Barriers to Care in Familial Hypercholesterolemia

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ABSTRACT

Background: Familial Hypercholesterolemia (FH) is associated with premature atherosclerotic cardiovascular disease caused by excessive accumulation of LDL-C in circulation. Early treatment can normalize life expectancy. There are many barriers to care in FH that may lead to low diagnosis rates and unfavourable patient outcomes. For example, despite recommendations of genetic screening for diagnosis of FH by several national organizations, it is not routinely available as part of clinical care in Canada. Additionally, sex has been identified as a potential barrier to optimal care in cardiovascular diseases, which needs to be further explored in FH specifically.

Methods/Results: First, the impact of unbiased genetic testing on re-classification of patients with a clinical diagnosis of FH in a single centre cohort in Québec was determined. Next-generation sequencing of the *LDLR*, *APOB* and *PCSK9* genes and multiplex ligation-dependent probe amplification of the *LDLR* gene to detect genetic variants, including copy number variants was performed. All mutations were reviewed by a geneticist and cross-referenced in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/). Among 335 FH cases seen at the lipid clinic of the McGill University Health Centre (55% men, 45% women), baseline LDL-C was 7.0 ± 1.8 mmol/L. Women were diagnosed 6 years later than men and presented with higher LDL-C and apoB levels. In 229 patients who underwent genetic testing, a pathogenic FH-causing variant was identified in 169 (74%) individuals. A majority had variants in the *LDLR* (86%) or *ABOP* (14%) genes. Interestingly, the genetic panels currently available in Québec, which includes 11 common variants in French Canadians, only accounted for 49% of identified mutations. Importantly, 67% of patients initially defined as “probable FH” were re-classified as “definite FH” following genetic screening.

Next, we investigated how sex can act as a barrier to care in FH, potentially leading to less-than-optimal patient outcomes. A preliminary retrospective registry analysis of 292 patients with FH at from the lipid clinic at the McGill University Health Centre was performed. In this cohort, less women were on high-intensity statins compared to men (35% vs. 74%, P=0.002) and less women reached an LDL-C target of ≤ 2.5 mmol/L compared to men (32% vs. 55%, P=0.02). To further investigate sex differences in treatment of FH, a global scale systematic review was performed. Publicly available databases were searched for peer-reviewed, English publications. Publications went through two rounds of screening in duplicate and were kept if the population was labelled as FH and data demonstrating a sex comparison in treatment was available. A thorough data extraction was performed. After duplicates were excluded, the search identified 3,979 records. After screening all items for inclusion criteria, 50 records remained.

Conclusion: Genetic testing in patients suspected of having FH provided diagnostic certainty and permitted re-classification of many individuals with a probable diagnosis of FH. The limited genetic panel offered by Québec, focusing only on common French Canadian variants provided incomplete data in half of the cases. Our data supports unbiased genetic testing for a diagnosis of FH. The preliminary results of our single centre registry analysis revealed important sex differences in treatment and lipid level achievement in FH. The final selection of records of our systematic review will allow us to compare and contrast existing data on sex differences in treatment of FH. This review has the potential to reveal sex as a barrier to optimal treatment in FH. Identifying these imbalances will allow us to reduce barriers in care through educational initiatives, adequate training, and public advocacy to improve the quality of life and life expectancy of all individuals with FH.

RÉSUMÉ

Contexte : L'hypercholestérolémie familiale (HF) est un désordre métabolique associé à la maladie cardiovasculaire athérosclérotique prématurée causée par une accumulation excessive de LDL-C en circulation. Il existe de nombreux obstacles dans le traitement de l'HF qui limitent le diagnostic et peuvent entraîner des résultats défavorables pour les patients. Par exemple, il n'est toujours pas inclus dans les soins cliniques de base des patients avec HF au Canada. De plus, le sexe a été identifié comme un obstacle potentiel dans le traitement des maladies cardiovasculaires, une limitation qui doit être explorée plus en détail dans le cadre du traitement de l'HF en particulier.

Méthodes/Résultats : Dans un premier temps, l'impact des tests génétiques sur la reclassification des patients ayant un diagnostic clinique d'HF a été déterminé. Un séquençage de nouvelle génération des gènes *LDLR*, *APOB* et *PCSK9* ont été réalisés afin de détecter les variants génétiques. Toutes les mutations détectées ont été examinées par un généticien et référencées dans ClinVar. Dans le groupe total de 335 patients avec HF (55% d'hommes, 45% de femmes), le LDL-C non traité était de 7.0 ± 1.8 mmol/L. Les patients non-index ont été diagnostiqués 11 ans plus tôt, et avec moins de facteurs de risque cardiovasculaire que les patients-index. Dans le groupe de 229 patients soumis aux tests génétiques, un variant causant l'HF a été identifié chez 169 (74%) individus, avec une majorité de variants dans les gènes *LDLR* (86%) ou *APOB* (14%). Les 11 variants communs chez les Canadiens français qui composent les panels génétiques actuellement disponibles au Québec ne représentaient que 49% des mutations identifiées. Il est important de noter que 67% des patients initialement définis comme « HF probable » ont été reclassés comme « HF définitif » à la suite du dépistage génétique.

Par la suite, nous avons étudié comment le sexe peut être un obstacle au traitement de l'HF. Une analyse préliminaire rétrospective du registre de 292 patients avec HF du CUSM a été réalisée. Dans cette cohorte, les résultats démontrent que les femmes ont été traitées de manière moins agressive et n'ont pas atteint les niveaux cibles de LDL-C comparativement aux hommes. Afin de mieux comprendre cette différence entre les sexes dans le traitement de l'HF, une revue systématique de la littérature a été réalisée. Les publications scientifiques révisées par les pairs et en anglais, disponibles dans les bases de données accessibles publiquement, ont été analysées. Les publications identifiées ont fait l'objet de deux cycles d'analyse et ont été conservées lorsque la population était identifiée comme HF et si des données comparant les sexes dans le traitement de l'HF étaient disponibles. La recherche initiale a identifié 3,979 publications, après exclusion des doublons. Après sélection des publications pour les critères d'inclusion, 50 publications ont été retenues.

Conclusion : Les tests génétiques réalisés chez les patients suspectés d'avoir une HF ont fourni une certitude diagnostique et permis la reclassification de nombreux individus. Le panel génétique limité offert au Québec a fourni des données incomplètes dans la moitié des cas étudiés. Nos données supportent l'utilité d'un test génétique sans biais pour le diagnostic d'HF. Les résultats préliminaires des analyses effectuées dans notre cohorte ont révélé un biais de sexe dans le traitement et les résultats de santé en HF. Les publications retenues suite à une revue systématique de la littérature dans ce domaine nous permettront de comparer et de contraster les données existantes sur les différences entre les sexes dans le traitement de l'HF. Cette revue de littérature permettra de mieux comprendre pourquoi le sexe est un obstacle potentiel au traitement optimal de l'HF et nous aidera à corriger les soins grâce à des initiatives éducatives pour améliorer la qualité de vie et l'espérance de vie de toutes les personnes atteintes d'HF.

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CONTRIBUTION OF AUTHORS:

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Chapter 2. Genetic testing for familial hypercholesterolemia in a single-centre cohort in Québec: Jean-Baptiste Rivière, Amanda Guerin, Isabelle Ruel, and Jacques Genest designed the study; Isabelle Ruel, Jean-Baptiste Rivière, and Linda Fri Ngufor acquired the data; Amanda Guerin, Iulia Iatan, and Isabelle Ruel analyzed the data; Amanda Guerin, Iulia Iatan, Isabelle Ruel, and Jacques Genest interpreted the results; Amanda Guerin drafted the written work. All authors revised the written work.

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Chapter 4. Sex differences in the treatment of FH: Systematic Review: Amanda Guerin, Iulia Iatan, Isabelle Ruel, and Jacques Genest designed the study and protocol;. With guidance from Amanda Guerin and Iulia Iatan, Lindsay Hales designed the search strategy; Amanda Guerin, Iulia Iatan, and Isabelle Ruel screened the records and acquired the data. Amanda Guerin, Iulia Iatan, Isabelle Ruel, and Jacques Genest interpreted the results. Amanda Guerin drafted the written work. Jacques Genest revised the written work.

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LIST OF ABBREVIATIONS

APOB: apolipoprotein B

ASCVD: atherosclerotic cardiovascular disease

CAD: coronary artery disease

CHD: coronary heart disease

DLCN: Dutch Lipid Clinics Network

FH: familial hypercholesterolemia

HDL-C: high density lipoprotein cholesterol

HeFH: heterozygous familial hypercholesterolemia

HoFH: homozygous familial hypercholesterolemia

HMG CoA: 3-hydroxy-3-methylglutaryl coenzyme A

LDL-C: low density lipoprotein cholesterol

LDLR: LDL receptor

LDLRAP1: LDLR adaptor-related protein 1

MEDPED: Make Early Diagnosis to Prevent Early Death

PCSK9: protein convertase subtilisin/kesin-9

PCSK9i: PCSK9 inhibitor

SB: Simon Broome

T.Chol: total cholesterol

Tg: triglycerides

VLDL: very low-density lipoprotein

1. INTRODUCTION

1.1 Diagnosis and presentation of FH

Familial hypercholesterolemia (FH), an inherited genetic condition characterized by elevated low-density lipoprotein cholesterol (LDL-C), is associated with premature atherosclerotic cardiovascular disease (ASCVD)(2, 3). Patients with FH typically present with elevated lipid panels, particularly, LDL-C and apolipoprotein B (apoB) levels. The high levels of LDL-C in circulation cause some of these patients to have physical features such as xanthomas in joints, tendon xanthomas, or xanthelasmas. These physical presentations are the result of a build-up of cholesterol underneath the skin. Accordingly, FH can be diagnosed clinically, genetically, or using a combination of the two, as seen in many clinical diagnostic tools. The most widely used clinical criteria algorithms are the Simon Broome Criteria (4), the Dutch Lipid Clinics Network (DLCN) criteria (5), the Make Early Diagnosis to Prevent Early Death (MEDPED) United States (US) criteria (6), or the new Canadian Definition of FH (7).

The Canadian Definition of FH, published in 2018 by Ruel et al, has been validated and is recommended for use by Quebec's Institute national d'excellence en santé et en services sociaux (INESSS). The clinical algorithm takes into consideration a patient's LDL-C level, the presence of a DNA variant or tendon xanthomas, as well as any family history of high LDL-C or premature ASCVD. Following the clinical algorithm, patients can be diagnosed as Definite FH, Probable FH, or Severe Hypercholesterolemia (**Figure 1)**. Similar to the Canadian Definition of FH, the Simon Broome Criteria takes into account a patient's LDL-C levels, presence of a DNA variant or tendon xanthoma, and family history of elevated LDL-C or premature ASCVD. The criteria also take into consideration a patient's family history of tendon xanthomas and total cholesterol level. Uniquely,

the Simon Broome Criteria allows for only two FH diagnoses, Definite FH or Possible FH (**Table 1**). The Dutch Lipid Clinic Network Score (DLCNS) for diagnosis of FH factors in very similar clinical characteristics as the Simon Broome criteria, such as LDL-C levels, physical signs, and family history of premature ASCVD. However, the DLCNS uses a point system to classify patients as Definite FH, Probable FH, Possible FH, or Unlikely FH (5) (table in **APPENDIX**).The United States MEDPED Criteria for FH diagnosis is very different from the other widely accepted diagnostic tools, as it only considers a patients total cholesterol and LDL-C levels according to their age group and family history of FH (table in **APPENDIX**).

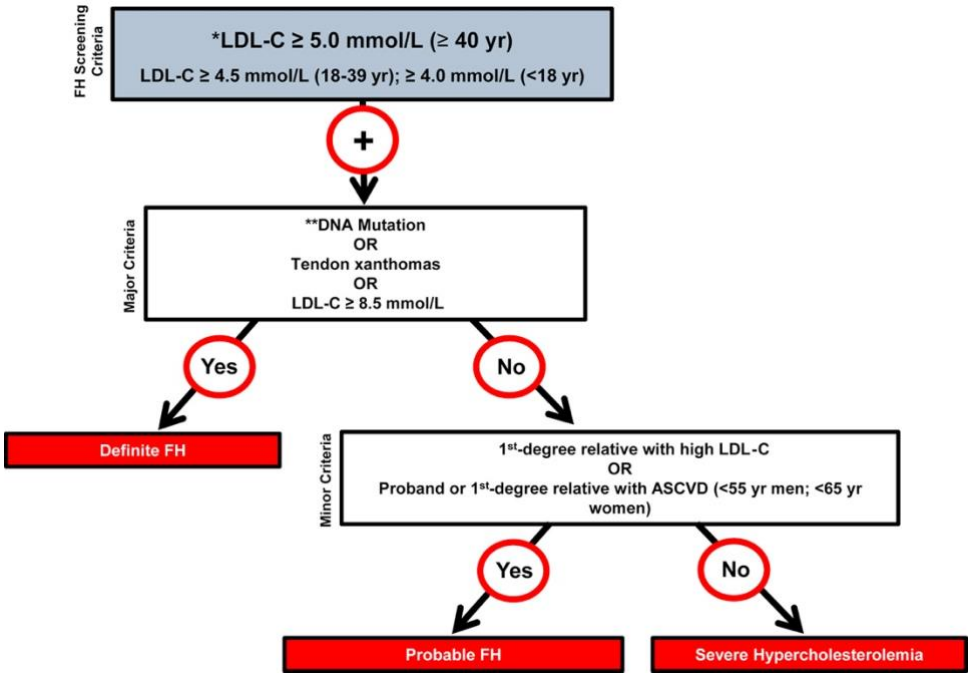

Figure 1. Canadian definition for the clinical diagnosis of familial hypercholesterolemia (FH). From Ruel et al. (7). LDL-C, low-density lipoprotein cholesterol.

Table 1. Simon Broome Criteria for the Diagnosis of FH (UK FH Registers Criteria)

Criteria	FH Diagnosis
- In adults: TC >7.5 mmol/L, or LDL-C >4.9 mmol/L - In pediatric patients (< 16 years): TC >6.7 mmol/L, or LDL-C >4 mmol/L **AND:**	Definite
- Tendon xanthoma in the patient or first/second-degree relative, **OR:**	
- Presence of LDL-R, ApoB, or PCSK9 variant	
- In adults: TC >7.5 mmol/L, or LDL-C >4.9 mmol/L - In pediatric patients (<16 years): TC >6.7 mmol/L, or LDL-C >4 mmol/L **AND:**	Possible
- Family history of MI <50 years old in second-degree relative or <60 years old in first-degree relative, **OR:**	
- Family history of TC >7.5 mmol/L in a first- or second-degree relative.	

ApoB, Apolipoprotein B, FH, familial hypercholesterolemia; LDL, low-density lipoprotein; LDL-R, low-density lipoprotein receptor; MI, myocardial infarction; PCSK9, proprotein convertase subtilisin/kexin type 9; TC, total cholesterol. Adapted from (4).

