Polymorphism in Genes Apolipoprotein C3 (APOC3) and Fatty Acid-Binding Proteins (FABP2) in Nondiabetic Dyslipidemics: A Tertiary Care Hospital-Based Pilot Study

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Abstract

Context Dyslipidemia is a multifactorial disease in which lipoproteins play an important role as one of the early markers for coronary heart disease (CHD). Mixed dyslipidemia is common in people with diabetes mellitus, but nondiabetic dyslipidemics (NDD) remain unidentified for the risk of developing dyslipidemia and eventually CHD.

Objectives This pilot study attempts to analyze the genetic basis of lipid metabolism alterations, emphasizing the association between fatty acid-binding protein-2 (FABP2-Ala54Thr) and apolipoprotein-C3 (APOC3-rs5128) genetic polymorphism, as a risk for developing dyslipidemia and CHD in NDD.

Methods and Design Total 90 subjects—30 DD, 30 NDD, and 30 apparently healthy subjects representing Central India—were included. Biochemical analysis and DNA genotyping were done by polymerase chain reaction restriction fragment length polymorphism.

Statistical Analysis The biochemical parameters were reported as means ± standard deviation. One-way analysis of variance test was used to compare biochemical parameters of three groups. Chi-squared test was done to compare genotype distributions. The strength of association was assessed by odds ratios (ORs) with 95% confidence intervals (CIs). All statistical analysis was done using SPSS-PC software and Graph Pad.

Results In NDD, maximum polymorphism was observed followed by DD and least polymorphism was observed in controls. There was a significant association of APOC3 G allele with occurrence of hypertriglyceridemia (p < 0.05); however, no such association was found for FABP2 A allele (p > 0.05). Logistic regression analysis revealed APOC3 polymorphism to be significantly associated with dyslipidemia (OR = 2.6667, 95% CI = 1.0510–6.7663, p = 0.0341); no such association was found for FABP2 polymorphism (OR = 0.4643, 95% CI = 0.1641–1.3136, p = 0.1347). The triglyceride and cholesterol values in individuals with homozygous genotype indicate

Keywords ➤ APOC3 ➤ dyslipidemia ➤ FABP2 ➤ nondiabetic ➤ polymorphism

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that genetic study is comparable to the biochemical findings in carriers of polymorphic allele than noncarriers, especially in NDD patients.

**Conclusions** Pilot study indicates that the presence of APOC3 gene polymorphism is associated with pro-atherogenic dyslipidemia in nondiabetic patients and may raise risk of CHD. This information could be used for preventive strategies in NDD group that may otherwise go unnoticed.

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**Introduction**

Dyslipidemia is a key health problem and concern for the clinicians' worldwide. It is characterized by any or a combination of the following: elevated low-density lipoprotein (LDL-C > 130 mg/dL), elevated total cholesterol (CHOL; > 200 mg/dL), elevated triglyceride (TG; > 150 mg/dL), or low high-density lipoprotein (HDL-C; < 40 mg/dL in men and < 50 mg/dL in women). These conditions play a major role in the progression of coronary heart diseases (CHD).

Hypercholesterolemia is a key independent modifiable risk factor for CHD and leading cause of death worldwide, India alone contributing to almost one-fifth (18.6%) of the global CHD burden. It accounts for 24% of all deaths among adults aged between 25 and 69 years. Although plasma lipid concentrations are strongly influenced by nutritional and lifestyle choices, 50 to 80% of the variation is influenced genetically.

Single nucleotide polymorphism in a few genes is associated with high triglyceride (TG) and CHOL levels significantly contributing to dyslipidemia. A gene associated with TG and CHOL level is the gene encoding for fatty acid–binding proteins (FABP2) located at the chromosome 4q2–q31, another gene apolipoprotein C3 gene (APOC3) located on the chromosome 11q23, allele *3238 C is also involved in the regulation of plasma TG levels.

The circulating lipid biomarkers, mainly polymorphism APOC3 (rs5128) and FABP2 (rs1799883), had been independently studied in diabetic patients, but the associations between these biomarkers and occurrence of dyslipidemia in nondiabetic patients had rarely been studied. There is no information regarding the genetic influence of dyslipidemia and their relations with biochemical estimations of circulating lipoproteins in the population representing Bhopal city (Central India). The present study explored the correlations of serum lipid levels and variations in the genes governing lipid metabolism very critically to find out their role with respect to increased serum lipid levels in the nondiabetic dyslipidemics (NDD) and how different they will be in comparison to the diabetic patients.

