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Short Communication

Common mutations of familial hypercholesterolemia patients in Taiwan: Characteristics and implications of migrations from southeast China

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ABSTRACT

Familial hypercholesterolemia (FH) is an autosomal dominant disease caused by mutations in low-density lipoprotein receptor (*LDLR*), apolipoprotein B-100 (*APOB*), and proprotein convertase subtilisin/kexin type 9 (*PCSK9*) genes. This study investigated FH patients carrying common mutations in Taiwan and compared them to FH southeastern Asians. Causal FH mutations were identified by exon-by-exon sequencing with/without multiplex ligation-dependent probe amplification among 208 Taiwanese with clinically diagnosed FH. Haplotype analyses among probands and family members were undertaken using TaqMan® Assays. Totally, *LDLR* mutations were found in 118 probands, consisting of 61 different loci, and *APOB* 10579C>T mutations in 12 probands. Three mutations (64delG, 1661C>T, and 2099A>G) were novel. *LDLR* 986G>A (13.1%), 1747C>T (10.8%), and *APOB* 10579C>T (9.2%) were common mutations with no differences in phenotypes. *LDLR* 1747C>T associated with one haplotype (CAAGCCCCATGG/(dTA)n-112nt); *LDLR* 986G>A with two. *APOB* 10579C>T associated with the same *LDLR* binding-domain pattern in Taiwanese and southeastern Asians. We concluded that *LDLR* 986G>A, 1747C>T and *APOB* 10579C>T are common mutations, with combined frequency of approximately 33%. The presence of different haplotypes associated with FH common mutations in Taiwan indicates multiple historical migrations, probable multiple recurrent origins from southern China, and haplotype homologies reflect the presence of common ancestors in southern China.

1. Introduction

Familial hypercholesterolemia (FH, OMIM #143890) is one of the most common inherited disorders of plasma lipoprotein metabolism, characterized clinically by an increased level of circulating low-density lipoprotein (LDL)-cholesterol that leads to lipid accumulation in tendons and arteries, premature atherosclerosis and increased risk of coronary heart disease (Ueda, 2005). FH is known to be associated with mutations in the LDL receptor (*LDLR*) gene, the apolipoprotein B-100 gene (*APOB*), and proprotein convertase subtilysin kexin 9 gene (*PCSK9*) (Abifadel et al., 2003; Brown and Goldstein, 1986; Soria et al., 1989). With the exception of a small number of founder populations where one or two mutations predominate, most geographically based-surveys of FH patients show a large number of mutations in a given population (Austin et al., 2004). Haplotype analysis of FH Caucasians improved the knowledge and understanding of the origin, spread by migrations and estimation of the ages of pathogenic mutation (Castillo et al., 2002; Miserez and Muller, 2000; Tejedor et al., 2010; Traeger-Synodinos et al., 1998), but no data has been reported concerning the Asian population.

Taiwanese, the major population group in Taiwan, are the descendants of early settlers from the southeast coast (Fuchien and Kwangton province) of China during the past 400 years or more in recent history (Lin et al., 2001). Recently, anthropological studies using genetic markers by human leukocyte antigen and microsatellites revealed that Taiwanese, with Singapore Chinese and Thai-Chinese, formed a southern Asian cluster with neighboring groups of Thais and Vietnamese, which is separate from the northern Asian cluster consisting of Koreans and Japanese (Chu et al., 1998; Lin et al., 2001; Yang et al., 2009). Although there have been reports of FH from the southern Chinese (Chang et al., 2003; Charng et al., 2006; Chiou and Charng, 2010; Chiu et al., 2005; Khoo et al., 2000; Mak et al., 1998a; Sun et al., 1994; Tai et al., 1998), the relationships need to be further investigated. Accordingly, we performed molecular genetic testing to investigate the frequency of mutations among unrelated FH patients in Taiwan, analyzed haplotype patterns among patients carrying common mutations, and further compared

Abbreviations: FH, familial hypercholesterolemia; *LDLR*, low-density lipoprotein receptor; *APOB*, apolipoprotein B; *PCSK9*, proprotein convertase subtilisin/kexin type 9; PCR, polymerase chain reaction; MLPA, multiple ligation-dependent probe amplification.

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the patterns with those of previous FH studies from the southeastern Asians.

2. Materials and methods

2.1. Study populations

Patients with LDL-cholesterol levels > 190 mg/dl and positive family history of hypercholesterolemia were recruited in a genetic screening program for FH in Taiwan from Sep, 2008 to Mar, 2011. All were clinically diagnosed as having FH by the Simon Broome Familial Hypercholesterolemia Register diagnostic criteria (Scientific Steering Committee on behalf of the Simon Broome Register Group, 1991). Totally, a series of 208 apparently unrelated Taiwanese residing throughout northern, central and southern Taiwan were enrolled, including 102 who had been surveyed in a previous study (Chiou and Charng, 2010). Patients with hypercholesterolemia due to secondary causes were excluded. Premature coronary artery disease and the risk factors for heart disease, including hypertension, diabetes mellitus, smoking habits and family history of premature coronary artery disease in first-degree relatives, were recorded. Family members of the probands with the identified mutations were also invited to participate in this investigation. The study complied with the Declaration of Helsinki of the World Medical Association, was approved by the institutional review board of the hospital, and informed consent was obtained from each patient.

