Autosomal Dominant Hypercholesterolemia: Needs for Early Diagnosis and Cascade Screening in the Tunisian Population

Awatef Jelassi1,*, Mohamed Najah1, Afeef Slimani1, Imen Jguirim1, Mohamed Naceur Slimane1 and Mathilde Varret2

1Research Unit of Genetic and Biologic Factors of Atherosclerosis, Faculty of Medicine, Monastir; Tunisia; 2INSERM U698, CHU Xavier Bichat, Université Paris Denis Diderot, France

Abstract: Autosomal dominant hypercholesterolemia (ADH) is characterized by an isolated elevation of plasmatic low-density lipoprotein (LDL), which predisposes to premature coronary artery disease (CAD) and early death. ADH is largely due to mutations in the low-density lipoprotein receptor gene (LDLR), the apolipoprotein B-100 gene (APOB), or the proprotein convertase subtilisin/kexin type 9 (PCSK9). Early diagnosis and initiation of treatment can modify the disease progression and its outcomes. Therefore, cascade screening protocol with a combination of plasmatic lipid measurements and DNA testing is used to identify relatives of index cases with a clinical diagnosis of ADH. In Tunisia, an attenuated phenotypic expression of ADH was previously reported, indicating that the establishment of a special screening protocol is necessary for this population.

Received on: June 21, 2012 - Revised on: November 02, 2012 - Accepted on: November 05, 2012

Keywords: Autosomal dominant hypercholesterolemia, Screening protocol, Molecular default.

INTRODUCTION

Autosomal dominant hypercholesterolemia (ADH) was firstly reported by Carl Müller in 1938 with the study of families presenting tendon xanthomas and heart disease due to a hypercholesterolaemia dominantly inherited [1]. In the 1970’s, several studies in patients and cultured cells revealed a defect in the receptor for the low-density lipoprotein and culminated in the Nobel Prize for Brown and Goldstein for their work on the regulation of the cholesterol metabolism [2, 3]. ADH (OMIM # 143890) is one of the most frequent inherited disorders in humans, with a frequency of 1 in 500 in western populations [4]. In some populations, the frequency of heterozygous ADH is considerably higher because of founder effect.

A founder effect occurs when a subpopulation is formed through the immigration of a small number of “founder” subjects, followed by population expansion. If some of the founders had ADH, then genetic drift could lead to a high proportion of affected subjects who share specific mutations introduced by founders. Such founder effect was noted in French Canadians [5], South African Afrikaners [6], Jews [7], Indians [8], Christian Lebanese [9], Finns [10] and Tunisians [11].

In Tunisia, the frequency was estimated at 1/165 for heterozygous, and beside the founder effect, the high birth rate and consanguinity marriage influenced this frequency [11].

ADH is characterized by a selective increase of low-density lipoprotein (LDL) particles in plasma giving rise to tendon xanthomas, arcus cornea, and premature mortality from cardiovascular complications [12].

ADH has proven to be genetically heterogeneous and is associated with defects in at least three different genes: LDLR, APOB and PCSK9 genes. Other ADH-genes are still unidentified [13-16].

In this review, we expose diagnosis protocol and molecular default causing ADH. We concluded on the necessity to establish special cutoff points for the Tunisian population.

BIOLOGICAL, CLINICAL ASPECTS AND SCREENING PROTOCOLS FOR ADH

Primary biological sign of ADH is elevated plasma cholesterol level [4, 17]. Plasma cholesterol level is largely modulated by different environmental factors. For these reasons, at least two measurements are required before a diagnosis of hypercholesterolemia can be made.

Plasma cholesterol levels vary with age, sex, hormonal status, some acute illness, and are population dependant [4, 17, 18]. The cutoff level for diagnosis of hypercholesterolemia should thus ideally be age, sex, and population specific.

Heterozygous ADH patients usually have a twofold increase in total and LDL-cholesterol level. Homozygous ADH patients are characterized by an elevation of LDL-cholesterol often greater than 15 mmol/L (581 mg/dL) [19].

Clinical signs for ADH are presence of tendon xanthomas and premature coronary heart disease (CHD). Homozy-
gous patients commonly have tendon xanthomas before the age of 10 years, and, if untreated, they develop severe atherosclerosis and CHD within their third decade [19]. In heterozygous patients tendon xanthomas are common after 25-30 years old, and the onset of CHD is mostly before 55 years old [20].

Diagnosis of ADH is mainly based on lipid levels, clinical signs, family history of dyslipidemia and/or premature CHD, and will be confirmed by genetic analysis. Three different diagnosis criteria were developed for ADH by the USMedPed (US Make early diagnosis Prevent early death) Program [18] (Table 1), the Simon Broome Register Group in the United Kingdom [21, 22] (Table 2), and the Dutch lipid Clinic Network [23] (Table 3).

Actually, in Tunisia, the Simon Broome Register criteria for ADH are mostly used to determine potential patient of ADH. Particularly the cutoff LDL-cholesterol of 4.9 mmol/L (190 mg/dL) is commonly used to determine heterozygote ADH patients.

