DNA INTEGRITY AND EXPRESSION OF PROTEASOMAL GENES IN MULTIPLE SCLEROSIS

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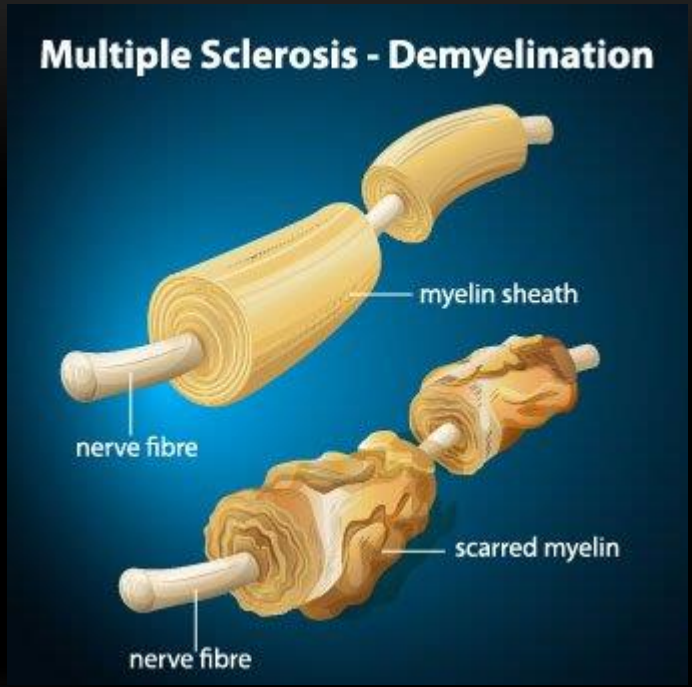
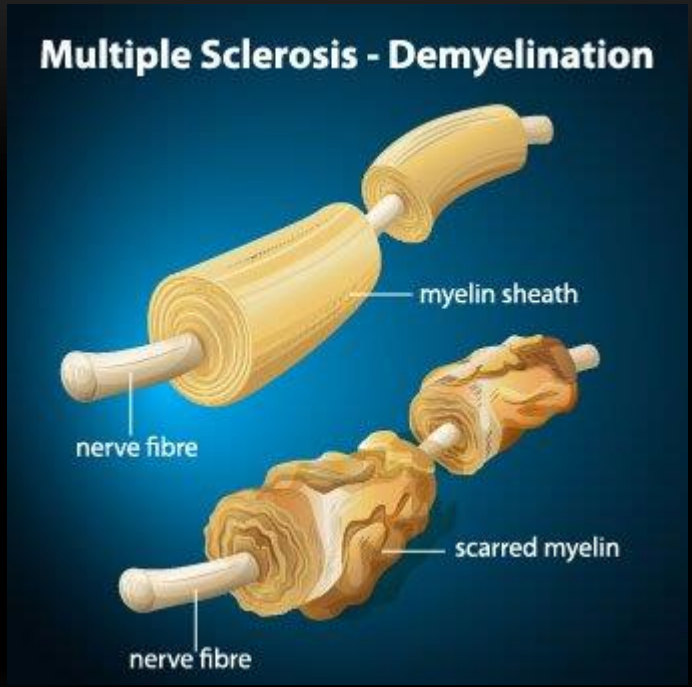
MULTIPLE SCLEROSIS

- Autoimmune disease
- Neurodegenerative disese
- Disease of the central nervous system - brain, spinal cord, and optic nerves
- Sclerosis means scarring
- Consequences - neurological disabilities



