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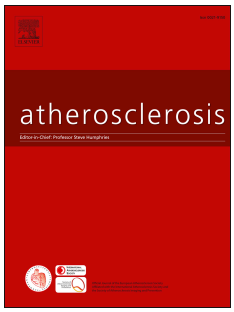

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Detecting familial hypercholesterolemia earlier in life by actively searching for affected children: The DECOPIN Project

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Abstract

Background and aims: Familial hypercholesterolemia (FH) is underdiagnosed in children. We assessed a combination of two screening methods. The first method was to detect hypercholesteraemic children and then study the parents (Ch-P pathway), and the second one was to study the offspring of FH-affected parents (P-Ch pathway).

Methods: In the Ch-P path, primary care paediatricians were asked to include lipid profiling or, at least, total cholesterol (TC) and then lipid profiling if TC was higher than 5.2 mmol/L in any clinically indicated blood test. Children with LDL-C ≥ 3.5 mmol/L, plus either a family history of early cardiovascular disease or one parent with severe hypercholesterolemia, were referred to the lipid unit where the parents, rather than their children, were studied. In parents with definite, clinical FH, a genetic study was performed. Focused genetic testing was performed on all offspring of genetically positive parents. The P-Ch path consisted of the active study of children from definite FH adults.

Results: Fifty-nine paediatricians covering a total population of 63,616 children agreed to participate in the project. Of the 216 children (122 Ch-P and 94 P-Ch) who were ultimately referred to the lipid unit, 87 children with FH (84% genetically positive) were identified. Additionally, 41 parents (from 40 families) were newly diagnosed with FH (63% genetically positive). Forty-nine different mutations were detected: 46 in the *LDLR*, 2 in the *PCSK9* and 1 in *APOB* gene.

Conclusions: The implementation of active strategies to detect FH in children, in close collaboration with primary care paediatricians, provides a high-performance method for early FH detection.

Key words: Familial hypercholesterolemia, Children FH, Opportunistic screening, Reverse cascade screening, Direct cascade screening.

INTRODUCTION

In Europe, it is estimated that approximately 4.5 million individuals are affected by familial hypercholesterolemia (FH), of whom 20-25% are children and adolescents [1]. Of those, less than 10% are diagnosed. FH is underdiagnosed, and moreover, it is generally detected late in adults [2], precluding any early management of lifestyle education during childhood or early pharmacological therapy if necessary [1]. According to the Safeheart cohort data, only 68.2% of FH patients younger than 18 y/o are on statins, and only 41.5% of patients had LDL-C < 3.4 mmol/L [3].

Unlike adults, the Dutch Lipid Clinic Network (DLCN) [4] and other clinical criteria for diagnosis cannot be applied in people younger than 18 years. Diagnosis in children should preferably be established by the detection of the FH causative mutation, which is considered the gold standard [5]. However, genetic testing is expensive and not always available. In that case, FH can be diagnosed according to phenotype criteria, such as the presence of very high LDL-C levels in a child from an FH family.

Because heterozygous FH (HeFH) is clinically silent during childhood and beyond, it is necessary to design and implement different strategies for detection [6,7]. Among these, the universal screening [8] of cholesterol concentrations or genetic mutations has been proposed [9]. On the other hand, several varieties of selective screening focusing on children from FH families have been recommended, considering they are more balanced cost-benefit methods [5,10,11]. In other words, there is no global consensus on FH detection strategies either in adults or children. Different countries apply screening methods based on opinions of local expert groups or scientific societies. For example, in the United States, selective screening is recommended beginning at the age of 2 years, and universal screening at 9-11 years [12,13], whereas in most European countries, selective cascade screening based on genetic testing is

recommended [14-16]. Therefore, the detection of FH in children remains a major challenge [17].

The objective of the present study is to evaluate an active search strategy in identifying FH children based on two parallel strategies. One implies the collaboration between specialized lipid units and primary care paediatricians. When one suspected case is detected, a child-to-parent study pathway is activated. The second pathway is based on the activation of a direct cascade screening from definite FH-parents.

