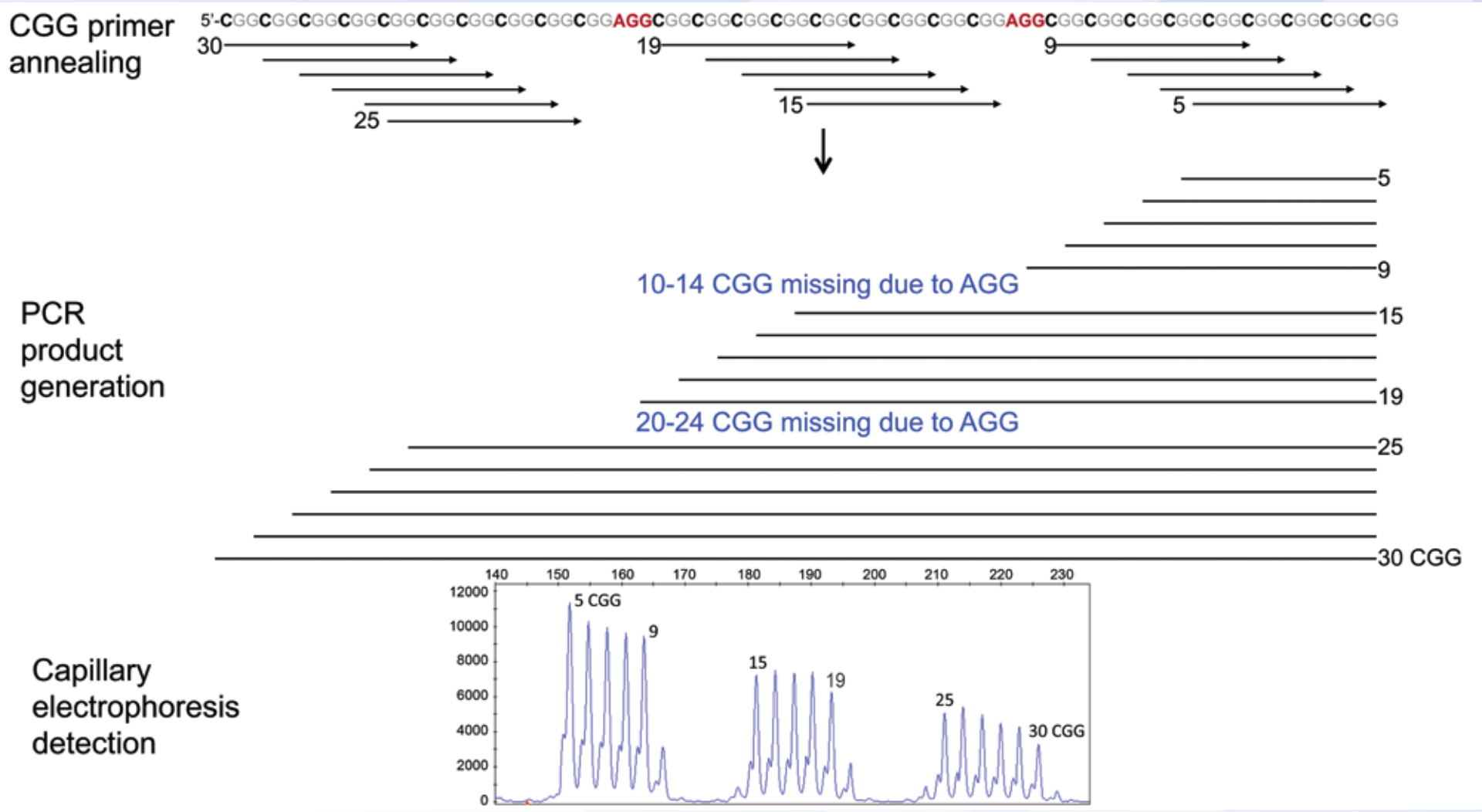
AGG Genotyping Assay



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Collaborative Study of AGG Structure and *FMR1* Allele Stability on Transmission

Completed During 2010-2011

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AGG Study – Design and Collaborators

- ***FMR1* genotyping of DNA from families with and without a history of FXS, using new assay**
- **Focus on 456 mother-to-child transmissions of alleles with 45-69 repeats**

Collaborator	Samples
Flora Tassone, PhD	39
Liz Berry-Kravis, MD, PhD	237
Stephanie Sherman, PhD	119
Sally Nolin, PhD	497
Total	**892**

New York State Institute for Basic Research

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AGG Study - Project Goals

- **To determine how knowledge of AGGs refines risk predictions for expansion of *FMR1* CGG repeat sequence**

  Evaluate based on:

  - **AGG number**
  - **Number of consecutive (uninterrupted) CGG repeats**

- **To determine implications of risk reclassification for individual patients**

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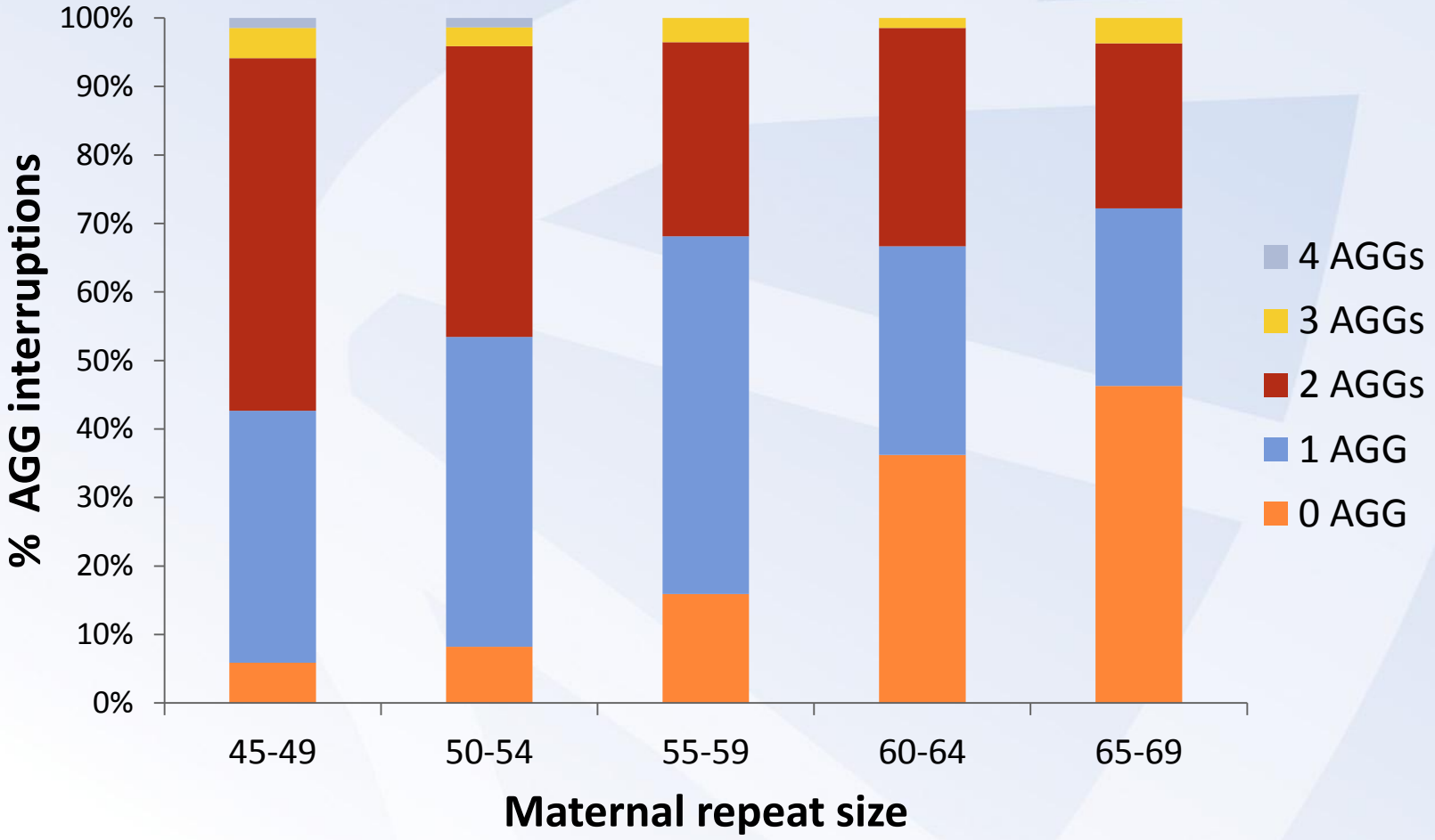
Number of Transmissions Studied for Each Maternal Repeat Size

