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Original article

Longitudinal evaluation of endothelial markers in children and adolescents with familial hypercholesterolemia

Patrizia Bruzzi, Barbara Predieri, Simona Filomena Madeo, Francesca Lami, Lorenzo Iughetti

Department of Medical and Surgical Sciences for Mothers, Children and Adults, University of Modena and Reggio Emilia, Modena, Italy

Abstract. *Background and aim:* Children with heterozygous familial hypercholesterolemia (heFH) are at risk of premature atherosclerosis. Aims of this study were: (a) to longitudinally evaluate the endothelial dysfunction, estimated through brachial flow mediated dilation (FMD), as first sign of subclinical atherogenesis in a group of children and adolescents affected by heFH in comparison to normo-lipidemic controls, and (b) to identify predictive factors influencing the endothelial function and its development in the same cohort of patients. *Methods:* This is a prospective, longitudinal and cross-sectional study. Physical examination, plasma lipid profile and brachial artery FMD were measured at baseline and after follow-up. *Results:* At baseline, FMD did not differ between heFH children (n.24, median age 9.71) and controls (n. 24, median age 10.29) (7.67 ± 9.26 vs. 11.18 ± 7.28 %, p 0.09). Nevertheless, during follow-up (median length of lipid-lowering diet 4.52 years), FMD got worse in 54% of heFH subjects and its worsening correlated to the increasing of low-density lipoprotein cholesterol (r -0.21, p < 0.05). Moreover, being male (β -0.46, p 0.03), undergoing puberty (β -0.61, p 0.03) and increasing of body mass index standard deviation score (β -0.39, p 0.03) were identified as main independent predictor factors of FMD drop. *Conclusions:* During the first decades of life, not only hypercholesterolemia, but also clusters of pro-atherogenic conditions and their persistence, could affect the endothelial function and its trend. (www.actabiomedica.it)

Key words: Flow mediated dilation, Familial hypercholesterolemia, Children, Endothelial function, Cardiovascular risk

Introduction

Familial hypercholesterolemia (FH) (OMIM 143690) is an inborn, autosomal and monogenic disorder of lipoprotein pathway. Affected patients presented high levels of total cholesterol (TC) and abnormal increased concentration of low-density lipoprotein (LDL) cholesterol (LDL-C). Clinically, even if rare in childhood and adolescence, symptoms of cholesterol deposits in tissues can be detected (planar and tuberous xanthomas on the extensor tendons of the hands and feet and xanthelasma palpebrarum) together with premature cardiovascular diseases (pCVD)[1]. The prevalence of heterozygous FH (heFH) in general population ranges from 1 in 300 to 1 in 500, although this figure rises within certain ethnic groups[2]. The most frequent cause of FH is attributable to mutations of the LDL receptor gene (LDL-R). More than 1700 mutations of this gene have been reported to date[3], ranging from single-nucleotide substitutions to large deletions, leading to a variety of gain-of-function or loss-of-function LDL-R[4,5]. In addition to LDL-R defects, a similar phenotype can be caused by a number of mutations in the apolipoprotein B100 (ApoB100)

gene (that disrupt the binding of the LDL particle to the LDL-R) and by gain of function mutations in the proprotein convertase subtilisin/kexin type 9 (PCSK9) gene (that increase LDL-R degradation) [6, 7].

It is already documented that elevated levels of LDL-C are one of the major risk factor of CVD [8]. Despite availability of an effective pharmaceutical approach, among FH patients, current age- and sex-standardized mortality due to CVD is about 5 times greater than general population[9]. Although the absolute risk of CVD during childhood is low [1], data coming from autopsy of healthy adolescents have revealed that early markers of atherosclerosis already exist at that age[10]. Nevertheless, only few data documenting early morphological (like fatty streak formation) and subclinical functional vascular abnormalities in FH children can be found[11,12]. Endothelial dysfunction, evaluated through flow-mediated dilation (FMD), seems to represent the earliest sign of atherogenesis and is documentable in patients at risk, even in absence of classical and clinical CVD signs [13].

