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# How could analyzing the activity of two matrix metalloproteinases unveil the cause of sudden cardiac death

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## Abstract

Sudden cardiac death is natural, unexpected death, related to cardiovascular disease. Its postmortem elucidation is significant, as the family of the deceased aspires to prevent other sudden deaths. Irrespective of the proper etiological entity, the myocardial collagen matrix remodels, associated with the progression of cardiovascular diseases. It has become evident that many mediators such as humoral factors, transforming growth factor (TGF)- $\beta$ 1 among them, are involved in the remodeling process. Cardiac remodeling is the balance of regenerative and eliminatory processes that include enzymes involved in the degradation of extracellular matrix (ECM) components. Enzymes capable of degrading native fibrillar collagen are interstitial collagenases, specifically matrix metalloproteinases (MMP)-1 and MMP-8. Here, we suggest a technique of visualizing turnover of collagen in cardiac tissue.

## Keywords

activity of collagenases, collagen, matrix metalloproteinases, sudden cardiac death

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Defining sudden cardiac death (SCD) is controversial without cardiac monitoring at the time of death.<sup>1</sup> Cardiovascular disease is the most common cause of death worldwide, and SCD is the leading cause of death in men aged 20–65 years.<sup>2</sup> Epidemiology of SCD suggests that we should not conclude the list of antecedent conditions,<sup>3</sup> i.e. potential collagen disorders that can result in SCD. Victims of SCD are often predisposed to detectable conditions. The cause of almost half of all dilated cardiomyopathies' fatal outcomes is sudden death.

The idiom “sudden cardiac death” is a public health conception. It does not explicitly refer to the cause or mechanism of death.

Cardiomyocytes occupy two-thirds of the total cardiac tissue volume. Fibroblasts and other non-myocytes together with the extracellular matrix (ECM) and tissue fluid fill the remaining one-third.<sup>4</sup> Mentioned ECM reflects hemodynamic conditions by continuous remodeling.<sup>5</sup> Maladaptive myocardial remodeling presents a structural basis for the development of cardiac disease.<sup>6,7</sup> Current

perspectives of ECM tells it is more than just an inert collection of macromolecules by standing as a frame for cells. ECM controls many cellular functions and serves as a substrate for adhesion of cells, the source of anti-apoptotic signals, a pool of growth factors, and a determinant of tissue mechanics.

Major ECM heart proteins are collagens,<sup>8</sup> precisely, isoforms I and III or fibrillar collagens. Those comprise approximately 85% of cardiac ECM and their production may be yet increased, e.g. by transforming growth factor-beta (TGF- $\beta$ ).<sup>6,7</sup>

Excessive collagen deposition leads to cardiac fibrosis. Since fibrosis has also been suggested to be arrhythmogenic, it is consequently associated

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with sudden death.<sup>8</sup> However, a close connection of fibrosis to SCD remains to be demonstrated. It could provide the underlying structural substrate, as in the findings of Polyakova et al.,<sup>9</sup> though in heart failure.

According to them, all values for collagens and markers of their metabolism, as well as matrix metalloproteinases (MMP) and tissue inhibitors of metalloproteinases (TIMPs), are low in control samples, and all are elevated in failing hearts. This refers equally to newly synthesized and mature collagens.<sup>9</sup> Moreover, they recognize the failing myocardium by increased numbers of fibroblasts and TGF- $\beta$ 1-positive cells, considering TGF- $\beta$ 1 a profibrotic agent.<sup>6</sup>

The accumulation of ECM is counterbalanced by a proportional increase in the degradation of the matrix through the action of so-called interstitial collagenases such as zinc-dependent endopeptidases or MMPs. Those are capable of degrading all kinds of ECM proteins, but can likewise process some bioactive molecules.<sup>7</sup> Activity of these zinc-dependent endopeptidases is tightly regulated by the amount of active protein and the concentration of explicit inhibitory molecules, TIMPs, essential in the regulation of connective tissue metabolism.

Evidence of MMP's role in the pathogenesis of circulatory disease increases rapidly. Specifically MMP-1 and MMP-8, "interstitial collagenases" are capable of degrading native fibrillar collagen, which is repeated by Aimes and Quigley, in spite of their finding that MMP-2 is capable of solubilizing interstitial collagen.<sup>10</sup> New evidence, however, points to another tier in the control of the MMP and suggests their wider regulatory role than merely inducing proteolysis of several ECM components. Indeed, MMP's activity is in full compliance with tissue remodeling, but they regulate cytokine activity as well and may initiate (and participate in) various cellular processes.

Considering all of the above, we propose a measurement of collagenase activity in fresh tissue samples obtained through autopsy.<sup>11</sup> In particular, the activity of MMP-1 and MMP-8 by collagen zymography.<sup>12</sup> Serial sections should be taken from areas of infarcts if any. Otherwise, tissue should be taken from SA and AV nodes as well as base and middle of the septum.<sup>13,14</sup>

Specimens should be prepared with extreme care as the result interpretation is sensitive to the

methodology. We propose collecting samples followed by inactivating non-metalloproteases. For preparation and loading of protein samples onto a gel for SDS-PAGE analysis, a protein sample should be diluted into sample loading buffer. Samples should be electrophoresed on polyacrylamide gels containing gelatin and casein. After electrophoresis, collagenase activity can be easily visualized, first by staining the gel with 0.25% Coomassie brilliant blue R250. Subsequently, zones of lysis could be visualized as clear bands against the blue background. The zymograms can then be digitized using a constant light intensity. Quantitative image analysis can then determine MMP activity.

Use of SDS-PAGE gelatinase zymography for MMP-1 and MMP-8 detection is not an optimal method because gelatin is not a favored substrate for these enzymes.<sup>15</sup> Therefore, this technique should go through some modifications for improved detection. This should be done by slotting a more suitable substrate in the gel.<sup>16,17</sup> Conventional methods using collagen as the substrate can only detect latent gelatinases.<sup>18</sup>

Considering that collagen zymography alone has a limited capacity to identify directly the causative mechanisms behind sudden death, recent unveiling of etiology behind Sorsby's fundus dystrophy suggests that more common genetic variation in MMP genes may establish a genetic propensity for the pathogenesis of various disorders.<sup>19</sup>

Using its forthrightness, gel zymography can, with some refinement, be used for quantitation of each MMP.<sup>7</sup>

With the controversial validity of serological markers in cardiac collagen turnover<sup>7,20</sup> and the reports of the reversibility of fibrosis in the liver,<sup>21</sup> our belief is that change in ECM components via visualization of MMP's activity might resolve the enigma surrounding SCD.

Reporting the results of any prospective validation experiments could, in principle, help improve approaches and contribute to creating appropriate datasets for future clinical management as well as prevention.

#### **Declaration of conflicting interests**

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