MATERIALS AND METHODS

Study design

During the period from July 2015 to December 2017, we applied a combination of 2 different screening methods in children: the first one was a combination of opportunistic screening and reverse cascade, and the second one was a direct cascade screening. In the first one, after detecting suspicious children, we studied the parents first (Children-to-parent pathway- Ch-P), while in the second, we studied the children from already diagnosed parents (parent-to-child pathway, P-Ch). To implement the Ch-P pathway, primary care paediatricians were asked to include, in any blood test indicated for children for clinical reasons, lipid profiling to determine LDL-C, or at least, a total cholesterol (TC) measurement, and if TC was higher than 5.2 mmol/L (for conversion to mg/dL multiply by 38.665), lipid profiling was performed. Children with LDL-C $\geq$ 4.9 mmol/L or $\geq$ 3.5 mmol/L plus one of the following conditions: early cardiovascular disease in a first- or second-degree relative; a parent with TC > 7.8 mmol/L (and/or on lipid lowering therapy) or one parent unknown, were sent to the Lipid Unit, or previously, to the hospital paediatric department if secondary dyslipidaemia was suspected. In the Ch-P protocol, we studied the families and parents,

instead of the index child, in order to identify the mandatory vertical transmission. When one of the parents had a DLCN > 8, a clinical FH diagnosis was established, and genetic testing was performed. In the case of a positive genetic diagnosis, genetic testing for the known mutation was carried-out on both the index child and all offspring (Figure 1). The P-Ch consisted of performing a directed genetic study of all offspring of genetically confirmed index cases or a clinical study of children from genetically negative definite FH parents (Figure 2). The Hospital Ethics Committee approved the study, and all parents provided their written consent to participate in the study. The study complied with the Declaration of Helsinki.

Clinical evaluation and diagnosis in parents and children

In the Ch-P pathway, parents underwent personal and family anamnesis and directed physical examination (searching for corneal arcus and tendinous xanthomas). Lipid profiling was performed if it was not available in the last two years, and the DLCN index was calculated.

In children, anamnesis and complete physical examination, including anthropometry data and a new lipid profile, were performed.

The FH diagnosis was established both in children and adults by a positive genetic test. Alternatively, the FH clinical diagnosis was established in adults with a DLCN > 8 (definite diagnosis). In children, the FH clinical diagnosis was established if they had an LDL-C > 3.9 mmol/L, and one of the parents had definite FH.

Genetic testing

In parents with definite FH, the presence of FH-associated mutations was studied by next-generation sequencing (NGS) (Liponext, SEQPRO LIPO RS, Roche

Diagnostics). The Liponext detects mutations in *LDLR, APOB, PCSK9, APOE, STAP1 (ADH) and LDLRAP1 (ARH)* genes, and copy-number variation in *LDLR*. A genetic study, focused on the known mutation, was performed for all offspring of positive cases detected from the Ch-P path or already-known FH patients detected in the P-Ch path.

Statistical analyses

The descriptive results are expressed as the mean ± SD for normally distributed data, the median (interquartile range, IQR) for data that were not normally distributed and the frequencies for categorical data. Because only descriptive results are shown, no additional statistical analyses were performed.

RESULTS

Child-to-parent pathway

A total of 59 primary care paediatricians from the hospital reference zone agreed to collaborate. These paediatricians treat a total population of 63,616 children. Approximately 13,000 TC tests and 3.540 complete lipid profiles were performed. In total, 127 children (3.6% of those with full lipid profile performed) fulfilled the hospital derivation criteria. Seventeen were excluded from the protocol due to several reasons (Fig. 1); thus, 110 children were considered index cases. Forty-one parents, from 40 different families, out of 220 had a DLCN > 8. The genetic study was carried out in these 41 parents, with a positive result in 26 (63%), two from the same family. A directed genetic study was carried out in “all” offspring of genetically positive parents. In total, 32 offspring from these 25 genetically positive FH families plus 8 children from 8 families with at least one unavailable parent (4 dead and 5 unknown, and two from the same family) were studied. Twenty-nine children were genetically positive for

FH (28 from positive FH families (87.5%) and 1 from families with some unknown parent), and among them were 2 homozygous individuals from the same family. Additionally, 9 children from genetically negative, but clinically definite FH parents were considered FH because of clinical criteria (definite FH parent + LDL-C >3.9 mmol/L). Therefore, by the Ch-P pathway, from 110 index children studied in the lipid clinic, a total of 79 new FH cases (38 children, 76% genetically positive, and 41 adults, 63% genetically positive) were detected (Fig. 1).

