Identification of Four Novel LDL Receptor Gene Mutations in the North-West Moroccan Population

F. Benyahya[1,2]
M. Bennani[2]
A. Barakat[2]
S. Blesa[3,4]
V.Gonzalez-Albert[3,4]
R. Carmena [4,5]
F.J. Chaves[3,4]
A.B. Garcia-Garcia[3,4]

[1]*Institute Pasteur of Morocco in Tangier. Tangier. Morocco.*

[2]*Laboratory of biochemical and molecular genetics, Faculty of Sciences and Technics à Tangier. Tangier. Morocco.*

[3]*Genotyping and Genetic Diagnosis Unit. Research Foundation of the University Clinical Hospital of Valencia. Valencia. Spain.*

[4]*CIBER of Diabetes and Metabolic Diseases (CIBERDEM). Spain*

[5]*Endocrinology Service. University Clinical Hospital of Valencia. Valencia. Spain.*

KEY WORDS: Familial hypercholesterolemia; Low density lipoprotein receptor; Coronary heart disease.

ABSTRACT

Familial Hypercholesterolemia (FH) and Familial Defective ApoB100 (FDB) are two clinically indistinguishable diseases with increased levels of LDL-cholesterol. Mutations in two different genes are responsible: LDLR for FH and APOB for FDB. We have a group of 46 individuals diagnosed clinically as ADH from the North-west Morocco, where genetic causes of this disease has not been well studied. We have analyzed LDLR and APOB genes by direct sequencing in order to find mutations causing this disease.

We have identified four LDLR mutations: p.C146X, p.R57H, p.V806F, and p.W789L in five probands (11% of the sample).

In conclusion, we have found a low prevalence of mutations in the two genes analyzed. Therefore, the main genetic causes of ADH in the population from the North-West of Morocco are probably due to genes different from LDLR or APOB.

INTRODUCTION

Autosomal Dominant Hypercholesterolemias (ADHs) (OMIM 143890) are a group of hereditary hypercholesterolemias with similar phenotype affecting about 1of 500 Caucasians. The clinical phenotype is marked by elevated cholesterol levels, tendom xanthomas, premature atherosclerosis, and a family history of premature coronary disease. The phenotype is more severe in homozygotes than heterozygotes. [1]

The clinical phenotype is similar for all the different ADHs, and they are distinguished by the genes that cause the disease,

which historically have received different names. Familial hypercholesterolemia (FH) results from mutations in the low density lipoprotein receptor gene (LDLR), located on chromosome 19p13.1-p13.3. [1] Familial ligand-defective ApoB (FDB, OMIN 144010) is caused by mutations in a determined zone of APOB gene (APOB), located on chromosome 2p23-24 [2,3]. Recently, a third gene, proprotein convertase subtilisin/kexin type 9 (PCSK9), causing autosomal dominant hypercholesterolemia Type 3 (OMIN 603776) has been described. This gene is located on chromosome 1p34.1-p32 and encodes NARC-1, a proprotein convertase. [4] More than 1,000 mutations in the LDLR gene have been reported worldwide [5,6] (http://www.ucl.ac.uk/fh and http://www.umd.necker.fr), up to sixmutations in APOB [2,3] and a small number of different mutations have been identified in PCSK9, [7] accounting for less than 1% of ADH patients. Prevalence of mutations in FH subjects from different populations and ethnic groups has been reviewed in the Human Genome Epidemiology Network. [8] This review showed that with the exception of selected genetically isolated populations, where a small number of mutations predominates due to founder effect, most populations have a large spectrum of different LDLR mutations, and each mutation is found only in a small number of individuals. Furthermore, studies often fail to detect the underlying mutation in at least 15–40 % of the subjects screened for LDLR and APOB, indicating additional genetic and/or environmental causes for the FH phenotype.

Few studies have been carried out in Morocco and a small number of mutations for ADH were identified. [9-11] The Moroccan population is composed by different ethnic groups, not uniformly distributed. The population from the North-West of Morocco could be different from other regions of the country, and therefore, previous studies may not be sufficient to describe the complete spectrum of LDLR gene mutations in this country. No data about FH mutations in the population from North-West of Morocco are available so far. Therefore, the purpose of this study was to determine the molecular basis of this disease in this population.

