Association between *ADD1* Gly460Trp Polymorphism and Essential Hypertension in Han Chinese

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Abstract

**Background:** The *ADD1* Gly460Trp polymorphism has been linked to essential hypertension (EH) in multiple populations, but the results were inconsistent. The goal of our study is to investigate the contribution of *ADD1* Gly460Trp polymorphism and environmental factors to the risk of EH.

**Methods:** We conducted a case-control study including 1020 hypertensive cases and 1020 controls, and the gender and age were well matched between hypertensive and control groups. Blood samples and participants information were also collected. Using the melting temperature shift technology, the *ADD1* Gly460Trp polymorphism was genotyped among all subjects. Multifactor dimensionality reduction (MDR) was used to identify the interactions among the *ADD1* Gly460Trp polymorphism and the nongenetic factors.

**Results:** Our results showed that body mass index (BMI), total cholesterol, triglycerides, and drinking were significantly associated with EH (P<0.05). In addition, the Gly460Trp polymorphism was found significantly associated with hypertension at allelic level (P<0.01; OR=0.85; 95%CI=0.75-0.96). A breakdown association analysis by gender showed the Gly460Trp polymorphism was associated with EH only in female (P<0.01; OR=0.79; 95%CI=0.68-0.92). MDR analysis indicated that there was an interaction among BMI, high density lipoprotein, drinking, and rs4961 involved in the risk of EH.

**Conclusion:** The present study indicated the Gly460Trp polymorphism was associated with EH in female Han Chinese, which might contribute to EH via interactions with non-genetic factors.

**Keywords:** Essential hypertension; *ADD1*; Polymorphism; Interaction

**Abbreviation:** EH: Essential Hypertension; *ADD1*: α-adducin; TC: Total Cholesterol; TG: triglycerides; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein

**Introduction**

Essential hypertension (EH) is an important worldwide public health issue which contributes to the burden of heart disease, stroke and kidney failure and premature mortality and disability. It disproportionately affects populations in low- and middle-income countries where health systems are weak. EH is a complex disorder resulting from genetic and environmental factors, as well as their interactions [1,2]. Approximately 20-60% of the blood pressure variability in general population is heritable [3].

Human *ADD1* gene, located on chromosome 4p16.3, encodes one of adducin subunits (α-adducin) [4]. Adducin modulates the surface expression of multiple transporters and ion pumps, and thus regulates cellular signal transduction and cytolemma ion transport [5]. Human and animal model studies have found that *ADD1* gene is a candidate gene for EH [5,6]. One well-studied polymorphism in *ADD1* gene is a missense mutation substituting thymine (T) for guanine (G) at position 614 of the 10th exon, resulting in an expressed *ADD1* with Trp in place of the wild type Gly at amino acid 460 (Gly460Trp, rs4961), which was first described by Cusi et al. [7]. Then, a large number of studies were conducted on the association of Gly460Trp with EH. Consequently, *ADD1* 460Trp allele was reported to be a risk factor for EH in South European [7], Japanese [8] and Mongolian [9], inversely a protective factor in Scandinavian [10] and UK [11], but not associated with EH in Australian [12] and South Korean [13]. However, epidemiological studies have shown that the contribution of *ADD1* Gly460Trp mutation to hypertension varies among different ethnic groups.

To convince the association of this mutation with EH, several meta-analyses were recently performed from different angle [14-17]. Most of these analyses fail to provide evidence for the genetic association between *ADD1* Gly460Trp mutation and EH, but it is suggested that the 460Trp allele might be a risk factor of EH in Han Chinese population [17]. Up to now, the studies performed to explore the association of *ADD1* Gly460Trp mutation with EH were mostly conducted in a small sample size. Therefore, aimed at clarifying the role of *ADD1* Gly460Trp in EH and exploring the interaction between this mutation and environmental factors on EH, we conducted a case-control study in a large, homogeneous sample of Han Chinese population.

**Materials and Methods**

**Sample collection**

This study comprised 1020 cases (mean age, 58.5 ± 6.4 years; female, 60.4%). The present study was approved by the Ethical Committee of the Zhejiang Provincial Key Laboratory of Pathophysiology of Ningbo University. All participants signed written informed consent.

