Atherosclerosis xxx (2017) 1–7

Contents lists available at ScienceDirect

Atherosclerosis

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

Greater preclinical atherosclerosis in treated monogenic familial hypercholesterolemia *vs.* polygenic hypercholesterolemia

Mahtab Sharifi [a, b], Elizabeth Higginson [c], Sven Bos [d], Angela Gallivan [b], Darren Harvey [b], Ka Wah Li [a], Amali Abeysekera [c], Angela Haddon [c], Helen Ashby [c], Kate E. Shipman [c], Jackie A. Cooper [a], Marta Futema [e], Jeanine E. Roeters van Lennep [d], Eric J.G. Sijbrands [d], Mourad Labib [c], Devaki Nair [b], Steve E. Humphries [a, *]

[a] Centre for Cardiovascular Genetics, University College London, The Rayne Institute, University Street, London, WC1E 6JF, UK
[b] Department of Clinical Biochemistry, The Royal Free London NHS Foundation Trust, Pond Street, London, NW3 2QG, UK
[c] Department of Clinical Biochemistry, Russells Hall Hospital, The Dudley Group NHS Foundation Trust, West Midlands, DY1 2HQ, UK
[d] Department of Internal Medicine, Rotterdam, The Netherlands
[e] Inherited Cardiovascular Diseases, Institute for Cardiovascular Science, University College London, Paul O'Gorman Building, 72 Huntley Street, London, WC1E 6DD, UK

ARTICLE INFO

Article history:
Received 29 January 2017
Received in revised form
27 April 2017
Accepted 12 May 2017
Available online xxx

Keywords:
Familial hypercholesterolemia
Polygenic hypercholesterolemia
Carotid intima media thickness
Coronary artery calcification
Preclinical atherosclerosis

ABSTRACT

Background and aims: Familial hypercholesterolemia (FH) is a common inherited disorder of low density lipoprotein-cholesterol (LDL-C) metabolism. It is associated with higher risk of premature coronary heart disease. Around 60% of patients with a clinical diagnosis of FH do not have a detectable mutation in the genes causing FH and are most likely to have a polygenic cause for their raised LDL-C. We assessed the degree of preclinical atherosclerosis in treated patients with monogenic FH *versus* polygenic hypercholesterolemia.
Methods: FH mutation testing and genotypes of six LDL-C-associated single nucleotide polymorphisms (SNPs) were determined using routine methods. Those with a detected mutation (monogenic) and mutation-negative patients with LDL-C SNP score in the top two quartiles (polygenic) were recruited. Carotid intima media thickness (IMT) was measured by B-mode ultrasound and the coronary artery calcium (CAC) score was performed in three lipid clinics in the UK and the Netherlands.
Results: 86 patients (56 monogenic FH, 30 polygenic) with carotid IMT measurement, and 166 patients (124 monogenic, 42 polygenic) with CAC score measurement were examined. After adjustment for age and gender, the mean of all the carotid IMT measurements and CAC scores were significantly greater in the monogenic than the polygenic patients [carotid IMT mean (95% CI): 0.74 mm (0.7–0.79) *vs.* 0.66 mm (0.61–0.72), $p = 0.038$ and CAC score mean (95%): 24.5 (14.4–41.8) *vs.* 2.65 (0.94–7.44), $p = 0.0004$].
Conclusions: In patients with a diagnosis of FH, those with a monogenic cause have a higher severity of carotid and coronary preclinical atherosclerosis than those with a polygenic aetiology.

1. Introduction

Familial hypercholesterolemia (FH) is a common autosomal dominant disorder and a well-known cause of premature coronary heart disease (CHD) [1]. It has a frequency of 1 in 200–500 in most European countries [2,3], and is caused by mutations in the low density lipoprotein receptor (*LDLR*) gene, the gene coding for apolipoprotein B (*APOB*) or the gene encoding protein convertase subtilisin/kexin 9 (*PCSK9*) [4]. The clinical diagnosis of definite FH is based on a low density lipoprotein-cholesterol (LDL-C) level >4.9 mmol/L and the presence of tendon xanthomata, while patients with a diagnosis of possible FH do not have xanthomata but have a family history of premature CHD and/or hypercholesterolemia [5].

