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Genetic testing increases the likelihood of a diagnosis of familial hypercholesterolaemia among people referred to lipid clinics: Danish national study

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ABSTRACT

Background and aims: It is unclear to what extent genetic testing improves the ability to diagnose familial hypercholesterolaemia (FH). We investigated the percentage with FH among individuals referred to Danish lipid clinics, and evaluated the impact of genetic testing for a diagnosis of FH.

Methods: From September 2020 through November 2021, all patients referred for possible FH to one of the 15 Danish lipid clinics were invited for study participation and >97% (n = 1488) accepted. The Dutch Lipid Clinical Network criteria were used to diagnose clinical FH. The decision of genetic testing for FH was based on local practice.

Results: A total of 1243 individuals were referred, of whom 25.9% were diagnosed with genetic and/or clinical FH. In individuals genetically tested (n = 705), 21.7% had probable or definite clinical FH before testing, a percentage that increased to 36.9% after genetic testing. In individuals with unlikely and possible FH before genetic testing, 24.4% and 19.0%, respectively, had a causative pathogenic variant.

Conclusions: In a Danish nationwide study, genetic testing increased a diagnosis of FH from 22% to 37% in patients referred with hypercholesterolaemia suspected of having FH. Importantly, approximately 20% with unlikely or possible FH, who without genetic testing would not have been considered having FH (and family

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screening would not have been undertaken), had a pathogenic FH variant. We therefore recommend a more widespread use of genetic testing for evaluation of a possible FH diagnosis and potential cascade screening.

1. Introduction

Familial hypercholesterolaemia (FH) is a common genetic disorder occurring in 1:250–1:300 individuals [1–3]. Patients with untreated FH have a significantly increased risk of premature atherosclerotic cardiovascular disease (ASCVD) in particular myocardial infarction and death from coronary heart disease as well as an increased risk of aortic stenosis [3–6]. Unfortunately, most individuals with FH remain undiagnosed and many are insufficiently treated [7–9]. Indeed, less than 20% of those expected to have FH have been identified in Denmark and in most other Western countries [9–11]. There is an urgent need to identify and treat more patients with FH to reduce ASCVD risk in this population.

In Denmark, individuals suspected of having FH are referred to one of 15 lipid clinics located throughout the country, with referral criteria being i) low-density lipoprotein cholesterol (LDL-C) ≥5 mmol/L (193 mg/dL) in individuals aged above 40 years, ii) LDL-C ≥4 mmol/L (155 mg/dL) in individuals at or below 40 years, or iii) LDL-C ≥4 mmol/L (155 mg/dL) in patients with early onset ASCVD [11–13]. The use of referral criteria is crucial to identify as many patients with FH as possible, and at the same time best utilize the resources in the lipid clinics. However, it is unclear to what extent genetic testing may improve the ability to diagnose patients with FH.

We investigated the proportion of individuals referred according to Danish clinical practice to Danish lipid clinics fulfilling a diagnosis of FH according to Dutch Lipid Clinical Network (DLCN) criteria [14]. We also evaluated the impact of genetic testing in clinical practice for a diagnosis of FH and whether recommendations for the rather limited use of genetic testing should be changed in Denmark. In addition, we investigated the proportion of individuals diagnosed with FH when adding information on premature ASCVD and/or elevated LDL-C in first-degree relatives.

2. Patients and methods

2.1. Study population

All 15 Danish lipid clinics covering the whole area of Denmark were invited and agreed to participate in the study. Individuals suspected of having FH were referred from general practitioners and from hospitals to these lipid clinics, and we aimed to include at least 1000 individuals above 18 years referred during a period of at least one year (both criteria to be fulfilled). The recommended criteria for referral on suspicion of FH in Denmark were based on the following [11–13]:

1. LDL-C ≥5 mmol/l (193 mg/dL) and age above 40 years
2. LDL-C ≥4 mmol/L (155 mg/dL) and age 18–40 years
3. LDL-C ≥4 mmol/L and premature (men <55 years, women <60 years) ASCVD
4. Cascade screening after detected FH in a first-degree relative.

Prior to referral to one of the lipid clinics, the Danish national recommendations regarding referral for suspected FH suggest that all individuals are screened for possible secondary causes of dyslipidemia defined as hypothyroidism, newly discovered or dysregulated diabetes mellitus, nephrotic syndrome, chronic renal failure, primary biliary cirrhosis, drug-induced dyslipidemia (e.g., high dose glucocorticoid, cyclosporine, and psychotropic drugs) or extreme diets known to cause severe elevation of plasma cholesterol. In addition, plasma LDL-C levels fulfilling criteria 1 to 3 should be documented by at least two lipid measurements obtained at least two weeks apart [15].