Maternal repeat size	# transmissions
45-49	81
50-54	82
55-59	140
60-64	88
65-69	65
Total	456

AGG Interruptions Among 374 Mothers



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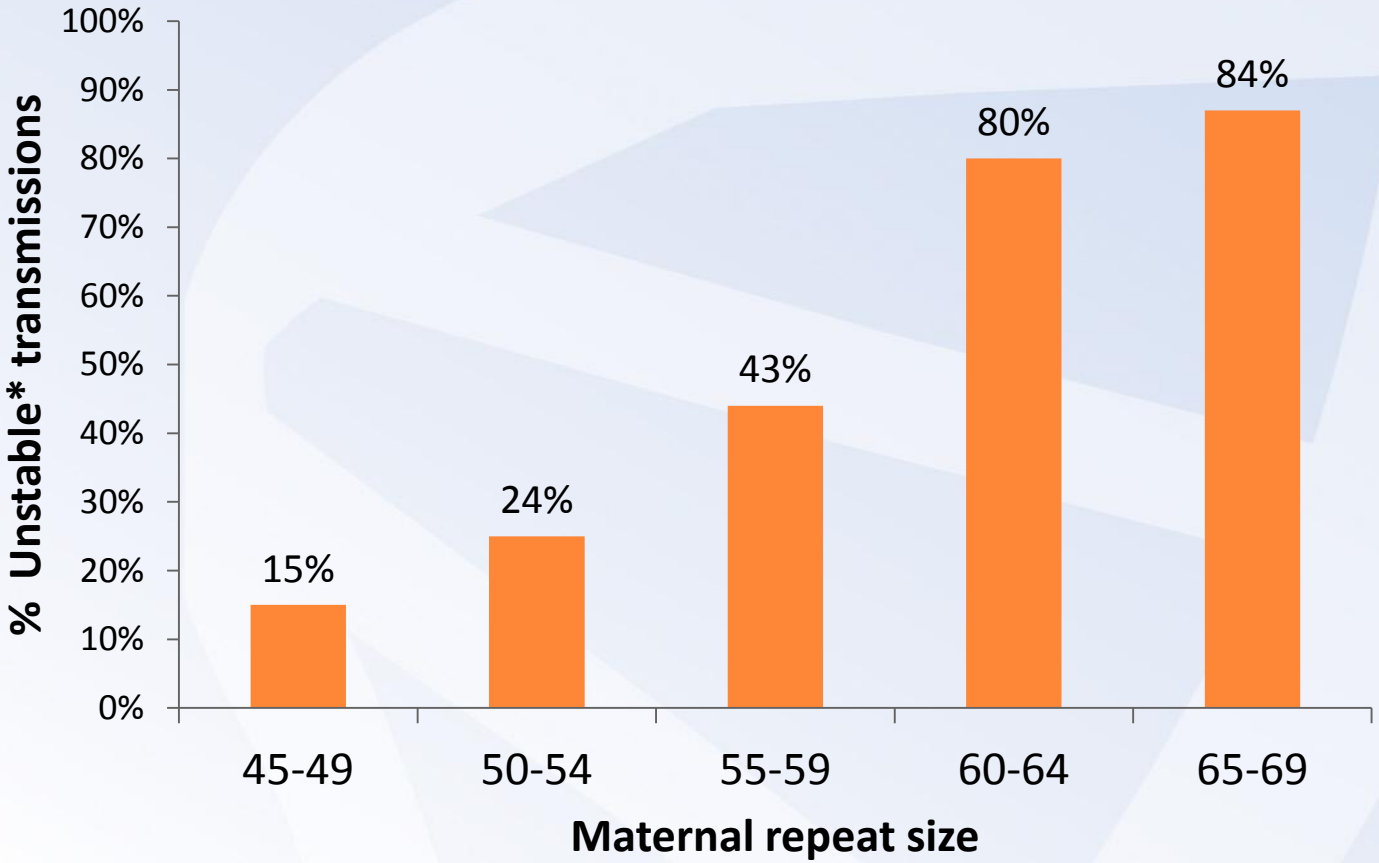
Unstable Transmissions by Maternal Repeat Size



*Unstable=change in 1 or more number of CGG repeats

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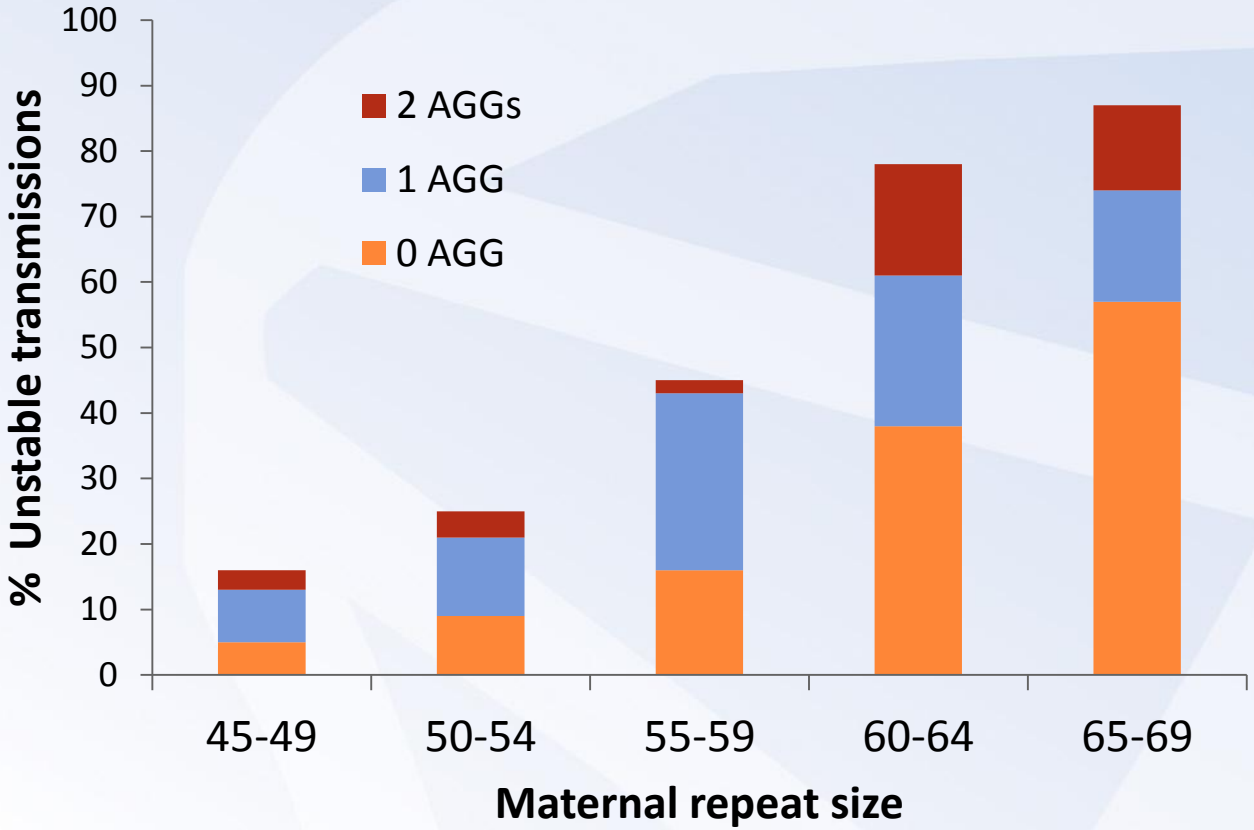
AGG Structure of Maternal Alleles for Unstable Transmissions



