

Research Article

Effects of Hinc II Polymorphism in the LDL Receptor Gene on Serum Lipid Levels of Jordanian Individuals with High Risk for Coronary Heart Disease

Nabil Ahmad Bashir* 

Department of Physiology and Biochemistry, Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan

Abstract

Coronary heart disease (CHD) has presented high prevalence in the Jordanian population. Nevertheless, studies of genetic risk factors for CHD in our country are insufficiently carried out. We are intended to investigated the effects of Hinc II (exon 12) polymorphisms at the low-density lipoprotein receptor (LDLR) gene on circulating lipids of 150 individuals with high risk for CHD (HRG) and 150 controls (C). Genomic DNA was extracted from white blood cells and amplified by polymerase chain reaction (PCR) and digested with HincII Restriction enzyme. LDL, TC (total cholesterol), TG (triglycerides), and HDL (high density lipoproteins) levels were measured in all subjects. RFLP (restriction fragment length polymorphism) analysis was conducted to identify genotype of LDLR gene in (HRG) and 150 controls (C). The results showed a significant correlation existed between this RFLP locus and (HRG), $X^2=10.6$, $P<0.05$; H^+ allele frequency: $X^2=7.88$, $P<0.05$., H^- allele frequency: $X^2=7.88$, $P<0.05$. Genotypes H^+H^+ and H^+H^- in high risk group are significantly associated with high levels of LDL, TC, TG, $P<0.05$ and with low level of HDL $P<0.05$., while H^-H^- is associated with normal values of serum lipids $P<0.05$. It is inferred that H^+ allele might be associated with high blood cholesterol level, and the H^- allele with normal level. This study suggests that the differences in LDLRG genotypes might affect the phenotype of lipid metabolism.

Keywords

Hypercholesterolemia, LDL, PCR-RFLP, Genotypes, Alleles

1. Introduction

Low density lipoprotein receptor has a central role in lipid metabolism and cholesterol homeostasis [1-6]. Mutations in the LDLR gene occur in approximately 1/500 individuals resulting in a dominant inherited disorder of plasma cholesterol metabolism, and causing elevated levels of serum LDL and consequently premature atherosclerosis known as familial hypercholesterolemia [3]. Familial hypercholesterolemia is

associated with defects in LDL receptor and other factors. Allele variation in LDL receptor plays a role in the clinical course of CHD. Polymorphic site in LDLR gene were reported to affect serum levels of lipids in normolipidic and hyperlipidemics [7-10]. Identification of genotypes and allele frequencies of LDL receptor gene in high risk group could be used as a marker for preclinical diagnosis and for better

*Corresponding author: nbashir@just.edu.jo (Nabil Ahmad Bashir)

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management. Specifically, we are going to identify allele variation of LDLR gene in coronary heart risk group and in control normal population,, also to investigate the allele variation on clinical parameters of lipid metabolism in the groups under investigation.

2. Materials and Methods

2.1. Subjects

The subjects consisted of 200 Jordanian males and females, 25 to 65 years (mean, 45 years) old who consulted the physician for regular health checkup, participants were interviewed and a questionnaire was completed, which included sex, age, presence of diabetes, family history CHD, family history of dyslipidemia, and any other metabolic diseases. All subjects gave informed consent to participate in the study. This work was approved by the IRB in Jordan University Of Science And Technology. From these subjects which were selected previously, 101 have cholesterol > 250 mg/dl representing the high risk group (HRG) and 99 controls (CG).

2.2. Methods

Venous blood samples were collected after 14 hour overnight fasting in plain and EDTA tubes, serum or plasma was obtained by low speed centrifugation at 1000 g for 15 minutes and stored at -20 °C until analysis. Ten ml blood was extracted by salting out method to prepare genomic DNA [11]. Primers and the gene amplifier kit were purchased from Promega to amplify exon 12 of LDLR gene [12, 13]. The sequence of the primers used for PCR amplification are shown:

5'-TCTCCTTATCCACTTGTGTGTCTAG-3'

5'-CTTCGATCTCGTACGTAAGCCACAC-3'

The PCR reaction conditions are: 35 cycles of 94 °C for 1 min, 56 °C for 1 min and 72 °C for 1 min. The size of the amplified fragment was 190 bp. PCR products were digested with HincII and separated on 3% agarose. The presence of 133 bp fragment was defined as allele H⁺ and that of 98 bp as allele H⁻. The frequencies of different alleles and genotypes in each group were calculated according to Hardy-Weinberg equilibrium. Total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) were determined by enzymatic colorimetric method CHOD-PAP using commercially available kits from Promega. Chi-square analysis was used for comparison of allele frequencies and genotype distribution between the studied groups, with the level of significance set at P< 5%.

