original article

Q192R polymorphism in the *PON1* gene and familial hypercholesterolemia in a Saudi population

Khalid Khalaf Alharbi,[a] May Salem Alnbaheen,[b] Fawiziah Khalaf Alharbi,[c] Rana M. Hasanato,[d] Imran Ali Khan[a]

From the [a]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia; [b]College of Science, Saudi Electronic University, Riyadh, Saudi Arabia; [c]Department of Biology Science, College of Science and Arts, Al-Qassim University, Al-Qassim, Saudi Arabia; [d]Department of Pathology College of Medicine and University Hospitals, King Saud University, Riyadh, Kingdom of Saudi Arabia

Correspondence: Dr. Imran Ali Khan · Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, PO Box 10219, Riyadh-11433, Saudi Arabia · T: +966-11-4693851 · imkhan@ksu.edu.sa · ORCID ID: orcid.org/0000-0002-9746-5300

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BACKGROUND: Familial hypercholesterolemia (FH) is an autosomal dominant condition characterized by abnormal levels of low-density lipoprotein (LDL) in the blood. FH is a risk factor for atherosclerosis and cardiovascular disease. The relationship between the paraoxonase 1 (*PON1*) gene, atherosclerosis and coronary artery disease has not been studied in Saudi patients.

OBJECTIVE: To investigate the genetic associations of the Q192R polymorphism in the *PON1* gene with FH in Saudi patients.

DESIGN: Case-control study.

SETTING: Tertiary care center, Riyadh.

METHODS: Two hundred Saudi patients were enrolled in this study, including 100 patients with FH and 100 healthy controls, during the period from January 2012 to March 2013. Serum was separated from coagulated blood (3 mL) and used for analysis of lipid profiles. Genomic DNA was isolated from anticoagulant-treated blood (2 mL). Genotyping for the Q192R polymorphism was performed by polymerase chain reaction-restriction fragment length polymorphism analysis, followed by 3% agarose gel electrophoresis.

MAIN OUTCOME MEASURE: The strength of association between the Q192R polymorphism and FH in the Saudi population.

RESULTS: We confirmed that QR versus QQ (odds ratio [OR]: 1.55; 95% confidence interval [CI]: 1.05–3.43; *P*=.03), QR+RR versus QQ (OR: 1.98; 95% CI: 1.13–3.49; *P*=.01), and R versus Q (OR: 1.68; 95% CI: 1.09–2.59; *P*=.01) in the Q192R polymorphism were associated with FH in the Saudi population.

CONCLUSION: In conclusion, the Q192R polymorphism in the *PON1* gene is associated with FH in the Saudi population. Our results confirmed that the R allele, QR, and dominant model genotypes were associated with FH.

LIMITATION: Only a single variant (Q192R) was analyzed, and the medical and family histories of the patients were not known.

Familial hypercholesterolemia (FH; OMIM# 143890) (Online Mendelian Inheritance in Man, *http://www.omim.org*) is a well-known autosomal dominant genetic disorder with a global prevalence of approximately 1:300–500.[1] FH is characterized by elevated low-density lipoprotein cholesterol (LDL; OMIM #606945), leading to lipid accumulation and increased risk of cardiovascular disease (CVD).[2-4] Premature coronary artery disease is also often encountered in patients with FH,[5] a well-known genetic disease. Additionally, LDL-C levels are associated with the risk of CVD.[6] In heterozygous familial hypercholesterolemia (HeFH), patients have high cholesterol levels, typically between 250 and 300 mg/dL, representing a risk factor for the

development of CVD. Men typically develop the disease from 30 to 50 years old, whereas women develop the disease from 40 to 60 years old.[7] Notably, most cases of FH are not appropriately diagnosed, leading to inappropriate treatment strategies. Early diagnosis and therapeutic intervention are essential for the prevention of FH-associated complications.