A girl affected with acid lysosomal lipase deficiency was diagnosed among the non-FH children.

Parent-to-child pathway

In the context of this project, 94 offspring from 61 FH patients (65% genetically positive), treated and controlled in our unit, were studied. From the 65 offspring of genetically positive families, 44 had a detected mutation (67.7%). Five out of 29 children from genetically negative FH families were considered to have clinical FH.

Overall, out of 216 children studied from Ch-P and P-Ch pathways, 87 children with FH (84% genetically positive) were detected. Additionally, 41 parents (40 families because in one family both parents were affected) were newly diagnosed with FH (63% genetically positive) (Fig. 2).

The clinical and biochemical characteristics of FH and non-FH children, sorted according the diagnostic pathway (P-Ch or Ch-P), are shown in Table 1.

We identified 49 different mutations: 46 in the *LDLR*, 2 in the *PCSK9* and 1 in *APOB* gene (Table 2).

Seven out of 73 FH children with a positive mutation had an LDL-C < 3.5 mmol/L; in other words, approximately 10% of genetically defined FH did not have the expected phenotype.

DISCUSSION

We report the impact of implementing an active FH search in children by combining two different screening strategies, Ch-P and P-Ch pathways.

While in the P-Ch pathway we studied offspring from definitive FH adults, in the Ch-P pathway, the first step was the opportunistic lipid measurement by paediatricians. We cannot exclude that the clinical indication for blood sampling would influence lipid levels in plasma. However, in general, blood testing was indicated as part of a global health screening or for minor health problems and cholesterol concentrations are quite constant in these circumstances. On the other hand, those children investigated in our unit had at least a new lipid profile measured in basal conditions.

Ultimately, 216 children were studied in our unit, and 87 of them had FH (84% genetically positive). Moreover, through the Ch-P pathway, 41 parents from 40 different families were newly diagnosed, for a total of 128 newly detected FH cases.

This screening resulted in being highly efficient in detecting genetically positive FH children. From 105 genetic tests performed in children, 73 were positive (70%). This percentage is well above that obtained from other strategies. The universal genetic screening, studied by others, will detect approximately 4 positive cases and 996 negative results per 1000 studies, according to the accepted prevalence of 1/250 [9,18]. Therefore, methods designed to perform genetic tests in selected children seem to be more cost-effective [5,10]. Among them, the Slovenian experience [8], based on the universal screening of cholesterol levels at the age of 5, is highly illustrative. By performing genetic testing on children with very high TC and/or a familial history of cardiovascular disease, they obtain a positivity rate of 57%. Even among clinically definite FH [19], the proportion of positive genetic tests is below 70%; therefore, ours

could be considered a high-yield strategy. An interesting aspect of our Ch-P pathway is that it is not exclusively based on LDL-C levels in children but also takes into account vertical transmission [20]. We performed 41 genetic tests on parents and obtained 26 positive results (63%) and then performed 32 genetic studies on children (all offspring) and obtained 28 (87.5%) positive results, two of them HoFH. Another important aspect is that in both Ch-P and P-Ch pathways, the genetic study in children can be focused on the parents' mutation saving time and money. This consideration is of particular importance in countries such as ours, where there is not a majority mutation responsible for the disease. We have detected 49 different mutations in 73 genetically positive FH children. None of them was responsible for more than 5 FH cases.

Our Ch-P strategy was implemented in collaboration with paediatricians, which was welcomed because it could be integrated into paediatricians' daily activities rather than added as an extra task for physicians to complete.

On the other hand, through the P-Ch pathway, we activated the direct cascade screening. Surprisingly, the number of relatives, both adults and children, of definitive FH patients assessed for FH is unacceptably low. There are several reasons, including organization mismatches due to the dependence of relatives on different health areas and physicians, and immigrant population, hindering the study of family members. Our data clearly show that a centralized management of family studies provides high performance. From our P-Ch pathway, we detected FH mutations in forty-four out of 65 (68%) offspring of genetically positive FH patients, again using a mutation-directed search strategy.

