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Autosomal recessive hypercholesterolemia in Spain

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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

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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

Table 1
Baseline clinical and genetic data.

Subject number	Gender	Genotype nucleotide substitution (c.DNA)	Genotype allele name (Protein)	Age at diagnosis (years)	Xanthomata	Total cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	Triglycerides (mg/dl)
1	Female	[c.603dupC];[c.603dupC]	p.[Ser202LeuFs*19];p.[Ser202LeuFs*19]	32	Yes	1056	50	901.6	522
2	Female	c.[431dupA]; [431dupA]	p.[His144Glnfs*27];[His144Glnfs*27]	12	Yes	570	46	502.6	107
3	Female	c.[207delC];[207delC]	p.Ala70ProfsX19;p.Ala70ProfsX19	2	No	565	37	511.2	84
4	Female	c. [345-?_459+?del];[345-?_459+?del]	NA	2 months	No	1200	40	1135	123
5	Male	c. [345-?_459+?del];[345-?_459+?del]	NA	4 months	No	1040	31	986	115
6	Male	c.[1A>G];c.[1A>G]	p.[Met1Val];p.[Met1Val]	48	No	621	60	535	130
7	Male	c.[653C>T][a];c.[863C>T][b]	p.[Thr218Ile];p.[Ser288Leu]	40	No	325	50	253	109

[a] Present in database www.ucl.ac.uk.
[b] Not described, possibly pathogenic *in silico* prediction.

Table 2
Current clinical data, treatment, post-treatment values of LDL-C and % of LDL-C reduction compared to baseline concentrations.

Subject number	Age (years)	BMI (kg/m^2)	ASCVD	Aortic stenosis	Lipid lowering treatment	Total cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	Triglycerides (mg/dl)	% LDL-C reduction
1	45	24.97	No	No	Atorvastatin 80 mg	221	63	150	51	83.4%
2	60	28	Yes, 55 y	No	Atorvastatin 80 mg + ezetimibe 10 mg + resins (4 g/d) + evolocumab 420/15 days	274	56	210	81	58.2%
3	11	18.3	No	No	Atorvastatina 40 mg + ezetimibe 10 mg	389	35	334	100	41.5%
4	27	22.3	No	No	Rosuvastatin 20 mg + Ezetimibe 10 mg	239	47	176	80	84.5%
5	33	27.03	No	No	Rosuvastatin 40 mg + Ezetimibe 10 mg + Evolocumab 140 mg/15 days	267	37	215	76	78.2%
6	50	29	No	No	Atorvastatin 80 + ezetimibe 10 mg + evolocumab 420 mg/15 days	176	44	60	107	88.8%
7	42	23.27	No	No	Atorvastatin 20 mg + ezetimibe 10 mg	223	53	149	107	41.1%

BMI, body mass index; ASCVD, atherosclerotic cardiovascular disease; y, years.

have atherosclerotic plaques in the carotid arteries. Current clinical data, lipid profile and lipid lowering treatment are shown in Table 2.

Concerning treatment, most patients were on combined therapy with a statin and ezetimibe, whereas one patient was on atorvastatin monotherapy. Three patients were receiving triple, combined treatment with PCSK9 inhibitors (evolocumab), obtaining heterogeneous responses. Specifically, case number 2 showed no response to evolocumab. This patient had previously been offered LDL apheresis, which she declined. Case number 5 showed only a small reduction (19%) in LDL-C concentrations, which remained very high (215 mg/dl), in spite of the PCSK9 inhibitor administration. Finally, in case number 6, who received higher doses of evolocumab, a more significant reduction of LDL-C (59%) was achieved (see Table 2). Regarding drug therapy, the starting age was 23.3 ± 15.8 years and the mean duration of the treatment was 13 ± 9 years.

After treatment, the achieved mean LDL-C level was 185 ± 79.3 mg/dl for the true homozygous patients and 149 mg/dl for the compound heterozygous subject.

Regarding the prevalence of ARH in Spain, this was estimated as approximately 1 case per 6.5 million people (7 cases out of over 46.5 million people in Spain at 1st January of 2017) [13].

In 3623 subjects who undergone LDLRAP1 sequencing with the clinical diagnosis of familial hypercholesterolemia, 19 *LDLRAP1* carriers with damaging or probably damaging mutations by bioinformatic analysis were detected. These data provide a frequency of *LDLRAP* carriers of 0.52%.

4. Discussion

ARH is a very rare disease affecting less than 1:1000,000 of the population [6], with the exception of Sardinia, a region in Italy with a founder effect [14,15], that has a prevalence of 1:40,000 including both true homozygotes and compound heterozygotes. The present report shows that the estimated prevalence of ARH in Spain is extremely low, with only 7 cases out of over 46.5 million people, representing a prevalence of approximately 1 case per 6.5 million people. Considering the extreme phenotype of ARH patients and the extensive case search in our study, it seems improbable that many subjects with ARH have remained undetected. Hence, our study provides an approximation to the prevalence of this disease in a population with wide genetic heterogeneity. This prevalence is in agreement with recently published data from the Sicilian population, with a c.432insA (p.H144QfsX26) mutation carrier status of 1:2500 [16]. Furthermore, except for two siblings, the ARH cases in Spain are unrelated and come from geographically spread Spanish regions.

