Proteasomal Protein Gene Expression and DNA Integrity in Multiple Sclerosis

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Background

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system. Most patients are diagnosed with a relapsing-remitting MS (RRMS) which eventually transition to secondary progressive MS (SPMS). Reactive oxygen species have been implicated as mediators of demyelination in MS and can cause DNA damage. Overproduction of NO in oligodendrocytes is considered to be a crucial step in the pathogenesis of MS. Accumulation of toxic protein aggregates is characteristic for MS patients and indicates dysfunction of ubiquitin-proteasome system (UPS), which is responsible for degradation of intracellular proteins and is one of the key factors in regulation of the immune system. Treatment with interferon β (IFNβ) is the most common therapy for MS patients.

Aim

The aim of the current study was to investigate proteasomal gene expression levels under conditions of different MS subtypes and IFNβ treatment, as well as DNA integrity and NO production in blood of MS patients and controls.

Methods

Groups of healthy subjects and MS patients were enrolled in the study. Single-stranded (ss-) DNA breaks were determined in whole blood and isolated peripheral blood mononuclear cell (PBMNC) samples of 28 patients and 15 controls by means of alkaline single cell gel electrophoresis (comet assay). NO level in blood of 22 MS patients and 22 controls was tested by applying electron paramagnetic resonance spectroscopy. Proteasomal gene expression levels were determined in blood of 127 MS patients and 17 controls by means of qPCR. The *PSMA6* (rs2277460 and rs1048990), *PSMC6* (rs2295826 and rs2294827) and *PSMA3* (rs2348071) proteasomal gene single nucleotide polymorphisms (SNPs) were genotyped on MS subtype and treatment efficiency association in 280 cases / 305 controls study.

Results

PSMA6 gene expression was significantly increased in MS patients compared to control group ($p<0.05$; Fig. 1). Also, patients with common genotypes at *PSMA6* rs1048990 locus had significantly increased *PSMA6* gene expression compared to the rest of the patients ($p<0.05$). IFNβ therapy showed tendency to increase *PSMA3*, *PSMA6* and *PSMC6* gene expression in patients with common genotypes at *PSMA3* rs2348071 locus, *PSMA6* rs2277460 and rs1048990 loci ($p<0.05$), and *PSMC6* rs2295826 and rs2294827 loci compared to non-treated patients (Fig.2). There was no difference in *PSMA3*, *PSMA6* and *PSMC6* gene expression between RRMS and SPMS patients (Fig. 3). The level of ssDNA breaks was increased in whole blood and isolated PBMNC samples of MS patients compared to controls ($p<0.05$; Fig. 4A). NO production was significantly elevated in the blood of MS patients ($p<0.05$; Fig. 4B).

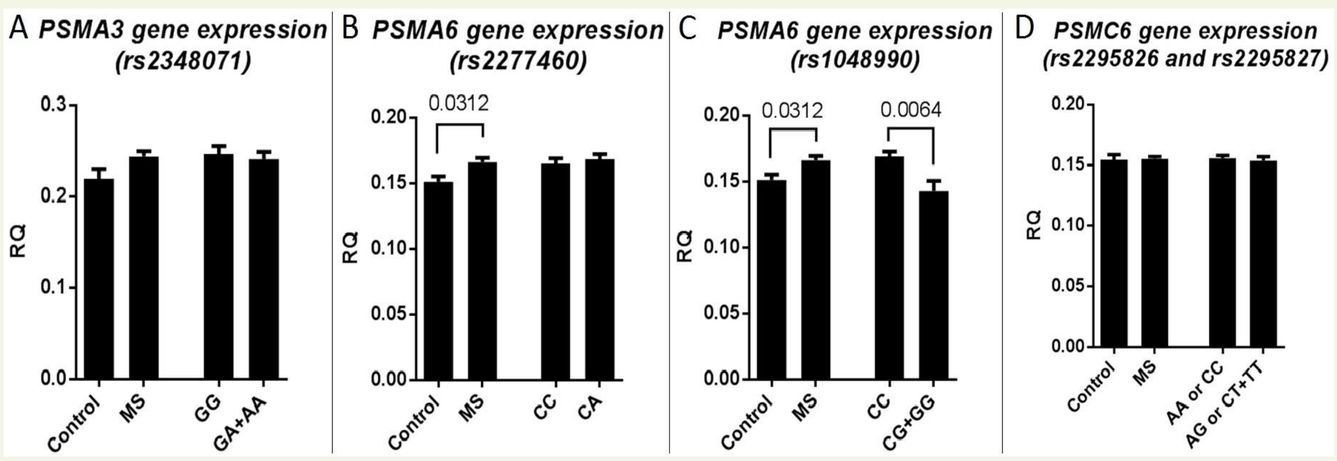

Figure 1. **Expression levels of (A) *PSMA3*, (B and C) *PSMA6* and (D) *PSMC6* genes.** First two bars – control group vs. MS patients; second two bars – common genotype vs. rare alleles and heterozygous genotypes in MS patients.

