



## Familial Hypercholesterolemia in Asia: A Review

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### Abstract

This review provides an update on recent studies conducted in the Asia-Pacific Region in the area of genotyping for SNP/Mutation association with Familial hypercholesterolemia (FH), diagnosis and management of subjects with Familial hypercholesterolemia.

It has always been thought that there are limited studies conducted on this complex disease in this part of the world but surprisingly there has been a growing interest in this disease and its awareness. FH is associated with premature coronary heart disease. Early detection and treatment of FH may save many lives because up to 30% of patients do not survive an initial myocardial infarction (MI). Unfortunately though clinically, FH is being identified, there are only few genetic studies reported from Asian countries and even though the spectrum of mutations is different from the European spectrum, there is no suitable product for DNA testing. Not enough genetic testing is being carried out on a large scale which can actually estimate the growing number of true FH patients.

The research being done on FH by individual groups have been encouraging. However, population frequency of prevalent haplotypes is not known and should be estimated and the appropriate genetic tests should be adopted by government on a national level for screening and further management of the disease.

**Key words:** FH, SNP, coronary, risk, Asia, studies

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### INTRODUCTION

Familial hypercholesterolemia is a complex multifactorial disease which follows Mendelian inheritance and today we have good knowledge about its detection, symptoms and management but this knowledge is restricted to the scientific community (Hobbs et al., 1992, Goldstein and Brown, 2009). The FH clinical manifestation has always been shown to be associated with increased coronary heart disease and premature death. The common man on the street is blissfully unaware of this malady. FH is one of the most common inherited disorders, with an estimated worldwide prevalence of 1 in 500 (0.2%), though the frequency is considerably higher in some populations because of a founder effect (Austin et al., 2004).

This estimate is based on the frequency observed in the Caucasian populations and it corresponds to approximately 13 million people worldwide and ~600,000 in the United States

who have FH. The overwhelming majority of affected persons are heterozygotes (those who have inherited one disease-causing mutation). Compound heterozygotes has an incidence of 1 in 200,000, while persons with homozygous FH are extremely rare (~1 in 1 million). Cholesterol is the main moiety which is the root cause of obesity and plaque formation which in turn leads to atherosclerosis, diabetes, cardiovascular disease, stroke etc. There is variation in the onset and severity of atherosclerotic disease in persons with FH, since a lot of exogenous factors like environmental, metabolic, and genetic factors influence the clinical phenotype (Austin et al., 2004) (Jansen et al., 2004) (Jansen et al., 2002).

So, if the genetic causes can be established, life style management can delay the otherwise early onset of FH. Prof. Steve Humphries rightly says in his article, it is very essential

that the knowledge acquired over the years be disseminated to the public (Hadfield and Humphries, 2007). Dissemination may not always be through seminars, journals or conferences. It can be through simple ways like print campaign, media campaign and through social networking sites. One could initiate awareness through education to immediate family members and close friends. This talk will motivate people to get them screened for FH.

Most of the knowledge we have today is based on studies carried out in the west. Some of these studies have even been translated into an innovative product such as Lipochip® by Progenika-Biopharma from Spain ([www.progenika.com](http://www.progenika.com)) which has since been incorporated as a genetic diagnostic platform in Europe (Tejedor et al., 2005). This genetic analysis complements the clinical diagnosis made by physicians.

However if one looks through the Asian studies there are only few FH studies and there is no product for screening and diagnosis except one, FH1536™ from INFOVALLEY® ([www.infovalley.net.my](http://www.infovalley.net.my)).

Currently there are no true estimates of people diagnosed with FH in Asia. Patients coming in to hospitals are being screened for their cholesterol levels (HDL/LDL) and even if their lipid levels are at the extremes they are given treatment and nobody bothers to even suspect if underlying FH could be the cause or not. There has been an international initiative to increase awareness and early screening but the move has not been so encouraging except for a few countries. Moreover, most often, studies are carried out on known genes and known mutations. Sequencing studies of index cases would reveal more insights but such studies have been limited in number. Nevertheless replication studies have generated better understanding of allelic heterogeneity in Asia and the prevalent risk alleles.

#### Asians:

Asians are people of the continent of Asia. The picture conjured in the minds of many of the word “Asia” comprises different combinations of countries. To make our understanding better let’s follow the following classification:

North Asians: Yakuts, Uralic, Ainu people

South Asians: Indians, Pakistanis, Bangladeshi, Sinhalese, Nepalese, Bhutanese

East Asians: Chinese, Japanese, Taiwanese, Koreans, Mongols, Tibetans

West Asians: Arabs, Turks, Iranians, Israelites

Central Asians: Uzbeks, Karkapak

South-East Asians: Thais, Vietnamese, Philipinos, Cambodians, people of Brunei, Malaysians, people of Laos, Singaporeans, Indonesians

With globalization and high migration rates, now we find Asians all over the world and there is interbreeding resulting in a mixing of gene pool.

