MEYREM- OSUM

INVESTIGATION OF RELATIONSHIP BETWEEN METHYLATION OF KLOTHO AND BMAL1 GENES AND

PhD THESIS

2024

NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF MEDICAL GENETICS

INVESTIGATION OF THE RELATIONSHIP BETWEEN METHYLATION OF KLOTHO AND BMAL1 GENES AND HYPERTENSION

PhD THESIS

Meyrem OSUM

Nicosia

June, 2024

NEAR EAST UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF MEDICAL GENETİCS

INVESTIGATION OF THE RELATIONSHIP BETWEEN METHYLATION OF KLOTHO AND BMAL1 GENES AND HYPERTENSION

PhD THESIS

Meyrem OSUM

Supervisor
Prof. Dr. Rasime KALKAN
Prof. Dr. Pınar TULAY

Nicosia
June, 2024

Approval

We certify that we have read the thesis submitted by Meyrem Osum titled **"Investigation of the Relationship Between Methylation of KLOTHO and BMAL1 Genes and Hypertension"** and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of PhD of Medical Genetics.

Examining Committee	Name-Surname	Signature
Head of the Committee:	Prof. Dr. Özlem Dalmızrak	.....
Committee Member*:	Assoc. Prof. Dr. Günnur Koçer	.....
Committee Member*:	Assoc. Prof. Dr. Emine İkbal Atlı	.....
Committee Member*:	Assist. Prof. Dr. Ebru Erzurumluoğlu Gökalp	.....
Committee Member*:	Assist. Prof. Dr. Özel Yürüker	.....
Supervisor:	Prof. Dr. Rasime Kalkan	.....
Supervisor:	Prof. Dr. Pınar Tulay	.....

Approved by the Head of the Department

11./06/2021

.....

Prof. Dr. Pınar Tulay

Head of the Department

Approved by the Institute of Graduate Studies

...../...../ 20...

Prof. Dr. Kemal Hüsnü Can Başer

Head of the Institute of Graduate Studies

Declaration of Ethical Principles

I declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of the Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

Meryem Osum

28/05/2024

Acknowledgements

First of all, I have endless gratitude for my parents, and brother, who have always supported me financially and morally throughout my education. Secondly, I would like to express my endless gratitude to my supervisor Dear Prof. Dr Rasime Kalkan, who shared her valuable knowledge with me throughout my doctoral education and provided all kinds of support in the determination and execution of my thesis project. Also, I have endless gratitude to her constant encouragement and helpful approach throughout my PhD program. I would also like to extend my appreciation to my supervisor Dear Prof. Dr. Pınar Tulay, who supported and helped me throughout my doctoral education. I would like to express my endless gratitude to Dear Assoc. Prof. Dr. Ozgur Tosun, who made the statistical analyses of my thesis project. Otherside, I would like to express my endless gratitude to Ozlem Soydam Yorgancı, Dervis Osum, Rasiha Minare who helped me to obtain hypertensive patient blood samples at Burhan Nalbantoglu State Hospital. I would like to express my gratitude to them for helping me with great effort despite their intense workload. Finally, I would also like to extend my endless appreciation to the head of Department of Molecular Biology and Genetics Dear Dr Selin Deliceırmak who supported me during PhD thesis study.

Meyrem Osum

Abstract

Investigation of the Relationship Between Methylation of *KLOTHO* and *BMAL1* Genes and Hypertension

Osum, Meyrem
PhD, Department of Medical Genetics
Supervisor: Prof. Dr. Rasime Kalkan
Prof. Dr. Pınar Tulay
June 2024, 102 pages

Hypertension is a multifactorial chronic disease that develops due to genetic and environmental factors. Several variations in *KLOTHO* and *BMAL1* genes and their altered expression due to different mechanisms play a significant role in the development of hypertension. Considering these findings, the effect of DNA methylation of these genes on hypertension should not be ignored. Therefore, this study aims to identify the methylation status of *KLOTHO* and *BMAL1* genes in hypertensive subjects. DNA samples were isolated from 78 hypertensive subjects and 49 control individuals. The Methylation-Sensitive High-Resolution Melting (MS-HRM) analysis was done to identify the methylation status of the *KLOTHO* and *BMAL1* gene promoter sites. As a result, a statistically significant association between the methylation patterns of the *KLOTHO* and *BMAL1* genes and hypertension was not found. Moreover, a statistically significant association between *KLOTHO* and *BMAL1* methylation and the fasting blood sugar, triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol, sodium (Na), creatinine (Cr), potassium (K), and urea levels was not detected in hypertensive subjects. However, a statistically significant difference between the methylated *KLOTHO* hypertensive subjects and the unmethylated *KLOTHO* control subjects for potassium (K) was identified. On the other hand, a statistically significant association was not observed between the methylation status of *KLOTHO* and *BMAL1* genes.

Keywords: hypertension, klotho, bmal1, DNA methylation, ms-hr

ÖZET

KLOTHO ve *BMAL1* Genlerinin Metilasyonu ile Hipertansiyon Arasındaki İlişkinin Araştırılması

Osum, Meyrem
Doktora, Tıbbi Genetik Anabilim Dalı
Danışmanlar: Prof. Dr. Rasime Kalkan
Prof. Dr. Pınar Tulay
Haziran 2024, 102 sayfa

Hipertansiyon genetik ve çevresel faktörlere bağlı olarak gelişen multifaktöriyel kronik bir hastalıktır. *KLOTHO* ve *BMAL1* genlerindeki çeşitli varyasyonlar ve bunların farklı mekanizmalara bağlı olarak değişen ifadeleri, hipertansiyon gelişiminde önemli rol oynamaktadır. Bu bulgular göz önüne alındığında bu genlerin DNA metilasyonunun hipertansiyon üzerindeki etkisi göz ardı edilmemelidir. Bu nedenle bu çalışmanın amacı hipertansif bireylerde *KLOTHO* ve *BMAL1* genlerinin metilasyon durumunu belirlemektir. Bunun için 78 hipertansif birey ve 49 kontrolden DNA örnekleri izole edildi. *KLOTHO* ve *BMAL1* gen promotör bölgelerinin metilasyon durumunu belirlemek için Metilasyona Özgü Yüksek Çözünürlüklü Erime Eğrisi (MS-HRM) analizi yapıldı. Sonuç olarak *KLOTHO* ve *BMAL1* genlerinin metilasyon paternleri ile hipertansiyon arasında istatistiksel olarak anlamlı bir ilişki bulunamadı. Ayrıca, hipertansif bireylerde *KLOTHO* ve *BMAL1* metilasyonu ile açlık kan şekeri, trigliseritler, toplam kolesterol, LDL-kolesterol, HDL-kolesterol, sodyum (Na), kreatinin (Cr), potasyum (K) ve üre seviyeleri arasında istatistiksel olarak anlamlı bir ilişki bulunmamıştır. Ancak, metillenmiş *KLOTHO* hipertansif bireyler ile metillenmemiş *KLOTHO* kontrol bireyleri arasında potasyum (K) açısından istatistiksel olarak anlamlı bir fark tespit edildi. Bunun dışında, *KLOTHO* ve *BMAL1* genlerinin metilasyon durumu arasında istatistiksel olarak anlamlı bir ilişki gözlemlenmemiştir.

Anahtar kelimeler: hipertansiyon, klotho, bmal1, DNA methylation, ms-hrm

Table of Contents

CHAPTER V

Discussion

CHAPTER VI

Conclusion and Recommendations

List of Tables

List of Figures

List of Graphics

Page

List of Abbreviations

HTN:	Hypertension
CpGs:	Cytosine-phosphate-guanine dinucleotides
5mC:	5-methyl-cytosine
BP:	Blood pressure
RAAS:	Renin-angiotensin-aldosterone system
SNS:	Sympathetic nervous system
KL:	KLOTHO
BMAL1:	Brain and muscle aryl hydrocarbon receptor nuclear translocator (ARNT)-like 1
COVID-19:	Coronavirus disease 19
SBP:	Systolic blood pressure
DBP:	Diastolic blood pressure
SNPs:	Single nucleotide polymorphisms
GWAS:	Genome-wide association studies
CYP11B1:	Cytochrome P450 Family 11 Subfamily B Member 1
CYP11B2:	Cytochrome P450 Family 11 Subfamily B Member 2
SCNN1B:	Sodium Channel Epithelial 1 Subunit Beta
SCNN1G:	Sodium Channel Epithelial 1 Subunit Gamma
WNK1:	Lysine deficient protein kinase 1
WNK4:	Lysine deficient protein kinase 4
AGT:	Angiotensinogen
ACE:	Angiotensin-converting enzyme
AT1:	Angiotensin II type I receptor
AT2:	Angiotensin type II receptor
ANG I:	Angiotensin I
ANG II:	Angiotensin II
NO:	Nitric oxide
ET-1:	Endothelin -1
eNOS:	Nitric oxide synthase
SAM:	S-adenosyl-L-methionine
CH3:	Methyl group

DNMTs:	DNA methyltransferases
DNMT1:	DNA methyltransferase 1
DNMT3a:	DNA (cytosine-5)-methyltransferase 3A
DNMT3b:	DNA-methyltransferase 3 beta
SHRs:	Spontaneously hypertensive rats
WKYs:	Wistar-Kyoto rats
MLPD:	Maternal low protein diet
ECE-1:	Endothelin converting enzyme-1
ADD:	Adducin
11bHSD2:	11 beta-hydroxysteroid dehydrogenase 2
NKCC1:	Na^+, K+, and $2Cl^-$ cotransporter 1
NET:	Norepinephrine transporter
GCK:	Glucokinase
ABCG4:	ATP-binding cassette G4
SULF:	Sulfatase 1
b1-AR:	b1-adrenergic receptor
TSS:	Transcription start site
Pi:	Phosphate
1,25(OH)2D3:	1,25-dihydroxyvitamin D3
FGF23:	Fibroblast growth factor 23
SP1:	Specificity protein 1
MMP9:	Matrix metallopeptidase 9
FGFRs:	FGF receptors
CYP27B1:	Cytochrome P450 Family 27 Subfamily B Member 1
CYP24A1:	Cytochrome P450 Family 24 Subfamily A Member 1
Wnt:	Wingless-related integration site
IGF-1:	Insulin-like growth factor-1
cAMP:	Cyclic adenosine monophosphate
ROMK:	Renal outer medullary potassium channel
TRPV5:	Transient receptor potential cation channel subfamily V member 5
K:	Potassium
Ca:	Calcium
LacNAc:	N-Acetyllactosamine

IFG-1:	Insulin-like growth factor-1
EH:	Essential hypertension
CAD:	Coronary artery disease
FoxO:	Forkhead box O
Mn-SOD:	Manganese superoxide dismutase
PBMCs:	Peripheral blood mononuclear cells
BMI:	Body mass index
PI3K:	Phosphoinositide 3-kinases
Akt:	Protein kinase B
SIRT1:	Sirtuin 1
SCN:	Suprachiasmatic nucleus
CLOCK:	Circadian locomotor output cycles kaput
PER:	Period
CRY:	Cryptochrome
TTO:	Transcription translation oscillating
BA-BMAL1-KO:	Adipocyte-specific *BMAL1* knockout
KO:	Knockout
PVAT:	Perivascular adipose tissue
MMP13:	Matrix metallopeptidase 13
TGR(mREN-2) 27 rats:	Hypertensive Ren-2 transgenic rat
STAT6:	Signal transducer and activator of transcription 6
IL4:	Interleukin 4
Gata-4:	GATA Binding Protein 4
FBS:	Fasting blood sugar
TC:	Total cholesterol
TG:	Triglycerides
HDL-C:	High-density lipoprotein cholesterol
LDL-C:	Low-density lipoprotein cholesterol
Na:	Sodium
Cr:	Creatinine
HRM:	High-resolution melting
dsDNA:	Double-stranded DNA
ssDNA:	Single-stranded DNA

MS-HRM:	Methylation Sensitive High-Resolution Melting
DNAm:	DNA methylation
6mA:	N6-methyladenine
N6AMT1:	N-6 adenine-specific DNA methyltransferase 1
8-oxoG:	8-oxoguanine
MBP:	Methyl-CpG binding protein
CKD:	Chronic Kidney Disease
SMCs:	Smooth Muscle Cells
VSMCs:	Vascular Smooth Muscle Cells
HASMCs:	Human Aortic Smooth Muscle Cells
CO:	Cardiac Output

CHAPTER I

Introduction

Hypertension (HTN) is one of chronic conditions in older individuals. However, it is expected to influence a large number of adult subjects worldwide in the future (Kanbay et al., 2021). It is implicated in diverse health issues such as renal failure and cardiovascular diseases etc. (Zhang et al., 2017). HTN generally exists as primary and secondary HTN. Approximately 95% of people with HTN possess primary HTN, whereas approximately 5% have secondary HTN. The primary HTN is developed due to genetic and environmental factors, regardless of different disease conditions. Genetic, epigenetic, age, overweight, smoking, diabetes, and arterial ageing are factors which contribute to primary HTN (Fang et al., 2021; Wang et al., 2010). Consequently, primary HTN is a complex, but its underlying mechanisms are fully unknown (Kanbay et al., 2021). However, the development of secondary HTN depends on several reasons such as endocrine disorders, kidney diseases, side effects of drugs, etc. (Pathare et al., 2020a).

Epigenetics is designated as a hereditary and reversible processes that alter gene expression without any changing in sequence of DNA molecule. It accounts for the transfer of stable gene expression profiles among cell generations and the formation of cell-specific gene expression profiles. Different epigenetic mechanisms exist that modify chromatin structures, such as histone modifications, DNA methylation, chromatin remodeling, and RNA-based mechanisms (Wise & Charchar, 2016). DNA methylation is a dynamic process participated in the regulation of gene expression by inserting methyl groups to the cytosine bases within the cytosine-phosphate-guanine dinucleotides (CpGs). Consequently, it reversibly regulates gene inhibition (Han et al., 2016; Pratamawati et al., 2023; Wise & Charchar, 2016). It arranges the expression of diverse genes implicated in the arrangement of blood pressure (BP) (Han et al., 2016; Pratamawati et al., 2023). Especially, it was reported that abnormal methylated genes involved in renal sodium retention system (RSRS), sympathetic nervous system (SNS), and andrenin-angiotensin-aldosterone system (RAAS), have a significant role in the HTN occurrence (Pei et al., 2015).

KLOTHO (*KL*) is an aging suppressor gene which is extremely conserved in diverse organisms from mice to human (Lanzani et al., 2020; Xu & Sun, 2015). The *KL* gene highly synthesize α-KL protein in kidney and brain (Donate-Correa et al

2013). *KL* gene variations such as G-395A (Gao et al., 2015; Wang et al., 2010), C1818T (Zhang et al., 2017), and F352V (Citterio et al., 2020) were declared to involve in HTN development. In the Indian population, the expression of *KL* gene was reported to decrease in subjects with primary HTN (Pathare et al., 2020). In addition, a decline in *KL* expression was reported in subjects with renal HTN. As a result, the *KL* gene was indicated to participate in the development of both primary and secondary HTN. Especially, it was postulated that KL could be applied as a considerable biomarker to determine kidney damage in patients with HTN (Kanbay et al., 2021).

Circadian clock is an extremely conserved in many organisms which enables organisms to adapt to environmental cues during the 24-hour period (Costello et al., 2023). It regulates the circadian rhythm of various physiological processes, including metabolism, renal function, immune response and BP etc. (Richards et al., 2014). It was proposed that disrupted function of circadian clock function can contribute to diverse diseases such as HTN (Costello et al., 2023). Brain and muscle aryl hydrocarbon receptor nuclear translocator (ARNT) -like 1 (*BMAL1*) is a factor of circadian clock that rhythmically arranges the expression of diverse target genes (Crislip et al., 2020). The BMAL1 rs9633835 and rs6486121 variations were revealed to be linked with HTN susceptibility (Kolomeĭchuk et al., 2011; Woon et al., 2007).

While several research have investigated the link between the BMAL1 and *KL* genes and HTN in terms of variant and expression analyses, none have examined the interactions between methylation of these genes and HTN and their relationship with biochemical variables in the HTN state. Consequently, the *KL* and *BMAL1* gene methylation was examined in this work in both hypertensive patients and control participants. Besides, the potential influence of *KL* and *BMAL1* methylation status on biochemical markers was investigated in both normal and hypertensive individuals. Consequently, the aims of this research are to:

(a) Identify the methylation of *BMAL1* and *KL* promoters
(b) Analyze the impact of the methylation of *KL* and *BMAL1* promoters on HTN
(c) Investigate the impact of the methylation of *KL* and *BMAL1* promoters on biochemical markers in HTN conditions.

In this study, the research question is;

(*) is there any link between the methylation of *KL* and *BMAL1* promoters and HTN?

CHAPTER II
General Information

2.1. Hypertension

2.1.1. Definition of Hypertension

HTN is a common chronic disorder associated with persistently high BP in arteries (Kjeldsen, 2018). HTN which mostly occurs as a significant health problem in advanced age is suggested to become increasingly widespread in pediatric patients (Yusuf et al., 2020). Particularly, it is presumed that hypertensive individuals will gradually raise and exceed 1.6 million in 2025 in the world (Mills et al., 2016). HTN is a crucial risk agent for Coronavirus disease 19 (COVID-19), cognitive impairment, kidney disease, stroke, and cardiovascular disorders including heart failure, and arrhythmias, thereby contributing substantially to mortality and morbidity (Alfonso Perez & Delgado Martinez, 2023; Gawałko et al., 2023). It has been predicted that HTN, especially during pregnancy, may lend to fetal and maternal morbidity and mortality (Alfonso Perez & Delgado Martinez, 2023). Consequently, although, it was suggested that HTN poses a considerable burden on health and economic areas, it is approved to be a preventable and treatable disease (Aydogdu et al., 2019).

HTN is known as a multifactorial disease due to various evidence showing that genetic, metabolic, and environmental factors are implicated in HTN development (Akbari et al., 2018). Various factors such as advanced age, gender, absence of physical activity, obesity, diabetes, extreme sodium and alcohol consumption have been associated with HTN occurrence (Türkiye Endokrinoloji ve Metabolizma Derneği, 2022). However, these factors haven't exactly put forward the knowledge about HTN development (Han et al 2016). Therefore, the etiology and pathogenesis of HTN have been fully unknown (Shih & Connor, 2008).

2.1.2. Epidemiology of Hypertension

The probability of a subject developing HTN during a lifetime in an industrialized country is suggested to be approximately 90% (Alfonso Perez & Delgado Martinez, 2023). The World Health Organization Report states that the HTN prevalence is rapidly increasing globally (World Health Organization, 2014).

The HTN risk was reported to gradually elevate with age in individuals (Connelly et al., 2022). However, the HTN prevalence was found to become higher in postmenopausal subjects relative to premenopausal subjects, and age-matched men. Approximately 75% of women aged 60 and over were declared to have HTN symptoms in the USA (Taddei, 2009). Moreover, according to Turkish HTN Prevalence study, it was shown that the prevalence of HTN was 36.1% in women, 27.7% in men, and 31.8% in adults age group (Türkiye Endokrinoloji ve Metabolizma Derneği, 2022). Consequently, gender has been determined to considerably affect the HTN prevalence (Ong et al., 2008). The prevalence of HTN was declared to vary according to race and geography. In the first comprehensive study focusing on the HTN in Turkey, it was determined that the prevalence of HTN was 33.7% and increased with age. In Turkey, it was found that, among those over 20, 41.4%, 22.1%, 15.3%, and 21.2% had normal BP, prehypertension, stage 1 HTN, and stage 2 HTN, respectively. It has been explained that the region with the highest HTN prevalence in Turkey is the Black Sea (Türkiye Endokrinoloji ve Metabolizma Derneği, 2022).

2.1.3. Categorization of Hypertension Based on BP Measurement and Etiology

2.1.3.1. Categorization of Hypertension Based on BP Measurement. In terms of guidance on the HTN diagnosis and treatment, the Turkish HTN Consensus Guideline was established in 2015 by 5 hypertension associations based on the European and American international hypertension guidelines (Williams et al., 2018). The updated international hypertension guidelines and new hypertension studies led to an update of the Turkish HTN Consensus Guideline in 2019. In the updated Turkish HTN Consensus Guideline, HTN is declared as systolic blood pressure (SBP)/diastolic blood pressure (DBP) to be ≥140 mmHg/ ≥90 mmHg based on repeated clinical measured by physicians in the population aged 18 and over. In guideline, the BP was classified according to levels of clinical BP in the general population (Table 1) (Aydogdu et al., 2019).

Table 1.

BP Classification

Category	(SBP)/(DBP) (mmHg)
Normal	(SBP<120)/ (DBP<80)
Elevated	(120<SBP<1139)/(80<DBP<89)
HTN	(SBP≥140)/(DBP≥90)
Stage 1	(140<SBP<159)/(90<DBP<99)
Stage 2	(SBP≥160)/(DBP≥100)
Stage 3	(SBP≥180)/(DBP≥ 110)

2.1.3.2. Categorization of Hypertension Based on Etiology. In general, HTN is categorized as primary (essential) HTN and secondary HTN. Primary HTN is a heterogeneous heritable health problem that is responsible for about 95% of hypertensive cases (Carretero & Oparil, 2000). It accounts for the occurrence several health condition, including peripheral vascular disease, renal disease, coronary heart disease, stroke etc. Environmental and genetic agents were reported to possess a crucial role in the primary HTN occurrence and progression. However, its exact genetic mechanisms haven't been fully determined (Gong & Hubner, 2006). It has been emphasized that various mechanisms, including oxidative stress take part a considerable role in the primary HTN occurrence (Pathare et al., 2020a). Animal and clinical research have declared the considerable role of oxidative stress in HTN development, as oxidants mediated BP elevation (González et al., 2014). However, secondary HTN arises from identifiable causes such as renal failure, renovascular disease, aldosteronism, pheochromocytoma, etc. It is responsible for about 5% of hypertensive cases (Onusko, 2003). Although it is rare in the population, its underlying causes can be identified easly and treated (Omura et al., 2004).

2.1.4. Hypertension Pathophysiology

The complex relationship of the kidneys, adrenal gland, vascular endothelium system, peripheral neurological system, and central nervous system is crucial for BP management. In addition, several other factors such as genetic factors, lifestyle, socioeconomic status, environmental factors, and demographic and metabolic characteristics influence BP. As a result, there are various pathophysiological

mechanisms responsible for the HTN occurrence, because of the implication of many interrelated factors in the BP regulation (Babalık, 2005; Harrap, 1999).

Parameters such as peripheral arterial resistance and cardiac output (CO) are responsible for the determination of BP level. Factors causing HTN increase CO and/or SVR. Generally, presence of high SVR and normal CO have been indicated in most of patients with primary HTN. The small arterioles containing smooth muscle cells (SMCs) determine peripheral resistance. It was suggested that prolonged contraction of SMCs enhances irreversibly peripheral resistance (Beevers et al., 2001).

Pathophysiological factors that account for HTN occurrence are genetic factors, fetal development, overactivity of the RAAS, dysregulated RSRS, increased SNS activity, endothelial dysfunction, and insulin resistance. Although multiple factors are involved in HTN development, its pathophysiology has still been unclear (Beevers et al., 2001; Carretero & Oparil, 2000).

2.1.4.1. Genetic Factors. Genetic alterations possess a main role in the HTN. Single-gene mutations associated with Liddle syndrome, aldosterone-producing adenomas, and pseudohypoaldosteronism was reported to be implicated in heritable Mendelian forms of HTN (Lifton et al., 2001). However, it has been indicated that these rare single-gene mutations cannot shed light on the common familial HTN in clinics (Harrison et al., 2021). It has been highlighted that multiple genes likely participate in HTN occurrence. As a result, it is immensely difficult to specify the effect of each gene on the HTN. On the other hand, people with a family history of HTN were stated to have 2-fold higher HTN risk (Beevers et al., 2001). In studies conducted among family members and twin children, the rate of genetic transmission has been detected to be 30-60% (Shih & Connor, 2008).

Mutations in mineralocorticoid, glucocorticoid, or sympathetic pathways have been informed to give rise to genetic forms of HTN (Raina et al., 2019). In addition, variations *in* Cytochrome P450 Family 11 Subfamily B Member 1/2 *(CYP11B1/2),* Sodium Channel Epithelial 1 Subunit Beta/Gamma *(SCNN1B/G),* lysine deficient protein kinase 1/4 *(WNK1/4)* genes have been correlated with HTN (Singh et al., 2016). Corin is responsible for the formation of the active natriuretic peptides that prevent HTN development. Mutations in the *Corin* gene was indicated to facilitate HTN development (Türkiye Endokrinoloji ve Metabolizma Derneği,

2022). In genome-wide association studies (GWAS), approximately 1000 single nucleotide polymorphisms (SNPs) were identified to contribute to HTN occurrence (Cabrera et al., 2019) (Cabrera et al., 2019).

