Screening of common genetic variants in the APOB gene related to familial hypercholesterolemia in a Saudi population

A case–control study

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Abstract

Familial hypercholesterolemia (FH) is a monogenic dominant inherited disorder of lipid metabolism characterized by elevated low-density lipoprotein levels, and is mainly attributable to mutations in low-density lipoprotein receptor (LDLR), apolipoprotein B (APOB), and pro-protein convertase subtilisin/kexin type 9 (PCSK9) genes. Next-generation and exome sequencing studies have primarily involved genome-wide association analyses, and meta-analyses and next-generation studies examined a few single-nucleotide polymorphisms (rs151009667 and Val2095Glu) in the APOB gene. The present study was conducted to investigate the association of APOB and patients with FH in a Saudi population.

We genotyped 100 patients with FH and 100 controls for 2 polymorphisms in APOB using polymerase chain reaction-restriction fragment length polymorphism, followed by 3% agarose gel electrophoresis. The strength of the association between the genotype and allele frequencies with the risk of developing FH was evaluated. Clinical details and genotype analysis results were recorded.

For the rs151009667 polymorphism, 18% of the CT genotypes were observed only in patients with FH. There was a positive association between CT and CC (odds ratio OR 45.07 [95% confidence interval CI] 2.67–759.1; P = .0001) and between T and C (OR 87.8 [95% CI, 5.34–144.2]; P < .0001). However, no Val2095Glu mutations were found in patients with FH or controls. There was also no correlation between clinical characteristics and the rs151009667 polymorphism.

In conclusion, we confirmed the association between the rs151009667 polymorphism and FH in a Saudi population. The Val2095Glu novel variant did not appear in either patients with FH or controls. Similar studies should be performed in different ethnic populations to rule out the role of this polymorphism in FH.

Abbreviations: ApoB = apolipoprotein B, BMI = body mass index, CI = conflict of interest, FH = familial hypercholesterolemia, Glu = glutamine, HDLC = high-density lipoprotein receptor, LDLR = low-density lipoprotein receptor, NGS = next-generation sequencing, PCSK9 = pro-protein convertase subtilisin-Kexin type 9, SD= standard deviation, TC = total cholesterol, TG = triglyceride, Val = valine.

Keywords: ApoB gene, familial hypercholesterolemia, rs151009667, Saudi population, Val2095Glu

1. Introduction

Familial hypercholesterolemia (FH; OMIM# 143890) is defined as a common genetic condition categorized by increased plasma levels of low-density lipoprotein-cholesterol (LDL-C) and premature atherosclerotic cardiovascular disease.[1] The disease FH was initially discovered in 1920 by Beeson,[2] and is typically considered to be a single-gene disorder.[3] The FH inheritance pattern was initially described by Khachadurian[4] in Lebanon, before the genes contributing to the disease were identified. FH is defined as an autosomal dominant disease with a clinical presentation based on the phenotype severity of homozygous and heterozygous forms, and with serum LDL-C levels that are 2- to 4-fold the normal level, respectively.[5] In FH, the frequency varies between heterozygous (1/500) and homozygous (1/1,000,000) FH. However, a recent population analysis estimated the prevalence to be as high as 1/250. FH develops between the ages of 30 and 50 years in men and 40 and 60 years in women. Patients with FH are not always diagnosed properly, leading to inappropriate treatment strategies. FH-associated complications are common because of premature diagnosis and therapeutic interventions.[7] FH may be caused by a gain-of-function mutation in the LDL receptor (LDLR), apolipoprotein B (APOB), and pro-protein convertase subtilisin-Kexin type 9
(PCSK9) genes. LDLR may suppress protein synthesis, which is translocated to the cell surface in monogenic mutations and by an additional mechanism that involves mutation affecting APOB, which encodes a key structural component of LDL and very low-density lipoprotein (VLDL). In addition, ApoE and LDLR adaptor protein 1 are required for LDLR and FH.

FH-affected mutations were present in 60% to 80% of patients with a clinical diagnosis of pure FH, and 20% to 30% of the affecting mutations may appear in conceivable FH. Genetic variations in LDLR are loss of function mutations, whereas APOB and PCSK9 show similar lipid profile homeostasis functional defects. To date, >1000 genetic variations in LDLR, APOB, and PCSK9 have been reported in the British Heart Foundation and other databases. The FH diagnostic criteria are based on the Simon Broome criteria (UK); Dutch Lipid Clinic Network Criteria (Netherlands); and MedPed criteria (USA). Various proteins, cholesterol internalization, and cellular metabolism have been connected to FH (e.g., ApoB-100, PCSK9, and LDLR).