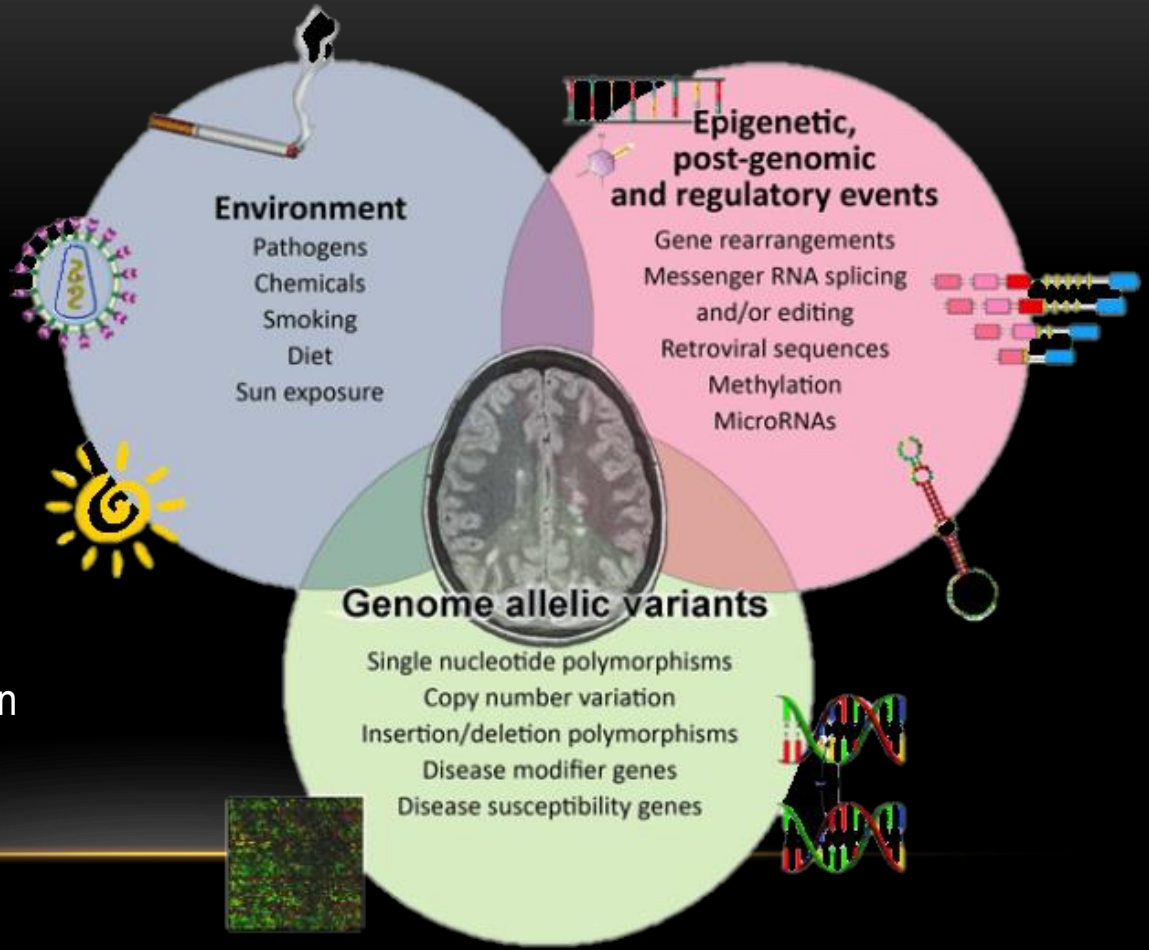
MULTIPLE SCLEROSIS

- Around 2.5 million patients worldwide
- The chances of developing the condition are highest between ages 20 and 50
- Women have about 3 times increased likelihood of developing MS compared with men



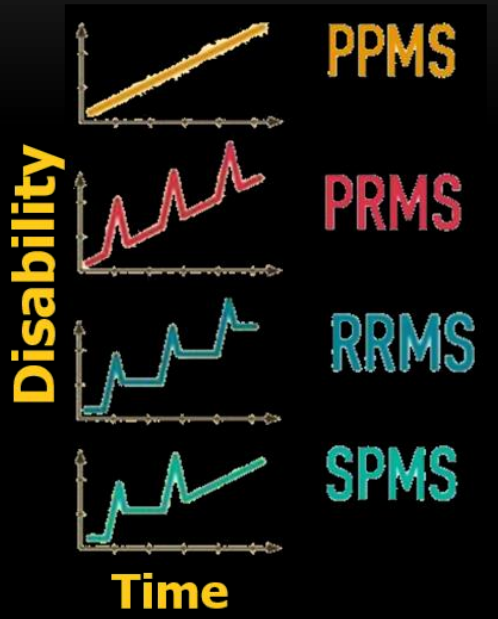
CAUSES

- The cause of MS is not clear
- Genetic susceptibility:
  - MS susceptible loci had been identified in the regions containing genes with immune, co-stimulatory, signal transduction functions and related to vitamin D function