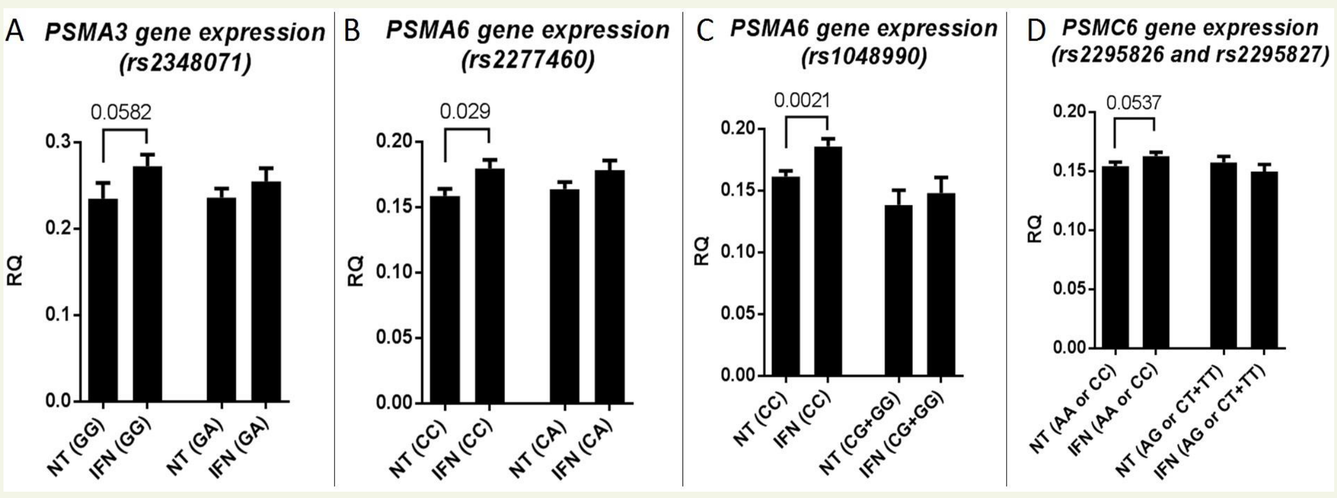

Figure 2. **Expression levels of (A) *PSMA3*, (B and C) *PSMA6* and (D) *PSMC6* genes in multiple sclerosis (MS) patients according to treatment and genotypes.** NT – group of MS patients without treatment; IFN – group of MS patients with interferon β treatment. First two bars – common genotype; second two bars – rare alleles and heterozygous genotypes.

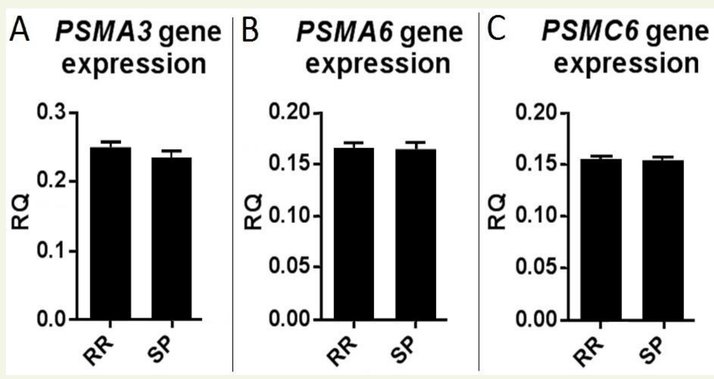

Figure 3. **Expression levels of (A) *PSMA3*, (B) *PSMA6* and (C) *PSMC6* genes according to multiple sclerosis (MS) subtypes**. RRMS – relapsing-remitting MS; SPMS – secondary progressive MS.

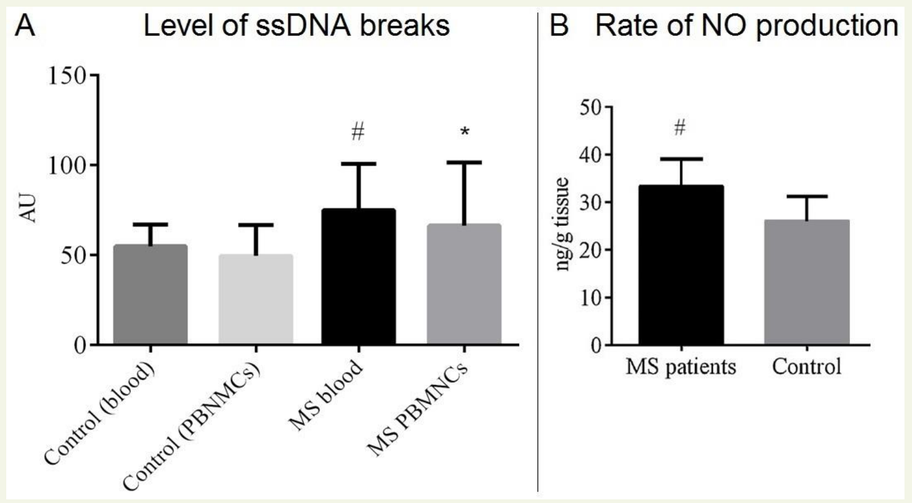

Figure 4. **(A) The level of ssDNA breaks in whole blood and isolated peripheral blood mononuclear cell (PBMNC) samples of MS patients and controls**. # and *- $p < 0.05$ of whole blood and PBMNCs of MS group vs. corresponding control group. **(B) Rate of NO production in blood of MS patients and controls.** # - $p < 0.05$ vs. control group.

Conclusions

- Overall group of MS patients have higher PSMA6 gene expression.
- IFNβ therapy increases PSMA3, PSMA6 and PSMC6 gene expression in patients with common genotypes compared to non-treated patients.
- Patients with MS have elevated level of DNA damage.
- Patients with MS have significantly higher level of NO in blood.

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