Atherosclerosis 224 (2012) 430–434

Contents lists available at SciVerse ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Genetic analysis of familial hypercholesterolaemia in Western Australia

Amanda J. Hooper [a,b,c], Lan T. Nguyen [a], John R. Burnett [a,b,c,d], Timothy R. Bates [b,d], Damon A. Bell [a,b,d], Trevor G. Redgrave [d], Gerald F. Watts [b,d], Frank M. van Bockxmeer [a,c,*]

[a] Cardiovascular Genetics Laboratory, Department of Core Clinical Pathology & Biochemistry, PathWest Laboratory Medicine WA, Royal Perth Hospital, Perth, Australia
[b] School of Medicine & Pharmacology, University of Western Australia, Perth, Australia
[c] School of Pathology & Laboratory Medicine, University of Western Australia, Perth, Australia
[d] Lipid Disorders Clinic, Department of Internal Medicine, Royal Perth Hospital, Perth, Australia

ARTICLE INFO

Article history:
Received 15 May 2012
Received in revised form
6 July 2012
Accepted 18 July 2012
Available online 27 July 2012

Keywords:
Familial hypercholesterolaemia
LDL-receptor
Mutations

ABSTRACT

Objective: To determine the spectrum of mutations associated with familial hypercholesterolaemia (FH) and their detection rate in the FH Western Australia (FHWA) Program.
Methods: Mutation testing of the *LDLR* gene, plus select regions in *APOB* and *PCSK9*, was performed in the first 343 patients considered to be phenotypic index cases of FH and classified on the basis of the Dutch Lipid Clinic Network Criteria (DLCNC) score as "possible", "probable", or "definite" FH.
Results: Overall, 86 different pathogenic (or likely pathogenic) mutations were identified in 129 patients, including four compound heterozygotes manifesting a more severe clinical phenotype. Fourteen of these mutations were novel and twelve (9.6%) were large deletions/duplications of the *LDLR*. The most common mutations were the familial defective apoB-100 mutation *APOB* p.Arg3527Gln (7.2%) and an *LDLR* intron 3 splice site mutation c.313 + 1G > A (4.8%). While 70% of 'definite' FH patients were found to carry a mutation, only 29% of 'probable' and 11% of 'possible' FH patients were mutation-positive.
Conclusion: This information provides a useful DNA database on which to base ongoing cascade screening for FH and future research into the genetic aetiology of FH in Western Australia. These findings suggest genetic testing should be prioritised to those with high DLCNC scores and offers a cost-effective family screening method from FH index cases, leading to detection of other previously undiagnosed and younger family members, enabling early instigation of intervention and preventative measures for premature coronary heart disease.

1. Introduction

It is well established that elevated plasma concentrations of low density lipoprotein (LDL) cholesterol are associated with increased risk of cardiovascular disease. Autosomal dominant familial hypercholesterolaemia (FH) with a prevalence of approximately one in 300–500 is one of the most common heritable human diseases, affecting at least 10 million people worldwide [1]. The high levels of LDL-cholesterol in FH are caused by reduced clearance of LDL from blood by the LDL-receptor pathway, leading to premature coronary heart disease (CHD). Untreated FH leads to premature coronary artery disease and in males, a 50% cumulative risk of myocardial infarction before the age of 50 years [1].

Lifetime exposure to high blood cholesterol concentrations causes the formation of cholesterol deposits not only in coronary arteries, but also in and around the eye as corneal arcus and xanthelasma, and in tendons, particularly the Achilles, as xanthomas [1–3]. These clinical characteristics together with raised LDL-cholesterol levels and a positive family history can be used in diagnostic algorithms such as the Dutch Lipid Clinic Network Criteria (DLCNC) to assign a probability of FH [4]. A DLCNC score of 9 or greater indicates 'definite' FH, 6–8 'probable' FH, and 3–5 'possible' FH.

FH is mainly caused by mutations in the LDL-receptor (*LDLR*) gene; over 1000 have been described [5,6]. In many countries, a large proportion of FH is accounted for by relatively few *LDLR* mutations [7]. Owing to significant European migration in the 1950s and Asian migration in the 1970s and 1980s, Australia has a genetically heterogeneous population, which poses challenges for

* Corresponding author. Cardiovascular Genetics Laboratory, Department of Core Clinical Pathology & Biochemistry, PathWest Laboratory Medicine WA, Royal Perth Hospital, GPO Box X2213, Perth WA 6847, Australia. Tel.: +61 8 9224 2322; fax: +61 8 9224 2491.
E-mail address: Frank.vb@health.wa.gov.au (F.M. van Bockxmeer).

