scientific reports

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# Frameshift variation in the HMG-CoA reductase gene and unresponsiveness to cholesterol-lowering drugs in type 2 diabetes mellitus patients

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**Dyslipidemia, an imbalance in blood lipid levels, is a frequent complication of type 2 diabetes mellitus (DM2) and heightens the risk of cardiovascular diseases (CVDs). Statins, which inhibit 3-hydroxy-3-methylglutaryl-CoA reductase, are potent competitive inhibitors that reduce plasma cholesterol levels. However, individual responses to statins can vary markedly, possibly due to genetic variations in the 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) gene. This study aimed to investigate the pharmacogenetic relationship between the HMGCR gene and hypercholesterolemia in type 2 diabetes mellitus patients who respond differently to atorvastatin, as well as in healthy individuals. Ninety participants were involved, including sixty with type 2 diabetes mellitus and hypercholesterolemia, and thirty healthy individuals. They were randomly assigned to three groups: responsive (received atorvastatin 40 mg), non-responsive (also received atorvastatin 40 mg), and control. Both responsive and non-responsive groups underwent fasting. Biochemical tests were conducted, followed by genetic analysis to identify mutations in the HMGCR gene. The effects of statins in each group were assessed using analysis of variance (ANOVA) and post hoc Tukey's Honestly Significant Difference (HSD) analysis. Atorvastatin 40 mg was administered to assess its efficacy in reducing cholesterol levels in patients with hypercholesterolemia and type 2 diabetes mellitus. The control group exhibited similar cholesterol levels to the responsive group (cholesterol < 200 mg/dl). However, both control and responsive groups significantly differed from the non-responsive group, which had markedly elevated cholesterol levels (> 240 mg/dl). Genetic analysis revealed a cytosine nucleotide insertion in the catalytic domain of the HMGCR gene in only two non-responsive participants to atorvastatin 40 mg therapy. These two patients showed non-responsiveness to atorvastatin 40 mg due to a genetic mutation in the HMGCR gene. This mutation altered the amino acid sequence in the flap domain, replacing isoleucine with a stop codon. As a result, translation was prematurely terminated, leading to the production of truncated proteins.**

**Keywords** Frameshift variation, HMG-CoA reductase, Hyperlipidemia, Atorvastatin

Diabetes is a heterogeneous, complex metabolic disorder, characterized by elevated blood glucose levels resulting from insulin resistance, insufficient insulin secretion, or a combination of both[1–3]. Hyperlipidemia which is a characterized by elevated levels of lipids and/or lipoproteins in the blood is often associated with diabetes[4]. Patients with DM2 commonly present a multitude of risk factors for cardiovascular disease (CVD), including hyperglycemia, abnormal lipid profiles, alterations in inflammatory mediators, coagulation/thrombolytic parameters, and other nontraditional risk factors often closely associated with insulin resistance[5,6]. Consequently, effectively managing CVD in diabetic individuals poses a significant challenge for clinicians[5,7,8].

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Diabetic patients were selected for this study due to the high prevalence of hypercholesterolemia and associated cardiovascular risks in this population. Understanding the genetic factors that influence the response to cholesterol-lowering drugs like atorvastatin in this group is crucial for optimizing treatment strategies and improving patient outcomes.

Statins, inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), are among the most widely prescribed drugs due to their function in inhibiting the production of mevalonic acid, a cholesterol precursor[9–12]. The advent of these inhibitors, commonly known as statins, has revolutionized the treatment of hypercholesterolemia. Statins are recommended as the initial pharmacological treatment for lowering LDL cholesterol levels[13,14]. This recommendation applies to patients with type 2 diabetes who either have overt cardiovascular disease or are over 40 years old with increased cardiovascular disease risk[15–18].

All statins competitively inhibit HMG-CoA reductase with respect to the binding of the substrate, HMG-CoA, but not for that of the coenzyme NADPH. This suggests that their HMG-CoA-like moieties bind to the HMG-CoA-binding portion of the enzyme's active site[19–21]. Until recently, Atorvastatin was the most effective statin available for decreasing LDL when administered in doses of 10–80 mg daily. Furthermore, the higher dose was shown to decrease serum triglycerides by 45% in individuals with hypertriglyceridemia[22–24].

However, treatment decisions should be individualized, considering factors such as age, comorbidities, lipid profile, cardiovascular risk, and medication tolerability[25,26]. Persistent dyslipidemia despite optimal treatment can pose a significant challenge[27]. This study highlights the novel discovery of a frameshift mutation in the HMGCR gene, which appears to significantly affect the efficacy of atorvastatin in type 2 diabetes mellitus patients. The focus has been narrowed to presenting only the most critical data, ensuring that the manuscript emphasizes this important finding. We hypothesize that certain genetic variations in the 3-hydroxy-3-methylglutaryl-CoA gene could promote unresponsiveness to statins.

It has been deduced that 37 different genetic loci contribute to an individual's response to statin therapy, including the gene encoding HMGCR[28–30]. Genetic factors are believed to significantly influence treatment outcomes, with several candidate genes associated with statin dose requirements and treatment responses[31,32]. However, a clinically relevant pharmacogenomic test to guide statin therapy has not yet emerged. Strong candidate genes such as cholesteryl ester transfer protein (CETP), HMGCR, SLCO1B1, ATP-binding cassette sub-family B member 1 (ABCB1), and Cytochrome P450 3A4 and Cytochrome P450 3A5 (CYP3A4/5) have been discussed regarding the challenges in developing much-needed statin pharmacogenomic biomarkers for predicting treatment outcomes[31].

HMG-CoA reductase enzymatically serves as the rate-limiting step in the synthesis of cholesterol, which is crucial for maintaining membrane fluidity and serves as a precursor for steroid hormones[33,34].

Most of the cholesterol biosynthesis takes place in the liver[35]. Structures of the catalytic portion of the human HMGCR complex with six statins, each containing an HMG-CoA moiety, demonstrate that the statins occupy a portion of the binding site of HMG-CoA, thereby blocking access of this substrate to the active site[21,36]. A comparison of substrate-bound and statins-bound HMGCR structures indicates that the flexibility of the flap domain is exploited by the statins to create a hydrophobic pocket for inhibition of binding[21].

Continued research into the molecular mechanisms of statins and genetic factors influencing their efficacy will ultimately improve the care and outcomes of diabetic patients at risk of cardiovascular disease[37,38]. Despite advancements in understanding and managing dyslipidemia, there remains a need for further development of pharmacogenomic biomarkers to guide treatment decisions and optimize outcomes[39–41]. By delving deeper into these areas, we can pave the way for more personalized approaches to care, enhancing treatment effectiveness and minimizing adverse effects. This represents an exciting frontier in medicine, with the potential to revolutionize how we approach treatment and management strategies, ultimately leading to better outcomes for patients[42,43].
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## Materials and methods

### Participants characteristics (subjects)

The study involved ninety participants, with 60 categorized as having type 2 diabetes mellitus (DM) and hypercholesterolemia, while the remaining 30 were healthy individuals. All participants were randomly assigned to one of three subgroups: the responsive group ($n = 30$), who received Atorvastatin 40 mg; the non-responsive group ($n = 30$), who also received Atorvastatin 40 mg but showed no response to the medication; and the control group ($n = 30$). Each subgroup underwent fasting conditions during the study. All experiments were performed in accordance with relevant guidelines and regulations. The Scientific and Ethical Committees of the University of Suleimani, College of Pharmacy, approved the experiments, including all relevant details. Furthermore, informed consent was obtained from all subjects recruited and enrolled from the research as a prerequisite for human-involved studies.

