REVIEW

Familial Hypercholesterolemia in the Czech Republic: More Than 17 Years of Systematic Screening Within the MedPed Project

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Summary

Familial hypercholesterolemia (FH) is the most common autosomal dominant disorder. It is characterized by a decrease in LDL cholesterol catabolism and an early clinical manifestation of atherosclerotic vessel damage. The aim of the MedPed (Make early diagnosis to Prevent early deaths) project is an early diagnosis of FH patients in order to profit from early treatment and prevent cardiovascular events. Till November 30, 2016 The Czech National MedPed Database has registered 7,001 FH patients from 5,223 different families that is 17.4 % of expected patients in the Czech Republic considering 1:250 FH prevalence. The improvement in diagnostic accuracy, patient cooperation and above all familial cascade screening is enabled by FH mutation detection using the modern technology of next-generation sequencing. FH still remain undiagnosed even though the Czech Republic is one of the most successful countries with respect to FH detection. The opportunities of international collaboration and experience sharing within international programs (e.g. EAS FHSC, ScreenPro FH etc.) will improve the detection of FH patients in the future and enable even more accessible and accurate genetic diagnostics.

Key words

Familial hypercholesterolemia • LDL-cholesterol • Cascade screening • MedPed

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Introduction

Familial hypercholesterolemia (FH) is the most common autosomal dominant disorder. It is characterized by a decrease in LDL cholesterol catabolism and an early clinical manifestation of atherosclerotic vessel damage (Vallejo-Vaz *et al.* 2016). For a long time, the prevalence of heterozygous FH in the developed countries used to be reported as 1:500 (Goldstein and Brown 1973, Goldstein *et al.* 2001). In the light of recent studies this value was corrected to 1:250. Thus, we may estimate there are more than 30 million people with this disease (Benn *et al.* 2014, Sjouke *et al.* 2015). The prevalence is even higher in populations with a founder effect and, also, with a high rate of consanguine marriages, for example in Lebanon, South Africa or in Canadian Quebec. The prevalence of homozygous FH was corrected from the previous value 1:1.000.000 to a new one of 1:300.000 (Sjouke *et al.* 2015).

For the first time, FH was described in 1938 as

familial xanthomatosis, when Müller connected familial xanthomas, high blood cholesterol level and ischemic heart disease with an inborn error of metabolism caused by a defect in one gene (Müller 1938). Next important moment in the history of FH was in 1963, when Khachadurian described heterozygous and homozygous phenotypes of FH in Lebanese patients and postulated the codominant type of inheritance (Khachadurian 1963). Ten years later, in 1973, Goldstein and Brown followed these works and described LDL receptor and its role in the catabolism of cholesterol. The defect in the LDL-receptor gene was recognized as the cause of FH (Brown and Goldstein 1973). For this discovery both the researchers were awarded the Nobel Prize in 1985.

If FH is not treated, there is much higher relative risk of early coronary events in FH patients than in the general population. Patients with homozygote FH (hoFH) typically suffer an acute cardiovascular event till 20 years of life and their life expectancy does not go beyond 30 (Huijgen *et al.* 2012, Cuchel *et al.* 2014). The situation of patients with FH improved tremendously after introducing statins on the market in 1987. A vast number of clinical studies as well as meta-analyses have proven statins are safe drugs reducing not only high concentrations of LDL-cholesterol in the circulation but, even more importantly, also the risk of cardiovascular events and cardiovascular mortality (CTT Collaboration 2010). Together with the therapeutic lifestyle changes, potent statins are treatment options of choice in FH patients. Particularly in this difficult-to-treat population it is frequently necessary to combine statins with other drugs such as cholesterol absorption blockers (ezetimibe) and/or bile acids sequestrants (resins). Recently, a new possibility for hypercholesterolemia treatment and (most probably, though outcome studies have not been published yet) also for the reduction of the risk of ischemic cardiovascular events – PCSK9 inhibitors – have been introduced. Clinical studies in FH population confirmed high therapeutic potential and added value of treatment with PCSK9 inhibitors (Raal *et al.* 2015, Kastelein *et al.* 2015).

