CHAPTER 1

INTRODUCTION

1.1 Background

Thoracic aortic aneurysm and dissection (TAAD) is a dilatation of thoracic aorta with diameter more than 1.5 times than that of the normal, in the presence or absence of the dissection.[1] It is one of 15th most leading cause of the death in USA and of the silent killer in the world.[2] Approximately 95% of the Thoracic Aortic Aneurysm (TAA) cases are asymptomatic.[3] Thoracic Aortic Aneurysm and Thoracic Aortic Dissection (TAD) are highly related in two ways because 16% patients with TAD have been known having TAA and the TAA increases the risk for developing dissection.[4] TAAD cases are diagnosed when the aneurysm is extremely large, dissection is occurred, or they are recognized accidentally in hospital because of the other disease. TAAD is found associated with other disease or syndrome like Marfan syndrome, Loeys-Dietz syndrome, and Ehler-Danlos syndrome vascular type. It is easier to recognize TAAD in syndromic TAAD than non-syndromic TAAD, since there are other features that lead the physicians to do examination of thoracic aorta. However, about 20% of TAAD is familial non-syndromic, which mean there is no another feature except TAAD and familial inheritance pattern in the patient.[5]

Several genes associated with TAAD have been recognized, including *FBN1, TGFβR1, TGFβR2, ACTA2, MYH11, COL3A1, MYLK* and *SMAD3*.[1,3,5-15] Up to 70% of Marfan syndrome patients which have mutation in *FBN1* develop

TAAD.[9] In familial non sydromic TAAD, mutation have been found in *ACTA2* (14%), *TGFβR1* (7%), *TGFβR2* (5%), *MYH11* (2%), and *MYLK* gene (3%).[10-14] However, many cases of familial TAAD have not been found for the mutation in those genes. Therefore, there are other genes involved in this disease still unknown.

The transforming growth factor beta (TGFβ) pathway is one of the pathways that explain the molecular mechanism of TAAD, besides of cytoskeletal and matrix metalloproteinase (MMP) pathways. In Marfan syndrome, mutation in *FBN1* gene increases release of TGFβ1 and correlates with TAA.[16-18] A recent study showed mutations in *TGFβ2* gene associated with mild systemic features of Marfan syndrome and cause a familial TAAD.[19]

In TGFβ pathway, TGFβ ligand attaches to TGFβRI and II complex and then phosphorylates SMAD2/3. SMAD4, as co-SMAD binds to the pSMAD2/3 to enter the nucleus as a transcription factor.[7,16,17,20,21] SMAD2 and SMAD3 have similar function as regulator SMAD in the TGFB pathway. Recently, pathogenic mutation found in *SMAD3* gene has been shown to cause aortic aneurysm.[15] Increase of pSMAD2 has been found in aortic tissue of TAA, and correlated with increase of elastic fiber fragmentation which is associated with pathogenesis of TAAD.[22] Therefore, *SMAD2* gene is likely to be candidate gene for TAAD patients.

Another protein in TGFβ pathway is SMURF2. SMURF2 is an enzyme which degrading TGFβR complex by E3 ubiquitynation.[23,24] Mutation in *SMURF2* gene is expected disturbing the function of the enzyme and increases the

level of TGFβR complex in the membrane, which increases TGFβ downstream signaling.

Screening of mutation in *FBN1, TGFβR1, TGFβR2, ACTA2* and *MYH11* genes have been done in patients with TAAD. And from previous study of TAAD patients in Caucasian population, mutation found in *MYLK*, *KLF2*, and *TGFβR3* genes, but not found in *TAGLN* and *CSRP2* genes (Ferdy KC *et al*, 2011, unpublished). In this study, mutation screening in *SMAD2, TGFβ2,* and *SMURF2* genes in TAAD patients will be done by using HRM technique. This technique is able to recognize mutations by distinguishing different melting point that can be seen as aberrant curve. HRM technique is fast, simple and inexpensive for screening a large number of samples.