**CLINICAL AND BIOLOGICAL ASPECTS OF ADH IN TUNISIA AND CASCADE SCREENING**

Studies on ADH in Tunisia started in 1993 with the work of NM Slimane and coworkers. They estimated a high frequency of this disease for heterozygous (about 1/165). Beside they noted an attenuated phenotypic expression of ADH [11, 24].

Indeed, the analysis of 91 ADH patients showed that the prevalence of CHD in Tunisian ADH heterozygous after 30 years old was 23.5% for men and 29.4% for women. All of them went through life without developing any tendon xanthomas (except one female aged 62). The mean total cholesterol level for heterozygous was 7.04 ± 1.40 mmol/L and was higher than the one reported in China (6.1 ± 1.2 mmol/L) [25], but lower than in Japan (8.8±2.0 mmol/L) [26], in UK (9.8±1.7 mmol/L) [27], in Afrikaners (10.8±1.8 mmol/L) [28], or in Italy (8.49±1.66 mmol/L) [29]. The same observation was made concerning LDL-cholesterol levels.

Concerning homozygous patients, xanthomas were present for all of them, CHD was present for 10% of them before 9 years old, for 71% between 10 and 19 years old, and for 100% above 20 years old. Therefore, CHD in Tunisian ADH homozygous appears to have a later onset than in other homozygous populations. Indeed, CHD occurs for 50% of the Afrikaners ADH homozygous patients before 9 years old. [6] and for 25% in Japan before 10 years old. [26]. Their mean life expectancy was 13 years old. compared with 17 years old in Japan (26) and 21 years old in Lebanon [29]. The mean total cholesterol level for homozygotes reported was 17.52±3.12 mmol/L [24], similar to those reported in other populations.

A recent study in Tunisia showed that 24% (9 out 38) of the ADH patients carrying an heterozygous mutation in the **LDLR** gene have a LDL-cholesterol level under the 60th percentile of an age-and gender-matched reference population [30]. This discrepancy between the clinico/biological and molecular phenotype observed reveals the existence of factors that decrease the severity of the disease. In a previous study, we identified one of these factors as the traditional Tunisian diet which is enriched in polyunsaturated fats [11]. This type of diet has been shown to have long-term beneficial therapeutic effects by reducing the incidence of recurrent cardiovascular events. To conclude, 15 years after the first study in 1993 [11] similar characteristics of a mild phenotype of ADH in Tunisia was reported, particularly for heterozygous patients [24]. Thus, it appears clearly that despite the change in the diet habit to a more western diet, the Tunisian population still has the same mild clinical expression of ADH. According to these characteristics of the Tunisian population, the establishment of specific cutoff point seems to be necessary.

**MOLECULAR DEFECTS**

The known genetic bases of the ADH phenotype are mutations in the **LDLR**, **APOB**, or **PCSK9** genes.

**In the LDL Receptor Gene (****LDLR***)**

The discovery of the LDL receptor and its defective function led to a great advance in the understanding of the pathophysiology of familial hypercholesterolemia (FH).

The LDL receptor is produced in the endoplasmic reticulum (ER) where the 21 amino acid signal peptide is cleaved and the protein glycosylated to give rise to a mature receptor [31]. The 160kDa transmembrane receptor (glycoprotein of 839 amino acids) is present at the surface of most cell types and mediates endocytosis thus playing a pivotal role in cholesterol homeostasis [31].

More than 1741 allelic variants have been identified in the **LDLR** gene and are distributed as presented in Fig. (1). All gene variants for **LDLR** are compiled online at two web sites: http://www.ucl.ac.uk/fh/ and http:// http://www.umd.be/LDLR/.

Functional **LDLR** mutations have been classified into five classes based on biosynthetic and functional studies of fibroblast cell [19, 32]. **Class 1** mutations are due to disruption of the promoter sequence, nonsense, frameshift or splicing mutations, all resulting in an absence of protein synthesis (null alleles). **Class 2** mutations, that primarily occur in the ligand binding and epidermal growth factor precursor domains, disrupt transport of the LDL receptor from the endoplasmic reticulum to the Golgi apparatus. **Class 3** mutations interfere with cell surface binding of the receptor to LDL, and these mutations are also primarily found in the ligand-binding and epidermal growth factor precursor domains. **Class 4** mutations appear in the cytoplasmic and membrane-spanning domains. They inhibit the clustering of the LDL receptor at the cell surface and the LDL internalization. **Class 5** mutations disrupt the recycling of the LDL receptor to the cell surface [19, 32].

