An isogenic fibroblast model of premutation-associated cellular phenotypes

Katarzyna A. Kościelska,
Sean Roenspie, Paul J. Hagerman, MD, PhD
UC Davis School of Medicine
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The need for an isogenic “case-control” human cell culture model

- Molecular mechanisms of premutation-associated cellular dysfunction need to be separated from background gene effects – e.g.,
  - Genetic effects that determine penetrance.
  - Familial clustering of symptoms
  - Childhood involvement
- Cell models can be used to study specific areas of dysfunction – e.g.,
  - Mitochondrial dysregulation
  - Inclusion formation
  - Threshold effects
- Such cells set the stage for production of neuronal cell models

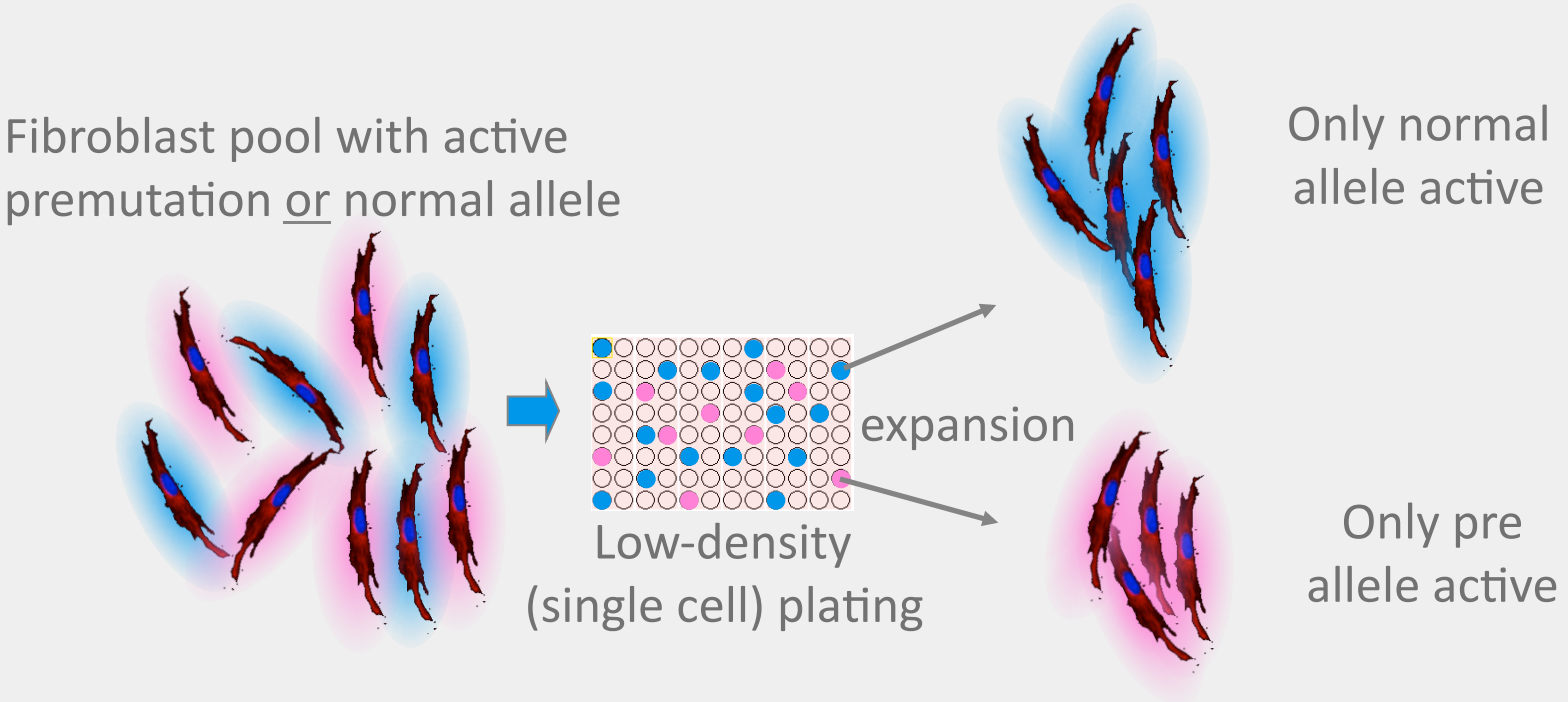
Fibroblasts as a cell model

- Skin/cord fibroblasts are easy to obtain, and are readily expandable in culture
- In 1995, Feng et al. cloned a male FXS mosaic to compare translation rates of different CGG repeats
- In 2010, Garcia-Arocena et al. described a fibroblast phenotype in premutation males
- In 2011, Yu et al. cloned RTT female fibroblasts by X-inactivation

