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Research Article

The Clinical Phenotype of Patients with the Heterozygous APOA5 rs3135506 S19W Variant: Findings from a Single Lipid Clinic

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Abstract

Background: Apolipoprotein A5 (ApoA5) plays a crucial role in regulating plasma triglyceride homeostasis. Any disruption in ApoA5 function due to common or rare genetic variants slows lipolysis of triglyceride-rich lipoproteins and causes dyslipidemia, a serious risk factor for atherosclerotic cardiovascular disease (ASCVD). **Methods:** Genetic testing was offered to patients presenting to a lipid clinic with pre-treatment LDL-C ≥190 mg/dL, fasting triglycerides ≥150 mg/dL, HDL-C <40 mg/dL in men or <50 mg/dL in women, lipoprotein(a) >50 mg/dL or >125 nmol/L, personal history of premature ASCVD (men <55 or women <65 years), or history of premature ASCVD in any first-degree relatives. Genetic testing was done using next-generation DNA sequencing of 127 genes known or suspected to cause dyslipidemia. We examined the clinical phenotype of patients found to carry heterozygous APOA5 rs3135506 risk variant. **Results:** Out of 355 patients who underwent genetic testing in this single lipid clinic, 43 (12.11%) were found to have a heterozygous APOA5 rs3135506 risk variant. The predominant baseline lipid abnormality was hypercholesterolemia with elevated total cholesterol and LDL-C. Among the patients with the APOA5 rs3135506 risk variant, eight (18.60%) had ASCVD events majority with premature disease (13.95%), and nine (20.93%) had objective evidence of preclinical atherosclerosis. **Conclusion:** Individuals with heterozygous APOA5 rs3135506 risk variant were found to have atherogenic dyslipidemia with a predisposition for premature ASCVD. The findings of this study encourage clinicians to be proactive with dyslipidemia treatment and have a low threshold for screening ASCVD in symptomatic patients found to have this risk variant on genetic testing.

Keywords: *genetics, variants, lipid, cardiovascular disease, prevention, apolipoprotein*

Introduction

Dyslipidemia is a well-established major risk factor for globally prevalent cardiovascular diseases [1]. The etiologies of dyslipidemia range from purely non-genetic (obesity, inactivity, medications, disease & disorders of metabolism) to purely genetic causes (familial hypercholesterolemia, familial chylomicronemia syndrome) [2]. Genetic testing technologies, such as next-generation genomic DNA sequencing, are increasingly available to researchers and clinicians. These testing methods are assisting in the detection of both rare and common DNA variants associated with dyslipidemias. Rare, large-effect DNA variants (mutations) cause monogenic or mendelian dyslipidemias, while common, small-effect DNA variants (single nucleotide polymorphisms or SNPs) in aggregate can cause susceptibility to dyslipidemias [3]. In some, extreme polygenic burden can contribute to similar or greater disease risk than monogenic variants [4]. While the phenotype of monogenic dyslipidemia has been better defined, there is a need for more information on the clinical importance of certain SNPs.

Apolipoprotein A5 (ApoA5) was first described in 2001. It was identified as an important determinant of plasma triglyceride (TG) levels, a serious risk factor for atherosclerosis and cardiovascular disease [5]. The APOA5 gene contains four exons and is located proximal to the apolipoprotein gene cluster of APOA1/C3/A4 on the long arm of

human chromosome 11q23 [6]. It is synthesized predominantly in the liver. ApoA5 is believed to play a crucial role in modulating TG homeostasis by affecting very-low-density lipoprotein (VLDL) production in the liver, stimulating proteoglycan-bound lipoprotein lipase at the endothelium of capillaries, and enhancing the clearance of TG-rich lipoproteins via liver receptors [7]. The absence of ApoA5 slows lipolysis of TG-rich lipoproteins and the removal of remnants by regulating their apoproteins content after secretion [8]. Since its discovery, a total of 87 APOA5 genetic variants have been reported [6]. APOA5 rs3135506 variant results in a C to G nonsynonymous substitution (56C>G) that changes serine to tryptophan at codon 19 (Ser19Trp) at position 116791691 (in human genome assembly GRCh38). The global minor allele frequency is 0.056 but found in nearly 1 in 6 people of Hispanic/Latino ancestry [9]. The APOA5 rs3135506 risk variant has been associated with dyslipidemia and atherosclerotic cardiovascular disease (ASCVD) [10,11]. Herein, we examine the clinical phenotype of patients found to have heterozygous APOA5 rs3135506 risk variant noted in a single lipid clinic.