In the current study, individuals referred for cascade screening, those with more than one referral criteria, and individuals with secondary dyslipidemia were excluded. We also excluded individuals awaiting results of genetic testing for FH.

The project was approved by the Danish Data Protection Agency (J. nr. 2019–899/10–0584) and all individuals provided written informed consent. All data were collected in an encrypted database Research Electronic Data Capture (REDCap), that met the authorities' security requirements and was approved by the Danish Data Protection Agency.

2.2. Data collection

All lipid clinics were provided with standardised sheets for data collection, and data were obtained at the first visit in one of the clinics. The referral cause and number of measurements of LDL-C levels fulfilling the referral criteria were registered. Measurements of height (centimetres), weight (kilograms), and waist circumference (centimetres), and examination for cholesterol deposits including tendon xanthomas, arcus cornealis, and xanthelasmatas was undertaken by experienced physicians. Body mass index was weight divided by height squared. Self-reported information regarding smoking status (never, former or current) and family history of premature ASCVD and elevated LDL-C were collected. Family history of premature ASCVD was defined as a first-degree relative with premature coronary and/or vascular disease (men aged <55 years, women aged <60 years). Family history of elevated LDL-C was defined as a first-degree relative with known LDL-C above the 95th percentile for age and sex [3,16]. The highest previously measured LDL-C was obtained by review of medical records and electronic laboratory systems and used in the DLCN classification. In individuals without registered untreated LDL-C measurements, the highest measured value was corrected for the lipid lowering treatment given at that time [17,18]. The correction factors used are shown in Supplemental Table 1 [18]. Personal history of premature ASCVD, lipid-lowering therapy, and history of hypertension and diabetes mellitus were obtained from medical records. Hypertension was defined as medical treatment for hypertension, while diabetes mellitus was defined by elevated haemoglobin A1c levels and/or treatment with antidiabetic drugs.

2.3. Laboratory analyses

Plasma total cholesterol, LDL-C, high-density lipoprotein cholesterol (HDL-C), and triglycerides were determined by routine methods at the local hospitals. All individuals were screened for major secondary dyslipidaemias including glucose status, thyroid, renal and liver function tests.

Pathogenic variants in the low-density lipoprotein receptor (*LDLR*), apolipoprotein B (*APOB*) and proprotein convertase subtilisin-like/kexin type 9 (*PCSK9*) genes were genotyped in individuals selected for genetic testing based on local practice in individual lipid clinics, reflecting usual care in Denmark of individuals with elevated LDL-C levels suggestive of FH. Genetic testing for pathogenic FH variants was carried out at five specialized laboratories. Three laboratories used next generation sequencing (NGS) for investigation of pathogenic variants in the *LDLR*, *APOB* and *PCSK9* genes in combination with multiplex ligation-dependent probe amplification (MLPA) analyses for investigation of potential copy number variations (CNVs) causative of FH. Two laboratories used Sanger sequencing for investigation of pathogenic variants in the *LDLR* and *APOB* genes in combination with MLPA analyses of CNVs causative of FH.

Table 1
Characteristics of the study population.

	Study population	Referral criteria 1 LDL-C ≥ 5.0 mmol/L and age >40 years	Referral criteria 2 LDL-C ≥ 4.0 mmol/L and age 18–40 years	Referral criteria 3 LDL-C ≥ 4 mmol/L and premature ASCVD
Median age (years)	52.4 (29.6–67.9)	56.4 (44.8–69.8)	32.2 (22.7–40.0)	53.1 (44.6–61.7)
Individuals, n (%)	1243 (100)	864 (69.5)	310 (24.9)	69 (5.6)
Men, n (%)	590 (47.5)	392 (45.4)	147 (47.4)	51 (73.9)
Women, n (%)	653 (52.5)	472 (54.6)	163 (52.6)	18 (26.1)
Body mass index (kg/m^2)	26.6 (22.0–33.5)	26.6 (22.2–33.1)	26.0 (21.4–34.8)	27.4 (24.2–34.0)
Smoking status, n (%)				
Never	606 (48.8)	386 (44.7)	195 (62.9)	25 (36.2)
Former	423 (34.0)	323 (37.4)	68 (22.9)	32 (46.4)
Current	212 (17.1)	153 (17.7)	47 (15.2)	12 (17.4)
Diabetes mellitus, n (%)	46 (3.7)	33 (3.8)	5 (1.6)	8 (11.6)
Hypertension, n (%)	265 (21.3)	224 (25.9)	12 (3.9)	29 (42.0)
History of ASCVD, n (%)	227 (18.3)	149 (17.3)	9 (2.9)	69 (100.0)
Highest untreated LDL-C, mmol/l	5.5 (4.9–6.9)	5.8 (5.1–7.0)	5.2 (4.4–6.6)	4.9 (4.2–6.5)
Lipoprotein(a), mg/l	83 (1–800)	83 (1–870)	83 (1–668)	94 (2–1274)
Lipid-lowering treatment, n (%)	338 (27.2)	247 (28.6)	49 (15.8)	42 (60.9)
Genetic test for FH, n (%)	705 (56.7)	477 (55.2)	176 (56.8)	52 (75.4)
No pathogenic FH variant	545 (77.3)	375 (78.6)	127 (72.2)	43 (84.6)
Pathogenic FH variant	160 (22.7)	102 (21.4)	49 (27.8)	9 (17.3)

