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APC Gene testing for Familial Adenomatosis Polyposis

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While genomics is destined to revolutionize clinical medicine, to date its beneficial effects have been somewhat muted. The promise and perils of genomics are emblemized by one of its flagship applications, namely the diagnosis of familial adenomatosis polyposis (FAP). FAP is an autosomal dominant syndrome characterized by numerous colonic adenomas which, without recognition and intervention, generally result in development of early-onset colorectal cancer (CRC), along with higher risks of several other malignancies (duodenal, pancreatic, thyroid, brain, etc.). The discovery that adenomatous polyposis coli (APC) tumor suppressor gene mutations, with concomitant over-activity in the Wingless-type mouse mammary tumour virus integration site (WNT) signaling cascade, were responsible for most (~80–90%) FAP cases occurred approximately two decades ago^{1,2}. More recently, a small subset of FAP cases were attributed to bi-allelic methylmalonyl CoA mutase (MUT) mutations, involved in preventing oxidizing DNA damage³.

Commercial assays for *APC* and *MUTYH* fostered rapid implementation of these scientific advances into clinical practice through widespread availability thereby substantially influencing patient care. Once the mutation has been identified in the proband (first affected family member to undergo medical evaluation), this information enables tailoring/ personalizing cancer preventive strategies for asymptomatic other family members. However, interpretation of mutational analysis in the proband can be vexing. If the test is positive, this is quite straightforward. However, if the proband's test is negative, this could mean that either the genes are not mutated (true negative) or that the diagnostic test currently available is unable to identify the pathogenic mutation (false negative). Further complicating matters is the not infrequent occurrence of indeterminate test results (mutations/ polymorphisms of unknown significance).

From a patient's perspective, the potential for unsatisfactory outcomes is accompanied by anxiety, expense and privacy/insurance considerations engendered by genetic testing. Thus identifying those most likely to benefit from gene testing is paramount. Towards this end, the report by Grover and colleagues in this issue of *JAMA*, is a much needed analysis on the *APC/MUTYH* mutational yield stratified by polyp number—the major indication for testing. This comprehensive, rigorously performed study provides insight into real life performance of these assays. In their investigation involving 8676 unrelated patients who underwent APC/MUTYH mutation analysis at a major commercial laboratory. The authors noted that *APC* mutations correlated with degree of polyposis whereas the frequency of *MUTYH* mutations was relatively constant, albeit low, over a wide range polyp numbers.

This study also underscores the problems associated with the current state-of-the-art for gene testing. Specifically, mutations were not detected in either genes in 18% of the patients

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with extensive polyposis (>1000 polyps) (22 out of 119) and ~one-third of those with 100–999 adenomas (473 out of 1338). For patients with a lower number of polyps, (10–100 polyps, the more common scenario encountered in practice), the majority of tests were negative. However, negative results may not necessarily portend low risk given the assay miss rate. Newer techniques such as deep intron sequencing may augment sensitivity⁴. Furthermore, *APC/MUTYH* mutation testing could be true negative with FAP phenotype resulting from mutations in novel or heretofore undiscovered genes, e.g. budding uninhibited by benzimidazoles 1 homolog beta *BUBR1* mutations or alterations in chromatin and other epigenetic mechanisms^{5,6}. These clinical dilemmas noted in the proband are accentuated in the management of members of the kindred.

Grover et al were able to improve diagnostic yield moderately for *APC/MUTYH* mutations via a multivariable model that incorporated age of first adenoma, personal or family history of CRC, and adenoma number. However, this may not be relevant for the common scenario of patients with 10–100 polyps without a family history (indeed, ~25% of FAP may be attributable to *de novo* mutations)⁷. From a management perspective, the phenotype of attenuated FAP is particularly vexing. For instance, the older age of onset means that recognition of syndrome may be delayed which can negatively impact extra-intestinal malignancy screening. Additionally, the more proximal adenoma distribution obligates frequent colonoscopy as opposed to classic FAP where flexible sigmoidoscopy suffices. Also, the performance of the proposed multivariable model performance may still be suboptimal for clinical use possibly because it does not account for myriad other putative neoplastic modulating factors including gene-environment interactions, modifier genes, etc. The importance of these variables is emerging in FAP but is better established in hereditary nonpolyposis colorectal cancer (HNPCC), the other major autosomal dominant CRC syndrome⁸.

Because the degree of polyposis appears to be a good but imperfect reflection of *APC/MUTYH* mutational status, this has led to exploration of other potential biomarkers. For instance, co-segregation of polyposis with congenital hypertrophy of the retinal pigmented epithelium (CHRPE) is commonly exploited given it is reasonably robust and inexpensive⁹. More recently, there has been interest in evaluating the uninvolved mucosa (field carcinogenesis) with numerous reports on cellular (apoptosis/proliferation), molecular (methylation, microRNA), biochemical and microarchitectural markers¹⁰. For instance, in murine models of FAP, there are profound gene expression and proteomic alterations in the histologically normal mucosa^{11,12}. Given the well-established APC-cytoskeletal interactions, submicron architectural alterations have been noted in the pre-dysplastic mucosa and can be used to reliably identify mutational status¹³. From a translational perspective, more human studies have focused on HNPCC (which is several fold more common than FAP) because the subtle pre-cancer manifestations (absence of polyposis) and variable CRC risk (~12–40% life-time risk of CRC) presents a clinical conundrum¹⁴. Ultrastructural biomarkers from the visually normal rectal mucosa (i.e. field carcinogenesis) range from crypt length to nanocytological (i.e. partial wave spectroscopic microscopy) have been shown to be associated with genetic risk in HNPCC patients^{15,16}. The biophotonic techniques have also been applied to field carcinogenesis detection in sporadic colon carcinogenesis given the importance of genetics and interactions with exogenous risk factors (obesity, diet, exercise, smoking)¹³. These approaches and others may represent potential adjunctive approaches to complement genomics.

In conclusion, the report by Grover and colleagues provides fundamental insights into the current performance of molecular diagnostics for FAP. While these applications of genomics are clearly a technological *tour de force*, from a clinical perspective more work is still required. In the future, these issues may be clarified by improved technologies such as

deep sequencing and more comprehensive datasets. Understanding the importance of splice site variants and epigenetic regulators may also provide the biological underpinnings to develop more effective diagnostic tools.

At this juncture, clinicians need to carefully consider the effect of a positive or negative test on patient management prior to making decisions regarding genetic testing. Appropriate patient education/informed consent prior to testing is mandatory, highlighting the integral nature of genetic counseling¹⁷. Until development of more robust genomic technologies for FAP detection, complementary approaches including careful assessment of family history and biomarkers may have utility. Furthermore, these considerations for FAP may serve as a model for evaluating the wider issues associated with practicing medicine at the front lines of the genomic revolution.

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