Genomic detection and characterization of a potential therapeutic biomarker in Bahraini Patients with multiple sclerosis

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Background:

MS is an immune mediated inflammatory demyelinating disease of the CNS, which usually begins in early adult life and pursues a variable course which may progress in many patients leading to significant morbidity

A variety of genetic, immunologic and environmental factors have been implicated in triggering the onset and progression of the disease

There is a universal increase in prevalence and incidence of MS over time

In the Middle East, and particularly the Gulf Region, increased prevalence has been recently reported due to genetic and environmental factors, with similar increase in Bahrain

To dig up better understanding of MS pathogenesis in a certain population and mark novel treatment approaches to identify patients with poor prognosis, a new broad approaches are needed such as gene expression

Objectives and Methods:

In order to examine such gene expression in Bahraini MS patients that might be related to pathogenesis of the disease for initiation of molecular targeted approaches to personalized therapy, the microarray technology was utilized to analyze differential gene upregulation in PBMCs isolated from MS patients Bahraini in comparison to healthy subject

The expression of the upregulated genes was confirmed by real time PCR

Gene cloning, protein expression and purification was done and cell proliferation assay in addition to cytokine mRNA detection using RT-PCR were carried out

The study included twenty five random Bahraini MS patients who were newly diagnosed at Salmaniya Medical Complex as relapse remission (RR) MS (male & female between the ages of 20-40 years)

Similar number of healthy Bahraini individuals were used as controls with matched age, sex, and without nervous system pathology

PBMCs were separated by density gradient centrifugation, cells were lysed and total RNA was purified by TRIzol Reagent. The quantity and purity of RNA samples were confirmed by spectrophotometer and agarose gel electrophoresis

Microarray experiments were carried out using the Affymetrix system

Data analysis was performed by Partek Genomics Suite software

Results:

Out of 50.000 gene transcripts, 493 were differentially expressed

Among these, 230 genes were upregulated while 263 were downregulated in MS patients compared to the healthy subjects

Most of the expressed genes are of known function in the databases and in some literatures, but we found one hypothetical protein with unknown function domain

This gene which is TMEM66 was differentially up-regulated in MS patients compared to Healthy individuals by 3 times (fold change = 3.106, $p<0.020$)

The expression of the upregulated genes was confirmed by real time PCR

Table 1: Up-regulated genes of MS patients compared to healthy subjects

Gene Symbol	Gene Title	Fold change	P-value
SMCHD1	structural maintenance of chromosomes flexible hinge domain containing 1	8.895	0.002
ANXA3	annexin A3	6.616	0.029
RGS1	regulator of G-protein signaling 1	4.926	0.006
MAFF	v-mafmusculoaponeuroticfibrosarcoma oncogene homolog F (avian)	4.897	0.012
TNFAIP3	tumor necrosis factor, alpha-induced protein 3	4.471	0.015
MALAT1	metastasis associated lung adenocarcinoma transcript 1 (non-protein coding)	4.371	0.022
CD69	CD69 molecule	3.906	0.006
CXCR4	chemokine (C-X-C motif) receptor 4	3.682	0.040
C12orf35	chromosome 12 open reading frame 35	3.637	0.045
CXCR4	chemokine (C-X-C motif) receptor 4	3.597	0.045
MXD1	MAX dimerization protein 1	3.585	0.008
PAPOLA	poly(A) polymerase alpha	3.558	0.030
KCNJ15	potassium inwardly-rectifying channel, subfamily J, member 15	3.515	0.041
NAMPT	nicotinamidephosphoribosyltransferase	3.442	0.004
HBP1	HMG-box transcription factor 1	3.269	0.030
MGAM	maltase-glucoamylase (alpha-glucosidase)	3.194	0.020
USP15	ubiquitin specific peptidase 15	3.151	0.028
PROK2	prokineticin 2	3.120	0.027
LOC100289246	**hypothetical protein LOC100289246**	**3.106**	**0.020**