**Subjects and Methods**

**Ethics Statement**

Ethics statement approval of the Institutional Ethics Committee was obtained before study commencement (IHEC approval number - LOP/2017/IM0147).

**Study Population**

The study was conducted on unselected population (in terms of their gender, occupation, ethnicity, socioeconomic status) coming to Dyslipidemia Clinic of Department of General Medicine. Clinical history was identified by physicians (Jan 2017 to Sep 2018). To identify the study population, risk assessment was done based on the following inclusion criteria: advancing age (35–70 years), diabetes mellitus, hypertension, family history of dyslipidemia; exclusion criteria: polycystic ovarian syndrome, hypothyroidism, cerebrovascular accidents, autoimmune diseases, tumors, and infectious diseases.

Written informed consent was obtained from all study subjects in English/Hindi language. All participants were adult between the age 35 to 70 years under the inclusion criteria and few were on statins (TG-lowering medication).

**Groups**

Based on the inclusion and exclusion criteria, 90 subjects were selected as study population and divided into three groups of 30 each (following a flat rule of thumb for pilot studies): Group 1: men and women aged between 35 and 70 years who are DD; Group 2: men and women aged between 35 and 70 years who are NDD; Group 3: men and women aged between 35 and 70 years who do not have diabetes and dyslipidemia (C or control group).

**Biochemical Analysis and Genotyping**

For lipid profile tests, 5 mL of fasting venous blood sample was collected in serum vial (yellow vial), and for fasting blood sugar (FBS) tests, 1 mL of blood was collected in fluoride vial (gray vial). For DNA analysis, 1 mL of blood was extracted in ethylenediaminetetraacetic acid vial (purple vial). Lipid profile tests, plasma glucose (FBS), and glycosylated hemoglobin (data not shown) were determined to confirm and classify the study population into DD, NDD, and C (healthy controls). All the biochemical analysis was done in automated high throughput analyzer Beckman Coulter AU680 (Auto Analyzer). Before starting any estimation, the machine was first standardized, and quality control was run.

Genomic DNA was isolated from peripheral blood samples using organic or phenol-chloroform method. Extracted DNA was run in 1% agarose gel, in 1X TAE buffer, for 25 to 30 minutes and visualized on Gel Doc system. Extracted DNA was subjected to polymerase chain reaction (PCR), artificial restriction site was introduced in the DNA. For FABP2,
primers used were F:5’-TACCGAGTTTTCTTCCCACC-3’, R:5’-TTAAATATCCTGCAATTGTGC-3’ product size 456bp, the denaturation temperature was 94°C, the annealing temperature was 59°C, and extension temperature was 72°C. For APOC3, primers used were F:5’- CCAGTGAAGTTGAGGGTG-3’, R:5’-ACCCACAGAACGCCCTCG-3’ product size 501bp, denaturation temperature was 94°C, the annealing temperature was 62°C, and extension temperature was 72°C. The products thus obtained were resolved using 2.5% agarose gel and then visualized in the Gel-Doc system.

The PCR products were digested with their respective restriction enzymes. For FABP2, enzyme HhaI was used, the size of the digested fragments 197/258bp; for APOC3, enzyme SacI was used, the size of the digested fragments 292/209bp. The digested fragments of gene FABP2 and APOC3 were resolved using 4% agarose gel and then visualized on the Gel-Doc system.

Statistical Analysis
The biochemical parameters of the individuals in various groups were reported as means ± standard deviation. To determine significant differences between the groups, analysis of variance test was used to compare biochemical parameters. Chi-squared test was applied to compare genotype distributions between the groups. For each genotype, Hardy–Weinberg equilibrium was tested with chi-squared test.

Results
The comparison of age and biochemical parameters: age, fasting sugar levels, and lipid profile data of the DD group, NDD group, and C group are given in Table 1. There were statistically significant differences between the groups DD, NDD, and C group for FBS, CHOL, TG, LDL-C, and very-low density lipoprotein. No significant differences between groups were detected for age and HDL-C.

