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The Effect of Genetic Variation of *CD36* Gene on Sample of Iraqi Patients with Essential Hypertension

Qusay A. Abdulameer[2], Ismail H. Aziz[1], Ismail A. Abdulhassan[1], Abdul-Jabbar A Ali[2]

[1]Institute of Genetic Engineering and Biotechnology for Postgraduate Studies / University of Baghdad / Baghdad / Iraq.
[2]Ministry of Science and Technology, Environment and Water Directorate, Baghdad-Iraq.

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Abstract: High blood pressure is defined as a systolic blood pressure of 140 mm Hg or more, or a diastolic blood pressure of 90 mm Hg or more. Blood pressure is the force exerted by the circulation of blood on the walls of the body's arteries, which are the main blood vessels in the body. High blood pressure is classified into normal, first stage, second stage, or third stage. Risk factors include lifestyle factors, environmental factors, and genetics. There are two types of high blood pressure, essential hypertension and non-essential hypertension. Blood samples were collected, representing 50 samples from patients with high blood pressure, in addition to 50 healthy samples. This study was conducted with the aim of studying the relationship between genetic variation of some genes and environmental factors associated with essential blood pressure in Iraqi patients. The studies were conducted in the laboratories of the Institute of Genetic Engineering - University of Baghdad, as well as in the research laboratories of the Ministry of Science and Technology. Total genomic DNA was extracted using a special kit from fresh unfrozen blood samples, and then normal polymerase chain reaction and PCR-RFLP were used to detect mutations in Cluster of differentiation 36 rs1761667 G>A gene using primers and specialized restriction enzyme is *HhaI*. The results of the GG wild-type Cluster of differentiation 36 gene showed a band (161+ 264 bp), while the heterozygous GA genotypes showed (161, 264, and 425 bp), respectively, and for the mutant AA genotypes (425 bp). Nitrogenous base sequence analysis (Sequencing) was conducted for both infected and healthy samples. The results of the study showed that the incidence of hypertension in the age group (20-65) years was higher in males than in females, reaching (60% and 40%), respectively. The study showed that stress, smoking and exposure to pollution all have a clear and dangerous effect on the percentage of people with high blood pressure. The results also showed that mutations between patients and apparently healthy people in the Cluster of differentiation 36 gene may cause an increase in blood pressure for the wild-type GG is (56%) *versus* (44%) a significant level ($p<0.05$), while for the heterozygous GA it is (20%) *versus* (24%)) found not significant, either for the homozygous mutant AA is (24%) *versus* (32%) with a level ($p<0.05$). These results indicate that genetic variation (G>A) may be a risk factor (GG)) for high blood pressure.

Keyword: *CD36* gene, *CD36* rs1761667 G>A SNP high blood pressure.

Corresponding author: (Email: qusay6666@yahoo.com).

Introduction

Essential hypertension (also known as primary hypertension or idiopathic hypertension) is the most prevalent kind of hypertension, affecting 95 percent of hypertensive individuals. It is often hereditary and is thought to be caused by a combination of environmental and genetic factors. Essential hypertension is more common as people become older, and people who have moderately high blood pressure at a young age are more likely to acquire hypertension later in life, which causes them a lot of pain. Hypertension raises the risk of stroke, heart attack, and kidney failure (1,2). Blood pressure rises in youth have been linked to etiological factors linked to hypertension in adulthood.

Intrauterine malnutrition, a family history of hypertension, obesity, especially excess abdominal fat, insulin resistance, high dietary sodium intakes, low dietary intakes of calcium, potassium, and magnesium, physical inactivity, high alcohol intakes, tobacco use, drug use (e.g., cocaine, ecstasy, anabolic steroids), emotional stress, diet pill use, and oral contraceptives are some of the factors linked to hypertension (3). In humans, Cluster of differentiation 36 (*CD36*) is a protein that is encoded by the differentiation cluster 36 (*CD36*). The Cluster of differentiation 36 gene, which is 32 kb long and has 15 exons, is found on chromosome 7 (q11.2). Cluster of differentiation 36 role in lipid metabolism, atherosclerosis, and blood pressure regulation. Primary (Essential) hypertension and secondary (additional) hypertension are the two basic types of hypertension. The most common type of hypertension is primary hypertension, which affects 90-95 percent of people with the condition. Secondary hypertension accounts for the remaining 5-10% of hypertension patients. Secondary hypertension occurs as a result of a known cause (4,5).

