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New approach for detection of LDL-hypercholesterolemia in the pediatric population: The Fr1dolin-Trial in Lower Saxony, Germany

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HIGHLIGHTS

- Combined early screening for hypercholesterolemia and type 1 diabetes is feasible.
- Fr1dolin offers capillary blood sampling to all children aged 2–6 years.
- Results show a 5-fold higher prevalence for LDL-hypercholesterolemia than expected in the general population.
- Fr1dolin provides a comprehensive counselling and follow-up plan for affected families.

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ABSTRACT

Background and aims: Lipid disorders are often detected very late, particularly in affected young children. We evaluated the feasibility of a screening for LDL-hypercholesterolemia (highLDL) among toddlers and preschoolers.

Methods: Population-based screening has been offered to all children (2–6 years) living in the State of Lower Saxony, Germany, with capillary blood sampling for detection of elevated LDL-cholesterol (LDL-C ≥ 135 mg/dL). Positive results were confirmed by a second measurement. Follow-up in specialized centers, including disease specific counselling and extended diagnostics, as well as evaluation of psychological distress of the parents, is carried out longitudinally.

Results: Up to March 2018, 5656 children have participated in the screening program. 5069/5656 children have completed the screening for highLDL (52.0% boys; median age: 4.0 years [Interquartile range, IQR 3.0–5.1]; mother age: 35 years [IQR 31–38]; father's age: 37 years; [IQR 33–42]). HighLDL was identified in 112 children (2.2%; 40.2% boys; LDL-C 157.6 ± 29.5 mg/dL, mean ± SD). In the total cohort, parents stated in 40.9% of the cases a positive family history for hyperlipidemia and in 29.9% a premature cardiovascular event. Children with highLDL had more often both risk factors in their family history; however, in 37% of them none of these factors were reported.

Conclusions: The first results of the screening program showed its feasibility and revealed high prevalence of highLDL in the general population. Furthermore, a large proportion of families of affected children were not aware about their lipid disorders.

1. Introduction

Elevated levels of low-density lipoprotein (LDL) characterize several genetic determined lipid disorders and particularly Familial Hypercholesterolemia (FH), which is a common genetic cause of premature coronary heart disease (CHD). For decades, the prevalence of FH was supposed to be 1:500 persons, but current studies postulate a higher prevalence of 1:200 in Caucasians, that means FH had been underestimated for a long time [1]. If diagnosed and treated in early-childhood, individuals with FH may have normal life expectancy.

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Familial hypercholesterolemia is diagnosed either on phenotypic criteria, i.e. an elevated low-density lipoprotein cholesterol (LDL-C) level or a family history of elevated LDL-C, premature coronary artery disease (men < 55 years, women < 65 years) and/or genetic diagnosis. Childhood is the optimal period for discrimination between FH and non-FH using LDL-C screening. An LDL-C ≥5 mmol/L (190 mg/dL), or an LDL-C ≥4 mmol/L (160 mg/dL) with a family history of premature CHD and/or high baseline cholesterol in one parent, leads to the phenotypic diagnosis (Simon Broome criteria for children). If one parent has a genetic predisposition, the LDL-C cut-off for the child is ≥ 3.5 mmol/L (130 mg/dL). A cascade screening of families is recommended by using a combined phenotypic and genotypic strategy. For children, testing is recommended beyond the age of five or earlier, if homozygous FH is suspected [1]. Other authors recommend screening starting with two years. This procedure is justified by steady accounts of blood lipids in this age (until the adolescence). An earlier screening is not recommended because low-fat nutrition cannot be started before the age of 2 years [2,3]. Several studies were able to show early signs of arteriosclerosis in childhood so an early treatment with low-fat nutrition and statins is the baseline for the treatment of heterozygous FH [4–6]. The safety and effectivity of statin treatment beyond an age of 8 years was shown in several studies [7].