The distribution of genotypes and alleles of apo-C3 polymorphism between the DD, NDD, and C groups is reported in Table 2. There was a significant difference in the genotype distribution between the NDD and control groups (chi-square = 12, d.f. = 2, p < 0.001). However, there was no significant difference in genotype distribution of DD and control groups (chi-square = 4.4, d.f. = 2, p > 0.001). CC genotype was significantly more frequent in the control group compared with the DD and NDD group (70 vs. 46.46%). Conversely, GG genotype was more prevalent in the NDD (10%) group compared with the DD (6.66%), and no GG genotype was seen in C groups. However, GG genotype was more prevalent in the DD group (46.66%), followed by the C group (30%) and NDD group (20%). There was a significant association of APOC3 G allele with hypertriglyceridemia (p < 0.05).

Logistic regression analysis revealed APOC3 polymorphism to be significantly associated with dyslipidemia (odds ratio [OR] = 2.6667, 95% confidence interval [CI] = 1.0510–6.7663, p = 0.0341; Table 3).

The distribution of genotypes and alleles of FABP2 polymorphism between the DD, NDD, and C groups is reported in Table 2. There was no significant difference in the genotype distribution between the DD, NDD, and C groups (chi-square = 2.22, d.f. = 2, p > 0.001). The distribution of AA, AG, and GG genotypes was found to be almost similar in all the three groups. No significant association of FABP2 A allele with hypertriglyceridemia was found (p > 0.05). Logistic regression analysis revealed no significant association of FABP2 polymorphism with dyslipidemia (OR = 0.4643, 95% CI = 0.1641–1.3136, p = 0.1347; Table 3).

Discussion
In the present pilot study, an unselected study population of 90 individuals consulting at AIIMS Bhopal Dyslipidemia Clinic (MP) was conducted, which is a cosmopolitan city with a diverse culture and different ethnic groups. Our aim was to identify genes associated with high serum levels of lipids in the NDD population by evaluating the correlation of polymorphism of each candidate gene to this condition. By the help of public databases namely PubMed (NCBI), we selected polymorphisms in two candidate genes—APOC3 (rs5128) and FABP2 (rs1799883)—since these polymorphisms were more prevalent in Indian population. Polymorphisms in both genes have been associated with dyslipidemia and may pose a risk factor for CHD in future.

Table 1 Comparison of age and biochemical parameters between DD, NDD, and C patients

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>DD</th>
<th>NDD</th>
<th>C</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>52.27 ± 11.00</td>
<td>50.37 ± 11.57</td>
<td>49.73 ± 11.27</td>
<td>0.66</td>
</tr>
<tr>
<td>FBS</td>
<td>180.36 ± 68.56</td>
<td>95.46 ± 15.21</td>
<td>95.76 ± 12.68</td>
<td>0.0001*</td>
</tr>
<tr>
<td>TC</td>
<td>225.53 ± 92.82</td>
<td>206 ± 49.66</td>
<td>158.8 ± 38.2</td>
<td>0.0004*</td>
</tr>
<tr>
<td>TG</td>
<td>222 ± 97.03</td>
<td>178.91 ± 60.66</td>
<td>100.03 ± 25.8</td>
<td>0.0001*</td>
</tr>
<tr>
<td>LDL-C</td>
<td>122.8 ± 32.7</td>
<td>125.46 ± 43.7</td>
<td>96.55 ± 30.79</td>
<td>0.0001*</td>
</tr>
<tr>
<td>HDL-C</td>
<td>45.25 ± 26.65</td>
<td>43.43 ± 10.52</td>
<td>42.46 ± 15</td>
<td>0.84</td>
</tr>
<tr>
<td>VLDL</td>
<td>64.90 ± 82.4</td>
<td>48.53 ± 54.44</td>
<td>19.96 ± 5.17</td>
<td>0.0097*</td>
</tr>
</tbody>
</table>

Abbreviations: C, control; DD, diabetic dyslipidemias; FBS, fasting blood sugar; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NDD, nondiabetic dyslipidemias; SD, standard deviation; TC, total cholesterol; TG, triglyceride; VLDL-C, very-low density lipoprotein cholesterol.

Data are reported as mean ± SD.