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**Abbreviations:** EH: Essential Hypertension; *ADD1*: α-adducin; TC: Total Cholesterol; TG: triglycerides; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein.

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including 339 males and 681 females) and 1020 controls (58.3 ± 6.5 years; 350 males and 670 females) collected from the community residents in Ningbo city of Zhejiang province, China. All individuals are Han Chinese living in Ningbo city for at least three generations, and their ages range from 35 to 70 years. Hypertensive patients were defined according to the golden standard [18]. All hypertensives have received antihypertensive medications for more than three months or have at least three consecutive records of systolic blood pressure (SBP) >140 mmHg and/or diastolic blood pressure (DBP) >90 mmHg (European Society of Hypertension-European Society of Cardiology Guidelines, 2003). Patients had SBP <120 mmHg and DBP <80 mmHg and had no family history of hypertension in the first degree relatives were recruited as controls. None of the controls has received antihypertensive therapy. The gender and age of controls were well matched with EH cases. All the individuals don’t have a history of diabetes mellitus, secondary hypertension, myocardial infarction, stroke, renal failure, drug abuse and other serious diseases. A calibrated mercury sphygmomanometer with appropriate adult cuff size was applied to measure blood pressures according to a standard protocol recommended by the American Heart Association [19]. Blood pressures were measured in supine position by two trained observers at an interval of at least 10 minutes. Blood samples were collected in 3.2% citrate sodium-treated tubes and then stored at -80 °C for DNA extraction. The study protocol was approved by the ethical committee of Ningbo University. The informed written consent was obtained from all subjects.

Phenotypes collection

Blood samples were obtained after a 12 h overnight fast from the antecubital vein using vacutainer tubes containing EDTA. Plasma levels of total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) concentrations were enzymatically measured using CX7 biochemistry analyzer (Beckman, Fullerton, CA). Clinical information including body mass index (BMI), and weekly alcohol and cigarettes consumption were also obtained. In this study, who drank ≥70 g alcohol per week for more than 1 year was defined as individuals with alcohol abuse. Moreover, who smoked ≥ 70 cigarettes per week for more than 1 year were defined as individuals with smoking habit.

Single Nucleotide Polymorphism (SNP) genotyping

Human genomic DNA was prepared from peripheral blood samples using the nucleic acid extraction automatic analyzer (Lab-Aid 820, Xiamen City, China). DNA was quantified using the PicoGreen® double strand DNA (dsDNA) Quantiﬁcation Kit (Molecular Probes, Inc. Eugene, USA). Amplification was performed on the ABI GeneAmp® PCR System 9700 Dual 96-Well Sample Block Module (Applied Biosystems, Foster City, CA, USA) for the polymerase chain reaction (PCR), and the standard 96-well plates (Bioplastics, Landgraaf, Netherlands) was sealed with Cyclerseal Sealing Film (Platemax). PCR conditions included an initial enzyme heat-activation step of 95°C for 15 min, followed by 35 amplification cycles (including 95°C for 20 sec, 59°C for 60 sec, and primer extension at 72°C for 30 sec), and a final extension for 7 minutes at 72°C. PCR product for genotyping was performed on LightCycler® 480 II Real-Time PCR (Roche Diagnostics Ltd., Rotkreuz, Switzerland) according to Melting Temperature shift method [20]. The PCR primers of SNP genotyping were described in Table S1.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) was analyzed using the Arlequin program (version 3.5) [21]. Statistical analyses were performed to investigate the association of ADD1 Gly460Trp polymorphism and metabolic profile with EH using the PASW Statistics 18.0 software (SPSS, Inc., Somers, NY, USA). Either Pearson chi-square or Fisher exact test was used for the association of EH with categorical variables including gender, smoking, drinking, genotype, and allele frequencies. The odds ratio (OR) with 95% confidence interval (95% CI) were calculated through an online tool (http://faculty.vassar.edu/lowry/odds2x2.html). Two sample t-test was applied for the association of EH with continuous variables including age, BMI, TC, TG, HDL, and LDL. Multifactor dimensionality reduction (MDR) was used to identify and characterize interactions among ADD1 Gly460Trp polymorphism and the nongenetic factors, including BMI, serum HDL, LDL, TC, and TG level, as well as the distribution of smoking and drinking [22]. The software used for MDR is distributed in a JAVA platform with a graphical user interface and is freely available (http://www.epistasis.org/mdr.html). A two-sided p-value <0.05 was considered statistically significant.