MATERIALS AND METHODS

Patients

Forty-six unrelated subjects from North-West of Morocco were studied. Diagnosis criteria for ADH probands followed the MEDPED guidelines: high plasma levels of total and LDL-cholesterol (total cholesterol (TC) > 250mg/dl and LDL-cholesterol (LDLc) > 190mg/dl), family history of hypercholesterolemia, presence of xanthomas (tendom, planar, and/or tuberous), and personal and/or family history of premature cardiovascular heart disease (CHD). Secondary causes of hypercholesterolemia, including diabetes, hypothyroidism, nephrotic syndrome, etc. were excluded.

Complete medical history and detailed family history data were obtained, when possible. A control group included 89 unrelated normocholesterolemic Moroccan individuals with no history of ADH, or premature CHD. All participants gave their written consent, and the study was approved by the ethical committee of the institution.

Biochemical analysis

Fasting plasma lipid concentrations were measured while subjects consumed their regular diet and before administering any hypolipidemic drug. TC, triglycerides, and HDL-cholesterol (HDLc) levels were measured enzymatically with commercial kits (Dade Behring, Dimension Clinical Chemistry System) using an automated analyzer (Dimension Xpand, Dade Behring). LDLc cholesterol was calculated by Friedewald's formula. ApoB levels were analysed in serum, separated, and kept at -70°C, enzymatically with a commercial kit (Dade Behring, Dimension Clinical Chemistry System) using the same automated analyzer.

Genetic analysis

Genomic DNA was isolated from frozen whole blood EDTA samples using the salting-out method. (12) The promoter and the 18 exons of the LDLR gene (including about

Table 1: *Biochemical data from patients and controls*

	PATIENTS N=46	CONTROLS N=89
Total Cholesterol	3.00 + 0.39	1.67 + 0.25*
Triglycerides	1.44 + 0.61	1.00 + 0.34*
LDLc	2.16 + 0.37	1.05 + 0.28*
HDLc	0.46 + 0.13	0.44 + 0.12
APOB	1.52 + 0.59	1.20 + 0.26*
BMI	28 + 4	25 + 3*

**p value <0.05*
All units are g/l, except for BMI (kg/m2)

50 bp of the intronic boundaries) and APOB gene regions (exons 26 and 29) were mutations causing FDB have been described were analyzed. When a mutation was detected, the containing fragment was reanalyzed using a new aliquot of genomic DNA from the patient. Found mutations were tested in the control population. Mutations were named according to Yamamoto et al., 1984. [13]

RESULTS

Clinical analysis

Biochemical and clinical data for patients and controls are shown in Table 1. Differences in TC and LDLc were significant between both groups, as expected.

Genetic analysis and phenotype relationship

After sequencing all the 18 exons, including the intron–exon boundaries and the promoter, four LDLR mutations were detected in heterozygosis: one nonsense mutation (p.C146X) in exon 4 and three missense mutations, one in exon 3 (p.R57H) and two in exon 17 (p.V806F and p.W789L). Mutations p.R57H, p.V806F and p.W789L had not been previously described. Although p.C146X had been previously described, it was detected for first time in our population. Patients carrying these four mutations did not present any other responsible variation. In addition, they have not been found in 89 normocholesterolemic subjects.

The p.C146X mutation causes a stop codon at cysteine 146 of exon 4, giving rise to a truncated LDL receptor. This mutation was found in heterozygosis in one proband, a woman who presented 382 mg/dl of LDLc even after 3 months of treatment. Her sister died from myocardial infarction at the age of 25. DNA sample was not available for this relative, but according to lipid values (490 mg/dl) and clinical data, she was considered as having the homozygous clinical form of FH. Proband's mother died at the age of 42 years from myocardial infarction. All three members of the family presented extensive tendon xanthomas.

The p.R57H is a G to A transition at nucleotide 233 (c.233G > A). It changes codon CGT 57 for arginine to codon CAT for histidine. The mutation was found in heterozygosis in one proband.

Mutation p.W789L consists in a G to T transition at nucleotide 2429 (c.2429G > T). It changes codon TGG 789 for tryptophan to TTG for leucine (p.W789L). The proband was a man, aged, 62, who presented 250mg/dl of LDLc and had coronary artery disease.

Mutation p.V806 F is caused by a G to T transition at nucleotide 2479 (c.2479G>T) (figure 4). A base substitution (GTC to TTC) caused an amino acid change from valine to phenylalanine. Two independent probands (C and D) carried this mutation, both in heterozygosis. Proband D was a man aged 57 who presented 265 mg/dL of LDLc and xanthomas. Proband C was a woman aged 46 who has not been treated and also presented xanthomas.