Although diagnosis of FH is possible and fairly common without screening for a disease-causing variant, genetic testing has been found to be the gold standard for accurate diagnosis of FH. In countries with the highest diagnostic rates of FH in the world, such as the Netherlands and Norway, genetic testing is offered to nearly all individuals suspected of having FH (8). Other European countries such as the UK, Spain, and Belgium have diagnosed only 2-10% of FH cases and roughly, only 30% of cases are diagnosed with genetic testing (8). Additionally, studies have reported that the presence of an FH-causing variant increases an individual's CVD risk, irrespective of LDL-C levels(9). In fact, knowledge of the type of variant present is equally as clinically useful, as null or negative variants impose a 2-fold higher CVD risk comparer to milder hypomorphic or defective variants(10). Therefore, genetic diagnosis of FH is recommended to best assess a patient's CVD risk and best course of treatment.

1.2 Historical aspects of familial hypercholesterolemia

FH was first described by Dr. Carl Müller, a Norwegian clinician, in 1938, which was the first clear description of how high cholesterol was linked to cardiovascular disease (CVD). His description was based on observations made among 17 families. He noted that 68 out of 76 family members showed signs of heart diseases and that their cholesterol levels were between 4-15 mmol/L(11). Based on his observations he suggested that the condition was hereditary, with autosomal dominant characteristics.

In years to follow, even before the causative genes were known, the patterns of inheritance of FH was first described by Dr. Khachadurian in 1964. The physician analyzed twelve patients from multiple Lebanese families with hypercholesterolemia and severe xanthomatosis. Based on his observations he described three classes of inheritance of FH: Homozygous Hypercholesterolemia, Heterozygous Dominant Hypercholesterolemia, and Heterozygous Recessive Hypercholesterolemia (ARH)(12). Historically, various names have been used for FH such as familial hyperlipoproteinemia type 2 and Fredrickson class 2a hyperlipidemia. In recent years it's most often referred to as either homozygous FH (HoFH) or heterozygous FH (HeFH).

The first FH-causative gene to be discovered was the low-density lipoprotein receptor (*LDLR)* gene, by Brown and Goldstein in the late 1970's. Through their experiments they first discovered that the cellular uptake of (LDL-C) requires the LDL-receptor (LDLR) and that patients with FH were lacking LDLRs(13). Roughly 20 years later, variants in the *APOB* gene were found to effect cholesterol levels, and was labelled an FH-causing gene(14). Lastly, a third gene, *PCSK9* was linked to autosomal dominant hypercholesterolemia in 2003(15).

1.3 Prevalence of FH and founder effects

FH is one of the most common genetic conditions in humans. Although the homozygous form (HoFH) of the condition is quite rare with a prevalence estimated at 1 in approximately 400 000 people (16, 17), the heterozygote form (HeFH) has recently been found to have a worldwide prevalence of 1:311 – 1:313, by two large meta-analyses conducted in 2020 (18, 19). Recently, it's been shown that the prevalence of HeFH among CAD or ASVCD populations is much higher than the general population. Many studies, such as the EUROSPIRE IV post-hoc analysis and a large meta-analysis by Hu, P et al. have reported a staggering prevalence rate of 1:10 – 1:17 in these patients, ultimately demonstrating the underlying association between FH and premature atherosclerosis (18, 20, 21).

Interestingly, certain distinct population groups in regions across the globe are known to have a higher prevalence of FH compared to the general population. These limited populations demonstrate what is known as a founder effect. This phenomena, first described by Ernst Mayr in 1942, is defined by a population with reduced genetic variability due to the establishment and expansion of a population in a new region originating from a small group of founding individuals (22, 23). For example, a founder effect present in the Amish community in Pennsylvania, USA, explains the elevated prevalence of a syndrome characterized by dwarfism (24). Additionally, the prevalence of a specific FH-causing variant in ApoB among the Amish community is the highest reported worldwide, with 12% of the secluded population carrying the R3500Q variant(25). Most relevant, the French-Canadian population in Quebec is a prime example of a population with founder effects and contains higher incidences of certain genetic disorders that are rare in the general population(26). This can be explained by a series of migrations of 8500 French settlers who arrived in Nouvelle-France between 1608 and 1759 (27), resulting in multiple population

bottlenecks. The later successive spread of descendants from these initial immigrants to other regions in Quebec led to regional founder effects (28). Specific for FH, a founder effect exists in regions of Quebec such as Kamouraska, Côte-Nord, and Saguenay-Lac-St-Jean. The prevalence of FH in these less genetically diverse regions were previously found to be as high as 1:80 (29), however a more recent review reported the prevalence of FH in Saguenay-Lac-St-Jean to be 1:120 (30).

1.4 Pathophysiology and genetics of FH

CVD is the leading cause of death globally, responsible for 32% of all deaths in 2019. It's well known that FH is the most common inherited genetic condition, characterized by elevated cholesterol in the blood, specifically LDL-C. Early exposure to high levels of LDL-C early on in life, often labelled cumulative LDL-C burden, leads to these patients being at an increased risk of premature ASCVD (1). The mechanisms connecting FH to high LDL-C and thus increased ASCVD risk are very well understood.

In humans, cholesterol is derived from two sources. Either through absorption of cholesterol in the intestine from dietary sources or through biosynthesis of cholesterol in cells. While most cells in the human body are capable of producing cholesterol, majority of the biogenesis takes place in hepatocyte cells in the liver(31). In hepatocytes, a complex metabolic mechanism synthesizes cholesterol through the use over 20 enzymes, including the rate-limiting enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase(32). Moreover, cholesterol deriving from dietary absorption is delivered to the liver by the chylomicron pathway and is then taken up by hepatocytes as cholesterol rich chylomicron remnants(33). Following it's endogenous production in or delivery to hepatocytes, cholesterol gets converted to very low-

density lipoprotein (VLDLs) particles within liver cells, which are then transported into circulation (34). Once in the bloodstream, VLDLs are further processed which generates VLDL remnants and intermediate-density lipoproteins (IDLs), some of which are further converted to LDL-C (35). Therefore, the liver plays a key role in production and circulation of LDL-C.

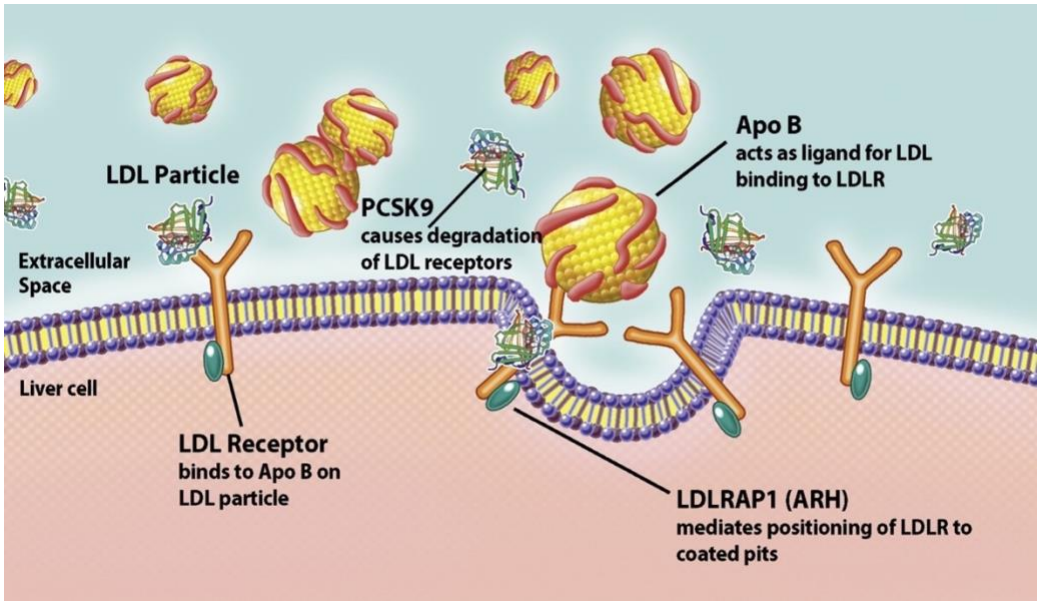

Figure 2. Major Molecular Causes of Familial Hypercholesterolemia**.** From Sniderman et al. (36).

The negative regulation of circulating LDL-C is also primarily controlled by the liver through the LDLR clearance pathway(37)(**Figure 2).** During normal homeostasis, LDL particles bind LDLR on the hepatocyte cell surface through their acting ligand, apoB(38). This initiates receptor mediated endocytosis of LDLR and LDL. Once inside the hepatocyte, LDL-C gets sent to the lysosome for degradation while LDLRs are released and recycled back to the cell surface to further transport more LDL particles. However, PCSK9 acts as a suppressor of LDLR recycling. In fact, studies have shown that PCSK9 binds to the LDLR and promotes its lysosomal degradation

in the cell(39). Thus, varying levels of PCSK9 and LDLR are kept in balance in order to maintain optimal levels of extracellular LDL-C (40) **(Figure 2)**.

It is now well understood that pathogenic variants in the *LDLR*, *APOB*, or *PCSK9* genes, integral components of the LDLR pathway, are all causative of FH(41). Their inheritance displays an autosomal co-dominant pattern. Most often observed are variants in the *LDLR* gene, responsible for roughly 80-90% of diagnosed FH cases(42). To date, more than 4970 FH-causing *LDLR* variants have been identified(43). Genetic variants of *LDLR* are classified as either *LDLR* defective variants, resulting in reduced LDLR activity, or *LDLR* negative (null) variants, which results in little to no LDLR protein production(44). With either type of variant, the ability of LDLR to bind ApoB on LDL particles and thus clear it from circulation is reduced or completely abolished, therefore causing increased LDL-C levels in the blood. FH can also be caused by variants in the *APOB* gene, a form also referred to as Familial Defective ApoB, which is phenotypically identical to "classical" FH(38). Although several variants in *APOB* associated with hypercholesterolemia have been identified, the most commonly found worldwide are the R3500Q and R3500W variants(45). Variants in *APOB* cause a poor interaction between LDLR and ApoB, resulting in reduced receptor-mediated endocytosis of circulating LDL-C. Thus, extracellular LDL-C levels become elevated. Lastly, initially described in 2003 by Abifadel et al, gain-of-function variants in the *PCKS9* gene have also been found to be causal towards FH (15). In recent years, studies have identified numerous *PCSK9* variants attributable to FH, with over 350 identified to date (43). Overall, variants in the *PCSK9* gene is the rarest, reported form of FH. Studies have reported the prevalence of *PCSK9* variants in FH populations to be anywhere from 0.1% to 6.4%, as it varies greatly depending on geographic location and population pool(46). FH-causing variants in *PCSK9* upregulate PCSK9 enzyme activity and therefore increases the binding of PCSK9 to LDLRs. In

turn, this increases degradation of LDLRs in the lysosome, reducing availability of LDLR at the cell surface to transport LDL-C into hepatocytes, leading to elevated LDL-C serum levels.

As described, variants in *LDLR*, *APOB*, or *PCSK9* genes are most commonly responsible for the FH phenotype. However, in very rare cases, a rare occurrence known as autosomal recessive hypercholesterolemia (ARH) that clinically resembles FH may be observed, when a variant is found in the LDLR adaptor-related protein 1 (*LDLRAP1*) gene(47). This affects the functionality of LDLR's at the cell surface, reducing its ability to clear LDL-C from the blood.

Variants in any of the aforementioned genes causes an upregulation of LDL-C in plasma, which is now well known to cause subsequent atherosclerosis (**Figure 3).** Some of the earlier studies demonstrating that elevated cholesterol in the blood is associate with increased CVD risk, include the 1961 Framingham study, and the Multiple Risk Factor Intervention Trial (MRFIT) (48, 49). Hundreds of studies have demonstrated the specific association between LDL-C levels in the blood and development of ASCVD(50). Importantly, to date, LDL-C is the only causal risk factor for ASCVD (51). The complex mechanism linking increased LDL-C to atherosclerotic plaques and subsequent ASCVD is well understood. Briefly, high levels of LDL in plasma result in increased permeability of the arterial endothelium, leading to increased influx and retention of LDL in the arterial wall(52). Following further LDL retention, LDL-C becomes oxidized in the artery wall, leading to endocytosis by macrophages, causing the formation of foam cells within the intima of arteries(53). The foam cells then progressively trigger an inflammatory response, causing the proliferation of smooth muscle cells and collagen production, forming plaque(54). These stable plaques can progress to occlusive atherosclerosis or lead to vulnerable plaque rupture, ultimately causing CVD events such as angina, myocardial infarction, or stroke(55, 56).

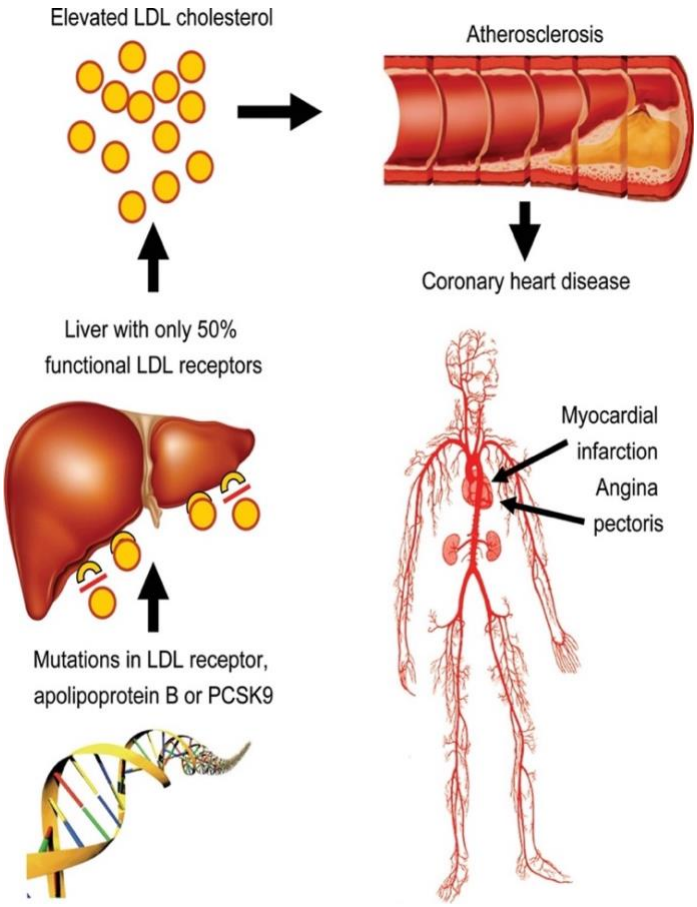

Figure 3. Pathophysiology of heterozygous familial hypercholesterolemia. From Nordestgaard et al (1). LDL, low-density lipoprotein; PCSK9, proprotein convertase subtilisin/kesin type 9.