Results

The baseline characteristics of all subjects are summarized in Table 1. Age, HDL, LDL, sex and smoking distribution showed no difference between hypertensive and control groups (P>0.05). However, BMI, TC and TG were significantly higher in the hypertensive group than in the control group (P<0.05). Additionally, drinking distribution was significantly different between hypertensive and control groups (P<0.01), and the corresponding OR (95%CI) was 2.43 (1.82, 3.26) (the data no showed in Table 1).

The genotypic and allelic frequency distributions of ADD1 Gly460Trp polymorphism were shown in Table 2. The genotype distribution was observed departure from the HWE in hypertensive cases (P<0.01). However, the genotype distribution was significantly different between hypertensive and control groups, and the Gly460Trp polymorphism was found significantly associated with hypertension at allelic level (P<0.01; OR=0.85; 95%CI=0.75-0.96). A breakdown association analysis by gender was also performed to explore the association between Gly460Trp polymorphism and EH (Table 2). Interestingly, the genotype distribution still deviated from the HWE in male hypertensive cases, but it was consistent with the HWE in female cases. No departure from the HWE was found in all control groups. The genotype distributions were still significantly different between hypertensive and control groups both in male (P<0.01) and in female (P<0.05). However, the Gly460Trp polymorphism was

| Variables | Hypertensive | Control | P value |
|-----------|--------------|---------|---------|
| Gender (M/F) | 1020 | 1020 | N/A |
| Age (years) | 58.5 ± 6.4 | 58.3 ± 6.5 | 0.58 |
| BMI (kg/m²) | 23.96 ± 2.89 | 22.94 ± 2.77 | <0.01 |
| TC (mmol/L) | 5.39 ± 1.01 | 5.28 ± 1.05 | 0.02 |
| TG (mmol/L) | 1.74 ± 1.40 | 1.52 ± 0.86 | <0.01 |
| HDL (mmol/L) | 1.68 ± 0.47 | 1.70 ± 0.50 | 0.57 |
| LDL (mmol/L) | 3.22 ± 0.80 | 3.16 ± 0.83 | 0.30 |
| Smoking (Y/N) | 155/865 | 148/872 | 0.66 |
| Drinking (Y/N) | 161/859 | 73/947 | <0.01 |

BMI: body Mass Index; TC: Total Cholesterol; TG: Triglyceride; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein
observed significantly associated with hypertension at allelic level in female (P<0.01; OR=0.79; 95%CI=0.68-0.92), but not in male (P=0.72; OR=0.96; 95%CI=0.78-1.19).

Further genetic tests under the dominant and recessive inheritance models were performed for SNP rs4961 T allele, and the results of these tests are shown in Table 3. In the dominant model, a significant association between the rs4961 GT+TT genotype and EH was detected (hypertension cases versus control group: P<0.01; OR=0.72; 95%CI=0.59-0.87). However, in the recessive model, the significant association was only observed in female (hypertension cases versus control group: P<0.02; OR=0.75; 95%CI=0.58-0.96). Finally, MDR was used to analyze the interaction among Gly460Trp polymorphism and non-genetic risk factors for hypertension. The genotype of Gly460Trp polymorphism together with information about BMI, TC, TG, HDL, LDL, smoking, and drinking were input, consequently the software outputs the best model for “BMI, HDL, drinking, rs4961” with 10/10 cross-validation consistency (Table 4).

