Title: *POLD1* and *POLE*: An Update on Phenotype Analysis

Authors: Marcy Richardson, Felicia Hernandez, Robert Huether, Shuwei Li, Carin R Espenschied, AJ Stuenkel, Tina Pesaran

1. Ambry Genetics, Aliso Viejo, CA 92656
2. Corresponding Author: mrichardson@ambrygen.com

Background: DNA Polymerase δ (*POLD1*) and DNA Polymerase ε (*POLE*) are two of the newest members of a growing list of genes in which mutations cause hereditary colorectal cancer. Early evidence indicates that mutations in the proofreading exonuclease domains lead to a highly penetrant clinical presentation of early-onset colorectal cancer, polyposis, possibly endometrial cancer and other cancers. Several recent publications have analyzed such families and have reported common mutations such as *POLD1* p.S478N, *POLD1* p.L474P and *POLE* p.L424V. In this work, we analyze a cohort of patients who have *POLD1* and *POLE* alterations identified as part of multi-gene panel testing.

Methods: As of June, 2016, approximately 45,000 individuals have been tested for *POLD1* and *POLE* alterations using one of three multigene panel tests at a single laboratory. The clinical histories of patients with reported *POLD1* and *POLE* loss-of-function (LOF) and exonuclease missense alterations were retrospectively reviewed. Co-segregation analysis was performed to quantify the disease causality by a logarithm of odds (LOD) score for *POLD1* p.L474P with penetrance rate estimated from the pedigree.

Results: *POLD1* and *POLE* alterations were reported with a frequency of 0.006%. To date, 167 *POLD1* and 151 *POLE* unique exonuclease domain alterations were detected. The recurrent *POLD1* p.S478N was not identified in this cohort. However, the *POLE* p.L424V pathogenic mutation has been detected in two unrelated probands in the absence of other pathogenic alterations. Neither p.L424V-carrier has a reported family history that satisfies Amsterdam II criteria. Interestingly, both probands have a reported personal history of brain malignancies. In addition, the pathogenic mutation *POLD1* p.L474P was identified in one large family that does meet Amsterdam II criteria and it segregated with disease with an LOD score of 3.035, implying strong evidence of pathogenicity. Loss-of-function (LOF) alleles were identified in 55 cases (15 and 40 in *POLD1* and *POLE*, respectively). Of the probands carrying LOF alleles 44% (24/55) had reported a personal or family history (in 1st or 2nd degree relatives) of colorectal cancer and/or endometrial cancer (27% (4/15) in *POLD1*; 50% (20/40) in *POLE*). Within the cohort of individuals with LOF alleles, only one family met Amsterdam II criteria.

Conclusions: In this update, we report the largest *POLD1* and *POLE* dataset to-date. The highly penetrant *POLD1* p.S478N, *POLD1* p.L474P and *POLE* p.L424V mutations are rare occurrences in this laboratory-based cohort, however the clinical phenotypes of these patients were consistent with what has been reported previously in the literature. Available clinical data for LOF allele-positive families are not consistent with highly penetrant *POLD1* and *POLE* mutations, and the clinical implications of these alterations warrant further investigation.