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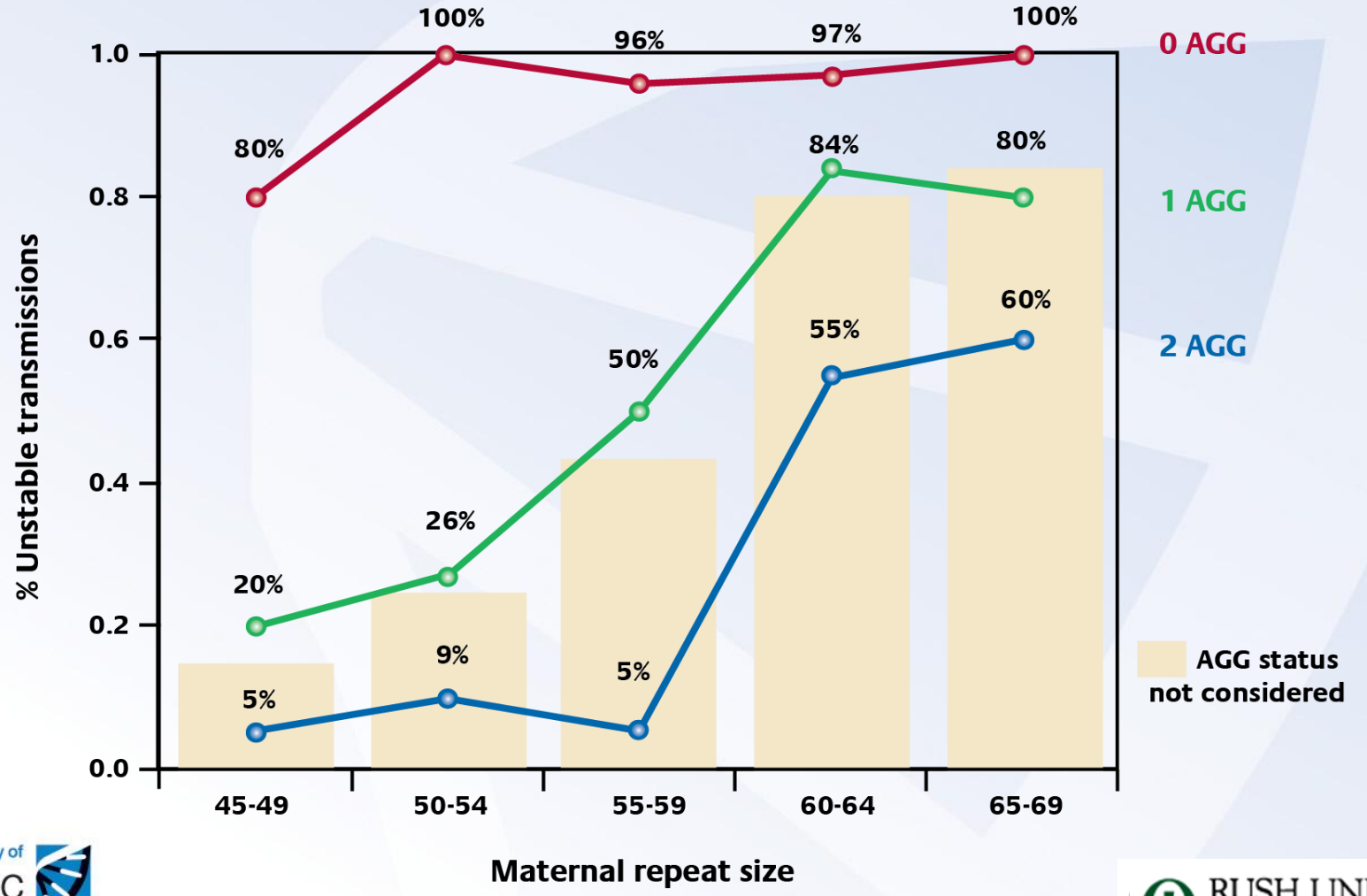
Comparison of the Maternal Repeat Length, # AGG Interruptions and Risk of Instability



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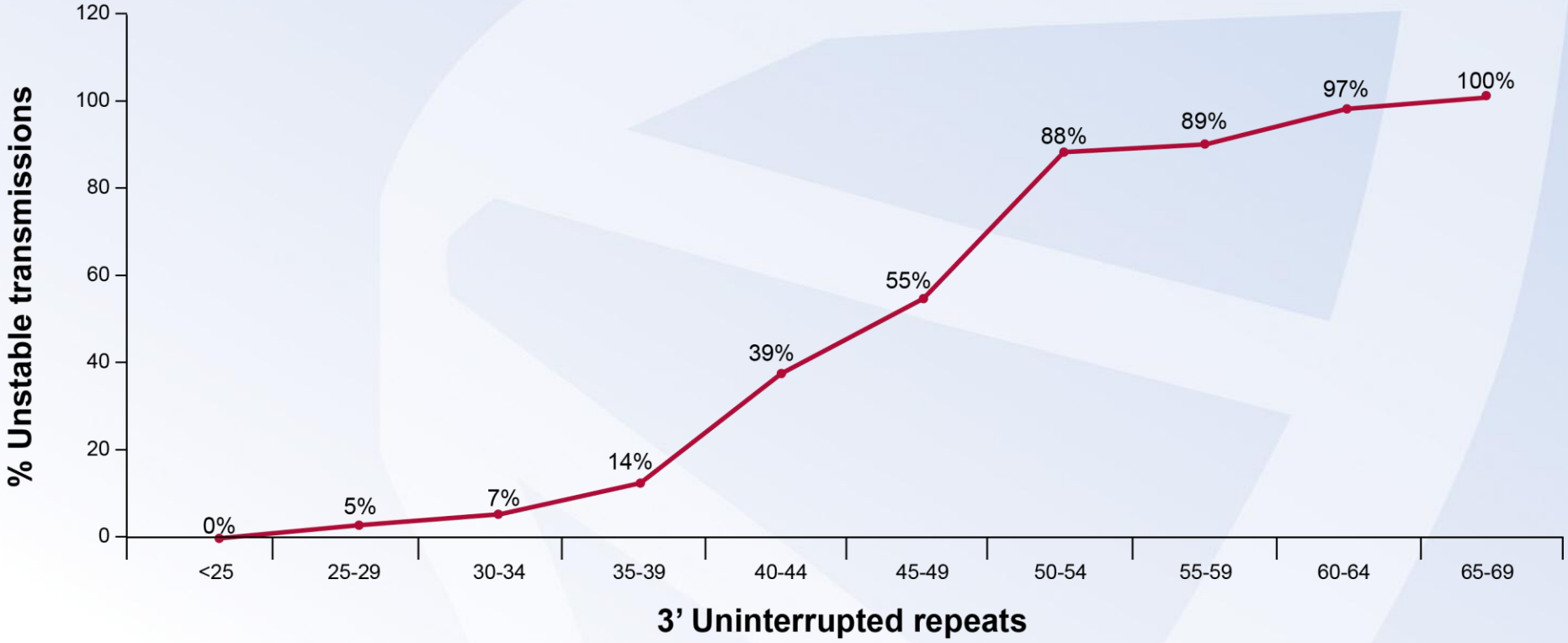
Percentage of Unstable Maternal Transmissions Based on the Length of 3' Uninterrupted Repeats



Longer 3' CGG repeats are associated with greater instability.