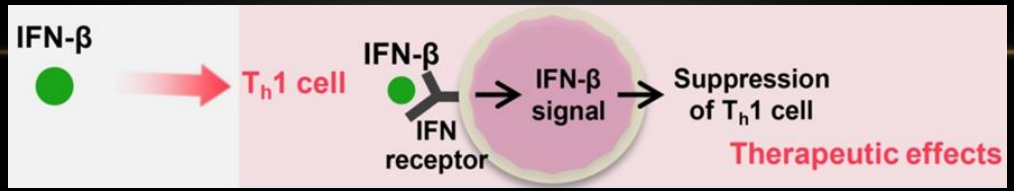
TYPES OF MS

- Primary-progressive (PPMS): 10-15%, MS slowly but steadily worsens.
- Progressive-relapsing (PRMS): 5%, the underlying disease steadily worsens. The patient has acute relapses, which may or may not remit.
- Relapsing-remitting (RRMS): 85%, symptoms flare during acute attacks, then improve nearly completely or "remit." This is the most common form of MS.
- Secondary-progressive (SPMS): Begins as relapsing-remitting type, then becomes progressive.
- In RRMS an inflammatory process predominates whereas in SPMS and PPMS a neurodegenerative process is more strongly expressed



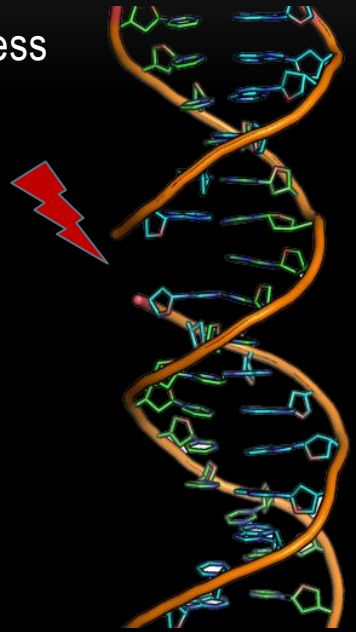
INTERFERON BETA THERAPY

- T cells - primary effectors of the autoimmune CNS inflammation
- Inside the CNS, activated T cells including T helper-1 (Th1) secrete proinflammatory cytokines
- Interferon β (IFNβ) is the first class of disease modifying therapies for MS
  - directly increases expression and concentration of anti-inflammatory agents
  - downregulates the expression of proinflammatory cytokines
- IFNβ treatment may
  - reduce the trafficking of inflammatory cells across the blood–brain barrier
  - increase nerve growth factor production
  - lead to a potential increase in neuronal survival and repair



DNA LESIONS IN MULTIPLE SCLEROSIS

- Reactive oxygen species (ROS), if produced in excess, lead to oxidative stress
- ROS have been implicated as mediators of demyelination in MS
- The brain is particularly vulnerable to oxidative damage due to:
  - elevated use of oxygen
  - low antioxidant levels
  - high phospholipid levels
- The excessiveness of ROS causes oxidative damage to DNA
- ROS attack the nitrogenous bases and the sugar phosphate backbone
  → single- and double-stranded DNA breaks



DNA LESIONS IN MULTIPLE SCLEROSIS

- Blood sera of MS patients contain DNA-hydrolysing antibodies
- Antigen-activated lymphocytes bear an unusual genomic stress
  - DNA damage-response signalling pathways are activated
  - A similar situation is in the lymphocytes of the MS patients
- There was found increased level of γ-H2AX and poly(ADP) ribose in nucleated blood cells of the MS patients.
- Alkaline single-cell electrophoresis or comet assay - a versatile and comparatively simple approach to detect DNA single-strand breaks