Genetic variations originating in the proteins include large rearrangements of intronic regions, coding, synonymous, non-synonymous substitutions, and mutations in regulatory regions or splicing sites. Missense mutations were the most frequent mutation type and were identified using second-generation sequencing techniques such as exome and next-generation sequencing (NGS) technologies within the exon coding region. Radovica-Spalvina et al previously performed NGS, and confirmed novel and documented variants in their cohort subjects. Our study was conducted to investigate the novel mutation Val2095Glu and familial variant rs151009667 in APOB in a case-control study of patients with FH in a Saudi population.

2. Materials and methods

2.1. FH participants

The Institutional Review Board of the College of Medicine at the King Saud University (KSU) provided ethics approval (E-12-829) for this study. All subjects who participated in study including patients with FH and control subjects signed an informed consent form. This study was performed according to the principles of the Declaration of Helsinki. As described in our prior publications, 100 patients with FH were recruited from King Khalid University Hospital (KKUH) at KSU. Inclusion criteria were as follows: FH diagnosis made according to the Dutch group criteria; male or female; subjects who underwent regular checkups; and subjects with no endocrine, metabolic, chronic, and other diseases. Exclusion criteria included the following: subjects with abnormal body mass index (BMI); subjects with diabetes; subjects with liver, renal, or thyroid disease or any other type of diseases; and subjects recruited outside the KKUH. Sex-matched controls (n=100) were recruited from contract-based KKUH staff who may or may not have been outpatients.

2.2. Blood collection

From each patient, 5 mL of the peripheral blood was collected into 2 tubes (plain and EDTA tubes) by an experienced nurse. A 3-mL sample was used for biochemical analysis of the lipid profile, such as triglycerides (TGs), total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), and LDL-C. The remaining 2 mL was used for molecular analysis and was collected into an EDTA tube. Biochemical indications were analyzed using an automated clinical chemistry analyzer (KoneLab, Espoo, Finland).

2.3. Molecular genotyping

Genomic DNA was extracted from the EDTA blood using a commercial human DNA kit as described by Alharbi et al. To quantify genomic DNA, a NanoDrop spectrophotometer (Thermo Fischer Scientific, Waltham, MA) was used and primers were designed for the selected APOB variants based on Radovica-Spalvina et al. The complete details of APOB variants are listed in Table 1. The primers were designed using Primer 3 software. For the Val2095Glu and rs151009667 variants, genotyping was performed by polymerase chain reaction with a 25-μL sample consisting of nuclease-free water, buffer, 2.5 μL MgCl2, 0.5 μL dNTPs, 0.5 μL Taq DNA polymerase, 100 pmol of both sense and antisense primers, and 50 ng quantified genomic DNA. Initial denaturation was carried out at 95°C for 5 min followed by 35 cycles of 30 s at 94°C (for both variants), and 95°C for 45 min for initial elongation. The final elongation step was carried out at 72°C for 5 min. For both variants, restriction fragment length polymorphism analysis was conducted. Both the RsaI (GT1AC) and Fnu4HI (GC1GCG) restriction enzymes (NEB England BioLabs, Ipswich, MA) were used to digest the samples for 18h at 37°C. The digestion products were separated on a 3 % agarose gel, which was stained with Ethidium bromide and visualized using ultraviolet light.

2.4. Statistical analysis

Clinical data between patients with FH and controls were evaluated using SAS software (version 9.3, SAS Institute, Cary, NC). Hardy-Weinberg equilibrium was tested between patients and controls. Descriptive characteristics of categorical variables are presented as the count (%), and continuous variables are presented as the mean and standard deviation. Between patients with FH and controls, the association was tested using 2 independent sample t tests. Genotype and allele frequencies between patients with FH and controls were assessed using Openepi software (version 2.3.1) to determine the odds ratios, Χ².
3. Results

3.1. Clinical analysis

The results of anthropometric and biochemical measurements of patients with FH and controls are presented in Table 2. The mean ages of patients with FH and controls were 51.66 ± 9.92 and 54.02 ± 6.29 years, respectively, which was significantly different (P = .0001). Patients with FH and controls had a nearly equal sex ratio with no significant difference between groups (P > .05). We measured the height (165.7 ± 7.53 cm) and weight (74.1 ± 9.40 kg) only of patients with FH, and the overall BMI was 27.1 ± 1.91 kg/m² for this group. Lipid profile analysis revealed a positive association between TC, TG, and LDL-C (P < .05), whereas HDL-C showed a negative association when comparing patients with FH and controls (P = .71).

