Molecular pathology selected abstracts

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Variants in *NUDT15*: association with thiopurine–induced myelosuppression

April 2019—The thiopurines mercaptopurine, thioguanine, and azathioprine are purine antimetabolites widely used as anticancer and immunosuppressive agents. Commonly prescribed in patients with inflammatory bowel diseases (IBD), such as Crohn's disease and ulcerative colitis, they are valuable steroid-sparing treatment options. Unfortunately, approximately 15 percent of IBD patients develop adverse drug reactions, such as thiopurine-induced myelosuppression (TIM), that necessitate drug withdrawal. TIM has a cumulative incidence of seven percent in IBD patients and an estimated mortality of one percent. A similar phenomenon is also described when this class of drug is used in antineoplastic protocols. The enzyme thiopurine methyltransferase (TPMT) metabolizes thiopurines, reducing their conversion to active drug. Genetic variation in the *TPMT* gene can result in decreased enzyme activity, increased active drug concentrations, and subsequent bone marrow suppression. *TPMT* variants are found in 25 percent of patients of European ancestry affected by TIM, suggesting the presence of other genetic and environmental determinants. Studies in patients of East Asian ancestry and other populations have identified variants in nudix hydrolase 15 (*NUDT15*) as risk factors for TIM. The authors of this study used genome-wide association studies (GWAS) and whole exome sequencing on a large case control cohort of patients to identify genetic variants associated with TIM in a population of European ancestry. Recruiting from 89 international sites during a four-year period, they tested 311 TIM-affected and 608 unaffected IBD patients using GWAS and 328 affected and 633 unaffected patients using whole exome sequencing. When comparing patients affected with TIM and those who were unaffected, the authors found no significant differences in gender, type of IBD, behavior of IBD, or extent of disease. Affected patients were noted to be younger at the time of IBD diagnosis and received a higher weight-adjusted thiopurine dose than unaffected patients. GWAS analysis confirmed the prior reported association of TIM with *TPMT* (variant rs11969064), seen in 30.5 percent (95 of 311) of affected patients compared with 16.4 percent (100 of 608) of unaffected patients in this study. No other genetic associations with TIM exceeded the a priori threshold for statistical significance. Exome sequencing to investigate the role of rare coding variants revealed a TIM association with a 6–base pair in-frame deletion in exon 1 of *NUDT15* (p.Gly17_Val18del) for 5.8 percent (19 of 328) of the affected patients compared with 0.2 percent (one of 633) of the unaffected patients. Variants in *NUDT15* or *TPMT*, or both in some patients, were enriched in patients affected with early-onset TIM, which occurs no more than eight weeks after starting the maximum thiopurine dose. The only other variant outside of *NUDT15* that was significantly associated with TIM in the exome sequencing data was within *TPMT*. Correlating functional assays with genetic data, TPMT enzyme activity levels were available for 75 percent of patients with exome analysis. All (10 of 10) patients with no TPMT activity and 73 percent of patients (80 of 109) with low TPMT activity carried various variant *TPMT* haplotypes. Overall, 4.9 percent (16 of 328) of affected patients and 0.2 percent (one of 633) of unaffected patients had two TIM-associated *TPMT* variant haplotypes. The median time to TIM was shorter for affected patients who carried *NUDT15* and double *TPMT* variants than for affected patients who did not carry risk variants. With the ubiquitous use of thiopurines across multiple diseases, the authors' findings are likely to have significance beyond IBD patients. According to population studies, variant *NUDT15* haplotypes can be found in 29.2 percent of East Asian, 20.7 percent of Latin American, and 13.4 percent of South Asian ancestry populations. In line with these findings, recent recommendations by the Clinical Pharmacogenetics Implementation Consortium advocate for pretreatment *TMPT* and *NUDT15* genotyping in patients slated to start thiopurine drugs.

Walker GJ, Harrison JW, Heap GA, et al. Association of genetic variants in *NUDT15* with thiopurine-induced myelosuppression in patients with inflammatory bowel disease. *JAMA.* 2019;321(8):773–785.

