Identification of proteasome related genetic markers for multiple sclerosis in Latvian population

Jolanta Kalniņa*[1]*, *Natālija Paramonova*[1]*, Gatis Melkus*[1]*, Kristīne Dokāne*[1]*, Nikolajs Sjakste*[1,2]

[1]*Genomics and Bioinformatics, Institute of Biology of the University of Latvia, Miera str. 3, LV2169, Salaspils, Latvia;*
[2]*Faculty of Medicine, University of Latvia , Riga*

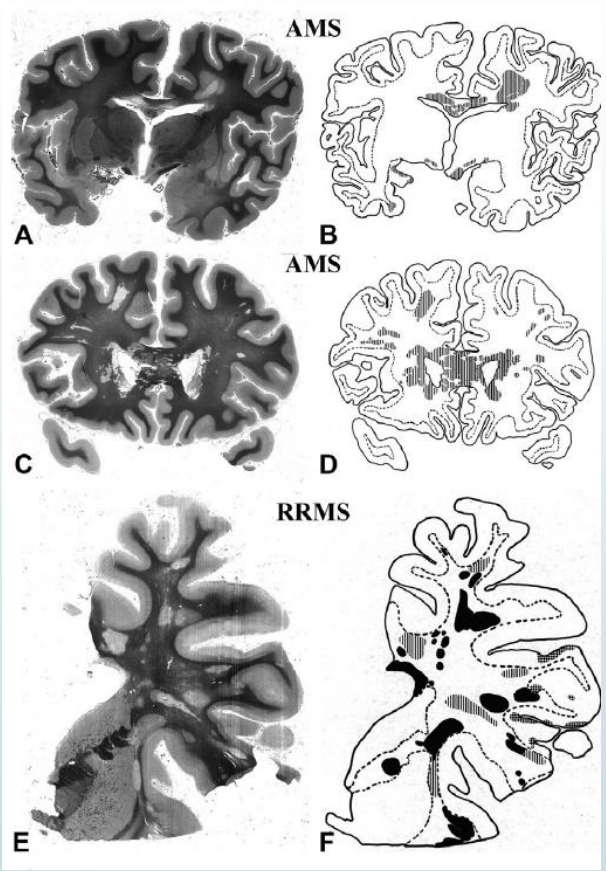
International Scientific Conference of Medicine
February 22, 2019
Riga

Acknowledgements

The study was funded from the UL research project ERAF SAM Nr. 1.1.1.1/16/A/016 project “Determination of proteasome-related genetic, epigenetic and clinical markers for multiple sclerosis”

Multiple sclerosis

- Most common chronic demyelinating disease of the CNS
- Multifactorial pathogenesis that includes a significant autoimmune component.
- Leading cause of permanent disability among young adults.
- 2.5 million cases worldwide, including ~2500 in Latvia.
- Studies thus far indicate a link between proteasomes and disease progression/treatment.



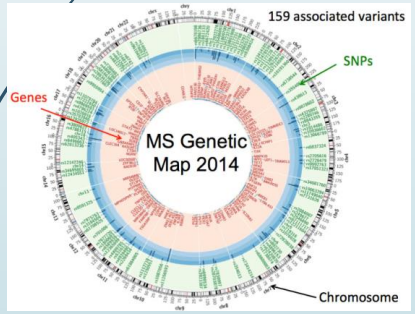
(Kutzelnigg et al. 2014)

Multiple sclerosis – A complex disease

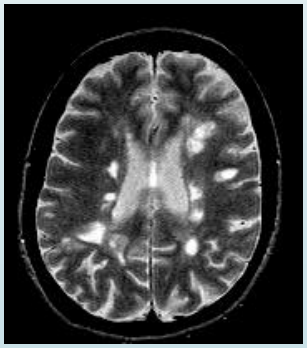
Genes

HLA-DRB1, HLA-DQB1,

IL-2R, IL-7R...(>140SNP)



