PHOSPHOPROTEOME ANALYSIS OF PERIPHERAL BLOOD MONONUCLEAR CELLS EVIDENCES AN ABNORMAL CALGRANULIN SIGNALING IN ANTIBODY-MEDIATED CHRONIC REJECTION

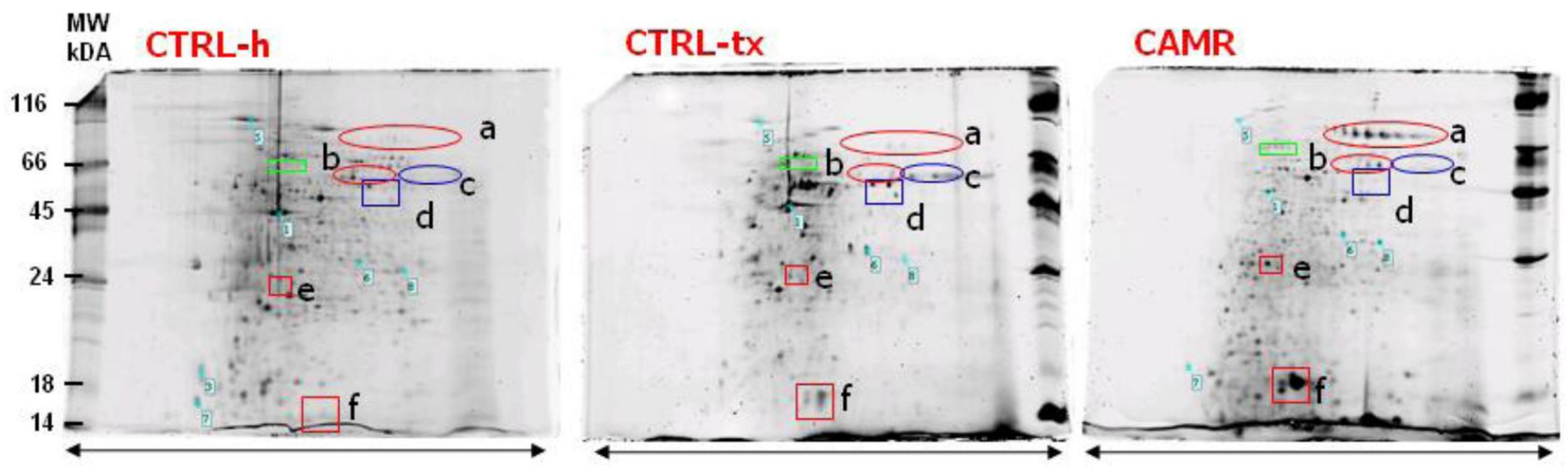
Maria Teresa Rocchetti[1], Paola Pontrelli[1], Federica Rascio[2], Marco Fiorentino[1], Anna Zito[1], Giovanni Stallone[2], Loreto Gesualdo[1], Giuseppe Grandaliano[2]

[1]Nephrology, Dialysis and Transplantation unit, Dept. of Emergency and Organ Transplantation (DETO), University of Bari
[2]Nephrology, Dialysis and Transplantation Unit, Dept. of Medical and Surgical Sciences, University of Foggia, Italy.

INTRODUCTION AND AIMS: Antibody-mediated chronic rejection (AMCR) represents one of the main causes of kidney transplant failure [1]**.** The molecular mechanisms underlying this event are still poorly defined and this lack of knowledge deeply influences the potential therapeutic strategies. [2]. In addition, specific markers for an early diagnosis of AMCR are currently missing. Proteomics can contribute to better define chronic pathological changes specifically attributable to alloimmunity and transplantation because it allow for the identification of molecular signatures (list of proteins) that confer significantly more information than the measurement of a single parameter.
In the attempt to identify potential diagnostic markers and to elucidate the signaling pathways involved in AMCR pathogenesis, we analyzed the peripheral blood mononuclear cells (PBMCs) phosphoproteome profile of biopsy proved CAMR patients to identify cellular signaling networks differentially activated in AMCR patients.

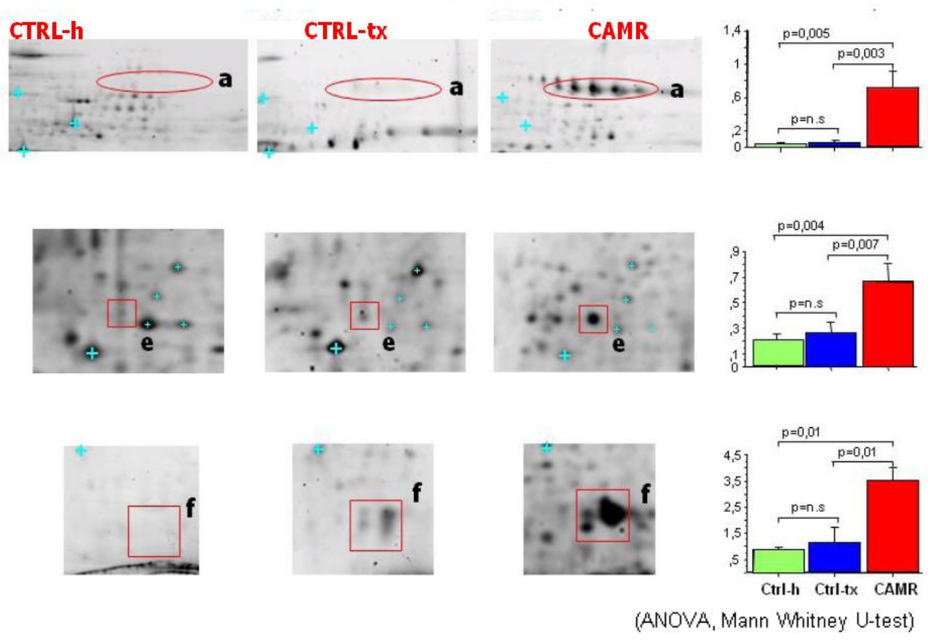
METHODS: PBMCs were harvested from 6 biopsy-proven AMCR according to Banff 2009 consensus, 7 renal transplant recipients with normal graft function and histology (tx-CTRL), and 6 healthy subjects (CTRL). Phosphoproteins were isolated by precipitation with lanthanum ions, separated by 2-D gel electrophoresis and stained by Sypro Red. Image Master Software was used to list the differentially expressed protein spots among the 3 groups. Phosphoproteins were identified by MALDI-TOF-MS/MS analysis. Western blot (WB) analysis was used to confirm proteomic data.

