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Türk Ailevi Hiperlipidemi Hastalarında Hiperlipidemi İlişkili Gen Paneli Kullanılarak Genotip ve Fenotip Analizi Yapılması

Genotype and Phenotype Analysis Using a Hyperlipidemia-Associated Gene Panel in Turkish Familial Hyperlipidemia Patients

Dilek Gun Bilgic[1]

[1]Manisa Celal Bayar Universitesi Tıp Fakültesi Tıbbi Genetik Anabilim Dalı Manisa Türkiye

e-mail: dr_dgun@yahoo.com
ORCID: 0000-0002-3971-9691

*Sorumlu Yazar / Corresponding Author: Dilek Gun Bilgic
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Öz

Amaç: Ailesel Hiperlipidemi (AH), monogenik kalıtılan hastalıklardan en sık görülenlerden biridir. Çok sayıda gen ailesel hiperlipidemi ile ilişkilendirilmiştir ve ailesel hiperlipidemili hastalarda gen paneli testi ile araştırılmaktadır.
Gereç ve Yöntem: Bu çalışmada, olası AH'li 25 hasta, 33 genden oluşan hedeflenmiş bir panel kullanılarak yeni nesil dizileme (NGS) ile değerlendirildi.
Bulgular: Beş hastada LDLR ve CETP genlerinde patojenik veya olası patojenik varyantlar tespit edildi.
Sonuç: Klinik bulgular literatür ile karşılaştırılarak bu varyantların genotip-fenotip korelasyonları tartışıldı.

Anahtar sözcükler: ailevi hiperlipidemi; LDLR; gen paneli dizileme testi

Abstract

Objective: Familial hyperlipidemia (FH) is one of the most frequent diseases with monogenic inheritance. Numerous genes have been associated with familial hyperlipidemia and are being investigated by gene panel testing in patients with familial hyperlipidemia.
Methods: In the present study, 25 patients with possible FH were evaluated via next-generation sequencing (NGS) using a targeted panel of 33 genes.
Results: Pathogenic or likely pathogenic variants were detected in five patients in the genes LDLR and CETP.
Conclusion: The genotype-phenotype correlations of these variants were discussed by comparing the clinical findings with the literature.

Keywords: familial hyperlipidemia; LDLR; gene panel sequencing test

1. Introduction

Clinical dyslipidemia includes patients with abnormal levels of low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol, triglycerides (TG), or lipoprotein(a) [1]. An abnormal level of each of these is the result of one or more genetic abnormalities or secondary to some underlying disease or environmental factors. Dyslipidemia includes different types of diseases: Type I 'Chylomicronemia syndrome', Type IIa, Type IIb, Type III, including familial dysbetalipoproteinemia, and Type IV, including familial hypertriglyceridemia and Type V including hyperprebetalipoproteinemia. Each type of

the dyslipidemia has different diseases. Type I includes "Lipoprotein lipase deficiency caused by LPL gene mutations" and ApoCII deficiency caused by APOC2 mutations. Type IIa includes "polygenic hypercholesterolemia" caused by multiple genetic defects or monogenic hypercholesterolemias. Unlike familial (monogenic) hypercholesterolemia, polygenic hypercholesterolemia is caused by several altered or faulty genes. Each faulty gene raises LDL cholesterol a little (LDL-C levels > 130 mg/dL). Familial hypercholesterolemias (FHC) is characterized by severely elevated LDL cholesterol (LDL-C) levels (LDL-C levels > 190 mg/dL) that lead to premature atherosclerosis and cardiovascular disease[2]. FHC was originally assumed to be an autosomal dominant trait. Later, autosomal recessive types were determined. However, studies on the families with the disorder, provided evidence consistent with a multigenic mode of inheritance [3]. An estimated 70%-95% of FHC results from a heterozygous pathogenic variant in one of three genes (APOB, LDLR, PCSK9) [4]. In FHC cases, the LDLR gene is responsible for 60-80%, the APOB gene for 1-5%, and the PCSK9 gene for 0-3%. In 20-40% of FH cases, the responsible gene is unknown [5]. In contrast, homozygous FHC results from biallelic (homozygous or compound heterozygous) pathogenic variants in one of the dyslipidemia related genes such as LDLRAP1 and severe coronary artery disease by their mid-20s. The rate of either death or coronary bypass surgery by the teenage years is high. Severe aortic stenosis is also common. Homozygous mutations in the APOB, LDLR, PCSK9 genes also cause extremely high LDL levels and coronary artery disease at an early age.[6].
Type IIb dyslipidemia includes familial combined hyperlipidemia (FCHL) which may present as mixed hyperlipidemia, isolated hypercholesterolemia, or hypertriglyceridemia. LPL, LIPC, APOA1 and APOE Familial dysbetalipoproteinemia is caused by APOE mutations, familial hypertriglyceridemia (triglyceride levels are between 250-1000 mg/dL) and hyperprebetalipoproteinemia are caused by APOA5 mutations [7] .

