T.C.
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DEPARTMENT OF MOLECULAR MEDICINE

"KLK1 GENE POLYMORPHISM EFFECT ON THE DEVELOPMENT OF CORONARY ARTERY DISEASE AND DIABETES MELLITUS TYPE 2"

DOCTOR OF PHILOSOPHY THESIS

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APPROVAL
This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated ...28/04/2021..... and numbered ...2021/04-05.....

Prof. Dr. Bayram YILMAZ
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DECLARATION

I declare that the all the information written in this thesis were collected by me according to the ethical and academic rules. The patients included in the study were volunteers whom signed the consent form. This is my own doctoral thesis study and I planned al the steps and details of this work.

M.D. Noor HUSSAIN

DEDICATION

This work belongs to my precious teacher Prof. Dr. Turgay İSBİR.

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My dear father, mother , my precious brothers, My beloved husband Mohammed, and my lovely daughters Nihan and Nil I am very lucky and proud to have you in my life.

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LIST OF SYMBOLS AND ABBREVIATIONS

AM:	Acute Marginal Artery
AV:	Atrioventricular
B2R:	Bradykinin 2 Receptor
BK:	Bradykinin
BMI:	Body Mass Index
CAD:	Coronary Artery Disease
CVD:	Cardiovascular Disease
CXA:	Circumflex Artery
DM:	Diabetes Mellitus
DNA:	Deoxyribonucleic Acid
EDTA:	Ethylenediaminetetraacetic Acid
GWAS:	Genome-Wide Association Study
HDL:	High-Density Lipoprotein
HMWK:	High Molecular Weight Kininogen
IMA:	Intermediate Artery
KKS:	Kallikrein-Kinin System
KLK1:	Kallikrein 1
LAD:	Left Anterior Descending Artery
LDL:	Low-Density Lipoprotein
LMCA:	Left Main Coronary Artery
MI:	Myocardial Infarction
OD:	Optical Density
PDA:	Posterior Descending Artery

RAAS:	Rennin-Angiotensin-Aldosterone System
RMCA:	Right Main Coronary Artery
RNA:	Ribonucleic Acid
RS:	Reference Single Nucleotide Polymorphism
RT-PCR:	Real Time Polymerase Chain Reaction
T2DM:	Type 2 Diabetes Mellitus
TG:	Triglyceride
VLDL:	Very Low-Density Lipoprotein

ABSTRACT

HUSSAIN. N. KLK1 Gene Polymorphism Effect on The Development of Coronary Artery Disease and Diabetes Mellitus Type 2. Yeditepe University Health Sciences Institute. Department of Molecular Medicine. Ph.D. Thesis. İstanbul, 2021.

Coronary artery disease is the most common cause of death worldwide. According to the statistics of the World Health Organization, 15 million patients were diagnosed with coronary artery disease in 2015. Diabetes disease is a metabolic chronic disease and is classified into three groups; type 1, type 2 and gestational. Type 2 diabetes is responsible for more than 90% of all diabetes cases. Both environmental and genetic factors play a role in the formation of these two diseases. The positive effects of the Klk1 gene on glucose balance, insulin synthesis and cardiovascular system have been shown in studies. In this study, the role of klk1 genetic polymorphism in these two diseases was investigated. This study included 4 groups of 174 people in total, the control group (n = 50), the coronary artery disease group (n = 48), the type 2 diabetes group (n = 50), and the coronary artery disease and type 2 diabetes group (n=26). There was no significant difference between these four groups in terms of gender and age criteria. When the body mass index values were examined, a significant difference was found ($p< 0,00001$). There was a significant difference in terms of HDL in the lipid profiles examination ($P< 0,00001$) but no significant difference was found in terms of cholesterol, LDL, VDL and TG. When the Klk1 genotypes were compared, the GG genotype differed significantly between the control and patient groups ($P=0.010$). Coronary artery disease was not significant in terms of Klk1 genotypes. When the genotypes of diabetic and non-diabetic individuals were compared, the AA genotype showed a significant difference ($P=0.029$). In terms of allele, the A allele showed a significant difference between people with and without diabetes ($P=0.029$). High body mass index poses a risk for these two diseases, and HDL is protective against coronary artery disease. These findings also revealed the protective effects of a healthy diet and physical activity against these diseases. The incidence of these 2 diseases is lower in people with the GG genotype. People with the AA genotype are at risk for type 2 diabetes. In these findings, mutations in the klk1 gene cause impairment of normal glucose metabolism and pose a risk for diabetes.

Key words: coronary artery disease, diabetes mellitus disease, KLK1, polymorphism, Real-time PCR

ÖZET

HUSSAIN. N. Klk1 Gen Polimorfizminin Koroner Arter Hastalığı ve Tip 2 Diyabetin Oluşumundaki Etkisi. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, moleküler tıp bölümü. Doktora tezi. İstanbul, 2021.

Koroner arter hastalığı bütün dünyadaki ölümlerin en sık sebebidir. Dünya sağlık örgütü istatistiklerine göre 2015 yılında 15 milyon hasta koroner arter hastalığıyla teşhis edilmiştir. Diyabet hastalığı metabolik bir kronik hastalıktır ve üç gruba sınıflandırılır; Tip 1, tip 2 ve gebelikle ilişkili. Tip 2 diyabet tüm diyabet vakalarının %90 fazlasında sorumludur. Bu iki hastalıkların oluşumunda hem çevresel hem kalıtımsal faktörler rol oynamaktadır. Klk1 Genin hem glikoz dengesinde ve insülin sentezinde hem de kardiyovasküler sistemi üzerinde olumlu etkileri çalışmalarda gösterilmiştir. Bu çalışmada klk1 genetik Polimorfizminin bu iki hastalıklardaki rolünü araştırılmıştır. Bu çalışma ya toplamda 174 kişiden oluşan dört grup dahil edilmiştir, Kontrol grubu (n=50), Koroner arter hastalığı grubu (n=48), tip 2 diyabet grubu (n=50) ve koroner arter hastalığı ve tip iki diyabet grubu (n=26).Bu dört grup arasında cinsiyet ve yaş kriterleri yönünden anlamlı bir fark saptanmamıştır. Vücut kitle indeksi değerlerine bakıldığında anlamlı bir fark bulunmuştur ($p< 0,00001$). Lipit profilleri incelemesinde, HDL yönünden anlamlı bir fark mevcut olup ($p< 0,00001$) kolestrol, LDL, VDL ve TG yönünden anlamlı bir fark saptanmamıştır. Klk1 genotipleri Karşılaştırıldığında GG genotipi kontrol ve hasta grupları arasında anlamlı düzeyde farklılık göstermiştir ($p=0.010$). Klk1 genotipleri yönünden koroner arter hastalığı anlamlılık bulunmamıştır. Diyabet ve diyabet olmayan kişilerin genotipleri karşılaştırıldığında ise AA genotipi anlamlı bir fark göstermiştir ($p=0.029$). Alel yönünden bakıldığında diyabet ve diyabet olmayan kişilerin arasında a aleli anlamlı fark göstermiştir ($p=0.029$). Yüksek vücut kitle indeksi bu iki hastalık için risk oluşturmakta olup HDL ise koroner arter hastalığı karşı koruyucudur. Bu bulgular da sağlıklı beslenmenin ve fiziksel aktivitenin bu hastalıklara karşı koruyucu etkilerini ortaya koymuştur. GG genotipindeki kişilerde bu 2 hastalıkların insidandsı daha düşüktür. AA genotipi taşıyan kişilerin tip iki diyabet yönünden risk altında olmaktadır. Bu bulgularda klk1 genindeki mutasyonlar normal glikoz metabolizmasının bozukluğuna yol açtığından diyabet için risk oluşturmaktadır.

Anahtar kelimeler: koroner arter hastalığı, diyabet hastalığı, KLK1, polimorfizm, Real-time PCR

1. INTRODUCTION

Cardiovascular disease (CVD) is a general term used to define a group of diseases affecting the cardiovascular system and it includes coronary heart disease (CHD), coronary artery disease (CAD), acute coronary syndrome (ACS), and other conditions (1). Besides environmental factors genetic factors also play an important role in the development of coronary artery disease. Genetic studies of families and twins estimated that the role hereditary factors in CAD development was ranging from 40% to 60% (2). For studying coronary artery disease the anatomy of these arteries must be understood. There are two main coronary arteries supplying the myocardium with oxygenated blood; The left and the right main coronary arteries (3). A study investigated human tissue kallikrein 1 effects on the neointimal formation of the carotid artery's in hypertensive rats showed that supplying rats with human tissue KLK1 had a potential effect to reduce the neointimal formation in the rats after balloon injury (4).

Diabetes mellitus is a widespread chronic metabolic disorder and the prevalence of this disorder is increasing by time in the globe. Type 2 diabetes mellitus is responsible for most of the diabetic cases (5). Besides the primary effects of this disorder; poor control of DM negatively affects the cardiovascular system (6). Environmental and genetic factors are related with the pathogenesis of diabetes mellitus. At past it was thought that environmental factors were the main cause of T2DM; nowadays GWAS cleared that more than 50 gene loci are closely associated with T2DM (7). A therapeutic study on diabetic rats stated that administrating rats with KLK1 gene therapy had beneficial effects in lowering blood glucose levels and this method may be a novel treatment of diabetes (8).

According to the literature findings we aimed in this study to investigate the possibility of KLK1 gene polymorphism relation with the incidence of CAD and T2DM. For this purpose we investigated KLK1 polymorphism (rs1054713) in 174 individuals distributed in 4 groups control (n=50), CAD (n=48), T2DM (n=50) and CAD+T2DM (n=26).We collected blood samples from each individual. DNA isolation from the blood samples was performed by using DNA isolation robot (iPrep Purelink, Invitrogen). Genotyping is performed by using 7500 fast-Real Time PCR (Applied Biosystems) real time PCR device. Three genotypes are available GG (homozygous wild type), GA (heterozygous) and AA (homozygous mutant). We investigated the genotype and allele frequencies in the study groups.

2. LITERATURE REVIEW

Coronary artery disease (CAD) is defined to be the major cause of death in the world (1). The World Health Organization (WHO) statistics showed that only in 2015 15 million people were diagnosed with CAD. Researching the genetics and pathophysiology of this disease may help to develop preventive and therapeutic strategies and consequently reduce the morbidity and mortality rates due to CAD (2).

Anatomy

The coronary arteries that provide the myocardium with oxygenated blood are composed of 2 main arteries: the left main coronary artery (LMCA) and the right main coronary artery (RMCA) (3).

The Left Main Coronary Artery (LMCA):

LMCA originates from the left sinus of valsalva and bifurcates into 2 branches

- The left anterior descending artery (LAD) which runs in the anterior interventricular sinus and supplies blood to the left ventricle and the atrioventricular (AV) bundle. LAD also gives diagonal and septal branches that supply blood to the anterior part of the interventricular septum and the anterior wall of the left ventricle (3, 4).
- The circumflex artery (CXA) which runs along the AV sulcus and supplies blood to the lateral wall of the left ventricle.
- Sometimes a third artery called the intermediate artery (IMA) may arise at the bifurcation of the LMCA.

The Right Main Coronary Artery (RMCA):

RMCA originates from the right sinus of valsalva and runs in the right atrioventricular sinus. RMCA gives the acute marginal branch (AM), AV node branch and the posterior descending artery (PDA). The AM supplies the lateral wall of the right ventricle and the PDA supplies the inferior wall of the left ventricle and the inferior part of the septum with blood (3, 4).

Coronary Artery Disease

Coronary artery disease (CAD) which is the most frequent heart disease is defined to be the major cause of mortality in the globe (1). The World Health Organization (WHO) statistics showed that only in 2015 15 million people were diagnosed with CAD (2). CAD is responsible for the mortality of about 7.2 people yearly and it's expected that CAD will be responsible for the death of about 11.1 people in 2020 (9-11).

CAD is a multifactorial disease and many risk factors like age, gender, life style, diet, hyperlipidemia, hypertension and diabetes mellitus are defined to be related with this disease. On the other hand, epidemiological studies state that genetic factors have a role in the development of this disease (12-14). Defining individuals with risk factors is the primary preventive strategy in many countries (15, 16).

Pathogenesis

CAD is a chronic inflammatory disease. In CAD the coronary arteries which provide the myocardium with oxygenated blood are narrowed. This disease can be presented by variable manifestations ranging from angina to myocardial infarction (MI) and death (17)

Atherosclerosis

Atherosclerosis is the major risk factor for CAD. Atherosclerosis develops due to genetic and environmental factors interaction. This chronic pathology is progressed silently by accumulation of fatty streaks in the artery wall (18). These fatty streaks are covered by a fibrous cap forming the atherosclerotic plaque. The process of atherosclerotic plaque formation is explained below (figure 2.1) (19-27).

According to the thickness of the fibrous plaque the atherosclerotic plaque is classified as stable and unstable. Stable plaques are covered by thick fibrous cap in which smooth muscle cells are supported by a matrix abundant with type I and III collagen fibers. Stable plaques may enlarge by time leading to arterial stenosis and consequently may cause tissue ischemia and angina (28). Unstable plaques are covered by thin fibrous cap formed by type I collagen fibers with few or no smooth muscle cells. Unstable plaques may rupture and release thrombi which may occlude arteries (22, 25).