### Biochemical studies

2.5 ml blood specimens obtained by venipuncture, from each patient. Of this, 1.5 ml of blood was drawn into serum gel separator tubes for lipid profile and fasting blood sugar analysis. The blood specimens were centrifuged using a Kokusan CH_19F centrifuge for 6 min at $40 \times 100$ rpm, followed by high-performance analysis on a

small footprint using the fully automated biochemistry device, Cobas C311, for 1 h. Subsequently, the results were obtained, documented, and saved.

### Laboratory measurements

Laboratory measurements included fasting plasma glucose levels (mmol/l), plasma triglycerides, total cholesterol, HDL cholesterol, low-density lipoprotein (LDL) cholesterol, and very low-density lipoprotein (VLDL) cholesterol for all participants. Total cholesterol was used as a parameter for plasma cholesterol (200 mg/dl). 1 ml of blood was collected into ethylenediaminetetraacetic acid (EDTA) tubes after a 12–14 h overnight fasting for HbA1c measurement. The sample was then shaken for 5 min, followed by high-performance analysis on a small footprint using the fully automated biochemistry device, Cobas C311, for 1 h. Subsequently, the results were obtained, documented, and saved. For the control group, LDL-C levels were considered (> 130 mg/dl). Diabetes was diagnosed when HbA1c was ≥ 6.5% (World Health Organization, 2011).

## Genomic DNA studies

Genomic DNA was isolated from 2.5 ml blood samples collected in EDTA tubes using a whole blood DNA kit, purified with a silica spin column, and stored at −70 °C. The concentration and purity of DNA from 90 participants were assessed using a Nanodrop spectrophotometer, revealing DNA concentrations of 35 µg/ml. The purity was evaluated by the absorbance ratio at 260/280 nm. A 1% agarose gel electrophoresis, using a 100 bp DNA size marker and 1× TBE buffer, was performed to visualize the DNA bands under a UV transilluminator.

### Polymerase chain reaction (PCR) and sequencing

Primers were designed as follows:

Forward primer: 5′-GCC ATT ACA GTT GCC CTG TTT-3′, with Tm 59.4 °C.

Reverse primer: 5′-CAG ATC TGA GGA GTC TGC ATG G-3′, with Tm 64.0 °C.

The expected PCR product size was 651 bp. Gradient PCR was used to optimize the annealing temperature, identifying 63.8 °C as optimal in 37 cycles. The PCR product was purified through a series of buffer washes and centrifugation steps, followed by elution. The final product was assessed via gel electrophoresis, and the remainder was stored at −70 °C. PCR products were sequenced using capillary electrophoresis on an ABI 3730xl. Forward and reverse DNA sequencing was prepared for all 180 samples.

### Bioinformatics' study

The DNA assembly program CAP3 was used to assemble a set of contiguous sequences (contigs)[45], essentially assembling the forward and reverse DNA sequences for each participant in each group. Multiple sequence alignment analyses were conducted using MEGA version 6, highlighting the differences between the sequences[46].

### Statistical analyses

Statistical analyses included a paired t-test to compare Baseline (participants' data three months earlier) with Post-Treatment (participants' data three months later), ANOVA to compare means between genders, and ANCOVA to evaluate differences among control, non-response, and response groups, with age and BMI as covariates. Significant ANOVA/ANCOVA results were further analyzed using Tukey's HSD test. Pearson's correlations were performed to investigate the relationships between genetic mutations in the HMGCR gene and various clinical parameters such as BMI, HbA1c, and lipid profiles. These correlations help elucidate potential mechanisms by which the frameshift mutation may influence atorvastatin responsiveness and overall lipid metabolism in DM2 patients. Data are presented as mean ± SD, and all analyses were performed using R version 3.0.2.

## Results

Ninety participants, comprising both females and males, were enrolled in the study: 60 participants diagnosed with type 2 diabetes mellitus (DM) and hypercholesterolemia, and 30 healthy participants.

### Group variation within females and males

Analysis of variance (ANOVA) was conducted to assess differences between male and female participants across various parameters in the control, non-response, and response groups. The results indicated no significant differences in cholesterol levels ($p > 0.05$), fasting blood sugar ($p > 0.05$), HDL ($p > 0.05$), LDL ($p > 0.05$), triglycerides ($p > 0.05$), VLDL ($p > 0.05$), and BMI ($p > 0.05$) between male and female participants in any of the three groups (Table 1).

However, a significant difference in HbA1c levels was observed between males and females in the control group ($p < 0.05$), with no significant differences found in the non-response and response groups ($p > 0.05$). These findings suggest that, aside from the significant difference in HbA1c levels within the control group, sex did not significantly influence baseline clinical characteristics or the response to atorvastatin treatment in this study population (Table 1).

The analysis of variance (ANOVA) was conducted to assess the differences in cholesterol, triglycerides, and HbA1c levels among the control, non-response, and response groups. Figure 1 illustrates the results of this analysis, showing significant differences in cholesterol (Fig. 1A), triglycerides (Fig. 1B), and HbA1c (Fig. 1C) levels between the groups. Specifically, the response group exhibited a significant reduction in cholesterol and HbA1c levels compared to the non-response group, while triglyceride levels remained relatively unchanged across the groups.

In addition, ANOVA was also performed to compare HDL, LDL, and BMI across the control, non-response, and response groups. As depicted in Fig. 2, HDL levels were significantly higher in the response group compared