Only in 60–80% of definitive FH and in 20–30% of possible FH cases, can a mutation be found. [6], and since possible FH cases usually represent around two-thirds of the lipid clinic patient

* Corresponding author. Centre for Cardiovascular Genetics, The Rayne Building, University College London, London, WC1E 6JF UK.
E-mail address: steve.humphries@ucl.ac.uk (S.E. Humphries).

http://dx.doi.org/10.1016/j.atherosclerosis.2017.05.015

group, this means that overall a mutation can be found in roughly only 40% of those with a clinical diagnosis of FH [6]. It has been shown that a significant proportion of the patients with a clinical diagnosis of FH, where no mutation is found, are likely to have a polygenic explanation for their raised LDL-C level [7,8]. A Global Genetic Consortium meta-analysis identified multiple loci where common variants associated with slight deviation in LDL-C levels [9]. Based on the common LDL-C raising single nucleotide polymorphisms (SNPs), a weighted SNPs score has been developed [7,8]. Using this score, it appears that at least 20% of FH patients without a mutation are likely to have a polygenic explanation for their LDL-C level of over 4.9 mmol/L. In contrast, in individuals with a low LDL-C SNP score, there is a possibility that there may be a yet unidentified monogenic cause.

The elevated risk for CHD in FH patients with a detected mutation has been convincingly confirmed by Khera et al. in a population-based analysis, which showed that patients with LDL-C >4.9 mmol/L and no FH mutation had a 6-fold higher risk for CHD and those with both LDL-C >4.9 mmol/L and an FH mutation had a 22-fold higher risk compared to subjects with normal LDL-C and no mutation [10]. The Simon Broome register showed that the Standardized Mortality Ratio (SMR) for CHD in patients with a clinical diagnosis of definite FH was higher than in patients with a possible diagnosis of FH [SMR = 2.94 (2.28–3.80) *vs.* 2.05 (1.45–2.82)] [11]. Since we now know that a mutation can be found in 60–80% of definite FH patients, this means that the majority of this group had a monogenic cause, while the detection rate in possible FH is only 20–30% and they were likely to have polygenic hypercholesterolemia. Humphries et al. also reported a significantly higher Odds Ratio (OR) for having CHD in FH patients with an *LDLR* mutation *versus* patients where no mutation was found [OR = 1.84 *vs.* 1.00, $p = 0.02$] [12]. Several case control studies also reported the raised CHD risk in monogenic FH patients compared to the patients with a high LDL-C with no mutation found and the general population by means of the imaging measurements such as angiography, CT scan and carotid ultrasounds [13–15].

The European guideline for cardiovascular risk stratification recommends imaging techniques for intermediate and high risk asymptomatic individuals such as patients with FH [16]. Coronary artery calcium (CAC) score has long been recognized as a surrogate marker for coronary atherosclerosis and a good predictor of future cardiovascular events and all-cause mortality in asymptomatic people [17,18]. Several clinical trials have shown that carotid IMT changes are sensitive to changes in the LDL-C levels [19]. A raised carotid IMT measurement is associated with increased risk of CHD and serves as an atherosclerotic surrogate end-point for therapeutic interventions [20].

While raised LDL-C is a known risk factor for atherogenesis [21], there are only limited data available to examine whether the extent of early atherosclerosis is higher in treated monogenic FH than in clinically diagnosed FH patients with the same level of LDL-C level but a polygenic cause. In this study, for the first time, we used the genetic testing to confirm the presence of the polygenic locus in individuals with a raised LDL-C, where no FH causing mutation was found and we compared the degree of preclinical atherosclerosis in these patients with monogenic FH patients.