The short-term goal of my work: to investigate the use of female single-cell-derived sub-clones

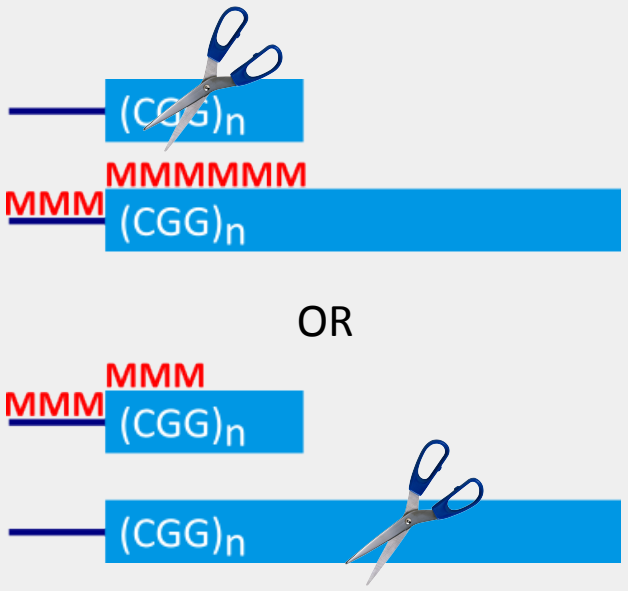
- Generation of fibroblast subclones from female premutation carriers as an isogenic “case-control” model (epi-isoautosomal; differing in the selection of active X chromosome)
- To determine whether allele size is stable during clonal expansion
- To determine whether X-inactivation is stable during single-cell clonal expansion (and iPSC reprogramming)
- If the lines are mitotically and epigenetically stable, one can treat the subclones as epi-isoautosomal case-control pairs (only one *FMR1* allele active in each clonal population)

Our cloning approach



Using X-inactivation to “epi-” genotype the clones

- The *FMR1* promoter is extensively methylated during X-inactivation
- The active (unmethylated) allele is cut by a methyl-sensitive enzyme and fails to amplify by CGG-repeat PCR