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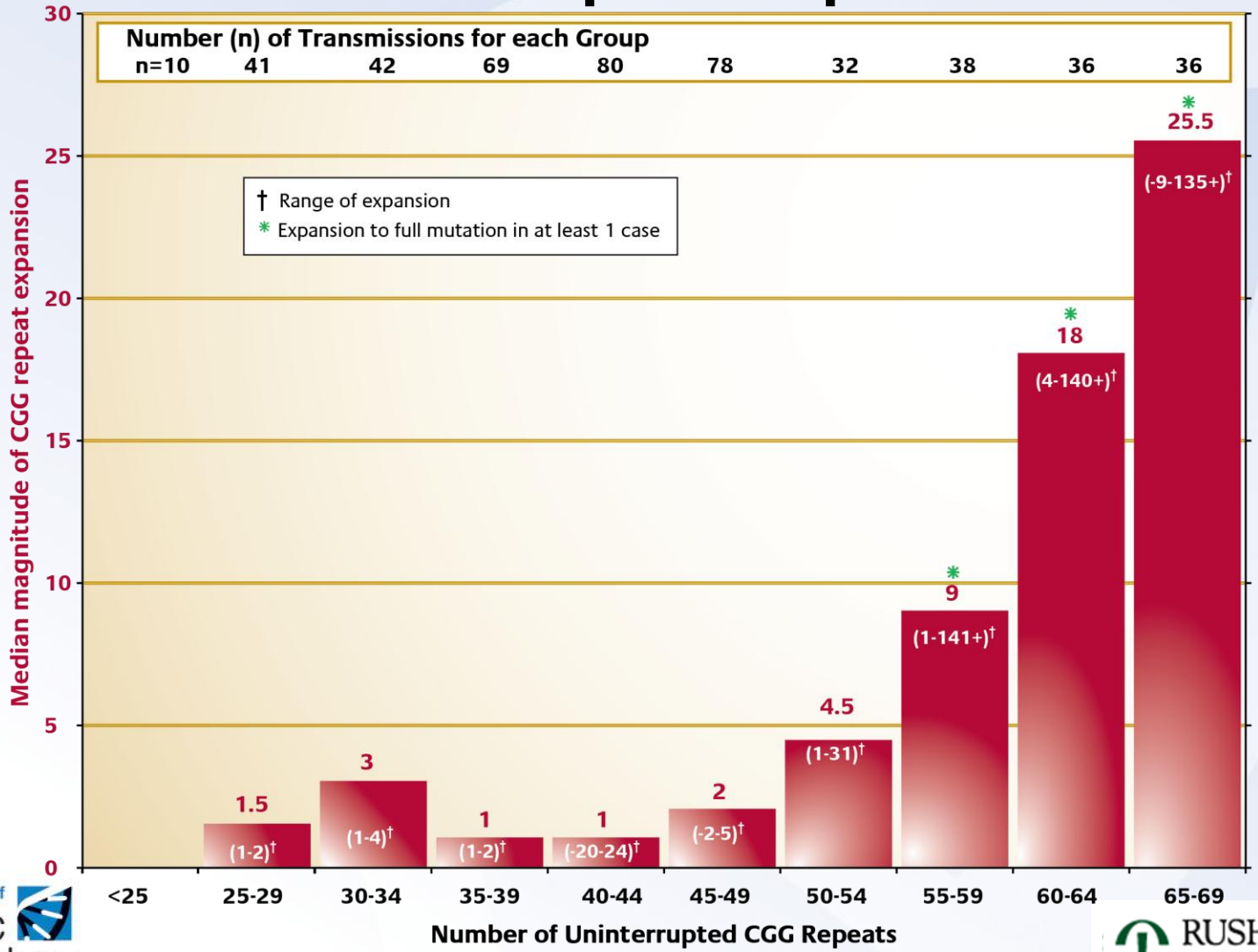
Magnitude of Instability of Unstable Maternal Transmissions Based on the Length of 3' Uninterrupted Repeats



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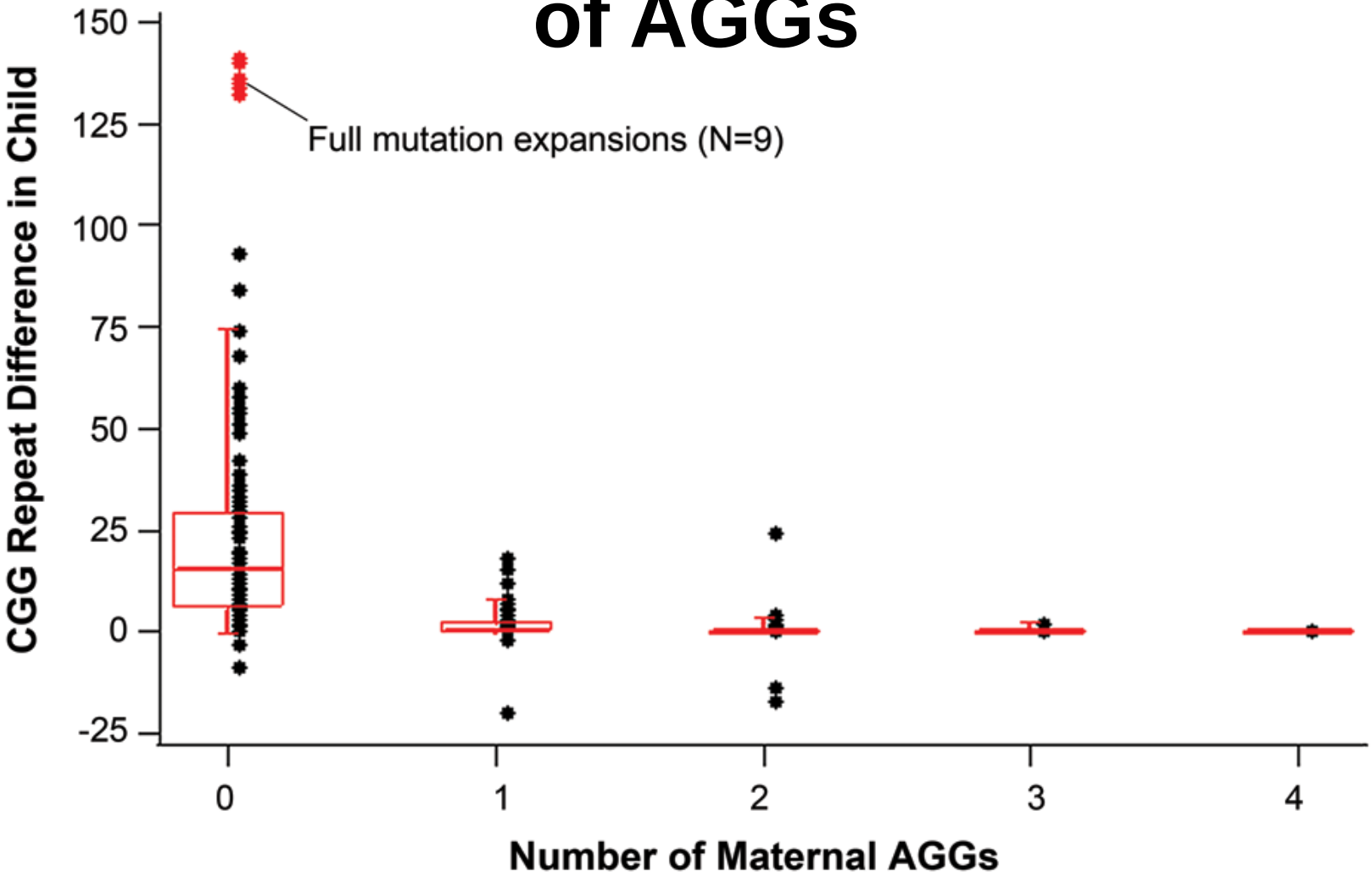
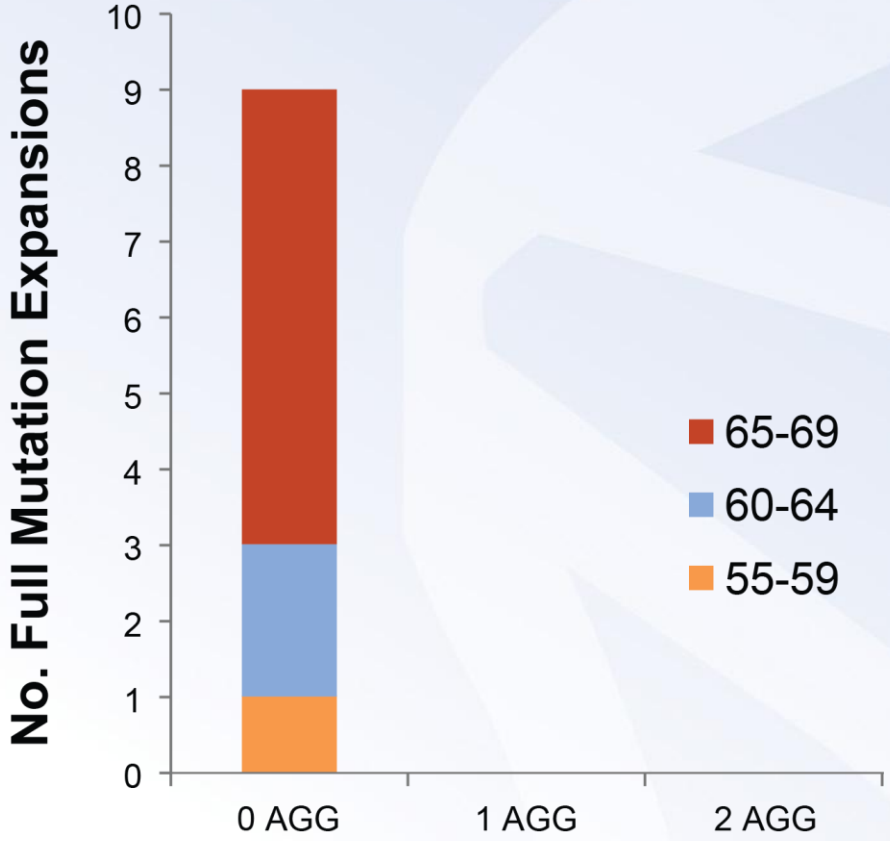
Magnitude of Instability of Maternal Transmissions Based on the Number of AGGs



The AGG Structures in Maternal Alleles Expanding to a Full Mutation



- All full mutations occurred in mothers with no AGG interruptions.
- The smallest maternal allele expanding to a full mutation contained 59 repeats.