Increased awareness, early identification, and optimal treatment from childhood on are keys for additional decades of healthy life for children and adolescents with FH [1]. Therefore, we initiated the Fr1dolin-Trial in the German Federal State of Lower Saxony aiming at a screening for LDL-hypercholesterolemia in early childhood. Experiences and findings based on the first 5000 participants are reported.

2. Materials and methods

The Fr1dolin-Trial is a feasibility study for a population-based screening offered to all children between 2 and 6 years in Lower Saxony, Germany during the compulsory routine check-ups as well as at any voluntary visits to the pediatrician's office. All pediatricians registered in Lower Saxony (n = 420) were invited per letter to participate in the Fr1dolin-Trial. According to the Federal Statistical Office (31.12.2014), 320,000 children in this age group are living in the State of Lower Saxony. Based on similar population-based screening programs in Germany e.g. Fr1da-Trial in Bavaria, a participation rate of 25–35% is expected. Based on an estimated prevalence of 1:300 for FH approximately 300 newly identified cases of LDL-hypercholesterolemia are expected if 100,000 children participate. The screening program started in November 2016 and will continue for at least three years. The Fr1dolin-Trial includes also a screening for the early detection of presymptomatic Type 1 diabetes in the same age group [8]; diabetes-related findings are not presented in the present paper.

2.1. Screening procedure

Sampling is done by primary care pediatricians who are registered to the Fr1dolin-Trial. They introduce the study to parents and obtain their written consent. The family is asked to complete a one-page questionnaire to collect master data, family history (dyslipidemia, premature cardiovascular disease in first and second grade of pedigrees) and information about nutrition during infancy. A known diagnosis of familial hypercholesterolemia is not an exclusion criterion as long as the participating child is not diagnosed with FH. A capillary blood sample (200–300 µL) is collected. Children have not to be in fasting conditions.

2.2. Screening methods

Blood samples are sent by mail or by lab transport facilities to the screening center at the Children's Hospital AUF DER BULT, Hannover. In case of total hemolysis or insufficient blood volume, a second sample is required.

LDL-C measurement (sample size 3 µL; method certified using the Low Density Lipoprotein (LDL) Cholesterol Method Evaluation Protocol by the Cholesterol Reference Method Laboratory Network (CRMLN) at Northwest Lipid Research Laboratories, University of Washington, Seattle, Washington, 98103) is performed at the laboratory of the Children's Hospital AUF DER BULT, by using the Dimension® Clinical Chemistry System (ALDL, Dimension® Clinical Chemistry System, Siemens Healthcare Diagnostics Ltd., Camberley, UK). The Automated Low Density Lipoprotein (ALDL) method is a direct assay not dependent on the Friedewald calculation and is referenced to the beta-quantification determination of LDL-C concentration. Briefly, the method is in a two reagent format and depends on the properties of detergent 1 which solubilizes only non-LDL particles. Cholesterol released is consumed by cholesterol esterase and cholesterol oxidase in a non-color forming reaction. Detergent 2 solubilizes the remaining LDL particles. The soluble LDL-C is then oxidized by the action of cholesterol esterase and cholesterol oxidase forming cholestenone and hydrogen peroxide (H_2O_2). The enzymatic action of peroxidase on H_2O_2 produces color in the presence of N,N-bis(4-sulfobutyl)-m-toluidine, disodium salt (DSBmT) and 4-aminoantipyrine (4-AA) that is measured using a bichromatic (540, 700 nm) endpoint technique. The color produced is directly proportional to the amount of LDL-C present in the sample. The average inter-assay coefficient of variation (CV%) was estimated from aliquots of Quality Control Materials and varies between 3.37% and 3.76% for LDL-C measurement range between 113 and 146 mg/dL. All lipoprotein methods are controlled quarterly by the accredited external quality assurance performed by the Reference Institute for Bioanalytics (RfB, Bonn, Germany, accredited to DIN EN ISO/IEC 17043) (participation number 0974010). The ALDL method reaches a success quote of 100%.