Although our study is mainly based on genetic diagnosis, we also included patients with clinical FH diagnosis. Although these patients are currently considered to have a serious form of polygenic hypercholesterolemia, they deserve the same clinical

management of patients with a monogenic form [21]. Current guidelines recommend that family studies of genetically negative FH patients not be performed [2]; however, the clinical evaluation of 49 offspring from clinical FH parents identified 14 additional clinical FH children (a parent with definite FH plus LDL-c > 3.9 mmol/L) suitable for lipid-lowering recommendations. Interestingly, clinical FH diagnosis in children was established in only 28.5% of the studied offspring, emphasizing that a second lipid profile after at least three months of diet is always needed in a child to distinguish highly probable from indeterminate FH.

Moreover, 7 positive FH children had normal LDL-C (10%). Wald et al. observed that 33% of genetically detected FH children had LDL-C < 95th percentile [18], reinforcing the utility of proactive protocols to detect affected offspring among members of FH families beyond TC values.

Despite the success of our Ch-P strategy, we detected only approximately one-third of the predicted 254 FH cases in 63,616 children. One of the reasons is that only one-third of children underwent a TC and/or a lipid profile measurement during this period of time. Actively maintaining this protocol will increase the detection rate; however, we agree that our data underline the need for additional protocols. Universal cholesterol screening during childhood associated with our Ch-P pathway could improve the currently unacceptable FH detection rate, and despite some doubts about the long-term clinical impact [22] of these early detection protocols, they will help to improve the lifelong prognosis of this disease.

The study has several limitations. The total number of children included is relatively low, although this number also represents the suspected children from a population of 63,616 individuals. We have also included patients with only the clinical criteria of FH, even when the genetic study was negative, which could be considered

inappropriate. However, in clinical practice, these patients are considered to have FH, and they have a genetic cause of hypercholesterolemia, probably polygenic, and should be managed as FH according to current guidelines [21].

In conclusion, we recommend that in addition to an active search of affected children from FH families, implementing a Ch-P strategy preceded by an opportunistic detection or universal screening of hypercholesterolemia in childhood, in collaboration with paediatricians, will provide a highly efficient strategy for the early detection of FH.

Conflict of interest

D.I. has received lecture fees from Sanofi, MSD and Rubio. C.RB. has received lecture fees from MSD. L.M. has received lecture and advisory board fees from Amgen, Sanofi and MSD. N.P. has received lecture fees from Amgen, MSD, Ferrer, Rubio and Alexion. The other authors have indicated they have no potential conflicts of interest to disclose.

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FIGURE LEGENDS

Figure 1. Child-to-parent pathway.

[a] We studied 220 parents from 110 index children. We sorted the families according to the presence of at least 1 parent with DLCN > 8 and families with at least 1 non-available parent. Two parents from the same family had DLCN > 8 and two parents from 1 family were not available.

Figure 2. Parent-to-child pathway.

Table 1. Characteristics of the studied children sorted by screening pathway and diagnosis.

	Parent to Child pathway (N=94)		Child to Parent pathway (N=119)[a]	
	FH (N=49)	**Non- FH (N=45)**	**FH (N=36)**	**Non- FH (N=83)**
Age	10±4	11±3	9±3	10±3
Gender (female, %)	40.8	44.4	54.1	46.6
Diagnosis age	9±4	11±3	8±3	10±3
Weight (kg)	40.6±18.5	45.3±16.5	36.3±16.4	38.6±16.0
Height (cm)	139.5±30.1	150.1±17.9	135.9±18.6	140.5±17.4
BMI score	0.16±0.90	0.03±0.94	0.28±0.99	0.06±1.06
Waist circumference (cm)	63.0±11.0	66.0±10.0	63.0±14.0	63.0±14.0
SBP (mmHg)	110±13	113±12	106±12	110±11
DBP (mmHg)	65±9	63±8	63±11	64±
TC (mmol/L)	7.1±1.4	4.6±0.7	6.7±1.2	5.2±0.8

LDL-C (mmol/L)	5.0±1.3	2.6±0.6	4.8±1.2	3.1±0.7
HDL-C (mmol/L)	1.7±0.4	1.7±0.4	1.5±0.3	1.8±0.5
TG (mmol/L)	0.6 (0.5-0.9)	0.6 (0.4-0.8)	0.9 (0.6-1.1)	0.7 (0.6-0.9)
Apo A1 (mg/dL)	147±26	156±23	146±25	159±30
Apo B100 (mg/dL)	136±29	79±16	137±31	97±18
Lp (a) (nmol/L)	36.0 (15.0-158.4)	20.0 (7.0-48.0)	47.0 (16.0-139.2)	36.0 (14.0-165.0)

[a]Two homozygous FH not included in the FH group. One girl with lysosomal acid lipase deficiency not included in the non-FH group.