| Characteristics | Responsive group ($n=30$) | Non- responsive group ($n=30$) | Control group ($n=30$) | Units |
|---|---|---|---|---|
| Age | 55.2 ± 9.31 (F)/53.0 ± 8.82 (M) | 59.8 ± 13.8 (F) 58.3 ± 19.1 (M) | 42.3 ± 7.22 (F)/45.0 ± 13.9 (M) | Years |
| Sex (M/F) | 9/21 | 8/22 | 16/14 | – |
| BMI | 23.9 ± 2.29 (F)/25.2 ± 3.64 (M) | 23.2 ± 1.61 (F)/24.4 ± 1.35 (M) | 23.6 ± 3.00 (F)/25.1 ± 1.46 (M) | $kg/m^2$ |
| FBS | 176 ± 45.8 (F)/187 ± 46.5 (M) | 196 ± 55.2 (F)/163 ± 50.1 (M) | 86.8 ± 2.99 (F)/90.6 ± 10.3 (M) | mg/dL |
| HbA1c | 9.81 ± 2.18 (F)/8.69 ± 1.67 (M) | 9.97 ± 1.73 (F)/9.63 ± 1.53 (M) | 5.81 ± 0.38 (F)/6.14 ± 0.19 (M) | % |
| Total cholesterol | 147 ± 26.6 (F)/153 ± 21.8 (M) | 257 ± 24.8 (F)/261 ± 23.7 (M) | 159 ± 18.1 (F)/156 ± 13.6 (M) | mg/dL |
| HDL | 60.1 ± 1.65 (F)/61.5 ± 4.44 (M) | 41.1 ± 4.00 (F)/38.5 ± 2.3 (M) | 64.2 ± 5.15 (F)/63.5 ± 5.88 (M) | mg/dL |
| LDL | 86.9 ± 16.4 (F)/90.1 ± 20.0 (M) | 180 ± 8.41 (F)/177 ± 8.59 (M) | 98.2 ± 18.2 (F)/97.4 ± 8.97 (M) | mg/dL |
| Triglycerides | 125 ± 28.6 (F)/141 ± 22.5 (M) | 156 ± 7.80 (F)/154 ± 7.35 (M) | 120 ± 27.2 (F)/112 ± 22.2 (M) | mg/dL |
| VLDL | 24.9 ± 5.71 (F)/28.0 ± 4.72 (M) | 27.1 ± 2.34 (F)/27.5 ± 2.35 (M) | 24.1 ± 5.44 (F)/22.3 ± 4.45 (M) | mg/dL |

**Table 1**. Demographic and baseline clinical characteristics of study participants. Descriptive statistics, including mean, standard deviation (SD), standard error (SE), minimum, maximum, and valid sample size, were reported for various parameters (age, weight, height, BMI, fasting blood sugar, HbA1c, cholesterol, HDL, LDL, triglycerides, and VLDL) across three groups: control, non-response, and response. A p-value of $< 0.05$ was considered significant for all comparison.

to the non-response group (Fig. 2A), while LDL levels remained largely unchanged between the groups (Fig. 2B). BMI comparisons (Fig. 2C) did not reveal significant differences among the three groups.

## Correlation analyses between variables regardless of the groups (control, non-response and response)

Pearson's correlation analysis revealed several significant relationships between clinical parameters. Notably, there was a moderately strong positive correlation between cholesterol and fasting blood sugar ($r=0.36$, $p<0.01$), indicating that participants with higher fasting blood sugar levels tend to have elevated cholesterol levels. Cholesterol also showed a positive correlation with HbA1c ($r=0.353$, $p<0.01$), suggesting a link between poor glycemic control and higher cholesterol levels. A particularly strong correlation was observed between cholesterol and LDL ($r=0.91$, $p<0.01$), highlighting the close association between these lipid measures. Additionally, HDL was negatively correlated with cholesterol ($r=-0.82$, $p<0.01$), consistent with the role of HDL in lipid metabolism. The strong positive correlation between triglycerides and VLDL ($r=0.87$, $p<0.01$) reflects the known relationship between these two variables.

Table 3 presents the mean cholesterol levels and HMGCR gene expression before and after atorvastatin treatment. In the responsive group, a significant reduction in cholesterol levels was observed, decreasing from 240 ± 15.2 mg/dL pre-treatment to 180 ± 14.3 mg/dL post-treatment. This reduction was accompanied by a decrease in HMGCR gene expression, from 1.0 ± 0.2 to 0.6 ± 0.1 relative expression units. In contrast, the non-responsive group showed no significant change in cholesterol levels (260 ± 18.1 mg/dL pre-treatment to 258 ± 17.9 mg/dL post-treatment) or HMGCR gene expression (1.1 ± 0.3 to 1.1 ± 0.2 relative expression units), suggesting resistance to atorvastatin's effects.

Genetic analysis revealed that two of the non-responsive participants were heterozygous for the cytosine nucleotide insertion in the catalytic domain of the HMGCR gene. This heterozygous frameshift mutation is predicted to produce truncated proteins due to a premature stop codon, potentially explaining the lack of response to atorvastatin in these individuals.

While Table 3 illustrates the direct effects of atorvastatin on cholesterol levels and HMGCR gene expression in the different study groups, Fig. 3 delves into the relationships between cholesterol and other key metabolic markers.

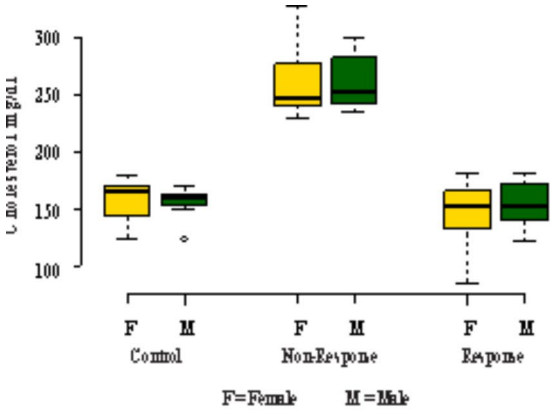
Figure 3A shows the relationship between cholesterol levels and HbA1c, revealing that higher cholesterol levels are generally associated with higher HbA1c percentages. This suggests a link between poor glycemic control and elevated cholesterol, emphasizing the interconnection between lipid metabolism and glucose regulation in the study population.

Figure 3B highlights the strong positive correlation between cholesterol and LDL levels, confirming the well-established relationship where elevated LDL is a major contributor to increased cholesterol levels. This relationship was consistent across all study groups.

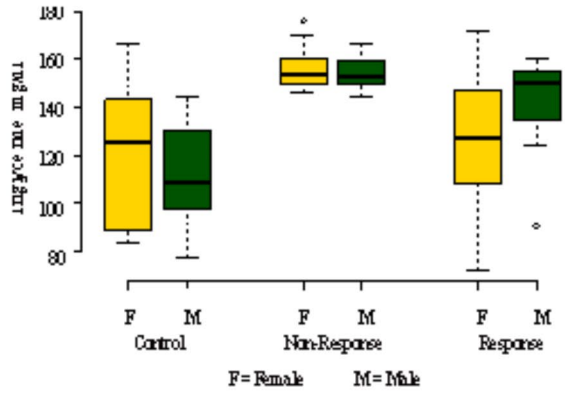
Furthermore, cholesterol was positively correlated with VLDL ($r=0.259$, $p<0.05$) and triglycerides ($r=0.505$, $p<0.01$), and negatively correlated with HDL ($r=-0.817$, $p<0.01$). These correlations align with the expected lipid profile changes, where higher cholesterol levels are typically associated with higher VLDL and triglycerides, and lower HDL levels. These relationships reinforce the importance of comprehensive lipid management in patients, particularly those resistant to atorvastatin treatment.