In order to overcome matrix related effects by capillary blood draw and the associated interference from hemolysis, we excluded all specimens with free hemoglobin levels above 1000 mg/dL based on Siemens validation according to CLSI/NCCLS EP7-P. Those screenings had to be repeated. Secondly, the Fr1dolin appraisal algorithm (Fig. 1) takes into consideration more sensitive levels of free hemoglobin concentration (50–250 mg/dL, 250–500 mg/dL and 500–1000 mg/dL) in order to prevent dilution effects in hemolytic capillary drawn samples.

HighLDL screening is considered positive if LDL-C is 135 mg/dL or higher or LDL-C levels are elevated in the presence of significant amounts of free hemoglobin (sample size 5 µL; Drabkin's Reagent, Sigma Product Code D 5941 [9]) and positive family history (Fig. 1). In that case, a second venous blood sample is requested and LDL-measurement is repeated. The above-mentioned appraisal algorithm incorporates the available information about the family history in those children with a moderate/significant level of hemolysis and elevated levels of LDL-C (i.e. higher than the age-dependent 75th centile (110 mg/dL, P75) or 95th centile (130 mg/dL, P95 [10]). In the second sample, total cholesterol (TC), HDL-Cholesterol (HDL-C) and triglycerides (TG) are also measured as well as free thyroxine (fT4) and thyroid-stimulating hormone (TSH) in order to exclude hypothyroidism as reason for the LDL-C elevation.

A child with two consecutive positive highLDL screening results is forwarded to a specialized tertiary Pediatric Lipid Center for further care, including molecular diagnosis and treatment counselling.

2.3. Communication of results

Pediatricians are informed per letter about the results. An abnormal result is discussed with the parents during a face-to-face session with the families' pediatrician based on a structured guideline. The pediatrician establishes the contact to the study center, which subsequently coordinates further consultation and education in a Pediatric Lipid Center nearby to child's home. Materials and guide brochures for parents, which have been developed specifically for the Fr1dolin-Trial, are provided to the teams of the local Pediatric Lipid Centers.