0021-9150/$ – see front matter
http://dx.doi.org/10.1016/j.atherosclerosis.2012.07.030

a screening program. Western Australians are mainly of Northern European ancestry (80%) with a significant proportion of the remainder from South-East Asia and Southern Africa.

Large deletions or insertions in the *LDLR* are estimated to occur in 10% of FH patients and although they cannot be readily determined by conventional DNA sequence analysis, they can now be tested for with a commercially available multiplex ligation-dependent probe amplification (MLPA) kit. MLPA combined with exon-by-exon sequencing of the 18 exons of the *LDLR* gene (plus part of *APOB* containing the familial ligand-defective apoB mutation p.Arg3527Gln and exon 7 of *PCSK9* containing p.Asp374Tyr) is a comprehensive means of mutation detection for FH.

Of the estimated 45,000 cases of FH in Australia, the large majority remain undiagnosed and inadequately treated [8]. DNA testing facilitates cascade screening for FH from index cases and previous studies have shown that genetic testing is superior to phenotypic measurement of LDL-cholesterol levels as a cascade screening tool [9]. The FH Western Australia (FHWA) pilot program was established to identify and treat individuals with FH in Western Australia, by cascade screening of families from index cases [10,11]. Recent guidelines for the screening, diagnosis and management of FH all focus on family or "cascade" DNA testing as the most cost-effective way for new case detection [12].

We present the first report of the mutation spectrum of FH in Australia and describe the findings of mutation testing performed in FH patients referred to a dedicated lipid disorders clinic in Perth, Western Australia.

2. Methods

Subjects were consecutive patients ($n = 343$) considered to have phenotypic FH who were referred for DNA testing from the Lipid Disorders Clinic at Royal Perth Hospital; this clinic takes referrals from the community via GPs and from medical specialists, particularly cardiologists. Informed written consent was obtained for DNA testing. DLCNC scores were calculated using phenotypic criteria alone by the physician requesting the genetic test [10–12]; when estimating this score pre-treatment, fasting plasma LDL-cholesterol concentrations were used for patients who were already on statins. A phenotypic diagnosis of FH was made in all cases after secondary causes of hypercholesterolaemia were excluded [12]. Genomic DNA was isolated from peripheral blood leukocytes using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA). The MLPA-LDLR kit P062 (MRC-Holland, The Netherlands) was used according to the manufacturer's instructions. Deletions/duplications were confirmed by a second MLPA reaction.

M13-tagged primers for the amplification of the 18 exons of the *LDLR* plus part of *APOB* exons 26 and 29 were designed *de novo* using CLC Main Workbench (CLC Bio, Denmark) with reference to the NCBI and the UCL databases. *PCSK9* exon 7 primers have been reported previously [13]. Sequencing was performed using Big Dye Terminator chemistry (Applied Biosystems) in the forward and reverse directions, and aligned using CLC Main Workbench; mutations were described using HGVS nomenclature and reference sequence NM_000384.2 (*APOB*), AY114155.1 (*LDLR*) or NM_174936.3 (*PCSK9*). Mutations were confirmed by sequencing of a second PCR product. Pathogenicity was assessed by reference to published data and for novel variants by *in-silico* methods using the online tools PolyPhen2 [14], SIFT [15], and MutationTaster [16], and determining whether other mutations had been reported at the same position.

T-tests were used to compare age, plasma LDL-cholesterol, triglyceride and HDL-cholesterol concentrations between patients found to carry a mutation and those in whom a mutation could not be detected, with statistical significance defined as $p < 0.05$.

Table 1
Compound heterozygous Western Australian FH patients referred to a lipid disorders clinic.

Compound heterozygote	Mutation 1		Mutation 2	
1	*LDLR* c.326G>A	p.Cys109Tyr	*LDLR* c.660delC	p.Asp221 Thrfs*44
2	*LDLR* c.417C>G	p.Asp139Glu[a]	*LDLR* c.693C>A	p.Cys231*
3	*LDLR* c.661G>A	p.Asp221Asn	*LDLR* c.681C>G	p.Asp227Glu
4	*APOB* c.10580G>A	p.Arg3527Gln	*LDLR* c.681C>G	p.Asp227Glu

[a] Novel, see Table 3.

