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**ISSN No: 2319-5886**

**International Journal of Medical Research & Health Sciences, 2017, 6(6): 100-105**

# Co-Segregation of *PCSK9* Gene I474V Variant with Diabetic and Hypercholesterolemic Subjects

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**ABSTRACT**

*PCSK-9 has a tremendous role in lipid metabolism. PCSK9 variants have been concomitantly linked to alterations in plasma lipid profile, leading to increased risk of cardiovascular disease and diabetes mellitus. The aim of our study was to determine the frequency of PCSK-9 I474V variant in hypercholesterolemic and diabetic subjects. Plasma lipid profile and fasting blood glucose were assayed in 30 healthy, 25 hypercholesterolemic, and 45 diabetic subjects. Genomic DNA was extracted from whole blood and PCR was run using specific primers to obtain an amplicon harbouring exon 9. The amplicon was later subjected to Sanger sequencing and. A missense mutation of PCSK9 I474V SNP was detected in the exon 9 of PCSK9 gene. Our results show a frequency of the PCSK9 I474V SNP in Hail region of Saudi Arabia and this frequency is higher among hypercholesterolemic and diabetic patients. To our best knowledge, this is the first report of mutation of this nature in Hail region of Saudi Arabia.*

**Keywords:** PCSK-9, I474V, hypercholesterolemia, diabetes, genomic DNA, mutation, Hail, Saudi Arabia.

## INTRODUCTION

Genetic and epidemiological records have revealed the link between plasma levels of LDL and HDL cholesterol, and cardiovascular disease incidence all over the world [1]; although lifestyle, diet, and physical activity have a role in shaping individual lipid profiles, it is clear that lipid profile is strongly linked to genetic variations.

Cardiovascular diseases inflict heavy economic load and social cost world-wide on human diseases including Saudi Arabia and significantly impart the morbidity and the mortality rates. Familial hypercholesterolemia has genetic determinants and is autosomal dominant and is a major risk factor for the development of CVD [2].

Familial hypercholesterolemia is frequently caused by mutations in the LDL receptor, which is responsible for hepatic clearance of low-density lipoprotein from the blood circulation. Also, it can be caused by some mutations in the Apo-lipoprotein B gene, which codes the ligand for LDL receptor [3] and recently Proprotein Convertase Subtilisin/Kexin type 9 (*PCSK9*) has been recognized as a major contributor to some form of FH [4,5].

Hypercholesterolemia and diabetes mellitus are highly prevalent diseases in Saudi Arabia [6]. The International Diabetes Federation (IDF) has categorized Saudi Arabia to be amongst the countries with the highest prevalence of diabetes [7]. Moreover, the incidence of some risk factors (e.g. hypercholesterolemia) for diabetes mellitus in Saudi Arabia has also been expected to be among the highest in the world [8].

Proprotein Convertase Subtilisin-Kexin type 9 (*PCSK-9*) plays a perilous role in cholesterol metabolism [9]. PCSK9 is expressed mostly in the liver, kidney, and small intestine [10]. PCSK9 is coded by a gene located on the short arm of chromosome 1 (1p32.3) [11]. PCSK9 is synthesized in the endoplasmic reticulum as a precursor of 692 amino acids and comprises 12 exons [12].

PCSK9 is an extremely polymorphic gene and several investigations revealed that missense mutations provoking elevation of PCSK9 activity cause familial hypercholesterolemia [13], while nonsense mutations inhibiting PCSK9 activity are accompanying with hypocholesterolaemia [14].

Among many genetic variants of PCSK9 identified to affect plasma lipid profiles, one of the common genetic variants of the *PCSK9* gene the most studied is I474V, and its link to the alterations in blood lipid profiles remains inconsistent [15].

In this present study, we attempt to explore the frequency of I474V polymorphism of *PCSK9* gene in hypercholesterolemic and diabetic patients in Hail region Saudi Arabia was determined.