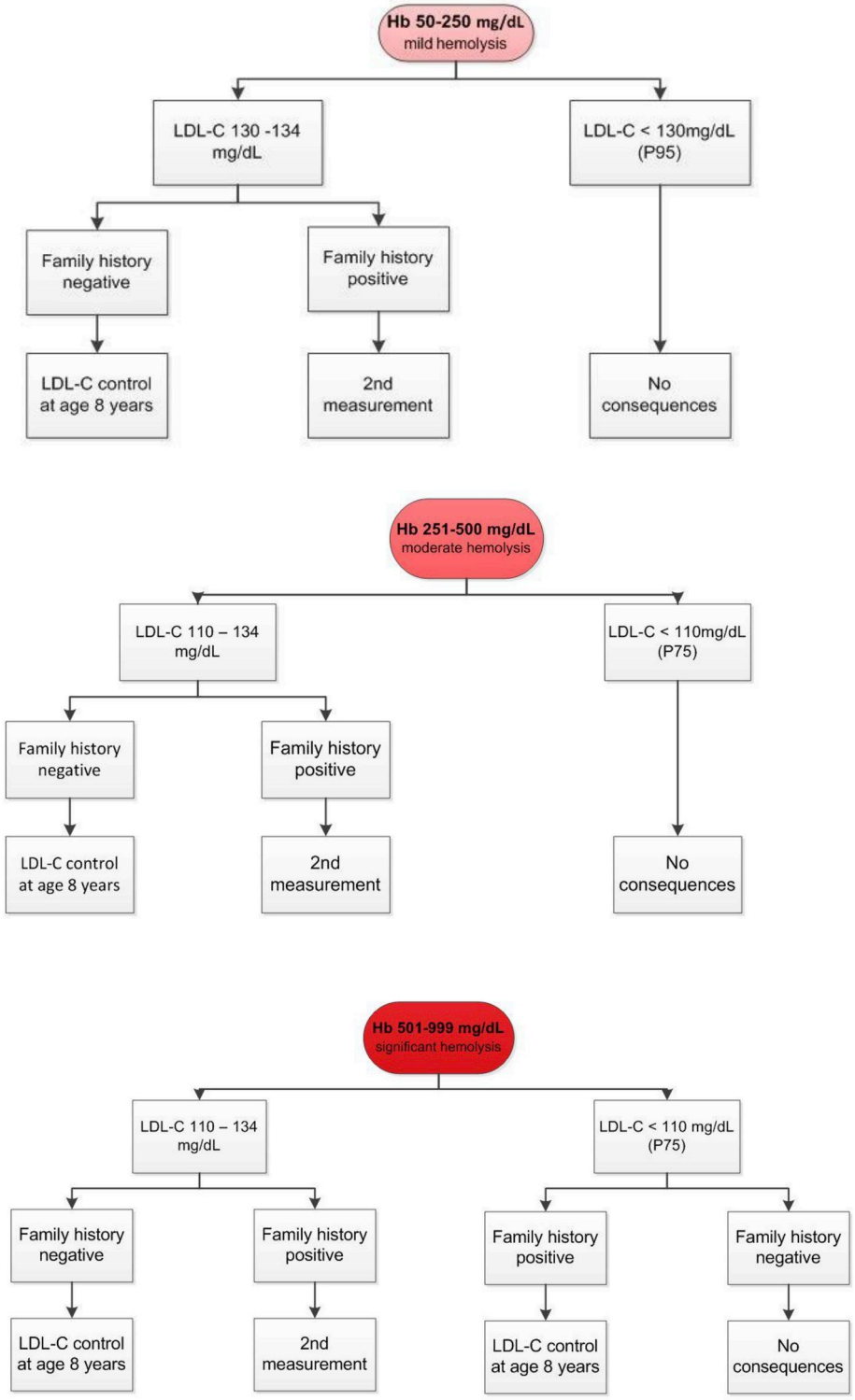

Fig. 1. Appraisal algorithm by incorporating the available information about the family history in those children with a moderate/significant level of hemolysis and relatively elevated levels of LDL-C (i.e. higher than the age-dependent 75th centile (110 mg/dL, P75) or 95th centile (130 mg/dL, P95) [17]).

2.4. Diagnosis of hypercholesterolemia

Significantly elevated LDL-C levels in two consequent blood samples confirm the diagnosis of LDL-hypercholesterolemia, suggesting genetic-determined lipid disorder as underlined disease. In the rare case of LDL-C levels ≥500 mg/dL, there is an urgent suspicion for homozygous FH and an immediate visit and treatment in a tertiary Lipid Center is organized. Otherwise, follow-up visits in the local Pediatric Lipid Centers are scheduled after 6 and 12 months and yearly afterwards.

In the Pediatric Lipid Center, extended diagnostic is performed under fasting conditions including lipid and cardiovascular risk factors profile (TC, LDL-C, HDL-C, TG, Apo B, Apo A-I, homocysteine, lipoprotein (a)) as wells as blood count, creatinine, GOT, GPT, blood glucose and urine analysis. Parents receive nutritional counselling and

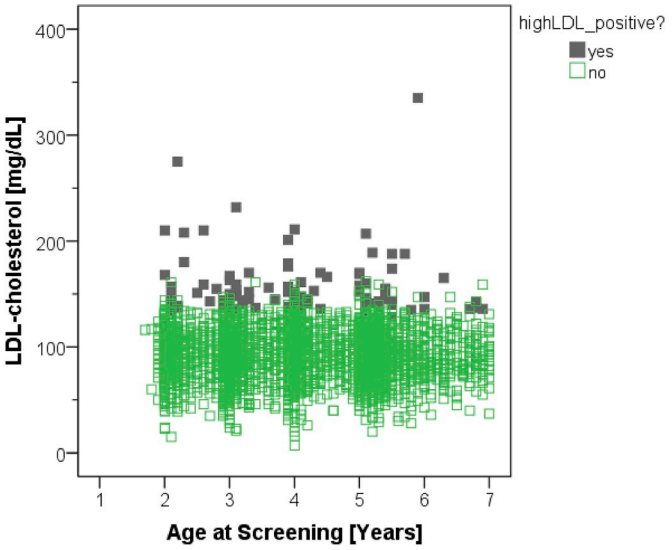

Fig. 2. Distribution of LDL-cholesterol values in 5069 children who participated and completed the Fr1dolin screening program for Hypercholesterolemia (highLDL) between November 2016 and March 2018 in Lower Saxony, Germany.