The first few defects in **LDLR** gene to be characterized were large deletions identified by southern blotting [33, 34]. Once amplification by PCR and direct automated sequencing of PCR products became possible the number of point mutation and minor deletions/insertions has greatly increased. The expanding use of multiplex ligation dependent probe amplification (MLPA), contributed to the evaluation of the exact contribution of major rearrangements. Recent sequencing into further intronic sequences has allowed identification of a large population of splice site mutations [35].
Table 1. US MedPed Program Diagnosis Criteria for Familial Hypercholesterolemia*

| Total Cholesterol Cutpoints (mmol/L) | First-degree relative with FH | Second-degree relative with FH | Third-degree relative with FH | General Population |
|--------------------------------------|-----------------------------|-------------------------------|-------------------------------|-------------------|
| Age (years)                          |                             |                               |                               |                   |
| <20                                  | 5.7                         | 5.9                           | 6.2                           | 7.0               |
| 20-29                                | 6.2                         | 6.5                           | 6.7                           | 7.5               |
| 30-39                                | 7.0                         | 7.2                           | 7.5                           | 8.8               |
| >40                                  | 7.5                         | 7.8                           | 8.0                           | 9.3               |

Diagnosis: FH is diagnosed if total cholesterol levels exceed the cutpoint.

*Williams et al. Diagnosing heterozygous familial hypercholesterolemia using new practical criteria validated by molecular genetics. Am J Cardiol 1993; 72:171-6(8).

Table 2. Simon Broome Familial Hypercholesterolemia Register Diagnostic Criteria for ADH*

| Description                                                                 | Criteria                                                                 |
|-----------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Total cholesterol concentration above 7.5 mmol/liter in adults or a total cholesterol concentration above 6.7 mmol/liter in children aged less than 16 years, or Low density lipoprotein cholesterol concentration above 4.9 mmol/liter in adults or above 4.0 mmol/liter in children | a                                                                        |
| Tendinous xanthomata in the patient or a first-degree relative               | b                                                                        |
| DNA-based evidence of mutation in the LDLR or APOB gene                     | c                                                                        |
| Family history of myocardial infarction before age 50 years in a second-degree relative or before age 60 years in a first degree relative | d                                                                        |
| Family history of raised total cholesterol concentration above 7.5 mmol/liter in a first- or second-degree relative | e                                                                        |

A ‘definite’ ADH diagnosis requires either criteria a and b or criteria c
A ‘probable’ ADH diagnosis requires either criteria a and d or criteria a and e

Risk of fatal coronary heart disease in familial hypercholesterolemia. Scientific Steering Committee on behalf of the Simon Broome Register Group. BMJ 1991; 303:893-6 (13).
Mortality in treated heterozygous familial hypercholesterolemia: implication for clinical management. Scientific Steering Committee on behalf of the simon Broome Register Group. Atherosclerosis 1999; 142:105-12 (14).

Table 3. Dutch Lipid Clinic Network Diagnosis Criteria for ADH*

| Points |
|--------|
| Criteria                                                                 | Points |
| First-degree relative with known premature (men:<55 years; women:<60 years) coronary and vascular disease, or | |
| First-degree relative with known LDLC above the 95th percentile | 1 |
| First-degree relative with tendinous xanthomata and/or arcus cornealis, or Children aged than 18 years with LDLC above the 95th percentile | 2 |
| Patient with premature (men:<55 years; women<60 years) coronary artery disease | 2 |
| Patient with premature (men:<55 years; women<60 years) cerebral or peripheral vascular disease | 1 |
| Tendinous xanthomata | 6 |
| Arcus cornealis prior to age 45 years | 4 |
| LDLC ≥ 8.5 | 8 |
(Table 3) contd....

| Points | LDLC 6.5-8.4 | LDLC 5-6.4 | LDLC 4.0 – 4.9 |
|--------|---------------|-------------|-----------------|
| 5      | 3             | 1           |

**DNA Analysis**

Functional mutation in the LDLR gene

| Diagnosis (based on the total number of points obtained) | Points |
|------------------------------------------------------|--------|
| A ‘definite’ ADH diagnosis requires more than 8 points | 8      |
| A ‘probable’ ADH diagnosis requires 6-8 points |        |
| A ‘possible’ ADH diagnosis requires 3-5 points |        |

World Health Organization, Familial hypercholesterolemia- report of a second WHO Consultation. Geneva, Switzerland: World Health Organisation, 1999. (WHO publication no. WHO/HGN/FH/CONS/99.2), (19)

**Fig. (1).** Distribution of molecular defects reported in the LDLR gene.

**In the Apolipoprotein B 100 Gene (APOB)**

The interaction between LDL and its receptor is fundamental for the regulation of plasma cholesterol in humans [31]. The only protein component of LDL is ApoB-100, which is the major ligand for the LDL receptor [36].

ApoB-100, a large protein of 550 kDa, is encoded on chromosome 2 and has 26 exons. The binding region is rich in positively charged amino acids and interacts with the binding domains of the LDL receptor [20]. The domain of apoB-100 that interacts with the LDL receptor has been defined using several approaches. The proposed model of this binding region, comprising two clusters [A (3147-3157) and B (3359-3367)] of basic amino acids that are linked through a disulfide bond between residues 3167 and 3297 [37, 38] has been further expanded though the discovery of the ADH causative mutation at residue 3500 [39, 40]. This has led to the general view that residues 3130-3630 are important for the binding of apoB-100 to the LDL receptor [41].

With the development of immune-electron microscopy studies, it was demonstrated that normal receptor binding involves an interaction between Arginine 3500 and Tryptophan 4369 in the carboxy- tail of apoB100 [42].