Data are n (%) for categorical variables and medians (10th; 90th percentile) for continuous variables. A total of 115 individuals had missing information on lipoprotein (a), while very few (single digits) had missing information on other covariates. ASCVD = atherosclerotic cardiovascular disease, LDL-C = low-density lipoprotein cholesterol; FH = familial hypercholesterolaemia.

2.4. Diagnostic criteria for FH

All individuals were categorised according to the DLCN criteria [7]. We further classified them into those fulfilling 1) a clinical diagnosis of FH defined as probable (6–8 point) or definite FH (>8 point) before genetic test results, 2) a genetically verified FH diagnosis, and 3) a clinical FH and/or a genetically verified FH diagnosis.

2.5. Statistical analyses

Descriptive statistics were presented as medians (10th; 90th percentiles) for continuous covariates and as numbers (percentages) for categorical variables. The DLCN criteria were calculated with and without the results of genetic testing for FH.

We calculated the percentages of patients diagnosed with clinical FH according to DLCN criteria, genetically verified FH, or genetic and/or clinical FH by dividing the number of individuals in each of these groups by the total number of referred individuals, categorised according to referral criteria and clinical characteristics. Clinical characteristics included age, sex, personal or family history of premature ASCVD, family history of elevated LDL-C, tendinous xanthomata, and elevated LDL-C. Subsequently, we evaluated the impact of genetic testing for a diagnosis of FH by calculating the proportion of patients with probable or definite FH before and after genetic test results were taking into account. We also calculated the percentage of referred individuals diagnosed with clinical FH, genetically verified FH, and clinical and/or genetically verified FH according to referral criteria 1, 2 and 3 in individuals with either LDL-C ≥5.0 mmol/l (193 mg/dL) or ≥6.5 mmol/l (250 mg/dL) in combination with a family history of premature ASCVD and/or a family history of elevated LDL-C. Finally, we determined the percentage of patients being diagnosed with FH among individuals from referral criteria 2 and 3 with LDL-C ≥4.0 mmol/L (155 mg/dL) and/or LDL-C ≥4.0 (155 mg/dL) mmol/L in combination with premature ASCVD, a family history of premature ASCVD, and/or a family history of elevated LDL-C. Data were analysed using Stata/MP version 17.

3. Results

We recruited participants between September 1, 2020 and November 30, 2021. A total of 1527 individuals were invited to participate, of whom 39 (2.6%) declined. For the present analysis, we excluded 245 individuals because they had either secondary dyslipidemia (n = 34), were referred for cascade screening (n = 173), had more than one criterion for referral fulfilled (n = 17), or awaited results of genetic testing for FH (n = 23), leaving 1243 individuals for study (Supplemental Fig. 1). Individuals referred according to referral criteria 1 (LDL-C ≥5 mmol/L and age >40 years) composed most of the study population (n = 864), while 310 and 69 individuals were referred due to referral criteria 2 (LDL-C ≥4 mmol/L and age 18–40 years) and 3 (LDL-C ≥4 mmol/L and premature ASCVD) (Supplemental Fig. 1).

