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FUNCTIONAL GENE SETS IN POST-TRAUMATIC STRESS DISORDER

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In the study principal component analysis and gene set enrichment analysis was carried out to evaluate genome wide expression and identify functional gene sets affected in peripheral blood mononuclear cells of patients with post-traumatic stress disorder and animal models of the disease. The results obtained indicate the significance of the genetic component in the pathogenesis of post-traumatic stress disorder, and provide strong evidence for the involvement of chronic inflammation, neuronal and cytokine/growth factor signaling pathways, in disease development and progression. In addition, the results obtained demonstrated that combination of principal component analysis with gene set enrichment analysis is an efficient strategy for discrimination of phenotype related gene expression variability from other factors.

***Keywords*:** gene principal component, gene set enrichment, post-traumatic stress disorder.

Introduction. Post traumatic stress disorder (PTSD) is a complex, severe and chronic psychiatric illness, an anxiety disorder (DSM-IV-TR code: 309.81) that may develop in a person, who has experienced, witnessed, or learned about a terrifying event or ordeal, in which grave physical harm occurred or was threatened. As estimated 70% of adults have experienced a traumatic event at least once in their life and up to 20% of these people go on to develop PTSD. It is predicted that in the near future 8–10% of the population will suffer PTSD at some time in their lives [1, 2].

The molecular pathomechanisms of PTSD are not well defined and only beginning to be understood and this lack of knowledge hampers our ability to find superior therapeutic approaches to the treatment of this disorder. Promising findings suggest that both environment and genetic factors are involved in PTSD-generation mechanisms, and that neuronal, endocrine, and immune alterations might be in a sufficient degree responsible for disease progression [2, 3]. However, due to insufficiency of relevant data, a molecular picture of generation and development of PTSD is yet unclear.

Genome wide expression analysis with DNA microarrays has become a mainstay of genomics research [4, 5]. The challenge no longer lies in obtaining gene expression profiles, but rather in interpreting the results to gain insight the

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molecular basis of diseased conditions. The common approach is to find a number of differentially and highly expressed genes responsible for the generation and progression of pathological changes. However, this kind of analysis has several limitations, from which the important one is that the information on disease-associated alterations in metabolic, regulatory or signaling pathways may be missed. Recently introduced Gene Set Enrichment Analysis (GSEA), which is designed to evaluate functional gene sets, overcomes these limitations [6]. Further extension of this approach may be its combination with the Principal Component Analysis (PCA). It is obvious, that variation in gene expression may be the result of combined action of several factors, often not connected to the phenotype of interest. PCA allows discrimination between these effects, as it is a linear dimensionality reduction technique, which identifies statistically uncorrelated orthogonal directions Principal Components (PC) of maximum variance in the original data. The component loadings of each gene may show to what extent differences in gene expression contribute to phenotypic diversity. Here we applied combined PCA and GSEA techniques to evaluate the functional gene sets affected in peripheral blood mononuclear cells (PBMC) of patients with PTSD at both early and advanced stages of the disease. In addition, we conducted comparative analysis of PTSD-related gene expression patterns in human and mouse model.

Materials and Methods.

Human and Mouse Datasets. We use two datasets (GDS1020 and GSE8870) available in the Gene Expression Omnibus repository (www.ncbi.nlm.nih.gov/geo/). Description of samples is available in the dataset summaries.

From human dataset probes that had at least 50% of present calls were chosen. For the mouse dataset probes containing less that 50% of missing values were chosen. The probe identifiers from each platform were converted to the HUGO-approved gene symbols, averaging log transformed expression values of multiple probes targeting the same gene. This resulted in 4545 and 32205 genes for human and mouse datasets respectively.

Principal Component Analysis and Phenotype Matching for Human Dataset. PCA was performed using MATLAB 7.13 software (Mathworks Inc., USA). We identified components, PCs that most accurately classify samples according to their phenotypes (PTSD vs. Controls). For that purpose, scores and phenotypes were assigned binary values (scores: positive – 1, negative – 0; phenotypes: PTSD – 1, Control – 0) to obtain 2×2 table. Further, the significance of phenotype differences in gene expression for each component was tested by Fisher's exact test. $p < 0.05$ was considered as significant. For PCs showing significant association with phenotypes we generated gene lists ranked from most positive to most negative factor loadings.

