International Journal of Biomedicine 12(3) (2022) 444-449
http://dx.doi.org/10.21103/Article12(3)_OA18

ORIGINAL ARTICLE

Mitochondrial DNA Mutations

INTERNATIONAL JOURNAL OF BIOMEDICINE

Mitochondrial tRNA$^{Leu(UUR)}$ Mutations in Patients with Essential Hypertension

Ikram Khan[1], An Lizhe[1*], Li Zhiqiang[2*]

[1]*Department of Microbiology, School of Life Sciences, Lanzhou University, Lanzhou, China*
[2]*Key Laboratory of School of Stomatology of State Ethnic Affairs Commission, Northwest Minzu University, Lanzhou, China*

Abstract

Arterial hypertension remains a major modifiable risk factor for cardiovascular disease. Previous studies have noted a maternal effect on blood pressure (BP). Mutations in mitochondrial DNA (mtDNA) have become an additional target of investigations on the missing BP heritability. The major objective of the present work was to investigate mutations in the tRNA$^{Leu(UUR)}$ gene in 20 Pakistani patients with essential hypertension (EH) and compare the amplified sequences to the mitochondrial reference sequence. DNA was extracted from their saliva, and the mitochondrial tRNA$^{Leu(UUR)}$ gene was amplified using PCR with specified primers. The present study did not find mutations in the tRNA$^{Leu(UUR)}$ gene in Pakistani EH patients. Further studies are needed for confirmation.(**International Journal of Biomedicine. 2022;12(3):444-449.**).

Keywords: essential hypertension • mitochondria • tRNA$^{Leu(UUR)}$ • mutations

For citation: Khan I, Lizhe A, Zhiqiang L. Mitochondrial tRNA$^{Leu(UUR)}$ Mutations in Patients with Essential Hypertension. International Journal of Biomedicine. 2022;12(3):444-449. doi:10.21103/Article12(3)_OA18

Abbreviations

AQ-seq, accurate quantification by sequencing; **BP**, blood pressure; **EH**, essential hypertension; **LVH**, left ventricular hypertrophy; **LVMI**, left ventricular mass index; **mtDNA**, mitochondrial DNA; **PCR**, polymerase chain reaction; **RAAS**, renin-angiotensin-aldosterone system; **ROS**, reactive oxidative species; **tRNA**, transfer RNA.

Introduction

Hypertension is an established risk factor for coronary heart disease, stroke, heart failure, renal dysfunction, and mortality. Worldwide, nearly 1.4 billion adults have hypertension.[1] Primary or essential hypertension (EH), hypertension due to undetermined causes, accounts for >90% of cases of hypertension. EH is a multifactorial disease involving interactions among genetic, environmental, and demographic factors.[2] It is well established that blood pressure (BP) is a heritable trait with genetic factors contributing about 30%-50% of its variance.[3,4] Improved techniques of genetic analysis, particularly genome-wide linkage analysis, have enabled the search for genes that contribute to the development of EH in the population.[5,6] However, most of the reported genetic variants were identified in studies of the nuclear genome;[6,7] only limited insights have been gained from investigations of the mitochondrial DNA (mtDNA). However, there is evidence to suggest that mitochondria and mtDNA may be important in hypertension. Mitochondria can regulate various aspects of vascular function, thereby being critical for the pathogenesis of hypertension.[8-10] Increasing evidence suggests that hypertensive myocardial injury is closely related to abnormal

Correspondence:

Li Zhiqiang, Lizhiqiang6767@163.com
An Lizhe, yxlzq@xbmu.edu.cn

mitochondrial function, energy metabolism, and homeostasis in cardiomyocytes.[11,12]

Reactive oxidative species (ROS) produced by mitochondria, as an important intracellular and intercellular regulatory factor, plays a key role in the pathogenesis of EH.[13-15] ROS can lead to the proliferation and migration of vascular smooth muscle cells and to damage of vascular endothelial cells, leading to vasodilatory dysfunction.[16] ROS is a major factor contributing to oxidative stress in the body. Several animal studies have shown that high BP can be alleviated by inhibiting the generation of mitochondrial ROS, providing clear evidence of the association between hypertension and oxidative stress.[17]