Characteristics of the total study population and individuals from each of the three referral criteria are given in Table 1. The median age was 52.4 years and women represented 52.5% of the study population. The median highest untreated value of LDL-C was 5.5 mmol/l. Genetic testing for FH was performed in 56.7% of the population with pathogenic FH variants found in 22.7% of the tested individuals. Less than 30% received lipid-lowering treatment in referral criteria 1, while this was given in 15.8% from referral criteria 2 and in 60.9% of those in referral criteria 3. The highest percentage of genetic tests was performed among those in referral criteria 3, but the highest percentage of pathogenic variants were found among individuals from referral criteria 2. Likewise, characteristics according to clinical information included in the DLCN criteria are shown in Supplemental Table 2. Very few had phenotypic signs of FH in terms of tendinous xanthomata (3.8%) and arcus cornealis (0.4%), and were therefore a minor cause of a clinical FH diagnosis in this population.

3.1. FH in the study population

The 1243 patients referred for hypercholesterolaemia suspected of FH, 25.9% had genetic and/or clinical FH. The percentage of patients diagnosed with clinical FH according to DLCN groups and referral criteria, both before and after genetic testing, are given in Supplemental Figs. 2–4. Among individuals from referral criteria 1, 26.0% (225/864) had genetic and/or clinical FH (Supplemental Fig. 2), while this was the case in 23.5% (73/310) and 34.8% (24/69) of the individuals from referral criteria 2 and 3, respectively (Supplemental Figs. 3 and 4).

Supplemental Fig. 2 also shows that in individuals from referral criteria 1, the percentage of patients with genetic and/or clinical FH increased from 26.0% (225/864) to 39.3% (173/440) when information

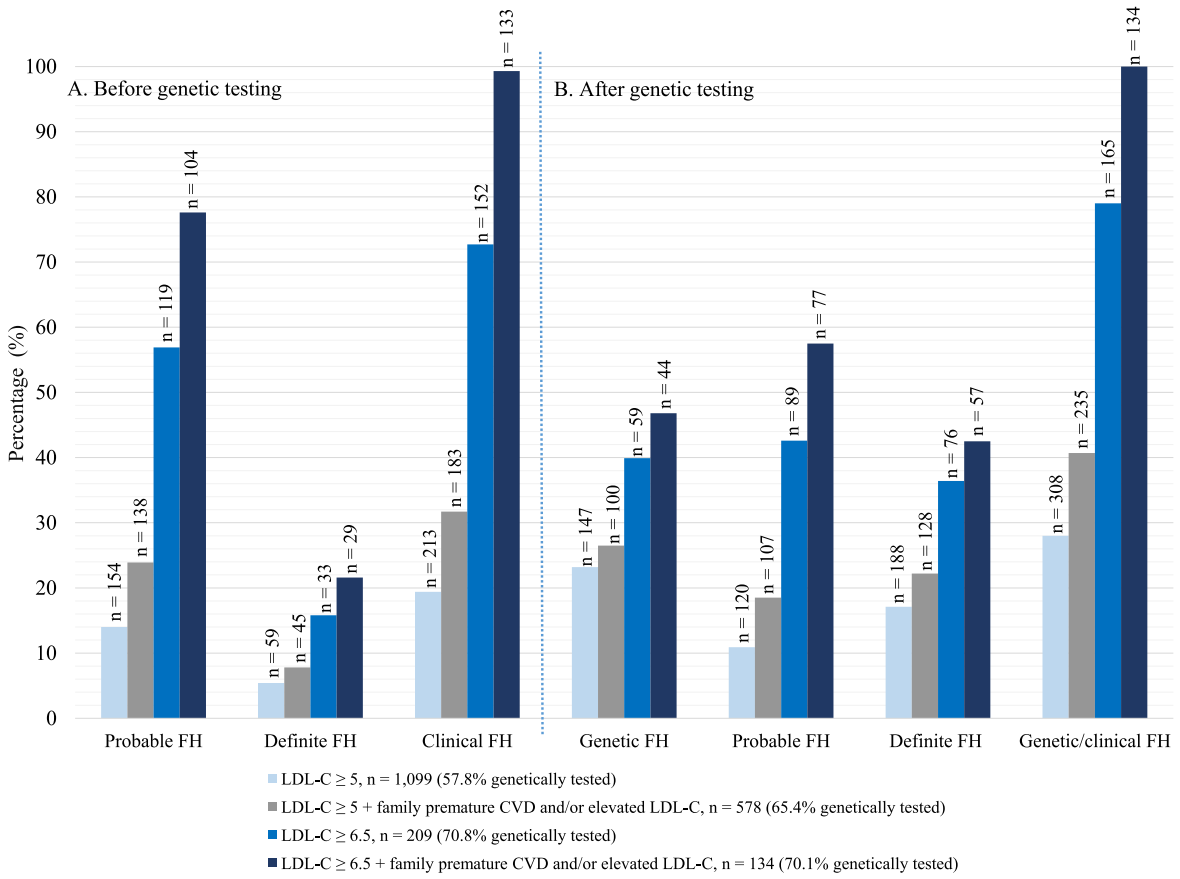

Fig. 1. Percentage of clinical FH (A) and genetic/clinical FH (B) according to DLCN criteria among individuals with LDL-C ≥5 mmol/L before and after genetic testing, respectively.
Clinical FH was defined as probable or definite FH. The percentages of individuals genetically tested are given in brackets.