In contrast to the numerous ADH causative mutations in the LDLR gene, only a very few mutations have been reported in the APOB gene. To date, 10 mutations in APOB gene were described. The most frequent one is p.Arg3500Gln. This form of ADH, due to APOB gene mutations, was previously called FDB for Familial ligand-Defective apolipoprotein B (OMIM #144010).

Compared with individual mutation in the LDLR gene, each of which is rare, the p.Arg3500Gln APOB mutation is common in Europe, where 2-5% of hypercholesterolemic are heterozygous carriers. The penetrance of the mutant APOB allele, however, is not 100%, so patients with familial ligand-defective apoB have a less-severe phenotypes than FH (Familial Hypercholesterolemia) patients with a LDLR mutation [43, 44].

**In the Proprotein Convertase Subtilisin Kexine Type 9 Gene**

The third locus causing ADH was identified to be a gene located at chromosome 1p32.3, and named proprotein convertase subtilisin kexine type 9 gene (PCSK9) [45].

PCSK9 encodes the ninth member of the subtilisine-like protein convertase family (PCs). PCs are implicated in limited proteolysis of protein precursors going through the secretory pathway such as prohormones or precursors of neuropetides [46].

This gene comprises 12 exons transcribed into a complementary DNA that spans 3617 bp. The preproPCSK9 is synthesized as a 694 amino acid long, that undergoes autocatalytic cleavage between the prodomain and catalytic do-
The observation that PCSK9 mutations cause dominant hypercholesterolemia suggests that mutations confer a gain-of-function [45]. This hypothesis was confirmed by studies in which wild-type and mutant PCSK9 (S127R, F216L) were expressed at high levels in the mice liver; hepatic LDL receptor fell dramatically in the mice receiving either the wild-type or mutant PCSK9 [50, 53]. No associated reduction in LDL receptor mRNA levels were observed. Thus overexpression of PCSK9, whether mutant or wild type, reduces the number of receptors through a post-transcriptional mechanism. However, the existence of a direct effect of PCSK9 on LDL receptor degradation has never been reported [54].

To confirm the hypothesis that loss-of-function mutant of PCSK9 would cause hypercholesterolemia, Cohen et al. [55] sequenced the coding region of PCSK9 in individuals with low levels of plasma LDL-cholesterol (<5th percentile). Surprisingly, one out of 50 African-Americans in the population had a nonsense mutation in PCSK9 that lowered LDL-cholesterol levels by ~40% [55]. Subsequently, additional PCSK9 mutations associated with a reduction in plasma levels of LDL-cholesterol have been found, including in-frame, and missense mutations [56, 57].

Until now, a total number of 101 unique variants were reported, covering the entire gene of PCSK9. [45, 50, 52 56, 57; http://www.ucl.ac.uk/ldlr/Current/index.php?select_db=PCSK9]

PCSK9, which interacts with the LDL receptor, is a promising therapeutic target for hypercholesterolemia and coronary artery disease. A clear link between PCSK9 and LDL-cholesterol is also observed in animal studies. Indeed PCSK9 knockout mice have decreased plasma LDL-cholesterol [58]. In non-human primates, PCSK9 knockdown by siRNA or inhibition by a monoclonal antibody also leads to decreased plasma LDL [59, 60].

A recent study showed that antibody 1B20, which binds to PCSK9 with high affinity, disrupts the PCSK9-LDL receptor interaction, and inhibits the effect of PCSK9 on cellular LDL uptake [61]. Moreover, treatment with the 1B20 antiPCSK9 monoclonal antibody in mice and rhesus monkeys led to robust LDL-cholesterol lowering in plasma decreased liver PCSK9 and LDL mRNAs, and transient increases in total plasma levels of PCSK9 [61].

To conclude, understanding the physiology of PCSK9 is important, and this protein has become a major new target for lipid lowering therapy.

Other Possible Genes for ADH

ADH has proven to be genetically heterogeneous and associated with defects in other still unknown genes. Indeed, in a Mexican population, no PCSK9 mutations were found in one large ADH family that showed positive linkage to the 1p34-32 locus. This indicates that genes other than PCSK9 in the locus may be involved [13]. Marques-Pinheiro et al. [14] showed in a large family with ADH phenotype, but with no mutations in the three known genes, the implication of a fourth loci that was named HCHOLA4. This locus is located at 16q22.1 in a 7.89 Mb interval containing 154 genes.

In the Chinese ADH population, after performing a genome-wide linkage analysis of a family pedigree without mutations in LDLR, APOB and PCSK9 genes, a two suggestive linkage loci were identified on chromosome 3q25.1-26.1 and 21q22.3 [15].

In ADH families from Spain, with no mutation in the known ADH-causing genes, Cenarro et al. demonstrated the implication of a new locus located in 8p.24-22 through linkage analyses [16].

Finally, a Portuguese ADH study found only 48% of its total received cases with clinical diagnosis of ADH had genetic defects on LDLR, APOB or PCSK9, leaving the other 52% of ADH cases with possible undiscovered genes mutations [62].

