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Cosegregation of serum cholesterol with cholesterol intestinal absorption markers in families with primary hypercholesterolemia without mutations in *LDLR*, *APOB*, *PCSK9* and *APOE* genes

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ABSTRACT

Background and aim: The genetic cause and pathogenic mechanism of approximately 20–40% of autosomal dominant hypercholesterolemias (ADH) are unknown. Increased cholesterol intestinal absorption has been associated to ADH. If this variation contributes to their pathogenesis is unknown.
Methods and results: We studied cholesterol absorption (phytosterols and cholestanol serum concentrations) and cholesterol synthesis (desmosterol serum concentration) in 20 families with ADH without causal mutations in *LDLR*, *APOB*, *PCSK9* or *APOE* genes (non-FH ADH) selected from 54 non-FH ADH probands with (non-cholesterol sterol concentrations above 75th percentile) and without (under 75th percentile) hyperabsorption. The concentrations of cholestanol, sitosterol, campesterol and stigmasterol were higher in affected than in non-affected subjects ($p = 0.003$, <0.001.<0.001, 0.002, respectively). There was a strong cosegregation of hyperabsorption with high LDL cholesterol within hyperabsorber families with odds ratio 6.80 (confidence interval 1.656–27.9), $p = 0.008$. In hyperabsorber families, 60.5% of subjects were hyperabsorbers and 76% of them had high LDL cholesterol versus 38.3% and 63% in non-hyperabsorber families, respectively.
Conclusion: Most hypercholesterolemic family members with a hyperabsorber proband are hyperabsorbers. These absorption markers are significantly and positively associated with LDL cholesterol, and predispose to high LDL cholesterol in family members. Our data suggest that complex interindividual variation in cholesterol absorption is involved in many non-FH ADH.

1. Introduction

Autosomal dominant hypercholesterolemias (ADH) are characterized by high levels of low-density lipoprotein (LDL) cholesterol, familial presentation and high risk of premature cardiovascular disease [1]. Most ADH have familial hypercholesterolemia (FH) due to mutations in the *LDLR* gene that encodes for the LDL receptor [2]. Approximately 2–15% of ADH subjects have familial defective apolipoprotein B-100 (FDB) due to mutations in the LDL receptor-binding domain coding region of the *APOB* gene, which encodes for apolipoprotein B-100 [3], or mutations in proprotein convertase subtilisin/kexin type 9 gene (*PCSK9*), a protein involved in the LDL receptor recycling [4]. Recently, a mutation in *APOE* (p.Leu167del) has been also associated with ADH [5,6]. Patients with mutations in these genes present an indistinguishable phenotype and are now included in the FH definition [2]. The genetic cause and pathogenic mechanism of approximately 20–40% of ADH, named in short as non-FH ADH, are unknown [7,8], and probably they are a heterogeneous group of diseases including some severe polygenic hypercholesterolemias [9].

Cholesterol concentration in plasma depends on the amount of cholesterol from the diet and its intestinal absorption, on *de novo*

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synthesis, and on its biliary excretion [10]. Previous studies have reported increased intestinal cholesterol absorption in non-FH ADH subjects that may partially explain plasma hypercholesterolemia in these subjects [11,12]. However, no familial cosegregation studies have been performed to study the linkage between hyperabsorption and high LDL cholesterol in non-FH ADH families.

Normal serum contains small but detectable amounts of non-cholesterol sterols, including plant sterols, also named phytosterols, and cholestanol, and their ratios to cholesterol are accepted surrogate markers for the efficiency of cholesterol intestinal absorption [13,14].

Efficiency of cholesterol intestinal absorption is a partly inherited phenomenon. Heredity of cholesterol absorption has been demonstrated in siblings of hypercholesterolemic probands with low and high serum cholestanol to cholesterol ratio [15].

Considering that some cases of non-FH ADH are associated with cholesterol intestinal hyperabsorption, the aim of our work was to determine if the efficiency of intestinal cholesterol absorption, measured by non-cholesterol sterol surrogate markers, cosegregates with LDL cholesterol concentration in non-FH ADH families.