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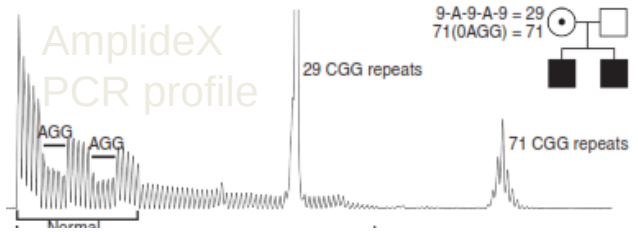
Separate Study: AGG interruptions refine risk of expansion to a full mutation from moderately sized PM alleles

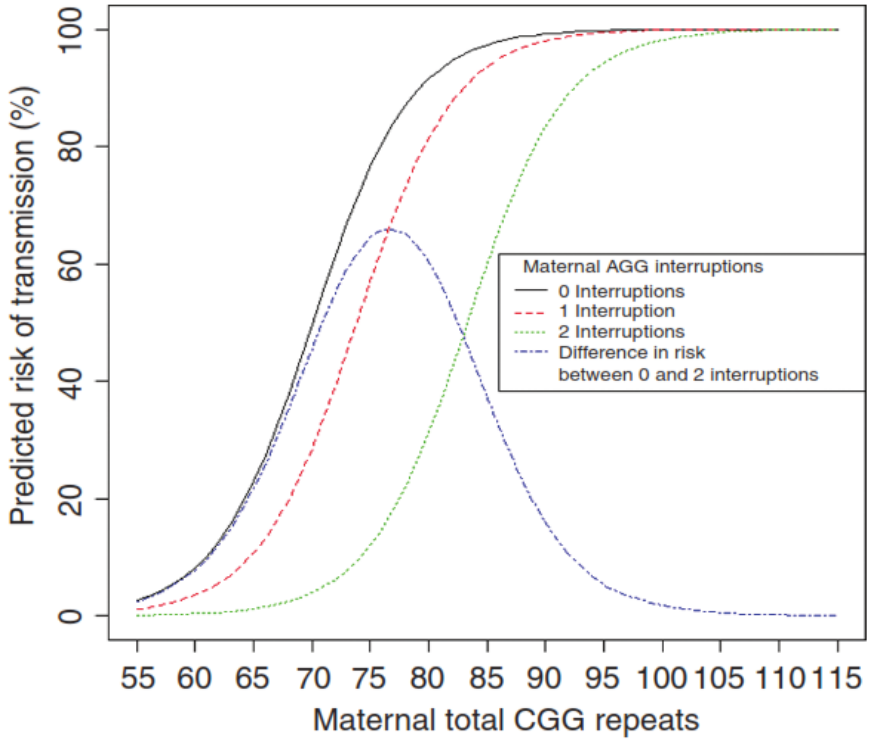
ORIGINAL RESEARCH ARTICLE | Genetics inMedicine

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]



- 267 mothers (55-175 CGG)
- 373 transmissions
- 296 expansions to full mutations
- AGG mapping using AmplideX technology *(enabled by Chen et al., 2010)*
- Reclassifies risk of expansion to full mutation
  - 75 CGG, 0 AGG=77%
  - 75 CGG, 2 AGG=12%



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

Key Conclusions from AGG Study

- The presence of AGG interruptions within the *FMR1* CGG repeats contribute to the stability of the alleles
- The uninterrupted 3' CGG length was 2 to 3X more correlated with the risk of expansion than the total repeat length
- A threshold of 35 3' consecutive CGG was associated with a statistically significant increasing risk of expansion
- The magnitude of repeat expansion was larger for alleles lacking AGG interruptions

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Case Studies Illustrate Implications of AGG Mapping for:

- Diagnostic Testing
- Understanding and Predicting Inheritance in Families
- Genetic Counseling

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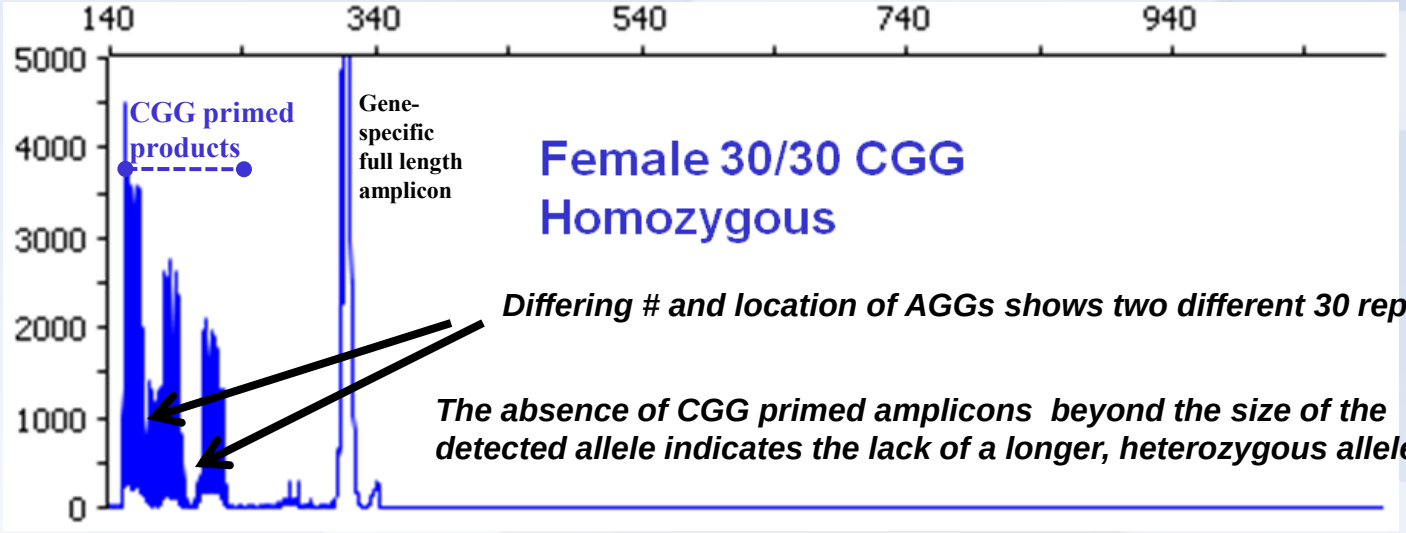
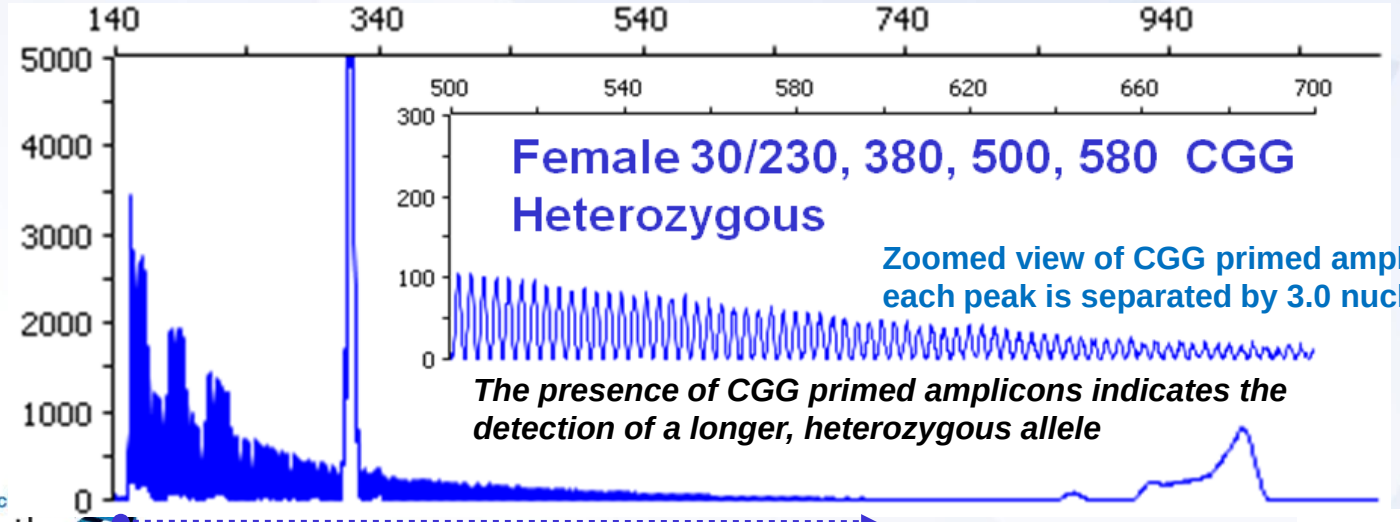
Case Study 1
Female Homozygous for 30 Repeat Allele - CGG Repeat Primed PCR and AGG Mapping Resolves Zygosity





