Author's response to reviews

Title: GSTM1*0/0 genotype and combined GSTM1*0/GSTA1*A genotype are associated with risk of cardiovascular death among hemodialysis patients

Authors:

Sonja Suvakov (sonja.suvakov@gmail.com)
Tatjana Damjanovic (damtanja@beotel.rs)
Tatjana Pekmezovic (pekmezovic@sezampro.rs)
Jovana Jakovljevic (jovanavjakovljevic@gmail.com)
Ana Savic-Radojevic (ana.savicradojevic@med.bg.ac.rs)
Marija Pljesa-Ercegovac (marijaercegovac@med.bg.ac.rs)
Slavica Radovanovic (s.radovanovic@sezampro.rs)
Dragan Simic (dvsimic@yahoo.com)
Steva Pljesa (spljesa@gmail.com)
Jasmina Mimic-Oka (okasn@rcub.bg.ac.rs)
Nada Dimkovic (dim@eunet.rs)
Tatjana Simic (tatjanasimic@med.bg.ac.rs)

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Dear Editor Maria Merrie Jul Ladag,

Thank you for your email. This is to confirm that we made changes you requested:

1. Name of Ethical Committee
   On page 7 “Study protocol was approved by the Institutional Review Board” is replaced with “Study protocol was approved by the University of Belgrade Faculty of Medicine Ethic Committee”

2. Consent
   On page 7 a line “All the participants provided written informed consent.” Is added.

3. Methodology/Genotyping
   On page 7 a section about GST genotyping is added
   “Genomic DNA was isolated from whole blood using the QIAGEN QIAmp kit (Qiagen, Inc., Chatsworth, CA).
   GSTA1 C-69T polymorphism was determined by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) [17]. Used primers were GSTA1 C-69T forward: 5’-TGTTGATTGTTTGCCTGAAATT-3’ and
GSTA1 C-69T reverse, 5’-GTTAAACGCTGTCACCCGTCCT-3’. Presence of restriction site resulting in two fragments (481bp and 385bp) indicated mutant allele (GSTA1*B/B) and if GSTA1*A/B polymorphism incurred it resulted in one more fragment of 96bp.

GSTM1 genotyping was performed by multiplex PCR [17]. Used primers were GSTM1 forward: 5’-GAACTCCCTGAAAAGCTAAAGC-3’ and GSTM1 reverse: 5’-GTTGGGCTCAAATATACGGTG-3’. Exon 7 of CYP1A1 gene was co-amplified and used as an internal control using following primers: CYP1A1 forward: 5’-GAAGTCCACTTCAGCTGTCT-3’ and CYP1A1 reverse: 5’-CAGCTGCATTGGGAAGTGCTC-3’. The presence of GSTM1-active genotype was detected by the band at 215bp, since the assay does not distinguish heterozygous or homozygous wild type genotypes.

GSTP1 Ile105Val polymorphism was analyzed using PCR-RFLP method [17]. Used primers were: GSTP1 Ile105Val forward: 5’-ACCCCAGGGCTCTATGGGAA-3’ and GSTP1 Ile105Val reverse: 5’-TGAGGGCAACAAGAGCCCT-3’. Presence of restriction site resulting in two fragments (91bp and 85bp) indicated mutant allele (Val/Val) while if Ile/Val polymorphism incurred it resulted in one more fragment of 176bp.

GSTT1 genotyping was performed by multiplex PCR [17]. Used primers were GSTT1-forward: 5’-TCACGGGATCATGGCCAGCA-3’ and GSTT1-reverse: 5’-TCACGGGATCATGGCCAGCA-3’. The assay does not distinguish between heterozygous or homozygous wild type genotypes, therefore the presence of 480bp bands was indicative for GSTT1-active genotype.”

Best regards,

Tatjana Simic, MD, PhD
Full Professor of Biochemistry
Institute of Medical and Clinical Biochemistry
Faculty of Medicine, University of Belgrade
Pasterova 2, 11000 Belgrade, Serbia
Phone: +381 11 3643250
Fax: +381 11 3643270
E-mail: tatjanasimic@med.ac.bg.rs