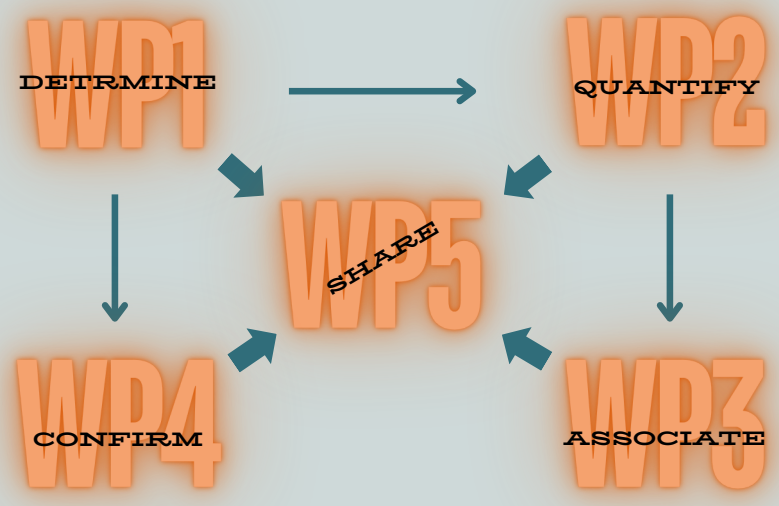




Welcome to FerroReg

♦ SCIENTIFIC RESULTS ♦

vol. 1



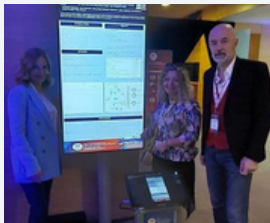
IN THIS NEWSLETTER YOU WILL FIND CONTENT AND LINKS:

- FerroRegs' conference presence
- Article publications
- Workshop
- Round Table

www.ferroreg.vin.bg.ac.rs

WP1. DETERMINE

Coordinated Genes–miRNAs expression determination, integrative analysis and validation in exosomes



Insight in miRNome of severe multiple sclerosis: Pilot study of distinctive relapse-onset MS phenotypes

Aleksandra Stankovic, Ivan Jovanovic, Evica Dincic, Slobodan Vojinovic, Ljiljana Stojkovic, Ana Djordjevic, Jovana Kuveljic, Maja Zivkovic

Members of the Ferroreg Project participated in the 17th World Congress on Controversies in Neurology - CONy - which was held on 23th-25th March 2023. in Dubrovnik, Croatia.

On that occasion, the preliminary results of the first research within the project were presented. The transcription level of micro RNA (miRNA), small molecules that carry out post-transcriptional regulation of gene expression, was determined. miRNA levels were determined by next-generation sequencing (NGS).

The crosstalk between circulating exosome carried miRNAs and ferroptosis related genes in multiple sclerosis

Jovana Kuveljic, Ivan Jovanovic, Maja Kosanovic, Natasa Macak, Tamara Djuric, Aleksandra Stankovic, Maja Zivkovic

Joint meeting of Austrian, Hungarian, Slovenian and Serbian Societies of Extracellular Vesicles took place on September 4th and 5th 2023. at the Medical University in Graz, Austria.

At this meeting, FerroReg team, as members of SrbEvs, have presented research on extracellular vesicles (EVs), their extraction and characterization. We have elaborated results of EVs miRNA cargo in relation to the MS course and severity.

FerroReg members attended workshops “Hands on gradient centrifugation” and “Hands on Flow cytometry: Set-up of the CytoFLEX platform for Extracellular Vesicle Measurement”, at the joint meeting Small New World 2.0.



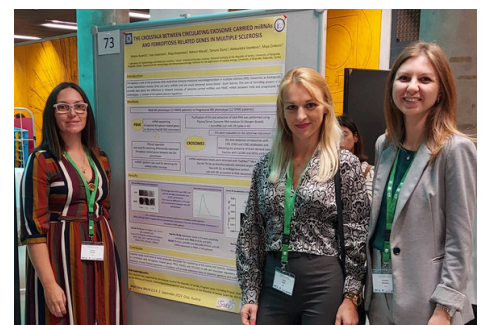
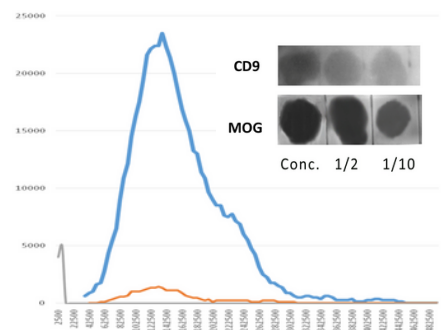
Small New World 2.0

