A Novel Mutation in the Kringle IV Domain of LPA Gene Leading to Familial Cardiovascular Diseases

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Research article

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Abstract

Background This study aims to investigate the clinical characterization and causative genetic defect of a four-generation Chinese Han family with cardiovascular diseases. Methods The combined use of next-generation sequencing and qPCR technique was performed to investigate genetic pathology of familial cardiovascular diseases. Results The clinical manifestations of the family members include coronaty artery disease, early-onset hypertension, lipoma, cerebral infarction and even unexplained sudden death, and a novel heterozygous deletion of 3-16 exon of LPA gene was identified to be causative for the symptoms in the family. Conclusions A novel deletion in the LPA gene was identified in a Chinese family associated with elevated Lp(a) levels and cardiovascular diseases, which expands the spectrum of the LPA mutation and its associated phenotypes. Keywords Copy number variation; Cardiovascular diseases Kringle IV; Lipoprotein(a); LPA;

Background

Coronary artery disease (CAD) has a wide range of clinical manifestations, from asymptomatic to stable coronary disease and acute coronary syndrome [1, 2]. The elevated plasma concentration of lipoprotein(a) [Lp(a)] has a strong association with coronary heart disease [3-4]. Most laboratories around the world recognize elevated Lp(a) as plasma levels above 30 mg/dl [5]. Lp(a) consists of a single large apolipoprotein(a) [apo(a)] which is attached covalently to the apolipoprotein B moiety of a cholesterol-rich low-density lipoprotein (LDL) cholesterol particle [1]. The biological function of Lp(a) involves interfering with plasminogen activation and its atherogenic potential serving as a lipoprotein particle after receptor-mediated uptake [2]. *LPA*, the gene coding for apo(a), located on chromosome 6q25-6q26, explains more than 90% of the Lp(a) variance and it genetically determines the circulating Lp(a) level [2]. Lp(a) concentration has been reported to be largely determined by genetic variation within the LPA locus, including both SNVs (single nucleotide variants) and CNVs (copy number variations) [6]. *LPA* gene consists of ten homologous Kringle IV domains, Kringle IV types 1 and 3-10 occur only once in every apo(a) gene, while the copy number of Kringle IV type 2 is variable among individual, and this copy number variation which represents the size polymorphism of Lp(a) is of pathological significance [7]. It has been reported that genetic diversity at the *LPA* locus, including the SNPs rs10455872 and rs3798220, is associated with raised plasma concentrations of Lp(a) and incident cardiovascular disease [8]. In the present study, a novel genetic mutation in the Kringle IV domain of *LPA* gene was identified in a Chinese family which causes cardiovascular disease (CVD).

Methods

The proband (III:1) (Figure 1.) aged 50 was found to have hypertension for 2 years before suffering cerebral infarction at 46 years old and also elevated Lp(a) with the maximum level up to 2,100 mg/L.The family history investigation showed that there were 6 other affected individuals in this Chinese Han four-generation family. Family members successively went through a series of diseases because of abnormal Lp(a) concentration.

Genetic analysis of the proband was performed using next-generation sequencing (NGS) (Figure 2.) with a panel covering 446 cardio-cerebrovascular diseases related genes followed by multiple lines of bioinformatics analysis (AmCare Genomics Lab). Additionally, the qPCR technique was used to detect and analyze the site-specific variation in other 15 family members including II, III and IV generations. The single-copy gene albumin was used to normalize for different concentrations of DNA in different samples. Reactions were performed in 10 ul final volume, using Bestar® SybrGreen qPCR Master Mix (with ROX) and primers. Primer sequences were as follows: KIV-2 forward 5'-ATCCAGATGCTGTGGCAGCT-3', KIV-2 reverse 5'-GCGACGGCAGTCCCTTCT-3'.

Results

Tthe family members suffered atherosclerotic cardiovascular disease (ASCVD): the grandmother of the proband (I:1) had unexplained ; her mother (II:1) was found to have hypertension at 40 years old and suffered unexplained at 73 years old; the maternal aunt of the proband (II:3) aged 73 had early-onset hypertension and coronary artery , (II:5) aged 69 had coronary artery disease and was diagnosed with large recurrent lipoma on the outer left thigh; her brother (III:3) aged 49 and her sister (III:8) aged 45 were diagnosed with coronary artery disease with elevated Lp(a) around 500 to 800mg/L. There was no other diseases or abnormalities in all the affects.