2.1.4.2. Fetal Development. Individuals with low birth weight due to fetal undernutrition were indicated to have a high risk of HTN as adults (Beevers et al., 2001). It has been put forward that intrauterine growth retardation can result in HTN by decreasing the number of nephrons and thus renal sodium excretion (Brenner & Chertow, 1994).

2.1.4.3. Renin Angiotensin Aldosterone System (RAAS). RAAS is a considerable hormonal mechanism adjusting blood volume and BP. This system contains angiotensinogen (AGT), and angiotensin II type I/II receptors (AT1/2R) and enzymes such as renin, and angiotensin converting enzyme (ACE) (Zhou et al., 2015b). Renin is an aspartic protease secreted from juxtaglomerular cells of kidneys due to under-perfusion pressure or a decrease in the salt intake or induction of the SNS is essential for RAAS activation (Beevers et al., 2001). The renin in systemic circulation converts angiotensinogen synthesized mainly from the liver to angiotensin-I (Ang I). Conversion of Ang I to angiotensin-II (Ang II), a active vasoconstrictor is catalysed by ACE (Morgan et al., 1996). The Ang II implicates its effects through AT1/2R. Ang II binding to the AT1R causes vasoconstriction in peripheral vessels and thus an increase in peripheral resistance, raising PB. Ang II triggers the retention of water and sodium and thus BP elevation by stimulating the aldosterone secretion from the adrenal glands. Ang II also increases BP by directly inducing sympathetic activation (Beevers et al., 2001; Laragh, 1992; Türkiye Endokrinoloji ve Metabolizma Derneği, 2022). High BP leads to a decrease in renin secretion through negative feedback. It has been reported that many primary hypertensive subjects exhibit different renin and Ang II levels. In particular, the renin level has been declared to be normal in 50%, low in 30%, and high in 20% of patients with primary HTN. It has been suggested that different renin levels in patients with primary HTN are based on nephron heterogeneity, excessive renin production or suppression of renin production in ischemic nephrons (Babalık, 2005; Laragh, 1992). RAAS blockers have been indicated to be not effective on subjects with primary HTN. Consequently, it has been

shown that the RAAS does not directly increase BP in patients with primary HTN (Beevers et al 2001).

2.1.4.4. Renal Sodium Retention System (RSRS). RSRS has a main impact on the HTN pathogenesis. Excessive sodium (Na^+) intake increases the BP and subsequently the excretion of renal Na^+ and water. This physiological process occurs under high BP in patients with HTN (Guyton et al 1972; Juraschek et al 2017). Defects in sodium excretion in hypertensive patients have been revealed (Surdacki et al., 1999). A decrease in the function and amount of nephrons and/or a decrease in the filtration surface of the glomerulus in nephrons based on congenital diseases and acquired diseases, acquired inhibition of the sodium pump, nephron heterogeneity and impairment of renal pressure natriuresis have been indicated to decrease sodium excretion and increase BP, contributing to HTN (Brenner & Chertow, 1994). According to nephron heterogeneity, the existence of ischemic nephron groups and disruption of homogeneity of renin secretion result in sodium retention and an increase in BP (Laragh, 1992). Pressure natriuresis normalizes an increase in plasma volume and BP by providing the excretion of Na^+ and water in normotensive subjects with high BP. However, pressure natriuresis was found to be negatively influenced in hypertensive patients. It has been suggested that renal salt excretion occurs at higher BP levels in hypertensive individuals with low renal salt excretion capacity (Türkiye Endokrinoloji ve Metabolizma Derneği, 2022).

2.1.4.5. Sympathetic Nervous System (SNS). SNS is a significant constituent of the autonomic nervous system that is involved in the HTN pathophysiology (Valensi, 2021). The SNS has been suggested to be necessary for short-term BP regulation (Guyton et al., 1972). The SNS activation based on a drop in arterial pressure increases both CO and SVR by triggering the secretion of epinephrine and norepinephrine, resulting in an elevation of blood volume and arterial pressure. The SNS activation also triggers constriction of renal afferent arterioles, resulting in sodium reabsorption, renin release, and subsequently an increase in BP (Di Bona and Kopp, 1997; Guyton et al., 1972; Valensi, 2021). It has been stated that the interactions between the SNS and RAAS play an important role in HTN occurrence (Miller & Arnold, 2019). It has been reported that several factors such as genetic, behavioral and lifestyle might provoke an imbalance in sympathetic/parasympathetic activity in

hypertensive patients (Mancia & Grassi, 2014). Although the impact of SNS on HTN has been declared to be undermined over time, it has been claimed that the persistence of high BP might be caused by SNS activation-related structural changes of the vascular wall and hypertrophy of vascular smooth muscle cells (VSMCs). Particularly, SNS overactivation was reported in young newly diagnosed hypertensive patients possessing high CO, and plasma norepinephrine levels. The norepinephrine levels have been indicated to gradually decline in hypertensive patients with age. As a result, it is claimed that SNS overactivity might be responsible for the emergence of HTN (Babalık, 2005).

2.1.4.6. Endothelial Dysfunction. Vascular endothelial cells participate in BP regulation by producing vasodilator nitric oxide (NO) and vasoconstrictor endothelin-1 (ET-1) that act on the VSMCs (Babalık, 2005). It was shown that endothelial dysfunction lend to the primary HTN occurrence, and the degree of endothelial dysfunction is positively linked to the severity of HTN (Benjamin et al., 2004). Endothelial dysfunction was linked to increased BP due to reduced NO that lends to heightened vascular contraction and peripheral resistance. Several studies have indicated that inhibition of nitric oxide synthase (eNOS) gives rise to HTN (Sander et al., 1999). ET-1 that prevents the NO-mediated relaxation of VSMC (Wagner et al., 1992), participates in the activation of the local renin-angiotensin system (Beevers et al., 2001; Türkiye Endokrinoloji ve Metabolizma Derneği, 2022). As normal ET-1 plasma levels have been identified in EH patients, it has been suggested that ET-1 might only contribute to renal HTN and salt-sensitive HTN (Delacroix et al., 2014).

2.1.4.7. Insulin Resistance. Insulin resistance is common in primary hypertensive patients. Especially, it has been reported that individuals with high fasting insulin levels have a higher HTN risk than control individuals (Haffner et al., 1992). Insulin exhibits both a BP-increasing effect through SNS activation and a BP-lowering effect through its vasodilator effect in normotensive subjects. However, it has been indicated that the vasodilator effect of insulin decreases, while the SNS stimulation effect of insulin increases in hypertensive patients, resulting in increasing in BP (Babalık, 2005).

2.2. Epigenetics

Epigenetics is designated as reversible and heritable modification on the chromatin structure that alter gene expression without any variation in DNA sequence. Different epigenetic mechanisms modify chromatin structures, including histone modifications, DNA methylation, chromatin remodelling, and RNA-based mechanisms (Wise & Charchar, 2016). These mechanisms are responsible for regulation of diverse processes such as replication, transcription, DNA repair, genomic imprinting and embryonic development (Tammen et al., 2013). They account for the transfer of stable gene activity from among cell generation, and the emergence of tissue and cell type-specific gene expression models (Wise & Charchar, 2016). Various factors such as chemicals, ageing, diet, and drugs can alter the gene expression by influencing different epigenetic mechanisms. Consequently, this leads to the unqiue epigenetic structures in identical twins (Fraga et al., 2005). In particular, it has been demonstrated that alterations of these epigenetic mechanisms lend to several health issues such as HTN occurrence (Wise & Charchar, 2016).

2.2.1. DNA Methylation

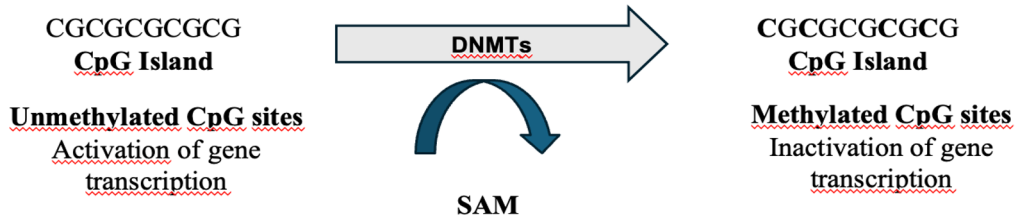
It is an epigenetic mechanism responsible for the reversible repression of gene expression in mammals. It is a process that involves the covalent incorporation of a methyl group (CH3) derived from S-adenosyl-L-methionine (SAM) to the carbon 5 of the cytosine rings within the cytosine-phosphate-guanine dinucleotides (CpGs) via DNA methyltransferases (DNMTs), and consequently causing to 5-methyl-cytosine (5mC). The "p" represents the phosphodiester bond formed between the cytosine base and subsequent guanine base in the same chain. In mammals, CpGs are mostly concentrated in gene promoter regions known as CpG-rich regions (CpG islands), but they also show a low rate distribution in non-promoter regions known as CpG-poor regions. Methylation of CpG islands (DNA hypermethylation) of genes inhibits gene transcription, while their demethylation (DNA hypomethylation) induces gene transcription (Figure 1). Inhibition of DNA methylation-mediated gene transcription is provided by inhibiting the recruiting of transcription factors to promoter sites, or by binding of methyl-CpG binding proteins (MBP) (Jones & Takai, 2001). In normal somatic cells, the CpG islands of genes are generally unmethylated, but the CpGs in the non-promoter regions undergo highly methylation (Han et al 2016; Pratamawati et al 2023; Wise and Charchar, 2016). DNA

methylation is included in the regulation of many processes such as genomic imprinting, inactivation of X-chromosome in females, and embryonic development (Huang et al., 2011).

DNA-methyltransferases (DNMTs) that catalyze DNA methylations are DNA methyltransferase 1 (DNMT1), DNA (cytosine-5)-methyltransferase 3A (DNMT3a), and DNA-methyltransferase 3 beta (DNMT3b). Particularly, the first identified DNA methyltransferase, DNMT1, ensures the maintenance of genome methylation by methylating hemi-methylated CpG sites on DNA molecules after replication. The DNMT3a/3b provide de novo methylation to create new methylation during fetal development (Han et al., 2016).

Figure 1.

Methylation and Demethylation Status of CpG Islands



2.2.2. DNA Methylation and Hypertension

DNA methylation is a crucial epigenetic mechanism participated in HTN pathogenesis (Han et al., 2016; Pratamawati et al., 2023). Kato et al. (2015) have found that SNPs at 12 genetic loci that modulate BP were correlated with methylation markers. Aberrant methylated genes of the RAAS, RSRS, and SNS have been determined to promote HTN occurrence. AT1R is component of RAAS involved in Ang II-mediated BP control and consists of two subunits, AT1a and AT1b. Abnormal AT1R function was reported to raise the impact of Ang II on the renal vasculature, thus exacerbating HTN development (Han et al., 2016). A study demonstrated that *AT1a* expression was significantly higher in male spontaneously

hypertensive rats (SHRs). In particular, it was proposed that hypomethylation of the *AT1a* promoter region accounted for an increase in *AT1a* expression in SHRs (Pei et al., 2015). Nicotine application in pregnant rats was shown to result in hypertensive offspring by upregulating *AT1a* expression through hypomethylation (Chaudhary, 2022; Xiao et al., 2014). Pregnant rats with a low protein diet have been indicated to have offspring with hypertension due to hypomethylation of the *AT1b* promoter and thus its overexpression (Bogdarina et al., 2007). It was suggested that levels of maternal glucocorticoid during early pregnancy in rat could cause hypomethylation of the *AT1b* promoter and thus upregulation of *AT1b* expression in offspring. Therefore, these findings suggest that abnormal methylated AT1R can have a crucial role in HTN pathogenesis (Bogdarina et al., 2010). ACE enzyme forms active Ang II by cleaving the inactive Ang I, causing a BP increase. A maternal low-protein diet (MLPD) was detected to cause to hypomethylated ACE-1 gene and alteration of its level in the fetal brain of rats (Goyal et al., 2010). A negative link between the ACE methylation and SBP levels was reported in low-birth-weight children (Rangel et al., 2014). Endothelin converting enzyme-1 (ECE-1) is a considerable component of the endothelin system, and accounts for the formation of an endothelin-1 (potent vasoconstrictor), regulating BP. In vitro methylation of *ECE-1c* in endothelial cells was found to suppress *ECE-1c* promoter activity. Therefore, this was suggested that methylation may be a crucial regulator for ECE-1 expression in HTN (Funke-Kaiser et al., 2003; Han et al 2016). Adducin (ADD) is a heterodimeric cytoskeleton protein containing αβ subunits or αγ subunits, and its α-subunit is synthesized from the *ADD1* gene (Matsuoka et al 2000). The *ADD1* gene has been strongly associated with EH (Hughes & Bennett,1995). Hypomethylated *ADD1* promoter was revealed to be related with high HTN susceptibility in patients with EH (Zhang et al., 2013). 11 beta-hydroxysteroid dehydrogenase 2 (11bHSD2) implicated in RSRS is a responsible enzyme for converting cortisol to cortisone (an inactive metabolite). Cortisol participates in Na+ reabsorption in the kidney by binding to mineralocorticoid receptors, thus playing a considerable role in regulation of arterial pressure. Especially, it has been put forward that reduced 11bHSD2 activity can lead to HTN by increasing BP (Han et al., 2016). It has been specified that the *11BHSD2* promoter was significantly hypermethylated in patients with EH. It has been suggested that *11BHSD2* promoter methylation can be a significant biomarker in hypertensive subjects (Friso et al., 2008). The Na^+, K+, and $2Cl^-$ cotransporter 1

(NKCC1) which is a member of the solute carrier family are implicated in the electroneutral symport of Na^+, K+, and $2Cl^-$. It was suggested that the overactivity of *NKCC1* in the aorta may lead to HTN occurrence in patients with HTN (Xu et al., 1994). Hypomethylated *NKCC1* promoter was identified to increase *NKCC1* expression in the heart and aorta of SHRs (Lee et al., 2010). The hypomethylation status of the *NKCC1* promoter has been suggested to be emerged following HTN occurrence in SHR (Cho et al., 2011). A norepinephrine transporter (NET) is known as a Na^+/Cl^- dependent transporter involved in the maintenance of norepinephrine homeostasis (Bonisch & Bruss,1994). It was indicated that the *NET* promoter region was highly methylated in patients with EH (Han et al., 2016). The hypomethylated glucokinase (*GCK*) gene body has been correlated with EH (Chaudhary, 2022; Fan et al., 2015).

Genome-wide DNA methylation analysis depends on investigation of the methylation of CpG sites in promoter and non-promoter regions. With this analysis, the methylation status of diverse genes linked to HTN has been identified. In particular, the level of 5mC throughout the genome was indicated to be importantly lower in EH patients relative to control individuals (Han et al., 2016). However, pre-eclampsia (hypertension during pregnancy) subjects were reported to exhibit higher global DNA methylation levels than control subjects (Chaudhary, 2022; Kulkarni et al 2011). According to genome-wide methylation analysis, among the genes, the CpG site of the sulfatase 1 (*SULF1)* gene was shown to be highly hypermethylated in subjects with HTN relative to healthy subjects, and this result was validated in different cohort studies. The ATP-binding cassette G4 (*ABCG4*) gene was shown to be significantly demethylated in hypertensive patients than in the control subjects, suggesting a significant role of *ABCG4* methylation in HTN occurrence (Han et al., 2016).

Several agents including age, gender, nutrition, smoking alcohol intake and obesity that take part in HTN pathogenesis have been suggested to contribute to DNA methylation variations. Nevertheless, a few studies exist to focus on the impact of these agents on methylation status in HTN (Han et al., 2016). For instance, an association between alcohol intake and *ADD 1* promoter methylation was indicated to alter EH susceptibility (Han et al., 2015).

It was specified that DNA methylation could cause different antihypertensive responses among patients carrying a similar b1-adrenergic receptor (*b1-AR*) variant.

Therefore, it was shown that DNA methylation can influence the efficacy of antihypertensive drugs and thus antihypertensive response among individuals (Jiang et al., 2009).

2.3. *KLOTHO* GENE

2.3.1. KLOTHO Gene Properties

Klotho (*KL*) was disovered to be an anti-ageing gene. The loss of *KL* function due to mutation has been demonstrated to cause multiple ageing-like phenotypes, including arteriosclerosis, infertility, soft tissue calcification, osteoporosis, etc. and short life span in mice (Kuro-o et al., 1997). The *KL* overexpression was indicated to retard the ageing related processes and extend the life span of mice (Masuda et al., 2005). Consequently, the *KL* gene was was put forward to be involved in the ageing process by influencing several cellular pathways (Kuro-o et al., 1997).

KL gene was indicated to be highly conserved in several animals such as mice, rats, and humans (Wang & Sun, 2009b). *KL* gene with 4 introns and 5 exons is located on chromosome 13 (13q12) in humans. Human GC-rich *KL* promoter site with 500-bp that is situated upstream of the *KL* transcription start site (TSS) (Turan & Ata, 2011) has been indicated to reside in a region sensitive to DNA methylation, suggesting that DNA methylation may be implicated in the management of *KL* activity (Azuma et al., 2012; Xu & Sun, 2015). The *KL* gene encodes α-KL protein mainly in the tubular segments of kidneys and the brain choroid plexus and furthermore in diverse tissues, such as epithelial, arterial, reproductive and endocrine tissues (Chang et al., 2015).

Two genes that have sequence similarity with *α-KL* are *γ-KL* and *β-KL* (Ito et al., 2002). The *γ-KL* and *β-KL* encode type 1 single-pass transmembrane protein (Hu et al., 2013b). While the adipose and liver tissues account for β-KL protein secretion (Kuro et al., 1997; Wang & Sun, 2009b), skin and kidney are responsible for γ-KL protein secretion (Ito et al., 2002). Both β- and α-KL proteins exhibit distinct functions. Especially, it has been indicated that α-KL protein acts as a regulator of 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] activity, and phosphate (Pi) absorption, while β-KL protein acts as a metabolic regulator (Xu & Sun, 2015). However, the γ-KL function has not been determined (Jamal et al., 2019).

The α-KL protein is present in two forms with different functions, a transmembrane and a circulating proteins. The extracellular region of transmembrane α-KL protein contains two separate KL1 and KL2 homologous glycosyl hydrolase domains determining its function (Donate-Correa et al., 2016). The circulating α-KL protein consists of shed- and secreted- α-KL proteins (Wang & Sun 2009b). The shed α-KL protein is formed by cleavage transmembrane α-KL protein via membrane-anchored proteases (Xu and Sun, 2015). The secreted α-KL protein containing KL1 domain only (~65kDa) is formed by alternative RNA splicing (Xu and Sun, 2015). It has been indicated that humans and mice possess all forms of α-KL protein, while rats have only transmembrane and shed forms of α-KL protein (Wang & Sun, 2009b).

Kidneys have been suggested to be the main source of circulating KL (Olauson et al., 2017). It has been reported that the circulating α-KL protein may participate in the regulation of functions of cells that do not produce any of the α-KL protein. Although *α-KL* is expressed in a few tissues, it is suggested that α-KL protein may have a crucial effect in regulating ageing phenotype intensely due to its hormonal function (Xu & Sun, 2015). Furthermore, as a secreted β-KL form has not been discovered, α-KL has been reported to exhibit more extensive functions (Xu & Sun, 2015).

Chronic HTN, chronic renal failure, diabetes mellitus, inflammatory stress, fibroblast growth factor 23 (FGF23), calcitonin gene-interrelated peptide, RAAS, and oxidative stress affect *α-KL* expression in kidneys (Koh et al., 2001; Sugiura et al., 2012). It has been claimed that the FGF23 and calcitonin gene-interrelated peptide may increase *α-KL* expression, while RAAS, oxidative stress, and inflammation may decrease *α-KL* expression (Jamal et al., 2019). Ang II may decrease *α- KL* expression by downregulating the specificity protein 1* (Sp1) gene expression, which is a positive regulator of the *α-KL* promoter region (Zhou et al 2010). The level of matrix metallopeptidase 9 (MMP9) have been negatively associated with *α-KL* expression level (Cheng et al., 2010).

2.3.2. KLOTHO Function

Three types of α-KL protein have distinct functions. Transmembrane α-KL protein acts as a co-receptor for a critical bone-derived hormone, FGF23, by interacting with FGF receptors (FGFRs), such as FGFR1c, FGFR3c, and FGFR4, in

the kidney (Urakawa et al., 2006). Among the three FGFRs, FGFR1c regulates Pi reabsorption, while FGFR3c and FGFR4 regulate vitamin D metabolism (Li et al., 2011). The interaction of transmembrane α-KL, FGFRs and FGF23 results in the inhibition of Pi reabsorption and the generation of $1,25(OH)_2D_3$ or active vitamin D, (Urakawa et al., 2006). Particularly, KL prevents the activity of 25-Hydroxyvitamin D 1α-hydroxylase ((Cytochrome P450 Family 27 Subfamily B Member 1) (CYP27B1)), a vitamin D-activating enzyme, and stimulates the activity of the 25-Hydroxyvitamin D 24-hydroxylase ((Cytochrome P450 family 24 subfamily A member 1) (CYP24A1)), a vitamin D-deactivating enzyme. Furthermore, vitamin D regulates *KL* gene expression. As a result, the functions of vitamin D and KL are extremely intertwined (Christakos et al., 2016). It has been indicated that transmembrane α-KL protein may diminish oxidative stress and prevent the Wingless-related integration site (Wnt) pathway (Xu & Sun, 2015). It has been specified that α-KL protein may block Wnt pathway by interacting to distinct Wnt ligands in mice (Wang & Sun, 2009b). Especially, it has been suggested that *KL* deficiency may support kidney injury due to activation of β-catenin/ Wnt pathway (Zhou et al., 2013).

The circulating α-KL affects several signaling pathways, such as cyclic adenosine monophosphate (cAMP), p21/p53, insulin-like growth factor-1 (IGF-1) signaling and Wnt pathway (Sopjani et al., 2015). It was suggested that sialidase activity of shed α-KL may raise the abundant of both the renal outer medullary potassium channel (ROMK) 1 and transient receptor potential cation channel subfamily V member 5 (TRPV5) that are involved in the potassium (K^+) and calcium (Ca^{2+}) reabsorption in kidney, respectively (Xu & Sun, 2015). It has been proposed that sialidase activity of shed-KL protein may expose the galectin-binding site of TRPV5 by removing α 2, 6-sialylated N-Acetyllactosamine (LacNAc) from the TRPV5 N-glycosylation branch, and thus preventing TRPV5 endocytic process (Leppänen et al., 2005). The deterioration of the α-KL gene has been linked with an increase in urinary Ca^{2+} excretion due to a reduction of TRPV5 expression in distal tubules of a transgenic mouse. Therefore, *α-KL* gene may arrange Ca^{2+} metabolism via TRPV5 (Xu & Sun, 2015). The shed α-KL has also been shown to physically interact with multiple Wnt ligands (Wnt1, Wnt4, Wnt7a), blocking the Wnt pathway (Zhou et al., 2013). Especially, loss of *KL* expression was suggested to inevitably cause to the Wnt pathway activation as well as kidney injury and renal insufficiency

(Hu et al 2013a). The secreted KL regulates several signaling pathways including protein kinase C, cAMP, p53/p21, Wnt pathways and IGF-1 signaling (Sopjani et al., 2015).

2.3.3. Impact of KLOTHO in Hypertension

It was put forward that *KL* expression significantly decreased in spontaneously hypertensive rats (SHR) (Tang et al., 2011), and *KL* gene delivery hindered the HTN and kidney damage in SHR (Wang and Sun, 2009a). It have been declared that diminished serum KL levels with age may result in renal damage and HTN (Akbari et al., 2018; Wang et al., 2010; Wang & Sun, 2009b). In patients with EH, the serum KL levels were stated to be considerably eradicated (Su & Yang, 2014). High serum KL levels have been correlated to a lower HTN incidence among subjects aged 70-79 years (Dew et al., 2021). Serum KL levels has been claimed to likely be used as a considerable biomarker for evaluating HTN risk in postmenopausal women possessing low serum KL levels (Yu et al., 2023).