GWAS - > 200 risk loci now identified



Environmental factors

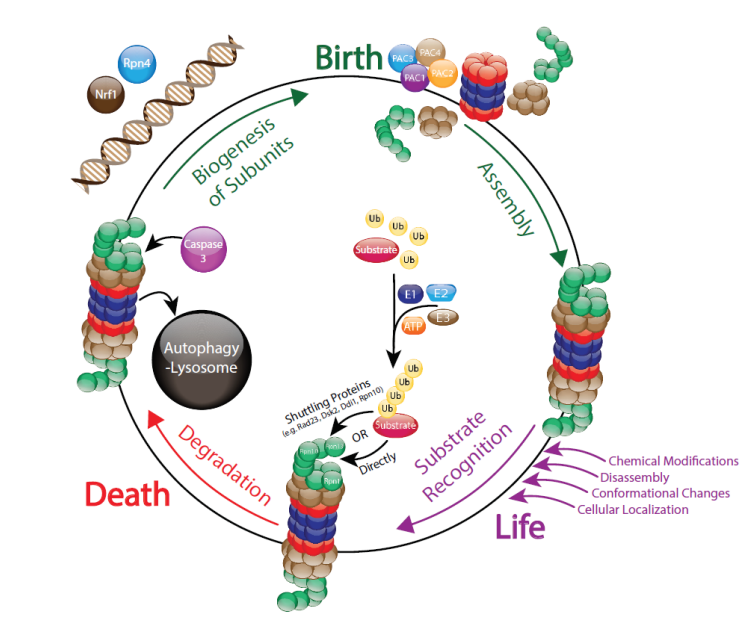
Infection with EBV

Sun exposure/ vitamin D status

Cigarette smoking

Giovannani and Ebers, 2007; Ebers, 2008; Handel *et al.*, 2010; Bagert, 2009; Pittas *et al.*, 2009; Wingerchuk DM, 2011; international Multiple Sclerosis Genetic Consortium, 2017.

Ubiquitin proteasome system (UPS)



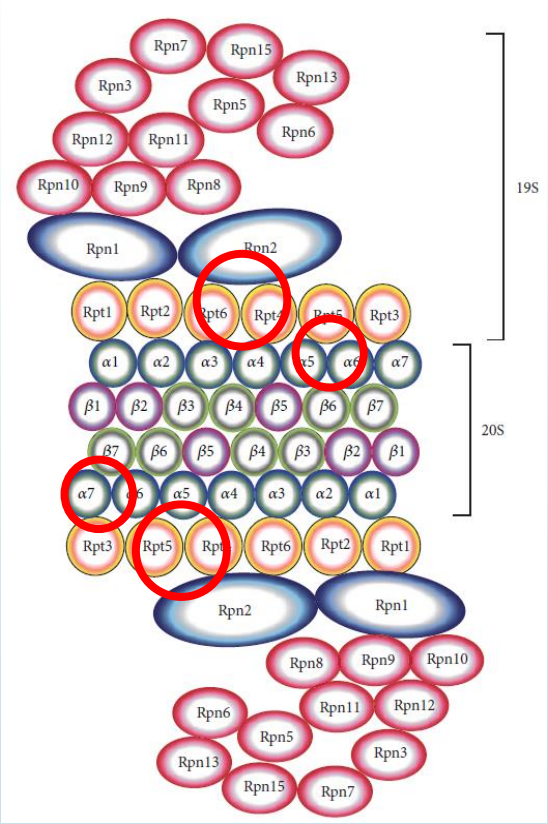
(Livneh et al. 2016)

- Is one of the main pathways of protein degradation in the human body, which is based on more than 80% of protein degradation in eucariots cells
- Knowledge of the UPS components that are specifically or preferentially involved in neurodegenerative disease will be critical in the development of targeted therapies which aim at limiting the accumulation of misfolded proteins without gross disturbance of this major proteolytic pathway (McKinnon and Tabrizi 2014, Dantuma and Bott 2014)
- The proteasome is a major autoantigen in multiple sclerosis
- The ubiquitin proteasoma system is important for immunity; its deregulation may affect the progression of MS (Mayo et al. 2002)

Proteasome genes

Eight different proteasome genes (PSMA6, PSMC6, PSMA3, PSME1, PSME2, PSMB5, PSMB11 and PSMC1) are found on the human 14 th chromosome, many of which have been previously studied in Latvia and other populations in relation to autoimmune diseases such as type 1 diabetes (Sjakste et al).. 2016), juvenile idiopathic arthritis (Sjakste et al. 2014), early asthma (Paramonova et al. 2014) and multiple sclerosis (Kalnina et al. 2014)