3. Results

Table 1 shows the serum lipid values of the participants, 101 HRG and 99 CG. About 80% of the patients were older

than 50 years, 50% were males and 50% were females. The mean values of cholesterol, LDL-cholesterol, and triglycerides in HRG are; 349.3±163.0 mg/dl, 223.3±59.9, mg/dl and 291.7±197.0 mg/dl, (p < 0.05), while HDL level was 150.6±75, (p < 0.05). The frequency of H+H+, H+H-, and H-H- genotypes in HRG subjects are 41%, 34%, 25%, respectively (Table 2) with H+ and H- allele frequency of 0.58 and 0.42, respectively (Table 2), while in the control group the frequencies of H+H+, H+H-, and H-H- genotypes are 3%, 36%, and 61%, respectively. With 0.2 and 0.8 H+ and H- allele frequency, respectively (Table 2).

Table 1. Characteristics of study participants.

Variable	CG(N=99)	HRG (N=101)
Age	25-65	25-65
Cholesterol	200.6±128.6	349.3±163.0
LDL-Cholesterol	93.5±38.8	223.3±59.9
HDL-Cholesterol	191.5±78.1	150.6 ±75
Triglycerides	115.0±31.0	291.7±197.0
Family history CHD	-	+
Family history dyslipidemia	-	+
Metabolic diseases	-	-

CG: control group, HRG: high risk group, CHD: coronary heart disease

The influence of Hinc II polymorphism and mutation at the LDLR gene on lipid profile of the HRG group is shown in Table 3. LDL levels are 248.4±132.5 mg/dl, 219.4±184.9 mg/dl, 186.6±164.5 mg/dl in H+H+, H+H-, and H-H- genotypes, respectively, while they are 118 ±32.5 mg/dl, 100.8±41.1 mg/dl, and 87.9± 37.0 mg/dl in control group. Total cholesterol levels are 368.2±148.9 mg/dl, 328.1±174.7 mg/dl, and 346.6±172.1 mg/dl in H+H+, H+H-, and H-H- genotypes, respectively, while they are 196.6± 53.2, mg/dl 182.8± 40.2 mg/dl, and 159.4± 48.5 mg/dl in the control group. Triglycerides levels are 345.7±235.1 mg/dl, 268.1±155.1 mg/dl, and 233.2±157.3 mg/dl in H+H+, H+H-, and H-H- genotypes, respectively, while they are 148.0± 8.8, 116.2±33.1 mg/dl, and 112.7±29.7 mg/dl in control group. HDL levels are 146.4± 84.5 mg/dl, 147.4± 69.6 mg/dl, and 162.0± 77.9 mg/dl, respectively, while they are 200.6±128.6 mg/dl, 196.1± 77.2 mg/dl, and 188.3± 77.5 mg/dl in the control group. The frequencies of genotypes and alleles H+H+, H+H-, H-H-, H+, and H- in control subjects who have LDL<150.5 mg/dl or TC<260 mg/dl or TG<166 mg/dl or HDL<352.5 mg/dl are 2.0%, 36%, 62%, 0.2, and 0.8%, respectively. (Table 4). In in coronary heart disease high risk group (HRG) the frequencies of genotypes and alleles H+H+,

H+H-, H-H-, H+, and H- in LDL>150.5 mg/dl subgroup are 51.5%, 28%, 20.5%, 0.6 and 0.4, respectively. While in LDL<150.5 mg/dl they are 21%, 45.5%, 33.5%, 0.4, and 0.6. (Table 5). In the TC>260 mg/dl subgroup the frequencies of genotypes and alleles H+H+, H+H-, H-H-, H+, and H- are 48%, 28%, 24%, 0.6, and 0.4, respectively. On the hand the

frequencies are 32%, 42%, 26%, 0.5 and 0.5, respectively,) in TC<260 mg/dl, (Table 5). In TG>166 mg/dl subgroup the frequencies of genotypes and alleles H+H+, H+H-, H-H-, H+, and H- are 40.4%, 40.2%, 19.4%, 0.6, and 0.4, respectively. On the other hand the frequencies are 45%, 17%, 38%, 0.5, and 0.5, respectively, in TG<166 mg/dl subgroup. (Table 5).

Table 2. Genotype distribution with and allele frequency of LDLR gene /HincII RFLP in coronary heart disease risk group (HRG) and control (CG) in Jordan.

Polymorphism	Genotype distribution			Allele frequency	
	%H+H+(n)	%H+H-(n)	%H-H-(n)	H+(n*)	H-(n*)
CG (n=99)	3 (3)	36 (36)	61 (60)*	0.2 (42)	0.8(156)
HRG(n=101)	41 (42)	34 (34)	25 (25)	0.58 (118)	0.42 (92)

H+H+: homozygous for the presence of HincII site (mutant); H-H-: homozygous for the absence of HincII site (normal allele). (n) represents the number of subjects. (n*) represents the number of alleles. There is a significant association between the genotype H+H+ and high risk group when compared to control; $\chi^2=10.6$, $P<0.05$; allele frequency: $\chi^2=7.88$, $P<0.05$.

