

# Outcomes of Chromosome Breakage Analysis as a Tool for *BRCA2* Variant Reclassification

Erin Mundt, MS, CGC<sup>1</sup>; Heather McElroy, BS<sup>1</sup>; Paris Vail, BS<sup>1</sup>; Lisa Esterling, PhD<sup>1</sup>; Susan B. Olson, PhD<sup>2</sup>; Susan Manley, MS, CGC, MBA<sup>1</sup>

1. Myriad Genetic Laboratories, Inc., Salt Lake City, UT    2. Oregon Health & Sciences University, Portland, OR

## OBJECTIVES

- The classification of rare variants identified during genetic testing can be challenging. For some classes of variants, additional testing may provide valuable supporting information.
- This is the case for variants of uncertain significance (VUS) identified *in trans* with a known pathogenic variant (PV) in *BRCA2*.
- The incidence of two PVs in *BRCA2* is typically limited to individuals with Fanconi anemia (Hirsch et al. *Blood*. 2004;103:2554), which can be ruled out using chromosome breakage analysis (CBA).
- In order to reclassify appropriate *BRCA2* variants from VUS to benign, the testing laboratory has developed a program to include CBA as an adjunct to genetic testing at no cost to qualifying patients.
- Here we assessed the impact of this program on variant reclassification by evaluating the number of CBA offers made, CBA completed, and resulting reclassifications.

## METHODS

### GENETIC TESTING

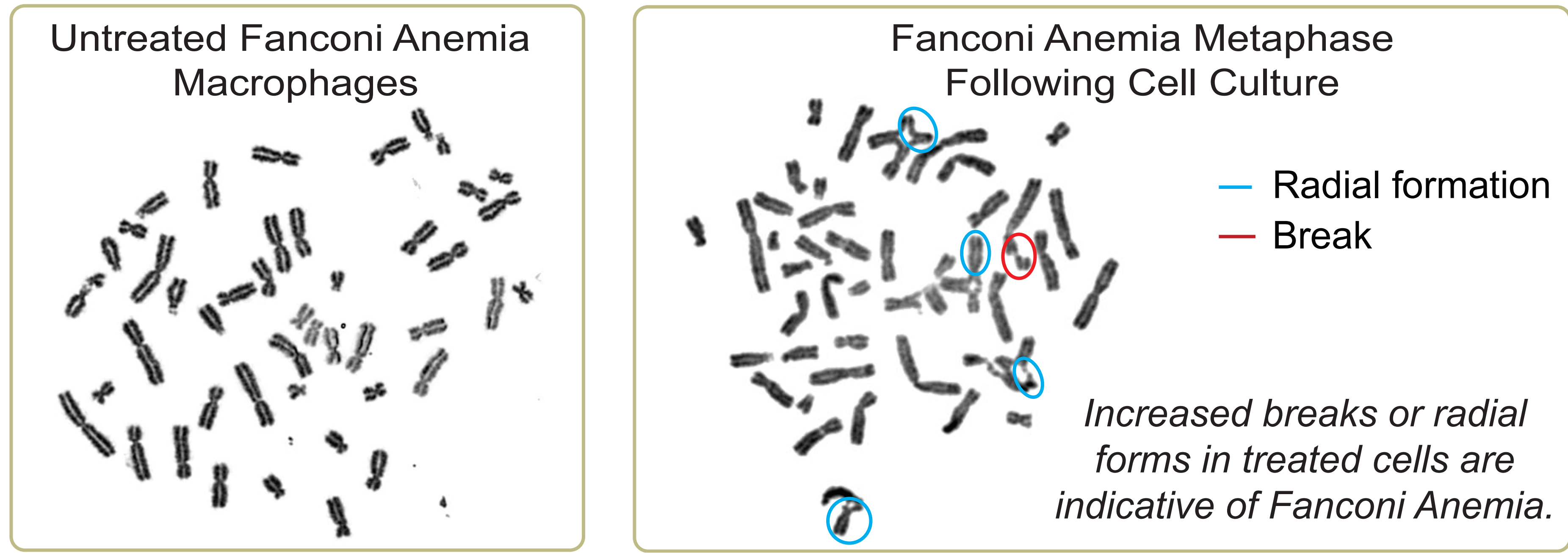
- Individuals who underwent *BRCA2* testing over a three year period (June 2011 – December 2014) and were eligible for CGA are included in this analysis.
- Individuals found to have a VUS *in trans* with a known PV in *BRCA2* and whose clinical presentation is inconsistent with Fanconi anemia are eligible for CBA.
- CBA for eligible patients is discussed with the ordering healthcare provider and blood samples from participating patients are sent to Oregon Health & Sciences University for analysis.

