



UNIVERSITI PUTRA MALAYSIA

***EXPRESSION ANALYSIS OF COMMD5, NPPA, SLC7A1 AND AT1R
GENES AMONG HYPERTENSIVE MALAYS***

BAN WAHEED HUSSEIN BDAIR

FPSK(M) 2018 11



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AMONG HYPERTENSIVE MALAYS**

By

BAN WAHEED HUSSEIN BDAIR

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Master of Science**

December 2017

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DEDICATION

I dedicate the thesis to the people who without them I won't have reached where I am today

My soul husband

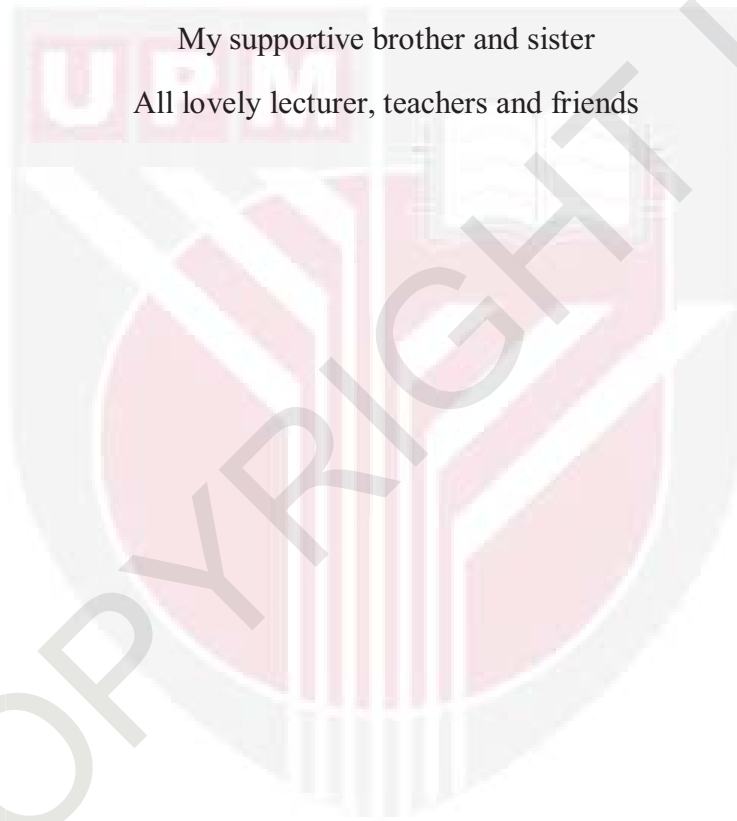
My beloved sons and daughters

My dear father

My caring mother

My supportive brother and sister

All lovely lecturer, teachers and friends



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

EXPRESSION ANALYSIS OF *COMMD5*, *NPPA*, *SLC7A1* AND *AT1R* GENES AMONG HYPERTENSIVE MALAYS

By

BAN WAHEED HUSSEIN BDAIR

December 2017

Chairman : Professor Patimah Ismail, PhD
Faculty : Medicine and Health Sciences

Hypertension is one of the most common and known risk factors for cardiovascular disease. This disease affects structures and functions of small muscles arteries, arterioles and other blood vessels. It is known to cause varying damages in different organs like brain, eye and kidney which may eventually lead to stroke, poor vision and renal disease. By 2025, it has been predicted that about 1.56 billion people are expected to have high blood pressure. Recently, Global Burden of Disease reported high-level blood pressure as the most significant risk factor for mortality. In the year 2011, National Health Morbidity Survey (NHMS) IV reported 32.7% as the prevalence rate of hypertension in Malaysia. Environmental risk factors such as sedentary lifestyle, dietary factors, smoking, and lack of physical activity in combination with genetic factors play important role in progression of hypertension.

Of recent, human genetic studies have reported several candidate genes such as *COMMD5* (COMM Domain-Containing Protein 5), which relates with calcium haemostasis and *NPPA* (Natriuretic Peptide A), a gene that play key roles in maintenance of cardiovascular homeostasis as well as vasodilation. Similarly, the *SLC7A1* (solute carrier family 7 member 1) gene which is involved in the transport of the cationic amino acids (arginine) and *AT1R* (Angiotensin II Receptor Type 1) that controls blood pressure/ volume in the cardiovascular system are also potent candidate genes. Although the functions of these genes have been identified, there is absence of detailed comprehensive analysis of the expression of these genes collectively in association with hypertension among Malays. In line with this, the present study aimed at determining the expression level of these genes (*COMMD5*, *NPPA*, *SLC7A1*, and *AT1R*) among hypertensive Malay subjects. Therefore, a total of 100 newly diagnosed hypertensive patients and 100 unrelated healthy individuals were recruited. Total RNA was extracted from whole blood specimen using RNA extraction kit and the target genes were quantitated using Real Time Quantitative Polymerase Chain Reaction (RT-

qPCR). General Linear Model analysis was performed using SPSS software and $p \leq 0.05$ was deemed significant and analysis of gene expression and relative expression in qPCR was performed using REST software. The demographic characteristic of the subject such as body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), low density lipoprotein (LDL), triglyceride (TG) and cholesterol (Chol) were shown to be differently significant ($p < 0.05$) in experimental subjects compared to control. Moreover, age and high density lipoprotein (HDL) did not show any significance ($p > 0.05$). Gene expression pattern was determined in hypertensive patients and compared with control. COMMD5 and AT1R genes were significantly upregulated in hypertensive patients compared with control ($p < 0.05$), while SLC7A1 and NPPA genes were downregulated in hypertensive patients compared with healthy individuals. This study demonstrated that COMMD5, NPPA, SLC7A1, and AT1R might be involved in pathogenesis of hypertension, and hence could be used as diagnostic biomarkers in the prediction of hypertension in Malay subjects.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**ANALISIS EXPRESI *COMMD5*, *NPPA*, *SLC7A1*, DAN *AT1R* GENESIS
DENGAN SUBJEK BAHARU HIPERTENSIF**

Oleh

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Disember 2017

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Hipertensi adalah salah satu faktor risiko yang paling dikenali dan boleh dirawat untuk penyakit kardiovaskular. Hipertensi memberi kesan kepada struktur dan fungsi arteri otot kecil, arterioli dan saluran darah yang lain. Ia diketahui menyebabkan kerosakan pada kadar berubah-ubah dalam organ-organ yang berbeza seperti; Buah pinggang, otak dan mata yang boleh membawa kepada penyakit buah pinggang yang akhirnya menyebabkan strok. Menjelang 2025, diharapkan bahawa 1.56 bilion orang akan mengalami hipertensi. Satu laporan baru-baru ini mengenai Kajian Beban Penyakit Global, BP peringkat tinggi sebagai faktor risiko yang paling penting untuk kematian. Menurut Kajian Kesihatan Morbiditi Nasional (NHMS) IV, prevalensi hipertensi di Malaysia adalah 32.7% pada tahun 2011. Faktor risiko alam sekitar seperti gaya hidup tidak aktif, faktor pemakanan, merokok, dan kurangnya aktiviti fizikal dalam kombinasi dengan faktor genetik memainkan peranan penting dalam Perkembangan hipertensi.

Baru-baru ini, kajian genetik manusia telah melaporkan beberapa gen calon seperti *COMMD5* (COMM Domain-Containing Protein 5) yang berkaitan dengan haemostasis kalsium, *NPPA* (Natriuretic Peptide A) yang memainkan peranan penting dalam homeostasis kardiovaskular melalui peraturan natriuresis, diuresis, dan vasodilation. *SLC7A1* (keluarga pembawa larut 7 anggota 1) yang terlibat dalam pengangkutan asid amino kationik (arginine) dan *AT1R* (Angiotensin II Receptor Type 1) yang berfungsi sebagai alat pengukur penting yang mengawal tekanan darah dan volum dalam sistem kardiovaskular. Tujuan kajian ini adalah untuk menentukan corak ekspresi gen ini (*COMMD5*, *NPPA*, *SLC7A1*, dan *AT1R*) di kalangan subjek Bahasa Melayu hipertensi. Sebanyak 100 pesakit hipertensi yang baru didiagnosis dan 100 orang yang tidak sihat yang sihat yang semuanya menjalani angiografi telah direkrut. Jumlah RNA diekstrak daripada spesimen darah keseluruhan menggunakan kit pengekstrakan. QPCR digunakan untuk menguatkan salinan cDNA sasaran RNA

yang diekstrak. Sensitif dan serba boleh, qPCR digunakan untuk mengambil dan mengklonkan terma mRNA 5' dan 3' dan menghasilkan pustaka cDNA yang besar dari sejumlah kecil mRNA. Analisa model Linear Umum dilakukan menggunakan perisian SPSS dan $p \leq 0.05$ dianggap penting dan analisis ekspresi gen dan ekspresi relatif dalam qPCR dilakukan menggunakan perisian REST. Ciri demografi subjek seperti indeks jisim badan (BMI), tekanan darah sistolik (SBP), tekanan darah diastolik (DBP), lipoprotein ketumpatan rendah (LDL), trigliserida (TG) dan kolesterol (Chol) Ketara ($p < 0.05$) dalam mata pelajaran eksperimen berbanding kawalan. Lebih-lebih lagi, umur dan lipoprotein ketumpatan tinggi (HDL) tidak menunjukkan sebarang kepentingan ($p > 0.05$). Corak ungkapan gen ditentukan dalam pesakit hipertensi dan dibandingkan dengan kawalan. GenSR dan gen AT1R telah dikawal dengan ketara dalam pesakit hipertensi berbanding dengan kawalan ($p < 0.05$), manakala gen SLC7A1 dan NPPA dikurangkan dalam pesakit hipertensi berbanding pesakit yang sihat. Kajian ini menunjukkan bahawa COMMD5, NPPA, SLC7A1, dan AT1R mungkin terlibat dalam patogenesis hipertensi, dan dengan itu boleh digunakan sebagai biomarker diagnostik dalam ramalan hipertensi dalam mata pelajaran Melayu.

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In the name of God, the Lord Majesty and Bounty, The Inspirer of Faith. Praised to Him for enlightening my path and surrounding me with wonderful people.

