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EDITORIAL COMMENT

Calcific Aortic Valve Disease "Omics" Is Timely, But Are We Looking Too Late?*

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Aortic stenosis (AS) due to calcific aortic valve disease (CAVD) is the most common valvular heart disease in developed countries and currently has no known pharmacotherapies despite its significant morbidity and mortality. AS is usually diagnosed too late, after the disease has reached a point of no return, with symptoms manifesting as hemodynamic changes in valve function. Depending on the hemodynamic metrics, the disease is classified as "mild," "moderate," or "severe," and is treated by surgical aortic valve replacement or transcatheter aortic valve implantation. Currently, there are no existing therapies that can slow or reverse the progression of CAVD, and by the time the patient reaches the clinic, invasive intervention is the only option.

One of the major challenges of drug target discovery for CAVD is the limited in vitro and in vivo animal models. Surgically removed aortic valve leaflets remain the main source to study and identify CAVD disease drivers. However, a current surge in transcatheter aortic valve replacement procedures will cut back the availability of already limited valve tissues provided by surgical aortic valve replacement. As a consequence, the time window to understand valvular pathologies is shortening all the while disease burden is increasing. Moreover, a near lack of appropriate nondiseased tissue leaves researches blinded to a true baseline phenotype of a normal aortic valve. Large-scale molecular screening methods that capture RNA, protein, or metabolite profiles are increasingly applied to CAVD. However, even if candidate disease drivers are identified, they likely represent the accumulation of several confounding metabolic steps leading to the end-stage disease and may not necessarily represent the initiating factors sought to treat CAVD. Treating CAVD in a timely manner will therefore heavily rely on bridging clinical and basic research interests to coordinate a systematic transfer of precious valve tissues from the bedside to bench. In addition, CAVD research must continue to exploit ever-evolving technologies such as mass spectrometry-enabled "omics" and computation-driven systems biology to extrapolate the findings described in end-stage disease molecular atlases back to the initiating events of CAVD.

In this issue of *JACC: Basic to Translational Science,* Surendran et al. (1) present the metabolome profile representing 106 patients' stenotic aortic valves leaflets classified by increasing disease severity (mild, moderate, and severe) and determined, through a targeted lipid profiling validation analysis, that lysophosphatidic acids associate with CAVD disease severity. Disease severity was assessed by clinically pertinent hemodynamic parameters, peak aortic jet velocity, mean pressure gradient (MPG), aortic valve area (AVA), and calcium score. Mildly diseased leaflets provided a baseline alternative to normal tissues. Aortic valves were categorized as mild if they had an AVA of >1.5 cm^2, a peak velocity of >2.5 m/s, or an MPG of >20 mm Hg. However, the AVA, peak velocity, and MPG for nondiseased aortic valves are 3.0 to 4.0 cm^2, ≤1.0 m/s and <15 mm Hg, respectively (2). Based on the hemodynamic criteria, the mild classification already manifests with significant changes to leaflet biology, associating with aortic valve sclerosis

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or leaflet thickening, a precursor to AS. Acquisition of nondiseased tissue is extremely rare. Recently, the proteome of stenotic aortic valves was investigated by Schlotter et al. (3), but instead of performing bulk proteomics on the whole leaflet tissue, the leaflets were subclassified by gross pathoanatomical features representing disease progression over time. Nondiseased portions were characterized as a thin transparent tissue, most resembling a normal leaflet tissue; a thicker and opaque tissue indicating stiffening was characterized as fibrotic or sclerotic; and a calcium-laden tissue at the leaflet base reflected calcification, the most advanced disease state (3). Moreover, this intraleaflet subclassification permitted the removal of proteome variance because of individual variability and clinical severity, to focus on protein signatures that specifically define a progressing valvular pathology (3). In contrast, the metabolic profile provided by Surendran et al. (1) was performed on whole leaflets without subdivision into portions representing disease progression. Thus, the tissue source of increased lysophosphatidic acid (lysoPA) lipids was unknown and could be a product derived from calcifying, fibrotic, or nondiseased tissue.