FH, familial hypercholesterolemia. BMI score, body mass index score; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglycerides; Apo A1, apolipoprotein A1; Apo B100, apolipoprotein B100; Lp (a), lipoprotein (a).

Data are expressed as mean ±SD for normally distributed data, median (IQR) for not normally distributed data or percentages for categorical data.

Table 2. Detected mutations in children.

Gene change	Location	N	Protein change	Pathogenicity
		LDLR		
Regulatory region				
c.-135C>G	Promoter	1		Yes
Missense/in frame				
c.1A>G	exon 1	1	p.Met1Val	Yes
c.241C>T	exon 3	1	p.Arg81Cys	Yes
c.274C>G[a]	exon 3	1	p.Gln92Glu	No
c.283T>G	exon 3	1	p.Cys95Gly	Yes
c.327C>G	exon 4	1	p.Cys109Trp	Yes
c.347G>A	exon 4	1	p.Cys116Tyr	Yes
c.518G>A	exon 4	1	p.Cys173Tyr	Yes

c.533A>G	exon 4	2	p.Asp178Gly	Yes
c.796G>A	exon 5	1	p.Asp266Asn	Yes
c.829G>A[a]	exon 6	2	p.Glu277Lys	No
c.1136G>A	exon 8	1	p.Cys379Tyr	Yes
c.1156G>T	exon 8	1	p.Asp386Tyr	Yes
c.1361C>A	exon 10	2	p.Thr454Asn	Yes
c.1414G>T	exon 10	2	p.Asp472Tyr	Yes
c.1567G>A	exon 10	1	p.Val523Met	Yes
c.1618G>A	exon 11	3	p.Ala540Thr	Yes
[c.1690A>C; c.2393_2401del][b]	[exon 11;17]	1	[p.Asn564His;p.Lys799_Phe801del]	Yes
c.1775G>A	exon 12	1	p.Gly592Glu	Yes
c.1816G>A	exon 12	1	p.Ala606Thr	possibly

c.1951G>A	exon 13	1	p.Asp651Asn	Yes
c.1952A>T	exon 13	1	p.Asp651Val	Yes
c.2051C>A	exon 14	2	p.Ala684Asp	Yes
c.2099A>G	exon14	1	p.Asp700Gly	Yes
c.2389G>A	exon 16	1	p.Val797Met	Yes
c.2475C>A	exon 17	4	p.Asn825Lys	Yes
Nonsense/Frameshift				
c.12G>A[a]	exon 1	4	p.Trp4*	Yes
c.97C>T	exon 2	1	p.Gln33*	Yes
c.460C>T	exon 4	1	p.Gln154*	Yes
c.518del	exon 4	1	p.Cys173Serfs*33	Yes
c.593C>A	exon 4	3	p.Ser198*	Yes
c.682G>T[c]	exon 4	5	p.Glu228*	Yes

c.925_931del	exon 6	2	p.Pro309LysfX59	Yes
c.1048C>T	exon 7	1	p.Arg350*	Yes
c.1448_1451dup	exon 10	1	p.Ile484Metfs*53	Yes
c.2416dup	exon 17	1	p.Val806Glyfs11	Yes
Intronic/Splicing				
c.313+1G>C[a]	intron 3	1	splicing	Yes
c.313+2T>C	intron 3	1	splicing	Yes
c.1187-10G>A	exon 9	1	splicing	Yes
c.1358+1G>A	exon 9	2	splicing	Yes
c.1845+1G>C	exon 12	1	splicing	Yes
c.2389+4A>G	intron 16	1	intronic	Yes
c.2390-1G>C	exon 17	5	splicing	Yes
Rearrangements				

dup exons 4-5		1		Yes
dup exons 4-15		2		Yes
del exon 12		2		yes
		APOB		
c.10580G>A	exon 26	2	p.Arg3527Gln	yes
		PCSK9		
c.60_65dup	exon 1	2	p.Leu22_Leu23dup	yes
c.1486C>T	exon 9	1	p.Arg496Trp	yes

[a]Three patients carry two mutations in the same allele of the *LDLR* gene. One child: c.274C>G + c.313+1G>C and two children: c.829G>A + c.12G>A.

