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Simplified Canadian Definition for Familial Hypercholesterolemia

Isabelle Ruel, PhD,[a] Diane Brisson, PhD,[b] Sumayah Aljenedil, MD,[a] Zuhier Awan, MD, PhD,[c] Alexis Baass, MD, MSc,[d,e] Alexandre Bélanger, BSc,[a] Jean Bergeron, MD, MSc,[f] David Bewick, MD,[g] James M. Brophy, MD, PhD,[a,h] Liam R. Brunham, MD, PhD,[i] Patrick Couture, MD, PhD,[f] Robert Dufour, MD, MSc,[j] Gordon A. Francis, MD,[i] Jiri Frohlich, MD,[k] Claude Gagné, MD,[f] Daniel Gaudet, MD, PhD,[b] Jean C. Grégoire, MD,[l] Milan Gupta, MD,[m] Robert A. Hegele, MD,[n] G.B. John Mancini, MD,[o] Brian W. McCrindle, MD,[p] Jing Pang, PhD,[q] Paolo Raggi, MD, PhD,[r] Jack V. Tu, MD, PhD,[s] Gerald F. Watts, DSc, MD,[q,t] and Jacques Genest, MD[a,h]

[a] Research Institute of the McGill University Health Centre, Royal Victoria Hospital, Montreal, Quebec, Canada; [b] Lipidology Unit, Community Genomic Medicine Centre and ECOGENE-21, Department of Medicine, Université de Montréal, Saguenay, Quebec, Canada; [c] Department of Clinical Biochemistry, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia; [d] Division of Experimental Medicine and Medical Biochemistry, Department of Medicine, McGill University, Quebec, Canada; [e] Nutrition, Metabolism and Atherosclerosis Clinic, Institut de recherches cliniques de Montréal, Quebec, Canada; [f] Lipid Research Centre, CHU de Québec-Université Laval, Québec City, Quebec, Canada; [g] Division of Cardiology, Department of Medicine, Dalhousie University, St John, New Brunswick, Canada; [h] Department of Medicine, McGill University, Royal Victoria Hospital, Montreal, Quebec, Canada; [i] Healthy Heart Program Prevention Clinic, St Paul's Hospital, Vancouver, British Columbia, Canada, Department of Medicine, University of British Columbia, Vancouver, British Columbia, and Centre for Heart Lung Innovation, Providence Health Care Research Institute, University of British Columbia, Vancouver, British Columbia, Canada; [j] Nutrition, Metabolism and Atherosclerosis Clinic, Institut de recherches cliniques de Montréal, Quebec, Canada, Department of Nutrition, Université de Montréal, Quebec, Canada; [k] Healthy Heart Program Prevention Clinic, St Paul's Hospital, Vancouver, British Columbia, Canada, Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada; [l] Montreal Heart Institute, Montreal, Quebec, Canada; [m] McMaster University, Hamilton, Ontario, Canada, Canadian Collaborative Research Network, Brampton, Ontario, Canada; [n] Departments of Medicine and Biochemistry, Schulich School of Medicine and Robarts Research Institute, Western University, London, Ontario, Canada; [o] Department of Medicine, Division of Cardiology, University of British Columbia, Vancouver, British Columbia, Canada; [p] Division of Cardiology, The Labatt Family Heart Centre, The Hospital for Sick Children, University of Toronto, Ontario, Canada; [q] School of Medicine, Faculty of Health and Medical Sciences, University of Western Australia, Perth, Australia; [r] Mazankowski Alberta Heart Institute, University of Alberta, Edmonton, Alberta, Canada; [s] Faculty of Medicine, University of Toronto, Institute for Clinical Evaluative Sciences, Schulich Heart Centre, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada; [t] Lipid Disorders Clinic, Department of Cardiology, Royal Perth Hospital, Perth, Australia

See editorial by McPherson, pages 1112–1113 of this issue.

ABSTRACT

Familial hypercholesterolemia (FH) is an autosomal codominant lipoprotein disorder characterized by elevated low-density lipoprotein cholesterol (LDL-C) and high risk of premature atherosclerotic cardiovascular disease.

RÉSUMÉ

L'hypercholestérolémie familiale (HF) est une maladie autosomique codominante caractérisée par un taux élevé de cholestérol à lipoprotéines de faible densité (cholestérol LDL) et un risque élevé de

Familial hypercholesterolemia (FH) is an autosomal dominant genetic lipoprotein disorder; the more common heterozygous form is characterized by a low-density lipoprotein cholesterol (LDL-C) > 95th percentile for age and sex within a family. Affected individuals might show clinical manifestations (premature corneal arcus, xanthomas, xanthelasmas), although these are seen less frequently in modern practice. FH is underdiagnosed and undertreated, in part because existing diagnostic criteria are complex and not widely used outside of specialty

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Corresponding author: Dr Isabelle Ruel, Research Institute of the McGill University Health Centre, 1001 Decarie Blvd, Block E, Office E01.2123, Montreal, Quebec H4A 3J1, Canada. Tel.: +1-514-934-1934 ×34852; fax: +1-514-933-6418.

E-mail: isabelle.ruel@mail.mcgill.ca

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Definitions for FH rely on complex algorithms that are on the basis of levels of total or LDL-C, clinical features, family history, and DNA analysis that are often difficult to obtain. We propose a novel simplified definition for FH. Definite FH includes: (1) elevated LDL-C ($\geq$ 8.50 mmol/L); or (2) LDL-C $\geq$ 5.0 mmol/L (for age 40 years or older; $\geq$ 4.0 mmol/L if age younger than 18 years; and $\geq$ 4.5 mmol/L if age is between 18 and 39 years) when associated with at least 1 of: (1) tendon xanthomas; or (2) causal DNA mutation in the *LDLR*, *APOB*, or *PCSK9* genes in the proband or first-degree relative. Probable FH is defined as subjects with an elevated LDL-C ($\geq$ 5.0 mmol/L) and the presence of premature atherosclerotic cardiovascular disease in the patient or a first-degree relative or an elevated LDL-C in a first-degree relative. LDL-C cut points were determined from a large database comprising > 3.3 million subjects. To compare the proposed definition with currently used algorithms (ie, the Simon Broome Register and Dutch Lipid Clinic Network), we performed concordance analyses in 5987 individuals from Canada. The new FH definition showed very good agreement compared with the Simon Broome Register and Dutch Lipid Clinic Network criteria (κ = 0.969 and 0.966, respectively). In conclusion, the proposed FH definition has diagnostic performance comparable to existing criteria, but adapted to the Canadian population, and will facilitate the diagnosis of FH patients.

Les définitions de l'HF reposent sur des algorithmes complexes basés sur les concentrations de cholestérol total ou de cholestérol LDL, les caractéristiques cliniques, les antécédents familiaux et les analyses de l'ADN souvent difficiles à obtenir. Nous proposons une nouvelle définition simplifiée de l'HF. Pour un diagnostic définitif d'HF, il faut : 1) un taux élevé de cholestérol LDL ($\geq$ 8,50 mmol/l) ou 2) un taux de cholestérol LDL $\geq$ 5,0 mmol/l (pour les 40 ans et plus); $\geq$ 4,0 mmol/l (pour les moins de 18 ans); $\geq$ 4,5 mmol/l (de 18 à 39 ans) lorsque associé à au moins l'une des caractéristiques suivantes : 1) un xanthome tendineux; ou 2) une mutation causale de l'ADN observée dans les gènes *LDLR*, *APOB* ou *PCSK9* chez le propositus ou les parents de premier degré. L'HF probable concerne les sujets qui ont un taux élevé de cholestérol LDL ($\geq$ 5,0 mmol/l) et qui montrent la présence d'une maladie cardiovasculaire athéroscléreuse prématurée chez le patient ou les parents de premier degré, ou un taux élevé de cholestérol LDL chez les parents de premier degré. Les seuils de cholestérol LDL ont été déterminés à partir d'une importante banque de données qui regroupait > 3,3 millions de sujets. En vue de comparer la définition proposée aux algorithmes actuellement utilisés (c.-à-d. le registre de Simon Broome et le Dutch Lipid Clinic Network), nous avons réalisé les analyses de concordance chez 5987 individus du Canada. La nouvelle définition de l'HF concorde très bien avec les critères du registre Simon Broome et du Dutch Lipid Clinic Network (κ = 0,969 et 0,966, respectivement). En conclusion, la définition proposée de l'HF possède une performance diagnostique comparable aux critères existants, tout en s'adaptant à la population canadienne, et facilitera le diagnostic des patients atteints d'HF.

clinics. The most commonly used diagnostic algorithms for FH are the Simon Broome Register (SBR) and the Dutch Lipid Clinic Network (DLCN) criteria, which incorporate LDL-C, clinical signs, and family history of premature atherosclerotic cardiovascular disease (ASCVD) and an elevated LDL-C > 95th percentile in a first-degree relative to generate a score that leads to classification of either "definite," "probable," or "possible" FH (Supplemental Tables S1 and S2). Detection of a pathogenic DNA mutation in an FH-related gene in a proband leads to a diagnosis of "definite FH." There are important limitations to the currently used algorithms: the clinical manifestations are infrequent; the baseline LDL-C (untreated) level is often unavailable because of the use of lipid-lowering therapies; and, family history is sometimes unavailable or unreliable. DNA testing is not readily available and not always concordant with the FH phenotype. Despite the complexities, diagnosis is important because untreated FH leads to premature ASCVD, whereas early identification and treatment can normalize risk.[1]

Heterozygous FH has a prevalence of approximately 1:250[2] and might be higher in populations with founder effects, as in the province of Québec. The homozygous form is rare and constitutes an orphan disease. Age of onset of ASCVD can vary considerably in FH subjects and in addition to sex, depends on the severity of the mutation and other risk factors. The increase in ASCVD risk remains across a broad range of elevated LDL-C levels and is at least sixfold higher even in the absence of documented FH-causing mutations. Currently used criteria are difficult to use. We therefore propose to redefine FH on the basis of simplified criteria as a genetic condition characterized by marked elevations in LDL-C and risk of early onset ASCVD (Supplemental Table S3). We provide Canada-specific LDL-C cut points and a validated calculation for an imputed LDL-C, on the basis of the type and intensity of lipid-lowering therapy.[3]

Methods

See the *Material and Methods* section of the Supplementary Material.