1.2 Research Question

Is there any pathogenic mutation in *SMAD2, TGFβ2,* and *SMURF2* genes in patients with TAAD who did not carry any mutation in *FBN1*, *TGFβR1, TGFβR2, ACTA2,* or *MYH11*?

1.3. Research Purposes

1.3.1 General Research Purposes

To identify the pathogenic mutation in *SMAD2, TGFβ2,* and *SMURF2* genes in patients with TAAD who did not carry any mutation in *FBN1*, *TGFβR1, TGFβR2, ACTA2,* or *MYH11* gene.

1.3.2 Specific Research Purposes

1. To identify the genetic variant that might be pathogenic mutation in *SMAD2, TGFβ2,* and *SMURF2* genes in TAAD patients by using HRM technique.
2. To analyze whether the genetic variant found in *SMAD2, TGFβ2,* and *SMURF2* genes in TAAD patients is pathogenic or not by using the mutation prediction software.

1.4 Research Benefits

The benefits of this study are:

1. To learn and develop the high resolution melting technique for doing the screening of *SMAD2, TGFβ2,* and *SMURF2* genes in TAAD patients.
2. To give a better understanding about possible genes that could be involved in pathogenesis of TAAD disease besides the genes which already establish for this disease, so the therapeutic prevention can be developed based on the molecular level.
3. To give more detail genetic counseling about the other genes that could be involved in TAAD disease besides of the genes which already establish for the disease.

1.5 Originality

This is the first study for screening *SMAD2* and *SMURF2* genes associated with TAAD patients. The previous researches have been done associated with this study.

Table 1. List of previous associated studies

No.	Author	Title of Publications	Method	Result
1.	Gomez D, Al Haj Zen A, Borges LF, Philippe M, Gutierrez PS, Jondeau G, et al.	Syndromic and non-syndromic aneurysms of the human ascending aorta share activation of the Smad2 pathway. J Pathol. 2009; 218(1): 131–42.	pSmad2 immunostaining on sections of aneurysmal and control of ascending aorta, quantification of Smad2 mRNA level.	Higher pSmad2 and Smad2 mRNA level in aneurysms aorta compared with control, in all types of aneurysm.
2.	de Laar IM, Oldenburg RA, Pals G, Roos-Hesselink JW, de Graaf BM, Verhagen JM, et al.	Mutations in SMAD3 cause a syndromic form of aortic aneurysms and dissections with early-onset osteoarthritis. Nat Genet. 2011 Feb; 43(2):121-6. Epub 2011 Jan 9.	Bidirectional sequencing of SMAD3 in aneurysm patients without mutation in FBN1, TGFBR1 and TGFBR2 genes, followed by clinical data collection.	Five novel SMAD3 mutations (one nonsense, two missense and two frame-shift mutations) found in five new AOS families. Almost 90% patients have cardiovascular abnormalities, and involved mainly aortic aneurysms and dissections.
3.	Boileau C, Guo DC, Hanna N, Regalado ES, Detaint D, Gong L, et al.	TGFB2 mutations cause familial thoracic aortic aneurysms and dissections associated with mild systemic features of Marfan syndrome. Nat Genet. 2012; 44(8): 916-21	Linkage analysis identified the candidate gene, followed by functional analysis	A frameshift mutation in exon 6 and a nonsense mutation in exon 4.
4.	Kavsak P, Rasmussen RK, Causing CG, Bonni S, Zhu H, Thomsen GH, et al.	Smad7 binds to Smurf2 to form an E3 ubiquitin ligase that targets the TGFb receptor for degradation. Mol Cell. 2000; 6(6): 1365–75.	Functional studies using Smurf2 transfected cells, followed by immunoblotting of TGFβR1 and TGβFR2	Endogenous Smurf2 and Smad7 participate in TGF-beta receptor downregulation.