Plasma Proteomics in Healthy Subjects with Differences in Tissue Glucocorticoid Sensitivity Identifies a Novel Proteomic Signature

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INTRODUCTION

Tissue sensitivity to glucocorticoids is characterized by significant inter-individual variation in terms of therapeutic response and susceptibility to several stress-related disorders (**1, 2**). Proteomics approaches, combined with appropriate bioinformatics analysis, offer a comprehensive description of molecular phenotypes with clear links to human disease pathophysiology **(3-5).**

AIM

To investigate the usefulness of plasma proteomics in identifying a proteomic signature that could distinguish glucocorticoid resistant from glucocorticoid sensitive subjects and provide clues of the underlying physiological differences.

METHODS

One hundred one (n=101) healthy volunteers were given a very low dose (0.25mg) of dexamethasone at midnight, and were polarized into the 10% most sensitive (S) and 10% most resistant (R) according to the 08:00h serum cortisol concentrations the following morning. One month later, DNA was isolated from peripheral blood mononuclear cells, and plasma samples were collected.

To identify any genetic defects in the *NR3C1* gene, the protein-coding sequences and the intron-exon junctions of the NR3C1 gene were PCR-amplified and sequenced.

The proteomic profile of plasma samples was determined using LC-MS/MS.

RESULTS

Clinical characteristics, biochemical and endocrinological parameters of the participants

The 11 participants (10% of the cohort) with the lowest cortisol concentrations and the 11 participants with the highest cortisol concentration were selected for further analysis as the most glucocorticoid sensitive (S) and most glucocorticoid resistant (R), respectively, of the group; [(mean serum cortisol concentrations ± SD: 34.4 ± 15 nmol/L in the S participants vs. 622.4 ± 93.7 nmol/L in the R participants, p <0.001); (mean plasma ACTH concentrations ± SD: 2.8 ± 2.4 pg/mL in the S participants vs. 31.6 ± 10.6 pg/mL in the R participants, p <0.001)]. The rest endocrinological and biochemical findings did not show any statistically significant differences.

NR3C1 gene sequencing revealed no polymorphisms or mutations in the 22 subjects

No genetic defects or polymorphisms were detected in the *NR3C1* gene of the 22 subjects.

Proteomics Analysis

In total, 2737 proteins were identified and quantified in at least one of the analyzed samples. After selecting those proteins with presence in at least 35% of the samples in one of the two groups (n = 466 features), significant proteins were further defined as the subset with a fold change greater than 1.5 (or less than 0.67). This counted for 66 proteins with higher abundance in the resistant and 110 proteins with higher abundance in the hypersensitive group (**Figure 1**). Among them, there were 21 proteins being present exclusively in only one of the two groups (**Table 1**).

In order to predict response to cortisol prior to administration, a random forest classifier was developed based on the proteomics data. After tuning for optimal parameters, the classifier showed promising results in correctly assigning random partitions of the training data to the studied groups, achieving an overall accuracy score of 0.86. The individual importance of each protein in the model was evaluated in terms of the Mean Decrease in Accuracy, the Mean Decrease in Gini index and the mean minimal depth. Out of the 14 proteins utilized for training, APOA4 and GSN were the most important variables in the classification (**Figure 2**).

A significant number of proteins with higher abundance in the sensitive group are involved in platelet activation and aggregation. The deregulated biological pathways in the sensitive group are presented in (**Table 2**).

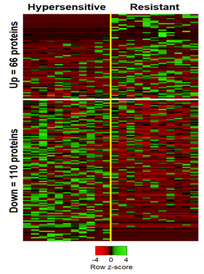

Figure 1: Heatmap (left) and Volcano plot (right) of proteins quantified in hypersensitive and resistant groups. Heatmap shows the abundance of proteins passing the ±0.585 log2 fold change threshold, in the two groups. Volcano plot illustrates the log_2 fold change (x axis) as a function of the Mann-Whitney p value (y axis). Red color marks proteins passing the 1.5 (or 0.67) fold change (equivalent to ±0.585 in the logarithmic scale).