4-5 September 2023, Graz, Austria

Joint Meeting of



Austrian Society for Extracellular Vesicles - ASEV
Hungarian Section for Extracellular Vesicles - HSEV
Slovenian Network for Extracellular Vesicles - SNA-EV
Serbian Society Extracellular Vesicles - SrbEVs



WP1. DETERMINE

Coordinated Genes–miRNAs expression determination, integrative analysis and validation in exosomes



International Journal of
Molecular Sciences

FerroReg
Research article
No.1

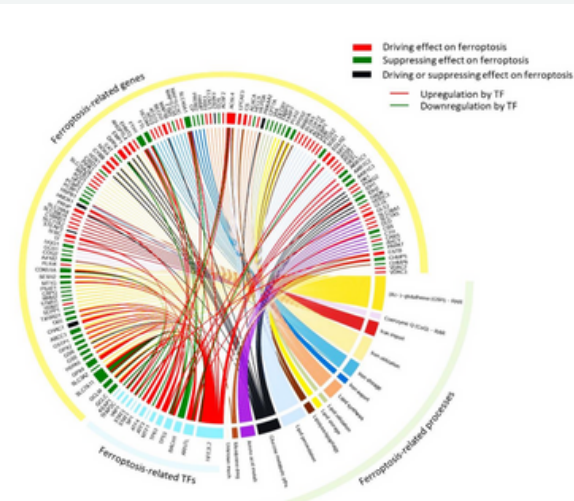


Article

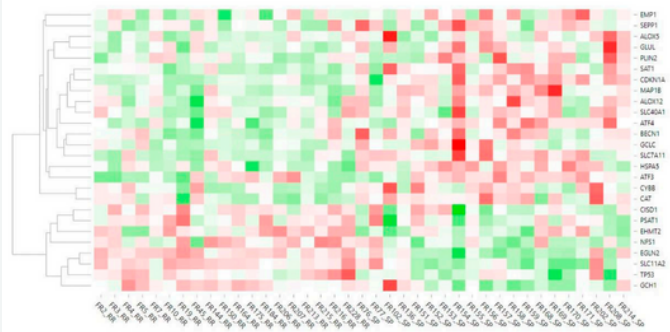
Targeted RNAseq Revealed the Gene Expression Signature of Ferroptosis-Related Processes Associated with Disease Severity in Patients with Multiple Sclerosis

Ljiljana Stojkovic ¹, Ivan Jovanovic ¹, Evica Dincic ^{2,3}, Ana Djordjevic ¹, Jovana Kuveljic ¹, Tamara Djuric ¹, Aleksandra Stankovic ¹, Slobodan Vojinovic ⁴ and Maja Zivkovic ^{1,*}

We have determined and analyzed the expression pattern/signature of a comprehensive set of 138 ferroptosis-related genes in highly homogenous groups of patients with relapsing-remitting (RR) (mild) and secondary progressive (SP) MS phenotypes.



The complete panel of selected genes with regard to the ferroptosis-related processes in which they are involved and their proposed effects on ferroptosis are presented in a Circos plot



Heatmap of gene expression data. The heatmap represents relative expression of ferroptosis-related DEGs across the samples and their clustering. Red color indicates overexpression while green color shows downregulation. White color shows the middle expression of the gene across the samples.

Analysis of the data obtained from the targeted sequencing of RR and SP MS patients led to the identification of 26 differentially expressed genes (DEGs) according to nominal p value. In the set of identified DEGs, 18 genes were upregulated while 8 genes were downregulated in SP patients compared to RR patients.

Our study integrates wide genetic signature and biochemical markers related to ferroptosis in the easily obtainable PBMCs of MS patients with clinical data and disease severity, thus providing novel molecular markers, which can complement disease-related changes in the brain and undergo further research as potential therapeutic targets.

