

# Connexion of Thrombomodulin Gene -33G/A Polymorphism and the Risk of Coronary Artery Disease

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## Abstract

Coronary Artery Disease (CAD) is caused by plaque buildup in the wall of the arteries that carry blood to the heart. Aim: This is an analytical case-control study carried out at Al-Neelain University and samples from Sudan Heart center, Khartoum, Sudan, during November 2018 and aimed to detect the association of thrombomodulin G33A polymorphism and CAD. A total of 80 subjects have participated in the current research. 40 of them were known Sudanese Patients diagnosed with Coronary Artery Disease, admitted to Sudan heart center, 21(52.5%) were males and 19 (47.5%) were females; their mean age is 56.6 years. Other 40 normal healthy volunteers similar in gender and age the case group used as a control group. 2.5 ml of EDTA anticoagulated blood was collected from all participants. DNA was extracted by the salting-out method. Then the detection of thrombomodulin G33A polymorphism using allele-specific Polymerase Chain Reaction. The data were obtained by using a directly structured interviewer questionnaire. Data were analyzed via (SPSS) software version 25. This study revealed that the thrombomodulin gene G33A polymorphism was statistically significantly associated with Sudanese patients with CAD ( $p$  value=0.00). This study concludes that the thrombomodulin G33A polymorphism predicts as a risk factor of the development of CAD in Sudanese patients.

**Keywords:** Thrombomodulin gene -33G/A, Coronary heart disease.

## Introduction

Thrombomodulin (TM) is a vascular endothelial cell-bound glycoprotein that has been described as a normal anticoagulant and whose main role is the attachment of thrombin. The

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interaction of thrombin with TM significantly prohibits the procoagulant action of thrombin and reduces thrombin development (Dittman and Majerus, 1990). TM often plays a major role in the process of protein C anticoagulation. Wu et al. found that a high level of dissolved thrombomodulin (sTM) indicated safety and a reduced risk of CAD. (Wu et al, 2003).

TM gene found on chromosome 20p11.2 has a single exon and is free of introns. This glycoprotein consists of 557 amino acids (AA) (60,300 Dalton) and is distributed mainly on the luminal surface of the vascular endothelium. The genetic structure of the TM gene consists of six domains which are N-terminal lectin-like domain (amino acid 1-154), a hydrophobic region (amino acid 155-222), six epidermal growth factors (EGF) such as sequences (amino acid 223-462), a threonine-and serine-rich region (amino acid 463-497), a transmembrane domain (amino acid 498-521) and a short cytoplasmic tail (amino acid 522-557) (Ireland et al, 1996).

The mutation G-33A in the promoter portion of the human TM gene was first described by Ireland et al. (Ireland et al., 1997). Systemic analysis of this mutation with a receptor gene assay showed that a variant had a substantial impact on the decreased activity of TM gene promoter. Also, the G-33A mutation reduced the amount of soluble TM in patients with CAD (Yi Heng Li et al, 2002). CAD is the most popular form of heart disease caused by the weakening or obstruction of coronary arteries caused by atherosclerosis, identified by the progressive development of fatty material and plaque within the wall of the arteries (Roger et al, 2012; Alzahrani, et al., 2019; Permadi, et al., 2020; Marzangi, et al., 2018; Elmasry, et al., 2019). While many conventional coronary risk factors, such as hypertension, diabetes mellitus, smoking, alcohol consumption, family history, and obesity, have taken a part in the progression of CAD, there is increased knowledge of the role of polymorphic gene variants as risk factors. (Ahmed et al, 2015; Kessler et al, 2013).

## Material and Methods:

### Study Design:

This is a case-control study that was conducted in September 2018 in Khartoum, Sudan . A random sample was collected from known Sudanese diagnosed with CAD. A total of 80 Sudanese subjects have participated in this study, 40 of them were known Sudanese patients diagnosed with CAD, further 40 were normal

subjects used as the normal control groups. The data was collected by using a directly structured interview questionnaire.

**Inclusions Criteria:**

Known Sudanese diagnosed with CAD of different ages and gender admitted to Sudan Heart Centre.

**Methodology:**

5ml of blood was collected in a container contain Ethylene diamine tetraacetic acid as an anticoagulant for DNA analysis. Genomic DNA was extracted by using the salting-out method. DNA samples were frozen below -20°C until analysis. The mutant allele was assessed by using allele-specific PCR reaction to detect the TM gene by using the sequence of the following primer: Forward (5' CCTTTTCCCGAACGTCC'). Reverse (5' GCCTCTCCTGTCCGTCC'3)

For detection of TM gene, G-33A mutation was amplified in 20 µL PCR reaction volume with 1µL and forward 1µL reverse, the reaction was performed at 95°C for 10 minutes, was followed by 36 cycles at 95°C for 30 seconds, annealing at 62°C for 30 seconds, and 72°C for 1 minute, and a final extension at 72°C for 5 minutes. PCR the amplification was checked by 2% Agarose gel electrophoresis was stain with ethidium bromide and was visualized by gel documentation system.