Taking into account all these considerations, the objectives of our study are: (a) longitudinal evaluation of the endothelial dysfunction, estimated through brachial FMD, as first sign of subclinical atherogenesis in a group of heFH children and adolescents in comparison to healthy controls and (b) identification of additional predictive factors of endothelial dysfunction, its progressive development and, consequently, the risk of premature CVD in the same cohort of children and adolescents.

Material and methods

Patients

This prospective, longitudinal, and cross-sectional study includes 24 Caucasian children and adolescents (median age 9.71, range 4.2 – 19.53 years, 12 boys) with genetically identified heFH and 24 normolipidemic healthy Caucasian controls, matched by sex and age (median age 10.29, range 2 – 16 years, 12 boys) attending the Paediatric Lipid Clinic of the University of Modena and Reggio Emilia, Italy. The clinical diagnosis of heFH was based on internationally recognized criteria[14] and then genetically confirmed. Exclusion criteria for both heFH patients and controls include: presence of acute infection, diabetes, hypertension or other metabolic and/or endocrine disease, use of any medications and smoking. After diagnosis, all heFH patients followed a lipid-lowering diet according to the American Heart Association (AHA) updated dietetic guidelines[15]. In the proposed Therapeutic Lifestyle Changes (TLC) diet, approximately 30% of calories have to derive from fat (less than 7% from saturated fat) and the total intake of cholesterol has to be limited to 200 mg/day[7]. None of the patients was on statin or other lipid-lowering therapy.

The study was conducted in accordance with the Declaration of Helsinki. Each subject and/or their parents gave informed consent to the study, which was approved by the local committee (Area Vasta Emilia Nord, Italy) on ethical practice protocol code 262/11.

Study design

A detailed medical history and a familial history of precocious CVD were obtained from all participants using a standardized report form. CVD happened before 55 years in males and 65 years in females were considered as premature CVD (pCVD) and, thus, the family history was defined as positive for pCVD.

Physical examination included an accurate auxological evaluation. Height was measured to the nearest 0.1 cm with a wall-mounted stadiometer (Harpenden, Crymych, UK). Body weight was measured to the nearest 0.1 kg and body mass index (BMI) was obtained from the weight in kg/height in meters squared and expressed, as well as height, as mean ± SDS with respect to chronological age and using national growth charts[16]. Pubertal development was determined using the grading system defined by Tanner[17].

Plasma lipid profile was performed in all patients at baseline. Blood samples were collected early in the morning after overnight fasting. Moreover, at baseline, all the enrolled patients and all the controls underwent an evaluation of endothelial function through brachial FMD. After follow-up (median duration 4.52 years), during which all heFH patients followed a lipid-lowering diet according to TLCs, physical examination and plasma lipid profile, brachial FMD were re-assessed in heFH patients.

Laboratory Methods

Plasma concentrations of TC, LDL-C, triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) were measured enzymatically using commercial kits (Cholesterol CHOD-PAD, HDL plus, Triglycerides GPO-PAP; Hitachi, Roche Diagnostic). The sensitivity of the TC, HDL-C, and LDL-C assay was 3 mg/ml. Intra- and inter-assay coefficients of variations (CVs) were less than 0.8% and 1.7%, respectively, for all examinations. The sensitivity of the TG assay was 4 mg/ml, while intra- and inter-assay CVs were 1.5% and 1.8%, respectively.

Apolipoprotein A (ApoA) and ApoB100 concentrations were measured using an in vitro human N-Antiserum anti-ApoA and ApoB100 kit (Dade Behring, Marburg, Germany). The sensitivity of the ApoA assay was 1.01 mg/ml. Intra- and inter-assay CVs were 2.2% and 5.7%, respectively. The sensitivity of the ApoB100 assay was 1.22 mg/ml, while intra- and inter-assay CVs were 1.9% and 2.4%, respectively.

A N Reagent Latex lipoprotein(a) [Lp(a)] kit was used for lipoprotein a [Lp(a)] (Dade Behring, Marburg, Germany). The sensitivity of the assay was 0.2 mg/dl. Intra- and inter-assay CVs were 2.1% and 2.8%, respectively.