2. Materials and methods

2.1. Probands

Selected subjects (n = 54) were unrelated adults 18–79 years of age with the clinical diagnosis of ADH: LDL cholesterol above the 95th percentile of the Spanish population [16], triglycerides below 200 mg/dL, primary cause, and familial presentation (at least one first-degree relative with the same phenotype) from the Lipid Clinic at Hospital Universitario Miguel Servet, Zaragoza, Spain. In all subjects, the presence of functional mutations in *LDLR*, *APOB* and *PCSK9*, and p.Leu167del in *APOE* were ruled out by DNA sequencing as previously described [5,7]. Secondary causes of hypercholesterolemia including: obesity (body mass index > 30 kg/m^2), poorly controlled type 2 diabetes (HbA1c > 8%), renal disease with glomerular filtration rate <30 mL/min and/or macroalbuminuria, liver diseases (ALT > 3 times upper normal limit), hypothyroidism (TSH > 6 mIU/L), pregnancy, autoimmune diseases and protease inhibitors were exclusion criteria. Subjects disclosing *APOE* ε2/ε2 genotype were not considered for this study. Subjects with previous cardiovascular disease or high risk for cardiovascular disease (>20% in the next 10 years) were excluded except if they were not on lipid-lowering drugs. Cardiovascular risk factors assessment, personal and family history of cardiovascular disease, consumption of drugs affecting intestinal or lipid metabolism and anthropometric measurements were performed in all participants. Dietary intake was determined by interview with one single nutritionist dietitian. In this interview, a Spanish validated 137-item food frequency questionnaire (FFQ) was used [17].

2.2. Biochemistry determinations

Fasting blood for biochemical profiles was drawn after at least 5–6 weeks without hypolipidemic drug treatment, plant sterols or fish oil supplements. Cholesterol and triglycerides were determined by standard enzymatic methods. HDL cholesterol was measured by a precipitation technique. Apo A1, apo B and lipoprotein (a) were determined by nephelometry using IMMAGE-Immunochemistry System (Beckman Coulter). LDL cholesterol was calculated using Friedewald's formula.

2.3. Intestinal absorption and synthesis markers

Serum phytosterols and cholestanol, all of them markers of cholesterol absorption, and cholesterol were quantified after 10 h of fasting. Subjects were without lipid lowering drugs or phytosterol supplements at least 5 weeks before blood extraction. Serum concentration of cholesterol, sitosterol, campesterol, stigmasterol, cholestanol and desmosterol were quantified using HPLC-MS/MS according to the method previously described [18], and were expressed as mg/dL as well as normalized to mg/dL of total cholesterol. Briefly, 100 μl of serum were transferred to a screw-capped vial and deuterium-labeled internal standard, [^{2}H6] cholesterol-26,26,26,27,27,27, (7.9 mM), was added to determine non-cholesterol sterols. Another 100 μl of serum were transferred to a screw-capped vial, deuterium-labeled internal standard, [^{2}H7] cholesterol-25,26,26,26,27,27,27, was added to determine cholesterol. Alkaline hydrolysis was performed for 20 min at 60 °C in an ultrasound bath and extracted twice with 3 ml of hexane. The extracts were loaded onto the SPE cartridge (1 mg, Discovery DSC-18, Supelco, Spain) which was preconditioned with 400 μl of methanol and gravity eluted. The non-cholesterol sterols and cholesterol were desorbed with 1.4 ml of 2-propanol by gravity and 40 μl of the final mixtures were injected into the HPLC-MS/MS system.

2.4. Definition of cholesterol intestinal hyperabsorption

We defined as hyperabsorber those subjects that showed ≥3 intestinal non-cholesterol sterols >75th percentile of the distribution in normolipidemic population. Subjects with serum phytosterol and cholestanol concentrations under 75th percentile were considered as non-hyperabsorbers. One hundred normolipidemic subjects (LDL cholesterol under the 75th percentile and triglycerides <200 mg/dL) were used to determine the normal non-cholesterol sterol distribution in our population. This group consisted of healthy, unrelated men and women volunteers aged 18–79 years, who underwent a medical examination at the Hospital Universitario Miguel Servet of Zaragoza. Exclusion criteria for normolipemic subjects were personal or parental history of premature cardiovascular disease or dyslipidemia, current acute illness, or use of drugs that might influence glucose or lipid metabolism.

2.5. Family studies

Available family members of all hyperabsorber probands and the same number of families with a non-hyperabsorber proband were studied for cosegregation analysis. Clinical, biochemical, and non-cholesterol sterol analyses were performed in family members as in probands, except for genetic studies that were not made in family members. The same exclusion criteria and hyperabsorption definition were used in family members as were used in probands. Family members were considered affected if LDL cholesterol was above 90th percentile of the Spanish distribution in absence of secondary causes.