*p < 0.05 = significant.
To our knowledge, this is the first study to test a significant association of APOC3 (rs5128) and FABP2 (rs1799883) polymorphism and abnormal serum lipid levels in NDD patients, but a comparable study was done to test the determinants of dyslipidemia in North Indians. The lipid profile values were highly variable among the population. Over 66% of the general adult population covered in this study have abnormalities in at least one of the lipid parameters. In the present study as expected, hypertriglyceridemia and hypercholesterolemia were most prevalent among DD group and NDD group and normal values were obtained for C group. Similar observations in the age range of 35 to 70 years were also reported in one such study. For APOC3, we got three genotypes (Table 2, Fig. 1): CC (wild type), CG (heterozygous), and GG (homozygous). In DD group, 6.66% of the population was found to be homozygous, 46.66% of the population was found to be heterozygous, and 46.66% population had wild-type genotype. In NDD group, maximum polymorphism was found; 33.33% population was found to be homozygous, 20% of the population was found to be heterozygous, and 46.66% population had wild-type genotype. In C group, the lowest polymorphism was found and no homozygotes were found; only 30% population was heterozygous and 70% population had wild-type genotype. In DD group and NDD group, it was found that homozygous individuals have significantly high TG and CHOL values (Table 3), followed by heterozygotes; this indicates that genetic study is comparable to the biochemical findings, and there was a significant association of APOC3 G allele with hypertriglyceridemia (p < 0.05). Logistic regression analysis suggested that polymorphism in APOC3 gene is significantly associated with the occurrence of dyslipidemia (OR = 2.6667, 95% CI = 1.0510–6.7663, p = 0.0341; Table 3).

### Table 2

The distribution of genotypes and alleles of APOC3 and FABP2 polymorphism between the DD, NDD, and C groups

<table>
<thead>
<tr>
<th>Genes</th>
<th>Genotype/Allele</th>
<th>DD (Group 1) n = 30</th>
<th>NDD (Group 2) n = 30</th>
<th>C (Group 3) n = 30</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>APOC3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td>GG</td>
<td>2 (6.66%)</td>
<td>10 (33.33%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td>CG</td>
<td>14 (46.66%)</td>
<td>6 (20%)</td>
<td>9 (30%)</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>14 (46.66%)</td>
<td>14 (46.66%)</td>
<td>21 (70%)</td>
</tr>
<tr>
<td>Allele</td>
<td>G</td>
<td>0.3</td>
<td>0.43</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.7</td>
<td>0.57</td>
<td>0.85</td>
</tr>
</tbody>
</table>

| **FABP2** | | | | |
| Genotype | AA | 10 (33.33%) | 12 (40%) | 11 (36.66%) |
| | AG | 9 (30%) | 8 (26.66%) | 13 (43.33%) |
| Allele | A | 0.48 | 0.53 | 0.58 |
| | G | 0.52 | 0.47 | 0.42 |

Abbreviations: APOC3; apolipoprotein C3; C, control; FABP2, fatty acid-binding proteins; DD, diabetic dyslipidemics; NDD, nondiabetic dyslipidemics. APOC3: X² = 12, d.f. = 2, *p < 0.05; FABP2: X² = 2.22, d.f. = 2, p > 0.05.

### Table 3

Logistic regression analysis of APOC3 (rs5128) and FABP2 (Ala54Thr) polymorphism and dyslipidemia

<table>
<thead>
<tr>
<th>Genes</th>
<th>Genotype</th>
<th>Group</th>
<th>OR</th>
<th>95% CI</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DD</strong></td>
<td><strong>NDD</strong></td>
<td><strong>C</strong></td>
<td><strong>OR</strong></td>
<td><strong>95% CI</strong></td>
<td><strong>p-Value</strong></td>
</tr>
<tr>
<td>APOC3</td>
<td>GG + CG</td>
<td>16</td>
<td>16</td>
<td>9</td>
<td>2.6667</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>14</td>
<td>14</td>
<td>21</td>
<td>0.4643</td>
</tr>
<tr>
<td>FABP2</td>
<td>AA + AG</td>
<td>19</td>
<td>20</td>
<td>24</td>
<td>0.1641–1.3136</td>
</tr>
</tbody>
</table>

Abbreviations: APOC3; apolipoprotein C3; C, control; CI, confidence interval; FABP2, fatty acid-binding proteins; DD, diabetic dyslipidemics; NDD, nondiabetic dyslipidemics; OR, odds ratio.

Note: * denotes that p-value is significant.

