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LETTER TO THE EDITOR

Gene expression profiling in the chronic fatigue syndrome

DEAR SIR,

Fatigue is a symptom found in many conditions of disease and illness. Although, unfrequently recognized by the medical profession, it is often of major importance for the patients. Chronic fatigue was reported by 5.9% of the Swedish population in a large telephone-based interview with 31 406 individuals in the Swedish twin registry (STR) [1]. The fatigue had lasted for more than 6 months and caused impairment, e.g. >25% reduction of working capacity. When at least four of eight criteria included in the current definition of chronic fatigue syndrome (CFS) [2] was added 2.4% reported that they suffered from a CFS-like illness.

This costly condition is still an intriguing issue for researchers and clinicians, and ambiguities in the definition have recently been focused upon [3, 4]. An empirical test of the definition was performed with data from the STR where five subgroups were identified: 'CFS-like', 'residual', 'rheumatic', 'depressive' and 'acute physical syndrome' [5].

We wanted to identify genes in peripheral blood mononuclear cells (PBMCs), which may play an important role in the pathogenesis and diagnostics of CFS, using microarray technology. PBMCs can serve as indicators of illness processes occurring in different parts of the human body. Patients with CFS from a clinic of infectious diseases at a university hospital were stratified according to the STR study findings [5] to sex, illness classification (ICD-10), illness onset type, illness duration and number of symptoms (Table 1).

Blood samples (heparin) were taken from 20 Swedish CFS patients, and 14 healthy age- and sex-matched volunteers from the hospital employees as controls (Table 1) after written informed consent (ethical approval, 130/02, Karolinska University Hospital, Huddinge, Sweden). PBMCs were immediately isolated (Lymphoprep™; Medinor, Oslo, Norway) and frozen in liquid nitrogen.

Total RNA was extracted (TRIzol Reagent; Invitrogen, Carlsbad, CA, USA) from quickly thawed cells. Two micrograms of total RNA was used for amplification (RiboAmp™; Arcturus, Mountain View, CA, USA). Labelled cDNA was synthesized from 0.5 μg of amplified RNA by indirect labelling with amino allyl-dUTP using SuperScript III enzyme system (Invitrogen), random hexamer primers and fluorophores (Cy3 and Cy5). Samples were hybridized to in-house manufactured 30 k cDNA microarrays (http://www.biotech.kth.se/molbio/microarray) utilizing indirect comparison with a common reference sample (Human reference RNA; Stratagene, La Jolla, CA, USA).

Scanned images were analysed with GenePix® Pro 5.1 (Axon Instruments, Union City, CA, USA). Generated data were analysed with the R environment for statistical computing (http://www.R-project.org). Data were loess print-tip group normalized and significant differentially expressed genes were identified using an empirical Bayes method for analysis of microarray data (*B*-test) [6]. The *B*-test is a modification of the *t*-test and it generates a ranking of genes, where genes most likely to be differentially expressed have the highest *B*-scores. Clustering was performed using Multi Experimental Viewer (TIGR MeV, Rockville, MD, USA).

Genes identified as differentially expressed were validated by real-time PCR of all samples, using the LightCycler system (Roche AB, Stockholm, Sweden) and gene-specific primers. GAPDH and 18S rRNA were used for normalization.

We found no indication of differential expression between the entire patient group and the control group including both sexes. Differences between the sexes were greater than between patients and controls, women clustered into one big group differentiated from men. However, comparing only female patients to female controls indicated

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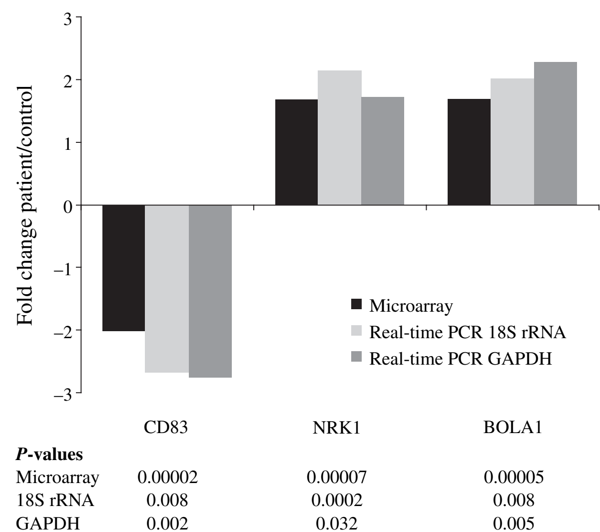
Table 1 Characteristics of chronic fatigue syndrome (CFS) patients and healthy controls

	CFS ($n = 20$)	Control ($n = 14$)
Age (years)[a]	37 (27–60)	37 (25–57)
Male/female	5/15	2/12
Illness duration (years)[a]	3¼ (1–27)	
Illness onset type[b]		
Gradual	11 (2/9)	
Sudden	9 (3/6)	
CFS classification[b]		
Noninfectious	11 (1/10)	
Infectious	9 (4/5)	
Number of symptoms		
4	7	
5	6	
6	0	
7	6	
8	1	
Onset triggers	Ehrlichiosis, herpes encephalitis, sarcoidosis, influenza, upper respiratory infection, urinary tract infection and tonsillitis	

[a]Median (range). [b]Number (male/female).