In previous studies, genetic analyses have been performed in patients with FH to identify disease-causing mutations.[8,9] Genetic association studies have confirmed that various genes associated with metabolism, renin-angiotensin system, inflammation, and blood coagulation are connected to human disease development.[10] For example, paraoxonase 1 (PON1) has been extensively studied in CVD, stroke, inflammation, and oxidative stress.[11] PON1, initially expressed with high-density lipoprotein (HDL), functions in lipoprotein phospholipid metabolism. However, PON1 inhibition by HDL and LDL may have applications in atherosclerosis prevention.[12] PON1 is a multifunctional enzyme involved in oxidant defense, and genetic variants in *PON1* are associated with CVD.[13] PON1 is a major antioxidant with a protective role in CVD, and PON1 enzyme activity is decreased in patients with CVD.[14] Coronary heart disease (CHD) and PON1 activity have been shown to be strongly associated in case-control studies.[15] In myocardial infarction, the concentration of serum PON1 activity is decreased when compared with that in controls, and lower serum PON1 activity is an independent risk factor for coronary events. However, various diseases, such as chronic liver impairment, chronic renal failure, Alzheimer's disease, and type 1 and type 2 diabetes mellitus, are associated with variations in the concentrations of PON1 circulation. PON1, aryldialkylphosphatase (EC 3.1.8.1), is a Ca2+-dependent serum esterase synthesized mainly by the liver that catalyzes the hydrolysis of many highly toxic xenobiotics.[16] PON1 has 355 amino acids (43–45 kDa), and the gene encoding PON1 is clustered on chromosome 7q21.3. The expression of *PON1* mRNA has been determined in human organs, including the liver, heart, kidney, lungs, brain, and small intestine.

In the human *PON1* gene, there are almost 160 polymorphisms in the coding sequence (exons), noncoding sequence (introns), and other regulatory parts of the gene.[17] A single nucleotide polymorphism (SNP) in the *PON1* gene, Q192R (rs662), causing an amino acid substitution from glutamine (Q) to arginine (R), was previously reported.[18] The presence of the R allele has been shown to be associated with higher PON1 hydrolytic activity and decreased total cholesterol, LDL-C, and ApoB,[19] whereas the Q allele can prevent LDL oxidation into paraoxon.[20] The polymorphism Q192R modulates enzymatic activity, and RR genotypes will have higher activity.[21] However, few studies have examined the association between FH and the *PON1* gene in the global population.[22] Accordingly, in this study, we evaluated the relationship between FH and the Q192R polymorphism in a Saudi population.

PATIENTS AND METHODS

Study population samples

In this study, 100 patients with FH were enrolled. The inclusion criterion for FH was diagnosed based on the Dutch Working Group classification criteria,[23-25] and both men and women were enrolled. The exclusion criteria for patients with FH included concurrent renal, liver, or thyroid diseases and recruitment from outside King Khalid University Hospital (KKUH). The inclusion criteria for control participants (n=100) included recruitment from regular and contract-based KKUH staff and outpatients. The control patients were also selected based on Dutch group criteria and received regular checkups but did not have chronic, endocrinological, metabolic, or other diseases. The exclusion criterion for control patients was recruitment from individuals not on the KKUH staff. All patients and controls provided informed consent for participation in the study and filled out a questionnaire. This study was conducted according to the principles of the Declaration of Helsinki. No formal sample size calculation was used in determining sample size. This study was approved by the ethical committee of the Institutional Review Board of King Saud University (approval no. E-12-829).

Blood and lipid profile

Trained nurses collected 5 mL peripheral blood and allowed 3 mL to coagulate for use in biochemical tests; the remaining 2 mL blood was mixed with an anticoagulant vacutainer of ethylenediaminetetraacetic acid (EDTA) for use in molecular assays. Serum samples were subjected to biochemical analyses, such as lipid profiling. Separated serum from the 3 mL of the coagulant blood was stored at -80°C for future use. Serum samples were used to analyze lipid profile parameters, including (i) total cholesterol (TC), (ii) triglyceride (TG), (iii) HDL-cholesterol (HDL-C), and (iv) LDL-C. Three milliliters of coagulant blood sample was analyzed with an automated clinical chemistry analyzer (KoneLab, Espoo, Finland).[23]

Genotyping

The technique described by Alharbi et al[23] was used to isolate genomic DNA with 2 mL of EDTA-treated blood. A