A novel heterozygous deletion of 3-16 exon of *LPA* gene was detected in the proband using next-generation sequencing (Table 1.). This copy number variation has not been reported in the related clinical cases, and the mutation is located in the Kringle IV area of *LPA* gene which has been reported to be associated with Lp(a) concentration in plasma [9]. So far, the frequency of this mutation is extremely low in the population genetic database and the mutation occurs in the Kringle IV region which plays an important role in functional activity of the protein. Further analysis revealed that the amino acid sequences of this region among different species are highly conserved, which further indicate the important role of Kringle IV region and the mutation in this region may therefore impair the protein function and cause related diseases.Similarly, the result using qPCR technique revealed a heterozygous deletion of 3-16 exon of *LPA* gene in 12 of the other 15 family members and 4 of them have had pre-existing symptoms.

Discussion

In the present study, part of the four-generation Chinese Han family members successively went through coronary artery disease, cerebral infarction and even unexplained sudden death with a common clinical manifestation of increased Lp(a) concentration in plasma of some members. novel heterozygous deletion of 3-16 exon in the Kringle IV domain of *LPA* gene was detected in 13 family members. Therefore, above results suggest that *LPA* gene may be the potential mechanism resulting in CVD and related with an elevated level of Lp(a). Above results are consistent with previous studies. Nonsize polymorphism in the *LPA* gene and apo(a) size polymorphism all influence Lp(a) levels [10-11]. Study by Zi-Kai Song et al. Showed that five SNPs (rs1367211, rs3127596, rs9347438, rs6415085, and rs9364559)

in the *LPA* gene were significantly associated with coronary artery disease (CAD) in Chinese Han population [12]. However, the result that deletion of 3-16 exon in the Kringle IV domain of *LPA* gene was associated with CVD in a Chinese Han family is reported for the first time.

Lp(a) is the preferential lipoprotein carrier for oxidized phospholipids (OxPL), a proatherogenic and proinflammatory biomarker. Lp(a) causes accelerated atherothrombosis and premature CAD through adversely affecting inflammation, fibrinolysis, plaque stability, endothelial function and oxidative stress. Genetic variants in the *LPA* gene that increases Lp(a) levels also increases CAD risk, indicating that Lp(a) is a risk factor for CAD. The 2018 Cholesterol Clinical Practice Guideline have recognized increased Lp(a) as an ASCVD risk enhancer for initiating or intensifying statin therapy [13]. Lp(a) value more than 30 mg/dl is strongly and independently associated with CVD complications in type 1 diabetic patients [14]. Studies suggest that Lp(a) levels more than 50 mg/dl is most pathogenic and supposed to signally increase CVD risk [15]. It has also been recommended to screen for elevated lipoprotein(a) concentration in patients with moderate or high-risk coronary heart disease, and available data increase priority for investigation of Lp(a) as a potential therapeutic target [16-17].

The present study expands the spectrum of the *LPA* mutation and its associated phenotypes. The combined use of NGS and qPCR technique is proved to be an effective approach to investigate genetic pathology of familial diseases. Missing Lp(a) results of some family members may be a limitation. Therefore, emphasis should be put on Lp(a) measurement to be routinely performed in clinical practice and screening strategies involving this important CVD risk factor

Conclusions

In conclusion, this study explored the association between a novel heterozygous deletion of 3-16 exon in the Kringle IV domain of *LPA* gene and risk of CVD in a four-generation Chinese Han family, expanding the spectrum of the *LPA* mutation and its associated phenotypes, and confirming that *LPA* gene determines the circulating Lp(a) level and elevated Lp(a) level is a risk factor for CVD, which agrees with previous studies.

Abbreviations

Lp(a): Lipoprotein(a)

LDL: Low-density lipoprotein

apo(a): Apolipoprotein(a)

CVD: Cardiovascular disease

CAD: Coronary artery disease

ASCVD: Atherosclerotic cardiovascular disease

SNVs: Single nucleotide variants

CNVs: Copy number variations

NGS: Next-generation sequencing

Declarations

Ethics approval and consent to participate

Written informed consent for the presentation of this study was obtained from all individual participants included in the study. The study was approved by the IRB of Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine (Reference numbers: 2014-368-64-01).

Consent for publication

An informed written consent for publication of the participants clinical details was obtained from each of the participants.

Availability of data and materials

The datasets used and analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YL, XZ, YW drafted the manuscript. FG, XY, TZ recruited, acquired, analyzed and interpreted the data. TZ and XY supervised the management of the study and approved the manuscript. All authors read and approved the final manuscript.