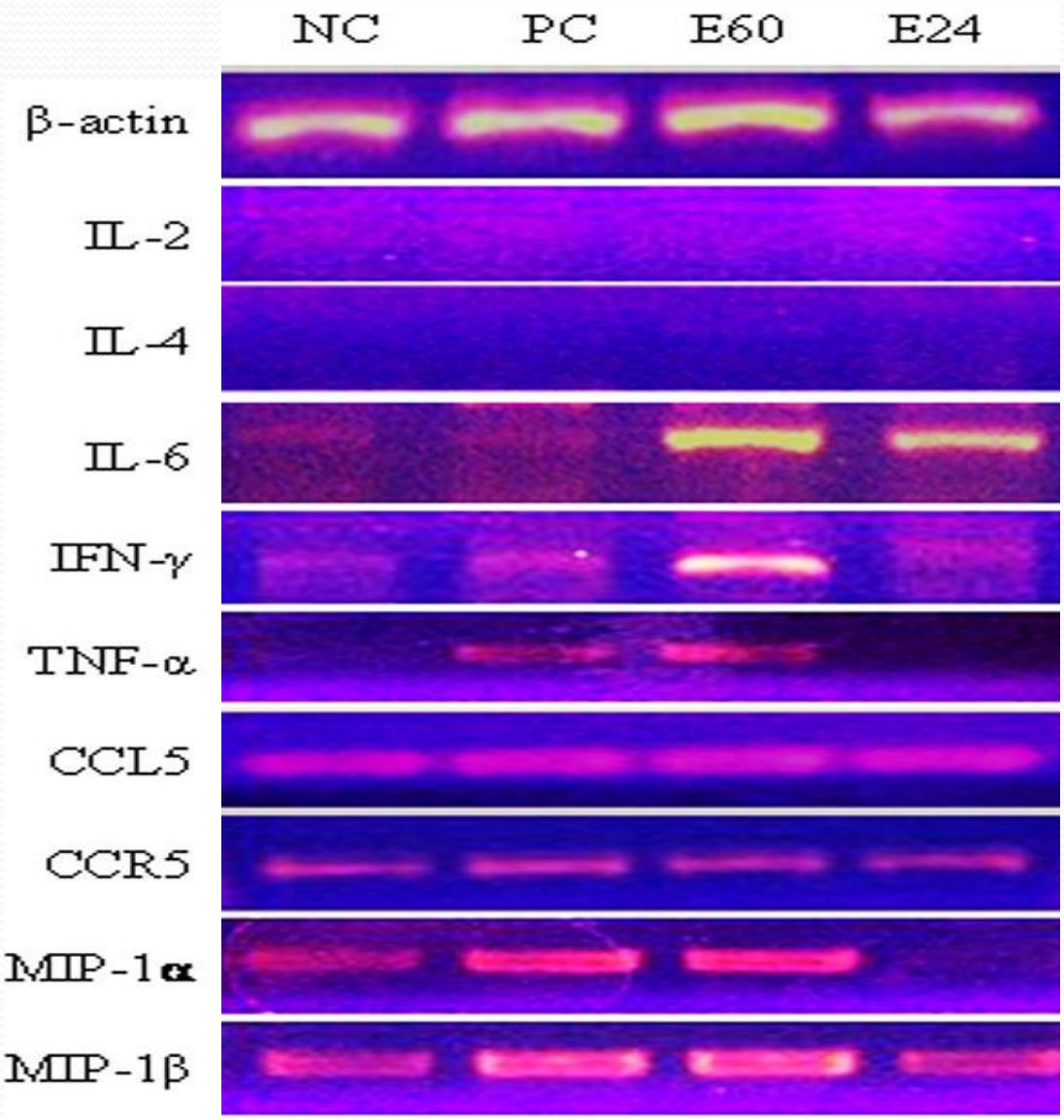
Table 2: Down-regulated genes of MS patients compared to healthy subjects

Gene Symbol	Gene Title	Fold change	P-value
DLEU2	deleted in lymphocytic leukemia 2 (non-protein coding)	-3.720	0.013
APOBEC3B	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B	-3.648	0.032
MCTP1	multiple C2 domains, transmembrane 1	-2.803	0.040
CLIC3	chloride intracellular channel 3	-2.744	0.018
SMAD1	SMAD family member 1	-2.534	0.043
ZNF880	zinc finger protein 880	-2.478	0.014
TMEM106B	transmembrane protein 106B	-2.421	0.049
DLEU2	deleted in lymphocytic leukemia 2 (non-protein coding)	-2.420	0.027
SRD5A3	steroid 5 alpha-reductase 3	-2.419	0.033
FAM43A	family with sequence similarity 43, member A	-2.330	0.004
RAPH1	Ras association (RalGDS/AF-6) and pleckstrin homology domains 1	-2.312	0.034
C7orf36	chromosome 7 open reading frame 36	-2.296	0.002
NBPF1	neuroblastoma breakpoint family, member 1	-2.295	0.013
ZEB2	zinc finger E-box binding homeobox 2	-2.243	0.016
GPR18	G protein-coupled receptor 18	-2.231	0.004
SOS1	son of sevenless homolog 1 (Drosophila)	-2.220	0.012
ADRB2	adrenergic, beta-2-, receptor, surface	-2.176	0.002
C12orf73	chromosome 12 open reading frame 73	-2.173	0.003
QSER1	glutamine and serine rich 1	-2.144	0.039
CTAGE5	CTAGE family, member 5	-2.140	0.042
PTGDS	prostaglandin D2 synthase 21kDa (brain)	-2.139	0.043
TRIM32	tripartite motif-containing 32	-2.128	0.001
MRPS14	mitochondrial ribosomal protein S14	-2.080	0.026
ARL17A / B	ADP-ribosylation factor-like 17A /B	-2.067	0.036
ZNF818P	zinc finger protein 818 (pseudogene)	-2.032	0.047