[b] These variants usually segregate together (counted as one change).

[c] Two homozygous children.

Figure 1

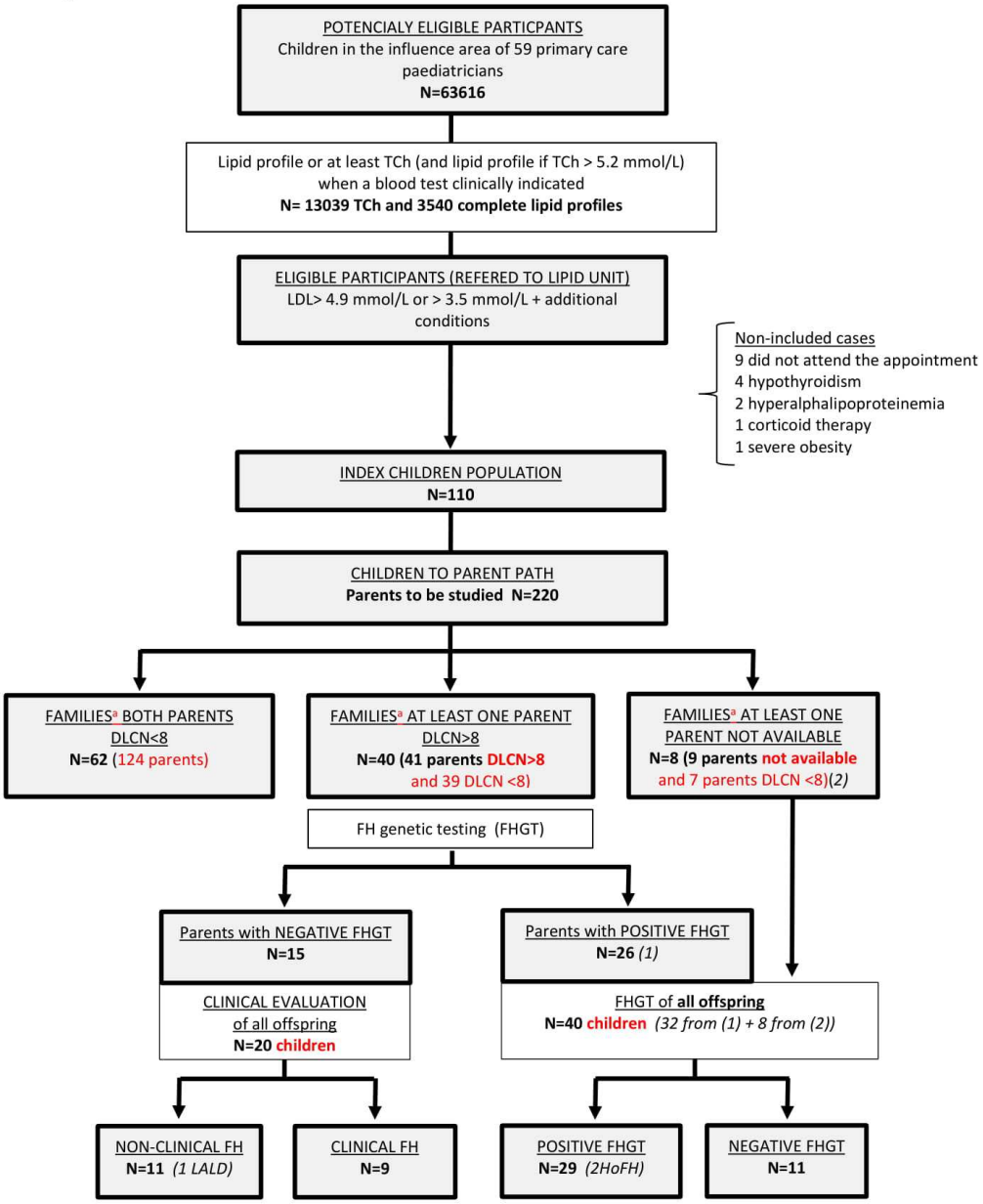
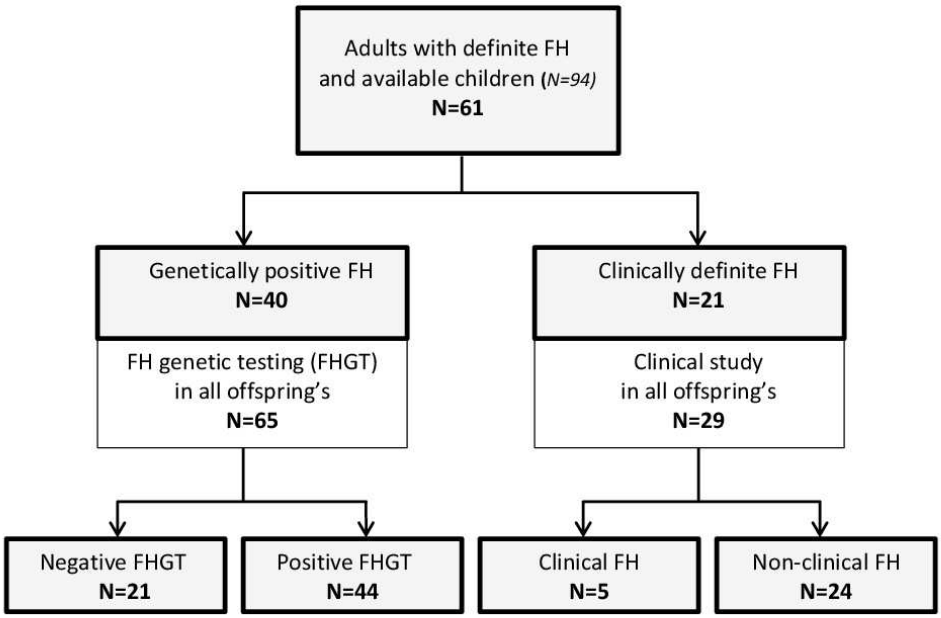