3. Results

Overall, 86 different pathogenic mutations were identified in 129 of the 343 patients referred on the basis of a clinical suspicion of FH, for DNA testing. Four of these patients were compound heterozygotes, including one patient heterozygous for a mutation in each of the *APOB* and *LDLR* genes (Table 1). The mean plasma LDL-cholesterol concentration in these patients was 12.9 mmol/L, ranging from 7.9 mmol/L (the individual carrying both the *APOB* p.Arg3527Gln and an *LDLR* mutation) to 16.8 mmol/L.

Of the 337 FH genetic testing requests where a DLCNC score was available, 38% were classified 'definite' (DLCNC score > 8), 26% 'probable' (DLCNC score 6–8), 33% 'possible' (score 3–5) and 3% 'unlikely' FH. While 70% of 'definite' FH patients were found to carry a mutation, only 29% of 'probable' and 11% of 'possible' FH patients were mutation-positive (Fig. 1).

Of the 125 heterozygous mutation-positive FH patients, the most common mutations were the familial defective apoB-100 mutation *APOB* p.Arg3527Gln (also known as R3500Q) and the *LDLR* intron 3 splice site mutation c.313+1G > A, found in 9 (7.2%) and 6 (4.8%) heterozygous mutation-positive patients, respectively (Table 2). Twenty-two patients (18%) carried a mutation in exon 4, the largest exon. Twelve *LDLR* gene deletions or duplications (9.6% of mutation-positive patients) were detected by MLPA analysis.

MLPA results suggesting reduced copy number in a single *LDLR* exon were sequenced for that exon; c.912C>G (p.Asp304Glu), c.1880C>A (p.Ala627Asp), and c.1885T>G (p.Phe629Val) were identified in this manner.

In total, 14 novel mutations were detected. Of these, there was one *APOB* variant of unknown pathogenicity, c.10477G>A (p.Glu3493Lys), found in an individual with a DLCNC score of 8. Four small *LDLR* deletions were found; three of these were frameshifts and one affected a single amino acid, p.Gly593del (Table 3). An *LDLR* promoter variant c.−121C>T was found and predicted to be pathogenic; a mutation affecting the adjacent nucleotide was

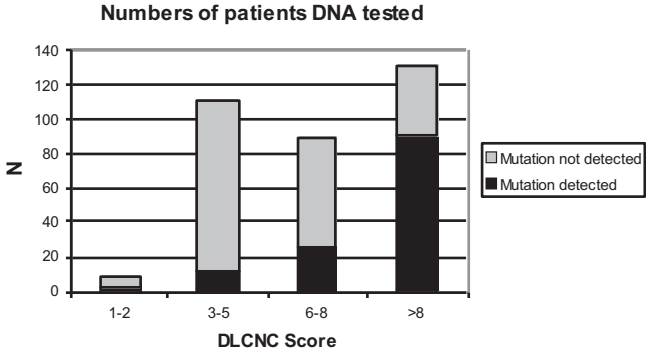

Fig. 1. Mutation detection in FH patients stratified by DLCNC score.

Table 2
Known pathogenic mutations identified in Western Australian heterozygous FH patients referred to a lipid disorders clinic. Human Genome Variation Society nomenclature is used.