Genomic DNA was extracted from peripheral blood by a standard procedure[18].

Type of LDLR Mutation

The LDL-R mutations were divided in 2 groups according to their residual function as previously reported in literature[18]: (1) the receptor-negative mutations or null alleles (R-N) contained all class 1 mutations, some missense class 2A mutations, large rearrangements (except the 2.5-kb deletion, which is a class 3 and 5 mutation), mutations resulting in a deletion of the translation initiation signal, and early stop codons; (2) the receptor-defective mutations (R-D) contained class 2B to 6 mutations[19]. The missense mutations were included in receptor-defective group if the residual receptor activity was > 55% in heFH[19].

Assessment of Endothelial-Dependent Flow-mediated Vasodilation of the Brachial Artery

A single experienced sonographer performed all the measurements after a fasting night, in a noiseless and properly temperate area. Subjects had not physical exercise and were instructed not to take caffeine for 24 h before testing. An ultrasound system ACUSON 7.0 mHz equipped with vascular software for two-dimensional (2D) imaging, color Doppler and high-frequency vascular transducer was used. The test performance followed standardized protocols for adult, due to the lack of specific guidelines for the child in literature[20].

The brachial artery was visualized longitudinally above the antecubital fossa. The diameter of the vessel was constantly monitored through an M mode. To generate a flow stimulus in the brachial artery, a sphygmomanometric cuff was positioned above the forearm and, after the acquisition of the rest image, inflated to a suprasystolic pressure to obstruct the artery for about 5 minutes. A following short reactive hyperemia provoked by the cuff deflation resulted in a dilation of the vessel. The longitudinal image of the artery was taped continuously from 30 seconds before to 3 minutes after cuff deflation. To measure the hyperemic flow, a mid-artery pulsed Doppler signal was acquired upon immediate cuff release and no later than 15 seconds after cuff deflation[21]. FMD was typically expressed as the change in post-stimulus diameter as a percentage of the baseline diameter[21].

Statistical Analysis

All normally distributed variables (height, BMI) were expressed as mean ± SDS. Data not normally distributed (age, TC, LDL-C, TG, HDL-C, ApoA, ApoB100, Lp(a), FMD) were log-transformed to approximate normality and expressed as mean ± SDS. The comparisons among auxological, biochemical and FMD data between heFH children and controls and in heFH subgroups (male vs. female; change in pubertal stage; R-D vs. R-N; positive family history pCVD vs. negative) were performed using *U* Mann-Whitney test. Wilcoxon matched-pairs test assessed the longitudinal differences in auxological, biochemical and

FMD data in each subgroup. Univariate analysis was performed to correlate variables and Pearson's *r* coefficient was calculated. In multiple regression analysis, we identified Δ FMD (variation of FMD values along time) as the dependent variable; independent variables were gender, presence of pCVD, changes in pubertal development, type of mutation (as a dichotomus variable) and the demographic and biochemical characteristics (duration of follow-up, variation in BMI-SDS and lipid parameters), which showed an association with the dependent variable in univariate analysis at 15% significance level.

Statistical significance was inferred at a p value of < 0.05.

Results

Table 1 enlisted baseline clinical and instrumental features of heFH patients and controls. Mean FMD values did not vary between groups.

Along follow-up, despite an improvement in TC (309 .29 ± 33.71 vs. 270.95 ± 48.96 mg/dl, p 0.001) and ApoB100 (153.57 ± 21.13 vs. 119.13 ± 25.18 mg/dl, p 0.004) levels, FMD got worse in the 54% (13/24) of heFH and, at the end of follow-up, the 66% (16/24) of heFH patients showed a pathological FMD.