Activity of TMEM66 protein on cytokine and chemokine gene expression

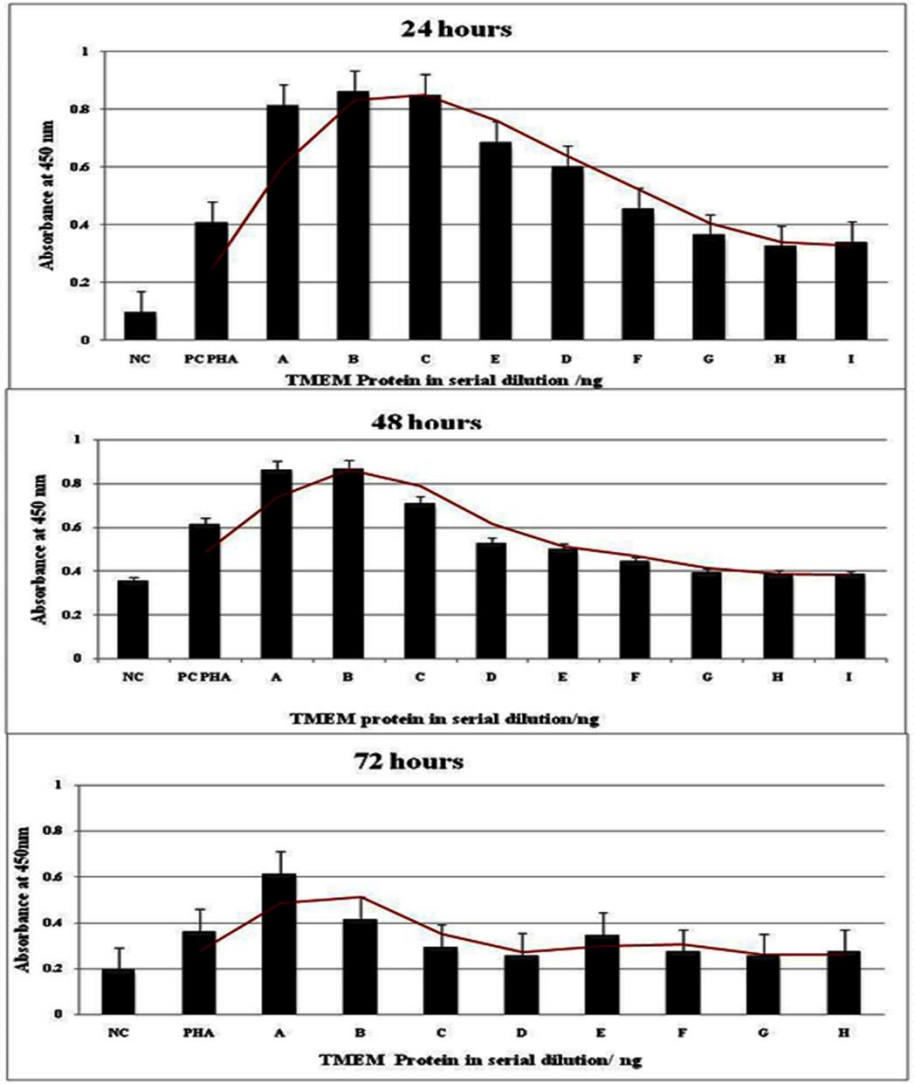
We cloned the TMEM66 gene and its protein showed marked immunologic activity relevant to MS since:
(a) augmented induction of the proinflammatory cytokines IL-6, IFN-γ, TNF-α and the chemokines CCL5/CCR5, MIP-1α, MIP-1β were recorded, but not the anti-inflammatory cytokine IL-4 or IL-2

(b) significant proliferative activity ($p<0.05$)



Effects of TMEM66 protein on hPBMCs proliferation

Negative control (unstimulated cells) and positive control (stimulated cells with PHA-M 5ug/ml) were included.
Letters from (A-H) represent serial dilution of the protein TMEM66 (A= 340, B = 170, C= 85, D= 42.5, E= 21.25, F= 10.6, G= 5.3, H= 2.66, and I= 1.33 ng).
Bars show the absorbance at 450nm after treating cells with WST-1 reagent. Higher concentration of Protein had a significant proliferative effect compared to unstimulated cells (negative control) in all three periods ($p<0.05$).



Conclusion:

TMEM66 may be related to the molecular events of MS in Bahraini patients and could be considered as MS biomarker during any future personalized medicine approaches.

Thank you

A very special thanks to my colleagues:

Dr. Safa Taha, Ms. Muna Aljishi and Dr. Isa Alsharoqi