Gene Set Enrichment Analysis. GSEA analysis was performed using GSEA 2.0 software [6]. As data source pre-ranked list of human genes and mouse gene expression dataset were used. This application scores a sorted list of genes with respect to their enrichment of selected functional categories (KEGG, Biocarta and GO). The significance of the enrichment score was assessed using 1000 permutations. For multiple testing adjustments, Benjamini and Hochberg's false discovery rate (FDR) < 25% was used. $p < 0.05$ (after FDR correction) were considered as significant.

Results and Discussion. First, we analyzed the gene expression profiles of PBMCs in PTSD affected subjects and controls. PCA reduced sample complexity of 4545 patterns of gene expression are available for PBMCs in the dataset, by grouping them into the sets of 32 orthogonal factors or PCs (Fig. 1, A).

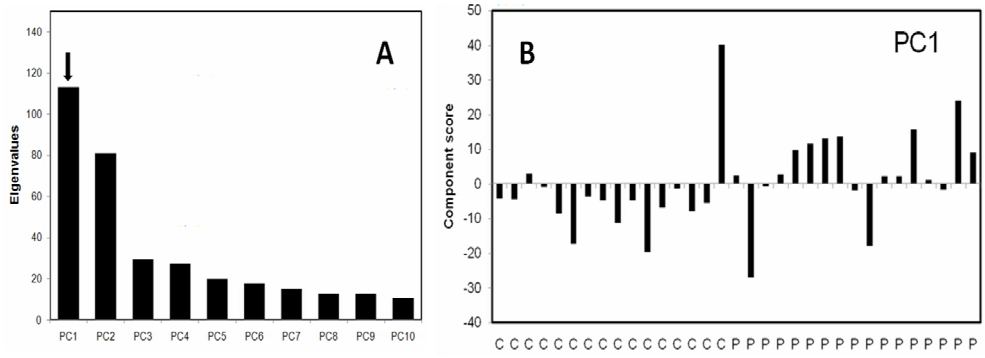

Fig. 1. The percentage of gene expression variability explained by PCs (A) and classification of phenotypes (PTSD vs. Controls) according to the first PC (B).

Next, we identified PCs associated with disease phenotype using Fisher's exact test. The results showed that the first PC associate with disease phenotype (Fig. 1, B; Tab. 1). Positive PC scores were associated with PTSD samples, whereas negative PC scores were associated with Control samples.

Table 1

Phenotype association with PC1 score and accuracy of classification

Phenotype	Component score	
	negative*	positive*
Control	14 (87.5)	2 (12.5)
PTSD	5 (29.4)	12 (70.6)**

Note: * data presented as count (percentage);
** $p = 0.001$ (two tailed Fisher's exact test).

In order to evaluate the biological significance of the identified PC, we performed GSEA using GSEA v 2.0 software [6]. Since the ranked gene lists were sorted in descending order, the top-enriched categories can be considered as up-regulated in PTSD and the opposite is true in case of down-regulated categories. The analysis revealed the 11 up-regulated functional gene sets at $p_{adjusted} < 0.05$ (Tab. 2) involved in inflammation, extracellular signaling and metabolism. For identification of genes, contributing to several pathways, we have performed leading edge analysis. Overall 356 genes contributed to the leading edge of up- and down-regulated datasets of which 29 genes appeared in two or more gene sets (Fig. 2). HRAS and MAPK1 were the most frequent genes and appeared in 11 and 10 gene sets respectively.

Table 2

The biological significance of PCs related to PTSD

Category	Number of genes in gene set	$p_{adjusted}$	Condition
Cell adhesion molecules	42	0.005	up-regulated
Hematopoietic cell lineage	47	0.018	up-regulated
Cytokine cytokine receptor interaction	65	0.050	up-regulated
ECM receptor interaction	20	0.076	up-regulated
Type I diabetes mellitus	20	0.078	up-regulated
Cell communication	17	0.076	up-regulated
EPO pathway	16	0.043	up-regulated
NGF pathway	16	0.079	up-regulated
IL2 pathway	19	0.041	up-regulated
Type II diabetes mellitus	17	0.075	up-regulated
Ribosome	46	0.004	up-regulated

The analysis of brain gene expression patterns in mouse models of PTSD revealed 74 significantly up-regulated gene sets (KEGG and Biocarta) involved in neurotransmission, extracellular signaling pathways, inflammation and general metabolic processes. The identified functional gene sets included the majority of categories up-regulated in PMBCs isolated from blood of PTSD subjects (Tab. 3).