regarding a family history of premature ASCVD or a family history of elevated LDL-C was added, whereas the percentage of patients with genetic and/or clinical FH was 75.6% (124/164) in those with LDL-C ≥6.5 mmol/L and 100.0% (102/102), when family history of premature ASCVD or a family history of elevated LDL-C also were present. We observed similar patterns in those from referral criteria 2 and 3 with a higher percentage of patients with genetic/clinical FH among those with LDL-C ≥6.5 mmol/L and/or in combination with a family history of premature ASCVD or a family history of elevated LDL-C (Supplemental Figs. 3 and 4).

Fig. 1 shows the percentage of patients with FH in all individuals (n = 1243) with LDL-C ≥5 mmol/L before and after genetic testing. Before genetic testing a total of 19.4% (213/1099) had FH, which increased to 28.0% (308/1099) after genetic testing. Among those with LDL ≥5 mmol/L plus a family history of premature ASCVD and/or a family history of elevated LDL-C, a total of 40.7% (235/578) had FH, while all individuals with LDL-C ≥6.5 mmol/L plus a family history of premature ASCVD and/or a family history of elevated LDL-C had FH. While the percentage with genetic and/or clinical FH was higher in those with severely elevated LDL-C and a family history of premature ASCVD or a family history of elevated LDL-C, the absolute number of identified patients with genetic/clinical FH markedly decreased (Fig. 1).

Among those genetically tested, we identified 21.4% with pathogenic FH variants in patients from referral criteria 1, while 27.8% and 17.3% had pathogenic FH variants in referral criteria 2 and 3, respectively (Table 1).

The percentage of patients diagnosed with clinical FH before genetic testing, genetic FH, and genetic and/or clinical FH are shown in Table 2. A total of 17.3% obtained a clinical FH diagnosis across referral criteria before results of genetic testing, a figure that increased to 25.9% after genetic testing. The percentage with genetic FH increased with increasing LDL-C levels (Table 2).

The percentage of genetic FH among those with a family history of premature ASCVD was 23.1%, whereas 28.1% of those with family history of elevated LDL-C had genetic FH (Table 2).

3.2. *Importance of genetic testing on FH diagnosis*

Genetic testing markedly increased the total number of patients with genetic and/or clinical FH (Fig. 2). In individuals undergoing genetic testing, 21.7% had probable or definite clinical FH before testing which increased to 36.9% after genetic testing. Corresponding values increased from 23.3% to 37.1% in patients with referral criteria 1, from 13.1% to 34.7% with referral criteria 2, and from 36.5% to 42.3% in patients with referral criteria 3. A higher percentage of those genetically tested had a personal history of ASCVD and more of them had probable or definite FH compared to those who did not receive genetic testing (Supplemental Table 3). Otherwise, there were only minor clinical differences between those genetically tested and those not tested. In individuals with unlikely clinical FH before genetic testing, 24.4% (10/41) had pathogenic FH variants while this value was 19.0% (97/511) in those with possible clinical FH (Fig. 3). Corresponding percentages of genetic FH were 31.8% (34/107) and 41.3% (19/46) in those with probable and definite clinical FH. For each of the three referral criteria, corresponding values are given in Supplemental Fig. 5.

Table 2
The probability of genetic FH or clinical FH (probable or definite) according to Dutch Lipid Clinical Network Score.