These studies confirm complex etiologies and suggest new genetic causal factors for the ADH disorder.

MOLECULAR DEFAULTS THAT CAUSE ADH IN TUNISIA

In Tunisia, ADH is one of the most frequent genetic disorders with a frequency of 1/165 for heterozygous [11]. This population had a mild phenotype of ADH, in particularly for heterozygous carriers.

Primary genetic studies were focused on LDLR gene. Recently, we started research on PCSK9 gene variation. Concerning APOB gene, studies were realized to search for the p.Arg3500Gln mutation. Studies were carried on 102 patients from 19 unrelated ADH families. Mutations identified in Tunisian ADH patients are presented in (Table 4).

In the LDLR gene, we identified 11 mutations in the different exons of the gene, from them 7 were novels. Mutations were nonsense, frame shift, missense and major rearrangement. The mutation p.Ser493ArgfsX44 in exon 10 appears to be the most frequent mutation. [11, 24, 30, 63, 64].

Concerning the PCSK9 gene, our team identified a novel missense mutation named c.520C>T (p.Pro174Ser) localized in exon 3. Study indicates that this new PCSK9 variant is able to reduce the severity of FH, very probably acting as a loss-of-function variant. This finding should be confirmed by in vitro experiments [30].

Moreover, four common polymorphisms of PCSK9 were identified in a sample of 13 unrelated FH patients: L10, L11 p.474Val and p.Glu670. Their frequencies were similar to those reported in previous study for different population [30].
No APOB Arg3500Gln was identified in all patients genotyped until now. This observation was also noted for the Lebanese [65] and Moroccan [66] population.

To conclude, the clinical expression of ADH in heterozygous patients is influenced by environmental factors as well as genetic factors in particular genes affecting lipoprotein metabolism such as APOE, MTP, HL, and ABCA1 genes. In unrelated ADH patients, the plasma LDL-cholesterol level is influenced by APOE, MTP and APOB polymorphisms, and the plasma High Density Lipoprotein (HDL)-cholesterol level is influenced by HL, FABP-2 and LPL polymorphisms [67]. The sequence analysis of these genes in Tunisian ADH patients may reveal genetic factors that are responsible of the mild clinical and biological phenotype of heterozygous ADH actually observed.

**CONCLUSION**

Special efforts are required to identify individuals with ADH in Tunisia as they are at high risk of premature coronary heart disease. The condition is seriously under diagnosed and the diagnosis is often made too late, in particular for heterozygous subjects, restricting the benefits of the treatments [68].

These patients can be treated to lower their cholesterol levels, before the installation of CHD, and thus avoid the complications and early death.

The attenuated phenotype of ADH in Tunisia was demonstrated and the establishment of a special diagnosis protocol is essential for the Tunisian population.

**CONFLICT OF INTEREST**

The author(s) confirm that this article content has no conflicts of interest.

**ACKNOWLEDGEMENTS**

Declared none.

**REFERENCES**

[1] Muller C. Xanthomata, hypercholesterolemia, angina pectoris. Acta Med Scand, 1938, 89 sup: 75-84.
[2] Brown, M.S; Goldstein, J,L. A receptor-mediated pathway for cholesterol homeostasis. Science, 1986, 232: 34-47.
[3] Goldstein, J.L; Brown, M.S. Regulation of the mevalonate pathway. Nature, 1990, 343: 425-30.
[4] Goldstein, J.; Brown, M. Familial hypercholesterolemia. In Scriver C, Beaudet A, Sly W, eds. The metabolic basis of inherited diseases. New York: Mc Graw-Hill, 1989: pp1215-1250.
[5] Moorjani, S.; Roy, M.; Gagne, C.; Davignon, J.; Brun, D.; Toussaint, M.; Lambert, M.; Campeau, L.; Blaych, S.; Lupien, P. Homozygous familial hypercholesterolemia among French Canadians in Quebec Province. Arteriosclerosis, 1989, 9: 211-216.
[6] Seftel, H.C.; Baker, S.G.; Sandler, P., Forman, M.B.; Joffe, B.I.; Mendelsohn, D.; Jenkins, T.; Miemy, C.J. A host of hypercholesterolaemic homozygotes in South Africa. Br Med J, 1980, 281: 633-636.
Goldstein, J.L.; Hobbs, H.H.; Brown, M.S. Familial hypercholesterolemia. In Scriver CR, Beaudet A, Sly W, eds. The metabolic basis of inherited diseases. 7th ed. New York: Mc Graw-Hill, 1995: p.1981-2030.

Robles-Osorio, L.; Huerta-Zepeda, A.; Ordonez, M.L.; Canizales-Quintan, S.; Diaz-Villarreal, A.; Gutiérrez-Aguilar, R.; Riba, L.B.; Huertas-Vázquez, A.; Rodríguez-Torres, M.; Gómez-Díaz, R.A.; Salinas, S.; Orga-Larios, L.; Codiz-Huerta, G.; More-Cabrera, M.; Mehta, R.; Gómez Pérez, F.J.; Rull, J.A.; Rabès, J.P.; Tusié-Luna, M.T.; Durán-Vargas, S.; Aguilar-Salinas, C.A. Genetic heterogeneity of autosomal dominant hypercholesterolemia in Mexico. Arch Med Res, 2006; 37: 102-108.