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Table

Table 1. Genetic features of the identified pathogenic variant

Gene name	Hereditary mode	HG19 location	Transcript	Nucleotide and amino acide changes
LPA	MU	chr6: 161032594-161067427	NM-005577	3-16 exon deletion

Figures

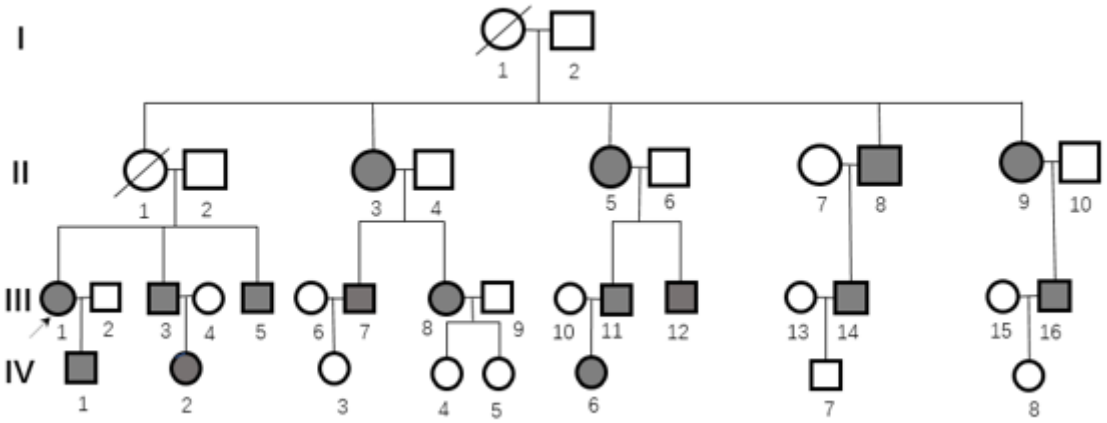

Figure 1

Pedigree of the family with CVD. Square indicates the male and circles indicates the female, arrow points out the proband, hollow symbols and solid symbols indicate normal individuals and family members who had qPCR analysis respectively.

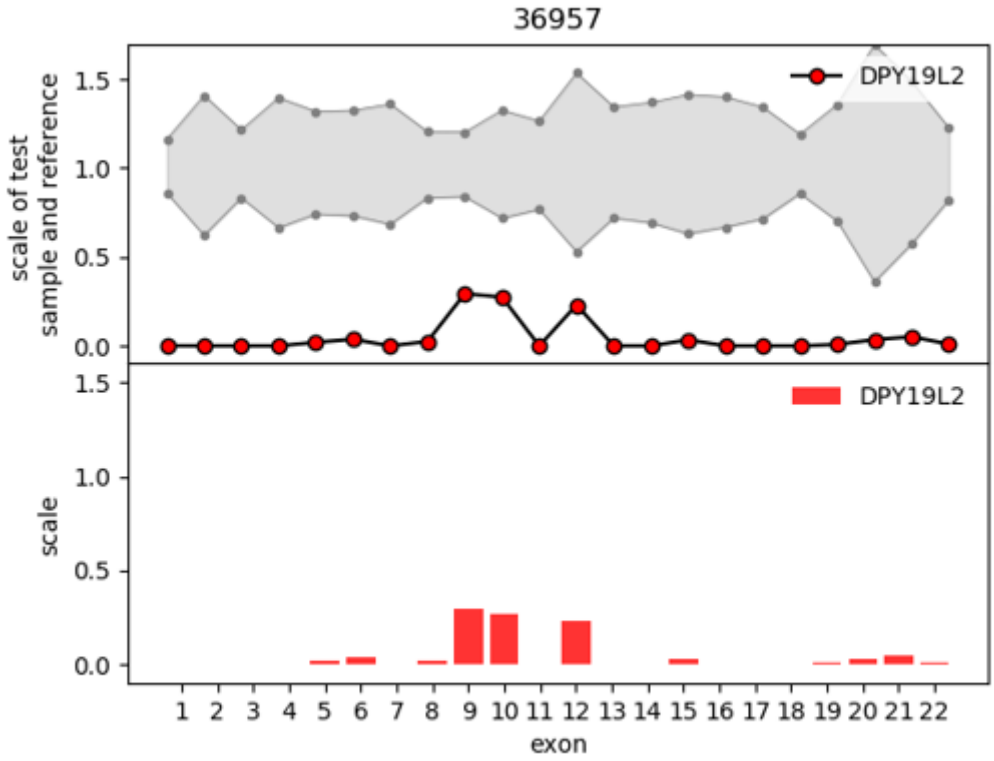

Figure 2

Copy number graph of NGS carried out on proband (III:I).