cDNA	Predicted effect on protein	Exon	Number of patients	Reference[a]
APOB				
c.10580G>A	p.Arg3527Gln	26	9	[24]
LDLR				
c.6delG	p.Trp4Glyfs*202	1	1	LDLR_00248
c.58G>A	p.Gly20Arg	1	1	LDLR_00868
c.81C>G	p.Cys27Trp	2	1	LDLR_00004
c.131G>A	p.Trp44*	2	1	LDLR_00007
c.190+4A>T		(Intron 2)	1	LDLR_01031
c.232C>T	p.Arg78Cys	3	1	LDLR_00251
c.246C>A	p.Cys82*	3	1	LDLR_00983
c.259T>G	p.Trp87Gly	3	1	LDLR_00011
c.269A>G	p.Asp90Gly	3	3	LDLR_00012
c.301G>A	p.Glu101Lys	3	3	LDLR_00013
c.311G>A	p.Cys104Tyr	3	1	LDLR_01117
c.313_313+1delCG		3	1	LDLR_00015
c.313+1G>A		(Intron 3)	6	LDLR_00163
c.326G>A	p.Cys109Tyr	4	2	LDLR_00646
c.427T>G	p.Cys143Gly	4	2	[18]
c.501C>A	p.Cys167*	4	2	LDLR_00250
c.551G>A	p.Cys184Tyr	4	4	LDLR_00236
c.589T>C	p.Cys197Arg	4	1	LDLR_00400
c.654_656delTGG	p.Gly219del	4	1	LDLR_00030
c.660delC	p.Asp221Thrfs*44	4	1	LDLR_00865
c.661G>A	p.Asp221Asn	4	1	LDLR_00490
c.662A>G	p.Asp221Gly	4	1	LDLR_00031
c.664T>C	p.Cys222Arg	4	1	LDLR_00707
c.680_681delAC	p.Asp227Glyfs*12	4	2	LDLR_00206
c.681C>G	p.Asp227Glu	4	2	LDLR_00036
c.798T>A	p.Asp266Glu	5	1	LDLR_00044
c.912C>G	p.Asp304Glu	6	1	LDLR_00048
c.938G>A	p.Cys313Tyr	6	1	LDLR_00210
c.986G>A	p.Cys329Tyr	7	1	LDLR_00380
c.1027G>A	p.Gly343Ser	7	1	LDLR_00056
c.1033C>T	p.Gln345*	7	2	LDLR_00213
c.1048C>T	p.Arg350*	7	4	LDLR_00215
c.1049G>C	p.Arg350Pro	7	2	LDLR_00214
c.1066G>T	Asp356Tyr	8	1	LDLR_00523
c.1121_1122insGT	p.Tyr374Serfs*39	8	1	LDLR_00923
c.1187-10G>A		(Intron 8)	2	LDLR_00882
c.1196C>A	p.Ala399Asp	9	1	LDLR_00217
c.1222G>A	p.Glu408Lys	9	1	LDLR_00065
c.1238C>T	p.Thr413Met	9	2	LDLR_00967
c.1285G>A	p.Val429Met	9	2	LDLR_00066
c.1330T>C	p.Ser444Pro	9	2	LDLR_01052
c.1358+2T>A		(Intron 9)	1	LDLR_00544
c.1432G>A	p.Gly478Arg	10	2	LDLR_00072
c.1444G>A	p.Asp482Asn	10	2	LDLR_00237
c.1618G>A	p.Ala540Thr	11	1	LDLR_00243
c.1637G>A	p.Gly546Asp	11	1	LDLR_00078
c.1705+1G>A		(Intron 10)	1	LDLR_00303
c.1715_1719delGTGGCinsA	p.Ser572Asnfs*92	12	1	LDLR_00494
c.1747C>T	p.His583Tyr	12	2	LDLR_00263
c.1775G>A	p.Gly592Glu	12	2	LDLR_00084
c.1784G>A	p.Arg595Gln	12	1	LDLR_00563
c.1814T>C	p.Leu605Pro	12	1	LDLR_00746
c.1955T>C	p.Met652Thr	13	1	[18]
c.2029T>C	p.Cys677Arg	14	2	LDLR_00090
c.2030G>T	p.Cys677Phe	14	1	LDLR_00659
c.2054C>T	p.Pro685Leu	14	2	LDLR_00094
c.2061dupC	p.Asn688Glnfs*29	14	1	LDLR_01158
c.2140 + 1G>A		(Intron 14)	1	LDLR_00853
c.2292delA	p.Ile764Metfs*2	15	1	LDLR_00097
c.2312-3C>A		(Intron 15)	1	LDLR_00948
PCSK9				
c.1120G>T	p.Asp374Tyr	7	1	PCSK9_00059
Large LDLR deletions and duplications				
c.68-?_940+?del		Deletion of exons 2 to 6	2	
c.191-?_1186+?dup		Duplication of exons 3 to 8	1	
c.314-?_694+?del		Deletion of exon 4	1	
c.314-?_1060+?del		Deletions of exons 4 to 7	1	
c.941-?_1186+?dup		Duplication of exons 7 and 8	1	
c.1587-?_1845+?del		Deletion of exons 11 and 12	2	
c.1846-?_2140+?del		Deletion of exons 13 and 14	1	