Methods

Participants

Candidates to undergo genetic testing were selected by a lipidologist in a single lipid clinic. Genetic testing was offered to patients meeting at least one of the following criteria:

1. Elevated low-density lipoprotein cholesterol (LDL-C): defined as pre-treatment LDL-C more than or equal to 190 mg/dL.
2. Elevated TG: defined as pre-treatment fasting TG more than or equal to 150 mg/dL.
3. Low high-density lipoprotein cholesterol (HDL-C): defined as HDL-C less than 40 mg/dL in men or less than 50 mg/dL in women
4. Elevated lipoprotein (a) (Lp(a)): defined as Lp(a) more than 50 mg/dL or 125 nmol/L
5. Personal history of premature ASCVD: defined as coronary artery disease, coronary revascularization procedure, ischemic stroke, or symptomatic peripheral artery disease in men less than 55 years or women less than 60 years.
6. Family history of premature ASCVD in a first-degree relative.

Genetic testing was primarily pursued to screen for monogenic inherited dyslipidemias. Pre-testing counseling was provided by an experienced lipidologist. Patients provided informed consent for genetic testing, and this study was approved by the University of Chicago Medical Center Institutional Review Board. Results were discussed in detail with each patient by the clinic's lipidologist. Cascade screening of first-degree relatives was recommended to patients diagnosed with monogenic inherited dyslipidemias.

Genetic test

Genetic test was performed using the GBinsight Comprehensive Dyslipidemia Panel (GB Lifesciences, San Diego, CA, USA). The participant's genomic DNA was sequenced using next-generation DNA sequencing at the regions targeted by GBinsight Panels on an Illumina HiSeq instrument. This panel is designed to analyze 127 genes known or suspected to be associated with dyslipidemia. Allele frequencies were estimated from 1000 Genomics Phase 3 Database [9]. The polygenic risk score was calculated using a proprietary algorithm developed by GB HealthWatch. It included both risk-increasing and risk-decreasing variants and calculated on a relative scale between 1 and 100, with 1 being the lowest genetic risk and 100 being the highest genetic risk compared to the reference population for patient's ethnicity. The final genetic analysis report generated for each individual shows pathogenic and likely pathogenic variants with allele frequency, variants of uncertain significance, and selected risk variants with allele frequency, polygenic disease risk, and pharmacogenomic associations.

Results

A total of 355 patients underwent genetic analysis in this single lipid clinic. While the relatively common autosomal dominant monogenic disorder, familial hypercholesterolemia, was found in 54 (15.21%) individuals (49 were heterozygous for an LDLR, 5 were heterozygous for the Arg3527Gln APOB variant), the heterozygous APOA5 rs3135506 risk variant was found in 43 (12.11%) individuals. Baseline characteristics of the 43 patients with the APOA5 rs3135506 risk variant are shown in Table 1.

Table 1. Baseline patient characteristics

Patient Characteristics	**n=43***
Age (in years)	60 (49, 71)
Male Sex	65%
Caucasians	90.60%
Body mass index (in kg/m^2)	26.29 (24.73, 28.56)
Statin use	72%
Ezetimibe use	37%
PCSK9 inhibitor use	25%
Prescription fish oil use	25%
Fibrate use	16%
Baseline total cholesterol (Reference <200 mg/dL)	208 (162, 268.5)
Baseline LDL-C (Reference <100 mg/dL)	112 (78, 161.5)
Baseline HDL-C (Reference >40 mg/dL in men and >50mg/dL in women)	45 (35.5, 51.5)
Baseline triglycerides (Reference <150 mg/dL)	140 (97, 280)
Individuals with Lp(a) more than 50 mg/dL or 125 nmol/L	n=13 (30.23%)
Individuals meeting more than one lipid profile abnormalities included in inclusion criteria	41.86%
LDL-C: Low-Density Lipoprotein Cholesterol; Lp(a): Lipoprotein (a); HDL-C: High-Density Lipoprotein Cholesterol; PCSK9: Proprotein Convertase Subtilisin/Kesin Type 9. *Continuous variables reported as median (25th quartile, 75th quartile)	

Clinical Events

Among the 43 patients with APOA5 rs3135506 risk variant, eight patients had ASCVD (seven had acute coronary artery events and/or coronary revascularization, one had symptomatic peripheral artery disease). Out of the eight patients with ASCVD, six had premature events (occurred in men less than 55 years or women less than 60 years). In the remaining 35 patients without known clinical ASCVD, nine patients had elevated coronary artery calcium score (range 40 to 97 percentile). The clinical event data is shown in Table 2.