## METHODS AND MATERIALS

In the current study, we analyzed 100 unrelated subjects; all participants were of Saudi Arabia origin and provided informed consent for our study, and the subjects were divided in three groups: 30 healthy control, 25 with clinically diagnosed as hypercholesterolemic (The diagnosis of hypercholesterolemia was made according to the following criteria total cholesterol above 4 mmol/L) and 45 diabetic subjects (The diagnosis of diabetes was according to American Diabetes Association patient have fasting glucose ≥7 mmol/L will be diabetic). Age of all subjects ranged between 22 to 58 years. Our study was carried out in accordance with the Institutional Scientific and Research Ethics Committees college of Medicine, Hail University, KSA.

### Biochemical analysis

Blood samples were taken for biochemical analysis following overnight fasting. Plasma lipid profile (total cholesterol, triglyceride, and HDL cholesterol levels) and blood glucose level were assayed using the routine clinical technique. VLDL-cholesterol and LDL-cholesterol levels were calculated according to the Friedewald equation [16].

### Genomic DNA analysis and Sanger sequencing

Genomic DNA was purified from whole blood using a Genomic DNA Purification Kit (QIA amp DNA Blood Mini Kit from Qiagen, Hilden, Germany).

PCSK9 SNP I474V was uncovered as followed; brief, we used specific PCSK9 primers: 5'-TGAGAGGAGGCT-GTCTTACCTC-3' (forward) and 5'-GAGTATGGAACTGCAAGTCAGG-3' (reverse) to amplify a fragment encompassing exon 8 to 10 as per Abifadel, et al. [4]. This fragment was separated on agarose gel, purified, and used as a template together with primers specific to exon 9 (forward) 5'-GTAAGGAGGATGATGCCACC-3' and (reverse) 5'-TTACAGAAGAGCTGGAGTCTGG-3' as per Abifadel, et al. [4] to detect the PCSK9 SNP I474V on a Sanger sequencer, Genetic Analyzer 3500XL automated sequencer with Big-Dye terminator cycle sequencing reagent (Applied Bio-system, Riyadh, Saudi Arabia).

### Statistical analysis

The data analysis was carried out using statistical software SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± standard deviation. Statistical analyses were performed using one-way ANOVA, and means were compared using Duncan's multiple range test as a post hoc test at the 5% probability level. $P<0.05$ was considered statistically significant.

## RESULTS

**Table 1 Levels of plasma total cholesterol and triglycerides in hypercholesterolemic, diabetic and healthy control subjects**

| Groups | Total cholesterol (mmol/L) | Fold change of control | Triglycerides (mmol/L) | Fold change of control |
|---|---|---|---|---|
| Healthy Control (n:30) | $3.47 \pm 0.44^{a}$ | 0 | $1.54 \pm 0.26^{a}$ | 0 |
| Hypercholesterolemic Subjects (n:25) | $7.91 \pm 0.91^{c}$ | 1.28 | $3.85 \pm 0.47^{c}$ | 1.5 |
| Diabetic Subjects (n:45) | $6.26 \pm 0.55^{b}$ | 0.8 | $3.00 \pm 0.39^{b}$ | 0.95 |
| F-ratio | 355.37 | - | 266.77 | - |
| p-value | 0 | - | 0 | - |

[a,b,c] The different letters indicate statistically different means according to Duncan multiple range test

Table 1 shows the levels of total cholesterol and triglycerides in all subjects included in the current study. For total cholesterol, one way ANOVA test showed a highly significant change in the different subjects ($F=355.37$, $p<0.001$). The healthy control subjects showed a mean total cholesterol level of 3.47 mmol/L ± 0.44 mmol/L. Whereas hypercholesterolemic subjects showed a mean total cholesterol level of 7.91 mmol/L ± 0.91 mmol/L, also diabetic subjects showed a mean total cholesterol level of 6.26 mmol/L ± 0.55 mmol/L. For Triglycerides, a very similar effect of hypercholesterolemia and diabetes was shown ($F=266.77$, $P<0.001$).