With respect to the mtDNA, a hallmark of its involvement is maternal inheritance. Multiple studies have identified strong maternal inheritance of BP,[18-20] suggesting that the mutation(s) in mtDNA is involved in the pathogenesis of hypertension.[10,21-24]

Mitochondrial tRNAs are the hot spots for mutations associated with hypertension.[25-30] Several hypertension-related tRNA mutations have been reported, including $tRNA^{Leu(UUR)}$ m.3243A>G and m.3253T>C; tRNAGly m.10003T>C; tRNAGlu m.14692A>G; and tRNAThr m.15909A>G. These mutations have structural and functional consequences, including the processing of RNA precursors, nucleotide modification, and aminoacylation.[31-34]

However, the molecular pathogenesis of hypertension in the Pakistani population remains poorly understood. The major objective of the present work was to investigate mutations in the $tRNA^{Leu(UUR)}$ gene in Pakistani EH patients and compare the amplified sequences to the mitochondrial reference sequence.

Materials and Methods

The study was approved by the Ethics Committee of the Hazara University (Mansehra, Khyber Pakhtunkhwa, Pakistan). Written informed consent was obtained from all participants.

The study included 20 male patients older than age 18 with EH who were enrolled in the cardiac ward at the Swat Medical Complex Hospital. Hypertension was defined as SBP ≥140 mmHg or DBP ≥90 mmHg, according to the Seventh Report of the Joint National Committee. All patients underwent the following examinations: assessment of traditional risk factors, physical examination, clinical and biochemical laboratory methods, 12-lead ECG, echocardiography, and 24-hour ambulatory blood pressure monitoring. Left ventricular hypertrophy (LVH) defined as LVMI of >115g/m^2 was detected in all patients.

Exclusion criteria were symptomatic hypertension, valvular heart disease, coronary artery disease, cardiac arrhythmia, diabetes, viral infections, chronic inflammatory and autoimmune diseases, and other co-morbidities

Collection of saliva sample

Before sample collection, the teeth of each patient were brushed, and a 5% sugar solution was given to them. Each patient received a brief instruction to spit the fluid into the sterile cups after rubbing it on their tongue for three to four minutes. The samples were transported to the Molecular Genetics Laboratory at Hazara University (Mansehra), where the samples were stored at -20 ºC for further analysis.

Molecular analysis

The whole DNA of buccal cavity epithelial cells was extracted, according to Aidar & Line [16]. For checking the quality of extracted DNA, agarose gel (0.5g of agarose dissolved in 29.4ml double distal water with 600ul of 50X TAE mixture) with 25ul ethidium bromide was used; then, 5µl of extracted DNA was dissolved with 2µl of loading dye, and the gel was run for 30 minutes at 60V before being photographed under ultraviolet light using the gel documentation system. After that, the DNA was kept at -20°C until it was time to process it.

PCR was used to amplify the desired gene. Initial denaturation was at 95ºC for 5 minutes, followed by denaturation at 95ºC for 5 minutes, annealing at 50ºC for 45 seconds, extension at 72ºC for 5 minutes, and final extension at 72ºC for 5 minutes; these were the thermal cycling conditions followed for 40 cycles. The final PCR findings were analyzed on a 1% agarose gel. The mixture was then treated with 12ml of ethidium bromide. For cooling, the melted mixture was kept at 25ºC. The agarose mixture previously had been put on the gel plate and allowed to solidify. We combined 15ml of PCR product with 2ml of DNA loading blue dye and put it into the agarose gel wells. A 60 Volute was supplied for 30 minutes in an electrophoresis approach until DNA fragments move from left to right. The bands that had been magnified were photographed and inspected under ultraviolet light. To purify the PCR amplification product from an agarose gel, a TIAN gel Midi purification Kit (Cat # DP20902) was used. PCR band including the $tRNA^{Leu(UUR)}$ gene was cut with a sterilized surgical blade and kept in labeled Eppendorf tubes until further processing.

Results

Each saliva sample was used to isolate genomic DNA, and we obtained reliable results from all the samples (Figure 1).