Table 3. Relation between LDLR/HincII RFLP and lipid in coronary heart disease high risk group (HRG) in Jordan.

Genotypes	n	LDL mg/dl	TC, mg/dl	TG, mg/dl	HDL mg/dl,
Control (mixed genotypes)	99	93.5±38.8	200.6±128.6	115.0±31.0	191.5±78.1
H+H+	3	118 ±32.5	196.6±53.2	148.0±8.8	200.6±128.6
H+H-	36	100.8±41.1	182.8±40.2	116.2±33.1	196.1±77.2
H-H-	60	87.9±37.0	159.4±48.5	112.7±29.7	188.3±77.5
Coronary heart disease High risk group HRG (mixed genotypes)	101	223.3±159.9	349.3±163.0	291.7±197.0	150.6 ±75.1
H+H+	42	248.4±132.5	368.2±148.9	345.7±235.1	146.4±84.5
H+H-	34	219.4±184.9	328.1±174.7	268.1±155.1	147.4±69.6
H-H-	25	186.6±164.5	346.6±172.1	233.2±157.3	162.0±77.9

LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; TC: total cholesterol; TG: triglycerides, H+H+: homozygous for allele H⁺; H-H-: homozygous for allele H⁻; H⁺H⁻: heterozygous.

Comparison of coronary heart disease High risk group (HRG) genotypes with the corresponding genotypes in the control:

H+H+: LDL: $P<0.05$, TC: $P<0.05$, TG: $P<0.05$, HDL: $P<0.05$.

H+H-: LDL: $P<0.05$, TC: $P<0.05$, TG: $P<0.05$, HDL: $P<0.05$.

H-H-: LDL: no significance, TC: no significance, TG: $P<0.05$, HDL no significance.

Table 4. LDLR genotypes and allele frequencies with LDL, TC, HDL, and TG subgroups in control group (CG).

Lipid subgroups	n	Genotypes Allele frequency			
		H+H+	H+H-	H-H-	H+ H-
♦LDL>150.5 mg/dl	2	50(1)*	50(1)	-	0.75(3)**
LDL<150.5 mg/dl	97	2.0(2)	36(35)	62(60)	0.2(39)

Lipid subgroups	n	Genotypes Allele frequency				
		H+H+	H+H-	H-H-	H+ H-	H-
♦♦TC>260 mg/dl	1	-	100(1)	-	0.5(1)	0.5(1)
TC<260 mg/dl	98	3(3)	36(35)	61(60)	0.2(41)	0.8(155)
***TG>166 mg/dl	1	-	100(1)	-	0.5(1)	0.5(1)
TG<166 mg/dl	98	3(3)	36(35)	61(60)	0.2(41)	0.8(155)
♣HDL>352.5 mg/dl	2	-	50(1)	50(1)	0.25(1)	0.75(3)
HDL<352.5 mg/dl	97	3(3)	36(35)	61(59)	0.2(41)	0.8(153)

H+H+: homozygous for allele 1; H-H-: homozygous for allele 2; H+H-: heterozygous; LDLR: low density lipoprotein receptor; HDL-C: high density lipoprotein cholesterol; (*): the number of each genotype; (**): the number of allele.

♦♦comparison with <150.5 mg/dl: genotype frequency, $X^2=10.6$, $P<0.05$, allelic frequency, no significance.

♦♦comparison with <260 mg/dl: genotype frequency, no significance. allelic frequency, no significance.

***comparison with <166 mg/dl: genotype frequency, no significance. allelic frequency, no significance.

♣comparison with <352.5 mg/dl: genotype frequency, no significance. allelic frequency, no significance.

Table 5. LDLR gene genotypes and allele frequencies with LDL, TC, HDL, and TG subgroups in coronary heart disease high risk group (HRG).

Lipid subgroups	n	Genotypes Allele frequency				
		H+H+	H+H-	H-H-	H+ H-	H-
♦LDL>150.5 mg/dl	68	51.5(35)*	28(19)	20.5(14)	0.6(89)**	0.4(47)
LDL<150.5 mg/dl	33	21(7)	45.5(15)	33.5(11)	0.4(25)	0.6(37)
♦♦TC>260 mg/dl	63	48(30)	28(18)	24(15)	0.6(78)	0.4(48)
TC<260 mg/dl	38	32(12)	42(16)	26(10)	0.5(40)	0.5(36)
***TG>166 mg/dl	72	40.4(29)	40.2(29)	19.4(14)	0.6(87)	0.4(57)
TG<166 mg/dl	29	45(13)	17(5)	38(11)	0.5(31)	0.5(27)
♣HDL>352.5 mg/dl	3	66.7(2)	-	33.3(1)	0.6(4)	0.4(2)
HDL<352.5 mg/dl	98	41(40)	34(34)	25(24)	0.6(114)	0.4(82)

H+H+: homozygous for allele 1; H-H-: homozygous for allele 2; H+H-: heterozygous; LDLR: low density lipoprotein receptor; HDL-C: high density lipoprotein cholesterol; (*): the number of each genotype; (**): the number of allele.

