Is Plasma Proteomics Able to Provide Alternative Paths to Better Understand Hypertension?

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See related article, pp 412–419

The history of hypertension research is sweet sour. Huge advances have been marred by the persistence of huge black boxes. The term primary hypertension epitomizes the issue: the underlying causes and pathophysiology of most cases of hypertension remain undefined. Furthermore, guideline panels are unable to agree on desirable target blood pressure values, and it may be the time to think about the cure of hypertension rather than just the control of blood pressure. The quote attributed to Einstein, "Insanity: doing the same thing over and over again and expecting different results" may provide some clues. It is likely that the next large conceptual leaps in our understanding of hypertension are based on the application of a novel technology not previously used for this purpose. In this regard, scientific advances depend to a large extent on technological advances that make them possible.[1] Hypothesis-based approaches must rely on current knowledge, and we are aware that this is limited. Systems biology provides a nonbiased approach that recognizes the current limitations of our understanding of hypertension. Gajjala et al[2] have used plasma proteomics to identify in a nonbiased manner potential molecular determinants that allowed the development of a model capable to discriminate between hypertensive and normotensive individuals. There are 2 main potential clinical consequences of these studies: the use of the proteomics panel as a biomarker and the identification of putative contributors to the pathophysiology of hypertension. As a biomarker of hypertension, the panel cannot, by definition, outperform a cheap and easy to use, noninvasive approach: measurement of blood pressure. So further studies are needed to explore whether the proteomics model may predict outcomes, response to therapy, define risk categories, or even more important, predict the development of clinical hypertension. In addition, as the authors point out, the identified molecular determinants, corresponding to 15 proteins and 1 amino acid, may be the starting point for further studies to clarify the molecular pathophysiology of hypertension.[2] Interestingly, the concentrations of fragments corresponding to 11 proteins and tryptophan were decreased in hypertension, whereas those corresponding to 4 proteins (osteocalcin, PRUNE, RAB13, and sarcolipin) were increased. A bioinformatics analysis could not integrate in a single pathway all the identified features. This may represent the involvement of multiple different pathways, current limitations in understanding the potential interconnections between these features or the presence of red herrings. Moreover, despite the statistical association with hypertension, it is currently unknown whether these features may contribute to the pathophysiology of hypertension or are the consequence of hypertension, its complications, associated comorbidities, or therapy. The identified fragments correspond to proteins diverse in their primary location (intracellular or extracellular), as well as in their known functions (enzymes, ion channels, transcription factors, and others). The authors are correct in avoiding any speculation on the potential relationship or function of these peptides in the hypertension context. The possibilities are too wide at this point. A first step would be to validate the findings in a similar hypertensive population. Functional in vivo studies are then required to assess causality.

Analysis of the results from a functional perspective is complex (Figure). Lower levels of the features do not necessarily mean downregulation of their function. The interpretation could be the complete opposite. Decreased protein fragments may represent decreased degradation of the full protein, that is, increased protein (and possibly activity) levels. Intracellular proteins were represented both among the upregulated and the downregulated features. This in itself provides some insight: the identified features do not appear to solely represent cell lysis, in which case most of the features associated with intracellular proteins may have increased, nor decreased catabolism (eg, decreased kidney catabolism for low molecular weight proteins) in which case most of the features would have been expected to be increased. For intracellular molecules, any hypothesis should explain how they reach the circulation. Peptide fragments may also represent evidence for the activity (or lack of thereof) of protein-processing enzymes. It would be interesting to explore whether the identified peptides share any canonical cleavage site. Identifying the enzymes responsible for their processing may provide clues because they may represent under- or overactivity of enzymes regulating blood pressure.

Despite these caveats, several features are worth discussing because they have previously been linked to blood pressure or cardiovascular disease.

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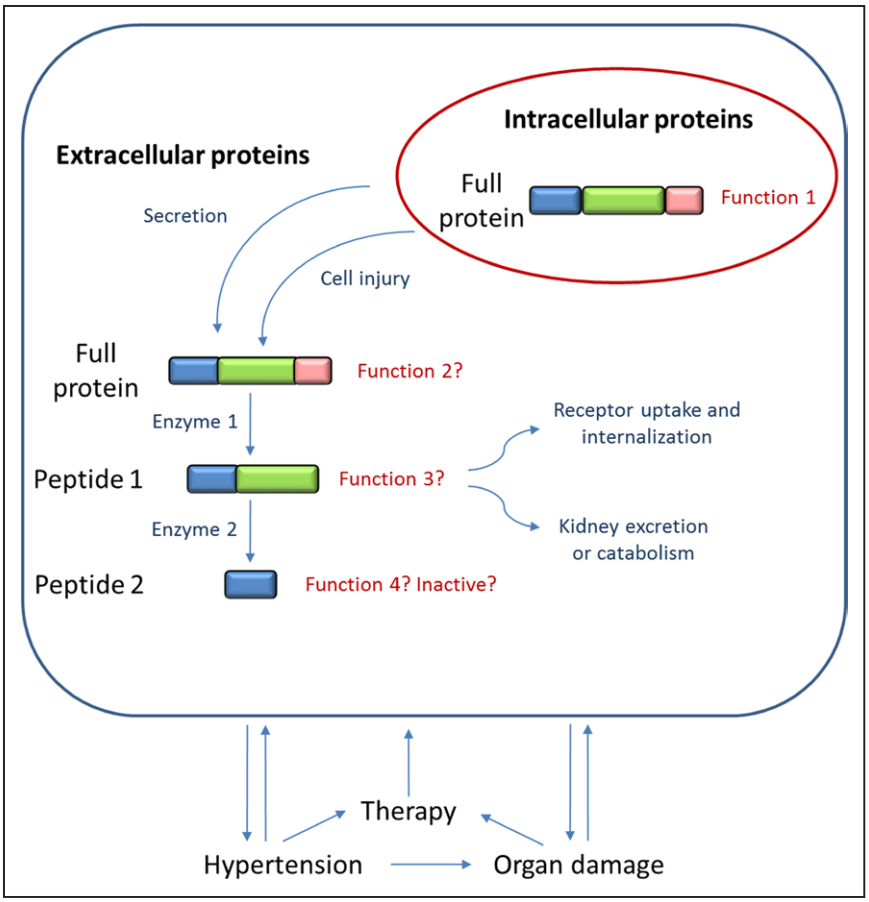