Results

Screening criteria for FH

The 95th percentile cut points for LDL-C are shown in Supplemental Figure S2; frequency distribution according to age and sex is shown in Supplemental Table S4. Overall, the 95th percentile for the population was 5.0 mmol/L in men and in women. The 95th percentile value for LDL-C in men younger than 18, 18-39, and older than 40 years were 3.67, 4.79, and 5.08 mmol/L, respectively. In women, these were 3.70, 4.27, and 5.18 mmol/L, respectively. We therefore selected the LDL-C cut points of $\geq$ 4.0 mmol/L for men and women younger than 18 years, $\geq$ 4.5 mmol/L for ages 18-39 years, and $\geq$ 5.0 mmol/L for subjects 40 years of age and older (Supplemental Table S3). These LDL-C levels constitute an obligatory major criterion for the diagnosis of FH and should be confirmed on repeat testing.

Along with the DLCN criteria, an LDL-C $\geq$ 8.5 mmol/L has > 99% specificity for a diagnosis of FH in genetically confirmed patients.

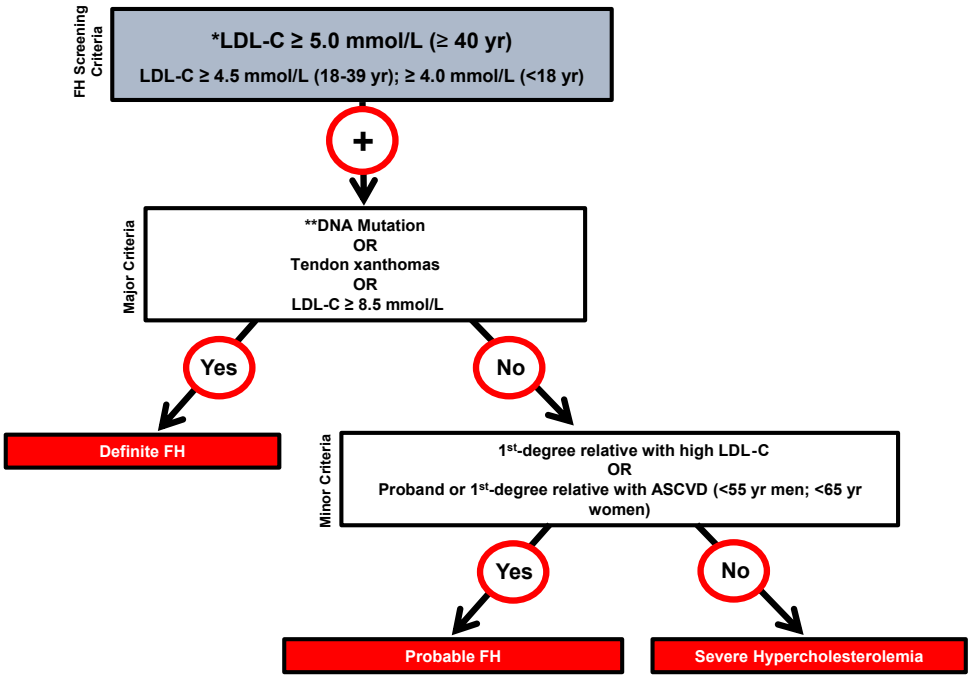

Figure 1. Canadian definition for the clinical diagnosis of familial hypercholesterolemia (FH). ASCVD, atherosclerotic cardiovascular disease; LDL-C, low-density lipoprotein cholesterol. * Secondary causes of high LDL-C should be ruled out (severe or untreated hypothyroidism, nephrotic syndrome, hepatic disease [biliary cirrhosis], medication, especially antiretroviral agents); LDL-C ≥ 4.0 mmol/L for age younger than 18 years; and LDL-C ≥ 4.5 mmol/L for age 18 years to younger than 40 years. ** Causal DNA mutation refers to the presence of a known FH-causing variant in the *LDLR*, *APOB*, or *PCSK9* gene on the basis of the presence of the variant in ClinVar, The Human Gene Mutation Database (HGMD), or Western Database of Lipid Variants (WDLV) databases, in the proband or a first-degree relative. FH diagnosis in a patient with a DNA mutation but normal LDL-C levels is unclear. Yearly follow-up of the proband is suggested and cascade screening of family members should be initiated. **Note:** In any case, cascade screening should be implemented; treatment decision should be at the discretion of the treating physician.

FH criteria: major

Xanthomas, corneal arcus and xanthelasmas. The prevalence of cutaneous manifestations of FH has decreased markedly in the statin era. In 268 new FH patients diagnosed according to the DLCN or SBR criteria examined in the Québec City Lipid clinic, CHU de Québec-Université Laval, and the Chicoutimi Hospital Lipid Clinic after 2012, only 20% had tendon xanthomas and none had premature corneal arcus or xanthelasmas (Supplemental Fig. S1). However, tendon xanthomas, which are highly specific of FH in subjects with genetic high LDL-C, are included in the DLCN and SBR criteria as a major clinical diagnostic criterion. Corneal arcus after age 45 and xanthelasma are not specific for FH and were not considered in the proposed definition of FH.

DNA mutation. The presence of a known pathogenic mutation in the *LDLR*, *APOB*, or *PCSK9* genes is a major criterion for FH. The availability of next-generation sequencing now allows the rapid and unbiased molecular diagnosis of FH using exome sequencing of the *LDLR*, *APOB*, or *PCSK9* and capture large insertion/deletion copy number variants in the *LDLR* gene. The FH diagnostic algorithm is shown in Figure 1. We do not recommend nor mandate DNA analysis systematically for all patients.

FH criteria: minor

There are 2 minor criteria: (1) a family history of elevated LDL-C > 95th percentile, according to the LDL-C criteria outlined in the *Screening Criteria for FH* section in a first-degree relative, according to age; and (2) a history of ASCVD in the proband or in a first-degree relative younger than 55 years for men or younger than 65 years for women. A diagnosis of "definite FH" is on the basis of the LDL-C criterion and 1 major criterion. "Probable FH" is on the basis of the LDL-C criterion and 1 minor criterion. "Severe hypercholesterolemia" refers to the LDL-C criterion (> 95th percentile), but without major or minor criteria for FH.

Sensitivity/specificity analyses

Agreement analyses were carried out using data from 2 large clinical databases in Canada and Australia, comparing the performance of the Canadian definition with that of SBR and the DLCN. Table 1 shows the sensitivity and specificity values for each set of data, the positive and negative predictive values, as well as the Cohen κ coefficient. Using the SBR criteria for comparison, the Canadian definition achieved 99.7% sensitivity and 98.9% specificity in the largest data set from Chicoutimi, Quebec, composed of 5987 subjects. Compared with the DLCN definition, the Canadian definition achieved 100% sensitivity and 98.8% specificity. The new Canadian definition of FH showed excellent agreement with the SBR and DLCN criteria, with κ coefficients of 0.969 and 0.966, respectively ($P < 0.0001$). Similar results were obtained in the Australian population, with the Canadian definition of FH showing excellent agreement with the SBR

Table 1. Agreement between proposed Canadian definition of FH and Simon Broome Register and DLCN criteria

	Canadian definition vs Simon Broome Register		Canadian definition vs DLCN	
	Canadian database (n = 5987)	Australian database (n = 947)	Canadian database (n = 5987)	Australian database (n = 947)
Sensitivity (95% CI), %	99.7 (99.2-99.9)	99.3 (97.6-99.9)	100 (99.6-100)	80.8 (76.5-84.6)
Specificity (95% CI), %	98.9 (98.6-99.2)	98.2 (96.8-99.0)	98.8 (98.4-99.1)	100 (99.4-100)
Positive predictive value (95% CI), %	95.3 (93.8-96.4)	96.1 (93.3-98.0)	94.5 (93-95.8)	100 (98.8-100)
Negative predictive value (95% CI), %	99.9 (99.8-100)	99.7 (98.9-100)	100 (99.9-100)	88.6 (85.9-91)
Cohen κ coefficient	0.969	0.966	0.966	0.834
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Sensitivity, specificity, and positive and negative predictive values as well as the Cohen κ coefficients were obtained from the comparison of the Canadian FH definition against the Simon Broome Register and DLCN criteria.