All subjects: non-FH ADH probands, normolipemic controls, and family members signed informed consent to a protocol previously approved by our local ethical committee (Comité Ético de Investigación Clínica de Aragón, Zaragoza, Spain).

2.6. Statistical analyses

Comparison of lipid variables among groups was performed using the Student's t test for data normally distributed and Mann–Whitney U test for skewed data. When significant differences were detected, multiple comparisons were made by using the

Bonferroni correction for normally distributed variables. The significance was set at $P < 0.05$ for the variables. Non-cholesterol sterol to cholesterol ratios were log transformed to achieve variance homogeneity. Data are presented as mean and standard deviation for continuous variables. Bivariate logistic regression was used to determine the ability of the hyperabsorber condition to discriminate between affected and non-affected family member patients. Age and gender were included in the analysis as predictor variables. Regression coefficients (B) and odds ratios (OR) for predictors variables are listed. A positive regression coefficient means that the hyperabsorption increases the probability to have hypercholesterolemia. The odds ratio is defined as the relative amounts by which the odds of presenting hypercholesterolemia increase when hyperabsorption is present. Sample size was calculated to detect differences of 25% in plasma phytosterols between affected and non-affected family members in each group of families, assuming similar variances, with a statistical power of 0.95 and a margin of error of 0.80. All statistical analyses were performed with SPSS software (version 15.0; SPSS, Chicago, IL, USA).

3. Results

The main clinical and biochemical characteristics of the 54 non-FH ADH probands are presented in Table 1. Probands were mostly healthy women (65%) with high total cholesterol and LDL cholesterol and normal triglycerides as expected due to inclusion and exclusion criteria. The concentration of the non-cholesterol sterols is represented by ratio to total cholesterol determined by HPLC-MS/MS.

Ten non-FH ADH probands fulfilled the diagnostic criteria of hyperabsorbers. Table 2 shows the main lipid characteristics of these subjects and of 10 non-hyperabsorber probands used for comparison. There were differences between groups in body mass index, waist circumference and triglycerides. Other clinical variables, including dietary characteristics, did not differ between hyperabsorber and non-hyperabsorber probands. Mean values of cholestanol, sitosterol, campesterol and stigmasterol showed statistically significant ($P = 0.003$ for cholestanol and $P < 0.001$ for phytosterols) between both groups.

Table 1
Clinical and biochemical characteristics of non-FH ADH probands[a].

	Proband (n = 54)
Age, years	52 ± 9.2
Females, n (%)	35 (64.8)
Systolic blood pressure, mm Hg	136 ± 15.7
Diastolic blood pressure, mm Hg	83.4 ± 9.1
Body mass index, kg/m^2	25.4 ± 3.2
Waist circumference, cm	87.9 ± 11.0
Total cholesterol, mg/dL	321 ± 54.7
HDL cholesterol, mg/dL	67.0 ± 18.1
LDL cholesterol, mg/dL	231 ± 47.4
Triglycerides, mg/dL	113.2 ± 62.0
Apolipoprotein A1, mg/dL	176 ± 37.8
Apolipoprotein B, mg/dL	159 ± 36.9
Lipoprotein(a), mg/dL	68.9 ± 93.1
C reactive protein, g/L	0.79 ± 0.15
Glucose, mg/dL	86.6 ± 7.1
GGT, IU/L	30.4 ± 24.7
ALT, IU/L	21.8 ± 6.8
Cholesterol by HPLC-MS/MS, mg/dL	347 ± 58.1
Cholestanol-to-TC x 10^3	2.14 ± 0.10
Sitosterol-to-TC x 10^3	2.08 ± 1.21
Campesterol-to-TC x 10^3	0.99 ± 0.52
Stigmasterol-to-TC x 10^3	0.17 ± 0.13

HDL denotes high density lipoprotein; LDL, low density lipoprotein; GGT, gamma glutamyl transpeptidase; ALT, alanine transaminase; HPLC, high-performance liquid chromatography; MS, mass spectrometry.

[a] Values are mean ± SD.