NanoDrop spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA) was used to determine both the quality and quantity of DNA. Genomic DNA was stored at -80°C for future use. Primers specific for the *PON1* Q192R polymorphism,[12,18,26] including the sense sequence 5′-GGGACCTGAGCACTTTTATGGC-3′ and antisense sequence 5′-CATCGGGTGAAATGTTGATTCC-3′, were used in polymerase chain reaction (PCR; Applied Biosystems, CA, USA) restriction fragment length polymorphism analyses. The final volume in each PCR tube was 25 µL, including 100 pmol of each forward and reverse primer, MgCl2 (2.5 µL), dNTP (0.5 µL), Taq DNA polymerase (0.5 µL), and genomic DNA (100 ng). The initial denaturation was carried out at 95°C for 5 min followed by 35 cycles of 30 s at 95°C, 30 s at 58°C, and 45 s at 72°C, with a final elongation step at 72°C for 5 min. The 176-bp undigested amplicon was visualized using 3% agarose gels. MboI (↓GATC; New England Biolabs, Ipswich, MA, USA) restriction enzyme was used to digest the Q192R products. The presence of the Q allele was confirmed when bands corresponding to 145 and 31 bp were observed. The R allele with the Q192R locus was confirmed when bands of 117, 31, and 28 bp were observed (**Figure 1**). Three PCR products with different variants were applied to Sanger sequencing (**Figure 2**).

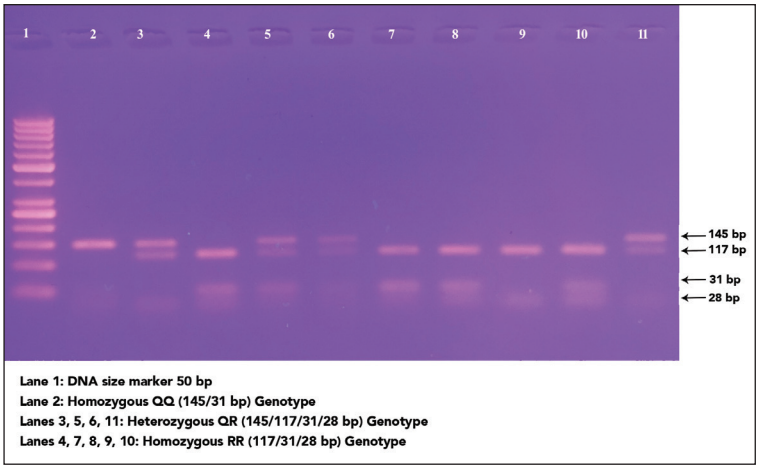

Figure 1. Gel documentation for genotyping analysis for Q192R polymorphism in *PON1* gene.

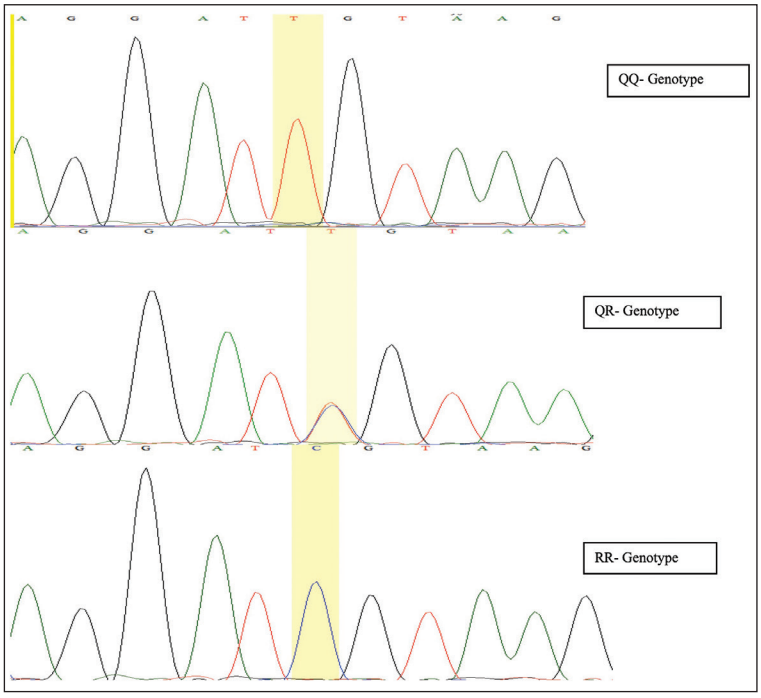

Figure 2. The DNA sequences of QQ, QR and RR genotypes in Q192R (rs662) polymorphism of the *PON1* gene.

