Atherosclerosis 269 (2018) 1–5

Contents lists available at ScienceDirect

Atherosclerosis

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

Autosomal recessive hypercholesterolemia in Spain

Rosa María Sánchez-Hernández [a, *], Pablo Prieto-Matos [b], Fernando Civeira [c], Eduardo Esteve Lafuente [d], Manuel Frías Vargas [e], José T. Real [f], Fernando Goñi Goicoechea [g], Francisco J. Fuentes [h], Miguel Pocovi [i], Mauro Boronat [a], Ana María Wägner [a], Luis Masana [j]

[a] Sección de Endocrinología y Nutrición, Complejo Hospitalario Universitario Insular Materno Infantil de Gran Canaria, Instituto Universitario de Investigaciones Biomédicas y Sanitarias (IUIBS) de la Universidad de Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain
[b] Unidad de Endocrinología Pediátrica, Hospital Universitario de Salamanca, Instituto de Investigación Biomédica de Salamanca, Spain
[c] Unidad Clínica y de Investigación en Lípidos y Arterioesclerosis, Hospital Universitario Miguel Servet, IIS Aragón, Centro de Investigación Biomedica en Red de Enfermedades Cardiovasculares (CIBERCV), Universidad de Zaragoza, Zaragoza, Spain
[d] Servicio Endocrinología y Nutrición, Hospital Universitari de Girona Dr. Josep Trueta, Spain
[e] Centro de Salud San Andrés, Madrid, Spain
[f] Servicio de Endocrinología y Nutrición, Hospital Clínico Valencia, Departamento de Medicina, Universidad de Valencia, INCLIVA, Centro de Investigación Biomedica en Red de Diabetes y Enfermedades Metabolicas Asociadas (CIBERDEM), Spain
[g] Servicio Endocrinología y Nutrición, Hospital Universitario de Basurto, Bilbao, Spain
[h] Hospital Universitario Reina Sofía, Universidad de Córdoba, Centro de Investigación Biomédica en Red de Fisiopatolgía de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III (ISCIII), Madrid, Instituto Maimónedes de Investigación Biomédica de Córdoba (IMIBIC), Spain
[i] Departamento de Bioquímica y Biología Molecular y Celular, Facultad de Ciencias, Universidad de Zaragoza, IIS Aragón, CIBERCV, Zaragoza, Spain
[j] Unidad de Medicina Vascular y Metabolica, Unidad de Investigación en Lipidos y Arterioesclerosis, Hospital Universitario "Sant Joan", Universitat Rovira i Virgili, IISPV, CIBERDEM, Reus, Madrid, Spain

ARTICLE INFO

Article history:
Received 17 October 2017
Received in revised form
21 November 2017
Accepted 5 December 2017
Available online 6 December 2017

Keywords:
Familial hypercholesterolemia
Autosomal recessive hypercholesterolemia
LDLRAP1

ABSTRACT

Background and aims: Autosomal recessive hypercholesterolemia (ARH) is a very rare disease, caused by mutations in LDL protein receptor adaptor 1 (*LDLRAP1*). It is characterized by high levels of low-density lipoprotein cholesterol (LDL-C) and increased risk of premature cardiovascular disease. We aimed to characterize ARH in Spain.
Methods: Data were collected from the Dyslipidemia Registry of the Spanish Atherosclerosis Society. A literature search was performed up to June 2017, and all diagnostic genetic studies for familial hypercholesterolemia of Spain were reviewed.
Results: Seven patients with ARH were identified, 6 true homozygous and one compound heterozygous with a novel mutation: *c.[863C>T];p.[Ser288Leu]*. High genetic heterogeneity was found in this cohort. True homozygous subjects for *LDLRAP1* have more severe phenotypes than the compound heterozygous patient, but similar to patients with homozygous familial hypercholesterolemia (HoFH). Cardiovascular disease was present in 14% of the ARH patients. LDL-C under treatment was above 185 mg/dl and the response to PCSK9 inhibitors was heterogeneous. Finally, the estimated prevalence in Spain is very low, with just 1 case per 6.5 million people.
Conclusions: ARH is a very rare disease in Spain, showing high genetic heterogeneity, similarly high LDL-C concentrations, but lower incidence of ASCVD than HoFH.

1. Introduction

Autosomal recessive hypercholesterolemia (ARH) (ARH; OMIM #603813) is a rare monogenic disease, characterized by very high levels of low-density lipoprotein cholesterol (LDL-C), usually above 400 mg/dl, and increased risk of premature atherosclerotic cardiovascular disease (ASCVD) [1]. ARH is caused by loss-of-function

* Corresponding author. Present/permanent address. Hospital Universitario Insular de Gran Canaria, Avenida Marítima Sin Número, CP: 35016, Las Palmas de Gran Canaria, Las Palmas, Spain.
E-mail address: rosamariasanher@gmail.com (R.M. Sánchez-Hernández).

https://doi.org/10.1016/j.atherosclerosis.2017.12.006
0021-9150/

mutations in *LDLRAP1*, a gene encoding an adaptor protein involved in the uptake of the LDL receptor (LDLr) and clearance of LDL particles. LDLRAP1 protein is an LDLr chaperone that binds to the LDLr, to allow the LDL/LDLr complex to be internalized in the clathrin coated pit [2]. The gene causing ARH is located on chromosome 1. It was identified in 2001 [3], although the disease had been clinically described by Khachadurian and Uthman in 1973, in four siblings from a Lebanese family, with a phenotype that reminded of homozygous familial hypercholesterolemia (HoFH), but whose parents were normolipemic [4]. Given its recessive inheritance pattern, two mutated alleles have to be present for the disease to develop. Although homozygous mutations in *LDLRAP1* are the most frequent cause of ARH, compound heterozygous mutations have also been described. Mutations causing ARH are null alleles with nonsense mutations resulting in a truncated or non-functional protein [3]. Heterozygous carriers of *LDLRAP1* mutations have normal LDL-C concentrations, because one functional copy of the gene is enough to maintain LDLr uptake.