CI, confidence interval; DLCN, Dutch Lipid Network Criteria; FH, familial hypercholesterolemia.

criteria (κ = 0.966) and the DLCN criteria (κ = 0.834; $P < 0.0001$ for both).

Discussion

This new definition of FH showed excellent agreement with the most widely used FH criteria, the SBR and DLCN criteria, and is well adapted to the Canadian population. The risk of developing ASCVD in mutation carriers with high LDL-C has been shown to be markedly elevated; identification and early treatment of subjects with FH has been shown to normalize life expectancy. Compared with normolipidemic individuals, ASCVD risk is increased 6-fold when LDL-C is > 5 mmol/L vs noncarriers having LDL-C levels < 3.4 mmol/L and up to 22-fold when a pathogenic DNA FH-causing mutation is present.[4] This is likely related to higher cumulative lifetime vascular exposure to atherogenic low-density lipoprotein particles. In this article, we propose a novel definition of FH and online or downloadable applications that should facilitate diagnosis (www.circl.ubc.ca).

We acknowledge limitations to this scheme but this simplified definition will provide physicians and health care professionals a reliable way to diagnose FH and to initiate treatment and cascade screening in affected patients so that appropriate treatment is initiated early and might prevent cardiovascular events and deaths. There is no "gold standard" for a definition of FH and therefore, comparison with existing diagnostic criteria are necessarily limited. We recognize that our LDL-C cut points are arbitrary and that the imputed LDL-C represents the average response to lipid-lowering agents. However, the new LDL-C cut points will minimize the underdiagnosis of FH in young adults as is the case in other criteria such as the SBR criteria.

Some subjects with a causal mutation in the *LDLR*, *APOB*, or *PCSK9* genes might have an LDL-C < 95th percentile. Nevertheless, a subject with a causal mutation in the *LDLR*, *APOB*, or *PCSK9* genes remains at elevated ASCVD risk and preventive therapies must be considered. DNA testing for FH is not widely available in Canada, might not detect all types of variants, and is costly. Although a DNA diagnosis is not mandated for a diagnosis of FH, it should be considered in "probable FH" or "severe hypercholesterolemia" cases, when this might influence therapeutic decisions, especially in younger subjects. Approximately 20% of FH patients have a polygenic form of the disease. These patients would not meet the DNA criterion, but might meet the LDL-C and ASCVD criteria, and still require aggressive treatment including a possible need for PCSK9 inhibitors.

Treatment decisions should be at the discretion of the physician and the patient and should follow the 2014 Canadian Cardiovascular Society position statement on FH,[5] and the 2016 Canadian Cardiovascular Society guidelines for the management of dyslipidemia (www.onlinecjc.ca/article/S0828-282X(16)30732-2/pdf). The proposed definition for FH will also be particularly useful as a guide to select patients suitable for genetic testing, which is becoming more widely available. Because of the worldwide prevalence of FH, this new definition might be useful in countries other than Canada. The absence of positive genetic testing does not imply lack of risk in patients with LDL-C > 95th percentile, and these individuals still require active treatment to reduce their risk. The opportunity for clinicians to initiate cascade screening from an index patient is a very cost-effective method to identify new patients and initiate treatment and might prove more effective than broad cholesterol screening in childhood.

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Disclosures

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Supplementary Material

To access the supplementary material accompanying this article, visit the online version of the *Canadian Journal of Cardiology* at www.onlinecjc.ca and at https://doi.org/10.1016/j.cjca.2018.05.015.

Simplified Canadian Definition for Familial Hypercholesterolemia

SUPPLEMENTARY FILES

- FULL MANUSCRIPT
- SUPPLEMENTARY TABLES AND FIGURES

FULL MANUSCRIPT

Simplified Canadian Definition for Familial Hypercholesterolemia

Isabelle Ruel PhD[1], Diane Brisson PhD[2], Sumayah Aljenedil MD[1], Zuhier Awan MD PhD[3], Alexis Baass MD MSc[4,5], Alexandre Bélanger BSc[1], Jean Bergeron MD MSc[6], David Bewick MD[7], James M. Brophy MD PhD[1,8], Liam R. Brunham MD PhD[9], Patrick Couture MD PhD[6], Robert Dufour MD MSc[10], Gordon A. Francis MD[9], Jiri Frohlich MD[11], Claude Gagné MD[6], Daniel Gaudet MD PhD[2], Jean C. Grégoire MD[12], Milan Gupta MD[13], Robert A. Hegele MD[14], GB John Mancini MD[15], Brian W McCrindle MD[16], Jing Pang PhD[17], Paolo Raggi MD PhD[18], Jack V. Tu MD PhD[19], Gerald F. Watts DSc MD[17,20], Jacques Genest MD[1,8].

Affiliations: [1]Research Institute of the McGill University Health Centre, Royal Victoria Hospital, Montreal, QC, Canada; [2]Lipidology Unit, Community Genomic Medicine Centre and ECOGENE-21, Department of Medicine, Université de Montréal, Saguenay, QC, Canada; [3]Department of Clinical Biochemistry, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia; [4]Division of Experimental Medicine and Medical Biochemistry, Department of Medicine, McGill University, QC, Canada; [5]Nutrition, Metabolism and Atherosclerosis Clinic, Institut de recherches cliniques de Montréal, QC, Canada; [6]Lipid Research Centre, CHU de Québec-Université Laval, Québec City, QC, Canada; [7]Division of Cardiology, Department of Medicine, Dalhousie University, St. John, NB, Canada; [8]Department of Medicine, McGill University, Royal Victoria Hospital, Montreal, QC, Canada; [9]Healthy Heart Program Prevention Clinic, St. Paul's Hospital, Vancouver, BC; Department of Medicine, University of British Columbia, Vancouver, BC; Centre for Heart Lung Innovation, Providence Health Care Research Institute, University of British Columbia, Vancouver, BC, Canada; [10]Nutrition, Metabolism and Atherosclerosis Clinic, Institut de recherches cliniques de Montréal, QC; Department of Nutrition, Université de Montréal, QC, Canada; [11]Healthy Heart Program Prevention Clinic, St. Paul's Hospital, Vancouver, BC; Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada; [12]Montreal Heart Institute, Montreal, QC, Canada; [13]McMaster University, Hamilton, ON, Canada; Canadian Collaborative Research Network, Brampton, ON, Canada; [14]Departments of Medicine and Biochemistry, Schulich School of Medicine and Robarts Research Institute, Western University, London, ON, Canada; [15]Department of Medicine, Division of Cardiology, University of British Columbia, Vancouver, BC, Canada; [16]Division of Cardiology, The Labatt Family Heart Centre, The Hospital for Sick Children, University of Toronto, ON, Canada; [17]School of Medicine, Faculty of Health and Medical Sciences, University of Western Australia, Perth, Australia; [18]Mazankowski Alberta Heart Institute, University of Alberta, Edmonton, Alberta, Canada; [19]Faculty of Medicine, University of Toronto; Institute for Clinical Evaluative Sciences; Schulich Heart Centre, Sunnybrook Health Sciences Centre, Toronto, ON, Canada; [20]Lipid Disorders Clinic, Department of Cardiology, Royal Perth Hospital, Perth, Australia.

Short title: Canadian Definition of FH

Address correspondence to:
Isabelle Ruel PhD
Research Institute of the McGill University Health Centre
1001 Decarie blvd, Block E, Office E01.2123
Montreal, Quebec, H4A 3J1
Tel: (514) 934-1934 ext 34852
e-mail: isabelle.ruel@mail.mcgill.ca

Brief summary

Familial hypercholesterolemia (FH) is characterized by elevated LDL-C and high risk of premature atherosclerotic cardiovascular disease (ASCVD). We propose a novel simplified definition for FH adapted to the Canadian population. The new definition shows excellent agreement with the most widely used FH criteria, the Simon Broome Register and DLCN criteria (κ=0.969 and 0.966, respectively), and should facilitate the diagnosis of FH and the identification of patients who are likely to benefit from preventive therapy.

Abstract

Background: Familial hypercholesterolemia (FH) is an autosomal co-dominant lipoprotein disorder characterized by elevated low-density lipoprotein-cholesterol (LDL-C) and high risk of premature atherosclerotic cardiovascular disease (ASCVD). Definitions for FH rely on complex algorithms that are based on levels of total or LDL-cholesterol, clinical features, family history and DNA analysis that are often difficult to obtain. We propose a novel simplified definition for FH.