## Comparison between previous and recent data obtained from non-response and response groups

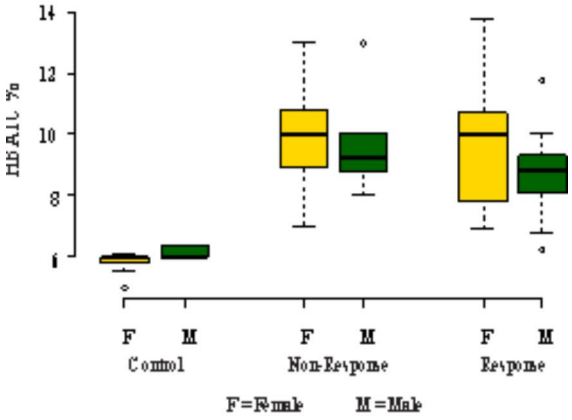
Data on BMI, fasting blood sugar, HbA1c, cholesterol, and triglycerides were collected for participants in both the non-response and response groups. Descriptive statistics for these variables are presented in Table 4. Paired t-tests were conducted to compare the data collected three months earlier (old datasets) with the data collected three months later (new datasets).




**A**: Cholesterol levels (mg/dL) in the control, non-response, and response groups.



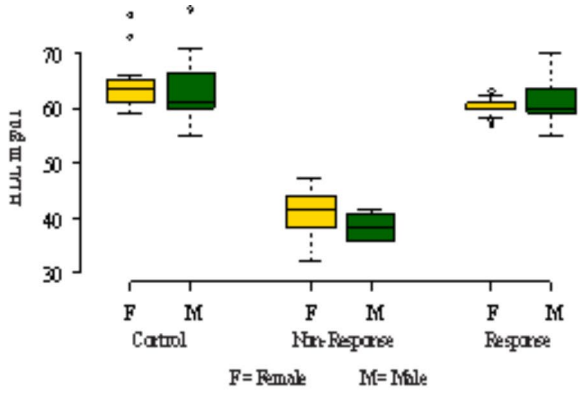

**B:** Triglyceride levels (mg/dL) in the control, non-response, and response groups.



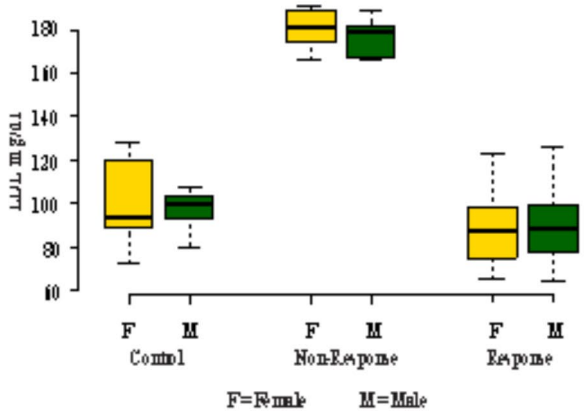

**C**: HbA1c levels (%) in the control, non-response, and response groups.

**Fig. 1**. Analysis of variance (ANOVA) for cholesterol, triglycerides, and HbA1c levels across control, non-response, and response groups.

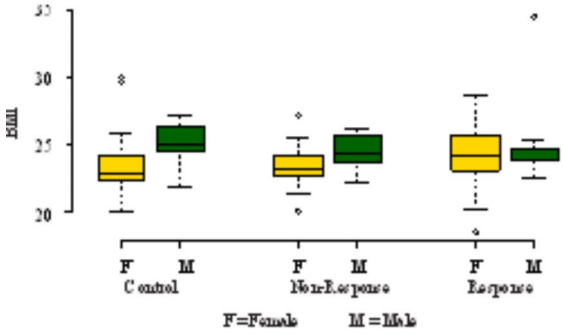
The characteristics of the participants in the response and non-response groups are summarized in Table 4. The response group had a slightly lower average age (55.4 years) compared to the non-response group (57.3 years), though this difference was not statistically significant ($p = 0.345$). Similarly, no significant difference was observed between the groups for BMI, fasting blood sugar, or HbA1c levels, although the non-response group showed slightly higher values across these parameters.




**2A:** HDL levels (mg/dL) in the control, non-response, and response groups.




**2B:** LDL levels (mg/dL) in the control, non-response, and response groups.




**2C:** BMI in the control, non-response, and response groups.

**Fig. 2**. Analysis of variance (ANOVA) for HDL, LDL, and BMI across control, non-response, and response groups.

Fasting blood sugar levels were higher in the non-response group (142.2 mg/dL) compared to the response group (130.5 mg/dL), with a p-value of 0.056, indicating a trend towards significance. The HbA1c levels were also slightly elevated in the non-response group (8.1%) compared to the response group (7.8%), but this difference was not statistically significant ($p = 0.124$). These results suggest that while there are observable differences between the groups, they are not large enough to reach statistical significance with the current sample size.

| Variables | FBS | HbA1c | Cholesterol | HDL | LDL | TG | VLDL |
|---|---|---|---|---|---|---|---|
| BMI | −0.2 | −0.06 | −0.16 | 0.1 | −0.17 | −0.21* | −0.16 |
| FBS | | 0.84** | 0.36** | −0.43** | 0.36** | 0.32** | 0.18 |
| HbA1c | | | 0.35** | −0.47** | 0.37** | 0.34** | 0.16 |
| Cholesterol | | | | −0.82** | 0.91** | 0.51** | 0.26* |
| HDL | | | | | −0.86** | −0.52** | −0.23* |
| LDL | | | | | | 0.58** | 0.31** |
| TG | | | | | | | 0.87** |

**Table 2**. Pearson's correlation coefficient values (r) between cholesterol, fasting blood sugar, HbA1c, HDL, LDL, triglyceride (TG), VLDL and BMI for all participants regardless of the groups. *Correlation is significant at < 0.05. **High Correlation is significant at < 0.01 level.

| Parameter | Responsive group ($n = 30$) | Non-responsive group ($n = 30$) | Control group ($n = 30$) | Units |
|---|---|---|---|---|
| Cholesterol (pre-treatment) | 240 ± 15.2 | 260 ± 18.1 | 159 ± 18.1 | mg/dL |
| Cholesterol (post-treatment) | 180 ± 14.3 | 258 ± 17.9 | 159 ± 18.1 | mg/dL |
| HMGCR expression (pre-treatment) | 1.0 ± 0.2 | 1.1 ± 0.3 | 1.0 ± 0.2 | Relative expression |
| HMGCR expression (post-treatment) | 0.6 ± 0.1 | 1.1 ± 0.2 | 1.0 ± 0.2 | Relative expression |

**Table 3**. Changes in cholesterol levels and hmgcr gene expression pre- and post-atorvastatin treatment in responsive, non-responsive, and control groups.