### Table 2: Distribution of genotypic and allelic frequencies between case and control.

| Rs4961 | Genotype frequencies | χ² | P   | HWE | Allele frequencies | χ² | P   | OR (95%CI) |
|--------|----------------------|----|-----|-----|-------------------|----|-----|-----------|
| Case   | GG 331 TT 245        | 11.69 | 0.003 | <0.01 | 933 1105 | 6.94 | 0.008 | 0.85 (0.75, 0.96) |
| Control | 262 GT 498 TT 260   |        | 0.45 | 1018 1022 |   |
| M-case | 116 GG 128 GT 94     | 15.00 | 0.001 | <0.01 | 316 360 | 0.13 | 0.72 | 0.96 (0.78, 1.19) |
| M-control | 91 GG 184 GT 75   |        | 0.34 | 334 366 |   |
| F-case | 215 GG 315 GT 151   | 8.37  | 0.015 | 0.09 | 617 745 | 8.93 | 0.003 | 0.79 (0.68, 0.92) |
| F-control | 171 GG 314 GT 185 |        | 0.11 | 664 656 |   |

### Table 3: Genetic analysis of ADD1 rs4961 mutation under dominant/recessive model.

| Rs4961 | Dominant model (GT + TT vs. GG) | P   | OR (95%CI) | Recessive model (TT vs. GG + GT) | P   | OR (95%CI) |
|--------|---------------------------------|-----|------------|---------------------------------|-----|------------|
| Case   | 688 vs. 331 | 245 vs. 774 | 0.47 | 0.93 (0.76, 1.13) | 16 vs. 94 | 0.003 | 0.79 (0.68, 0.92) |
| Control | 758 vs. 262 | 260 vs. 760 | 0.47 | 0.93 (0.76, 1.13) | 16 vs. 94 | 0.003 | 0.79 (0.68, 0.92) |
| M-case | 222 vs. 116 | 94 vs. 244 | 0.47 | 0.93 (0.76, 1.13) | 16 vs. 94 | 0.003 | 0.79 (0.68, 0.92) |
| M-control | 259 vs. 91 | 75 vs. 275 | 0.06 | 1.41 (0.99, 2.00) | 16 vs. 94 | 0.003 | 0.79 (0.68, 0.92) |
| F-case | 466 vs. 215 | 151 vs. 530 | 0.06 | 1.41 (0.99, 2.00) | 16 vs. 94 | 0.003 | 0.79 (0.68, 0.92) |
| F-control | 499 vs. 171 | 185 vs. 485 | 0.02 | 0.75 (0.58, 0.96) | 16 vs. 94 | 0.003 | 0.79 (0.68, 0.92) |

### Table 4: MDR analysis of gene-environment interaction.

| BMI      | Testing accuracy | Testing sensitivity | Testing odds ratio | Testing χ² | Cross-validation consistency |
|----------|------------------|---------------------|-------------------|------------|-----------------------------|
| BMI      | 0.60             | 0.46                | 2.69 (0.95, 7.64) | 3.57 (p = 0.06) | 10/10                       |
| BMI, drinking | 0.63             | 0.54                | 3.09 (1.13, 8.50) | 4.94 (p = 0.03) | 10/10                       |
| BMI, HDL, drinking | 0.64             | 0.51                | 3.77 (1.31, 10.88) | 6.33 (p = 0.01) | 10/10                       |
| BMI, HDL, drinking, rs4961 | 0.64             | 0.54                | 3.57 (1.27, 10.03) | 6.10 (p = 0.01) | 10/10                       |

**Discussion**

To evaluate the role of ADD1 Gly460Trp and environmental factors in EH and clarify their interactions on EH, we conducted a case-control study in a large, homogeneous sample of Han Chinese population. The gender and age were well matched between hypertensive and control groups. We found that BMI, TC, TG, and drinking were significantly associated with EH, and they might be the risk factor for EH. However, we did not find HDL, LDL, and smoking were associated with EH. To our knowledge, this is the first study that a large-scale case-control study focusing on the association of ADD1 Gly460Trp with EH was performed in Chinese Han population, moreover, ADD1 Gly460Trp was found to be associated with EH in the present study. Additionally, Gly460Trp genotype distribution deviated from the HWE in hypertensive cases, which might opportunely clarify the association of Gly460Trp polymorphism with EH.
hypertension risk [34,35]. Disorders in the metabolism of HDL and TG play a key role in EH progression [36,37]. In the current study, we detected the association of EH with BMI, TC, TG and drinking, but not with HDL, LDL and smoking. However, the MDR analysis in this study demonstrated that BMI, HDL and drinking interacted with rs4961, whichconjunctively contributed to EH. Thereby, the present interaction analysis gave a little more information than the single genetic study.