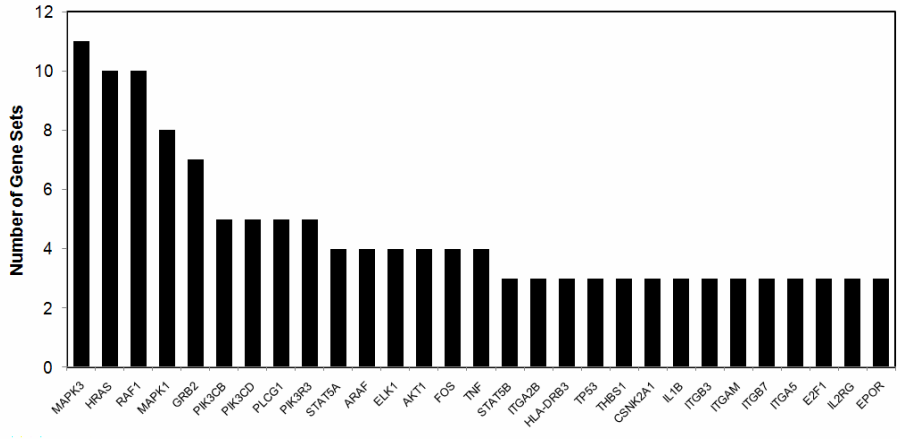

Fig. 2. Genes appearing in two or more gene sets.

The results obtained demonstrated that PBMCs of PTSD affected subjects bear specific hallmark of gene expression compared with non-affected subjects. Importantly, our data showed significant overlap of functional sets between human and animal model data, which indicates their importance in the development and progression of PTSD. Our findings are in line with previous data indicating the involvement of neuro–immune–endocrine system in pathogenesis of this disease. Characteristic suppression of the hypothalamic-pituitary adrenal axis in sustained PTSD suppresses the action of endogenous cortisol on proinflammatory cytokine production [7, 8]. Recently, it was shown that the levels of several inflammatory cytokines, CRP and other inflammatory molecules are increased in the blood of PTSD patients, as well as changes in WBC subpopulations have been noticed

[9–11]. This persisting chronic inflammation may, in turn, affect the function of central nervous system. It is known that the pro-inflammatory cytokines IL-6 and TNF-α, together with IL-1 are the most important mediators of the so-called sickness response, which is described as a series of depression like symptoms that occur during peripheral immune system activation [12].

Table 3

Functional gene sets up-regulated in the mouse model of PTSD similar to gene sets identified in human

Category	Number of genes in gene set	$p_{adjusted}$	Condition
Cell adhesion molecules	101	0.000	up-regulated
Cytokine cytokine receptor interaction	216	0.000	up-regulated
Type I diabetes mellitus	42	0.000	up-regulated
Neuroactive ligand receptor interaction	224	0.004	up-regulated
Lysine degradation	42	0.004	up-regulated
ECM receptor interaction	81	0.006	up-regulated
Cell communication	78	0.012	up-regulated
IL2 RB pathway	34	0.018	up-regulated
VEGF signaling pathway	66	0.037	up-regulated

The PCA technique allows reducing the overall variability of microarray data to a relatively small number of components with some biological meanings [13, 14]. We demonstrated that with this approach it is possible to identify components that are responsible for disease-related changes in gene expression. In this study, we have found that only 30% of gene expression variability was related to the development of PTSD. The remaining variability may be defined by a number of other factors, including heterogeneity of cell populations, age, gender, ethnic origin, etc. GSEA directly scores pre-defined gene sets for differential expression and especially aims to identify gene sets with "subtle but coordinated" expression changes that cannot be detected by cutoff methods (for review see 15). The key principle is that even weak expression changes in individual genes gathered to a large gene set can show a significant pattern. By changing the focus from individual genes to a set of genes or pathways, the GSEA approach enables understanding of cellular processes as an intricate network of functionally related components [15].

Conclusion. In this paper we used two-step approach for evaluation of genome wide expression associated with PTSD in human subjects and animal models. In the first step PCA was applied to identify the contribution of gene expression changes to PTSD phenotype, and at the second step GSEA was used to target functional gene sets related to disease phenotype. The results of the present study clearly indicate the significance of the genetic component in the pathogenesis of PTSD, and provide strong evidence for involving alterations of neuronal, endocrine and immune system at the gene expression level.

In addition, the results of the present study demonstrate that the combination of PCA with GSEA is an efficient tool to discriminate phenotype related gene expression variability from other influencing factors and to have a more complex outlook into molecular mechanisms underlying a pathological condition.

In conclusion, our results give biological insights into the molecular processes occurring in PTSD and introduce a combined approach to discriminate phenotype effects on gene expression from influencing factors.

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