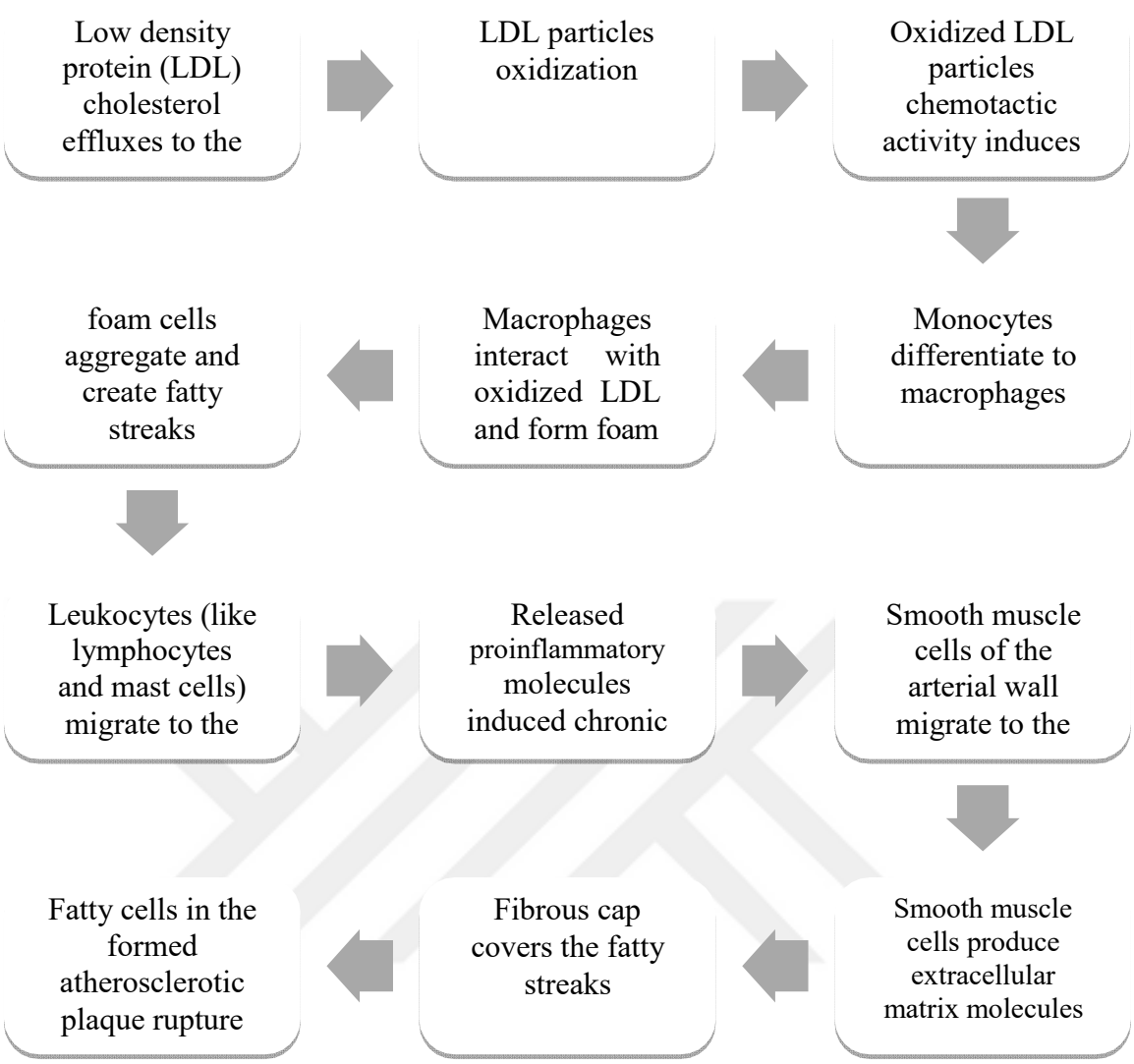


Figure 2.1: The process of atherosclerotic plaque formation (19-27)

Genetic Variation and Coronary Artery Disease

A meta-analysis of many genome-wide association studies established in 2013 stated that about 40 genetic variants were related with about 6% of the CAD inheritance (Table 2.1.). Some of these variants are related with blood pressure and lipid metabolism. On the other hand, no overlap was found between CAD and type 2 Diabetes loci. Additionally, many CAD loci are related to atherosclerosis (29).

Table 2.1.: Genetic variants in Coronary Artery Disease (29)

Known loci	Published lead SNP or proxy	Chr.	Effect/non-effect allele (frequency)
SORT1	rs602633 (tagging rs599839;*r*2 = 1.00)	1	C/A (0.77)
PCSK9	rs11206510	1	T/C (0.84)
WDR12	rs6725887	2	C/T (0.11)
MRAS	rs9818870	3	T/C (0.14)
TCF21	rs12190287	6	C/G (0.59)
SLC22A3-LPAL2-LPA	rs3798220	6	C/T (0.01)
ZC3HC1	rs11556924	7	C/T (0.65)
CDKN2BAS1	rs1333049	9	C/G (0.47)
ABO	rs579459	9	C/T (0.21)
CYP17A1-CNNM2-NT5C2	rs12413409	10	G/A (0.89)
KIAA1462	rs2505083	10	C/T (0.42)
PDGFD	rs974819	11	A/G (0.29)
SH2B3	rs3184504	12	T/C (0.40)
COL4A1-COL4A2	rs4773144	13	G/A (0.42)
HHIPL1	rs2895811	14	C/T (0.43)
RAI1-PEMT-RASD1	rs12936587	17	G/A (0.59)
LDLR	rs1122608	19	G/T (0.76)
Gene desert *(KCNE2)*	rs9982601	21	T/C (0.13)
PPAP2B	rs17114036	1	A/G (0.91)
ANKS1A	rs12205331 (tagging rs17609940; *r*2 = 0.85)	6	C/T (0.81)
PHACTR1	rs9369640 (tagging rs12526453; *r*2 = 0.90)	6	A/C (0.65)
CXCL12	rs501120	10	A/G (0.83)
LIPA	rs2246833 (tagging rs1412444; *r*2 = 0.98)	10	T/C (0.38)
UBE2Z	rs15563 (tagging rs46522; *r*2 = 0.93)	17	C/T (0.52)
SMG6	rs2281727 (tagging rs216172; *r*2 = 0.96)	17	C/T (0.36)
ApoE-ApoC1	rs2075650	19	G/A (0.14)
MIA3	N/A	1	T/G (0.87)
7q22	N/A	7	A/C (0.19)
ZNF259-APOA5-APOA1	N/A	11	C/G (0.10)
ADAMTS7	N/A	15	T/C (0.58)

Epidemiology

The American Heart Association (AHA) reported in the 2016 Heart Diseases and Stroke update Statistics that 15.5 million ≥20 years old persons were diagnosed with CAD in USA. CAD prevalence was reported to be increased with age. Gender is another factor which affects the prevalence of CAD; and MI is more prevalent in males than females (30).

According to observations of data collected from 44 years follow-up Framingham study and 20 years surveillance of their offspring; lifetime risk of developing CAD was 49% in males and 32% in females aged 40 years, and 35% in males and 24% in females aged 70 years. For both genders the risk was found to be increased with age. Comparing 65-94 and 35-64 age groups the incidence was found to be more than double in males and triple in females respectively. The morbidity and mortality rates due to CAD were found to be higher in postmenopausal than premenopausal women at the same age (31-33).

In developed countries the overall CAD incidence has decreased by time and it was observed that mortalities due to CAD is lower in countries with advanced economies and health care systems like USA (32). CAD is still the major cause of adult death in every country and CAD mortality is expected to increase between 1990 and 2020; the estimated rates of increase in developed countries are 48% in males and 29% in females while the rates in developing countries are 137% in males and 120% in females approximately (34, 35).

In 2014 a study about 49 countries in Europe and northern Asia performed by using data from WHO stated that more than 4 million were died yearly due to CAD (9). CAD risk factors could not be well explained in India (36). In Beijing (China) higher cholesterol levels were explained to be responsible for the increase in CAD mortality (5). Also, decrease in CAD rates were less in Latin America compared to USA, this difference was attributed to be due to unhealthy life style, obesity and smoking (6).

Diabetes Mellitus Type 2

Diabetes Mellitus (DM) is a prevalent metabolic disorder characterized by hyperglycemia and constitutes a source of a prevalent health problem worldwide. As DM is becoming more prevalent rapidly it's necessary to understand the factors contributing to the development of this disorder. The incidence of DM is expected to increase by 55% by 2035 (7). According to the 2017 National Diabetes Statistics Report; 30.3 million people had diabetes in USA (9.4% of the population). DM is generally classified as type 1, type 2 and gestational. Type 2 Diabetes Mellitus (T2DM) is the most common type and composes more than 90% of DM cases (37). T2DM patients must be followed-up and managed lifetime. T2DM patients management strategies involve many aspects like diet, exercise and drug therapies (38).

Etiology

T2DM is a multifactorial disease caused by combination of genetic and environmental factors.. Genetic and environmental factors interact and result in increased body adiposity leading to obesity and insulin resistance (Figure 2.2.)(39).

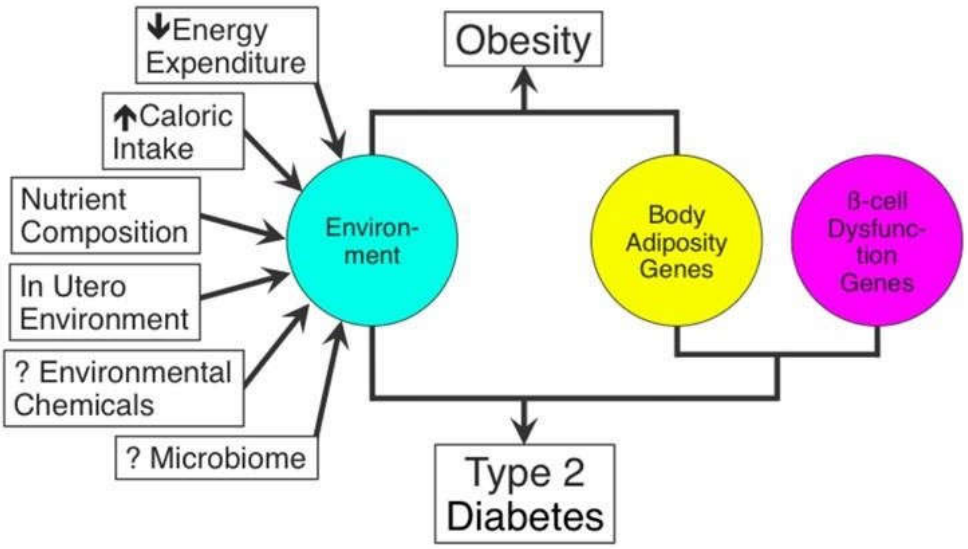


Figure 2.2.: Roles of genes and the environment as causative agents in obesity and type 2 diabetes development (39)

Genetic Factors

Family history of diabetes is proved to be related with T2DM development. Also, the significant higher concordance between monozygotic twins than between dizygotic twins is another evidence of the genetic role in T2DM. Genetic disturbances in the glucose metabolism regulatory system are accepted to be associated with the pathogenesis of T2DM. In 2008, a GWAS performed by Unoki H. et.al. identified that KCNQ1 gene mutation is related to abnormal insulin secretion and as a consequent plays important role in the pathogenesis of diabetes in the Asian ethnic group . Approximately 30% of the genetic factors of diabetes are defined nowadays (40).

2.3.1.2. Environmental Factors

Obesity; especially visceral obesity is a major risk factor for T2DM. Aging, smoking, alcohol, overeating (particularly simple sugars) and low energy consumption due to lack of exercise are common environmental factors that attribute risk for increased visceral fat and as a consequence may lead to disturbed glucose intolerance. Mild obesity (BMI<25) when accompanied by increased visceral fat mass constitutes increased diabetes risk for about 5 times (39).

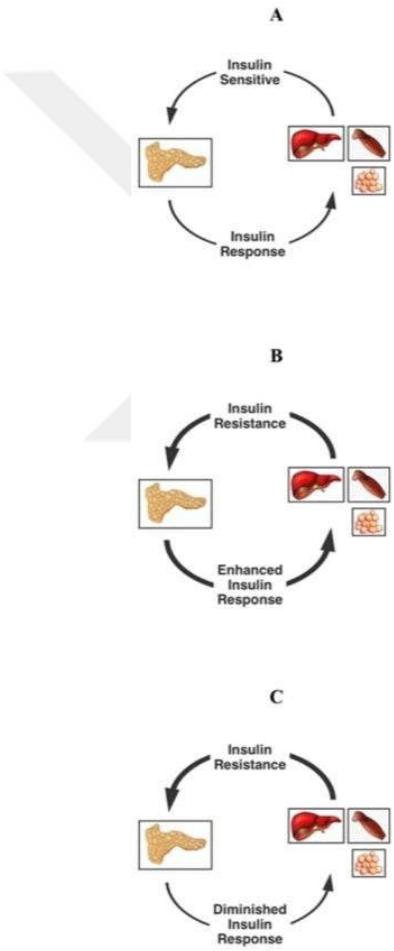
Pathophysiology

Insulin Resistance

Insulin resistance begins before the clinical onset of the disease. In T2DM despite the secretion of insulin by pancreatic β-cells; hyperglycemia occurs due to unresponsive insulin resistant target cells. Studies about the molecular mechanisms of insulin action explained the relation of genetic and environmental factors with the insulin resistance. In addition to insulin receptor gene and insulin receptor substrate gene polymorphisms many genes like the uncoupling protein gene and β3 adrenergic gene are proved to be associated with the development of visceral obesity leading to insulin resistance. By the progression of the disease glycolipo-toxicity impairs insulin secretion gradually (39).

Impaired Insulin Secretion

Animal experiments showed that insulin resistance progresses by time leading to gluco- and lipo-toxicity which in turn damage pancreatic β-cells decreasing the number of these cells and the insulin secretion capacity consequently. At early stages of T2DM hyperglycemia is only postprandial as the elevated glucose levels (due to resistance) induces pancreatic β-cell to secrete more insulin. As the disease progresses damaged β-cells will not be able to secrete insulin leading to permanent hyperglycemia (figure 2.3.) (39).