More than 10 human *KL* SNPs were correlated to the EH (Arking et al., 2002; Shimoyama et al., 2009). A human KL-VS variant contains 2 SNPs which lend amino acid substitutions C370S and F352V, and thus altering KL function *in vivo* (Arking et al., 2002; 2005). The V/V genotype carriers of the F352V variant were reported to possess a decrease high-density lipoprotein cholesterol (HDL-C) and high SBP compared to V/F genotype carriers (Arking et al., 2002; 2005). But, this result was indicated to contradict the findings of the research done in the French people (Nzietchueng et al., 2011). The *KL* C1818T polymorphism (rs564481) has been related to decreased risk of HTN in Iran population. Particularly, it was stated that the risk of HTN and coronary artery disease (CAD) was reduced in T allele carriers (Akbari et al., 2018). In contrast, the link between *KL* C1818T and an elevated risk of HTN and CAD was reported in the Asian people (Rhee et al., 2006). It has been put forward that these contradictory results may depend on distinct genetic and environmental properties (Akbari et al., 2018). The G395A SNP in the *KL* promoter has been detected to be related to EH for individuals over 60 years, females and nonsmokers in the Chinese Han population. For the *KL* G-395A, it was shown that people carrying the GA or AA genotype possess a considerably lower HTN risk than people with the GG genotype. Especially, it has been proposed that A allel of the *KL* G395A may raise KL gene activity, thus protecting against EH (Gao et al., 2015;

Hao et al., 2018; Wang et al., 2010). Constrary, A allele carriers of *KL* G395A SNP were reported to exhibit higher mean SBP relative to non-carriers in Korean females with healthy (Rhee et al., 2006). Furthermore a significant difference was not found for mean SBP between individuals carrying the A allele and non-carriers in Japanese healthy individuals (Shimoyama et al., 2009). It has been suggested that this circumstance might correlate with conducting of these studies in distinct populations and existing of different genetic backgrounds in distinct populations (Gao et al., 2015; Wang et al., 2010). According to a meta-analysis, it has been indicated that G-395A and F352V variants of the *KL* gene might be important factors contributing to HTN occurrence (Donate-Correa et al., 2016). The *KL* variant rs650439 (A/T) was shown to be significantly related with a stroke in hypertensive subjects. It was determined that the stroke incidence in hypertensive patients carrying the TT genotype of *KL* SNP rs650439 was significantly higher than in hypertensive patients carrying the AA and AT genotypes. Plasma KL concentrations were reported to become significantly lower in individuals carrying the TT genotype relative to those carrying the AA or AT genotype in elderly population (Yokoyama et al., 2018).

Research have proved that *KL* deficiency can contribute to HTN occurrence in different ways (Drew et al., 2021). It has been reported that *KL* deficiency may be a factor that triggers oxidative stress in HTN (Izbeki et al., 2010). The functional KL has been declared to improve endothelial dysfunction and decrease BP by promoting NO-mediated pathways (Rhee et al 2006). It was demonstrated that serum IGF-1 levels became high in HTN patients, and KL could block IGF-1 signaling to prevent oxidative stress in animal models (Sopjani et al., 2015). It has been indicated that KL may diminish oxidative stress by downregulating IGF-1 signaling that ihibits the Forkhead box O (FoxO) -mediated augment of the manganese superoxide dismutase (Mn-SOD) expression (Yamamoto et al., 2005). However, the receptor to which α-KL binds to inhibit the IGF-1 receptor has not been identified yet (Pathare et al., 2020b). In the Indian population, expression of *KL* and catalase genes have been indicated to be lower in peripheral blood mononuclear cells (PBMCs) of individuals with recently diagnosed EH relative to age-, body mass index (BMI), and gender-, matched normotensive healthy subjects. However, Mn-SOD expression has been observed to not change in the patient group. It has been suggested that apart from the IGF-1 pathway, α-KL may account for regulation of Mn-SOD expression by inhibiting Phosphoinositide 3-kinases/Protein kinase B (PI3K/Akt) mediated

FOXO3a phosphorylation in mitochondria. The KL expression was proven to be considerably linked with catalase expression in subjects with HTN (Pathare et al., 2020a). In another study, the positive association between the *FOXO1* gene expression and expressions of *α-KL* and catalase genes in patients with EH have also been demonstrated. It has been suggested that α-KL may affect catalase expression in EH by affecting *FOXO1* expression (Pathare et al., 2020b). Therefore, these studies have shown that the *KL* gene has an antioxidant defence effect in EH (Pathare et al., 2020a; 2020b).

Overactivity of RAAS negatively influences *KL* levels (Kale et al., 2023). The renal KL deficiency, increase in multiple RAAS proteins, and elevated BP have been indicated in a mouse model with chronic kidney disease (CKD). Particularly, it has been considered that KL may inhibit Wnt pathway-mediated RAAS activation, normalizing BP (Zhou et al., 2015a). Arterial stiffness that is related with progressive missing of elastin fibre and deposition of collagen fibres in large arteric media lend HTN occurence (Sun, 2015). KL insufficiency has been reported to increase superoxide formation, collagen level and elastin degradation in the aortic media. Sirtuin 1 (SIRT1) that acts as a deacetylase exhibits anti-oxidant, anti-apoptotic, and anti-inflammatory impacts in the endothelium (Zarzuelo et al., 2013). *KL* deficiency has been suggested to downregulate *SIRT1* in the VSMCs and aortic endothelium of mice, leading to arterial stiffness and HTN (Gao et al., 2016).

2.4. Circadian Clock

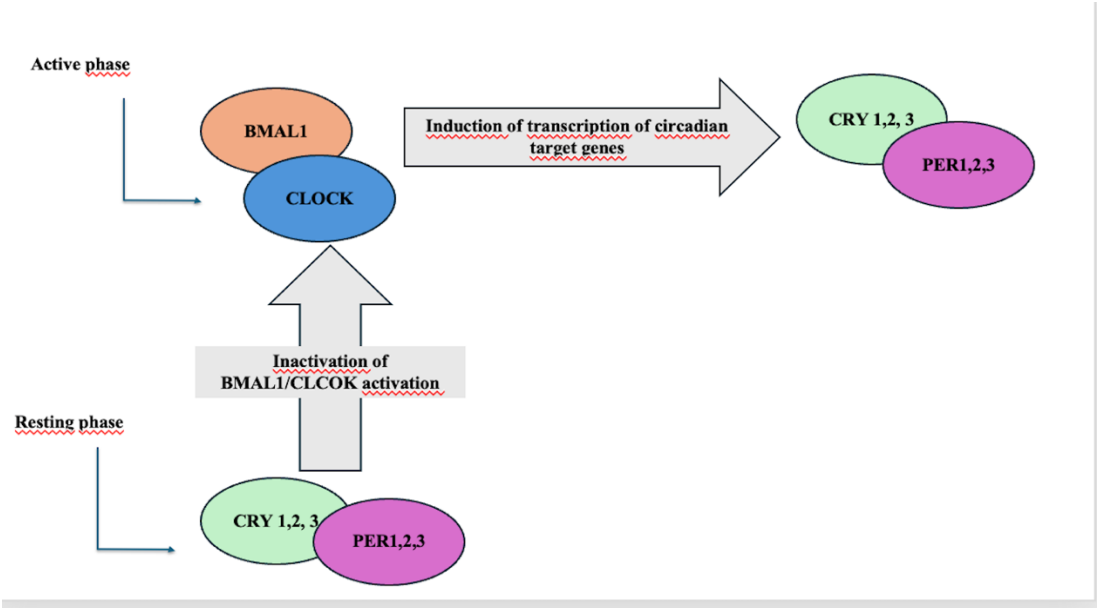
The ‘’circadian’ arised from Latin words circa revealing about, and dies revealing day. Almost all organisms, from archaea to humans have a circadian clock or biological clock (Loudon, 2012). The circadian clock forms endogenous circadian rhythms with a 24 hour-period (Miyazaki et al., 2011), and thus ensuring the regulation of several processes in a rhythmic manner, including sleep-wake cycles, metabolism, immune system, renal function and BP (Richards & Gumz, 2013). Generally, the circadian clock composed of two parts that are the central clock within the suprachiasmatic nucleus (SCN) of the brain, and the peripheral clock within nearly all tissues (Albrecht & Eichele, 2003). Both circadian clocks regulate the circadian rhythms (Costello et al., 2023). The central clock and peripheral clocks are synchronized by the light-dark cycle, and feeding-fasting cycle, respectively. In particular, the synchronized central circadian clock (known as a pacemaker) in turn

leads to the synchronized peripheral clocks by sending neural and hormonal signals (Richards et al., 2014). Synchronization of the circadian clocks contributes to an adaptation of organisms to daily environmental alterations such as temperature, light and humidity (Woelfle et al., 2004). The molecular machinery of clocks contains core clock genes that are Circadian locomotor output cycles kaput (CLOCK), BMAL1, Cryptochrome1/2/3 (Cry1/2/3), and Period1/2/3 (Per1/2/3). CLOCK and BMAL1, constituents of the positive arm of the functional circadian clock, create a heterodimer complex and induce transcription of circadian target genes such as Per and Cry genes during the active phase. The Cry and Per proteins, constituents of the negative arm of the functional circadian clock, interact to block the activity of the CLOCK/BMAL1 complex in the nucleus during the resting phase (Figure 2). The CLOCK/BMAL1- related induction of circadian target genes, and Per/Cry-mediated inhibition of CLOCK/BMAL1 activation (negative feedback loop) result in a transcription-translation oscillating (TTO) loop model and thus circadian rhythms in physiological processes (Richards et al., 2014).

The circadian clock has a critical function in the arrangement of BP in rhythm manner. It provides that BP drops at night and rises in the morning in healthy people, contributing to the protection of human health (Fang et al., 2021). It has been suggested to regulate BP by influencing processes in the kidney, heart and vasculature. Consequently, it has been proposed that the dysfuntional circadian clock can cause a misalignment among circadian rhythms and environmental cues such as light, resulting in several pathologies such as HTN (Richards et al., 2014). Especially, it has been suggested that subjects undergoing shift work, or chronic jet lag possess circadian disruption, and thus several health problems such as HTN (Costello & Gumz, 2021). Furthermore, according to epidemiological research, it was indicated an important link between circadian clock genes and HTN (Fang et al., 2021; Woon et al., 2007).

Figure 2.

Molecular Constituents of Circadian Clock



2.4.1. BMAL1 Gene Properties

BMAL1, also known as *MOP3*, *ARNTL*, *ARNTL1*, and *ARNTL3*, is a core circadian clock component (Huo et al., 2022; Zhang et al., 2022). *BMAL1* gene resides on human chromosome 11p15.2 (Woon et al., 2007). Especially, the *BMAL1* gene resided within hypertension susceptibility regions in rats (Chang et al 2018). *BMAL1* is produced significantly in the brain, heart, and skeletal muscle (Znag et al., 2022).

2.4.2. BMAL1 Function

BMAL1/CLOCK complex ensures rhythmic expression of clock-target genes, regulating the metabolic, cardiovascular, and immune systems (Huo et al., 2022; Zhang et al., 2022). Global *BMAL1* knockout mice have been reported to exhibit several premature ageing phenotypes, including cataracts, subcutaneous fat loss, muscle loss, and organ atrophy. It has been indicated that *BMAL1* inhibits transposase activation and cellular senescence and contributes to the maintenance of genomic stability. Consequently, it has an anti-aging effect. It has been found that *BMAL1* is involved in the attenuation of inflammation and therefore acts as a mediator between the immune system and biological clock (Zheng et al., 2023).

Furthermore, it has been shown that BMAL1 have a regulator role in insulin sensitivity and mitochondrial functions (Fan et al., 2022).

2.4.3. Impact of BMAL1 in Hypertension

The critical function of the *BMAL1* gene in arrangement of BP level has been declared in animals and human research (Chang et al., 2018). Global BMAL1 knockout (KO) male mice have been reported to have a decrease in BP level during the active phase, and furthermore a disrupted BP daily rhythm. Further, it has been observed vascular dysfunction and an increase in superoxide production in these mice (Anea et al., 2012; Huo et al., 2022). Male mice with vascular smooth muscle-specific *BMAL1* deletion have been shown to have a decrease in BP during the active period and less impaired circadian rhythm of BP (Xie et al., 2015). Based on the balance between contractile and noncontractile effects of perivascular adipose tissue (PVAT), vessel homeostasis and BP can be arranged. Male mice in which the perivascular brown fat cell-specific BMAL1 gene (BA-BMAL1-KO) was knocked out have been shown to have reduced BP during the inactive period, except the active period. Angiotensinogen is highly expressed rhythmically in PVAT, and it is the transcriptional target of *BMAL1*. Particularly, the BMAL1-Agt-Ang II complex has been reported to act as a significant regulator of BP during inactive period (Chang et al., 2018). Kidney-specific *BMAL1* deletion has been noticed to reduce BP in male mice without affecting BP rhythm. Therefore, it was suggested that kidney *BMAL1* regulates BP in a rhythm-independent manner. Application of a diet with K^+ deficiency has been indicated to increase sodium (Na^+) retention and restrict the K^+ loss in the urine. During feeding of a diet with K+-deficient, male kidney *BMAL1* KO mice were shown to possess less Na+ retention relative to controls. Therefore, it has been shown that *BMAL1* controls Na+ retention in male mice possessing K+ restriction (Crislip et al., 2020; Zhang et al., 2020). It has been indicated that adrenal-specific *BMAL1* KO male mice exhibit altered BP rhythm in a stressor condition in which serum corticosterone increases (Costello et al., 2023). In another study with hypertensive Ren-2 transgenic rat TGR (mREN-2) 27 rats, high *BMAL1* levels have been linked BP increase (Fang et al., 2021). The *BMAL1* deletion in myeloid cells such as monocytes and macrophages, has been shown to exacerbate SBP and DBP increase in mice infused by Ang II. Myeloid cell-specific *BMAL1* deletion has been reported to induce the pro-fibrotic M2 macrophage accumulation

in the vascular wall, by promoting interleukin 4 (IL4)-mediated signal transducer and activator of transcription 6 (STAT6) overactivation in mice with Ang infusion mediated HTN. Matrix metallopeptidase 13 (MMP13) overproduction followed by MMP9 overactivation in these fibrotic M2 macrophage cells has been indicated to exacerbate vascular dysfunction and wall thickening, enhancing BP increase. Consequently, myeloid cells with *BMAL1* deletion has been found to elevate BP in hypertensive mice infused with Ang-II by increasing vascular stiffness (Huo et al., 2022). Older type 2 diabetic (T2D) mice have been shown to develop HTN along with impaired circadian rhythms of BP and heart rate, because of dampened oscillation of the *BMAL1* gene in vasculature system. Therefore, it has been considered that T2D is related with a disrupted BP daily rhythm, resulting in HTN risk. However, conflicting results have been shown in a few studies. These contradictory results have been associated with differences in age and genetic background of mice and using of distinct BP measurement methods (Su et al., 2008). A case-control research has indicated that expression of *BMAL1* in women with HTN are considerably lower than in women with non-HTN. Furthermore, the *BMAL1* levels were declared to be negatively related with both SBP and DBP levels in these women. It has been proposed that age and gender may influence the expression levels of circadian-target genes (Quintela et al., 2015). Therefore, it has been speculated that gender may influence the *BMAL1*-mediated BP regulation. By this suggestion, the significant association between *BMAL1* and HTN has only been demonstrated in women (Fang et al., 2021).

Several studies have put forward that SNPs in the *BMAL1* gene have been correlated with HTN occurrence. *BMAL1* rs9633835 and rs6486121 SNPs have been notified to be linked with HTN susceptibility (Woon et al., 2007). According to a study, it has been detected that patients with HTN carrying the GG genotype of the *BMAL1* A1420G SNP may have a high risk of developing high nighttime SBP (Fang et al., 2021; Li et al., 2020). The G allele of *BMAL1* promoter 18477-T/G SNP in SHRs has been found to significantly impair GATA Binding Protein 4 (Gata-4) mediated transcriptional activation of *BMAL1 in vitro*. Particularly, it has been proposed that this SNP may influence *BMAL1* levels in organs that regulate BP. However, it has been suggested that the effect of this *BMAL1* promoter variant on BP regulation in vivo remains unclear (Woon et al., 2007). A research in patients with EH, GG genotype carriers of *CLOCK* 257TG variant were shown to possess lower

BMAL1 transcript levels compared to the other genotype carriers at 9.00, 13.00, and 17.00-time points. However, the EH patients with heterozygotes for *CLOCK* 257TG variant have been demonstrated to exhibit a higher *BMAL1* expression at the 17:00 time point (Kurbatova et al., 2014). Consequently, all these results support that BMAL1 is an important candidate gene for HTN development.

CHAPTER III
Methodology

3.1. Materials

3.1.1. Study Cases

In this research, a total of 78 hypertensive patients (47 male and 31 female) and 49 non-hypertensive individuals (13 male and 36 female) were incorporated. The blood samples of individuals were collected from the Department of Cardiovascular Surgery in Dr. Burhan Nalbantoglu Government Hospital (November 2022 - April 2023). Hypertension was diagnosed by the criteria based on the SBP/ DBP >140/90 mmHg or using of an anti-hypertensive drug for declining high BP (Akbari et al., 2018). In this study, patients with EH were included. People who were not satisfied to participate in this research, and subjects with cancer, cerebral infarction, respiratory diseases, congenital heart disease, autoimmune diseases, and chronic kidney diseases or diabetes mellitus were excepted from the research. The medical history of all individuals was interrogated. Clinical analysis of this research were performed regarding the principles of the Declaration of Helsinki. The Scientific Research Ethics Committee of the Near East University approved this study (YDU/2020/80-1066). Participants approved and signed the written informed consent form.

3.1.2. Solutions

Molecular Biology Grade Ethanol ≥ 99,8% (Sigma-Aldrich)
EpiTect Control DNA Set, Cat No./ID 59568 (QIAGEN)

3.1.3. Laboratory Consumables

Micropipette Tips
1.5ml Eppendorf Tubes
0.2ml PCR Tubes
2ml Vacuum Blood Collection Tubes-K2EDTA

3.1.4. Kits

AllPrep DNA/RNA/Protein Mini Kit (QIAGEN)
EpiTect Bisulfite Modification Kit (QIAGEN)

EpiTect HRM PCR Kit (QIAGEN)

3.1.5. Devices

Eppendorf Research Micropipettes
Eppendorf 5418 Centrifuge
NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific)
HLC BioTech Ditabis TH21 Heating Block
Real-Time PCR System
MasterCycler Gradient Thermal Cycler PCR
Vortex

3.2. Methods

3.2.1. Analysis of the Biochemical Parameters

The blood specimen of individuals were taken after an overnight fast. To obtain serum, all blood specimen were centrifuged at 2000 rpm, 4 °C for 20 minutes. The levels of total cholesterol (TC), fasting blood sugar (FBS), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), triglycerides (TG), creatinine (Cr), potassium (K), sodium (Na), and urea were measured by using an automated clinical biochemistry analyzer (Abbott Architect C8000).

3.2.2. DNA Extraction

DNA extraction of blood specimen of all participants was performed via the AllPrep isolation kit (Qiagen GmbH, Hilden, Germany).

1. Incubation of blood specimen containing erythrocyte lysis buffer (EL buffer) were performed at +4º C for 15 minutes.
2. Centrifugation of the specimen were done at 1000xg, +4ºC for 15 minutes, and their supernatants were discarded.
3. Following addition of 5ml EL Buffer in the pellets, centrifugation of specimens was performed at 1000xg at +40C for 10 minutes. After the supernatants were discarded, the pellets were dried.
4. The cells were broken up via 350-600 µl buffer RLT, and then the cell lysates were homogenized by vortexing.

5. After addition of cell lysates to a spin columns resided in a 2ml collection tubes, centrifugation was done at 14 000 rpm for 3 minutes.
6. Tubes containing 500 µl AW1 buffer were centrifuged at 8000xg for 15sec. In the case of some filtrate in columns, the centrifugation was repeated. After centrifugation, spin columns were placed in clean collection tubes.
7. All tubes to which 500 µl AW2 was incorporated were centrifuged at 20 000 x g for 2 minutes.
8. Afterwards, 100 µl EB buffer was added to the central region of all columns placed in 1.5 ul collection tubes. After the incubation of specimens at room temperature for 2 minutes, centrifugation was done at 8000 x g for 1min, to elute the DNA.

The assessment of DNA quality and quantity were done via the NanoDrop ND-1000 Spectrophotometer. The analysis of DNA quantity via Nanodrop ND-1000 Spectrophotometer:

1. The software connected with the NanoDrop spectrophotometer was turned up. DNA application was selected.
2. The hinged arm on the NanoDrop spectrophotometer instrument was gently lifted.
3. The pedestal on which the sample was placed was gently cleaned with DNase-free water.
4. 1µL blanking solution (EB buffer) was pipetted on the pedestal.
5. The hinged arm was gently lowered and "measure blank" was clicked.
6. The arm was lifted and cleaned.
7. 4-6 steps were repeated for all specimens.

A260/280 ratio was measured for the determination of specimen contamination. The A260/A280 ratio is lower than 1.8 showing protein contamination, while higher than 2.0 indicates RNA contamination. If the A260/280 ratio is between 1.7-2.0, this shows pure DNA. The formula for identification of DNA concentration is [DNA] = 50ug/ml (ng/ul) x A260 x dilution factor (Wilfinger et al., 1997).

3.2.3. Bisulfite Modification

Sodium bisulfite modification is identified as an effective tool that enables identification of DNA methylation. After treatment of DNA with sodium bisulfite, unmethylated cytosines are converted to uracil, but methylated cytosines remains unaltered. Thus, two different DNA sequences are originated from unmethylated and methylated DNA samples (Table 2).

Table 2.

Sodium Bisulfite Conversion of Methylated and Unmethylated DNA Sequence (Qiagen, 2014)

	Original DNA sequence	**Modified DNA sequence with sodium bisulfite**
Unmethylated DNA	G-C-G-A-T-C-G-A-T	G-U-G-A-T-U-G-A-T
Methylated DNA	G-**C**-G-A-T-**C**-G-A-T	G-**C**-G-A-T-**C**-G-A-T

Completely transformation of unmethylated cytosine bases is required for the accurate identification of the methylation of DNA sequences. Therefore, DNA samples are incubated with a high concentration of sodium bisulfite at low pH and high temperature. These conditions often result in highly fragmented DNA and subsequent losses of DNA in the purification step. The purification step is applied to remove chemicals and sodium bisulfite, that interfere with subsequent procedures. A high percentage of DNA samples is required because of DNA degradation and loss during the bisulfite modification process. However, the process usually results in low DNA yield and fragmented DNA. The fragmentation of the DNA sample is hindered by the DNA Protect Buffer including a pH-indicator dye that provides approval of the accurate pH value for the conversion of cytosine residues.

The DNA bisulfite modification was performed by following the ‘’Sodium Bisulfite Conversion of Unmethylated Cytosines in DNA from Low-Concentration Solutions’’ protocol of EpiTect Bisulfite Kit (Qiagen).

Procedure

The steps of bisulfite DNA conversion

1. DNA samples were thawed before the application of the bisulfite modification reactions. Bisulfite Mixtures were vortexed to dissolve completely.
2. Bisulfite reactions were prepared by adding each component to 200µl PCR tubes according to order in Table 3 (Table 3).

Table 3.

Constituents of Bisulfite Conversion Reaction (Qiagen, 2014)

Constituent	Volume per reaction (µl)
1-500 ng DNA	10
RNase Free water	30
Bisulfite Mıx	85
DNA Protect Buffer	15
Total	**140**

3. After the incorporation of DNA Protect Buffer into Bisulfite Mix, the buffer was turned from green to blue. Therefore, this indicated an adequate mixture and accurate pH value for bisulfite conversion.
4. Bisulfite modification was performed via a thermal cycler which was programmed based on table 4.

Table 4.

Conditions of Thermal Cycler for Bisulfite Conversion (Qiagen, 2014)

Step	Temperature/Time
Denaturation	95^0/5min
Incubation	60^0/25min
Denaturation	95^0/5min
Incubation	60^0C/85 min-1h 25min
Denaturation	95^0C/5min
Incubation	60^0C/175min -2h 55min
Hold	20^0C/∞

5. PCR tubes including the bisulfite reactions were put into a thermal cycler and then following with incubation.

Cleanup bisulfite bisulfite-modified DNA samples

6. After bisulfite conversion was completed, each bisulfite reaction was mixed with 560 µl Buffer BL including carrier RNA (10 µg/ml) in clean 1.5ml microcentrifuge tubes. Each sample was briefly centrifuged.
7. EpiTect spin columns containing mixtures were placed into collection tubes, following with centrifugation at full speed for 1minute and throwing the flow-through.
8. Centrifugation of every spin column containing 500ul Buffer BW (wash buffer) was performed at full speed for 1 minute, and following with discardation of the flow-through.
9. Each spin column containing 500 µl Buffer BD was incubated at 15-25°C for 15 minute. Then, centrifugation of each spin column was performed at full speed for 1 minute, and following with discardation of the flow-through.
10. The centrifugation of spin column containing 500 ul Buffer BW was carried out at full speed for 1 minute, then throwing of flow-through.
11. Step 13 was repeated once.
12. Centrifugation of each spin column placed into 2 ml collection tubes was performed at full speed for 1 minute until removing of all liquid residue.
13. 200ul Buffer EB was transfered in the central region of spin columns placed in clean 1.5ml microcentrifuge tubes, then following with centrigufation at approximately 15 000 x g for 1minute to elute pruified DNA.
14. To increase eluted DNA yield, an additional 20µl Buffer EB was transfered in central region of each tube and then centrifugation was done at full speed for 1min.

