Trends in **Molecular Biology** • Special issue

Abstract Book

CoMBoS2

2nd Congress of Molecular Biologist of Serbia

ISBN-978-86-82679-15-8

Belgrade • 2023

CoMBoS2 – the Second Congress of Molecular Biologists of Serbia, Abstract Book – Trends in Molecular Biology, Special issue
06-08 October 2023, Belgrade, Serbia
Online Edition
https://www.imgge.bg.ac.rs/lat/o-nama/kapacitet-i-oprema/istrazivacka-delatnost
https://indico.bio.bg.ac.rs/e/CoMBoS2

IMPRESSUM

PUBLISHER:
Institute of Molecular Genetics and Genetic Engineering (IMGGE), University of Belgrade

FOR THE PUBLISHER:
Dr. Sonja **Pavlović**

EDITOR:
Dr. Zorana **Dobrijević**

EDITORIAL REVIEW BOARD:
Prof. Dr. Silvana **Andrić**
Dr. Valentina **Ćirković**
Dr. Ivica **Dimkić**
Prof. Dr. Branko **Jovčić**
Prof. Dr. Gordana **Matić**
Ass. Prof. Dr. Milena **Milutinović**
Dr. Aleksandra **Stanković**
Dr. Nemanja **Stanisavljević**
Dr. Maja **Stoiljković**

EDITOR IN CHIEF:
Prof. Dr. Dušanka **Savić-Pavićević**

DESIGN:
Ivan **Strahinić**

Institute of Molecular Genetics and Genetic Engineering (IMGGE),
University of Belgrade
Belgrade, 2023
ISBN 978-86-7078-173-3

CoMBoS2

Content

RECONSTITUTION OF NON-CARRIER, HETEROZYGOUS AND HOMOZYGOUS PROTHROMBIN BELGRADE MUTATION CARRIER PLASMA USING RECOMBINANT PROTEINS

Sofija Dunjic Manevski,[1] Marija Cumbo,[1] Maja Gvozdenov,[1] Branko Tomic,[1] Dusan Usjak,[1] Valentina Djordjevic[1]

[1]*Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia*

Introduction: The prothrombin Belgrade variant (c.1787G>A, p.Arg596Gln) is a rare mutation found in Serbia, Japan, China, America, India and leads to antithrombin resistance. Prothrombin Belgrade mutation influences thrombin-antithrombin interactions and leads to impaired inactivation of mutated thrombin. Also, it affects sodium binding site in thrombin, which is important for switching from fast thrombin configuration (coagulant properties) to slow configuration (anticoagulant properties). It has only been found in a heterozygous state, which could mean that homozygous carriers are incompatible with life. By using prothrombin (FII) deficient plasma, we could reconstitute plasma of wild type, heterozygous and homozygous carrier, which could give more insight into the mechanism of this mutation.

Methods: Recombinant wild type and mutated prothrombin were generated by transient transfection in HEK293T cell line. Western blot analysis was performed to test the efficiency of transfection. Human Prothrombin ELISA (Nordic BioSite, Sweden) was used in order to measure recombinant prothrombin concentration. Overall Hemostasis Potential (OHP) assay was performed to assess recombinant protein activity. Recombinant wild type and mutated prothrombin were added to FII deficient plasma (Siemens, Germany) in order to create reconstituted plasma, in the final concentration of 0.1 mg/mL, as it is approximately the level of prothrombin in human plasma.

Results: Reconstituted plasma samples that correspond to non-carrier, heterozygous carrier, and homozygous mutation carrier plasma were reconstructed. Recombinant proteins tested by OHP assay were functional.

Conclusion: Reconstituted plasma samples allow us to examine the mechanism of prothrombin Belgrade mutation in various assays and in homozygous form as well.

Keywords: Transfection; Recombinant protein; Prothrombin; Mutation; Prothrombin Belgrade

Acknowledgments: This study was supported by the Pro-Belgrade - Could Prothrombin Belgrade mutation affect blood vessels? A new insight into thrombosis mechanism, Serbian Science and Diaspora Collaboration Program: Knowledge Exchange Vouchers, Science Fund of the Republic of Serbia, 6476784, 2020-2022.