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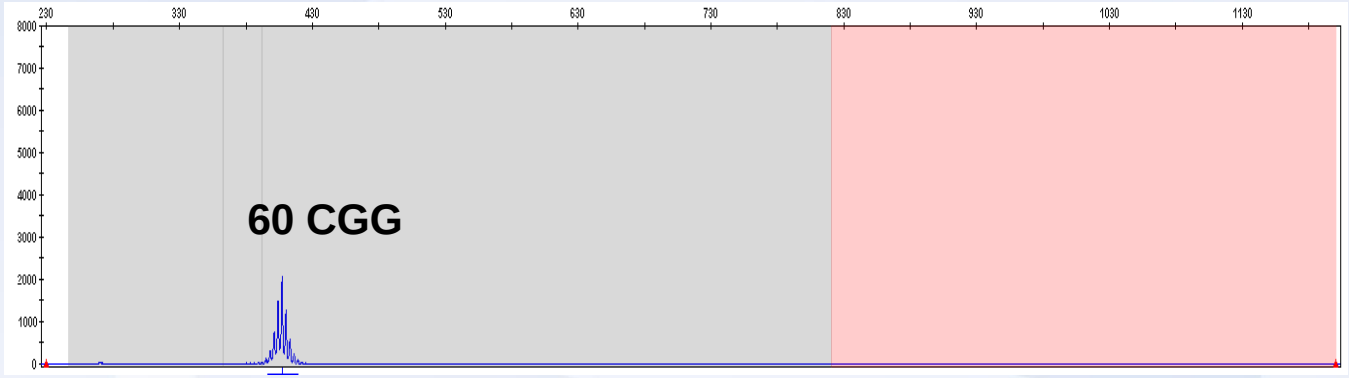
*Preliminary research data. The performance characteristics of this assay have not yet been established.

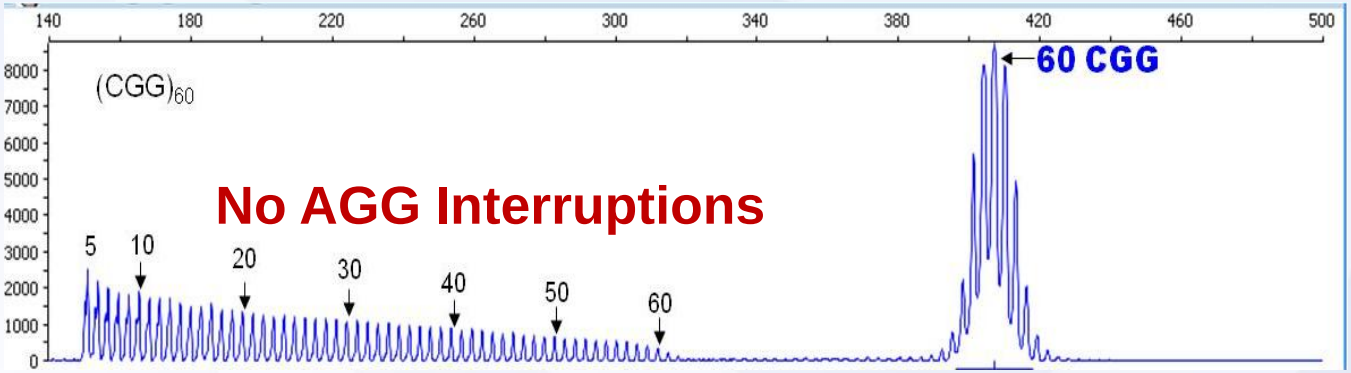
Case Study 2

Phenotype: **Male sample with 60 CGG. Allele expanded to 78 CGG in daughter and then to a full mutation in grandson.**

Gene-specific



CGG RP



***Outcomes*:** **CGG PCR can highlight consecutive CGG and flag samples likely to expand rapidly to full mutation.**

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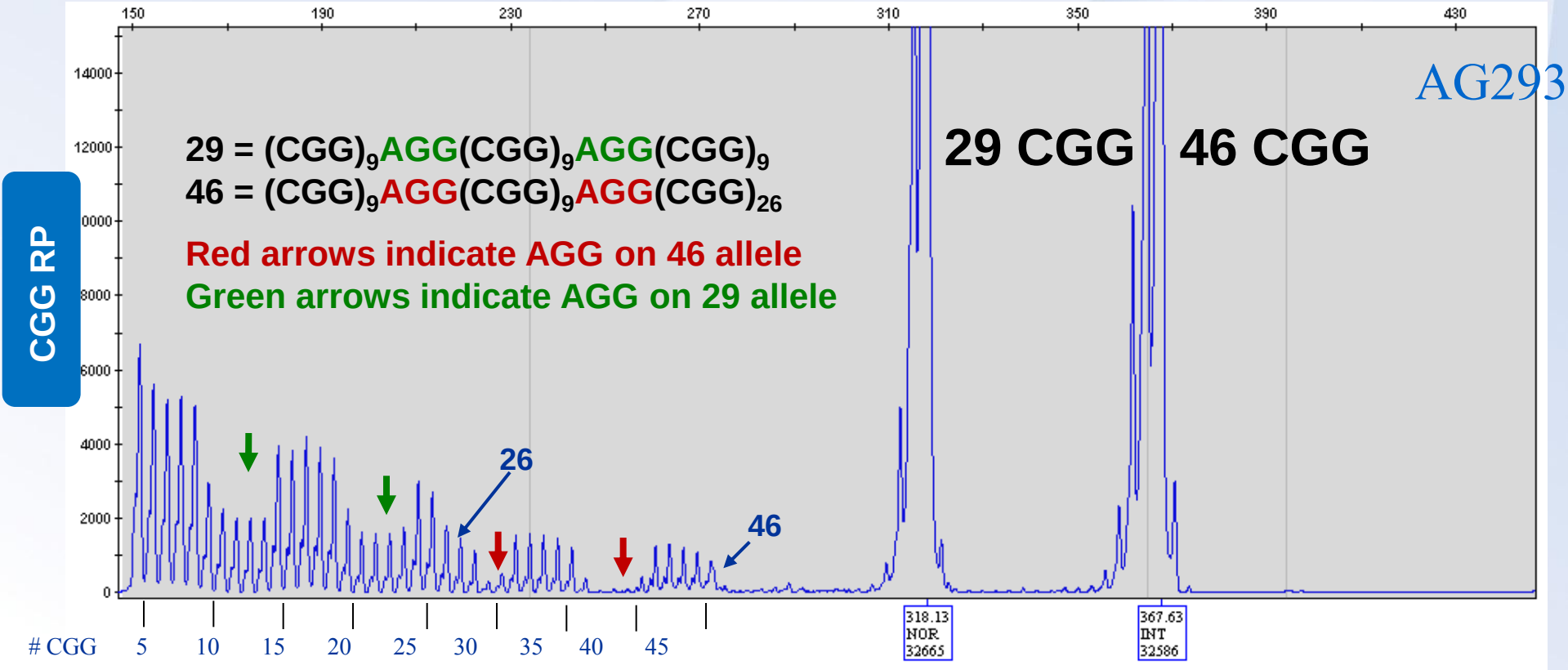
Case Study 3

***Phenotype*: Female sample 29 and 46 CGG. Referred from fertility clinic prior to IVF due to gray zone allele and concerns about expansion.**



***Outcomes*: CGG PCR shows location of AGGs at 10 and 20, only 26 consecutive CGGs, suggest only 5% risk of size change of only 1-2 repeats, allele likely to be stable, thus assist with risk prediction for patient in IVF decision.**