Next-generation DNA sequencing (NGS) is a high-throughput technique that allows the quick production of large volumes of sequence data [4]. The list of genes that cause FH is expanding, thanks to the ability to analyze multiple gene panels with NGS. However, analyzing the effect of variants on phenotype is often challenging. There is a great need for genotype–phenotype correlation studies in the interpretation of variants arising from sequence data. In the present study, 25 possible FH patients were evaluated via NGS using a targeted panel consisting of 33 genes. The study aimed to provide a genetic diagnosis of the patients in this cohort and discuss genotype-phenotype correlations of the patients according to the literature.

2. Materials And Methods

2.1. Patients

Informed consent was obtained from all the patients. A total of 25 patients who presented with possible FH as their primary complaint at their first visit to our hospital were recruited. Patients with LDL-C levels > 190 mg/dL or TG levels > 250 mg/dL were choosed for genetic testing. Variants of the analyzed genes and patients' clinical data, including age, symptoms, laboratory findings, and family history were analyzed. Ethics Committee approval was obtained from our hospital's Ethics Board (Date: 25/05/2022, Decision: 1378) and written informed consent was obtained from each patient.

2.2 Sample collection and DNA isolation

Genomic DNA was isolated from peripheral blood specimens of patients using the QIAamp DNA Blood Mini Kit (Qiagen), according to the manufacturer's instructions.

2.3 Next-generation sequencing

Multiple amplicon DNA libraries were obtained using an assay kit (QIAseq Targeted DNA Panel) according to the manufacturer's instructions. DNA libraries were prepared as in the literature [8]. The libraries were then aggregated and sequenced using the Illumina NGS system (MiniSeq®, Illumina MiniSeq). Table 1 shows the genes included in the panel.

2.4 Analysis of next-generation sequencing data

FASTQ files were downloaded from BaseSpace and uploaded to the QIAGEN Data Analysis Centre, and the variants were called and annotated using the QIAGEN Ingenuity® Variant Analysis software (QCI®-A). The variants were filtered for the following criteria: call quality of at least 30, read depth of at least 50, and mutant allele fraction of at least 30%. The variants evaluated with the QIAGEN Clinical Insight® (QCI) Interpret database. VarSome Clinical® database was used as a secondary database to check "The American College of Medical Genetics and Genomics criteria (ACMG)".

3. Results

The study population included 20 females and 5 males. Of the 25 patients studied, six patients were found to carry pathogenic or likely pathogenic variants, with four in the LDLR gene, and one each in the LIPC, CETP genes. Four patients were found to carry a variant of unknown significance, with two in the GPD1 gene and one one each in the LPL, APOB, APOA5 genes. The ages of the patients ranged from 20 to 61 years. The diseases of the patients included ischemic stroke, myocardial infarction, pancreatitis, polycystic ovary syndrome. (Table 2).