	Study population		Clinical DLCN criteria for FH BEFORE genetic testing			Genetic FH	Genetic/linical FH
			Probable FH	Definite FH	Clinical FH		
	n (%)	Genetically tested, n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Total	1243 (100)	705 (56.7)	154 (12.4)	61 (4.9)	215 (17.3)	160 (22.7)	322 (25.9)
Men	590 (47.5)	344 (58.3)	75 (12.7)	28 (4.8)	103 (17.5)	80 (23.3)	154 (26.1)
Women	653 (52.5)	361 (55.3)	79 (12.1)	33 (5.1)	112 (17.2)	80 (22.2)	168 (25.7)
Age							
Age ≤40 years	292 (23.5)	169 (57.9)	23 (7.9)	11 (3.8)	34 (11.7)	51 (30.2)	73 (25.0)
Age >40 years	951 (76.5)	536 (56.4)	131 (13.8)	50 (5.3)	181 (19.1)	109 (20.3)	249 (26.2)
Family history							
First-degree relative with premature[a] ASCVD	384 (30.9)	242 (63.0)	72 (18.8)	29 (7.6)	101 (26.4)	56 (23.1)	135 (35.2)
First-degree relative with elevated[b] LDL-C	346 (27.8)	224 (64.7)	79 (22.8)	25 (7.2)	104 (30.1)	63 (28.1)	134 (38.7)
Clinical history							
Individuals with premature[a] CAD	117 (9.4)	84 (71.8)	43 (36.8)	10 (8.6)	53 (45.3)	12 (14.3)	56 (47.9)
Individuals with premature[a] cerebral or peripheral vascular disease	17 (1.4)	8 (47.1)	5 (29.4)	2 (11.8)	7 (41.2)	1 (12.5)	7 (41.2)
Physical examination							
Tendinous xanthomata	47 (3.8)	37 (78.7)	0 (0)	47 (100.0)	47 (100.0)	13 (35.1)	47 (100.0)
LDL-C, highest untreated							
4.0–4.9 mmol/L	144 (11.6)	70 (48.6)	0 (0)	2 (1.4)	2 (1.4)	13 (18.6)	14 (9.7)
5.0–6.4 mmol/L	890 (71.6)	487 (54.7)	35 (3.9)	26 (2.9)	61 (6.8)	88 (18.1)	143 (16.1)
6.5–8.4 mmol/L	186 (15.0)	132 (71.0)	113 (61.8)	16 (8.6)	129 (69.4)	50 (37.9)	142 (76.3)
≥8 mmol/L	23 (1.9)	16 (69.6)	6 (26.1)	17 (73.9)	23 (100.0)	9 (56.3)	23 (100.0)

Clinical FH was defined as possible or definite FH according DLCN. [a]Men aged <55 years; women aged <60 years. [b]Above the 95th percentile for age and gender. ASCVD = atherosclerotic cardiovascular disease. CAD = coronary artery disease.

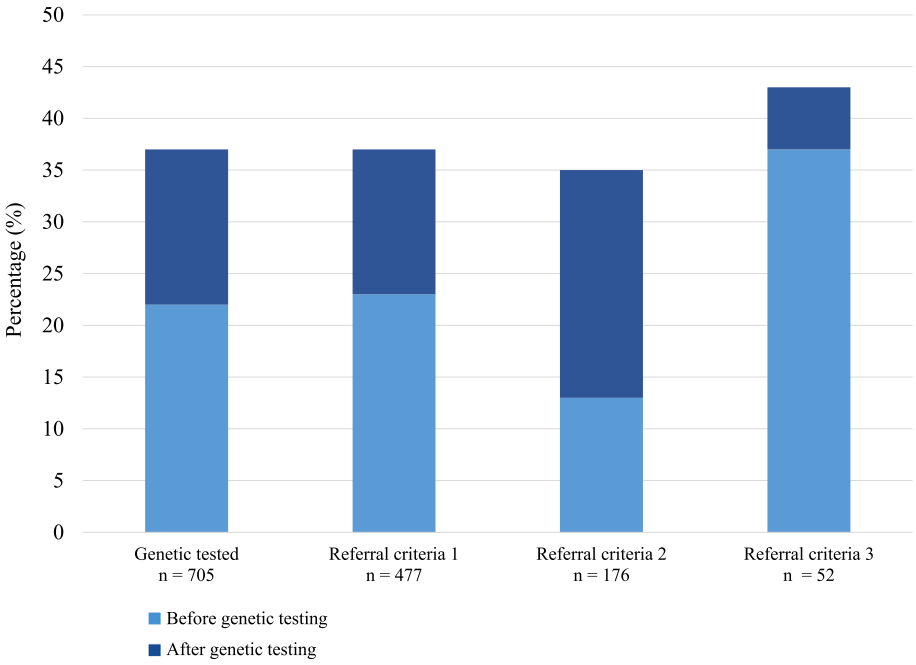

Fig. 2. The proportion of individuals with clinical FH (probable or definite FH) according to DLCN: before and after genetic testing.