In spite of clinical and laboratory definition, advanced diagnostic possibilities and sufficient information of high risk of premature manifestation of atherosclerosis related to lifelong increased levels of LDL-cholesterol and, also, in spite of availability an effective lipid lowering therapy, FH still remains underdiagnosed, untreated or treated inadequately (Vallejo-Vaz *et al.* 2016, Nordestgaard *et al.* 2013).

Moreover, even using all currently available treatment options to lower LDL-C, most FH patients do not reach recommended target values (Pijlman *et al.* 2010, Perez de Isla *et al.* 2016). This holds true also for the Czech Republic, although the country belongs to the most successful countries in the detection of FH because of the systematic efforts of the MedPed project.

Diagnosing familial hypercholesterolemia: so easy, so difficult

Diagnosis of FH is based on a repeated measurement of serum concentrations of LDL-cholesterol (when available pre-treatment values should be considered) and a detailed family and medical history. LDL-cholesterol level above the 95th percentile of the age and gender specific values for the population studied supports the possibility of FH diagnosis. Premature manifestation of atherosclerotic vascular complications (especially ischemic heart disease) and/or hypercholesterolemia in the proband, one of his parents, grandparents or siblings is a significant factor supporting the diagnosis of FH. Similarly, occurrence of tendon xanthomas or arcus lipoides corneae before the age of 40 years are suggestive of FH. The concentration of triglycerides is usually normal, but higher levels do not exclude the possibility of FH diagnosis. Obviously, secondary etiology of hypercholesterolemia (e.g. hypothyroidism or nephrotic syndrome) must be ruled out. Diagnosis of FH can be confirmed by identification of a causal mutation in one of the genes, which have been recognized as causal in the development of FH – LDL-receptor (LDLR), apolipoprotein B (APOB), proprotein convertase subtilisin-kexin 9 (PCSK9) or some very rare variants of other genes (e.g. APOE or STAP1) (Nordestgaard *et al.* 2013).

Currently, the most widely accepted and, also for our conditions the most suitable diagnostic criteria of FH are the Dutch Lipid Clinic Network Criteria, which use point system to classify patients into categories of definite, probable, possible or unlikely diagnosis of FH (Van Aalst-Cohen *et al.* 2006). These criteria take into account the medical history (hypercholesterolemia or premature cardiovascular disease), clinical signs (xanthomas, arcus lipoides corneae before the age of 40), LDL-cholesterol or identification of a causal mutation in one of the FH-causing genes, the latter being the strongest single criterion.

The differential diagnosis of FH comprises

familial combined hyperlipidemia – a polygenic disorder characterized by various lipid phenotypes, often associated with insulin resistance – or polygenic hypercholesterolemia. Severe form of heFH or hoFH can be misdiagnosed with sitosterolemia, which has an autosomal recessive type of inheritance and can be confirmed by assessment of markedly increased phytosterol concentrations in the plasma and, also, by identification of responsible mutations in the genes encoding for ABCG5 or ABCG8 transporters (Hubacek *et al.* 2001, Watts *et al.* 2014).

The increased risk of premature clinical manifestation of atherosclerosis in FH is mostly attributable to lifelong elevation of LDL-cholesterol, but there are factors significantly modifying the risk of an FH patient. FH patients with symptomatic or subclinical atherosclerosis, smokers, men, those initiating lipid lowering therapy at age over 40 years, patients with hypertension, diabetes or patients with high concentrations of lipoprotein (a) have increased cardiovascular disease risk compared to FH individuals without these characteristics (Santos *et al.* 2016).

Molecular aspects of FH

Most cases of FH are caused by defective gene for LDL receptor (LDLR) or apolipoprotein B-100 (APOB), rarely by a mutation in the gene for PCSK9 leading to the overproduction of this protein (gain-of-function mutations). Other mutations in genes causing FH phenotype have been described sporadically; these were for example gene variations in STAP1 or APOE loci (Santos *et al.* 2016). The phenotype of hoFH can also be caused by mutations in the LDLRAP1 gene, which are associated with an autosomal recessive form of the disease.