The quality and quantity of bisulfite-converted DNA specimens were measured by the NanoDrop Spectrometer.

3.2.4. Methylation Sensitive High-Resolution Melting (MS-HRM) Analysis

High-resolution melting (HRM) analysis is a technique that enables the analysis of the methylation of bisulfite-converted DNA samples according to their melting behavior during the conversion of double stranded DNA to single stranded DNA (ssDNA) with raising the temperature, after PCR amplification. Prior to HRM analysis, bisulfite-converted DNA samples are amplified in the existence of EvaGreen fluorescent dye. The dye only interacts with dsDNA samples, emitting

bright fluorescence. Fluorescence alteration allows measurement of the raising in DNA concentration during PCR and subsequent direct measurement of DNA melting due to temperature increase by HRM. HRM analysis is performed by increasing the temperature. Measurement of EvaGreen fluorescence is done continuously during the increase in temperature. As a result, fluorescence against the temperature is plotted. The amplification process causes high fluorescence at the initiation of the HRM analysis. The release of EvaGreen during melting of dsDNA causes the decrease of fluorescence to background level.

In this work, the methylation of *KLOTHO* and *BMAL1* promoter regions was determined by MS-MRM analysis according to EpiTect® HRM™ PCR Handbook protocol (Rotor-Gene Q-QIAGEN). The design of primers was performed according to EpiTect® MS-HRM PCR Handbook. Primers allowing specific amplification of bisulfite-converted DNA were designed. As the strands could no longer be complementary to each other following bisulfite treatment of the DNA, primers were designed to anneal to either the forward or reverse strands. The melting temperature (Tm) of primers was arranged to be at least 62°C. Primer pairs with similar Tm values were formed (Qiagen, Manchester, UK).

Universal methylated and universal unmethylated DNA samples (EpiTect Control DNA Set, Cat No./ID: 59568) were utilized as controls (Kalkan & Becer, 2019).

Procedure of MS-HRM Analysis

1. The EpiTect Master Mix, RNase-free water, primers, bisulfite converted DNA templates, and the control DNA samples were thawed.
2. The preparation of the reaction mixture was done based on the table below (Table 5).

Table 5 (Continued).

Reaction Constituents of EpiTect HRM PCR Master Mix (Qiagen, 2009)

Constituent	Volume per 10 ul reaction	Final concentration
(2X) EpiTect HRM PCR Master Mix	5 µl	1X

10 µM each primer mix	0.75 µl	0.75 µM F (forward) primer and R (reverse) primer
RNase Free Water	2ul	-
DNA template	1.5ul	5-10ng/reaction
Total per reaction	10µl	

3. The thoroughly mixed reaction mixture was dispensed in appropriate volumes into PCR tubes, following with addition of bisulfite-converted template DNA samples to the individual PCR tubes.

4. The real-time cycler was programmed based on the steps in the table 6 (Table 6).

Table 6.

Optimized Cycling Protocol of HRM Analysis on the Rotor-Gene Q (Qiagen, 2009)

	Temperature	**Duration**
Initial PCR activation step	95^0C	5 min
3 step cycling		
Denaturation	95^0C	10s
Annealing	60^0C for *BMAL1* gene 58^0C for *KLOTHO* gene	30s
Extension	72^0C	10s
40-45 cycles		
Denaturation	95^0C	30s
Pre-hold	50^0C	30s
HRM Analysis for Rotor-Gene Q	65-95^0C 0.1^0C increments	2s

5. PCR tubes placed in the real-time cycler were subjected to PCR and HRM analyses, respectively.

3.3. Statistical Analysis

Descriptive statistics were calculated for quantitative (arithmetic mean, standard deviation) and qualitative variables (frequency and percentage). Fisher's exact test or Pearson's Chi-Square test was chosen to determine the relationship between the methylation of genes and characteristics of patients. The association of methylation in both genes was investigated by using the McNemar test. The Two-Way Analysis of Variance test was used to investigate the impact of HTN and gene methylation on biochemical parameters. In case of statistical significance, Sidak's posthoc test was preferred to analyze the pairwise differences. For all statistical analyses, the Statistical Package for Social Science (SPSS) (Demo Version 26.0 for Mac), and GraphPad Prism (Demo Version 9.51 for Mac) software were preferred. The significance level was admitted to be 0.05.

CHAPTER IV

Result

The measurement results of DNA quantity and quality of a few DNA samples were indicated in Table 1S (Appendices).

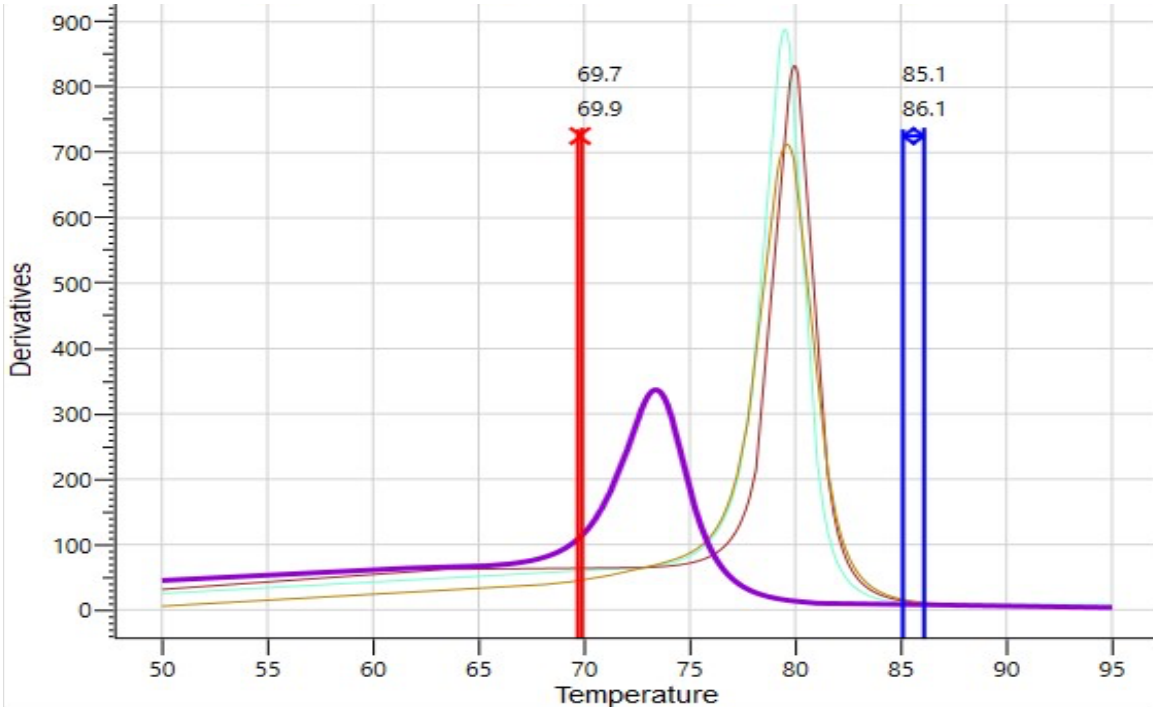
The average age of 76 patients with HTN was 60.04 ± 13.57, while the average age of 49 control individuals was 44.45± 13.94.

4.1. Interaction Between *KLOTHO* Promoter Methylation and Hypertension

The *KLOTHO* promoter methylation was identified in hypertensive patients and control subjects. The universal unmethylated and universal methylated controls were indicated as purple, and red, respectively. *KLOTHO* was identified to be methylated in patients 25 (turquoise line) and 12 (light green line) (Figure 3).

Figure 3.

Methylated KLOTHO Promoter in Hypertensive Patients



A total of 62 of the 100 subjects with methylated promoter sites of the *KLOTHO* gene were hypertensive (62.0%), while 16 of the 27 subjects with unmethylated promoter sites of the *KLOTHO* gene were hypertensive (59.3%). As a result, no statistically significant relationship between *KLOTHO* promoter methylation and HTN was determined (Table 7) ($p > 0.05$).

Table 7.

Methylation Status of KLOTHO Promoter in Subjects

		Subjects			
KLOTHO		**Hypertension**	**Control**	**Total**	
Methylated	**Count**	62	38	100	
	% within column	62.0%	38.0%	100.0%	p value
Unmethylated	**Count**	16	11	27	p>0.05
	% within	59.3%	40.7%	100.0%	
Total	**Count**	78	49	127	
	% within column	61.4%	38.6%	100.0%	

4.2. Interaction Between Gender and *KLOTHO* Promoter Methylation

While a total of 50 of the 100 subjects with methylated promoter site of the *KLOTHO* gene were female (50.0%), 17 of the 27 subjects with unmethylated promoter sites of the *KLOTHO* gene were female (63.0%). As a result, no statistically significant relationship between *KLOTHO* promoter methylation and gender was determined (Table 8) ($p > 0.05$).

Table 8.

Methylation Status of KLOTHO Promoter According to Gender

		Subjects			
KLOTHO		**Female**	**Male**	**Total**	
Methylated	**Count**	50	50	100	
	% within column	50.0%	50.0%	100.0%	p value
Unmethylated	**Count**	17	10	27	p>0.05
	% within column	63.0%	37.0%	100.0%	
Total	**Count**	67	60	127	
	% within column	52.8%	47.2%	100.0%	

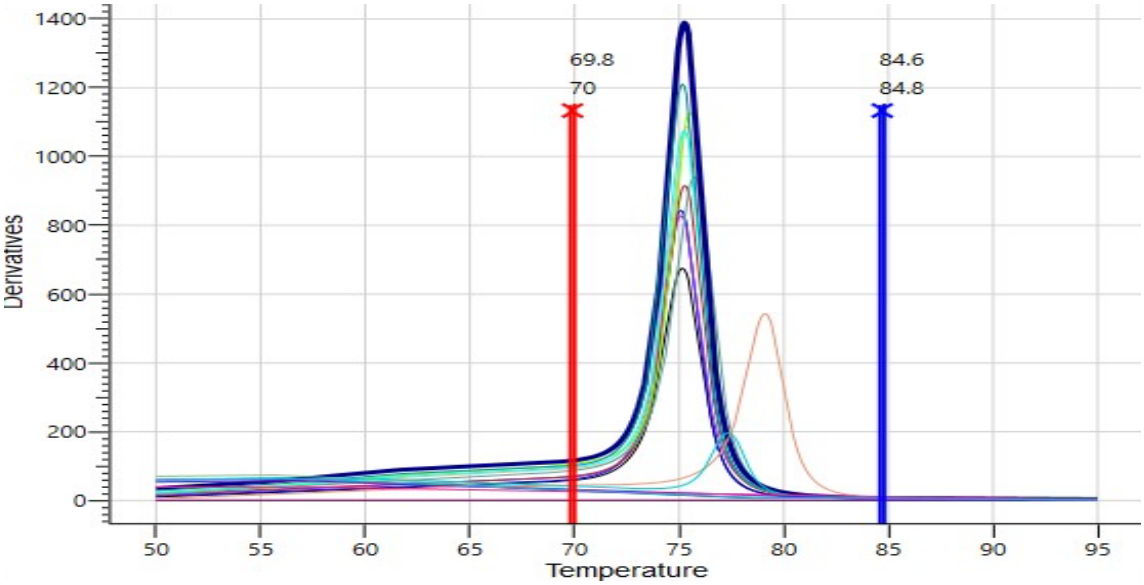
4.3. Interaction Between *BMAL1* Promoter Methylation and Hypertension

The *BMAL1* methylation was identified in hypertensive patients and control subjects. The universal unmethylated and universal methylated controls were indicated as blue and orange, respectively. *BMAL1* was identified to be methylated in

patients 13 (purple line), 33 (green line), 45 (black line), 49 (turquoise line), and 67 (red line) (Figure 4).

Figure 4.

Unmethylated BMAL1 Promoter in Hypertensive Patients



While a total of 46 of the 67 subjects with methylated promoter sites of the *BMAL1* gene were hypertensive (68.7%), 27 of the 48 subjects with unmethylated promoter sites of the *BMAL1* gene were hypertensive (56.2%). As a result, no statistically significant relationship between the *BMAL1* promoter methylation and HTN was detected (Table 9) ($p > 0.05$).

Table 9.

Methylation Status of BMAL1 Promoter in Subjects

		Subjects			
BMAL1		**Hypertension**	**Control**	**Total**	
Methylated	**Count**	46	21	67	
	% within column	68.7%	31.3%	100.0%	
Unmethylated	**Count**	27	21	48	p value
	% within column	56.2%	43.8%	100.0%	p>0.05
Total	**Count**	73	42	115	
	% within column	63.5%	36.5%	100.0%	

4.4. Interaction Between Gender and *BMAL1* Promoter Methylation

While a total of 36 of the 67 subjects with methylated promoter sites of the *BMAL1* gene were female (53.7%), 25 of the 48 subjects with unmethylated promoter sites of the *BMAL1* gene were female (52.1%). As a result, no statistically significant relationship between the *BMAL1* promoter methylation and gender was detected ($p > 0.05$) (Table 10).

Table 10.

Methylation Status of BMAL1 Promoter According to Gender

		Subjects			
BMAL1		**Female**	**Male**	**Total**	
Methylated	**Count**	36	31	67	
	% within column	53.7%	46.3%	100.0%	
Unmethylated	**Count**	25	23	48	p value
	% within column	52.1%	47.9%	100.0%	p>0.05
Total	**Count**	61	54	115	
	% within column	53.0%	47.0%	100.0%	

4.5. Comparison of Biochemical Parameters According to Hypertension and Methylation Status for *KLOTHO*

The differences in TG, FBS, LDL-C, HDL-C, TC, Na, Cr, K and Urea levels according to hypertension and methylation status for the *KLOTHO* gene were analyzed. No statistically significant difference between subjects with methylated *KLOTHO* promoter site and subjects with unmethylated *KLOTHO* promoter site in terms of TG, FBS, LDL-C, HDL-C, TC, Cr, Na, and Urea levels was identified (P1>0,05). A statistically significant difference for K was determined between subjects with methylated promoter site of *KLOTHO* gene and subjects with unmethylated promoter site of *KLOTHO* gene (P1 = 0,019) (Table 11). No statistically significant difference was identified between subjects with hypertension and controls for levels of FBS, LDL-C, TC, HDL-C, and Urea (P2>0,05). A statistically significant difference was identified between hypertensive subjects and controls for levels of TG, Na, K, and Cr (P2<0,05) (Table 11).

Table 11.

Comparison of Biochemical Parameters for KLOTHO

Parameter	Methylated Status		Unmethylated Status		P value	
	HTN patients	**Control Subjects**	**HTN patients**	**Control subjects**	**P1**	**P2**
FBS - mg/dL	129,96 ± 83,08 (54)	97,97 ± 12,43 (38)	117,20 ± 34,03 (15)	98,18 ± 5,23 (11)	0,6333	0,0544
TG-mg/dL	166,82 ±91,28 (38)	122,22 ± 31,10 (37)	183,93 ±104,06 (14)	132,00±28 ,43 (11)	0,4205	**0, 0046**
TC-mg/dL	195,97 ± 61,63 (36)	170,59 ±43,12 (37)	197,07 ± 49,94 (14)	191,09 ± 42,03 (11)	0,3711	0,1951
HDL-C-mg/dL	45,84 ± 14,31 (37)	52,19 ± 12,72 (37)	46,50 ± 14,34 (14)	49,64 ± 6,83 (11)	0,7573	0,1232
LDL-C-mg/dL	131,92 ± 65,55 (37)	129,62 ± 28,28 (37)	125,71 ± 60,77 (14)	130,27 ± 33,02 (11)	0,8137	0,9235
Na - mmol/L	137,98 ± 4,29 (53)	138,26 ± 7,50 (38)	136, 60 ± 12,98 (15)	143, 27 ± 8,60 (11)	0,2764	**0,0383**
K -mmol/L	4, 45 ± 0,60 (53)	4,88 ± 1,11 (38)	4,71± 0,75 (15)	5,55 ± 1,20 (11)	**0,019**	**0,0017**
Cr-mg/dL	1,11± 0,59 (54)	0,66 ± 0,14 (38)	1,06 ± 0,95 (15)	0,65 ± 0,11 (11)	0,8179	**0,0005**
Urea - mg/dL	28,66 ± 23,10 (53)	15,18 ± 3,40 (38)	21,14 ± 17,82 (14)	20,91 ± 13,40 (11)	0,822	0,0874

P1: Comparison for methylation, **P2:** Comparison for hypertension

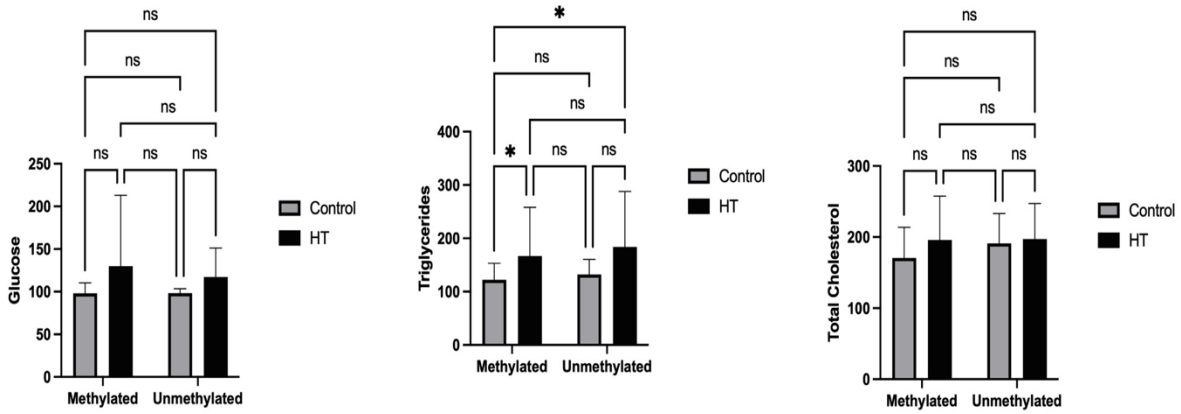
4.6. Difference in Biochemical Parameters Between Methylation Levels of *KLOTHO* Promoter

The differences in FBS, LDL-C, HDL-C, TC, TG, K, Na, Cr, and Urea levels between methylation levels of *KLOTHO* promoter were analyzed in this study. No statistically significant difference for levels of FBS, HDL-C, TC, LDL-C, TG, Na, Cr, K, and Urea between methylation levels of the *KLOTHO* promoter was detected

(p>0.05) (Graphic 1, 2, 3). A statistically significant difference between hypertensive subjects with methylated promoter site of *KLOTHO* gene and control subjects with methylated promoter site of *KLOTHO* gene for TG was detected (p=0,0485). A statistically significant difference for TG was detected between control subjects with methylated promoter site of *KLOTHO* gene and hypertensive subjects with unmethylated promoter site of *KLOTHO* gene (p=0.0423). These differences were detected to be statistically dependent on hypertension (p = 0.0046) (Graphic 1).

Graphic 1.

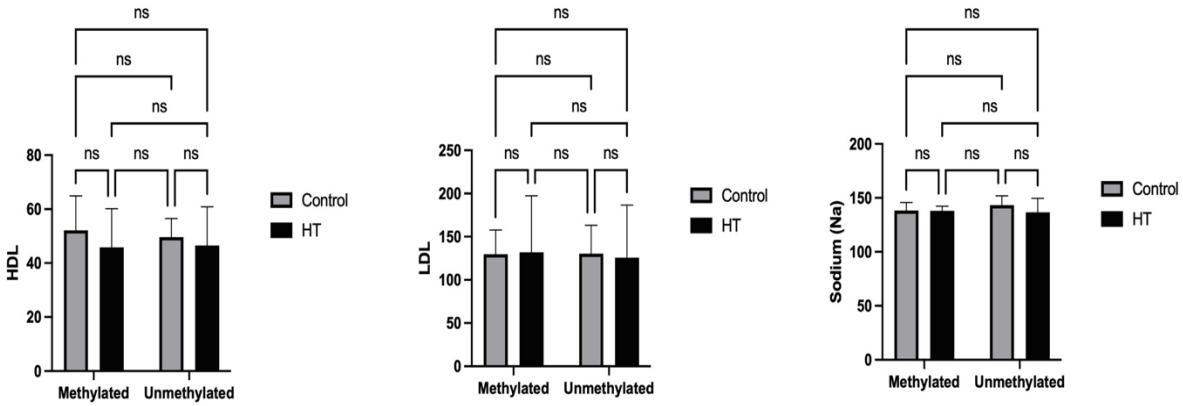
Difference in Levels of FBS, TG, and TC Between Methylation Level of KLOTHO Promoter



Statistically significant difference for levels of HDL, LDL, and Na between methylation levels of the *KLOTHO* promoter were no identified (p>0.05) (Graphic 2).

Graphic 2.

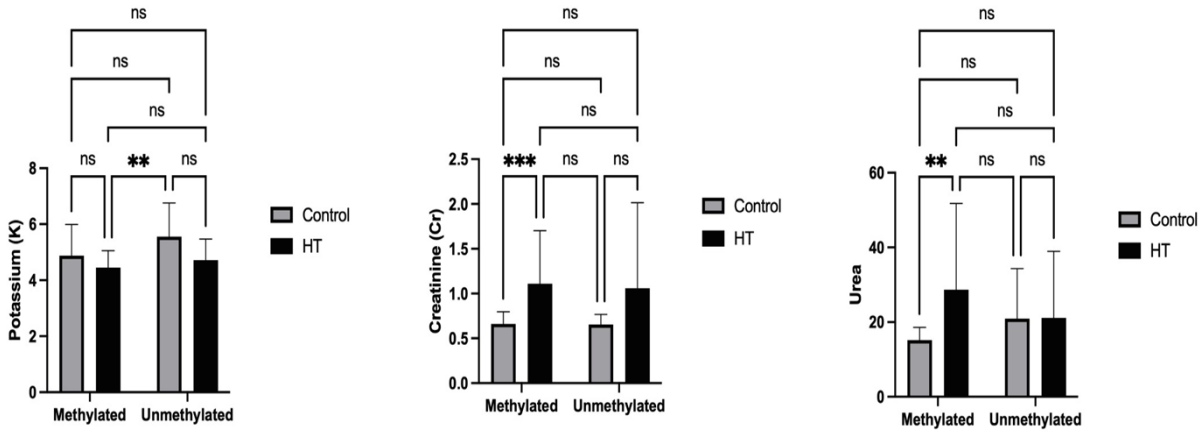
Difference in Levels of HDL, LDL, and Na Between Methylation Levels of KLOTHO Promoter



The statistically significant difference between hypertensive patients with methylated promoter site of *KLOTHO* gene and control subjects with unmethylated promoter site of *KLOTHO* gene in terms of K was identified (p=0,0014). This difference was determined to be statistically dependent on hypertension (p=0,0017) and methylation status (p = 0.019) (Graphic 3). The statistically significant difference between hypertensive patients with methylated promoter site of *KLOTHO* gene and control subjects with methylated promoter site of *KLOTHO* gene in terms of Cr was identified (p=0.0007). This difference was detected to be statistically dependent on hypertension (p= 0,0005) (Graphic 3). The statistically significant difference was detected between hypertensive patients with methylated promoter site of *KLOTHO* gene and control subjects with methylated promoter site of *KLOTHO* gene in terms of Urea (p= 0,0025) (Graphic 3).

Graphic 3.

Difference in Levels of K, Cr, and Urea Between Methylation Level of KLOTHO Promoter



4.7. Comparison of Biochemical Parameters According to Hypertension and Methylation Status for *BMAL1*

The differences in FBS, LDL-C, HDL-C, TC, TG, K, Na, Cr and Urea levels according to hypertension and methylation status for the *BMAL1* gene were analyzed. No statistically significant difference between subjects with methylated promoter site of *BMAL1* gene and subjects with unmethylated promoter site of *BMAL1* gene in terms of FBS, LDL-C, HDL-C, TC, TG, K, Cr, Na and Urea was identified (P1>0,05) (Table 12). The statistically significant difference was not identified between patients with hypertension and control subjects in terms of TC,

LDL-C, and Na levels for the *BMAL1* (P2>0,05). The statistically significant difference was detected between patients with hypertension and control subjects in terms of FBS, TG, HDL-C, K, Cr, and Urea levels for the *BMAL1* gene (P2<0,05) (Table 12).