Statistical analysis

We used SAS, version 9.3 (SAS Institute, Cary, NC, USA) software to analyze the data. The Hardy-Weinberg Equilibrium was tested in patients with controls. Descriptive characteristics of categorical variables were presented as count (%), and continuous variables were presented as mean (standard deviation). Association between these groups was tested using two sample independent *t*-test. Genotype and allelic frequencies were carried out using chi-square (χ^2) tests, and odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by multiple logistic regression for genotype frequencies. One-way analysis of variance (ANOVA) was also performed with baseline characteristics and variable genotypes through stratified analysis and assessed by the goodness-of-fit tests. Association between the three groups of genotypes was compared using post-hoc tests. Multiple logistic regression analysis was carried out for FH cases (Q192R variants) and lipid profile parameters. Differences with *P* values of less than .05 were considered significant.

RESULTS

Clinical characteristics

Patients with FH were older (51.6 [9.92] years) than in controls (44.0 [6.92] years) (**Table 1**) and age was positively associated with FH (*P*=.0001). TC and LDL-C levels were significantly higher in the FH cases than in controls (**Table 1**).

Genotype analysis

No deviation from Hardy-Weinberg equilibrium was detected in the control group (chi-square=0.16., *P*=.68)

There was a positive association between rs662

Table 1. Baseline characteristics and biochemical profile of selected participants.

S. No		FH cases (n=100)	Healthy controls (n=100)	*P*
1	Age (Years)	51.66 (9.92)	44.02 (6.29)	.0001
2	Gender: Male/Female	37:63	40:60	.62
3	Height (kg)	165.7 (7.53)	NA	NA
4	Weight (cms)	74.1 (9.40)	NA	NA
5	BMI (kg/m^2)	27.1 (1.91)	NA	NA
6	TG (mmol/L)	2.2 (1.2)	1.6 (0.99)	.009
7	TC (mmol/L)	5.4 (1.1)	4.8 (0.73)	.003
8	HDL-C (mmol/L)	0.7 (0.2)	0.6 (0.27)	.71
9	LDL-C (mmol/L)	4.5 (0.9)	3.7 (0.72)	.003

FH=Familial Hypercholesterolemia, TG=Triglycerides, TC=Total Cholesterol, HDL-C=High Density Lipoprotein Cholesterol, LDL-C=Low Density Lipoprotein Cholesterol and NA=Not analysed.

Table 2. Genotype and allele frequencies of Q192R polymorphisms in FH cases and control subjects.

Genotypes/ alleles	Cases (n=100)	Controls (n=100)	χ^2	OR (95%CI)	*P*
QQ	40 (40%)	57 (57%)		Reference	
QR	48 (48%)	36 (36%)	4.53	1.55 (1.05-3.43)	.03
RR	12 (12%)	7 (7%)	3.06	2.44 (0.88-6.74)	.07
QR+RR	60 (60%)	43 (43%)	5.75	1.98 (1.13-3.49)	.01
Q	128 (0.64)	150 (0.75)		Reference	
R	72 (0.36)	50 (0.25)	5.69	1.68 (1.09-2.59)	.01

Table 3. Association between Q192R genotypes and clinical characteristics in the FH cases.