Figure. Plasma proteomics and human hypertension. The finding of a hypertension signature in the human plasma proteome may be used to explore new pathophysiological pathways. However, decreased or increased serum levels of a specific protein fragment may result from different potential mechanisms beyond increased or decreased synthesis of the original protein. For proteins known to be intracellular, changes in the mechanisms leading to the extracellular location of the peptide, differences in the activities of enzymes generating or degrading the peptides, or changes in clearance of the peptides may all contribute to the observed peptide levels in hypertensive patients. Furthermore, the identified peptides may share the known function of the full protein, may have a different totally unrelated unction, or may even behave as antagonists of common receptors or functions or be inactive fragments. Finally, the observed peptide pattern may be a cause or consequence of hypertension or, despite statistical support for an association with hypertension, it may be related to other hypertension-associated features, including therapy for hypertension.

Tryptophan is a precursor of several uremic toxins with adverse effects on the heart and vasculature and in uremia tryptophan processing to some of these toxins in enhanced (reviewed in [3]). In patients with chronic renal failure, lower tryptophan levels as observed by Gajjala et al[2] for hypertensive individuals were associated with lower systolic blood pressure.[4] Furthermore, studies in the 1990s observed that pharmacological doses of tryptophan reduced blood pressure in patients with mild to moderate essential hypertension.[5]

In addition, genetic studies have already associated some for these proteins to cardiovascular disease. Mutations in nexilin and phospholamban result in cardiomyopathy in humans and overexpression of sarcolipin in mice.[6] The findings may also point to disturbances in intracellular calcium regulation. Thus, sarcolipin and phospholamban are highly homologous intracellular proteins expressed mainly in the heart and skeletal muscle that share a role in the regulation of cardiac muscle sarcoplasmic reticulum Ca^{2+} ATPase. Sarcolipin fragments were upregulated and phospholamban ones downregulated in hypertensive individuals. Interestingly, phospholamban is also expressed in smooth muscle cells. Anoctamin 10 (downregulated) also regulates intracellular calcium, as well as cell volume and chloride fluxes.[7]

Phosphoinositide 3-kinase regulator 1 is a regulatory subunit of phosphoinositide 3-kinase, which binds to estrogen-activated estrogen receptors to increase phosphoinositide 3-kinase and endothelial nitric oxide synthase activity.[8] Humanin fragments were reduced. Humanin is a circulating peptide encoded in the mitochondrial genome by the 16S ribosomal RNA gene, which has cytoprotective and antioxidant functions in diverse settings, and lower levels have been associated with human endothelial dysfunction.[9] Both osteocalcin single-nucleotide polymorphism and circulating protein levels have been previously associated with hypertension. Mannose-6-phospho isomerase and RAB13 regulate intracellular protein trafficking. Mannose-6-phospho isomerase maintains the supply of D-mannose derivatives required for many glycosylation reactions, and mannose-6-phosphate is a signal required for trafficking of lysosomal proteins. Several mechanisms may link altered protein trafficking to hypertension.

How does information from plasma proteomics compare with that obtained from other systems biology approaches, such as genome-wide association studies? As discussed for proteomics, the functional impact of the findings of either approach may not be immediately apparent. In addition, genome-wide association studies provide a static image of the individual, based on the genetic background that it does not reflect environmental influences known to impact hypertension and cardiovascular risk, such as dietary habits and lifestyle differences. In this regard, proteomics provides a dynamic view of the ultimate consequences of gene expression, being able to differentiate diverse gene products encoded by single gene. Although genome-wide association studies are unresponsive to therapy, proteomics may be used to assess the response to therapy and the dynamic nature of the disease process.

Unlike genomics, proteomics focuses in the gene products already present in the animal or in the biological

material studied. The use of proteome analysis in unraveling the mechanisms leading to or associated with hypertension is far younger than that of genome. Great expectation is being placed on the foreseen contribution of proteomics in improving the understanding of hypertension and therefore its treatment and prevention.[10] The study by Gajjala et al[2] paves the way to new research which will show whether the findings obtained with this new approach are meaningful and may allow us to start thinking about curing hypertension.

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Disclosures

None.

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