3.2. Genetic analysis of the rs151009667 polymorphism

No deviation from Hardy-Weinberg equilibrium was detected in the control group for both variants. Allele and genotype analyses for FH patients and controls are presented in Table 3. All alleles and genotypes in both variants were adjusted by Yates correction. The rs151009667 genotype frequencies for CC and CT were 82% and 18%, respectively, for patients with FH patients, whereas the genotypes in the control group were all CC. However, a strongly significant association was observed only for CT versus CC (odds ratio [OR] 45.07 [95% confidence interval (CI), 2.67–759.1]; P = .0001) and T vs C: (OR 87.8 [95% CI, 5.34–144.2]; P < .0001). No mutations were detected for CT genotypes within control subjects. No homozygous variants appeared in either patients with FH or control subjects for rs151009667.

3.3. Val2095Glu allele and genotype frequencies

A 470 bp polymerase chain reaction product encompassing the novel variant Val2095Glu was digested using the FNU4H1 restriction enzyme, which yielded a 241/147/82 bp fragment, confirming the presence of the C allele. Only the CC genotype was detected in patients with FH and controls. No significant association was observed between the alleles and genotypes for CT vs CC (OR 1.00 [95% CI, 0.01–50.88]; P = .99) and T vs C (OR 1.00 [95% CI, 0.01–50.63]; P = .99). Thus, this locus was not further analyzed. Sanger sequencing was performed on 30 FH samples, which showed the same results.

3.4. Analysis of variance

Anthropometric measurements and the lipid profiles of patients with FH and controls with the CC, CT, and TT genotypes of the rs151009667 polymorphism were compared (Table 4). No FH patient characteristics showed a positive association between the rs151009667 polymorphism and age (P = .31), BMI (P = .41), TG (P = .48), HDL-C (P = .47), and LDL-C (P = .95).

4. Discussion

No case-control studies have investigated rs151009667 and the novel Val2095Glu variant in the global population. This is the first study to analyze these factors in a Saudi population and the world. We investigated the association between 2 genetic variants

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### Table 2

Clinical features of familial hypercholesterolemia (FH) patients and controls.

| No. | Features         | FH patients (n = 100) | Controls (n = 100) | P   |
|-----|------------------|-----------------------|--------------------|-----|
| 1   | Age, y           | 51.66 (9.92)          | 44.02 (6.29)       | .0001|
| 2   | Sex (male:female)| 37.63                 | 40.60              | .62 |
| 3   | Weight, kg       | 74.1 (9.40)           | NA                 | NA  |
| 4   | Height, cm       | 165.7 (7.53)          | NA                 | NA  |
| 5   | BMI, kg/m²       | 27.1 (1.91)           | NA                 | NA  |
| 6   | TC, mM           | 5.4 (1.1)             | 4.8 (0.73)         | .003|
| 7   | TG, mM           | 2.2 (1.2)             | 1.6 (0.99)         | .009|
| 8   | HDL-C, mM        | 0.7 (0.2)             | 0.6 (0.27)         | .71 |
| 9   | LDL-C, mM        | 4.5 (0.9)             | 3.7 (0.72)         | .003|

BMI = body mass index, HDL-C = high-density-lipoprotein-cholesterol, LDL-C = low-density-lipoprotein-cholesterol, NA = not analyzed/not applicable, TC = total cholesterol, TG = triglycerides, and P values with and without Yates correction. Analysis of variance was also performed between the FH genotypes for rs151009667 and clinical characteristics.