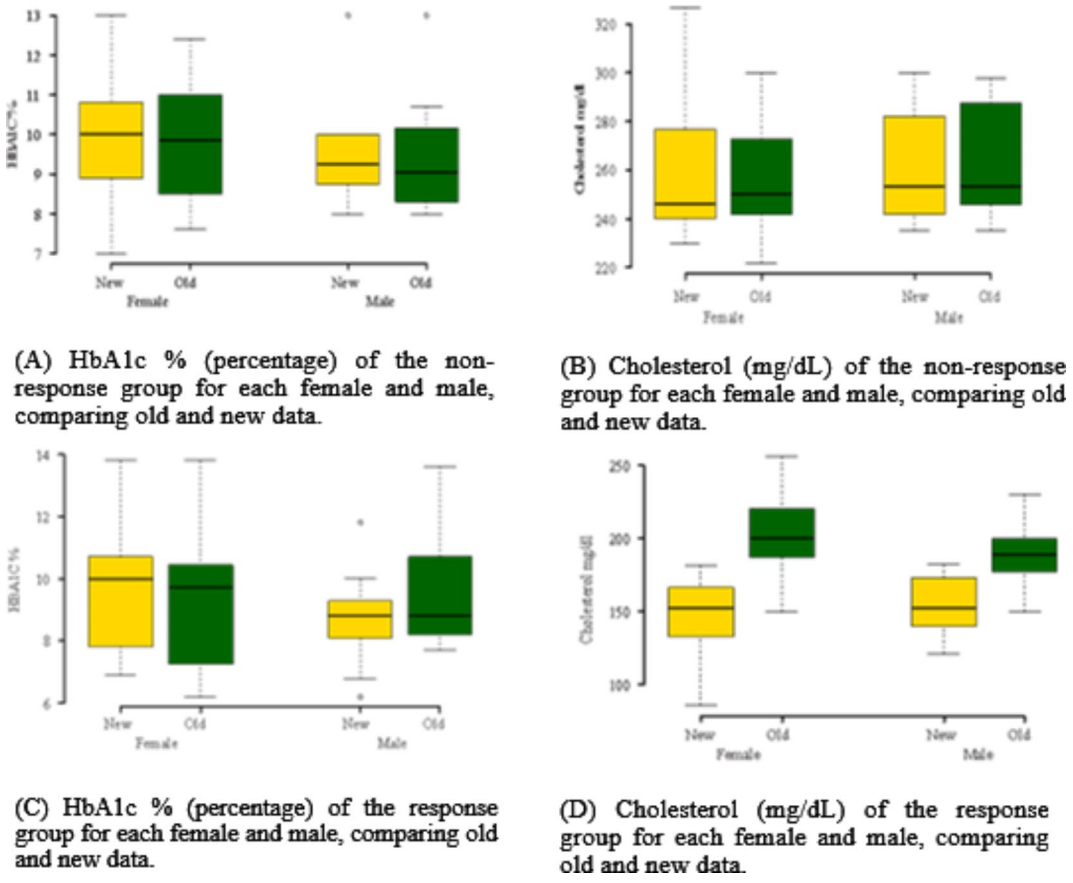



**Fig. 3**. Relationship between cholesterol (mg/dl) and HbA1c (%), (**A**). Relationship between cholesterol (mg/dl) and LDL (mg/dl), (**B**). Cholesterol was positively correlated with VLDL ($r = 0.259$, $p < 0.05$) and triglycerides ($r = 0.505$, $p < 0.01$), and negatively correlated with HDL ($r = -0.817$, $p < 0.01$).

| Group | Variables (Units) | Post-Treatment Female | Post-treatment Male | Baseline Female | Baseline Male |
|---|---|---|---|---|---|
| Response Group | Age (Years) | 55.2 ± 9.31 (*N* = 21) | 53 ± 8.82 (*N* = 9) | 55.2 ± 9.31 (*N* = 21) | 53 ± 8.82 (*N* = 9) |
| | Weight (kg) | 63.2 ± 5.5 | 79.2 ± 8.59 | 63.2 ± 5.5 | 79.2 ± 8.59 |
| | Height ($m^2$) | 163 ± 4.28 | 177 ± 3.36 | 163 ± 4.28 | 177 ± 3.36 |
| | BMI ($kg/m^2$) | 23.9 ± 2.29 | 25.2 ± 3.64 | 23.9 ± 2.29 | 25.2 ± 3.64 |
| | FBS (mg/dL) | 176 ± 45.8 | 187 ± 46.5 | 184 ± 51.5 | 172 ± 44.1 |
| | HbA1c (%) | 9.81 ± 2.18 | 8.69 ± 1.67 | 9.41 ± 2.19 | 9.70 ± 2.05 |
| | Cholesterol (mg/dL) | 147 ± 26.6 | 153 ± 21.8 | 202 ± 23.7 | 190 ± 25.3 |
| | TG (mg/dL) | 125 ± 28.6 | 141 ± 22.5 | 142 ± 31.3 | 170 ± 24.4 |
| Non- Response Group | Age (Years) | 54.3 ± 10.2 (*N* = 18) | 54 ± 9.45 (*N* = 11) | 54.3 ± 10.2 (*N* = 18) | 54 ± 9.45 (*N* = 11) |
| | Weight (kg) | 62.5 ± 6.1 | 78.5 ± 9.2 | 62.5 ± 6.1 | 78.5 ± 9.2 |
| | Height ($m^2$) | 162 ± 4.35 | 176 ± 3.45 | 162 ± 4.35 | 176 ± 3.45 |
| | BMI ($kg/m^2$) | 23.5 ± 2.42 | 24.9 ± 3.58 | 23.5 ± 2.42 | 24.9 ± 3.58 |
| | FBS (mg/dL) | 175 ± 44.3 | 186 ± 45.7 | 183 ± 50.6 | 171 ± 43.2 |
| | HbA1c (%) | 9.75 ± 2.22 | 8.65 ± 1.65 | 9.38 ± 2.24 | 9.68 ± 2.08 |
| | Cholesterol (mg/dL) | 149 ± 25.7 | 155 ± 22.3 | 200 ± 24.6 | 188 ± 26.1 |
| | TG (mg/dL) | 128 ± 29.5 | 143 ± 23.1 | 145 ± 30.4 | 173 ± 23.6 |

**Table 4**. Descriptive statistics for non-response and response groups: baseline (participants' data three months earlier) and post-treatment (participants' data three months later). P value is significant at < 0.05.

