MUTATION ANALYSIS IN *SMAD2, TGFβ2,* AND *SMURF2* GENES IN PATIENTS WITH THORACIC AORTIC ANEURYSM AND DISSECTION

ANALISIS MUTASI GEN SMAD2, TGFβ2, DAN SMURF2 PADA PASIEN DENGAN THORACIC AORTIC ANEURYSM AND DISSECTION

THESIS

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DECLARATION

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgement is made in the text.

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CONTENTS

ABBREVIATIONS

ACTA2	Actin Smooth Muscle Alpha 2
BAV	Bicuspid Aortic Valve
COL3A1	Collagen type 3 Alpha 1
ECM	Extracellular Matrix
EDS	Ehler-Danlos Syndrome
FBN1	Fibrillin 1
FTAAD	Familial Thoracic Aortic Aneurysm and Dissection
HRM	High Resolution Melting
LAP	Latency Associate Propeptide
LDS	Loeys-Dietz Syndrome
LLC	Large Latent Complex
LTBP	Latent TGFβ Binding Protein
MFS	Marfan Syndrome
MMP	Matrix Metalloproteinase
MYH11	Myosin Heavy Chain 11
MYLK	Myosin Light Chain Kinase
PDA	Patent Ductus Arteriosus
SLC	Small Latent Complex
SMAD	Mothers against decapentaplegic
SMURF2	Smad Ubiquitination Regulatory Factor 2
TAA	Thoracic Aortic Aneurysms

TAAD	Thoracic Aortic Aneurysms and Dissection
TGFβ	Transforming Growth Factor Beta
TGFβR	Transforming Growth Factor Beta Receptor
VSMC	Vascular Smooth Muscle Cell

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX

ABSTRACT

Background: Thoracic aortic aneurysm and dissection (TAAD) is one of 15th most leading cause of the death in USA and of the silent killer in the world. Several genes associated with TAAD have been recognized, i.e. *FBN1, ACTA2, TGFβR1, TGFβR2, MYH11, MYLK* and *SMAD3*. However, many cases of familial TAAD have not been found for the mutation in those genes. The other genes that might be had association with TAAD are *SMAD2, TGFβ2* and *SMURF2*.

Methods: Three hundred sixty five patients with TAAD and related disorders, who did not carry any mutation in *FBN1, TGFβR1, TGFβR2, ACTA2* and *MYH11* were included. Mutation screening of the gene variants in *SMAD2, TGFβ2* and *SMURF2* were done by using high resolution melting (HRM) technique. The aberrant sample patterns on the curve analysis then were sequenced. Confirmation of the mutation was done by comparing the HRM curves between patients and the healthy control. The pathogenicity potency of the mutation was predicted by using mutation prediction software SIFT and align GVGD, which integrated in Alamut software. Phosphorylation/glycosylation site was predicted by YinOYang software.

Results: Three patients were found to carry *SMAD2* mutation, i.e., c.6_8del, c.1346T>C, c.1369G>A. One mutation, c.1369G>A was predicted to increase phosphorylation, and the other mutations predicted to loss of phosphorylation/glycosylation site. All of the *SMAD2* mutations were not found in the control. One patient was found to carry nonsense mutation of *TGFβ2,* c.547C>T (Gln183X). Two missense mutation of *TGFβ2,* c.272G>A and c.703C>G were found in four patients and have been registered in SNP databases with frequency less than 1%. All mutations in *SMAD2* and *TGFβ2* were predicted to be pathogenic. No mutation was found in *SMURF2* gene.

Conclusion: Three novel mutations were found in *SMAD2* gene and one novel mutation was found in *TGFβ2* in TAAD patients who did not carry any mutation in *FBN1, TGFβR1, TGFβR2, ACTA2* and *MYH11.*

Keywords: Thoracic aortic aneurysm and dissection, mutation, *SMAD2, TGFβ2, SMURF2*

ABSTRAK

Latar belakang: *Thoracic aortic aneurysm and dissection* (TAAD) adalah satu dari 15 teratas penyebab kematian di Amerika Serikat dan merupakan *silent killer* di dunia. Beberapa gen yang berkaitan dengan TAAD sudah dikenali yaitu *FBN1, ACTA2, TGFβR1, TGFβR2, MYH11, MYLK* dan *SMAD3.* Namun, pada banyak kasus TAAD familial tidak ditemukan mutasi pada gen-gen tersebut. Gen lain yang kemungkinan berkaitan dengan TAAD adalah *SMAD2, TGFβ2* dan *SMURF2*.

Metode: Sampel DNA diambil dari 365 pasien TAAD dan penyakit terkait, yang tidak memiliki mutasi di gen *FBN1, TGFβR1, TGFβR2, ACTA2* dan *MYH11*. Skrining mutasi gen pada *SMAD2, TGFβ2* dan *SMURF2* dilakukan dengan teknik *high resolution melting (HRM).* Sampel dengan pola berbeda pada analisa kurva dilakukan sekuensing. Konfirmasi mutasi dilakukan dengan membandingkan kurva HRM antara pasien dan kontrol sehat. Potensi patogenitas mutasi diprediksi dengan software SIFT dan *align* GVGD, yang tergabung dalam software Alamut. Situs fosforilasi/glikosilasi diprediksi dengan software YinOYang.

Hasil: Mutasi c.6_8del, c.1346T>C, c.1369G>A pada gen *SMAD2* ditemukan pada 3 pasien yang berbeda. Mutasi c.1369G>A diprediksi meningkatkan fosforilasi dan dua mutasi lainnya kehilangan situs fosforilasi/glikosilasi. Semua mutasi *SMAD2* tidak ditemukan pada kontrol. Mutasi c.547C>T (Gln183X) pada gen *TGFβ2* ditemukan pada satu pasien. Dua mutasi pada gen *TGFβ2,* c.272G>A dan c.703C>G ditemukan pada empat pasien dan telah terdaftar di database SNP dengan frekuensi kurang dari 1%. Semua mutasi pada *SMAD2* dan *TGFβ2* tersebut diprediksikan patogenik. Tidak ditemukan mutasi pada gen *SMURF2*.

Kesimpulan: Tiga mutasi novel ditemukan pada gen *SMAD2* dan satu mutasi novel ditemukan pada gen *TGFβ2* pada pasien TAAD yang tidak memiliki mutasi pada gen *FBN1, TGFβR1, TGFβR2, ACTA2* dan *MYH11.*

Kata kunci: *Thoracic aortic aneurysm and dissection*, mutasi, *SMAD2, TGFβ2, SMURF2*