| Mutation | Genotypes | FH cases (n = 100) | Controls (n = 100) | λ² | OR (95% CI) | P   |
|----------|-----------|--------------------|--------------------|----|-------------|-----|
| rs151009667 | CC       | 82 (82%)           | 100 (100%)         | R  | R           | R   |
|           | CT       | 18 (18%)           | 0 (0%)             | 16.79 | OR 45.07 [2.67–759.1] | .0001 |
|           | TT       | 0 (0%)             | 0 (0%)             | 0.05 | OR 0.02 [0.0002–3.35] | .03  |
|           | CT+TT vs CC | 18 (18%)           | 0 (0%)             | 0.23 | OR 0.22 [0.004–11.67] | .41  |
|           | CT vs CC+TT | 18 (18%)           | 0 (0%)             | 0.0009 | OR 201 [1.64–245.0] | .0005 |
|           | TT vs CC+CT | 0 (0%)             | 0 (0%)             | 0.0009 | OR 201 [1.64–245.0] | .0005 |
|           | C        | 164 (82%)          | 200 (100%)         | R  | R           | R   |
|           | T        | 36 (18%)           | 0 (0%)             | 37.09 | OR 87.8 [5.34–144.2] | <.0001 |
| Mutation | Genotypes | FH cases (n = 100) | Controls (n = 100) | λ² | OR (95% CI) | P   |
|----------|-----------|--------------------|--------------------|----|-------------|-----|
| Val2095Glu | CC       | 100 (100%)         | 100 (100%)         | R  | R           | R   |
|           | CT       | 0 (0%)             | 0 (0%)             | 1.005 | OR 1 [0.01–50.88] | .99  |
|           | TT       | 0 (0%)             | 0 (0%)             | —   | —           | —   |
|           | CT+TT vs CC | 0 (0%)             | 0 (0%)             | —   | —           | —   |
|           | CT vs CC+TT | 0 (0%)             | 0 (0%)             | —   | —           | —   |
|           | TT vs CC+CT | 0 (0%)             | 0 (0%)             | —   | —           | —   |
|           | C        | 200 (100%)         | 200 (100%)         | R  | R           | R   |
|           | T        | 0 (0%)             | 0 (0%)             | 1.002 | OR 1 [0.01–50.63] | .99  |

CI = conflict of interest, R = reference.
and patients with FH in a Saudi population. To confirm the findings of this study, we screened the Val2095Glu and Arg1689His (rs151009667) variants in patients with FH and controls. The rs151009667 SNP was found to be associated in patients with FH compared to controls (P < .05). However, Val2095Glu showed no heterozygous (CT) and homozygous variant (TT) genotypes in patients with FH or controls (P > .05). These 2 variants were screened by exome sequencing analysis, as used in a previous study of a Latvian population of patients with FH. Generally, heterozygous variants detected in FH disease assists with detection using a sequence base as the prime methodology, and NGS/ES techniques have made these methods easier to use for diagnostic purposes.[3]

Alharbi et al[7] defined FH as a well-known autosomal dominant disorder with an earlier increased risk of coronary heart disease. FH is the only disease diagnosed based on cascade screening of genetic mutations such as LDLR, APOB, and PCSK9 and based on high cholesterol levels and a family history. It is recommended to diagnose FH as soon as possible to prevent morbidity and mortality. However, the latest statistics in pharmacotherapy have helped to lower LDL-C levels, decreasing morbidity and mortality related to cardiovascular disease.[14] A recent study by Ghaleh et al[17] confirmed that ApoE is an additional causative gene that contributes to screening for FH. Genetic variants in LDLR, APOB, APOE, and PCSK9 are present in 80% of patients with FH. Genetic risk profiling improved after genome-wide screening was introduced and can simultaneously screen millions of polymorphisms for any complex disease.[18] In France, genetic cascade screening is currently used to help diagnose FH. NGS techniques enable examination of the monogenic and polygenic origins of elevated LDL-C levels observed in patients with FH.[19] There are a few common misconceptions in case control studies, such as matching in these studies does not always eliminate confounding factors; and matching analysis may not always be required. A previous study by Pearce[20] clarifies the misconceptions in case–control studies. In this study, we selected sex-based samples in the recruited subjects. Patients with FH included 37% male and 63% female subjects, whereas controls included 40% male and 60% female subjects. However, age-matched controls were not recruited in this study because FH can develop at any age.