2.2. DNA analyses

Genomic DNA was isolated from the leukocytes of peripheral blood of patients. First, exon-by-exon sequence analysis was performed as previously described on both strands of all 3 FH-causing genes—*LDLR*, *APOB*, and *PCSK9T* (Chiou and Charng, 2010). Next, if exon-by-exon sequence failed to detect mutations, DNA samples were subsequently analyzed for large deletions or insertions using the multiplex ligation-dependent probe amplification (MLPA) assay with the SALSA P062-B *LDLR* MLPA kit obtained from MRC-Holland (Amsterdam, the Netherlands) according to manufacturer instructions (http://www.mrc-holland.com). The nomenclature and classification of the found mutations are based on the findings in the updated FH mutation databases (http://www.ucl.ac.uk/ldlr/Current/index.php?select_db=LDLR, accessed 11 October 2011). The effects of novel nucleotide variants were subjected to online computer program using Poly Phen-2 to predict possible impact of an amino acid substitution on the structure and function of the newly discovered mutation (http://genetics.bwh.harvard.edu/pph2/).

2.3. Genotyping LDLR and APOB polymorphisms

To gain more insight into FH Taiwanese, the haplotypes of the common mutations in the study cohort were compared with those of FH Caucasians and southeastern Asians. The particular polymorphisms were selected according to both previous FH studies having haplotype analysis (Castillo et al., 2002; Chang et al., 2003; Mak et al., 1998a; Miserez and Muller, 2000; Tai et al., 1998; Zuliani and Hobbs, 1990) and the haplotype website (www.hapmap.com). Probands and their family members carrying the most common mutations were genotyped for their haplotype pattern. *LDLR* was genotyped at 13 polymorphic markers within or flanking the *LDLR* gene: rs2228671 [C/T], rs2304183 [C/T], rs12983082 [A/C], rs12710260 [C/G], rs5929 [C/T], rs4508523 [C/T], rs688 [C/T], rs2738450 [A/C], rs2738452 [A/G], rs5925 [C/T], rs5927 [A/G], rs5742911 [A/G], and hypervariable region of TA dinucleotide repeat marker at the 3′ end. *APOB* was genotyped at 17 polymorphic sites within or flanking the *APOB* gene: rs1367117 [A/C/G/T], rs13306198 [C/T], rs1469513 [C/T], rs679899 [A/C/G/T], rs3828293 [A/G], rs3791981 [A/G], rs11676704 [G/T], rs12713956 [C/T], rs2854725 [A/C], rs693 [A/C/G/

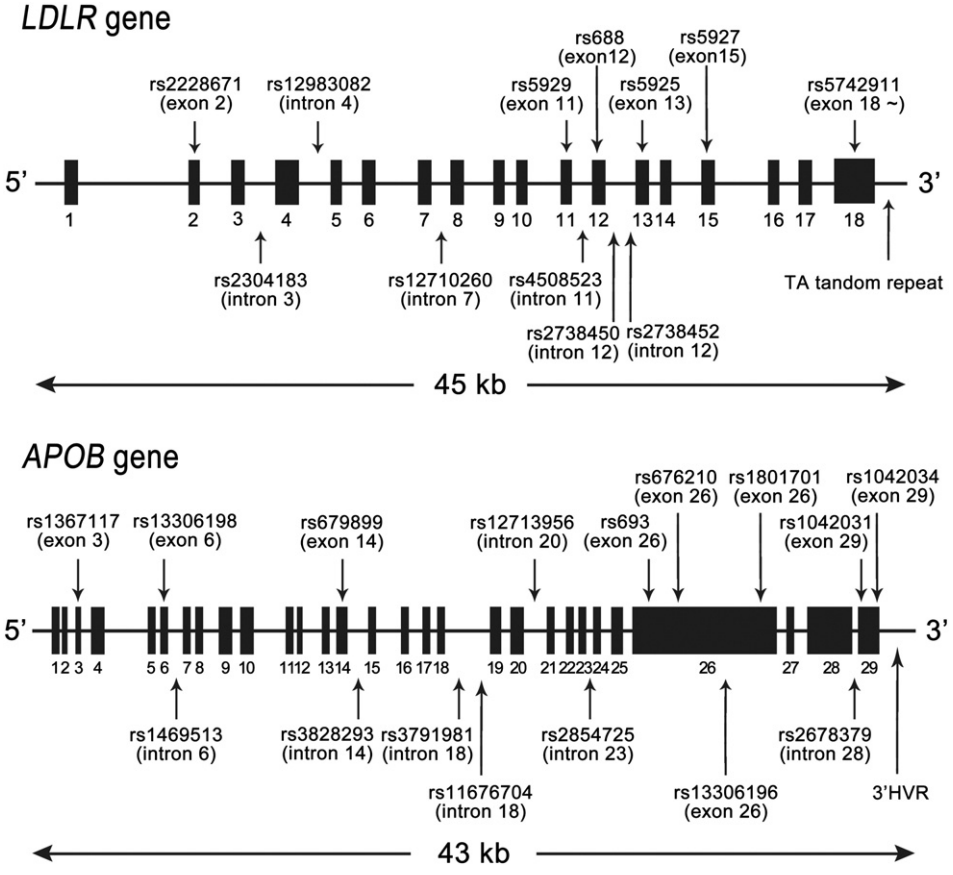

Fig. 1. Haplotype markers and positions for the *LDLR* gene (Panel A) and *APOB* gene (Panel B).