A total of 16 different mutations were detected among heFH patients. We found 10 null alleles in 13 children and 6 receptor-defective mutations in 11 children. At baseline, no anthropometric and biochemical parameters differed between groups (R-N vs. R-D) (table 2), but FMD mean values was documented pathological only in R-N (table 3). On diet, TC (Δ TC: -36.53 ± 41.71 and -40.45 ± 49.09 mg/dl in R-N and R-D, respectively) and Apo-B100 levels (Δ ApoB100: -31.66 ± 23.67 and -9.14 ± 56.56 mg/dl in R-N and R-D, respectively) significantly improved in both groups (table 4). Surprisingly, FMD mean values deteriorated only in R-D (table 3).

According to gender, at baseline only heFH males presented a non-pathological mean FMD value (table 3) even if their LDL-C levels were higher than in females (M vs. F: 239.91± 37.65 vs. 205.52 ± 41.98 mg/dl, p 0.040) (table 2). Nevertheless, along time, mean FMD values attenuated in both genders

Table 1. Baseline anthropometric and vascular features of heFH patients and healthy controls.

	heFH (N= 24)	Controls (N= 24)	p-value
% males	50%	50%	/
% puberty	8/24 (34%)	10/24 (41%)	/
Age (years) (median; range)	9.71 (4.2 – 19.53)	10.29 (2 - 16.00)	0.39
BMI SDS (mean ± SDS)	0.24 ± 1.21	0.43 ± 0.94	0.45
FMD (%) (mean ± SDS)	7.67 ± 9.26	11.18 ± 7.28	0.09

Table 2. Baseline BMI SDS and lipid profile according to heFH-subgroups.

	Gender		LDL-R mutation		CVD Family history	
	M	F	R-D	R-N	pCVD+	pCVD-
BMI SDS	0.47 ± 1.24	-0.01 ± 1.18	-0.04 ± 1.26	0.47 ± 1.16	-0.08 ± 1.12	0.69 ± 1.24
TC (mg/dl)	313.58 ± 39.23	305.00 ± 28.23	319.18 ± 32.31	300.92 ± 33.80	299.92 ± 32.12	322.40 ± 32.97
LDL-C (mg/dl)	239.91± 37.65	205.52 ± 41.98*	239.78 ± 35.55	208.27 ± 44.28	210.40 ± 47.43	239.96 ± 29.32
HDL-C (mg/dl)	51.25 ± 13.78	53.66 ± 10.37	47.72 ± 9.48	56.46 ±12.76	52.64 ± 11.32	52.20 ± 13.50
TG (mg/dl)	90.83 ± 38.88	78.41 ± 19.42	86.27 ± 42.23	83.23 ± 17.84	75.28 ± 20.31	97.70 ± 38.60
ApoA (mg/dl)	123.58 ± 22.02	136.11 ± 16.98	121.80 ± 18.32	135.45 ± 21.09	126.41 24.07±	132.33 ± 15.38
ApoB100 (mg/dl)	151.33 ± 25.74	156.55 ± 13.69	159.50 ± 19.52	148.18 ± 21.97	148.58 ± 19.51	160.22 ± 22.48
Lp(a) (mg/dl)	47.54 ± 29.96	39.80 ± 31.86	50.81 ± 33.88	36.20 ± 25.45	42.72 ± 25.89	45.10 ± 36.04

*Data are reported as mean±SD. Legend * p< 0.05 between groups.*

Table 3. Baseline and longitudinal FMD according to heFH-subgroup.

	Gender		LDL-R mutation		CVD Family history	
	M	F	R-D	R-N	pCVD+	pCVD-
FMD at baseline (%)	11.55 ± 7.89	3.80 ± 9.18*	10.00 ± 9.32	5.70 ± 9.10	7.30 ± 9.11	8.19 ± 9.95
FMD at follow-up (%)	6.73 ± 7.49	6.77 ± 7.50	2.95 ± 8.02	9.96 ± 4.99 *	7.95 ± 7.09	5.07 ± 7.70
ΔFMD	- 4.81 ± 10.90	2.97 ± 12.99	-7.05 ± 12.69	4.26 ± 9.80	0.65 ± 12.36	-3.12 ± 12.73

*Data are reported as mean±SD. Legend * p< 0.05 between groups.*

(table 3). No other anthropometric and biochemical differences were detected at baseline and at the end of follow-up between genders (table 2 and 4). Only among females, TC (305.00 ± 28.23 vs. 261.33 ± 32.72 mg/dl, p 0.004) and ApoB100 (156.55 ± 13.69 vs. 111.37 ± 13.87 mg/dl, p 0.04) levels significantly decreased on diet (table 4).