Table 12.

Comparison of Biochemical Parameters for BMAL1

Parameter	**Methylated status**		**Unmethylated status**		**P value**	
	HTN patients	**Control Subjects**	**HTN patients**	**Control subjects**	**P1**	**P2**
FBS-mg/dL	120,00 ± 70,48 (42)	96,67 ± 15,54 (21)	142,65 ± 87,50 (23)	98,14 ± 6,02 (21)	0,3253	**0,0065**
TG -mg/dL	177,58 ±98,15 (33)	132,50 ± 31,30 (20)	172,06±91,38 (16)	120,57±28,13 (21)	0,5893	**0, 0035**
TC -mg/dL	197,58 ± 59,89 (33)	179,60 ±59,04 (20)	195,07 ± 52,06 (14)	168,14 ± 28,81 (21)	0,5542	0,0595
HDL-C -mg/dL	47,55 ± 15,91 (33)	48,55 ± 8,75 (20)	41,87 ± 10,33 (15)	53,48 ± 12,15 (21)	0,8946	**0,0285**
LDL-C-mg/dL	128,30 ± 60,90 (33)	134,65 ± 35,32 (20)	136,93 ± 71,65 (15)	128,24 ± 26,19 (21)	0,9229	0,9184
Na-mmol/L	137,38 ± 8,36 (42)	138,67 ± 6,63 (21)	138, 00 ±4,57 (22)	141, 00 ± 9,11 (21)	0,3383	0,1656
K-mmol/L	4, 45 ± 0,70 (42)	5,02 ± 1,05 (21)	4,59± 0,57 (22)	5,11 ± 1,30 (21)	0,532	**0,0034**
Cr-mg/dL	1,00± 0,51 (42)	0,64 ± 0,15 (21)	1,07 ± 0,52 (23)	0,68 ± 0,12 (21)	0,5002	**<0,0001**
Urea-mg/dL	26,78 ± 23,29 (40)	17,95 ± 10,51 (21)	23,22 ± 13,49 (23)	15,76 ± 2,95 (21)	0,3916	**0,0166**

P1: Comparison for methylation**, P2:** Comparison for hypertension

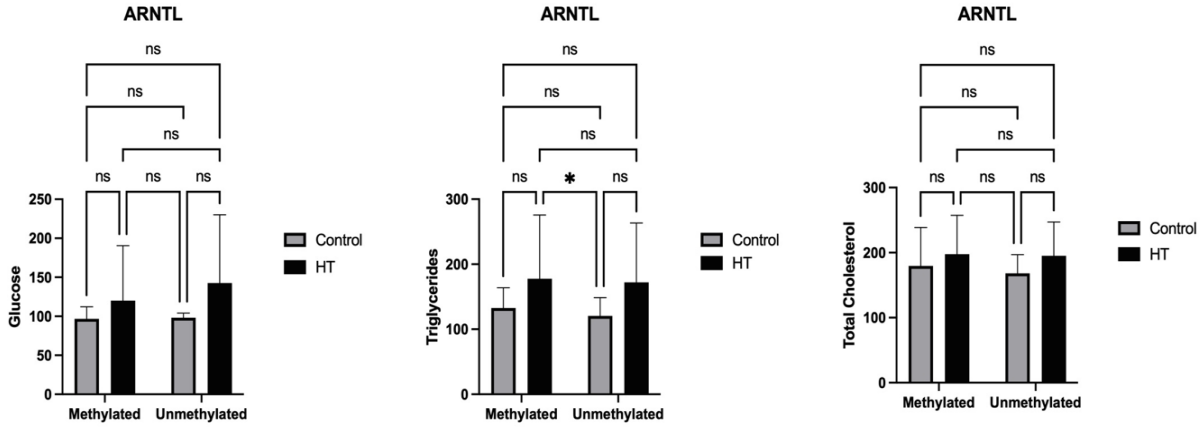
4.8. Difference in Biochemical Parameters Between Methylation Levels of *BMAL1* Promoter

The differences in FBS, LDL-C, HDL-C, TC, TG, K, Na, Cr and Urea levels between methylation levels of the *BMAL1* gene were analyzed in this study. No

statistically significant difference for levels of FBS, LDL-C, HDL-C, TC, TG, K, Na, Cr, and Urea between methylation levels of the *BMAL1* gene was detected (p>0.05) (Graphic 4, 5, 6). The statistically significant difference was determined between hypertensive subjects with methylated promoter site of *BMAL1* gene and control subjects with unmethylated promoter site of *BMAL1* gene in terms of TG (p= 0,0407). These differences were detected to be statistically dependent on hypertension (p = 0.0035) (Graphic 4).

Graphic 4.

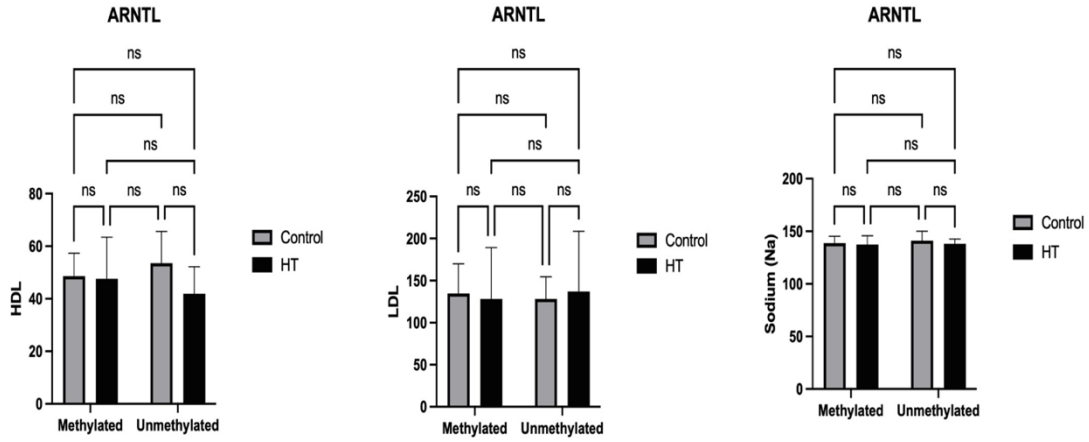
Difference in Levels of FBS, TG, and TC Between Methylation Level of BMAL1 Promoter



The statistically significant difference was not detected for levels of HDL, LDL, and Na between methylation levels of the BMAL promoter (p>0.05) (Graphic 5).

Graphic 5.

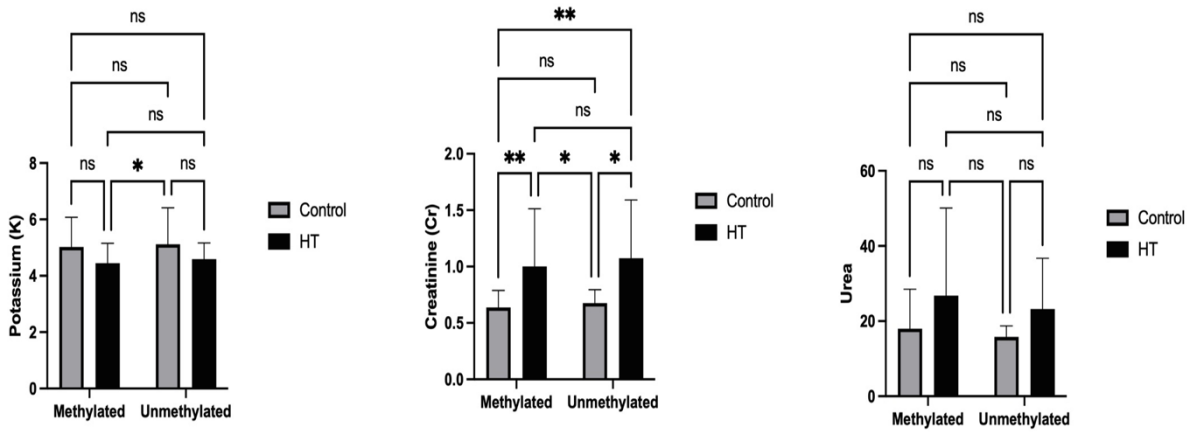
Difference in Levels of HDL, LDL, and Na Between Methylation Levels of BMAL1 Promoter



The statistically significant difference between hypertensive patients with methylated promoter site of *BMAL1* gene and control subjects with unmethylated promoter site of *BMAL1* for K was detected (p=0.0415). This difference was detected to be statistically dependent on hypertension (p = 0.0034) (Graphic 6). The statistically significant difference between hypertensive patients with methylated promoter site of *BMAL1* gene and control subjects with methylated promoter site of *BMAL1* gene for Cr was detected (p= 0,008) (Graphic 6). The statistically significant difference between hypertensive patients with methylated promoter site of *BMAL1* gene and control subjects with unmethylated promoter site of *BMAL1* gene for Cr was identified (p=0.0234) (Graphic 6). The statistically significant difference between control subjects with methylated promoter site of *BMAL1* gene and hypertensive patients with unmethylated promoter site of *BMAL1* gene for Cr was identified (p=0.0039) (Graphic 6). The statistically significant difference between hypertensive patients with unmethylated promoter site of *BMAL1* gene and control subjects with unmethylated promoter site of *BMAL1* gene for Cr was detected (p=0.0106) (Graphic 6). These differences were detected to be statistically dependent on hypertension (p<0.0001).

Graphic 6.

Difference in Levels of K, Cr, and Urea Between Methylation Level of BMAL1 Promoter



4.9. Correlation of Methylation Status of *BMAL1* and *KLOTHO* Promoters

The correlation of methylation of *BMAL1* and *KLOTHO* promoters was analyzed. Both methylated *BMAL1* and methylated *KLOTHO* promoters were in 53 out of 115 subjects (46.1%), while both unmethylated *BMAL1* and unmethylated

KLOTHO promoters were in 10 out of 115 subjects (8.8%) (Table 13). Both unmethylated *BMAL1* and methylated *KLOTHO* promoters were in 38 out of 115 subjects (33.0%) while both methylated *BMAL1* and unmethylated *KLOTHO* promoters were in 14 out of 115 subjects (12.1%) (Table 13). Therefore, no statistically significant relationship was detected between the methylation of the *KLOTHO* and *BMAL1* promoters (Table 13) ($p>0.05$).

Table 13.

Correlation of Methylation of KLOTHO and BMAL1 Promoters in Subjects

Methylation status of genes		***KLOTHO* Methylated**	***KLOTHO* Unmethylated**	**Total**	
***BMAL1* Methylated**	**Count**	53	14	67	p value $p>0.05$
	% within column	79.1%	20.9%	100.0%	
***BMAL1* Unmethylated**	**Count**	38	10	48	
	% within column	79.2%	20.8%	100.0%	
Total	**Count**	91	24	115	
	% within column	79.1%	20.9%	100.0%	

Both methylated *BMAL1* and methylated *KLOTHO* promoters were in 36 out of 73 hypertensive patients (49,3%) while unmethylated *BMAL1* and unmethylated *KLOTHO* were in 4 out of the 73 hypertensive patients (5,48%) (Table 14). Both unmethylated *BMAL1* and methylated *KLOTHO* promoters were in 23 out of 73 hypertensive patients (31.5%) while both methylated *BMAL1* and unmethylated *KLOTHO* promoters were in 10 out of 73 hypertensive patients (13.7%) (Table 14). Therefore, no statistically significant relationship was detected between the methylation of the *KLOTHO* and *BMAL1* promoters in hypertensive patients ($p>0.05$) (Table 14).

Table 14 (Continued).

Correlation of Methylation of KLOTHO and BMAL1 Promoters in Hypertensive Patients

Methylation status of genes		***KLOTHO* Methylated**	***KLOTHO* Unmethylated**	**Total**	
***BMAL1* Methylated**	**Count**	36	10	46	
	% within column	78.3%	21.7%	100.0%	

BMAL1	**Count**	23	4	27	p value
Unmethylated	**% within column**	85.2%	14.8%	100.0%	p>0.05
	Count	59	14	73	
Total	**% within column**	80.8%	19.2%	100.0%	

Both methylated *BMAL1* and methylated *KLOTHO* promoters were in 17 out of 42 controls (40.5%) while both unmethylated *BMAL1* and unmethylated *KLOTHO* promoters were in 6 out of the 42 controls (14.3%) (p>0,05) (Table 15). Both unmethylated *BMAL1* and methylated *KLOTHO* promoters were in 15 out of 42 controls (35.7%) while both methylated *BMAL1* and unmethylated *KLOTHO* promoters were in 4 out of 42 controls (9.5%) (Table 15). Therefore, no statistically significant relationship was detected between the methylation of the *KLOTHO* and *BMAL1* promoters in controls (p>0.05) (Table 15).

Table 15.

Correlation of Methylation of KLOTHO and BMAL1 Promoters in Controls

Methylation status of genes		***KLOTHO*** **Methylated**	***KLOTHO*** **Unmethylated**	**Total**	
BMAL1	**Count**	17	4	21	
Methylated	**% within column**	81.0%	19.0%	100.0%	
BMAL1	**Count**	15	6	21	p value
Unmethylated	**% within column**	71.4%	28.6%	100.0%	p>0.05
Total	**Count**	32	10	42	
	% within column	76.2%	23.8%	100.0%	

CHAPTER V

Discussion

HTN which is a multifactorial disease was related with the diverse disease occurrence, including kidney, and cardiovascular diseases (Kanbay et al., 2021; Zhang et al., 2017). Although HTN is a common in older people, it has become gradually widespread in pediatric patients, recently (Viridis et al., 2011; Yusuf et al., 2020). Several factors such as age, gender, genetics and epigenetics factors, absence of physical activity, extreme sodium and alcohol consumption, obesity and diabetes have a critical role in the HTN pathogenesis (Türkiye Endokrinoloji ve Metabolizma Derneği, 2022). Generally, it is divided into two types, primary HTN and secondary HTN. While almost 95% of the cases are primary hypertensive subjects, almost 5 % are secondary hypertensive subjects. The primary HTN develops due to genetic and environmental factors, independent of different disease states and its developmental mechanisms are still unclear. However, oxidative stress was noted to contribute to the HTN development (Pathare et al., 2020a). Secondary HTN develops due to different conditions such as kidney problems, endocrine disease, and drugs' side effects (Kanbay et al., 2021, Pathare et al., 2020a). Both genders possess an HTN risk increased with age (Connelly et al., 2022). However, there is a distinct between genders in terms of HTN risk. Generally, the HTN incidence was determined to be higher in individuals with post-menopause than in individuals with pre-menopause, and age-matched men (Yu et al., 2023). Particularly, the decreased estrogen level, increased RAAS activity, and obesity-mediated sympathetic activity have been associated with postmenopausal hypertension. However, the exact mechanisms underlying postmenopausal hypertension have been suggested to be unclear yet (Di Giosia et al., 2018; Fu, 2019; Sabbatini & Kararigas, 2020).

Epigenetic modification is a state of heritable and reversible change in gene expression without any variation of DNA sequence (Wise & Charchar, 2016). Among the epigenetic processes, DNA methylation process is a reversible that includes the incorporation of CH3 groups to the cytosines in the CpGs of the DNA sequence, causing to 5-methyl-cytosine (5mC). In mammalians, the CpGs are densely dispersed in the gene promoter regions (CpG islands), but also non-densely found in non-promoter regions. In healthy somatic cells, while the CpG islands of genes are generally unmethylated, CpGs in the other genomic region are methylated (Pratamawati et al., 2023). Abnormal DNA methylation has been proven to

participate in HTN occurrence (Hiltunen et al., 2002). Many studies have focused on investigating promoter methylation alterations of genes implicated in the HTN. However, it has specified that influences of DNA methylation of other regions of genes (excluding promoter sites) and other DNA regions on HTN have been unknown yet. (Han et al., 2016). Furthermore, as HTN is a multifactorial disease, it has been declared to be difficult to determine the specific DNA methylation modifications linked to the HTN occurrence and progression by investigating the methylation of single genes or single pathways. Hence, methylation mapping analysis identifying the methylation status of all sequencing such as genomic DNA, gene promoter sites, and interspersed repeat is recommended (Saco et al., 2014). As both internal and external factors influence DNA methylation, it is emphasized that the impact of these factors should also be not ignored. Generally, hypertensive patients possess several complications such as stroke, aneurysm, kidney problems, metabolic syndrome, eye problems and dementia. It was suggested that these complications may cause abnormal DNA methylation or may be promoted by abnormal DNA methylation. Consequently, that criteria is recommended to be considered in future studies. It is reported that tumour-associated methylations can be identified with circulating cell-free DNA in the blood (Earl et al., 2015). Yet, it has been proposed that promoter methylation of genes in blood samples may not directly affect BP. As a result, it has been proposed that methylation status of genes in blood samples may difficultly reflect HTN susceptibility (Han et al., 2016). But conflicting findings have recently been presented showing the intriguing relationships between blood DNA methylation and HTN (Wang et al., 2023). It was specified that genome-wide DNA methylation (DNAm) changes with ageing, which may indicate molecular alterations related to the development of HTN (Kresovıch et al., 2023). Women possessing high biological age was stated to be more likely to develop HTN in later years, indicating a crucial risk agent of biological age for HTN development (Wang et al., 2023). Biological age metrics (epigenetic clocks), derived from genome-wide DNA methylation data in whole blood samples have been suggested to be effective tools for predicting age-related physiological alteration, risk of diseases, and mortality. The biological age metrics are DunedinPACE (estimator of ageing paces) and PhenoAgeAccel or GrimAgeAceel (estimator of difference between biological and chronological ages) developed by selecting sets of CpG regions. DNA methylation-based biological age metrics (PhenoAgeAccel, GrimAgeAccel,

DunedinPACE) have been positively associated with HTN. It has been reported that biological age metrics can be used in HTN risk classification. The existence of elevated biological age metrics even though successful treatment of HTN suggested that biological age metrics can reflect age-linked molecular conditions causing HTN (Kresovıch et al., 2023). DNA methylation-depedent biological age metrics were also emphasized to be crucial tools in terms of early HTN intervention and the development of personalized prevention strategies for HTN. Risk factors that can be used in the early prediction of HTN have still been unclear. Recently, most researchers have focused on the identification of several targets such as circular RNA and metabolites that could be utilized for the prognosis, diagnosis, and treatment of HTN. It has been emphasized that DNA methylation-based biological age metrics have been highlighted to play a considerable role in discovering epigenetic targets for HTN. Consequently, it is proposed that combined usage of blood pressure measurements with DNA methylation-based biological age metrics could provide an effective prediction of HTN (Wang et al., 2023).

Many research to date have targeted DNA methylation analysis of genes that may be related to HTN (Table 16). Because of several studies indicating the impact of *KL* and *BMAL1* genes on HTN, and the absence of any *KL* and *BMAL1* gene promoter methylation analysis in HTN, in this research, we aimed to detect the impact of *KL* and *BMAL1* gene promoter methylation status on HTN by the first time.

α-KL is an anti-ageing gene, and its expression has been indicated to gradually decline with age (Xiao et al., 2004). Various research have highlighted that *α-KL* possesses a considerable role in the correlation of HTN with ageing (Pathare et al., 2020b). *KL*-deficient mice have been indicated to exhibit disrupted vasculogenesis and endothelial dysfunction (Shiraki et al 2000). SHRs have been reported to exhibit significantly decreased *KL* expression than control WKY rats (Cheng et al., 2010). The inhibition of the *KL* gene was correlated with elevated aldosterone levels in human adrenocortical cells and HTN development (Zhou et al., 2016). Human α-KL protein is present in two forms that are transmembrane and soluble α-KL proteins. Kidneys are the main sources of soluble α-KL protein that are present in the cerebrospinal fluid, blood, and urine (Lanzani et al., 2020; Xu & Sun 2015). Particularly, it has been suggested that either transmembrane or soluble KL protein can normalize BP by inhibiting Wnt/β-mediated RAAS activation in CKD

patients (Zhou et al., 2015a). In the control individuals of Indian population, it has indicated that the soluble α-KL levels are positively linked with HDL-C, but negatively linked with TG. Besides, the levels of soluble α-KL have been negatively associated with BMI in primary hypertensive subjects (Pathare et al., 2020a). It has been considered that low soluble KL levels can lead to BP by increasing total body sodium in CKD patients (Lanzani et al., 2020). Since postmenopausal hypertensive women have been found to exhibit low serum KL concentration, it has been proposed that serum KL level can be utilized as a biomarker for prediction risk of HTN in postmenopausal individuals (Yu et al., 2023). Various studies have reported that low KL concentration increases the HTN risk by augmenting BP (Su & Yang, 2014; Zhou et al., 2018). By contrast, significant difference in terms of serum KL concentration was no detected between HTN patients and controls in the Chinese individuals (Liang et al., 2021). In a research conducted with a larger population, it was reported that no related between serum KL level and BP (Semba et al., 2011). The *KL* promoter region is sensitive to DNA methylation (King et al., 2012). Therefore, we focused on the relation between *KL* methylation and HTN in this research. However, we didn't detect statistically significant relation between *KL* methylation and HTN ($P>0.05$). It is thought that the disparity between the studies' results could have arisen from the age of the population or sample size. Because *KL* expression is altered during ageing, handling large-scale research focusing on the correlation between *KL* methylation, KL concentration, and HTN in patients with distinct diseases and age-matched controls will be beneficial to show the link between *KL* methylation and HTN. Since the main contributor of soluble KL protein is kidneys, it has been emphasized that alteration of KL gene expression in other tissues may not indicate KL insufficiency in patients with normal soluble KL concentration because of normal kidney function. Consequently, despite the normal soluble KL levels, it has been considered that distinct factors, such as *KL* genetic variants and *KL* epigenetic alteration may decrease *KL* expression in other tissues (Donate-Correa et al., 2016). Therefore, this situation should be considered in future studies. Especially, it has been proposed that the influence of *KL* polymorphisms on BP regulation should be discounted (Liang et al., 2021). Subjects carrying the GA or AA genotype of the *KL* G-395A variant have been indicated to possess lower BP compared to subjects carrying the GG genotype (Hao et al., 2018). As a result, it has been indicated that the A allele of *KL* G-395A may hinder the EH by augmenting the

KL expression (Gao et al., 2105; Wang et al., 2010). *KL* expression has been reported to be inhibited via an indefinite mechanism that influences the human *KL* promoter region in HEK293 cells (Turan & Ata, 2011). Especially, although the *KL* promoter site is sensitive to DNA methylation, other epigenetic processes may also possess a considerable role for inhibition of *KL* expression (Azuma et al., 2012; King et al., 2012). The *KL* gene variations have been suggested to alter serum levels of biological parameters such as HDL-C, fasting insulin and fasting glucose. The KL-VS variant has been correlated with increased SBP and serum cholesterol in Baltimore Caucasian and African American subjects (Arking et al., 2002; 2003; 2025). The *KL* rs1207568 + rs9536314, or rs564481+ rs9536314 have been related with a rise in HDL-C in female hospitalized older patients (Paroni et al., 2012). The KL rs564481 variant has been positively associated with low-density lipoprotein (LDL) in the Kazak population (Xu et al., 2015). It has been claimed that KL-related pathways such as hormonal alterations, and inflammation may decrease HDL-C plasma concentrations during ageing (Van der Westhuyzen et al., 2007). Nevertheless, according to the cross-sectional studies, levels of HDL-C have been indicated to slightly increase during ageing (Barziali et al., 2003). It has been proposed that increased levels of HDL interfere with the detrimental effect of decrease of KL level (Arking et al., 2003; 2005). KL and HDL have been stated to regulate the same signaling pathways, including the induction of angiogenesis and NO synthesis, and inhibition of insulin signaling and apoptosis in vitro (Nofer et al., 2002). The *KL* rs564481 and rs9536314 have been correlated with fasting glucose in female hospitalized older patients (Paroni et al., 2012). Higher fasting glucose has been found in women carrying T allele of *KL* rs564481 relative to non-carrier women in Japan, (Shimoyama et al 2009) and Korea (Rhee et al., 2006). In addition, KL rs9527025 and rs9536314 variants have been indicated to be positively correlated with fasting blood glucose in the Kazak population (Xu et al., 2015). The *KL* SNP rs9536314 has been indicated to be correlated with serum Cr levels in hospitalized older female patients (Paroni et al., 2012). KL is crucial for normal structure and function of kidneys (Koh et al., 2001). Particularly, the KL was reported to decrease serum creatinine. It has been highlighted that KL levels decrease, and serum creatinine levels increase in kidney damage (Aiello & Noris 2010). Many studies have put forward an association between KL variants and several biochemical parameters. However, in this research, no statistically significant

association among the KL methylation and levels of FBS, TC, TG, LDL-C, HDL-C, Na, Cr, K, and Urea were found ($p > 0.05$). It is thought that these conflicting findings may be because of distinct genetic backgrounds in populations or generally no influence of *KL* methylation on *KL* expression and biochemical parameters in hypertensive patients. Nevertheless, a statistically significant difference was detected between the hypertensive subjects with methylated KL and control individuals with unmethylated KL for K level ($p= 0,0014$). This difference was detected to be statistically dependent on hypertension ($p=0,0017$) and methylation status ($p = 0.019$).