Table 2 (*continued*)

cDNA	Predicted effect on protein	Exon	Number of patients	Reference[a]
c.1846-?_2140+?dup		Duplication of exons 13 and 14	1	
c.2141-?_3429+?del		Deletion of exons 15 to 18	1	
c.2390-?_2547+?del		Deletion of exon 17	1	

[a] LDLR and PCSK9 numbers refer to ID on the UCL LDLR Mutation Database (www.ucl.ac.uk/ldlr).

shown to affect LDLR expression *in vitro* [17]. Five novel missense mutations (p.Pro84Leu, p.Asp139Glu, p.Asp178Val, p.Ala627Asp, and p.Tyr828Ser) were likely to be pathogenic and three (p.Gln125Lys, p.Phe629Val and p.Tyr828Ser) were likely to be benign (Table 3). In addition p.Gly516Ser was detected, which has been previously considered to be benign [18].

The mean highest LDL-cholesterol in heterozygous mutation-positive patients was significantly higher than in patients without a detectable mutation (6.6 ± 2.2 vs. 4.9 ± 1.5 mmol/L, $p = 3 \times 10^{-15}$). Of interest, 96% of patients with highest recorded LDL-cholesterol above 8.5 mmol/L ($n = 24$) were found to carry a mutation. Patients with heterozygous mutations were also younger at age of referral than those where no mutation could be detected (47 vs. 51 years, $p = 0.02$). In addition, triglyceride values at the time of highest recorded LDL-cholesterol were higher in mutation-negative patients (2.1 ± 1.2 vs. 1.6 ± 0.9 mmol/L in mutation-positive patients; $p = 0.002$).

4. Discussion

In this study, 86 different pathogenic mutations were identified in 129 patients of the 343 tested. These included four compound heterozygotes manifesting a much higher mean LDL-cholesterol level than heterozygotes (12.9 vs. 6.6 mmol/L), suggesting a "trans" rather than "cis" mode of inheritance of the mutations in each of the compound heterozygotes. The most common mutations were *APOB* p.Arg3527Gln (7.2% of mutation-positive patients) and *LDLR* c.313 + 1G > A (4.8%). Overall, 70% of 'definite' FH patients were found to carry a mutation; only 29% of 'probable' and 11% of 'possible' FH patients were mutation-positive. These results are similar to those obtained in Northern European screening centres using comprehensive sequence-based strategies. Taylor et al. had an overall mutation detection rate of 36.5% (comparable to our overall detection rate of 37.6%), with a detection rate of 56% in definite FH and 28% in possible FH (by Simon Broome FH criteria) [19].

Only one patient heterozygous for *PCSK9* p.Asp374Tyr was identified (0.8% of mutation-positive FH patients), which is similar to the 1.7% observed in the UK [19]. Although *PCSK9* mutations are rare, it is important to identify these patients, as they generally have a more severe phenotype than FH patients carrying *LDLR* mutations [20]. It has been shown that *PCSK9* p.Asp374Tyr mutation carriers are younger at presentation, have higher pre-treatment serum cholesterol levels, and are affected by premature CHD more than 10 years earlier than the *LDLR* patients [20]. Of interest, the highest recorded LDL-cholesterol in our study (excluding compound heterozygotes) was seen in a patient heterozygous for *PCSK9* p.Asp374Tyr, at 12.4 mmol/L. Full exon-by-exon sequencing of *PCSK9* is underway in patients classed as 'definite' FH in whom we could not detect a causative mutation.

Patients who carry a mutation have higher LDL-cholesterol levels and CHD risk than patients in whom a mutation is not detected, and null-allele carriers have higher LDL-cholesterol levels compared with receptor-defective mutations [21,22]. Our study also showed that heterozygous mutation-positive patients were younger and had a significantly higher LDL-cholesterol than in patients without a detectable mutation.

Table 3
LDLR variants associated with FH that are novel and/or of unknown pathogenicity.