Analyzing data according to pubertal changes, heFH group was divided in: patients already pubertal at baseline and patients still in pre-puberty at the end of follow-up (U, unchanged; 11 patients; 4 males) and patients who experienced puberty during follow-up (C, changed; 13 patients; 8 males). At the end of follow-up, C presented an unfavorable lipid levels, in particular lower HDL-C and ApoA and higher TG and ApoB100 levels than U (table 4). Nevertheless, mean FMD values did not differ between groups (U vs. C: 8.70 ± 4.46 vs. 5.10 ± 8.94, p 0.32).

14 heFH patients (58%) have a positive familial history of pCVD. They presented a significant reduction of TC (Δ TC: -35.35 ± 45.20 mg/dl, p 0.01) and ApoB100 levels (Δ ApoB100: -24.75 ± 24.60, p 0.035) along follow-up, but LDL-C concentrations significantly improved only in pCVD- group (Δ LDL-C: - 43.36 ± 49.42, p 0.02) (table 4). No differences in FMD mean values were detected between groups at baseline and at the end of follow-up (table 3).

In heFH group, Δ FMD correlated negatively only with Δ LDL-C (r -0.21, p < 0.05).

Multiple regression analysis (model SE 8.81, R^2 0.49, p 0.025) identified gender (level of effect: male; β -0.46, p 0.03), puberty (level of effect: progression in

Table 4. BMI SDS and lipid profile according to heFH-subgroups at the end of follow-up.

	Gender		**LDL-R mutation**		**CVD Family history**		**Pubertal change**	
	M	F	R-D	R-N	pCVD+	pCVD-	U	C
BMI SDS	0.48 ± 1.41	-0.05 ± 1.22	-0.15 ± 1.50	0.52 ± 1.10	-0.12 ± 1.46	0.69 ± 0.97	-0.14 ± 1.29	0.51 ± 1.31
TC (mg/dl)	280.58 ± 61.16	261.33 ± 32.72°	278.72 ± 51.08°	264.38 ± 48.16°	264. 57 ± 37.89°	279.90 ± 62.45	253.45 ± 37.31	285.76 ± 54.00
LDL-C (mg/dl)	197.05 ± 56.23	179.66 ± 47.11	197.96 ± 49.07	180.23 ± 54.05	182.47 ± 40.82	196.60 ± 65.14°	170.60 ± 43.52	203.38 ± 54.52
HDL-C (mg/dl)	49.25 ± 11.94	53.16 ± 15.18	49.45 ± 10.31	52.69 ± 15.99	48.92 ± 12.04	54.40 ± 15.41	58.09 ± 13.21	45.38 ± 11.13*
TG (mg/dl)	104.50 ± 58.23	91.75 ± 38.58	87.27 ± 53.01	107.30 ± 44.86	102.57 ± 47.01	91.90 ± 52.97	74.18 ± 37.02	118.38 ± 49.42*
ApoA (mg/dl)	130.85 ± 18.88	138.37 ± 19.66	128.14 ± 17.98	140.75 ± 18.99	136.6 ± 15.65	131.40 ± 26.28	153.33 ± 8.40	122.55 ± 12.72*
ApoB100 (mg/dl)	128.00 ± 32.90	111.37 ± 13.87 °	126.85 ± 20.93°	112.37 ± 27.93°	121.60 ± 21.60°	114.2 ± 33.51	103.66 ± 9.68	129.44 ± 27.42*
Lp(a) (mg/dl)	40.83 ± 34.86	41.87 ± 28.99	48.71 ± 37.30	34.14 ± 21.88	32.90 ± 27.17	62.75 ± 30.41	42.00 ± 34.88	41.00 ± 28.97

*Data are reported as mean±SD. Legend: * p< 0.05 between groups; ° p < 0.05 vs. baseline*

puberty; β -0.61, p 0.03) and Δ BMI SDS (β -0.39, p 0.03) as main independent predictor factors of ΔFMD.