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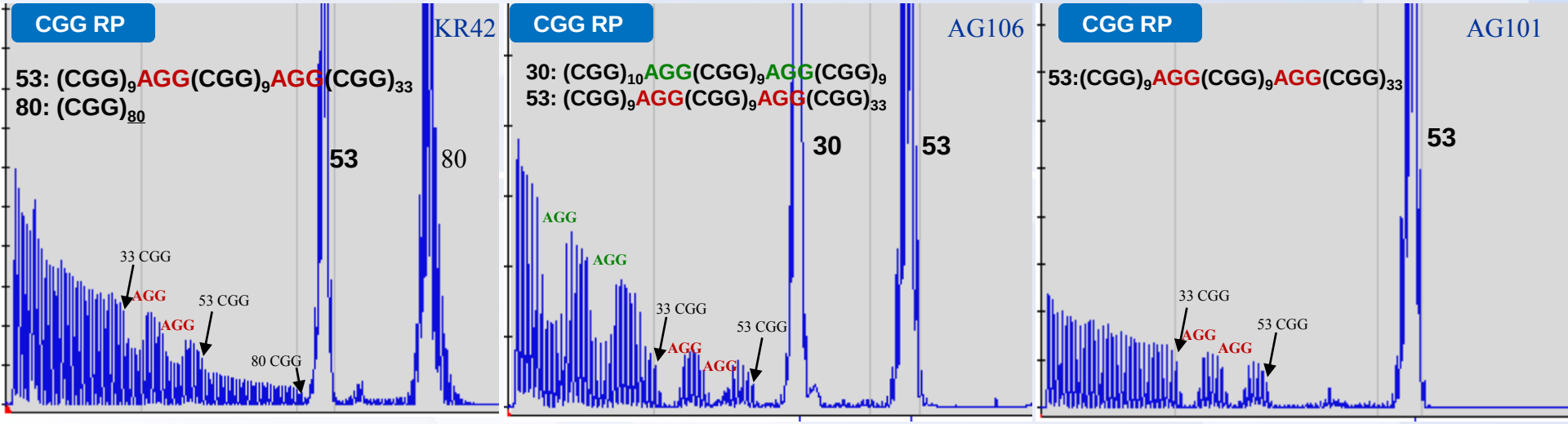
Case Study 4

Phenotype: **Sample from grandmother (left, 53 and 80 CGG) of proband with full mutation FXS (mother has premutation) and other children of grandmother (aunt [middle, 30 and 53 CGG] and uncle [right, 53 CGG] of proband), aunt has infertility and concerned about IVF risks.**

GRANDMOTHER **AUNT** **UNCLE**



Outcomes: **CGG PCR shows location of AGGs at 10 and 20 in 53 repeat allele, with 33 consecutive CGGs (7% risk) and 2 AGGs for this size (9% risk) of size change of only 1-4 repeats, allele will often be stable, thus assist with risk prediction for patient in IVF decision. Note lack of AGGs in 80 repeat allele have contributed to instability leading to full mutation.**

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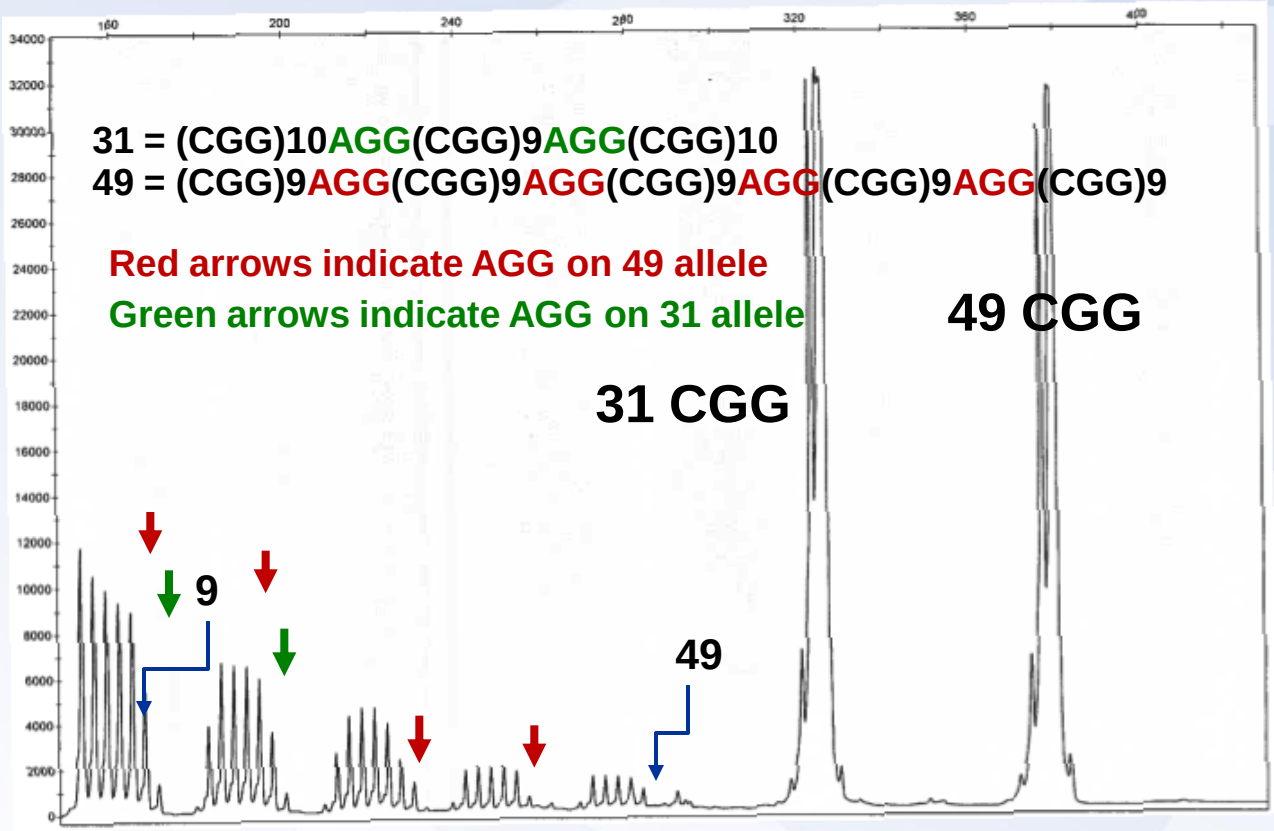
Case Study 5

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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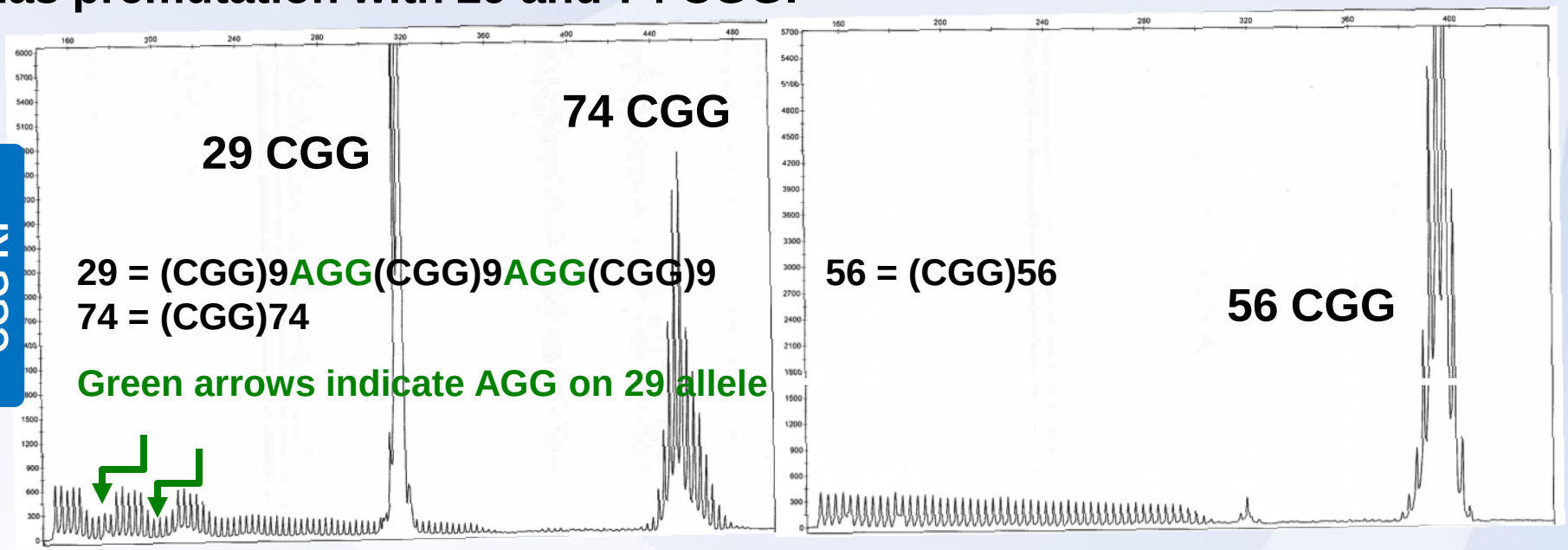
Case Study 6

