THE PROTEASOME TO IMMUNOPROTEASOME SWITCH IN IGA NEPHROPATHY AND ITS GENETIC CONTROL: A POST-VALIGA EUROPEAN RESEARCH STUDY.

Licia Peruzzi[1], Elisa Loiacono[2], Monica Bodria[3], Alessandro Amore[1], Luca Vergano[1], Sigrid Lundberg[4], Agnieszka Perkowska-Ptasińska[5], Dimitris Goumenos[6], Kresimir Galesic[7], Aik Papagiannis[8], Maria Stangou[8], Ali Gharavi[9], Krzysztof Kiryluk[9], Malgorzata Mizerska-Wasiak[10], Maria Roszkowska-Blaim[10], Dita Maixerova[11], Vladimir Tesar[11], Loreto Gesualdo[12], Lucio Eustacchio Montemurno[12], Rosanna Coppo[1] on behalf of the Immunonephrology Working Group of the ERA-EDTA

[1]Regina Margherita University Hospital, Nephrology, Dialysis and Transplantation, Turin, ITALY, [2]Fondazione Giovanni Goria, Fondazione Giovanno Goria, Turin, ITALY, [3]Columbia University, Medicine, New York, NY, [4]Karolinska University Hospital, Nephrology, Stockholm, SWEDEN, [5]Warsaw Medical University, Transplantation Medicine and Nephrology, Warsaw, POLAND, [6]University Hospital of Patras, Nephrology, Patras, GREECE, [7]University Hospital Dubrava, Nephrology, Zagreb, CROATIA, [8]Hippokration Hospital, Nephrology, Thessaloniki, GREECE, [9]Columbia University, Nephrology, New York, NY, [10]Warsaw Medical University,[11]Department of Nephrology, 1st Faculty of Medicine, General University Hospital, Charles University, Prague, Czech Republic , [12] Department of Nephrology, Dialysis and Transplantation Unit 'Aldo Moro' University of Bari, Bari, Italy

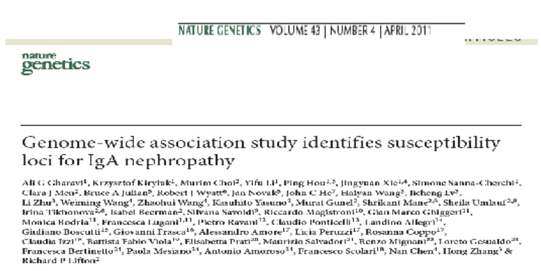
OBJECTIVES

Under the influence of α - and γ-interferons, the proteasome (PS) β type catalytic subunits PSMB5, PSMB6 and PSMB7 are substituted with PSMB8, PSMB9 and PSMB10 leading to the formation of immunoproteasomes (IPS).

This switch favours optimal MHC 1 peptide presentation and T-cell response.

Mutations and single nucleotide polymorphisms (SNPs) in the IPS catalytic subunit PSMB8 are associated with several inflammatory and autoimmune diseases.

The locus encoding PSMB8 and PSMB9 genes has previously been associated with increased risk of IgA nephropathy (IgAN) in genome-wide association studies (GWAS).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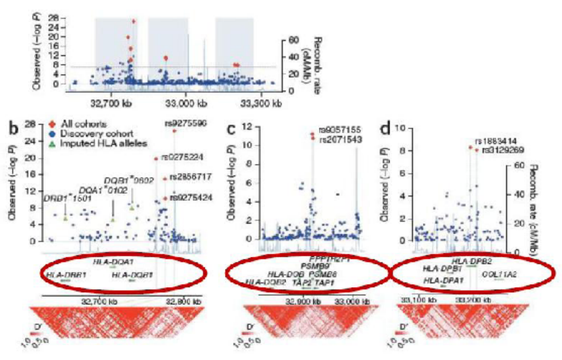
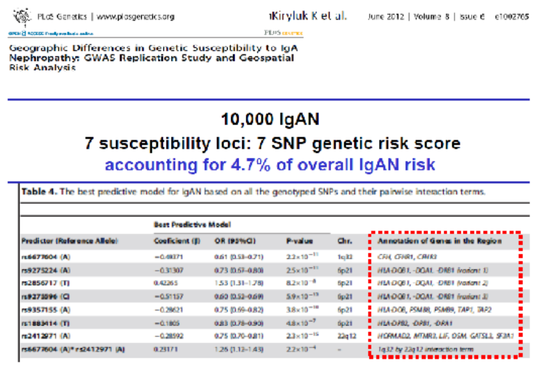
Genome-wide association study identifies susceptibility loci for IgA nephropathy

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Geographic Differences in Genetic Susceptibility to IgA Nephropathy: GWAS Replication Study and Geospatial Risk Analysis

10,000 IgAN

7 susceptibility loci: 7 SNP genetic risk score

accounting for 4.7% of overall IgAN risk

Table 4. The best predictive model for IgAN based on all the genotyped SNPs and their pairwise interaction terms.