In summary, the present study indicated that ADD1 Gly460Trp polymorphism was associated with EH in female Han Chinese. However, EH is a complex and polygenic disease, and ADD1 Gly460Trp polymorphism may play a tiny role in the pathogenesis of EH. In addition, our interaction analysis confirmed the interaction existed between genetic and non-genetic factors, suggesting that single genetic study is not enough for hypertension. In the future study, the interaction of genetic and environmental factors needs more attention to clarify the pathogenesis of this complex disease.

Supporting Information

Table S1: Primer sequences for ADD1 Gly460Trp polymorphism

Acknowledgement

There are no conflicts of interest.

References

1. GenSalt Collaborative Research Group (2007) GenSalt: rationale, design, methods and baseline characteristics of study participants. J Hum Hypertens 21: 639-646.

2. Svetkey LP, Harris EL, Martin E, Vollmer WM, Meltsen GT, et al. (2011) Modulation of the BP response to diet by genes in the renin-angiotensin system and the adrenergic nervous system. Am J Hypertens 24: 209-217.

3. Kurtz TW, Spence MA (1993) Genetics of essential hypertension. Am J Med Sci 305: 195-205.

4. Matsuoka Y, Li X, Bennett V (2000) Adducin: structure, function and regulation. Cell Mol Life Sci 57: 884-895.

5. Tripodi G, Valtorta F, Torielli L, Chierigati E, Salardi S, et al. (1996) Hypertension-associated point mutations in the adducin alpha and beta subunits affect actin cytoskeleton and ion transport. J Clin Invest 97: 2815-2822.

6. Casari G, Barlassina C, Cusi D, Zagato L, Muirhead R, et al. (1995) Association of the alpha-adducin locus with essential hypertension. Hypertension 25: 320-326.

7. Cusi D, Barlassina C, Azzani T, Casari G, Citterio L, et al. (1997) Polymorphisms of alpha-adducin and salt sensitivity in patients with essential hypertension. Lancet 349: 1353-1357.

8. Tamaki S, Iwai N, Tsujiya Y, Nakamura Y, Kinosita M (1998) Polymorphism of alpha-adducin in Japanese patients with essential hypertension. Hypertension Res 21: 29-32.

9. Wang C, Sun G, Yan X, Ding Y (2007) Study of a-adducin and endothelial nitric oxide synthase gene polymorphism in patients with essential hypertension in mongolia population. Journal of Clinical Cardiology 23: 525-527.

10. Melander O, Bengtsson K, Orho-Melander M, Lindblad U, Forsblom C, et al. (2000) Role of the Gly460Trp polymorphism of the alpha-adducin gene in primary hypertension in Scandinavians. J Hum Hypertens 14: 43-46.

11. Clark CJ, Davies E, Anderson NH, Farmer R, Friel EC, et al. (2000) alpha-adducin and angiotensin I-converting enzyme polymorphisms in essential hypertension. Hypertension 36: 990-994.

12. Alam S, Liyou N, Davis D, Tresillian M, Johnson AG (2000) The 460Trp polymorphism of the human alpha-adducin gene is not associated with isolated systolic hypertension in elderly Australian Caucasians. J Hum Hypertens 14: 198-203.

13. Shin MH, Chung EK, Kim HN, Park KS, Nam HS, et al. (2004) Alpha-adducin Gly460Trp polymorphism and essential hypertension in Korea. J Korean Med Sci 19: 812-814.

14. Liu K, Liu J, Huang Y, Liu Y, Lou Y, et al. (2010) Alpha-adducin Gly460Trp polymorphism and hypertension risk: a meta-analysis of 22 studies including 14303 cases and 15961 controls. PLoS One 5.

15. Niu W, Qi Y (2011) Association of Iso-adducin and G-protein β3 genetic polymorphisms with hypertension: a meta-analysis of Chinese populations. PLoS One 6: e17052.

16. Liu K, Liu J, Liu J, Wang Z, Lou Y, et al. (2011) Iso-adducin Gly460Trp polymorphism and essential hypertension risk in Chinese: a meta-analysis. Hypertens Res 34: 389-399.

17. Li YY (2012) Iso-Adducin Gly460Trp gene polymorphism and essential hypertension in a Chinese population: a meta-analysis including 10,983 subjects. PLoS One 7: e30214.