educational material, and are offered a molecular genetic assessment for FH-associated mutations. In further follow-up, an ultrasound of the common carotid artery with measuring of the intima media thickness might be also performed. If necessary, lipid lowering drug therapy will be initiated according to the current clinical guidelines [11]. Bottom-up cascade screening is offered additionally to the other family members.

2.5. Psychological stress assessment

One important aim of the study is to assess to what extent families are stressed by the diagnosis of highLDL and the screening procedure. The psychological impact, e.g. depression and anxiety symptoms as well as diagnosis related burden are monitored using a standardized questionnaire (Patient Health Questionnaire (PHQ-D) and disease-specific distress) given on the diagnosis of highLDL, at 6 and 12 months [12]. A free hotline makes sure that parents can contact the study team with all questions and worries they might have. To reduce psychological burden, initial counselling of families, printed information and regularly follow-up visits in a specialized center play an important role. If necessary and required, psychological support is offered to the families.

2.6. Statistics

Descriptive statistics are presented. Data are presented as mean ± SD, median and interquartile range (IQR) or numbers and percentages. The statistical evaluation was performed using the statistical software package IBM SPSS 23.0 (Armonk, NY). Differences between groups regarding categorical variables were analyzed with Chi-square test, Student's *t*-test for non-categorical variables with normal distribution and Mann–Whitney test for non-categorical variables which did not follow a normal distribution. A statistically significant difference was assumed with a two-sided level of $p < 0.05$ for each test.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee of Hannover Medical School (MHH, Number of Approval 7089/20.01.2016) and with the 1964 Helsinki declaration and its amendments or comparable ethical standards. A written consent of at least one parent or other primary caregiver was required.

3. Results

Up to March 2018, 130 pediatricians and primary care professionals have been registered to the Fr1dolin screening program. They have contributed in average with 60 children per pediatrician, ranging from 1 to 544 children. In the first six months, average weekly recruitment equaled 45.3 ± 23.7 samples per week, afterwards this rate increased to 87.5 ± 31.7 samples. Recruitment has been lower particularly during the public school-holidays.

Currently, 5656 children have participated in the Fr1dolin screening program. 5069/5656 children have completed the screening for highLDL (2638 boys and 2431 girls; median age: 4.0 years [IQR 3.0–5.1]). Their mothers (n = 5045) were 35 years old [IQR 31–38], their fathers (n = 5005) were 37 years old [IQR 33–42].

The distribution of the LDL-C levels in the screened population is depicted in Fig. 2. Boys had significantly lower LDL-C levels than girls (91.4 ± 22.8 mg/dL vs. 96.5 ± 22.8 mg/dL, p < 0.001). According to the Fr1dolin algorithm, significantly elevated levels of LDL-C were found in 112 children, resulting to a prevalence of LDL-hypercholesterolemia of 2.2%. Their demographic, clinical characteristics and lipid profiles are summarized in Table 1. This group consisted of 45 boys and 67 girls. No gender-specific differences in LDL-C levels were found: 157.3 ± 33.6 mg/dL in boys vs. 153.0 ± 26.0 mg/dL in girls (p = 0.445). 11/112 children (8.9%) showed also elevated triglycerides levels above 150 mg/dL. No child had hypothyroidism as cause for LDL-hypercholesterolemia (TSH: 2.55 ± 1.02 μU/mL, free T4: 1.08 ± 0.12 ng/dL).

Table 1
Summary of clinical and family history data as well as of lipid profiles in 5069 children who participated and completed the Fr1dolin screening program for HighLDL between November 2016 and March 2018 in Lower Saxony.