Methods: Definite FH includes 1) Elevated LDL-C (≥8.50 mmol/L); or 2) LDL-C ≥5.0 mmol/L (for age ≥40; ≥4.0 mmol/L if age <18; and ≥4.5 mmol/L if age is between 18-39 years) when associated with at least one of a) tendon xanthomas; or b) causal DNA mutation in the *LDLR*, *APOB* or *PCSK9* genes in the proband or first-degree relative. Probable FH is defined as subjects with an elevated LDL-C (≥5.0 mmol/L) and the presence of premature ASCVD in the patient or a first-degree relative or an elevated LDL-C in a first-degree relative. LDL-C cut-points were determined from a large database comprising over 3.3M subjects. To compare the proposed definition with currently used algorithms, i.e. the Simon Broome Register and Dutch Lipid Clinic Network (DLCN), we performed concordance analyses in 5987 individuals from Canada.

Results: The new FH definition showed very good agreement when compared to the Simon Broome Register and DLCN criteria (κ=0.969 and 0.966, respectively).

Conclusions: The proposed FH definition has diagnostic performance comparable to existing criteria, but adapted to the Canadian population, and will facilitate the diagnosis of FH patients.

Introduction

Familial hypercholesterolemia (FH) has traditionally been defined as an autosomal dominant genetic lipoprotein disorder; the more common heterozygous form is characterized by low-density lipoprotein cholesterol (LDL-C) >95th percentile for age and sex within a family. Affected individuals may show clinical manifestations (e.g. premature corneal arcus, xanthomas, xanthelasmas) although these are seen less frequently in modern practice with earlier diagnosis and treatment.[1] Worldwide, including in Canada, FH is underdiagnosed and undertreated, in part because existing diagnostic criteria are complex and not widely used outside of specialty lipid clinics.[2]

FH was first characterized in the 1930's by the Norwegian physician Carl Mueller.[3] There is no "gold standard" to define FH, and working definitions have evolved throughout the past decades, taking into account the molecular basis for the disease, long-term cardiovascular risk and the need for family screening. With rapid advances in genomic medicine, it is likely that these definitions will be updated. The most commonly used diagnostic algorithms for FH are the Simon Broome Register[4] and the Dutch Lipid Clinic Network (DLCN) criteria,[5] which incorporate LDL-C levels, clinical signs and family history of premature atherosclerotic cardiovascular disease (ASCVD) and an elevated LDL-C >95th percentile in a first-degree relative to generate a score that leads to classification of either "definite" or "probable" or "possible" FH, with several other less commonly used criteria.[6,7] Detection of a pathogenic DNA mutation in a FH-related gene in a proband leads to a diagnosis of "definite FH". Head-to-head comparisons suggest that the Simon Broome Register and DLCN criteria perform similarly well in diagnosing FH patients.[8] There are important limitations to the currently used algorithms: the clinical manifestations of FH, such as premature corneal arcus, xanthelasmas and tendon xanthomas are infrequently present; the baseline LDL-C (untreated) level is often unavailable due to use of lipid lowering therapies; and, family history is sometimes unavailable or unreliable. In addition, DNA testing is not readily available and not always concordant with the FH phenotype.[9] Despite the complexities, diagnosis is important because untreated FH leads to premature ASCVD (before the fourth and fifth decade in men and women, respectively),[2] while early identification and treatment can normalize risk.[10]

Heterozygous FH (HeFH) has a prevalence of approximately 1:250 based on a recent meta-analysis[11] and may be higher in populations with founder effects, as observed in the province of Québec.[12] The homozygous form (HoFH) is rare and constitutes an orphan disease. Age of onset of ASCVD can vary considerably in FH subjects and in addition to sex, depends on the severity

of the mutation, other concomitant cardiovascular risk factors, and gene-gene and gene-environment interactions.[13,14] This increase in ASCVD risk remains across a broad range of elevated LDL-C levels and is at least 6-fold higher even in the absence of documented FH-causing mutations.[15] Currently used criteria are difficult to use in the clinic and, as a consequence, many patients at very high risk of developing ASCVD may be missed. We therefore propose to redefine FH on the basis of simplified criteria as a genetic condition characterized by marked elevations in LDL-C and risk of early onset ASCVD. We provide Canada-specific LDL-C cut-points and a validated calculation for an imputed LDL-C, based on the type and intensity of lipid-lowering therapy.[16] We acknowledge limitations to this scheme but this simplified definition will provide physicians and health care professionals a reliable way to diagnose FH and to initiate treatment and cascade screening in affected patients so that appropriate treatment is initiated early may prevent cardiovascular events and deaths.

Material and methods

Baseline LDL-C. In all cases, secondary cases of elevated LDL-C (severe or untreated hypothyroidism, nephrotic syndrome, hepatic disease [primary biliary cirrhosis], medication, especially antiretroviral agents) were excluded.[17] Baseline LDL-C levels were available for most patients. When baseline LDL-C level was missing, an imputed baseline LDL-C was calculated according to the type and dose of statin (lovastatin 10, 20, 40 mg; pravastatin 10, 20 and 40 mg; simvastatin 10, 20, 40 and 80 mg; atorvastatin 10, 20, 40 and 80 mg; rosuvastatin 5, 10, 20 and 40 mg; and ezetimibe, 10 mg/day). Details of the analysis are reported elsewhere[16] but briefly, the correction factors from the meta-analysis of Hou *et al.*[18] were used to impute the LDL-C from the on-treatment LDL-C and validated this imputation in 951 Canadian patients with FH.[16] The untreated LDL-C at the time of diagnosis and the LDL-C obtained within a period of 18 months were used.

LDL-C cut-points. Data from the Gamma Dynacare Medical Laboratories (GDML) database were obtained. These data were used to generate the 95th percentile data. Details of this cohort have been previously published.[19] The 95th percentile for LDL-C was determined in 3,366,046 unique patients examined by GDML from 2002 to 2013 in the province of Ontario. The calculation of LDL-C was performed using the Friedewald formula when the plasma triglyceride level was <4.5 mmol/L; otherwise, the LDL-C was not used. For subjects with multiple testing, a single value, the highest level of LDL-C, was kept. Based on a retrospective analysis of data from the lipid clinics in Chicoutimi, Québec City and Clinical Research Institute of Montreal, all patients with a baseline LDL-C>8.5 mmol/L or with tendinous xanthomas with an elevated LDL-C has a mutation of the *LDLR* or *APOB* genes. Thus, these constitute criteria for "definite" FH. In accordance with the DLCN and Simon Broome Register criteria, a family history of elevated LDL-C in a first-degree relative or a family history of premature ASCVD in a first-degree relative constitute minor criteria for a diagnosis of "probable". These set of criteria correspond to the "probable" FH category from the Simon Broome Register and both the "possible" and "probable" FH categories as seen in the DLCN. An elevated LDL-C in the absence of other criteria constitutes a third category of "severe hypercholesterolemia".

Xanthomas, corneal arcus and xanthelasmas. The clinical manifestations of FH, such as premature corneal arcus (onset <45 years old), xanthelasmas and tendon xanthomas were visually determined in a large lipid clinic (Québec City Lipid clinic, CHU de Québec-Université Laval and

the Chicoutimi Hospital Lipid Clinic, QC, Canada) in three time periods (prior to 1979; 1980-2011 and 2012 and later).[20]

Canadian FH algorithm. We based a diagnosis of "definite" FH on the presence of the LDL-C screening criteria and one or more of the following major criteria (**Supplemental Table S3**): 1) the presence of extensor tendon xanthomas; 2) the identification of a mutation in the *LDLR, APOB* or *PCSK9* genes known to cause FH in the proband or a first-degree relative; or, 3) an LDL-C level ≥8.5 mmol/L.[5] A "probable" FH diagnosis relies on the presence of one or both of the minor criteria: 1) the presence of an LDL-C ≥95th percentile (as described above) in a first-degree relative; or, 2) the presence of premature ASCVD, as defined in the 2016 update of the Canadian Cardiovascular Society guidelines for the management of dyslipidemia in the adult[21] in the proband or in a first-degree relative (<55 and <65 years in men and women, respectively). Patients who only have the LDL-C criterion have a "severe hypercholesterolemia" diagnosis, and remain at a risk of ASCVD 6-fold that of age and gender-matched subjects with LDL-C levels <3.4 mmol/L.[15,22]

Statistical analysis and validation. The validation of the conversion factors used to impute baseline LDL-C has been previously published.[16] Descriptive statistics and statistical analysis were performed using Stata, version 13.1 (Texas, USA). Patients with a "possible" or "probable" diagnosis were designated as negative cases for the purpose of calculating sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The Cohen's kappa (κ) coefficient was applied to evaluate the agreement between the new Canadian FH definition and both the Simon Broome Register or DLCN criteria (**Supplemental Tables S1 & S2**) using data from the Lipidology Unit at the Community Genomic Medicine Centre in Chicoutimi, Québec, Canada (n=5,987), the largest database currently available on FH in Canada. Data from the FH Western Australia program (n=947) were also used to provide an international comparator.[23-25] The extent of agreement among the κ values was interpreted according to the terminology by Landis and Koch;[26] specifically, κ >0.8 indicated excellent agreement, 0.6–0.8 indicated good agreement, 0.4–0.6 indicated moderate agreement, and <0.4 indicated poor agreement.