1.5 Treatment of FH

Over three decades ago, there were very little effective treatment options available for patients with FH, which resulted in an excess of premature ASCVD and reduced life expectancy for this population. The first very effective lipid-lowering treatment brought into care was statins. The extensive clinical trials by Yamamoto et al. (57), and Merk Research Laboratories, lead to FDA approval of lovastatin in 1987 (58). To date, there are a variety of statins available for use

such as Rosuvastatin, Atorvastatin, Pravastatin, and Simvastatin. All statins' drugs are effective at lowering LDL-C in circulation by inhibiting HMG-CoA reductase activity in hepatocytes (59, 60). By inhibiting activity of HMG-CoA reductase, HMG-CoA conversion to mevalonic acid, a cholesterol precursor, is blocked, and thus intracellular cholesterol is reduced. This mechanism results in the upregulation of LDL-Rs at the cell surface and consequently, increased removal of LDL-C from the circulation (61). Many large-scale randomized controlled clinical trials have demonstrated the ability of statins to reduce T.Chol, LDL-C, triglycerides, and even ApoB(62, 63). Importantly, clinical outcomes trials have shown that intensive therapy with statins reduces the risk of stroke, major coronary events, and coronary heart disease deaths (64).

After the discovery and introduction of statins into regular practice, the first new treatment for hypercholesterolemia, Ezetimibe, was discovered in the 1990's (65). The drug was approved for use in the United States in 2002 and shortly after in Canada in 2003 (66, 67). Although statins and Ezetimibe both reduce LDL-C in the bloodstream, Ezetimibe's mechanism of action is distinct, as it specifically inhibits intestinal absorption of cholesterol and phytosterol (68). More specifically, it selectively blocks the Niemann-Pick C1-like 1 protein (NPC1L1), essential for sterol transport, expressed in the jejunal brush border(30, 69). Although the lipid-lowering effect of ezetimibe when used alone is milder, shown to lower LDL-C by roughly 18% and TGs by 5-10% (70), it has proven to be a very useful tool when combined with statin therapy. It's been reported that adding Ezetimibe to statin therapy can have an additional LDL-C reducing effect of roughly 20%(71). Additionally, studies have demonstrated that the use of Ezetimibe whether used alone or with other lipid lowering therapies, lowers CVD risk. In fact a meta-analysis by Savarese et al compared the results of 7 trials and found that ezetimibe significantly reduced the risk of MI and stroke by 13.5% and 16.0% respectively(72).

More recently, another class of lipid-lowering drugs has been approved for use known as anti-PCSK9 monoclonal antibodies (PCSK9 mAbs), also known as PCSK9 inhibitors (PCSK9i). The first treatment of its kind, Evolocumab, was approved for use in Canada on September 2015, with Alirocumab approved the following year(67). They both function similarly, by binding and consequently inhibiting PCSK9 in circulation, thus preventing PCKS9 from binding LDL-R on the hepatocyte cell surface. This results in less LDL-R degradation in the lysosome, increased LDL-R recycling, and thus upregulation of LDL-C uptake from circulation(73). The lipid-lowering effect of PCSK9i's has been well studied in the literature. According to reports from numerous randomized clinical trials, patients treated with PCSK9i's alone benefit from a reduction of LDL-C anywhere from 26% up to 67% depending on the dose strength and frequency taken(74). The typical recommended dose of Evolocumab is 140 mg every 2 weeks or 420 mg monthly and when given as monotherapy, has been reported to reduce LDL-C by roughly 60% according to the MENDEL-2, Phase III clinical trial(75). PCKS9i's are also recommended for use in combination with statins for those who require more aggressive treatment, which has shown even greater efficacy at reducing LLD-C levels in hypercholesterolemia patients(76). Since PCSK9i's have been approved for use, there are now multiple large-scale clinical outcomes trials, demonstrating their protective effect and ability to reduce risk of CVD events(77). For example, the FOURIER trial, which was comprised of 27, 564 patients with ASCVD, demonstrated that Evolocumab treatment reduced the risk of the primary endpoint of major cardiovascular events compared to the placebo group (hazard ratio, 0.85; 95% CI, 0.79-0.92, $P<0.001$)(78). Based off of evidence in the literature, PCSK9i's have proven to be one of the most effective treatment available for reducing LDL-C and reducing risk of ASCVD in higher risk patients.

Interestingly, a novel type of PCKS9i, has recently been discovered as a highly effective treatment for lowering LDL-C in FH patients. As opposed to a monoclonal antibody, inclisiran is an injection based small interfering RNA drug, shown to inhibit gene expression of PCSK9, reducing synthesis of PCSK9 in hepatocytes. Therefore, this causes upregulation of LDLR recycling and clearance of LDL-C from circulation. Multiple phase III clinical trials have shown effective reduction of LDL-C from use of inclisiran(79, 80). The novel drug was approved by Health Canada in July 2021, followed by the FDA in January 2022(66, 67). Concrete clinical outcomes trials are still in progress.

Depending on FH patients' LDL-C or ApoB levels, presence of other risk factors, and clinical family history, different types, dosages, and combinations of lipid-lowering medications (LLMs) may be recommended. Specifically for statins, varying intensity classifications exist to guide dosage recommendations. According to the American College of Cardiology (ACC) and the American Heart Association (AHA) Classifications, high-intensity statins comprises atorvastatin 40mg and 80mg and rosuvastatin 20mg and 40 mg. Moderate or low-intensity statins includes atorvastatin 10mg and 20 mg, rosuvastatin 5mg and 10mg, and other statins such as simvastatin, pravastatin, lovastatin, fluvastatin, and pitvastatin(81). If a patient is at higher risk or is having difficulty lowering their LDL-C levels, the addition of Ezetimibe may be recommended. Additionally, PCSK9i's may be suggested either as monotherapy or in combination with statins depending on aggressivity of treatment required to achieve optimal results.

Recommendations of LLM types or dosages are recommended based on FH patients LDL-C targets and their progress to date. Current guidelines have clear LDL-C goals for FH patients based on their current lipid levels, presence of risk factors, and their total CVD risk. The treatment target guidelines in the United States and the European Society of Cardiology (ESC) and the

European Atherosclerosis Society (EAS) guidelines are fairly similar. The 2019 ESC/EAS Guidelines for the management of dyslipidemias recommend treating FH patients to reach an LDL-C goal of <2.5 mmol/L for high risk subjects or even <1.8 mmol/L and/or ≥ 50% reduction if patients have a history of CVD or are very CV high risk (82). The 2021 Canadian Cardiovascular Society guidelines reports similar recommendations (83).

With multiple advances of effective lipid lowering medications available for use in FH patients, studies have shown that early diagnosis and initiation of lipid lowering treatment can alter the natural history of FH by reducing the risk of premature ASCVD and normalizing life expectancy among FH patients(1). In fact, a study investigating long-term effect (>30years of treatment) of cholesterol-lowering regimens on FH patients lipid levels and CVD outcomes, found significant reductions in LDL-C and CVD risk(84). Additionally, it's been shown that the earlier patients begin treatments, such as statins, the more delayed the onset of CHD, due to reduced cumulative LDL-C burden throughout their life (**Figure 4)**(85).

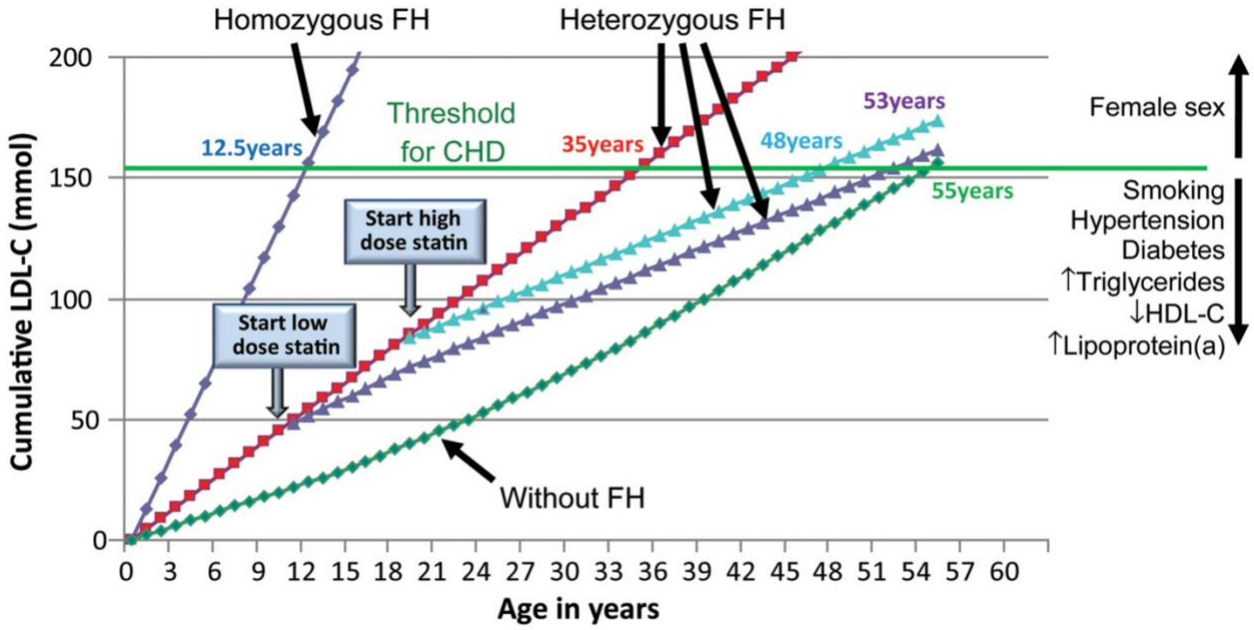

Figure 4. LDL cholesterol burden in individuals with or without familial hypercholesterolemia as a function of age of initiation of statin therapy. From Nordestgaard et al. (1). LDL-C, LDL cholesterol; HDL-C, high-density lipoprotein cholesterol; CHD, coronary heart disease; FH, familial hypercholesterolemia

1.6 Underdiagnosis and undertreatment of FH

Despite widely accepted diagnostic criteria and many available effective treatments, FH remains underrecognized and undertreated worldwide. Globally, estimated diagnosis rates range from < 1% to 40%, with most countries having an estimated diagnostic rate <1% (1). Currently, the Netherlands and Norway lead the world with diagnosis rates estimated at 71% and 43% respectively. Additionally, even the FH patients that are diagnosed, are diagnosed too late, resulting in less-than-optimal care and outcomes. For example, a study by Rizos et al., using data from the Hellenic FH Registry (HELLAS-FH) found that the median age of diagnosis was 42.4 years (86). Another large study by deGoma et al. analyzed data from the CASCADE FH Registry

and the median age of FH diagnosis for patients in the United States was 47 years. Various other studies have repeatedly reported that FH is far too underdiagnosed and diagnosed too late (87, 88).

As described earlier, current guidelines have clear LDL-C targets for FH patients based on their CVD history and current risk. Many studies have demonstrated that despite the availability of effective treatments, many FH patients are not achieving recommended LDL-C targets in real-world scenarios. For example, the 5-year Spanish Familial Hypercholesterolemia Cohort Study (SAFEHEART) Registry follow-up study found that only 11.2% of patients reached a target LDL-C of <100 mg/dl (2.5 mmol/L)(89). Additionally, a study from the Netherlands who used the PHARMO Database Network found that only 53% of FH patients were on lipid-lowering therapy and only 13% were on a high-potency statin. The undertreatment of these FH patients is most likely why only 23% of FH patients attained a LDL-C goal of <100 mg/dl (2.5 mmol/L)(90). A similar study done in the Czech Republic and Slovakia revealed that only 54.6% of FH patients were on a high-intensity lipid lowering therapy and importantly only 15.4% of patients achieved their guideline recommended LDL-C target (91). To date, multiple studies across the globe have demonstrated similar findings (92, 93). This highlights that despite how well understood FH appears to be, and how guidelines for treatment are fairly accessible, FH patients remain undertreated, and many are not reaching LDL-C targets to optimally reduce their CVD risk.

Due to the global underdiagnosis and undertreatment of patients with FH, various national FH registries have been created around the world. Some of the largest national registries in the world are the Familial hypercholesterolemia foundation Cascade Screening for Awareness and Detection of Familial Hypercholesterolemia (CASCADE FH) Registry in the US and the Spanish SAFEHEART registry (89, 94). Most relevant, is the FH Canada Registry, founded in 2014 with the main aim to “improve the detection and management of individuals and families with FH in

Canada" (www.FHcanada.net). In 2018, they reported that the Canadian registry includes 19 academic centres across Canada and had recruited 3000 patients (95). Most recent data reveals that over 5000 patients have now registered. There are many other national FH registries which have successfully lead to publications of patient studies, including registries in the Netherlands and Japan (96). Additionally, an impressive collaborative effort to assess gaps in care and improve FH management and outcomes has resulted in the creation of the global European Atherosclerosis Society Familial Hypercholesterolaemia Studies Collaboration (EAS FHSC), a global pooling of registry data from over 60 countries (97). This initiative has been quite successful and has led to a publication reviewing current FH care in countries across the globe as well as a large cross-sectional study (98, 99).

Over the years, another method put in place to improve identification and diagnosis rates of FH is the practice of cascade screening. This mechanism is when individual is found to have genetically confirmed diagnosis of FH, physicians offer a genetic screening for all of their 1st degree family members to further identify FH cases. This method typically has a diagnosis yield of 50% considering the autosomal dominant pattern of the condition, also making it a very cost-effective method of FH identification. Studies have proven cascade screening to be an effective method of FH identification (100). Cascade screening is recommended by the National Institute for Health and Clinical Excellence in the UK(101) and by the Canadian Cardiovascular Society(102).

1.7 Sex differences in care in cardiovascular disease

It's well known that within FH, patients are underdiagnosed and undertreated worldwide (1). However, the striking underdiagnosis, undertreatment, and less than optimal outcomes of this disease may weigh more heavily on certain groups than others. Clinical research surrounding

cardiovascular disease in general has explored how varying demographics has large impact on the quality of care received by the health care system. In fact, the impact of sex on quality of care for cardiovascular diseases has been of interest in the research and health care community.

Firstly, many studies have investigated treatment use among patients with CVD and found important sex differences, in both primary and secondary care. Multiple studies have reported that among patients with CVD, less women have been prescribed or are taking lipid-lowering medications compared to men. For example, a study by Gil Metser et al demonstrated that in primary prevention care, women were less likely than men to receive a prescription for statin therapy during outpatient care (adjusted odds ratio, 0.79; 95% CI, 0.71-0.88)(103). As well, a study by Nanna et al investigated guideline-recommended statin use among statin-eligible patients and reported similar findings. Notably, less women were prescribed a statin compared to men (67.0% vs. 78.4%, $P<0.001$) and less women received guideline-recommended statin intensity prescriptions (36.7% vs. 45.2%, $P<0.001$). Equally important, they also found that less women reported ever being offered a statin by their physician compared to men(104). Sex differences in treatment have even been reported among patients in secondary care. For example a study by Lee et al reported that women with premature ASVCD (≤ 55 years) and extremely premature ASCVD (≤ 55 40 years) were less likely to be prescribed statins or antiplatelet medications compared to men (105). Similarly, a large cohort study found that women with CVD were less likely than men with CVD to receive LLMs(106). Therefore collectively, studies have shown that in primary prevention or secondary prevention care for CVD, women are not treated as well.