Fig. 1 Three types of banding pattern observed for apolipoprotein C3 (APOC3) and fatty acid-binding proteins (FABP2) after restriction fragment length polymorphism (RFLP). M = 100 bp ladder, 1–8 = digested polymerase chain reaction products. (A) Three types of banding pattern for gene FABP2 after RFLP—458 bp = wild type (GG genotype), 456/258/192 bp = heterozygous (GA genotype), 258/192 bp = homozygous (AA genotype). (B) Three types of banding pattern of gene APOC3 after RFLP—501 bp = wild type (CC genotype), 501/292/209 bp = heterozygous (GC genotype), 292/209 = homozygous (GG) genotype.
Similar findings have been demonstrated that carriers of G allele had higher levels of TG and CHOL in comparison to non-carriers.\textsuperscript{1} The overall frequency of occurring of GG genotype was found to be very low in the population followed by GC; similar observations were also suggested in the study.\textsuperscript{12} Thus, it can be hypothesized that polymorphism in APOC3 gene (GG genotype) is contributing to high TG and CHOL values in NDD group individuals in the study; similar results have been found in a multiethnic study conducted on US adults, which supports the fact that carriers of polymorphism have high TG and to CHOL levels.\textsuperscript{13}

In the present study, investigations have clearly specified that mean value of TG in NDD group is 178 ± 0.66 mg/dL as compared with the DD group with 222 ± 97.03 mg/dL in clinically maintained cases with TG lowering medicaments.

Analysis of APOC3 gene can suggest here that DD group has high TG (mean value 222 ± 97.03 mg/dL) and CHOL (225.53 ± 92.82 mg/dL), mainly due to diabetes mellitus, but they are also carriers of polymorphic allele (GC). In NDD group, high TG (178.9 ± 60.66 mg/dL) and CHOL (206 ± 49.66 mg/dL) and maximum polymorphism indicates polymorphism in lipid metabolizing genes as one of the important factors. In C group, TG (100.03 ± 25.8mg/dL) and CHOL (158.8 ± 38.2mg/dL) were all in normal range. In C group, the few polymorphisms are observed as carriers for the polymorphic allele (GC); they may also be at a risk of developing dyslipidemia in future.

For gene FABP2, we got three genotypes (→Table 2, →Fig. 1): GG (wild-type), GA (heterozygous), and AA (homozygous). All three genotypes were found in all the three groups. All the groups contained an almost equal number of homozygotes, heterozygotes, and wild type genotype. In DD group, 33.33% of the population was found to be homozygous, 43.33% of the population was found to be heterozygous, and 23.33% population had wild-type genotype. In NDD group, 40% population was found to be homozygous, 26.66% of the population was found to be heterozygous, and 33.33% population had wild-type genotype. In C group for FABP2 gene, 36.66% of the population was found to be homozygous, 43.33% population was heterozygous, and 23.33% population had wild-type genotype. In NDD group, high TG (178.9 ± 60.66 mg/dL) and CHOL (206 ± 49.66 mg/dL) and maximum polymorphism indicates polymorphism in lipid metabolizing genes as one of the important factors. In C group, TG (100.03 ± 25.8mg/dL) and CHOL (158.8 ± 38.2mg/dL) were all in normal range. In C group, the few polymorphisms are observed as carriers for the polymorphic allele (GC); they may also be at a risk of developing dyslipidemia in future.

In conclusion, the present study has strongly directed that there is difference in patterns of dyslipidemia in diabetic and nondiabetic population. The results mainly showed an association of APOC3 polymorphism with variation in serum lipid levels among the NDD group. The fact that even young adults are affected is distressing and thus screening from younger ages may help to promote lifestyle changes that can prevent or slow development of dyslipidemia for the population that comes under this group. Results of this study might facilitate in the understanding of the pathophysiology of dyslipidemia and multifactorial disorders like CHD in NDD patients. Since lipid levels may be genetically controlled, the identification of genetic variants linked with plasma lipid concentrations can provide beneficial information related to genotype–phenotype relationships. These genetic variations may be useful in the development of diagnostic/prognostic markers for dyslipidemia in nondiabetic individuals. However, further studies with large sample size are required to validate the results from present study.

### Authors’ Contribution

Rashmi Chowdhary was involved in concepts, design, definition of intellectual content, literature search, clinical studies, experimental studies, data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing, and manuscript review. Neha Masarkar was involved in design, definition of intellectual content, literature search, clinical studies, experimental studies, data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing, and manuscript review. Sagar Khadanga was involved in concepts, design, clinical studies, data acquisition, and manuscript review. Rashmi Chowdhary has given guarantee for this manuscript.

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Statement of Approval
This study has been approved by Institutional Human Ethics Committee.

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Conflict of interest
Authors declare no conflicts of interest.

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