Table 3 shows the clinical and biochemical characteristics of the family members in both, hyperabsorber and non-hyperabsorber families. In families with a hyperabsorber proband, there were significant differences between affected and non-affected subjects for total cholesterol, LDL cholesterol, non-HDL cholesterol and apolipoprotein B, without differences in BMI. Intestinal cholesterol absorption markers were higher in affected subjects, with significant differences for cholestanol. In non-hyperabsorber families, there were similar lipid differences than in hyperabsorber families and affected subjects had higher BMI than non-affected subjects. The concentration of cholesterol absorption markers was not significantly different in the non-hyperabsorber families, except for cholestanol. No difference was found in desmosterol between affected and non-affected subjects in hyperabsorber families and significant difference was found between affected and non-affected subjects in non-hyperabsorber families ($P = 0.004$), probably associated with a significantly higher BMI. Desmosterol, was significantly lower in affected subjects from hyperabsorber families than in affected subjects from non-hyperabsorber families (2.76 (2.26–2.94) vs. 2.96 (2.67–3.32), $P = 0.035$). An opposite pattern although without reaching statistical significance was found for intestinal cholesterol absorption markers (6.87 (5.43–8.75) vs. 5.90 (4.90–6.77), $P = 0.083$).

Spearman's rank correlations between LDL cholesterol levels and cholesterol absorption markers are reported in Table 4. Sitosterol and stigmasterol in hyperabsorber families had a positive correlation with LDL cholesterol. However, no correlation was found for cholesterol absorption markers in non-hyperabsorber families. When subjects were divided in hyperabsorbers and non-hyperabsorbers, there was a significant association between LDL cholesterol and cholestanol and sitosterol only in hyperabsorber subjects (Supplementary Figure). The diagnosis of hyperabsorber in the families was associated with a higher risk to be affected by high LDL cholesterol with OR 3.47 (confidence interval 1.60–7.51) and B 1.24 ($P = 0.002$). This risk was substantially increased when only family members from families with an hyperabsorber proband were considered ((OR = 6.80 (confidence interval 1.656–27.9), B = 1.917, $P = 0.008$)).

In hyperabsorber families the percentage of affected hyperabsorber subjects was higher (76.5%) than in families with non-hyperabsorber probands (Fig. 1). Among non-affected subjects, the number of hyperabsorber and non-hyperabsorber subjects was identical. In non-hyperabsorber families, the percentage of non-affected non-hyperabsorber subjects was higher (73.2%) than in hyperabsorber families. The 60.5% of subjects in hyperabsorber families were hyperabsorbers and 76% of them were affected. The 38.3% of the subjects in non-hyperabsorber families were hyperabsorbers and 63% of them were affected (Fig. 1).

4. Discussion

This is the first study, to our knowledge, to analyze the cholesterol intestinal absorption in families with non-FH ADH. Our results show that 78% of the family members with high LDL cholesterol with a hyperabsorber proband show an increase in serum non-cholesterol sterols of intestinal origin, with a positive correlation between these sterols and LDL cholesterol levels. Because phytosterols and cholestanol are a well established tool to study cholesterol intestinal absorption, our results highly support that inter-individual variation in the efficiency of cholesterol absorption plays an important role in the pathophysiology of non-FH ADH. Previous reports had established that serum cholesterol and phytosterols are increased in this population [11,12], consequently, these data all together suggest that genetic variation in cholesterol

Table 2
Clinical and biochemical characteristics of selected probands and control subjects[a].