We searched for studies done on FH, Low Density Lipoprotein Receptor gene (LDLR), Apolipoprotein B-100 gene (APOB), Proprotein Convertase Subtilisin Kexin Type 9 (PCSK9) and coronary heart disease from PUBMED and other online libraries. The table (Table-1) shows a complete lack of participation in FH studies from North Asia, Central Asia and West Asia.

#### Major genes being studied in FH:

##### LDLR

LDLR encoding the LDL receptor was first discovered by Goldstein et al. (Goldstein and Brown, 1973). This disease was named FH for Familial Hypercholesterolemia (MIM# 606945) and its heterozygous prevalence was estimated at 1/500. Mutations of the LDLR are by far the most common molecular cause of FH. The LDLR is located on chromosome 19 and has 18 exons. Thus far, more than 1500 different mutations have been identified in the LDLR gene. LDLR mutations can be classified according to the effect they have on LDL receptor protein function (Hobbs et al., 1992). The LDLR protein is a cell surface receptor that removes LDL particles from the plasma. In class 1 mutations, the LDL receptor protein is not synthesized; in class 2 mutations, the LDL receptor is not transported to the Golgi; in class 3 mutations, the LDL receptor does not properly bind with the LDL particles; in class 4 mutations, bound surface receptors are not internalized; and in class 5 mutations, the internalized LDL particles are not released in the endosome. The majority of mutations identified to date are class 2 or class 3 mutations occurring in the ligand-binding and epithelial growth factor precursor regions of the gene (Heath et al., 2001). Class 1 mutations are also called “receptor-negative” mutations in the literature Heath, et al., 2001), whereas mutations from classes 2–5 are termed “receptor defective.”

##### APO-B

Sometimes no mutations are seen in LDL receptor in FH patients, but they have defective clearance of LDL due to mutations in APOB gene. In such patients, cholesterol concentrations in plasma can vary from those found in heterozygous FH to only modest hypercholesterolemia (Simons et al., 1975). The defect lies in the inability of the LDL to bind to LDLR receptor due to defective APOB, the protein moiety of LDL (Soutar et al., 1977). Apolipoprotein B-100 gene (APOB), is located on chromosome 2p23-24 (Arca et al., 2002) (Ceska et al., 2000) and codes for the protein component of LDL particles (Schumaker et al., 1994).