Polymorphisms of these genes are potentially usefull as biomarkers in evaluating the risk of these multifactorial diseases



(Gomes 2013)

PSMA6 single nucleotide polymorphisms (SNP) associations

Marker	Gene	Associations discovered	
		Disease	References
rs2277460	*PSMA6 (-110C/A)*	T2DM	Sjakste et al., 2007
		JIA, BA T1DM	Sjakste et al., 2009; Sjakste et al., 2014b; Paramonova et al., 2014b; Sjakste et al., 2016
rs1048990	*PSMA6 (-8C/G)*	T2DM	Sjakste et al., 2007; Barbieri et al., 2008
		CVD	Ozaki et al., 2006; Sjakste et al., 2007; Takashima et al., 2007; Ikeda et al., 2012; Heckman et al., 2013; Wang et al., 2013 Yu et al. 2016
		BA	Paramonova et al., 2014b;
		JIA BA Cancer T1DM	Sjakste et al, 2014b Paramonova et al., 2014b; Zemeckiene et al. 2015 Bachman et al., 2010; Sjakste et al., 2016

BA - bronchial asthma; T2DM and T1DM - type 2 and type 1 diabetes mellitus; JIA - juvenilais idiopatiskais artrits; CVD – cardiovascular disease; OB - obesity.

The aim of the study

- The aim of the current study was to investigate an association between *PSMA6* proteasome gene polymorphisms with MS and with treatment efficiency in MS patient's groups

Goup	Subgroup	Sex	Number (%)	Age ±SD(years)
Case	All pacients	All	280(100)	42,77±11,10
		Female	198(70,71)	43,21±10,87
		Male	82 (29,29)	41,51±11,65
	RRMS	All	187 (66,79)	38,80±9,50
		Female	131 (46,79)	39,42±9,47
		Male	56 (20,00)	37,30±9,50
	SPMS	All	93 (33,21)	50,56±9,88
		Female	67 (23,93)	50,61±9,53
		Male	26 (9,29)	50,42±10,92
Control	-	All	305 (100)	38,80 ± 10,54
		Female	179 (58,69)	38,78 ± 11,52
		Male	126 (41,31)	37,22 ± 9,33

RRMS – relapsing remitting multiple sclerosis, SPMS –secondary progressive multiple sclerosis, SD – standard deviation

Methods

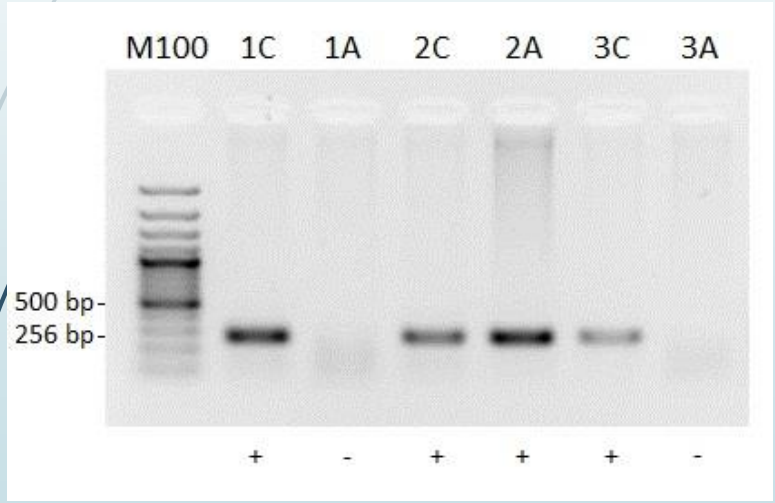
NEDA

- No evidence of disease activity defined as:[1,2]
  - No relapses
  - No sustained disability progression
  - No MRI activity
    - No new or enlarging T2 leasions
    - No Gd-enhancing lesions

