Alkaptonuria: a far much complex disease than thought so far

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BACKGROUND and AIM

Alkaptonuria (AKU) is an ultra-rare inborn error of metabolism due to a deficient activity of homogentisate 1,2-dioxygenase leading to accumulation of homogentisic acid (HGA). The disease shows with ochronosis (melanin-like pigment deposition) involving the musculoskeletal, respiratory, airway, cardiovascular, genitourinary, cutaneous, and ocular systems. The processes that govern toxicity and deposition of ochronotic pigment at specific sites are not well understood, although possible factors include:

i. co-presence of amyloidosis and angiogenesis [1,2]
ii. oxidative and inflammatory effects of HGA [3]

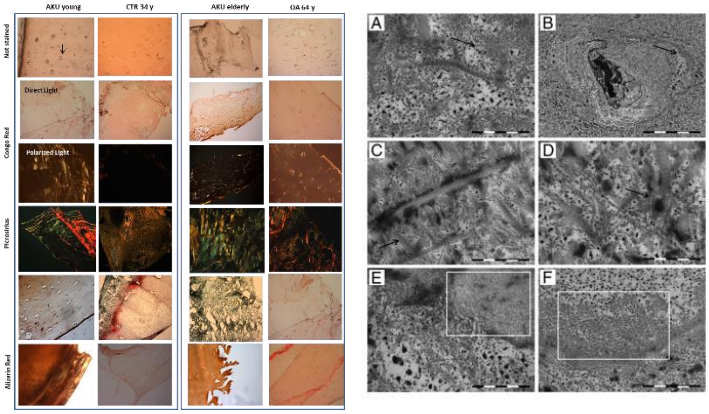
on tissues and cells .

Thanks to a range of *in vitro* and *ex vivo* models, we investigated possible pathogenic mechanisms of AKU in different decades of life. Overall, we provide the first detailed overview of AKU, emphasizing phenotype heterogeneity and complexity.

METHODS

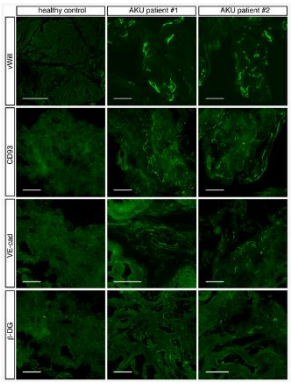
A complete microscopic and ultrastructural analysis for angiogenesis and amyloid in AKU cartilage and synovia was performed by Congo Red, Immunofluorescence, and TEM Microscopy. The expression of LC3-II protein (autophagy marker) was investigated by Immunofluorescence and Western Blot in AKU primary chondrocytes. TEM analysis was also used to detect morphological changes related to chondroptosis and autophagy in AKU chondrocytes.

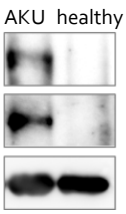
Congo Red staining and TEM - amyloidosis



Congo Red staining of articular cartilage from young and elderly AKU patients showed massive presence of amyloid deposits in correspondence to pigmented areas (left panel) [4]. TEM microscopy (right panel) confirmed the presence of amyloid (arrows) and the fibrillar nature of the deposits (white squares). Scale bars: A: 200 nm; B: 5 µm; C: 500 nm; D: 200 nm; E: 200 nm; F: 500 nm [5].

Western blot & immunofluorescence - angiogenesis

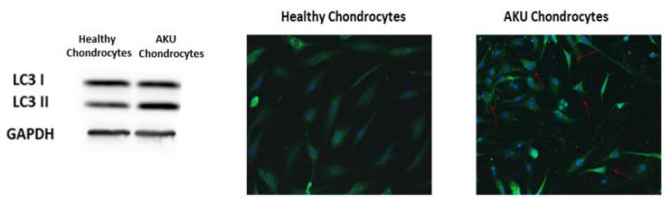




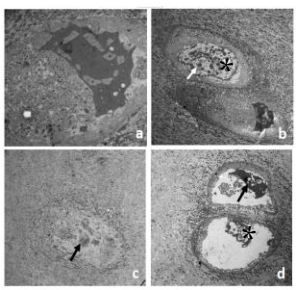
Confocal immunofluorescence showed an increased presence of angiogenesis markers [Von Willebrand (VW) factor, VE cadherin, CD93 and BDG] in AKU synovium compared to control (left panel) [2].

Western blot revealed VE cadherin and CD31 expression in all the tested AKU synovial specimens, which were lacking in control samples (right panel) [2].

Western blot, immunofluorescence and TEM - autophagy



Western blot (left) and immunofluorescence (right) showed increased expression of LC3 (autophagy marker) in AKU chondrocytes [Galderisi et al, manuscript in preparation].



TEM microscopy showed chondrocytes with secretory phenotype arising from the deep zone of pigmented AKU cartilage, fragmented nuclear condensation with convoluted nuclei (parts b and d, asterisks), and abundant autophagic vacuoles (parts c and d, arrows) [6].

CONCLUSIONS

We provided evidence on the presence of secondary amyloidosis even in young asymptomatic AKU patients, as well as on the presence of synovial angiogenesis, suggesting that synovial inflammation is directly implicated in the initiation and progression of AKU. We also showed increased autophagy in AKU; this may be a compensatory response to prolonged HGA-induced cellular stress and lead to chondroptosis (cell death). Overall, our findings depict a novel biological framework in which AKU may be viewed either as a systemic disease or as a metastatic disorder leading to multi-organ failure, representing a fierce challenge requiring a multi organ approach to patient management.

References

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