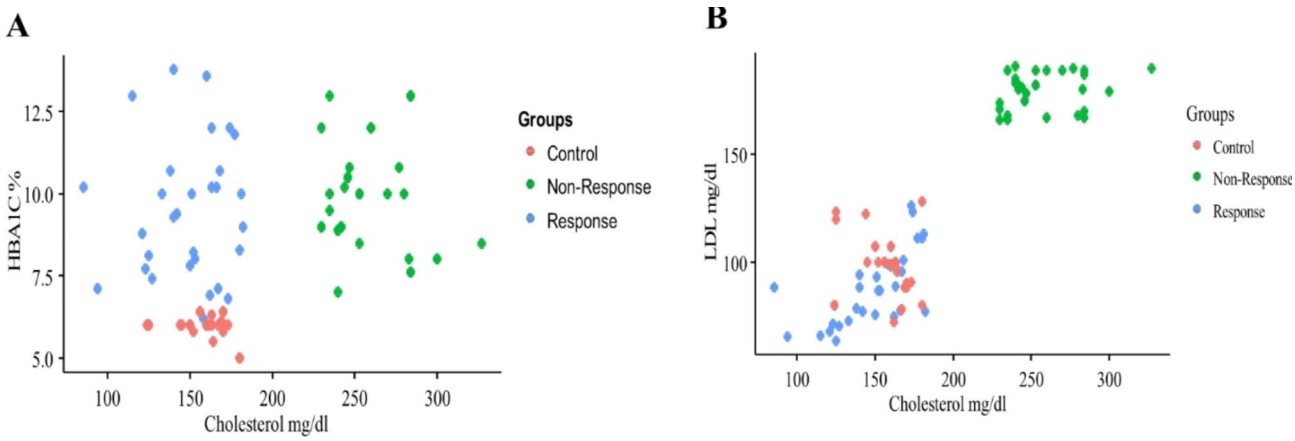



**Fig. 4**. Comparison of non-response group with type-2 diabetes using paired t-test between old and new data. (**A**) HbA1c %; (**B**) cholesterol (mg/dL). Comparison of response group with type-2 diabetes using paired *t*-test between old and new data. (**C**) HbA1c %; (**D**) cholesterol (mg/dL).

## Comparison of non-response group using paired t-test

Age, weight, height, and BMI of the participants did not change significantly over the three-month study period in both the non-response and response groups. There were no significant differences between baseline and post-treatment data for HbA1c ($t = 0.994$, $p > 0.05$; see Fig. 4A). Similarly, there were no significant differences for cholesterol between baseline and post-treatment data ($t = 0.419$, $p > 0.05$; see Fig. 4B). However, there was a significant difference in triglyceride levels ($t = -3.162$, $p < 0.05$).

## Comparison of response group using paired t-test

Paired t-test results showed that fasting blood sugar and HbA1c were similar between Baseline and Post-Treatment data (Fasting Blood Sugar: $t = 0.17919$, $p > 0.05$; HbA1c: $t = -0.074735$, $p > 0.05$; see Fig. 4C). However, there were significant differences for cholesterol ($t = -6.573$, $p < 0.05$; see Fig. 4D) and triglyceride ($t = -4.374$, $p < 0.05$) between Baseline and Post-Treatment data.

## Sequencing analysis of mutant nonresponse group

Sequencing was conducted to identify mutations within the nonresponse group. The results revealed an insertion mutation in the HMGCR gene. Specifically, the chromatogram for sample 3 N-F of the nonresponse group showed an insertion of a cytosine (C) nucleotide. This insertion is highlighted in the red row of the chromatogram.

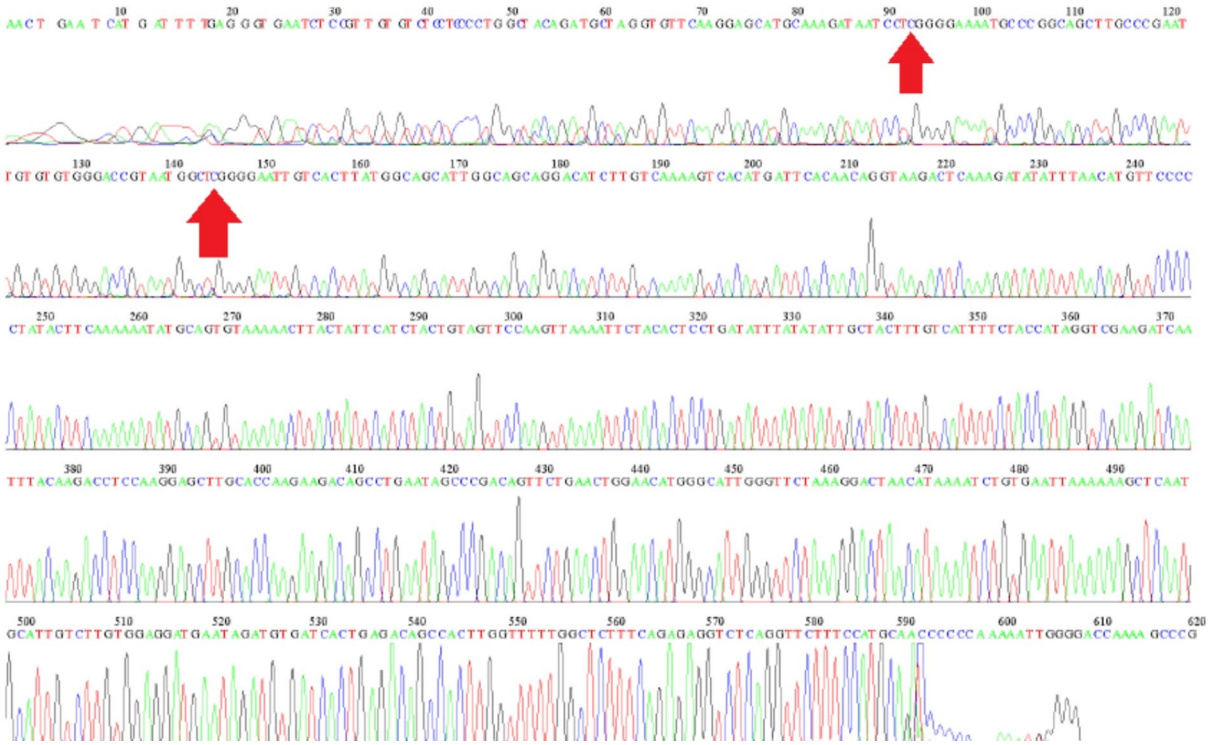


**Fig. 5**. Chromatogram show sequencing of the mutant nonresponse group (sample 3 N-F HMGCR), the red row shows the insertion of cytosine (c) nucleotide.

Figure 5 displays the chromatogram of the sequencing results for the mutant nonresponse group (sample 3 N-F HMGCR), clearly showing the insertion of the cytosine (C) nucleotide.

Additional analyses and data are provided in the supplementary materials (Supplementary Figs. 1, 2, 3, 4, 5, 6, 7, 8).

## Genetics values

A genetic study was conducted on the control, response, and non-response groups to identify any mutations in the sequences of the 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR) gene. This study focused on the region from the glutamic acid at position 671 in the catalytic domain to the alanine at position 888 in the flap domain of the HMGCR gene.

## Genetic sequence analysis for control group

In the control group of 30 participants, six individuals exhibited an insertion mutation at two positions (insertion of cytosine nucleotide) in the target DNA sequence. This mutation caused a shift in the subsequent nucleotides (Supplementary Fig. 1), leading to changes in the amino acid sequences during translation (Supplementary Fig. 2).

The amino acid sequence was altered due to the change in the target DNA sequence. In 24 participants with the same DNA sequence, the amino acid at position 694 is valine. In six mutated participants, arginine substituted for valine at the same position (Supplementary Fig. 2).