18. (2003) 2003 european society of hypertension-european society of cardiology guidelines for the management of arterial hypertension. J Hypertens 21:1011-1053

19. Perloff D, Grim C, Flack J, Frohlich ED, Hill M, et al. (1993) Human blood pressure determination by sphygmomanometry. Circulation 88: 2460-2470.

20. Yuan F, Xu J, Li BD, Fei LJ, Liu PP, et al. (2012) [Application of Tm-shift genotyping method in genetic studies], Yi Chuan 34: 1484-1490.

21. Excoffier L, Lischer HE (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10: 564-567.

22. Motsinger AA, Ritchie MD (2006) The effect of reduction in cross-validation intervals on the performance of multifactor dimensionality reduction. Genet Epidemiol 30: 546-555.

23. Ferrandi M, Salardi G, Tripodi G, Barassi P, Rivera R, et al. (1999) Evidence for an interaction between adducin and Na(+)-K(+)ATPase: relation to genetic hypertension. Am J Physiol 277: H1338-1349.

24. Bianchi G, Tripodi G, Casari G, Salardi S, Barber BR, et al. (1994) Two point mutations within the adducin genes are involved in blood pressure variation. Proc Natl Acad Sci U S A 91: 3999-4003.

25. Ferrandi M, Tripodi G, Salardi S, Florio M, Modica R, et al. (1996) Renal Na-K-ATPase in genetic hypertension. Hypertension 28: 1018-1025.

26. Castejon AM, Aller A, Hoffmann IS, Rathinavelu A, Cebenedu LX (2003) Alpha-adducin polymorphism, salt sensitivity, nitric oxide excretion, and cardiovascular risk factors in normotensive Hispanics. Am J Hypertens 16: 1018-1024.

27. Staessen JA, Bianchi G (2005) Adducin and hypertension. Pharmacogenomics 6: 665-669.

28. Gilbert JS, Niljand MJ (2008) Sex differences in the developmental origins of hypertension and cardioirenal disease. Am J Physiol Regul Integr Comp Physiol 295: R1941-1952.

29. Fisher ND, Ferri C, Bellini C, Santucci A, Gleason R, et al. (1997) Age, gender, and non-modulation. A sexual dimorphism in essential hypertension. Hypertension 29: 980-985.

30. Dzudie A, Kengne AP, Muna WF, Ba H, Menanga A, et al. (2012) Prevalence, awareness, treatment and control of hypertension in a self-selected sub-Saharan African urban population: a cross-sectional study. BMJ Open 2.

31. Coadet-mcollet-Taglioni G1, Dausse JP, Giudicelli Y, Ribière C (2002) Gender difference in diet-induced obesity hypertension: implication of renal alpha2-adrenergic receptors. Am J Hypertens 15: 143-149.

32. Silva-Antonialli MM1, Tostes RC, Fernandes L, Fio-Chadi DR, Akamine EH, et al. (2004) A lower ratio of AT1/AT2 receptors of angiotensin II is found in female than in male spontaneously hypertensive rats. Cardiovasc Res 62: 587-593.

33. Wang JG, Staessen JA, Barlassina C, Fagard R, Kuznetsova T, et al. (2002) Association between hypertension and variation in the alpha- and beta-adducin genes in a white population. Kidney Int 62: 2152-2159.

34. Binder A (2007) A review of the genetics of essential hypertension. Curr Opin Cardiol 22: 176-184.
35. Whelton PK, He J, Appel LJ, Cutler JA, Havas S, et al. (2002) Primary prevention of hypertension: clinical and public health advisory from The National High Blood Pressure Education Program. JAMA 288: 1882-1888.

36. Zhang X, Giovannucci EL, Wu K, Smith-Warner SA, Fuchs CS, et al. (2012) Magnesium intake, plasma C-peptide, and colorectal cancer incidence in US women: a 28-year follow-up study. Br J Cancer 106: 1335-1341.

37. Tohidi M, Hatami M, Hadaegh F, Azizi F (2012) Triglycerides and triglycerides to high-density lipoprotein cholesterol ratio are strong predictors of incident hypertension in Middle Eastern women. J Hum Hypertens 26: 525-532.