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I certify that a Thesis Examination Committee has met on 18 December 2017 to conduct the final examination of Ban Waheed Hussein on her thesis entitled "Expression Analysis of *COMMD5*, *NPPA*, *SLC7A1* and *AT1R* Genes Among Hypertensive Malays" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

ACE	Angiotensin-converting enzyme
Ang I	angiotensin I
Ang II	angiotensin II
ANOVA	Analyze of Variance
AT1R	Angiotensin II Receptor Type 1
AT2R	angiotensin II type 2 receptor
BMI	body mass index
BP	Blood pressure
CAT-1	Cationic Amino Acid Transporter 1
CD	cardiovascular disease
cDNA	Complementary Deoxyribonucleic acid
Chol	cholesterol
CI	confidence interval
COMMD5	COMM Domain-Containing Protein 5
CT	Cycle Threshold for Real- Time PCR analysis
CVD	Cardiovascular Disease
DBP	diastolic blood pressure
EH	Essential hypertension
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
GWAS	Genome -Wide Association Studies
HbA1c	Glycated Haemoglobin
HCaRG	hypertension-related, calcium-regulated gene
HDL	high density lipoprotein
Kg	Kilogram
LDL	low density lipoprotein
ml	milliliter
mm Hg	millimetre of mercury
ng	Nano gram
NHMS	National Health Morbidity Survey
NMRR	National Medical Research Register

NPPA	Natriuretic Peptide A
NTC	No Template Control
OD	optical densities
qPCR	quantitative Polymerase Chain Reaction
RAAS	renin-angiotensin-aldosterone system
REST	Relative Expression Software Tool
RIN	RNA Integrity Number
RNA	Ribonucleic Acid
S1	Stage 1 hypertension
S2	Stage 2 hypertension
SBP	systolic blood pressure
SLC7A1	solute carrier family 7 member 1
SNP	Single Nucleotide Polymorphism
SPSS	statistical package for social sciences
TG	triglyceride
UV	Ultraviolet
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Hypertension or increase in blood pressure (BP) is a major risk factor for cardiovascular diseases (CVD). Hypertension have been reported to affect over one billion people worldwide (Kearney *et al.*, 2005) , leading to heart attacks and strokes. It has also been estimated that increase in blood pressure currently kills 9 million people every year (WHO, 2013). Hypertension is diagnosed from the force of arterial heartbeat-induced blood pressure. In normal individuals, the blood pressure is usually 120/80mmHg which implies a systolic blood pressure of 120 mmHg and a diastolic pressure of 80 mmHg. Blood pressures $\leq 130/89$ mmHg are considered to exceed the normal ranged and are termed as pre-hypertension. Hypertension however is characterised by a blood pressure $\geq 140/80$ mmHg (Glasser *et al.*, 2013; Weber *et al.*, 2014). Two broad categories are employed for the classification of hypertension, these are as Essential Hypertension (EH) or Secondary Hypertension (SE). About 95% of hypertensive cases are EH and its diagnosed when there is no evidence of high blood pressure susceptibility to medical conditions (Go *et al.*, 2013). However, SE constitutes a small number of cases and is associated with diseases such as Cushing's syndrome, chronic renal failure or Conn's syndrome (Schaefer and Mehls, 2004; Ceccato and Boscaro, 2016) .

Many factors contribute to the presence of high blood pressure, these factors include lifestyle, genetic factors, eating habits and other medical complications (Niiranen *et al.*, 2017). Elevated BP has been associated with obesity, smoking, less physical activity, too much alcohol consumption and high salt intake (Carretero and Oparil, 2000; Weber *et al.*, 2014) While the role of environment is known to impact on the BP, genetic factors can not be ignored as it may form the underlying basis of this condition. A complex form of genetic traits resulting from multiple genes being controlled by interactions between one gene and another as well as between a gene and its environment is widely referred to as polygenic mutation and this have been shown to be the primary culprit in EH (Chern and Chiang, 2004; Singh *et al.*, 2016).

It has been suggested that hypertension has a basis in genetics as interactions between genes and external factors as well as within genes have led to diabetes in already susceptible individuals (Williams *et al.*, 1991; Chandra *et al.*, 2015). In an effort to maintain physiological homeostasis, a specific genetic system interacts with candidate genes to regulate blood pressure. Renin angiotensin aldosterone system (RAAS) and in association with candidate genes. Atrial natriuretic peptide (*NPPA*) and Angiotensin-II Type-1 Receptor (*AT1R*) are RAAS mediators with significant roles in hypertension through regulation of aldosterone secretion and cardio renal homeostasis, respectively. Endothelium dysfunction leading to hypertension could result from variations in the *SLC7A1* gene as reported by genetic studies.

Recently, the Qiagen Company indicated how *COMMD5* gene is involved and associated with hypertension (Matsuda *et al.*, 2014). *COMMD5* was previously known as HCaRG, first identified in spontaneously hypertensive rats (SHR) (Solban *et al.*, 2001). Gene expression is the most basic level in which the genotype of an organism causes the phenotype. A good way to consider gene expression is to interpret the information stored in the cell DNA as a mediator to produce a phenotypic output by gene transcription and mRNA progression. The ultimate effect on the phenotype is primarily through the synthesis of proteins, some of which structurally control the shape and characteristics of an organism, while others may be enzymes responsible for catalyzing specific metabolic pathways (Morley *et al.*, 2004). Therefore, the present study focused on the gene expression levels of key genes (*AT1R*, *NPPA*, *SLC7A1* and *COMMD5*) associated with hypertension amongst Malays. It is important to state however, that this study ensured that the respondents are three-generational genetic hierarchical Malays with no Chinese, Indian or other race within the generations. This will eliminate to a high extent, the effect of genetic variations that may arise as a result of inter-racial marriages.

1.2 Problem statement

In developed and developing countries, hypertension has been identified as a serious threat to public health. It is one of the risk factors for cardiovascular death (Kishore *et al.*, 2016). Globally, cardiovascular disease cause for about 17 million deaths a year (WHO, 2013). Hypertensive complications are reported in the world every year with 9.4 million deaths (Lim *et al.*, 2012). Hypertension causes heart disease that lead to at least 45% dead while 51% die due to stroke deaths (WHO, 2013).

There were 1.33 billion people living with high BP in the year 2015, and most of them in middle income and low income countries. Females aged 18 years and older have around 20% prevalence of high BP while the prevalence in males is around 24% (Collaboration, 2016). In Malaysia, the National Health Morbidity Survey (NHMS) IV conducted in 2011, reported 43.5% as the prevalence rate of hypertension in adults ≥ 30 years (Naing *et al.*, 2016), which implies a continuous increase in the prevalence level when compared to 32.9% reported in NHMS II 1996 (Lim and Morad, 2004) and 42.6 % reported in NHMS III 2006 (Nor *et al.*, 2008) . Another study conducted in 2016, highlights the shocking situation, almost half of adults older than 30 years old suffered from high BP. Overall, the hypertension prevalence was 47.9 % and was higher in men (43.5 %) than women (41.0 %) (Abdul-Razak *et al.*, 2016).

Genetics plays a major role in hypertension development and progression as many studies are currently focusing on gene therapy in the treatment of metabolic and cardiovascular disorders (Padmanabhan *et al.*, 2015). Additionally, there is a gap in the availability of research materials collectively associating *AT1R*, *NPPA*, *SLC7A1* and *COMMD5* genes with hypertension especially among Malays. This is a novel study on the gene expression of *NPPA*, *SLC7A1* and *COMMD5* in association with hypertension among Malays. Hence, this is the first study to particularly identify these genes among hypertensive Malay ethnic group in Malaysian population.

1.3 Significance of the study

The Cross sectional study attempt to determine the presence of variation within candidate genes (*AT1R*, *NPPA*, *SLC7A1* and *COMMD5*) which might be associated with the pathogenesis of hypertension among hypertensive Malay as compared to non-hypertensive Malays. Analysis of these genes provides better approach for identifying the level of expression and their possible correlation with the disease. The physicians can recognize the onset of hypertension in high risk individuals which can be prevented or delayed. Results of this study will be compared with other studies worldwide and would form a deposit database that could be used for Malaysian genetic database for future references.

1.4 Hypothesis

There is an association between *AT1R*, *NPPA*, *SLC7A1* and *COMMD5* genes expression among hypertensive subjects.

1.5 General objective

To identify the expression levels of *AT1R*, *NPPA*, *SLC7A1* and *COMMD5* genes and analyse their association with the severity of hypertension amongst Malay subjects.

1.6 Specific objectives

1. To correlate the demographic, physical and cardiological description of respondents with their hypertensive condition.
2. To determine the expression levels of *AT1R*, *NPPA*, *SLC7A1* and *COMMD5* genes in patients with hypertension and compare with non-hypertensive subjects.
3. To evaluate the relationship between the expression levels of *AT1R*, *NPPA*, *SLC7A1* and *COMMD5* genes and severity of hypertension.
4. To identify changes in some biochemical indices and associate these changes with gene expression in the hypertensive patients.