In addition to lipid lowering treatment use, studies have also investigated how well CVD patients, particularly those with high cholesterol, successfully lower their LDL-C to guideline recommended levels. Many studies have reported that women with CVD were less likely than men

to reach target LDL-C levels. For example a study by Cooke et al demonstrated that within their cohort of CHD patients, more men (51.0%)than women (36.7%) reached a target LDL-C of <100mg/dL (<2.59 mmol/L)(107). As well, a large cohort study performed in China reported that women with established CVD has less well-controlled LDL-C (OR 0.66 [95% CI, 0.57-0.76]) compared to men with CVD. Many other studies report similar findings(108, 109).

The reasons surrounding these important sex differences in care and outcomes continue to be discussed. Many have suggested that these disparities exist partially due to the lack of knowledge of women's health in physicians(110). Accordingly, available literature suggests that many physicians have long lacked knowledge and awareness of specific sex differences in heart disease(111). In fact, a study by McDonell et al investigated the knowledge, beliefs, and practices regarding women's heart health in physicians in Canada. Their results demonstrated that Canadian physician's lack awareness regarding the prevalence, identification, and treatment of heart disease in women(112). In regard to lower statin use among women compared to men, many factors may contribute. According to a study that analyzed data from the PALM registry, women were more likely to report never being offered a statin (113). However, personal beliefs and reluctancy could partially be to blame, and should be factored in.

1.8 Rational and research objectives

Genetic Testing in Quebec

While genetic testing is not required for the diagnosis of FH, it is nevertheless considered the 'gold standard' and is recommended by several professional organizations, including the Canadian Cardiovascular Society, the US Centers for Disease Control Office of Public Health Genomics, the International Atherosclerosis Society, and by the United Kingdom National

Institutes for Clinical Excellence (NICE)(83, 102, 114, 115). Despite these recommendations, it is not routinely available as part of clinical care in Canada, which may contribute to our low diagnosis rates (1, 116). A few academic medical centres in Canada perform complete DNA analysis of the main aforementioned genes causing FH, but these are on a research basis. In the province of Québec, the *Ministry of Health and Social Services* (MSSS) offers genetic screening for the 11 most commonly known French-Canadian variants in *LDLR*, but the *APOB* and *PCSK9* genes are not included. To address this, we set-up the first clinically certified genetic screening of the *LDLR*, *APOB* and *PCSK9* genes in Canada (Clinical Laboratory Improvement Amendment (CLIA) certification). The objective of this single-centre cohort study was therefore to examine the impact of an unbiased full next-generation sequencing (NGS) on re-classification of patients with a clinical diagnosis of FH in Québec, based on the Canadian definition of FH, in comparison with the partial genetic panel currently offered by the MSSS. We also sought to investigate if any sex differences exist in baseline characteristics, variant prevalence, or re-classification.

Sex Differences in Treatment of FH Systematic Review

The undertreatment of women with cardiovascular diseases has been established. It's been shown that less statin-eligible women are offered statins compared to stain-eligible men(104). Several organizations have been created to promote improved women's cardiovascular health care such as the Women's Healthy Heart Initiative at the McGill University Health Centre, the National Coalition for Women with Heart Disease, and the Canadian Women's Heart Health Alliance, which stresses the important need of reducing health disparities in women. While sex has been shown to influence the quality of care and outcomes for cardiovascular diseases, this topic has not been explored extensively within FH specifically. Additionally, systematic reviews or meta-analyses focused on sex differences in care in FH are lacking, in order to gather a large-scale global

perspective on the topic. Therefore, the objective of this study was to conduct a systematic review and meta-analysis to determine sex differences in treatment and lipid level target achievement in FH.

2. GENETIC TESTING FOR FAMILIAL HYPERCHOLESTEROLEMIA IN A SINGLE-CENTRE COHORT IN QUEBEC

2.1 Methods

2.1.1 Participants and study design

A retrospective cohort study was conducted on the patients seen in the Preventive Cardiology/Lipid Clinic of the McGill University Health Centre at the Royal Victoria Hospital in Montreal, Québec, Canada, between September 2017 to September 2021. The McGill University Health Centre Preventive Cardiology/Lipid clinic is one of the 19 academic FH Canada Registry participating sites (95). Patients were recruited into the study, and biochemical and DNA samples were collected. Individuals were recruited into the FH Canada Registry if they were referred to the clinic for an LDL-C > 95th percentile for age/sex, or from cascade screening of family members from an index-patient previously seen at the clinic. The inclusion criteria for this study required participants to be adults (18 years or older), to be seen in the McGill University Health Centre Preventive Cardiology/Lipid Clinic and have consented to be in the study. Only participants with a clinical diagnosis of "definite FH", "probable FH", or "severe hypercholesterolemia" according to the Canadian Definition of FH (7) were included. A cohort of 335 consecutive HeFH participants with a mean age of 50 ± 15 years was included in this retrospective analysis.

2.1.2 Data sources

Clinical data. The data was extracted from the FH Canada Registry database, as described previously (95). Briefly, data on patients' demographics, medical history and medication, family history of premature CVD and dyslipidemia, physical signs of FH such as tendon xanthomas and

untreated lipid profile was obtained by a cardiologist with expertise in FH. Secondary causes of high LDL-C were ruled out(7). Patients who were diagnosed with FH through family cascade screening protocols were classified as cascade patients (non-index), while all other diagnoses were classified as index patients. Standard non-fasting blood collection was performed, and total cholesterol, HDL-cholesterol, triglycerides, apolipoprotein B (Apo B), lipoprotein (a) (Lp(a)) levels, thyroid stimulating hormone, hepatic transaminases, creatinine and creatinine kinase were measured by standard automated assays performed by the OptiLab Montreal-McGill University Health Centre biochemistry laboratory. LDL-C was calculated by the Friedewald formula. When untreated LDL-C levels were unavailable, they were imputed based on the LDL-C levels on treatment and the actual dose and type of the lipid-lowering therapy used at the time of analysis, as previously described(117). Clinical data for each patient was used to generate an FH score according to the Canadian Definition for FH(7), before and after genetic testing. The high LDL-C cut-off points in this criterion are ≥ 5.0 mmol/L for ages 40 and over; ≥ 4.5 mmol/L for ages 18-39 and ≥ 4.0 mmol/L for ages less than 18. Briefly, patients were classified as having "definite FH" if they had high untreated LDL-C combined with a causal DNA variant (*LDLR*, *APOB* or *PCSK9*) or tendon xanthomas, or alternatively if they had an untreated LDL-C ≥ 8.5 mmol/L. Patients were diagnosed as "probable FH" if they had high LDL-C and a 1st-degree relative with high LDL-C or premature ASCVD. Lastly, patients were diagnosed as having "severe hypercholesterolemia" if the only diagnostic criteria they presented with was high LDL-C(7).

Genetic testing. Out of 335 FH patients, 106 did not undergo genetic screening due to personal preference, inadequate sample for analysis, and loss of contact etc. Full FH genetic testing was done for 229 patients. Next-generation sequencing (NGS) of the *LDLR*, *PCSK9* and *APOB* genes and multiplex ligation-dependent probe amplification (MLPA) (118) for detection of CNVs in the

LDLR gene were carried out at the Core Molecular Diagnostic Lab (CMDL) of the McGill University Health Centre. The CMDL is currently the only CLIA-certified clinical molecular genetics laboratory for FH in Canada (Clinical Laboratory Improvement Amendments - CLIA certification from FDA, CMS and CDC). Briefly, all coding bases and splice junctions of the *LDLR*, *APOB* and *PCSK9* genes were amplified at a sequencing depth of at least 20X using custom multiplex PCRs. Standard bioinformatics software and databases were used for data analysis, from management of raw sequencing data to clinical annotation of identified variants. DNA variants were classified according to the 2015 American College of Medical Genetics guidelines(119). Several databases were cross-referenced including dbSNP (https://www.ncbi.nlm.nih.gov/projects/SNP/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), and the Genome Aggregation Database (http://gnomad.broadinstitute.org/). In case of ambiguity, Sanger sequencing was used to confirm variants detected by NGS.

The list of FH variants identified in the present cohort from the McGill University Health Centre, Montreal, Québec using our new clinical genetic diagnosis protocol was compared with the list of French-Canadian variants currently available from the only provincially approved genetic assay. Currently, the Provincial Québec Health Ministry (MSSS) reimburses genetic screening for specific variants of the *LDLR* gene only, which are variants commonly seen in the French-Canadian population: two copy number variants (CNVs; del 5 Kb, del >15 Kb) and nine single-nucleotide variants (Trp66Gly, Cys646Tyr; Glu207Lys, Cys152Trp, Arg329Xaa, Cys347Arg, Tyr468Xaa, Tyr354Cys, 681ins7). The two genotyping panels are described on the MSSS' website (http://www.msss.gouv.qc.ca/repertoires/biomed/index.php).

2.1.3 Statistical Analysis

Participant demographic characteristics and lipid profiles were presented using standard descriptive statistics, including mean and standard deviation, median and interquartile range (IQR), and frequency with percentage. Statistical testing was used to compare differences between index-patients and cascade screening patients, and between males and females. A Student's *t*-test was used to compare continuous variables with a normal distribution while a Mann-Whitney *U* test was used to compare continuous variables with a skewed distribution (triglycerides and Lp(a)). Lastly, a Chi-square test was used to compare categorical variables. Significance level was set at *p* less than 0.05. SPSS Statistics 24 (IBM SPSS Canada) was used for all analyses.

2.1.4 Ethics Approval

The study was approved by the Research Ethics Board of the McGill University Health Centre (REB#13-292-BMD) and all patients signed informed consent forms for data collection and genetic analysis. The FH Canada registry is registered at www.ClinicalTrials.gov (NCT02009345).

2.2 Results

2.2. 1 Patient Characteristics

Data on a total of 335 patients with "severe hypercholesterolemia", "probable FH" or "definite FH" according to the Canadian definition of FH (7) was collected between 2017-2021. **Table 2** describes the major baseline characteristics of this cohort. For all patients, mean age at time of registration was 50 ± 15 years, with 55% being men and 45% women. In this cohort of FH patients, 23% had hypertension, 29% had coronary artery disease (CAD), and 24% presented with tendon xanthomas. A majority of patients were from European descent (82%) with, 55% of

patients self-describing as French-Canadians. At the time of registration, the mean LDL-C was 3.61 ± 2.05 mmol/L, with 76% of patients already on lipid-lowering therapy. However, the mean recorded baseline LDL-C was 6.96 ± 1.79 mmol/L.

Table 2. Patient Characteristics

Variable	N	All Patients (n=335)	N	Index Patients (n=288)	N	Cascade Screening Patients (n=47)	*P* value
Men	335	184 (54.9%)	288	157 (54.5%)	47	27 (57.4%)	0.708
Age at registration (y)	335	50 ± 15	288	51 ± 15	47	41 ± 15	**<0.0001**
Age at diagnosis (y)	324	40 ± 16	277	42 ± 16	47	30 ± 17	**<0.0001**
Smoker	334	31 (9.3%)	288	27 (9.4%)	46	4 (8.7%)	0.882
Hypertension	334	75 (22.5%)	287	71 (24.7%)	47	4 (8.5%)	**0.013**
Diabetes	335	33 (9.9%)	288	31 (10.8%)	47	2 (4.3%)	0.127
CAD	335	97 (29.0%)	288	93 (32.3%)	47	4 (8.5%)	**<0.001**
Tendon xanthomas	335	80 (23.9%)	288	70 (24.3%)	47	10 (21.3%)	0.652
On lipid lowering therapy at registration	335	255 (76.1%)	288	223 (77.4%)	47	32 (68.1%)	0.164
Family history of CAD	327	238 (72.8%)	280	201 (71.8%)	47	37 (78.7%)	0.323
Family history of dyslipidemia	318	283 (89.0%)	271	236 (87.1%)	47	47 (100%)	**0.009**
Fully genetically tested	335	229 (68.4%)	288	189 (65.6%)	47	40 (85.1%)	**0.008**
Self-reported ethnicity							
European	314	257 (81.8%)	268	222 (82.8%)	46	35 (76.1%)	0.273
French-Canadian descent	314	172 (54.8%)	268	149 (55.6%)	46	23 (50.0%)	0.481
Middle Eastern	314	25 (8.0%)	268	18 (6.7%)	46	7 (15.2%)	0.071
Southeast Asian	314	9 (2.9%)	268	6 (2.2%)	46	3 (6.5%)	0.153
African/African American	314	7 (2.2%)	268	6 (2.2%)	46	1 (2.2%)	0.978
Latin American	314	4 (1.3%)	268	4 (1.5%)	46	0	0.259
Other or mixed ethnicity	314	12 (3.8%)	268	12 (4.4%)	46	0	**0.050**
Lipid profile at registration							
Total cholesterol (mmol/L)	335	5.73 ± 2.22	288	5.66 ± 2.22	47	6.14 ± 2.16	0.165
LDL cholesterol (mmol/L)	333	3.61 ± 2.05	286	3.51 ± 2.02	47	4.27 ± 2.15	**0.018**
Triglycerides (mmol/L)	335	1.30 (1.14)	288	1.32 (1.20)	47	1.23 (0.89)	0.107
HDL cholesterol (mmol/L)	335	1.31 ± 0.37	288	1.31 ± 0.37	47	1.28 ± 0.37	0.552
ApoB (g/L)	290	1.19 ± 0.48	246	1.16 ± 0.46	44	1.34 ± 0.57	**0.021**
Lp(a)	271	349 (746)	227	342 (740)	44	411 (878)	0.527
Untreated lipid profile*							
Total cholesterol (mmol/L)	288	8.99 ± 1.75	248	9.05 ± 1.76	40	8.67 ± 1.66	0.214
LDL cholesterol (mmol/L)**	335	6.96 ± 1.79	288	6.95 ± 1.79	47	6.99 ± 1.77	0.897
Triglycerides (mmol/L)	281	1.63 (1.32)	242	1.71 (1.33)	39	1.30 (1.25)	**0.009**
HDL cholesterol (mmol/L)	284	1.29 ± 0.36	244	1.31 ± 0.36	40	1.20 ± 0.30	0.063
ApoB (g/L)	135	1.90 ± 0.49	114	1.88 ± 0.48	21	2.01 ± 0.55	0.261

Data are presented as n (%), mean ± (SD) or median ± (IQR). CAD, Coronary artery disease; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ApoB, Apolipoprotein B; Lp(a), Lipoprotein(a). *Based on data available from chart review. **Used imputed baseline LDL-C when untreated LDL-C values were missing (for n = 39). P value obtained from *T*-test , Mann-Whitney *U* test, or Chi-Square Test.

This cohort included 288 index patients (mean age at time of registration, 51 ± 15 years; 55% men) and 47 individuals identified through cascade screening family members of index patients (mean age at time of registration, 41 ± 15 years, 57% men). At time of first diagnosis of FH, index patients were 12 years older than patients from cascade screening (42 years vs. 30 years, P <0.0001) and were more likely to have a history of hypertension (25% vs 9%, P =0.013) and CAD (32% vs 9%, P <0.001). At registration, more index patients were on lipid lowering therapy compared to cascade screening patients, however this was not significant (77% vs. 68%, P =0.164). Index patients had significantly lower LDL-C (3.51 ± 2.02 vs. 4.27 ± 2.15 mmol/L, P =0.018) and ApoB (1.16 ± 0.46 vs. 1.34 ± 0.57 mmol/L, P =0.021) compared to cascade screening patients. Baseline untreated lipid profiles revealed that index patients had significantly higher triglycerides compared to cascade screening patients at first diagnosis (**Table 2**).

