

Association between Gly460Trp Polymorphism of α -Adducin Gene and Risk Factors in Hypertensive Pakistani Population

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Abstract

Objective: To determine possible association between α -adducin gene G460W polymorphism and risk factors like age & BMI in hypertensive Pakistani population.

Material & Methods: Deoxyribonucleic acid (DNA) samples from 200 patients with essential hypertension and 200 normotensive healthy individuals from Pakistani population were genotyped for G460W polymorphism by Polymerase Chain Reaction and Restriction Fragment Length Polymorphism. Logistic regression analysis was used to find association between ADD1 gene G640W polymorphism and risk factors like age and BMI (Body mass index) in hypertensive Pakistani population.

Results: No significant association was found between G640W polymorphism and risk factors like age and obesity.

Conclusion: This study showed that there is no significant association between ADD1 gene G640W polymorphism and risk factors in our selected samples from Pakistani population.

Key words: Alpha adducin gene, Body mass index, Hypertension, rs4961

Introduction

Blood pressure is a quantitative trait, which is influenced by multiple genetic and environmental factors.¹ Essential hypertensive subjects either inherit aggregate of hypertension causing genes or are exposed to exogenous factors that predispose them to hypertension.² More than 100 genetic variants contributing to BMI have been indicated in different studies. According to genomic

association studies, about 15 loci are identified that effect BMI.³ Different studies suggest simultaneous emergence of interacting proteins with increase in age. Proteins are synthesized by gene transcription; but despite the importance of gene duplication in genome, evolution with age, none consider protein family dynamics as a contributing factor.⁴ Human ADD1 gene contain 16 exons and is located on chromosome 4p16.3 and encodes α -adducin. Adducin is a heterodimer cytoskeleton protein containing two subunits i.e. α units with β or γ subunit. Adducin binds with high affinity to Ca^{2+} / Calmodulin and is a substrate for protein kinases A and C which increases calcium absorption and subsequently increases weight.⁵ Alpha subunit of adducin plays an important role in sodium retention in renal tubules thus G460W polymorphism is thought to be associated with risk factors like age and obesity.⁶

A series of parallel studies in human indicated that replacement of glycine (Gly) to tryptophan (Trp) at position 460 leads to higher activity of Na^+ - K^+ pump.⁷ The association of risk factors like age and BMI with ADD1 gene polymorphism have been reported in Asian population.⁸ Amino acid substitution from glycine to tryptophan in adducin results in drastic change effecting the protein assembly and stability resulting in increased water and sodium retention inside renal tubules which ultimately causes high blood pressure. So, ADD1 gene is considered causative factor for increase in BMI.⁹ The results of various studies carried out in different nations to elucidate possible involvement of G460W polymorphism as a risk factor are widely inconsistent, so no definite conclusion can be drawn. However, in Hans Chinese population G460W polymorphism was strongly associated with risk factors of hypertension.¹⁰ In the Japanese population, G460W polymorphism has no evidence for a positive association with age.¹¹ In Pakistani population, the tendency of obesity is considered contributing factor for many diseases. The

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present study was designed to find association between ADD1 gene polymorphism with age and BMI.

Methodology

In this study, 200 patients with essential hypertension and 200 healthy subjects (normotensive) were recruited from Pakistan Institute of Medical Sciences Islamabad, Benazir Bhutto Hospital Rawalpindi and Federal Government Services Hospital Islamabad. Parameters like age, weight, blood sugar fasting, systolic blood pressure, diastolic blood pressure, height and body mass index were recorded. For measuring blood pressure, mercurial sphygmomanometer was used. Total lipid profile and blood glucose level was measured using microlab 300 semi-automated chemistry analyzer. Subjects of both genders with age limit 25-55 years were included in the study. Already known patients with diabetes mellitus, dyslipidemia and renal disorders were excluded from study groups. Blood samples were collected and genomic DNA was extracted from blood samples using phenol-chloroform method.¹² Quantification of isolated DNA was performed on spectrophotometer. Extracted DNA was amplified by using primers designed from flanking regions of the polymorphism (Forward primer 5'-CTC CTT TGC TAG TGA CGG TGA TTC-3' and reverse primer 5'-GAC TTG GGA CTG CTT CCA TTC TGCC-3'). Total volume of reaction mixture was 20 µl containing: PCR master mixture 5µl, forward primer and reverse primer 4µl (stock concentration 100 µmolar, working conc.10 µmolar), PCR Grade pure water 3.2 µl, genomic DNA 1µl, Betain 2.6 µl. Restriction fragment length polymorphism was done by using *Sau96I* restriction enzyme. For calculating genomic frequency direct gene counting method was employed¹³. Logistic regression analysis was applied to find out possible association between risk allele and essential hypertension. All statistical analysis was performed with SPSS version 22.