Phenotype: **Normal boy who has brother with full mutation FXS, mother has premutation with 29 and 74 CGG.**

CGG RP



***Outcomes*:** **CGG PCR highlights consecutive CGG and shows this boy has deleted allele from mothers premutation (not expansion from normal allele) based on lack of AGGs. Risk of size change for this allele will be 100% based on size and lack of AGGs, 88% based on consecutive CGGs, for expansion of 1-31 repeats.**

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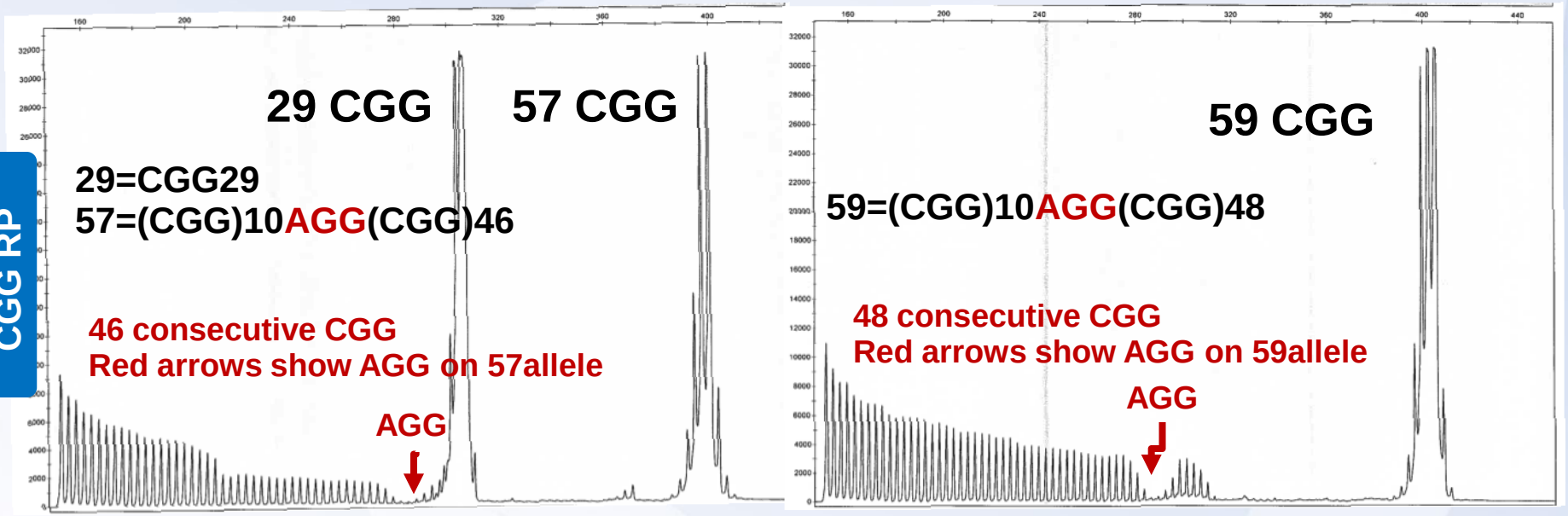
Case Study 7 – Testing the Study Predictions

Phenotype: **Non-study sample from mother with small premutation, AGG mapping shows 29 and 57 repeats, 46 consecutive CGG.**

CGG RP



Outcomes: **Model predicts: based on 1 AGG, 57 repeats 50% risk of size change; or based on 46 consecutive CGG 55% risk of size change; average size change of 2 repeats. CGG PCR highlights size change in son of 2 repeats to 59 repeat allele with 1 AGG and 48 consecutive CGG, consistent with model.**

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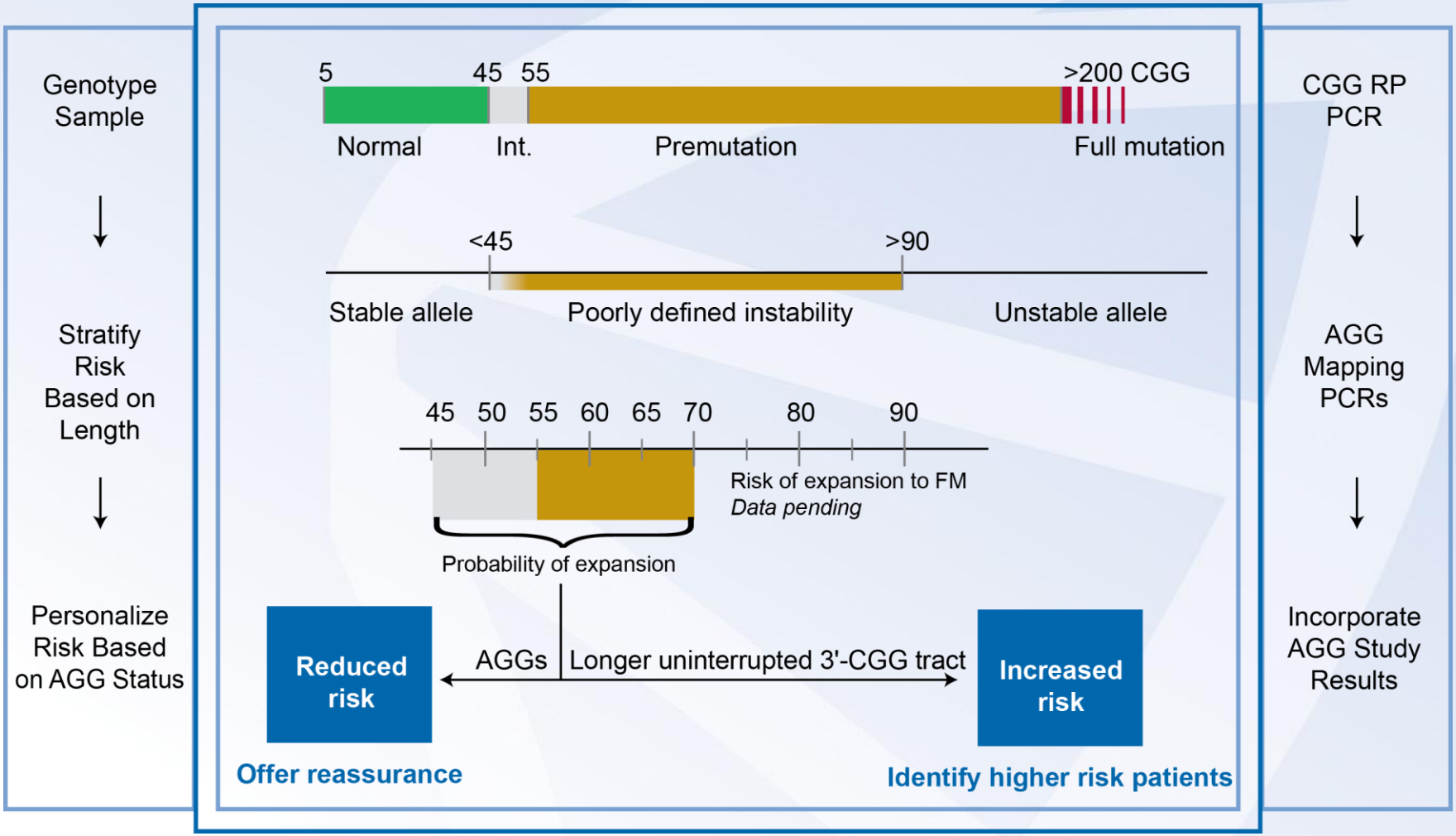
AGG Mapping has potential to be integrated into a comprehensive fragile X carrier testing protocol to aid genetic counseling.



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QUESTIONS AND ANSWERS

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