SENSITIVITY OF THE CLINICAL CRITERIA FOR SUSPECTED FAMILIAL HYPERCHOLESTEROLEMIA IN THE DETECTION OF DIAGNOSTIC MUTATIONS OF THE DISEASE

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Background: Familial Hypercholesterolemia (FH) is characterized by high LDLc levels and high cardiovascular risk, being caused by mutations *in LDLR, APOB, PCSK9, APOE, STAP1* or *LDLRAP1* genes. The European Atherosclerosis Society (EAS) has recommended some criteria of clinical suspicion, but their sensitivity and efficiency have not been studied. EAS criteria include the presence of any of the following:

- Total plasma cholesterol ≥ 310 mg/dL in adults or > 95th percentile adjusted for age and sex
- Premature CHD (<55 years male and <65 years female)
- Sudden premature coronary death in a family member
- Tendon xanthomas

Objective: To establish the frequency of subjects with EAS criteria of clinical suspicion of FH who are carriers of mutations in candidate genes in a cohort from Aragon, Spain.

Material and Methods: The Aragon Work Health Study (AWHS) is a prospective study in which 5676 workers from a factory are being studied from 2009. From this cohort, we selected a group of subjects with one of these inclusion criteria:

1. Total cholesterol ≥ 310 mg/dL in at least two determinations between 2009 and 2014 in absence of secondary causes OR LDLc > 95 percentile of the distribution of the Spanish population. Subjects on statin treatment with LDLc > 130 mg/dL
2. Premature coronary heart disease (< 55 years in males and < 65 years in females)

Secondary causes of exclusion:
a. TSH > 6 ng/mL
b. Direct bilirubin > 1 mg/dL
c. Diabetes mellitus poorly controlled (HbA1c > 8%)
d. Obesity grades II and III (BMI > 35 kg/m2)
e. Triglycerides > 500 mg/dL
f. Creatinine > 2 mg/dL
g. Macroalbuminuria
h. Intake of drugs that increase cholesterol

In all selected subjects, *LDLR, APOB, PCSK9, APOE, STAP1* and *LDLRAP1* genes were sequenced by NGS.

Results

Table 1. Clinical characteristics of the selected subjects.

	2009	2010	2011	2012	2013	2014
N=257						
Age, years	44,1 ±7,69	45,1 ± 7,69	46,1 ± 7,69	47,1 ± 7,69	48,1 ± 7,69	49,1 ± 7,69
Weight, kg	79,4 ± 10,4	80,0 ± 10,7	80,0 ± 10,3	80,2 ± 10, 4	80,3 ± 10,8	80,1 ± 10,7
BMI, kg/m^2	27,2 ± 2,94	27,4 ± 2,99	27,4 ± 2,91	27,5 ± 3,01	27,4 ± 3,13	27,5 ± 3,05
TC, mg/dL	249 ± 44, 1	253 ± 44,6	255 ± 47,7	249 ± 46,0	244 ± 45,8	228 ± 42,9
LDLc, mg/dL	159 ± 37,9	165 ± 41, 0	168 ± 41,1	164 ± 41,2	161 ± 40,8	143 ± 37,1
TG, mg/dL	159 ± 74,3	151 ± 79,3	160 ± 96,3	151 ± 71,4	145 ± 68,9	141 ± 75,0
HDLc, mg/dL	52,7 ± 9,89	55, 2 ± 11,0	53,7 ± 10,2	54,3 ± 10,7	54,5 ± 10,74	56,1 ± 11,0
Lp(a), mg/dL	50,0 ± 47,9	-	-	-	-	-
ApoB, mg/dL	132 ± 30,3	-	-	-	-	-
TSH, mg/dL	1,75 ± 0,88	1,64 ± 0,80	1,65 ± 0,68	1,64 ± 0,79	-	-
Glucose, mg/dL	91,1 ± 13,9	-	-	-	-	-
HbA1c, %	5,31 ± 0,14	5,46 ± 0,35	-	5,54 ± 0,41	-	-
Lipid lowering treatment, n (%)	73 (28,4%)	76 (29,6%)	96 (37,3%)	112 (43,6%)	115 (44,7%)	135 (52,5%)
Premature CVD, n (%)	22					

BMI: Body Mass Index, TC: Total cholesterol; LDLc: Low density lipoprotein cholesterol; TG: Triglycerides; HDLc: High density lipoprotein colesterol; Lp(a): Lipoprotein(a); ApoB: Apolipoprotein B; TSH: Thyroid-Stimulating Hormone; HbA1c: Glycated haemoglobin; Premature CVD: Cardiovascular disease before 55 years male, 65 years female. Variables are expressed as media ± standard deviation or n (%).

