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Microduplication of 10q26.3 in a Chinese hypertriglyceridemia patient

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ABSTRACT

Hypertriglyceridemia (HTG) plays an important role in the development and progression of atherosclerosis. It is inherited in an autosomal dominant pattern with a frequency of approximately 1:1,000,000 worldwide. Previous study has demonstrated that more than six genes underlie this disorder. In addition, copy number variants (CNVs) including disease-causing genes also play a crucial role in it. In this study, we have employed SNP-ARRAY chip technology to detect the pathogenic CNVs in a HTG patient who carried no meaningful mutations in HTG candidate genes. And we identified a de novo CNV interstitial 134.7 kb duplication of chromosome region 10q26.3 containing *CYP2E1*. And this CNV also has been confirmed by Real-time PCR. CYP2E1 is a member of cytochrome P450 superfamily of enzymes which play an important role in fatty acid metabolism. Our study is consistent with previous research and further claimes that CNVs containing *CYP2E1* may be related to HTG and obesity. Our study not only further confirmes the hypothesis that the *CYP2E1* is a plausible candidate gene for HTG, but also may contribute to the diagnosis and treatment of these genomic diseases.

1. Introduction

Hypertriglyceridemia (HTG) is a major public health problem and is one of the most severe lipid metabolism disorders, characterized by elevated levels of plasma triglyceride (TG > 1.7 mmol/L). HTG is an important contributory factor to development of atherosclerosis, and as such is recognized as a major risk factor for coronary artery disease. The prevalence of HTG has increased several times over the past few decades worldwide [1–3]. Previous study has demonstrated that both environment and genetic factors may increase the levels plasma TG [4]. When the levels of TG > 10 mmol/L, genetic factors may conduct a dominating role in HTG [5].

To date, more than five genes include *APOA5* (NM_001166598), *APOC2* (NM_000483), *LPL* (NM_000237), *LMF1* (NM_022,773), *GPIHBP1* (NM_001301772), *GPD1*(NM_001257199), etc [6–9] have been identified in HTG patients. In addition, several copy number variants (CNVs) also have been detected in HTG, obesity and obesity-related syndromes, such as 1p21.3 deletion, 19q13.2 duplication and so on [10,11]. At the same time, some rare CNVs such as 10q26.3, 5q31.3 also have been reported to relate to HTG and obesity [12–14].

In this study, we employed SNP-ARRAY chip technology to detect pathogenic CNVs in a HTG patient who carried no meaningful mutations in HTG candidate genes including *LPL*, *APOA5*, *APOC2*, *LMF1*, *GPD1* and *GPIHBP1*. And we identified a de novo CNV interstitial 134.7 kb duplication of chromosome region 10q26.3 containing *CYP2E1* (NM_000773). Real-time PCR was performed to confirm this CNV. Our finding suggests that the de novo CNV containing *CYP2E1* may be the genetic factor of the HTG patient with obesity.

2. Materials and methods

This study was performed in accordance with the Helsinki Declaration and approval of the ethics boards of the Second Xiangya Hospital of the Central South University. All subjects have consented to this study.

2.1. Subjects

We collected a female HTG patient with very high level of serum TG (TG = 11.2 mmol/L). Her body mass index (BMI) is 26.4 kg/m^2. In addition, her four family members including her father and mother also have been enrolled in this study.

2.2. DNA extraction

All subjects joined this study signed informed consent. Genomic DNA was extracted from peripheral blood of all patients using a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA) on the QIAcube automated

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Table 1
Primer sequences used for mutation sequencing ofAPOA5, APOC2, GPIHBP1, LPL, GPD1 and LMF1 genes.