The circadian clock or biological clock is a highly conserved system in most organisms and regulates several physiological processes, including metabolism and immune response by forming endogenous circadian rhythms with a 24 hour-period (Loudon, 2012; Richards & Gumz, 2013). There are two types of circadian clocks, central and peripheral clocks (Albrecht & Eichele, 2003). The central circadian clock synchronizes peripheral clocks according to environmental cues (Richards et al 2014). Synchronization of the circadian clocks contributes to an adaptation of organisms to daily temperature, light, and humidity etc. alteration (Woelfle et al., 2004). BP is under the control of the circadian clocks. Therefore, BP level exhibits diurnal alteration with an increase during daytime and a decrease at nighttime. The circadian rhythm of BP has been revealed to be disrupted with age (Woon et al., 2007). It was suggested that NO administration could ameliorate the age-mediated decrease in circadian oscillation in BP (Richards et al., 2014). Circadian clock proteins are composed of CLCOK, BMAL1, Pers, and Crys. The CLOCK and BMAL1 create a heterodimer complex and induce transcription of circadian target genes such as Per and Cry genes in active phase. The Per and Cry complex blocks the BMAL1/CLOCK activity in the nucleus during the resting phase. The CLOCK/BMAL1-dependent activation of circadian target genes, and Per/Cry-mediated inhibition of CLOCK/BMAL1 activation (negative feedback loop) result in a TTO loop model, and thus circadian rhythms in physiological processes. These circadian clock proteins regulate BP by ensuring the arrangement of diverse processes in the kidney, vasculature, and heart (Richards et al., 2014). It was reported that the circadian clock disruption launches in the pre-hypertensive SHRs (Miyazaki et al., 2011). It has been indicated that oscillation of *BMAL1*, *CLOCK*, *PER2*, and angiotensinogen, renin, *ACE*, *AT1a,* and *AT2* receptors genes were

increased in the heart tissue of SHRs. Particularly, it has been implicated that Ang II is a vigorous inducer of circadian oscillation of *BMAL1* and *PER2* expression in cultured VSMCs (Rudic & Fulton, 2009). In rats, the *BMAL1* gene has been declared to exist in hypertension susceptibility region. It has been suggested that SNPs in the *BMAL1* gene are linked with hypertension susceptibility (Woon et al., 2007). It has been declared that *BMAL1* levels are decreased in female patients with HTN (Fang et al., 2021). Non-dipper hypertensive individuals who exhibit high BP during nighttime carry a high risk of cardiovascular mortality and morbidity (Cuspidi et al., 2004). Especially the *BMAL1* rs3816358 variant has been indicated to have a considerable role in the generation of the non-dipper phenotype in patients with early-onset EH. Consequently, it is proposed that variations in circadian genes could influence diurnal BP chancing in HTN (Leu et al., 2015). Diabetic subjects are known to have a high risk of developing HTN and the existence of HTN causes to more severe progression of diabetic complications. Diabetic subjects have been indicated to have a decrease in nighttime BP dip, consequently leading to non-dipper diabetic patients. However, mechanisms of underlying the increased HTN risk in diabetic patients were unclear (Su et al., 2008). Nevertheless, it is considered that functional disrupted circadian clock genes may be implicated in the occurrence of a non-dipper diabetic state. CRY-deficient mice were indicated to lack BP dip during the rest period, consequently abolishing the circadian rhythm in BP (Rudic & Fulton, 2009). While global *BMAL1* KO male rats had a normal BP circadian rhythm (Kaneko et al., 1968), global *BMAL1* KO male mice had a disrupted BP circadian rhythm (Anea et al., 2012). It has been proposed that the role of *BMAL1* is different among species, and therefore the influence of *BMAL1* on the arragement of BP level and rhythm is complex. It has been implicated that *BMAL1* knockout female rodents did not exhibit BP alteration. On the other hand, premenopausal women have been indicated to have less likely blunted BP rhythm and lower HTN risk than men. Furthermore, non-dipping BP has been declared to be more common in postmenopausal subjects relative to premenopausal subjects. Therefore, it has been proposed that ovarian hormones may override the impact of *BMAL1* on BP levels and rhythms in female rodents and women (Costello & Gumz, 2021). Although several studies revealed the impact of BMAL1 on HTN, in this research, no statistically significant relationship was detected between *BMAL1* gene methylation and HTN ($p > 0.05$). Additionally, it was found that the relationship between the

BMAL1 methylation and levels of TG, FBS, LDL-C, TC, HDL-C, Cr, K, Na and Urea were identified to be not statistically significant ($p>0.05$).

In briefly, the related between methylation of *KL* and *BMAL1* genes and HTN and, their relation with FBS, LDL-C, HDL-C, TC, TG Cr, Na, Urea and K levels were shown to be not statistically significant in hypertensive subjects. However, a statistically significant difference was detected for K between the methylated KL subjects with hypertension and unmethylated KL control individuals ($p= 0,0014$). This difference was detected to be statistically dependent on hypertension ($p=0,0017$) and methylation status ($p = 0.019$). Considering this study, there are several limitations including the age discordance between patients with HTN and control individuals, the small study population, study restriction with the Cyprus population, and the lack of the measurement of serum KL levels and *KL* expression. Therefore, these findings should be replicated in large cohorts. Various factors including gender, age, diabetes, and obesity are considerable risk factors for HTN. Therefore, it is emphasized that patients and control individuals matched in terms of age, gender, and BMI should be involved in further investigations to attenuate the influence of these different factors on the findings (Pathare et al., 2020a). Consequently, these conditions should not be disregarded in further large population investigations.

In terms of DNA methylation, not only cytosine residues at CpG sites on DNA structure undergo methylation, but adenine bases can also be methylated to give rise to N6-methyladenine (6mA). The 6mA is a common in prokaryotes, but with the development of sequencing techniques, eukaryotic organisms have also been indicated to possess this modification (Chaudhary, 2022). The 6mA modification sites were shown to mostly exist in the intergenic and intronic sites of human genome. The N-6 adenine-specific DNA methyltransferase 1 (N6AMT1) and AlkB homolog 1, histone H2A dioxygenase (ALKBH1) provide methylation and demethylation of human 6mA DNA, respectively (Xiao et al., 2018). DNA 6mA modification that is involved in DNA damage repair prevents DNA polymerase-mediated mispairing of 8-oxo guanine (8-oxoG) with adenine (Zhang et al 2021). Recently, the association between 6mA DNA modification and HTN has been indicated. The methylation level of 6mA was indicated to diminish in the leukocytes of subjects with HTN, but its methylation level was found to return to normal after HTN treatment. Decreased 6mA DNA level was reported to be correlated with

increased DBP, SBP, LDL, TG, and TC, whereas it was associated with decreased HDL in hypertensive subjects. Notably, 6mA DNA level decreased due to increased ALKBH1 levels in human aortic smooth muscle cells (HASMCs) exposed to Ang II and ET-1. Consequently, the 6mA level was suggested as a significant biomarker for HTN diagnosis (Guo et al., 2020). These results have revealed the significant role of 6mA level on HNT pathogenesis. Further research is recommended to identify the role of this methylation in HTN occurrence (Chaudhary, 2022).

Table 16 (Continued).

Gene-specific and Genome-wide DNA Methylation in HTN

Gene	DNA methylation	Effects	Subjects	Reference
CORIN	Hypermethylation	Lower probability of prevalent HTN	Human peripheral blood mononuclear cells	Shi et al., 2022
ACEII, SCNN1B, CKG, IFN-γ	Hypermethylation	HTN risk	Meta-analysis	Holmes et al., 2019
TLR2, IFN-γ, ADD1, AGTR1, GCK	Hypomethylation	HTN risk	Meta-analysis	Holmes et al., 2019
AT1aR	Hypomethylation	HTN risk	Age-matched SHRs and WKY rats	Pei et al., 2015
AT1a	Hypomethylation	HTN risk	Offsprings of pregnant rats with nicotine exposure	Xiao et al., 2014
AT1b	Hypomethylation	HTN risk	Offsprings of pregnant rats with low protein diet	Bogdarina et al., 2007

AT1b	Hypomethylation	HTN risk	Virgin female Wistar rats	Bogdarina et al., 2010
ACE	Hypomethylation	HTN risk	MLPD in fetal rats	Goyal et al., 2010
ACE	Hypomethylation	HTN risk	Low-birth weight children	Rangel et al., 2014
ECE-1c	Hypermethylation	Regulator for HTN	Vascular endothelial cells	Funke-Kaiser et al., 2003
ADD1	Hypomethylation	HTN risk	Patients with EH	Zhang et al., 2013
11bHSD2	Hypermethylation	HTN risk	Patients with EH	Friso et al., 2008
NKCC1	Hypomethylation	HTN risk	SHRs	Lee et al., 2010; Cho et al., 2011
NET	Hypermethylation	HTN risk	Patients with EH	Han et al., 2016
GCK	Hypomethylation (Gene-body methylation status)	HTN risk	Patients with EH	Fan et al., 2015
SULF1 GWS)	Hypermethylation (Non-CpG island)	HTN risk	Patients with HTN	Wang et al., 2013
ABCG4 (GWS)	Hypomethylation	HTN risk	Patients with HTN	Guo et al., 2011

CHAPTER VI

Conclusion and Recommendations

Many investigations have indicated the impact of *KL* and *BMAL1* variations and their changed gene expressions on HTN. Considering these findings, in this study, we tried to reveal the significance of *KL* and *BMAL1* methylations in HTN and their association with different biochemical parameters. Although we could not identify an important difference in *KL* and *BMAL1* methylations among hypertensive subjects and control subjects, this study will provide an opportunity for the analysis of other epigenetic modifications for *BMAL1* and *KL* genes. Moreover, except for BMAL1, as other circadian clock genes may contribute to the HTN, epigenetic analysis of them may ensure further knowledge for the identification of the mechanisms participating in the HTN progress. Since HTN is a multifactorial disease, genome-wide methylation studies should be considered in future studies to identify specific DNA methylation mechanisms playing a considerable role in the HTN. Additionally, the biological age of some individuals may differ from their chronological age. Notably, subjects with higher biological age have been suggested to be more likely to develop HTN in later years. Consequently, DNA methylation-based biological age metrics should be considered in future studies in terms of early HTN intervention, and the development of personalized prevention strategies for HTN.

REFERENCES

Aiello, S., & Noris, M. (2010). Klotho in acute kidney injury: biomarker, therapy, or a bit of both?. *Kidney international*, *78*(12), 1208–1210. https://doi.org/10.1038/ki.2010.367

Albrecht, U., & Eichele, G. (2003). The mammalian circadian clock. *Current opinion in genetics & development*, *13*(3), 271–277. https://doi.org/10.1016/s0959-437x(03)00055-8

Alfonso Perez, G., & Delgado Martinez, V. (2023). Epigenetic Signatures in Hypertension. *Journal of personalized medicine*, *13*(5), 787. https://doi.org/10.3390/jpm13050787

Anea, C. B., Cheng, B., Sharma, S., Kumar, S., Caldwell, R. W., Yao, L., Ali, M. I., Merloiu, A. M., Stepp, D. W., Black, S. M., Fulton, D. J., & Rudic, R. D. (2012). Increased superoxide and endothelial NO synthase uncoupling in blood vessels of Bmal1-knockout mice. *Circulation research*, *111*(9), 1157–1165. https://doi.org/10.1161/CIRCRESAHA.111.261750

Arking, D. E., Krebsova, A., Macek, M., Sr, Macek, M., Jr, Arking, A., Mian, I. S., Fried, L., Hamosh, A., Dey, S., McIntosh, I., & Dietz, H. C. (2002). Association of human aging with a functional variant of klotho. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(2), 856–861. https://doi.org/10.1073/pnas.022484299

Arking, D. E., Becker, D. M., Yanek, L. R., Fallin, D., Judge, D. P., Moy, T. F., Becker, L. C., & Dietz, H. C. (2003). KLOTHO allele status and the risk of early-onset occult coronary artery disease. *American journal of human genetics*, *72*(5), 1154–1161. https://doi.org/10.1086/375035

Arking, D. E., Atzmon, G., Arking, A., Barzilai, N., & Dietz, H. C. (2005). Association between a functional variant of the KLOTHO gene and high-density lipoprotein cholesterol, blood pressure, stroke, and longevity. *Circulation research*, *96*(4), 412–418. https://doi.org/10.1161/01.RES.0000157171.04054.30

Aydoğdu, S., Güler, K., Bayram, F., Altun, B., Derici, Ü., Abacı, A., Tükek, T., Sabuncu, T., Arıcı, M., Erdem, Y., Özin, B., Sahin, İ., Ertürk, Ş., Bitigen, A., & Tokgözoğlu, L. (2019). Türk Hipertansiyon Uzlaşı Raporu 2019 [2019 Turkish Hypertension Consensus Report]. *Turk Kardiyoloji Dernegi arsivi :*

Turk Kardiyoloji Derneginin yayin organidir, *47*(6), 535–546. https://doi.org/10.5543/tkda.2019.62565

Azuma, M., Koyama, D., Kikuchi, J., Yoshizawa, H., Thasinas, D., Shiizaki, K., Kuro-o, M., Furukawa, Y., & Kusano, E. (2012). Promoter methylation confers kidney-specific expression of the Klotho gene. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, *26*(10), 4264–4274. https://doi.org/10.1096/fj.12-211631

Babalık, E. (2005). Hipertansiyon Patofizyolojisi. *Kilinik Gelişim Dergisi,* 18(2), 25-32.

Beevers, G., Lip, G. Y., & O'Brien, E. (2001). ABC of hypertension: The pathophysiology of hypertension. *BMJ (Clinical research ed.)*, *322*(7291), 912–916. https://doi.org/10.1136/bmj.322.7291.912

Benjamin, E. J., Larson, M. G., Keyes, M. J., Mitchell, G. F., Vasan, R. S., Keaney, J. F., Jr, Lehman, B. T., Fan, S., Osypiuk, E., & Vita, J. A. (2004). Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham Heart Study. *Circulation*, *109*(5), 613–619. https://doi.org/10.1161/01.CIR.0000112565.60887.1E

Bogdarina, I., Haase, A., Langley-Evans, S., & Clark, A. J. (2010). Glucocorticoid effects on the programming of AT1b angiotensin receptor gene methylation a and expression in the rat. *PloS one*, *5*(2), e9237. https://doi.org/10.1371/journal.pone.0009237

Bogdarina, I., Welham, S., King, P. J., Burns, S. P., & Clark, A. J. (2007). Epigenetic modification of the renin-angiotensin system in the fetal programming of hypertension. *Circulation research*, *100*(4), 520–526. https://doi.org/10.1161/01.RES.0000258855.60637.58

Bönisch, H., & Brüss, M. (1994). The noradrenaline transporter of the neuronal plasma membrane. *Annals of the New York Academy of Sciences*, *733*, 193–202. https://doi.org/10.1111/j.1749-6632.1994.tb17269.x

Brenner, B. M., & Chertow, G. M. (1993). Congenital oligonephropathy: an inborn cause of adult hypertension and progressive renal injury?. *Current opinion in nephrology and hypertension*, *2*(5), 691–695.

Cabrera, C. P., Ng, F. L., Nicholls, H. L., Gupta, A., Barnes, M. R., Munroe, P. B., & Caulfield, M. J. (2019). Over 1000 genetic loci influencing blood pressure with multiple systems and tissues implicated. *Human molecular genetics*, *28*(R2), R151–R161. https://doi.org/10.1093/hmg/ddz197

Carretero, O. A., & Oparil, S. (2000). Essential hypertension. Part I: definition and etiology. *Circulation*, *101*(3), 329–335. https://doi.org/10.1161/01.cir.101.3.329

Chang, J. R., Sun, N., Nan, Y., Yu, W., & Qi, Y. F. (2015). *Sheng li ke xue jin zhan [Progress in physiology]*, *46*(4), 245–249.

Chang, L., Xiong, W., Zhao, X., Fan, Y., Guo, Y., Garcia-Barrio, M., Zhang, J., Jiang, Z., Lin, J. D., & Chen, Y. E. (2018). Bmal1 in Perivascular Adipose Tissue Regulates Resting-Phase Blood Pressure Through Transcriptional Regulation of Angiotensinogen. *Circulation*, *138*(1), 67–79. https://doi.org/10.1161/CIRCULATIONAHA.117.029972

Chaudhary M. (2022). Novel methylation mark and essential hypertension. *Journal, genetic engineering & biotechnology*, *20*(1), 11. https://doi.org/10.1186/s43141-022-00301-y

Cheng, X., Zhou, Q., Lin, S., & Wu, R. (2010). Fosinopril and valsartan intervention in gene expression of Klotho, MMP-9, TIMP-1, and PAI-1 in the kidney of spontaneously hypertensive rats. *Zhong nan da xue xue bao. Yi xue ban = Journal of Central South University. Medical sciences*, *35*(10), 1048–1056. https://doi.org/10.3969/j.issn.1672-7347.2010.10.004

Cho, H. M., Lee, H. A., Kim, H. Y., Han, H. S., & Kim, I. K. (2011). Expression of Na+-K+ -2Cl- cotransporter 1 is epigenetically regulated during postnatal development of hypertension. *American journal of hypertension*, *24*(12), 1286–1293. https://doi.org/10.1038/ajh.2011.13

Christakos, S., Dhawan, P., Verstuyf, A., Verlinden, L., & Carmeliet, G. (2016). Vitamin D: Metabolism, Molecular Mechanism of Action, and Pleiotropic Effects. *Physiological reviews*, *96*(1), 365–408. https://doi.org/10.1152/physrev.00014.2015

Citterio, L., Delli Carpini, S., Lupoli, S., Brioni, E., Simonini, M., Fontana, S., Zagato, L., Messaggio, E., Barlassina, C., Cusi, D., Manunta, P., & Lanzani, C. (2020). Klotho Gene in Human Salt-Sensitive Hypertension. *Clinical journal of the American Society of Nephrology : CJASN*, *15*(3), 375–383.

https://doi.org/10.2215/CJN.08620719

Connelly, P. J., Currie, G., & Delles, C. (2022). Sex Differences in the Prevalence, Outcomes and Management of Hypertension. *Current hypertension reports*, *24*(6), 185–192. https://doi.org/10.1007/s11906-022-01183-8

Costello, H. M., Crislip, G. R., Cheng, K. Y., Lynch, I. J., Juffre, A., Bratanatawira, P., Mckee, A., Thelwell, R. S., Mendez, V. M., Wingo, C. S., Douma, L. G., & Gumz, M. L. (2023). Adrenal-Specific KO of the Circadian Clock Protein BMAL1Alters Blood Pressure Rhythm and Timing of Eating Behavior. *Function (Oxford, England)*, *4*(2), zqad001. https://doi.org/10.1093/function/zqad001

Costello, H. M., & Gumz, M. L. (2021). Circadian Rhythm, Clock Genes, and Hypertension: Recent Advances in Hypertension. *Hypertension (Dallas, Tex. : 1979)*, *78*(5), 1185–1196. https://doi.org/10.1161/HYPERTENSIONAHA.121.14519

Crislip, G. R., Douma, L. G., Masten, S. H., Cheng, K. Y., Lynch, I. J., Johnston, J. G., Barral, D., Glasford, K. B., Holzworth, M. R., Verlander, J. W., Wingo, C. S., & Gumz, M. L. (2020). Differences in renal BMAL1 contribution to Na^+ homeostasis and blood pressure control in male and female mice. *American journal of physiology. Renal physiology*, *318*(6), F1463–F1477. https://doi.org/10.1152/ajprenal.00014.2020

Cuspidi, C., Meani, S., Salerno, M., Valerio, C., Fusi, V., Severgnini, B., Lonati, L., Magrini, F., & Zanchetti, A. (2004). Cardiovascular target organ damage in essential hypertensives with or without reproducible nocturnal fall in blood pressure. *Journal of hypertension*, *22*(2), 273–280. https://doi.org/10.1097/00004872-200402000-00010

Delacroix, S., Chokka, R.G., Worthley S. (2014). Hypertension: Pathophysiology and Treatment. *Journal of Neurology & Neurophysiology*.5:6

Donate-Correa, J., Martín-Núñez, E., Martínez-Sanz, R., Muros-de-Fuentes, M., Mora-Fernández, C., Pérez-Delgado, N., & Navarro-González, J. F. (2016). Influence of Klotho gene polymorphisms on vascular gene expression and its relationship to cardiovascular disease. *Journal of cellular and molecular medicine*, *20*(1), 128–133. https://doi.org/10.1111/jcmm.12710

Donate-Correa, J., Mora-Fernández, C., Martínez-Sanz, R., Muros-de-Fuentes, M., Pérez, H., Meneses-Pérez, B., Cazaña-Pérez, V., & Navarro-González, J. F.