	Total Cohort	HighLDL yes	HighLDL no	Significance *p* value
Population (N)	5069	112	4957	–
Boys	2638	45	2593	0.013
Age (years)	4.0 ± 1.2	4.0 ± 1.3	4.0 ± 1.2	0.796
Height (cm)	103.8 ± 11.0	103.1 ± 10.6	103.9 ± 11.0	0.435
Weight (kg)	17.5 ± 5.4	17.5 ± 4.6	17.6 ± 5.4	0.853
Mother age (years)	34.5 ± 5.3	33.8 ± 5.3	34.5 ± 5.3	0.029
Father age (years)	37.8 ± 6.6	36.8 ± 6.9	37.9 ± 6.5	0.106
Family history positive for hypercholesterolemia	1986	59	1927	0.004
Family history positive for premature CVD or stroke	1454	36	1418	0.528
LDL-C (mg/dL) (1st measurement)	93.9 ± 23.0	154.7 ± 29.2	92.5 ± 20.8	< 0.001
LDL-C (mg/dL) (2nd measurement)	n. a.	157.6 ± 29.5	n. a.	n. a
Total Cholesterol (mg/dL) (2nd measurement)	n. a.	231.1 ± 32.8	n. a.	n. a
HDL-C (mg/dL) (2nd measurement)	n. a.	56.4 ± 13.7	n. a.	n. a
Triglycerides (mg/dL) (2nd measurement)	n. a.	96.3 ± 58.9	n. a.	n. a

Extended lipid profile measurement in fasting conditions (2nd measurement) including total cholesterol, HDL-cholesterol (HDL-C), and triglycerides was performed only in those children with elevated LDL-cholesterol (LDL-C) in the screening (1st measurement).
SD: standard deviation; CVD: cardiovascular disease; n. a. = not applicable. Data are presented as mean ± SD.

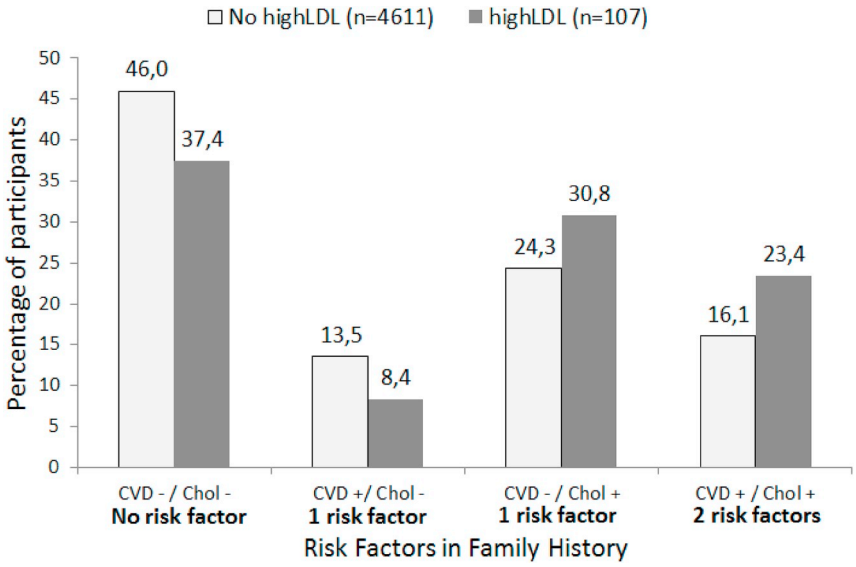

Fig. 3. Distribution of familial risk factors as hypercholesterolemia (Chol) and premature cardiovascular disease (CVD) in participants in the Fr1dolin-Trial stratified according to the screening findings in children: highLDL = positive screening, no highLDL = negative screening.

Table 2
Clinical characteristics and lipid profiles in 107 out of 112 children with HighLDL stratified according to their reported family history for hypercholesterolemia or premature cardiovascular disease or stroke in first and second degree relatives.