(A) Insulin acts in the liver to suppress glucose production, and in the muscle and adipose tissue to stimulate the uptake of glucose, amino acids and fatty acids. The amount of insulin released to maintain normal glucose homeostasis is determined by the prevailing insulin sensitivity. This feedback is likely mediated through neuronal and humoral mechanisms, but the exact mediators are still not known. (B) When insulin resistance develops in the insulin-sensitive tissues, feedback to the β-cell ensures that it increases insulin output to maintain normal glucose tolerance. (C) When the β-cell is incapable of increasing insulin output in the presence of insulin resistance, the result is the development of elevated glucose levels, initially manifest as impaired glucose tolerance. As β-cell dysfunction progresses, further elevations in glycaemia occur and diabetes is the eventual result.

Figure 2.3.: Feedback loop between the islet β-cell and the insulin sensitive tissues (39)

Kallkrein 1

Kallikreins are group of proteins that generate from 15 genes located on the 19q13 chromosome. These genes are the largest gene group of proteases in the human genome. Kallikreins are produced and secreted as pro-enzymes that are converted to mature enzymes by proteolysis (41).

Kallikrein-1 (KLK1) is a glycoprotein composed by 238 acids (42). KLK1 is also called tissue kallikrein and is located in cardiovascular tissues, pancreas, kidney and salivary glands (41).

KLK1 and CAD

KLK1 is essential for a healthy cardiovascular system and its deficiency is associated with cardiovascular disorders. KLK1 plays role in releasing active bradykinin (BK) by cleaving High molecular weight kininogen (HMWK). Humans with mutated KLK1 gene are observed to have impaired vascular response to blood flow changes (43). KLK1 regulates blood flow by cleaving HMWK to BK, this mechanism is named kallikrein-kinin system (KKS). KKS is considered to be a major vasodilatory mechanism. Also, kinins reduce the vasoconstriction activity of the sympathetic nervous system by inhibiting nor-epinephrine release (44).

KLK1 and T2DM

It is suggested that KLK1 have a role in glucose homeostasis and insulin sensitization. As mentioned above klk1 plays role in the cleavage HMWK to release BK, BK in turn stimulates insulin dependent cellular glucose uptake by activating the bradykinin 2 receptor (B2R). In a study made on rats it had been observed that B2R antagonists administration caused a decrease in the glucose uptake as a result of insulin insensitivity (8). At the same time compared to wild type, rats with impaired kininogen are noticed to be glucose intolerant and insulin resistant (38).

3. MATERIALS AND METHODS

Identification of Samples in The Study Population

4 groups were included in the study; healthy control group (50), CAD group (48), T2DM group (50) and CAD-T2DM group (26). Age, gender, body mass index and lipid profile of each case was stated in order to obviate the demographic characteristics of the study population.

5 ml blood samples were collected from each case. The blood samples were collected in EDTA tubes. DNA samples were obtained from the leukocytes of the blood samples by using DNA isolation robot (iPrep TM Purification Instrument, Invitrogen, Thermo Fischer Scientific Inc.). Genotyping of the kallikrein 1 location that we aimed to amplify was done by Real Time Polymerase Chain Reaction (RT-PCR).

Materials and Devices Used

DNA isolation kit (iPrep Purelink) and DNA isolation robot (iPrep Purelink, Invitrogen), NanoDrop 2000 Spectrophotometer (Thermo Scientific), TaqMan Genotyping Assay and TaqMan SNP Genotyping Master Mix, Real time PCR (Fast Real Time 7500 , Applied Biosystems), Mini Centrifuge (Wealtec), Plate Centrifuge (Hettich), Microplate washing device(WHYM200,Powen Medical co) , Ultra-pure H2O (Pure lab option Q,Elga), Vortex (V.I. plus Biosan), +4°C Refrigerator(Haier),-20°C Refrigerator (Haier), pipette Kit (Thermo Fisher Scientific Inc.).

Methods

DNA Isolation from Blood

The blood samples were collected from the groups included in the study in EDTA tubes. The collected samples were stored at +4°C. In order to isolate DNA from the blood iPrep DNA isolation kit and iPrep DNA isolation robot were used. The working principles of the iPrep DNA isolation kit and iPrep DNA isolation robot are explained below;

- 13 blood samples can be processed at a time.
- 350 µl blood is used in the kit (for each sample).
- Solutions needed to isolate DNA from the blood cells are included in the kit. These solutions are used automatically when needed.
- The robot can isolate 150 µl DNA from each of the 13 samples.
- The isolated DNA samples are stored at +4°C until used.

DNA Purity Identification

The purity and concentration of the DNA isolated by iPrep DNA Isolation kit and robot is identified by spectrophotometer. We used NanoDrop device for this purpose. Wavelengths used to measure the DNA purity are 260 nm and 280 nm. Values measured at the 260 nm wavelengths are divided by the values measured at the 280 nm wavelengths to obtain the DNA purity value. When this value is 1.8 it means that the isolated DNA is pure while when the value is below 1.8 protein concentration and when above 1.8 RNA existence is mentioned.

For DNA concentration identification the below calculation is used;

DNA concentration: OD260 x 50 µl/ml x dilution rate (100)

The value of DNA at the 260 nm is accepted to be 1 optic density (OD)

Genotyping by Real Time PCR

Genotyping is performed by using 7500 fast-Real Time PCR (Applied Biosystems) real time PCR device. Klk1 gene [chr19:50820245(Rs1054713)] location was genotyped and "TaqMan Genotyping Assay" as a specific primer and probe set was used for this purpose (FORWARD: CCTTCACAGCATCTGTGATGGTATCAGCAG. REVERSE: CTGCTGATACCATCACAGATGC5 (GRCh38.p12)

3.3.3.1 Real Time PCR Protocol

a. Reaction mix (20 ml) (Table 3.1.).

Table 3.1.: PCR reaction mix contents

THE MATERIAL USED	**QUANTİTY**
Master Mix	10µl
TaqMan Assay	0.5µl
DNAse,RNAse, Free water	8.5µl
Tamplet DNA	1µl

b. Real time PCR conditions:

10 minutes waiting (hold) at 95°C and table 3.2 for each cycle (Table 3.2.)

Table 3.2.: Real Time PCR Protocol

40 cycle			
	Hold	duration	Annealing/Elongation
temperature	95°C	92°C	60°C
period	10 min.	15 sec.	1 min.

Statistical Analysis

Statistical analysis of data obtained from the genotyping was performed using spss 13.0 program. Student T test, Chi Square test and Fisher's Exact test are the tests used for this purpose. Significance value was accepted to be $p<0.05$.

4. RESULTS

4.1.Working Groups Demographic Data

Advanced control with Chi-squared and student-t test analysis and coronary artery disease patient groups compared in terms of demographic features (Table 4.1.);

The study included control (n=50), CAD (n=48), T2DM (n=50) and CAD+T2DM (n=26) groups. The distribution of Demographic characteristics and descriptive information in the CAD, T2DM, CAD+T2DM and control groups are given in table 4.1. All groups were found to be homogenous in Gender (p=0.068), Age (p=0.264), Cholesterol (p=0.570), TG (p=0.413), LDL (p=0.476) and VDL (p=0.508) presence between groups not showing any difference and not significant. A significant difference between the groups in term of BMI (p<0.00001) and HDL (p<0.00001) was observed.

Table 4.1.: Demographic characteristics of the study population

		N	Mean	P value
Gender F / M	Control	50	23/27	0, 068
	CAD	48	18/30	
	T2DM	50	29/21	
	T2DM+CAD	26	15/11	
Age, x ± SD (years)	Control	50	55,80±10,89	0,264
	CAD	48	63,94±8,85	
	T2DM	50	55,62±12,80	
	T2DM+CAD	26	64,12±11,22	
Body Mass Index (kg/m2)	Control	50	25,13±2,88	< 0,00001*
	CAD	48	26,91±4,68	
	T2DM	50	29,93±6,64	
	T2DM+CAD	26	30,18±6,92	
Cholesterol	Control	50	197,36±38,06	0,570
	CAD	48	186,69±48,14	
	T2DM	44	195,43±55,40	
	T2DM+CAD	26	184,62±53,67	

TG	Control	50	163,06±102,96	0,413
	CAD	48	148,48±58,09	
	T2DM	47	176,81±130,04	
	T2DM+CAD	26	144,31±56,52	
LDL	Control	50	127,96±35,19	0,476
	CAD	48	119,04±40,30	
	T2DM	48	116,25±45,41	
	T2DM+CAD	26	116,35±43,43	
HDL	Control	50	42,16±9,5	< 0,00001*
	CAD	48	37,71±7,3	
	T2DM	44	49,64±13,75	
	T2DM+CAD	26	40,85±11,49	
VDL	Control	50	28,16±18,26	0,508
	CAD	48	29,79±11,63	
	T2DM	26	32,16±20,37.	
	T2DM+CAD	26	28,00±8,74	

n: number of samples, $\bar{x} \pm$ SD**:** mean value ± Standard deviation, , student t test used for comparison of mean values and chi square and other Demographic characteristics, * =significantly different ($p< 0.05$), NS= non significant ($p>0.05$).

Control and Patient Groups Risk Factor Related to the KLK1 Results

Total plasma cholesterol, LDL-cholesterol, HDL-cholesterol, Triglycerides and body mass index parameters in coronary artery disease related to KLK1 genotype findings (Table 4.2.).

As the result BMI (p=0.139), plasma cholesterol (p=0.296), triglycerides (p=0.735), low density lipoprotein (p=0.144), high density lipoprotein (p=0.199), not showing any significant.

Table 4.2.: Total plasma cholesterol, LDL-cholesterol, HDL-cholesterol, Triglycerides and body mass index parameters in coronary artery disease related to KLK1 genotype in all study groups

Genotype of KLK1				
	GG $\bar{x} \pm SD$ (n=71)	GA $\bar{x} \pm SD$ (n=74)	AA $\bar{x} \pm SD$ (n=29)	P Value
Body Mass Index (kg/m2)	26.82±4.47	28.11±5.58	29.13±5.64	0.139 (NS)
Plasma Cholesterol (mg/dl)	197.53±41.70	190.47±54.19	181.00±47.27	0.296 (NS)
Triglycerides (mg/dl)	160.08±84.12	164.47±90.65	147.64±84.31	0.735 (NS)
Low-density lipoprotein (mg/dl)	125.59±35.39	119.00±46.52	110,62±37.09	0.253 (NS)
High-density lipoprotein (mg/dl)	43.57±12.12	40.89±10.84	41.19±10.83	0.199 (NS)

The results are shown as **n**: number of individuals, $\bar{x}$ **± SD:** mean of value ± Standard deviation, , one way ANOVA TEST used for comparison of KLK1 genotypes and lipid parameters , GG : Homozygote Wild Type, GA: Heterozygote, AA: Homozygote Mutant, ***** =significantly different ($p< 0.05$), (NS)= non significant ($p>0.05$).

The Genotype Frequencies of KLK1 in All Study Groups

Three genotypes of KLK1 gene are present (GG, GA, AA). Control group genotype distribution was 28 GG, 16 GA and 6 AA. CAD group distribution was 18 GG, 25 GA and 5 AA. T2DM distribution was 17 GG, 22 GA and 11 AA. CAD + T2DM distribution was 8 GG, 11 GA and 7 AA (Table 4.3.).

There was no significant difference among the genotypes comparing the 4 groups (p=0.095). GG (p=0.071), GA (p=0.250) and AA (p=0.163)

Table 4.3.: The Genotype Frequencies of KLK1 in all study groups

Genotype of KLK1 p (0.095 NS) Chi square (10.79)			
	GG (n=71)	GA (n=74)	AA (n=29)
Control (n=50)	28 56.0 %	16 32.0 %	6 12.0 %
CAD (n=48)	18 37.5 %	25 52.1 %	5 10.4 %
T2DM (n=50)	17 34.0 %	22 44.0 %	11 22.0 %
T2DM+CAD (n=26)	8 30.8%	11 42.3%	7 26.9 %
p value	0.071 NS	0.250 NS	0.163 NS
Chi square	7.039	4.105	5.127

The results are shown as **n**: number of individual, chi square used to determine KLK1 genotypes in groups. GG: Homozygote Wild Type, GA: Heterozygote, AA: Homozygote Mutant, ***** =significantly different ($p< 0.05$), (NS)= non significant ($p>0.05$).

The Allele Frequencies of KLK1 Genotypes in All Study Groups

Control group allele frequencies were 88.0 % G carrier, 12.0 % Non G carrier, 44.0 % A carrier and 56.0 % Non A carrier. CAD group allele frequencies were 89.6 % G carrier, 10.4 % Non G carrier, 62.5 % A carrier and 37.5 % Non A carrier. T2DM group allele frequencies were 78.0 % G carrier, 22.0 % Non G carrier, 66.0 %A carrier and 34.0 % Non A carrier. CAD+T2DM group allele frequencies was 73.1 % G carrier, 26.9 % Non G carrier, 69.2 % A carrier and 30.8 % Non A carrier (Table 4.4.). Comparing the G and the A allele in these groups didn't show a significant difference. G allele (p=0.163) and A allele (p=0.071).

Table 4.4.: The allele frequencies of KLK1 genotypes in all study groups

	G allele		A allele	
	G carrier	Non G carrier	A carrier	Non A carrier
Control (n=50)	44 88.0 %	6 12.0 %	22 44.0 %	28 56.0 %
CAD (n=48)	43 89.6 %	5 10.4 %	30 62.5 %	18 37.5 %
T2DM (n=50)	39 78.0 %	11 22.0 %	33 66.0 %	17 34.0 %
T2DM+CAD (n=26)	19 73.1 %	7 26.9 %	18 69.2 %	8 30.8 %
p value	0.163 NS		0.071 NS	
Chi square	5.127		7.039	

The results are shown as **n**: number of individuals, chi square used to determine KLK1 genotypes in groups. G allele: Wild Type, A allele Mutant, ***** =significantly different ($p < 0.05$), (NS) = non significant ($p > 0.05$).