4. Discussion

In this nationwide study involving all Danish lipid clinics, one in four of those referred had FH, and the percentage with definite or probable FH was higher in those with LDL-C ≥6.5 mmol/L and when a family history of premature ASCVD and/or a family history of elevated LDL-C was also present. In those genetically tested (56.7% of the population), the percentage with FH increased from 21.7% to 36.9%. The impact of genetic testing was even higher in the youngest individuals aged 18–40 years with LDL-C ≥4 mmol/L where the percentage of FH increased from 13.1% to 34.7% after genetic test results. Interestingly, we found that 24.4% of those with unlikely clinical FH and 19.0% with possible FH according to DLCN had genetic FH. Our findings of pathogenic FH variants in patients with clinical FH were in the same

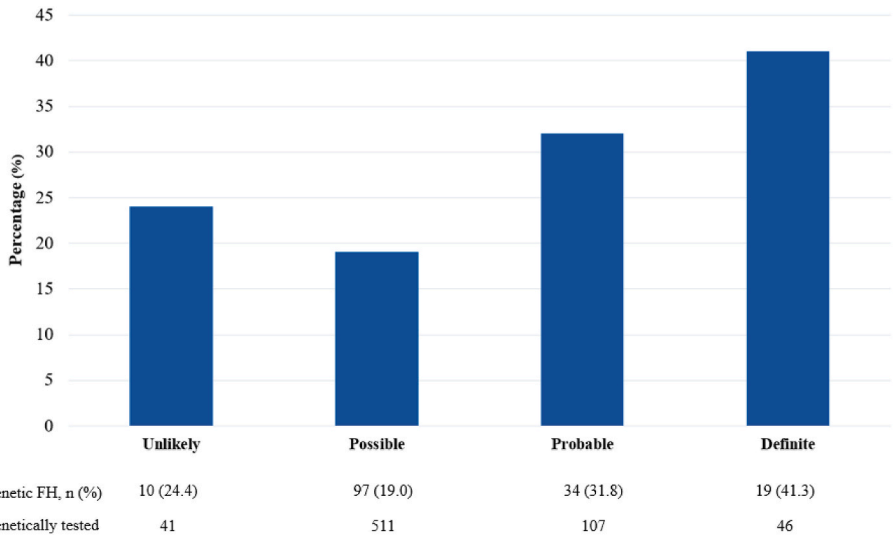

Fig. 3. The percentage of pathogenic FH variants among genetically tested individuals (n = 705) according to Clinical DLCN criteria (without taking into account genetic test results).

order of magnitude as reported previously [19] with a higher percentage of pathogenic FH variants the higher the scores were according to DLCN criteria [19–21]. Finally, approximately 20% classified with unlikely or possible FH had pathogenic mutations, which is in line with the results by other groups [21,22].

A major strength of this study was that subjects (n = 1527) referred to all Danish Lipid Clinics throughout the country during a 15-month period were included and very few (2.6%) declined to participate. Also, data collection was standardized throughout all participating lipid clinics, and all individuals were examined and categorised according to DLCN criteria. However, this study also had limitations that warrant consideration. Decision on genetic testing was individually considered and decided by lipid specialists as part of local clinical practice, and only 56.7% underwent genetic testing. This may imply that the proportion of patients with genetic FH has been underestimated as pathogenic FH variants were quite frequent among individuals with unlikely and possible FH according to the DLCN criteria. Therefore, genetic testing would have been preferable in all individuals for finding as many patients with FH as possible, but on the other hand the results represent a real-life investigation of individuals referred to Danish lipid clinics on suspicion of FH. Reasons for not performing a genetic test for FH might include a physician's decision based on the belief that the patient was unlikely to have FH or that the finding of a pathogenic FH variant would not contribute to the treatment or detection of FH in the individual or in the family. The Healthcare system in Denmark covers the costs for genetic testing free of charge for the patients, but genetic testing is costly, and the decision has to be taken by physicians in the lipid clinic, who at the same time works under responsibility for expenses in the healthcare system. We suppose that economic issues were likely important determinants for the (restricted) use of genetic testing. Furthermore, the study population consisted of Danish individuals aged above 18 years and the vast majority were Caucasians, so the results may not apply to younger individuals or patients of other ethnicities. Another limitation was that we did not correct measured LDL-C levels for concentrations of plasma lipoprotein(a) as this was not clinical practice in Denmark. Also, the optimal correction factor for adjustment of LDL-C levels for lipoprotein(a)-cholesterol has not yet been determined, and the importance of adjustment for LDL-C levels for lipoprotein(a)-cholesterol in clinical practice warrants further investigation.