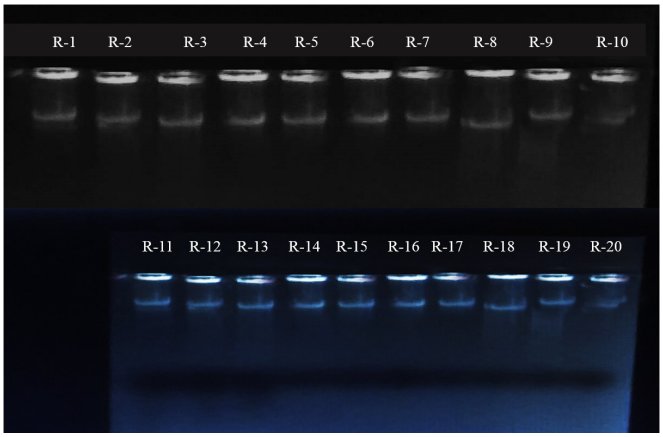

Fig. 1. *Total genomic DNA extracted from samples.*

Amplification of the tRNA$^{Leu(UUR)}$ gene

PCR was done using the common primer pair (Forward 5'-CAAATTCCTCCCTGTACGAAAGG-3';Reverse 5'-AATGAGGAGTAGGAGGTTGGCC-3') specific for a region containing tRNA$^{Leu(UUR)}$ gene. Figure 2 shows the amplification of a 279-base-pair fragment.

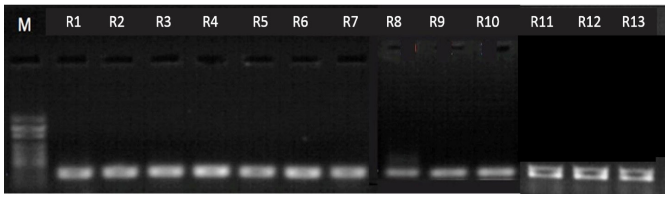

Fig. 2. *PCR results of samples (R-1 to R-13).* Amplification of a 279 bp fragment. *M, molecular size ladder, 1000 bp DNA ladder*

Sequencing analysis

Ten eluted DNA samples were sent to Macrogen Inc., Korea, for sequencing. The sequence was aligned with revised Cambridge Reference Sequence (rCRS) accession # NC-012920.1. The alignment was analyzed for mutations in tRNA$^{Leu(UUR)}$ gene.

Sequencing results

No mutations were discovered in any patient's tRNA$^{Leu(UUR)}$ gene**.** Although there was a two-nucleotide mismatch in the alignment when we studied the chromatogram of this sequencing result, we found that it was a technical error rather than an exact miss-match (Figure 3).

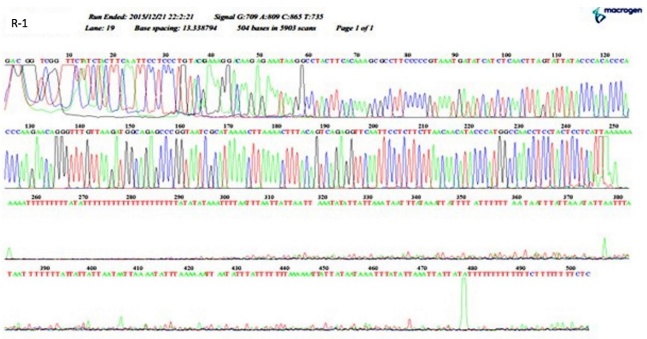

Fig. 3*. Chromatogram of the R-1 sample as a sequencing result provided by Macrogen Inc. (South Korea).*

The alignment of the patient's AQ-seq data is shown in Figure 4. Herein, we only show one sample of results because there were no mutations discovered in any EH patient's tRNA$^{Leu(UUR)}$ gene**.**

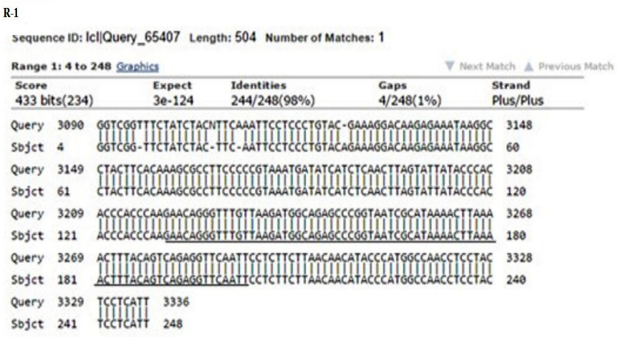

Fig. 4. *The alignment of the R-1 AQ-seq data. The Cambridge reference accession number was used to compare the sequence. The highlighted region indicates the tRNA$^{Leu(UUR)}$ gene.*