NITRIC OXIDE IN MULTIPLE SCLEROSIS

- Overproduction of nitric oxide (NO) by inducible NO synthase (iNOS) in glia

↓

subsequent lesions of mitochondrial DNA and demyelination

↓

a crucial step in the pathogenesis of MS

- Increased amount of nitrates and nitrites in the blood serum of MS patients

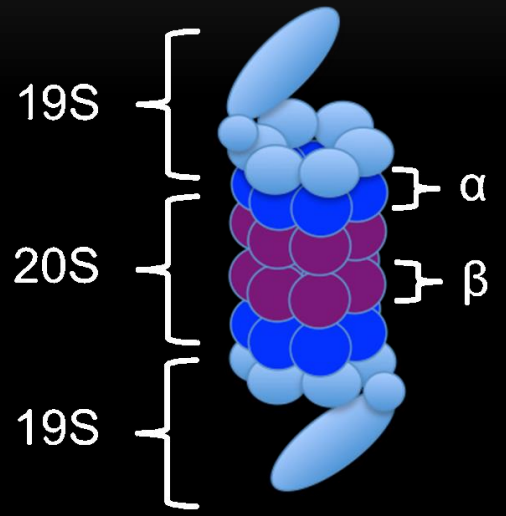
PROTEASOMES

- Accumulation of toxic protein aggregates can be observed in MS patients
- Ubiquitin-proteasome system (UPS) is responsible for degradation of intracellular proteins
- UPS is one of the key factors in regulation of the immune system
- MS ↔ dysfunction of UPS
- IFNβ therapy:
  - increased proteasome concentration
  - decreased proteasome activity
  - improved clinical course of MS

PSMA3, *PSMA6* AND *PSMC6*

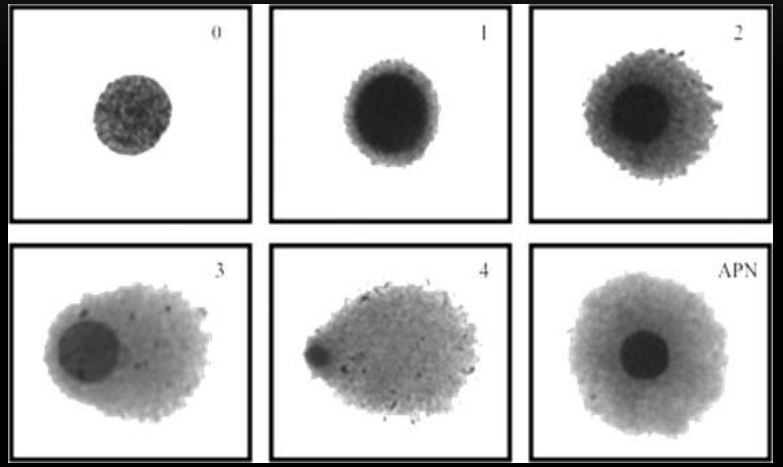
- Susceptibility of *PSMA3*, *PSMA6* and *PSMC6* genotypes involving rare alleles with autoimmune diseases
- Susceptibility of the *PSMA3* rs2348071 heterozygous genotype to multiple sclerosis
- Association of the *PSMA6* rs2277460 heterozygous genotype with MS in female group
- Association between *PSMC6* rs2295826 and rs2294827 loci and MS, for genotypes involving rare alleles

  (*PSMC6* rs2295826 and rs2294827 loci are in complete linkage disequilibrium)



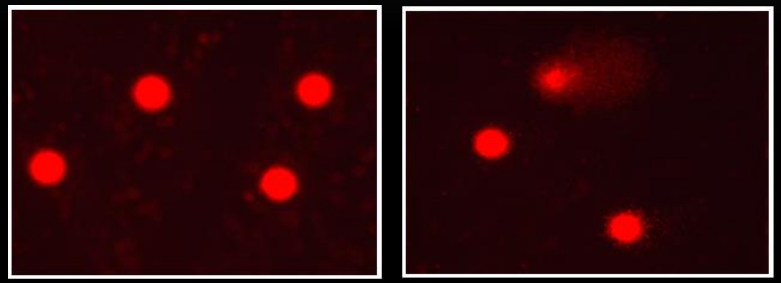
METHODS

- Comet assay
- Direct measurements of the NO radical by electron paramagnetic resonance (EPR) spectroscopy
- qPCR
  - *PSMA3*
  - *PSMA6*
  - *PSMC6*