APOB is one of the major genes used for the molecular diagnosis of FH. The chromosomal region is located in the 2p24.1 region.[21] APOB was initially discovered in 1987 in phenotypic patients with FH, which revealed that FH is not related to mutations in LDLR.[22] APOB has a specific role in FH disease, and variants in APOB typically occur in the exons. Patients with FH with a familial deficiency in APOB may have a milder form of the disease, which can affect LDLR mutation-

### Table 4

| Variables          | CC (n = 82) | CT (n = 18) | TT (n = 00) | P   |
|--------------------|------------|------------|------------|-----|
| Age, y             | 51.73 ± 10.14 | 51.33 ± 9.10 | 0.00 ± 0.00 | .57 |
| BMI, kg/m²         | 27.00 ± 2.40  | 26.43 ± 2.04  | 0.00 ± 0.00 | .41 |
| TC, mMM            | 5.33 ± 1.03   | 5.89 ± 1.24   | 0.00 ± 0.00 | .31 |
| TG, mMM            | 2.08 ± 1.32   | 2.25 ± 1.15   | 0.00 ± 0.00 | .48 |
| HDL-C, mMM         | 0.70 ± 0.21   | 0.65 ± 0.24   | 0.00 ± 0.00 | .47 |
| LDL-C, mMM         | 3.76 ± 0.86   | 3.60 ± 0.85   | 0.00 ± 0.00 | .95 |

BMI = body mass index, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, TC = total cholesterol, TG = triglycerides.
results will reveal the genetic role of the diagnostic markers. Future studies should also include NGS/ES studies in the Saudi population. Large-scale studies would be required from different areas and races to screen such a polymorphism.

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References

[1] Paquette M, Genest J, Baass A. Familial hypercholesterolemia: experience from the French-Canadian population. Curr Opin Lipidol 2018;29:59–64.

[2] REESON BB, Albrecht P. A contribution to the study of xanthoma tuberosum, with report of a case. Arch Dermatol Syphilol 1923;8:695–710.

[3] Radovicova Spalvina L, Lakosovska G, Silamukels L et al. Next-generation sequencing-based identification of familial hypercholesterolemia-related mutations in subjects with increased LDL-C levels in a Latvian population. BMC Med Genet 2015;16:86.

[4] Khachadurian AK. The inheritance of essential familial hypercholesterolemia. Am J Med 1964;37:402–7.

[5] Fahed AC, Nemer GM. Familial hypercholesterolemia: the lipids or the genes? Nutr Metab 2011;8:23.

[6] BAILA-RUEDA I, LAMIQUI-MONIO J, JARAPTA E et al. Association between non-cholesterol sterol concentrations and Achilles tendon thickness in patients with genetic familial hypercholesterolemia. J Transl Med 2018;16:6.

[7] ALHARBI KK, ALNBAHEEN MS, ALHARBI FK et al. Q192R polymorphism in the po1ni gene and familial hypercholesterolemia in a Saudi population. Ann Saudi Med 2017;37:425–32.

[8] Durst R, Ible UK, Shpitzten S et al. Molecular genetics of familial hypercholesterolemia in Israel revisited. Atherosclerosis 2017;257:55–63.

[9] Mollazadeh H, Carbone F, Montecucco F et al. Oxidative burden in familial hypercholesterolemia. J Cell Physiol 2018;233:5716–25.

[10] SHARI’I M, WALUS-MIARKA M, IDZIOR-WALUS B et al. The genetic spectrum of familial hypercholesterolemia in south-eastern Poland. Metabolism 2016;65:48–53.

[11] LEE SH, CHAHL JG, BAGLI P et al. Genetic polymorphisms in LDLR, APOB, PCSK9 and other lipid related genes associated with familial hypercholesterolemia in Malaysia. PLos One 2013;8:e60729.

[12] Sharifi M, Futema M, Nair D et al. Genetic architecture of familial hypercholesterolemia. Curr Cardiol Rep 2017;19:44.

[13] Anzargica VE, Orozco M, Sanchez J. Exploring the complete mutational space of the LDL receptor LAG5 domain using molecular dynamics: linking SNPs with disease phenotypes in familial hypercholesterolemia. Hum Mol Genet 2016;25:1233–46.

[14] Alhbari KK, Kashour TS, Al-Hussaini W et al. Association of angiotensin converting enzyme gene insertion/deletion polymorphism and familial hypercholesterolemia in the Saudi population. Lipids Health Dis 2013;12:177.

[15] Alharbi KK, Kashour TS, Al-Hussaini W et al. Screening for genetic mutations in LDLR gene with familial hypercholesterolemia patients in the Saudi population. Acta Biochim Pol 2015;62:559–62.

[16] Hovingh GK, Davidson MH, Kastelein JJ et al. Diagnosis and treatment of familial hypercholesterolaemia. Eur Heart J 2013;34:962–71.

[17] Ghaleh Y, Elbistar S, El Khoury P et al. Usefulness of the genetic risk score to identify phenocopies in families with familial hypercholesterolemia? Eur J Hum Genet 2018;26:570–8.