**Data analysis:**

Data were analyzed by (SPSS) software Version 25

**Ethical Approval:**

This study was approved by the ethical committee board of the faculty medical laboratory science, Al Neelain University, and written consent was taken from each participant before sample collection.

**Results**

This is a case-control study, done in Khartoum, Sudan, within a period from September to October (2018). A total of 80 Sudanese subjects were enrolled in this study, 40 of them were known Sudanese diagnosed with CAD as case group; 21 (52.5%) were males, and 19 (47.5%) were females; their mean age is 56.6 years. Further 40 normal healthy individuals were used as normal control, their age and gender were matched with the case group (Figure 1).

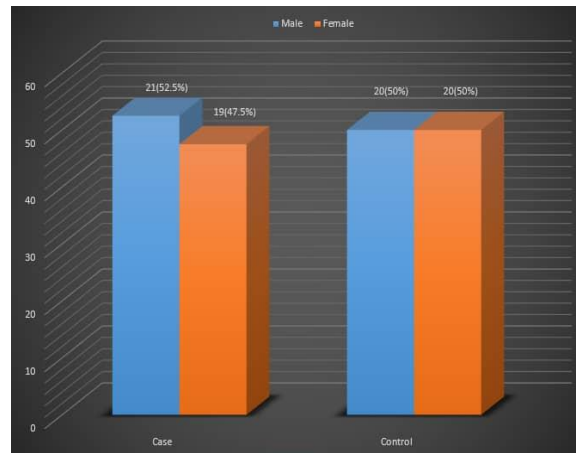
Detection of G33A mutation of the TM gene was done by allele-specific PCR. This study showed that the frequency of G33A mutation was (65% vs 0.00 %) among the CAD group (Figure 2).

The statistical analysis of this study exhibited that there is an association between TM -33G/A polymorphism and the risk of CHD was significant with P value (0.00). (Table 1), and the

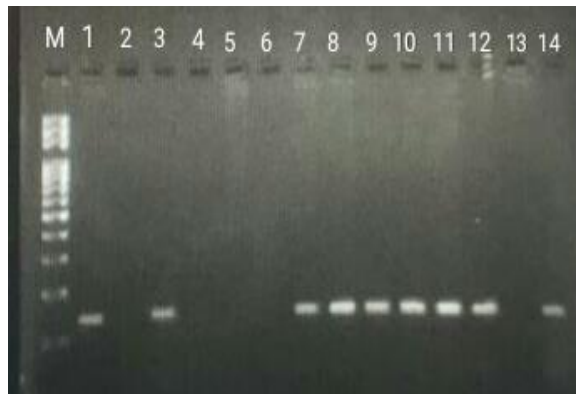
frequency of G33A mutation was significantly higher in the CAD group (65% vs 0.00 %, odds ratio [OR] 3.857, P =0.00).

This current study revealed that there is no significant correlation between TM-33G/A polymorphism in CAD and Gender, Age, Family History, Hypertension, Diabetes Mellitus, Smoking, and Obesity, P. value (0.461), (0.383), (0.249), (0.605), (0.429), (0.371), (0.196) respectively (Table 1).

On another hand, this research revealed that the mutation was an independent risk factor for CAD, as was hypertension (OR 0.953), diabetes mellitus (OR 0.1417), smoking (OR 0.444), obesity (OR 1.556), family history (OR 0.491), age (OR 0.595), gender (OR 0.750) (Table 2).



**Figure 1:** Distribution of study group according to gender



**Figure 2:** Agarose gel showing the G33A mutation of TM gene product in the sample from a population of CAD patients. M: 100bp ladder (1,3,7,8, 9,10,11,12,14) present band (positive) (pb:137bp). (2,4,5,6,13) absent the band.(Negative).