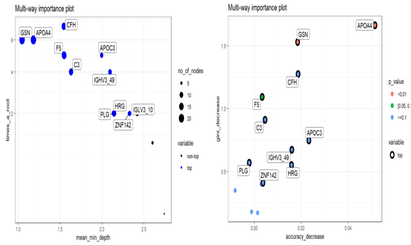

Figure 2: Variable importance for the 14 proteins used to train the random forest classifier so as to distinguish between responders (hypersensitive) and non-responders (resistant) to cortisol. Multiway importance plots depicting the mean decrease in accuracy as a function of the mean minimal depth (left) and of the mean decrease in the Gini index (right).

Table 1: Proteins identified in only one of the two groups

Protein	Description	Present
KIF28P	Kinesin-like protein KIF28P	Only in resistant
MRPS34	28S ribosomal protein S34, mitochondrial	Only in resistant
PRPF8	Pre-mRNA-processing-splicing factor 8	Only in resistant
MYH11	Myosin-11	Only in resistant
MLH1	DNA mismatch repair protein Mlh1	Only in resistant
ARHGAP21	Rho GTPase-activating protein 21	Only in resistant
EMC10	ER membrane protein complex subunit 10	Only in resistant
ZSWIM9	Uncharacterized protein ZSWIM9	Only in resistant
FANCB	Fanconi anemia group B protein	Only in resistant
CDADC1	Cytidine and dCMP deaminase domain-containing protein 1	Only in resistant
ACSS3	Acyl-CoA synthetase short-chain family member 3, mitochondrial	Only in resistant
IGHV3-66	Immunoglobulin heavy variable 3-66	Only in hypersensitive
IGLV5-39	Immunoglobulin lambda variable 5-39	Only in hypersensitive
LCP1	Plastin-2	Only in hypersensitive
DOCK4	Dedicator of cytokinesis protein 4	Only in hypersensitive
SLC38A3	Sodium-coupled neutral amino acid transporter 3	Only in hypersensitive
RTN4	Reticulon-4	Only in hypersensitive
CFAP97	Cilia- and flagella-associated protein 97	Only in hypersensitive
POLK	DNA polymerase kappa	Only in hypersensitive
ANKRD50	Ankyrin repeat domain-containing protein 50	Only in hypersensitive

Table 2: Deregulated pathways for the hypersensitive group. P value corresponds to the Benjamini-Hochberg correction.

Reactome pathway	P value	% Associated Genes	Associated Genes Found
O2/CO2 exchange in erythrocytes	0.000194	23.1	[CA1, CA2, HBA1]
G-protein mediated events	0.004559	5.5	[CAMKK2, ITPR1, ITPR2]
PLC beta mediated events	0.004452	5.6	[CAMKK2, ITPR1, ITPR2]
DAG and IP3 signaling	0.002614	7.1	[CAMKK2, ITPR1, ITPR2]
Signaling by VEGF	0.000892	4.7	[CDH5, CRK, ITGB3, ITPR1, ITPR2]
Platelet activation, signaling and aggregation	1.5E-06	4.6	[CRK, F8, FLNA, ITGB3, ITPR1, ITPR2, PFN1, PPBP, QSOX1, RARRES2, TUBA4A, VCL]
Fcgamma receptor (FCGR) dependent phagocytosis	0.002295	4.7	[CRK, FCGR3A, ITPR1, ITPR2]
Platelet degranulation	1.18E-06	7.0	[F8, FLNA, ITGB3, PFN1, PPBP, QSOX1, RARRES2, TUBA4A, VCL]
Role of phospholipids in phagocytosis	0.000858	12.0	[FCGR3A, ITPR1, ITPR2]

CONCLUSIONS

A proteomic profile indicating erythrocyte gas exchange and platelet activation was observed in the S compared to the R group, suggesting a state of the organism that is more capable to respond to stressful stimuli.

Our findings also indicate that a proteomics signature may differentiate the most glucocorticoid resistant from the most glucocorticoid sensitive subjects, and may be useful in clinical practice. In addition, it may provide clues of the underlying molecular mechanisms of the chronic stress-related diseases, including myocardial infarction, stroke and Alzheimer's disease.

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