The p.A370T polymorphism was also detected in our population, as in other studies. The five silent mutations p.C6C, p.R450R, p.R723R, p.N570N and p.V632V described previously in Moroccan population (9,10) were also detected in our sample. In addition, one novel variation p.P518P was detected in the first time in Moroccan population by our group, although it had previously been reported in other populations and is considered not responsible for the disease.

DISCUSSION

More than 1,000 mutations have been described in the LDLR gene in different populations. [5,6] This gene is composed by 18 exons that encode a protein involving five domains: the ligand binding domain, the epidermal growth factor (EGF) precursor homology domain, the domain with O-linked carbohydrates, the membrane spanning domain, and the cytoplasmatic part of the receptor. The ligand binding domain consists of seven repeats, each containing six cysteine residues, which form disulphide bonds within each repeat. [1]

Up to now, few studies about hypercholesterolemia in Moroccan population have been carried out. Due to possible differences between ethnic groups and the different regions of Morocco, it is interesting to analyze mutations present in the North-West of Morocco, whose principal city is Tangier. We have selected a group of 46 subjects from this region who had been diagnosed as ADH by standard criteria, 39% of them clinically diagnosed of "certain" ADH. The results of our genetic study have shown that only five patients carried four mutations in the LDLR gene. Moreover, as far as we know, three out of these four mutations are described here for first time, and neither of them were detected in the control population. Unfortunately, no relatives were available to perform cosegregation analysis. The remaining subjects did not presented any mutation in the analyzed genes. The five patients with LDLR gene mutations had been clinically classified as "certain" ADH. Therefore, 28% of "certain" ADH patients carry a mutation in LDLR gene.

The C>T substitution at codon 146 (p.C146X) described in this paper represents a novel mutation in the LDLR gene in Moroccan population, although it has been described in other samples. This exon encodes part of the ligand-binding domain of the LDL receptor protein. (1) This mutation yields a stop codon, and therefore the protein synthesis is supposed to be stopped at this point.

Mutations p.W789L and p.V806F are located in exon 17 and, as far as we know, they are described here for first time. Both mutations are within the cytoplasmic domain of the LDLR, which is important for the localization of the receptor in coated pits on the cell surface[1]. It is therefore proposed that mutations p.W789L and p.V806F may prevent the localization of the LDLR in pits, failing internalization or recycling to the cell membrane. Furthermore, mutation p.V806F can be compared with p.V806I (known as variant FH New York -5), [14] involving the same codon 806.

Mutation p.R57H has been described for first time in this work. The altered base of this mutation (position 233 of cDNA) is conserved in 11 mammals, including 3 Hominidae (orangutan, chimpanzee and Human), suggesting p.R57H has occurred on an evolutionary conserved residue. Other change in this position, p.R57C, has been described in Indian population of South Africa, [15] and is likely to cause a binding- or transport defective allele.

Our study confirms the LDLR mutation variability suggested by other group in Moroccan population. [9,10] The first study described six probands carrying different LDLR mutations out of a sample of 8 unrelated subjects. The second study (including the two individuals without mutation from the previous study) identifies five (out of six) subjects carrying some LDLR mutation. However, the prevalence of LDLR gene mutations is very different. Approximately 90% compared to our 11% which is probably due to the small number of individuals studied

by the other group. An important limitation of these two studies [9,10] acknowledged by the author was the small sample size (12 subjects). We have performed the genetic screening in 46 non-related probands. We also believe differences between studies could be due to the different ethnic origin of the analyzed populations (Rabat and Casabalanca, and North West Morocco).

In conclusion, our results show that ADH in North-West Morocco is not mainly due to neither LDLR nor APOB gene mutations. It is interesting to point out that endogamy degree in Moroccan population is high, giving rise to the possibility of a founder effect of a mutation in a gene so far unknown. More studies should be performed looking for other responsible genes (for instance, PCSK9) in order to expand our knowledge about the molecular basis of ADH in Morocco.

This work has been funded by grants from the Spanish Agency for International Cooperation (Ref. A/7087/06) (Spanish Ministry of International Affairs and Cooperation), CIBER of Diabetes and Associated Metabolic Diseases (CIBERDEM), PROMETEO/2009/029 (Generalitat Valenciana, Valencia, Spain) and CIT-00100-2007-36 (Spanish Ministry of Science and technology). FJ Chaves is supported by contract FIS02/3047. AB Garcia-Garcia is supported by CIBERDEM.

