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# Identification of Novel Mutation delC336 and insC376 in Exon 4-of *LDLR* Gene in Vietnamese Patients with High-Blood-Cholesterol

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**KEYWORDS** Binding Domain Repeat III. Low Density Lipoprotein Receptor. PCR-Sequencing. Translational Frameshift. Vietnamese Population

**ABSTRACT** The *LDLR* (*Low density lipoprotein receptor*) gene encodes the low density lipoprotein receptor. It has been reported to function as a carrier of cholesterol in the blood. Further, exon 4 of *LDLR* gene has been found to be a hotspot of mutation. However, *LDLR* gene mutation has not been studied in Vietnam. Therefore, the aim of the current study is to identify mutations located in exon 4 of *LDLR* gene in Vietnamese high blood cholesterol patients by PCR-sequencing method. Based on the results, two novel mutations including (1) deletion "C" at position 336 and (2) insertion "C" at position 376 (insC376) were detected in 2 of 17 clinical samples (accounting for 11.76%) and 14 of 17 clinical samples (accounting for 82.35%), respectively. Both delC336 and insC376 are located at the binding domain repeat III. The observed mutations were separately predicted to cause a translational frameshift leading to the abnormal LDLR proteins that may have lost their function, resulting in the high blood cholesterol levels in patients.

## INTRODUCTION

The *low-density lipoprotein receptor* (*LDLR*) gene is located in the 19p13.1-p13.3 region, spans 45 kb, includes 18 exons, and encodes a glycoprotein of 839 amino acids, which is the cell surface receptor that plays a key role in cholesterol homeostasis; this receptor mediates the specific uptake of low-density lipoprotein (LDL) particles from circulation and transfers them into cells via endocytosis (Brown and Goldstein 1974; Lindgren et al. 1985; Phuong et al. 2018a; Phuong et al. 2018b).

The mutation in the *LDLR* gene is considered as the genetic cause of monogenic familial hypercholesterolemia (FH) (Brown and Goldstein 1974; Lindgren et al. 1985). This condition results in very high blood low-density lipoprotein cholesterol level, which can lead to various human diseases, including premature cardiovascular disease (CVD), premature coronary heart disease (CHD), xanthomas and/or corneal arcus (Austin et al. 2004; Karalis 2009; Arraíz et al. 2010). Presently, a large number of mutations have been reported and catalogued worldwide. Based on the LOVD database (http://www.ucl.ac.uk/ldlr/LOVDv.1.1.0/), a total of 1741 different mutations have been reported, and most of them are substitutions (accounting for 73.5%); insertions and deletions have also been reported (Fokkema et al. 2005). Mutations in *LDLR* gene are distributed along the full gene length; however, a high mutation frequency has been reported in the exon 4 coding region of the ligand-binding domain (Arraíz et al. 2010). The explanation could be that exon 4 encodes three of the seven cysteine-rich ligand binding repeats of the LDLR that plays an essential role (required for LDL binding via apolipoprotein B) in receptor binding activity (Neff et al. 2003; Phuong et al. 2018b; Defesche 2004). In a recent study, a systematic review by Jiang et al. (2015), 131 mutations of LDLR were identified. Approximately sixty percent of the mutations were identified as missense mutations, and thirty mutations were identified as new mutations that were not recorded in the database of LDLR mutations. The database may provide information that is specific to China for further studies that aim to explore the treatment of FH (Jiang et al. 2015).

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## Aim

In Vietnam, the molecular basis as well as the mutation of *LDLR* gene has not been studied yet; therefore, the aim of the current study is to screen and investigate the mutations in exon 4 of *LDLR* gene in Vietnamese patients with high plasma cholesterol levels by PCR-sequencing method.

## METHODOLOGY

### Sample Collection and DNA Isolation

A total of 17 blood samples were collected from unrelated hypercholesterolemia patients with informed consent, and all existing ethical regulations were followed. The samples were collected at Hospital Thu Duc District, Ho Chi Minh City, Vietnam. The patients were diagnosed with increased serum cholesterol concentration (> 5.2 mmol/L) without tendon xanthomas.

Total DNA was isolated from clinical samples by 700 µl lysis buffer (NaCl 5 M, Tris-HCl 1 M, EDTA 0.5 M, SDS 10% and Proteinase K 1 mg/ml). The samples were incubated at 56°C overnight. The purification of DNA was performed by Phenol (pH = 8)/Chloroform extraction and ethanol precipitation. Finally, the DNA solution was stored at EDTA 0.5M at -20°C for further use.