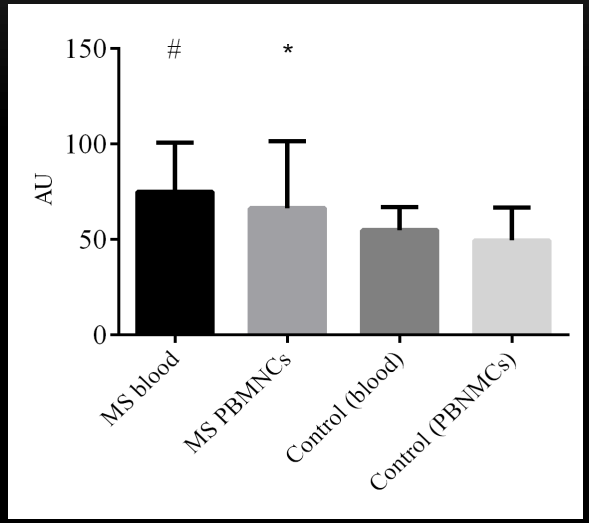


Comet types with the increasing damage. 0 – healthy cell and 4 extremely damaged cell. APN – apoptic nuclei (from: Collins et al. *Environ Mol Mutagen*. 1997;**30**, 139–146)

DNA INTEGRITY

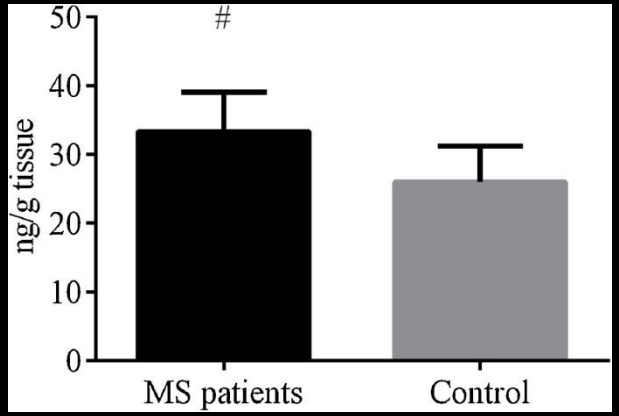


Left: Type 0 comets in the sample of a healthy patient. Right: Types 0, 1 and 3 comets in the sample of a patient with MS. Stained with EtBr. 40x magnification.



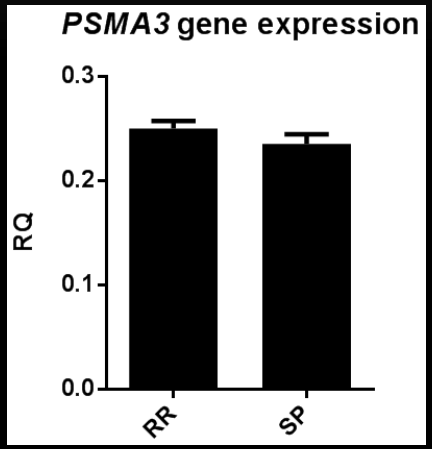
and *- $p < 0.05$ of whole blood and PBMNCs of MS group *vs.* control group