Table 2. Clinical events in the cohort [23]

Event	**n=43**
Total ASCVD events	8 (18.60%) (7 CAD and/or revascularization + 1 symptomatic PAD)
Total premature ASCVD events	6 (13.95%)
Total ASCVD events in patients with a pathogenic and likely pathogenic variant	3 (6.9%) Including LDLR, CBS, CPT2 mutation*
Total individuals with abnormal CAC score	9 (20.93%) (CAC percentile ranging from 40 to 97)
ASCVD: Atherosclerotic Cardiovascular Disease; CAC: Coronary Artery Calcium Score; CAD: Coronary Artery Disease; CBS: Cystathionine Beta-Synthase; CPT2: Carnitine Palmitoyltransferase II; LDLR: Low-Density Lipoprotein Receptor; PAD: Peripheral Artery Disease -ASCVD is defined as coronary artery disease, revascularization procedure, ischemic stroke, or symptomatic peripheral artery disease - Premature ASCVD events are defined as ASCVD in men less than 55 years or women less than 60 years	

Dyslipidemia

Among the 43 patients with APOA5 rs3135506 risk variant, median baseline total cholesterol (TC), LDL-C were elevated, and HDL-C was low-normal as shown in Table 1. About 13 individuals (30.23%) had elevated Lp(a), but mean or median Lp(a) couldn't be calculated due to use of variable assays (mg/dL and nmol/L). The predominant baseline lipid abnormality was hypercholesterolemia with elevated TC (208 (162, 268.5) mg/dL; reference <200 mg/dL) and LDL-C (112 (78, 161.5) mg/dL; reference <100 mg/dL). Six patients (13.95%) had severe TG >500 mg/dL at baseline. Among patients with ASCVD (n=8), median baseline lipid panel was TC 153 (138, 185.75) mg/dL, HDL-C 41 (33.75, 51) mg/dL (reference HDL-C >40 mg/dL), TG 115.5 (92.75, 182.25) mg/dL (reference TG <150 mg/dL), and LDL-C 75 (62.5, 94.5) mg/dL. Among patients with objective evidence of preclinical atherosclerosis (n=9), median baseline lipid panel was TC 221 (187, 240) mg/dL, HDL-C 48 (40, 51) mg/dL, TG 106 (73, 140) mg/dL, and LDL-C 150.5 (104.5, 165) mg/dL.

Genetic findings

Out of the 43 patients with APOA5 rs3135506 risk variant, nine had pathogenic/likely pathogenic variants, as shown in Table 3. In the cohort examined, a total of 23 variants of uncertain significance, 239 other risk variants, 9 protective variants, and 23 pharmacogenomic associations were found, as shown in Table 4.

Table 3. Pathogenic and likely pathogenic variants in the cohort [23]

Gene	Number of patients	variant	Molecular sequence
ABCC8	1	rs151344623	3989-9G>A
CBS	1	rs121964964	Ala114Val
CPT2	1	rs74315294	Ser113Leu
HFE	1	rs749553271	Glu298Ter
LDLR	1	rs121908031	Cys681Ter
LDLR	1	rs137853966	Gly218ValfsTer47
LDLR	1	rs121908029	Glu228Lys
LDLR	1	EX3-6DEL (FH Aarhus-2)	Leu64_Gly314delinsTrp
LDLR	1	Exon16 Duplication	Uncertain

ABCC8: ATP-Binding Cassette Transporter sub-family C member 8; CBS: Cystathionine Beta-Synthase; CPT2: Carnitine Palmitoyl Transferase II; HFE: Homeostatic Iron Regulator; LDLR: Low-Density Lipoprotein Receptor

Table 4. Other genetic findings in the cohort [23]

Analysis	Number of individuals with positive results	Most common mutation identified	Number of patients with most common mutation
Pathogenic and likely pathogenic variants	9	LDLR	5
Variants of uncertain significance	23	ApoB c11477C>T(pThr3826Met) rs61744153	3
Other risk variants	239	ZPR1 rs964184	41
Protective variants	9	LIPG c1187A>G(pAsn396Ser) rs77960347	3
		TM6SF2 p.Glu167Lys rs58542926	3
Pharmacogenomic associations	23	SLCO1B1 c521T>C(pVal174Ala) rs4149056	17

LDLR: Low-Density Lipoprotein Receptor; APOB: Apolipoprotein B; ZPR1: Zinc Finger Protein 1; LIPG: Endothelial Lipase Gene; TM6SF2: Transmembrane 6 Superfamily 2; SLCO1B1: Solute Carrier Organic Anion Transporter Family Member 1B1