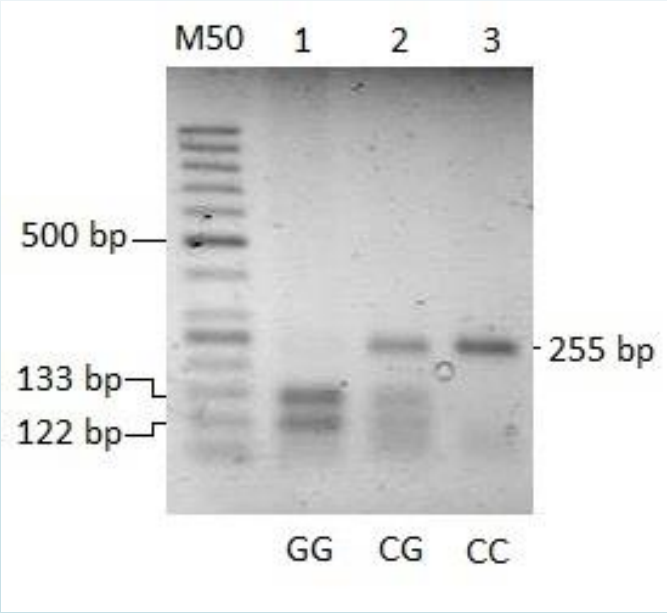
1.Havrdova E, et al. Lancet Neurol 2009; 8:254-260. 2.Giovannoni G, et al. Lancet Neurol 2011; 10:329-337.

Methods

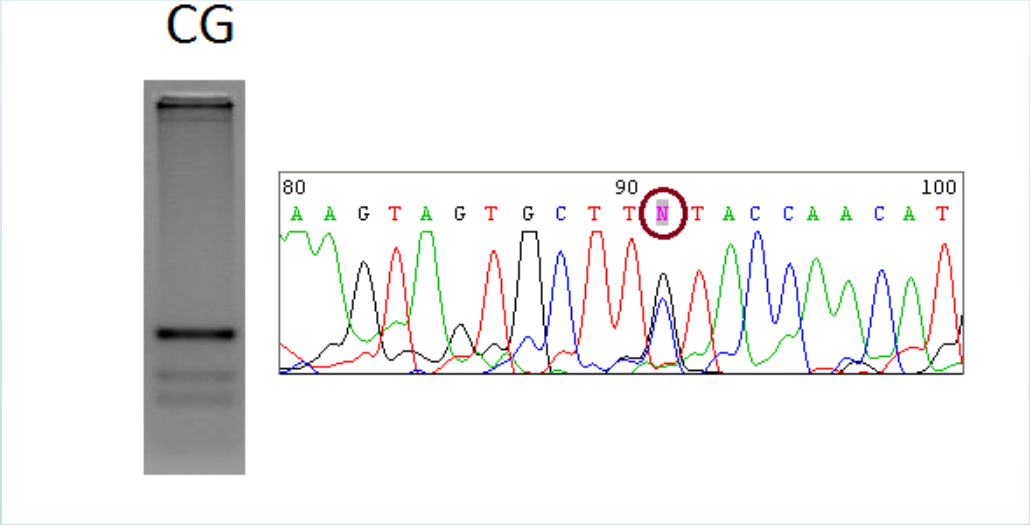
PSMA6 gene rs2277460 was analysed with *allele specific (ASA) polymerase chain reaction (*PCR)



PSMA6 gene rs1048990 was analysed with restriction site polymorphism (RESP) method



- We tested the results of genotyping by randomly selecting eight samples that we supplemented with amplixone sequencing in both directions with Applied Biosystems 3130 xl Genetic Analyzer (Thermo Fisher Scientific, USA), producing fully relevant results for restriction genotypes