Discussion

EH is a multifactorial disease arising from the combined action of many genetic, environmental, demographic, vascular, and neuroendocrine factors.[35-37] EH is likely to be a polygenic disorder that results from the inheritance of a number of susceptibility genes. Candidate genes for hypertension include genes involved in regulating the RAAS (*AGT, ACE*), genes involved in sodium transport, signal transduction pathway, endothelium functioning.

The inheritance of hypertension has been debated for many years. Cross-sectional analysis has shown a familial aggregation of BP despite environmental factors.[38,39] The rate of genetic variance associated with EH has been estimated to range from 30% to 50%, and both maternal and paternal patterns of inheritance have been reported.[40-43] Correlations between BP in mothers and their offspring, as well as maternally inherited hypertension, have been reported in several studies.[42,44] Austin et al.[45] described a five-generation family with the A8344G mutation in the tRNALys gene and 19 affected members with a variant neurologic syndrome of ataxia, myopathy, hearing loss, and neuropathy. Along with axial lipomas and diabetes mellitus, hypertension was a frequent somatic feature, suggesting that mitochondrial mutations may contribute to hypertension in these patients. The mitochondrial genomes of 58 black Americans with hypertension who have progressed to end-stage renal disease (H-ESRD) and 58 normotensive individuals were analyzed in a study by Watson et al.[46]An A10398G DdeI mutation in the ND3 gene was significantly increased in the H-ESRD cohort, as was an HaeIII T6620C/G6260A double mutation in the CO1 gene.

Previous studies identified mtDNA mutations that are associated with maternally inherited hypertension, including tRNA^{Ile} m.4263A>G, m.4291T>C, m.4295A>G, tRNA^{Met} m.4435A>G, tRNA^{Ala} m.5655A>G, and tRNA^{Met}/tRNA^{Gln} m.4401A>G.[21,32-34,47-49] Majamaa-Voltti et al.[50] found that LVH was the most common cardiac abnormality in pedigrees with the 3243A>G mtDNA mutation.

Investigations of mitochondrial genome polymorphism are a fast-growing area of research. Each year, new links between mtDNA mutations and particular human diseases are discovered. It is not surprising that many of the reported mtDNA variants that affect mitochondrial function are associated with cardiovascular diseases because cells of the cardiovascular system are known for their high energy consumption and are significantly dependent on proper mitochondrial activity. Some of these correlations were

discovered to be potent enough to allow for the diagnostic use of mtDNA mutations. Future research will be necessary to turn the knowledge we have collected about mtDNA mutations linked to hypertension into brand-new treatments for hypertension and related conditions. Selective targeting of mitochondrial oxidative stress is one potential avenue for such treatments, and it also appears promising to rectify mitochondrial dynamics.

Conclusion

Recent studies have identified the mitochondria as the target and origin of major pathogenic pathways that lead to the progression of hypertension. Screening for mtDNA mutations in EH patients may provide further insight into the understanding of the pathophysiology of maternally inherited hypertension. However, the present study did not find mutations in the $tRNA^{Leu(UUR)}$ gene in Pakistani EH patients. Further studies are needed for confirmation.

Acknowledgments

We thank the individuals and their families for their participation in this study.

Data availability statement

The sequences have been uploaded to the National Center for Biotechnology Information using accession numbers: NC-012920.1.

Sources of Funding

The study was supported by the National Natural Science Foundation of China and the project approval number is 31760159.

Competing Interests

The authors declare that they have no competing interests.