REFERENCES

- Abdul-Razak, S., Daher, A. M., Ramli, A. S., Ariffin, F., Mazapuspavina, M. Y., Ambigga, K. S. & Ng, K. K. (2016). Prevalence, awareness, treatment, control and socio demographic determinants of hypertension in Malaysian adults. *BMC public health*, 16(1), 351.
- Agachan, B., Isbir, T., Yilmaz, H., & Akoglu, E. (2003). Angiotensin converting enzyme I/D, angiotensinogen T174M-M235T and angiotensin II type 1 receptor A1166C gene polymorphisms in Turkish hypertensive patients. *Experimental & molecular medicine*, 35(6), 545.
- Agarwal, A., Williams, G. H., & Fisher, N. D. (2005). Genetics of human hypertension. *Trends in Endocrinology & Metabolism*, 16(3), 127-133.
- Agyemang, C., Addo, J., Bhopal, R., de Graft Aikins, A., & Stronks, K. (2009). Cardiovascular disease, diabetes and established risk factors among populations of sub-Saharan African descent in Europe: a literature review. *Globalization and health*, 5(1), 7.
- Akintunde, A. A. (2010). Epidemiology of conventional cardiovascular risk factors among hypertensive subjects with normal and impaired fasting glucose. *SAMJ: South African Medical Journal*, 100(9), 594-597.
- Akuyam, S. A., Isah, H. S., & Ogala, W. N. (2007). Evaluation of serum lipid profile of under-five Nigerian children. *Annals of African medicine*, 6(3), 119.
- Albritton, L. M., Bowcock, A. M., Eddy, R. L., Morton, C. C., Tseng, L., Farrer, L. A., Cunningham, J. M. (1992). The human cationic amino acid transporter (ATRC1): physical and genetic mapping to 13q12-q14. *Genomics*, 12(3), 430-434.
- Anbazzhagan, K., Sampathkumar, K., Ramakrishnan, M., Gomathi, P., Gomathi, S., & Selvam, G. S. (2009). Analysis of polymorphism in renin angiotensin system and other related genes in South Indian chronic kidney disease patients. *Clinica Chimica Acta*, 406(1), 108-112.
- Anderson, K. M., Wilson, P., Odell, P. M., & Kannel, W. B. (1991). An updated coronary risk profile. A statement for health professionals. *Circulation*, 83(1), 356-362.
- Backlund, M. (2016). Regulation of angiotensin II type receptor by its messenger RNA-binding proteins. *Dissertationes Scholae Doctoralis Ad Sanitatem Investigandam Universitatis Helsinkiensis*.
- Bader, M. (2010). Tissue renin-angiotensin-aldosterone systems: targets for pharmacological therapy. *Annual review of pharmacology and toxicology*, 50, 439-465.

- Bader, M., & Ganten, D. (2008). Update on tissue renin–angiotensin systems. *Journal of Molecular Medicine*, 86(6), 615.
- Ball, J. R., & Micheel, C. M. (Eds.). (2010). *Evaluation of biomarkers and surrogate endpoints in chronic disease*. National Academies Press.
- Bartuzi, P., Hofker, M. H., & van de Sluis, B. (2013). Tuning NF- κ B activity: a touch of COMMD proteins. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, 1832(12), 2315-2321.
- Bautista, L. E., Vargas, C. I., Orostegui, M., & Gamarra, G. (2008). Population-based case-control study of renin-angiotensin system genes polymorphisms and hypertension among Hispanics. *Hypertension Research*, 31(3), 401-408.
- Beevers, D. G., Lip, G. Y., & O'Brien, E. T. (2014). *ABC of Hypertension*: John Wiley & Sons.
- Belly, M. J., Tiede, H., Morty, R. E., Schulz, R., Voswinckel, R., Tanislav, C., . . . Reichenberger, F. (2012). HbA 1c in pulmonary arterial hypertension: a marker of prognostic relevance? *The Journal of Heart and Lung Transplantation*, 31(10), 1109-1114.
- Bergsma, D. J., Ellis, C., Kumar, C., Nuthulaganti, P., Kersten, H., Elshourbagy, N., & Aiyar, N. (1992). Cloning and characterization of a human angiotensin II type 1 receptor. *Biochemical and biophysical research communications*, 183(3), 989-995.
- Bernabé-Ortiz, A., Carrillo-Larco, R. M., Gilman, R. H., Checkley, W., Smeeth, L., Miranda, J. J., & CRONICAS Cohort Study Group. (2017). Impact of urbanisation and altitude on the incidence of, and risk factors for, hypertension. *Heart*, 103(11), 827-833.
- Bettinaglio, P., Galbusera, A., Caprioli, J., Orisio, S., Perna, A., Arnoldi, F. & BENEDICT Study Group. (2002). Single strand conformation polymorphism (SSCP) as a quick and reliable method to genotype M235T polymorphism of angiotensinogen gene. *Clinical biochemistry*, 35(5), 363-368.
- Blankstein, R., Budoff, M. J., Shaw, L. J., Goff, D. C., Polak, J. F., Lima, J. & Nasir, K. (2011). Predictors of coronary heart disease events among asymptomatic persons with low low-density lipoprotein cholesterol: MESA (Multi-Ethnic Study of Atherosclerosis). *Journal of the American College of Cardiology*, 58(4), 364-374.
- Bonnardeaux, A., Davies, E., Jeunemaitre, X., Fery, I., Charru, A., Clauser, E. & Soubrier, F. (1994). Angiotensin II type 1 receptor gene polymorphisms in human essential hypertension. *Hypertension*, 24(1), 63-69.
- Briet, M., & Schiffrin, E. L. (2010). Aldosterone: effects on the kidney and cardiovascular system. *Nature Reviews Nephrology*, 6(5), 261-273.

- Burstein, E., Hoberg, J. E., Wilkinson, A. S., Rumble, J. M., Csomos, R. A., Komarck, C. M. & Duckett, C. S. (2005). COMMD proteins, a novel family of structural and functional homologs of MURR1. *Journal of Biological Chemistry*, 280(23), 22222-22232.
- Bush, W. S., & Moore, J. H. (2012). Genome-wide association studies. *PLoS computational biology*, 8(12), e1002822.
- Bustin, S. A., Benes, V., Nolan, T., & Pfaffl, M. W. (2005). Quantitative real-time RT-PCR—a perspective. *Journal of molecular endocrinology*, 34(3), 597-601.
- Calderon, K. S., Yucha, C. B., & Schaffer, S. D. (2005). Obesity-related cardiovascular risk factors: intervention recommendations to decrease adolescent obesity. *Journal of Pediatric Nursing*, 20(1), 3-14.
- Calhoun, D. A., Jones, D., Textor, S., Goff, D. C., Murphy, T. P., Toto, R. D., . . . Sica, D. (2008). Resistant hypertension: diagnosis, evaluation, and treatment. *Circulation*, 117(25), e510-e526.
- Carey, R. M. (2010). Aldosterone and cardiovascular disease. *Current Opinion in Endocrinology, Diabetes and Obesity*, 17(3), 194-198.
- Carretero, O. A., & Oparil, S. (2000). Essential hypertension. *Circulation*, 101(3), 329-335.
- Ceccato, F., & Boscaro, M. (2016). Cushing's Syndrome: Screening and Diagnosis. *High Blood Pressure & Cardiovascular Prevention*, 23(3), 209-215.
- Ceolotto, G., Papparella, I., Bortoluzzi, A., Strapazon, G., Ragazzo, F., Bratti, P. & Semplicini, A. (2011). Interplay between miR-155, AT1R A1166C polymorphism, and AT1R expression in young untreated hypertensives. *American journal of hypertension*, 24(2), 241-246.
- Chan, S. H., Wang, L. L., Tseng, H. L., & Chan, J. Y. (2007). Upregulation of AT1 receptor gene on activation of protein kinase C β /nicotinamide adenine dinucleotide diphosphate oxidase/ERK1/2/c-fos signaling cascade mediates long-term pressor effect of angiotensin II in rostral ventrolateral medulla. *Journal of hypertension*, 25(9), 1845-1861.
- Chandra, S., Saluja, D., Narang, R., Bhatia, J., & Srivastava, K. (2015). Atrial natriuretic peptide and aldosterone synthase gene in essential hypertension: A case-control study. *Gene*, 567(1), 92-97.
- Chapman, M. J. (2006). Therapeutic elevation of HDL-cholesterol to prevent atherosclerosis and coronary heart disease. *Pharmacology & therapeutics*, 111(3), 893-908.
- Chern, T. H., & Chiang, F. T. (2004). Molecular genetic study of hypertension. *Acta Cardiologica Sinica*, 20(3), 129-138.

- Chobanian, A. V., Bakris, G. L., Black, H. R., Cushman, W. C., Green, L. A., Izzo Jr, J. L. & Roccella, E. J. (2003). The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure: the JNC 7 report. *Jama*, 289(19), 2560-2571.
- Churchill, P. C., Churchill, M. C., Bidani, A. K., Griffin, K. A., Picken, M., Pravenec, M., Wang, N. (1997). Genetic susceptibility to hypertension-induced renal damage in the rat. Evidence based on kidney-specific genome transfer. *Journal of Clinical Investigation*, 100(6), 1373.
- Cifu, A. S., & Davis, A. M. (2017). Prevention, detection, evaluation, and management of high blood pressure in adults. *Jama*, 318(21), 2132-2134.
- Collaboration, N. R. F. (2016). Worldwide trends in blood pressure from 1975 to 2015: A pooled analysis of 1479 population-based measurement studies with 19· 1 million participants. *Lancet*, 389(10064), 37-55.
- Collaboration, P. S. (2002). Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *The Lancet*, 360(9349), 1903-1913.
- Colledge, N. R., Walker, B. R., & Ralston, S. H. (2010). *Davidson's Principles and Practice of Medicine*. illust. Robert Britton . Edinburgh: Churchill Livingstone.
- Conen, D., Glynn, R. J., Buring, J. E., Ridker, P. M., & Zee, R. Y. (2007). Natriuretic peptide precursor a gene polymorphisms and risk of blood pressure progression and incident hypertension. *Hypertension*, 50(6), 1114-1119.
- Crabbe, J. C., & Goldman, D. (1992). Alcoholism: A complex genetic disease. *Alcohol Research and Health*, 16(4), 297.
- Crump, C., Sundquist, J., Winkleby, M. A., & Sundquist, K. (2016). Interactive effects of physical fitness and body mass index on the risk of hypertension. *JAMA internal medicine*, 176(2), 210-216.
- De Bold, A. J. (1985). Atrial natriuretic factor: a hormone produced by the heart. *Science*, 230(4727), 767-770.
- De Bold, A. J., Borenstein, H. B., Veress, A. T., & Sonnenberg, H. (1981). A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. *Life sciences*, 28(1), 89-94.
- De Vriese, A. S., Verbeuren, T. J., Van de Voorde, J., Lameire, N. H., & Vanhoutte, P. M. (2000). Endothelial dysfunction in diabetes. *British journal of pharmacology*, 130(5), 963-974.
- Deedwania, P. C. (1997). The progression from hypertension to heart failure. *American journal of hypertension*, 10(S7), 280S-288S.
- Desai, M., Stockbridge, N., & Temple, R. (2006). Blood pressure as an example of a biomarker that functions as a surrogate. *The AAPS journal*, 8(1), E146-E152.