Results

Screening criteria for FH:

Baseline LDL-C. When baseline LDL-C was unavailable, an imputed value for FH diagnosis was used, based on the average response to statins and ezetimibe.[16] The use of a downloadable application (www.FHCanada.net; www.circl.ubc.ca/) facilitates the imputation of LDL-C. The correlation between baseline LDL-C and imputed LDL-C has been previously published (r=0.76, $p<0.001$).[16]

LDL-C cut-points. The 95th percentile cut-points for LDL-C were determined in 3,366,046 subjects from the province of Ontario[19] and are shown in **Figure 1;** frequency distribution according to age and sex is shown in **Supplemental Table S4**. Overall, the 95th percentile for the population was 5.0 mmol/L in men and in women. The 95th percentile value for LDL-C in men <18, 18-39 and >40 years were 3.67, 4.79 and 5.08 mmol/L, respectively. In women, these were 3.70, 4.27 and 5.18 mmol/L, respectively. We therefore selected the LDL-C cut-points of ≥4.0 mmol/L for men and women <18 years, ≥4.5 mmol/L for ages 18-39 and ≥5.0 mmol/L for subjects ≥40 years of age. These LDL-C levels constitute an obligatory major criterion for the diagnosis of FH and should be confirmed on repeat testing.

Along with the DLCN criteria, examination of existing Canadian databases confirms that LDL-C levels ≥8.5 mmol/L has >99% specificity for a diagnosis of FH in genetically confirmed patients (data not shown). However, the sensitivity of this criterion is weak. In many cases, the baseline (untreated) LDL-C level is either based on historical values or is unknown because the patient was started on lipid-lowering therapy and often high intensity statin after an acute coronary syndrome.

FH criteria: Major

Xanthomas, corneal arcus and xanthelasmas. The prevalence of cutaneous manifestations of FH has decreased markedly in the statin era. In 268 new FH patients diagnosed according to the DLCN or Simon Broome Register criteria examined in the Québec City Lipid clinic, CHU de Québec-Université Laval and the Chicoutimi Hospital Lipid Clinic after 2012, only 20% had tendon xanthomas and none had premature corneal arcus or xanthelasmas (**Supplemental Figure S1**). However, tendon xanthomas, which are highly specific of FH in subjects with genetic high LDL-C, are included in both the DLCN and Simon Broome Register criteria as a major clinical diagnostic criterion[27] (**Supplemental Tables S1 & S2**). Similarly, examination of the Clinical Research Institute of Montreal database showed a 98.7% specificity of xanthomas for FH (data not

shown), which were therefore included in the Canadian algorithm as a major criterion for FH. However, corneal arcus after age 45 and xanthelasma are not specific for FH[7] and were not considered in the proposed definition of FH**.**

DNA mutation. The presence of a known pathogenic mutation in the *LDLR*, *APOB* or *PCSK9* genes is a major criterion for FH. Several other genes have been shown to cause the biochemical phenotype of FH, but these are rare and will not be discussed further. In geographical areas with genetic founder effects, especially in the province of Québec, a panel of 10 molecular defects in the *LDLR* gene that capture ~85% of FH causing mutations in patients of French-Canadian descent is available at low cost.[28] The availability of next generation sequencing (NGS) now allows the rapid and unbiased molecular diagnosis of FH by exome sequencing of the *LDLR*, *APOB* or *PCSK9* and capture large insertion/deletion copy number variants in the *LDLR* gene.[29,30] The FH diagnostic algorithm is shown in **Figure 2**. DNA sequence variants can be validated using several databases including the Western Database of Lipid Variants (WDLV);[31] the Human Gene Mutation Database (HGMD)[32] and ClinVar from the National Center for Biotechnology Information; or for novel variants, according to accepted criteria for pathogenicity.[33,34] We do not recommend nor mandate DNA analysis systematically for all patients.[17]

FH criteria: Minor

There are two minor criteria: 1) a family history of elevated LDL-C >95th percentile, according to the criteria outlined below in a first-degree relative, according to age; and 2) a history of ASCVD in the proband or in a first-degree relative <55 for men or <65 years for women. A diagnosis of "definite FH" is based on the LDL-C criterion and one major criterion. "Probable FH" is based on the LDL-C criterion and one minor criterion. "Severe hypercholesterolemia" refers to the LDL-C criterion (>95th percentile), but without major or minor criteria for FH.

Sensitivity/Specificity analyses.

Agreement analyses were carried out using data from two large clinical databases in Canada and Australia, comparing the performance of the Canadian definition with that of Simon Broome Register and the DLCN. **Table 1** shows the sensitivity and specificity values for each set of data, the positive and negative predictive values as well as the Cohen's kappa coefficient. Using the Simon Broome Register criteria for comparison, the Canadian definition achieved 99.7% sensitivity and 98.9% specificity in the largest dataset from Chicoutimi, QC, composed of 5,987 subjects. When compared with the DLCN definition, the Canadian definition achieved 100% sensitivity and 98.8% specificity (**Table 1**). The new Canadian definition of FH showed excellent

agreement with both the Simon Broome Register and DLCN criteria, with kappa coefficients of 0.969 and 0.966, respectively (p <0.0001). Similar results were obtained in the Australian population, with the Canadian definition of FH showing excellent agreement with both the Simon Broome Register criteria ($\kappa = 0.966$) and the DLCN criteria ($\kappa = 0.834$; p <0.0001 for both).

Discussion

To facilitate the diagnosis of FH and the identification of patients who are likely to benefit from preventive therapy, we have first established LDL-C cut-points for a large population in Canada and determined major and minor criteria for FH in the Canadian context. We propose a simplified Canadian definition for FH that relies on 1) LDL-C levels; 2) major criteria of the presence of xanthomas, LDL-C ≥8.5 mmol/L or DNA mutation causing FH in proband or a first-degree relative; and 3) minor criteria of premature ASCVD (<55 years in men, <65 years in women) in proband or a first-degree relative or elevated LDL-C in a first-degree relative. This new Canadian definition of FH showed excellent agreement with the most widely used FH criteria, the Simon Broome Register and DLCN criteria, and is well-adapted to the Canadian population.

The diagnosis of FH has evolved over the past decades, owing to clarification of the genetic basis, the changing phenotype and awareness of the clinical implications. Once considered a relatively uncommon disorder with a prevalence of 1:500, a more recent meta-analysis of published studies shows a prevalence of ~1:250, making FH the most common monogenic disorder encountered in clinical practice.[11] The risk of developing ASCVD in mutation carriers with high LDL-C has been shown to be markedly elevated;[15,35] identification and early treatment of subjects with FH has been shown to normalize life expectancy.[2] Compared to normolipidemic individuals, ASCVD risk is increased 6-fold when LDL-C is >5 mmol/L versus non-carriers having LDL-C levels <3.4 mmol/L and up to 22-fold when a pathogenic DNA FH-causing mutation is present.[15,22,35] This is likely related to higher cumulative lifetime vascular exposure to atherogenic LDL particles. Yet, the diagnosis of FH remains the province of specialized physicians, especially lipidologists. Here, we propose a novel definition of FH and on-line or downloadable applications that should facilitate diagnosis.[36,37] This new simplified definition has a remarkably high degree of agreement with the Simon Broome Register and DLCN criteria.

We acknowledge limitations to the present study. There is no "gold standard" for a definition of FH and therefore, comparison to existing diagnostic criteria are necessarily limited. We recognize that our LDL-C cut-points are arbitrary and that the imputed LDL-C represents the average response to lipid-lowering agents and are based on branded and not generic agents. However, the new LDL-C cut-points will minimize the under-diagnosis of FH in young adults as is the case in other criteria such as the Simon Broome Register criteria. For children, we kept the LDL-C cut-point of >4.0 mmol/L although an LDL-C >3.5 mmol/L is strongly predictive of FH in this age-group,[38] for which the issue of definite diagnosis is important since it infers an LDL

risk that is present starting at birth and extending across the lifespan. Early treatment has been shown to be more effective than later treatment, and a lifetime of low risk is necessary to achieve normal vascular health across the lifespan. Detection, diagnosis and treatment of FH early in life is, therefore, essential.

Some subjects with a causal mutation in the *LDLR*, *APOB* or *PCSK9* genes may have an LDL-C <95th percentile.[8] Nevertheless, a subject with a causal mutation in the *LDLR*, *APOB* or *PCSK9* genes remains at elevated ASCVD risk and preventive therapies must be considered.[15,35] DNA testing for FH is not widely available in Canada, may not detect all types of variants, and is costly. While a DNA diagnosis is not mandated for a diagnosis of FH, it should be considered in "probable FH" or "severe hypercholesterolemia" cases, where this may influence therapeutic decisions especially in younger subjects. Furthermore, a molecular diagnosis of FH would mandate an aggressive therapeutic approach. A DNA diagnosis in a subject with LDL-C levels ≥8.5 mmol/L carries a near 100% certainty of identifying a mutation, and therefore, may not influence clinical decisions. Finally, approximately 20% of FH patients have a polygenic form of the disease.[39,40] These patients would not meet the DNA criterion, but may meet the LDL-C and ASCVD criteria, and still require aggressive treatment including possible need for PCSK9 inhibitors.