References

1. Mills KT, Bundy JD, Kelly TN, Reed JE, Kearney PM, Reynolds K, Chen J, He J. Global Disparities of Hypertension Prevalence and Control: A Systematic Analysis of Population-Based Studies From 90 Countries. Circulation. 2016 Aug 9;134(6):441-50. doi: 10.1161/CIRCULATIONAHA.115.018912. PMID: 27502908; PMCID: PMC4979614.
2. Kunes J, Zicha J. Developmental windows and environment as important factors in the expression of genetic information: a cardiovascular physiologist's view. Clin Sci (Lond). 2006 Nov;111(5):295-305. doi: 10.1042/CS20050271. PMID: 17034366.
3. Wang X, Ding X, Su S, Harshfield G, Treiber F, Snieder H. Genetic influence on blood pressure measured in the office, under laboratory stress and during real life. Hypertens Res. 2011 Feb;34(2):239-44. doi: 10.1038/hr.2010.218. Epub 2010 Nov 11. PMID: 21068740; PMCID: PMC3713474.
4. Wang X, Ding X, Su S, Yan W, Harshfield G, Treiber F, Snieder H. Genetic influences on daytime and night-time blood pressure: similarities and differences. J Hypertens. 2009 Dec;27(12):2358-64. doi: 10.1097/HJH.0b013e328330e84d. PMID: 19657283; PMCID: PMC3713486.
5. Fan JB, Chen X, Halushka MK, Berno A, Huang X, Ryder T, Lipshutz RJ, Lockhart DJ, Chakravarti A. Parallel genotyping of human SNPs using generic high-density oligonucleotide tag arrays. Genome Res. 2000 Jun;10(6):853-60. doi: 10.1101/gr.10.6.853. PMID: 10854416; PMCID: PMC310915.
6. Oliphant A, Barker DL, Stuelpnagel JR, Chee MS. BeadArray technology: enabling an accurate, cost-effective approach to high-throughput genotyping. Biotechniques. 2002 Jun;Suppl:56-8, 60-1. PMID: 12083399.
7. Padmanabhan S, Melander O, Johnson T, Di Blasio AM, Lee WK, Gentilini D, et al.; Global BPgen Consortium, Munroe P, Caulfield MJ, Zanchetti A, Dominiczak AF. Genome-wide association study of blood pressure extremes identifies variant near UMOD associated with hypertension. PLoS Genet. 2010 Oct
8. Dromparis P, Michelakis ED. Mitochondria in vascular health and disease. Annu Rev Physiol. 2013;75:95-126. doi: 10.1146/annurev-physiol-030212-183804. Epub 2012 Nov 16. PMID: 23157555.
9. Eirin A, Lerman A, Lerman LO. Mitochondria: a pathogenic paradigm in hypertensive renal disease. Hypertension. 2015 Feb;65(2):264-70. doi: 10.1161/HYPERTENSIONAHA.114.04598. Epub 2014 Nov 17. PMID: 25403611; PMCID: PMC4289015.
10. Marian AJ. Mitochondrial genetics and human systemic hypertension. Circ Res. 2011 Apr 1;108(7):784-6. doi: 10.1161/CIRCRESAHA.111.242768. PMID: 21454791.
11. Eirin A, Lerman A, Lerman LO. Mitochondrial injury and dysfunction in hypertension-induced cardiac damage. Eur Heart J. 2014 Dec 7;35(46):3258-66. doi: 10.1093/eurheartj/ehu436. Epub 2014 Nov 10. PMID: 25385092; PMCID: PMC4258226.
12. Gong S, Peng Y, Jiang P, Wang M, Fan M, Wang X, Zhou H, Li H, Yan Q, Huang T, Guan MX. A deafness-associated tRNAHis mutation alters the mitochondrial function, ROS production and membrane potential. Nucleic Acids Res. 2014 Jul;42(12):8039-48. doi: 10.1093/nar/gku466. Epub 2014 Jun 11. PMID: 24920829; PMCID: PMC4081083.
13. Wallace DC. Mitochondrial diseases in man and mouse. Science. 1999 Mar 5;283(5407):1482-8. doi: 10.1126/science.283.5407.1482. PMID: 10066162.
14. Hirano M, Davidson M, DiMauro S. Mitochondria and the heart. Curr Opin Cardiol. 2001 May;16(3):201-10. doi: 10.1097/00001573-200105000-00008. PMID: 11357017.
15. Lassègue B, Griendling KK. Reactive oxygen species in hypertension; An update. Am J Hypertens. 2004 Sep;17(9):852-60. doi: 10.1016/j.amjhyper.2004.02.004. PMID: 15363831.
16. Taniyama Y, Griendling KK. Reactive oxygen species in the vasculature: molecular and cellular mechanisms. Hypertension. 2003 Dec;42(6):1075-81. doi: 10.1161/01.HYP.0000100443.09293.4F. Epub 2003 Oct 27. PMID: 14581295.