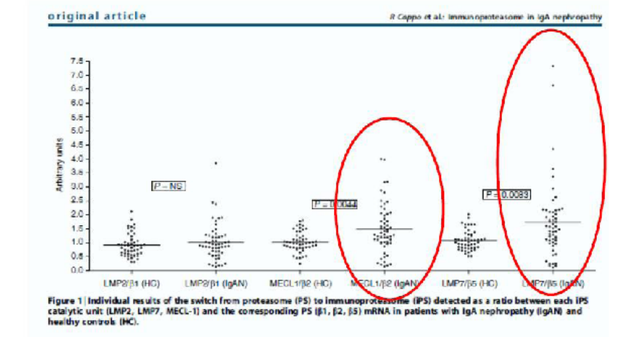
Best Predictive Model					
Predictor (Reference Allele)	Coefficient (β)	OR (95%CI)	P-value	Chr.	Annotation of Genes in the Region
rs6677604 (A)	−0.68371	0.61 (0.53–0.71)	2.3×10^{-11}	1q32	CR1, CFHR1, CFHR3
rs9275224 (A)	−0.31307	0.73 (0.67–0.80)	2.5×10^{-11}	6p21	HLA-DQB1, -DQA1, -DRB1 (region 1)
rs2856717 (T)	0.42265	1.53 (1.31–1.78)	8.2×10^{-8}	6p21	HLA-DQB1, -DQA1, -DRB1 (region 2)
rs9272896 (C)	−0.51137	0.60 (0.52–0.69)	5.9×10^{-19}	6p21	HLA-DQB1, -DQA1, -DRB1 (region 3)
rs9357155 (A)	−0.29621	0.75 (0.69–0.82)	3.8×10^{-10}	6p21	HLA-DOB, PSMB8, PSMB9, TAP1, TAP2
rs1883414 (T)	−0.1805	0.83 (0.78–0.90)	4.8×10^{-7}	6p21	HLA-DPB1, -DPA1
rs2412971 (A)	−0.28592	0.75 (0.70–0.81)	2.3×10^{-10}	22q12	HORMAD2, MTMR3,LIF, OSM, GATSL3, SF3A1
rs6677604 (A)* rs2412971 (A)	0.25171	1.29 (1.13–1.45)	2.2×10^{-4}	-	1q32 by 22q12 interaction term

The aim of this study is to validate our observations of increased switch from PS to IPS in PBMC in a large cohort of European patients with IgAN (VALIGA: validation study of Oxford classification of IgAN) and to test if the top SNP at the PSMB8/PSMB9 locus (rs9357155) contributes to the genetic control of this process.

METHODS

We analyzed mRNA expression of the constitutive (PSMB 5, -6, -7) and the corresponding IPS subunits (PSMB 8, -9, -10) by RT-PCR in PBMCs from 150 VALIGA patients with IgAN, of which 77 patients had DNA available for rs9357155 genotyping.

We have also previously reported up-regulation of PSMB8 mRNA expression in peripheral mononuclear cells (PBMC) of patients with IgA nephropathy (IgAN)