The Genotype Frequencies of KLK1 in Control and Patients Groups

The frequencies of the KLK1 genotype in control group were 56.0 % GG, 32.0 % GA and 12.0 % AA while the frequencies in patient group were 34.6 % GG, 46.7 % GA and 18.5 % AA (Table 4.5.).

GA and AA genotypes showed no significant difference [GA (p=0.074), AA (p=0.163)] while there was a significant difference between control and patient groups in terms of GG genotype (p=0.010)

Table 4.5.: The Genotype Frequencies of KLK1 in control and patient groups

Genotype of KLK1 p (0.0348*) Chi square (6.71)			
	GG	GA	AA
Control (n=50)	28 56.0 %	16 32.0 %	6 12.0 %
Patient (n=124)	43 34.6 %	58 46.7 %	23 18.5 %
p value	0.010*	0.074 NS	0.294 NS
Chi square	6.707	3.182	1.101
Odds Ratio	0.147	1.867	1.670
Confidence Interval 95%	0.213-0.815	0.936-3.727	0.636-4.386

The results are shown as **n**: number of individuals, chi square used to determine KLK1 genotypes in groups. GG: Homozygote Wild Type, GA: Heterozygote, AA: Homozygote Mutant, ***** =significantly different ($p < 0.05$), (NS)= non significant ($p > 0.05$).

The Allele Frequencies of KLK1 Genotypes in Control and Patient Groups

KLK1 genotype allele frequencies in control group were 88.0 % G carrier, 12.0 % non G carrier, 44.0 % A carrier and 56.0 % non A carrier while in patient group were 81.4 % G carrier, 18.6% non G carrier, 34.7% A carrier and 65.3% non A carrier (Table 4.6.). Comparing the G and the A allele between control and patient groups didn't show a significant difference. G allele (p=0.294) and A allele (p=0.249).

Table 4.6.: The allele frequencies of KLK1 genotypes in control and patient group

	G allele		A allele	
	G carrier	Non G carrier	A carrier	Non A carrier
Control (n=50)	44 88.0 %	6 12.0 %	22 44.0 %	28 56.0 %
Patient (n=124)	101 81.4 %	23 18.6 %	43 34.7 %	81 65.3 %
p value	0.294 (NS)		0.249 (NS)	
Chi square	1.100		1.323	
Odds Ratio	0.599		0.147	
Confidence Interval 95%	0.228-1.573		0.213-0.815	

The results are shown as **n**: number of individuals, chi square used to determine KLK1 genotypes in groups. G allele: Wild Type, A allele Mutant, ***** =significantly different ($p< 0.05$), (NS)= non significant ($p>0.05$).

The Genotype Frequencies of KLK1 in Non-Diabetes and Diabetes Groups

The frequencies of the KLK1 genotype in non-diabetes group were 46.9 % GG, 41.8 % GA and 11.3 % AA while the frequencies in diabetes group were 32.9 % GG, 43.4 % GA and 23.7 % AA (Table 4.7.).

GG and GA genotypes showed no significant difference [GG (p=0.062), GA (p=0.834)] while there was a significant difference between non-diabetes and diabetes groups in terms of AA genotype (p=0.029).

Table 4.7.: The Genotype Frequencies of KLK1 in non-diabetes and diabetes groups

Genotype of KLK1 p (0.047*) Chi square (6.08)			
	GG	GA	AA
Non-diabetes (n=98)	46 46.9 %	41 41.8 %	11 11.3 %
T2DM (n=76)	25 32.9 %	33 43.4 %	18 23.7 %
p value	0.062 NS	0.834 NS	0.029*
Chi square	3.495	0.044	4.785
Odds Ratio	0.554	1.067	2.455
Confidence Interval 95%	0.298-1.032	0.582-1.955	1.081-5.575

The results are shown as **n**: number of individuals, chi square used to determine KLK1 genotypes in groups. GG: Homozygote Wild Type, GA: Heterozygote, AA: Homozygote Mutant, ***** =significantly different ($p< 0.05$), (NS)=non significant ($p>0.05$).

The Allele Frequencies of KLK1 Genotypes in Non-Diabetes and Diabetes Groups

KLK1 genotype allele frequencies in non-diabetes group were 88.8 % G carrier, 11.2 % non G carrier, 53.0 % A carrier and 47.0 % non A carrier while in diabetes group were 76.3 % G carrier,23.7 % non G carrier, 67.1 % A carrier and 32.9 % non A carrier (Table 4.8.). There was a significant difference between the two groups in terms of G allele (p=0.029). No significant difference was found in terms of A allele.

Table 4.8.: The allele frequencies of KLK1 genotypes in non-diabetes and diabetes groups

	G allele		A allele	
	G carrier	Non G carrier	A carrier	Non A carrier
Non-diabetes (n=98)	87 88.8 %	11 11.2 %	52 53.0 %	46 47.0 %
T2DM (n=76)	58 76.3 %	18 23.7 %	51 67.1%	25 32.9 %
p value	0.029*		0.061 (NS)	
Chi square	4.785		3.495	
Odds Ratio	0.407		1.805	
Confidence Interval 95%	0.179-0.925		0.969-3.360	

The results are shown as **n**: number of individuals, chi square used to determine KLK1 genotypes in groups. G allele: Wild Type, A allele Mutant, ***** =significantly different ($p< 0.05$), (NS)= non significant ($p>0.05$).

Assessment of Real Time PCR

The allelic discrimination was defined by fluorescent probe 7500 Fast-Real Time PCR device. The results were interrupted automatically by the software (Figure 4.1.).

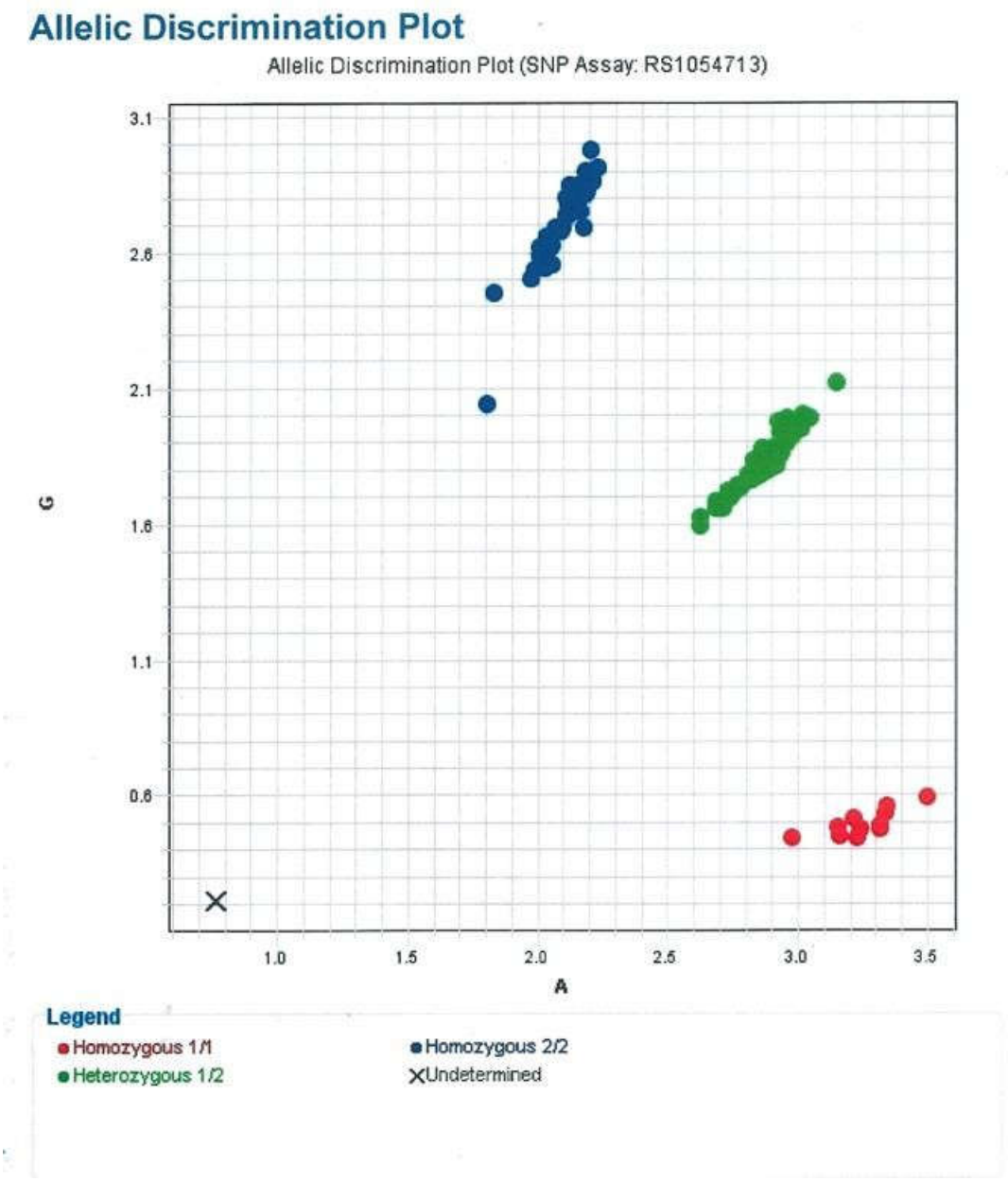


Figure 4.1.: Allelic discrimination plot

GG: Homozygous Wild Type, GA: Heterozygous, AA: Homozygous Mutant

5. DISCUSSION AND CONCLUSION

CAD which is the leading cause of mortality and morbidity in the world is a complex disease in which both lifestyle and genetic factors play role in its development (45, 46). Lifestyle factors such as diet and exercise are modifiable and interventions to change the unhealthy habits can potentially reduce the CAD incidence rates (47-49). To understand the complex genetic architecture of CAD GWAS and next generation sequencing studies identified many genetic loci associated with CAD (50, 51). In spite of all the studies about CAD risk factors the interaction between genetic and lifestyle factors is still not fully cleared (52).

Studies about the genetics of CAD stated that the heritability of CAD is about 40-50% (53). In 2018, van der Hast et al. performed a GWAS in which 122,733 CAD patients and 424,528 controls were included. van der Hast et al. study reported about 160 significant gene loci ($P < 5 \times 10\text{-}8$) related to CAD. On the other hand, genetic pathways thought to be associated with other disorders were investigated and lipids, blood pressure, diabetes mellitus, renal function, inflammatory markers and anthropometric measures were found to be related with CAD development (51).

A study included 3229 Swedish twins by Rappaport et al. stated that only 21.6% of CAD deaths were due to genetic factors and the rest 78.4% deaths were due to modifiable factors like lifestyle and environmental exposures, these results highlighted the importance of lifestyle modification as a primary preventive strategy in CAD (54).

According to the INTERHEART study tobacco smoking, alcohol consumption, coffee consumption, physical activity and diet are the main modifiable factors of MI (55).

Although mechanisms of tobacco smoking in CAD pathophysiology are unclear, tobacco smoking is a leading cause of CAD (56, 57). Besides, tobacco smoking beginning, heaviness and surcease are influenced by genetic factors (58, 59).

While low-moderate alcohol consumption may reduce CAD risk, heavy intake is a CAD risk factor. Same as the situation in smoking alcohol consumption is also affected by genetics. Excessive intake of coffee increases CAD risk wile moderate coffee consumption is thought to be protective against CAD (59-63).

Physical activity is inversely related with CAD risk and has an effective role in both primary and secondary prevention of CAD (64). According to genetic studies in families and twins physical activity and sedentary lifestyle may be inherited (65).

Vegetables, fruits and nuts consumption may reduce the risk of CAD (66). Also diet may induce epigenetic change like DNA methylation (67).

Combination of non-modifiable genetic factors and modifiable lifestyle factors together influences the CAD incidence risk. About people with genetic risk factors the CAD risk is higher when lifestyle is unhealthy and individuals with both high genetic risk and unhealthy lifestyle are at the highest risk of CAD (68).

According to the International Diabetes Federation about 629 million adults will be diabetic by 2045. T2DM compromises more than 90% of the total DM cases. The T2DM pathophysiology can be summarized generally by two mechanisms; insulin resistance in target tissues and impaired insulin secretion from the pancreas (69).

Although the mechanisms of T2DM pathway are still not fully cleared, T2DM is a complex disorder in which both genetic and environmental factors play role to develop it (70). As obesity is an insulin resistant state, the diabetes epidemic is closely related with the obesity epidemic. Sedentary lifestyle, unhealthy food consumption and other lifestyle factors may increase the risk of T2DM development by insulin resistance. Compared to environmental factors, the association of genetic factors with T2DM is relatively week (71).

Many non-genetic risk factors had been defined to compose risk for T2DM. Age, gender, ethnicity, BMI especially abdominal obesity, tobacco smoking, unhealthy diet, poor physical activity, dyslipidemia and hypertension are common risk factors for T2DM (69).

About 50% of the T2DM patients are at the same time hypertensive (72). In 2018, the prevalence of hypertension was 23.2% and DM was 10.9% in China and about 290 million Chinese adults had CVD according to the National Report of Cardiovascular Disease 2018 (73).

The exact mechanism of T2DM development due to hypertension is complex and includes many factors such as inflammation, disturbed rennin-angiotensin-aldosterone system (RAAS) activation, impaired renal sodium management, increased

sympathetic output, endothelial dysfunction due to insulin resistance and oxidative stress. Both T2DM and hypertension pathophysiologies share similar features (74, 75).