**Table 1** A list of FH and FH related studies from Asia for past decade

No	Study Title	Journal	Authors
<b>JAPAN</b>			
1	Update of Japanese common LDLR gene mutations and their phenotypes: Mild type mutation L547V might predominate in the Japanese population.	Atherosclerosis. 2009 Mar; 203(1): 153-60. Epub 2008 Jul 15.	Miyake Y et al
2	Efficacy of colestimide coadministered with atorvastatin in Japanese patients with heterozygous familial hypercholesterolemia (FH).	Circ J. 2005 May; 69(5): 515-20.	Kawashiri MA et al
3	Clinical features of familial hypercholesterolemia in Japan in a database from 1996-1998 by the research committee of the ministry of health, labour and welfare of Japan.	J Atheroscler Thromb. 2004; 11(3): 146-51.	Bujo H et al
4	Molecular genetic analysis of familial hypercholesterolemia: spectrum and regional difference of LDL receptor gene mutations in Japanese population.	Atherosclerosis. 2002 Dec; 165(2): 335-42.	Yu W et al
5	Japanese familial hypercholesterolemia with a 327insC mutation in the LDL receptor gene.	Ann Clin Biochem. 2002 Sep; 39(Pt 5): 526-30.	Hirota R et al
6	Eight novel mutations and functional impairments of the LDL receptor in familial hypercholesterolemia in the north of Japan.	J Hum Genet. 2002; 47(2): 80-7.	Hattori H et al
7	Effects of low-dose pravastatin on plasma levels of lipids and apolipoproteins in Japanese type II hyperlipoproteinemic subjects with apolipoprotein E phenotype E3/2, E3/3, and E4/3.	J Clin Pharmacol. 2001 Oct; 41(10): 1055-8.	Kobayashi T et al
8	Association between LDLR polymorphism and diseases in the Japanese population: aging and distribution of the polymorphism.	Forensic Sci Int. 2000 Sep 11; 113(1-3): 133-7.	Hara M et al
9	[Genotype-phenotype correlations in familial hypercholesterolemia]	Nihon Rinsho. 1999 Dec; 57(12): 2770-5.	Kajinami K et al
10	Identification of recurrent and novel mutations in the LDL receptor gene in Japanese familial hypercholesterolemia. Mutation in brief no. 248. Online.	Hum Mutat. 1999; 14(1): 87.	Hattori H et al
<b>KOREA</b>			
1	Novel and recurrent mutations of the LDL receptor gene in Korean patients with familial hypercholesterolemia.	Mol Cells. 2004 Aug 31; 18(1): 63-70.	Kim JH et al
2	Polymorphic DNA haplotypes at the human low-density lipoprotein receptor gene locus in Koreans.	Hum Biol. 2001 Feb; 73(1): 105-19.	Chae JJ et al
3	Identification of four novel mutations of the low-density lipoprotein receptor gene in Korean patients with familial hypercholesterolemia.	Clin Genet. 2000 Mar; 57(3): 225-9.	Shin JA et al
4	Three novel small deletion mutations of the LDL receptor gene in Korean patients with familial hypercholesterolemia.	Clin Genet. 1999 May; 55(5): 325-31.	Chae JJ et al
<b>CHINA</b>			
1	Identification and characterization of LDL receptor gene mutations in hyperlipidemic Chinese.	Journal of Lipid Research Volume 44, 2003	Jui-Hung Chang et al
2	Mutations in the LDL receptor gene in four Chinese homozygous familial hypercholesterolemia phenotype patients.	Nutr Metab Cardiovasc Dis. 2009 Jul; 19(6): 391-400. Epub 2008 Dec 13.	Wang L et al
3	Two mutations in LDLR gene were found in two Chinese families with familial hypercholesterolemia.	Mol Biol Rep. 2009 Nov; 36(8): 2053-7. Epub 2008 Nov 20.	Cheng X et al
4	Primary hypercholesterolemia, carotid atherosclerosis and insulin resistance among Chinese.	Lipids. 2008 Feb; 43(2): 117-24. Epub 2007 Dec 15.	Chien KL et al
5	Two novel mutations 685del 1 and D129G in the low-density lipoprotein receptor gene in a compound heterozygote Chinese family with familial hypercholesterolemia.	Metabolism. 2007 May; 56(5): 636-40.	Chen K et al
6	Apolipoprotein B is associated with metabolic syndrome in Chinese families with familial combined hyperlipidemia, familial hypertriglyceridemia and familial hypercholesterolemia.	Int J Cardiol. 2007 Mar 20; 116(2): 194-200. Epub 2006 Jul 10.	Pei WD et al
7	Apolipoprotein B is associated with metabolic syndrome in Chinese pedigrees with familial hyperlipidemia	Zhonghua Yi Xue Za Zhi. 2005 Feb 2; 85(5): 313-7.	Pei WD et al
8	Screening for low-density lipoprotein receptor gene mutations in familial hypercholesterolemia Chinese	Zhonghua Nei Ke Za Zhi. 2004 Sep; 43(9): 665-8.	Pang QF et al
9	Identification of a novel splice mutation of low density lipoprotein receptor gene in a Chinese family with familial hypercholesterolemia.	Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2004 Feb; 21(1): 14-8.	Lin J et al
10	Analysis of low-density lipoprotein receptor gene mutations in a Chinese patient with clinically homozygous familial hypercholesterolemia.	Chin Med J (Engl). 2003 Oct; 116(10): 1535-8	Cao S et al
11	Analysis of low density lipoprotein receptor function and gene mutation in familial hypercholesterolemic patients.	Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2003 Apr; 20(2): 138-42.	Guan X et al
12	A gene analysis of the low density lipoprotein receptor in Chinese with homozygous familial hypercholesterolemia.	Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2001 Aug; 18(4): 279-82.	Wang D et al
13	A Chinese homozygote of familial hypercholesterolemia: identification of a novel C263R mutation in the LDL receptor gene.	J Hum Genet. 2001; 46(3): 152-4.	Wang D et al