Characteristics	QQ N=40	QR N=48	RR N=12	F values	*P*
Age in years, mean (SD)	52.9 (9.6)	51.5 (10.8)	48.0 (5.6)	6.84	.32
Male, n (%)	19 (47)	15 (31)	3 (25)	1.23	.19
Female, n (%)	21 (53)	33 (69)	9 (75)	1.46	.23
BMI (kg/m^2), mean (SD)	27.8 (1.5)	25.9 (2.7)	27.6 (1.7)	1.42	.0004
TG (mmol/L), mean (SD)	1.84 (1.1)	2.16 (1.4)	2.88 (1.2)	1.82	.047
TC (mmol/L), mean (SD)	5.13 (0.9)	5.62 (1.1)	5.67 (1.3)	1.40	.080
HDL-C (mmol/L), mean (SD)	0.67 (0.2)	0.69 (0.2)	0.78 (0.2)	1.62	.28
LDL-C (mmol/L), mean (SD)	3.9 (0.8)	3.5 (0.9)	3.9 (0.9)	1.28	.13

Abbreviations: QQ=Normal genotype; QR=Heterozygous genotype; RR=Variant/mutant genotype; TG=Triglycerides; TC=Total Cholesterol; HDL-C=High Density Lipoprotein Cholesterol; LDL-C=Low Density Lipoprotein Cholesterol; BMI=body mass index.

(Q192R) and FH risk (R versus Q; odds ratio: 1.68 [95% CI: 1.09–2.59]; *P*=.01 and QR+RR versus QQ; odds ratio: 1.98 [95% CI: 1.13–3.49]; *P*=.01) (**Table 2**). To more accurately assess the association with FH, we compared the QR and RR genotypes with the QQ genotype. From this analysis, we confirmed the positive association between the QR and QQ genotypes (QR versus QQ; OR: 1.55 [95% CI: 1.05–3.43]; *P*=.03). However, we could not find a significant association between the RR and QQ genotypes (RR versus QQ; odds ratio: 2.44 [95% CI: 0.88–6.74]; *P*=.07).

ANOVA

Comparisons of different genotypes in the Q192R variant with anthropometric and lipid profile parameters are documented in **Table 3**. There were no statistically significant differences between the rs662 polymorphism and patient characteristics, such as age (*P*=.32), sex (*P*=.19), HDL-C (*P*=.28), and LDL-C (*P*=.13). TG and TC were positively associated with BMI (*P*<.05) in Saudi patients with FH.

Logistic regression analysis

Logistic regression analysis was performed for lipid profiles and Q192R genotypes (**Table 4**). Statistical analysis revealed associations of QR genotypes with both TG (odds ratio: 2.22 [95%CI:1.00-4.96]; *P*=.05) and TC (odds ratio: 2.32 [95%CI: 1.04-5.17]; *P*=.04).In the RR grp2, only TG was associated (odds ratio: 5.80 [95%CI:1.79-18.82]; *P*=.034). The other variants were not associated with HDL-C and LDL-C for the Q192R genotypes.

DISCUSSION

In this study, we investigated the baseline characteristics, allele frequencies, and genotype distribution in Saudi patients with FH. We found that there were significant associations among allele, genotype, and the dominant model in the Q192R analysis. However, ANOVA and logistic regression analysis showed associations among the Q192R genotype, baseline characteristics, and lipid profiles. Earlier studies found that the Q192R polymorphism is not associated with FH or hypercholesterolemia.[22,27,28] However, in our study, these polymorphisms were associated with FH, possibly due to differences in ethnicity, and the disease appeared to occur in younger patients. FH is also related to high cholesterol, which affects lifetime cholesterol levels and can lead to an early crisis of CAD due to the formation of cholesterol plaque in the arteries. Indeed, these changes are associated with the supply of blood and oxygen to the major blood vessels in the heart, and conversion to the diseased form of the vessels.[29]

Some genetic studies have confirmed that the R allele is often observed in patients with CAD,[30-33] suggesting that the Q192R variant may be a risk factor for atherosclerosis.[34,35] Notably, in the Saudi population, the Q192R polymorphism is associated with CAD, glucose 6 phosphate dehydrogenase, gestational diabetes, and type 2 diabetes mellitus.[12,18,26,31] Results from other studies have shown that there is a positive association between the R allele in the Q192R polymorphism and CAD development.[34,36] Moreover, individuals with the RR genotype have a high risk of CAD; in contrast, patients with other *PON1* polymorphisms have a low-

Table 4. Adjusted logistic regression analyses of association between genotypes/alleles, TG, TC, HDL-C, and LDL-C.