Results

rs1048990 associations in MS case/control groups

SNP ID	Salīdzinātās grupas		Asociācijas dati				
	1. grupa (n)	2. grupa (n)	Ģenētiskais modelis	Riska faktoru skaits (%)		P_{χ} ($P_{\chi c}$)	OR [95% CI]
				1. grupa (%)	2. grupa (%)		
rs1048990	K (305)	MS (280)	G vs. C	57 (9,34)	50 (8,93)	**n.s.**	-
			CG+GG vs. CC	55 (18,03)	47 (16,79)	**n.s**	-
	K-F (179)	MS-F (198)	G vs. C	34 (9,50)	34 (8,59)	**n.s.**	-
			CG+GG vs. CC	33 (18,44)	32 (16,16)	**n.s**	-
	K-M (126)	MS-M (82)	G vs. C	23 (9,13)	16 (9,76)	**n.s.**	-
			CG+GG vs. CC	22 (18,25)	15 (18,29)	**n.s**	-
	K-F (179)	SPMS-F (67)	CG+GG vs. CC	33 (18,44)	11 (16,42)	**n.s**	-
	K-M (126)	SPMS-M (26)	G vs. C	23 (9,13)	3 (5,77)	**n.s.**	-
			CG+GG vs. CC	22 (18,25)	3 (11,54)	**n.s**	-

When looking at the breakdowns of rs1048990 alles and genotypes in case and control groups with χ 2 or Fisher direct tests, we did not reveal any statistically relevant ($p < 5 \times 10\text{-}2$) differences in compared group options monitoring/MS, control/SPMS, control/RRMS, each looking at all patients together, and separating women and men

K - kontroles, MS - multiplās sklerozes gadījumi, M - vīrieši, F - sievietes, RRMS - recidivējošā-remitējošā MS, SPMS - sekundārā progresīvā MS, P_{χ}- asociācijas statistiskais būtiskums pēc χ^2 testa, $P_{\chi c}$ - asociācijas būtiskuma korekcija ar Monte Karlo metodi, izmantojot 10 000 simulācijas, n.s. - nav būtisks ($p>0,05$)

Results of analysis of *PSMA6* rs2277460 Associations by Experiment Groups

SNP ID	Compared groups		Assosation data				
	1. group (n)	2. group (n)	Genetic model	Number of risk factors (%)		P_X (P_{XC})	OR [95% CI]
				1. grupa (%)	2. grupa (%)		
rs2277460	K (305)	MS (280)	A vs. C	44 (7,21)	51 (9,11)	n.s.	-
			CA vs. CC	44 (14,43)	51 (18,21)	n.s	-
	K-F (179)	**MS-F (198)**	**A vs. C**	22 (6,14)	42 (10,61)	**< 0.5**	1,72 [1,02-3,01]
			CA vs. CC	22 (12,29)	42 (21,21)	**< 0.5**	1,91 [1,10-3,41]
	C-F (179)	**SPMS-F (67)**	**A vs. C**	22 (6,14)	20 (14,93)	**< 0.002**	2,68 [1,39-5,11]
			CA vs. CC	22 (12,29)	20 (29,85)	**< 0.002**	3,02 [1,51-6,05]

C - control, MS – multiple sclerosis, M - male, F - female, RRMS – relapsing remitting MS, SPMS – secondary progressive MS, P_X- statistical relevance of the association following the χ 2 test
, P_{XC} - the association's materiality adjustment by Monte Carlo method, using 10,000 simulations
, n.s. – not relevant (p>0,05)

Distribution of *PSAM6* gene rs1048990 polymorphism allele and genotypes depending on the response to interferon-β therapy

	n (%)		Statistics		
	R	NR	Design	p_F	OR [95% CI]
Allele					
C	70 (94,59)	194 (87,39)	G vs C	**0,59**	2,53 [0,90–7,08]
G	**4 (5,41)**	**28 (12,60)**	G vs CC	**0,56**	2,69 [0,92–7,84]
Genotype					
CC	33 (89,19)	86 (77,48)	CG+GG vs CC	**0,69**	2,40 [0,78–7,42]
CG	**4 (10,81)**	**22 (19,82)**			
GG	–	**3 (2,70)**			

R - responder, NR – not responder; p_F - one-sided P value after Fisher's direct test, OR –odds ratio, n - number

Conclusion

- PSMA6 gene rs227746 rare allele and heterozigotes could detect an increased risk of MS in women and particularly secondary progressive multiple sclerosis (SPMS) in the female group

- Our results highlight a possible relationship between the Rs1048990 variation of PSMA6 gene rs1048990 and the response to interferon treatment for multiple sclerosis patients in the Latvian group

THANK YOU FOR YOUR ATTENTION!