### Amplification and Analysis of *LDLR* Gene

The application and analysis of *LDLR* gene was performed by PCR-sequencing method. The forward (LDLR-L) and reverse primer (LDLR-R) sequences were 5'ACTGCGGCAGCGTCCCCG-GC3' and 5'TGGGGGAGCCCAGGGACAGG3' respectively. The amplification was done in a total volume of 15 µl, containing 10 ng DNA template. PCR reaction was subjected to initial denaturation at 95 °C for 5 min, followed by 35 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, and finally 72 °C for 10 min. PCR products were directly loaded onto a two percent agarose gel, stained with ethidium bromide, and directly visualized under UV illumination. The PCR product was sequenced to identify whether there were mutations in exon 4 of *LDLR* gene in comparison to the reference transcript of exon 4 of *LDLR* gene (ENST00000558518.5, location: 19p13.2 11,105,220 – 11,105,660, length 381 bps).

## RESULTS

The PCR product was observed in a total of 17 clinical samples, yielding a PCR product of 527 bps, as shown in Figure 1. The amplification of *LDLR* gene's exon 4 fragments was determined by Sanger sequencing.

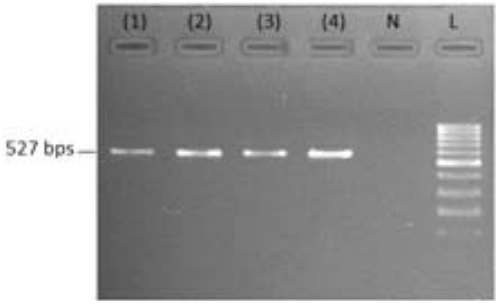


**Fig. 1. Agarose gel electrophoresis of the PCR products of (1) (4): representative clinical samples. N: negative control; L: 100-bps DNA marker**

Based on the comparison to the referent sequence, the deletion "C" nucleotide at position 336 (delC336) (Fig. 2a) and the insertion "C" nucleotide at position 376 (insC376) (Fig. 2b) were detected in the current study. Regarding the frequency, delC336 and insC376 were detected in 2 of 17 clinical samples (accounting for 11.76%) and 14 of 17 clinical samples (accounting for 82.35%), respectively. Notably, both delC336 and insC376 were not observed in the reversed sequence; therefore, these mutations were heterozygous.

Mutations delC336 and insC376 were located in the LDL-receptors class A domains and separately predicted to code the truncated LDLR protein. In details, delC336 mutation in our hypercholesterolemia patients led to the loss of all three important disulfide bonds, and the reading frame was shifted, leading to the amino acid being changed from amino acid at 121 to create a stop codon at 205 position (L205*), as shown in Figure 3a. InsC376 mutation encoded the truncated protein within the creation of stop codon downstream the insertion site (D129*), as shown in Figure 3b.

## DISCUSSION

According to the LOVD database (http://www.ucl.ac.uk/ldlr/LOVDv.1.1.0/), the largest num-

