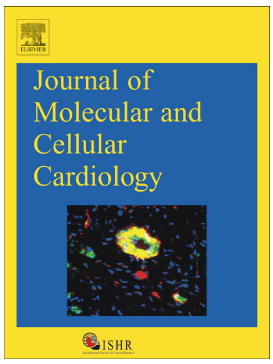

Developing potential biomarkers through bedside-to-bench translation

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Developing potential biomarkers through bedside-to-bench translation

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The vast majority of therapies for heart failure (HF) are based on randomised clinical trials of highly heterogenous groups of patients. Mortality and morbidity remain substantial even in patients who are optimally treated with such therapies. There is therefore significant interest in identifying biomarkers that might enable earlier or more refined diagnosis, staging, prognostication and personalisation of therapy. The only biomarker that is routinely employed in clinical practice is NT-proBNP but this is useful mainly in informing the likelihood of HF rather than guiding therapy.

Pre-clinical approaches to identify new biomarkers that have wider utility typically focus on specific molecular signalling pathways or biological processes known to be involved in HF (e.g. inflammation), followed by the testing of markers of such processes in patients. However, bench-to-bedside translation of this type has generally been unsuccessful, possibly because most pre-clinical models that are utilised are too far removed from the clinical HF syndrome [1, 2]. An alternative approach is to undertake global screens (e.g. with 'omics approaches) in patients with HF in the hope that markers that are strongly associated with a particular aspect of HF may lead to clinically useful biomarkers. This approach has also, so far, failed to identify useful biomarkers – possibly because the markers chosen for further study are not necessarily causatively linked to disease pathogenesis or pathophysiology. In a recent article published in the journal, Caillard et al employ a reverse translation 'bedside-to-bench' approach to address this gap [3]. These investigators previously used plasma proteomic studies to identify Quiescin Q6 sulfhydryl oxidase 1 (Qsox1) as a potential diagnostic biomarker of acute decompensated HF when used in conjunction with BNP [4]. In the current study, they have now studied the potential biological link(s) between Qsox1 and HF in a pre-clinical model, an approach that may strengthen the relevance of the marker and suggest how it might best be used, before returning to additional clinical studies (**Fig 1**).

An important feature of the heart`s response to stress is alterations of redox signalling [5]. In HF, redox homeostasis is perturbed due both to increased ROS generation by several sources (e.g. mitochondria, NADPH oxidases, monoamine oxidase) and altered antioxidant enzymes and molecules (e.g. thioredoxin [Trx], peroxiredoxin [Prx]) [5]. For example, circulating Trx1 is increased in patients with HF and correlates with disease severity [6, 7]. Qsox1 is a redox enzyme that contains a Trx domain (Trp-Cys-Gly-His-Cys) and has FAD-dependent sulfhydryl oxidase activity that is thought to be involved in protein folding in the endoplasmic reticulum (ER). Qsox1 may coordinate in this function with other thiol oxido-reductase isomerases such as protein disulfide isomerase, ER oxidase-1 and Prx [8, 9]. Caillard and colleagues [3] developed and characterised a global Qsox1 knockout mouse model in an attempt to determine the role of Qsox1 in HF. They found that $Qsox1^{-/-}$ mice have cardiac dilatation at baseline, with very mild systolic impairment, a reduced SERCA2a content and altered calcium homeostasis. After induction of acute stress with isoproterenol, $Qsox1^{-/-}$ mice developed worse contractile impairment than wild-type controls and this persisted for longer – suggesting that Qsox1 is involved in the acute stress response.

Protein folding in the ER may be compromised under stress conditions ("ER stress") and triggers a compensatory response known as the unfolded protein response (UPR) which increases the expression of ER chaperones and other proteins that restore normal protein homeostasis. If there is a failure to restore normal protein folding, cells will tend to develop oxidative stress and may enter a downward spiral that leads to cell death [9-11]. Caillard *et al.* [3] found that $Qsox1^{-/-}$ mice had evidence of UPR activation at baseline and that after isoproterenol stress, this activation was enhanced and persistent and was accompanied by oxidative stress and evidence of inflammation.

These data would be consistent with the notion that the absence of Qsox1 impairs protein folding especially during acute stress, and that this in turn leads to prolonged detrimental activation of the UPR, thereby contributing to cardiac impairment. One interesting question in this study is the mechanistic inter-relationship between altered sarcoplasmic reticulum calcium levels and ER stress. It is known that deranged intracellular calcium handling provokes ER stress and activates the UPR but also that ER stress impairs calcium homeostasis [12]. Therefore, the primary defect in Qsox1$^{-/-}$ mice could either be in calcium handling due to the decreased Serca2a levels (with secondary ER stress and UPR activation) or could be a direct general effect on protein folding leading to ER stress (with secondary effects on calcium homeostasis). Further work is necessary to dissect this question. The data in the study by Caillard et al [3] would be explained by the actions of Qsox1 in cardiomyocytes but it is conceivable that other cell types might also be involved. Qsox is expressed during development and, in addition to its cellular location, it is present in foetal serum and the extracellular space, where it plays a role in extracellular matrix formation [8].

This study convincingly suggests an adaptive role of increased Qsox1 in the setting of acute adrenergic crisis and explains why Qsox1 may have emerged as a biomarker in acute HF. Whether circulating Qsox1 in the blood has any pathophysiological role is an intriguing question. It would also be of interest to investigate the possible contribution of Qsox1 to ventricular remodelling.

The study by Caillard and colleagues adds to the growing body of evidence for a role of redox mechanisms in HF and highlights Qsox1 as a potential novel target. It also provides a demonstration of the utility of bedside-to-bench studies to illuminate clinically relevant mechanisms, potentially leading both to novel biomarkers and innovative therapies for HF.

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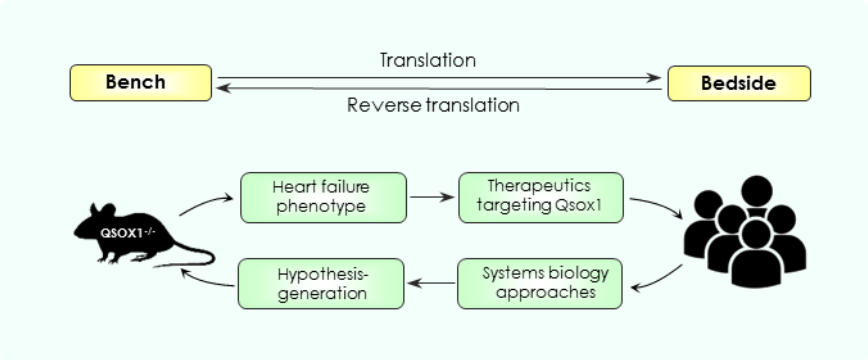
Figure 1



A schematic of forward and reverse translation, using Qsox1 as an example.

Systems biology approaches, including proteomics, can generate hypotheses for investigation in pre-clinical models. This knowledge can then be used to develop new approaches to prognostication, diagnosis and treatment in patients. Reverse translation has the advantage of focusing pre-clinical investigation on mechanisms that may be more likely to have clinical relevance.