Discussion

APOA5 rs3135506 risk variant and ASCVD

In this single lipid clinic, among the 355 individuals who underwent genetic testing due to clinical suspicion for monogenic inherited dyslipidemia, 43 (12.11%) were found to have heterozygous APOA5 rs3135506 risk variant. Among the patients with APOA5 rs3135506 risk variant, eight (18.60%) had ASCVD events majority with premature disease (13.95%), and nine (20.93%) had objective evidence of preclinical atherosclerosis. Heterozygous APOA5 rs3135506 risk variant has been found to have a possible link with various cardiovascular disease risk factors such as dyslipidemia, obesity, metabolic syndrome, and an increase in systolic blood pressure [12,13]. It is negatively correlated to body mass index [14]. Among patients with obstructive sleep apnea, APOA5 rs3135506 heterozygote risk variant has a possible link with type 2 diabetes mellitus (adjusted OR 2.64; 95% CI, 1.38 to 5.04; p=0.003) [15]. However, there is limited evidence assessing preclinical and clinical ASCVD events in individuals with this variant. APOA5 rs3135506 risk variant has been associated with intima-media thickness defined subclinical atherosclerosis [16,17]. In a study involving 61 subjects with APOA5 rs3135506 (five with homozygous mutation), this variant was found more frequently in patients with CAD [11]. Nonsynonymous mutations of APOA5 have shown a 2.2-fold increased risk of myocardial infarction [18]. When occurring in combination with low-density lipoprotein receptor mutations, the risk for myocardial infarction amplifies [18]. APOA5 rs3135506 risk variant revealed an increased risk of ischemic stroke (1.97-fold higher risk) [10,19].

APOA5 rs3135506 risk variant and dyslipidemia

APOA5 plays an important role in determining plasma TG levels in humans. It is necessary for the activation of lipoprotein lipase that decreases TG levels. ApoA5 contains 366 amino acids and includes the signal peptide, lipid-binding domain, receptor binding domain, and the C-terminal [12]. The APOA5 rs3135506 risk variant affects the signal peptide, the coding region of the ApoA5 gene that disrupts the localization and function of ApoA5 protein [20]. Due to disruption in protein function or expression, patients with this variant can have an increased risk of hypertriglyceridemia. The APOA5 rs3135506 risk variant has also been associated with increased levels of larger VLDL and increased size of intermediate-density lipoprotein (IDL, precursors of small dense LDL), leading to hypertriglyceridemia and ASCVD risk [14,21,22]. ApoA5 is positively correlated with HDL-C, and mutations can reduce large HDL particles [14,16]. In our cohort, the predominant baseline lipid abnormality was hypercholesterolemia with elevated TC and LDL-C suggestive of atherogenic dyslipidemia. Six patients (13.95%) had severe TG >500 mg/dL at baseline. Contrary to expectation, the median baseline lipid panel was normal among patients with ASCVD (n=8). This discrepant observation could be due to the small sample size, with or without potential contribution by unmeasured residual risk factors such as VLDL, IDL, Apolipoprotein B, LDL particle size, and number. Among patients with objective evidence of preclinical atherosclerosis (n=9), median TC and LDL-C were elevated, suggestive of atherogenic dyslipidemia.

The findings of this study might help encourage clinicians to be proactive with dyslipidemia control and have a low threshold for screening ASCVD in symptomatic patients found to have APOA5 rs3135506 risk variant on genetic testing. Our study has some limitations. As the sample size is small and the study included only high-risk patients with clinical suspicion of monogenic inherited dyslipidemia, the findings might not apply to the general population. In patients with hypertriglyceridemia (TG >400 mg/dL), calculated LDL-C might be under-estimated or incalculable in patients with severe hypertriglyceridemia. The influence of other innate known and unknown genetic factors on the clinical presentation cannot be differentiated. Hence, while the study provides a potential genetic tool for suspecting ASCVD risk and prompting early intervention, there is a need for caution with the interpretation and clinical applicability of the findings.

In conclusion, individuals with heterozygous APOA5 rs3135506 risk variant were found to have atherogenic mixed hyperlipidemia with a predisposition for premature ASCVD. Increasing availability and advances in genetic testing are facilitating the identification of common and rare genetic variants associated with dyslipidemias. Understanding the association between an individual's genetic variants and disease risk will assist in patient-specific counseling, cascade screening, and early therapeutic intervention to decrease cardiovascular disease risk.

Conflicts of interest

Mendel Roth: Employee at Genben Lifesciences and Michael H. Davidson, MD, FACC, FNLA: Consultant and Speaker for Amgen, Esperion, New Amsterdam, Novo Nordisk, Piper Biosciences, Regeneron, Sanofi.

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