Results

Clinical data of cases and controls such as systolic and diastolic blood pressure, weight, cholesterol level, lipid profile, BMI and BSF (blood sugar fasting) were recorded. Table 1 shows the comparison of these variables in cases and controls. Association analysis of G460W polymorphism of ADD1 gene and age revealed no significant relationship (p = 0.15). Similarly, no significant relationship was found between ADD1 polymorphism and BMI (p= 0.26).

Study of parameter	Controls (n 200) (Mean ± SD)	Cases (n 200) (Mean ± SD)
Age (year)	28.84 ± 5.80	41.19 ± 5.92
Systolic (mm Hg)	109.82 ± 6.97	138.05 ± 3.64
Diastolic (mm Hg)	71.17 ± 6.06	97.84 ± 3.68

Weight (kg)	77.11 ± 8.76	82.37 ± 7.48
BMI (kg/m ²)	26.88 ± 0.876	27.04 ± 1.02
BSF (mg/dl)	78.15 ± 10.19	76.82 ± 10.28
TC (mg/dl)	164.8 ± 26.30	325.90 ± 50.93
TG (mg/dl)	134.31 ± 23.63	437.95 ± 79.50
HDL(mg/dl)	55.05 ± 7.19	29.12 ± 13.69
LDL (mg/dl)	80.30 ± 11.24	313.20 ± 95.76

Table 2 shows the comparison of allele frequency with risk factors like age and obesity.

Parameter	Risk allele odds ratio (G/G vs. T/G-T/T)	p-value
Age	1.98(0.78-5.03)	0.15*
BMI vs. Overweight	0.44(0.10-2.05)	0.26*

*p < 0.05 (Significant)

Discussion

The W allele of G460W polymorphism in adducin-1 gene has been occasionally associated with increased blood pressure.^{9,10} The aim of this study was to analyze whether the G460W variant is associated with age and BMI in Pakistani population or not. In our studies, no significant correlation was developed between G460W polymorphism and age or BMI. A meta-analysis conducted on Chinese Hans population showed a strong association between ADD1 gene polymorphism with age, a finding which was not comparable with those reported in our studies.^{9, 14} A study conducted in the South China showed a significant association between *ADD1* gene and age.⁹ In a meta-analysis of the *ADD1* gene G460W polymorphism association has been reported with age in Asians, but not in Blacks and Caucasians, however, the results of our studies are not supporting their findings.¹⁵ In another study conducted in a North Indian population showed a significant association between the Gly460Trp gene polymorphism of α -adducin and age.¹⁶ Another study elucidated a significant association between obesity and rs4961 and results indicate that the SNP rs4961 has a protective role in the development of obesity.¹⁷

A significant association have been proven in South China, especially Hans, Mongolian and North Indian population and those reported in some meta-analysis, which support our study results.^{9,14,16} Meta-analysis results still did not reveal strong association between G460W polymorphism and age in studies conducted in Caucasians and East Asians. This meta-analysis failed to provide evidence for the genetic association of α -adducin gene Gly460Trp polymorphism with age.¹⁸

In another study conducted on Caucasians and Asians, no significant association of G460W polymorphism with age

was found.¹⁹ The cause of controversial results from different areas of the world are due to the difference in race, living style and may be due to different gene pool. A study involving proteomics and genomics might be more informative for future prediction regarding the onset of hypertension. Further studies need to be done to find association of G460W polymorphism with ae and BMI in different ethnic groups with larger sample size in Pakistan.

Conclusion

There is no genetic association between α -adducin gene G460W polymorphism and age and BMI in our selected samples from Pakistani population. Further studies are required with large sample size in various regions of Pakistan.

Conflict of Interest

This study has no conflict of interest as declared by authors

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Authorship Contribution:

Author 1: Active participation in active methodology, Interpretation & analysis

Author 2: Concept and planning

Author 3,4: Discussion