This simplified definition of FH should enable physicians to recognize and treat a frequent monogenic lipoprotein disorder that carries a very high risk of ASCVD in affected subjects. Treatment decision should be at the discretion of the physician and the patient and should follow the 2014 Canadian Cardiovascular Society position statement on familial hypercholesterolemia,[17] the 2016 Canadian Cardiovascular Society Guidelines for the Management of Dyslipidemia for the Prevention of Cardiovascular Disease in the Adult[21] and the NHLBI Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents.[41] The proposed definition for FH will also be particularly useful as a guide to select patients suitable for genetic testing, which is becoming more widely available in the country. Given the worldwide prevalence of FH, this new definition might be useful in countries other than Canada. The absence of positive genetic testing does not imply lack of risk in patients with LDL-C >95th percentile, and these individuals still require active treatment to reduce their risk. Worldwide, FH is underdiagnosed and considerable efforts are being implemented to raise awareness internationally.[42-44] The opportunity for clinicians to initiate cascade screening from an index-patient is a very cost-effective method to identify new patients and initiate treatment[45-48] and may prove more effective than broad cholesterol screening in childhood.[49] The role of registries for FH stems from the European

experience (especially the Netherlands and Norway)[2,50] and such a registry is being implemented in Canada (www.FHCanada.net). The experience of the British Columbia FH Registry shows the importance of learning from such a registry.[51]

Conclusions

To provide physicians and health care professionals a reliable way to detect FH and to initiate treatment and cascade screening in affected patients, we propose a pragmatic, simplified definition of FH. The proposed definition is adapted to the Canadian population, and shows diagnostic performance comparable to existing criteria. We expect that it will facilitate the identification of FH patients and help prevent cardiovascular events and deaths associated with this condition.

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Disclamer

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Table 1. Agreement between Proposed Canadian Definition of Familial Hypercholesterolemia and Simon Broome Register and DLCN criteria.

	Canadian definition versus Simon Broome Register		Canadian definition versus DLCN	
	Canadian database (n=5987)	Australian database (n=947)	Canadian database (n=5987)	Australian database (n=947)
Sensitivity, % (95% CI)	99.7 (99.2-99.9)	99.3 (97.6-99.9)	100 (99.6-100)	80.8 (76.5-84.6)
Specificity, % (95% CI)	98.9 (98.6-99.2)	98.2 (96.8-99.0)	98.8 (98.4-99.1)	100 (99.4-100)
Positive Predictive Value, % (95% CI)	95.3 (93.8-96.4)	96.1 (93.3-98.0)	94.5 (93-95.8)	100 (98.8-100)
Negative Predictive Value, % (95% CI)	99.9 (99.8-100)	99.7 (98.9-100)	100 (99.9-100)	88.6 (85.9-91)
κ coefficient	0.969	0.966	0.966	0.834
p value	<0.0001	<0.0001	<0.0001	<0.0001

This table shows the sensitivity, specificity, and positive and negative predictive values as well as the Cohen's kappa coefficients obtained from the comparison of the Canadian FH definition against the Simon Broome Register and DLCN criteria. DLCN: Dutch Lipid Network Criteria.

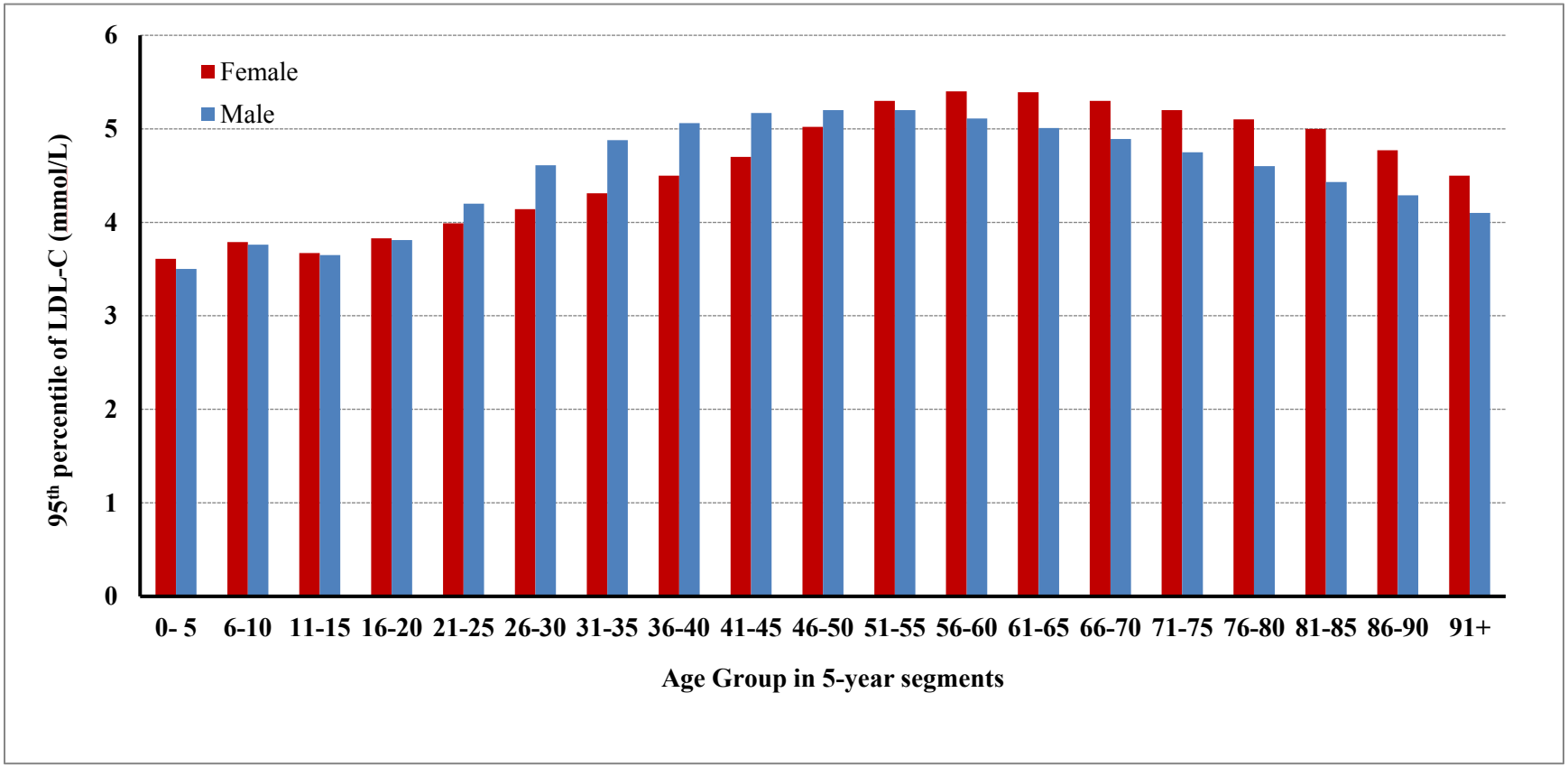

Female
Male
95th percentile of LDL-C (mmol/L)
0
1
2
3
4
5
6
0- 5
6-10
11-15
16-20
21-25
26-30
31-35
36-40
41-45
46-50
51-55
56-60
61-65
66-70
71-75
76-80
81-85
86-90
91+
Age Group in 5-year segments

Figure 1. Characterization of the 95th percentile of LDL-C levels in the Canadian population.

Data from the GDML database were used to generate the 95th percentile data for LDL-C in 3,366,046 unique patients from 2002 to 2013 in the province of Ontario. For subjects with multiple testing, a single value, the highest level of LDL-C, was kept.

LDL-C: low-density lipoprotein cholesterol.

