Genetic Association of LDLR rs688 TT Genotype with Obesity in FH Patients

ORIGINAL ARTICLE

LDLR gene variant rs688 TT genotype; genetic association with obesity in Familial Hypercholesterolemia patients

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Abstract

The predominant genetic risk factor for familial hypercholesterolemia along with obesity is LDLR allele status. These receptors maintain cellular cholesterol homeostasis. For instance, a common SNP rs688 in different populations has been reported to be associated with elevated plasma LDL, resulting in hypercholesterolemia. The potential role of variant rs688 with obesity in FH patients was observed. This is a case-control study with 120 blood samples of clinically diagnosed obese familial hypercholesterolemia patients by physicians and 120 healthy individuals’ blood samples were recruited for the study. Genomic DNA was extracted from blood samples. By using Tetra ARMS PCR, genotyping of LDLR rs688 (C>T) exon 12 gene variation was performed. Moreover, BMI, BP, and BSL of obese FH patients were also obtained; a chi-square test was performed on the obtained data to evaluate the association between rs688 and obese FH patients. Genotype CC, CT, and TT frequencies reported in the obese FH patients’ group were 0.33, 0.41, and 0.25, while in healthy individuals were 0.37, 0.41, and 0.21 respectively. Chi–square values showed significant association with BMI, BP, and BSL. It was assessed that there is a 4% increased susceptibility of FH development along with obesity in individuals with TT genotype. So, for obese familial hypercholesterolemia, the LDLR rs688 C>T gene variation can be used as a predisposing genetic marker.
1. INTRODUCTION

Familial Hypercholesterolemia (FH) is an autosomal dominant genetic disorder. It is an imbalance condition in which the body has too much Low-Density Lipoproteins (LDL) cholesterol but a very small number of High-Density Lipoproteins (HDL) cholesterol. A mutation on chromosome 19 triggers FH (Raal, 2020). Due to this mutation, the body is incapable of low-density lipoprotein cholesterol evacuation from the blood (Wang, 2020). Thus, in blood, the LDL level elevates. Due to this at an early age narrowing of the supply routes from atherosclerosis occurs. Approximately 2,000 mutations are reported in this gene (Bjornsson, 2021). The number of low-density lipoprotein receptors delivered inside cells decreases due to this hereditary mutation. Also, the receptor's capacity to evacuate LDLs from the blood is disturbed by mutations (Benito-Vicente, 2018). Some other mutations disturb (Flores, 2020). Exceptionally raised blood cholesterol levels are observed as a consequence, in individuals with defects within the LDLR gene. In the supply routes that transport blood to the heart (coronary arteries), the abundant cholesterol is unusually stored there (Baniaamam, 2021). An individual's chance of having a premature stroke increases due to an accumulation of cholesterol within the walls of arteries. Individuals suffering from familial hypercholesterolemia acquire the LDLR gene with a single changed allele from a diseased parent and a single normal allele of the gene from the other parent (Nomura, 2004). Regularly starting at the age of the '40s & '50s, an expanded hazard of early heart disorders begins. Seldom, is an individual is congenital with familial hypercholesterolemia having an LDLR gene with two mutated alleles (Rosenson, 2020). This circumstance happens when each of the diseased parents passes on one defective allele of the gene to the offspring, as both parents have mutant LDLR genes. This occurrence of mutations in both LDLR genes comes about in an extra serious frame of hypercholesterolemia that as a rule shows up in childhood (Sanogo, 2019). LDLR gene is responsible for providing commands for the production of a low-density lipoprotein receptor protein. The function of these receptors is to tie up with the particles present in the circulation termed low-density lipoproteins. The essential transporters of cholesterol within the blood are LDLs (Grundmann, 2020). Fat-like waxy constituent termed cholesterol is delivered inside the body and gotten as nourishment having an origin in animals. The family of low-density lipoprotein receptor genes comprises cell surface proteins included in a mechanism called receptor-mediated endocytosis specifically of particular ligands. Numerous sorts of cells have low-density lipoprotein residing on their external surfaces. Here they choose up circulating LDLs within the circulation system, leading them inside the cell (Luo, 2020). The LDL is smashed down to discharge cholesterol when it reaches inside the cell. At this point, cell utilization of cholesterol occurs and if needed the body evacuates it. Subsequently, LDLRs after dropping off their batch are reused again on the external surface of cells to choose up more LDLs from the circulation. The sum of total cholesterol levels in the circulation is directed by the Low-density lipoprotein (Lucarelli, 2020). The liver is the body organ whose cells contain an excess of LDLRs as the liver clears the most abundant amount of cholesterol out of the body. As soon as Low-density lipoprotein bounds upon the external surface of cells and is transported into the cell for lysosome processing. The protein is degraded in the lysosome, and then the cholesterol is made completely accessible (Boren, 2020). The main aim of this present study was to explore whether the single nucleotide polymorphism rs688 in the LDLR gene is associated with the development of obesity in familial hypercholesterolemia patients. The variant rs688 of LDLR gene plays rule as a modulator for alternative exon splicing, due to which there occurs shift in the reading frame along with gene transcript alteration and have been testified to cause familial hypercholesterolemia (McIntosh, 2021). Therefore, this study figures out the frequency of LDLR (rs688C > T) gene polymorphism in obese familial hypercholesterolemia patients.