Marques-Pinheiro, A.; Marduel, M.; Rabès, J.P.; Devillers, M.; Villeger, L.; Allard, D.; Weissenbach, J.; Guerin, M.; Zair, Y.; Erlich, D.; Junien, C.; Munich, A.; Kempf, M.; Abifadel, M.; Jais, J.P.; French Research Network on ADH, Boileau, C.; Varret, M. A fourth locus for autosomal dominant hypercholesterolemia maps at 16q22.1. Eur J Hum Genet, 2010; 18: 1236-1242.

Wang, X.; Li, X.; Zhang, Y.B.; Zhang, F.; Sun, L.; Lin, J.; Wang, D.M.; Wang, L.Y. Genome-wide linkage scan of a pedigree with familial hypercholesterolemia suggests susceptibility loci on chromosomes 3q25-26 and 2q22. PLoS One, 2011; 6: e24388.

Cenarro, A.; Garcia-Otin, A.L.; Tejedor, M.T.; Solanas, M.; Jarauta, E.; Junquera, C.; Ros, E.; Mozas, P.; Puzo, J.; Pocovi, M.; Civeira, F. A presumptive new locus for autosomal dominant hypercholesterolemia maps to 8q24.22. Clin Genet, 2011; 79: 475-481.

Marks, D.; Thorogood, M.; Neil, H.A.; Humphries, S.E. A review on the diagnosis, natural history, and treatment of familial hypercholesterolemia. Atherosclerosis, 2003; 168: 1-14.

Williams, R.R.; Hunt, S.C.; Schumacher, M.C.; Hegele, R.A.; Leppert, M.F.; Ludwig, E.H.; Hopkins, P.N. Diagnosing heterozygous familial hypercholesterolemia using new practical criteria validated by molecular genetics. Am J Cardiol, 1993; 72: 171-176.

Goldstein, J.L.; Hobbs, H.H.; Brown, M.S. Familial hypercholesterolemia. In Scriver CR, Beaudet A, Sly W, eds. The metabolic and molecular basis of inherited diseases. 7th ed. New York: Mc Graw-Hill, 1995: pp, 1853-1886.

Bertolini, S.; Cantafora, A.; Averna, M.; Cortese, C.; Motti, C.; Martini, S.; Pes, G.; Postiglione, A.; Stefanutti, C.; Blotta, I.; Pisciotta, L.; Rolli, M.; Langheim, S.; Ghisellini, R.; Rabbone, I.; Calandra, S. Clinical expression of familial hypercholesterolemia in clusters of mutations of the LDL receptor gene that cause a receptor-defective or receptor-negative phenotype. Arterioscler Thromb Vasc Biol, 2000; 20: e41–52.

Slimani, N.; Jelas, A.; Jiguirim, I.; Najah, M.; Rebbi, L.; Omezzine, A.; Luda, K.B.; Kaem, M.; Rabès, J.P.; Abifadel, M.; Boileau, C.; Rouis, M.; Slimane, M.N.; Varret, M. Effect of mutations in LDLR and PCSK9 genes on phenotypic variability in Tunisian familial hypercholesterolemia patients. Atherosclerosis, 2012; 222: 158-166.

Goldstein, J.L.; Brown, M.S.; Anderson, R.G.; Russell, D.W.; Schneider, W.J. Receptor-mediated endocytosis: concepts emerging from the LDL receptor system. Annu Rev Cell Biol, 1985; 1: 1-39.

Hobbs, H.H.; Russell, D.W.; Brown, M.S.; Goldstein, J.L. The LDL receptor locus and familial hypercholesterolemia: mutational analysis of a membrane protein. Annu Rev Genet, 1990; 24: 133-170.

Yamamoto, T.; Davis, C.G.; Brown, M.S.; Schneider, W.J.; Casey, M.L.; Goldstein, J.L.; Russell, D.W. The human LDL receptor: a cysteine-rich protein with multiple Alu sequences in its mRNA. Cell, 1984; 39: 27-38.

Soutar, A.K.; Naoumova, R. Mechanisms of disease: genetic causes of familial hypercholesterolemia. Nature, 2007; 4: 214-225.

Graham, C.A.; Mellhton, B.P.; Krik, C.W.; Beattie, E.D.; Lyttle, M.; McCarthy, B.J. Association between a specific apolipoprotein B gene polymorphism and familial hypercholesterolemia. Arterioscler Thromb Vasc Biol, 2012; 32: 1460-1468.

Krauss, R.M.; Vega, G.L.; Grundy, S.M.; Stein, J.L.; Krauss, R.M.; Vega, G.L.; Grundy, S.M.; et al. Familial defective apolipoprotein B-100. N Engl J Med, 1987; 317: 1460-1468.