For more results on the subject, discussion and conclusions
download our open-access research article



[Int. J. Mol. Sci. 2024. https://doi.org/10.3390/ijms25053016](https://doi.org/10.3390/ijms25053016)

WP2. QUANTIFY

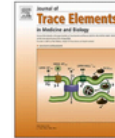
Quantification of the molecular indicators of key ferroptosis related processes



Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb



Essential trace element levels in multiple sclerosis: Bridging demographic and clinical gaps, assessing the need for supplementation

Aleksandar Stojsavljević^{a,*}, Jovana Jagodić^b, Sladan Pavlović^c, Evica Dinčić^{d,e}, Jovana Kuveljić^f, Dragan Manojlović^b, Maja Živković^f

More details you can find



J Trace Elem Med Biol. 2024.
<https://doi.org/10.1016/j.jtemb.2024.127421>

Serum Essential Trace Element Levels in Multiple Sclerosis Patients: A Comparative Analysis of Relapsing Remitting (RRMS), Secondary Progressive (SPMS), and Primary Progressive (PPMS) Multiple Sclerosis, measured in µg/L.

µg/L	RRMS n = 150	SPMS n = 52	PPMS n = 13
Cr	4.808 ± 1.191	4.852 ± 1.364	4.699 ± 1.132
Mn	0.270 ± 0.274	0.332 ± 0.306	0.337 ± 0.300
Co	0.586 ± 0.249	0.494 ± 0.194	0.638 ± 0.189 ^A
Cu	855.711 ± 204.102	852.406 ± 195.148	1033.351 ± 186.050 ^B
Zn	709.336 ± 246.269	729.444 ± 244.258	724.508 ± 292.047
Se	72.428 ± 17.223	67.838 ± 18.820	67.517 ± 22.122

Kruskal-Wallis ANOVA and Post-Hoc Dunn's test were used to seek differences between groups.

Data are expressed as Mean ± SD. ^A RRMS vs. PPMS progressive and ^B SPMS secondary progressive vs PPMS.

Significant difference at P < 0.05. n – number of patients.



FerroReg members attended the world's largest meeting dedicated to multiple sclerosis - ECTRIMS 2024 -

organized by European Committee for Treatment and Research in Multiple Sclerosis (<https://ECTRIMS.eu/>) on 18th-22th Sep 2024. in Copenhagen, Denmark.

Preliminary results regarding serum and plasma levels of molecular components related to ferroptosis were presented.

Correlation of multiple sclerosis severity, expression of ferroptosis genes and products of lipid peroxidation in patients

Maja Živković, Ivan Jovanović, Ljiljana Stojković, Milan Stefanović, Evica Dinčić, Slobodan Vojinović, Ana Đorđević, Jovana Kuveljić, Aleksandra Stanković

Relations of circulating long chain polyunsaturated fatty acids, lipid peroxidation product and FADS2 gene variants differentiate between patients with relapsing-remitting and secondary progressive multiple sclerosis

Ljiljana Stojković, Milan Stefanović, Slavica Ranković, Ana Đorđević, Jovana Kuveljić, Evica Dinčić, Aleksandra Stanković, Maja Živković

WP2. QUANTIFY

Quantification of the molecular indicators of key ferroptosis related processes



International Journal of
Molecular Sciences



Article

Circulatory Indicators of Lipid Peroxidation, the Driver of Ferroptosis, Reflect Differences between Relapsing–Remitting and Progressive Multiple Sclerosis

Ljiljana Stojkovic ^{1,*}, Ana Djordjevic ¹, Milan Stefanovic ¹, Aleksandra Stankovic ¹, Evica Dincic ^{2,3}, Tamara Djuric ¹ and Maja Zivkovic ^{1,*}

We analyzed circulatory molecular indicators of the main ferroptosis-related processes, lipid peroxidation and iron metabolism, to estimate their contribution to the clinical manifestation of MS and differences between relapsing–remitting (RRMS) and progressive (PMS) disease course.

Plasma and serum molecular indicators were quantified in patients with RRMS and PMS.

We found significantly decreased plasma levels of 4-HNE, total glutathione, and GSSG in patients with PMS compared to RRMS.

The results indicate changes in redox processes associated with ferroptosis, supporting the involvement of ferroptosis in key neuropathogenic mechanisms.