About half of the hypertensive patients also exhibit hyperinsulinemia or insulin resistance (76). Hyperglycemia due to insulin resistance induces pancreatic cells to secrete more insulin. Hyperinslinemia in turn causes hypertension by various mechanisms like endothelial/smooth muscle inflammation of the vascular wall, increased sympathetic output and reduced arterial compliance (77). Otherwise, disturbed endothelium-dependant vasodilatation may also aggravate insulin resistance (74-76, 78, 79).

For both T2DM and hypertension obesity (especially increased visceral fat mass) is a major risk factor (80). Adipocytes release a lipid-soluble factor that enhances aldosterone secretion from the zona glomerulosa of the adrenal glands (81, 82). Aldosterone in turn elevates blood pressure by sodium retention (83).

Adiponektin is a protein which carries anti-hypertensive properties and associated with insulin resistance and the blood levels of this protein are inversely related to BMI (84-86). Leptin elevation in individuals with high BMI activates the leptin receptors in the central nervous system leading to increased sympathetic output (77).

Family and twin studies revealed that genetic factors compose an important component in the etiology of T2DM. when both parents have T2DM the risk is about 70% and when one parent has T2DM the risk is about 40% (87). In twins T2DM risk ranges from 26% to 73%. In monozygotic twins the risk may be up to 76% (88).

Kallikreins are a family of proteins composed of 15 serin proteases which have trypsin-like and chymotrypsin-like characters. The kallikreins were discovered at the 1930s in the pancreatic extracts. Kallikrein are generally divided into two groups; plasma kallikreins and tissue kallikreins. Plasma kallikreins are secreted from the pancreas and circulate in the blood. Tissue kallikreins are expressed in the cells of different tissues. The tissue kallikreins are composed of 15 proteases. Genes that encode tissue kallikreins are located on chromosome 19q13 (89).

Each kallikrein is constricted by 244-253 residues forming a single polypeptide. Kallikreins are structurally identical to each other by about 40%.Tissue kallikreins belong to the chymotrypsin-like S1 family and the latter is a large family that includes

several essential proteases like chymotrypsin, trypsin, thrombin, matriptase and elastase. Many pathologies like cancer, pulmonary disorders, schizophrenia, anxiety, neurodegenerative disorders, pathological inflammation and skin barrier disruption are related with klk activity disturbance (90).

Klks are released from the pancreas as pre-pro-proteins and the main mechanism of tissue klks activity regulation is the activation of these pre-pro-proteins. Klks are activated by a cleavage process, this process occurs in the extracellular space (91, 92). In the extracellular space klks can be inhibited by several factors like plasma protease inhibitors and zinc cation (Zn2+) (93).

Klk1 is the first identified tissue kallikrein and exhibits both trypsin-like and chymotrypsin-like specificity. In patients with asthma or chronic bronchitis klk1 is found in the bronchoalveolar lavage fluid in a higher concentration (94). Klk1 has beneficial effects on the cardiovascular system and the renal system. As a result of its proteolytic activity; klk1 releases bradykinin from HMWK and subsequently klk1 activates bradykinin 2 receptor (B2R). klk1 is found to be effective in preventing T2DM and hypertension in rodents through its ability to activate B2R (95). Also klk1 plays role in the RAAS activity control (96).

In our study 4 groups were included; control group (n=50), CAD group (n=48), T2DM group (n=50) and CAD+T2DM group (n=26). Demographic characteristics of the study population were analyzed by using student t test and chi square test. Gender and age characteristics didn't show a significant difference between control and patient groups so these 2 factors were not been found to be effective in the pathogenesis of CAD and T2DM in patients included in our study. In maddona et.al study that was published in 2019 data from several centers from USA, Italy and Hungary showed that females are at lower risk than males in term of CAD. Madonna et.al stated that female hormones (specifically premenopausal females) may be protective against CAD. In the same study it was found that premenopausal females with diabetes mellitus were at a higher risk of CAD (97). Another study also stated that T2DM who are <60 years especially females are at a higher risk of cardiovascular disorders(98). In a meta-analysis published by WHO in 2013 it was demonstrated that comparing genders males are at higher risk of impaired fasting glycemia and females are at higher risk of impaired glucose tolerance but in terms of the DM prevalence there was no significant difference between males and females (99).According to Rodgers JL et.al; in old aged

population CVD is an important health issue. Age is not a direct risk for CVD but with again predisposing factors for CVD increase leading indirectly with the development of this disorder (100). Xu G et.also study compared the prevalence of type 1 and type 2 DM in US adults; the prevalence was 5.6% for type 1 and 91.2% for type 2 (101).

In our study we found that BMI is a predisposing factor for CAD and DM. Alkhawam H et.al stated that people with ≥30 BMI are at a higher risk of CAD when compared with people with BMI lower than 30 (102). Gupta s et.al study demonstrated that in comparison with normal weighted people, overweighed people are at a higher risk in terms of being diabetic or prediabetic (103).

Investigating lipid profiles in our study groups we found a significant difference in terms of HDL levels and we think that HDL has a protective role against CAD and DM. Shepra LY et.al also stated that HDL has an important role in averting CAD especially in females (104). Femlak M et.al study stated that normal levels of HDL are protective against atherosclerotic events. On the other hand, even at normal levels HDL may be dysfunctional due to disturbances in the lipids/proteins components and the enzymatic activities related with HDL. In DM HDL functions are distributed due to many different reasons like glycation and/or oxidation. Also, HDL protein may be transformed to a pro-inflammatory protein in diabetic patients. And as a result of the disturbed function of HDL; HDL will not be protective in diabetics and in turn these patients are at higher risk of inflammatory and atherogenetic effects (105).

Analyzing the genotypes of KLK1 (rs1054713) three genotypes are available; GG (homozygous wild type), GA (heterozygous) and AA (homozygous mutant). No correlation between lipid profile and BMI and KLK1 genotype of the CAD group. Comparing our study groups in terms of KLK1 genotypes didn't show any significance. Also, the allele frequencies of KLK1 genotypes in all study groups were not found to be significant. Comparing KLK1 genotype frequencies in control and patients groups no significant difference was found in GA and AA genotypes while GG genotype showed a significant difference between the groups. In terms of allele frequencies of KLK1 genotypes A allele and G allele were not significant. Comparing diabetic and non-diabetic groups; KLK1 genotypes frequencies showed a significant difference in terms of AA genotype and no significance in terms of GG and GA. We also found that G allele was significantly different between diabetic and non diabetic groups with no significance in terms of A allele. According to these findings we think that KLK1 gene

polymorphism (rs1054713) is not a risk for CAD while this polymorphism is related with T2DM. As a result of our genotypic and allelic analysis we think that G allele is protective against T2DM and A allele is not directly related to T2DM development.

As a result of our study;

- The number of males with CAD is higher than females, but the difference is not significant. While in patients with DM and CAD+T2DM the number of females is insignificantly higher.
- The mean age of the CAD and CAD+T2DM is higher than the mean age of the control and T2DM groups but the difference is not significant.
- The BMI is higher in T2DM and CAD+T2DM groups than the other 2 groups, and CAD group BMI is higher than control group.
- Analyzing the lipid profile parameters; TG and VLDL levels are higher in T2DM group. LDL levels are higher in the control group. HDL levels are lower in CAD. The differences of TG, VLDL and LDL are not significant. The HDL difference is significant.
- GG KLK1 genotype is significantly different between control and patient groups.
- The AA genotype is significantly different between diabetic and non diabetic groups
- The G allele frequency is significantly different between diabetic and non diabetic groups.

According to our study BMI is a risk factor for T2DM and at a lower extent for CAD. HDL is protective against CAD. Lifestyle habits like diet and physical activities have effects on the development of these diseases and improving lifestyle is an important preventive management. KLK1 genetic polymorphism analyses in our study had no effect on CAD in our study population. KLK1 gene polymorphism is related with T2DM and patients with this mutation are more prone to have this disease. The mutant A allele difference between diabetic and non diabetic groups is not significant while the G allele is significantly different. The polymorphism of this study is related indirectly with T2DM. Studies with larger numbers of people will be useful for understanding this genetic polymorphism roles and mechanisms in the development of T2DM.

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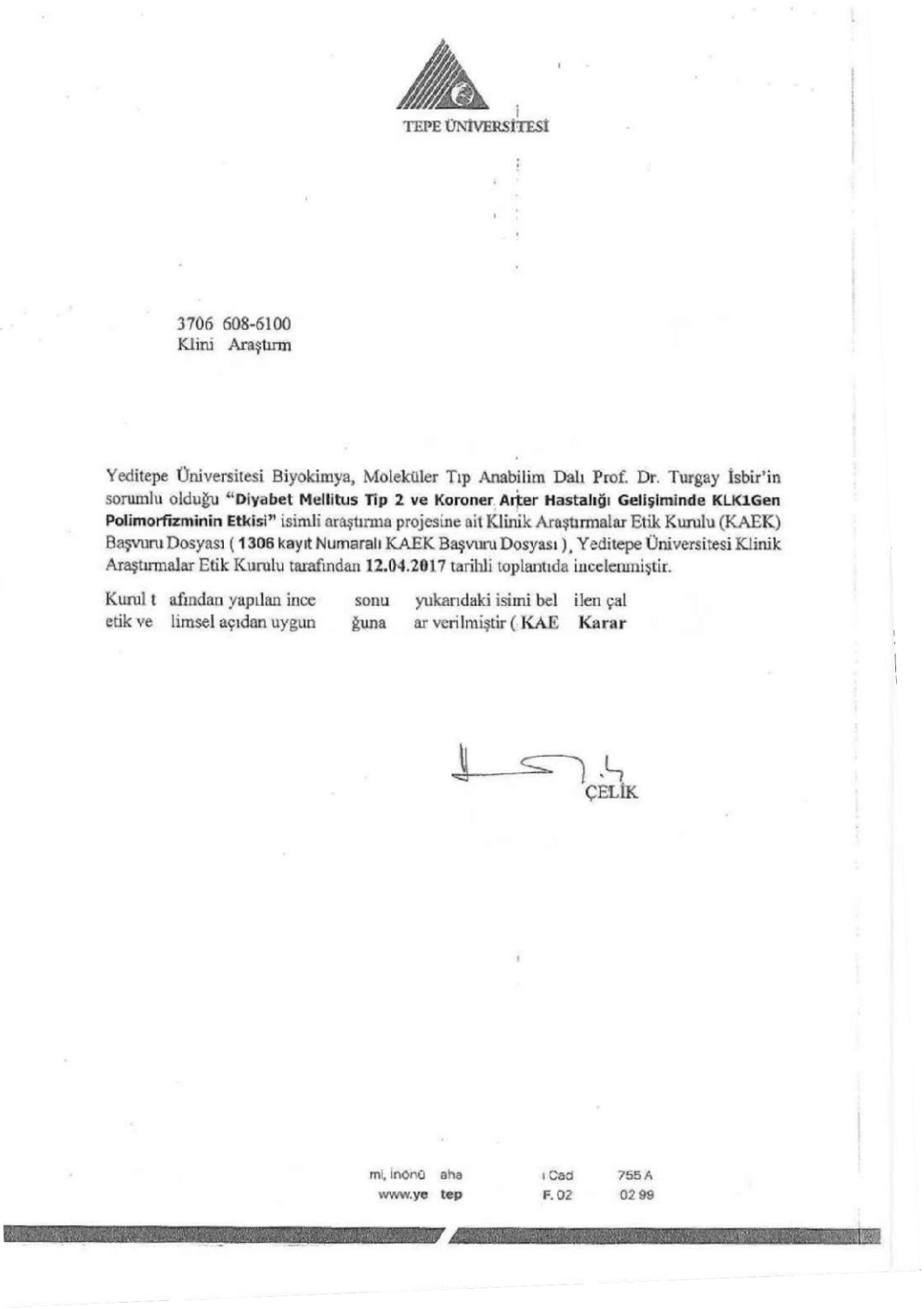
7. APPENDICES

Ethical Approval



TEPE ÜNİVERSİTESİ

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Klini Araştırm

Yeditepe Üniversitesi Biyokimya, Moleküler Tıp Anabilim Dalı Prof. Dr. Turgay İsbir'in sorumlu olduğu **"Diyabet Mellitus Tip 2 ve Koroner Arter Hastalığı Gelişiminde KLK1Gen Polimorfizminin Etkisi"** isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KAEK) Başvuru Dosyası (**1306** kayıt Numaralı KAEK Başvuru Dosyası), Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından **12.04.2017** tarihli toplantıda incelenmiştir.