Apolipoprotein B in patients with familial hypercholesterolemia. Arterioscler Thromb Vasc Biol, 2009; 29: 1637-1646.
No. 1

32 Current Genomics, 2013, Vol. 14, No. 1

Jelassi et al.

[43] Myant, N.B. Familial defective apolipoprotein B-1a review, including some comparisons with familial hypercholesterolemia. Arteriosclerosis, 1993, 104, 1-18.

[44] Vrablik, M.; Ceska, R.; Horinek, A. Major apolipoprotein B-100 mutations in lipoprotein metabolism and atherosclerosis. Physiol Res, 2001, 50: 337-343.

[45] Abifadel, M.; Varret, M.; Rabès, J.P.; Allard, D.; Ogugurren, K.; Devillers, M.; Cruaud, C.; Benjannet, S.; Wickham, L.; Erlich, D.; Derré, A.; Villégé, L.; Farnier, M.; Beucler, I.; Bruckert, E.; Chambaz, J.; Chau, B.; Lecerf, J.M.; Luc, G.; Moulin, P.; Weissenbach, J.; Prat, A.; Krempf, M.; Junien, C.; Seidah, N.G.; Boileau, C. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nat Genet, 2003, 34: 154-156.

[46] Seidah, N.G.; Chretien, M. Proprotein and prohormone convertases: a family of subtilases generating diverse bioactive polypeptides. Brain Res, 1999, 848: 45-62.

[47] Seidah, N.G.; Benjannet, S.; Wickham, L.; Marcinkiewicz, J.; Jasmin, S.B.; Stifani, S.; Basak, A.; Prat, A.; Chretien, M. The secretory proprotein convertase neutral apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation. Proc Natl Acad Sci U S A, 2003, 100: 928-933.

[48] Naureckiene, S.; Ma, L.; Sreekumar, K.; Parandare, U.; Lo, C.F.; Huang, Y.; Chiang, L.W.; Grenier, J.M.; Ozenberger, B.A.; Jacobson, J.S.; Kennedy, J.D.; DiStefano, P.S.; Wood, A.; Bingham, B. Functional characterization of NARC 1, a novel proteinase related to proteinases K. Arch Biochem Biophys, 2003, 420: 55-67.

[49] Basak A. Inhibitors of proprotein convertases. J Mol Med (Berl), 2005, 83: 844-855.

[50] Benjannet, S.; Rhaïnds, D.; Hamelin, J.; Nassoury, N.; Seidah, N.G. The proprotein convertase (PC) PCSK9 is inactivated by furin and/or PCS5A: functional consequences of natural mutations and post-translational modifications. J Biol Chem, 2006, 281: 30561-30572.

[51] Moore, K.L. The biology and enzymology of protein tyrosine O-sulfation. J Biol Chem, 2003, 278: 24243-24246.

[52] Timms, K.M.; Wagner, S.; Samuels, M.E.; Forbey, K.; Goldfine, H.; Jammulapati, S.; Skolnick, M.H.; Hopkins, P.N.; Hunt, S.C.; Shattuck, D.M. A mutation in PCSK9 causing autosomal-dominant hypercholesterolemia in an Utah pedigree. Hum Genet, 2004, 114: 349-353.

[53] Park, S.W.; Moon, Y.A.; Horton, J.D. Post-transcriptional regulation of low density lipoprotein receptor protein by proprotein convertase subtilisin/kexin type 9a in mouse liver. J Biol Chem, 2004, 279: 50630-50638.

[54] Varret, M.; Abifadel, M.; Rabès, J.P.; Boileau, C. Genetic heterogeneity of autosomal dominant hypercholesterolemia. Clin Genet, 2008, 73: 1-13.

[55] Cohen, J.; Pertsemidis, A.; Kotowski, I.K.; Graham, R.; Garcia, C.K.; Hobbs, H.H. Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. Nat Genet, 2005, 37: 161-165.

[56] Cameron, J.; Holla, O.L.; Ranheim, T.; Kulseth, M.A.; Berge, K.E.; Leren, T.P. Effect of mutations in the PCSK9 gene on the cell surface LDL receptors. Hum Mol Genet, 2006, 15: 1551-1558.

[57] Kotowski, I.K.; Pertsemidis, A.; Luke, A.; Cooper, R.S.; Vega, G.L.; Cohen, J.C.; Hobbs, H.H. A spectrum of PCSK9 alleles contributes to plasma levels of low-density lipoprotein cholesterol. Am J Hum Genet, 2006, 78: 410-422.

[58] Rashid, S.; Curtis, D.E.; Garuti, R.; Anderson, N.N.; Bashmakov, Y.; Ho, Y.K.; Hammer, R.E.; Moon, Y.A.; Horton, J.D. Decreased plasma cholesterol and hypersensitivity to statins in mice lacking Pkcs9. Proc Natl Acad Sci U S A, 2005, 102: 5374-5379.

