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Letters

Prioritizing Proteomics Assay Development for Clinical Translation

The human genome consists of more than 20,000 genes; about one-half have been found in microarray experiments to be expressed in the heart. We asked which of the ~10,000 cardiac proteins are most studied in and are thus essential to cardiac research, for which, perhaps surprisingly, no objective measures yet existed. This question is relevant to clinical investigations, basic research, and biomarker discovery, because high-quality quantification assays are available for relatively few proteins. Selected reaction monitoring (SRM) is a targeted mass spectrometry technique that allows the quantification of virtually any user-specified proteins with high sensitivity (~20 amol) and specificity (1). SRM assays have important advantages over conventional immunoblots or enzyme-linked immunosorbent assays in their precision, throughput, and ability to target post-translational modifications, but adoption rates in basic/clinical research remain poor. It is thought that challenges for method developers to strategize development to prioritize important and popular proteins contribute to a bottleneck in technology dissemination (2).

To assess protein importance objectively, we performed a large-scale bibliometric analysis of the 24 million research papers curated on PubMed. We used the search terms "heart [MeSH term/All fields] or cardiac [All fields]" to retrieve cardiac-related articles, then tallied the occurrences of each protein being referenced to the retrieved papers on NCBI Gene using a new software tool (BD2KPubMed). In total, we retrieved ~1.4 million cardiac-related papers referenced to 8,325 distinct human proteins. **Table 1** lists the top 15 cardiac proteins with the most publications.

The data underline an intense focus of the majority of cardiac research on relatively few proteins. The 50 most-studied proteins accounted for 19% of all referenced cardiac publications, averaging 150 studies each. Publications declined precipitously after the 50th protein, with the next 50 proteins having one-third as many publications, whereas 84% of the investigated proteins had ≤5 publications. The most-studied human protein is natriuretic peptide B (BNP/NPPB), a routinely utilized clinical marker for evaluation and risk stratification of heart failure patients, which alone accounts for 1.3% of all publications referenced to cardiac proteins. Following BNP are angiotensin-converting enzyme and $Na_V1.5$ (sodium channel protein type 5), accounting for 1.0% and 0.9% of referenced publications, respectively.

Intriguingly, there exists a marked discrepancy between the most studied proteins in humans and in mice. Among the top 50 mouse and human proteins, only 17 are shared. BNP ranks 61st in mouse studies, which instead featured homeobox protein NKX2-5/tinman as the top protein. NKX2-5 is a master regulator of cardiac differentiation/development that accounts for 1% of publications in mice but ranks 29th in humans. We suggest that this discrepancy reflects different priorities between basic and clinical investigations. Many human proteins have clinical values prior to further reports on mechanisms of action. The top 20 human proteins alone contained 9 secreted proteins that are known disease biomarkers, versus 4 in mice. The popularity of BNP increased following its adoption as a clinical biomarker of heart failure circa 2003. In contrast, top mouse proteins include signal transducers in developmental pathways that have not been sufficiently translated to humans. Gene ontology analysis corroborates functional differences: the top 50 human proteins are significantly enriched in regulation of transport ($p < 4.7 \times 10^{-21}$) and contractility ($p < 8.3 \times 10^{-18}$), whereas the top 50 mouse proteins are enriched in heart morphogenesis ($p < 2.1 \times 10^{-35}$) and cardiac muscle development ($p < 4.2 \times 10^{-29}$).

The measurement of protein abundance is essential to biomedical research, where the availability of high-quality protein quantification tools can dictate the pace of discovery (3). There are currently few experimentally verified SRM assays, and most available assays await further validation in relevant cohorts. We present here a resource to comprehend research trends, and a list of high-priority proteins that merit expedited assay development and clinical translation. These most-studied proteins provide opportunities for developers to target community interests in cardiovascular research, but complementary metrics may also be employed (e.g., hub proteins in biological