Materials and Methods

Genomic DNA was extracted using *gSYNC* Genomic DNA Extraction Kit (Geneaid/ Taiwan). PCR was performed using *AccuPower* PCR PreMix (Bioneer, South Korea) and specific primers Table (1). The programmed was initial denaturation at 95 °C for 5 min, followed by 35 cycles of amplification including denaturation at 95 °C, annealingat 60 °C, extension at 72 °C (each comprising 30 s), and the final extension at 72 °C for 5 min.

Table (1): Sequences of primers used in this study *CD36* gene (6).

Forward	Reverse	Size / bp
CAAGGTCTGGTATCCACCTGTT	ATGAAGCTTCCCGCCTTAGAA	425

RFLP-PCR

PCR product was digested using restriction enzyme *HhaI*. The reaction was done as showed in Table (2).

Table (2): *HhaI* Restriction enzyme, *CD36* gene.

Component	Quantity (µl)
PCR product	10
Buffer enzyme	2
HhaI Enzyme	1
D.W	2
Total volume	15

Incubate for 3hr at 37(°C).

Results and Discussion

The targeted fragment was amplified using PCR, which was done with specific primers. The *CD36* gene's flanking rs1761667 (G > A) SNP in intron1 was amplified as a 425bp fragment. as shown in Figure (1).

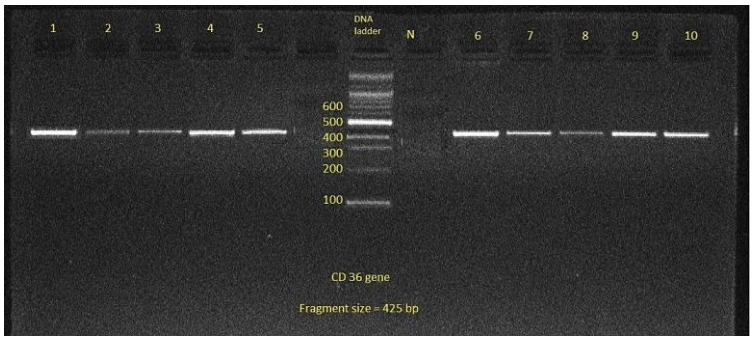

Figure (1): PCR product (425bp) of rs1761667 (G>A) SNP visualized under UV light after staining with ethidium bromide. The electrophoresis was on 2% agarose gel at 70 volt for 1hour. DNA ladder=100bp; N= negative control.

PCR-RFLP with the *HhaI* restriction enzyme was used to identify the *CD36* gene polymorphism at SNP rs1761667 (G > A). The studied SNP rs1761667 (G > A) was located within the enzyme target sequence of the targeted fragment, which contained one restriction site for the *HhaI enzyme*

($\begin{matrix} GCG^{\downarrow}C \\ C_{\uparrow}GCG \end{matrix}$).

PCR fragments containing adenine (A) were left undigested (425 bp), while those containing guanine (G) at the same location were split into two fragments (264 and 161bp) (Figure 2). According to Figure 2, AA genotype carriers had one fragment (425 bp), GA genotype carriers (heterozygous) had three fragments (425, 264, and 161 bp), and GG genotype carriers (wild-type) had two fragments (264 and 161 bp). Due to a single nucleotide transformation in the two strands of DNA, both sequences of *HhaI* restriction sites in the DNA of the target fragment had modified.

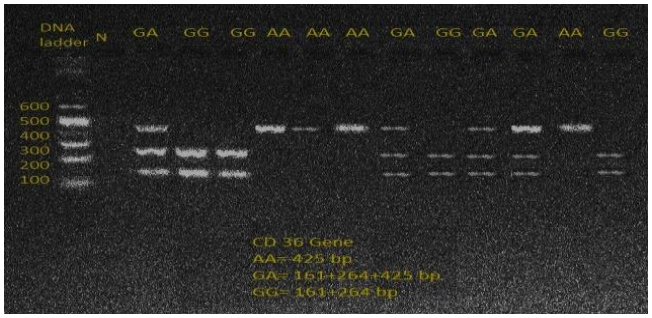

Figure (2): PCR-RFLP analysis of the *HhaI*digest of the PCR product that contains SNP rs1761667 (G>A) SNP of the *CD36* gene separated on a 2% agarose gel. DNA ladder= 100 bp; AA= homozygous mutant, GA= heterozygous and GG= wild type.

The genotypes and allele frequency distributions of the rs1761667 (G > A) SNP of the *CD36* gene in hypertensive patients and apparently healthy controls were compared. are shown in Table (3).

There was an association between GG genotype and the incidence of essential hypertension (EH) (56% *versus* 44% for EH patients and control subjects,

Respectively, OR=0.782, χ^2=4.794, p>0.05). The AA genotype carriers were less essential hypertension (EH) incidence than GG genotype carriers (24% *versus* 32%, respectively).