**Table 1(Continue)** A list of FH and FH related studies from Asia for past decade

<b>THAILAND</b>			
1	Hypercholesterolemia in Thai primary school children: relation to maternal and nutritional factors.	Pediatr Int. 2008 Aug; 50(4): 557-62.	Yamborisut U et al
2	Detection of a known mutation M412T in the LDL receptor in a Chinese Thai FH family.	Clin Chim Acta. 2006 Mar; 365(1-2): 211-6. Epub 2006 Jan 9.	Pongrapeeporn KU et al
3	Screening for mutations in exons encoding the ligand-binding domain of the LDL receptor gene using PCR-RFLP and PCR-SSCP.	J Med Assoc Thai. 2001 Dec; 84 Suppl 3: S619-27	Pongrapeeporn KU et al
4	Mutation analysis of exon 9 of the LDL receptor gene in Thai subjects with primary hypercholesterolemia.	J Med Assoc Thai. 2000 Nov; 83 Suppl 2: S81-8.	Yamwong P et al
5	Screening for mutations in exon 4 of the LDL receptor gene in Thai subjects with primary hypercholesterolemia: detection of a novel mutation D151Y by PCR-CFLP.	J Med Assoc Thai. 2000 Nov; 83 Suppl 2: S66-73.	Pongrapeeporn KU et al
<b>MALAYSIA</b>			
1	Ala519Thr Mutation in Exon 11 of LDL Receptor Gene in Members of a Malaysian Family with Hypercholesterolemia.	AsPac J. Mol. Biol. Biotechnol., Vol. 11 (2), 2003	Sarni Mat Junit et al
2	Genetic causes of familial hypercholesterolemia in a Malaysian population.	Med J Malaysia. 2000 Dec;55(4): 409-18	Khoo KL et al
<b>PHILIPPINES</b>			
1	Low density lipoprotein--receptor (LDL-R) gene mutations among Filipinos with familial hypercholesterolemia.	J Atheroscler Thromb. 2005; 12(5): 276-83	Punzalan FE et al
<b>INDIA</b>			
1	The challenge produced by familial homozygous hypercholesterolemia when treating premature coronary arterial disease in the young.	Cardiol Young. 2009 Jun; 19(3): 257-63. Epub 2009 Apr 6.	Shankarappa RK et al
2	Genetic analysis of Indian subjects with clinical features of possible type IIa hypercholesterolemia.	Clin Lab Anal. 2007; 21(6):375-81.	Kondkar AA et al
3	Gene polymorphism and coronary risk factors in Indian population.	Clin Chem Lab Med. 2002 Oct; 40(10): 975-85	Ashavaid TF et al
<b>SINGAPORE</b>			
1	Compound Heterozygous Familial Hypercholesterolemia and Familial Defective Apolipoprotein B-100 Produce Exaggerated Hypercholesterolemia.	Clinical Chemistry. 2001; 47: 438-443	E. Shyong Tai et al
<b>TAIWAN</b>			
1	Relation of C-reactive protein and carotid intima media thickness in Taiwanese with familial hypercholesterolemia.	Am J Cardiol. 2008 Jul 15; 102(2): 184-7. Epub 2008 May 28.	Ye ZX et al
2	LDLR and ApoB are major genetic causes of autosomal dominant hypercholesterolemia in a Taiwanese population.	Formos Med Assoc. 2007 Oct; 106(10): 799-807.	Yang KC et al
3	Identification and characterization of novel low-density lipoprotein receptor mutations of familial hypercholesterolemia patients in Taiwan.	Eur J Clin Invest. 2006 Dec; 36(12): 866-74.	Chang MJ et al
<b>PAKISTAN</b>			
1	Estimation of heritability of familial hypercholesterolemia among 335 family members of five hypercholesterolemic probands of Pakistani population.	J Ayub Med Coll Abbottabad. 2009 Jan-Mar; 21 (1): 58-61.	Imtiaz F et al

In contrast to LDLR, only a small number of functional mutations have been identified in *APOB*. The clinical phenotype associated with *APOB* mutations is termed “Familial Defective Apolipoprotein B-100” or “FDB”. Mutations are found only in the LDL binding domain of *APOB*, which consists of exon 26 and 27 of *APOB* gene.

**PCSK-9**

Another gene causing autosomal dominant hypercholesterolemia was identified to be Proprotein Convertase Subtilisin / Kexin type IX (*PCSK9*) (Abifadel et al., 2009). It was recently identified on chromosome 1p32 (Abifadel et al., 2003). *PCSK9* is a serine protease that regulates cholesterol metabolism through low-density

lipoprotein receptor (*LDLR*) degradation. To date, no epidemiologic research has investigated mutations in *PCSK9*. This gene encodes for a protein of 694 amino acids belonging to a family of Proprotein Convertase Subtilase. *PCSK9* is secreted by hepatocytes and appears to down regulate the density of functional *LDL* receptors in hepatocytes by promoting endosomal degradation rather than recycling of the receptor on the surface. Hypercholesterolemia is caused by gain-of-function mutations in the *PCSK9* gene (Cameron et al., 2006). Since its discovery, various mis-sense mutations in *PCSK9* have been shown to co-segregate with severe hypercholesterolemia in many families in several countries. It was observed that Apolipoprotein E (*APOE*) genotypes exerted their respective effects on *LDL* cholesterol in an

additive manner to that of the PCSK9 variants (Davignon et al., 2010).