2. METHODOLOGY

To conduct this research, 240 samples were collected the sample size was calculated with a 95% confidence level (https://www.openepi.com/SampleSize/SSCC.htm). They were sorted into two groups. One group had 120 samples of patients diagnosed with familial hypercholesterolemia while the other had 120 samples of controls. Duration of the study was one year, from 2022 to 2023. Blood samples were obtained from Punjab Cardiology and Sheikh Zaid Hospital, Lahore Pakistan and ethical approval was also taken from there (ERC: 14/9/23/1189). Permission of study subjects was obtained before sample collection. After 12 hours of fasting the blood samples of hypercholesterolemia
patients were collected. Blood samples were gathered from only those subjects who consented to contribute to the research and filled out the consent form. Performa was also designed to obtain history relevant to the disease.

Individuals with any genetic disease were excluded from commencing the research. Individuals who were diagnosed with infectious diseases, for example, AIDS, HCV hepatitis, and HBV hepatitis were not included in the research. The next step is the extraction of DNA, after the collection of samples. High-yield DNA was extracted by using the isoamyl-chloroform method (https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/phenol-chloroform-extraction). Gene polymorphism LDLR-rs688C > T was detected using Tetra ARMS PCR for SNP detection (https://pubmed.ncbi.nlm.nih.gov/24519268/). In this PCR four primers (inner forward, inner reverse, outer forward and outer reverse) were used for the amplification of desired bands. The primers used for amplification were

IF 5'TCAGCTCATCTAAGCATGATGTC 3’
IR 5'CCAAGATGTTCTTCCGGTGCCCACA 3’
OF 5'GTCTAGATCTCCTCAGTTGGCCGCC 3’
OR 5'TATCCGCCACCTAAGTGCTTGCATCTCG3’

The genotyping primers of LDLR-rs688C/T were designed by PRIMER3 software. In this amplification procedure, the annealing temperature is very crucial as it is the temperature at which primers melt. The reaction mixture was prepared by adding the master mix (6µl), the four primers that were “outer forward” and “outer reverse”, “inner forward” and “inner reverse”, 1µl each, water 0.5 µl, and DNA sample 1.5 µl. Taq Polymerase was added to about 0.01 µl in the PCR mixture. For analysis of the association between LDLR polymorphism and the susceptibility to obesity in familial hypercholesterolemia patients, a Chi-square (χ²) test was done. T-test was applied to check the association of different clinical-pathological features with high cholesterol in FH patients.

3. RESULTS & DISCUSSION

The study is to check the variants of the LDLR gene in association with the development of obesity in familial hypercholesterolemia patients. 240 individuals were included among them 120 were patients diagnosed with FH, and 120 were controls. A difference was observed in genotype distributions among the obese FH cases and matched healthy controls (Figure 1).

All three genotypes of CC, CT, and TT frequencies reported in the FH patients’ group were 33%, 41%, and 25%, while in healthy individuals were 37%, 41%, and 21% respectively (Table 1).

Table 1: LDLR SNP rs688 allele frequency among obese FH patients and controls.

<table>
<thead>
<tr>
<th>Allele</th>
<th>FH Patients</th>
<th>Healthy Controls</th>
<th>Chi-square</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>110 (0.46)</td>
<td>101 (0.42)</td>
<td>0.685</td>
<td>0.40787</td>
</tr>
</tbody>
</table>

*Designates a significant association
The SNP, rs688 was detected in 65 (54%) patients. It was assessed that there is a 4% increased susceptibility of FH development in individuals with TT genotype (Table 2). According to an observation, LDLR rs688 modulates the splicing efficiency as it alters the exon splicing enhancers (Gallo, 2020). Moreover, rs688 is recognized as a functional variant of the LDLR gene associated with clinically relevant phenotypes. An approximate twice the increase in LDL-cholesterol is observed with the subsequent loss of a single LDLR allele. So rs688 is associated with increased plasma LDL-cholesterol levels.

**Table 2:** LDLR SNP rs688 Genotype Frequency among obese FH patients and controls.

<table>
<thead>
<tr>
<th>Alleles</th>
<th>FH Patients</th>
<th>Healthy Controls</th>
<th>Chi-square</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n(f)</td>
<td>n(f)</td>
<td>X²</td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>30 (0.25)</td>
<td>26 (0.21)</td>
<td>0.5899</td>
<td>0.744557</td>
</tr>
<tr>
<td>T/C</td>
<td>50 (0.416)</td>
<td>49 (0.41)</td>
<td>0.5899</td>
<td>0.744557</td>
</tr>
<tr>
<td>C/C</td>
<td>40 (0.33)</td>
<td>45 (0.37)</td>
<td>0.5899</td>
<td>0.744557</td>
</tr>
</tbody>
</table>

*Designates a significant association

The high cholesterol level of FH patients was studied for the association with different parameters i.e., body mass index (BMI), blood pressure, and blood sugar level. By performing a chi-square test on each of these variables a p-value obtained was < 0.01 for each respectively. This represents a remarkable association of the blood cholesterol
levels with BMI, BP, and blood sugar levels of patients as shown in Table 3.