Molecular Parameters	RRMS n = 153	PMS n = 69	p-Value
Malondialdehyde (MDA, ng/mL)	98.94 ± 40.71	103.77 ± 67.50	0.89
4-Hydroxynonenal (4-HNE, pg/mL)	1848.24 ± 1115.45	1712.27 ± 1289.24	0.03
Hexanoyl-lys adduct (HEL, nmol/L)	12.68 ± 3.93	13.17 ± 3.77	0.39
Glutathione peroxidase 4 (GPX4, pg/mL)	2503.18 ± 1606.99	2442.53 ± 1349.04	0.83
* Total glutathione (GSH + GSSG, μmol/L)	44.46 ± 6.48	42.03 ± 11.26	0.006
* Reduced glutathione (GSH, μmol/L)	15.77 ± 7.30	16.35 ± 8.13	0.91
* Oxidized glutathione (GSSG, μmol/L)	14.35 ± 2.47	12.84 ± 3.41	0.003
* Reduced/oxidized glutathione (GSH/GSSG)	1.18 ± 0.69	1.34 ± 0.69	0.17
Iron (Fe, μmol/L)	14.63 ± 5.34	14.60 ± 4.63	0.85
Transferrin (Tf, g/L)	2.53 ± 0.36	2.42 ± 0.43	0.07
Ferritin (Ft, ng/mL)	65.71 ± 61.58	75.39 ± 116.71	0.56

RRMS—relapsing–remitting multiple sclerosis; PMS—progressive multiple sclerosis; n—number of patients; values of continuous parameters are presented as means ± standard deviations; p-value (Mann–Whitney U test); comparison PMS vs. RRMS; p-values < 0.05 were considered statistically significant; * analysis performed in a subgroup of 100 patients, 63 RRMS and 37 PMS.

For more results and thoughts on the subject, download our open-access research article

Int. J. Mol. Sci. 2024, <https://doi.org/10.3390/jms252011024>

WP3. ASSOCIATE

Identification of regulatory SNPs



CONGRESS OF THE SERBIAN GENETIC SOCIETY

October
2024 | 2 - 5
ZLATIBOR · SERBIA



Association of genetic variant rs3176326 residing in ferroptosis-related CDKN1A with multiple sclerosis severity

Djuric Tamara, Stefanovic Milan, Kuveljic Jovana, Djordjevic Ana, Kolakovic Ana, Dincic Evica, Zivkovic Maja

FerroReg members attended the 7th Congress of the Serbian Genetic Society, held on October 2nd-5th 2024, on Zlatibor, Serbia.

Preliminary results regarding SNPs identification were presented. Public databases available for scientific research (GTEx, Regulome, Haploreg, ENSEMBLE, Encode, Genome browser, Phenoscanner) and recent literature findings have been implemented to prioritize the most relevant non-coding SNPs, suggested to be involved in the regulation of target genes transcription or function.

We have genotyped 20 SNPs on 1000 MS patients and results are in publishing process.

Ferroptosis-Related Processes Gene Expression and Gene Variants: Relevance for Disease Severity in Patients with Multiple Sclerosis

Djuric Tamara, Zivkovic Maja



WP4. CONFIRM

Functional validation of exosome and miRNAs effects



2nd MOVE Symposium

8-11 October 2024, Belgrade, Serbia

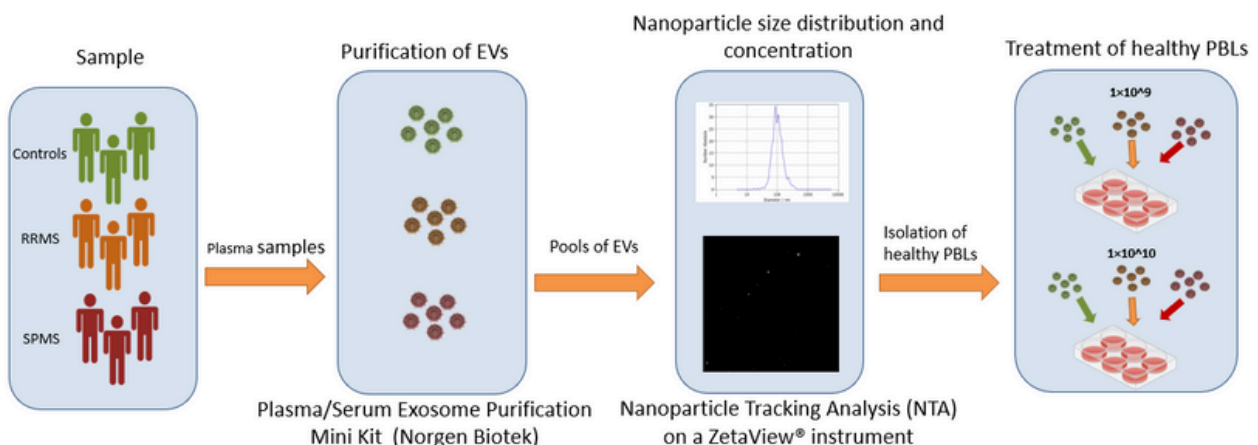
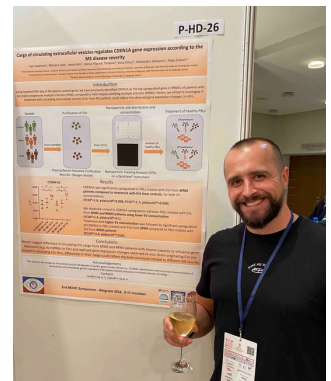
Cargo of circulating extracellular vesicles regulates CDKN1A gene expression according to the MS disease severity