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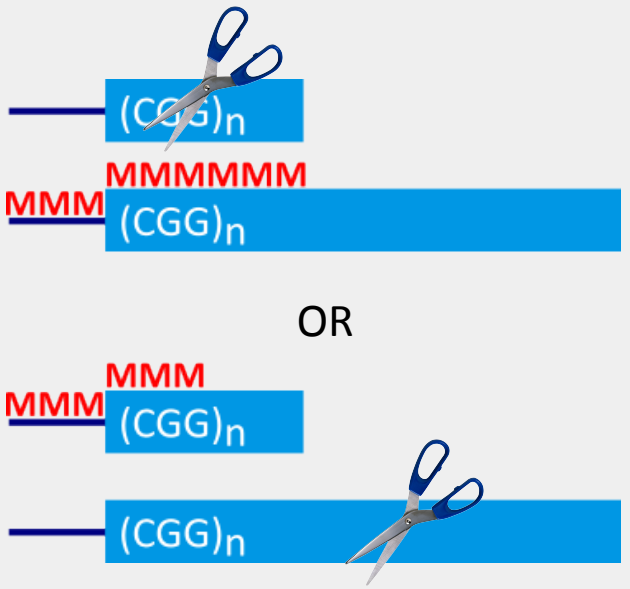
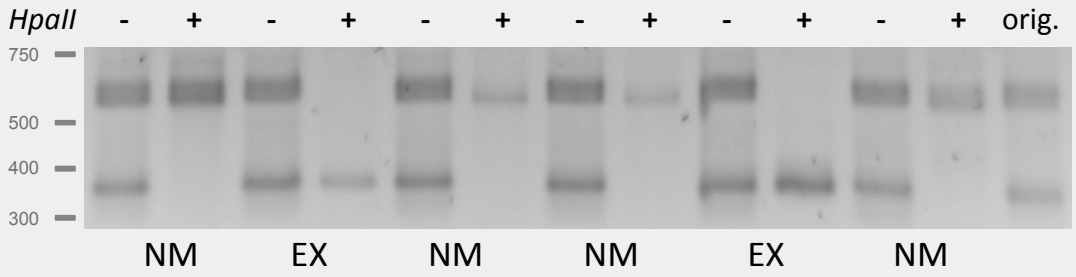
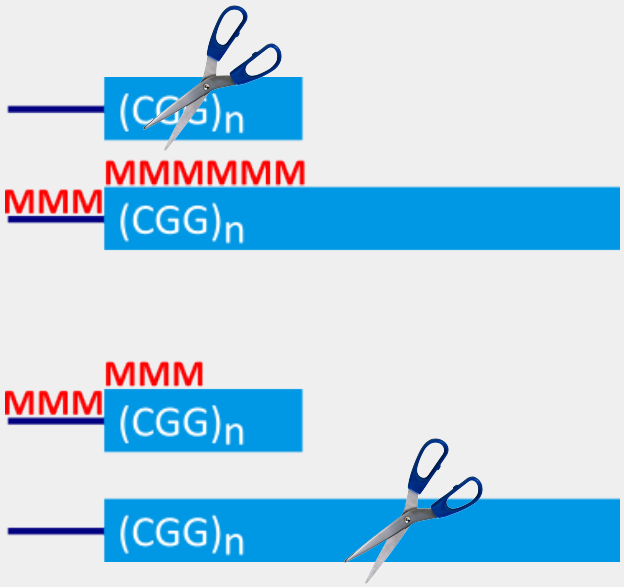
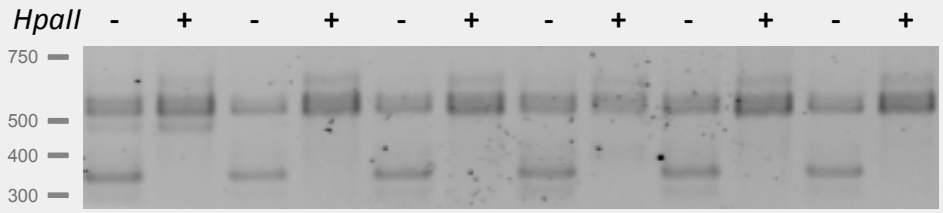


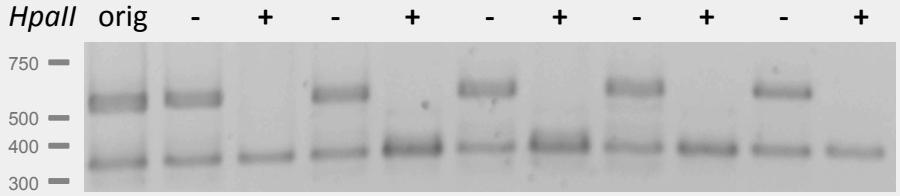


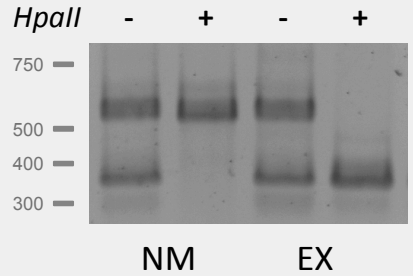
X-inactivation remains stable in iPSCs and neurons





(iPSC clones)





(differentiated neurons)

Liu, Koscielska, Cao et al., 2012

CGG repeat size also remains stable

- Evidence for mitotic stability of CGG repeats: cloned fibroblasts retain the same repeat size after 20+ doublings (*even clones of clones*)
- The issue of Xi stability during iPSC reprogramming has been controversial; our findings show that Xi remains unchanged

Multiple subclones generated so far

Fibroblast Line	Clones
female FXTAS, 35 y	4 NM, 0 EX
female pre, autoimmune, 51 y	5 NM, 5 EX
female pre, 55 y	3 NM, 2 EX
female pre, 54 y	7 NM, 5 EX, also iPSCs and neurons
female pre, 63 y.	4 NM
male mosaic, 20 y	2 ~45, 1 ~60, 29 ~70, 15 ~745 CGGs (and several in-between)
male normal, 14 y	~10 NM