Kurul t afından yapılan ince sonu yukarıdaki isimi bel ilen çal
etik ve limsel açıdan uygun ğuna ar verilmiştir (KAE **Karar**

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Forms

Biological Materials Transfer Form

YEDİTEPE ÜNİVERSİTESİ HASTANESİ

KLİNİK ARAŞTIRMALARDA KULLANILACAK BİYOLOJİK MATERYAL TRANSFER FORMU

Araştırmanın Açık Adı: KLK1 genetic polymorphism effect on the development of coronary artery disease and diabetes mellitus type 2

Araştırmanın Özeti:

Coronary artery disease (CAD) also known as atherosclerotic heart disease,[1] coronary heart disease,[2] or ischemic heart disease (IHD),[3] is the most common type of heart disease and cause of heart attacks.[4] Diabetes mellitus type 2 (formerly noninsulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes) is a metabolic disorder that is characterized by hyperglycemia (high blood sugar) in the context of insulin resistance and relative lack of insulin.[5] This is in contrast to diabetes mellitus type 1, in which there is an absolute lack of insulin due to breakdown of islet cells in the pancreas.[6] Diabetes mellitus (DM) is an important risk factor for coronary heart disease [7], and it is associated with a high prevalence of coronary artery disease (CAD) and an unfavorable prognosis [8]. The age-adjusted cardiovascular mortality is at least 2-fold higher in diabetic men than in nondiabetic subjects in the presence of any number of major risk factors. [9] The survival after myocardial infarction is worse in diabetic men and women. [10]

Kallikreins are secreted serine proteases that are synthesized as pre-proenzymes, the signal peptide of which is cleaved for secretion; the proenzyme is also cleaved for full activation [11]. Human plasma kallikrein (PK), encoded by the KLKB1 gene on human chromosome 4q34-35, is synthesized in the liver as an inactive precursor and circulates in the plasma. [12] Tissue kallikrein (TK) is a serine proteinase that releases vasoactive kinin peptides from a low-molecular-weight kininogen substrate. [13] KLK1 (tissue kallikrein 1) is a member of the tissue kallikrein family of serine proteases, which, in humans, comprises 15 proteases and constitutes the largest contiguous cluster of any family of proteases on the human genome .[14,15] (*KLK1*) gene is located on chromosome 19 (19q13.2- q13.4) together with several homologous genes coding for non-kinin-forming serine proteases or unidentified protein products. The *KLK1* gene spans 5.2 kb containing 5 exons and codes for an inactive prokallikrein form activated by the intracellular proteolysis of a short amino-terminal peptide [16] .Animal studies have shown that the *KLK1* gene is involved in bradykinin coronary outflow [17] and carotid artery neointima formation after balloon angioplasty [18]

İş bu anlaşma ile, biyolojik materyali gönderen araştırmacı ve kurum : **'Association of KLK1 gene polymorphism with the development of CAD and DM2**..isimli araştırmada kullanılmak üzere gönderilecek ....**10ml**.....miktarda ve ....**araştırma** ..... amaçla kullanılacak biyolojik materyali .... **26 Ağustos Yerleşimi Yeditepe Üniversitesi, Kayışdağı / İstanbul** adresindeki .....**Yeditepe Üniversitesi Moleküler Tıp Anabilim Dalında'ndaki** merkeze göndermeden önce ALICI kurumdan aşağıdaki koşulları kabul etmesi istenmektedir:

1. Gönderilen biyolojik materyaller yalnızca yukarıda yazılı amaç için, ya da gönderici kurumun yeniden yazılı iznini almak koşulu ile ikincil amaç için kullanılabilir.
2. ALICI biyolojik materyali gönderici kurumun yazılı izni olmadan üçüncü şahıslara vermeyecektir. ALICI üçüncü şahıslardan gelebilecek istekleri GÖNDERİCİ'ye bildirecektir.
3. Biyolojik materyaller GÖNDERICI tarafından bireyin kimlik bilgileri olmaksızın ALICI'ya gönderilecektir.
4. ALICI biyolojik materyalleri Birleşmiş Milletler İnsan Genomu ve İnsan Hakları Evrensel Beyannamesine uygun olarak kullanacaktır.
5. Biyolojik materyaller ALICI'ya gönderilmeden önce biyolojik materyalin sağlandığı kişilere ait Sağlık Bakanlığı'nın ve Etik Kurul'un onayladığı bilgilendirilmiş gönüllü olur formunun her bir gönüllüden alınmış olması gerekmektedir.

6. Bu anlaşma ile gönderilecek biyolojik materyalin araştırma için kullanılacak olduğu ve biyolojik materyal kullanımının bazı tehlikeli özelliklerinin var olduğu ALICI tarafından kabul edilmektedir. Biyolojik materyali sağlayan kurum bu konuda sorumlu değildir.
7. GÖNDERİCİ ve ALICI yapılacak ortak bir yayınla ya da doğabilecek patent hakkı ve ticari gelişmelerle ilgili haklarını araştırma başlangıcında karşılıklı olarak belirleyecektir.
8. Bu anlaşma aşağıdaki iki maddeden herhangi birinin gerçekleşmesi halinde son bulacaktır.
   a. Araştırmanın sonlanması durumunda,
   b. Taraflardan herhangi birinin diğerine gönderdiği yazılı uyarıyı takiben 30 (otuz) gün içinde Anlaşma kurallarına uymama; patent haklarının ihlali veya sağlık tehdidi oluşturan riskler dışında bu anlaşma 8 (b) koşulunda materyali sağlayan tarafın yazılı uyarısı ile bitirilecek olursa ALICI'nın araştırmasının engellenmemesi için ve ALICI'nın isteği üzerine materyali sağlayan araştırmacı 1 (bir) yıla kadar varan bir süre içinde anlaşmanın sonlanacağı bir tarih belirleyebilir.
9. ALICI bu anlaşmanın bitiminde bütün materyalleri geri vermeyi veya ortadan kaldırmayı ve bunu belgelemeyi kabul eder.
10. GÖNDERİCİ biyolojik materyali toplama, hazırlama ve göndermek için bir ücret talep ediyorsa bu ücret burada belirtilecektir.
11. Bu anlaşmanın yürümesinde ALICI ve GÖNDERİCİ kurum amirleri ile destekleyici sorumludur. Anlaşmazlık halinde ihtilafın çözümü için her iki ülke mahkemeleri de yetkilidir.

BİYOLOJİK MATERYALİ GÖNDEREN ARAŞTIRMACI BİLGİSİ

Adı Soyadı ve Unvanı:	Adı Soyadı: Selim ISBIR
Uzmanlık Alanı:	Unvan (Dr., ...): Prof. Dr.
Kurumu:	Uzmanlık alanı: kardiyovaskuler cerrahisi
Adresi:	İş adresi: Marmara universitesi hastanesi,mimar sinan caddesi no: 41 üst kaynarca fevziçakmak mah. / pendik / istanbul
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Adı Soyadı ve Unvanı:	Adı Soyadı: Hasan Aydın
Uzmanlık Alanı:	Unvan (Dr., ...): Doç. Dr.
Kurumu:	Uzmanlık alanı: İç Hastalıkları
Adresi:	İş adresi: Yeditepe Üniversitesi Hastanesi, İçerenköy Mahallesi Hastane Yolu Sokak no:102-104 Ataşehir-İstanbul
Telefon:	Telefon numarası: (0216) 578 40 00
Faks:	-

YEDİTEPE ÜNİVERSİTESİ HASTANESİ	KLİNİK ARAŞTIRMALARDA KULLANILACAK BİYOLOJİK MATERYAL TRANSFER FORMU

E-posta:	E-posta **adresi**: haydin@yeditepe.edu.tr

BİYOLOJİK MATERYALİ ALAN ALICI BİLGİSİ

Adı Soyadı ve Unvanı:	M.D. Noor HUSSAIN
Uzmanlık Alanı:	
Kurumu:	Yeditepe üniversitesi Moleküler Tıp Anabilim Dalı
Adresi:	26 Ağustos yerleşkesi Kayışdağı /İstanbul
Telefon:	0544 284 12 02
Faks:	(0216) 578 00 00 (pbx)
E-posta:	noora_289@yahoo.com

Bu anlaşmada belirtilen koşulları okudum ve anladım. Gönderilen materyalde bu anlaşmada belirtilen koşullara uyacağımı taahhüt ederim.

	Gönderen Araştırmacı	Gönderen **Destekleyici Firma Yetkilisi veya Yasal Temsilcisi**	**Klinik Şefi / Ana Bilim Dalı Başkanı**	**Kurum Amiri / Rektör veya Yetkilendirdiği Makam**	**Alıcı Kurum Yetkilisi**
El Yazısı ile Adı Soyadı Unvanı					
Tarih					
İmza					

Not: Bu anlaşmada yer alan alıcı kurum yetkilisinin imzası yerine alıcı kurum tarafından verilecek olan ve içerik olarak bu anlaşmadaki hükümlere benzer hükümleri içeren imzalı "end use certificate" "son kullanım sertifikası" de kabul edilir.

Volunteer Form

YEDİTEPE ÜNİVERSİTESİ HASTANESİ	Klinik Araştırmalar Etik Kurulu Bilgilendirilmiş Gönüllü Olur Formu

Hastanın veya yerine onam verecek kişinin okuma, anlama, konuşma, dil sorunu mevcut mu? Evet ☐ Hayır ☐ Cevabınız EVET ise Hasta İlişkileri Sorumlusu ile iletişim kurunuz.	**Tercüman gerektiyse;** Tercümanın adı _____ İmza _____ Tarih _____

Sayın Hastamız,

- Bu belge bilgilendirilme ve aydınlatılmış onam haklarınızdan yararlanabilmenizi amaçlamaktadır.
- Size gerçekleştirilebilecek klinik araştırmalar amaçlı girişimler konusunda, tüm seçenekler ile bu girişimlerin yarar ve muhtemel zararları konusunda anlayabileceğiniz şekilde **bilgi alma hakkınız ve bir kopyasını isteme hakkınız** vardır.
- Yasal ve tıbbi zorunluluk taşıyan durumlar dışında **bilgilendirmeyi reddedebilirsiniz.** Yazılı bildirmek koşulu ile bilgi almama veya yerinize güvendiğiniz bir kimsenin bilgilendirilmesini talep etme hakkına sahipsiniz.
- klinik araştırmalara katılım konusunda bilgilendirildikten sonra bunu kabul edebilirsiniz. Ya da **karar verebilmek için uygun zaman talep edebilirsiniz.**
- Hayatınız veya hayati organlarınız tehlikede olmadığı sürece onamınızı (yazılı talep etme koşulu ile) **dilediğiniz zaman geri alabilir** ya da önceden kabul etmediğiniz herhangi bir tanı/tedavi amaçlı girişimi **tekrar talep edebilirsiniz.**
- Hastanemizde verilen hizmetleri **Hastane Tanıtım Broşürü**nden edinebilirsiniz. Ayrıca Hastanemiz personeli hakkında http://www.yeditepehastanesi.com.tr/ web sayfamızdan daha detaylı bilgilere ulaşabilirsiniz.
- Burada belirtilenlerden başka sorularınız varsa bunları yanıtlamak görevimizdir.

VEDİTEPE ÜNİVERSİTESİ HASTANESİ	Klinik Araştırmalar Etik Kurulu Bilgilendirilmiş Gönüllü Olur Formu

TANIMLAMA

Araştırmanın Adı / KLK1 genetic polymorphism effect on the development of coronary artery disease and diabetes mellitus type 2

Araştırma Konusu KLK1 genetic polymorphism effect on the development of coronary artery disease and diabetes mellitus type 2

Araştırmaya Katılımcı Sayısı : 150

Bu araştırmanın

Amacı detecting the effect of KLK-1 genetic polymorphism on developping coronary artery disease and diebetes mellitus type 2

Süresi 1 years

İzlenecek Yöntem / Yöntemler KLK1 genotype will be analyzed by using PCR-RFLP method.

Araştırma Sonunda Beklenen Fayda

Alternatif Tedavi Veya Girişimler

Araştırma Sırasında Karşılaşılabilecek;

Riskleri	*Rahatsızlıklar*
a)	a)
b)	b)
c)	c)
d)	d)
e)	e)
f)	f)
g)	g)

Risk / rahatsızlık durumlarında yapılması gerekenler

Aşağıdaki özel durumlara ait katılımcı var mı?

	EVET*	HAYIR
Çocuk		x
Mahkum		x
Gebe		x
Mental yetersizlik		x
Sosyoekonomik eğitim olarak yetersiz		x

*Ancak çocuklarda, hamilelik, lohusalık ve emzirme dönemlerinde ve kısıtlılık durumunda; gönüllüler yönünden araştırmadan doğrudan fayda sağlanacağı umuluyor ve araştırma gönüllü sağlığı açısından öngörülebilir ciddi bir risk taşımıyor ise, usulüne uygun bir şekilde alınmış bilgilendirilmiş gönüllü olur formu ile birlikte ilgili etik kurulun onayı ve Bakanlık izni alınmak suretiyle araştırmaya izin verilebilir.

ONAM (RIZA)

Bilgilendirilmiş Gönüllü Olur Formundaki tüm açıklamaları okudum. Bana, yukarıda konusu ve amacı belirtilen araştırma ile ilgili yazılı ve sözlü açıklama aşağıda adı belirtilen hekim tarafından yapıldı. Araştırmaya gönüllü olarak katıldığımı, istediğim zaman gerekçeli veya gerekçesiz olarak araştırmadan ayrılabileceğimi ve kendi isteğime bakılmaksızın araştırmacı tarafından araştırma dışı bırakılabileceğimi biliyorum. Bu durumda hastanenin çalışma düzeni ve hastalara verilen bakımda aksaklık olmayacağı konusunda bilgilendirildim. Bu araştırmaya katılırken zorlama, maddi çıkar ve ast üst ilişkisine dayalı herhangi bir baskı olmaksızın bu çalışmaya katıldığımı beyan ederim. Bu bilimsel çalışmanın devamı esnasındaki süreçle ilgili olarak ayrıca eklenen çalışma protokolü ile bilgilendirildim.

YEDİTEPE ÜNİVERSİTESİ HASTANESİ	Klinik Araştırmalar Etik Kurulu Bilgilendirilmiş Gönüllü Olur Formu

Söz konusu araştırmaya, hiçbir baskı ve zorlama olmaksızın kendi rızamla katılmayı kabul ediyorum.

Gönüllünün Adı / Soyadı / İmzası / Tarih

Açıklamaları Yapan Kişinin Adı / Soyadı / İmzası / Tarih

Gerekiyorsa Olur İşlemine Tanık Olan Kişinin Adı / Soyadı / İmzası / Tarih

Gerekiyorsa Yasal Temsilcinin Adı / Soyadı / İmzası / Tarih

24 Saat ulaşılabilir iletişim bilgiler

Bilgilendirilmiş Gönüllü Onam Formu asgari olarak yukarıda belirtilen başlıkları içermelidir.