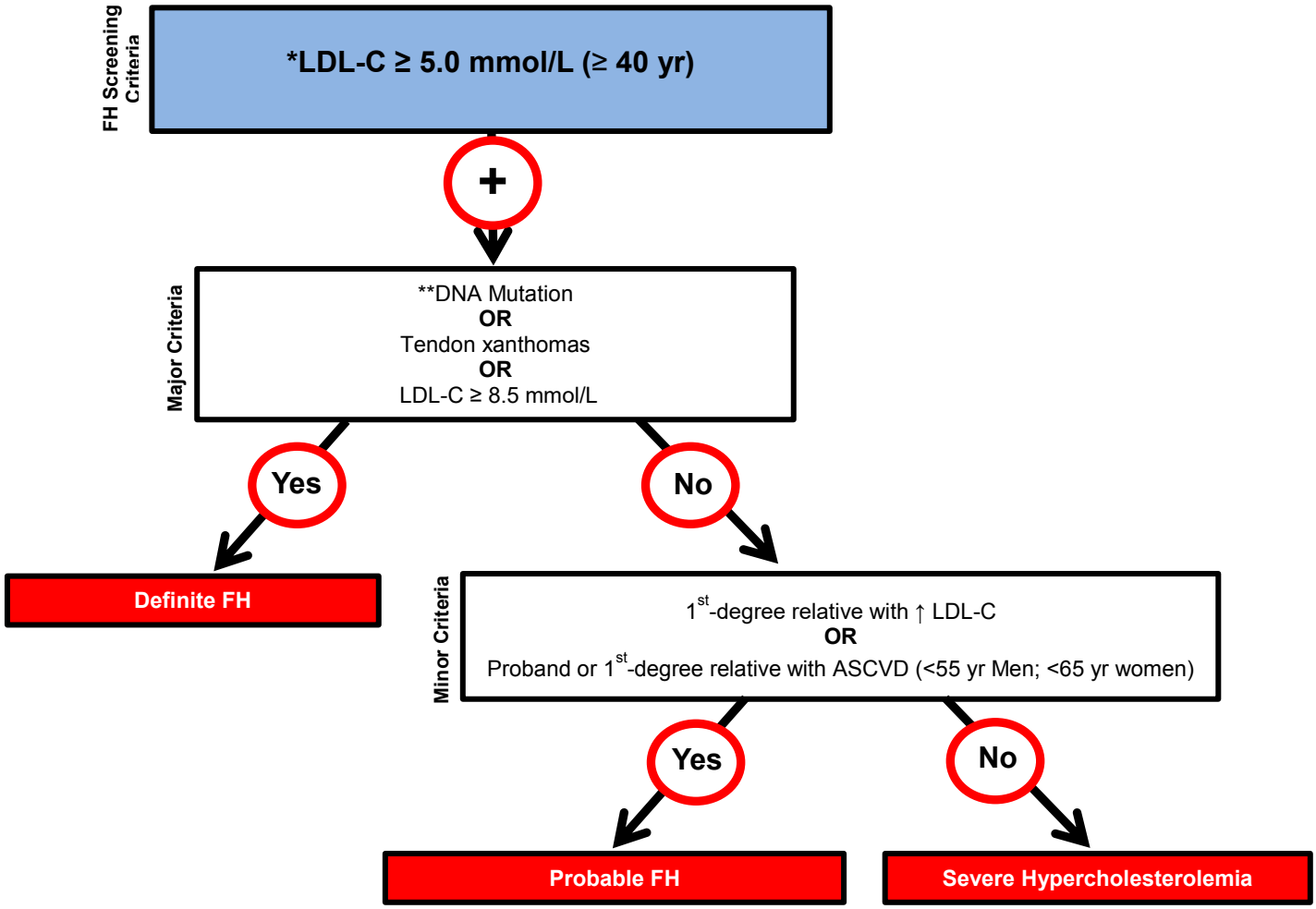


Figure 2. Canadian definition for the clinical diagnosis of FH.

* Secondary causes of high LDL-C should be ruled out (severe or untreated hypothyroidism, nephrotic syndrome, hepatic disease (biliary cirrhosis), medication especially antiretroviral agents);

LDL-C ≥ 4.0 mmol/L for age < 18 yr;

LDL-C ≥ 4.5 mmol/L for age ≥ 18 yr and < 40 yr.

** Causal DNA mutation refers to the presence of a known FH-causing variant in the *LDLR*, *APOB* or *PCSK9* gene based on presence of the variant in ClinVar, HGMD or WDLV databases, in the

proband or a first-degree relative. FH diagnosis in a patient with a DNA mutation but normal LDL-C levels is unclear. Yearly follow-up of the proband is suggested and cascade screening of family members should be initiated. Note: In any case, cascade screening should be implemented; treatment decision should be at the discretion of the treating physician.

LDL-C: low-density lipoprotein cholesterol; yr: year; DNA: deoxyribonucleic acid; ASCVD: atherosclerotic cardiovascular disease.

SUPPLEMENTARY TABLES AND FIGURES

Supplemental Table S1. Simon Broome Register criteria for the clinical diagnosis of FH.

Presence of DNA mutation known to cause FH (*LDLR*, *APOB*, *PCSK9* genes)			**Definite**
LDL-C > 4.9 mmol/L (> 4.0 mmol/L in children under 16yr) **or** Total cholesterol > 7.5 mmol/L (> 6.7 mmol/L in children under 16yr)	**+**	Tendon xanthomas or evidence of these signs in first- or second-degree relative	**Definite**
LDL-C > 4.9 mmol/L (> 4.0 mmol/L in children under 16yr) **or** Total cholesterol > 7.5 mmol/L (> 6.7 mmol/L in children under 16yr)	**+**	Family history of MI under 50 yr in a second-degree relative or under 60 yr in a first-degree relative **or** Family history of raised total cholesterol concentration > 7.5 mmol/L in a first- or second-degree relative or > 6.7 mmol/L in children under 16 yr	**Possible**

FH: familial hypercholesterolemia; DNA: deoxyribonucleic acid; LDLR: low-density lipoprotein receptor; APOB: apolipoprotein B; PCSK9: Proprotein convertase subtilisin/kexin type 9; LDL-C: low-density lipoprotein cholesterol; yr: year; MI: myocardial infarction.

Adapted from Reference #4: Risk of fatal coronary heart disease in familial hypercholesterolaemia. Scientific Steering Committee on behalf of the Simon Broome Register Group. BMJ 1991;303:893-6.

Supplemental Table S2. Dutch Lipid Clinic Network criteria for the clinical diagnosis of FH.

Group 1: Family history	
• First-degree relative known with premature coronary and vascular disease (men under 55 yr, women under 60 yr) **or** • First-degree relative known with LDL-C > 95th percentile	**1 point**
• First-degree relative with tendon xanthomata and/or arcus cornealis **or** • Children under 18 yr with LDL-C > 95th percentile	**2 points**
Group 2: Clinical history	
• Patient has premature (men under 55 yr, women under 60 yr) CAD	**2 points**
• Patient has premature (men under 55 yr, women under 60 yr) cerebral or peripheral vascular disease	**1 point**
Group 3: Physical examination	
• Tendon xanthomata	**6 points**
• Corneal Arcus under 45 yr	**4 points**
Group 4: Laboratory analysis	
• LDL-C > 8.5 mmol/L	**8 points**
• LDL-C 6.5 - 8.50 mmol/L	**5 points**
• LDL-C 5.0 - 6.49 mmol/L	**3 points**
• LDL-C 4.0 - 4.99 mmol/L	**1 point**
Group 5: DNA analysis	
• Functional mutation known to cause FH	**8 points**
FH DIAGNOSIS	
• **Definite**	**9 or more points**
• **Probable**	**6-8 points**
• **Possible**	**3-5 points**

The highest score per group should be applied

FH: familial hypercholesterolemia; yr: year; LDL-C: low-density lipoprotein cholesterol; CAD: coronary artery disease; DNA: deoxyribonucleic acid.
Adapted from Reference #5: World Health Organization. Familial Hypercholesterolemia - Report of a Second WHO Consultation. Geneva, Switzerland 1999.

Supplemental Table S3. Proposed Canadian Definition for the Diagnosis of Familial Hypercholesterolemia.

		Variable	FH Diagnosis
Step 1	**FH screening criterion***	LDL-C ≥ 4.0 mmol/L for age under 18 yr LDL-C ≥ 4.5 mmol/L for age between 18 yr and 39 yr LDL-C ≥ 5.0 mmol/L for age 40 yr and over	
Step 2	**Major criteria**	Requires one of the following: •Tendon xanthomas in proband •FH causing DNA mutation in proband or in a first-degree relative** •High LDL-C (≥8.5 mmol/L) in proband	**Definite**
Step 3	**Minor criteria**	Requires one of the following: •First-degree relative with high LDL-C (not due to secondary causes)* •Proband or First-degree relative with early onset ASCVD (men under 55yr; women under 65 yr)	**Probable**
Step 4	**None of the criteria from step 2 and 3**		**Severe Hypercholesterolemia**

* Secondary causes of high LDL-C should be ruled out (severe or untreated hypothyroidism, nephrotic syndrome, hepatic disease [primary biliary cirrhosis], or medication especially antiretroviral agents);