REFERENCES:

1. Goldstein JL, Hobbs H, Brown MS. (2001). Familial Hypercholesterolemia. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. The metabolic and molecular basis of inherited disease. New York: McGraw-Hill. pp.2863-2913
2. Soria LF, Ludwig EH, Clarke HRG, Vega GL, Grundy SM, McCarthy BJ (1989). "Association between a specific apoB mutation and familial defective apoB100". *Proc. Natl. Acad. Sci*. USA. 86: 587-591.
3. Fouchier SW, Kastelein JJ, Defesche JC. Update of the molecular basis of familial hypercholesterolemia in The Netherlands. *Hum Mutat*. 2005;26:550-6.
4. Abifadel M, Varret M, Rabes JP, Allard D, Ouguerram K, Devillers M, Cruaud C, Benjannet S, Wickham L, Erlich D, Derre A, Villeger L, Farnier M, Beucler I, Bruckert E, Chambaz J, Chanu B, Lecerf JM, Luc G, Moulin P, Weissenbach J, Prat A, Krempf M, Junien C, Seidah NG, Boileau C. (2003) "Mutations in PCSK9 cause autosomal dominant hypercholesterolemia". *Nat Genet*. ;34:154-
5. Leigh, S.E., Whittal, R.A., Hubbart ,C.S. and Humphries, S.E. (2008). Update and analysis of University College London low density lipoprotein receptor gene familial hypercholesterolemia database, *Ann Hum Genet* 72:485-498.
6. Varret M, Abifadel M, Rabès JP, Boileau C. (2008). Genetic heterogeneity of autosomal dominant hypercholesterolemia. *Clin Genet*. 73:1-13.
7. Abifadel M, Rabès JP, Devillers M, Munnich A, Erlich D, Junien C, Varret M, Boileau C. Mutations and polymorphisms in the proprotein convertase subtilisin kexin 9 (PCSK9) gene in cholesterol metabolism and disease. *Hum Mutat*. 2009;30:520-9.
8. Austin MA, Hutter CM, Zimmern RL, Humphries SE. Genetic causes of monogenic heterozygous familial hypercholesterolemia: a HuGE prevalence review. *Am J Epidemiol.* 2004 Sep 1;160(5):407-20.
9. El Messal, M., Aı¨t Chihab, K. Chater,R., Joan Carles,V., Bennis, F., Hafidi, A., Ribalta,J., Varret, M., Loutfi, M., Rabe`s, J.P., Kettani, A., Boileau, C., Masana, L., Adlouni, A. (2003). Familial. hypercholesterolemia in Morocco: first report of mutations in the LDL receptor gene. *J Hum Genet* 48:199–203
10. Chater, R., Aït Chihab, K., Rabès, J.P., Varret, M., Chabraoui, L., El Jahiri, Y., Adlouni, A., Boileau, C., Kettani ,A. and El Messal, M.. (2006) Mutational heterogeneity in low-density lipoprotein receptor gene related to familial hypercholesterolemia in Morocco. *Clinica* 373: 62-69
11. Ait Chihab, K. Chater, R., Cenarro, A., Kettani, A., Castillo, S., Loutfi M., Ribalta,J., Adlouni, A., Pocovi, M., And El Messal, M.: (2007) Familial hypercholesterolemia associated with severe hypoalphalipoproteinemia in a Moroccan family. *J Genet* 86: 159-163.
12. Miller, S.A., Dykes, D.D. and Polesky, H.F. (1988). A simple salting out procedure for extracting DNA from nucleated cells, *Nucleic Acids Res* 16: 1215.
13. Yamamoto, T., Davis, C.G., Brown, M.S., Scheider, W.J. , Casey, M.L. and Goldstein J.L. (1984). The human LDL receptor: a cysteine-rich protein with multiple Alu sequences in its mRNA, Cell 39: 27–38.
14. Zakharova,F.M., Damgaard, D., Mandelshtam1, M.Y., Golubkov, V.I., Nissen, P.H., Nilsen, G.G., Stenderup, A., Lipovetsky, B. M., Konstantinov,V. O., Denisenko, A.D., Vasilyev, V. B and Faergeman, O. (2005). Familial hypercholesterolemia in St.-Petersburg: the known and novel mutations found in the low density lipoprotein receptor gene in Russia. *BMC Medical Genetic*s 6:6
15. Callis, M., Jansen, S., R. Thiart, J. Nicol de Villiers, P., Raal, F.J., Kotze, M. J. (1998). Mutation analysis in familial hypercholesterolemia patients of different ancestries: identification of three novel LDLR gene mutations. *Molecular and Cellular Probes* 12: 149–152