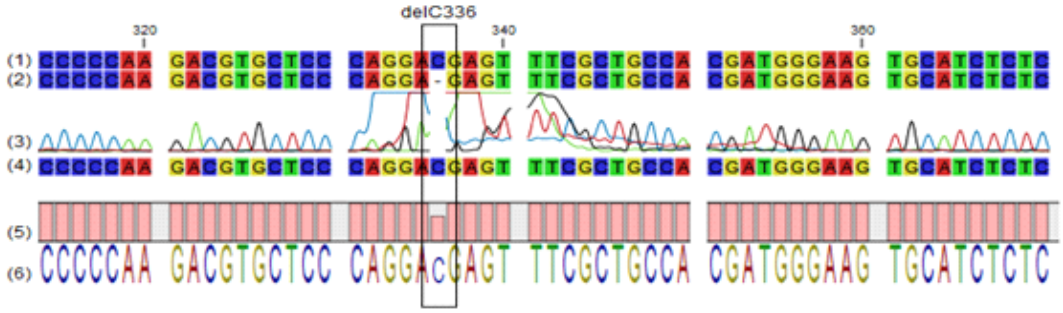


**Fig. 2(a)**

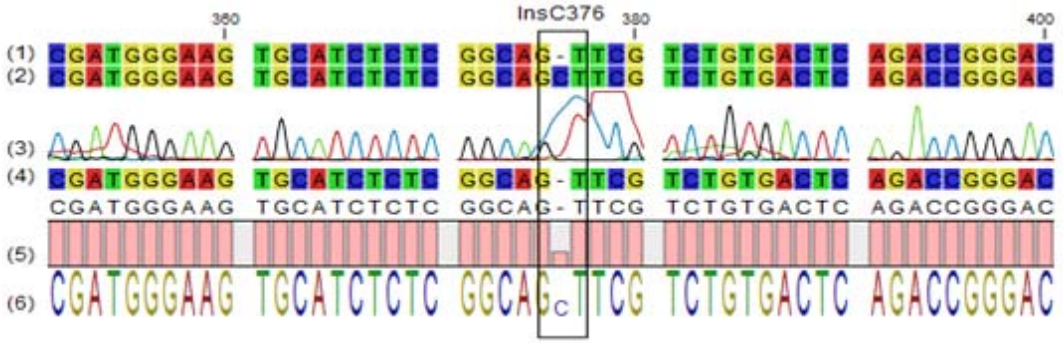


**Fig. 2(b)**

**Fig. 2. Diagram of detected (A) delC336 mutation and (B) insC376 mutation in exon 4 of *LDLR* gene. (1) Referent sequence: ENST00000558518.5; (2) (3): A partial exon 4 forward sequence of represented clinical samples; (4) A partial exon 4 of reversed sequence of represented clinical samples; (5) Bar of conservation; (6) Consensus sequence logo. Analyzed by CLC Workbench 8.0.1**

ber of mutations has been reported in exon 4; however, the mutations of *LDLR* gene are difficult to analyze and observe by full gene sequencing. The explanation could be that the mutations in *LDLR* full gene were difficult to identify by Sanger sequencing method because mutation identification by Sanger sequencing is difficult due to the complexity of the mutation itself; sequencing data contains overlapping signals. Additionally, the mutation of *LDLR* gene has not been studied yet. Therefore, in the current study, the researchers initially focused on the LDL-receptor class A domain belonging to exon 4 of LDLR in high blood cholesterol levels of Vietnamese patients. In the current study, the frequencies of mutations delC336 and ins C376 are 11.76 percent and 82.35 percent, respectively in exon 4 of *LDLR* gene in Vietnamese high blood cholesterol patients. To the researchers' understanding, by screening based on LOVD database, both mutations (delC336 and insC376) are novel mutations of LDL-receptor class A domain belonging to the *LDLR* gene's mutation in the researchers' region. According to protein structure, exon 4 of *LDLR* gene codes for the fifth repeat of LDL receptor, which is required for LDL binding via apolipoprotein; therefore, the variants within this region are associated with hypercholesterolemia (Neff et al. 2003; Defesche 2004). In the original LDLR, the LDL-receptor class A domain contains six disulfide-bound cysteines, which forms three disulfide bonds (Fig. 3), including the disulfite bond between cysteine 109 – cysteine 121, cysteine 116 – cysteine 134, cysteine 128 – cysteine 143, which are important for high affinity binding of positively charged sequences in LDLR ligands (Mahley 1988). In the current study, the deletion of "C" nucleotide in the position 336 and insertion of "C" nucleotide in the position 376 in exon 4 of *LDLR* gene were located in the LDL-receptors