[18] Nurnberg ST, Zhang H, Hand NJ et al. From loci to biology: functional genomics of genome-wide association for coronary disease. Circ Res 2016;118:586–606.

[19] Rabes JP, Beliard S, Carrie A. Familial hypercholesterolemia: experience from France. Curr Opin Lipidol 2018;29:65.

[20] Pearce N. Analysis of matched case-control studies. BMJ 2016;352:i969–20.

[21] Innenarty TL, Weisgraber KH, Arnold KS et al. Familial defective apolipoprotein B-100: low density lipoproteins with abnormal receptor binding. Proc Natl Acad Sci 1987;84:6919–23.

[22] Henderson R, O’Kane M, McGillian V et al. The genetics and screening of familial hypercholesterolaemia. J Biomed Sci 2016;23:39.

[23] Youngblom E, Faziani M, Knowles JW. Familial hypercholesterolemia: 2016.

[24] Whitfield AJ, Barrett PHR, Van Bockxmeer FM et al. Lipid disorders and mutations in the APOB gene. Clin Chem 2004;50:1725–32.

[25] Johansen CT, Wang J, Lanktree MB et al. Excess of rare variants in genes identified by genome-wide association study of hyperglycemia-ldemia. Nat Genet 2010;42:684–7.

[26] Al-Allaf FA, Athar M, Abduljalil Z et al. Next generation sequencing to identify novel genetic variants causative of autosomal dominant familial hypercholesterolemia associated with increased risk of coronary heart disease. Gene 2015;565:76–84.

[27] Aksoyamen LE, Genest J, Shan SD et al. Estimating the prevalence of heterozygous familial hypercholesterolaemia: a systematic review and meta-analysis. BMJ Open 2017;7:e016461.

[28] Elsami SM, Nikkar S, Ghasemi M et al. Does Evoloclumab, as a PCSK9 Inhibitor, Ameliorate the Lipid Profile in Familial Hypercholesterolemia Patients? A Meta-Analysis of Randomized Controlled Trials. J Pharm Pharm Sci 2017;20:81–96.

[29] Peng W, Qiang F, Peng W et al. Therapeutic efficacy of PCSK9 monoclonal antibodies in statin-nonresponswe patients with hypercholesterolemia and dyslipidemia: A systematic review and meta-analysis. Int J Cardiol 2016;222:119–28.

[30] Khan L, Gao Y, Zhang YM et al. Therapeutic efficacy and safety of PCSK9 monoclonal antibodies on familial hypercholesterolemia and statin-intolerant patients: A meta-analysis of 15 randomized controlled trials, Sci Rep 2017;7:238.

[31] Tada H, Kawasaki MA, Okada H et al. A Rare Coincidence of Sitosterolemia and Familial Mediterranean Fever Identified by Whole Exome Sequencing. J Atheroscler Thromb 2016;23:884–90.

[32] Faiz F, Alcock RJ, Hooper AJ et al. Detection of variations and identifying genomic breakpoints for large deletions in the LDLR by Ion Torrent semiconductor sequencing. Atherosclerosis 2013;230:249–55.

[33] Hinchcliffe M, Le H, Fimmel A et al. Diagnostic validation of a familial hypercholesterolaemia cohort provides a model for using targeted next generation DNA sequencing in the clinical setting. Pathology 2014;46:60–9.

[34] Kim HN, Kweon SS, Shin MH. Detection of familial hypercholesterolemia using next generation sequencing in two population-based cohorts. Chonnam Med J 2018;54:31–3.

[35] Reiman A, Pandey K, Lloyd KL et al. Molecular testing for familial hypercholesterolaemia-associated mutations in a UK-based cohort: development of an NGS-based method and comparison with multiplex polymerase chain reaction and oligonucleotide arrays. Ann Clin Biochem 2016;53:65–62.

[36] Schafer EJ, Tsinoda F, Diiffenderer M, De Groot LJ, Chroulos G, Dungan K, Feingold KR, Grossman A, Hershman JM et al. The measurement of lipids, lipoproteins, apolipoproteins, fatty acids, and sterols, and next generation sequencing for the diagnosis and treatment of lipid disorders. Endotext South Dartmouth, MA: MDText.com, Inc; 2000.

[37] Zeggin E, Ioanidis JP. Meta-analysis in genome-wide association studies. Pharmacogenomics 2009;10:191–201.