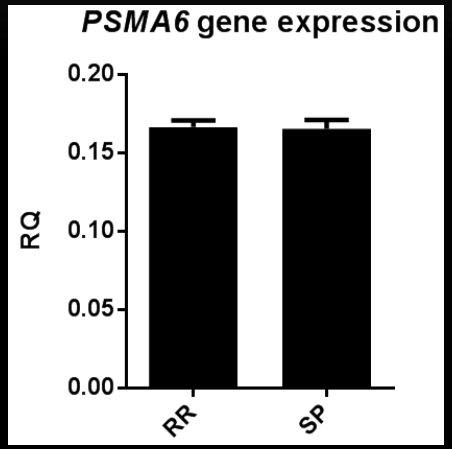
NO LEVEL IN BLOOD

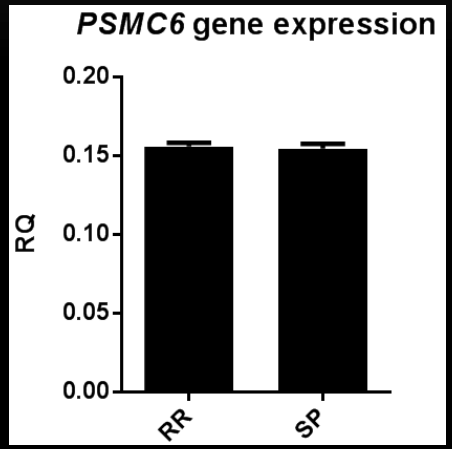


- p < 0.05 *vs.* control group

MS SUBTYPES

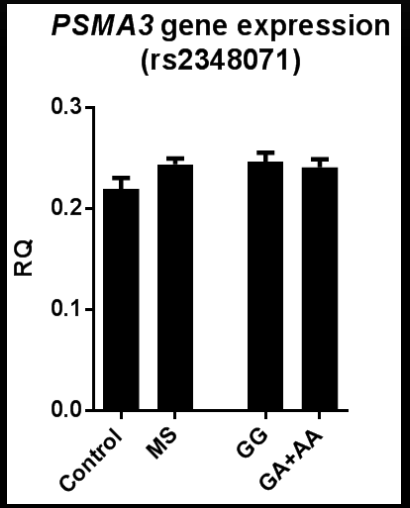


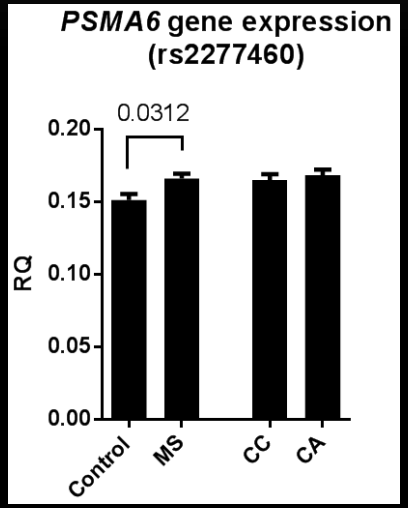


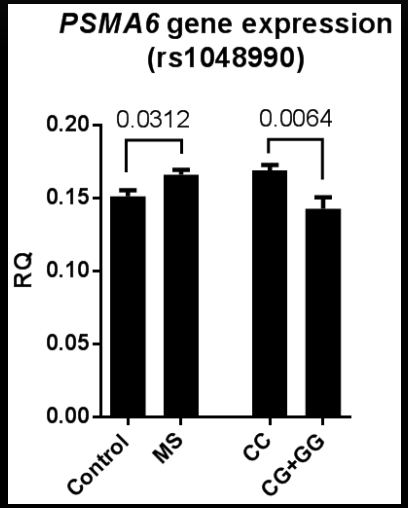


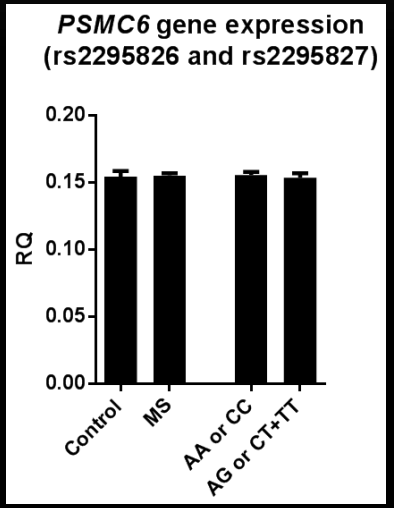
RR - Relapsing-remitting multiple sclerosis
SP - Secondary-progressive multiple sclerosis

GENOTYPES

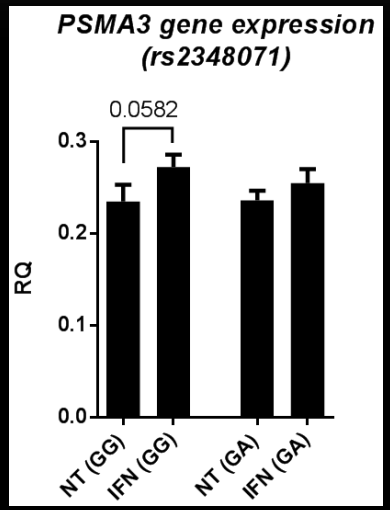

PSMA3 gene expression
(rs2348071)
RQ
0.3
0.2
0.1
0.0
Control
MS
GG
GA+AA

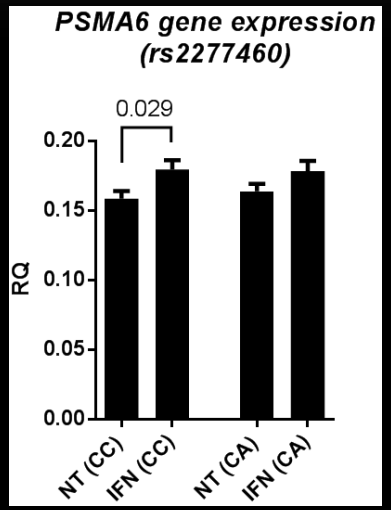

PSMA6 gene expression
(rs2277460)
0.0312
RQ
0.20
0.15
0.10
0.05
0.00
Control
MS
CC
CA