17. Liang M. Hypertension as a mitochondrial and metabolic disease. Kidney Int. 2011 Jul;80(1):15-6. doi: 10.1038/ki.2011.84. PMID: 21673736.
18. Hutchinson J, Crawford MH. Genetic determinants of blood pressure level among the Black Caribs of St. Vincent. Hum Biol. 1981 Sep;53(3):453-66. PMID: 7309028.
19. DeStefano AL, Gavras H, Heard-Costa N, Bursztyn M, Manolis A, Farrer LA, Baldwin CT, Gavras I, Schwartz F. Maternal component in the familial aggregation of hypertension. Clin Genet. 2001 Jul;60(1):13-21. doi: 10.1034/j.1399-0004.2001.600103.x. PMID: 11531965.
20. Yang Q, Kim SK, Sun F, Cui J, Larson MG, Vasan RS, Levy D, Schwartz F. Maternal influence on blood pressure suggests involvement of mitochondrial DNA in the pathogenesis of hypertension: the Framingham Heart Study. J Hypertens. 2007 Oct;25(10):2067-73. doi: 10.1097/HJH.0b013e328285a36e. PMID: 17885549.
21. Wilson FH, Hariri A, Farhi A, Zhao H, Petersen KF, Toka HR, Nelson-Williams C, Raja KM, Kashgarian M, Shulman GI, Scheinman SJ, Lifton RP. A cluster of metabolic defects caused by mutation in a mitochondrial tRNA. Science. 2004 Nov 12;306(5699):1190-4. doi: 10.1126/science.1102521. Epub 2004 Oct 21. PMID: 15498972; PMCID: PMC3033655.
22. Watson B Jr, Khan MA, Desmond RA, Bergman S. Mitochondrial DNA mutations in black Americans with hypertension-associated end-stage renal disease. Am J Kidney Dis. 2001 Sep;38(3):529-36. doi: 10.1053/ajkd.2001.26848. PMID: 11532685.
23. Zinner SH, Levy PS, Kass EH. Familial aggregation of blood pressure in childhood. N Engl J Med. 1971 Feb 25;284(8):401-4. doi: 10.1056/NEJM197102252840801. PMID: 5540477.
24. Havlik RJ, Feinleib M. Epidemiology and genetics of hypertension. Hypertension. 1982 Sep-Oct;4(5 Pt 2):III121-7. doi: 10.1161/01.hyp.4.5_pt_2.iii121. PMID: 7049926.
25. Ruiz-Pesini E, Lott MT, Procaccio V, Poole JC, Brandon MC, Mishmar D, Yi C, Kreuziger J, Baldi P, Wallace DC. An enhanced MITOMAP with a global mtDNA mutational phylogeny. Nucleic Acids Res. 2007 Jan;35(Database issue):D823-8. doi: 10.1093/nar/gkl927. Epub 2006 Dec 18. PMID: 17178747; PMCID: PMC1781213.
26. Abbott JA, Francklyn CS, Robey-Bond SM. Transfer RNA and human disease. Front Genet. 2014 Jun 3;5:158. doi: 10.3389/fgene.2014.00158. PMID: 24917879; PMCID: PMC4042891.
27. Liu C, Yang Q, Hwang SJ, Sun F, Johnson AD, Shirihai OS, Vasan RS, Levy D, Schwartz F. Association of genetic variation in the mitochondrial genome with blood pressure and metabolic traits. Hypertension. 2012 Oct;60(4):949-56. doi: 10.1161/HYPERTENSIONAHA.112.196519. Epub 2012 Sep 4. PMID: 22949535; PMCID: PMC3753106.
28. Liu Y, Li Y, Wang X, Ma Q, Zhu C, Li Z, Yin T, Yang J, Chen Y, Guan M. Mitochondrial tRNA mutations in Chinese hypertensive individuals. Mitochondrion. 2016 May;28:1-7. doi: 10.1016/j.mito.2016.02.007. Epub 2016 Feb 26. PMID: 26923935.
29. Jia Z, Wang X, Qin Y, Xue L, Jiang P, Meng Y, Shi S, Wang Y, Qin Mo J, Guan MX. Coronary heart disease is associated with a mutation in mitochondrial tRNA. Hum Mol Genet. 2013 Oct 15;22(20):4064-73. doi: 10.1093/hmg/ddt256. Epub 2013 Jun 4. PMID: 23736300; PMCID: PMC3781636.
