Title: An Inside Look at Mosaic Findings in Cancer Susceptibility Genes During One Year at a Clinical Diagnostic Laboratory

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Objective: With recent advances in technology, commercial laboratories are able to detect genetic alterations at frequencies lower than expected for heterozygous findings, often referred to as mosaic findings. Mosaic findings may be detected in blood or saliva for a variety of reasons, including: the presence of a true mosaic syndrome in the patient; a somatic mutation induced by hematologic malignancy/pre-malignancy, secondary to chemotherapy, or originating from circulating tumor cells; or age-related clonal hematopoiesis. These findings add to the complexity of result disclosure and management for clinicians and their patients, not to mention the patient's family members. This study examines multigene panel testing (MGPT) results in cancer susceptibility genes and assesses gene-specific frequencies of mosaic mutations and likely pathogenic variants (VLPs). We also aim to shed light on the further clarification and follow-up of these mosaic cases.

Methods: Test results with allele frequencies <30% on next-generation sequencing (NGS) with either Sanger sequencing or multiplex ligation-dependent probe amplification (MLPA)/chromosomal microarray confirmation were reviewed for individuals undergoing MGPT in cancer susceptibility genes during 2016 at one clinical diagnostic laboratory. Individuals underwent NGS and deletion/duplication analysis of between 5-67 genes, depending on the MGPT ordered. Clinical history information was obtained from test requisition forms and clinician notes.

Results: A total of 108 (0.15%) mosaic mutations/VLPs were reported in >70,000 individuals undergoing MGPT in cancer susceptibility genes in 2016. Mosaic mutations/VLPs were most frequently detected in *TP53* (58%, N=62), followed by *CHEK2* (17%, N=17), *NF1* (16%, N=14) and *ATM* (12%, N=11). Genes with ≤2 mosaic occurrences included *APC, BRCA2, MLH1, MRE11A, RAD50,* and *SUFU*. Of the 108 individuals with a mosaic mutation/VLP, 62% (N=66) were ≥60 years old, while only 18% (N=20) were under age 50. In addition, 18/108 (17%) went on to pursue follow-up fibroblast testing and 19/108 (18%) also had some form of follow-up familial testing. Of the 18 individuals who pursued fibroblast testing, four (22%) also carried the mosaic alteration in cultured fibroblasts. Their phenotypes included breast cancer at age 19 (mosaic *TP53* mutation), colon cancer at age 26 and 37 (mosaic *MLH1* mutation), breast cancer at age 35 (mosaic *TP53* VLP), and breast cancer at age 35 (mosaic *TP53* mutation). The 14 individuals that pursued cultured fibroblast testing and were negative were primarily individuals with later onset breast cancer and had mosaic mutations/VLPs in *TP53* (N=8), *CHEK2* (N=3), and *ATM* (N=3), with 1 also having a history of Hodgkin's Lymphoma. None of the family members that pursued familial testing (N=19) for the mosaic mutations/VLPs tested positive.

Conclusion: MGPT in cancer susceptibility genes has led to an increase in the detection of mosaic gene alterations. Current diagnostic testing methodologies cannot differentiate between germline vs. somatic alterations detected in blood or saliva samples; however, fibroblast follow-up analysis and familial testing may help determine the origin and significance of these results. Follow-up genetic testing in four individuals from our cohort demonstrated the presence of the mosaic mutation/VLP in cultured fibroblasts, which aided in ruling out most somatic etiologies. Furthermore, all four individuals presented with phenotypes that supported the presence of true mosaic syndromes. While detection of the mutation/VLP in a family member would confirm germline origin, familial testing among 19 families in our cohort revealed that none had identifiable heritable etiologies. As MGPT in cancer susceptibility

genes continues to surge, clarifying the underlying etiologies of these mosaic findings is imperative for clinicians, patients, and their families.