2. Materials and methods

2.1. Subjects

Data from two outpatient lipid clinics in the UK, the Royal Free Hospital in London and the Russells Hall Hospital in Dudley, and an outpatient lipid clinic in the Netherlands, the Erasmus Medical Centre in Rotterdam, were included in this study over the period 2014–2016.

The following clinical diagnostic criteria for FH were used: an LDL-C level above the 95th percentile for gender and age in combination with the presence of tendon xanthomas in the patient or in a first degree relative, or an LDL-C level above the 95th percentile for gender and age in a first degree relative, or a proven coronary artery disease in a first degree relative under the age of 60. No patients had proven CHD or had any symptoms suggestive of ischemic heart disease.

All patients with secondary causes of hypercholesterolemia such as renal disease, liver disease and thyroid disease were excluded from the study. All the patients with a CHD disease or any symptoms of ischemic heart disease, renal insufficiency (serum creatinine > 120 mmol/L), known contrast allergy or atrial fibrillation were excluded from the study.

All patients had a genotyping test to confirm their monogenic or polygenic cause (see below) and they all had a CT scan to measure coronary calcium score or a carotid ultrasound to measure carotid intima media thickness. All patients were clinically asymptomatic, meaning they had no cardiac symptoms or any history of CHD. The inclusion age for the study varied from 30 to 70 years to have a carotid ultrasound and 40 to 70 for CT scan. All patients gave written informed consent. The ethical approval was obtained from the relevant ethics committees (13/LLO/1334).

Data from all the monogenic patients and the patients with no mutation and a gene score in the top two quartiles of *LDL-C* gene scoring were included in the final analysis of this study. The data from the patients with no mutation in genotyping and a low gene score were excluded from the analysis. The patients at the Rotterdam and Russells Hall hospital in the UK only underwent a CT scan to measure the CAC score, while the patients at the Royal Free hospital had only carotid IMT measurement. From a total number of 312 patients (94 patients at the Russells Hall and 97 at the Royal Free hospital in the UK and 121 patients in the Netherlands), data from 166 patients with a CAC score and 86 patients with a carotid IMT measurement were included in the final analysis.

2.2. Molecular analysis

2.2.1. FH genotyping

All participants had FH mutation testing for all 18 exons of the *LDLR* gene, a fragment of exon 26 of *APOB* to cover the area for the common mutation p.Arg3527Gln, and exon 7 of *PCSK9* to cover p.Asp374Tyr using direct sequencing analysis of PCR products [22–24]. Multiplex Ligation-dependent Probe Amplification to detect gross deletions and insertions in *LDLR,* according to the manufacturer's protocol on all samples (MRC-Holland, Amsterdam, the Netherlands), and *in silico* prediction of pathogenicity of identified variants were also performed [25].

2.2.2. LDL-C gene score calculations for polygenic hypercholesterolemia

The patients with no mutation detected in their FH genotyping test were genotyped for six LDL-C-raising SNPs (*CELSR2* (rs629301), *APOB* (rs1367117), *ABCG8* (rs4299376), *LDLR* (rs6511720), and *APOE* (rs429358, and rs7412)) at the Cardiovascular Genetics Lab at UCL in the UK. KASPar™ PCR technique (Kbiosciences, UK Hoddesdon, Herts, UK) or TaqMan® assays (Life Technologies, Carlsbad, California, US) and genotype calls for all assays was carried out using an automated system, the results of which were checked manually by study personnel using SNP viewer® software (Supplementary Table 1) as previously described [7,8]. Patients were grouped into quartiles of the gene score based on those reported by Futema et al. for a healthy UK population. It has been estimated using probability calculations that patients in the top three quartiles of the score have

Please cite this article in press as: M. Sharifi, et al., Greater preclinical atherosclerosis in treated monogenic familial hypercholesterolemia *vs.* polygenic hypercholesterolemia, Atherosclerosis (2017), http://dx.doi.org/10.1016/j.atherosclerosis.2017.05.015