Only a partial correlation between the genotype and phenotype has been reported in FH subjects. Higher LDL-cholesterol and more severe phenotype are associated with so called “null mutations” in the LDLR gene, which lead to a decrease of LDLR activity below 2 % of normal as compared to so called “defective mutations”, in which the LDLR activity remains between 2 and 25 % normal function.

Identification of a causal mutation in one of the genes responsible for the development of FH confirms the diagnosis of FH and thereby, also, lifelong elevation of LDL-cholesterol. Most importantly, identification of the mutation is crucial for successful cascade screening

which enables unambiguous confirmation or excluding of FH in the proband’s family members. Moreover, knowledge of the mutation in the family increases compliance of family members to undergo the examination, what is supported by findings from the Czech national database. In families with a known causal mutation, the number of FH patients per family is on average 1.77, while in families without this information it is only 1.18. More information regarding the genetic analysis of the Czech MedPed cohort can be found in the article by Tichy *et al.* (2017).

Recent community studies from the USA underline the benefits of genetic diagnostics as these have shown that identification of a mutation was an independent predictor of overt cardiovascular disease in hypercholesterolemic patients (Khera *et al.* 2016). It is necessary to highlight, that a negative result of the genetic examination does not completely rule out the possibility of FH. Such a result can be caused by low sensitivity of the methods used, a position of the mutation out of the analyzed part of the gene or by a defect located in another gene. Some patients with clinical diagnosis of FH can have polygenic hypercholesterolemia, which is caused by multiple polymorphisms in a number of different genes. Each of these variants leads to a small increase of LDL-cholesterol and as these minor effects sum up, they may result in a marked increase of LDL-cholesterol and, thus, mimic the FH. Talmud *et al.* (2013) have identified 12 common variants of small effect with the largest relative contribution to LDL-cholesterol levels. Combinations of these small effect variants determine so called LDL-score, which is a measure of polygenic effect on LDL-concentrations. The value of LDL-score can help to differentiate patients with FH (low LDL-score) from patients with polygenic hypercholesterolemia (high LDL score). However, this approach needs be verified by more studies with higher number of patients. It is likely up to 30 % of patients with the clinical diagnosis of FH may have polygenic hypercholesterolemia in fact (Futema *et al.* 2014).

Recently, the diagnostic possibilities have improved significantly with the use of next generation sequencing. In a recent study using whole-exome sequencing approach, the causal mutation was discovered in 20 % of patients with definite clinical diagnosis of FH in whom previous DNA analysis found no mutation (Futema *et al.* 2014). On the other hand, targeted NGS in a cohort of hypercholesterolemic patients in a primary care setting revealed an FH-causing mutation in only 2 %

of the individuals examined (Norsworthy *et al.* 2014). Thus, appropriate selection of patients for the genetic analysis still remains a crucial step in FH identification.

Coordinated efforts to improve FH identification: the Czech MedPed project

The Czech Republic joined the MedPed project in 1998. However, full implementation throughout the country did not occur until the project was accepted by the Czech Society for Atherosclerosis (CSAT). A network of national and regional centers, specialized centers and professional collaborators has gradually been established under the auspices of CSAT, all of which are dedicated to identification and treatment of FH patients. Project management and coordination have been entrusted to the Centre of Molecular Biology and Gene Therapy (CMBGT) at University Hospital Brno, and, since 2003, at the Centre for Cardiovascular Surgery and Transplantation (CCST) in Brno. The clinical part of the project primarily falls under the coordination and supervision of two national centers in Prague at General University Hospital's 3rd Internal Medicine Clinic; and in

Brno at St. Anne's University's Department of Clinical Biochemistry. Essential factors for project success include involving coordinators who ensure contact with all collaborators, and are dedicated to project promotion and database compilation.