**Table 1:** Association between TM G33A polymorphism in a patient with coronary artery disease and control group

| Gene     | Group       |             | OR    | P-value |
|----------|-------------|-------------|-------|---------|
|          | Case        | Control     |       |         |
| Positive | 26 (65.0%)  | 0 (0.0%)    | 3.857 | 0.000   |
| Negative | 14 (35.0%)  | 40 (100.0%) |       |         |
| Total    | 40 (100.0%) | 40 (100.0%) |       |         |

OR= Odd ration, P≤ 0.001 highly significant

**Table 2:** Association between thrombomodulin G33A polymorphism in a patient with CAD and patient's gender, age, family history, hypertension, Diabetes mellitus, obesity, and smoking.

| Variable                 | Result     |            | P-value | OR    |
|--------------------------|------------|------------|---------|-------|
|                          | Positive   | Negative   |         |       |
| <b>Gender</b>            |            |            |         |       |
| Male                     | 13 (61.9%) | 8 (38.1%)  | 0.461   | 0.750 |
| Female                   | 13 (68.4%) | 6 (31.6%)  |         |       |
| <b>Age</b>               |            |            |         |       |
| <40 Years                | 5 (55.6%)  | 4 (44.4%)  | 0.383   | 0.595 |
| >40 Years                | 21 (67.7%) | 10 (32.3%) |         |       |
| <b>Family History</b>    |            |            |         |       |
| Yes                      | 7 (53.8%)  | 6 (46.2%)  | 0.249   | 0.491 |
| No                       | 19 (70.4%) | 8 (29.6%)  |         |       |
| <b>Hypertension</b>      |            |            |         |       |
| Yes                      | 9 (64.3%)  | 5 (35.7%)  | 0.605   | 0.953 |
| No                       | 17 (65.4%) | 9 (34.6%)  |         |       |
| <b>Diabetes Mellitus</b> |            |            |         |       |
| Yes                      | 17 (68.0%) | 8 (32.0%)  | 0.429   | 1.417 |
| No                       | 9 (60.0%)  | 6 (40.0%)  |         |       |
| <b>Obesity</b>           |            |            |         |       |
| Yes                      | 14 (70.0%) | 6 (30.0%)  | 0.371   | 1.556 |
| No                       | 12 (60.0%) | 8 (40.0%)  |         |       |
| <b>Smoking</b>           |            |            |         |       |
| Yes                      | 8 (53.3%)  | 7 (46.7%)  | 0.196   | 0.444 |
| No                       | 18 (72.0%) | 7 (28.0%)  |         |       |

## Discussion:

CHD is a leading cause of mortality worldwide. The burden of CAD is growing fast in Africa and has become a public health problem. Heart disease is prevalent in Sudan, with at least 2.5% of the population affected, which is one of the main causes of hospital deaths. Several studies have shown that TM G-33A polymorphism predicts the risk of developing CAD. No further published data are considered that the correlation between TM G-33A and CAD has been identified in Sudan, so this study has been conducted to fill this void. This study was conducted to detect the association of TM gene G-33A polymorphism and the risk of CAD among Sudanese patients.

TM is an endothelial glycoprotein that decreases thrombin function and stimulates protein C. (WA et al., 1990). Recent studies have shown that the G33A promoter mutation of the TM gene decreases the amount of soluble TM in patients with CAD. (Yi Heng Li et al, 2000). In this study, we analyzed the distribution of the G-33A mutation in the TM gene in the Sudanese population and determined whether the mutation could pose a risk to CAD.

Interesting results from this research have shown that there is a statistically significant association between the G33A polymorphism of TM and the risk of CAD ( $P = 0.00$ ). This result is similar to that made in Taiwan (2000) in 520 patients, 320 with CAD and 200 regulars, Li YH, et al., who demonstrate that there is a substantial association of G33A mutations in the TM gene and CAD with p-value (0.31) (Li et al., 2000).

Our finding was corroborated with a study done in China (2014), in which studied the TM 33G/A, and Ala455Val polymorphism in a meta-analysis study that includes 14 case-control studies, and found that the TM 33G/A and Ala455Val polymorphism were risk factors of CAD (Zhang et al., 2014).

Another meta-analysis study agreed with our finding was done in (2015) by Wang, Hongxia; Dong, and Pingshuan in 13 case-control types of research on the correlation between TM -33G/A and Ala455Val polymorphism and risk factor of CAD, who showed that association between the TM -33G/A and the risk factor of CAD was significant with (OR= 1.65;  $P < 0.01$ ). (Wang and Dong, 2015).

The finding of this result disagrees with a study done in the Han Chinese population (2005) by Jianguo Zhao, et al. Who has studied the Relationship analysis of TM -33G/A polymorphism with CAD and myocardial infarction in the Chinese Han population in 808 patients with CAD and 813 normal control and has finally reported that there is no statistically significant association of TM 33G/A polymorphism and CAD, MI with P value (0.249) And this can be due to a different method or ethnic origin (Zhao, et al 2005).

## Conclusions:

This study concludes the TM gene G33A polymorphism predicts as a risk factor for developing CAD in Sudanese patients.

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