Table-1: Analysed genes in the panel
ABCA1, ABCG5, ABCG8, ANGPTL3, APOA1, APOA5, APOB, APOC2, APOC3, APOE, CETP, CH25H, CYP7A1, GCKR, GPD1, GPIHBP1, INSIG2, LCAT, LDLRAP1, LDLR, LIPA, LIPC, LMF1, LPL, MTTP, NPC1, NPC1L1, NPC2, OSBPL5, PCSK9, SAR1B, SLCO1B1, STAP1

Table 2: patients and detected variants

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CN	Age		LDL level mg/dL	TG mg/dL	disease	gene	variation	significance	zygosity
1	20	m	495		CABG	LDLR	NM_000527.5:c.1285G>A p.V429M	P	hom
2	35	f	199		-	-	-	--	-
3	20	f	556		-	-	-	-	-
4	36	f	96	1118	pancreatitis	LIPC	NM_000236.3:c.1214C>T p.T405M	VUS	het
5	40	f	207	117	-	GPD1	NM_001257199.2:c.521T>C p.V174A	VUS	Het
6	47	m	224	184	-	GPD1	NM_001257199.2: c.208C>T p.P70S	VUS	Het
7	44	f	369	86	NSTEMI	LDLR	NM_000527.5: c.1135T>C p.C379R	P	Het
8	48	f	165	290	-	-			
9	44	f	108	250	-	LPL	NM_000237.3:c.574G>A p.A192T	VUS	Het
10	55	m	95	252	-	APOA5	NM_052968.5: c.64C>T p.Q22*	VUS	Het
11	30	f	160	147	inability to lose weight	CETP	NM_000078.3:c.853C>T p.R285*	P	Het
12	48	f	66 Vldl:330	1648	pancreatitis	-			
13	28	m	226	94	-	LDLR	NM_000527.5: c.1478_1479delCT p.S493fs*42	P	Het
14	36	f	51	1739 VLDL:278	-	APOB	NM_000384.3: c.1753C>A (ekzon13) p.Q585K	VUS	Het
15	45	f	141	1477 VLDL:354	-	-			
16	33	f	165	144	PCOS				
17	57	f		1418 Nonhdl 418	-	-			
18	34	f	147	264	-	-			
19	50	m		1205 Nonhdl 234	-	-			
20	40	f	241	217	-	-			
21	37	f	171	278	CVD	-			
22	37	f	239	180	-				
23	57	f	225	137	-	LDLR	NM_000527.5: c.1678A>T p.I560F	P	het
24	61	f	196	112	-	-			
25	52	f	191	62	-	-			
	CN: case no; CABG: coronary artery bypass graft; hom: homozygous ; het: heterozygous; NSTEMI: non-ST-elevation myocardial infarction ; CVD: cerebrovascular disease; PCOS: polycystic ovary syndrome								

4. Dıscussıon

A 20-year-old female patient presented with a high level of LDL cholesterol (495 mg/dl) and a history of coronary artery bypass graft surgery. The patient was found to carry the NM_000527.5:c.1285G>A (p.V429M) pathogenic homozygous variant in the LDLR gene. This variant had been determined pathogenic in the Clinvar database. The mutation was first described by Leitersdorf et al. (1989) in the Afrikaner population and occurs at a high frequency of 30% [9]. A possible explanation for the high frequency of this mutation in South Africa is the founder hypothesis. Defesche et al. (1993) concluded that this mutation in all likelihood originated in the Netherlands and was introduced into South Africa in the seventeenth century by Dutch settlers [10]. While LDL is >190mg/dL in heterozygous LDLR mutations, LDL can be >500 mg/dL in homozygous LDLR mutations. LDL level was found to be 495mg/dl in this patient and coronary artery disease was detected at the age of 20 years. In patients aged 44, 58 and 27 years old with other heterozygous LDLR mutations, NM_000527.5: c.1135T>C (p.C379R), NM_000527.5: c.1478_1479delCT (p.S493fs*42), NM_000527.5: c.1678A>T (p.I560F) previously reported pathogenic variants were detected respectively. LDL levels were 369, 226, 225 mg/dL, respectively. The patients had no known disease. Since 2.5-10% of the mutations in the LDLR gene are large deletions and duplications, gene-targeted deletion/duplication analysis should be performed in patients with negative sequence analysis. This constitutes a limitation of the study as this analysis was not performed in this study.