The Raw Data

```
CROSSTABS
  /TABLES=Grup BY Genotip_rs1054713 GG GA AA G A
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ RISK
  /CELLS=COUNT ROW COLUMN TOTAL
  /COUNT ROUND CELL.
```

Crosstabs

Notes

Output Created		18-SEP-2018 16:01:35
Comments		
Input	Data	C:\Users\seda.gulec\Desktop\No or Tez- KLK1- KAH- Diyabet.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	174
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each table are based on all the cases with valid data in the specified range(s) for all variables in each table.
Syntax		CROSSTABS /TABLES=Grup BY Genotip_rs1054713 GG GA AA G A /FORMAT=AVALUE TABLES /STATISTICS=CHISQ RISK /CELLS=COUNT ROW COLUMN TOTAL /COUNT ROUND CELL.
Resources	Processor Time	00:00:00,02

	Elapsed Time	00:00:00,03
	Dimensions Requested	2
	Cells Available	524245

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Grup * Genotip_rs1054713	174	100,0%	0	0,0%	174	100,0%
Grup * GG	174	100,0%	0	0,0%	174	100,0%
Grup * GA	174	100,0%	0	0,0%	174	100,0%
Grup * AA	174	100,0%	0	0,0%	174	100,0%
Grup * G	174	100,0%	0	0,0%	174	100,0%
Grup * A	174	100,0%	0	0,0%	174	100,0%

Grup * Genotip_rs1054713

Crosstab

			Genotip_rs1054713			
			GG	GA	AA	Total
Grup	Kontrol	Count	28	16	6	50
		% within Grup	56,0%	32,0%	12,0%	100,0%
		% within Genotip_rs1054713	39,4%	21,6%	20,7%	28,7%
		% of Total	16,1%	9,2%	3,4%	28,7%
	KAH	Count	18	25	5	48
		% within Grup	37,5%	52,1%	10,4%	100,0%
		% within Genotip_rs1054713	25,4%	33,8%	17,2%	27,6%
		% of Total	10,3%	14,4%	2,9%	27,6%
	Diyabet	Count	17	22	11	50

		% within Grup	34,0%	44,0%	22,0%	100,0%
		% within Genotip_rs1054713	23,9%	29,7%	37,9%	28,7%
		% of Total	9,8%	12,6%	6,3%	28,7%
	KAH+Diyabet	Count	8	11	7	26
		% within Grup	30,8%	42,3%	26,9%	100,0%
		% within Genotip_rs1054713	11,3%	14,9%	24,1%	14,9%
		% of Total	4,6%	6,3%	4,0%	14,9%
Total		Count	71	74	29	174
		% within Grup	40,8%	42,5%	16,7%	100,0%
		% within Genotip_rs1054713	100,0%	100,0%	100,0%	100,0%
		% of Total	40,8%	42,5%	16,7%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	10,799[a]	6	,095
Likelihood Ratio	10,560	6	,103
Linear-by-Linear Association	7,202	1	,007
N of Valid Cases	174		

a. 1 cells (8,3%) have expected count less than 5. The minimum expected count is 4,33.

Risk Estimate

	Value
Odds Ratio for Grup (Kontrol / KAH)	[a]

a. Risk Estimate statistics cannot be computed. They are only computed for a 2*2 table without empty cells.

Grup * GG

Crosstab

			GG		
			Yok	Var	Total
Grup	Kontrol	Count	22	28	50
		% within Grup	44,0%	56,0%	100,0%
		% within GG	21,4%	39,4%	28,7%
		% of Total	12,6%	16,1%	28,7%
	KAH	Count	30	18	48
		% within Grup	62,5%	37,5%	100,0%
		% within GG	29,1%	25,4%	27,6%
		% of Total	17,2%	10,3%	27,6%
	Diyabet	Count	33	17	50
		% within Grup	66,0%	34,0%	100,0%
		% within GG	32,0%	23,9%	28,7%
		% of Total	19,0%	9,8%	28,7%
	KAH+Diyabet	Count	18	8	26
		% within Grup	69,2%	30,8%	100,0%
		% within GG	17,5%	11,3%	14,9%
		% of Total	10,3%	4,6%	14,9%
Total		Count	103	71	174
		% within Grup	59,2%	40,8%	100,0%
		% within GG	100,0%	100,0%	100,0%
		% of Total	59,2%	40,8%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	7,039[a]	3	,071
Likelihood Ratio	6,993	3	,072
Linear-by-Linear Association	5,745	1	,017
N of Valid Cases	174		

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 10,61.

Risk Estimate

	Value
Odds Ratio for Grup (Kontrol / KAH)	[a]

a. Risk Estimate statistics cannot be computed.
They are only computed for a 2*2 table without
empty cells.

Grup * GA

Crosstab

			GA		
			Yok	Var	Total
Grup	Kontrol	Count	34	16	50
		% within Grup	68,0%	32,0%	100,0%
		% within GA	34,0%	21,6%	28,7%
		% of Total	19,5%	9,2%	28,7%

	KAH	Count	23	25	48
		% within Grup	47,9%	52,1%	100,0%
		% within GA	23,0%	33,8%	27,6%
		% of Total	13,2%	14,4%	27,6%
	Diyabet	Count	28	22	50
		% within Grup	56,0%	44,0%	100,0%
		% within GA	28,0%	29,7%	28,7%
		% of Total	16,1%	12,6%	28,7%
	KAH+Diyabet	Count	15	11	26
		% within Grup	57,7%	42,3%	100,0%
		% within GA	15,0%	14,9%	14,9%
		% of Total	8,6%	6,3%	14,9%
Total		Count	100	74	174
		% within Grup	57,5%	42,5%	100,0%
		% within GA	100,0%	100,0%	100,0%
		% of Total	57,5%	42,5%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	4,105[a]	3	,250
Likelihood Ratio	4,151	3	,246
Linear-by-Linear Association	,748	1	,387
N of Valid Cases	174		

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 11,06.

Risk Estimate

	Value
Odds Ratio for Grup (Kontrol / KAH)	[a]

a. Risk Estimate statistics cannot be computed.
They are only computed for a 2*2 table without
empty cells.

Grup * AA

Crosstab

			AA		
			Yok	Var	Total
Grup	Kontrol	Count	44	6	50
		% within Grup	88,0%	12,0%	100,0%
		% within AA	30,3%	20,7%	28,7%
		% of Total	25,3%	3,4%	28,7%
	KAH	Count	43	5	48
		% within Grup	89,6%	10,4%	100,0%
		% within AA	29,7%	17,2%	27,6%
		% of Total	24,7%	2,9%	27,6%
	Diyabet	Count	39	11	50
		% within Grup	78,0%	22,0%	100,0%
		% within AA	26,9%	37,9%	28,7%
		% of Total	22,4%	6,3%	28,7%
	KAH+Diyabet	Count	19	7	26
		% within Grup	73,1%	26,9%	100,0%
		% within AA	13,1%	24,1%	14,9%
		% of Total	10,9%	4,0%	14,9%

Total	Count	145	29	174
	% within Grup	83,3%	16,7%	100,0%
	% within AA	100,0%	100,0%	100,0%
	% of Total	83,3%	16,7%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	5,127[a]	3	,163
Likelihood Ratio	5,045	3	,169
Linear-by-Linear Association	4,056	1	,044
N of Valid Cases	174		

a. 1 cells (12,5%) have expected count less than 5. The minimum expected count is 4,33.

Risk Estimate

	Value
Odds Ratio for Grup (Kontrol / KAH)	[a]

a. Risk Estimate statistics cannot be computed.
They are only computed for a 2*2 table without
empty cells.

Grup * G

Crosstab

		G	Total

			Yok	Var	
Grup	Kontrol	Count	6	44	50
		% within Grup	12,0%	88,0%	100,0%
		% within G	20,7%	30,3%	28,7%
		% of Total	3,4%	25,3%	28,7%
	KAH	Count	5	43	48
		% within Grup	10,4%	89,6%	100,0%
		% within G	17,2%	29,7%	27,6%
		% of Total	2,9%	24,7%	27,6%
	Diyabet	Count	11	39	50
		% within Grup	22,0%	78,0%	100,0%
		% within G	37,9%	26,9%	28,7%
		% of Total	6,3%	22,4%	28,7%
	KAH+Diyabet	Count	7	19	26
		% within Grup	26,9%	73,1%	100,0%
		% within G	24,1%	13,1%	14,9%
		% of Total	4,0%	10,9%	14,9%
Total		Count	29	145	174
		% within Grup	16,7%	83,3%	100,0%
		% within G	100,0%	100,0%	100,0%
		% of Total	16,7%	83,3%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	5,127[a]	3	,163
Likelihood Ratio	5,045	3	,169
Linear-by-Linear Association	4,056	1	,044

N of Valid Cases	174		

a. 1 cells (12,5%) have expected count less than 5. The minimum expected count is 4,33.

Risk Estimate

	Value
Odds Ratio for Grup (Kontrol / KAH)	[a]

a. Risk Estimate statistics cannot be computed.
They are only computed for a 2*2 table without
empty cells.

Grup * A

Crosstab

			A		
			Yok	Var	Total
Grup	Kontrol	Count	28	22	50
		% within Grup	56,0%	44,0%	100,0%
		% within A	39,4%	21,4%	28,7%
		% of Total	16,1%	12,6%	28,7%
	KAH	Count	18	30	48
		% within Grup	37,5%	62,5%	100,0%
		% within A	25,4%	29,1%	27,6%
		% of Total	10,3%	17,2%	27,6%
	Diyabet	Count	17	33	50
		% within Grup	34,0%	66,0%	100,0%

	% within A	23,9%	32,0%	28,7%
	% of Total	9,8%	19,0%	28,7%
KAH+Diyabet	Count	8	18	26
	% within Grup	30,8%	69,2%	100,0%
	% within A	11,3%	17,5%	14,9%
	% of Total	4,6%	10,3%	14,9%
Total	Count	71	103	174
	% within Grup	40,8%	59,2%	100,0%
	% within A	100,0%	100,0%	100,0%
	% of Total	40,8%	59,2%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	7,039[a]	3	,071
Likelihood Ratio	6,993	3	,072
Linear-by-Linear Association	5,745	1	,017
N of Valid Cases	174		

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 10,61.

Risk Estimate

	Value
Odds Ratio for Grup (Kontrol / KAH)	[a]

a. Risk Estimate statistics cannot be computed.

They are only computed for a 2*2 table without

empty cells.

```
CROSSTABS
  /TABLES=hasta_kontrol BY Genotip_rs1054713 GG GA AA G A
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ RISK
  /CELLS=COUNT ROW COLUMN TOTAL
  /COUNT ROUND CELL.
```

Crosstabs

Notes

Output Created		18-SEP-2018 16:01:51
Comments		
Input	Data	C:\Users\seda.gulec\Desktop\No or Tez- KLK1- KAH- Diyabet.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	174
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each table are based on all the cases with valid data in the specified range(s) for all variables in each table.

Syntax		CROSSTABS /TABLES=hasta_kontrol BY Genotip_rs1054713 GG GA AA G A /FORMAT=AVALUE TABLES /STATISTICS=CHISQ RISK /CELLS=COUNT ROW COLUMN TOTAL /COUNT ROUND CELL.
Resources	Processor Time	00:00:00,02
	Elapsed Time	00:00:00,03
	Dimensions Requested	2
	Cells Available	524245

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
hasta_kontrol * Genotip_rs1054713	174	100,0%	0	0,0%	174	100,0%
hasta_kontrol * GG	174	100,0%	0	0,0%	174	100,0%
hasta_kontrol * GA	174	100,0%	0	0,0%	174	100,0%
hasta_kontrol * AA	174	100,0%	0	0,0%	174	100,0%
hasta_kontrol * G	174	100,0%	0	0,0%	174	100,0%
hasta_kontrol * A	174	100,0%	0	0,0%	174	100,0%

hasta_kontrol * Genotip_rs1054713

Crosstab

			Genotip_rs1054713			
			GG	GA	AA	Total
hasta_kontrol	kontrol	Count	28	16	6	50
		% within hasta_kontrol	56,0%	32,0%	12,0%	100,0%

		% within Genotip_rs1054713	39,4%	21,6%	20,7%	28,7%
		% of Total	16,1%	9,2%	3,4%	28,7%
	hasta	Count	43	58	23	124
		% within hasta_kontrol	34,7%	46,8%	18,5%	100,0%
		% within Genotip_rs1054713	60,6%	78,4%	79,3%	71,3%
		% of Total	24,7%	33,3%	13,2%	71,3%
Total		Count	71	74	29	174
		% within hasta_kontrol	40,8%	42,5%	16,7%	100,0%
		% within Genotip_rs1054713	100,0%	100,0%	100,0%	100,0%
		% of Total	40,8%	42,5%	16,7%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	6,716[a]	2	,035
Likelihood Ratio	6,649	2	,036
Linear-by-Linear Association	5,329	1	,021
N of Valid Cases	174		

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 8,33.