Our cohort include 184 men (mean age at registration 48± 14 years) and 151 women (mean age at registration 52 ± 17 years). Men patients were diagnosed 5 years earlier on average compared to women (37 years vs. 43 years, P=0.001) and were more likely to have a history of CAD (37% vs. 19%, P<0.001). At registration, a higher proportion of men were on lipid lowering therapy compared to women (84% vs. 66%). Interestingly, men had significantly lower total cholesterol, LDL-C, HDL-C, and ApoB compared to women at registration (**Table 3).** Alternatively, baseline lipid profiles revealed that men and women had similar lipid levels at first diagnosis, with the exception of men having higher triglycerides (1.75 ± 1.21 vs. 1.49 ± 1.37 mmol/L, P=0.018) and lower HDL-C (1.16 ± 0.26 vs. 1.44 ± 0.39 mmol/L, P<0.001).

Table 3. Patient characteristics according to sex

Variable	N	All Patients (n=335)	N	Men (n=184)	N	Women (n=151)	*P* value
Index Patients	335	288 (86.0%)	184	157 (85.3%)	151	131 (86.8%)	0.708
Age at registration (y)	335	50 ± 15	184	48 ± 14	151	52 ± 17	**0.01**
Age at diagnosis (y)	324	40 ± 16	178	37 ± 15	146	43 ± 17	**0.001**
Smoker	334	31 (9.3%)	183	21 (11.5%)	151	10 (6.6%)	0.305
Hypertension	334	75 (22.5%)	184	42 (22.8%)	150	33 (22.0%)	0.857
Diabetes	335	33 (9.9%)	184	20 (10.9%)	151	13 (8.6%)	0.490
CAD	335	97 (29.0%)	184	68 (37.0%)	151	29 (19.2%)	**<0.001**
Tendon xanthomas	335	80 (23.9%)	184	49 (26.6%)	151	31 (20.5%)	0.193
On lipid lowering therapy at registration	335	255 (76.1%)	184	155 (84.2%)	151	100 (66.2%)	**<0.001**
Family history of CAD	327	238 (72.8%)	178	126 (70.8%)	149	112 (75.2%)	0.375
Family history of dyslipidemia	318	283 (89.0%)	177	161 (91.0%)	140	122 (87.1%)	0.350
Fully genetically tested	335	229 (68.4%)	184	126 (68.5%)	151	103 (68.2%)	0.958
Self-reported ethnicity							
European	314	257 (81.8%)	175	139 (79.4%)	139	118 (84.9%)	0.212
French-Canadian descent	314	172 (54.8%)	175	97 (55.4%)	139	75 (54.0%)	0.795
Middle Eastern	314	25 (8.0%)	175	17 (9.7%)	139	8 (5.8%)	0.198
Southeast Asian	314	9 (2.9%)	175	6 (3.4%)	139	3 (2.2%)	0.503
African/African American	314	7 (2.2%)	175	4 (2.3%)	139	3 (2.2%)	0.939
Latin American	314	4 (1.3%)	175	2 (1.1%)	139	2 (1.4%)	0.816
Other or mixed ethnicity	314	12 (3.8%)	175	7 (4.0%)	139	5 (3.6%)	0.853
Lipid profile at registration							
Total cholesterol (mmol/L)	335	5.73 ± 2.22	184	5.29 ± 2.19	151	6.26 ± 2.14	**<0.001**
LDL cholesterol (mmol/L)	333	3.61 ± 2.05	182	3.21 ± 1.94	151	4.10 ± 2.09	**<0.001**
Triglycerides (mmol/L)	335	1.30 (1.14)	184	1.36 (1.25)	151	1.24 (0.97)	0.206
HDL cholesterol (mmol/L)	335	1.31 ± 0.37	184	1.20 ± 0.29	151	1.44 ± 0.40	**<0.001**
ApoB (g/L)	290	1.19 ± 0.48	161	1.09 ± 0.47	129	1.31 ± 0.48	**<0.001**
Lp(a)	271	349 (746)	152	354 (797)	119	342 (727)	0.989
Untreated lipid profile*							
Total cholesterol (mmol/L)	288	8.99 ± 1.75	152	9.01 ± 1.80	136	8.98 ± 1.70	0.880
LDL cholesterol (mmol/L)**	335	6.96 ± 1.79	184	7.11 ± 1.81	151	6.77 ± 1.74	0.088
Triglycerides (mmol/L)	281	1.63 (1.32)	151	1.75 (1.21)	130	1.49 (1.37)	**0.018**
HDL cholesterol (mmol/L)	284	1.29 ± 0.36	151	1.16 ± 0.26	133	1.44 ± 0.39	**<0.001**
ApoB (g/L)	135	1.90 ± 0.49	74	1.97 ± 0.53	61	1.82 ± 0.44	0.087

Data are presented as n (%), mean ± (SD) or median ± (IQR). CAD, Coronary artery disease; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ApoB, Apolipoprotein B; Lp(a), Lipoprotein(a). *Based on data available from chart review. **Used imputed baseline LDL-C when untreated LDL-C values were missing (on n = 39). P value obtained from *T*-test, Mann-Whitney *U* test, or Chi-Square Test.

2.2.2 Genetic Testing

Genetic testing was performed on 229 patients. An FH variant was identified in 169 (74%) of patients tested. Within the patients with a positive genetic test, majority were found to have variants within the *LDLR* gene (87%), the *APOB* gene (13%) or the *PCSK9* gene (5%), including 11 patients with variants on more than one gene (**Table 4**). The *LDLR* del 15Kb, known as the "French Canadian" mutation, was identified in 33% of patients. The second most prevalent variant was the *LDLR* p.cys681*, predominantly found in patients of Christian Lebanese descent (120) (**Table 4**). Interestingly, the genetic panel offered by Québec's Health Ministry (MSSS), which includes 11 common mutations in French Canadians, accounted for only 49% of identified mutations. Even in patients self-describing as French Canadians with an FH-causing variant, 16% did not have a common mutation listed in the panel. Notably, a higher proportion of men had a PCSK9 variant than women (7% vs. 1%, *P*=0.04) (**Table 5).** No other significant differences in prevalence of variants were found between men and women. **Table 4** shows the FH-causing variants identified with a frequency > 2% in our cohort as well as the MSSS genetic panel, while **Table 6** lists all the variants identified, including variants of uncertain significance or newly identified. In total, 69 unique variants were identified in this cohort of FH patients **(Table 6).**

2.2.3 FH diagnosis

We then determined whether genetic testing allowed for reclassification of patients according to the Canadian definition of FH(7). **Table 7** shows the number of patients entering with a "severe hypercholesterolemia", "probable FH" and "definite FH" phenotype and their re-classification after genetic testing. Before genetic testing, a majority of patients were diagnosed as "probable FH" (58.3%). Genetic testing allowed for re-classification to "definite FH" for 30.8%

of patients initially classified as "Severe Hypercholesterolemia" and 90 (67.2%) of patients initially "probable FH" (**Table 7, Figure 5).** Additionally, when comparing the reclassification of FH patients according to sex, no significant differences were found (**Table 8**). In 7 "definite FH" patients, we did not identify a mutation in the *LDLR, APOB* or *PCSK9* genes **(Table 9**). Further sequencing in research laboratories identified mutations in the *ABCG5/8* or *APOE* genes for 3 of these patients.

Table 4. Main FH variants and MSSS French-Canadian variants identified in our cohort

Patients Screened (N_{total}=229)		
Patients With a FH Variant Identified 169^{+} (73.8%)	**Patients With No FH Variant Identified 60 (26.2%)**	
Gene – Variant	**Known Name**	**Number of Patients (N_{total}=169^{+})**
LDLR		**146 (86%)**
p.Cys681*	C660*	15 (9%)
p.Ala431Thr	A410T	6 (4%)
p.Asp90Asn	D69N	4 (2%)
p.Gly592Glu	G571E	3 (2%)
MSSS French-Canadian variants		82 (49%)
Delta 15kb	Delta 15 kb (*FH French Canadian -1*)	55 (33%)
Delta 5kb	Delta 5 kb (*FH French Canadian-5*)	0
p.Trp87Gly	W66G (*FH French Canadian-4*)	10 (6%)
p.Cys667Tyr	C646Y (*FH French Canadian-2*)	6 (4%)
p.Cys173Trp	C152W	0
p.Glu228Lys	E207K (*FH French Canadian-3*)	4 (2%)
p.Arg350*	R329* (FH Fossum)	2 (1.2%)
p.Cys368Arg	C347R	4 (2%)
p.Tyr375Cys	Y354C	0
p. Tyr489*	Y468*	0
681ins7		0
Other		41 (24%)
APOB		**24 (14%)**
p.Thr3496Ala	-	3 (2%)
p.Arg3527Gln	-	3 (2%)
Other		20 (12%)
PCSK9		**6 (4%)**

APOB, gene encoding apolipoprotein B; FH, familial hypercholesterolemia; LDLR, gene encoding the LDL receptor. Includes 11 patients with multiple variants. 2 patients have multiple APOB variants and 2 patients have multiple LDLR variants. Main Variant is defined as ≥ 2%. $^{+}$ Includes a patient with an APOE variant identified after additional genetic analysis.

Table 5. Frequency of FH variants according to sex

Patients Screened (N_{total}=229)				
Patients With a FH Variant Identified 169^{+} (73.8%)		**Patients With No FH Variant Identified 60 (26.2%)**		
Gene – Type/Variant	**Known Name**	**Females (N=74)**	**Males (N=95^{+})**	**P-value**
LDLR		**63 (85.12. 89%)**	**83 (87.37%)**	0.674
Null		34 (54.0% of LDLR variants)	57 (67% of LDLR variants)	0.09
p.Cys681*	C660*	3 (4.05%)	12 (14.46%)	0.052
p.Ala431Thr	A410T	3 (4.05%)	3 (3.16%)	0.756
p.Asp90Asn	D69N	2 (2.70%)	2 (2.11%)	0.801
p.Gly592Glu	G571E	1 (1.35%)	2 (2.11%)	0.709
MSSS French-Canadian variants				
Delta 15kb	Delta 15 kb (*FH French Canadian-1*)	22 (29.73%)	33 (34.74%	0.491
Delta 5kb	Delta 5 kb (*FH French Canadian-5*)	0	0	
p.Trp87Gly	W66G (*FH French Canadian-4*)	4 (5.41%)	6 (6.32%)	0.790
p.Cys667Tyr	C646Y (*FH French Canadian-2*)	3 (4.05%)	3 (3.16%)	0.756
p.Cys173Trp	C152W	0	0	
p.Glu228Lys	E207K (*FH French Canadian-3*)	2 (2.70%)	2 (2.11%)	0.801
p.Arg350*	R329* (FH Fossum)	0	2 (2.11%)	0.127
p.Cys368Arg	C347R	3 (4.05%)	1 (1.05%)	0.199
p.Tyr375Cys	Y354C	0	0	
p. Tyr489*	Y468*	0	0	
681ins7		0	0	
APOB		**8 (10.81%)**	**16 (16.84%)**	0.265
p.Thr3496Ala	-	1 (1.35%)	2 (2.11%)	0.709
p.Arg3527Gln	-	1 (1.35%)	2 (2.11%)	0.709
PCSK9		**5 (6.76%)**	**1 (1.05%)**	**0.042**

APOB, gene encoding apolipoprotein B; FH, familial hypercholesterolemia; LDLR, gene encoding the LDL receptor. Includes 4 Females and 7 Males with multiple variants; 2 males have multiple APOB variants and 2 females have multiple LDLR variants. $^{+}$ Includes a patient with an APOE variant identified after additional genetic analysis. P value obtained from Chi-Square Test.

Table 6. List of all unique variants identified in our FH cohort

Gene and type of defect	Nucleotide change	Protein change	Known Name	Location in protein (start point)	Variant type	*LDLR* null or defective	Pathogenicity (Clinvar, LOVD[3], gnomAD, etc)	Frequency (number of patients)	PMID #
LDLR, large rearrangement	c.(?_187)_(2311+1_2312-1)del	p.0?		Promoter	Deletion (removes Promoter to Exon 15)	Null	Pathogenic	1	23375686
LDLR, large rearrangement	c.(?_-187)_(67+1_68-1)del	p.0?	FH French Canadian-1; FH Denver-1	Promoter	Deletion (removes Promoter and Exon 1)	Null	Pathogenic	55	3627182; 3343347
LDLR, point mutation	c. -135C>G	p.0?	FH Columbia-2	Promotor	Missense (nucleotide change)	Defective	Pathogenic/Likely Pathogenic	1	15241806, 18096825, 19007590, 19411563, 1301956
LDLR, point mutation	c.11G>A	p.Trp4Ter	W-18X; FH Columbia-1	Exon 1	Nonsense	Null	Pathogenic	2	16314194; 1301956; 10206683
LDLR, point mutation	c.81C>G	p.Cys27Trp	C6W; FH San Francisco	Exon 2	Missense (nucleotide change)	Defective	Pathogenic	1	25463123
LDLR, point mutation	c.173A>G	p.Glu58Gly	E37G	Exon 2	Missense (nucleotide change)	Defective	Likely pathogenic	1	19446849
LDLR, small insertion/deletion	c.190+4A>T	p.?		Intron 2	Intronic insertion – affecting splicing	Null	Pathogenic	1	15199436; 16205024; 16250003; 21418584; 27765764; 19208450
LDLR, point mutation	c.245G>T	p.Cys82Phe	C61F	Exon 3	Missense (nucleotide change)	Defective	Pathogenic/Likely pathogenic	1	12417285
LDLR, point mutation	c.259T>G	p.Trp87Gly	W66G; FH French Canadian-4	Exon 3	Missense (nucleotide change)	Defective	Pathogenic	10	9272705
LDLR, point mutation	c.268G>A	p.Asp90Asn	D69N	Exon 3	Missense (nucleotide change)	Defective	Pathogenic	4	12837857
LDLR, small insertion/deletion	c.313+1G>A	p.? suggested; p.Leu64_Pro105delinsSer	FH Elverum; FH Olbia	Intron 3	Intronic insertion – affecting splicing	Null	Pathogenic	1	7718019; 7616128
LDLR, point mutation	c.458T>G	p.Phe153Cys	F132C	Exon 4	Missense (nucleotide change)	Defective	Likely pathogenic	1	15199436
LDLR, point mutation	c.518G>A	p.Cys173Tyr	C152Y	Exon 4	Missense (nucleotide change)	Defective	Likely pathogenic	1	23375686
LDLR, small insertion/deletion	c.654_656delTGG	p.Gly219del	G197del; FH Lithuania; FH Piscataway	Exon 4	Deletion	Defective	Pathogenic	1	9744476; 11309683; 1867200
LDLR, point mutation	c.661G>T	p.Asp221Tyr	D200Y; FH Finn-3	Exon 4	Missense (nucleotide change)	Defective	Pathogenic	2	7573037; 10206683; 23375686; 23375686
LDLR, point mutation	c.682G>A	p.Glu228Lys	E207K; FH French Canadian-3	Exon 4	Missense (nucleotide change)	Null	Pathogenic	4	2318961
LDLR, small insertion/deletion	c.694+25C>T	p.=		Intron 4	Intronic insertion - benign	Defective	Uncertain significance	1	11668640; 23375686