Discussion

Even if atherosclerosis is clinically evident only in adulthood, atherogenesis starts early in childhood [22]. Hypercholesterolemia is a significant risk factor for the early structural changes of atherosclerosis and for late CVD and mortality. Fatty streak, considered the first sign of evident atherosclerosis, can be seen in fetuses if exposed to maternal hypercholesterolemia[5, 23]. Later in life, not only genetic background but also environmental modifiable risk factor such as obesity and diet could increase the atherosclerotic risk.

Endothelial dysfunction is an essential early event in atherogenesis[24, 25]. In animal models and in humans, both spatial and temporal correlations between endothelial dysfunction and coronary atherosclerosis have been already demonstrated[25, 26]. Moreover, experiments have already documented the induction of endothelial injuries by hypercholesterolemia, particularly by the LDL component[27, 28].

To measure the degree of atherosclerosis, several surrogate markers, appearing long before clinical symptoms arise, as FMD, have been evolved. Our results show that endothelial function, investigated through FMD, is not worse in children as young as pre-scholar and scholar age with an altered lipid profile in comparison to healthy normo-lipidemic controls. Nevertheless, it seems to deteriorate rapidly along follow-up in more than half of heFH children and adolescents, despite an improvement of lipid profile. Data in literature are conflicting. Among studies that investigated FMD in children and adolescents with FH, several have found significantly impaired FMD in the superficial femoral [11,24] or in the brachial artery[29-33]. On the contrary, two studies have reported no differences in FMD between children with FH and healthy controls[34, 35]. Comparing our data with previous ones[11, 24, 29-33], we suppose that children in our study might be too young to achieve differences in the markers of atherosclerosis. Moreover, our study supports the hypothesis that in early ages not only the presence of a major risk factor (e.g. hypercholesterolemia) but maybe a cluster of pro-atherogenic conditions and their persistence along time, could influence the endothelial function. In fact, on one side, we demonstrate a significant correlation between LDL-C variation and FMD progression in youths with heFH, as expressed in the correlation analysis. Similar results were already reported in adult heFH patients [33, 36]. On the other side, data confirm that the lipid profile and the development of CVD can be variable among FH individuals, even when carrying similar functional mutations. Subgrouping our children and adolescents according to type of mutation, FMD mean values was documented pathological only in R-N at baseline. This was an expected result: Bertolini et al., investigating 264 children with heFH, already demonstrated in 2009 that patients carrying a LDL-R-N mutation had a more severe phenotype, in terms of plasma lipid levels and intima-media-thickness and a higher prevalence of premature CVD in first-degree relatives[37]. Nevertheless, in our study, along follow-up, FMD mean values deteriorated only in R-D, despite a significant improvement in TC and Apo-B100 levels and, thus, regardless the functional type of mutations. Interestingly, as presented by Vlahos et al., also in our study, lipid parameters cannot explain solely the complete variability of FMD within the subgroups of heFH children[33]. Other data coming from adult patients do not clarify yet the effect of different LDL-R mutations on CVD[38]. These data have not to be interpreted as conflicting: the better a patient controls LDL-C levels across time, the better she/he could preserve her/his vascular function. Nevertheless, it is imperative to better understand how elevated LDL-C levels can induce a proinflammatory, proatherogenic state or which other mechanisms (e.g. inflammatory, immune and haemostatic mechanisms) and/or mediators are involved. FH subjects with maternal inheritance have higher all-cause mortality than FH subjects with paternal inheritance due to an exposition to an unfavorable "*in utero*" environment [39]. Considering our results, we could speculate that the majority of our R-D patients may have a maternal FH inheritance (*data not shown*).