A 36-year-old male patient presented with a high level of TG, (1118 mg/dl). The patient was found to carry the heterozygous NM_000236.3:c.1214C>T (p.T405M) variant of unknown significance in the LIPC gene encoding hepatic lipase and a history of recurrent pancreatitis. Hepatic lipase deficiency is caused by homozygous or compound heterozygous mutation in the LIPC gene. Hepatic lipase deficiency is characterized by premature atherosclerosis, elevated total cholesterol, TG and very low density lipoprotein (VLDL), as well as TG rich LDL and high density lipoprotein (HDL) subfractions [11]. Durstenfeld et al. (1993) investigated two variants of the LIPC gene, S267F and T383M. They analysed the HL function by examining secretion and lipase activity in vitro cells positive for these mutations. Although heterozygous samples showed low HL activity in response to heparin, there was no statistically significant difference between the two mutations: 3.88 and 7.18U/mL for S267F and T383M heterozygotes, respectively, compared with 9.71 U/mL for normal subjects (mean±SEM). No HL activity was observed in the plasma of compound heterozygotes after heparin administration [12]. Hegele et al. (1993) reported that simple heterozygotes for either HL mutant did not have a discrete lipoprotein abnormality, except for relative triglyceride enrichment of lipoprotein fractions with d > 1.006 g/mL[13]. This variant was reported and counselled to be follow up for current literature data.

A 55-year-old male patient presented with a high level of TG (212 mg/dl). The patient was found to carry the NM_052968.5: c.64C>T (p.Q22*) heterozygous variant in the APOA5 gene encoding Apolipoprotein A-5. The variant is annotated as variant of unknown significance according to ACMG criteria. In his family history his daughter had hypertriglyceridemia (1500mg/dl). Status of the variant of the daughter was unknown. Mutations of the APOA5 cause Hypertriglyceridemia, susceptibility to (MIM: 145750) disease characterised by Apolipoprotein C-II deficiency, precocious atherosclerosis and hypertriglyceridemia [14]. This variant can be classified as likely pathogenic if family segregation is complete.

A 30-year-old female patient presented with inability to lose weight. The patient was found to carry the NM_000078.3:c.853C>T (p.R285*) pathogenic heterozygous variant in the CETP gene encoding cholesteryl ester transfer protein. The patient had a LDL level of 160 mg/dL, a triglycerid level of 147 mg/dL and a HDL level of 58 mg/dL (40-60 mg/dL). Hyperalphalipoproteinemia is caused by heterozygous mutations in the CETP gene and characterized by cholesteryl ester transfer protein deficiency [15]. CETP mediates the transfer of cholesteryl esters from HDL to VLDL, IDL and LDL in exchange for triglycerides. CETP deficiency causes mildly elevated HDL in heterozygotes (2-6x in homozygotes) [16]. Although the risk of cardiovascular disease is low in those with low HDL levels, the opposite has not been demonstrated [17].

5. Conclusıon

This is the first study in the Turkish FH patients to perform targeted exome sequencing. The absence of mutations in the FH classical genes strongly demonstrates the polygenic nature of FH and further underlines the importance of targeted exome sequencing to identify mutations in the FH cases.

References

1. https://reader.elsevier.com/reader/sd/pii/S0140673603142341?token=AF3D34AF95AF070560AD9B163FAEBD417AF0C3C225FFCE3176729 108EB7E06EEB35EF4F2FB66B83CABE2024413327642&originRegion=eu-west-1&originCreation=20220404122724, (accessed 04.04.2022)
2. Awan Z, Choi HY, Stitziel N, Ruel I, Bamimore MA, Husa R, et al. APOE p.Leu167del mutation in familial hypercholesterolemia. *Atherosclerosis*. 2013;231(2):218–22.
3. Veerkamp MJ, de Graaf J, Bredie SJH, Hendriks JCM, Demacker PNM, Stalenhoef AFH.