Variant	Effect on protein	DLCNC score of patient	MutationTaster[a]	PolyPhen2[a]	SIFT[a]	Comments
Novel deletions likely to be pathogenic						
c.196_197delGT	p.Val66Hisfs*63	11				
c.1162delC	p.His388Thrfs*25	14				
c.1776_1778del	p.Gly593del	6				
c.1954_1955delAT	p.Met652Glyfs*16	19				
Novel mutations likely to be pathogenic						
c.−121T>C	−	6	n/a	n/a	n/a	Other mutation reported at adjacent nucleotide [17]
c.251C>T	p.Pro84Leu	10	Disease-causing	Probably damaging	Protein function affected	Other mutations reported at same position (Pro84Ser, Pro84Arg)
c.417C>G	p.Asp139Glu	17[b]	Disease-causing	Probably damaging	Protein function affected	
c.533A>T	p.Asp178Val	9	Disease-causing	Probably damaging	Protein function affected	Other mutations reported at same position (Asp178Asn, Asp178Tyr, Asp178Gly, Asp178Gly)
c.1880C>A	p.Ala627Asp	16	Disease-causing	Possibly damaging	Protein function affected	
c.2483A>C	p.Tyr828Ser	16	Disease-causing	Probably damaging	Protein function affected	Other mutation reported at same position (Tyr828Cys)
Mutations of unknown pathogenicity						
c.373C>A	p.Gln125Lys	1	Polymorphism	Benign	Benign	Novel, probably benign
c.1546G>A	p.Gly516Ser	6	Polymorphism	Probably damaging	Protein function affected	Reported as 'benign' in [18]
c.1885T>G	p.Phe629Val	3	Disease-causing	Probably damaging	Benign	Novel
c.2289G>T	p.Glu763Asp	9	Polymorphism	Possibly damaging	Benign	Novel

[a] MutationTaster – http://www.mutationtaster.org/; PolyPhen2 – http://genetics.bwh.harvard.edu/pph2/; SIFT – http://sift.jcvi.org/.
[b] Compound heterozygote with Cys231*.

The triglyceride concentrations at the time of highest recorded LDL-cholesterol, were higher in mutation-negative than mutation-positive patients, suggesting possible inclusion of patients with familial combined hyperlipidaemia in this group [23].

The mutation detection rate was comparable to that reported in other countries, and the distribution of *LDLR* mutation types (57% missense, 9.7% nonsense, 9.7% frameshift, 1.6% small in-frame deletions, 11.3% splice, 0.8% 5′UTR, 9.7% major rearrangements) similar to that reported in France [18]. The proportion of large *LDLR* deletions and duplications that we observed was twice that observed in the UK (∼5%) [19], reflecting a possible ascertainment bias in Western Australia due to the introduction of genetic testing for FH only in the last few years. Possible reasons for not finding a mutation include the presence of polygenic rather than monogenic hypercholesterolaemia, intronic mutations occurring outside of amplified regions or within primer binding sites, a balanced rearrangement of the *LDLR* (no net gain or loss of *LDLR* exons and hence unable to be detected by MLPA), or the involvement of other, as yet unidentified, genes influencing cholesterol metabolism.

5. Conclusions

In summary, we present the findings of FH mutation screening in Western Australia. It should be noted that the clinic spectrum of patients initially enrolled in the program may not accurately reflect the community spectrum of disease and mutations causative of FH in Western Australia, and that further studies are required in unselected patients and samples. These findings suggest genetic testing should be prioritised to those with high DLCNC scores and that it offers a cost-effective family screening method from FH index cases. Family mutation cascade screening from affected individuals is ongoing and should identify further family members at a much younger age than these probands thereby providing the additional benefit of initiation of preventative measures at an earlier age and optimising cardio-protective intervention. However, in the case of novel mutations (particularly missense mutations) and those of questionable pathogenicity by *in silico* methods will need to be interpreted with caution requiring further *in vitro* or pedigree studies to confirm their pathogenic role.

Acknowledgements

FHWA is supported by grants from the Health Department of Western Australia and the University of Western Australia. JRB is supported by a Practitioner Fellowship from the Royal Perth Hospital Medical Research Foundation.