Ivan Jovanovic, Mariana Seke, Ivana Kolic, Jelena Filipovic Trickovic, Evica Dincic, Aleksandra Stankovic, Maja Zivkovic

As members of Serbian Society for Extracellular Vesicles, SrbEVs, FerroReg team attended 2nd MOVE Symposium organized by 8 European National EV Societies on 8th-11th October 2024 in Belgrade, Serbia

Using targeted RNA-Seq of ferroptosis-related genes we have previously identified top upregulated genes in PBMCs of patients with secondary progressive (SPMS) compared to mild relapse-remitting (RRMS) MS. Herein, we investigated if treatment with circulating extracellular vesicles (EVs) from MS patients could induce the observed gene expression changes in healthy peripheral blood lymphocytes (PBLs), *in vitro*.

Results of this experiment are soon to be published.



WP5. SHARE

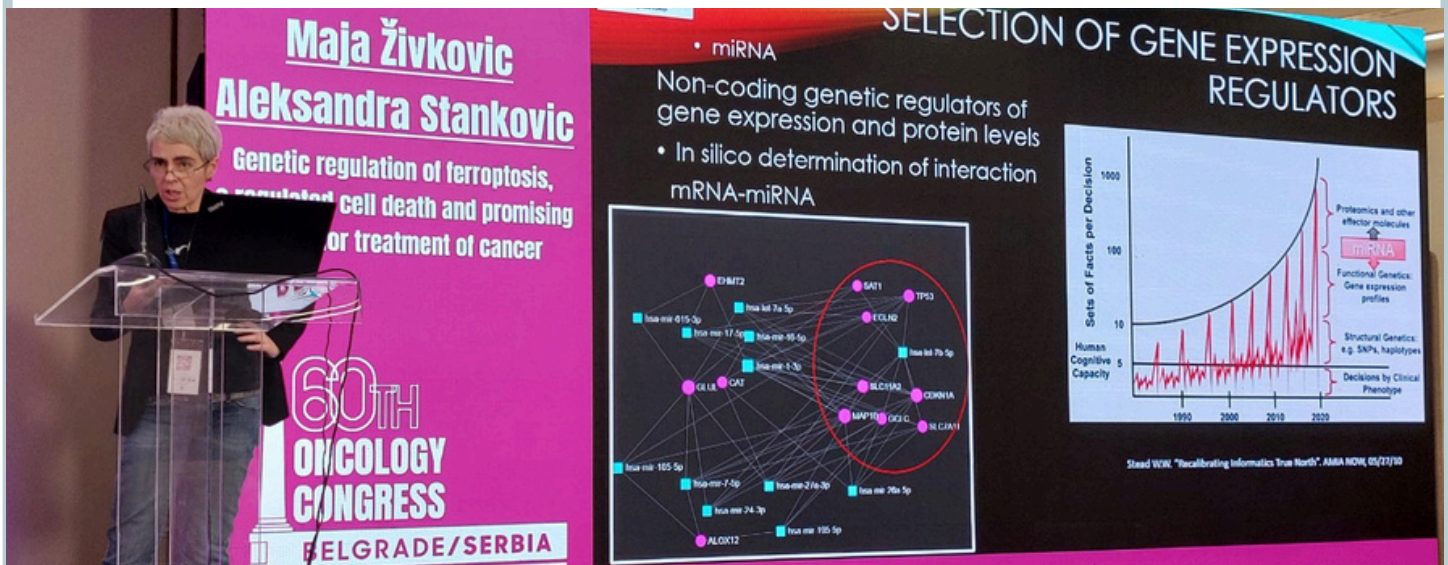
Communication, Dissemination & Exploitation