Diagnosis of Familial Combined Hyperlipidemia Based on Lipid Phenotype Expression in 32 Families. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 2002,22(2),274–82.
4. Khera AV, Won HH, Peloso GM, Lawson KS, Bartz TM, Deng X, et al. Diagnostic Yield of Sequencing Familial Hypercholesterolemia Genes in Severe Hypercholesterolemia. *Journal of the American College of Cardiology*. 2016, 7,67(22),2578–89.
5. Youngblom E, Pariani M, Knowles JW. Familial Hypercholesterolemia. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJ, Gripp KW, et al., editors. GeneReviews® 1993 [cited 2022 Jun 24]. http://www.ncbi.nlm.nih.gov/books/NBK174884/
6. Cuchel M, Bruckert E, Ginsberg HN, Raal FJ, Santos RD, Hegele RA, et al. Homozygous familial hypercholesterolaemia: new insights and guidance for clinicians to improve detection and clinical management. A position paper from the Consensus Panel on Familial Hypercholesterolaemia of the European Atherosclerosis Society. *European Heart Journal* 2014,21,35(32),2146–57.
7. Bello-Chavolla OY, Kuri-Garcia A, Rios-Rios M, Vargas-Vazquez A, Cortes-Arroyo J E, Tapia-Gonzalez G, et al. Familial combined hyperlipidemia: current knowledge, perspectives, and controversies. *Revista de Investigacion Clinica* 2018,70: 224-236.
8. Bilgic GD, Genotype and Phenotype Analysis Using a Hypertrophic Cardiomyopathy-Associated Gene Panel in Turkish Cardiomyopathy Patients. *Celal Bayar Üniversitesi Sağlık Bilimleri Enstitüsü Dergisi,2022, 9(2),* 268-272.
9. Schuster H, Fischer HJ, Keller C, Wolfram G, Zöllner N. Identification of the 408 valine to methionine mutation in the low density lipoprotein receptor in a German family with familial hypercholesterolemia. *Human Genetics*. 1993,91(3),287–9.
10. Defesche JC, van Diermen DE, Lansberg PJ, Lamping RJ, Reymer PWA, Hayden MR, et al. South African founder mutations in the low-density lipoprotein receptor gene causing familial hypercholesterolemia in the Dutch population. *Human Genetics*. 1993,1;92(6),567–70.
11. Carlson LA, Holmquist L, Nilsson-Ehle P. Deficiency of hepatic lipase activity in post-heparin plasma in familial hyper-alpha-triglyceridemia. *Acta Medica Scandinavica*. 1986,219(5),435–47.
12. Durstenfeld A, Ben-Zeev O, Reue K, Stahnke G, Doolittle MH. Molecular characterization of human hepatic lipase deficiency. In vitro expression of two naturally occurring mutations. *Arteriosclerosis and Thrombosis: A Journal of Vascular Biology*. 1994,14(3),381–5.
13. Hegele RA, Little JA, Vezina C, Maguire GF, Tu L, Wolever TS, et al. Hepatic lipase deficiency. Clinical, biochemical, and molecular genetic characteristics. *Arteriosclerosis and Thrombosis: A Journal of Vascular Biology*. 1993,13(5),720–8.
14. Breckenridge WC, Little JA, Steiner G, Chow A, Poapst M. Hypertriglyceridemia associated with deficiency of apolipoprotein C-II. *The New England Journal of Medicine*. 1978,8;298(23),1265–73.
15. Brousseau ME, Schaefer EJ, Wolfe ML, Bloedon LT, Digenio AG, Clark RW, et al. Effects of an inhibitor of cholesteryl ester transfer protein on HDL cholesterol. *The New England Journal of Medicine.* 2004,8,350(15),1505–15.
16. Zannis VI, Fotakis P, Koukos G, Kardassis D, Ehnholm C, Jauhiainen M, et al. HDL biogenesis, remodeling, and catabolism. *Handbook of Experimental Pharmacology*. 2015,224,53–111.
17. Kardassis D, Thymiakou E, Chroni A. Genetics and regulation of HDL metabolism. Biochimica et Biophysica Acta (BBA) - *Molecular and Cell Biology of Lipids.* 2022,1,1867(1),159060.