These metabolic changes offer a snapshot in time of the disease stages of CAVD, but they still do not provide information into the inciting events. Early changes to the metabolome may be critical driving factors for valvular interstitial cell osteogenesis or may point toward mitochondrial dysfunction. It would therefore be of great benefit to the field to know the metabolic signature of initiating events. However, a comprehensive molecular map or molecular "atlas," whether it be of a metabolome or a proteome, is never complete. Metabolites, for instance, comprise a vast range of structures with diverse physicochemical properties. A given extraction strategy therefore offers only a subset of small molecules (4). For the initial unbiased profiling performed by Surendran et al. (1), metabolites were extracted from whole aortic valve leaflets using an aqueous-based solvent that enriched a variety of small molecules including amino acids, pyrimidines and benzenoids, and a subset of small or amphipathic lipids representing 4 of the 8 major lipid classes (5), including the lysoPA-containing glycerophospholipids. The subsequent interest in lysoPA lipid subclass for targeted lipid analysis of valve and plasma thus warranted an extraction solvent more suited to enrich a broader range of lipids. Without a universal extraction method and an unlimited tissue source, only 1 of many possible workflows is often prioritized. The pros and cons of enriching a biased molecular profile must thus be equally and carefully weighed in the data interpretation.

The metabolomics field is experiencing a research boom as of recent, in particular due to rapidly evolving advances in mass spectrometry (4). Therefore, it is time for the CAVD field to expeditiously embark in "omics" strategies, and Surendran et al. (1) take advantage of these technological developments. Latest-generation instruments feature faster, deeper, and increased sensitivity capabilities. Despite these advances, a single shot into the mass spectrometer is not sufficient to detail the true depth of biological molecules present in the sample. As is the case by Surendran et al. (1), metabolite profiling requires a multipronged analytical workflow. Multiple chromatographic separation and mass spectrometric acquisition methods are often used to analyze a single sample. However, running multiple acquisition strategies introduces a detrimental source of technical variability: time. Many metabolites have a low shelf life, decaying in signal across the collection period. There are several acquisition and computational strategies to account for signal decay (4). Awareness of such downstream issues is one reason why clinicians and analytical scientists must work closely on early study design. For instance, validation experiments are best performed on freshly processed samples; thus, tissue collection and banking procedures should be prepared accordingly.

In addition to sample and mass spectrometric workflow considerations are those pertaining to data analysis and interpretation. Metabolite mass spectra yield several thousands of signals referred to as "features," yet most of these features are nonbiological of origin or redundancies produced by molecular adducts (4). Moreover, small molecules are reactive, and their products are readily detected by the mass spectrometer. Once these artifacts are accounted for, only ~10% of collected features are left to be identified. However, identification itself is not absolute. A calculated feature mass can be derived from 1 of hundreds of candidate structural isomers; thus, a list of candidate molecules is provided in standard metabolite software. The inclusion of small molecule standards, fragmentation steps in the mass spectrometric analysis, and spectral libraries in the data analysis workflow can provide confident identification. However, the final number of presentable small molecules is whittled down to just 1% of the original dataset. Features with less certain identifications must therefore be validated, using targeted mass spectrometry and

compound references (4). These data interpretation caveats also reinforce that there remains a large portion of unidentified small molecules. In the meantime, we will likely continue to see the "usual suspects" in our metabolite studies. Pathway enrichment analyses can be elegantly presented in a variety graphics; however, the relevance of their findings will have to be scrutinized carefully. It is intriguing that bile acids were enriched in valvular tissue (1); therefore, how they accumulate in the valve and contribute to CAVD is particularly interesting.

The study presented by Surendran et al. (1) will undoubtedly inspire others from the cardiovascular field. The unbiased to target validation study design follows what would be expected from metabolite discovery studies. Yet, these very same unbiased datasets harbor several yet-to-be discovered molecules. Therefore, it will be only be a matter of time that annotation workflows improve enough to uncover the identities of these anonymous features, and potentially change the trajectory of CAVD research.

AUTHOR DISCLOSURES

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