TABLE 1 Top 15 Most-Studied Cardiac Proteins in Human/Mouse

Rank	No. of Publications	UniProt	Gene Name	Protein Name	Cardiovascular Relevance	MS Method	Expt. Assay
				Human			
1	532	P16860	NPPB	Natriuretic peptides B	Secreted heart failure marker	●	●
2	379	P12821	ACE	Angiotensin-converting enzyme	Vasoconstrictor activity	●	●
3	343	Q14524	SCN5A	Sodium channel protein type 5 subunit alpha	EC coupling	●	●
4	325	Q12809	KCNH2	Potassium voltage-gated channel H2	Repolarization, ventricular action potential	●	
5	287	P02741	CRP	C-reactive protein	Inflammation marker	●	●
6	283	P02649	APOE	Apolipoprotein E	Cholesterol and triglyceride regulation	●	●
7	254	P51787	KCNQ1	Potassium voltage-gated channel KQT1	Modulate action potential duration	●	
8	237	P29474	NOS3	Nitric oxide synthase, endothelial	Vasoprotection against atherosclerosis	●	●
9	218	P45379	TNNT2	Troponin T, cardiac muscle	Infarct marker in plasma; contractility	●	●
10	211	P19429	TNNI3	Troponin I, cardiac muscle	Infarct marker in plasma; contractility	●	●
11	209	P01375	TNF	Tumor necrosis factor	Inflammatory marker	●	●
12	202	P05231	IL6	Interleukin-6	Inflammatory marker	●	●
13	174	P42898	MTHFR	Methylenetetrahydrofolate eductase	Association with congenital heart defects	●	
14	168	P07550	ADRB2	Beta-2 adrenergic receptor	Catecholamine signaling	●	
15	166	P08588	ADRB1	Beta-1 adrenergic receptor	Catecholamine signaling	●	
				Mouse			
1	377	P42582	Nkx2-5	Homeobox protein Nkx-2.5	Tissue development and differentiation	●	
2	337	Q08481	Pecam1	Platelet endothelial cell adhesion mol	Angiogenesis	●	
3	253	Q08369	Gata4	Transcription factor GATA-4	Gene expression control	●	
4	253	P62737	Acta2	Actin, aortic smooth muscle	Cell structure	●	
5	252	P05125	Nppa	Natriuretic peptides A	Cardiovascular homeostasis	●	
6	249	P08226	Apoe	Apolipoprotein E	Cholesterol and triglyceride regulation	●	
7	244	P70313	Nos3	Nitric oxide synthase, endothelial	Vasoprotection against atherosclerosis	●	
8	224	P51667	Myl2	Myosin light chain 2, ventricular/cardiac	Regulation of myosin ATPase activity	●	
9	206	P23242	Gja1	Gap junction alpha-1 protein	Major protein of gap junctions in the heart	●	
10	176	P11531	Dmd	Dystrophin	Stabilization of actin filaments	●	
11	144	P61372	Isl1	Insulin gene enhancer protein ISL-1	Regulation of insulin signaling	●	
12	142	Q01231	Gja5	Gap junction alpha-5 protein	Gap junction component	●	
13	140	Q02566	Myh6	Myosin-6	Muscle contractility	●	
14	139	P04202	Tgfb1	Transforming growth factor beta-1	Cellular proliferation and differentiation	●	
15	139	P70326	Tbx5	T-box transcription factor TBX5	Cardiomyocyte differentiation	●	

Availability of SRM methods and assays are from SRMAtlas (5).

EC = excitation-contraction; Expt. = experimental; MS = mass spectrometry; UniProt = Universal Protein Resource.

networks). Developmental priority may further be influenced by the scale required in clinical applications (50 to 5,000+ samples), which often lies beyond the domain of basic research (10 to 100 samples). To accelerate translation, we suggest that more concerted basic clinical collaborations to optimize sample and data sharing pipelines are in order.

Users interested in identifying high-impact proteins in other topics can download BD2KPubMed and additional methodology information (4).

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Adenosine and Clinical Forms of Neurally-Mediated Syncope

Central or peripheral baroreceptor reflex abnormalities, alterations in neurohumoral mechanisms, or both, are thought to play a role in causing neurally-mediated syncope. Because adenosine and its receptors are involved in some forms of syncope (1–3), we evaluated the purinergic profile of 4 common forms of syncope: typical vasovagal syncope (VVS); situational syncope (which occurs in specific circumstances after micturition, defecation, coughing, swallowing, or gastrointestinal stimulation); carotid sinus syncope (CSS); and syncope without prodromes or with very short (2 to 3 s) prodromes and a normal heart (no prodromes). We compared patients with neurally-mediated syncope with healthy control subjects to test the hypothesis that the adenosine profile differs with the different clinical presentation.

The purinergic profile included an assay of the baseline adenosine plasma level (APL) and characterization of A_{2A} adenosine receptor (A_{2A} R) expression and single nucleotide c.1083 C>T polymorphism (SNP), which is the most common SNP in the A_{2A} R gene. The method was previously described (1–4). Clinical and biological characteristics of patients and control subjects are given in **Table 1**.

Thus, these findings demonstrate an association between adenosine plasmatic levels and unexplained syncope in patients without prodromes, CSS, and VVS, who have profiles different from normal control subjects. The clinical manifestation of adenosine depends on its concentration, on adenosine receptor expression level, and on the presence of receptor reserve. However, the causal role of this interplay in the mechanism of syncope is yet to be determined. Conversely, adenosine is not associated with situational syncope, which is mainly triggered by well identifiable afferent neural reflexes. Patients with situational syncope showed APL values similar to those in normal control subjects, although they had high A_{2A} R expression and a higher rate of the TT variant. The purinergic profile of situational syncope patients was never investigated.

Syncope without prodromes and CSS (which is a similar form of syncope without prodromes or very short prodromes and an absence of known triggers) have a similar distinct profile. In these 2 forms, the role of adenosine may potentially be important in causing syncope. When APL values are very low, as in these clinical forms, and are mainly below or approximately at the K_D value for A_{1A} adenosine receptor (A_1 R) of 0.7 μM, even a modest acute increase in APL may recruit a sufficient number of A_1 R, which is known to be located within the sinus node and in the atrioventricular node. Their activation causes sinus bradycardia and/or atrioventricular block.

For patients with typical VVS, a combination of neural outflow and purinergic activation is likely.