References

[1] Goldstein JL, Hobbs HH, Brown MS. Familial hypercholesterolemia. In: Scriver CR, Beaudet AL, Sly WS, et al., editors. The metabolic and molecular bases of inherited disease. New York: McGraw-Hill; 2001. p. 2863–913.
[2] Burnett JR, Hooper AJ. Common and rare gene variants affecting plasma LDL cholesterol. Clin Biochem Rev 2008;29:11–26.
[3] Yuan G, Wang J, Hegele RA. Heterozygous familial hypercholesterolemia: an underrecognized cause of early cardiovascular disease. CMAJ 2006;174:1124–9.
[4] World Health Organization. Familial hypercholesterolemia. Report of a second WHO consultation. WHO/HGN/FH/CONS/99.2. 1999.
[5] UCL LDLR Database. http://www.ucl.ac.uk/ldlr/LOVDv.1.1.0/. [accessed 20.04.12].
[6] Humphries SE, Norbury G, Leigh S, Hadfield SG, Nair D. What is the clinical utility of DNA testing in patients with familial hypercholesterolaemia? Curr Opin Lipidol 2008;19:362–8.
[7] Liyanage KE, Burnett JR, Hooper AJ, van Bockxmeer FM. Familial hypercholesterolemia: epidemiology, Neolithic origins and modern geographic distribution. Crit Rev Clin Lab Sci 2011;48:1–18.
[8] Burnett JR, Ravine D, van Bockxmeer FM, Watts GF. Familial hypercholesterolaemia: a look back, a look ahead. Med J Aust 2005;182:552–3.
[9] Leren TP, Finborud TH, Manshaus TE, Ose L, Berge KE. Diagnosis of familial hypercholesterolemia in general practice using clinical diagnostic criteria or genetic testing as part of cascade genetic screening. Community Genet 2008;11:26–35.
[10] Watts GF, van Bockxmeer FM, Bates T, Burnett JR, Juniper A, O'Leary P. A new model of care for familial hypercholesterolaemia from Western Australia: closing a major gap in preventive cardiology. Heart Lung Circ 2010;19:419–22.
[11] Familial Hypercholesterolaemia Western Australia Program Committee. Model of care – familial hypercholesterolaemia, http://www.genomics.health.wa.gov.au/fh/docs/FH_Model_of_care.pdf; August 2008 [accessed 20.04.12].
[12] Watts GF, Sullivan DR, Poplawski N, et al. Familial hypercholesterolaemia: a model of care for Australasia. Atheroscler Suppl 2011;12:221–63.
[13] Leren TP. Mutations in the PCSK9 gene in Norwegian subjects with autosomal dominant hypercholesterolemia. Clin Genet 2004;65:419–22.
[14] Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. Nat Methods 2010;7:248–9.
[15] Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc 2009;4:1073–81.
[16] Schwarz JM, Rodelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods 2010;7:575–6.
[17] Francova H, Trbusek M, Zapletalova P, Kuhrova V. New promoter mutations in the low-density lipoprotein receptor gene which induce familial hypercholesterolaemia phenotype: molecular and functional analysis. J Inherit Metab Dis 2004;27:523–8.
[18] Marduel M, Carrie A, Sassolas A, et al. Molecular spectrum of autosomal dominant hypercholesterolemia in France. Hum Mutat 2010;31:E1811–24.
[19] Taylor A, Wang D, Patel K, et al. Mutation detection rate and spectrum in familial hypercholesterolaemia patients in the UK pilot cascade project. Clin Genet 2010;77:572–80.
[20] Naoumova RP, Tosi I, Patel D, et al. Severe hypercholesterolemia in four British families with the D374Y mutation in the PCSK9 gene: long-term follow-up and treatment response. Arterioscler Thromb Vasc Biol 2005;25:2654–60.
[21] Humphries SE, Cranston T, Allen M, et al. Mutational analysis in UK patients with a clinical diagnosis of familial hypercholesterolaemia: relationship with plasma lipid traits, heart disease risk and utility in relative tracing. J Mol Med 2006;84:203–14.
[22] Junyent M, Gilabert R, Zambon D, et al. Femoral atherosclerosis in heterozygous familial hypercholesterolemia: influence of the genetic defect. Arterioscler Thromb Vasc Biol 2008;28:580–6.
[23] de Graaf J, van der Vleuten G, Stalenhoef AF. Diagnostic criteria in relation to the pathogenesis of familial combined hyperlipidemia. Semin Vasc Med 2004;4:229–40.
[24] Soria LF, Ludwig EH, Clarke HR, Vega GL, Grundy SM, McCarthy BJ. Association between a specific apolipoprotein B mutation and familial defective apolipoprotein B-100. Proc Natl Acad Sci USA 1989;86:587–91.