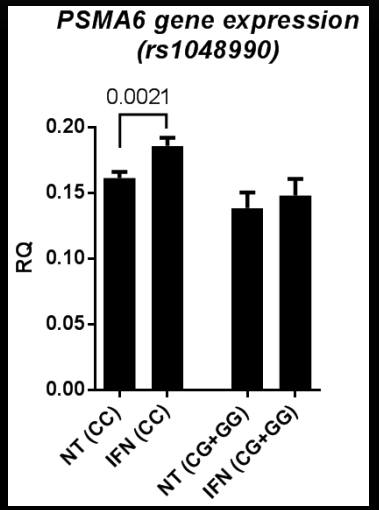

PSMA6 gene expression
(rs1048990)
0.0312
0.0064
RQ
0.20
0.15
0.10
0.05
0.00
Control
MS
CC
CG+GG

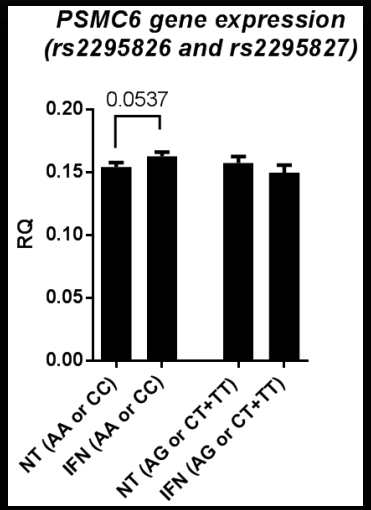

PSMC6 gene expression
(rs2295826 and rs2295827)
RQ
0.20
0.15
0.10
0.05
0.00
Control
MS
AA or CC
AG or CT+TT

INTERFERON β THERAPY AND GENOTYPES





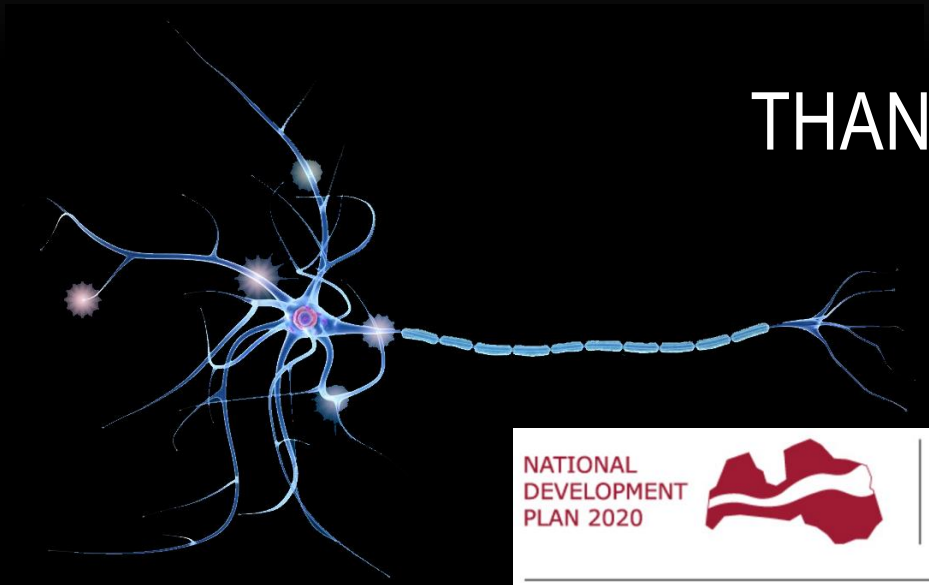




NT – no treatment; IFN - interferon β therapy

CONCLUSION

- Patients with MS have elevated level of DNA damage
- Patients with MS have significantly higher level of NO in the blood
- 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



THANK YOU!

NATIONAL DEVELOPMENT PLAN 2020

EUROPEAN UNION
European Regional Development Fund

INVESTING IN YOUR FUTURE

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