(2013). Expression of FGF23/KLOTHO system in human vascular tissue. *International journal of cardiology*, *165*(1), 179–183. https://doi.org/10.1016/j.ijcard.2011.08.850

Drew, D. A., Katz, R., Kritchevsky, S., Ix, J. H., Shlipak, M. G., Newman, A. B., Hoofnagle, A. N., Fried, L. F., Sarnak, M., Gutiérrez, O. M., Semba, R. D., & Neyra, J. A. (2021). Soluble Klotho and Incident Hypertension. *Clinical journal of the American Society of Nephrology : CJASN*, *16*(10), 1502–1511. https://doi.org/10.2215/CJN.05020421

Earl, J., Garcia-Nieto, S., Martinez-Avila, J. C., Montans, J., Sanjuanbenito, A., Rodríguez-Garrote, M., Lisa, E., Mendía, E., Lobo, E., Malats, N., Carrato, A., & Guillen-Ponce, C. (2015). Circulating tumor cells (Ctc) and kras mutant circulating free Dna (cfdna) detection in peripheral blood as biomarkers in patients diagnosed with exocrine pancreatic cancer. *BMC cancer*, *15*, 797. https://doi.org/10.1186/s12885-015-1779-7

Fan, R., Peng, X., Xie, L., Dong, K., Ma, D., Xu, W., Shi, X., Zhang, S., Chen, J., Yu, X., & Yang, Y. (2022). Importance of Bmal1 in Alzheimer's disease and associated aging-related diseases: Mechanisms and interventions. *Aging cell*, *21*(10), e13704. https://doi.org/10.1111/acel.13704

Fan, R., Wang, W. J., Zhong, Q. L., Duan, S. W., Xu, X. T., Hao, L. M., Zhao, J., & Zhang, L. N. (2015). Aberrant methylation of the GCK gene body is associated with the risk of essential hypertension. *Molecular medicine reports*, *12*(2), 2390–2394. https://doi.org/10.3892/mmr.2015.3631

Fang, Z., Zhu, L., Jin, Y., Chen, Y., Chang, W., & Yao, Y. (2021). Downregulation of Arntl mRNA Expression in Women with Hypertension: A Case-Control Study. *Kidney & blood pressure research*, *46*(6), 741–748. https://doi.org/10.1159/000518669

Fraga, M. F., Ballestar, E., Paz, M. F., Ropero, S., Setien, F., Ballestar, M. L., Heine-Suñer, D., Cigudosa, J. C., Urioste, M., Benitez, J., Boix-Chornet, M., Sanchez- Aguilera, A., Ling, C., Carlsson, E., Poulsen, P., Vaag, A., Stephan, Z., Spector, T. D., Wu, Y. Z., Plass, C., … Esteller, M. (2005). Epigenetic differences arise during the lifetime of monozygotic twins. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(30), 10604–10609. https://doi.org/10.1073/pnas.0500398102

Friso, S., Pizzolo, F., Choi, S. W., Guarini, P., Castagna, A., Ravagnani, V., Carletto,

A., Pattini, P., Corrocher, R., & Olivieri, O. (2008). Epigenetic control of 11 beta-hydroxysteroid dehydrogenase 2 gene promoter is related to human hypertension. *Atherosclerosis*, *199*(2), 323–327. https://doi.org/10.1016/j.atherosclerosis.2007.11.029

Funke-Kaiser, H., Thomas, A., Bremer, J., Kovacevic, S. D., Scheuch, K., Bolbrinker, J., Theis, S., Lemmer, J., Zimmermann, A., Zollmann, F. S., Herrmann, S. M., Paul, M., & Orzechowski, H. D. (2003). Regulation of the major isoform of human endothelin-converting enzyme-1 by a strong housekeeping promoter modulated by polymorphic microsatellites. *Journal of hypertension*, *21*(11), 2111–2124. https://doi.org/10.1097/00004872-200311000-00021

Gao, D., Zuo, Z., Tian, J., Ali, Q., Lin, Y., Lei, H., & Sun, Z. (2016). Activation of SIRT1 Attenuates Klotho Deficiency-Induced Arterial Stiffness and Hypertension by Enhancing AMP-Activated Protein Kinase Activity. *Hypertension (Dallas, Tex. : 1979)*, *68*(5), 1191–1199. https://doi.org/10.1161/HYPERTENSIONAHA.116.07709

Gao, L. L., Ding, X., Xie, D. M., Yang, M., & Dong, B. R. (2015). G-395A polymorphism in the promoter region of the KLOTHO gene and hypertension among elderly (90 years and older) Chinese individuals. *Genetics and molecular research : GMR*, *14*(4), 15444–15452. https://doi.org/10.4238/2015.November.30.22

Gawałko, M., & Linz, D. (2023). Atrial Fibrillation Detection and Management in Hypertension. *Hypertension (Dallas, Tex. : 1979)*, *80*(3), 523–533. https://doi.org/10.1161/HYPERTENSIONAHA.122.19459

Goyal, R., Goyal, D., Leitzke, A., Gheorghe, C. P., & Longo, L. D. (2010). Brain renin-angiotensin system: fetal epigenetic programming by maternal protein restriction during pregnancy. *Reproductive sciences (Thousand Oaks, Calif.)*, *17*(3), 227–238. https://doi.org/10.1177/1933719109351935

Gong, M., & Hunber, N. (2006). Molecular genetics of human hypertenson. Clinical science (London, England:1979). 110(3), 315-326. http://doi.org/10.1042/CS20050208

González, J., Valls, N., Brito, R., & Rodrigo, R. (2014). Essential hypertension and oxidative stress: New insights. *World journal of cardiology*, *6*(6), 353–366. https://doi.org/10.4330/wjc.v6.i6.353

Guo, Y., Pei, Y., Li, K., Cui, W., & Zhang, D. (2020). DNA N^6-methyladenine modification in hypertension. *Aging*, *12*(7), 6276–6291. https://doi.org/10.18632/aging.103023

Guyton, A. C., Coleman, T. G., Cowley, A. V., Jr, Scheel, K. W., Manning, R. D., Jr, & Norman, R. A., Jr (1972). Arterial pressure regulation. Overriding dominance of the kidneys in long-term regulation and in hypertension. *The American journal of medicine*, *52*(5), 584–594. https://doi.org/10.1016/0002-9343(72)90050-2

Haffner, S. M., Ferrannini, E., Hazuda, H. P., & Stern, M. P. (1992). Clustering of cardiovascular risk factors in confirmed prehypertensive individuals. *Hypertension (Dallas, Tex. : 1979)*, *20*(1), 38–45. https://doi.org/10.1161/01.hyp.20.1.38

Han, L., Liu, P., Wang, C., Zhong, Q., Fan, R., Wang, L., Duan, S., & Zhang, L. (2015). The interactions between alcohol consumption and DNA methylation of the ADD1 gene promoter modulate essential hypertension susceptibility in a population- based, case-control study. *Hypertension research : official journal of the Japanese Society of Hypertension*, *38*(4), 284–290. https://doi.org/10.1038/hr.2014.172

Han, L., Liu, Y., Duan, S., Perry, B., Li, W., & He, Y. (2016). DNA methylation and hypertension: emerging evidence and challenges. *Briefings in functional genomics*, *15*(6), 460–469. https://doi.org/10.1093/bfgp/elw014

Hao, Q., Wang, Y., Ding, X., Dong, B., Yang, M., Dong, B., & Wei, Y. (2018). G-395A polymorphism in the promoter region of the KLOTHO gene associates with frailty among the oldest-old. *Scientific reports*, *8*(1), 6735. https://doi.org/10.1038/s41598-018-25040-4

Harrison, D. G., Coffman, T. M., & Wilcox, C. S. (2021). Pathophysiology of Hypertension: The Mosaic Theory and Beyond. *Circulation research,* 128(7), 847-863. https://doi.org/10.1161/CIRCRESAHA. 121.318082.

Hiltunen, M. O., Turunen, M. P., Häkkinen, T. P., Rutanen, J., Hedman, M., Mäkinen, K., Turunen, A. M., Aalto-Setälä, K., & Ylä-Herttuala, S. (2002). DNA hypomethylation and methyltransferase expression in atherosclerotic lesions. *Vascular medicine (London, England)*, *7*(1), 5–11. https://doi.org/10.1191/1358863x02vm418oa

Holmes, L., Jr, Lim, A., Comeaux, C. R., Dabney, K. W., & Okundaye, O. (2019). DNA Methylation of Candidate Genes (ACE II, IFN-γ, AGTR 1, CKG, ADD1, SCNN1B and TLR2) in Essential Hypertension: A Systematic Review and Quantitative Evidence Synthesis. *International journal of environmental research and public health*, *16*(23), 4829. https://doi.org/10.3390/ijerph16234829

Hu, M. C., Kuro-o, M., & Moe, O. W. (2013a). Klotho and chronic kidney disease. *Contributions to nephrology*, *180*, 47–63. https://doi.org/10.1159/000346778

Hu, M. C., Shiizaki, K., Kuro-o, M., & Moe, O. W. (2013b). Fibroblast growth factor 23 and Klotho: physiology and pathophysiology of an endocrine network of mineral metabolism. *Annual review of physiology*, *75*, 503–533. https://doi.org/10.1146/annurev-physiol-030212-183727

Huang, J. B., Liang, J., Zhao, X. F., Wu, W. S., & Zhang, F. (2013). Epigenetics: novel mechanism of pulmonary hypertension. *Lung*, *191*(6), 601–610. https://doi.org/10.1007/s00408-013-9505-1

Hughes, C. A., & Bennett, V. (1995). Adducin: a physical model with implications for function in assembly of spectrin-actin complexes. *The Journal of biological chemistry*, *270*(32), 18990–18996. https://doi.org/10.1074/jbc.270.32.18990

Huo, M., Cao, X., Zhang, H., Lau, C. W., Hong, H., Chen, F. M., Huang, Y., Chawla, A., & Tian, X. Y. (2022). Loss of myeloid Bmal1 exacerbates hypertensive vascular remodelling through interaction with STAT6 in mice. *Cardiovascular research*, *118*(13), 2859–2874. https://doi.org/10.1093/cvr/cvab336

Ito, S., Fujimori, T., Hayashizaki, Y., & Nabeshima, Y. (2002). Identification of a Novel mouse membrane-bound family 1 glycosidase-like protein, which carries an atypical active site structure. *Biochimica et biophysica acta*, *1576*(3), 341–345. https://doi.org/10.1016/s0167-4781(02)00281-6

Izbeki, F., Asuzu, D. T., Lorincz, A., Bardsley, M. R., Popko, L. N., Choi, K. M., Young, D. L., Hayashi, Y., Linden, D. R., Kuro-o, M., Farrugia, G., & Ordog, T. (2010). Loss of Kitlow progenitors, reduced stem cell factor and high oxidative stress underlie gastric dysfunction in progeric mice. *The Journal of*

physiology, *588*(Pt 16), 3101–3117. https://doi.org/10.1113/jphysiol.2010.191023

Jamal, T., Liang, W. Y., Li, L. H. (2019). Klotho, Hypertension and Arterial Stiffness: A Review. Austin J Nephrol Hypertens, 6(2): 1082.

Jiang, Q., Yuan, H., & Huang, Z. (2009). DNA methylation might be the epigenetic Molecular mechanism of different antihypertensive efficacy of metoprolol in beta1-AR gene-directed therapy. *Medical hypotheses*, *72*(3), 364–365. https://doi.org/10.1016/j.mehy.2008.10.013

Jones, P. A., & Takai, D. (2001). The role of DNA methylation in mammalian epigenetics. *Science (New York, N.Y.)*, *293*(5532), 1068–1070. https://doi.org/10.1126/science.1063852

Juraschek, S. P., Woodward, M., Sacks, F. M., Carey, V. J., Miller, E. R., 3rd, & Appel, L. J. (2017). Time Course of Change in Blood Pressure From Sodium Reduction and the DASH Diet. *Hypertension (Dallas, Tex. : 1979)*, *70*(5), 923–929. https://doi.org/10.1161/HYPERTENSIONAHA.117.10017

Kale, A., Sankrityayan, H., Anders, H. J., & Gaikwad, A. B. (2023). Klotho in kidney diseases: a crosstalk between the renin-angiotensin system and endoplasmic reticulum stress. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association*, *38*(4), 819–825. https://doi.org/10.1093/ndt/gfab340

Kanbay, M., Demiray, A., Afsar, B., Covic, A., Tapoi, L., Ureche, C., & Ortiz, A. (2021). Role of Klotho in the Development of Essential Hypertension. *Hypertension (Dallas, Tex. : 1979)*, *77*(3), 740–750. https://doi.org/10.1161/HYPERTENSIONAHA.120.16635

Kaneko, M., Zechman, F. W., & Smith, R. E. (1968). Circadian variation in human peripheral blood flow levels and exercise responses. *Journal of applied physiology*, *25*(2), 109–114. https://doi.org/10.1152/jappl.1968.25.2.109

Kato, N., Loh, M., Takeuchi, F., Verweij, N., Wang, X., Zhang, W., Kelly, T. N., Saleheen, D., Lehne, B., Leach, I. M., Drong, A. W., Abbott, J., Wahl, S., Tan, S. T., Scott, W. R., Campanella, G., Chadeau-Hyam, M., Afzal, U., Ahluwalia, T. S., Bonder, M. J., … Chambers, J. C. (2015). Trans-ancestry genome-wide association study identifies 12 genetic loci influencing blood pressure and implicates a role for DNA methylation. *Nature genetics*, *47*(11), 1282–1293. https://doi.org/10.1038/ng.3405

Kjeldsen S. E. (2018). Hypertension and cardiovascular risk: General aspects. *Pharmacological research*, *129*, 95–99. https://doi.org/10.1016/j.phrs.2017.11.003

King, G. D., Rosene, D. L., & Abraham, C. R. (2012). Promoter methylation and Age-related downregulation of Klotho in rhesus monkey. *Age (Dordrecht, Netherlands)*, *34*(6), 1405–1419. https://doi.org/10.1007/s11357-011-9315-4

Koh, N., Fujimori, T., Nishiguchi, S., Tamori, A., Shiomi, S., Nakatani, T., Sugimura, K., Kishimoto, T., Kinoshita, S., Kuroki, T., & Nabeshima , Y. (2001). Severely reduced production of klotho in human chronic renal failure kidney. *Biochemical and biophysical research communications*, *280*(4),1015–1020. https://doi.org/10.1006/bbrc.2000.4226

Kolomeĭchuk, S. N., Makeeva, I. V., Topchieva, L. V., Korneva, V. A., & Nemova, N. N. (2011). Association of T3111C poly- morphism in 3'-untranslated region of the CLOCK gene with the risk of essential arterial hypertension and coronary artery disease in the Russian population Karelia. *Genetika*, *47*(10), 1411–1415.

Kresovich, J. K., Sandler, D. P., & Taylor, J. A. (2023). Methylation-Based Biological Age and Hypertension Prevalence and Incidence. *Hypertension (Dallas, Tex. : 1979)*, *80*(6), 1213–1222. https://doi.org/10.1161/HYPERTENSIONAHA.122.20796

Kulkarni, A., Chavan-Gautam, P., Mehendale, S., Yadav, H., & Joshi, S. (2011). Global DNA methylation patterns in placenta and its association with maternal hypertension in pre-eclampsia. *DNA and cell biology*, *30*(2), 79–84. https://doi.org/10.1089/dna.2010.1084

Kurbatova, I. V., Topchieva, L. V., Korneva, V. A., Kolomeichuk, S. N., & Nemova, N. N. (2014). Expression of circadian rhythm genes CLOCK, BMAL1, and PER1 in buccal epithelial cells of patients with essential arterial hypertension in dependence on polymorphic variants of CLOCK and BMAL1 genes. *Bulletin of experimental biology and medicine*, *157*(3), 360–363. https://doi.org/10.1007/s10517-014-2566-1

Kuro-o, M., Matsumura, Y., Aizawa, H., Kawaguchi, H., Suga, T., Utsugi, T., Ohyama, Y., Kurabayashi, M., Kaname, T., Kume, E., Iwasaki, H., Iida, A., Shiraki-Iida, T., Nishikawa, S., Nagai, R., & Nabeshima, Y. I. (1997).

Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature*, *390*(6655), 45–51. https://doi.org/10.1038/36285

Lanzani, C., Citterio, L., & Vezzoli, G. (2020). Klotho: a link between Cardiovascular and non-cardiovascular mortality. *Clinical kidney journal*, *13*(6), 926–932. https://doi.org/10.1093/ckj/sfaa100

Laragh J. H. (1992). Lewis K. Dahl Memorial Lecture. The renin system and four Lines fo hypertension research. Nephron heterogeneity, the calcium connection, the prorenin vasodilator limb, and plasma renin and heart attack. *Hypertension (Dallas, Tex. : 1979)*, *20*(3), 267–279. https://doi.org/10.1161/01.hyp.20.3.267

Lee, H. A., Baek, I., Seok, Y. M., Yang, E., Cho, H. M., Lee, D. Y., Hong, S. H., & Kim, I. K. (2010). Promoter hypomethylation upregulates Na+-K+-2Cl-cotransporter 1 in spontaneously hypertensive rats. *Biochemical and biophysical research communications*, *396*(2), 252–257. https://doi.org/10.1016/j.bbrc.2010.04.074

Leppänen, A., Stowell, S., Blixt, O., & Cummings, R. D. (2005). Dimeric galectin-1 Binds with high affinity to alpha2,3-sialylated and non-sialylated terminal N-acetyllactosamine units on surface-bound extended glycans. *The Journal of biological chemistry*, *280*(7), 5549–5562. https://doi.org/10.1074/jbc.M412019200

Li, G. Y., Wang, H., & Chen, H. (2020). Association of insulin resistance with Polymorphic variants of Clock and Bmal1 genes: A case-control study. *Clinical and experimental hypertension (New York, N.Y. : 1993)*, *42*(4), 371–375. https://doi.org/10.1080/10641963.2019.1676769

Li, H., Martin, A., David, V., & Quarles, L. D. (2011). Compound deletion of Fgfr3 and Fgfr4 partially rescues the Hyp mouse phenotype. *American journal of physiology. Endocrinology and metabolism*, *300*(3), E508–E517. https://doi.org/10.1152/ajpendo.00499.2010

Liang, W. Y., Wang, L. H., Wei, J. H., Li, Q. L., Li, Q. Y., Liang, Q., Hu, N. Q., & Li, L. H. (2021). No significant association of serum klotho concentration with blood pressure and pulse wave velocity in a Chinese population. *Scientific reports*, *11*(1), 2374. https://doi.org/10.1038/s41598-021-82258-5

Lifton, R. P., Gharavi, A. G., & Geller, D. S. (2001). Molecular mechanisms of Human hypertension. *Cell*, *104*(4), 545–556. https://doi.org/10.1016/s0092-8674(01)00241-0

Loudon A. S. (2012). Circadian biology: a 2.5 billion year old clock. *Current biology : CB*, *22*(14), R570–R571. https://doi.org/10.1016/j.cub.2012.06.023

Mancia, G., & Grassi, G. (2014). The autonomic nervous system and hypertension. *Circulation research*, *114*(11), 1804–1814. https://doi.org/10.1161/CIRCRESAHA.114.302524

Masuda, H., Chikuda, H., Suga, T., Kawaguchi, H., & Kuro-o, M. (2005). Regulation of multiple ageing-like phenotypes by inducible klotho gene expression in klotho mutant mice. *Mechanisms of ageing and development*, *126*(12), 1274–1283. https://doi.org/10.1016/j.mad.2005.07.007

Matsuoka, Y., Li, X., & Bennett, V. (2000). Adducin: structure, function and regulation. *Cellular and molecular life sciences : CMLS*, *57*(6), 884–895. https://doi.org/10.1007/PL00000731

Miller, A. J., & Arnold, A. C. (2019). The renin-angiotensin system in cardiovascular autonomic control: recent developments and clinical implications. *Clinical autonomic research : official journal of the Clinical Autonomic Research Society*, *29*(2), 231–243. https://doi.org/10.1007/s10286-018-0572-5

Mills, K. T., Bundy, J. D., Kelly, T. N., Reed, J. E., Kearney, P. M., Reynolds, K., et al. (2016). Global Disparities of Hypertension Prevalence and Control: A Systematic Analysis of Population-Based Studies From 90 Countries. *Circulation*, *134*(6), 441–450. doi.org/10.1161/CIRCULATIONAHA.115.018912

Miyazaki, M., Schroder, E., Edelmann, S. E., Hughes, M. E., Kornacker, K., Balke, C. W., & Esser, K. A. (2011). Age-associated disruption of molecular clock expression in skeletal muscle of the spontaneously hypertensive rat. *PloS one*, *6*(11), e27168. https://doi.org/10.1371/journal.pone.0027168

Morgan, L., Broughton Pipkin, F., & Kalsheker, N. (1996). Angiotensinogen: molecular biology, biochemistry and physiology. *The international journal of biochemistry & cell biology*, *28*(11), 1211–1222. https://doi.org/10.1016/s1357-2725(96)00086-6

Nofer, J. R., Kehrel, B., Fobker, M., Levkau, B., Assmann, G., & von Eckardstein, A. (2002). HDL and arteriosclerosis: beyond reverse cholesterol transport. *Atherosclerosis*, *161*(1), 1–16. https://doi.org/10.1016/s0021-9150(01)00651-7

Nzietchueng, R., El Shamieh, S., Benachour, H., Labat, C., Herbeth, B., Ndiaye, N. C., Masson, C., Visvikis-Siest, S., & Benetos, A. (2011). Klotho KL-VS genotype is involved in blood pressure regulation. *Clinica chimica acta; international journal of clinical chemistry*, *412*(19-20), 1773–1777. https://doi.org/10.1016/j.cca.2011.05.032

Olauson, H., Mencke, R., Hillebrands, J. L., & Larsson, T. E. (2017). Tissue expression and source of circulating αKlotho. *Bone*, *100*, 19–35. https://doi.org/10.1016/j.bone.2017.03.043

Omura, M., Saito, J., Yamaguchi, K., Kakuta, Y., & Nishikawa, T. (2004). Prospective study on the prevalence of secondary hypertension among hypertensive patients visiting a general outpatient clinic in Japan. *Hypertension research : official journal of the Japanese Society of Hypertension*, *27*(3), 193–202. https://doi.org/10.1291/hypres.27.193

Ong, K. L., Tso, A. W., Lam, K. S., & Cheung, B. M. (2008). Gender difference in blood pressure control and cardiovascular risk factors in Americans with diagnosed hypertension. *Hypertension (Dallas, Tex. : 1979)*, *51*(4), 1142–1148. https://doi.org/10.1161/HYPERTENSIONAHA.107.105205

Onusko E. (2003). Diagnosing secondary hypertension. *American family physician*, *67*(1), 67–74.

Paroni, G., Seripa, D., Panza, F., Addante, F., Copetti, M., D'Onofrio, G., Pellegrini, F., Fontana, L., & Pilotto, A. (2012). Klotho locus, metabolic traits, and serum hemoglobin in hospitalized older patients: a genetic association analysis. *Age (Dordrecht, Netherlands)*, *34*(4), 949–968. https://doi.org/10.1007/s11357-011-9273-x

Pathare, G., Raju, S., Mashru, M., Shah, V., & Shalia, K. (2020a). Gene expression of klotho & antioxidative enzymes in peripheral blood mononuclear cells of essential hypertension patients in Indian population. *The Indian journal of medical research*, *152*(6), 607–613. https://doi.org/10.4103/ijmr.IJMR_2112_18

Pathare, G., Raju, S., Mashru, M., Shah, V., & Shalia, K. (2020b). α-Klotho and catalase expression in essential hypertension. *Integrative Molecular Medicine*,7, 2056-6360

Pei, F., Wang, X., Yue, R., Chen, C., Huang, J., Huang, J., Li, X., & Zeng, C. (2015). Differential expression and DNA methylation of angiotensin type 1A receptors in vascular tissues during genetic hypertension development. *Molecular and cellular biochemistry*, *402*(1-2), 1–8. https://doi.org/10.1007/s11010-014-2295-9

Pratamawati, T. M., Alwi, I., & Asmarinah (2023). Summary of Known Genetic and Epigenetic Modification Contributed to Hypertension. *International journal of hypertension*, *2023*, 5872362. https://doi.org/10.1155/2023/5872362

Qiagen. (2009). 'EpiTect ® HRM PCR Handbook', (May 2009), pp. 1–26

Qiagen. (2014). 'Sample & Assay Technologies EpiTect ® Bisulfite Handbook', (December). Available at: www.qiagen.com

Quintela, T., Sousa, C., Patriarca, F. M., Gonçalves, I., & Santos, C. R. (2015). Gender associated circadian oscillations of the clock genes in rat choroid plexus. *Brain structure & function*, *220*(3), 1251–1262. https://doi.org/10.1007/s00429-014-0720-1

Raina, R., Krishnappa, V., Das, A., Amin, H., Radhakrishnan, Y., Nair, N. R., & Kusumi, K. (2019). Overview of Monogenic or Mendelian Forms of Hypertension. *Frontiers in pediatrics*, *7*, 263. doi.org/10.3389/fped.2019.00263

Rangel, M., dos Santos, J. C., Ortiz, P. H., Hirata, M., Jasiulionis, M. G., Araujo, R. C., Ierardi, D. F., & Franco, M.doC. (2014). Modification of epigenetic patterns in low birth weight children: importance of hypomethylation of the ACE gene promoter. *PloS one*, *9*(8), e106138. https://doi.org/10.1371/journal.pone.0106138

Rhee, E. J., Oh, K. W., Lee, W. Y., Kim, S. Y., Jung, C. H., Kim, B. J., Sung, K. C., Kim, B. S., Kang, J. H., Lee, M. H., Kim, S. W., & Park, J. R. (2006). The Differential effects of age on the association of KLOTHO gene polymorphisms with coronary artery disease. *Metabolism: clinical and experimental*, *55*(10), 1344–1351. https://doi.org/10.1016/j.metabol.2006.05.020

Richards, J., & Gumz, M. L. (2013). Mechanism of the circadian clock in physiology. *American journal of physiology. Regulatory, integrative and comparative physiology*, *304*(12), R1053–R1064. https://doi.org/10.1152/ajpregu.00066.2013

Richards, J., Diaz, A. N., & Gumz, M. L. (2014). Clock genes in hypertension: novel insights from rodent models. *Blood pressure monitoring*, *19*(5), 249–254. https://doi.org/10.1097/MBP.0000000000000060

Sabbatini, A. R., & Kararigas, G. (2020). Estrogen-related mechanisms in sex differences of hypertension and target organ damage. *Biology of sex differences*, *11*(1), 31. https://doi.org/10.1186/s13293-020-00306-7

Saco, T. V., Parthasarathy, P. T., Cho, Y., Lockey, R. F., & Kolliputi, N. (2014). Role of epigenetics in pulmonary hypertension. *American journal of physiology. Cell physiology*, *306*(12), C1101–C1105. https://doi.org/10.1152/ajpcell.00314.2013

Sander, M., Chavoshan, B., & Victor, R. G. (1999). A large blood pressure-raising effect of nitric oxide synthase inhibition in humans. *Hypertension (Dallas, Tex. : 1979)*, *33*(4), 937–942. https://doi.org/10.1161/01.hyp.33.4.937

Semba, R. D., Cappola, A. R., Sun, K., Bandinelli, S., Dalal, M., Crasto, C., Guralnik, J. M., & Ferrucci, L. (2011). Plasma klotho and cardiovascular disease in adults. *Journal of the American Geriatrics Society*, *59*(9), 1596–1601. https://doi.org/10.1111/j.1532-5415.2011.03558.x

Shi, J., Wu, L., Chen, Y., Zhang, M., Yu, J., Ren, L., He, Y., Li, J., Ma, S., Hu, W., & Peng, H. (2022). Association between CORIN methylation and hypertension in Chinese adults. *Postgraduate medical journal*, pmj-2022-141802. Advance online publication. https://doi.org/10.1136/pmj-2022-141802

Shih, P. A., & O'Connor, D. T. (2008). Hereditary determinants of human hypertension: strategies in the setting of genetic complexity. *Hypertension (Dallas, Tex. : 1979)*, *51*(6), 1456–1464. https://doi.org/10.1161/HYPERTENSIONAHA.107.090480

Shimoyama, Y., Nishio, K., Hamajima, N., & Niwa, T. (2009). KLOTHO gene polymorphisms G-395A and C1818T are associated with lipid and glucose

metabolism, bone mineral density and systolic blood pressure in Japanese healthy subjects. *Clinica chimica acta; international journal of clinical chemistry*, *406*(1-2), 134–138. https://doi.org/10.1016/j.cca.2009.06.011

Shiraki-Iida, T., Iida, A., Nabeshima, Y., Anazawa, H., Nishikawa, S., Noda, M., Kuro-o, M., & Nabeshima, Y. (2000). Improvement of multiple pathophysiological phenotypes of klotho (kl/kl) mice by adenovirus-mediated expression of the klotho gene. *The journal of gene medicine*, *2*(4), 233–242. https://doi.org/10.1002/1521-2254(200007/08)2:4<233::AID-JGM110>3.0.CO;2-5

Singh, M., Singh, A. K., Pandey, P., Chandra S., Singh, K.A., & Gambhir, I.S. (2016). Molecular genetics of essential hypertension. *Clinical and experimental hypertension (New York, N.Y.:1993),* 38(3), 268-277. Doi.org/10.3109/10641963.2015.1116543.