These six participants also shared the same position of the stop codon as found in the mutant non-response patients. They had the same stop codon in the flap domain of the 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR) gene (Supplementary Fig. 3) when compared to the wild type.

## Genetic sequence analysis for non-response group

In the non-response group, two participants exhibited two insertion mutations (cytosine nucleotide) in the DNA sequence target at positions (Supplementary Fig. 4). Consequently, the amino acid sequence was altered at position 684 of the catalytic domain, where glycine was substituted with arginine (Supplementary Fig. 5). This insertion resulted in a frameshift mutation. Additionally, the stop codon in the flap domain (Supplementary Fig. 6) was affected, and this position of the stop codon coincided with that of the stop codon in the six participants from the control group.

## Genetic sequence analysis for response group

The responsive patients exhibited similar DNA sequences (Supplementary Fig. 7), with no variations observed among the sequences for all participants and their respective amino acid sequences (Supplementary Fig. 8). The

amino acid sequence of the catalytic domain (and flap domain) of the wild-type 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) in Homo sapiens is available in the NCBI reference [NCBI Reference Sequence: NG_011449.1]. (Supplementary Fig. 9).

### Group sequences comparison

Control group A, non-response group A, and the response group exhibit sequences similar to the wild-type 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) amino acid sequence of the catalytic domain (and flap domain) found in the NCBI reference for human (Homo sapiens, NCBI Reference Sequence: NG_011449.1). However, control group B and non-response group B differ from the wild-type HMGCR sequence due to the formation of a stop codon in the flap domain (Supplementary Fig. 10).

## Discussion

This study identified a novel frameshift mutation in the HMGCR gene that is associated with unresponsiveness to atorvastatin in DM2 patients. The presence of this mutation may lead to the production of truncated HMG-CoA reductase proteins, potentially impairing the drug's efficacy in lowering cholesterol levels[47]. These findings align with previous research suggesting genetic factors play a crucial role in statin responsiveness[48]. Understanding these genetic variations can inform personalized treatment strategies, optimizing cholesterol management in diabetic populations[49,50]. However, the study is limited by its small sample size, and further research with larger cohorts is necessary to validate these results. Future studies should also explore the functional consequences of the identified mutation in vitro and its prevalence in diverse populations.

## Data availability

The gene sequencing data associated with this study has been deposited in the European Nucleotide Archive (ENA) under the study ID PRJEB77623 (ERP162000), titled 'Frameshift Variation in the HMG-CoA Reductase Gene and Unresponsiveness to Cholesterol-Lowering Drugs in Type 2 Diabetes Mellitus Patients'. The data became publicly available on 16-Jul-2024 and can be accessed via the ENA Browser (https://www.ebi.ac.uk/ena/browser/support).

Received: 6 July 2024; Accepted: 4 October 2024
Published online: 02 January 2025

## References

1. Karalliedde, J. & Gnudi, L. Diabetes mellitus, a complex and heterogeneous disease, and the role of insulin resistance as a determinant of diabetic kidney disease. *Nephrol. Dial. Transplant.*. **31**, 206–213 (2016).
2. Banday, M. Z., Sameer, A. S. & Nissar, S. Pathophysiology of diabetes: an overview. *Avicenna J. Med.* **10**, 174–188 (2020).
3. Punthakee, Z., Goldenberg, R. & Katz, P. Definition, classification and diagnosis of diabetes, prediabetes and metabolic syndrome. *Can. J. Diabetes*. **42**, S10–S15 (2018).
4. Goldberg, I. J. Diabetic dyslipidemia: causes and consequences. *J. Clin. Endocrinol. Metab.* **86**, 965–971 (2001).
5. Kalofoutis, C. et al. Type II diabetes mellitus and cardiovascular risk factors: current therapeutic approaches. *Experimental Clin. Cardiol.* **12**, 17 (2007).
6. Ormazabal, V. et al. Association between insulin resistance and the development of cardiovascular disease. *Cardiovasc. Diabetol.* **17**, 1–14 (2018).
7. Sugandh, F. et al. Advances in the management of diabetes mellitus: A focus on personalized medicine. *Cureus* **18**; 15(8):e43697 (2023).
8. Askari, B. et al. Study on a novel polymorphism in the VKORC1 promoter region using bioinformatic tools and warfarin dosing data. *Thromb. Res.* **158**, 76–78 (2017).
9. Willey, J. Z. & Elkind, M. S. 3-Hydroxy-3-methylglutaryl–coenzyme A reductase inhibitors in the treatment of central nervous system diseases. *Arch. Neurol.* **67**, 1062–1067 (2010).
10. Liao, J. K. Effects of statins on 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibition beyond low-density lipoprotein cholesterol. *Am. J. Cardiol.* **96**, 24–33 (2005).
11. Bonetti, P., Lerman, L., Napoli, C. & Lerman, A. Statin effects beyond lipid lowering—are they clinically relevant? *Eur. Heart J.* **24**, 225–248 (2003).
12. Rosales-Acosta, B. et al. Simvastatin and other inhibitors of the enzyme 3-hydroxy-3-methylglutaryl coenzyme a reductase of Ustilago maydis (Um-Hmgr) affect the viability of the fungus, its synthesis of sterols and mating. *Rev. Iberoam. Micol.* **36**, 1–8 (2019).
13. Endo, A. A historical perspective on the discovery of statins. *Proc. Japan Acad. Ser. B* **86**, 484–493 (2010).
14. Feingold, K. R. Cholesterol lowering drugs. (2016).
15. Leon, B. & Maddox, T. Diabetes and cardiovascular disease: Epidemiology, biological mechanisms, treatment recommendations and future research. *World J. Diabetes* **6** (13): 1246-58. *Am J Cardiol* 34, 29–34 (1974). (2015).
16. Martín-Timón, I., Sevillano-Collantes, C. & Segura-Galindo, A. Del Cañizo-Gómez, F. J. Type 2 diabetes and cardiovascular disease: have all risk factors the same strength? *World J. Diabetes*. **5**, 444 (2014).
17. Buse, J. B. et al. Primary prevention of cardiovascular diseases in people with diabetes mellitus: a scientific statement from the American Heart Association and the American Diabetes Association. *Circulation*. **115**, 114–126 (2007).
18. Fox, C. S. et al. Update on prevention of cardiovascular disease in adults with type 2 diabetes mellitus in light of recent evidence: a scientific statement from the American Heart Association and the American Diabetes Association. *Circulation*. **132**, 691–718 (2015).
19. Murphy, C., Deplazes, E., Cranfield, C. G. & Garcia, A. The role of structure and biophysical properties in the pleiotropic effects of statins. *Int. J. Mol. Sci.* **21**, 8745 (2020).
20. Ucar, M., Mjörndal, T. & Dahlqvist, R. HMG-CoA reductase inhibitors and myotoxicity. *Drug Saf.* **22**, 441–457 (2000).
21. Istvan, E. S. & Deisenhofer, J. Structural mechanism for statin inhibition of HMG-CoA reductase. *Science*. **292**, 1160–1164 (2001).
22. Sadeghi, R., Asadpour-Piranfar, M., Asadollahi, M., Taherkhani, M. & Baseri, F. The effects of different doses of atorvastatin on serum lipid profile, glycemic control, and liver enzymes in patients with ischemic cerebrovascular accident. *ARYA Atheroscler.* **10**, 298 (2014).
23. Adams, S. P., Tsang, M. & Wright, J. M. Atorvastatin for lowering lipids. *Cochrane Database Syst. Reviews* **12**; 2015(3):CD008226 (2015).