Table 3: Effect of High Cholesterol on BMI, Blood Pressure & Blood Sugar Level of FH patients.

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Parameters</th>
<th>Mean ± SD</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Body Mass Index (kg/m²)</td>
<td>29.24 ± 7.89</td>
<td>-29.351625</td>
<td>&lt; 0.00001*</td>
</tr>
<tr>
<td>2.</td>
<td>Systolic Blood Pressure (mg/dL)</td>
<td>119.89 ± 16.94</td>
<td>-8.611198</td>
<td>&lt; 0.00001*</td>
</tr>
<tr>
<td>3.</td>
<td>Diastolic Blood Pressure (mg/dL)</td>
<td>75.8 ± 9.81</td>
<td>-18.919582</td>
<td>&lt; 0.00001*</td>
</tr>
<tr>
<td>4.</td>
<td>Blood Sugar Level (mg/dL)</td>
<td>234.09 ± 98.32</td>
<td>6.002622</td>
<td>&lt; 0.00001*</td>
</tr>
</tbody>
</table>

*Designates a significant association

Familial hypercholesterolemia is a widespread disease. It proves to be a big challenge for healthcare officers these days. According to estimates, FH affects 1 in 200 to 1 in 250 people in major countries (Hu, 2020). It is also considered the most common inherited condition that affects the heart and blood vessels. More than 10,000 FH cases are prevailing in Pakistan and are under-diagnosed still (Ali, 2019). A critical role is played by the low-density lipoprotein receptor in sustaining the homeostasis of cellular cholesterol (Hassan, 2019). It also functions in the uptake and binding of plasma LDL particles from circulation. SNP rs688 of LDLR gene has been testified to be linked with elevated total cholesterol along with LDL cholesterol in numerous populations. rs688 is chief to raised levels of plasma LDL, after a higher hazard for developing premature atherosclerosis along with obesity and hypercholesterolemia (Nicchio, 2021). In the present study, the role of rs688 in obese hypercholesterolemia patients was explored. A noteworthy dissimilarity was detected in the distribution of genotype between the obese hypercholesterolemia patients and corresponding healthy controls with a p-value <0.05. All three genotype frequencies were reported as CC, CT, and TT 38% 42%, and 22% inpatient samples respectively. The LDLR genotype TT was found associated with obese FH in Pakistan. Similarly, a study conducted in India, shows that the genotype TT and majorly T allele of SNP rs688 was associated with obesity in FH in Indian patients (Pasdar, 2022). Similar research conducted in Taiwan obese adults with FH, showed the comparable minor allele T incidence in patients (Liu, 2020). Recent research conducted in China in 2022 suggests that elevated levels of LDLs and any mutation in LDLR rs688 are correspondingly independent risk factors for the development of obesity and hypercholesterolemia (Chen, 2022). Studies suggest the pivotal inheritance of rs688 in LDLR. The TC level heritability has been assessed to be about 40–60% (Pourzargar, 2020). The prominence is confirmed by the information on the LDLR that the underlying cause of familial hypercholesterolemia is the genetic abnormalities within the LDLR gene. Increased LDL cholesterol levels are displayed by these patients. Furthermore, atherosclerosis and coronary artery disease (CAD) are at affiliated increased risk. It was observed that the efficiency of splicing exons is affected by this variant. Moreover, it was presented that due to the negative effect on exon-splicing, the cholesterol levels are altered. So, we can use LDLR SNP rs688 gene variation C>T as a prognostic genetic marker for familial hypercholesterolemia and different cardiovascular diseases. High cholesterol levels in FH patients affect the BMI, BP, and BSL (Liang, 2022). Any disturbance in lipid metabolism gives rise to problems like high blood pressure, obesity, and diabetes.

4. CONCLUSION

This study represents many scientific aspects of FH. The synonymous variant rs688 of the LDLR gene can be used as a predisposing genetic marker for familial hypercholesterolemia. The decrease in LDLR exon splicing efficiency due to rs688 causes elevated plasma LDL levels and thus obesity. The results prove the risk factor for FH is the TT genotype and T allele, while the protective factor against FH is the CC genotype and C allele. So LDLR rs688 TT genotype and the T allele are associated with increased susceptibility for the development of obesity in FH patients.
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Competing Interests
The authors have no competing interests.

Author Contributions
Muneeba Afzal: Design the Proposal, Experimental Design, Wrote the Paper; Hamza Altaf: Collection of data, Wrote the paper, Submission process; Tayyaba Faiz: Data and analysis tools, Contributed to wet lab work, Financial contribution; Rabia Zafar: Literature review, Reference formatting, Financial contribution; Ruqaya Shoukat: Data and analysis tools, sample collection; Muhammad Waseem: Proof reading, Article revision; Dr. Shahid Aziz: External supervisor, Aid in funding, Aid in research methodology.

5. REFERENCES


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