### CHROMOSOME BREAKAGE ANALYSIS

- Cells are exposed to the clastogens mitomycin C and diepoxybutane, slides prepared, and 50 metaphase cells from each treatment scored for increased breakage and chromosome radial formation (see Figure 1).
- Positive test results (increased breakage and radial formation) is diagnostic of Fanconi anemia. Negative results allow reclassification of a VUS to benign.

## RESULTS

Figure 1. CBA Testing



- 37 individuals with co-occurrences in *BRCA2* were eligible for CBA.
  - Eligibility was based on clinical presentation, age at testing, and confirmation of *in trans* co-occurrence by haplotype analysis and/or family testing.
  - Testing was performed for 11 individuals, including 2 siblings (Figure 2).
- Of the 11 participating individuals, 9 had negative CBA results and 2 had inconclusive results.
- As a result, 7 variants were reclassified from VUS to benign.
  - This includes c.559G>A (p.Glu187Lys) (Table 1), which has been observed in 3 tested individuals.
- The reclassification of these variants impacted a total of 89 individuals who underwent hereditary cancer testing.

Figure 2. CBA Program Overview

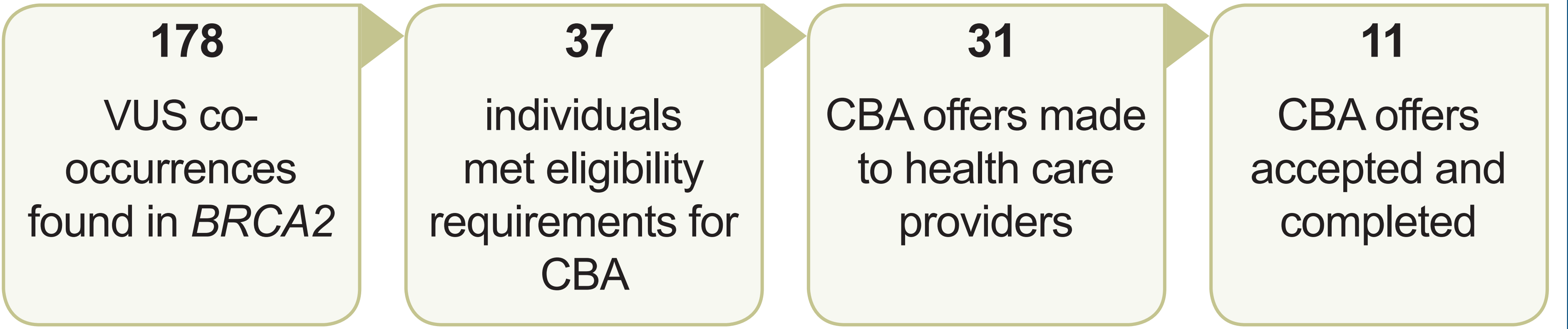


Table 1. Case Study of c.559G>A (p.Glu187Lys)

Proband	Personal diagnosis of breast cancer (age 27)
Genetic Testing Results	<i>BRCA2</i> c.559G>A (p.Glu187Lys) is found <i>in trans</i> with deleterious variant c.2808_2811del (p.Ala938Profs*21)
Family Analysis	Free single site testing for c.559G>A (p.Glu187Lys) and c.2808_2811del (p.Ala938Profs*21) offered to first degree relatives  Mother of proband tested positive for c.559G>A (p.Glu187Lys) and negative for c.2808_2811del (p.Ala938Profs*21)  Variant is <i>in trans</i> with a deleterious variant by family analysis
CBA	Negative - No increased breaks or radial forms
Reclassification	c.559G>A (p.Glu187Lys) is reclassified from a VUS to benign

## CONCLUSIONS

- Our analysis reveals that CBA is an effective and necessary tool for reclassifying rare VUSs identified in *BRCA2*, leading to more definitive classifications for providers and patients.
- Continued education and improvements (including use with other genes associated with FA) to the process for providers and patients will likely lead to greater uptake.