	Control n = 100	Proband		*P*
		Hyperabsorber n = 10	Non-hyperabsorber n = 10	
Age, years	46 ± 17	56 ± 4	48 ± 11	0.060
Females, n (%)	55 (55)	8 (80)	5 (50)	0.160
Systolic blood pressure, mm Hg	126 ± 22	133 ± 11	140 ± 19	0.353
Diastolic blood pressure, mm Hg	80 ± 13	82 ± 11	84 ± 7	0.636
Body mass index, kg/m^2	25 ± 5.1	23.9 ± 1.5	26.9 ± 3.8	0.039
Waist circumference, cm	89 ± 13	82 ± 9	94 ± 10	0.010
Total cholesterol, mg/dL	198 ± 31.5	326 ± 34.2	316 ± 71.4	0.697
LDL cholesterol, mg/dL	128 ± 25.8	235 ± 37.2	228 ± 57.6	0.735
HDL cholesterol, mg/dL	53.7 ± 12.6	74.7 ± 8.8	59.4 ± 22.1	0.065
Triglycerides, mg/dL	83.3 ± 37.3	77.8 ± 28.1	148.7 ± 67.2	0.010
Apolipoprotein A1, mg/dL	159 ± 28.9	191 ± 31.7	161 ± 38.6	0.070
Apolipoprotein B, mg/dL	99 ± 21.6	168 ± 33.6	150 ± 39.8	0.306
Lipoprotein(a), mg/dL	24.3 ± 24.9	69.4 ± 98.6	68.4 ± 92.5	0.981
Glucose, mg/dL	87.4 ± 13.7	87.5 ± 6.7	85.7 ± 7.4	0.584
GGT, IU/L	23.7 ± 25.2	23.9 ± 17.8	36.9 ± 29.5	0.249
ALT, IU/L	21.6 ± 12.4	19.8 ± 5.0	23.9 ± 8.0	0.188
Cholesterol HPLC-MS, mg/dL	191.4 ± 28.9	352.6 ± 46.4	341.8 ± 70.1	0.690
Cholestanol-to-TC x 10^3	2.16 ± 0.79	2.68 ± 0.71	1.59 ± 0.74	0.003
Sitosterol-to-TC x 10^3	1.78 ± 0.85	3.06 ± 0.85	1.09 ± 0.44	<0.001
Campesterol-to-TC x 10^3	0.93 ± 0.45	1.42 ± 0.34	0.56 ± 0.24	<0.001
Stigmasterol-to-TC x 10^3	0.083 ± 0.052	0.26 ± 0.13	0.088 ± 0.038	0.002
Desmosterol-to-TC x 10^3	2.62 ± 1.14	2.17 ± 0.64	2.69 ± 0.73	0.153

HDL denotes high density lipoprotein; LDL, low density lipoprotein; GGT, gamma glutamyl transpeptidase; ALT, alanine transaminase; HPLC, high-performance liquid chromatography; MS, mass spectrometry.
[a] Values are mean ± SD. *P* refers to differences calculated by Student's t test between hyperabsorber and non-hyperabsorber probands.

Table 3
Clinical and biochemical characteristic of family members[a].

	Hyperabsorber families n = 43		*P*	Non-hyperabsorber families n = 60		*P*
	Affected n = 17	Non-affected n = 26		Affected n = 41	Non-affected n = 19	
Age, years	42 (26–49)	35 (29–52)	0.982	45 (32–51)	38 (25–52)	0.710
Males, n (%)	8 (38)	13 (62)	0.850	12 (44)	15 (56)	0.054
Systolic blood pressure, mm Hg	126 (109–133)	122 (115–141)	0.728	128 (121–146)	120 (109–147)	0.357
Diastolic blood pressure, mm Hg	75 (68–87)	76 (68–84)	0.881	81 (76–84)	76 (67–89)	0.572
Body mass index, kg/m^2	23.3 ± 3.8	24.3 ± 5.3	0.508	27.5 ± 7.2	24.6 ± 3.5	0.043
Waist circumference, cm	85.3 ± 9.3	87.9 ± 13	0.443	96.6 ± 16.5	89.6 ± 10.3	0.104
Total cholesterol, mg/dL	266 ± 43.7	203 ± 33.9	<0.001	278 ± 42.6	198 ± 36.8	<0.001
LDL cholesterol, mg/dL	182 ± 38.0	127 ± 28.7	<0.001	186 ± 38.0	122 ± 32.5	<0.001
HDL cholesterol, mg/dL	62.3 ± 17.4	59.3 ± 13.0	0.529	52.2 ± 12.3	56.5 ± 13.3	0.243
Non-HDL cholesterol, mg/dL	203 ± 37.8	144 ± 31.0	<0.001	220 ± 39.4	141 ± 36.8	<0.001
Triglycerides, mg/dL	89 (69–114)	81 (64–98)	0.371	168 (137–247)	91 (61–117)	<0.001
Apolipoprotein A1, mg/dL	163 (155–192)	159 (138–182)	0.210	152 (129–180)	157 (140–181)	0.313
Apolipoprotein B, mg/dL	127 ± 23.2	92.0 ± 19.9	<0.001	142 ± 30.2	42.4 ± 23.2	<0.001
Lipoprotein(a), mg/dL	28.3 (57.7–15.2)	28.2 (18.5–50.9)	0.948	16.4 (2.7–57.6)	24.2 (7.5–66.1)	0.685
Glucose, mg/dL	87 (80–91)	84 (75–93)	0.593	85 (77–100)	83 (79–93)	0.660
Cholesterol HPLC-MS, mg/dL	270 ± 41.5	212 ± 34.0	<0.001	271 ± 48.5	204 ± 38.9	<0.001
Cholestanol-to-TC x 10^3	2.95 (2.43–3.25)	2.35 (1.95–2.77)	0.042	2.55 (2.22–3.22)	2.12 (1.85–2.54)	0.001
Sitosterol-to-TC x 10^3	2.73 (1.84–3.58)	1.82 (1.44–2.66)	0.070	2.09 (1.55–2.48)	1.69 (1.48–2.01)	0.076
Campesterol-to-TC x 10^3	1.36 (1.06–1.80)	1.12 (0.86–1.41)	0.059	1.18 (0.91–1.26)	0.96 (0.74–1.27)	0.088
Stigmasterol-to-TC x 10^3	0.17 (0.11–0.22)	0.11 (0.09–0.16)	0.098	0.12 (0.08–0.15)	0.10 (0.09–0.14)	0.243
All cholesterol absorption markers	6.87 (5.43–8.75)	5.46 (4.43–7.52)	0.060	5.90 (4.90–6.77)	5.24 (4.20–5.77)	0.020
Desmosterol-to-TC x 10^3	2.76 (2.26–2.94)	2.85 (2.46–3.05)	0.371	2.96 (2.67–3.32)	2.43 (2.00–2.93)	0.004