**Table 2 Levels of plasma HDL-cholesterol and LDL-cholesterol in hypercholesterolemic, diabetic and healthy control subjects**

| Groups | HDL-cholesterol (mmol/L) | Fold change of control | LDL-cholesterol (mmol/L) | Fold change of control |
|---|---|---|---|---|
| Healthy Control | 1.34 ± 0.08[c] | 0 | 1.42 ± 0.47[a] | 0 |
| Hypercholesterolemic Subjects | 0.60 ± 0.09[a] | -0.55 | 5.56 ± 0.96[c] | 2.92 |
| Diabetic Subjects | 0.74 ± 0.09[b] | -0.45 | 4.15 ± 0.50[b] | 1.92 |
| F-ratio | 657.66 | - | 307.25 | - |
| p-value | 0 | - | 0 | - |
| [a,b,c] The different letters indicate statistically different means according to Duncan multiple range test | | | | |

With respect to HDL-cholesterol and LDL-cholesterol were studied. For HDL-cholesterol level, one way ANOVA test showed a highly significant difference between the different subjects (F=657.66, p<0.001). The healthy control subjects showed HDL-cholesterol of 1.34 mmol/L ± 0.08 mmol/L; in hypercholesterolemic subjects, a value was reduced to 0.60 mmol/L ± 0.09 mmol/L. Whereas in diabetic subjects HDL-cholesterol showed 0.74 mmol/L ± 0.09 mmol/L. LDL-cholesterol showed a highly significant difference between the different subjects (F=307.25, p<0.001) as shown (Table 2).

**Table 3 Levels of plasma VLDL-cholesterol and fasting glucose in hypercholesterolemic, diabetic and healthy control subjects**

| Groups | VLDL-cholesterol (mmol/L) | Fold change of control | Fasting glucose (mmol/L) | Fold change of control |
|---|---|---|---|---|
| Healthy Control | 0.70 ± 0.12[a] | 0 | 4.62 ± 0.48[a] | 0 |
| Hypercholesterolemic Subjects | 1.75 ± 0.21[c] | 1.5 | 6.86 ± 0.68[b] | 0.48 |
| Diabetic Subjects | 1.36 ± 0.18[b] | 0.94 | 7.76 ± 0.78[c] | 0.68 |
| F-ratio | 265.69 | - | 196.68 | - |
| p-value | 0 | - | 0 | - |
| [a,b,c] The different letters indicate statistically different means according to Duncan multiple range test | | | | |

Table 3 shows the levels of VLDL-cholesterol and fasting glucose in different subjects. For VLDL-cholesterol, one way ANOVA test showed a highly significant difference in the different subjects (F=265.69, p<0.001). The healthy control subjects showed a mean VLDL-cholesterol level of 0.70 mmol/L ± 0.12 mmol/L. in hypercholesterolemic subjects, a value was elevated to 1.75 mmol/L ± 0.21 mmol/L. Whereas in diabetic subjects VLDL-cholesterol showed 1.36 mmol/L ± 0.18 mmol/L. For fasting glucose level, one way ANOVA test showed a highly significant difference in the different subjects (F=196.68, p<0.001). The healthy control subjects showed a mean fasting glucose level of 4.62 mmol/L ± 0.48 mmol/L. Whereas hypercholesterolemic subjects showed a mean fasting glucose level of 6.86 mmol/L ± 0.68 mmol/L, also diabetic subjects showed a mean fasting glucose level of 7.76 mmol/L ± 0.78 mmol/L.

**Table 4 Distribution and frequency of PCSK9- I474V mutation in our study**

| Subjects | Number of Subjects with I474V | PCSK9-I474V % |
|---|---|---|
| Healthy control (n:30) | 2 | 6.67% |
| Hypercholesterolemic Subjects (n:25) | 10 | 40% |
| Diabetic Subjects (n:45) | 25 | 55.56% |
| Total (n:100) | 37 | 37% |

The frequencies of PCSK9-I474V SNP in hypercholesterolemic group were higher than those in healthy control group, and the frequencies of PCSK9-I474V mutation in diabetic group were also higher than those in healthy control group and hypercholesterolemic one (Table 4).