XIX KONGRES NEUROLOGA SRBIJE
Niš, Srbija. 21. 9. – 24. 9. 2023



FerroReg principal investigator dr Maja Živković presented the FerroReg project at the XIX Neurology congress held on September 21st - 24th 2023. in Niš, Serbia. The congress was organized by the Serbian Society of Neurologists, gathering eminent neurology and pharma experts from the country and the region.

It was of great importance for FerroReg team to present the project to national and international medical, pharmaceutical and scientific stakeholders from the field of multiple sclerosis and neurodegenerative diseases.



FerroReg team has been honored to be invited to participate at the 60th Oncology Congress, organized by the Oncology Section of Serbian Medical Society, held from November 29th to December 2nd, 2023 in Belgrade, Serbia. This is one of the oncology congresses with the longest tradition in Serbia and region, with diverse, multidisciplinary scientific program and speakers of great international impact.

FerroReg PI dr Maja Živković presented the FerroReg project to the oncology-related national and international medical and scientific stakeholders, also its preliminary results and principle role of ferroptosis in cancer and in neurodegenerative/neuroinflammatory diseases.

WP5. SHARE

Communication, Dissemination & Exploitation

WORKSHOP

“Targeted RNA sequencing using the Illumina platform and analysis of upstream regulators”



FerroReg Project organized a workshop, conducted by dr Ivan Jovanović at the Second Congress of Molecular Biologists of Serbia - CoMBos2 - which was held from October 6th to 8th, 2023.

The workshop consisted of a theoretical and practical part. During the theoretical part of the workshop, the participants of the course were introduced to the new generation sequencing technology (NGS), which is based on massive parallel sequencing.

Also, the principles and methodology of panel generation for determining the expression of candidate genes by targeted RNA sequencing was presented.

Special emphasis was placed on quality control and data analysis during primary, secondary and tertiary processing. At the end, the concept and goal of identifying upstream regulators and their importance in the interpretation of the obtained profiles of differentially expressed genes were clarified.

In the second part of the workshop, through practical work on the computer, the course participants went through all the steps of the explained analysis following simple “step-by-step” instructions.

At the following discussion members of the FerroReg team answered questions posed by the course participants.

An anonymous survey concluded that the course was very successful and that the participants were satisfied with both, the lecturers and the newly acquired knowledge.

WP5. SHARE

Communication, Dissemination & Exploitation



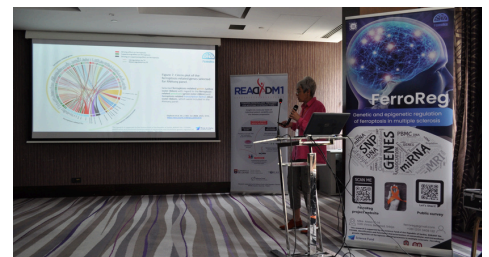
ROUND TABLE

Belgrade Bioinformatics Conference

FerroReg Project is funded by the Science Fund of the Republic of Serbia. Together with other two projects from the Program "Sharing Ideas", we organized round table at the 5th Belgrade Bioinformatics Conference - BelBi2024 - held on 17th-20th June 2024. in Belgrade, Serbia.

PI of the FerroReg Project dr Maja Živković presented project goals, current results and impact. The panel was attended by international scientific, technological, medical and governmental stakeholders.

Afterwards, there was a discussion about projects, the methodology used, strengthening of the collaboration between teams and future challenges.



◆ ACKNOWLEDGEMENTS ◆



„VINČA” Institute of Nuclear Sciences –
National Institute of the Republic of Serbia,
University of Belgrade

Laboratory for radiobiology and molecular genetics



The Medical Faculty of
the Military Medical Academy



Institute for Medical Research,
National Institute of the Republic of Serbia,
University of Belgrade

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functional characterization of extracellular and intracellular genetic
regulators of ferroptosis related processes in multiple sclerosis” -

FerroReg