	Family history	Significance	*p*-value
	Positive	Negative	
Population (N)	82	25	–
Boys/Girls	36/46	7/18	0.171
Age (years)	4.3 ± 1.4	3.9 ± 1.2	0.128
Mothers' age (years)	35.2 ± 5.3	32.9 ± 5.3	0.116
Fathers' age (years)	37.8 ± 6.5	36.6 ± 7.1	0.437
LDL-C (mg/dL) (1st measurement)	159.8 ± 35.2	153.3 ± 27.3	0.903
LDL-C (mg/dL) (2nd measurement)	165.3 ± 38.8	155.3 ± 26.2	0.424
Total cholesterol (mg/dL) (2nd measurement)	238.2 ± 41.9	229.2 ± 29.9	0.261
HDL-C (mg/dL) (2nd measurement)	53.2 ± 16.6	57.3 ± 12.7	0.301
Triglycerides (mg/dL) (2nd measurement)	111.8 ± 98.6	91.7 ± 40.2	0.669

In 5 children, no information about the family history was available.
SD: standard deviation. Data are presented as mean ± SD.

In the total cohort, parents stated in 1986 cases (40.9%) a positive family history for hyperlipidemia and in 1454 cases (29.9%) for a premature cardiovascular event; information on familial risk factors was not available in 208 children (4.1%), mostly in adopted children. Fig. 3 reports of the familial risk factors for children with and without highLDL in the total cohort. As expected, children with highLDL had more often both risk factors (premature CVD and hyperlipidemia) reported in their family history. On the other hand, in a large proportion of children with highLDL (37%) these risk factors were not reported.

Among the 112 children with highLDL, 50 families reported hyperlipidemia in any family member. Interestingly, only in 24 cases father and/or mother were aware about their own condition. Positive history for premature CVD was reported in 37 families, whereas only 9 parents were affected. We found not difference in the lipid levels between children with reported positive family history and those with negative family history. The clinical and laboratory findings in children with highLDL stratified according to their family history status are summarized in Table 2.

87 parents answered the questionnaires on psychological impact after getting the diagnosis of highLDL in their child. 19.5% reported mild, 2.3% moderate and one parent (1.2%) of severe depressive symptoms. Two parents (2.3%) reported severe anxiety or panic symptoms. The burden due to the diagnosis was reported as mild (mean 0.94 ± 1.02) on a rating scale from 0 to 4. The parents with mild to severe depressive and/or panic symptoms were approached by the psychological team members by phone. There was no indication for further psychological intervention.

4. Discussion

Fr1dolin is the first population-based screening program after the newborn age for the early detection of LDL-hypercholesterolemia in toddlers and preschoolers in Germany. The first results of the screening procedure using very small amounts of blood demonstrate its feasibility. Blood sampling can be performed by capillary lancing in the pediatrician's office also in non-fasting conditions. However, long-lasting transport by mail in extremely varying temperatures may lead to a high level of hemolysis in the sample. This problem can be avoided by transporting the samples via professional lab couriers. The measurement of LDL-C may be affected by the grade of hemolysis. In order to reduce the number of false negative results regarding the screening for highLDL and reduce the number of repetitive blood sampling, we developed an appraisal algorithm by incorporating the available information about the family history in those children with a moderate/significant level of hemolysis and relatively elevated levels of LDL-C [10]. In future, by incorporating the LDL-C screening in the routine care, the issue of hemolysis shall be rather overcome by proper logistics and handling of the samples.

The Fr1dolin program is focusing in the age group of toddlers and preschoolers aiming to detect monogenic dyslipidemias in a very young age. The advantage of this age is that elevated LDL cholesterol levels are mainly due to a genetic disease, as environmental and dietary factors for lipid elevation are rare in this young age. Screening in older age groups might be confounded by life style factors influencing lipid levels. With this strategy we have a greater chance to pick-up children with a monogenic lipid disorder very early in life. In addition, introducing an appropriate diet and life style as early as possible would allow intervention before clinically relevant consequences of hyperlipidemia occur. Arguably young families are more open to professional advice and education with a higher chance of sustainable modification of family's life style and diet.