- Devlin, A. M., Solban, N., Tremblay, S., Gutkowska, J., Schürch, W., Orlov, S. N. & Tremblay, J. (2003). HCaRG is a novel regulator of renal epithelial cell growth and differentiation causing G 2 M arrest. *American Journal of Physiology-Renal Physiology*, 284(4), F753-F762.
- Dheda, K., Huggett, J. F., Bustin, S. A., Johnson, M. A., Rook, G., & Zumla, A. (2004). Validation of housekeeping genes for normalizing RNA expression in real-time PCR. *Biotechniques*, 37(1), 112-119.
- Diaz, K. M., Booth, J. N., Seals, S. R., Abdalla, M., Dubbert, P. M., Sims, M. & Shimbo, D. (2017). Physical Activity and Incident Hypertension in African Americans Novelty and Significance. *Hypertension*, 69(3), 421-427.
- Dussault, A. A., & Pouliot, M. (2006). Rapid and simple comparison of messenger RNA levels using real-time PCR. *Biological procedures online*, 8(1), 1-10.
- Dzida, G., Sobstyl, J., Puzniak, A., Golon, P., Mosiewicz, J., & Hanzlik, J. (2001). Polymorphisms of angiotensin-converting enzyme and angiotensin II receptor type 1 genes in essential hypertension in a Polish population. *Medical Science Monitor*, 7(6), 1236-1241.
- El Hader, C., Tremblay, S., Solban, N., Gingras, D., Beliveau, R., Orlov, S. N., & Tremblay, J. (2005). HCaRG increases renal cell migration by a TGF- α autocrine loop mechanism. *American Journal of Physiology-Renal Physiology*, 289(6), F1273-F1280.
- Engeli, S., Negrel, R., & Sharma, A. M. (2000). Physiology and pathophysiology of the adipose tissue renin-angiotensin system. *Hypertension*, 35(6), 1270-1277.
- Erne, P., & Hermsmeyer, K. (1989). Intracellular vascular muscle Ca²⁺ modulation in genetic hypertension. *Hypertension*, 14(2), 145-151.
- Ezzati, M., Lopez, A. D., Rodgers, A., Vander Hoorn, S., Murray, C. J., & Comparative Risk Assessment Collaborating Group. (2002). Selected major risk factors and global and regional burden of disease. *The Lancet*, 360(9343), 1347-1360.
- Fleming, T. R., & Powers, J. H. (2012). Biomarkers and surrogate endpoints in clinical trials. *Statistics in medicine*, 31(25), 2973-2984.
- Foulds, H. J., Bredin, S. S., & Warburton, D. E. (2012). The relationship between hypertension and obesity across different ethnicities. *Journal of hypertension*, 30(2), 359-367.
- Franco, E., Palumbo, L., Crobu, F., Anselmino, M., Frea, S., Matullo, G., & Bergerone, S. (2007). Renin-angiotensin-aldosterone system polymorphisms: a role or a hole in occurrence and long-term prognosis of acute myocardial infarction at young age. *BMC medical genetics*, 8(1), 27.
- FRAUMAN, A. G., JOHNSTON, C. I., & FABIANI, M. E. (2001). Angiotensin receptors: distribution, signalling and function. *Clinical Science*, 100(5), 481-

- Freeman, V., Fraser, H., Forrester, T., Wilks, R., Cruickshank, J., Rotimi, C., & Cooper, R. (1996). A comparative study of hypertension prevalence, awareness, treatment and control rates in St Lucia, Jamaica and Barbados. *Journal of hypertension*, 14(4), 495-502.
- Fuh, M. M., Shieh, S. M., Wu, D. A., Chen, Y. I., & Reaven, G. M. (1987). Abnormalities of carbohydrate and lipid metabolism in patients with hypertension. *Archives of internal medicine*, 147(6), 1035-1038.
- Fukuyama, K., Ichiki, T., Takeda, K., Tokunou, T., Iino, N., Masuda, S., & Kanaide, H. (2003). Downregulation of vascular angiotensin II type 1 receptor by thyroid hormone. *Hypertension*, 41(3), 598-603.
- Fusco, G., & Minelli, A. (2010). Phenotypic plasticity in development and evolution: facts and concepts.
- Gallagher, J., Watson, C., Zhou, S., Ryan, F., Ledwidge, M., & McDonald, K. (2017). B-Type Natriuretic Peptide and Ventricular Dysfunction in the Prediction of Cardiovascular Events and Death in Hypertension. *American journal of hypertension*, hpx153.
- Gardner, D. G., Chen, S., Glenn, D. J., & Grigsby, C. L. (2007). Molecular biology of the natriuretic peptide system. *Hypertension*, 49(3), 419-426.
- Gheissari, A., Salehi, M., Dastjerdi, S. B., Jahangiri, M., Hooman, N., Otookesh, H., & Shahidi, S. (2008). Angiotensin- converting enzyme gene polymorphism and the progression rate of focal segmental glomerulosclerosis in Iranian children. *Nephrology*, 13(8), 708-711.
- Ghodsian, N., Ismail, P., Ahmadloo, S., Eskandarian, N., & Etemad, A. (2016). Genetic Analysis of the Atrial Natriuretic Peptide Gene Polymorphisms among Essential Hypertensive Patients in Malaysia. *BioMed research international*, 2016.
- Gibbs, R. A., Belmont, J. W., Hardenbol, P., Willis, T. D., Yu, F. L., Yang, H. M., & Tam, P. K. H. (2003). The international HapMap project.
- Giles, T. D., Berk, B. C., Black, H. R., Cohn, J. N., Kostis, J. B., Izzo, J. L., & Weber, M. A. (2005). Expanding the definition and classification of hypertension. *The journal of clinical hypertension*, 7(9), 505-512.
- Glasser, S. P., Khodneva, Y., Lackland, D. T., Prineas, R., & Safford, M. M. (2013). Prehypertension and incident acute coronary heart disease in the REasons for Geographic and Racial Differences in Stroke (REGARDS) study. *American journal of hypertension*, 27(2), 245-251.
- Go, A. S., Bauman, M., King, S. M. C., Fonarow, G. C., Lawrence, W., Williams, K. A., & Sanchez, E. (2013). An effective approach to high blood pressure control. *Hypertension*,

- Goldstein, I. B., Shapiro, D., & Weiss, R. E. (2008). How family history and risk factors for hypertension relate to ambulatory blood pressure in healthy adults. *Journal of hypertension*, 26(2), 276-283.
- González, M., Gallardo, V., Rodríguez, N., Salomón, C., Westermeier, F., Gutiérrez, E. G., & Sobrevia, L. (2011). Insulin-stimulated L-arginine transport requires SLC7A1 gene expression and is associated with human umbilical vein relaxation. *Journal of cellular physiology*, 226(11), 2916-2924.
- Group, A. C. (2008). Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl J Med*, 2008(358), 2560-2572.
- Guo, X., Cheng, S., Taylor, K. D., Cui, J., Hughes, R., Quiñones, M. J., & Hsueh, W. (2005). Hypertension genes are genetic markers for insulin sensitivity and resistance. *Hypertension*, 45(4), 799-803.
- Haider, A. W., Larson, M. G., Franklin, S. S., & Levy, D. (2003). Systolic blood pressure, diastolic blood pressure, and pulse pressure as predictors of risk for congestive heart failure in the Framingham Heart Study. *Annals of internal medicine*, 138(1), 10-16.
- Hajjar, I., Miller, K., & Hirth, V. (2002). Age-related bias in the management of hypertension: a national survey of physicians' opinions on hypertension in elderly adults. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 57(8), M487-M491.
- Halimi, J. M., Giraudeau, B., Cacès, E., Nivet, H., & Tichet, J. (2002). The risk of hypertension in men: direct and indirect effects of chronic smoking. *Journal of hypertension*, 20(2), 187-193.
- Hamet, P., Pausova, Z., Dumas, P., Sun, Y. L., Tremblay, J., Pravenec, M., & Kren, V. (1998). Newborn and adult recombinant inbred strains: a tool to search for genetic determinants of target organ damage in hypertension. *Kidney international*, 53(6), 1488-1492.
- Hanachi, P. (2008). The Association of Hypertension with Major Risks Factors among University Putra Malaysia Retirees. *J. Med. Sci*, 8(3), 254-261.
- Harvey, J. M., & Beevers, D. G. (1990). Biochemical investigation of hypertension. *Annals of clinical biochemistry*, 27(4), 287-296.
- Hatzoglou, M., Fernandez, J., Yaman, I., & Closs, E. (2004). Regulation of cationic amino acid transport: the story of the CAT-1 transporter. *Annu. Rev. Nutr.*, 24, 377-399.
- He, D. H., Zhang, L. M., Lin, L. M., Ning, R. B., Wang, H. J., Xu, C. S., & Lin, J. X. (2014). Effects of Losartan and Amlodipine on Left Ventricular Remodeling and Function in Young Stroke-Prone Spontaneously Hypertensive Rats. *Acta Cardiologica Sinica*, 30(4), 316.