LDLR, point mutation	c.910G>A	p.Asp304Asn	D283N; FH Denver-2	Exon 6	Missense (nucleotide change)	Defective	Pathogenic	1	1301956, 9664576, 11810272, 12436241, 21418584, 21310417
LDLR, point mutation	c.917C>T	p.Ser306Leu	S285L; FH Amsterdam	Exon 6	Missense (nucleotide change)	Defective	Pathogenic	1	1301956
LDLR, point mutation	c.932A>G	p.Lys311Arg	K290R	Exon 6	Missense (nucleotide change) – Double variant allele with Cys313Trp	Defective	Pathogenic	1	1301940; 11810272; 11737238; 16250003
LDLR, point mutation	c.939C>G	p.Cys313Trp	C292W	Exon 6	Missense (nucleotide change) - Double variant allele with Lys311Arg	Defective	Pathogenic	1	16250003; 12436241
LDLR, point mutation	c.1048C>T	p.Arg350Ter	R329X; FH Fossum	Exon 7	Nonsense	Null	Pathogenic	2	7709162; 9039985; 21382890; 22390909
LDLR, point mutation	c.1102T>C	p.Cys368Arg	C347R	Exon 8	Missense (nucleotide change)	Defective	Pathogenic	4	9452094; 1301940
LDLR, point mutation	c.1285G>A	p.Val429Met	V408M; FH Afrikaner-2	Exon 9	Missense (nucleotide change)	Null	Pathogenic	1	2569482
LDLR, point mutation	c.1291G>A	p.Ala431Thr	A410T; FH-Algeria	Exon 9	Missense (nucleotide change)	Defective	Pathogenic	6	12837857
LDLR, point mutation	c.1529C>T	p.Thr510Met	T489M	Exon 10	Missense (nucleotide change)	Defective	Likely pathogenic	1	22698793; 16542394; 15199436
LDLR, point mutation	c.1576C>T	p.Pro526Ser	P505S; FH Cincinnati-3	Exon 10	Missense (nucleotide change)	Defective	Pathogenic/Likely pathogenic	2	1301956; 9259195; 11462246
LDLR, point mutation	c.1595A>G	p.Tyr532Cys		Exon 11	Missense (nucleotide change)	Unknown	Uncertain significance	1	28379029
LDLR, point mutation	c.1618G>A	p.Ala540Thr	A519T	Exon 11	Missense (nucleotide change)	Defective	Pathogenic	1	9409298
LDLR, point mutation	c.1747C>T	p.His583Tyr	H562Y	Exon 12	Missense (nucleotide change)	Defective	Pathogenic	1	27206935; 20538126; 16205024
LDLR, point mutation	c.1775G>A	p.Gly592Glu	G571E; FH Sicily; FH Foggia-1; FH Naples4	Exon 12	Missense (nucleotide change)	Defective	Pathogenic	3	1301956
LDLR, point mutation	c.1784G>A	p.Arg595Gln	R574Q	Exon 12	Missense (nucleotide change)	Defective	Pathogenic/Likely pathogenic	2	11737238; 15359125; 16250003
LDLR, large rearrangement	c.(1845+1_1846-1)_(2140+1_2141-1)del	p.? suggested: p.(Asp616Argfs*16)	FH Vancouver-1; FH London-1; FH Italy-1; FH Amsterdam-4	Intron 12	Deletion (removes Exons 13-14)	Null	Pathogenic	1	3549308; 2837085; 3343347
LDLR, point mutation	c.1860G>T	p.Trp620Cys	W599C	Exon 13	Missense (nucleotide change)	Defective	Likely pathogenic	1	17539906
LDLR, point mutation	c.1868T>C	p.Ile623Thr		Exon 13	Missense (nucleotide change)	Unknown	Uncertain significance	1	27765764
LDLR, point mutation	c.2000G>A	p.Cys667Tyr	C646Y; FH French Canadian-2	Exon 14	Missense (nucleotide change)	Null	Pathogenic	6	2318961; 11810272; 7979249

LDLR, point mutation	c.2043C>A	p.Cys681Ter	C660X; FH Lebanese	Exon 14	Nonsense	Null	Pathogenic	15	19319977
LDLR, point mutation	c.2054C>T	p.Pro685Leu	P664L; FH Gujerat; FH Frosinone1; FH Kanazawa-2	Exon 14	Missense (nucleotide change)	Defective	Pathogenic	1	2726768
LDLR, point mutation	c.2093G>A	p.Cys698Tyr	C677Y	Exon 14	Missense (nucleotide change)	Null	Pathogenic/Likely pathogenic	2	7979249; 11933210; 15241806; 32015373
LDLR, point mutation	c.2113G>T	p.Ala705Ser	A684S	Exon 14	Missense (nucleotide change)	Defective	Likely pathogenic	1	17142622
LDLR, small insertion/deletion	c.2311+1G>T	p.?		Intron 15	Intronic insertion – affecting splicing	Defective	Pathogenic/Likely pathogenic	1	20809525
LDLR, small insertion/deletion	c.2312-3C>A	p.? suggested: p.Ala771_Ile796del	IVS15-3C>A	Intron 15	Intronic insertion – skipping of exon 16	Defective	Pathogenic	2	11317362; 11668640; 11317362; 21865347
LDLR, point mutation	c.2389G>A	p.Val797Met	V776N	Exon 16	Missense (nucleotide change)	Defective	Pathogenic	1	23375686; 20538126; 19411563
APOB, point mutation	c.35T>C	p.Leu12Pro		Exon 1	Missense (nucleotide change)	-	Uncertain significance	1	29036232
APOB, point mutation	c.2968G>A	p.Ala990Thr		Exon 19	Missense (nucleotide change)	-	Uncertain significance	1	No PMID; in ClinVar
APOB, point mutation	c.2996C>T	p.Thr999Ile		Exon 19	Missense (nucleotide change)	-	Uncertain significance	2	Newly identified
APOB, point mutation	c.3178T>C	p.Leu1060=		Exon 21	Synonymous	-	Uncertain significance – likely benign	1	-
APOB, point mutation	c. 4149C>A	p.Phe1383Leu		Exon 25	Missense (nucleotide change)	-	Uncertain significance	1	Newly identified
APOB, point mutation	c.6917_6918delinsTC,	p.Ser2306Phe		Exon 26	Deletion-insertion (nucleotide change)	-	Uncertain significance	1	Newly identified
APOB, point mutation	c.7696G>A	p.Glu2566Lys		Exon 26	Missense (nucleotide change)	-	Uncertain significance	2	26036859, 27765764
APOB, point mutation	c. 8462C>A	p.Pro2821Gln		Exon 26	Missense (nucleotide change)	-	Uncertain significance	1	Newly identified
APOB, point mutation	c.8608A>G	p.Ser2870Gly		Exon 26	Missense (nucleotide change)	-	Uncertain significance	1	No PMID; in ClinVar
APOB, point mutation	c.8693T>C	p.Leu2898Pro		Exon 26	Missense (nucleotide change)	-	Uncertain significance	1	No PMID; in ClinVar
APOB, point mutation	c.8882A>G	p.Asn2961Ser		Exon 26	Missense (nucleotide change)	-	Uncertain significance	1	No PMID; in ClinVar
APOB, point mutation	c.9811G>A	p.Gly3271Ser		Exon 26	Missense (nucleotide change)	-	Uncertain significance	1	No PMID; in ClinVar
APOB, point mutation	c.10061C>G	p.Ala3354Gly		Exon 26	Missense (nucleotide change)	-	Uncertain significance	1	No PMID; in ClinVar
APOB, point mutation	c.10294C>A	p.Gln3432Lys		Exon 26	Missense (nucleotide change)	-	Uncertain significance	1	Newly identified
APOB, point mutation	c.10486A>G	p.Thr3496Ala		Exon 26	Missense (nucleotide change)	-	Uncertain significance	3	No PMID; in ClinVar

APOB, point mutation	c.10580G>A	p.Arg3527Gln	R3500Q	Exon 26	Missense (nucleotide change)	-	Pathogenic	3	3771801; 3477815; 2563166; 26643808; 24404629
APOB, point mutation	c.10780T>C	p.Trp3594Arg		Exon 26	Missense (nucleotide change)	-	Uncertain significance	1	23064986; 23833242
APOB, point mutation	c.12245A>G	p.Tyr4082Cys		Exon 29	Missense (nucleotide change)	-	Uncertain significance	1	Newly identified
APOB, point mutation	c.12809G>T	p.Arg4270Met		Exon 29	Missense (nucleotide change)	-	Uncertain significance	1	Newly identified
APOB, point mutation	c.13096G>A	p.Glu4366Lys		Exon 29	Missense (nucleotide change)	-	Uncertain significance	1	No PMID; in ClinVar
PCSK9, point mutation	c.*171C>T	p.?		3'UTR	Missense (nucleotide change)	-	Uncertain significance	2	No PMID; in ClinVar
PCSK9, small insertion/deletion	c.1260delC	p.Asp422Metfs		Exon 8	Frameshift	-	Uncertain significance	2	No PMID; in ClinVar
PCSK9, point mutation	c.1486C>T	p.Arg496Trp		Exon 9	Missense (nucleotide change)	-	Uncertain significance	1	23375686, 26374825, 27206942, 16183066
PCSK9, point mutation	c.1978G>A	p.Asp660Asn		Exon 12	Missense (nucleotide change)	-	Uncertain significance	1	29259136
APOE, deletion	c.497TCC(1)	p.Leu167del		Exon 4	Deletion	-	Pathogenic	1	11095479, 16094309, 24267230, 22949395
ABCG8, point mutation	c.619A>G	p.Asn207Asp		Exon 5	Missense (nucleotide change)	-	Uncertain significance	2	No PMID; in ClinVar

APOB: gene encoding apolipoprotein B; LDLR: gene encoding the LDL receptor; PCSK9: gene encoding the Proprotein convertase subtilisin/kexin type 9; APOE: gene encoding the apolipoprotein E; ABCG8: gene encoding the ATP Binding Cassette Subfamily G Member 8

Table 7. Impact of genetic testing on the re-classification of a clinical FH diagnosis

Pre-Genetic Testing			**Post Genetic Testing**	
Clinical FH Diagnosis	No. of Patients (n=229)		Clinical FH Diagnosis	No. of Patients
Severe Hypercholesterolemia	13 (5.7%)	➡		(n=13)
			Severe Hypercholesterolemia	9 (69.2%)
			Probable FH	0
			Definite FH	4 (30.8%)
Probable FH	134 (58.3%)	➡		(n=134)
			Severe Hypercholesterolemia	0
			Probable FH	44 (32.8%)
			Definite FH	90 (67.2%)
Definite FH	82 (35.8%)	➡		(n=82)
			Severe Hypercholesterolemia	0
			Probable FH	0
			Definite FH	82 (100%)

FH, familial hypercholesterolemia. Data are presented as n (%). Clinical FH Diagnosis is according to the Canadian Definition of FH (9).

Table 8. Re-classification of FH patients according to sex

Women				Men				
Pre-Genetic Testing		**Post Genetic Testing**		**Pre-Genetic Testing**		**Post Genetic Testing**		
Clinical FH Diagnosis	No. of Women (n=103)	Clinical FH Diagnosis	No. of Women	Clinical FH Diagnosis	No. of Men (n=126)	Clinical FH Diagnosis	No. of Men	***P* value**
			(n=5)				(n=8)	
Severe Hypercholesterolemia	5 (4.8%) →	Severe Hypercholesterolemia	4 (80.0%)	Severe Hypercholesterolemia	8 (6.4%) →	Severe Hypercholesterolemia	5 (62.5%)	0.498
		Probable FH	0			Probable FH	0	
		Definite FH	1 (20.0%)			Definite FH	3 (37.5%)	
			(n=64)				(n=70)	
Probable FH	64 (62.1%) →	Severe Hypercholesterolemia	0	Probable FH	70 (55.6%) →	Severe Hypercholesterolemia	0	0.996
		Probable FH	21 (32.8%)			Probable FH	23 (32.9%)	
		Definite FH	43 (67.2%)			Definite FH	47 (67.1%)	
			(n=34)				(n=48)	
Definite FH	34 (33.0%) →	Severe Hypercholesterolemia	0	Definite FH	48 (38.1%) →	Severe Hypercholesterolemia	0	N/A
		Probable FH	0			Probable FH	0	
		Definite FH	34 (100%)			Definite FH	48 (100%)	

FH, familial hypercholesterolemia. Data are presented as n (%). Clinical FH Diagnosis is according to the Canadian Definition of FH (9).

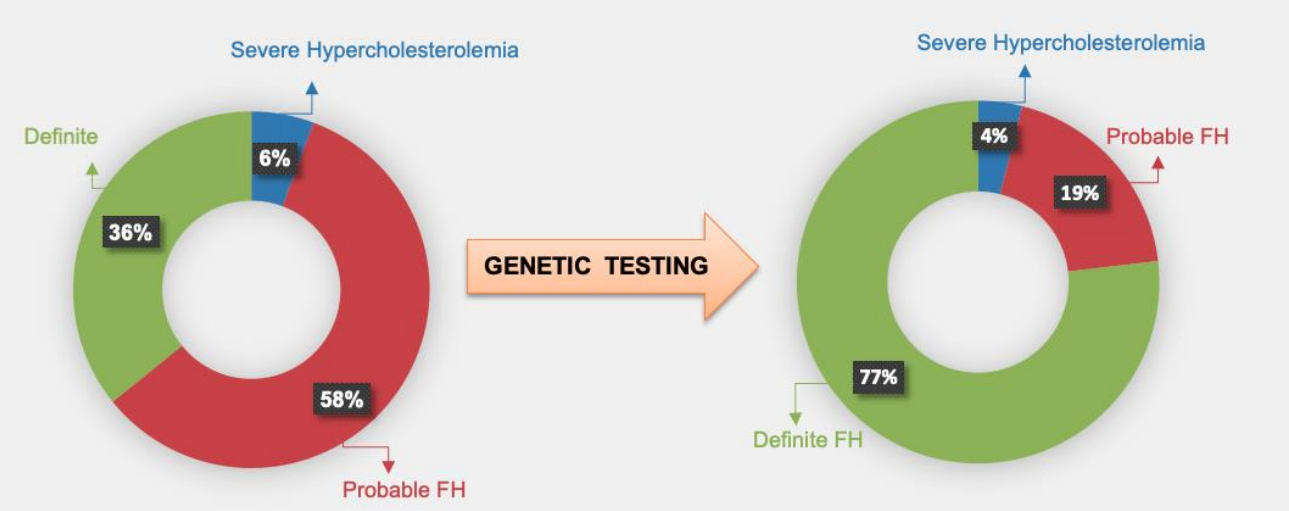

Figure 1. Re-classification of FH diagnosis after genetic testing

Table 9. Classification of FH patients according to genetic testing result

Patients with a FH variant identified		**Patients with no FH variant identified**	
Clinical FH Diagnosis	**No. of Patients (n=169)**	**Clinical FH Diagnosis**	**No. of Patients (n=60)**
Severe Hypercholesterolemia	0	Severe Hypercholesterolemia	9 (15.0%)
Probable FH	0	Probable FH	44 (73.3%)
Definite FH	169 (100%)	Definite FH	7 (11.7%)

FH, familial hypercholesterolemia. Data are presented as n (%). Clinical FH Diagnosis is according to the Canadian Definition of FH (9).

