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Editors: Donna E. Hansel, MD, PhD, chief, Division of Anatomic Pathology, and professor, Department of Pathology, University of California, San Diego; John A. Thorson, MD, PhD, associate professor of pathology, director of the Clinical Genomics Laboratory, Center for Advanced Laboratory Medicine, UCSD; Sarah S. Murray, PhD, associate professor, Department of Pathology, and director of genomic technologies, Center for Advanced Laboratory Medicine, UCSD; and James Solomon, MD, PhD, resident, Department of Pathology, UCSD.

Ability of cell-free circulating tumor DNA to reflect genomic changes in cancer deposits

Analysis of cell-free circulating tumor DNA is an emerging precision medicine technology that may be used to assess molecular alterations in cancer-derived DNA present in the blood, as well as to monitor cancer genomic changes over time and assess genomic changes and resistance following cancer therapy. This approach detects small amounts of DNA in the blood and obviates the need to isolate and analyze individual, intact circulating tumor cells. Cell-free circulating tumor DNA (ctDNA) is derived from cancer cells that undergo apoptosis or necrosis and that subsequently release DNA into the peripheral blood. The authors of this study compared ctDNA to cancer tissue obtained through biopsy from patients with metastatic breast cancer to determine if ctDNA can mirror the full genomic landscape of the parent tumor and its derived metastases. The study expands on prior work by numerous authors who have demonstrated that ctDNA can be isolated in the vast majority of patients with metastatic breast cancer and that there appears to be a relationship between ctDNA and patient outcomes in this population. The authors of this study evaluated 45 patients with metastatic breast cancer, the majority of whom were diagnosed with ductal carcinoma of the breast. Using two different commercially available next-generation sequencing (NGS) tests to analyze gene alterations in ctDNA and tissue, respectively, the authors compared the genes shared in common between these two platforms. When specifically analyzing these shared gene alterations, the concordance for detecting DNA alterations was 94.2 percent. However, much of the concordance was likely due to wild-type/wild-type agreement. The different genomic test panels showed a significant lack of overlapping genes to be tested. When the authors analyzed the subset of genes with genomic alterations identified in either one of the assays (232 genes total), the concordance on identified genomic alterations was only 10.8 percent. Even when analyzing only genomic alterations that had the potential to be identified in both assays, the concordance was 15.1 percent. CtDNA alterations with a mean variance allele frequency greater than one percent showed a significantly higher concordance rate of 72.7 percent. Copy number variance concordance was much lower, at 3.5 percent. The time frame between tissue biopsy and ctDNA analysis did not appear to alter concordance rates in this study. An important takeaway from this study is that genomic testing platforms do not frequently overlap in the large panels of genes tested, which may make it difficult to understand how well ctDNA predicts tissue genomic alterations in metastatic tumor deposits. This study also made use of large gene panel platforms, whereas many prior studies of ctDNA have focused on single genes or a small panel of hotspot genes. This explains the differences in concordance rates but also raises the question of how well the entire genomic landscape can be monitored through ctDNA applications. The authors concluded that only cancer cells that release DNA into the peripheral blood can be assessed, and the heterogeneity of cancer subclones may complicate this analysis. Although ctDNA analysis appears to hold promise for precision medicine approaches in breast cancer patients, the specifics of which genomic alterations and how many genes should be analyzed requires further study.

Chae YK, Davis AA, Jain S, et al. Concordance of genomic alterations by next-generation sequencing in tumor tissue versus circulating tumor DNA in breast cancer [published online ahead of print April 26, 2017]. *Mol Cancer Ther.* doi:10.1158/1535-7163. MCT-17-0061.

Correspondence: Dr. Young Kwang Chae at young.chae@northwestern.edu

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Association of SNPs with serum calcium levels and risk of CAD and heart attack

Studies have shown an association between elevated serum calcium levels and an increased risk of cardiovascular events, including myocardial infarction. Randomized clinical trials have shown that calcium supplementation can lead to increased levels of serum calcium, activation of calcification mechanisms, and production of insoluble calcium-protein particles in the blood. In these studies, calcium supplement use was shown to increase the risk of heart attack by 24 percent, although these findings are somewhat controversial. To address the importance of serum calcium relative to the risk of cardiovascular events, the authors studied the association between single nucleotide polymorphisms (SNPs) and serum calcium levels, risk of coronary artery disease (CAD), and heart attack. They used a Mendelian randomization approach that assumes the genetic variants analyzed are key variables associated with the risk factor and that they are not associated with confounding factors and will not affect the outcome through any way other than the risk factor itself. As the first step in their study, the authors identified SNPs associated with serum calcium levels by analyzing publicly available de-identified data from large-scale genome-wide association studies (GWAS). They reviewed data from as many as 61,079 people and identified seven SNPs associated with serum calcium levels. One SNP was subsequently removed due to confounding effects. The remaining six SNPs were analyzed for their association with CAD and heart attack using a meta-analysis of genome-wide associations in a separate patient database of up to 184,305 people. The researchers found that the odds ratio per 0.5 mg/dL increase in serum calcium levels for these SNPs was 1.25 (95 percent confidence interval [CI], 1.08–1.45; $P = .003$) for CAD and 1.24 (95 percent CI, 1.05–1.42; $P = .009$) for heart attack. By comparison, this risk is approximately equivalent to the risk associated with triglyceride levels for CAD and heart attack. The 0.5 mg/dL increase in serum calcium represents one standard deviation of serum calcium change, which also corresponds to peak blood serum calcium levels four hours after ingestion of a 1,000-mg calcium supplement or six hours after ingestion of a 500-mg calcium supplement. In line with the results of prior studies that showed that the short-term serum calcium increases caused by supplements are associated with an increased risk of CAD and heart attack, the current study demonstrates similar results from presumed long-term increases in calcium levels due to a genetic predisposition in some people. However, this study lacks complete information on gender and did not replicate the analysis in an independent data set. Because of factors such as these, additional studies will be needed to confirm the findings.

Larsson SC, Burgess S, Michaelsson K. Association of genetic variants related to serum calcium levels with coronary artery disease and myocardial infarction. *JAMA.* 2017;318(4):371–380.

Correspondence: Dr. Susanna C. Larsson at susanna.larsson@ki.se