30. Xue L, Wang M, Li H, Wang H, Jiang F, Hou L, Geng J, Lin Z, Peng Y, Zhou H, Yu H, Jiang P, Mo JQ, Guan MX. Mitochondrial tRNA mutations in 2070 Chinese Han subjects with hypertension. Mitochondrion. 2016 Sep;30:208-21. doi: 10.1016/j.mito.2016.08.008. Epub 2016 Aug 17. PMID: 27544295.
31. Zhou M, Wang M, Xue L, Lin Z, He Q, Shi W, Chen Y, Jin X, Li H, Jiang P, Guan MX. A hypertension-associated mitochondrial DNA mutation alters the tertiary interaction and function of $tRNA^{Leu(UUR)}$. J Biol Chem. 2017 Aug 25;292(34):13934-13946. doi: 10.1074/jbc.M117.787028. Epub 2017 Jul 5. PMID: 28679533; PMCID: PMC5572913.
32. Li R, Liu Y, Li Z, Yang L, Wang S, Guan MX. Failures in mitochondrial tRNAMet and tRNAGln metabolism caused by the novel 4401A>G mutation are involved in essential hypertension in a Han Chinese Family. Hypertension. 2009 Aug;54(2):329-37. doi: 10.1161/HYPERTENSIONAHA.109.129270. Epub 2009 Jun 22. PMID: 19546379; PMCID: PMC2907155.
33. Wang S, Li R, Fettermann A, Li Z, Qian Y, Liu Y, Wang X, Zhou A, Mo JQ, Yang L, Jiang P, Taschner A, Rossmanith W, Guan MX. Maternally inherited essential hypertension is associated with the novel 4263A>G mutation in the mitochondrial tRNAIle gene in a large Han Chinese family. Circ Res. 2011 Apr 1;108(7):862-70. doi: 10.1161/CIRCRESAHA.110.231811. PMID: 21454794.
34. Jiang P, Wang M, Xue L, Xiao Y, Yu J, Wang H, Yao J, Liu H, Peng Y, Liu H, Li H, Chen Y, Guan MX. A Hypertension-Associated tRNAAla Mutation Alters tRNA Metabolism and Mitochondrial Function. Mol Cell Biol. 2016 Jun 29;36(14):1920-30. doi: 10.1128/MCB.00199-16. PMID: 27161322; PMCID: PMC4936059.
35. Gong M, Hubner N. Molecular genetics of human hypertension. Clin Sci (Lond). 2006 Mar;110(3):315-26. doi: 10.1042/CS20050208. PMID: 16464173.
36. Abais-Battad JM, Dasinger JH, Fehrenbach DJ, Mattson DL. Novel adaptive and innate immunity targets in hypertension. Pharmacol Res. 2017 Jun;120:109-115. doi: 10.1016/j.phrs.2017.03.015. Epub 2017 Mar 20. PMID: 28336371; PMCID: PMC5497567.
37. Zhang RM, McNerney KP, Riek AE, Bernal-Mizrachi C. Immunity and Hypertension. Acta Physiol (Oxf). 2021 Jan;231(1):e13487. doi: 10.1111/apha.13487. Epub 2020 Jun 25. PMID: 32359222; PMCID: PMC7606441.
38. Pérusse L, Rice T, Bouchard C, Vogler GP, Rao DC. Cardiovascular risk factors in a French-Canadian population: resolution of genetic and familial environmental effects on blood pressure by using extensive information on environmental correlates. Am J Hum Genet. 1989 Aug;45(2):240-51. PMID: 2757030; PMCID: PMC1683357.
39. Fuentes RM, Notkola IL, Shemeikka S, Tuomilehto J, Nissinen A. Familial aggregation of blood pressure: a population-based family study in eastern Finland. J Hum Hypertens. 2000 Jul;14(7):441-5. doi: 10.1038/sj.jhh.1001049. PMID: 10918549.
40. Longini IM Jr, Higgins MW, Hinton PC, Moll PP, Keller JB. Environmental and genetic sources of familial aggregation of blood pressure in Tecumseh, Michigan. Am J Epidemiol. 1984 Jul;120(1):131-44. doi: 10.1093/oxfordjournals.aje.a113862. PMID: 6741914.
41. Hunt SC, Hasstedt SJ, Kuida H, Stults BM, Hopkins PN,