POTENCIALY ELIGIBLE PARTICPANTS
Children in the influence area of 59 primary care paediatricians
N=63616
Lipid profile or at least TCh (and lipid profile if TCh > 5.2 mmol/L) when a blood test clinically indicated
N= 13039 TCh and 3540 complete lipid profiles
ELIGIBLE PARTICIPANTS (REFERED TO LIPID UNIT)
LDL> 4.9 mmol/L or > 3.5 mmol/L + additional conditions
Non-included cases
9 did not attend the appointment
4 hypothyroidism
2 hyperalphalipoproteinemia
1 corticoid therapy
1 severe obesity
INDEX CHILDREN POPULATION
N=110
CHILDREN TO PARENT PATH
Parents to be studied N=220
FAMILIES[a] BOTH PARENTS DLCN<8
N=62 (124 parents)
FAMILIES[a] AT LEAST ONE PARENT DLCN>8
N=40 (41 parents DLCN>8 and 39 DLCN <8)
FAMILIES[a] AT LEAST ONE PARENT NOT AVAILABLE
N=8 (9 parents not available and 7 parents DLCN <8)(2)
FH genetic testing (FHGT)
Parents with NEGATIVE FHGT
N=15
Parents with POSITIVE FHGT
N=26 (1)
CLINICAL EVALUATION of all offspring
N=20 children
FHGT of all offspring
N=40 children (32 from (1) + 8 from (2))
NON-CLINICAL FH
N=11 (1 LALD)
CLINICAL FH
N=9
POSITIVE FHGT
N=29 (2HoFH)
NEGATIVE FHGT
N=11

Figure 2


Adults with definite FH
and available children (N=94)
N=61
Genetically positive FH
N=40
Clinically definite FH
N=21
FH genetic testing (FHGT)
in all offspring's
N=65
Clinical study
in all offspring's
N=29
Negative FHGT
N=21
Positive FHGT
N=44
Clinical FH
N=5
Non-clinical FH
N=24

HIGHLIGHTS

- Detection of Familial Hypercholesterolemia in children remains a major challenge.
- To implement strategies for FH detection in children is necessary.
- ~~Opportunistic~~ Active children to parent and parent to children FH screening pathways ~~reverse and direct cascade screening~~ increase early FH detection.
- Close collaboration with paediatricians provides high-performance detection method.