P-values	CD83	NRK1	BOLA1
Microarray	0.00002	0.00007	0.00005
18S rRNA	0.008	0.0002	0.008
GAPDH	0.002	0.032	0.005

Fig. 1 CD83, NRK1 and BOLA1 were identified with significant differential gene activity in peripheral blood mononuclear cells, comparing female CFS patients with noninfectious and gradual illness onset ($n = 8$) to healthy controls ($n = 12$). Genes were identified with microarray technology and verified with real-time PCR using two different genes for normalization (18S rRNA and GAPDH). CD83 has lower activity level in patients compared with controls, whilst NRK1 and BOLA1 are overexpressed.

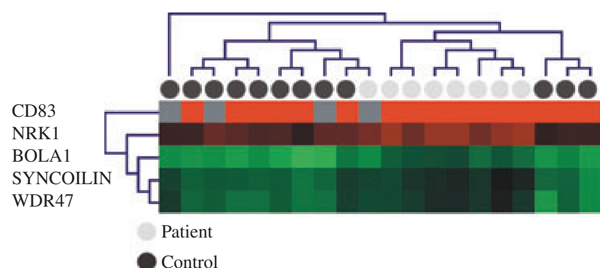


Fig. 2 Clustering of all female patients with noninfectious and gradual illness onset and healthy female controls using the five genes, identified as differentially expressed using the *B*-test, differentiated all patients but one from the controls (hierarchical clustering with complete alignment and Pearson correlation distance using MeV). This patient followed the control pattern in both microarray experiments and real-time PCR. The controls divided into two main clusters with three controls being closer to the major patient cluster and one outlier.

significant differential gene activity. Further analysis was performed using only female study participants.

Patients with no previous documented infection and gradual illness onset ($n = 8$) showed significant gene expression differences compared with healthy female controls ($n = 12$). Of the top eight genes, identified by the *B*-test, five were known and used for further investigation. Significant gene activity difference was detected in three of the five genes (CD83, BOLA1 and NRK1) (Fig. 1) with real-time PCR. Significance was achieved using both normalization genes, 18S rRNA and GAPDH.

The five genes differentiated all but one of the female patients with noninfectious and gradual illness onset from the healthy controls (Fig. 2). This patient followed the gene activity pattern of the controls in both microarray and real-time PCR experiments, and was considered not to belong to the group and excluded from calculations. Male patients and controls, and female patients from other subgroups did not follow the clustering pattern (data not shown).

Differences in gene activity were indicated within the patient group, between the illness classification subgroups and illness onset subgroups. However, no statistical significance was achieved. A greater number of patients within some subgroups is probably required. Indications of gene activity differences were also observed between patients with previous infection and sudden illness onset compared with healthy controls.

No changes in gene activity over time of CFS illness duration were observed. No differences were observed between patients fulfilling four to five

symptoms of the CFS criteria [2] compared with patients fulfilling seven to eight symptoms.

Conclusively, no differential gene expression was identified with classification of patients according to the STR study [5]. No data were found to support the current definition from 1994, where at least four of eight specified symptoms need to be present for the diagnosis of CFS.

The function of the human CD83 gene is not fully known. Studies indicate an important functional role in the immune system. It is present in both dendritic cells (DCs), where it serves as a maturation marker, and in B lymphocytes. Nicotinamide has been used as medical treatment for CFS patients. NRK1 is an enzyme involved in the synthesis of NAD+ through nicotinamide mononucleotide using nicotinamide riboside as the precursor. BOLA1 (previously denoted as CGI-143) has unknown function. CD83 expression is lower in the patient group (noninfectious and gradual illness onset) compared with healthy controls. NRK1 and BOLA1 are overexpressed in the patient group. To clarify the role of the three genes in CFS pathogenesis, further studies on a protein level are needed.

A strength of this study is confirmation of the significant mRNA expression level differences found by microarray using real-time PCR. Also, this patient cohort has been diagnosed in a uniform way (as noticed in the biological findings). The patient population is thus quite unique to its character. It would have been desirable with an even larger number of patients; however, for clinical research this is an unusually large number of well characterized and subgrouped patient in this category.

These findings indicate the need of subgrouping of patients with fatiguing illness in search for pathogenic mechanisms.

Conflict of interest statement

No conflict of interest was declared.

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