- He, D. H., Zhang, L. M., Ning, R. B., Wang, H. J., Xu, C. S., & Lin, J. X. (2013). Differential effects of antihypertensive treatments on apoptosis, oxidative stress, and expression of angiotensin receptors in the cerebral cortex from the onset of prehypertension and hypertension in stroke-prone spontaneous hypertensive rats. *NeuroReport*, 24(16), 911-917.
- He, J., & Whelton, P. K. (1997). Epidemiology and prevention of hypertension. *Medical Clinics of North America*, 81(5), 1077-1097.
- Hemmens, B., & Mayer, B. (1998). Enzymology of nitric oxide synthases. *Nitric oxide protocols*, 1-32.
- Henderson, S. O., & Bretsky, P. (2003). Common variants in CYP11B2 and AGTR1 could account for excess hypertension in African Americans. *Academic Emergency Medicine*, 10(5), 560.
- Hooper, T., & Mellor, A. (2011). Cardiovascular physiology at high altitude. *Journal of the Royal Army Medical Corps*, 157(1), 23-28.
- Horiuchi, M., Lehtonen, J. Y., & Daviet, L. (1999). Signaling mechanism of the AT2 angiotensin II receptor: crosstalk between AT1 and AT2 receptors in cell growth. *Trends in Endocrinology & Metabolism*, 10(10), 391-396.
- Houweling, A. C., van Borren, M. M., Moorman, A. F., & Christoffels, V. M. (2005). Expression and regulation of the atrial natriuretic factor encoding gene *Nppa* during development and disease. *Cardiovascular research*, 67(4), 583-593.
- Hsu, C. C. C., Bray, M. S., Kao, W. L., Pankow, J. S., Boerwinkle, E., & Coresh, J. (2006). Genetic variation of the renin-angiotensin system and chronic kidney disease progression in black individuals in the atherosclerosis risk in communities study. *Journal of the American Society of Nephrology*, 17(2), 504-512.
- Hu, B. C., Li, Y., Liu, M., Li, L. H., Sheng, C. S., Zhang, Y., & Wang, J. G. (2014). Blood pressure and urinary sodium excretion in relation to 16 genetic polymorphisms in the natriuretic peptide system in Chinese. *Endocrine journal*, 61(9), 861-874.
- Huai, P., Xun, H., Reilly, K. H., Wang, Y., Ma, W., & Xi, B. (2013). Physical Activity and Risk of Hypertension. *Hypertension*, HYPERTENSIONAHA-113.
- Imaizumi, T., Ando, M., Nakatochi, M., Maruyama, S., Yasuda, Y., Honda, H., & Nakashima, T. (2017). Association of interactions between dietary salt consumption and hypertension-susceptibility genetic polymorphisms with blood pressure among Japanese male workers. *Clinical and experimental nephrology*, 21(3), 457-464.
- Inagami, T. (1994). Atrial natriuretic factor as a volume regulator. *The Journal of Clinical Pharmacology*, 34(5), 424-426.

- Inoue, K., Naruse, K., Yamagami, S., Mitani, H., Suzuki, N., & Takei, Y. (2003). Four functionally distinct C-type natriuretic peptides found in fish reveal evolutionary history of the natriuretic peptide system. *Proceedings of the National Academy of Sciences*, 100(17), 10079-10084.
- Istrail, S., Sutton, G. G., Florea, L., Halpern, A. L., Mobarry, C. M., Lippert, R., & Flanigan, M. J. (2004). Whole-genome shotgun assembly and comparison of human genome assemblies. *Proceedings of the National Academy of Sciences*, 101(7), 1916-1921.
- Jeunemaitre, X., Soubrier, F., Kotelevtsev, Y. V., Lifton, R. P., Williams, C. S., Charu, A., & Corvol, P. (1992). Molecular basis of human hypertension: role of angiotensinogen. *Cell*, 71(1), 169-180.
- Jiang, Z., Zhao, W., Yu, F., & Xu, G. (2001). Association of angiotensin II type 1 receptor gene polymorphism with essential hypertension. *Chinese medical journal*, 114(12), 1249-1251.
- John, S. W., Krege, J. H., Oliver, P. M., Hagaman, J. R., Hodgin, J. B., Pang, S. C., & Smithies, O. (1995). Genetic decreases in atrial natriuretic peptide and salt-sensitive hypertension. *SCIENCE-NEW YORK THEN WASHINGTON-*, 679-679.
- Jujić, A., Leosdottir, M., Östling, G., Gudmundsson, P., Nilsson, P. M., Melander, O., & Magnusson, M. (2013). A genetic variant of the atrial natriuretic peptide gene is associated with left ventricular hypertrophy in a non-diabetic population—the Malmö preventive project study. *BMC medical genetics*, 14(1), 64.
- Kamha, E. S., Abdelmonsif, D. A., & Abdeldaim, T. M. (2013). Angiotensin Converting Enzyme (ACE) and Angiotensin II Type I Receptor (ATIR) Polymorphisms in Egyptian Preeclamptic Patients. *Clinical Medicine and Diagnostics*, 3(5), 123-128.
- Kang, Y. S. (2013). Obesity associated hypertension: new insights into mechanism. *Electrolytes & Blood Pressure*, 11(2), 46-52.
- Kannel, W. B. (1996). Blood pressure as a cardiovascular risk factor: prevention and treatment. *Jama*, 275(20), 1571-1576.
- Karppanen, H., & Mervaala, E. (2006). Sodium intake and hypertension. *Progress in cardiovascular diseases*, 49(2), 59-75.
- Kawaguchi, N., Hatta, K., & Nakanishi, T. (2013). 3D-culture system for heart regeneration and cardiac medicine. *BioMed research international*, 2013.
- Kearney, P. M., Whelton, M., Reynolds, K., Muntner, P., Whelton, P. K., & He, J. (2005). Global burden of hypertension: analysis of worldwide data. *The lancet*, 365(9455), 217-223.

- Kim, S., & Iwao, H. (2000). Molecular and cellular mechanisms of angiotensin II-mediated cardiovascular and renal diseases. *Pharmacological reviews*, 52(1), 11-34.
- Kishore, J., Gupta, N., Kohli, C., & Kumar, N. (2016). Prevalence of hypertension and determination of its risk factors in rural Delhi. *International journal of hypertension*, 2016.
- Klag, M. J., Whelton, P. K., Randall, B. L., Neaton, J. D., Brancati, F. L., Ford, C. E., & Stamler, J. (1996). Blood pressure and end-stage renal disease in men. *New England Journal of Medicine*, 334(1), 13-18.
- Kruzliak, P., Kovacova, G., Pechanova, O., & Balogh, S. (2013). Association between angiotensin II type 1 receptor polymorphism and sudden cardiac death in myocardial infarction. *Disease markers*, 35(5), 287-293.
- Kumar, A. (2014). Correlation between anthropometric measurement, lipid profile, dietary vitamins, serum antioxidants, lipoprotein (a) and lipid peroxides in known cases of 345 elderly hypertensive South Asian aged 56–64 y—A hospital based study. *Asian Pacific journal of tropical biomedicine*, 4, S189-S197.
- Kunes, J., & Zicha, J. (2009). The interaction of genetic and environmental factors in the etiology of hypertension. *Physiological research*, 58, S33.
- Kunz, R., Kreutz, R., Beige, J., Distler, A., & Sharma, A. M. (1997). Association between the angiotensinogen 235T-variant and essential hypertension in whites. *Hypertension*, 30(6), 1331-1337.
- Landsberg, L., Aronne, L. J., Beilin, L. J., Burke, V., Igel, L. I., Lloyd-Jones, D., & Sowers, J. (2013). Obesity-related hypertension: Pathogenesis, cardiovascular risk, and treatment—A position paper of the The Obesity Society and the American Society of Hypertension. *Obesity*, 21(1), 8-24.
- Laragh, J. H. (1962). Hormones and the pathogenesis of congestive heart failure: vasopressin, aldosterone, and angiotensin II. *Circulation*, 25(6), 1015-1023.
- Lassere, M. N., Johnson, K. R., Schiff, M., & Rees, D. (2012). Is blood pressure reduction a valid surrogate endpoint for stroke prevention? An analysis incorporating a systematic review of randomised controlled trials, a by-trial weighted errors-in-variables regression, the surrogate threshold effect (STE) and the Biomarker-Surrogacy (BioSurrogate) Evaluation Schema (BSSES). *BMC medical research methodology*, 12(1), 27.
- Lauer, M. S., Pashkow, F. J., Harvey, S. A., Marwick, T. H., & Thomas, J. D. (1995). Angiographic and prognostic implications of an exaggerated exercise systolic blood pressure response and rest systolic blood pressure in adults undergoing evaluation for suspected coronary artery disease. *Journal of the American College of Cardiology*, 26(7), 1630-1636.
- Lee, Y. Y., Lee, L., Hong, L., Welluppillai, V., & Kamarudin, A. (2015). Trio H's (Hyperglycemia, Hypertension, Hyperlipidemia): Undiagnosed Modifiable

- Risk Factors In Malaysia Rural Community. *Value in Health*, 18(7), A620-A621.
- Lemeshow, S., Hosmer, D. W., Klar, J., Lwanga, S. K., & World Health Organization. (1990). Adequacy of sample size in health studies.
- Levin, E. R., Gardner, D. G., & Samson, W. K. (1998). Natriuretic peptides. *New England Journal of Medicine*, 339(5), 321-328.
- Levin, K. A. (2006). Study design V. Case-control studies. *Evidence-based dentistry*, 7(3), 83.
- Li, Y. C., Qiao, G., Uskokovic, M., Xiang, W., Zheng, W., & Kong, J. (2004). Vitamin D: a negative endocrine regulator of the renin–angiotensin system and blood pressure. *The Journal of steroid biochemistry and molecular biology*, 89, 387-392.
- Lifton, R. P. (1995). Genetic determinants of human hypertension. *Proceedings of The National Academy of Sciences*, 92(19), 8545-8551.
- Lim, S. S., Vos, T., Flaxman, A. D., Danaei, G., Shibuya, K., Adair-Rohani, H., ... & Aryee, M. (2012). A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *The lancet*, 380(9859), 2224-2260.
- Lim, T. O., & Morad, Z. (2004). Prevalence, awareness, treatment and control of hypertension in the Malaysian adult population: results from the national health and morbidity survey 1996. *Singapore medical journal*, 45(1), 20-27.
- Lin, S. J., Lee, K. T., Lin, K. C., Cheng, K. H., Tsai, W. C., Sheu, S. H., & Lai, W. T. (2010). Prevalence of prehypertension and associated risk factors in a rural Taiwanese adult population. *International journal of cardiology*, 144(2), 269-273.
- Longo, D. L., Fauci, A. S., Kasper, D. L., Hauser, S. L., Jameson, J. L., & Loscalzo, J. (2012). *Harrison's Principles of Internal Medicine 18E Vol 2 EB*. McGraw Hill Professional.
- Lynch, A. I., Boerwinkle, E., Davis, B. R., Ford, C. E., Eckfeldt, J. H., Leiendecker-Foster, C., & Arnett, D. K. (2008). Pharmacogenetic association of the NPPA T2238C genetic variant with cardiovascular disease outcomes in patients with hypertension. *Jama*, 299(3), 296-307.
- Määttä, K., Kunnas, T., & Nikkari, S. T. (2013). Contribution of SLC7A1 genetic variant to hypertension, the TAMRISK study. *BMC medical genetics*, 14(1), 69.
- Maine, G. N., & Burstein, E. (2007). COMMD proteins: COMMMing to the scene. *Cellular and molecular life sciences*, 64(15), 1997-2005
- MacMahon, S., Peto, R., Collins, R., Godwin, J., Cutler, J., Sorlie, P., ... & Stamler, J. (1990). Blood pressure, stroke, and coronary heart disease: part 1, prolonged

differences in blood pressure: prospective observational studies corrected for the regression dilution bias. *The Lancet*, 335(8692), 765-774.