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Use of genomic landscape of esophageal adenocarcinoma to define biomarkers

Esophageal cancer is the eighth most common cancer worldwide and the sixth most common cause of cancer-related death. Esophageal adenocarcinoma (EAC), the predominant subtype in the West, is highly aggressive and generally resistant to chemotherapy, and it has an overall five-year survival rate of less than 15 percent. In comparison to other cancer types, EAC is characterized by very high mutation rates but, paradoxically, a paucity of recurrently mutated genes. Knowledge of which genetic events drive the development of EAC is limited. Consequently, there are few molecular biomarkers for prognosis or targeted therapeutics. A study by Frankell, et al., accumulated a cohort of 551 newly sequenced and previously characterized EACs. Cases had high quality clinical annotation, associated whole genome sequencing (WGS), and RNA sequencing (RNA-seq) data. From these 551 samples, the authors identified 11,813,333 single-nucleotide variants (SNVs) and small insertions or deletions (indels), 286,965 copy number alterations (CNAs), and 134,697 structural variants. They used an armamentarium of bioinformatic tools to assess recurrent mutations within a gene (dNdScv, ActivedriverWGS, and MutSigCV2), high-functional-impact mutations (OncodriveFM and ActivedriverWGS), mutation clustering (OncodriveClust, eDriver, and eDriver3D), and recurrent amplification or deletions of genes (GISTIC; genomic identification of significant targets in cancer) undergoing concurrent over- or underexpression. Seventy-six EAC driver genes were discovered, 71 percent of which had not been detected in EAC and 69 percent of which are known drivers based on published pancancer analyses. The authors discovered 21 noncoding driver elements in the study cohort, including known elements, such as the enhancer on chromosome 7, which is linked to *TP53TG1*, and new elements found in the 5′ untranslated region of *MMP24*. Using GISTIC, they identified 149 recurrently deleted or amplified loci. To determine which genes within these loci confer a selective advantage, the authors correlated cases with matched RNA-seq to detect changes in expression. Although the study may have been underpowered to detect small expression changes, the authors were able to identify significant changes in 17 cancer genes, including *ERBB2, KRAS*, and *SMAD4*. Some loci also showed extremely high copy number amplification, commonly more than 100 copies. In one example, circularization and amplification initially occurred around *MYC* but subsequently incorporated *ERBB2* from a different chromosome. Such a pattern of extrachromosomal amplification via double minutes has been previously noted in EAC. The authors also detected several cases of overexpression or complete expression loss without associated copy number changes, reflecting nongenetic mechanisms for driver dysregulation. Novel drivers of particular interest included *B2M*, which encodes a core component of the MHC class I complex and is a marker of acquired resistance to immunotherapy, and *ABCB1*, which encodes a channel pump protein associated with multiple instances of drug resistance. *TP53* was found to be a critical tumor suppressor in EAC, although 28 percent of cases remain *TP53* wild type. Amplification and overexpression of MDM2, an E3 ubiquitin ligase that targets p53 for degradation, is mutually exclusive with *TP53* mutation, suggesting that its degradation can functionally substitute for the effect of *TP53* mutation. Mutually exclusive relationships were observed among *KRAS* and *ERBB2*, *GATA4* and *GATA6*, and cyclin genes (*CCNE1, CCND1,* and *CCND3*). The authors also identified co-occurring relationships between *TP53* and *MYC, GATA6* and *SMAD4*, and *Wnt* and immune pathways. In univariate analysis, events in two genes were associated with significantly poorer prognosis after multiple-hypothesis correction—*GATA4* amplification and *SMAD4* mutation or homozygous deletion. The authors presented a detailed catalog of genomic events that have been selected for during the evolution of EAC. This

catalog of biologically important gene alterations was used to identify prognostic biomarkers and actionable genomic events. This study should help pave the way for evidence-based clinical trials for esophageal adenocarcinoma.

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