[a] Values are mean ± SD or median (interquartile range). *P* refers to differences calculated by Student's t test for data normally distributed and Mann–Whitney U test for skewed data.

Table 4
Correlations between LDL-cholesterol and cholesterol absorption markers.

	Hyperabsorber families n = 53		Non-hyperabsorber families n = 70		All family members n = 103	
	Coefficient	*P*	Coefficient	*P*	Coefficient	*P*
Cholestanol	0.232	0.094	0.108	0.377	0.305	0.002
Sitosterol	0.268	0.050	−0.067	0.583	0.145	0.145
Campesterol	0.216	0.120	−0.148	0.224	0.126	0.208
Stigmasterol	0.333	0.016	−0.058	0.638	0.066	0.516
All cholesterol absorption markers	0.266	0.057	−0.015	0.902	0.214	0.032

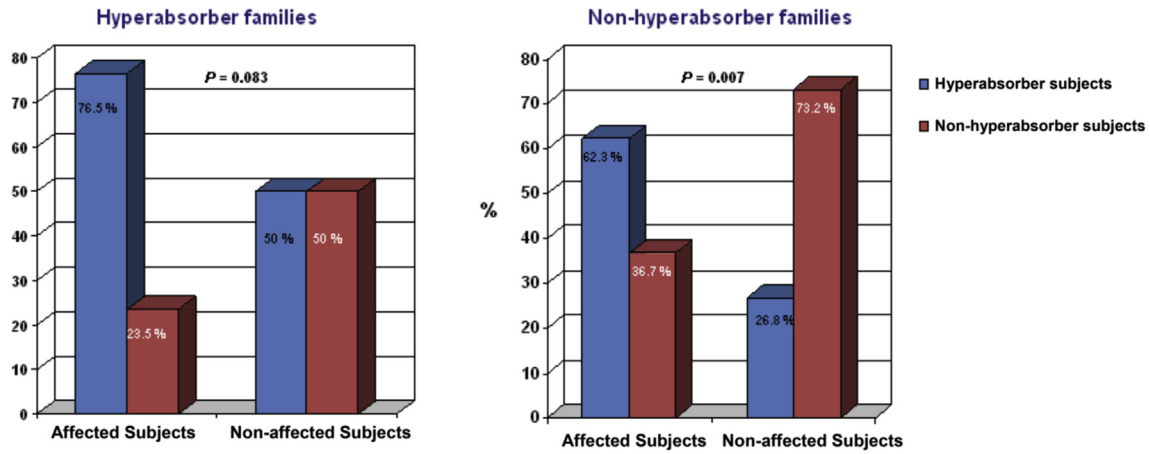

Figure 1. Affected and non-affected subjects distribution in hyperabsorber and non-hyperabsorber families.

absorption is involved in non-FH ADH families.