### APOE

It is the pivotal protein in the modulation of metabolism of highly atherogenic APOB containing lipoproteins. Coronary heart disease has been often associated with apolipoprotein E (APOE) (Eichner et al., 2002). Three common alleles,  $\epsilon_4$ ,  $\epsilon_3$  and  $\epsilon_2$  code for three major isoforms i.e. APOE4, E3 and E2 respectively. Six different APOE phenotypes: E3/3, E4/4, E2/2, E4/3, E3/2 and E4/2 occur in the general population with varying frequencies. It has been estimated that APOE polymorphisms may account for 2-16% of the variability of LDL cholesterol levels. The E4 allele is associated with higher and the E2 allele is associated with lower levels of plasma apolipoprotein B-containing proteins such as LDL (Davignon et al., 1988).

### Global Heterogeneity in allele frequency

From association studies it is evident that there is heterogeneity within populations and it is important to compare the allelic differences to optimize the treatment strategies (Hegele, 2002). For instance the frequency of the mutation C646Y is 18 percent in northeastern Quebec (Simard et al., 1994) but only 5 percent in Montreal (Leitersdorf et al., 1990). This reflects a difference within the founder population itself. The common APOB mutation, R3500Q, is observed at high frequency in Poland, Switzerland, and the Czech Republic, at lower frequency in other European populations, and virtually absent from Asian and South African populations (Hegele, 2002). This mutation harbours a rare haplotype defined by eight variable sites in the APOB gene and its flanking region (Ludwig and McCarthy, 1990). On the basis of this distribution and haplotype analysis, the original R3500Q mutation is postulated to have occurred approximately 6,750 years ago (Myant et al., 1997).

Exogenous factors like climate, diet lifestyle etc. play an important role in allelic differences. Migrant studies of Asian populations to Western countries generally show increases in CHD, type 2 Diabetes Mellitus (DM) and atherosclerosis. Migrant studies of Japanese in the United States, e.g., the Honolulu Heart Program, show that Japanese Americans have higher CHD rates than Japanese people living in Japan (Yano et al., 1984), whereas stroke rates in Japanese Americans are lower than in the Japanese in Japan and in whites (Kagan et al., 1980). Likewise, migrant studies of Asian Indians show an increase in CHD among those living in Western countries. Migrant Asian populations, including Chinese, Indians and Japanese have a higher prevalence of type 2 DM not only when compared with their counterparts in their home countries but also compared with whites living in countries where Asians have migrated.

Environmental factors play an important role in determining the phenotypic expression of heterozygous FH geographically (Hegele, 2002). For example, total and LDL cholesterol levels in FH heterozygotes of similar genetic background vary in different parts of the world (Kotze et al., 1997) (Lehrman et al., 1987) (Slimane et al., 2001) even after controlling for differences in the underlying mutation. Chinese FH subjects in Canada were matched to FH heterozygotes in China with similar LDLR mutations (Pimstone et al., 1998). Subjects residing in Canada had higher concentrations of LDL cholesterol and an increased prevalence of tendinous xanthomas and coronary heart disease.

### Common/Novel mutations found in Asia:

Most of the studies thus far have been conducted on LDLR, APO-B and PCSK-9. A study on Indians in South Africa found mutations in exons 3, 4, 9 and 14 of LDLR gene. The mutations majorly occurred in the CpG rich regions of the gene making it a mutational hotspot in South African Indians (Kotze et al., 1997). Unrelated families had different mutations. Ashavaid et al. in a study in Mumbai identified four previously known mutations and two novel insertion mutations in LDLR gene in Indian subjects (Henney et al., 1991). In a study conducted in 40 subjects with clinical features of FH in India, none of the patients or controls showed the R3500W mutation in exon 26 of APOB. This suggests that common mutations in APOB are not associated with hypercholesterolemia among Indians (Kroon et al., 1995). R3500W has been reported only from Taiwan and Singapore. Malaysia has a report of a novel mutation, R3500Q (Table-2).