♦comparison with <150.5 mg/dl: genotype frequency, $X^2=5.9$, $P<0.05$, allelic frequency $X^2=7.8$, $P<0.05$.

♦♦comparison with <260 mg/dl: genotype frequency, no significance. allelic frequency, no significance.

***comparison with <166 mg/dl: genotype frequency, $X^2=5.9$, $P<0.05$, allelic frequency $X^2=7.8$, $P<0.05$.

♣comparison with <352.5 mg/dl: genotype frequency, no significance. allelic frequency, no significance.

4. Discussion

The presented data provides valuable insights into the association between HincII polymorphism in the LDLR gene and serum lipid profiles in both high-risk coronary heart disease group (HRG) and the control group (CG). The study reveals significant variations in lipid levels and genotypic frequencies, shedding light on the potential influence of genetic factors on lipid metabolism.

In HRG, the H+H+ genotype is predominant (41%), fol-

lowed by H+H- (34%) and H-H- (25%). The corresponding allelic frequencies are 0.58 for H+ and 0.42 for H-. In contrast, the CG exhibits a different distribution, with H-H- being the most prevalent (61%), followed by H+H- (36%), and H+H+ (3%), and allelic frequencies of 0.8 for H- and 0.2 for H+.

The observed differences in genotypic and allelic frequencies between HRG and CG suggest a potential association between HincII polymorphism and coronary heart disease risk. The higher prevalence of H+ alleles in HRG implies a genetic predisposition that may contribute to altered lipid metabolism.

The mean values of total cholesterol, LDL-cholesterol, and

triglycerides are significantly elevated in HRG compared to CG, suggesting a potential role of HincII polymorphism in influencing lipid homeostasis in high-risk individuals. The HDL levels, however, are lower in HRG

Analyzing the impact of HincII polymorphism on lipid profiles within HRG reveals genotype-specific differences. In HRG, individuals with H+H+ genotype exhibit higher LDL levels (248.4 ± 132.5 mg/dl), total cholesterol (368.2 ± 148.9 mg/dl), and triglycerides (345.7 ± 235.1 mg/dl) compared to H+H- and H-H- genotypes. This trend is consistent with the notion that certain genotypes may predispose individuals to dyslipidemia, potentially elevating the risk of coronary heart disease. [14]. Further stratification based on lipid levels reveals interesting patterns. In the LDL > 150.5 mg/dl subgroup, H+H+ genotype is more prevalent (51.5%), supporting the association between HincII polymorphism and elevated LDL levels. Similarly, in the TC > 260 mg/dl subgroup and TG > 166 mg/dl subgroup, H+H+ genotype shows higher frequencies, emphasizing the potential impact of this genetic variation on hypercholesterolemia and hypertriglyceridemia.

The presented data provides compelling evidence suggesting a potential association between HincII polymorphism in the LDLR gene and altered lipid profiles in high-risk coronary heart disease individuals [15]. The findings underscore the importance of genetic factors in influencing lipid metabolism and, subsequently, cardiovascular health [16]. Further research is warranted to elucidate the underlying molecular mechanisms and to explore the potential for personalized interventions based on genetic risk profiling.

5. Conclusion

It is inferred that H⁺ allele might be associated with high blood cholesterol level, and the H⁻ allele with normal level. This study suggests that the differences in LDLRG genotypes might affect the phenotype of lipid metabolism.

Abbreviations

CHD	Coronary Heart Disease
LDLR	Low-Density Lipoprotein Receptor
HRG	High Risk for CHD
LDL	Low Density Lipoprotein
PCR	Polymerase Chain Reaction
RFLP	Restriction Polymorphism
TG	Triglycerides
HDL	High Density Lipoprotein
C	Control Group
H+H+	Homozygous for Allele 1
H-H-	Homozygous for Allele 2
H+H-	Heterozygous
HDL-C	High Density Lipoprotein Cholesterol

TC Total Cholesterol

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Author Contributions

Nabil Ahmad Bashir is the sole author. The author read and approved the final manuscript.

Copyright Transfer

Our manuscript has not been published or submitted for publication elsewhere.

Conflicts of Interest

The author declares no conflicts of interest.

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