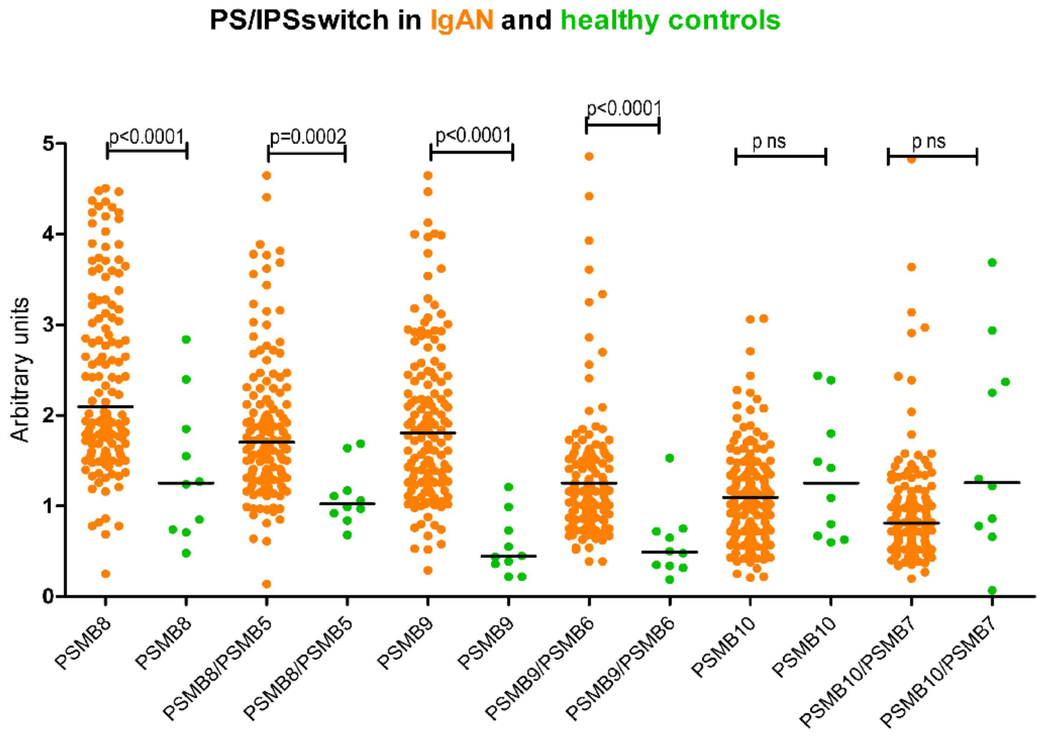

Upregulation of the immunoproteasome in peripheral blood mononuclear cells of patients with IgA nephropathy

Rosanna Coppo[1], Roberta Camilla[1], Alda Alfarano[2], Sabrina Balegno[1], Domenico Mancuso[1], Licia Peruzzi[1], Alessandro Amore[1], Antonio Dal Canton[1], Vincenzo Sepe[1] and Pierangelo Tovo[1]

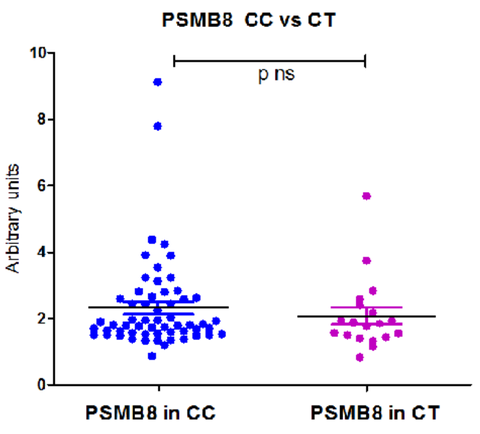
Figure 1 | Individual results of the switch from proteasome (PS) to immunoproteasome (IPS) detected as a ratio between each IPS catalytic unit (LMP2, LMP7, MECL-1) and the corresponding PS (β1, β2, β5) mRNA in patients with IgA nephropathy (IgAN) and healthy controls (HC).

RESULTS

We detected a highly significant increase in comparison to healthy controls (HC) in the expression of IPS subunit PSMB8 and corresponding PS/IPS switch.
(PSMB8:IgAN median value 2.09, IQR 1.6- 3.2;HC 1.2, 0.7-1.9, p< 0.0001;PSMB8/PSMB5 IgAN 1.70, 1.3-2.3; HC 1.02, 0.9-1.2, p=0.0002) and of IPS subunit PSMB9 and corresponding PS/IPS switch (PSMB9 IgAN 1.80, 1.2 -2.4; HC 0.44, 0.3-0.7, p<0.0001; PSMB9/PSMB6 IgAN 1.25, 0.9-1.9, HC 0.49. 0.3-0.7 , P< 0.0001).
The expression of PSMB10 and PSMB10/PSMB7 was similar to HC.



The frequency of IgAN risk allele rs9357155-C was 0.87 in our cases, as compared to 0.83 in the CEU HapMap population (European controls).
Patients with CC genotype tended to have higher IPS switch in comparison to CT/TT genotypes, but these preliminary results did not reach statistical significance



CONCLUSIONS

In conclusion, this large multicenter European study confirms enhanced immunoproteasome activation in PBMCs from IgAN patients. We also observed a trend for increased PS/IPS switch in homozygotes for the GWAS risk allele at the PSMB8/PSMB9 locus, although low power of our genetic association study precludes a definitive conclusion; we expect to confirm this trend as we continue to increase the sample size for the genetic arm of this study.

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