Genotypes/ alleles	TG			TC			HDL-C			LDL-C		
	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
QQ *grp10*	Reference			Reference			Reference			Reference		
QR grp1	2.22	1.00-4.96	**.050**	2.32	1.04-5.17	**.040**	1.41	0.64-3.10	.396	0.65	0.29-1.43	.285
RR grp2	5.80	1.79-18.82	**.034**	2.75	0.87-8.68	.084	2.53	0.80-7.96	.112	1.16	0.37-3.59	.803
Age	0.96	0.92-1.00	.075	0.99	.95-1.04	.794	0.98	0.94-1.02	.40	0.98	0.94-1.02	.409
Gender	1.27	0.55-2.90	.572	1.31	0.57-3.00	.517	1.42	0.62-3.24	.409	1.96	0.85-4.52	.114
BMI	1.11	0.94-1.31	.199	1.01	0.86-1.19	.890	1.02	0.87-1.21	.743	1.01	0.86-1.19	.889

QQ=normal genotype; QR=heterozygous genotype; RR=variant/mutant genotype (risk genotype); TG=triglycerides; TC=total cholesterol; HDL-C=high density lipoprotein cholesterol; LDL-C=low density lipoprotein cholesterol; BMI=body mass index. - 2 log likelihood statistic: TG: 869.593,TC=886.490, HDL-C: 756.867, LDL-C: 814.864

Bold values are significant.

er risk of HDL and LDL oxidation.[37,38] In our study, we found that 12% of patients with FH had the RR genotype. There are common variants associated with CAD and FH in the global population.[23,24,39-45] However, there are other variants that are associated with CAD other than the *PON1* gene in the global population.[46-49]

The Q192R variant in the *PON1* gene modifies the ability of the enzyme to protect LDL from oxidation in vitro through the protective Q allele.[50] PON1 protein acts as a lipophilic antioxidant in both the liver and serum by binding to HDL and plays a protective role against oxidative stress in the lungs.[51] Recently, meta-analyses have become important approaches to confirm the accuracy of findings in the global population and to facilitate complex analyses. Unfortunately, few meta-analyses of the Q192R polymorphism have been performed in patients with FH,[52,53] and no meta-analyses have described the few case-control studies of the Q192R variant in patients with FH. However, in two meta-analyses in the Q192R variant in patients with CAD, the association of this variant with lower PON1 activity and increased risk of CHD was confirmed.[54,55]

Environmental factors, such as dieting, exercise, and smoking, have been largely ascribed to influence the development of FH. However, in our study, we did not have access to patient health records, and the role of environmental factors in the development of FH in the Saudi population is still unknown. Moreover, other studies from Saudi Arabia have also not documented the effects of environmental factors.[56-60] If patients with FH develop diabetes, obesity, metabolic syndrome, or any other diseases, the risk of CAD, which is a major health problem in adult Saudis, could be increased.[59]

The strengths of the current study were that we selected all Saudi subjects, and then analyzed the association of FH with the Q192R polymorphism. Finally, we collected a minimum of 100 FH cases and 100 controls to perform a thorough case-control study. However, there are several limitations. For example, we investigated only a single SNP and did not obtain information on medications or clinical details for enrolled patients with FH. We also could not collect data on smoking status. Another important limitation of our study was that we did not document exercise and diet habits. Finally, we also did not analyze the mRNA expression of *PON1*.

In conclusion, we confirmed a positive association of FH with the Q192R polymorphism in the Saudi population. However, additional studies with larger sample sizes and younger patients of different ethnicities are needed to further demonstrate the role of the *PON1* gene. Meta-analyses of the relationship between FH and the *PON1* gene are also needed to determine the risk of developing the disease within the Saudi population and overall global population. Comparisons between Saudi and non-Saudi populations, which may yield conclusions regarding the significance of Q192R genetic variance in the *PON1* gene, are also necessary. Additionally, studies with a large sample size and samples from individuals of different age groups can further validate this positive association.

Conflict of interest

All the authors declare that there is no conflict of interest.

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