Mineralization of soft connective tissues, also known as ectopic calcification, is a pathologic process that may occur in different contexts. During aging, for instance, it could be the consequence of atherosclerotic lesions or it may be associated to osteoporosis that, through the calcification paradox, lead to increased levels of circulating ions. Hydroxyapatite can therefore precipitate within tissues mainly affecting the vascular system. Similarly, ipercalcemia- and/or iperphosphatemia-mediated calcification is present in hormonal diseases and in patients suffering from chronic kidney disorders. Furthermore, there are a group of genetic diseases in which, as a consequence of defects in different genes, some related to bone metabolism (MGP, ENPP1, GCGX) some apparently unrelated to the calcification process (ABCC6, HB), ectopic calcification takes place. Even though many key regulators have been found to be abnormally expressed in mineralized areas within soft connective tissues, pathogenic mechanisms are elusive and it is still puzzling whether calcification affects peculiar matrix components in specific organs/tissues, whereas other areas remain unaffected.

Nowadays many informative data can be obtained by imaging mass spectrometry (IMS) enabling the combined identification and localization of molecules directly on tissue section in a single experiment. As an experimental model to test the potential of this approach, we have used the skin from a patient affected by Pseudoxanthoma elasticum (PXE), a rare genetic disorder mainly affecting skin, eyes and the cardiovascular system due to progressive calcification. Interestingly, calcification does not involve the whole tissue as in the skin, where mineral deposits specifically accumulate in the middle reticular dermis. IMS was performed on mineralised and non-mineralised areas of the same PXE biopsy, in order to reveal differences in protein distribution and content, and data have been also compared to those from healthy skin. Analysis of the ion density maps demonstrates that mineralized and non-mineralized areas within the PXE dermis are characterized by a distinct protein profile. Interestingly, among the proteins which are differently localised compared to the normal dermis, it has to be highlighted, for instance, thymosin-beta4 (TB4), being surprisingly absent from mineralized areas. This is a pleiotropic molecule exhibiting, within connective tissues, protective and regenerative properties. It could be suggested that tissues devoid of TB4 are more susceptible to damaging noxae, favouring mineral precipitates. Since, TB4 has been never associated to PXE, present findings could open new pathogenic pathways that should be further investigated.

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Fig. 1: Von Kossa staining, used to reveal the presence of calcification, confirms that mineralised areas (MA) are present only in Pseudoxanthoma elasticum (PXE) (b, arrow). Sections stained with haematoxylin eosin (c and d) underwent IMS analysis to identify proteins differently expressed and localized in MA, as in the case of thymosin-beta4 (e and f).