RESULTS: 2-D gel image analysis detected 554±68 (mean±SD) protein spots (CV=26%) in CTRL, 418±119 protein spots (CV=35%) in AMCR and 475±75 protein spots (CV=20%) in tx-CTRL. A protein signature of 10 protein spots, corresponding to 4 proteins (**a**, **b**, **e**, **f**), discriminated AMCR patients from CTRL and tx-CTRL, and 5 protein spots, corresponding to 2 proteins, distinguished tx-CTRL from AMCR and CTRL. One of the protein (**f**) discriminating AMCR patients from CTRL and tx-CTRL was identified as Calgranulin, and its increase in AMCR was confirmed by WB analysis.



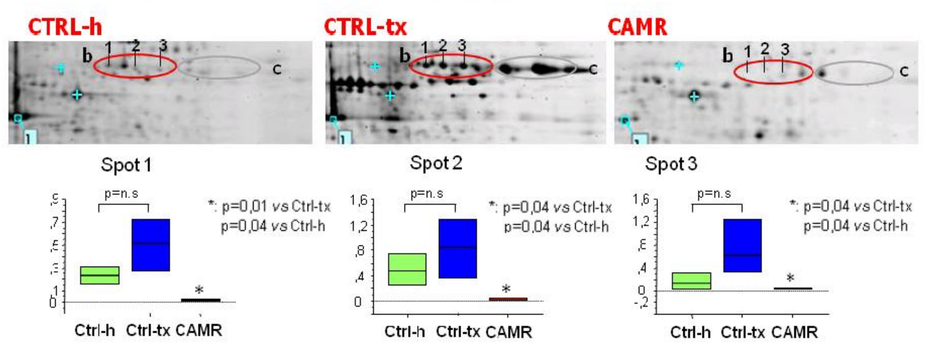
Phosphoproteome signature of CAMR

•3 proteins were up regulated (a, e, f)

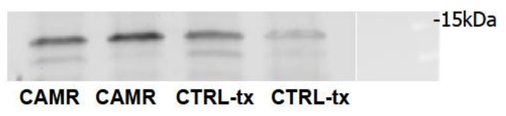


(ANOVA, Mann Whitney U-test)

•1 protein was down regulated (b)



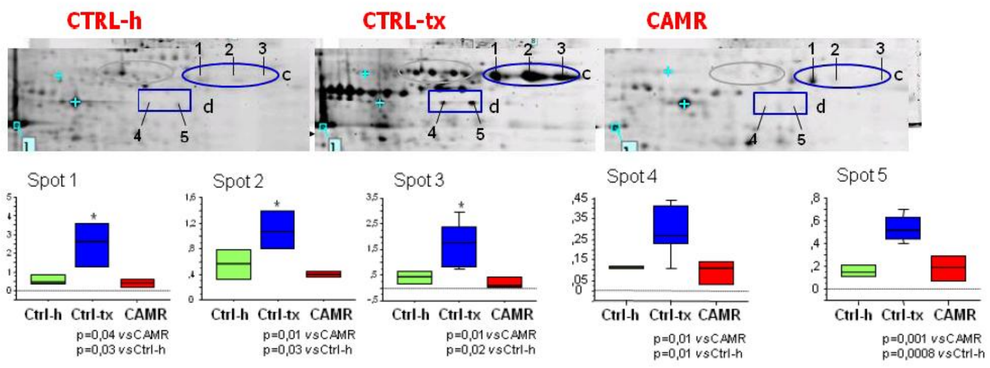
Protein spots **f** were identified by MALDI-TOF-MS/MS as Calgranulin



WB: 4-15% Polyacrylamide gel; 20 ug of total phosphoproteins were loaded on each lane, and blotted with anti-calgranulin monoclonal ab (Abcam)

Phosphoproteome signature of CTRL-tx

•5 proteins spots were up regulated (c,d)



CONCLUSIONS

•PBMC phosphoproteome might help to distinguish biopsy-proven AMCR patients from healthy subjects and stable renal transplant recipients

•Exists an abnormal calgranulin signaling in PBMCs of CAMR, a protein with a prominent role in the regulation of inflammatory processes and immune response.

REFERENCES

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2. Porcheray F, *et al.* Transplantation 89(10):1239-46, 2010.

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N) Renal Transplantation. N1) Transplantation basic science and immune-tolerance of allogenic and xenogenic transplants.
Maria Teresa Rocchetti
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