- Maine, G. N., & Burstein, E. (2007). COMMD proteins: COMMMing to the scene. *Cellular and molecular life sciences*, 64(15), 1997-2005.
- Malik, B., Price, S. R., Mitch, W. E., Yue, Q., & Eaton, D. C. (2006). Regulation of epithelial sodium channels by the ubiquitin-proteasome proteolytic pathway. *American Journal of Physiology-Renal Physiology*, 290(6), F1285-F1294.
- Mancia, G., De Backer, G., Dominiczak, A., Cifkova, R., Fagard, R., Germano, G., & Narkiewicz, K. (2007). 2007 Guidelines for the management of arterial hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *European heart journal*, 28(12), 1462-1536.
- Mann, C. J. (2003). Observational research methods. Research design II: cohort, cross sectional, and case-control studies. *Emergency medicine journal*, 20(1), 54-60.
- Mann, G. E., Yudilevich, D. L., & Sobrevia, L. (2003). Regulation of amino acid and glucose transporters in endothelial and smooth muscle cells. *Physiological reviews*, 83(1), 183-252.
- Manolio, T. A., Brooks, L. D., & Collins, F. S. (2008). A HapMap harvest of insights into the genetics of common disease. *The Journal of clinical investigation*, 118(5), 1590.
- Marfo, A. F., Owusu-Daaku, F. T., Addo, M. O., & Saana, I. I. (2014). Ghanaian hypertensive patients understanding of their medicines and life style modification for managing hypertension. *Int J Pharm Pharm Sci*, 6(4), 165-170.
- Matsuda, H., Cossette, S., Hamet, P., & Tremblay, J. (2012). Hypertension-related, calcium-regulated gene (HCaRG/COMMD5) inhibits kidney cancer development: A novel link between hypertension and risk of kidney cancer. *J Hypertens*, 30, e218. Matsuda, H., Hamet, P., & Tremblay, J. (2014). Hypertension-related, calcium-regulated gene (HCaRG/COMMD5) and kidney diseases: HCaRG accelerates tubular repair. *Journal of nephrology*, 27(4), 351-360.
- Matsuda, H., Hamet, P., & Tremblay, J. (2014). Hypertension-related, calcium-regulated gene (HCaRG/ COMMD5) and kidney diseases: HCaRG accelerates tubular repair. *J Nephrol*, 3(360), 27:351. doi:10.1007/s40620-014-0054-3
- McAllister, A., Atkinson, A., Johnston, G., Hadden, D., Bell, P., & McCance, D. (1999). Basal nitric oxide production is impaired in offspring of patients with essential hypertension. *Clinical science*, 97(2), 141-147.
- McArdle, W. D., Katch, F. I., & Katch, V. L. (2010). Exercise physiology: nutrition, energy, and human performance. Lippincott Williams & Wilkins.

- McCarthy, M. I., Abecasis, G. R., Cardon, L. R., Goldstein, D. B., Little, J., Ioannidis, J. P., & Hirschhorn, J. N. (2008). Genome-wide association studies for complex traits: consensus, uncertainty and challenges. *Nature reviews. Genetics*, 9(5), 356.
- Mechanic, L. E., & Hutter, C. M. (2015). Gene-environment interactions in human health. In *Environmental epigenetics* (pp. 229-249). Springer London.
- Mehio Sibai, A., Nasreddine, L., Mokdad, A. H., Adra, N., Tabet, M., & Hwalla, N. (2010). Nutrition transition and cardiovascular disease risk factors in Middle East and North Africa countries: reviewing the evidence. *Annals of Nutrition and Metabolism*, 57(3-4), 193-203.
- Mein, C. A., Caulfield, M. J., Dobson, R. J., & Munroe, P. B. (2004). Genetics of essential hypertension. *Human molecular genetics*, 13(suppl_1), R169-R175.
- Mercure, C., Yogi, A., Callera, G. E., Aranha, A. B., Bader, M., Ferreira, A. J., & Reudelhuber, T. L. (2008). Angiotensin (1-7) blunts hypertensive cardiac remodeling by a direct effect on the heart. *Circulation research*, 103(11), 1319-1326.
- Messner, B., & Bernhard, D. (2014). Smoking and cardiovascular disease. *Arteriosclerosis, thrombosis, and vascular biology*, 34(3), 509-515.
- Modesti, P. A., Morabito, M., Bertolozzi, I., Massetti, L., Panci, G., Lumachi, C., & Orlandini, S. (2006). Weather-Related Changes in 24-Hour Blood Pressure Profile. *Hypertension*, 47(2), 155-161.
- MOHAMED, A. B. (2012). National Health and Morbidity Survey 2011 (NHMS 2011). Vol 1: Methodology and general Findings.
- Mohd Yunus, A., Sherina, M. S., MZ, N. A., Rampal, L., & Tiew, K. H. (2004). Prevalence of cardiovascular risk factors in a rural community in Mukim Dengkil, Selangor. *Malaysian journal of nutrition*, 10(1), 5-11.
- Mokdad, A. H., Ford, E. S., Bowman, B. A., Dietz, W. H., Vinicor, F., Bales, V. S., & Marks, J. S. (2003). Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. *Jama*, 289(1), 76-79.
- Morley, M., Molony, C. M., Weber, T. M., Devlin, J. L., Ewens, K. G., Spielman, R. S., & Cheung, V. G. (2004). Genetic analysis of genome-wide variation in human gene expression. *Nature*, 430(7001), 743.
- Moser, M., & Franklin, S. S. (2007). Hypertension management: results of a new national survey for the hypertension education foundation: Harris interactive. *The Journal of Clinical Hypertension*, 9(5), 316-323.
- Moutsianas, L., & Morris, A. P. (2014). Methodology for the analysis of rare genetic variation in genome-wide association and re-sequencing studies of complex human traits. *Briefings in functional genomics*, 13(5), 362-370.

- Mulrow, P. J., & Ganong, W. F. (1961). Stimulation of Aldosterone Secretion by Angiotensin II: A Preliminary Report. *The Yale journal of biology and medicine*, 33(5), 386.
- Murray, C. J., & Lopez, A. D. (1997). Mortality by cause for eight regions of the world: Global Burden of Disease Study. *The lancet*, 349(9061), 1269-1276.
- Naing, C., Yeoh, P. N., Wai, V. N., Win, N. N., Kuan, L. P., & Aung, K. (2016). Hypertension in Malaysia: an analysis of trends from the National Surveys 1996 to 2011. *Medicine*, 95(2).
- National Health And Morbidity Survey. (2011). fact sheet: cardiovascular diseases, hypertension.
- Newton-Cheh, C., Johnson, T., Gateva, V., Tobin, M. D., Bochud, M., Coin, L., ... & Papadakis, K. (2009). Genome-wide association study identifies eight loci associated with blood pressure. *Nature genetics*, 41(6), 666-676.
- Niiranen, T. J., McCabe, E. L., Larson, M. G., Henglin, M., Lakdawala, N. K., Vasani, R. S., & Cheng, S. (2017). Heritability and risks associated with early onset hypertension: multigenerational, prospective analysis in the Framingham Heart Study. *bmj*, 357, j1949.
- Niu, W. (2011). The relationship between natriuretic peptide precursor a gene T2238C polymorphism and hypertension: a meta-analysis. *International journal of hypertension*, 2011.
- Nor, M., Safiza, N., Khor, G. L., Shahar, S., Kee, C. C., Haniff, J., & Ab Rahman, J. (2008). The Third National Health and Morbidity Survey (NHMS III) 2006: nutritional status of adults aged 18 years and above. *Malaysian Journal of Nutrition*, 14(2), 1-87.
- Norihiro, K., Sugiyama, T., Morita, H., Nabika, T., Kurihara, H., Yamori, Y., & Yazaki, Y. (2000). Genetic analysis of the atrial natriuretic peptide gene in essential hypertension. *Clinical science*, 98(3), 251-258.
- Nwankwo, T., Yoon, S. S., Burt, V., & Gu, Q. (2013). Hypertension among adults in the United States: National Health and Nutrition Examination Survey, 2011-2012. *NCHS data brief*, (133), 1-8.
- Nygren, P., Gylfe, E., Larsson, R., Johansson, H., Juhlin, C., Klareskoq, L., ... & Rastad, J. (1988). Modulation of the Ca²⁺-sensing function of parathyroid cells in vitro and in hyperparathyroidism. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, 968(2), 253-260.
- O'connor, D. T., Kailasam, M. T., Kennedy, B. P., Ziegler, M. G., Yanaihara, N., & Parmer, R. J. (2002). Early decline in the catecholamine release-inhibitory peptide catestatin in humans at genetic risk of hypertension. *Journal of hypertension*, 20(7), 1335-1345.