Most patients were true homozygotes carrying null allele mutations. Indeed, the vast majority of ARH subjects reported so far are true homozygotes with a premature termination codon or truncated protein [3], leading to complete loss of function of the LDLRAP1 [17]. This suggests that complete abolition of LDLRAP1 is needed to express the disease, in agreement with *in vitro* studies demonstrating that partial LDLRAP1 activity restores LDL/LDLr internalization in hepatic cells [18], and explains the discrepancy between the prevalence of *LDLRAP1* mutation carriers and ARH subjects. Probably, severe, biallelic mutations in LDLRAP1 are required for the full phenotype of ARH to be expressed. The high prevalence of mutations carriers in our study, approximately 1:200 subjects with primary hypercholesterolemia, would suggest that non-severe mutations in *LDRAP1* may play a role in the pathogenesis of some forms of polygenic hypercholesterolemia.

Except in Sardinia, where 3 alleles are responsible for most of the cases [15], ARH is due to multiple, different mutations along the *LDLRAP1* gene [3]. Great genetic heterogeneity was also observed in the Spanish patients, among whom only two siblings had the same mutations in homozygosis, while the rest of cases carried different pathogenic mutations. The compound heterozygous patient has a new, previously undescribed variant: *c.[863C>T]*, which was probably pathogenic according to *in silico* predictions, although no functional study was performed.

Regarding ASCVD, only one (14.3%) female ARH patient suffered from premature ASCVD at the age of 55 and no patient had developed aortic stenosis during follow-up. This represents a low frequency of ASCVD in comparison to previous reports. In a study performed in Sardinian patients with ARH, 11 out of 28 had evidence of coronary atherosclerosis and 3 had suffered a myocardial infarction [19] and another Italian study found a prevalence of 40% of premature ASCVD in ARH [6]. Regarding HoFH subjects, in a Spanish cohort, the ASCVD prevalence was higher in carriers of null allele mutations of *LDLR* (50%) and defective allele mutations (43.8%) [20]. It must be considered that our ARH group is composed of quite young patients, with clinical diagnosis at an early age, and potent lipid-lowering treatment for many years, all of which could explain the low prevalence of ASCVD, but also emphasizes the good prognosis of these patients with appropriate lipid-lowering treatment begun early in life.

Although affected patients have approximately 9-fold lower risk of ASCVD than HoFH and aortic stenosis appears later [6], ARH has a lipid phenotype similar to HoFH [21]. In this regard, patients in our cohort showed very high LDL-C at diagnosis, approaching concentrations seen in patients with HoFH with a defective allele [1]. However, in the Spanish HoFH cohort LDL-C at diagnosis was lower in defective allele carriers than in ARH patients (488 mg/dl vs 689.2 mg/dl) [20]. The more favorable clinical prognosis of ARH patients is probably explained by their response to classical lipid-lowering treatment, compared to that of patients with HoFH. The LDLRAP1 protein is necessary for the LDLr uptake by hepatic cells and lymphocytes, but the lack of LDLRAP1 does not affect the internalization of LDLr in fibroblasts [2]. These facts could also contribute to the milder ASCVD phenotype in ARH compared with HoFH.

The LDL-C reduction with statins in ARH is variable. Some series have reported decreases of up to 60% [5,19,22], ranging between 60 and 80% when high potency statins are combined with ezetimibe [14,22,23]. In the present report, the LDL-C decrease with lipid-lowing therapy ranged from 41 to 84%, confirming a much larger lipid response than HoFH and very similar to that observed in general population. Nevertheless, our patients' mean LDL-C after treatment was 185 mg/dl, an unacceptably high level that implies the need of new treatments to achieve the recommended LDL-C goals. A heterogeneous reduction of LDL-C levels response to PCSK9 inhibitor was observed in this case report. In agreement with previous reports, showing a moderate (15−32%) reduction of LDL-C in ARH [24,25], one patient here treated with this drug, experienced a mild decrease in LDL-C (−19%). Besides, another patient did not show any response at all. However, one homozygous patient experimented a better response (−59%). PCSK9 reduces LDLr expression in the cell surface, and activates monoclonal antibodies against PCSK9 enhance LDL-LDLr uptake by increasing the LDLr activity in the presence of normal LDLRAP1 protein, hence with a predictably mild response in ARH subjects who lack functional LDLRAP1 [25]. Lomitapide, an inhibitor of the microsomal triglyceride transfer protein, could be an alternative treatment for ARH, because it acts independently of the LDLr. An Italian study reported 5 ARH patients treated with lomitapide achieving an important reduction (68.2 ± 24.8%) in LDL-C [26].

Nevertheless, the small number of patients limits the conclusions that can be drawn from the results on genotype-phenotype correlation and response to therapy.

4.1. Conclusions

ARH is a rare disease with a very low prevalence in Spain, with only seven cases diagnosed so far. It is a disease with great genetic heterogeneity, similarly high LDL-C concentrations but lower incidence of ASCVD than HoFH. In spite of the strong LDL-C lowering effect of statins and ezetimibe, LDL-C is not adequately controlled in this population.

Conflict of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Author contributions

RMS drafted the manuscript, LM, PPM, EE, JTR, FF, FG and MF contributed with patient data, MP performed genetic analysis. RMS, FC, MB and AMW contributed to the interpretation of the data and writing the manuscript. All of the authors have read and accept the final version of the paper.

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