Table (3): The Distribution and allele frequency of *CD36* gene in control and patients groups.

Genotype (*CD36*)	Control (N= 50) No. (%)	Patients (N= 50) No. (%)	O.R.	Chi-Square (χ^2)
GG: Wild	22 (44.00%)	28 (56.00%)	0.782	4.794 *
GA: Hetro.	12 (24.00%)	10 (20.00%)	0.385	1.095 NS
AA: Mutant	16 (32.00%)	12 (24.00%)	0.607	4.075 *
AA+GA	28 (56.00%)	22 (44.00)	0.782	4.794 *
Allele	**Frequency**			
G	0.56	0.66	--	--
A	0.44	0.34	--	--
* ($P \leq 0.05$).				

Momeni-Moghaddam *et al.* (6) found an association between *CD36* rs1761667 polymorphism and susceptibility to hypertension in southeastern Iranian population. The *CD36* gene, which is 32 kb long and has 15 exons, is located on chromosome 7 (q11.2). Yun *et al.* (7). Exon 1A is flanked by SNP rs1761667 (G > A) in the *5′* intron (8). *CD36* polymorphisms are thought to increase hypertension sensitivity because of *CD36*'s functions in lipid metabolism, atherosclerosis, and blood pressure control (9, 10, 11, 6) revealed that the A allele may be linked to hypertension; nevertheless, in the current research, the A allele frequency was 0.34 versus 0.44 for patients with essential hypertension versus apparently healthy subjects. Pravenec *et al.* (12) decreased nitric oxide (NO) activity in the renal medulla has been linked to hypertension, implying that reduced *CD36* in renal cells may be linked to hypertension (10). Solakivi *et al.* (13). In Finnish topics, there was no connection between the *CD36* rs1761667 gene polymorphism and hypertension. Our findings matched those of Momeni-Moghaddam *et al*. (6). Who discovered that A allele carriers of the rs1761667 SNP had a protective impact against hypertension. Our findings revealed that the A allele frequency of the *CD36* rs1761667 gene polymorphism was 0.44 in the control group, which was close to 0.46 percent in a study on healthy Egyptian subjects (9), while in healthy Chinese subjects (0.32 percent) (14). Fujii *et al.* (15) in contrast to our findings, which showed that the AA genotype frequency was slightly lower in essential hypertension patients than in apparently healthy subjects (24 %versus 32%, respectively), the AA genotype of rs1761667 in the *CD36* gene was associated with a lower risk of hypertension in a Japanese population. Love-Gregory *et al*. (16) discovered that the AA genotype of rs1761667 is linked to lower *CD36* mRNA expression (15) found that rs1761667 carriers with the AA genotype had lower blood pressure than those with the GG genotype, which is consistent with the findings of the current study, where the AA genotype frequency was 24% in essential hypertension patients versus 32 % in apparently healthy subjects. Direct sequencing and genotype detection were used to investigate the relationship between *CD36* gene single nucleotide polymorphisms (SNPs) and essential hypertension in northeastern Han Chinese (17). *CD36* is found on the surfaces of platelets, endothelial cells, macrophages, dendrite cells, adipocytes, striated muscle cells, and hematopoietic cells, and it plays an important role in the regulation of hypertension (18, 19).

DNA Sequencing

In Figure (3) show the matching and the peaks of sequencing for fragment in *CD36* gene that flanking rs1761667 SNP (g.18436 G>A) and appear A instead of G in the position. DNA sequencing in South korea-macrogen Company.

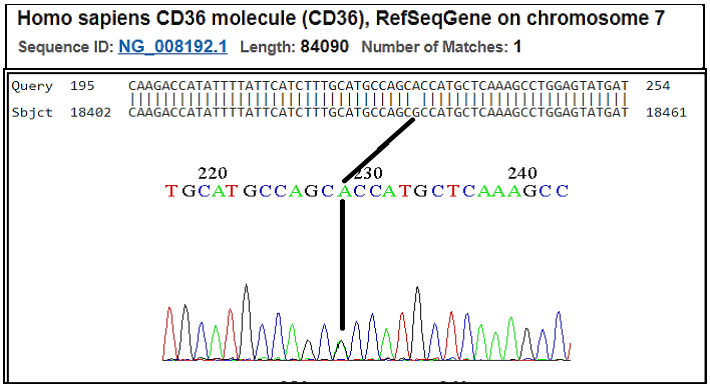

Figure (3): Sequence flanking rs1761667 SNP (g.18436 G>A) in *CD36* gene.

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