To our knowledge, only few studies evaluated longitudinally FMD in the same population of FH children and adolescents[30]. In these previous papers, the study period lasted usually no more than few months[30],

while the median length in our study was 4.52 years. This is one of the major strength of the present study together with the homogeneity of the population. All our patients underwent dietary therapy (TLCs diet); the key-treatment of every dyslipidemia, and no one was on statin therapy. If few studies in heFH pediatric populations demonstrated improvement in vascular function with statin therapy[30, 40], in our study dietary treatment in heFH childhood does not seem to prevent the gradual deterioration of endothelial function, as similarly supposed in previous publications[41].

In our study, living in a family with heritage of pCVD does not influence longitudinal changes of FMD in childhood. Our data are far from what has been already demonstrated in literature: in 2002, de Jongh et al. documented a more impaired FMD in FH patients (14.6 ± 2.2 years) with positive family history of pCVD than those without[31]; Guardamagna et al. in 2009 concluded that children with heFH and family history of pCVD had higher LDL-C and ApoB100 levels and greater aortic and carotid intima media thickness than those with negative family history[37].

Considering the early age of our population, no differences between genders were expected. In the present study, after follow up, mean FMD values did not vary between males and females, even if the trend of its development was opposite across time: in males, FMD level deteriorated progressively, while females showed an amelioration from baseline concomitant to an improvement of TC and ApoB100 levels. Moreover, being male was identified as one of the independent predictive factors of FMD attenuation across time, together with the experience of pubertal changes and the worsening of BMI SDS, as multiple regression analysis showed. Taking into account the changes of lipid assessment during puberty, we have to consider that, among the study population, 8 males (67%) and only 5 females (42%) experienced puberty during follow-up. In the general population, throughout puberty, levels of TC, LDL-C and non-HDL-C decreased, TG in males increased, and HDL-C and TG in females showed no changes[42]. In particular, LDL-C levels rose during stage 3 of development in males[43]. Nevertheless, in our study, subjects getting into puberty presented an unfavorable lipid levels and no changes in FMD evaluation in comparison to patients still in pre-puberty at the end of follow-up or already in puberty at baseline. Therefore, puberty could hardly justify the trend of FMD in males.

As many other chronic conditions, FH requires early intervention as well as careful surveillance to maximize long-term vascular consequences-free survival. Recommendations have encouraged prescribing statins from the age of 8 years or younger in selected patients [44]. Our findings further support these recommendations: later the prevention of atherosclerosis is begun, less effective it would be, because it has not only to target the ongoing risk factors, but also to treat and to regress an already existing pathology.

Study limitations

There are some limitations in our study, as mentioned above. Firstly, data interpretation should consider the exiguity of our population and its youngness (median age 9.71 years) that could seem apparently precocious to detect even subclinical CV alteration. Moreover, the lack of a standard method to monitor dietary adherence of patients, do not allow us a clear deduction of the effect of diet alone on FMD progression and, therefore, in later CV rescue. On the other side, major strengths include the homogeneity of our population together with the length of the follow-up (median 4.52 years). In fact, in our study, the same population that at baseline was accurately and completely characterized in anamnestic, anthropometric and biochemical terms was longitudinally evaluated and we could speculate that, over follow up, potential confounding environmental factors remain constant.

Conclusions

Although monogenic, FH is characterized by a substantial variation in the onset and severity of atherosclerotic disease symptoms. Our data support that additional metabolic atherogenic risk factors (being male, BMI-SDS) and the effect of environment, in association with the genetic defect, are supposed to influence the clinical phenotype of FH maybe through synergistic interactions or additive effects.

In FH, the baseline atherosclerotic risk, its progression and the response to treatments could be serially assessed through a noninvasive subclinical instrument as FMD. Actually, we could only speculate that, if a clear correlation between FMD changes and cardiovascular outcomes will be defined independently of LDL-C levels, the clinical practice could be enriched by the routinely use of such surrogates.

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

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Correspondence:
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Lorenzo Iughetti, MD, PHD, Prof Paediatric Unit,
Departments of Medical and Surgical Sciences of Mothers, Children and Adults,
Azienda Ospedaliero-Universitaria of Modena
Via del Pozzo, n. 71
Modena, 41124 Italy
Phone: +39-594225382
E-mail: iughetti.lorenzo@unimore.it