There is a large interindividual difference in intestinal cholesterol absorption of cholesterol that is mainly due to genetic variation [15,19]. Single nucleotide variation (SNV) in *NPC1L1* and, *ABCG5* and *ABCG8* key modulators of cholesterol influx and efflux into intestinal mucosal cells, respectively, have been associated with the LDL cholesterol concentration in several populations including subjects with hypercholesterolemia [20,21]; may explain interindividual variations in LDL cholesterol level in response to ezetimibe treatment [22,23], and several rare genotype variations in NPC1L1 are associated with mild reductions in sterol absorption, circulatory LDL cholesterol concentrations and cardiovascular disease [24]. In contrast, obligate heterozygous subjects with severe mutations in *ABCG5* or *ABCG8* causing sitosterolemia, show normal LDL cholesterol levels [25]; and extensive sequencing analysis of these genes in subjects with ADH has not detected causative mutations [11]. All together indicate that the genetic interindividual variation in cholesterol absorption is not monogenic, but complex and probably polygenic, resulting from the effects of multiples SNVs common in the population, rather than the effects of rare mutation with substantial impact on cholesterol absorption [20]. According with this concept, cholesterol hyperabsorption is a common phenomenon among family members from a hyperabsorber proband, both in subjects with high and with normal LDL cholesterol; and hyperabsorption is more common in affected subjects with high LDL cholesterol than in normolipemic individuals independently of the proband. Consequently, increased intestinal cholesterol absorption is a risk factor for the development of primary hypercholesterolemia but can not discriminate between affected and non-affected individuals.

The genetic basis of non-FH is a relevant and controversial issue. In two different whole exome sequencing in ADH patients negative for *LDLR*|*APOB*|*PCSK9* mutations [26] and individuals selected for extreme LDL cholesterol (>98th percentile) [27] no major novel *locus* for FH was detected. A new locus at 4p13 associated with ADH was found in a family from The Netherlands, and the study of 400 additional unrelated ADH probands detected 4 missense variants in the STAP1 gene, encoding the protein signal transducing adapter family member 1, a new candidate gene for ADH, but in any case being responsible for a small proportion of non-FH ADH [28]. A recent study in patients with non-FH ADH from two different countries has found that high LDL cholesterol concentrations in some of these subjects might have a polygenic cause, which would difficult a precise diagnosis and the efficiency of cascade testing in family members [9]. Some non-hyperabsorber family members showed high TG what suggests the existing of overlapping between non-FH ADH and FCH. Furthermore, patients with a detected causal mutation in the candidate genes have a substantial polygenic contribution that might contribute to the variable penetrance of the disease and to the large overlap in LDL cholesterol concentrations in mutation-carrier and non-carrier relatives [9]. Our study supports the concept that what we had previously considered ADH, in most cases, are in fact complex metabolic diseases in which different mechanism are involved, one of them being hyperabsorption, and probably with a polygenic contribution, and with environmental interactions. Affected subjects with hyperabsorption show lower BMI than affected non-hyperabsorber subjects in our study (Table 3). This relationship between plasma phytosterols and BMI has been already established, and leaner individuals have increased phytosterols plasma levels than subjects with obesity or metabolic syndrome (MetS) [29]. The mechanism of this association has not been fully established. The weak correlation between LDL cholesterol and intestinal cholesterol markers would support the complex and probably polygenic background of the association. Furthermore, the diagnosis of ADH should be redefined because with the present diagnostic criteria [1,2] many subjects could be diagnosed of FH, suggesting an inexistent monogenic disorder.

In conclusion, serum phytosterols and cholestanol, markers of cholesterol intestinal absorption efficiency, are frequently increased in non-FH ADH subjects. There is a high percentage of first-degree relatives of hyperabsorber probands that present a hyperabsorber phenotype, and this percentage of hyperabsorber subjects is higher in families with hyperabsorber probands than in families with non-hyperabsorber probands. Most hypercholesterolemic family members with a hyperabsorber proband are hyperabsorbers. These absorption markers are significantly and positively associated with LDL cholesterol, and predispose to high LDL cholesterol in family members. However, the cosegregation of both phenotypes is not compatible with a monogenic defect in the intestinal cholesterol absorption mechanism. Our data suggest that complex interindividual variation in cholesterol absorption is involved in many non-FH ADH.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2016.01.005.

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