Sopjani, M., Rinnerthaler, M., Kruja, J., & Dermaku-Sopjani, M. (2015). Intracellular signaling of the aging suppressor protein Klotho. *Current molecular medicine*, *15*(1), 27–37. https://doi.org/10.2174/1566524015666150114111258

Su, W., Guo, Z., Randall, D. C., Cassis, L., Brown, D. R., & Gong, M. C. (2008). Hypertension and disrupted blood pressure circadian rhythm in type 2 diabetic db/db mice. *American journal of physiology. Heart and circulatory physiology*, *295*(4), H1634–H1641. https://doi.org/10.1152/ajpheart.00257.2008

Su, X. M., & Yang, W. (2014). Klotho protein lowered in elderly hypertension. *International journal of clinical and experimental medicine*, *7*(8), 2347–2350.

Sugiura, H., Yoshida, T., Shiohira, S., Kohei, J., Mitobe, M., Kurosu, H., Kuro-o, M., Nitta, K., & Tsuchiya, K. (2012). Reduced Klotho expression level in kidney aggravates renal interstitial fibrosis. *American journal of physiology. Renal physiology*, *302*(10), F1252–F1264. https://doi.org/10.1152/ajprenal.00294.2011

Sun, B., Dronma, T., Qin, W. J., Cui, C. Y., Tse, D., Pingtso, T., Liu, Y., & Qiu, C. C. (2004). Polymorphisms of renin-angiotensin system in essential hypertension in Chinese Tibetans. *Biomedical and environmental sciences : BES*, *17*(2), 209–216.

Sun, F., Cui, J., Gavras, H. and Schwartz, F. (2003).A novel class of tests for the detection of mitochondrial DNA-mutation involvement in diseases. Am. J.Hum. Genet. 72, 1515–1526

Sun Z. (2015). Aging, arterial stiffness, and hypertension. *Hypertension (Dallas, Tex. : 1979)*, *65*(2), 252–256. https://doi.org/10.1161/HYPERTENSIONAHA.114.03617

Surdacki, A., Nowicki, M., Sandmann, J., Tsikas, D., Boeger, R. H., Bode-Boeger, S. M., Kruszelnicka-Kwiatkowska, O., Kokot, F., Dubiel, J. S., & Froelich, J. C. (1999). Reduced urinary excretion of nitric oxide metabolites and increased plasmalevels of asymmetric dimethylarginine in men with essential hypertension. *Journal of cardiovascular pharmacology*, *33*(4), 652–658. https://doi.org/10.1097/00005344-199904000-00020

Taddei S. (2009). Blood pressure through aging and menopause. *Climacteric : the journal of the International Menopause Society*, *12 Suppl 1*, 36–40. https://doi.org/10.1080/13697130903004758

Tammen, S. A., Friso, S., & Choi, S. W. (2013). Epigenetics: the link between nature and nurture. *Molecular aspects of medicine*, *34*(4), 753–764. https://doi.org/10.1016/j.mam.2012.07.018

Tang, R., Zhou, Q., Liu, Z., Xiao, Z., & Pouranan, V. (2011). Effects of fosinopril and losartan on renal Klotho expression and oxidative stress in spontaneously hypertensive rats. *Zhong nan da xue xue bao. Yi xue ban = Journal of Central South University. Medical sciences*, *36*(1), 27–33. https://doi.org/10.3969/j.issn.1672-7347.2011.01.005

Turan, K., & Ata, P. (2011). Effects of intra- and extracellular factors on anti-aging klotho gene expression. *Genetics and molecular research : GMR*, *10*(3), 2009–2023. https://doi.org/10.4238/vol10-3gmr1261

Türkiye Endokrinoloji ve Metabolizma Derneği.(2022). Hipertansiyon Tanı ve Tedavi Kılavuzu,

Urakawa, I., Yamazaki, Y., Shimada, T., Iijima, K., Hasegawa, H., Okawa, K., Fujita, T., Fukumoto, S., & Yamashita, T. (2006). Klotho converts canonical FGF receptor into a specific receptor for FGF23. *Nature*, *444*(7120), 770–774. https://doi.org/10.1038/nature05315

Valensi P. (2021). Autonomic nervous system activity changes in patients with hypertension and overweight: role and therapeutic implications. *Cardiovascular diabetology*, *20*(1), 170. https://doi.org/10.1186/s12933-021-01356-w

van der Westhuyzen, D. R., de Beer, F. C., & Webb, N. R. (2007). HDL cholesterol transport during inflammation. *Current opinion in lipidology*, *18*(2), 147–151. https://doi.org/10.1097/MOL.0b013e328051b4fe

Wagner, O. F., Christ, G., Wojta, J., Vierhapper, H., Parzer, S., Nowotny, P. J., Schneider, B., Waldhäusl, W., & Binder, B. R. (1992). Polar secretion of endothelin-1 by cultured endothelial cells. *The Journal of biological chemistry*, *267*(23), 16066–16068.

Wang, H. L., Xu, Q., Wang, Z., Zhang, Y. H., Si, L. Y., Li, X. J., Yang, Q. H., & Xiao, H. (2010). A potential regulatory single nucleotide polymorphism in the promoter of the Klotho gene may be associated with essential hypertension in the Chinese Han population. *Clinica chimica acta; international journal of clinical chemistry*, *411*(5-6), 386–390. https://doi.org/10.1016/j.cca.2009.12.004

Wang, X. M., Wang, H. P., Chen, H. Z., & Liu, D. P. (2023). Epigenetic Clock: Future of Hypertension Prediction?. *Hypertension (Dallas, Tex. : 1979)*, *80*(8), 1569–1571. https://doi.org/10.1161/HYPERTENSIONAHA.123.21197

Wang, Y., & Sun, Z. (2009a). Klotho gene delivery prevents the progression of spontaneous hypertension and renal damage. *Hypertension (Dallas, Tex. : 1979)*, *54*(4), 810–817. https://doi.org/10.1161/HYPERTENSIONAHA.109.134320

Wang, Y., & Sun, Z. (2009b). Current understanding of klotho. *Ageing research reviews*, *8*(1), 43–51. https://doi.org/10.1016/j.arr.2008.10.002

Wilfinger, W. W., Mackey, K., & Chomczynski, P. (1997). Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. *BioTechniques*, *22*(3), 474–481. https://doi.org/10.2144/97223st01

Wise, I. A., & Charchar, F. J. (2016). Epigenetic Modifications in Essential Hypertension. *International journal of molecular sciences*, *17*(4), 451. https://doi.org/10.3390/ijms17040451

Williams, B., Mancia, G., Spiering, W., Agabiti Rosei, E., Azizi, M., Burnier, M., Clement, D. L., Coca, A., de Simone, G., Dominiczak, A., Kahan, T., Mahfoud, F., Redon, J., Ruilope, L., Zanchetti, A., Kerins, M., Kjeldsen, S. E., Kreutz, R., Laurent, S., Lip, G. Y. H., … ESC Scientific Document Group (2018). 2018 ESC/ESH Guidelines for the management of arterial hypertension. *European heart journal*, *39*(33), 3021–3104. https://doi.org/10.1093/eurheartj/ehy339

Woelfle, M. A., Ouyang, Y., Phanvijhitsiri, K., & Johnson, C. H. (2004). The adaptive value of circadian clocks: an experimental assessment in cyanobacteria. *Current biology : CB*, *14*(16), 1481–1486. https://doi.org/10.1016/j.cub.2004.08.023

Woon, P. Y., Kaisaki, P. J., Bragança, J., Bihoreau, M. T., Levy, J. C., Farrall, M., & Gauguier, D. (2007). Aryl hydrocarbon receptor nuclear translocator-like (BMAL1) is associated with susceptibility to hypertension and type 2 diabetes. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(36), 14412–14417. https://doi.org/10.1073/pnas.0703247104

World Health Organization.(2014). Global Status Report on Noncommunicable Diseases.http://apps.who.int/iris/bitstream/10665/148114/1/9789241564854_eng.pdf?ua1⁄41

Xiao, C. L., Zhu, S., He, M., Chen, D., Zhang, Q., Chen, Y., Yu, G., Liu, J., Xie, S. Q., Luo, F., Liang, Z., Wang, D. P., Bo, X. C., Gu, X. F., Wang, K., & Yan, G. R. (2018). N^6-Methyladenine DNA Modification in the Human Genome. *Molecular cell*, *71*(2), 306–318.e7. https://doi.org/10.1016/j.molcel.2018.06.015

Xiao, D., Dasgupta, C., Li, Y., Huang, X., & Zhang, L. (2014). Perinatal nicotine exposure increases angiotensin II receptor-mediated vascular contractility in adult offspring. *PloS one*, *9*(9), e108161. https://doi.org/10.1371/journal.pone.0108161

Xiao, N. M., Zhang, Y. M., Zheng, Q., & Gu, J. (2004). Klotho is a serum factor related to human aging. *Chinese medical journal*, *117*(5), 742–747.

Xie, Z., Su, W., Liu, S., Zhao, G., Esser, K., Schroder, E. A., Lefta, M., Stauss, H. M., Guo, Z., & Gong, M. C. (2015). Smooth-muscle BMAL1 participates in blood pressure circadian rhythm regulation. *The Journal of clinical investigation*, *125*(1), 324–336. https://doi.org/10.1172/JCI76881

Xu, J. C., Lytle, C., Zhu, T. T., Payne, J. A., Benz, E., Jr, & Forbush, B., 3rd (1994). Molecular cloning and functional expression of the bumetanide-sensitive Na-K-Cl cotransporter. *Proceedings of the National Academy of Sciences of the United States of America*, *91*(6), 2201–2205. https://doi.org/10.1073/pnas.91.6.2201

Xu, X., Liang, X., Hu, G., Zhang, J., & Lei, H. (2015). Renal function and klotho gene polymorphisms among Uygur and Kazak populations in Xinjiang, China. *Medical science monitor : international medical journal of experimental and clinical research*, *21*, 44–51. https://doi.org/10.12659/MSM.891213

Xu, Y., & Sun, Z. (2015). Molecular basis of Klotho: from gene to function in aging. *Endocrine reviews*, *36*(2), 174–193. https://doi.org/10.1210/er.2013-1079

Yamamoto, M., Clark, J. D., Pastor, J. V., Gurnani, P., Nandi, A., Kurosu, H., Miyoshi, M., Ogawa, Y., Castrillon, D. H., Rosenblatt, K. P., & Kuro-o, M. (2005). Regulation of oxidative stress by the anti-aging hormone klotho. *The Journal of biological chemistry*, *280*(45), 38029–38034. https://doi.org/10.1074/jbc.M509039200

Yokoyama, S., Oguro, R., Yamamoto, K., Akasaka, H., Ito, N., Kawai, T., Kusunoki, H., Takeya, Y., Takeya-Onishi, M., Yamamoto-Hanasaki, H., Sugimoto, K., Ikebe, K., Gondo, Y., Ohishi, M., Kamide, K., & Rakugi, H. (2018). A *klotho* gene single nucleotide polymorphism is associated with the onset of stroke and plasma klotho concentration. *Aging*, *11*(1), 104–114. https://doi.org/10.18632/aging.101728

Yu, J., Li, J., Li, M., Wang, L., Xu, X., & Li, M. (2023). Association between serum Klotho concentration and hypertension in postmenopausal women, a cross-sectional study from NHANES 2013-2016. *BMC geriatrics*, *23*(1), 466. https://doi.org/10.1186/s12877-023-04191-8

Yusuf, S., Joseph, P., Rangarajan, S., Islam, S., Mente, A., Hystad, P., Brauer, M., Kutty, V. R., Gupta, R., Wielgosz, A., AlHabib, K. F., Dans, A., Lopez-Jaramillo, P., Avezum, A., Lanas, F., Oguz, A., Kruger, I. M., Diaz, R., Yusoff, K., Mony, P., … Dagenais, G. (2020). Modifiable risk factors, cardiovascular disease, and mortality in 155 722 individuals from 21 high-income, middle-income, and low-income countries (PURE): a prospective cohort study. *Lancet (London, England)*, *395*(10226), 795–808. https://doi.org/10.1016/S0140-6736(19)32008-2

Zarzuelo, M. J., López-Sepúlveda, R., Sánchez, M., Romero, M., Gómez-Guzmán, M., Ungvary, Z., Pérez-Vizcaíno, F., Jiménez, R., & Duarte, J. (2013). SIRT1 inhibits NADPH oxidase activation and protects endothelial function in the rat aorta: implications for vascular aging. *Biochemical pharmacology*, *85*(9), 1288–1296. https://doi.org/10.1016/j.bcp.2013.02.015

Zhang, D., Jin, C., Obi, I. E., Rhoads, M. K., Soliman, R. H., Sedaka, R. S., Allan, J. M., Tao, B., Speed, J. S., Pollock, J. S., & Pollock, D. M. (2020). Loss of circadian gene *Bmal1* in the collecting duct lowers blood pressure in male, but not female, mice. *American journal of physiology. Renal physiology*, *318*(3), F710–F719. https://doi.org/10.1152/ajprenal.00364.2019

Zhang, H., Shi, Y., Ma, F., Wang, L., Hou, Y., & Zhu, Z., Association of Klotho single nucleotide polymoprhisms with cardiovascular diseases: a systematic review and meta-analysis. Int J Clin Exp Med. 2017; 10(3): 5721-5741.

Zhang, L. N., Liu, P. P., Wang, L., Yuan, F., Xu, L., Xin, Y., Fei, L. J., Zhong, Q. L., Huang, Y., Xu, L., Hao, L. M., Qiu, X. J., Le, Y., Ye, M., & Duan, S. (2013). Lower ADD1 gene promoter DNA methylation increases the risk of essential hypertension. *PloS one*, *8*(5), e63455. https://doi.org/10.1371/journal.pone.0063455

Zhang, X., Blumenthal, R. M., & Cheng, X. (2021). A Role for N6-Methyladenine in DNA Damage Repair. *Trends in biochemical sciences*, *46*(3), 175–183. https://doi.org/10.1016/j.tibs.2020.09.007

Zhang, W., Xiong, Y., Tao, R., Panayi, A. C., Mi, B., & Liu, G. (2022). Emerging insight Into the Role of Circadian Clock Gene BMAL1 in Cellular Senescence. *Frontiers in endocrinology*, *13*, 915139. https://doi.org/10.3389/fendo.2022.915139

Zheng, Y., Pan, L., Wang, F., Yan, J., Wang, T., Xia, Y., Yao, L., Deng, K., Zheng, Y., Xia, X., Su, Z., Chen, H., Lin, J., Ding, Z., Zhang, K., Zhang, M., & Chen, Y. (2023). Neural function of Bmal1: an overview. *Cell & bioscience*, *13*(1), 1. https://doi.org/10.1186/s13578-022-00947-8

Zhou, L., Li, Y., Zhou, D., Tan, R. J., & Liu, Y. (2013). Loss of Klotho contributes to kidney injury by derepression of Wnt/β-catenin signaling. *Journal of the American Society of Nephrology : JASN*, *24*(5), 771–785. https://doi.org/10.1681/ASN.2012080865

Zhou, L., Mo, H., Miao, J., Zhou, D., Tan, R. J., Hou, F. F., & Liu, Y. (2015a). Klotho Ameliorates Kidney Injury and Fibrosis and Normalizes Blood Pressure by Targeting the Renin-Angiotensin System. *The American journal of pathology*, *185*(12), 3211–3223. https://doi.org/10.1016/j.ajpath.2015.08.004

Zhou, Q., Lin, S., Tang, R., Veeraragoo, P., Peng, W., & Wu, R. (2010). Role of Fosinopril and Valsartan on Klotho Gene Expression Induced by Angiotensin II in Rat Renal Tubular Epithelial Cells. *Kidney & blood pressure research*, *33*(3), 186–192. https://doi.org/10.1159/000316703

Zhou, X., Chen, K., Lei, H., & Sun, Z. (2015b). Klotho gene deficiency causes salt-sensitive hypertension via monocyte chemotactic protein-1/CC chemokine receptor 2-mediated inflammation. *Journal of the American Society of Nephrology : JASN*, *26*(1), 121–132. https://doi.org/10.1681/ASN.2013101033

Zhou, X., Chen, K., Wang, Y., Schuman, M., Lei, H., & Sun, Z. (2016). Antiaging Gene Klotho Regulates Adrenal CYP11B2 Expression and Aldosterone Synthesis. *Journal of the American Society of Nephrology : JASN*, *27*(6), 1765–1776. https://doi.org/10.1681/ASN.2015010093

Zhou,Y., Xue,Q., Peng, H., Zhu, Z., Xu,Q., Zhang,Q, & Li, H.(2018). The relationship between serum klotho protein and hypertension. Chin. J. Hypertens. 26:265–270.

APPENDICES

Table 1S.

DNA Quantity and Quality of a few DNA Samples

Sample	DNA concentration	A260/A280	Sample	DNA concentration	A260/A280
2HTN	44.3ng/ul	1.72	**44HTN**	45.2ng/ul	1.86
4HTN	35.5ng/ul	1.68	**46HTN**	38.5ng/ul	1.83
11HTN	36.1ng/ul	1.93	**47HTN**	52.4ng/ul	1.81
23HTN	32.9ng/ul	1.91	**55HTN**	66.2ng/ul	1.80
30HTN	36.7ng/ul	1.77	**59HTN**	39.9ng/ul	1.78
34HTN	36.8ng/ul	1.84	**66HTN**	49ng/ul	1.84
35HTN	43.2ng/ul	1.85	**69HTN**	31.9ng/ul	1.78
40HTN	31.3ng/ul	1.89	**54HTN**	62.7ng/ul	1.65
42HTN	33.7ng/ul	1.90	**53HTN**	45.2ng/ul	1.79
43HTN	69.4ng/ul	1.87	**75HTN**	27.5ng/ul	1.76

YAKIN DOĞU ÜNİVERSİTESİ
BİLİMSEL ARAŞTIRMALAR ETİK KURULU

ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU

Toplantı Tarihi : 25.06.2020
Toplantı No **:** 2020/80
Proje No **:**1066

Yakın Doğu Üniversitesi Tıp Fakültesi öğretim üyelerinden Doç. Dr. Rasime Kalkan'ın sorumlu araştırmacısı olduğu, YDU/2020/80-1066 proje numaralı ve **"Klotho gen metilasyonu ve polimorfizlerinin hipertansiyon ile ilişkisinin araştırılması"** başlıklı proje önerisi kurulumuzca online toplantıda değerlendirilmiş olup, etik olarak uygun bulunmuştur.

Prof. Dr. Rüştü Onur
Yakın Doğu Üniversitesi
Bilimsel Araştırmalar Etik Kurulu Başkanı

Meryem Osum Thesis

ORİJİNALLİK RAPORU

%13	%10	%11	%
BENZERLİK ENDEKSİ	İNTERNET KAYNAKLARI	YAYINLAR	ÖĞRENCİ ÖDEVLERİ

BİRİNCİL KAYNAKLAR

1	www.ncbi.nlm.nih.gov İnternet Kaynağı	%2
2	docs.neu.edu.tr İnternet Kaynağı	%1
3	www.mdpi.com İnternet Kaynağı	<%1
4	academic.oup.com İnternet Kaynağı	<%1
5	link.springer.com İnternet Kaynağı	<%1
6	Systems Biology of Free Radicals and Antioxidants, 2014. Yayın	<%1
7	hdl.handle.net İnternet Kaynağı	<%1
8	www.frontiersin.org İnternet Kaynağı	<%1
9	www.qiagen.com İnternet Kaynağı	<%1

CURRICULUM VITAE

1. **Name- Surname:** Meyrem Osum
2. **Title:** Research Assistant
3. **Educational Background:** Master's

YEAR	DEGREE	UNIVERSITY	FIELD OF LEARNING
2010-2014	Bachelor	TC. Halic University	Molecular Biology and Genetics
2015-2018	Master	TC. Halic University	Molecular Biology and Genetics
2018-	PhD	Near East University	Medical Biology and Genetics

4. **Master's/PhD Thesis**

4.1. Master's Thesis Title and Thesis Advisor (s):

Thesis Title: Expression Analysis of *miR-200a-3p*, *miR-223-5p*, and *miR-29a-3p* in Polymorphic *Helicobacter Pylori CagA* Genotypes in Gastric Cancer

Advisor: Assoc. Prof. Dr. Meliha Burcu Irmak Yazıcıoğlu

4.2. PhD Thesis Title and Thesis Advisor(s):

Thesis Title: Investigation of the Relationship Between Methylation of *KLOTHO* and *BMAL1* Genes and Hypertension

Advisor: Prof. Dr. Rasime Kalkan & Prof. Dr. Pınar Tulay

5. **Academic Titles:**

Date of Assistant Professorship:

Date of Associate Professorship:

Date of Professorship:

6. Supervised Master's and PhD Theses:

6.1. Master's Theses

6.2. PhD Theses

7. Publications

7.1. Articles Published in International Peer-Reviewed Journals (SCI, SSCI, AHCI, ESCI, Scopus)

1. Osum, M., & Serakinci, N. (2020). Impact of circadian disruption on the heath; SIRT1 and Telomeres. DNA repair, 96, 102993.
2. Oztenekecioglu, B., Mavis, M., Osum, M., & Kalkan, R. (2021). Genetic and Epigenetic Alterations in Autism Spectrum Disorder. Global medical genetics, 8(4), 144-148.

7.2. Articles Published in Other International Peer-Reviewed Journals

7.3. Papers Presented at International Scientific Conferences and Published in Conference Proceedings

7.4. National/international Books or Book Chapters

1. Osum, M.,& Kalkan, R. (2023). Cancer Stem Cells and Their Therapeutic Usage. Advances in experimental medicine and biology, 1436, 69-85.

7.5. Articles Published in National Peer-Reviewed Journals

8. Art and Design Activities

9. Projects

10. Administrative Responsibilities

11. Memberships in Scientific and Professional Organizations

12. Awards

13. Undergraduate and Graduate Courses Taught in the Last Two Years

Academic Year	Semester	Course Name	Weekly Hours	
			Theoretical	Practical
2022-2023 And 2023-2024	Fall	MBG101 General and Cell Biology	3	3
	Fall	TMG101 Genel ve Hücre Biyolojisi	3	3
	Fall	MBG503 Aging	3	0
	Fall	TMG503 Yaşlanma	3	0
	Fall	MBG512 Microbiome	3	0
	Fall	TMG512 Mikrobiyom	3	0
2022-2023 And 2023-2024	Spring	TMG104 Moleküler Hücre Biyolojisi	3	2
	Spring	MBG521 Stem Cell Biology	3	0
	Spring	TMG521 Kök Hücre Biyolojisi	3	0
	Spring	MBG207 Human Genetics and Genomics	3	2
	Spring	TMG207 İnsan Genetiği ve Genomik	3	2