Gene	Forward	Reverse
APOA5-1	TGTCCCTTCGTCTCCTTCTT	CTGTGGAGAGGGACTAGGTAAT
APOA5-2	CTGATTACCTAGTCCCTCTCCA	AACAGCTACGGAGTTGTCAAG
APOA5-3-1	GGGACAAAGGAGATGATGGA	TCGGCGTATGGGTGGAAG
APOA5-3-2	CTGAAGCCCTACACGATGGA	GCGGAAAGCCTGAAGTCG
APOA5-3-3	GGCACTGGGACTGAGGAAG	GACAAGGAGCTGGGAATGG
APOC2-1	TGGGAAACTTGACTGGGACA	GGCTGGGAAGATGCTTGG
APOC2-2	CCTGGTATTGGGATTTGGT	AACTTCTGGGTCCTGGATG
APOC2-3	CCCCTCCTCCCTCTAACCA	GTGCCATCCATGAGAAGCAA
GPIHBP1-1	ATGCCCTTCATCCCACTTACC	GCTTCCATCCATGCTGCTCT
GPIHBP1-2	GTAGGGTGTTCAGGGTAGGG	CAGAATGCTCCAGGCAGAT
GPIHBP1-3	CTCACCAGGCTAGGCTTTGG	TGGAGTGGGTGGTCAGGAGG
GPIHBP1-4	CGCCCATCCTCAGCACTT	CGCCCAAGACACTCCAAATC
LPL-1	GGAAAGCTGCCCACTTCTA	TTCCTTCTTCTCATCCTCAGTTC
LPL-2	TGGTTGCCTGTGAACCTAAA	CCTGAGCCAGAACTGTCTTTAT
LPL-3	GACAAGTGGTAGGTGGGTATTT	CCACGCTGATTCTGAAGATTTG
LPL-4	GGCAGAACTGTAAGCACCTT	CCTAATAAAGAGCCCTACAATGAGATA
LPL-5	GCCAGTGCATTCAAATGATGAG	TGGGTCAATAAGGGTTAAGGATAAG
LPL-6	ATGCCAAATGAAACACTC	TTAGAAGCCTCAGACAAA
LPL-7	CTTCCGGTTTGAGTGCTAGT	TGCTCAGACCAAGGGTTATG
LPL-8	TGAGTTCTTTGTTGGACA	CTGAAATACAGCCCCTAG
LPL-9	TCCTGACAGAACTGTACCTTTG	GGATGCCCAGTCAGCTTTA
GPD1-1	TCCTTTCCCTGGCTCTGC	CCTCCTACCCACCTCTGTCTT
GPD1-2	GGGACTATTTGTCATGGGAGT	GAGGCACCTGTTGAGTAAGG
GPD1-3	CCAAACAAGCCTTCCTGC	CTCCTTGCTTCACCCACC
GPD1-4	TGGCCTCCTCACAGCAAA	TCCCAGCCTCCTTCACCT
GPD1-5	TAAGCCCAGGAGTTTGAG	CACGGTCTGATGATGAATAA
GPD1-6	GTCACGGCTGATGAAATGA	CAAGCACCTCTACCTGGAT
GPD1-7	TCTGTAGGCATCCAGGTAG	AGATTTGTGGCAGGTTTAG
GPD1-8	GGAGGGTTAGGCAGTGAG	TTTCTGGCAAATGTGGTG
LMF1-1	AACTGCGAAGGAGGCAGGC	GCGGAGGAGTCTCGAGGGAG
LMF1-2	TGCCTCGCCCCGCATTCT	AGCTCCGACCGCCCCATT
LMF1-3	GGTTGAAACAAGCCAAAGTGT	AGATCACAAGCGCCCATC
LMF1-4	CTTGCGTGTCGATGTTGA	GGTTAGAAGAGCCACCGTTA
LMF1-5	CCCTTCTCAAATTCTGCCTTCC	TGATGCGACAGCTCACCAG
LMF1-6	CTCTTAGCGTGGCAGGTGG	CAAACGAAGGCTGGGGAG
LMF1-7	GGCACAGCTGGGTTTCA	TGAGCCACCTACCGAATCT
LMF1-8	GCGTGCCAGGAACAAGGT	TGTCCAGGCCCGGTAGTG
LMF1-9	GGGCCACAGTTCCCAAA	CGTTCTAGAAACCTGCCATCTAT
LMF1-10	GAACCCACCTCCAGGAAAG	TGATGCCAAGGCTGATGT
LMF1-11	TTGCTGCGCTGTTCACT	GCTGGGTCTTCGCCTTTATT

DNA extraction robot (Qiagen, Hilden, Germany).

2.3. Sanger sequencing

Through polymerase chain reaction (PCR; primer sequences were shown in the Table 1), we amplified several genes, including *LPL*, *APOA5*, *APOC2*, *LMF1*, *GPD1* and *GPIHBP1*. The sequences of the PCR products were obtained using the ABI 3100 genetic analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA).

2.4. SNP array analysis

Genomic DNA samples of patients were adjusted to a final concentration of 50 ng/μL. The HumanOmni1-Quad Chip (Illumina Inc., San Diego, USA) and the Illumina BeadScan genotyping system (Beadstation Scanner) were employed to obtain the signal intensities of SNP probes. HumanOmni1-Quad Beadchip contains over 1.1 million loci across the human genome, including markers derived from the three HapMap, the 1000 Genomes Project and recently published studies. The GenomeStudio V2011 software was used to analyze the genotypes (human genome build 37/Hg19 for analysis) and evaluate the experimental quality as previously described [15]. The call rates of the samples are greater than 99.0%.

2.5. Real-time PCR

To validate variable copy numbers, real-time quantitative PCR (qPCR) was performed using the 7500 Fast Real-Time PCR systems (Applied Biosystems, Foster City, California). For potentially pathogenic CNV, two primer sets were designed within the boundaries of the CNV region. Primer pairs were designed by an online PrimerQuest tool of Integrated DNA Technology (IDT) (http://www.idtdna.com/Primerquest/Home/Index). The forward and reverse primers are 5′-CTGGACTACAAGGACGAGTTC-3′ and 5′-CCTTCCAGGTAGGTCCATTATT-3′, respectively. PCR reactions were prepared with the SYBR Premix Ex Taq II PCR reagent kit (TaKaRa Bio, Dalian, China) according to the manufacturer's protocol. Amplification levels were calculated with the $2^{-\Delta\Delta CT}$ method [16].

3. Results

3.1. Clinic data

The proband, a 29-year-old officer from Hunan province of Central-South China, had extremely high serum level of HTG (TG = 11.2 mmol/L). Her BMI was 26.4 kg/m^2 and had no diabetes mellitus and acute pancreatitis. All the four family members showed no HTG and obesity.

3.2. Genetic analysis

Sanger sequencing showed no meaningful mutations in *LPL*, *APOA5*, *APOC2*, *LMF1*, *GPD1* and *GPIHBP1*. Then we suspected that CNVs may be the genetic lesion of this patient. So we applied SNP array system