R3500W mutation in Apo B-100 heterozygotes were identified in hyperlipidemic Chinese subjects. P664L, a missense mutation in exon 14 of LDLR has been found in Japanese, Korean and Taiwanese patients. Japan, Korea and China have done immense research to investigate the mutant alleles prevalent in their population. There are several mutations which are unique to a population and are worth investigating e.g. 1845+2 T >C of Japan and 1846-1G >A of Korea.

Frequency of APOE  $\epsilon_3$  allele was found to be high (0.913) in people of north India (Beaumont et al., 1976). In Asian Indians, these allele frequencies observed were 0.031-0.094 for  $\epsilon_2$ ; 0.803-0.968 for  $\epsilon_3$  and 0.000-0.133 for  $\epsilon_4$  (Gagne et al., 1979). In another study on Asian Indians, individuals with at least one  $\epsilon_4$  allele were considered to be at risk to develop premature myocardial infarction, independent of other conventional risk factors (Heiberg and Berg, 1976). However, to date 10 different LDLR mutations in immigrants from India have been reported in the literature. Most of these mutations have been reported in exons 3, 4, 9, and 14 among Indians settled in South Africa, which suggests an increased

frequency of FH in India (Kotze et al., 1997). More than 80% of the 150,000 Indians who immigrated to South Africa between 1860 and 1911 originated from diverse areas in India

(Ashavaid et al., 2000). This group has remained isolated as a whole and also within different communities, primarily as a result of religious and cultural practices.

**Table 2** A list of mutations reported from Asia

Mutation	Gene	Country
Asp. 407 lys	Exon-9 (LDLR)	Singapore
Arg 3500 Trp	Apo B 100	Singapore
Ala 519 Thr	Exon-11 LDLR	Malaysia
Arg3500Gln	APOB	Malaysia
Arg3531Cys	APOB	Malaysia
Asp100Asp	LDLR	Malaysia
Asp139His	LDLR	Malaysia
Arg471Gly	LDLR	Malaysia
C.1705+117 T>G	LDLR	Malaysia
C.1186+41T>A	LDLR	Malaysia
1705+112C>G	LDLR	Malaysia
Trp666ProfsX45	LDLR	Malaysia
rs688	LDLR exon-12	Pakistan
rs5925	LDLR exon-12	Pakistan
M412T	LDLR	Thailand
D151Y	LDLR	Thailand
M391T	LDLR	Thailand
C317S	LDLR	Japan
F382L	LDLR	Japan
W512X	LDLR	Japan
P664L	LDLR	Japan
C337R	Exon-8 LDLR	Japan
W556C	Exon-12 LDLR	Japan
A410T	LDLR	Japan
L547V	LDLR	Japan
E693K	LDLR	Japan
K790X	LDLR	Japan
Tyr358Cys	LDLR	Japan
Ser587Pro	LDLR	Japan
C83Y	LDLR	Korea
C675X	LDLR	Korea
941 -1G>A	LDLR	Korea
-136C>T	LDLR	Korea
F382L	LDLR	Korea
R574Q	LDLR	Korea
1846-1G>A	LDLR	Korea
P664L	LDLR	Korea
E119K	LDLR	Korea
E207X	LDLR	Korea
E207K	LDLR	Korea
Glu161X	LDLR	Korea
Cys210Tyr	LDLR	Korea
Pro584Leu	LDLR	Korea
C112Y	LDLR	China
T383I	LDLR	China
D129G	LDLR	China
C210R W462X	LDLR	China
C122Y and T383I	LDLR	China
p.W483X	LDLR	China
p.A627T	LDLR	China
C263R	LDLR	China
C107Y	LDLR	Taiwan
D69N	LDLR	Taiwan
R385W	LDLR	Taiwan
W462X	LDLR	Taiwan
G170X	LDLR	Taiwan
V408M	LDLR	Taiwan
T3540M	APOB	Taiwan
R3500W	APOB	Taiwan
M510K	LDLR	Taiwan
W512R	LDLR	Taiwan
I420T	LDLR	Taiwan
C660W	LDLR	Taiwan
H562Y	LDLR	Taiwan
A606T	LDLR	Taiwan
P664L	LDLR	Taiwan

Eight gene sequence variants were reported for the first time from Malaysia and they were noticed in familial hypercholesterolemic patients (p.Asp100Asp, p.Asp139His, p.Arg471Gly, c.1705+117 T>G, c.1186+41T>A, 1705+112C>G, Dup exon 12 and p.Trp666ProfsX45). The incidence of the p.Arg471Gly variant was 11% (Al-Khateeb et al., 2011).