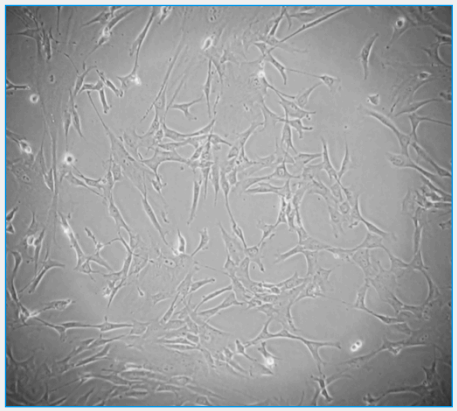
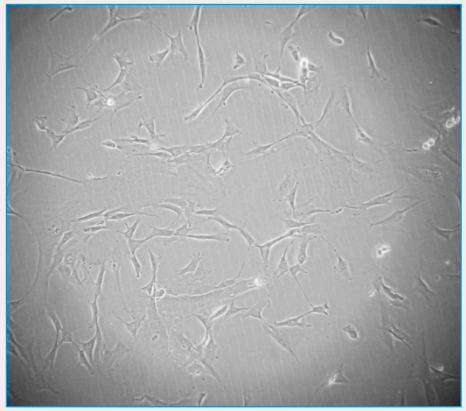
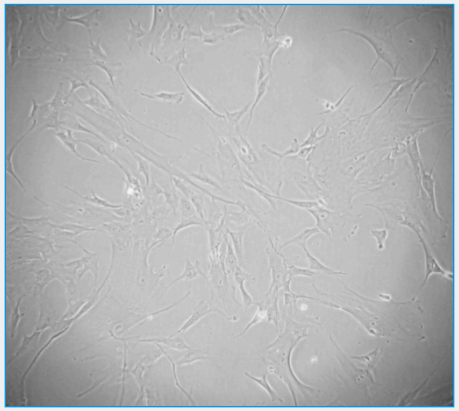
Summary

- Created an isogenic, epi-isoautosomal, case-control model for molecular studies of premutation using female fibroblasts
- Cloned cells maintain same allele size and X-inactivation, throughout long-term culture and iPSC reprogramming/ differentiation

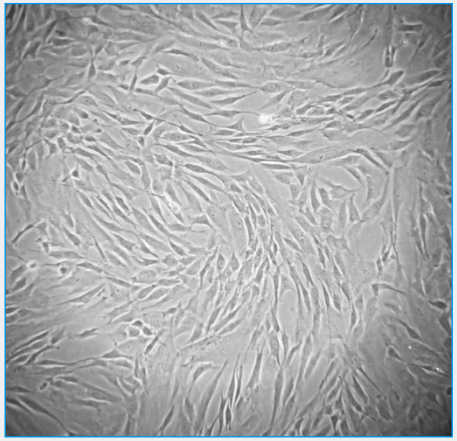
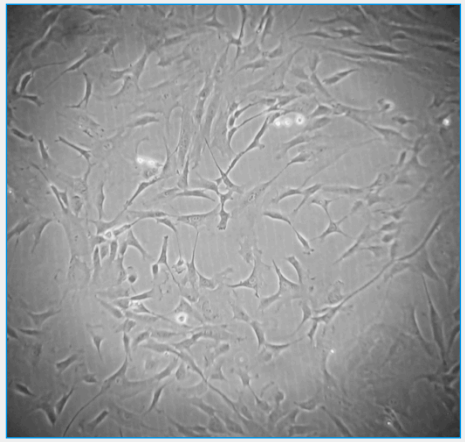
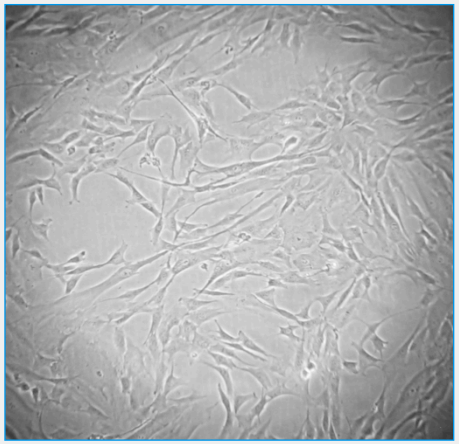
An important additional observation:

Lowered O_2 improves cell growth

FXTAS male | premutation female | premutation male mosaic

20% O_2

5% O_2

Future goals

- Extensively characterize the subclones from the molecular standpoint
- Use them in live-cell assays (e.g. mitochondrial activity)
- Create isogenic human neurons… more about it next!

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