LDL particle clearance rate is similar in ARH and HoFH patients who lack the LDLr, but much lower than in normolipemic patients. Since most ARH forms are clinically indistinguishable from HoFH, the former is considered a clinical subtype of HoFH [1]. Its manifestations include extremely high LDL-C levels, very extensive xanthomas, aortic stenosis and premature ASCVD, although less aggressive phenotypes have also been described [5]. Furthermore, in spite of similarly low LDL particle uptake, and for reasons that are still to be elucidated, ARH patients have lower rates of ASCVD and better response to lipid lowering treatment than HoFH [6].

The real prevalence of ARH remains undetermined and could be, as described for HoFH, higher than previously reported. Our present study aimed to establish an estimation of ARH prevalence, phenotype variability, genotype-phenotype correlation and response to lipid-lowering treatment in all ARH cases diagnosed in Spain.

2. Materials and methods

2.1. Patients

The identification of all potential ARH patients was performed by several approaches:

1. A search on the Dyslipidemia Registry of the Spanish Atherosclerosis Society, an active on-line registry, in which 50 certified lipid units throughout all Spanish regions enter cases with different types of primary hyperlipidemia. These lipid units are the facilities in the Spanish Public National Health System where severe primary hyperlipidemias are usually referred for diagnosis and treatment.
2. Extensive literature search up to June 2017 of all PubMed recorded publications from Spain in which any of the following words were included: Homozygous Familial Hypercholesterolemia/ARH/Autosomal recessive hypercholesterolemia/LDLRAP1.
3. Review of all diagnostic genetic studies for FH performed in Spain from 1996 to June 2017. All genetic tests were performed at one of the following six Spanish centers: Zaragoza University, Progenika-Biopharma SA (Derio, Vizcaya), Hospital Clínico in Valencia, Hospital Santa Creu i Sant Pau in Barcelona, Hospital La Paz in Madrid and Laboratorio de Análisis Clínico-Genéticos, Gendiag.exe, Barcelona.

2.2. Molecular diagnosis

Diagnosis of ARH was defined by the presence of two documented pathogenic mutations in *LDLRAP1*, including true homozygous and compound heterozygous mutations. Double heterozygous subjects, with mutations in other FH candidate genes (*LDLR*, *APOB*, *PCSK9*), were excluded. Next generation sequencing of the promoter, exon, and intron-exon boundaries of the *LDLRAP1* gene was performed [7]. The heterozygous status, defined as the presence of one pathogenic variant of *LDLRAP1*, was evaluated in all the molecular studies where the *LDLRAP1* gene was sequenced. Predict-SNP [8], Polyphen-2 [9] and SIFT [10] bioinformatic tools were used to predict functionality of previously unknown mutations.

2.3. Clinical and biochemical measurements

The clinical data recorded were family history of hypercholesterolemia and premature ASCVD, personal history of hypercholesterolemia and ASCVD, presence of aortic stenosis, current lipid-lowering treatment, and a physical examination at diagnosis, including the presence of arcus cornealis and xanthomata. All data were collected directly by the patient's attending physician or from the Dyslipidemia Registry of the Spanish Arteriosclerosis Society.

Results of blood tests were also recorded, including a fasting lipid profile (total cholesterol, HDL-cholesterol, LDL-C and triglycerides) without and with current lipid lowering treatment. Samples were processed at the standardized laboratories in the participating centers.

All patients who underwent molecular diagnosis were informed and signed a written, informed consent form. In each center, the study was approved by the local ethics committee.

2.4. Statistical analysis

Prevalence was calculated as the number of ARH cases divided by the mean total number of population for the whole period. Mean population was estimated using demographic data provided by the Spanish National Institute for Statistics (Instituto Nacional Estadística).

Data are expressed as mean ± SD for numeric variables that followed a normal distribution or as median and range for other numeric variables. Between-group comparisons were performed using Student's *t* or Mann–Whitney's U tests. Differences were considered significant when the 2-tailed *p* value was <0.05.

3. Results

Seven ARH patients were identified, 6 true homozygous and one compound heterozygous. Their clinical and genetic features are displayed in Table 1. Four were female and their mean age at diagnosis was 19.2 years. Xanthomata were present in 2 patients. Mean LDL-C at diagnosis was 689.2 ± 319.5 mg/dl. Regarding mutations in *LDLRAP1,* except for siblings, all were different among patients, and were previously described [11,12]. The mutation *c.[863C>T];p.[Ser288Leu]* was a novel mutation, not previously described, possibly pathogenic according to *in silico* predictions (PredictSNP 87%, PolyPhen 0.806, SIFT 0) identified in the compound heterozygous patient in heterozygosis with *c.[653C>T]; p.[Thr218Ile]*, which is present in the Human Gene Mutation Database (www.ucl.ac.uk). Furthermore, most homozygous patients had null allele mutations. Interestingly, the compound heterozygous subject presented a milder phenotype, with much lower baseline LDL-C concentrations and later diagnosis than most true homozygous. Regarding cardiovascular disease, only one patient had suffered from ASCVD (at 55), although the age at the time of this report was 38.3 years and 5 subjects were below 50 years of age. No patient developed aortic stenosis and only two subjects