As epidemiological data on genetic lipid disorders are not available in Germany, we have expected a prevalence of highLDL of around 1:300 based on the literature for Familial Hypercholesterolemia. In our cohort of toddlers and preschoolers, we have found a prevalence of LDL-hypercholesterolemia of 2.2%, (1:45) which is significantly higher than expected. Although the definitive diagnosis of FH is still lacking, as molecular diagnosis will take place later on during the follow-up visits at the Pediatric Lipid Centers, we may explain this discrepancy through the high percentage of children from families with positive family history in our sample. It may be speculated that the sensitivity and awareness of families with known higher cardiovascular risks are higher. These families are more responsive to screening programs. However, only in 20% of the detected children hyperlipidemia was known in their parents and in 45% of their 2nd grade family members. This underlines the potential of the Fr1dolin strategy in detecting not only affected children, but also further affected relatives in a “reverse cascade screening strategy” [13]. This strategy is also followed by the Slovenian screening program for Familial Hypercholesterolemia, where universal hypercholesterolemia screening is performed in pre-school children at the primary care level (measurement of total cholesterol up to age of 5 years) and genetic FH screening in children referred to the tertiary care level according to clinical guidelines with additional cascade screening of family members [14]. Currently, the general recommendations for FH screening in Germany are focused on an index-case driven cascade strategy. More recently, a recommendation for a single total cholesterol measurement during childhood (for example at the age of 5 years) or early adolescence (for example between 12 and 14 years) as screening instrument was added [11]. In the US, the recommendations by the National Heart, Lung, and Blood Institute Expert Panel include a universal 2-step lipid screening between the ages 9 and 11 years and again at ages 17–21 years, as many children with heterozygous FH are missing by relying on the selective strategy and stayed undiagnosed during childhood [15]. On the other hand, the benefits of a child-parent screening for familial hypercholesterolemia in primary care was recently shown by Wald and colleagues [16]. For every 1000 screened children at the age 1–2 years they identified 8 persons (4 children and 4 parents) as having positive screening results for FH.

To our opinion, we need a larger number of Fr1dolin participants as well as the results of the molecular genetic analysis in order to evaluate our Fr1dolin appraisal algorithm and the LDL-C threshold of 135 mg/dL in a reverse cascade screening. Comparable cohorts and screening strategies are not available. To our knowledge, the only lipid data available for German children were measured in the KiGGS study. In that study, the LDL-cholesterol values measured in 14,233 German children from the general population were also higher than from previous studies reported [17]. Furthermore, Pang et al. who performed a genetic testing in children of affected parents with FH in Australia found that a LDL-cholesterol threshold of 3.5 mmol/L (i.e. 135 mg/dL) was the best indicator for underlying FH-associated mutation with a sensitivity of 92.8% and specificity of 96.6% [18].

The first results of the Fr1dolin-Trial demonstrate the feasibility of a public health screening in collaboration with primary care pediatricians and the introduction of a screening test within the context of regular compulsory checkups, followed by education, counselling and follow-up assessments in specialized centers. Families sensitized to the screened diseases are well responsive to this offer. In the current sample, we do not capture extended socio-economic data of participating families. During the first months of the screening program we have had an increasing number of participants, with currently around 400 participants per month, which is similar to previous studies [19,20]. Furthermore, in the present feasibility study no economic analysis of the screening including costs for counselling, follow-up of children, follow-up of parents etc. against long-term savings in health care costs has been performed. This is scheduled for the second step of the study, which will include final data on molecular genetic analysis in affected children and their family members.

In conclusion, the Fr1dolin-Trial, if successful, could have a major impact on the implementation of a screening for lipid disorders by using simple and reliable screening methods in the preventive health program in Germany and elsewhere. This strategy does not only aim the early detection of lipid disorders in young children, but also in many adult family members with unrecognized lipid abnormalities, who are at high risk for cardiovascular disease.

Conflicts of interest

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

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