[59] Frank-Kamenetsky, M.; Greghorst, A.; Anderson, N.N.; Racie, T.S.; Bramlage, B.; Akine, A.; Butler, D.; Charisse, K.; Dorkin, R.; Fan, Y.; Gamba-Vitalo, C.; Hadwiger, P.; Jayaraman, M.; John, M.; Jayaprakash, K.N.; Maier, M.; Nechev, L.; Rajeev, K.G.; Read, T.; Röhl, I.; Soutschek, J.; Tan, P.; Wong, J.; Wang, G.; Zimmermann, T.; de Fruegoleres, A.; Vonnlocher, H.P.; Langer, R.; Ander- son, D.G.; Manoharan, M.; Koteliansky, V.; Horton, J.D.; Fitzgerald, K. Therapeutic RNAi targeting PCSK9 acutely lowers plasma cholesterol in rodents and LDL cholesterol in nonhuman pri-mates. Proc Natl Acad Sci U S A, 2008, 105: 11915-11920.

[60] Comstock, D.G.; Teshima, T.; Teshima, H.; Teshima, K.; Teshima, Y. A mutation in PCSK9 causing autosomal-dominant hypercholesterolemia. Hum Genet, 2003, 114: 349-353.

[61] Wang, S.P.; Johns, D.G.; Volpari, C.; Hamuro, L.; Chin, J.; Huang, L.; Zhao, J.Z.; Vitelli, S.; Haytko, P.; Wisniewski, M.; Mitnaul, L.J.; Sparrow, C.P.; Hubbard, B.; Carfì, A.; Sitlani, A. A proprotein convertase subtilisin/kexin type 9 (PCSK9)-binding antibody that structurally mimics the EGF(A) domain of LDL-receptor reduces free circulating PCSK9 and LDL-cholesterol. J Lipid Res, 2011, 52: 78-86.

[62] Zhang, L.; McCabe, T.; Condra, J.H.; Ni, Y.G.; Peterson, L.B.; Wang, W.; Fang, P.; Pandit, S.; Hammond, H.A.; Rosa, R.; Cummings, R.T.; Wood, D.D.; Liu, X.; Bottomley, M.J.; Shen, X.; Cubbon, R.M.; Wang, S.P.; Johns, D.G.; Volpari, C.; Hamuro, L.; Chin, J.; Huang, L.; Zhao, J.Z.; Vitelli, S.; Haytko, P.; Wisniewski, M.; Mitnaul, L.J.; Sparrow, C.P.; Hubbard, B.; Carfì, A.; Sitlani, A. An anti-PCSK9 antibody reduces LDL-cholesterol on top of a statin and suppresses hepatocyte SREBP-regulated genes. Int J Biol Sci, 2012, 8: 310-327.

[63] Medeiros, A.M.; Alves, A.C.; Francisco, V.; Bourbon, M. Investigators of the Portuguese FH Study Update of the Portuguese Familial Hypercholesterolaemia Study. Atherosclerosis, 2010, 212: 552-558.

[64] Jelassi, A.; Najah, M.; Jguirim, I.; Maatouk, F.; Lestavel, S.; Laroussi, O.S.; Rouis, M.; Boileau, C.; Rabès, J.P.; Varret, M.; Slimane, M.N. A novel splice site mutation of the LDL receptor gene in a Tunisian hypercholesterolemic family. Clin Chim Acta, 2008, 392: 25-29.

[65] Jelassi, A.; Jguirim, I.; Najah, M. Abid, A.M.; Boughamoura, L.; Maatouk, F.; Rouis, M.; Boileau, C.; Rabès, J.P.; Slimane, M.N.; Varret, M. Limited mutational heterogeneity in the LDLR gene in familial hypercholesterolemia in Tunisia. Atherosclerosis, 2009, 203: 449-453.

[66] Abifadel, M.; Rabès, J.P.; Jambart, S.; Habary, G.; Gannagé-Yared, M.H.; Sarkis, A.; Beaino, G.; Varret, M.; Salem, N.; Corbani, S.; Aydénian, H.; Junien, C.; Munnich, A.; Boileau, C. The molecular basis of familial hypercholesterolemia in Lebanon: spectrum of LDLR mutations and role of PCSK9 as a modifier gene. Hum Mutat, 2009, 30: E682-91.

[67] Chater, R.; Ait Chihab, K.; Rabès, J.P.; Varret, M.; Chabrouai, L.; El Jahiri, Y.; Adlouni, A.; Boileau, C.; Kettani, A.; El Messal, M. Mutational heterogeneity in low-density lipoprotein receptor gene related to familial hypercholesterolemia in Morocco. Clin Chim Acta, 2006, 373: 62-69.

[68] Bertolini, S.; Pisciotta, L.; DiScala, L.; Langheim, S.; Bellochio, A.; Masturzo, P.; Cantafota, A.; Martini, S.; Averna, M.; Pes, G.; Stefanutti, C.; Calandra, S. Genetic polymorphisms affecting the phenotypic expression of familial hypercholesterolemia. Atherosclerosis, 2004, 174: 57-65.

[69] Seita, N.; Verma, I.C.; Khan, B.; Arora, A. Premature Coronary Artery Disease and Familial Hypercholesterolemia: Need for Early Diagnosis and Cascade Screening in the Indian Population. Cardiol Res Pract, 2012, 658526.