24. Laufs, U., Karmann, B. & Pittrow, D. Atorvastatin treatment and LDL cholesterol target attainment in patients at very high cardiovascular risk. *Clin. Res. Cardiol.* **105**, 783–790 (2016).
25. Khaleqsefat, E., Khalaj-Kondori, M., Bonyadi, M. J., Soraya, H. & Askari, B. The contribution of VKORC1 and CYP2C9 genetic polymorphisms and patients' demographic characteristics with warfarin maintenance doses: a suggested warfarin dosing algorithm. *Iran. J. Pharm. Res. IJPR.* **19**, 77 (2020).
26. Berberich, A. J. & Hegele, R. A. A modern approach to dyslipidemia. *Endocr. Rev.* **43**, 611–653 (2022).
27. Hendrani, A. D. et al. Dyslipidemia management in primary prevention of cardiovascular disease: current guidelines and strategies. *World J. Cardiol.* **8**, 201 (2016).
28. Medina, M. W. & Krauss, R. M. The role of HMGCR alternative splicing in statin efficacy. *Trends Cardiovasc. Med.* **19**, 173–177 (2009).
29. Chu, A. Y. et al. Differential genetic effects on statin-induced changes across low-density lipoprotein–related measures. *Circulation: Cardiovasc. Genet.* **8**, 688–695 (2015).
30. Kuang, Y. L., Theusch, E., Krauss, M., Medina, W. & R. & Identifying genetic modulators of statin response using subject-derived lymphoblastoid cell lines. *Pharmacogenomics*. **22**, 413–421 (2021).
31. Kitzmiller, J. P. et al. Statin pharmacogenomics: pursuing biomarkers for predicting clinical outcomes. *Discov. Med.* **16**, 45 (2013).
32. Canestaro, W. J., Austin, M. A. & Thummel, K. E. Genetic factors affecting statin concentrations and subsequent myopathy: a HuGENet systematic review. *Genet. Sci.* **16**, 810–819 (2014).
33. Schumacher, M. M. & DeBose-Boyd, R. A. Posttranslational regulation of HMG CoA reductase, the rate-limiting enzyme in synthesis of cholesterol. *Annu. Rev. Biochem.* **90**, 659–679 (2021).
34. Cerqueira, N. M. et al. Cholesterol biosynthesis: a mechanistic overview. *Biochemistry*. **55**, 5483–5506 (2016).
35. Holdgate, G., Ward, W. & McTaggart, F. Molecular mechanism for inhibition of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase by rosuvastatin. *Biochem. Soc. Trans.* **31**, 528–531 (2003).
36. Istvan, E. Statin inhibition of HMG-CoA reductase: a 3-dimensional view. *Atheroscler. Suppl.* **4**, 3–8 (2003).
37. Galicia-Garcia, U. et al. Statin treatment-induced development of type 2 diabetes: from clinical evidence to mechanistic insights. *Int. J. Mol. Sci.* **21**, 4725 (2020).
38. Ganesh, S. K. et al. Genetics and genomics for the prevention and treatment of cardiovascular disease: update: a scientific statement from the American Heart Association. *Circulation*. **128**, 2813–2851 (2013).
39. Berinstein, E. & Levy, A. Recent developments and future directions for the use of pharmacogenomics in cardiovascular disease treatments. *Expert Opin. Drug Metab. Toxicol.* **13**, 973–983 (2017).
40. Stein, R., Ferrari, F. & Scolari, F. Genetics, dyslipidemia, and cardiovascular disease: new insights. *Curr. Cardiol. Rep.* **21**, 1–12 (2019).
41. Khaleqsefat, E., Khalaj-Kondori, M., Bonyadi, M. J., Soraya, H. & Askari, B. Frequency of two VKORC1 gene variants and its correlation with warfarin maintenance dose. *Nova Biol. Rep.* **5**(3): 237-243 (2018).
42. Khaleqsefat, E., Khalaj-Kondori, M., Jabbarpour, B. & Battaloğlu, E. Warfarin maintenance dose associated with genetic polymorphisms of CYP2C9. *Hippokratia*. **21**, 93 (2017).
43. Borghetti, G. et al. Diabetic cardiomyopathy: current and future therapies. Beyond glycemic control. *Front. Physiol.* **9**, 414750 (2018).
44. Benes, L. B., Brandt, D. J., Brandt, E. J. & Davidson, M. H. How genomics is personalizing the management of dyslipidemia and cardiovascular disease prevention. *Curr. Cardiol. Rep.* **20**, 1–7 (2018).
45. Huang, X. & Madan, A. CAP3: a DNA sequence assembly program. *Genome Res.* **9**, 868–877 (1999).
46. Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* **30**, 2725–2729 (2013).
47. Langsjoen, P. H. & Langsjoen, A. M. The clinical use of HMG CoA-reductase inhibitors and the associated depletion of coenzyme Q10. A review of animal and human publications. *Biofactors*. **18**, 101–111 (2003).
48. Mangravite, L., Thorn, C. & Krauss, R. Clinical implications of pharmacogenomics of statin treatment. *Pharmacogenom. J.* **6**, 360–374 (2006).
49. Gloyn, A. L. & Drucker, D. J. Precision medicine in the management of type 2 diabetes. *Lancet Diabetes Endocrinol.* **6**, 891–900 (2018).
50. Strianese, O. et al. Precision and personalized medicine: how genomic approach improves the management of cardiovascular and neurodegenerative disease. *Genes*. **11**, 747 (2020).


## Acknowledgements

We acknowledge the University of Suleimani, College of Pharmacy, for their support and resources.

## Author contributions

Jamil Baban participated in study design and data analysis, and extensively reviewed the manuscript.Esmat Khaleqsefat, Khder Hussein Rasul, and Ramiar Kamal Kheder conducted a literature search, analyzed the data, and drafted the manuscript.Sonia Baban conducted a literature search, provided medical consultation, and participated in reviewing the manuscript.

## Declarations

## Competing interests

The authors declare no competing interests.

## Ethics approval and consent to participate

All experiments were performed in accordance with relevant guidelines and regulations. The Scientific and Ethical Committees of the University of Suleimani, College of Pharmacy, approved the experiments, including all relevant details. Written informed consent to participate was obtained from all participants as necessary.

## Additional information

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1038/s41598-024-75461-7.

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