Two novel LDLR mutations, D151Y and M391T, located in the fourth cysteine repeat of the ligand-binding domain and in the sixth YWTD repeat of the epidermal growth factor precursor homology domain, respectively were identified in unrelated Thai patients with heterozygous FH (Jeenduang et al., 2010).

Gain-of-function and loss-of-function mutations within PCSK9 gene lead to hypercholesterolemia or hypocholesterolemia respectively. Studies in the U.S. and Canada reported a correlation between multiple metabolic factors and circulating PCSK9 concentrations. However, there is no data available on circulating PCSK9 levels in Asians. A sandwich ELISA assay was applied to measure serum PCSK9 levels in a Chinese population of 2719 adults from Nanjing district, China, which represents a large and uniform ethnic population of Han Chinese. Serum PCSK9 levels ranged from 12.85 to 222.50ng/ml with a mean concentration of 69.35ng/ml in this population. Serum PCSK9 levels were slightly higher in women than in men. Compared to premenopausal women, postmenopausal women had significantly higher PCSK9 levels. Serum PCSK9 levels were correlated with multiple metabolic variables including age, BMI, total cholesterol, LDL cholesterol, triglycerides, fasting blood glucose, systolic blood pressure (SP) and diastolic blood pressure (DP) in this population. After stepwise regression analysis, there was a significant positive association between serum PCSK9 levels and total cholesterol, triglycerides and SP in men. In women, there was a positive correlation between PCSK9 levels and total cholesterol, age and DP. Serum PCSK9 level may be a biomarker of metabolic status and cardiovascular disease (Cui et al., 2010).

An E670G polymorphism of the exon 12 of the Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) gene was recently found to be associated with increased plasma low-density lipoprotein cholesterol (LDL-C) levels and severity of coronary atherosclerosis. However in a case-control study in Taiwan, involving 202 CAD patients and 614 unrelated controls, it was seen that this polymorphism modulates plasma LDL-C levels, but is not a risk variant for CAD (Hsu et al., 2009).

Studies from Japan have shown that common variants intron 1/ C(-161)T and exon 9/ I474V in PCSK9 significantly affect TC and LDL-C levels in the general population in Japan (Shioji et al., 2004)

#### **Risks associated with FH:**

People with FH mutations have a high risk of coronary heart disease which has been well established in people of European descent.

The explicit prevalence of CVD in Asians is mainly due to increased incidence of fatty and spicy diet, and an unhealthy lifestyle. Investigations of the genetic causes for this increased trend have been largely unexplored and the contribution of Familial hypercholesterolemia (FH) is unknown. High cholesterol level, the major modifiable risk factor for heart disease has both an environmental as well as a genetic component. Premature CAD in the Indian population might be due to an unhealthy lifestyle alone or due to genetic factors in combination with an unhealthy lifestyle. The genetic component has been largely ignored in India although it has the highest number of deaths due to heart disease.

In addition, to the best of our knowledge there are no published reports on systematic analyses of mutations underlying FH in India, China and other Asian countries as being done in UK and Spain.

#### **Treatment**

Cascade screening is recommended by The National Institute for Health and Clinical Excellence in the United Kingdom followed by DNA testing if available and affordable. Cascade screening is a mechanism for identifying people at risk for a genetic condition by a process of systematic family tracing. Guidelines have been developed in UK for diagnosis and treatment of patients of FH and cascade testing among family members.

A person diagnosed with FH molecularly and clinically is called an index case. If an index case has been discovered it is very easy to screen the first, second and third degree relatives for FH. FH is very often associated with high levels of cholesterol in the serum and this can lead to a greatly elevated risk for coronary heart disease (CHD) and death (Austin et al., 2004). In fact, for those with heterozygous FH, the cumulative risk for CHD is greater than 50% in men by the age of 50 and at least 30% in women by the age of 60 (Neil et al., 2008). Persons with homozygous FH manifest an even more severe form of the disorder.

Identifying and contacting biological relatives of a person diagnosed with FH (the index case) and then systematically testing these relatives (first-, second-, third-, etc. degree) using a combination of serum LDL cholesterol concentration measurements and a variety of mutation detection or screening assays for mutations in the LDLR, APOB, or PCSK9 genes is the best screening method.

The majorities of FH/FDB subjects are being diagnosed late in life and are not being adequately treated. In order to prevent them from contracting premature coronary heart disease, it is important that levels of LDL cholesterol are

normalized from a young age and that sufficient doses of lipid-lowering drugs are used (Leren and Berge, 2011).