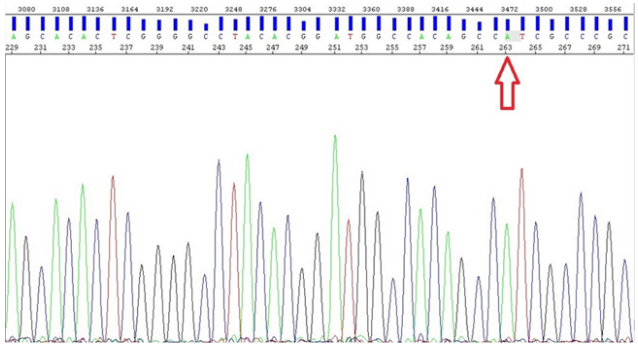

**Figure 1 A part of DNA analysis representative sequence data highlighting the presence of a missense mutation in the exon 9**

## DISCUSSION

Elevated low-density lipoprotein cholesterol levels are known as the main risk factor for CVD [17]. PCSK9 has an important role in the regulation of plasma low-density lipoprotein (LDL) cholesterol [18].

PCSK9 regulates plasma levels of LDL-cholesterol by directing cell-surface LDL receptors to the lysosomes for degradation, causing reduced clearance and accumulation of LDL-C in the circulation [19]. PCSK9 mutations that increase the degradation of LDLR are referred to as gain-of-function mutations and cause autosomal dominant hypercholesterolemia [20].

In the present study, our results showed a highly significant elevation in levels of total cholesterol, triglycerides and LDL-cholesterol in hypercholesterolemic and diabetic subjects as compare to healthy control subjects whereas there is a highly significant decrease in plasma level of HDL-cholesterol in hypercholesterolemic and diabetic subjects as compare to healthy control subjects. Our results in agreement with other studies [21].

There was a strong association between high level of triglycerides and low level of HDL-cholesterol as reported by Toth [22].

In diabetes mellitus, Hyperglycaemia progressively increases the transfer of cholesterol esters from HDL-cholesterol to VLDL-cholesterol particles which lead to elevation of VLDL-cholesterol in diabetic subjects.

Hyperlipidaemia caused in diabetes mellitus is due to excess mobilization of fat from the adipose tissue, increase VLDL secretions by the liver and stimulate VLDL formation by the intestine, decreasing hepatic lipoprotein lipase activity that inhibit the removal of plasma VLDL and leading to an increase plasma VLDL level [21].

The association of PCSK9 variant SNPs and plasma lipid profile is still controversial. Some previous studies propose that PCSK9 affects plasma LDL-C levels by altering hepatic LDLR levels [23]. Also, other studies suggested that mutations in PCSK9 affect plasma LDL-C levels by altering the rate of secretion of VLDL from the liver [24]. This controversial observation in variation in PCSK9 SNPs with plasma lipid profile is due to different gene pools studied so far.

Per our genomic DNA analysis, our study reported the presence of a missense mutation in the *PCSK9* gene in exon 9. This mutation is characterized by substitution of G to A as depicted in Figure 1. The frequency of this mutation is more frequent in hypercholesterolemic and diabetic subjects (Table 4) who have high levels of total cholesterol and LDL-cholesterol as shown in Tables 1 and 2. To our knowledge, no study has described the frequency of PCSK9 variation frequencies in Hail region.

Strangely, previous studies reveal that I474V is not associated with any changes in LDLC concentration [25,26]. However, other studies reveal a significant association between the appearance of I474V mutation and increased total cholesterol and LDLC levels and this in agreement with our finding [27-29].

## CONCLUSION

Our study highlights the frequency of PCSK9 I474V among hypercholesterolemic and diabetic subjects in Hail region Saudi Arabia, and this is the first study of this nature reporting the frequency of PCSK9 variant I474V in Hail region. Further studies are needed to determine and find other variations in PCSK-9 in Hail region and its association with common diseases in this population.

## CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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