At present, the Czech MedPed project comprises 69 active centers and collaborators. It consists of 2 adult and 2 pediatric national centers (2 in Prague, and 2 in Brno); 15 regional centers for adults and 10 for children; 21 specialized centers for adults and 5 for children; and additional 14 collaborating physicians. The network is dynamic – new centers are continuously being accepted to join the project, while some end their participation. All important information pertaining to project operations, its organization, and network of participating centers, can be found on the CSAT website (<http://athero.cz/cze/projekt-medped>).

Project organization is as follows: when clinicians of different specialties (general practitioners, cardiologists, internists etc.) encounter a suspected FH patient, they may refer the patient to the network center. As of November 30, 2016, the database contained 7,001 registered patients from 5,223 families (Fig. 1).

Fig. 1. Numbers of subjects included in the Czech MedPed project.

The number of patients with FH/FDB included in the database amounts to 17.4 % of the estimated 40,000 patients living in the Czech Republic (when assuming the prevalence of 1:250). DNA samples were available in 4,399 unrelated patients, and mutations in the gene for the LDL receptor or *APOB* were found in 1,322. Moreover, the causal mutation was found in 712 pediatric patients (below the age of 18). Causal mutation in the gene for PCSK9 has not been detected in the Czech FH

population. Fourteen patients with homozygous FH or FDB have been registered in the database.

The data are being continuously monitored and updated and as of September 1, 2016 a subset of 3067 adult patients with clinical FH was analyzed. Basic characteristics of the patients' group is given in Table 1. The clinical and biochemical description of the patients' group according to the result of genetic analysis is provided in Table 2.

Table 1. Basic characteristics of a group of adult FH patients with updated records in the Czech MedPed registry as of September 1, 2016.

	Total	Males	Females
<i>N</i>	3067	1107	1960
<i>Age (years)</i>	52.6 ± 17.1	48.8 ± 16.4	54.8 ± 17.6
<i>BMI (kg/m²)</i>	26.9 ± 5.1	26.3 ± 4.8	27.2 ± 5.8
<i>Premature atherosclerosis (%)</i>	5.1	8.0	3.5
<i>T2DM (%)</i>	6.8	6.0	7.3
<i>Smoking (%)</i>	14.1	14.5	13.7
<i>Tendon xanthomatosis (%)</i>	2.7	3.4	2.2
<i>Xanthelasma palpebrarum (%)</i>	5.6	5.7	5.6
<i>TC (mmol/l ± SD)</i>	8.59 ± 1.79	8.41 ± 1.45	8.69 ± 2.0
<i>TG (mmol/l ± SD)</i>	1.69 ± 1.33	1.8 ± 1.52	1.63 ± 1.21
<i>HDL-C (mmol/l ± SD)</i>	1.55 ± 0.98	1.37 ± 0.91	1.65 ± 1.18
<i>LDL-C (mmol/l ± SD)</i>	6.26 ± 1.71	6.21 ± 1.48	6.29 ± 1.92

BMI – body mass index, TC – total cholesterol, TG – triglycerides, HDL-C – high density lipoprotein cholesterol, LDL-C – low density lipoprotein cholesterol, T2DM – type 2 diabetes mellitus, premature atherosclerosis was defined as an atherothrombotic event in personal medical history before the age of 55 in males and before the age of 60 in females.

Table 2. Characteristics of a group of adult FH patients with updated records in the Czech MedPed registry as of September 1, 2016 according to the results of genetic analysis.