- Ono, K., Mannami, T., Baba, S., Yasui, N., Ogihara, T., & Iwai, N. (2003). Lack of association between angiotensin II type 1 receptor gene polymorphism and hypertension in Japanese. *Hypertension Research*, 26(2), 131-134.
- Oparil, S., Zaman, M. A., & Calhoun, D. A. (2003). Pathogenesis of hypertension. *Annals of internal medicine*, 139(9), 761-776.
- Osuji, C. U., Omejua, E. G., Onwubuya, E. I., & Ahaneku, G. I. (2012). Serum lipid profile of newly diagnosed hypertensive patients in Nnewi, South-East Nigeria. *International journal of hypertension*, 2012.
- Padmanabhan, S., Caulfield, M., & Dominiczak, A. F. (2015). Genetic and molecular aspects of hypertension. *Circulation research*, 116(6), 937-959.
- Padwal, R., Straus, S. E., & McAlister, F. A. (2001). Evidence based management of hypertension: cardiovascular risk factors and their effects on the decision to treat hypertension: evidence based review. *BMJ: British Medical Journal*, 322(7292), 977.
- Papathanasiou, G., Zerva, E., Zacharis, I., Papandreou, M., Papageorgiou, E., Tzima, C., ... & Evangelou, A. (2015). Association of high blood pressure with body mass index, smoking and physical activity in healthy young adults. *The open cardiovascular medicine journal*, 9, 5.
- Pardo, R., Málaga, S., Coto, E., Navarro, M., Alvarez, V., Espinosa, L., & Braga, S. (2003). Renin-angiotensin system polymorphisms and renal scarring. *Pediatric Nephrology*, 18(2), 110-114.
- Pauku, K., Backlund, M., De Boer, R. A., Kalkkinen, N., Kontula, K. K., & Lehtonen, J. Y. (2012). Regulation of AT1R expression through HuR by insulin. *Nucleic acids research*, 40(12), 5250-5261.
- Poch, E., González, D., Giner, V., Bragulat, E., Coca, A., & de la Sierra, A. (2001). Molecular basis of salt sensitivity in human hypertension. *Hypertension*, 38(5), 1204-1209.
- Pocock, S. J. (2013). *Clinical trials: a practical approach*. John Wiley & Sons.
- Porrello, E. R., D'amore, A., Curl, C. L., Allen, A. M., Harrap, S. B., Thomas, W. G., & Delbridge, L. M. (2009). Angiotensin II type 2 receptor antagonizes angiotensin II type 1 receptor-mediated cardiomyocyte autophagy. *Hypertension*, 53(6), 1032-1040.
- Potter, L. R., Abbey-Hosch, S., & Dickey, D. M. (2005). Natriuretic peptides, their receptors, and cyclic guanosine monophosphate-dependent signaling functions. *Endocrine reviews*, 27(1), 47-72.
- Primatesta, P., Falaschetti, E., Gupta, S., Marmot, M. G., & Poulter, N. R. (2001). Association between smoking and blood pressure. *Hypertension*, 37(2), 187-193.

- Pushkarev, D., Neff, N. F., & Quake, S. R. (2009). Single-molecule sequencing of an individual human genome. *Nature biotechnology*, 27(9), 847-850.
- Raihan, K., & Azmawati, M. N. (2013). Cigarette smoking and cardiovascular risk factor among male youth population. *Malaysian Journal of Public Health Medicine*, 13(1), 28-36.
- Ramachandran, V., Ismail, P., Stanslas, J., Shamsudin, N., Moin, S., & Mohd Jas, R. (2008). Association of insertion/deletion polymorphism of angiotensin-converting enzyme gene with essential hypertension and type 2 diabetes mellitus in Malaysian subjects. *Journal of the Renin-Angiotensin-Aldosterone System*, 9(4), 208-214.
- Rampal, L., Rampal, S., Azhar, M. Z., & Rahman, A. R. (2008). Prevalence, awareness, treatment and control of hypertension in Malaysia: a national study of 16,440 subjects. *Public health*, 122(1), 11-18.
- Rampal, L., Rampal, S., Khor, G. L., Zain, A. M., Ooyub, S. B., Rahmat, R. B., ... & Krishnan, J. (2007). A national study on the prevalence of obesity among 16,127 Malaysians. *Asia Pacific journal of clinical nutrition*, 16(3), 561-566.
- Rasool, A., Rahman, A., Ismail, R., Hatim, S., Abdullah, A., Singh, R., & Haron, R. (2000). Ethnic differences in response to non-selective beta-blockade among racial groups in Malaysia. *International journal of clinical pharmacology and therapeutics*, 38(5), 260-269.
- Reckelhoff, J. F. (2001). Gender differences in the regulation of blood pressure. *Hypertension*, 37(5), 1199-1208.
- Reckelhoff, J. F., & Fortepiani, L. A. (2004). Novel mechanisms responsible for postmenopausal hypertension. *Hypertension*, 43(5), 918-923.
- Rehman, A., Rasool, A., Naing, L., Roshan, T., & Rahman, A. (2007). Influence of the angiotensin II type I receptor gene 1166A> C polymorphism on BP and aortic pulse wave velocity among Malays. *Annals of human genetics*, 71(1), 86-95.
- Reja, V., Goodchild, A. K., Phillips, J. K., & Pilowsky, P. M. (2006). Upregulation of angiotensin AT1 receptor and intracellular kinase gene expression in hypertensive rats. *Clinical and experimental pharmacology and physiology*, 33(8), 690-695.
- Reza, C. M., Kabir, A. S. M. A., Biswas, T., Choudhury, K. N., Rahman, M. Z., Hussain, D. A., & Ghosh, S. K. (2014). Status of Lipid Profile among the Hypertensive Patients in Bangladesh. *University Heart Journal*, 9(1), 13-17.
- Riggen, S., & Agarwal, R. (2014). Hypertension and Chronic Kidney Disease. In *Management of Chronic Kidney Disease* (pp. 57-69). Springer Berlin Heidelberg.
- Roberts, R. (2014). Genetics of coronary artery disease. *Circulation research*, 114(12),

1890-1903.

- Rüster, C., & Wolf, G. (2006). Renin-angiotensin-aldosterone system and progression of renal disease. *Journal of the American Society of Nephrology*, 17(11), 2985-2991.
- Sandberg, K., & Ji, H. (2012). Sex differences in primary hypertension. *Biology of sex differences*, 3(1), 7.
- Schaefer, F., & Mehls, O. (2004). Hypertension in chronic kidney disease *Pediatric Hypertension* (pp. 371-387): Springer.
- Schlaich, M. P., Parnell, M. M., Ahlers, B. A., Finch, S., Marshall, T., Zhang, W.-Z., & Kaye, D. M. (2004). Impaired L-arginine transport and endothelial function in hypertensive and genetically predisposed normotensive subjects. *Circulation*, 110(24), 3680-3686.
- Senior, H., Anderson, C. S., Chen, M. H., Haydon, R., Walker, D., Fourie, D., ... & Gommans, J. (2006). Management of hypertension in the oldest old: a study in primary care in New Zealand. *Age and ageing*, 35(2), 178-182.
- Sethupathy, P., Borel, C., Gagnebin, M., Grant, G. R., Deutsch, S., Elton, T. S., ... & Antonarakis, S. E. (2007). Human microRNA-155 on chromosome 21 differentially interacts with its polymorphic target in the AGTR1 3' untranslated region: a mechanism for functional single-nucleotide polymorphisms related to phenotypes. *The American Journal of Human Genetics*, 81(2), 405-413.
- Sham, P. C., & Purcell, S. M. (2014). Statistical power and significance testing in large-scale genetic studies. *Nature reviews. Genetics*, 15(5), 335.
- Shihab, H. M., Meoni, L. A., Chu, A. Y., Wang, N. Y., Ford, D. E., Liang, K. Y., ... & Klag, M. J. (2012). Body mass index and risk of incident hypertension over the life course: the Johns Hopkins Precursors Study. *Circulation*, CIRCULATIONAHA-112.
- Shintani, M., Ikegami, H., Fujisawa, T., Kawaguchi, Y., Ohishi, M., Katsuya, T., ... & Ogihara, T. (2002). Leptin gene polymorphism is associated with hypertension independent of obesity. *The Journal of Clinical Endocrinology & Metabolism*, 87(6), 2909-2912.
- Shukla, A. N., Madan, T., Thakkar, B. M., Parmar, M. M., & Shah, K. H. (2015). Prevalence and predictors of undiagnosed hypertension in an apparently healthy western indian population. *Advances in Epidemiology*, 2015.
- 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*, 38(3), 268-277.
- Singh, R. B., Suh, I. L., Singh, V. P., Chaithiraphan, S., Laothavorn, P., Sy, R. G., ... & Sarraf-Zadigan, N. (2000). Hypertension and stroke in Asia: prevalence,

control and strategies in developing countries for prevention. *Journal of human hypertension*, 14(10/11), 749.

Solban, N., Dumas, P., Gossard, F., Sun, Y., Pravenec, M., Kren, V., ... & Tremblay, J. (2001). Chromosomal mapping of HCaRG, a novel hypertension-related, calcium-regulated gene. *Folia biologica*, 48(1), 9-14.

Solban, N., Jia, H. P., Richard, S., Tremblay, S., Devlin, A. M., Peng, J., ... & Lewanczuk, R. (2000). HCaRG, a novel calcium-regulated gene coding for a nuclear protein, is potentially involved in the regulation of cell proliferation. *Journal of Biological Chemistry*, 275(41), 32234-32243.

Song, W., Wang, H., & Wu, Q. (2015). Atrial natriuretic peptide in cardiovascular biology and disease (NPPA). *Gene*, 569(1), 1-6.

Sorof, J. M., Lai, D., Turner, J., Poffenbarger, T., & Portman, R. J. (2004). Overweight, ethnicity, and the prevalence of hypertension in school-aged children. *Pediatrics*, 113(3), 475-482.

Sowers, J. R., Epstein, M., & Frohlich, E. D. (2001). Diabetes, hypertension, and cardiovascular disease. *Hypertension*, 37(4), 1053-1059.

Staessen, J. A., Kuznetsova, T., Wang, J. G., Emelianov, D., Vlietinck, R., & Fagard, R. (1999). M235T angiotensinogen gene polymorphism and cardiovascular renal risk. *Journal of hypertension*, 17(1), 9-17.

Staessen, J. A., Wang, J.-G., Brand, E., Barlassina, C., Birkenhäger, W. H., Herrmann, S.-M., Bianchi, G. (2001). Effects of three candidate genes on prevalence and incidence of hypertension in a Caucasian population. *Journal of hypertension*, 19(8), 1349-1358.

Stanković, A., Živković, M., Glišić, S., & Alavantić, D. (2003). Angiotensin II type 1 receptor gene polymorphism and essential hypertension in Serbian population. *Clinica chimica acta*, 327(1), 181-185.

Suzana, S., Kee, C. C., Jamaludin, A. R., Noor Safiza, M. N., Khor, G. L., Jamaiyah, H., ... & Ahmad Fauzi, Y. (2012). The Third National Health and Morbidity Survey: prevalence of obesity, and abdominal obesity among the Malaysian elderly population. *Asia Pacific Journal of Public Health*, 24(2), 318-329.