Referral criteria and their use by general practitioners and physicians at hospitals are crucial to improve detection of individuals with FH to ensure effective treatment and cascade screening for identification of family members with FH [23]. We found the highest percentage (34.8%) of FH in those with LDL-C ≥4 mmol/L and premature ASCVD (referral criteria 3), while the percentage of FH was 26.1% in those aged above 40 years with LDL-C ≥5 mmol/L (referral criteria 1) and 23.6% in those aged 18–40 years with LDL-C ≥4 mmol/L (referral criteria 2). The percentage with FH was as expected higher among those with severely elevated LDL-C and a family history of premature ASCVD and those with a family history of elevated LDL-C. While referral of individuals with these characteristics would increase the probability of FH among the referred individuals, data from this study suggest, that less than half of the patients would then be diagnosed with FH, and therefore such an approach cannot be recommended.

In the present study we investigated individuals fulfilling specific referral criteria built upon knowledge from several other studies suggesting that LDL-C ≥5 mmol/L may reflect both FH due to pathogenic genetic variants and common hypercholesterolaemia not based on pathogenic variants [3,24–26]. Our results are novel and reflect a Danish model of care with an investigation of FH among individuals admitted to specialized lipid clinics, while different strategies are used in other countries to identify patients with FH [10,27].

The vast majority of our study population (>88%) had LDL-C ≥5.0 mmol/L and approximately 23% of those referred that underwent genetic testing carried a pathogenic FH variant. Interestingly, less than 2% of individuals with LDL-C ≥5.0 mmol/L identified in the Copenhagen General Population Study had a pathogenic FH variant [16]. Also, less than 2% of individuals included in the CHARGE and MIGen Consortia with LDL-C ≥4.9 mmol/L carried a pathogenic FH variant [28]. The percentage with a pathogenic FH variant in individuals with definite FH was lower than observed in some previous studies, which have reported to be 60–80% in those with definite FH, while a pathogenic FH variant typically were identified in 20–45% of those with possible FH [24]. Thus, we found that 41.3% had a pathogenic variant in those with definite FH, while this was the case in 31.8% and 19.0% of those with probable and possible FH, respectively. In summary, the main findings of the study were that among individuals referred because of LDL-C ≥5 mmol/l (≥4 mmol/l in subjects ≤40 years) and/or premature ASCVD the risk of having FH was approximately one in four. This is a minimum figure as only 56.7% were genetically tested for FH. Importantly, a diagnosis of FH could not be ruled out in patients classified as having unlikely or possible FH according to DLCN criteria. Such patients would commonly not be offered genetic testing for FH, but in our study as many as 19.4–24.4% of such patients in fact had a pathogenic FH variant. Our results therefore suggest that a low clinical DLCN score cannot reliably rule out genetic FH in particular in the young. We recommend a more

widespread use of genetic testing for FH when the genetic test results may be of importance in clinical decision making for diagnosing FH and for cascade screening purposes.

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CRediT authorship contribution statement

Berit Storgaard Hedegaard: Data gathered in all lipid clinics, Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Visualization, Project administration. **Christian Sørensen Bork:** Data gathering, Conceptualization, Methodology, Formal analysis, Writing – review & editing, Visualization, Supervision. **Helle Lynge Kanstrup:** Data gathering, Writing – review & editing. **Kristian Korsgaard Thomsen:** Data gathering, Writing – review & editing. **Merete Heitmann:** Data gathering, Writing – review & editing. **Lia Evi Bang:** Data gathering, Writing – review & editing. **Finn Lund Henriksen:** Data gathering, Writing – review & editing. **Lars Juel Andersen:** Data gathering, Writing – review & editing. **Thomas Gohr:** Data gathering, Writing – review & editing. **Mette Rauhe Mouridsen:** Data gathering, Writing – review & editing. **Anne Merete Boas Soja:** Data gathering, Writing – review & editing. **Frank-Peter Elpert:** Data gathering, Writing – review & editing. **Tomas Joen Jakobsen:** Data gathering, Writing – review & editing. **Anette Sjøl:** Data gathering, Writing – review & editing. **Albert Marni Joensen:** Data gathering, Methodology, Formal analysis, Writing – review & editing, Supervision. **Børge Grønne Nordestgaard:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Supervision. **Ib Christian Klausen:** Data gathering, Conceptualization, Funding acquisition, Methodology, Formal analysis, Supervision. **Erik Berg Schmidt:** Data gathering, Conceptualization, Methodology, Resources, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Talks sponsored by Sanofi, Amgen and Novartis

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Appendix A. Supplementary data

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