	All adult FH	LDLR mutation	APOB mutation	No mutation
<i>N</i>	3067	831	454	1782
<i>Age (years ± SD)</i>	52.6 ± 17.1	49.9 ± 17.1	47.2 ± 19.1	56.2 ± 16.7
<i>BMI (kg/m² ± SD)</i>	26.9 ± 5.1	26.2 ± 4.8	27.8 ± 5.2	27.0 ± 4.7
<i>Premature atherosclerosis (%)</i>	5.1	5.3	2.9	5.6
<i>T2DM (%)</i>	6.8	4.1	3.5	8.9
<i>Hypertension (%)</i>	17.0	11.9	11.2	20.8
<i>Tendon xanthomatosis (%)</i>	2.7	5.1	0.7	2.1
<i>Xanthelasma palpebrarum (%)</i>	5.6	7.5	3.7	5.2
<i>TC (mmol/l ± SD)</i>	8.59 ± 1.80	8.33 ± 2.12	7.81 ± 1.45	8.67 ± 1.47
<i>TG (mmol/l ± SD)</i>	1.69 ± 1.39	1.6 ± 1.58	1.23 ± 1.16	1.86 ± 2.1
<i>HDL-C (mmol/l ± SD)</i>	1.55 ± 0.44	1.44 ± 0.42	1.57 ± 0.41	1.60 ± 0.42
<i>LDL-C (mmol/l ± SD)</i>	6.26 ± 1.74	6.67 ± 2.0	5.68 ± 1.36	6.22 ± 1.47

BMI – body mass index, TC – total cholesterol, TG – triglycerides, HDL-C – high density lipoprotein cholesterol, LDL-C – low density lipoprotein cholesterol, T2DM – type 2 diabetes mellitus, premature atherosclerosis was defined as an atherothrombotic event in personal medical history before the age of 55 in males and before the age of 60 in females, LDLR – low density lipoproteins receptor, APOB – apolipoprotein B.

It is worth emphasizing that, with respect to the proportion of FH patients diagnosed, the Czech Republic has quite a good standing among the international community and ranks 3rd place behind the Netherlands and Norway. The key factors for successful management of the MedPed project in the Czech Republic can be summarized as follows:

- support from professional associations;
- creation of a network of MedPed centers that covers the entire Czech Republic and is supported by a professional society (CSAT);
- use of an activity coordinator;
- access to DNA diagnostics.

Challenges and future directions

Despite the above mentioned significant successes of the FH screening program MedPed in the Czech Republic, still approximately 83 % of the predicted number of FH patients in the country have not been included in the database. Therefore, the next goal is to increase medical community awareness of FH and the active search for patients; this should lead to an increase in the number of diagnosed and well-managed patients, and, even more importantly, a substantial increase in the number of examined members of affected families.

Lots of initiatives focusing on FH detection have been launched recently. In Australia, Pacific and South America “Ten Countries Study” was successfully conducted by Watts *et al.* (2016). Another rapidly developing FH project creating a platform for mutual interaction of FH patients and health care professionals – “The FH Foundation” – has been developing since 2011 in the USA (O’Brien *et al.* 2014). In Europe, the project “FH Studies Collaboration” led by K. Ray and supported by the European Atherosclerosis Society (EAS) has evolved into a multinational project aiming at providing a consolidated data on FH in the region together with creation of a universal database platform for data collection (Vallejo-Vaz *et al.* 2015). The ScreenProFH project, embedded in the FHSC initiative, helps to enhance FH screening activities in the region of Central, Eastern and Southern Europe as well as Central Asia and is described in detail in a separate article (Ceska *et al.* 2017). Czech MedPed project is actively participating at and/or collaborating with all these international activities. Undoubtedly, these coordinated international efforts should increase the chances to achieve the principal goal – to identify, diagnose and provide treatment for all FH patients early enough to prevent development of

atherosclerotic vascular complications and avoid unnecessary premature death.

Conclusion

Familial hypercholesterolemia is the most common hereditary metabolic disease leading to early deaths due to rapid progression of atherosclerotic vascular disease. Early diagnosis and effective treatment can significantly improve the prognosis of FH patients. Thus, initiatives enhancing early detection and appropriate therapeutic approach in this population are vital. The Czech MedPed project has been working to the above-mentioned aims for more than 17 years. The opportunities of international collaboration and experience sharing within international programs (e.g. EAS FHSC, ScreenPro FH etc.) will improve the detection of FH patients in the future including more accessible and accurate genetic diagnostics.

Conflict of Interest

There is no conflict of interest.

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