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Characterization of the protein components of matrix stones: rare and enigmatic soft renal calculi

V. Marzano[1], L. Huang[1], C. Martelli[1], F. Iavarone[1], F. Vincenzoni[1], C. Desiderio[2], I. Messana[3], N. Buchholz[4], F. Zattoni[5], P. Beltrami[5], P.M. Ferraro[6], M. Castagnola[1], G. Gambaro[6]

[1] *Institute of Biochemistry and Clinical Biochemistry, Catholic University, Rome, Italy*
[2] *Institute of Chemistry of Molecular Recognition, National Research Council CNR, Rome, Italy*
[3] *Department of Life and Environmental Science, Cagliari University, Cagliari, Italy*
[4] *U-Merge, London, United Kingdom*
[5] *Division of Urology, University Hospital, University of Padova, Padova, Italy*
[6] *Division of Nephrology, Department of Internal Medicine and Medical Specialties, Catholic University, Rome, Italy*

Objective: Among the different types of kidney stones, matrix stone is an uncommon soft form of urinary calculi composed of a mucus-like, pliable, amorphous and radiolucent substance with very little crystalline component. In order to identify the protein components involved in their formation, we analyzed the proteomic profiles of five surgically removed matrix stones by means of two complementary proteomic platforms: top-down and bottom-up.
Materials-Methods: For the top-down strategy, the proteins were extracted with an acidic water/acetonitrile solution, separated and analyzed as naturally occurring proteins and peptides by a C8 HPLC column coupled to an ESI-LTQ-Orbitrap-tandem mass spectrometry (MS/MS) instrument. For the bottom-up approach, samples were treated with a strong denaturing buffer followed by homogenization and sonication. After mono-dimensional SDS-PAGE separation and trypsin digestion samples were analyzed by C18 liquid chromatography and ESI-LTQ-Orbitrap MS/MS.
Results: We identified a total of 142 non-redundant proteins and peptides across all samples. Among these proteins, Neutrophil defensin 1, 2, 3 and 4 together with Protein S100-A8 (Calgranulin A) and S100-A9 (Calgranulin B) were the major components of these particular renal calculi. Among other less represented proteins Thymosin β4, Ubiquitin, and Granulin 4 were also characterized.
Conclusion: The use of two different proteomic approaches allowed the identification of 9 proteins common to all investigated stones. Interestingly, they all belong to inflammatory cascades and wound repair mechanisms which suggests that an inflammatory process is the initial event of soft calculi formation and not the consequence. While in calcium stones the matrix contains some of the inflammatory proteins observed in matrix stones, none of the counter-regulatory, anti-inflammatory proteins Thymosin β4, Ubiquitin, and Granulin 4 have been observed which implies that there is no involvement of leukocytes and ongoing major inflammatory processes in the pathogenesis of Calcium renal stones.