3. TRANSITION: PRELIMINARY REGISTRY ANALYSIS OF SEX DIFFERENCES IN FH

Investigating how an unbiased genetic testing allows for improved and more precise FH diagnosis, revealed how inadequate access to a full genetic screening may act as a barrier to precise diagnosis. Although we found no significant differences in prevalence of genetic variants or in the yield of re-classification according to sex, when we stratified our patient characteristics according to sex, there were some significant differences that warranted further investigation. In fact, we found that within our FH cohort, women were diagnosed 6 years later than men. As well, we found that at registration women had higher T.Chol, LDL-C, and ApoB than men **(Table 3)**. We sought to investigate this in further detail to see if sex acts as a barrier to care in FH specifically. The aim of the preliminary analysis was to determine if there are any sex differences in treatment, and lipid level target achievement in FH patients at the McGill University Health Centre.

A preliminary retrospective registry analysis of 292 HeFH patients at the McGill University Health Centre was performed. Data was obtained from the FH Canada Registry. Data such as untreated lipids, 1st clinic visit data, and most recent clinic visit data was extracted. Patients were included in the study if they were HeFH adults and were diagnosed as either "Definite FH", "Probable FH", or "Possible FH", according Simon-Broome criteria (4), Dutch Lipid Clinic Network criteria (5), or the new Canadian definition of FH(7). As well, only patients with multiple clinic visits were included. Differences between men and women were calculated using a *t*-test or chi-squared test. All analyses were performed in RStudio.

There were 127 women and 162 men from the McGill University Health Centre included in the analysis. The mean age at registration was 49±17 years for women and 45±16 years for men (p=0.04). Average follow-up time was similar between sexes (women: 3.4 ± 6.9 years, men: 4.2 ±

15.7 years, P=0.244). Among patients with multiple visits, at the most recent clinic visit, similar proportions of men and women were taking lipid lowering treatments (94.0 % vs. 85.9 %, *P*=0.55), yet only 35% of women were on high-intensity statins, compared to 74% of men (*P*=0.002) (**Table 10).** Less women were taking other types of lipid-lowering medications such as Ezetimibe or PCSK9 inhibitors compared to men as well, although this was found to be insignificant, perhaps due to the limited sample number. Interestingly, statin intolerance was reported in 40% of women and 22% of men (p=0.02). We then examined guideline-recommended lipid target achievement between both sexes. At baseline, men and women had similar mean LDL-C levels of 6.9±2.2 mmol/L and 6.7±1.6 mmol/L respectively (p=0.7). Despite this, at the most recent visit, 55% of men reached a target LDL-C of ≤ 2.5 mmol/L compared to just 32% of women (p=0.02). As well, from baseline to most recent visit, women reduced their LDL-C by 51%, whereas men lowered their LDL-C by 62% (p=0.01). Therefore, overall, we found that less women were reaching guideline recommended LDL-C levels compared to men (**Table 11).**

Table 10. Lipid-lowering treatment use among FH patients at the McGill University Health Centre

Variable	Women (n=85)	Men (n=116)	*P* value
Lipid lowering treatment	85.9%	94.0%	0.552
Statin	76.5%	88.8%	0.704
High-intensity statin	35.3%	74.1%	0.002*
Low-intensity statin	41.2%	14.7%	<0.001*
Ezetimibe	44.7%	59.5%	0.383
Statin + Ezetimibe	40.0%	57.8%	0.221
PCSK9 inhibitors	16.5%	25.9%	0.231
Statin intolerance	40.0%	21.6%	0.017*

Data reported as n (%)

Table 11. Change in LDL-C levels from baseline to most recent visit

	Women (n=81)	**Men (n=110)**	***P* value**
Follow-up time, y	5.1 ± 6.2	5.9 ± 6.9	0.373
Baseline LDL-C, mmol/L	6.7 ± 1.6	6.9 ± 2.2	0.724
Follow-up LDL-C, mmol/L	3.1 ± 1.5	2.4 ± 1.3	0.006*
Average % reduction	-51.6% ± 25.4	-62.1% ± 20.5	0.011*
≤ 1.8 mmol/L at follow-up	17.3%	32.7%	0.039*
≤ 2.5 mmol/L at follow-up	32.1%	54.6%	0.022*
≥ 50% reduction	54.3%	74.6%	0.089
> 4.0 mmol/L at follow-up	21.0%	7.3%	0.01*

Data presented as % or mean ± SD.

Our preliminary analysis of patients at the McGill University Health Centre revealed that women were not being treated as intensely as men and less women were reaching LDL-C targets compared to men. Importantly, investigators at the University of British Columbia found similar findings in their FH cohort. Ryzhaya et al reported that women were treated less intensively and accordingly, had a lower rate of target achievement in lipid levels compared to men (121). They also found that women were diagnosed roughly 3 years later than men. Investigating sex differences such as these are important to understand more clearly how sex may act as a barrier to care and optimal outcomes. To look into this issue further, we decided to pursue a global scale systematic review investigating sex differences in treatment of FH**.**

4. SEX DIFFERENCES IN THE TREATMENT OF FH: A SYSTEMATIC REVIEW

4.1 Methods

The design and methods used for this systematic review comply with the Centre for Review and Dissemination Guidelines and is reported in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses – Protocol (PRISMA-P)(122, 123). Eligibility criteria were created using the PICO guidelines. The final written protocol was registered with PROSPERO.

4.1.2 Information sources

We employed database-specific search strategies designed to identify relevant English-language publications from inception to July 11th, 2020. Electronic databases, clinical trial registries, grey literature, and conference proceedings were searched. Electronic databases included MEDLINE, Embase, The Cochrane Controlled Register of Trials (CENTRAL), PubMed, Scopus, Africa-Wide (via EBSCO), Biosis (Web of Science), and Global Health (Ovid) were searched. Clinical trial registries included ClinicalTrials.gov, International Clinical Trials Registry Platform, the International Standard Randomised Controlled Trial Number (ISRCTN) registry, the UK Clinical Trials Gateway, and ProQuest Dissertations. Grey literature was searched from Google Scholar and Open Gray. Conference proceedings were search from American Heart Association Canadian Lipoprotein Conference, Canadian Cardiovascular Conference/Congress, European Society of Cardiology Congress, Canadian Lipid & Vascular Summit, Arteriosclerosis, Thrombosis and Vascular Biology (ATVB), and Canadian Society of Internal Medicine.

4.1.3 Search strategy

The following databases were searched for relevant studies: MEDLINE (via Ovid 1946 to 2020/07/21); Embase Classic + Embase (via Ovid 1947 to 2020/07/21); The Cochrane Central Register of Controlled Trials (via The Cochrane Library, Issue 7 of 12, January 2020); PubMed (National Library of Medicine 2020/07/14 – Current) ; PsychInfo (via OVID 1987 – 2020/07/21) and Scopus (via Elsevier 1788 – 2020/07/21). The search strategies designed by a librarian used text words and relevant indexing to identify studies on sex or gender differences in the diagnosis and treatment of patients with familial hypercholesterolemia. The full MEDLINE strategy (**Table 12)** was applied to all databases, with modifications to search terms as necessary. No language limits were applied. Search strategies were peer-reviewed by two librarians. The full search strategy for each database is available in the **APPENDIX**.

Searches were also completed of clinical trial registries including: clinicaltrials.gov, International Clinical Trials Registry Platform , UK Clinical Trials Gateway and ProQuest Dissertations and Theses. Google Scholar and Open Grey were also searched. The full search strategy for each of these searches is available in the **APPENDIX**.

The Medline strategy was recently rerun, and 328 relevant studies were found. These studies will undergo two rounds of screening using the same protocols as in the initial screening of records. Further studies will be identified in Scopus by carrying out citation searches for the reference lists of included studies.

Table 12. Medline (Ovid) Search Strategy (July 21, 2020)

#	Searches	Results
1	Hyperlipoproteinemia Type II/	6707
2	Hypercholesterolemia/ or Hyperlipidemia, Familial Combined/ or Hyperlipidemias/ge [Genetics]	28853
3	limit 2 to yr="1966 - 1979"	3659
4	((familia* or type* 2 or type* 2s or type* ii or type iis or type* iia* or type* iib* or essential* or autosomal dominant or genetic*) adj3 (hypercholesterolemi* or hypercholesterolaemi* or hyperlipoproteinemi* or hyperlipoproteinaemi* or hyper-cholesterolemi* or hyper-cholesterolaemi* or hyper-lipoproteinemi* or hyper-lipoproteinaemi* or apolipoprotein-b* or dyslipidemi*)).tw,kf.	10219
5	(HoFH or HFH or HzFH or HeFH or HHF).tw,kf.	1057
6	((extreme* or rare* or severe* or homozyg* or homo-zygo* or heterozygo* or hetero-zygo*) adj3 (hypercholesterolemi* or hypercholesterolaemi* or hyperlipoproteinemi* or hyperlipoproteinaemi* or hyper-cholesterolemi* or hyper-cholesterolaemi* or hyper-lipoproteinemi* or hyper-lipoproteinaemi* or apolipoprotein-b*)).tw,kf.	3249
7	(hyperbetalipoproteinemi* or hyperbetalipoproteinaemi* or hyper-beta-lipoproteinemi* or hyper-beta-lipoproteinaemi* or ((lipoproteinemi* or lipoproteinaemi*) adj3 (hyper-low* or hyper-beta* or hyperlow* or hyperbeta*)) or ldl receptor disorder*).tw,kf.	154
8	lipoid gout*.tw,kf.	4
9	(tendon* adj2 (xanthoma* or xanthogranulomatos*)).tw,kf.	404
10	((heterozygo* or hetero-zygo*) adj2 FH).tw,kf.	607
11	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10	39819
12	Sex Characteristics/	54164
13	Sex/	7649
14	Sex ratio/	9234
15	Sex Factors/	263493
16	((sex* or gender* or man or men or male* or woman or women or female*) adj3 (difference* or different or characteristic* or ratio* or factor* or imbalanc* or issue* or both or specific* or disparit* or dependen* or gap or gaps or influenc* or discrepan* or distribut* or composition* or variability or comparison* or accept* or barrier* or perception* or perceiv* or between* or treat* or alirocumab or evolocumab or statin or atorvastatin or rosuvastatin or simvastatin or ezetimibe or ezetimib or niacin or enduracin or nicamin or nicobid or nicocap or nicolar or nicotinate or nicotinic or bile acid sequestrant or bempedoic acid or lomitapide or mipomersen or apheresis or PCSK9 inhibitor or anticholesteremic* or hypocholesteremic* or hmg-coa or hydroxymethylglutaryl or hydroxymethylglutaryl-coa or hydroxymethylglutaryl-coenzyme or (cholesterol adj2 inhibitor*))).tw,kf.	606145
17	((men or men's) adj2 women*).tw,kf.	127692
18	(gender*-related or gender*-based).tw,kf.	8281
19	or/12-18	874971

20	11 and 19	3040
Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations and Daily <1946 to July 17, 2020>		

4.1.4 Study selection and data extraction

Titles, abstracts and full texts were evaluated in triplicate by three independent reviewers (AG, II, IR). During the screening stages, disagreements between reviewers were resolved by discussion and another review of the title, abstract or full text until consensus was reached.

During screening stages, we included any English language study published in 1987 or later, with data demonstrating a sex comparison in treatment in heterozygous adults (>18 years) with FH. Studies were excluded if there was no mention of sex and/or gender or there was no data stratified by sex. Studies were included if all of the subjects or a clear subgroup of subjects were defined as FH according to one of the following criteria: (1) Molecular diagnosis due to variants of LDLR, ApoB, or PCSK9 genes; (2)Simon Broome Registry (SBR) Criteria; (3) Dutch Lipid Clinic Network (DLCN) Criteria; (4) Making Early Diagnosis to Prevent Early Death (MEDPED) Criteria; or (5) LDL-C levels in the 95th percentile for age: LDL-C > 5 mmol/L or 190 mg/dL (> 40 yrs) or LDL-C > 4.5 mmol/L or 174 mg/dL (18-39 yrs). Lastly papers were marked as having data on treatment if they reported data on one of the following sections: (1) proportion of patients taking LLT's; (2) Different LLT's taken such as statins, ezetimibe, PCKS9i; (3) type of statin taken (high or low intensity); (4) patients LDL-C reduction or LDL-C target attainment; (5) Treatment prescription, initiation, adherence, or tolerance. All detailed inclusion/exclusion criteria are presented in **Table 13.**

Once papers were screened, and the final included records list was obtained, data extraction was performed. Three reviewers independently extracted the data from studies that met the

eligibility criteria in duplicate. During the data extraction, disagreements between reviewers were resolved by discussion and another review of the full text until consensus was reached. Data extracted from studies included general study characteristics such as study authors, year of publication, country/location, recruitment setting, and duration of follow-up. Additionally, characteristics of the study population were collected such as number of participants with FH enrolled and analyzed, mean/median age, number of female and male participants, mean LDL-C, and percent of patients on LLT. Finally, we collected definitions of FH diagnosis used and study outcomes according to sex such as proportion of patients on LLT , mean LDL-C reduction, proportion of patients reaching LDL-C targets, and effect of treatment on lipid profiles (LDL-C, T.Chol, HDL-C, Tg, apoB, Lp(a)).

Table 13. Detailed inclusion/exclusion criteria for sex differences in treatment of FH

1) Full-text peer-reviewed publication?
   - Yes → include
   - No → exclude

2) English language publication?
   - Yes → include
   - No → exclude

3) Published in 1987 or later?
   - Yes → include
   - No → exclude

4) Live human subjects or study participants?
   - Yes → include
   - No → exclude

5) Is the study in Heterozygous familial hypercholesterolemia?
   - Yes → include
   - No (HoFH) → exclude
   - Can't decide → include

6) Are study participants adults (18 years or older)?
   - Yes → include
   - No → exclude
   - Can't decide → include

7) What type of study is reported in the article?
   - Cohort/registry → include
   - Other observational studies (Case Control, Cross-sectional, Survey, etc.) → include
   - Controlled clinical trial findings → include (separate)
   - Meta-analyses/systematic reviews → exclude
   - Practice/treatment guideline → exclude
   - Academic/Narrative Review, Comment, Editorial, Letter, Note, Patient Handout, Study Design Description → exclude

8)

A) Does the study answer one of the following questions?
   - Are men and women reaching target LDL-C levels?
   - Are men and women treated equally with lipid-lowering treatments?
   - Does lipid-lowering treatments affect men and women the same in a controlled clinical trial?
   - Is treatment adherence/tolerance the same in men and women?