DNA platforms are being increasingly used currently in screening but it is important to note that with discovery of new mutations across populations, population specific tools also need to be made to detect all the mutations. An older version of a chip may miss out the novel mutations. For instance there is a significant allelic heterogeneity between Spanish and French populations. So the older version of Lipochip could only detect 40% of carriers. The Lipochip used to screen clinically diagnosed FH patients in Spain was able to detect mutations in 78% of all carriers (Alonso et al., 2009). If the Lipochip (version 8) had been used to screen this French cohort, it only would have detected 40% of the mutation carriers, thus indicating the need for specific national screening strategies (Marduel et al., 2010).

It is unfortunate that though cascade screening of relatives of FH index cases is fairly easy, people are ignorant

and there is no active participation by governments.

This is true especially for Asian countries. Though there are a large number of FH research studies going on in the academia, it is a pity that there are no studies or products or tests applied directly in the field.

**Summary**

There has been an international drive to implement screening in all countries for FH but very few countries have heeded to the call. The need of the hour is to engage in systematic sequencing studies of index patients to discover novel mutations and also simultaneously to implement cascade screening of relatives of index cases so that the people affected are traced early and treated properly. A survey of 2010 and 2011 papers from PUBMED shows the lack of active involvement in FH research from many of the Asian countries (Table-3).

**Table 3** A list of FH/FH related studies of 2010/2011 from Asia

No	Country	Study Title	Journal	Authors
1	Japan	Molecular genetic epidemiology of homozygous familial hypercholesterolemia in the Hokuriku district of Japan.	Atherosclerosis. 2011 Feb; 214(2): 404-7. Epub 2010 Nov 13.	Mabuchi H et al
2	Japan	A novel Thr56Met mutation of the autosomal recessive hypercholesterolemia gene associated with hypercholesterolemia.	J Atheroscler Thromb. 2010 Feb 26; 17(2): 131-40. Epub 2010 Feb 3.	Harada K et al
3	Thailand	Two novel D151Y and M391T LDLR mutations causing LDLR transport defects in Thai patients with familial hypercholesterolemia.	Clin Chim Acta. 2010 Nov 11; 411(21-22): 1656-61. Epub 2010 Jun 26.	Jeendum N et al
4	Malaysia	Analysis of sequence variations in low-density lipoprotein receptor gene among Malaysian patients with familial hypercholesterolemia.	BMC Med Genet. 2011 Mar 19; 12:40.	Al-Khateeb A et al
5	Taiwan	Detection of mutations and large rearrangements of the low-density lipoprotein receptor gene in Taiwanese patients with familial hypercholesterolemia.	Am J Cardiol. 2010 Jun 15; 105(12): 1752-8. Epub 2010 May 4.	Chiou KR et al
6	Pakistan	The genetic characterization of familial hypercholesterolemia in Pakistan	Journal of Basic and Applied Sciences Vol. 7, No. 1, 21-25, 2011	Sobia Rafiq et al
7	Pakistan	Identification of a recurrent insertion mutation in the LDLR gene in a Pakistani family with autosomal dominant hypercholesterolemia	Mol Biol Rep. 2010 Dec; 37(8): 3869-75. Epub 2010 Mar 10.	Muhammad Ajmal et al
8	Iran	Molecular Characterization of Iranian Patients with Possible Familial Hypercholesterolemia	Ind Journal Clin Biochemistry, 2011 10.1007/s12291-011-0113-7	E. Farrokhi, et al
9	China	A novel mutation in proprotein convertase subtilisin/kexin type 9 gene leads to familial hypercholesterolemia in a Chinese family	Chin Med j. 2010 123(9):1133-8	Lin.J et al
10	Korea	Genetic loci associated with lipid concentrations and cardiovascular risk factors in the Korean population	J. Med Genet. 2011 Jan; 48(1):10-5. Epub 2010 Oct 23.	Park MH, et al
11	Taiwan	Detection of mutations and large rearrangements of the low-density lipoprotein receptor gene in Taiwanese patients with familial hypercholesterolemia	Am J Cardiol. 2010, Jun 15; 105(12):1752-8. Epub 2010 May 4.	Chiou.KR et al

Haplotyping and population frequency of haplotypes needs to be estimated in Asia. The sensitivity and specificity of each DNA test for FH will depend on the particular mutations being assayed and on the population(s) tested. Initially mutations that are most prevalent in a particular

population should be tested followed by more uncommon ones. Direct sequencing of index cases and relatives is the best approach but cost still remains a challenge for Asian countries.

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