Tabel, Y., Berdeli, A., Mir, S., Serdaroğlu, E., & Ytlmaz, E. (2005). Effects of genetic polymorphisms of the renin-angiotensin system in children with nephrotic syndrome. *Journal of the Renin-Angiotensin-Aldosterone System*, 6(3), 138-144.

Takei, Y., Kawakoshi, A., Tsukada, T., Yuge, S., Ogoshi, M., Inoue, K., ... & Miyano, S. (2006). Contribution of comparative fish studies to general endocrinology: structure and function of some osmoregulatory hormones. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, 305(9), 787-798.

- Tall, A. R., & Yvan-Charvet, L. (2015). Cholesterol, inflammation and innate immunity. *Nature reviews. Immunology*, 15(2), 104.
- Tang, F. Y., Liu, F. Y., & Xie, X. W. (2008). Association of angiotensin-converting enzyme and endothelial Nitric Oxide synthase gene polymorphisms with vascular disease in ESRD patients in a Chinese population. *Molecular and cellular biochemistry*, 319(1-2), 33-39.
- Thompson, M. D., Siminovitch, K. A., & Cole, D. E. (2008). G protein-coupled receptor pharmacogenetics. *Pharmacogenomics in Drug Discovery and Development: From Bench to Bedside*, 139-185.
- Touyz, R. M., & Schiffrin, E. L. (2000). Signal transduction mechanisms mediating the physiological and pathophysiological actions of angiotensin II in vascular smooth muscle cells. *Pharmacological reviews*, 52(4), 639-672.
- Tremblay, J., Verissimo, T., Champion, C. G., Cossette, S., & Hamet, P. (2016). [PS 01-20] Role of hcar9/commd5 in epithelial-mesenchymal transition during hypoxia. *Journal of hypertension*, 34, e101.
- Ueshima, H., Zhang, X. H., & Choudhury, S. R. (2000). Epidemiology of hypertension in China and Japan. *Journal of human hypertension*, 14(10/11), 765.
- Urbina, E. M., Khoury, P. R., McCoy, C. E., Dolan, L. M., Daniels, S. R., & Kimball, T. R. (2013). Triglyceride to HDL-C ratio and increased arterial stiffness in children, adolescents, and young adults. *Pediatrics*, 131(4), e1082-e1090.
- van Leeuwen, R., Ikram, M. K., Vingerling, J. R., Wittman, J. C., Hofman, A., & de Jong, P. T. (2003). Blood pressure, atherosclerosis, and the incidence of age-related maculopathy: the Rotterdam Study. *Investigative ophthalmology & visual science*, 44(9), 3771-3777.
- Verdecchia, P., Schillaci, G., Borgioni, C., Ciucci, A., Zampi, I., Battistelli, M., ... & Porcellati, C. (1995). Cigarette smoking, ambulatory blood pressure and cardiac hypertrophy in essential hypertension. *Journal of hypertension*, 13(10), 1209-1216.
- Vesely, D. L. (2006). Which of the cardiac natriuretic peptides is most effective for the treatment of congestive heart failure, renal failure and cancer?. *Clinical and experimental pharmacology and physiology*, 33(3), 169-176.
- Vesely, D. L., Norris, J. S., Walters, J. M., Jespersen, R. R., & Baeyens, D. A. (1987). Atrial natriuretic prohormone peptides 1-30, 31-67, and 79-98 vasodilate the aorta. *Biochemical and biophysical research communications*, 148(3), 1540-1548.
- Vincent, J. L., & Su, F. (2008). Physiology and pathophysiology of the vasopressinergic system. *Best practice & research Clinical anaesthesiology*, 22(2), 243-252.

- Walker, B. R., & Colledge, N. R. (2013). *Davidson's Principles and Practice of Medicine E-Book*: Elsevier Health Sciences.
- Wang, J.-G., & Li, Y. (2012). Characteristics of hypertension in the Chinese population. *Current hypertension reports*, 14(5), 410-415.
- Wang, L., Li, N., Yao, X., Chang, G., Zhang, D., Heizhati, M., . . . Kong, J. (2017). Detection of Secondary Causes and Coexisting Diseases in Hypertensive Patients: OSA and PA Are the Common Causes Associated with Hypertension. *BioMed research international*, 2017.
- Wang, W., Zee, R. L., & Morris, B. J. (1997). Association of angiotensin II type 1 receptor gene polymorphism with essential hypertension. *Clinical genetics*, 51(1), 31-34.
- Weber, M. A., Schiffrin, E. L., White, W. B., Mann, S., Lindholm, L. H., Kenerson, J. G., ... & Cohen, D. L. (2014). Clinical practice guidelines for the management of hypertension in the community. *The journal of clinical hypertension*, 16(1), 14-26.
- Weir, M. R., & Dzau, V. J. (1999). The renin-angiotensin-aldosterone system: a specific target for hypertension management. *American journal of hypertension*, 12(S9), 205S-213S.
- Whelton, P. K. (1994). Epidemiology of hypertension. *The lancet*, 344(8915), 101-106.
- Whelton, P. K. (2015). The elusiveness of population-wide high blood pressure control. *Annual review of public health*, 36, 109-130.
- Wilkening, S., Chen, B., Bermejo, J. L., & Canzian, F. (2009). Is there still a need for candidate gene approaches in the era of genome-wide association studies?. *Genomics*, 93(5), 415-419.
- Wilkins, M. R., Redondo, J., & Brown, L. A. (1997). The natriuretic-peptide family. *The Lancet*, 349(9061), 1307-1310.
- Williams, R. R., Hunt, S. C., Hasstedt, S. J., Berry, T. D., Barlow, G. K., Stults, B. M., & Kuida, H. (1988). Definition of Genetic Factors in Hypertension: A Search for Major Genes, Polygenes, and Homogeneous Subtypes. *Journal of cardiovascular pharmacology*, 12, S7-20.
- Williams, R. R., Hunt, S. C., Hasstedt, S. J., Hopkins, P. N., Wu, L. L., Berry, T. D., ... & Lifton, R. P. (1991). Are there interactions and relations between genetic and environmental factors predisposing to high blood pressure?. *Hypertension*, 18(3 Suppl), I29.
- Wolf-Maier, K., Cooper, R. S., Banegas, J. R., Giampaoli, S., Hense, H. W., Joffres, M., ... & Stegmayr, B. (2003). Hypertension prevalence and blood pressure levels in 6 European countries, Canada, and the United States. *Jama*, 289(18), 2363-2369.

- World Health Organization, & International Society of Hypertension Writing Group. (2003). 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. *Journal of hypertension*, 21(11), 1983-1992.
- World Health Organization. (1996). *Cancer pain relief: with a guide to opioid availability*. World Health Organization.
- World Health Organization. (2001). *The World Health Report 2001: Mental health: new understanding, new hope*. World Health Organization.
- World Health Organization. (2002). *The world health report 2002: reducing risks, promoting healthy life*. World Health Organization.
- World Health Organization. (2013). *A global brief on hypertension: silent killer, global public health crisis: World Health Day 2013*.
- Wu, Q., Xu-Cai, Y. O., Chen, S., & Wang, W. (2009). Corin: new insights into the natriuretic peptide system. *Kidney international*, 75(2), 142-146.
- Yagil, C., & Yagil, Y. (2009). The genomics of hypertension. *Essentials of genomic and personalized medicine*, 259-268.
- Yamazaki, T., Komuro, I., & Yazaki, Y. (1995). Molecular mechanism of cardiac cellular hypertrophy by mechanical stress. *Journal of molecular and cellular cardiology*, 27(1), 133-140.
- Yang, Z., & Kaye, D. M. (2006). Endothelial dysfunction and impaired L-arginine transport in hypertension and genetically predisposed normotensive subjects. *Trends in cardiovascular medicine*, 16(4), 118-124.
- Yang, Z., & Kaye, D. M. (2009). Mechanistic insights into the link between a polymorphism of the 3' UTR of the SLC7A1 gene and hypertension. *Human mutation*, 30(3), 328-333.
- Yang, Z., Venardos, K., Jones, E., Morris, B. J., Chin-Dusting, J., & Kaye, D. M. (2007). Identification of a Novel Polymorphism in the 3' UTR of the L-Arginine Transporter Gene SLC7A1. *Circulation*, 115(10), 1269-1274.
- Yen, S. T., Tan, A. K., & Feisul, M. I. (2016). Awareness and prevalence of diabetes, hypertension, and hypercholesterolemia in Malaysia. *Journal of diabetes*.
- Yin, M., Augustin, B., Fu, Z., Yan, M., Fu, A., & Yin, P. (2016). Geographic Distributions in Hypertension Diagnosis, Measurement, Prevalence, Awareness, Treatment and Control Rates among Middle-aged and Older Adults in China. *Scientific reports*, 6.
- Yu, X.-H., Fu, Y.-C., Zhang, D.-W., Yin, K., & Tang, C.-K. (2013). Foam cells in atherosclerosis. *Clinica chimica acta*, 424, 245-252.
- Zhang, H., Mo, X. B., Xu, T., Bu, X. Q., Lei, S. F., & Zhang, Y. H. (2015). Novel genes affecting blood pressure detected via gene-based association analysis.

G3: Genes, Genomes, Genetics, 5(6), 1035-1042.

Zhang, S., Mao, G., Zhang, Y., Tang, G., Wen, Y., Hong, X & Xu, X. (2005). Association between human atrial natriuretic peptide Val7Met polymorphism and baseline blood pressure, plasma trough irbesartan concentrations, and the antihypertensive efficacy of irbesartan in rural Chinese patients with essential hypertension. *Clinical therapeutics*, 27(11), 1774-1784.

Zhang, Y., Tang, W., Zhang, Y., Liu, L., & Zhang, L. (2017). Effects of integrated chronic care models on hypertension outcomes and spending: a multi-town clustered randomized trial in China. *BMC public health*, 17(1), 244.

Zhu, X., Chang, Y. P. C., Yan, D., Weder, A., Cooper, R., Luke, A., ... & Chakravarti, A. (2003). Associations between hypertension and genes in the renin-angiotensin system. *Hypertension*, 41(5), 1027-1034.