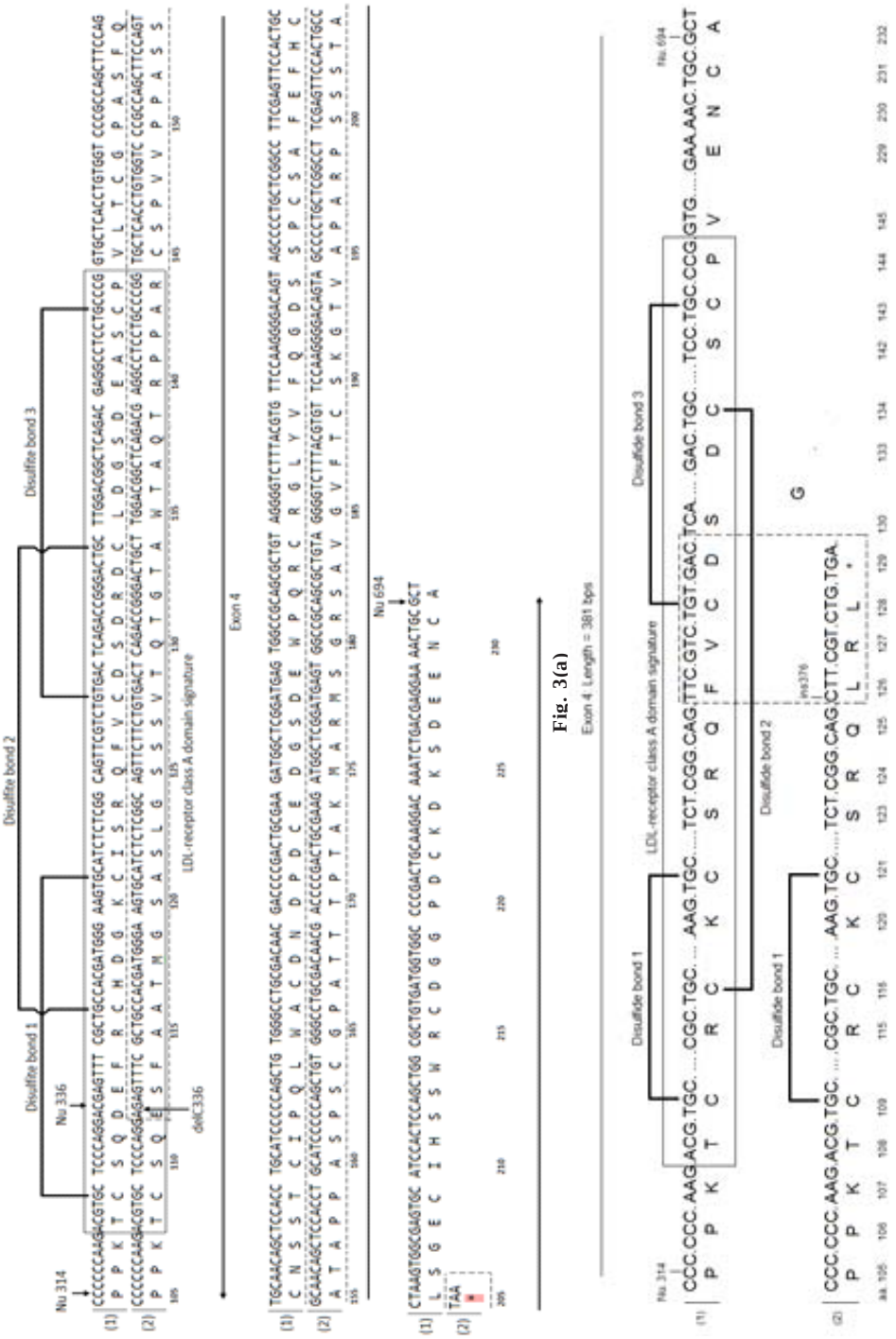


Fig. 3(b)

**Fig. 3. The predicted truncated LDLR protein in the exon 4 region. (A): The case of delC336; and (B) insC376. The LDLR protein encoded from (1) referent *LDLR* gene sequence; (2) mutation exon 4 sequence. The mutated amino acids are annotated in dotted frame**

class A domains, which is necessary in forming the binding site for LDL, thereby affecting the transportation of cholesterol. As a result, the reading frame is shifted, and incorrect amino acids that have probably lost their function are encoded following the frameshift mutation, leading to the high plasma cholesterol (Yamamoto et al. 1984). In details, the delC336 mutation led to the loss of all three important disulfide bonds, and the reading frame was shifted, leading to the amino acid being changed from amino acid at 121 to create a stop codon at position 205. In the case of insC376, it affected the formation of disulfide bonds between cysteine 116 – 134 and cysteine 128 – 143 due to the creation of stop codon at 129. These mutations resulted in the conformation change of the ligand-binding domain of receptor. The generation of truncated proteins that have probably lost their function led to the high plasma cholesterol. For further study, the amount of samples could be increased to characterize the mutations that occurred in exon 4 of *LDLR* gene. Additionally, these results are considered as the foundation for the establishment of PCR allele specific assay for rapid detection of LDLR point mutation, such as delC336 and insC376, which could be further applied in clinical diagnosis.

## CONCLUSION

In the current study, two novel mutations (delC336 and insC376 mutations) were identified in Vietnamese hypercholesterolemia patients by PCR-sequencing method. Regarding the frequency, delC336 and insC376 mutations were detected in 2 of 17 clinical samples (accounting for 11.76%) and 14 of 17 clinical samples (accounting for 82.35%), respectively. Additionally, the delC336 and insC376 mutations were identified to be located at the LDL-receptor class A domain due to the creation of stop codon at 205 and 129 respectively, leading to the conformation of defective protein, which results in the high plasma cholesterol.

## RECOMMENDATIONS

In continuation of this current initial study, the size of samples should be expanded. Additionally, the mutations in other exon of *LDLR* gene should be considered to characterize the pattern mutation of *LDLR* gene in Vietnamese high blood cholesterol patients.



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