Williams RR. Genetic heritability and common environmental components of resting and stressed blood pressures, lipids, and body mass index in Utah pedigrees and twins. Am J Epidemiol. 1989 Mar;129(3):625-38. doi: 10.1093/oxfordjournals.aje.a115175. PMID: 2916556.
42. DeStefano AL, Gavras H, Heard-Costa N, Bursztyn M, Manolis A, Farrer LA, Baldwin CT, Gavras I, Schwartz F. Maternal component in the familial aggregation of hypertension. Clin Genet. 2001 Jul;60(1):13-21. doi: 10.1034/j.1399-0004.2001.600103.x. PMID: 11531965.
43. Uehara Y, Shin WS, Watanabe T, Osanai T, Miyazaki M, Kanase H, Taguchi R, Sugano K, Toyo-Oka T. A hypertensive father, but not hypertensive mother, determines blood pressure in normotensive male offspring through body mass index. J Hum Hypertens. 1998 Jul;12(7):441-5. doi: 10.1038/sj.jhh.1000646. PMID: 9702929.
44. Bengtsson B, Thulin T, Scherstén B. Familial resemblance in casual blood pressure--a maternal effect? Clin Sci (Lond). 1979 Dec;57 Suppl 5:279s-281s. doi: 10.1042/cs057279s. PMID: 540443.
45. Austin SA, Vriesendorp FJ, Thandroyen FT, Hecht JT, Jones OT, Johns DR. Expanding the phenotype of the 8344 transfer RNAlysine mitochondrial DNA mutation. Neurology. 1998 Nov;51(5):1447-50. doi: 10.1212/wnl.51.5.1447. PMID: 9818878.
46. Watson B Jr, Khan MA, Desmond RA, Bergman S. Mitochondrial DNA mutations in black Americans with hypertension-associated end-stage renal disease. Am J Kidney Dis. 2001 Sep;38(3):529-36. doi: 10.1053/ajkd.2001.26848. PMID: 11532685.
47. Li Z, Liu Y, Yang L, Wang S, Guan MX. Maternally inherited hypertension is associated with the mitochondrial tRNA(Ile) A4295G mutation in a Chinese family. Biochem Biophys Res Commun. 2008 Mar 21;367(4):906-11. doi: 10.1016/j.bbrc.2007.12.150. Epub 2008 Jan 3. PMID: 18177739.
48. Liu Y, Li R, Li Z, Wang XJ, Yang L, Wang S, Guan MX. Mitochondrial transfer RNAMet 4435A>G mutation is associated with maternally inherited hypertension in a Chinese pedigree. Hypertension. 2009 Jun;53(6):1083-90. doi: 10.1161/HYPERTENSIONAHA.109.128702. Epub 2009 Apr 27. PMID: 19398658; PMCID: PMC2907152.
49. Liu Y, Chen Y. Mitochondrial tRNA Mutations Associated With Essential Hypertension: From Molecular Genetics to Function. Front Cell Dev Biol. 2021 Jan 22;8:634137. doi: 10.3389/fcell.2020.634137. PMID: 33585472; PMCID: PMC7874112.
50. Majamaa-Voltti K, Peuhkurinen K, Kortelainen ML, Hassinen IE, Majamaa K. Cardiac abnormalities in patients with mitochondrial DNA mutation 3243A>G. BMC Cardiovasc Disord. 2002 Aug 1;2:12. doi: 10.1186/1471-2261-2-12. PMID: 12150714; PMCID: PMC119851.