Table 2.

Selection Criteria	N
1. Subjects with TC ≥ 310 mg/dL or LDLc>95th percentile adjusted by age and sex in at least 2 determinations	128
2. Subjects with personal history of premature CVD (<55 male, <65 female)	22
3. Subjects under statin treatment and LDLc > 130 mg/dL in two or more occasions	39
4. Subjects who take statin in some occasion and with LDLc >95th percentile in one or more determinations and also with LDLc>130 mg/dL under statin treatment	113

257 subjects met one or more criteria:

- 43 subjects met 2 criteria
- 1 subject met 3 criteria

Table 3. Mutations identified in selected subjects.

ID	Gene	Nucleotide change	Translation	Pathogenicity	Selection criteria
1	*LDLR*	c.(-135)C>G	NA	Pathogenic	1 + 4
2	*LDLR*	c.[274C>G;313+1G>C]	p.(Gln92Glu); p.?	Pathogenic	3 + 4
3	*LDLR*	c.1247G>A	p.(Arg416Gln)	Pathogenic	4
4	*LDLR*	c.1529C>T	p.(Thr510Met)	Possibly pathogenic	1
5	*LDLR*	c.1586+5G>A	NA	Possibly pathogenic	1 + 4
6	*LDLR*	c.1775G>A	p.(Gly592Glu)	Pathogenic	4
7	*LDLR*	c.1775G>A	p.(Gly592Glu)	Pathogenic	1 + 4
8	*LDLR*	c.1816G>A	p.(Ala606Thr)	Possibly pathogenic	1
9	*LDLR*	c.530C>T	p.(Ser177Leu)	Pathogenic	1 + 4
10	*LDLR*	c.826T>G	p.(Cys276Gly)	Pathogenic	1 + 4
11	*LDLR*	c.862G>A	p.(Glu288Lys)	Pathogenic	1 + 4
12	*LDLR*	c.941-?_1845+?del	Deletion exons 7-12	Pathogenic	3
13	*PCSK9*	c.60_65dupGCTGCT	p.(Leu22_Leu23dup)	Pathogenic	1
14	*PCSK9*	c.60_65dupGCTGCT	p.(Leu22_Leu23dup)	Pathogenic	1
15	*PCSK9*	c.60_65dupGCTGCT	p.(Leu22_Leu23dup)	Pathogenic	1
16	*PCSK9*	c.60_65dupGCTGCT	p.(Leu22_Leu23dup)	Pathogenic	2
17	*STAP1*	c.(-60)A>G	NA	NA	4
18	*STAP1*	c.(-60)A>G	NA	NA	1
19	*STAP1*	c.619G>A	p.(Asp207Asn)	Pathogenic	1
20	*STAP1*	c.803T>C	p.(Ile268Thr)	Possibly pathogenic	3

Table 4. Lipid levels without treatment of subjects carrying a FH mutation

	Subjects carrying a *LDLR* mutation (N=12)	Subjects carrying a *PCSK9* mutation (N=4)	Subjects carrying a *STAP1* mutation (N=4)	*p*
Total cholesterol, mg/dL	356 ± 78.1	308 ± 42.5	247 ± 37.2	0.0348
HDL cholesterol, mg/dL	56.1 ± 9.32	59.8 ± 21.1	53.5 ± 9.00	0.769
LDL cholesterol, mg/dL	267 ± 86.4	215 ± 36.8	173 ± 23.9	0.0849
Triglycerides, mg/dL	134 ± 44.7	151 ± 74.9	111 ± 23.2	0.509

p: ANOVA 3 groups mutation carriers

Conclusions: Using EAS criteria of clinical suspicion of FH, 12 subjects were carriers of a *LDLR* mutation, 4 subjects were carriers of a *PCSK9* mutation, and 4 subjects were carriers of a *STAP1* mutation. Expected and observed frequencies of *LDLR* mutations were very similar (1/500 and 12/5676= 0,002), suggesting that criteria used are very sensitive but with low specificity. *LDLR* mutations cause a more severe phenotype than *PCSK9* or *STAP1* mutations. Subjects carrying *STAP1* mutations have a lipid profile similar to that of subjects without mutation, what makes doubt about pathogenicity of these mutations.

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