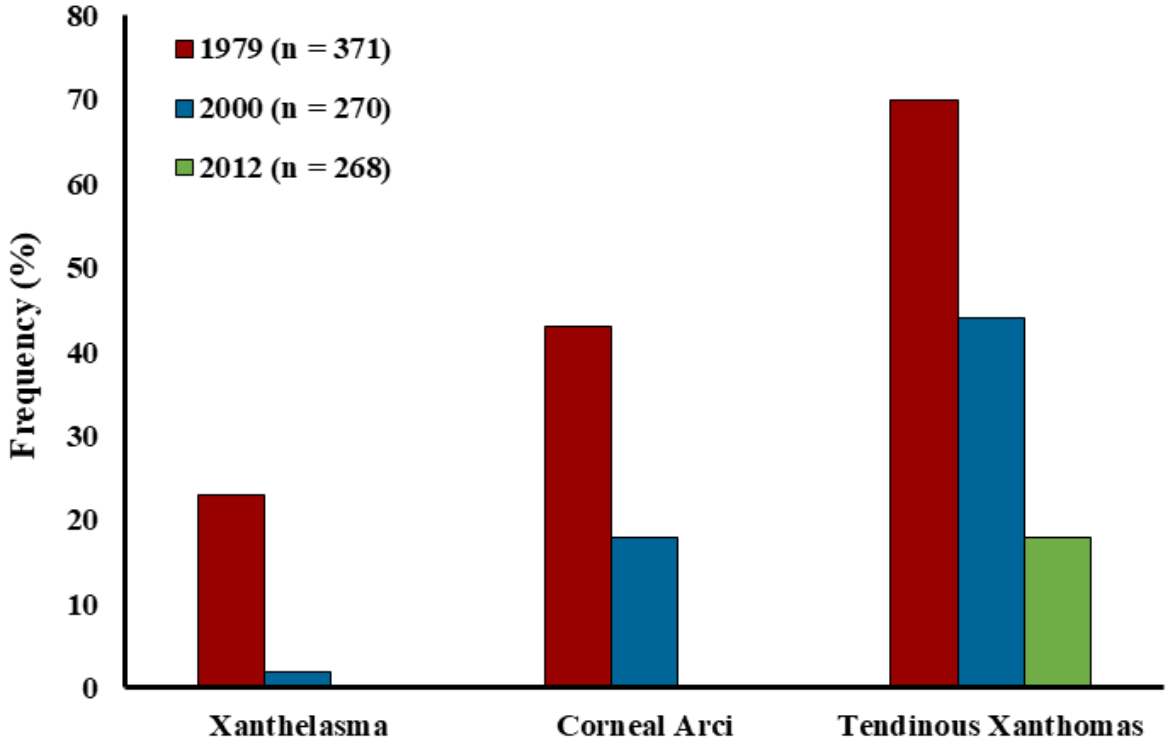
** FH diagnosis in a patient with a DNA mutation but normal LDL-C levels is unclear. Yearly follow-up of the proband is suggested and cascade screening of family members should be initiated. Note: In any case, treatment decision should be at the discretion of the treating physician.

FH: familial hypercholesterolemia; LDL-C: low-density lipoprotein cholesterol; yr: year; DNA: deoxyribonucleic acid; ASCVD: atherosclerotic cardiovascular disease.

Supplemental Table S4. Data groups used to characterize the 95th percentile of LDL-C levels in the Canadian population.

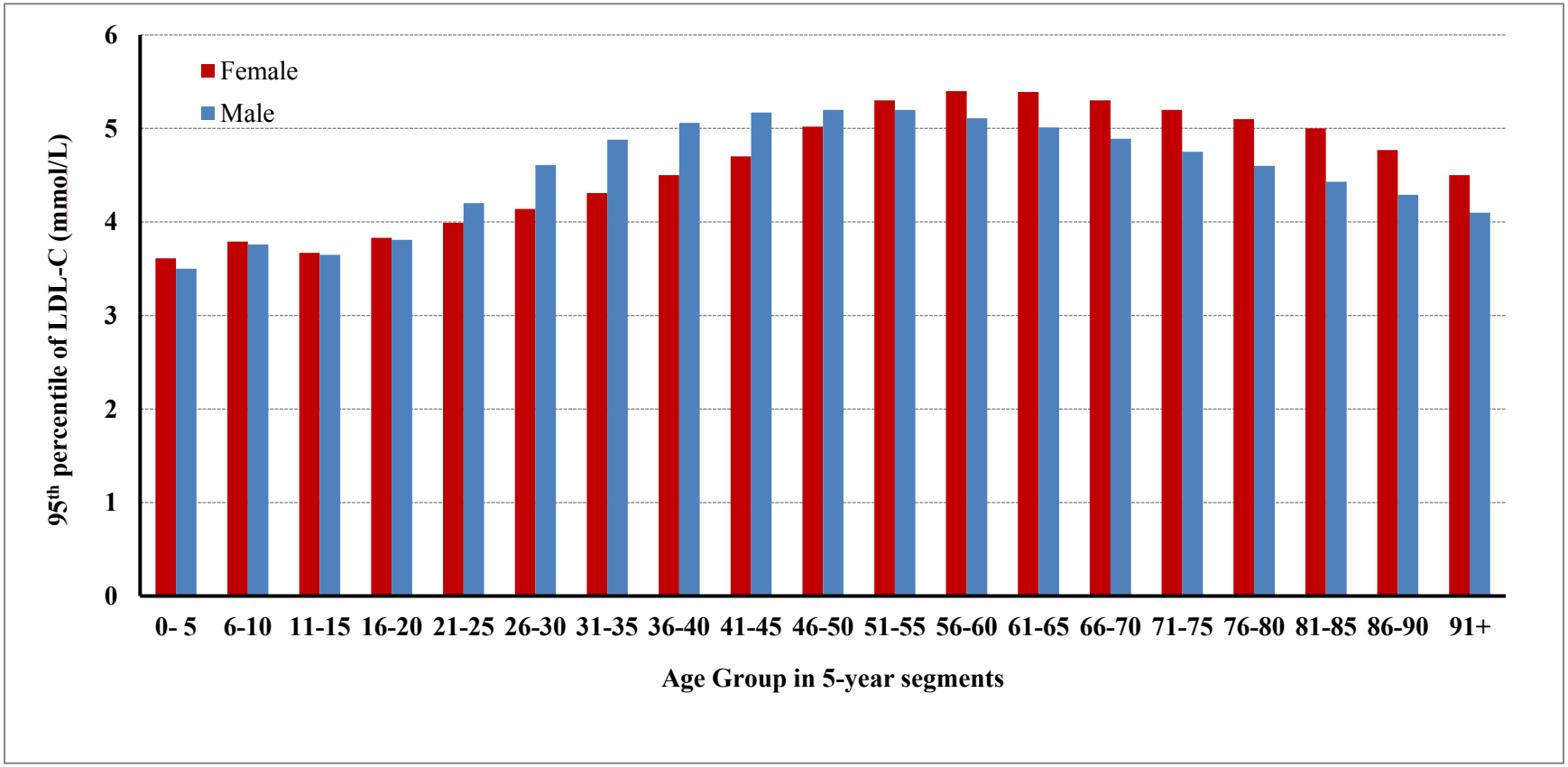
Group	Sex	Age Group (yr)	Total N	Missing N	Mean	Median	Min	Max	95th Percentile of LDL-C (mmol/L)
Overall*			3,366,067	21	3.26	3.20	0.20	18.33	**5.00**
	-	0-18	92,278	1	2.41	2.32	0.20	18.33	**3.69**
By Age	-	18-39	892,738	2	2.93	2.82	0.20	17.83	**4.53**
	-	40+	2,381,051	18	3.42	3.39	0.20	18.30	**5.12**
By Sex	Female	-	1,828,280	7	3.23	3.14	0.20	18.33	**5.00**
	Male	-	1,537,787	14	3.29	3.26	0.20	18.30	**5.00**
	Female	0-18	44,275	0	2.43	2.35	0.28	18.33	**3.70**
By Sex and Age	Female	18-39	501,141	0	2.78	2.70	0.20	17.83	**4.27**
	Female	40+	1,282,864	7	3.44	3.39	0.20	16.80	**5.18**
	Male	0-18	48,003	1	2.38	2.30	0.20	12.80	**3.67**
By Sex and Age	Male	18-39	391,597	2	3.12	3.04	0.20	14.44	**4.79**
	Male	40+	1,098,187	11	3.40	3.39	0.20	18.30	**5.08**

Data from the Gamma Dynacare Medical Laboratories (GDML) database were used to generate the 95th percentile data for LDL-C in 3,366,046 unique patients examined by from 2002 to 2013 in the province of Ontario. For subjects with multiple testing, only the highest level of LDL-C was kept.
LDL-C: low-density lipoprotein cholesterol; yr: year.



Supplemental Figure S1. Comparison of heterozygous FH clinical signs at baseline visit in time.

The clinical manifestations of FH, such as premature corneal arcus (onset <45 years old), xanthelasmas and tendinous xanthomas were determined at the Québec City Lipid clinic (CRML), CHU de Québec-Université Laval, Québec city (<1979; 1980-2011 and 2012) and at the Chicoutimi Hospital Lipid Clinic (2000-2012).
Updated from Gagné C, Gaudet D. Les dyslipoprotéinémies: l'approche clinique – 3e édition. Québec; 2007, 305 pages



Supplemental Figure S2. Characterization of the 95th percentile of LDL-C levels in the Canadian population.

Data from the GDML database were used to generate the 95th percentile data for LDL-C in 3,366,046 unique patients from 2002 to 2013 in the province of Ontario. For subjects with multiple testing, a single value, the highest level of LDL-C, was kept.

LDL-C: low-density lipoprotein cholesterol.