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Misclassification of genetic variants associated with hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy has a variable clinical presentation and may lead to sudden cardiac death. In many cases, it is associated with pathogenic genetic variants, enabling screening of relatives and, possibly, the ability to individualize treatment strategies through lifestyle modification or invasive procedures. However, the importance of accurately classifying such genetic variants cannot be understated, as misidentification could potentially cause inappropriate management, not only for patients but also for their relatives. Yet there is also a significant amount of background-normal genetic variation in the population. Therefore, the assignment of pathogenicity can be strongly influenced by observed variant frequencies in control populations. The authors hypothesized that some of the high-frequency variants classically associated with hypertrophic cardiomyopathy may be due to normal genetic variation in groups not traditionally represented in historical population-based sequencing. They predicted that many of these false-positives would occur in patients of African ancestry. The authors examined publicly available data from multiple sources, including exome data from 4,300 people of European ancestry (EA) and 2,203 people of African ancestry (AA), as well as whole genome data from 1,092 people from 14 ethnic populations, and SNP data for 938 people from 51 ethnic populations. They reviewed clinical data from patients who had been diagnosed with hypertrophic cardiomyopathy and included any patients with a genetic variant classified as pathogenic, presumed pathogenic, pathogenicity debated, or unknown significance. The patient population included 94 variants associated with hypertrophic cardiomyopathy, five of which were found to be high-frequency variants, defined as having a minor allele frequency of greater than one percent in the population. These five high-frequency variants—*TNNT2* (p.K247R), *OBSCN* (p.R4344Q), *TNNI3* (p.P82S), *MYBPC3* (p.G278E), and *JPH2* (p.G505S)—accounted for 75 percent of the genotypic prevalence of hypertrophic cardiomyopathy. In the publicly available data, all five of these high-frequency variants had significantly higher frequencies in the AA when compared to the EA groups, ranging from 1.5 to 14.9 percent in the AA group and 0.01 to 1.5 percent in the EA group, respectively. The remaining 89 variants associated with hypertrophic cardiomyopathy showed no significant frequency differences between the AA and EA ancestry groups. The study also found that even if these high-frequency variants were pathogenic, they would have very low penetrance, considering their prevalence in the general population. Multiple patients had positive genetic reports, which, in some cases, reported only these high-frequency variants that were later reclassified as benign. The authors found that for a few of the initial studies in the medical literature that first identified pathogenic variants associated with hypertrophic cardiomyopathy, the control sample sizes were too small and included no people of African ancestry. With statistical models, the authors demonstrated that even small studies can exclude pathogenicity of variants if small proportions of diverse populations are used. New resources, such as the Exome Aggregation Consortium database, include sufficiently diverse populations to provide enough power to rule out pathogenicity for rare variants. In addition, these large-scale efforts allow for the retrospective assessment of previously determined associations between genetic variants and disease. The authors concluded that the classification of variants should be a constantly evolving process as new information is obtained to guide interpretation.

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Analysis of colorectal neuroendocrine carcinomas with glandular components

Neuroendocrine carcinomas are relatively infrequent in the colorectum and are characterized by neuroendocrine morphology and expression of neuroendocrine markers, such as synaptophysin, chromogranin A, and CD56. They appear to be biologically distinct from the less aggressive neuroendocrine tumors and more biologically related to adenocarcinoma. Furthermore, neuroendocrine carcinomas are often spatially proximate to conventional adenocarcinoma components and can sometimes be classified as mixed adenoneuroendocrine carcinomas if they meet the requisite criteria. The authors conducted a study in which they identified a series of 15 cases of colorectal neuroendocrine carcinomas with glandular components, 10 of which were classified as mixed adenoneuroendocrine carcinomas. After identifying the pure glandular and pure neuroendocrine components by morphologic and immunohistochemical examination, the separate components were microdissected for genetic analysis. In all cases, an abrupt morphologic transition from glandular to neuroendocrine components occurred. KRAS gene mutation analysis revealed identical mutational status in the glandular and neuroendocrine components of all cases, suggesting a common clonal origin. Ten cases were examined by a next-generation sequencing panel of 50 cancer-related genes. In the tumors analyzed, 130 mutations were identified, 55 of which were seen in pure glandular components and 75 in neuroendocrine components. Of these mutations, 32.6 percent were shared among the two components, 40.7 percent were exclusive to the neuroendocrine components, and 26.7 percent were exclusive to the glandular components. The most frequent mutations were seen in *TP53, KRAS,* and *APC*, with 62 percent of these shared among the two components. Also seen were PIK3CA mutations, occurring more often in the glandular components, and RB1 and *MET* mutations, more often seen in the neuroendocrine components, although these differences were not significant. Mutations identified in *TP53, KRAS, APC, RB1, MET, BRAF, ERBB4,* and *PTPN11* could be classified as founding clone mutations, as determined by identifying mutations seen in nearly 100 percent of tumor cells. However, mutations in other genes, including *PIK3CA*, had lower allele fractions and were, therefore, determined to be a later event that defined some subclones. The founding mutations were more likely to be shared between the neuroendocrine and glandular components. Whole exome sequencing performed on three cases identified shared mutations in all of them, but the shared mutations were only nine percent of all mutations seen, while 44.4 percent were exclusive to the glandular components and 46.6 percent exclusive to the neuroendocrine components. While histologic examination showed an abrupt transition, the genetic findings in this study appear to demonstrate a common clonal progenitor due to identical KRAS mutational status and additional shared mutations identified by next-generation panel and exome sequencing. However, the findings of mutations exclusive to glandular or neuroendocrine components show divergent evolution likely early in tumor development. In addition, this study demonstrates that the genetic basis of neuroendocrine carcinomas is distinct from that of neuroendocrine tumors or carcinoids.

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