Risk Estimate

	Value
Odds Ratio for hasta_kontrol (kontrol / hasta)	[a]

a. Risk Estimate statistics cannot be computed.
They are only computed for a 2*2 table without
empty cells.

hasta_kontrol * GG

Crosstab

			GG		
			Yok	Var	Total
hasta_kontrol	kontrol	Count	22	28	50
		% within hasta_kontrol	44,0%	56,0%	100,0%
		% within GG	21,4%	39,4%	28,7%
		% of Total	12,6%	16,1%	28,7%
	hasta	Count	81	43	124
		% within hasta_kontrol	65,3%	34,7%	100,0%
		% within GG	78,6%	60,6%	71,3%
		% of Total	46,6%	24,7%	71,3%
Total		Count	103	71	174
		% within hasta_kontrol	59,2%	40,8%	100,0%
		% within GG	100,0%	100,0%	100,0%
		% of Total	59,2%	40,8%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	6,707[a]	1	,010		
Continuity Correction[b]	5,853	1	,016		
Likelihood Ratio	6,638	1	,010		
Fisher's Exact Test				,011	,008
Linear-by-Linear Association	6,668	1	,010		
N of Valid Cases	174				

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 20,40.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for hasta_kontrol (kontrol / hasta)	,417	,213	,815
For cohort GG = Yok	,674	,480	,944
For cohort GG = Var	1,615	1,144	2,279
N of Valid Cases	174		

hasta_kontrol * GA

Crosstab

			GA		
			Yok	Var	Total
hasta_kontrol	kontrol	Count	34	16	50
		% within hasta_kontrol	68,0%	32,0%	100,0%
		% within GA	34,0%	21,6%	28,7%
		% of Total	19,5%	9,2%	28,7%
	hasta	Count	66	58	124
		% within hasta_kontrol	53,2%	46,8%	100,0%
		% within GA	66,0%	78,4%	71,3%
		% of Total	37,9%	33,3%	71,3%
Total		Count	100	74	174
		% within hasta_kontrol	57,5%	42,5%	100,0%
		% within GA	100,0%	100,0%	100,0%

% of Total	57,5%	42,5%	100,0%	

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	3,182[a]	1	,074		
Continuity Correction[b]	2,606	1	,106		
Likelihood Ratio	3,245	1	,072		
Fisher's Exact Test				,091	,052
Linear-by-Linear Association	3,164	1	,075		
N of Valid Cases	174				

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 21,26.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for hasta_kontrol (kontrol / hasta)	1,867	,936	3,727
For cohort GA = Yok	1,278	,993	1,643
For cohort GA = Var	,684	,438	1,068
N of Valid Cases	174		

hasta_kontrol * AA

Crosstab

	AA	Total

			Yok	Var	
hasta_kontrol	kontrol	Count	44	6	50
		% within hasta_kontrol	88,0%	12,0%	100,0%
		% within AA	30,3%	20,7%	28,7%
		% of Total	25,3%	3,4%	28,7%
	hasta	Count	101	23	124
		% within hasta_kontrol	81,5%	18,5%	100,0%
		% within AA	69,7%	79,3%	71,3%
		% of Total	58,0%	13,2%	71,3%
Total		Count	145	29	174
		% within hasta_kontrol	83,3%	16,7%	100,0%
		% within AA	100,0%	100,0%	100,0%
		% of Total	83,3%	16,7%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1,100[a]	1	,294		
Continuity Correction[b]	,679	1	,410		
Likelihood Ratio	1,160	1	,281		
Fisher's Exact Test				,372	,207
Linear-by-Linear Association	1,094	1	,296		
N of Valid Cases	174				

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 8,33.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for hasta_kontrol (kontrol / hasta)	1,670	,636	4,386
For cohort AA = Yok	1,080	,946	1,233
For cohort AA = Var	,647	,280	1,493
N of Valid Cases	174		

hasta_kontrol * G

Crosstab

			G		
			Yok	Var	Total
hasta_kontrol	kontrol	Count	6	44	50
		% within hasta_kontrol	12,0%	88,0%	100,0%
		% within G	20,7%	30,3%	28,7%
		% of Total	3,4%	25,3%	28,7%
	hasta	Count	23	101	124
		% within hasta_kontrol	18,5%	81,5%	100,0%
		% within G	79,3%	69,7%	71,3%
		% of Total	13,2%	58,0%	71,3%
Total		Count	29	145	174
		% within hasta_kontrol	16,7%	83,3%	100,0%
		% within G	100,0%	100,0%	100,0%
		% of Total	16,7%	83,3%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1,100[a]	1	,294		
Continuity Correction[b]	,679	1	,410		
Likelihood Ratio	1,160	1	,281		
Fisher's Exact Test				,372	,207
Linear-by-Linear Association	1,094	1	,296		
N of Valid Cases	174				

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 8,33.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for hasta_kontrol (kontrol / hasta)	,599	,228	1,573
For cohort G = Yok	,647	,280	1,493
For cohort G = Var	1,080	,946	1,233
N of Valid Cases	174		

hasta_kontrol * A

Crosstab

	A		
	Yok	Var	Total

hasta_kontrol	kontrol	Count	28	22	50
		% within hasta_kontrol	56,0%	44,0%	100,0%
		% within A	39,4%	21,4%	28,7%
		% of Total	16,1%	12,6%	28,7%
	hasta	Count	43	81	124
		% within hasta_kontrol	34,7%	65,3%	100,0%
		% within A	60,6%	78,6%	71,3%
		% of Total	24,7%	46,6%	71,3%
Total		Count	71	103	174
		% within hasta_kontrol	40,8%	59,2%	100,0%
		% within A	100,0%	100,0%	100,0%
		% of Total	40,8%	59,2%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	6,707[a]	1	,010		
Continuity Correction[b]	5,853	1	,016		
Likelihood Ratio	6,638	1	,010		
Fisher's Exact Test				,011	,008
Linear-by-Linear Association	6,668	1	,010		
N of Valid Cases	174				

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 20,40.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for hasta_kontrol (kontrol / hasta)	2,397	1,227	4,684
For cohort A = Yok	1,615	1,144	2,279
For cohort A = Var	,674	,480	,944
N of Valid Cases	174		

```
CROSSTABS
  /TABLES=diyabet_kontrol BY Genotip_rs1054713 GG GA AA G A
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ RISK
  /CELLS=COUNT ROW COLUMN TOTAL
  /COUNT ROUND CELL.
```

Crosstabs

Notes

Output Created		18-SEP-2018 16:02:04
Comments		
Input	Data	C:\Users\seda.gulec\Desktop\No or Tez- KLK1- KAH- Diyabet.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	174
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.

	Cases Used	Statistics for each table are based on all the cases with valid data in the specified range(s) for all variables in each table.
Syntax		CROSSTABS /TABLES=diyabet_kontrol BY Genotip_rs1054713 GG GA AA G A /FORMAT=AVALUE TABLES /STATISTICS=CHISQ RISK /CELLS=COUNT ROW COLUMN TOTAL /COUNT ROUND CELL.
Resources	Processor Time	00:00:00,00
	Elapsed Time	00:00:00,04
	Dimensions Requested	2
	Cells Available	524245

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
diyabet_kontrol * Genotip_rs1054713	174	100,0%	0	0,0%	174	100,0%
diyabet_kontrol * GG	174	100,0%	0	0,0%	174	100,0%
diyabet_kontrol * GA	174	100,0%	0	0,0%	174	100,0%
diyabet_kontrol * AA	174	100,0%	0	0,0%	174	100,0%
diyabet_kontrol * G	174	100,0%	0	0,0%	174	100,0%
diyabet_kontrol * A	174	100,0%	0	0,0%	174	100,0%

diyabet_kontrol * Genotip_rs1054713

Crosstab

			Genotip_rs1054713			
			GG	GA	AA	Total
diyabet_kontrol	kontrol	Count	46	41	11	98
		% within diyabet_kontrol	46,9%	41,8%	11,2%	100,0%
		% within Genotip_rs1054713	64,8%	55,4%	37,9%	56,3%
		% of Total	26,4%	23,6%	6,3%	56,3%
	diyabet	Count	25	33	18	76
		% within diyabet_kontrol	32,9%	43,4%	23,7%	100,0%
		% within Genotip_rs1054713	35,2%	44,6%	62,1%	43,7%
		% of Total	14,4%	19,0%	10,3%	43,7%
Total		Count	71	74	29	174
		% within diyabet_kontrol	40,8%	42,5%	16,7%	100,0%
		% within Genotip_rs1054713	100,0%	100,0%	100,0%	100,0%
		% of Total	40,8%	42,5%	16,7%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	6,081[a]	2	,048
Likelihood Ratio	6,089	2	,048
Linear-by-Linear Association	5,789	1	,016
N of Valid Cases	174		

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 12,67.

Risk Estimate

	Value
Odds Ratio for diyabet_kontrol (kontrol / diyabet)	[a]

a. Risk Estimate statistics cannot be computed.
They are only computed for a 2*2 table without
empty cells.

diyabet_kontrol * GG

Crosstab

			GG		
			Yok	Var	Total
diyabet_kontrol	Control	Count	52	46	98
		% within diyabet_kontrol	53,1%	46,9%	100,0%
		% within GG	50,5%	64,8%	56,3%
		% of Total	29,9%	26,4%	56,3%
	Diyabet	Count	51	25	76
		% within diyabet_kontrol	67,1%	32,9%	100,0%
		% within GG	49,5%	35,2%	43,7%
		% of Total	29,3%	14,4%	43,7%
Total		Count	103	71	174
		% within diyabet_kontrol	59,2%	40,8%	100,0%
		% within GG	100,0%	100,0%	100,0%
		% of Total	59,2%	40,8%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	3,495[a]	1	,062		

Continuity Correction[b]	2,938	1	,087		
Likelihood Ratio	3,526	1	,060		
Fisher's Exact Test				,065	,043
Linear-by-Linear Association	3,475	1	,062		
N of Valid Cases	174				

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 31,01.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for diyabet_kontrol (kontrol / diyabet)	,554	,298	1,032
For cohort GG = Yok	,791	,620	1,009
For cohort GG = Var	1,427	,972	2,095
N of Valid Cases	174		

diyabet_kontrol * GA

Crosstab

			GA		
			Yok	Var	Total
diyabet_kontrol	kontrol	Count	57	41	98
		% within diyabet_kontrol	58,2%	41,8%	100,0%
		% within GA	57,0%	55,4%	56,3%
		% of Total	32,8%	23,6%	56,3%
	diyabet	Count	43	33	76

	% within diyabet_kontrol	56,6%	43,4%	100,0%
	% within GA	43,0%	44,6%	43,7%
	% of Total	24,7%	19,0%	43,7%
Total	Count	100	74	174
	% within diyabet_kontrol	57,5%	42,5%	100,0%
	% within GA	100,0%	100,0%	100,0%
	% of Total	57,5%	42,5%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,044[a]	1	,834		
Continuity Correction[b]	,003	1	,956		
Likelihood Ratio	,044	1	,834		
Fisher's Exact Test				,878	,478
Linear-by-Linear Association	,044	1	,834		
N of Valid Cases	174				

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 32,32.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for diyabet_kontrol (kontrol / diyabet)	1,067	,582	1,955
For cohort GA = Yok	1,028	,794	1,332
For cohort GA = Var	,964	,681	1,363

N of Valid Cases	174		

diyabet_kontrol * AA

Crosstab

			AA		
			Yok	Var	Total
diyabet_kontrol	kontrol	Count	87	11	98
		% within diyabet_kontrol	88,8%	11,2%	100,0%
		% within AA	60,0%	37,9%	56,3%
		% of Total	50,0%	6,3%	56,3%
	diyabet	Count	58	18	76
		% within diyabet_kontrol	76,3%	23,7%	100,0%
		% within AA	40,0%	62,1%	43,7%
		% of Total	33,3%	10,3%	43,7%
Total		Count	145	29	174
		% within diyabet_kontrol	83,3%	16,7%	100,0%
		% within AA	100,0%	100,0%	100,0%
		% of Total	83,3%	16,7%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4,785[a]	1	,029		
Continuity Correction[b]	3,929	1	,047		
Likelihood Ratio	4,757	1	,029		
Fisher's Exact Test				,040	,024
Linear-by-Linear Association	4,757	1	,029		

N of Valid Cases	174				

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 12,67.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for diyabet_kontrol (kontrol / diyabet)	2,455	1,081	5,575
For cohort AA = Yok	1,163	1,008	1,343
For cohort AA = Var	,474	,238	,943
N of Valid Cases	174		

diyabet_kontrol * G

Crosstab

			G		
			Yok	Var	Total
diyabet_kontrol	Control	Count	11	87	98
		% within diyabet_kontrol	11,2%	88,8%	100,0%
		% within G	37,9%	60,0%	56,3%
		% of Total	6,3%	50,0%	56,3%
	Diyabet	Count	18	58	76
		% within diyabet_kontrol	23,7%	76,3%	100,0%

	% within G	62,1%	40,0%	43,7%
	% of Total	10,3%	33,3%	43,7%
Total	Count	29	145	174
	% within diyabet_kontrol	16,7%	83,3%	100,0%
	% within G	100,0%	100,0%	100,0%
	% of Total	16,7%	83,3%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4,785[a]	1	,029		
Continuity Correction[b]	3,929	1	,047		
Likelihood Ratio	4,757	1	,029		
Fisher's Exact Test				,040	,024
Linear-by-Linear Association	4,757	1	,029		
N of Valid Cases	174				

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 12,67.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for diyabet_kontrol (kontrol / diyabet)	,407	,179	,925
For cohort G = Yok	,474	,238	,943
For cohort G = Var	1,163	1,008	1,343
N of Valid Cases	174		

diyabet_kontrol * A

Crosstab

			A		
			Yok	Var	Total
diyabet_kontrol	Control	Count	46	52	98
		% within diyabet_kontrol	46,9%	53,1%	100,0%
		% within A	64,8%	50,5%	56,3%
		% of Total	26,4%	29,9%	56,3%
	Diyabet	Count	25	51	76
		% within diyabet_kontrol	32,9%	67,1%	100,0%
		% within A	35,2%	49,5%	43,7%
		% of Total	14,4%	29,3%	43,7%
Total		Count	71	103	174
		% within diyabet_kontrol	40,8%	59,2%	100,0%
		% within A	100,0%	100,0%	100,0%
		% of Total	40,8%	59,2%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	3,495[a]	1	,062		
Continuity Correction[b]	2,938	1	,087		
Likelihood Ratio	3,526	1	,060		
Fisher's Exact Test				,065	,043
Linear-by-Linear Association	3,475	1	,062		
N of Valid Cases	174				

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 31,01.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for diyabet_kontrol (kontrol / diyabet)	1,805	,969	3,360
For cohort A = Yok	1,427	,972	2,095
For cohort A = Var	,791	,620	1,009
N of Valid Cases	174		