B) Which groups are compared?
- FH Women & FH Men → Include
- FH Women with control women & FH men with control men → include
- FH women before vs after & FH men before vs after (pre-post study) → include (separate)

4.1.5 Quality assessment (future methods)

Three reviewers (II, AG, IR) will independently assess the quality of the included studies using the Effective Public Health Practice Project (EPHPP) Quality Assessment Tool for Quantitative Studies (http://www.ephpp.ca/tools.html)(124). Disagreements on ratings will be resolved through discussion until consensus, with a fourth reviewer only if raters are unable to arrive at an agreement. Data from the risk of bias assessments will be put together in a summary table where summary assessments of risk of bias both within individual studies and across all studies will be derived.

4.2 Preliminary results

The flow diagram demonstrating identification of records, each stage of screening, and reported excluded is shown in **Figure 6**. A total of 4, 981 records from databases and 237 records from register and grey literature searching were identified. Following automatic duplicate removal, 76% of records were kept. In total of 3, 979 records went through the first round of screening abstracts. After the first stage of screening titles and abstract, 415 articles did not fit exclusion criteria and were sought for retrieval. The full text papers of 394 records were found and assessed for eligibility according to our inclusion criteria. After screening full text papers, we excluded 344. Majority of papers were excluded due to missing outcomes of interest (no treatment in FH data), no sex comparisons, or due to ineligible publications such as reviews, editorials, commentaries etc. After the full-text screening round, 50 studies remain.

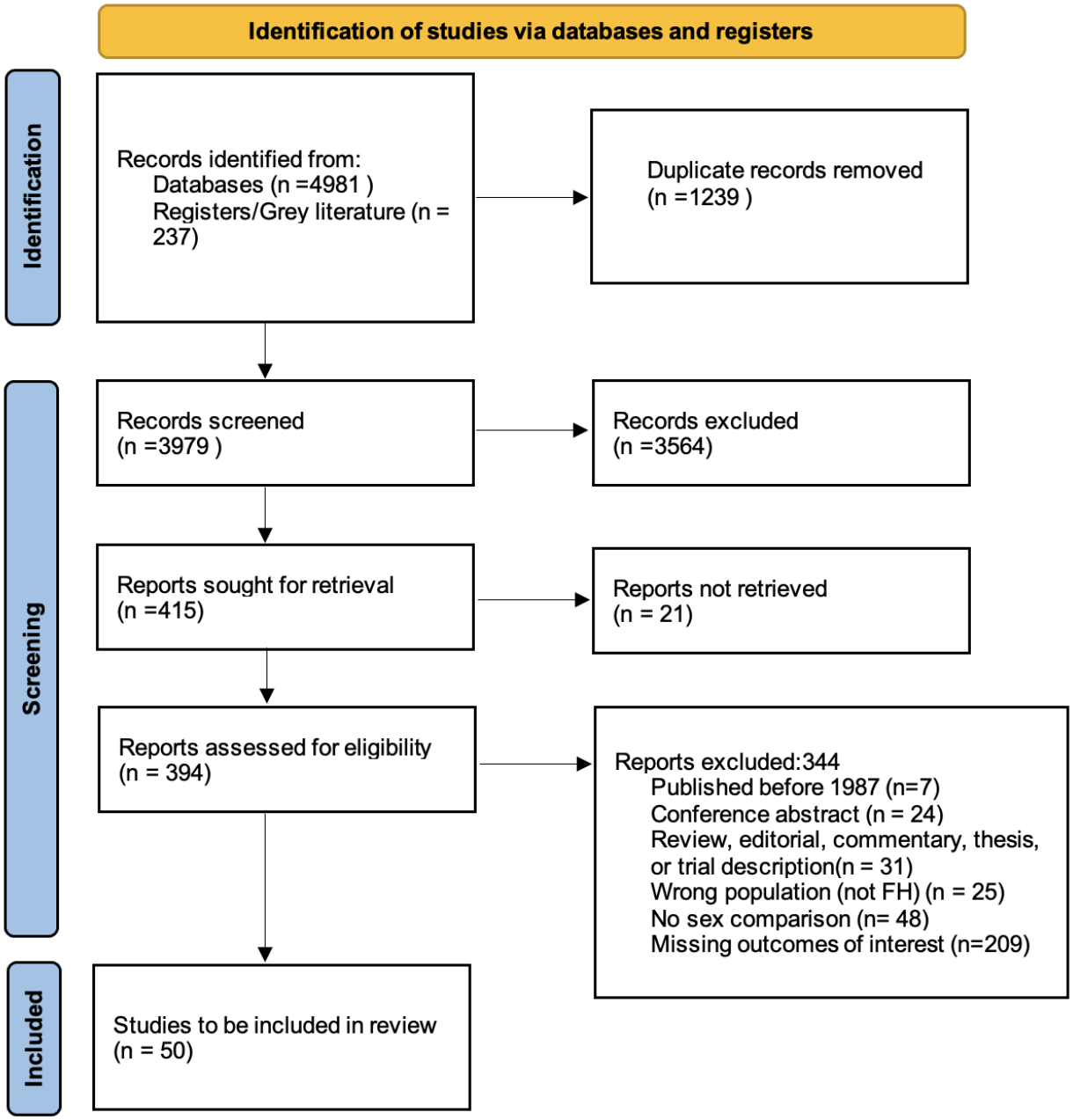

Figure 2. Flow diagram for record screening (PRISMA 2020 Flow Diagram)

Currently 50 studies remain to be included in the systematic review. Of the 50 records, 12 are clinical drug trials, 11 are registry studies, and 27 are other types of observational studies including cohort studies, cross-sectional studies, or case-control studies. A sample table of 10 randomly selected included papers with their respective study characteristics are presented in **Table 14**. The final count of included papers is to be confirmed as an updated search of the databases and registers using the original search strategy will be performed to check for newly published studies. Additionally, the quality/bias assessment still needs to be done for each study, which may result in fewer included records.

Table 14. Sample table of characteristics of studies included in systematic review of sex differences of treatment in heterozygous FH

First Author	Year	Title	Study Design	Location	Recruitment Setting	Diagnostic Method	Sample Number	Women	Men	Mean Age
Nestruck	1987	Apolipoprotein E polymorphism and Plasma Cholesterol Response to Probucol	Retrospective cross-sectional study	Canada	Lipid clinic	DLCN	50	30	20	44 ± 11
Pérez García	2018	Familial hypercholesterolemia: Experience in the Lipid Clinic of Alava	Retrospective, study	Spain	Lipid Clinic at Hospital Universitario Araba	Genetic	133	66	67	45 ± 16
Pérez-Calahorra	2016	Value of the Definition of Severe Familial Hypercholesterolemia for Stratification of Heterozygous Patients	Registry study	Spain	Dyslipidemia Registry of 50 lipid units in Spain	DLCN	1,732	881	851	52± 20
Raal	2015	PCSK9 inhibition with evolocumab (AMG 145) in heterozygous familial hypercholesterolemia (RUTHERFORD-2): a randomised, double-blind, placebo-controlled trial	Randomized, double-blind, placebo-controlled clinical trial	Global	Rutherford-2 protocol, in participating clinics worldwide	SB	331	139	192	51 ± 13
Razek	2018	Attainment of Recommended Lipid Targets in Patients With Familial Hypercholesterolemia: Real-World Experience With PCSK9 Inhibitors	Registry study	Canada	Canadian BC Registry	DLCN	275	154	121	Not reported

Rodriguez	2018	Frequency of Statin Use in Patients With Low-Density Lipoprotein Cholesterol above 190 mg/dl from the Veterans Affairs Health System	Retrospective cohort study	United States	Council of Teaching Hospitals, outpatients	LDL-C	63,576	9,536	54,040	55 ± 13
Smilde	2000	The effect of cholesterol lowering on carotid and femoral artery wall stiffness and thickness in patients with familial hypercholesterolaemia	Clinical Trial	The Netherlands	One hospital	DLCN	45	29	16	46 ± 10
Waluś-Miarka	2017	Carotid artery plaques – Are risk factors the same in men and women with familial hypercholesterolemia?	Prospective cohort study	Poland	Outpatient Lipid Clinic University Hospital, Krakow	SB	154	91	63	Not reported
Zamora	2017	Familial hypercholesterolemia in a European Mediterranean population—Prevalence and clinical data from 2.5 million primary care patients	Registry study	Spain	Catalan Institute of Health	LDL-C	14,699	7,952	6,747	61 ± 15
Zhao	2019	Genetic Determinants of Myocardial Infarction Risk in Familial Hypercholesterolemia	Bidirectional cohort study	Canada	Lipid Clinic at University Hospital, London Health Sciences Center	Canadian Definition, Genetic.	182	102	80	No reported

DLCN, Dutch Lipid Clinics Network; LDL-C, Low-density lipoprotein cholesterol; SB, Simon Broome

4.3 Plan of analysis:

Our preliminary plan of analysis includes five sections. The first section will pool together data from clinical drug trials to see if lipid lowering treatments such as statins, ezetimibe, or PCSK9i's have the same effect on lipids in men and women. The rest of the analyses will be performed on all the registry studies and other observational studies pooled together. Clinical trial studies and other studies need to be analyzed separately due to the core nature of their design. Since clinical trials often have very strict inclusion and exclusion criteria, they often exclude certain patient populations. Additionally, men and women are often matched for age or for baseline LDL-C. This results in data that is not comparable to other observational study data.

<u>The analyses include:</u>

1) Clinical Trials:

- Comparing the effect of lipid-lowering medications on LDL-C reduction in men and women: Statins, ezetimibe, PCSK9is.

2) **Registry/Observational studies:**

2.1) Sex comparisons of untreated lipid profile: LDL-C, T.Chol, HDL-C, apoB, Lp(a), Tg.

2.2) Sex comparisons of proportion of patients on any LLT.

2.3) Sex comparisons of type of LLT use: statins, ezetimibe,PCKS9i, combinations, high intensity statins, low intensity statins.

2.4) Sex comparison of change/reduction of LDL-C: % reduction of LDL-C, LDL-C target attainment (<1.8mmol/L, <2.0 mmol/L, <2.5 mmol/L, ≥ 50% reduction).

- Based on guideline recommended target LDL-C levels for FH patients(83).

2.5) Possible reasons for sex differences in treatment: sex differences in treatment initiation, adherence, tolerance, or side effects, and barriers or enablers to treatment.

5. DISCUSSION

5.1 Genetic testing study

In our FH cohort, 74% of total patients tested and 76% of patients initially classified as "probable FH" or "definite FH", were found to have a genetic variant known to cause FH **(Table 4)**, well in keeping with data showing that ~20% of patients with a presumed diagnosis of FH may have a polygenic form of the disorder (125). Compared to index patients, individuals identified through cascade screening were diagnosed 11 years younger and presented with less cardiovascular risk factors at registration. In addition, most of the variants identified in our cohort were in the *LDLR*, *APOB*, and *PCSK9* genes, half of which were not covered by the MSSS' genetic panels. Remarkably, our genetic testing protocol allowed for a majority of patients clinically diagnosed as "probable FH" to be re-classified as "definite FH".

It is well known that FH is underdiagnosed and thus, undertreated, with less than 15% of cases diagnosed in Canada (1). In fact, it is estimated that in Québec more than 27,600 to 34,400 individuals have FH, and data from the FH Canada Registry has revealed that less than 10% of these patients have thus far been identified. In order to further facilitate diagnosis, the Canadian FH definition was recently implemented based on simplified clinical criteria or genetic testing for variants in one of the 3 aforementioned genes, with subsequent cascade screening to identify affected relatives effectively (7). Previous studies have found that genetic screening is highly effective in identifying patients and improving follow-up rates (1, 10, 126), and is considered the gold standard for the diagnosis of FH (100). The clinically certified genetic panels from the Québec MSSS only covers screening of 11 variants in *LDLR* commonly identified in French Canadians due to the presence of a founder effect. However, in our FH cohort, less than half of patients with

a positive genetic result had variants listed in these panels **(Table 4**). In fact, only 33% of patients with a positive genetic test were found to have the *LDLR* 15Kb del, known as the "French Canadian" variant, less frequent than previously described (127). The search for variants only in the *LDLR* gene is not enough considering that 18% of variants identified were on the *APOB* and *PCSK9* genes (**Table 4**). Therefore, widening the scope of genetic screening offered by Québec's health services across the province has potential to increase the identification of genetic variants in FH patients. Improving identification of genetic variants in patients with FH will also allow for a better assessment of CVD risk in these individuals, and thus a potential change in treatment, even in patients already classified as "definite FH". Previous studies have found that regardless of LDL-C levels, patients with a confirmed pathogenetic FH variant have an elevated risk of CAD (9). In fact, loss-of-function variants were found to be associated with a 2-fold higher CAD risk than hypomorphic variants (10). Therefore, it is essential that patients with a presumptive clinical diagnosis of FH undergo a complete, unbiased genetic screening for FH in *LDLR*, *PCSK9* and *APOB* genes.

Where genetic screening may be most useful is in cases where the clinical diagnosis of "probable FH" or "severe hypercholesterolemia" is made. Often times a patient's clinical diagnosis can be incomplete due to the absence of specific diagnostic criteria such as untreated LDL-C, or family history of CVD, dyslipidemia or xanthomas (7). Our genetic screening protocol allowed for a significant improvement in identification and re-classification of FH patients, with one third of "severe hypercholesteremia" patients and majority of "probable FH" patients being re-classified as "definite FH" (**Table 7**). This improved diagnosis may improve quality of care, improve compliance, encourage cascade screening, and facilitate access to new drugs (e.g., PCSK9is,

evolocumab or alirocumab (REPATHA® or PRALUENT®)) for which the high cost justifies limited use within the province.

Several studies have demonstrated that targeted family cascade screening with DNA analysis is highly effective in identifying patients with hypercholesterolemia (126, 128). In our cohort, patients identified through cascade screening were diagnosed significantly earlier than index patients and consequently presented at the clinic in a healthier state. Compared to index patients, cascade screening patients presented with less hypertension and CAD, along with lower baseline untreated triglyceride levels (**Table 2**). These findings coincide with previous studies that state the importance of an early diagnosis of FH for normal life expectancy (100). Studies have shown that once identified through cascade screening, most affected patients seek treatment and are successfully started on cholesterol-lowering therapies to lower their risk of premature CVD early (126). Ideally, cascade screening should be systematic, co-ordinated in specialized centres, and patients should be offered genetic counseling and long-term follow-up (1).

Results from the genetic study also revealed some compelling sex differences of our FH patients. By presenting our patient characteristics according to sex, we found that women were diagnosed roughly 6 years later than men **(Table 3**). Similarly, a study by Amrock et al, reported that FH women in the CASCADE-FH patient registry were diagnosed 7 years later than men (129). Thus, it appears that women are consistently diagnosed many years later than men. This can drastically impact women's cardiovascular risk, as it's been shown that the earlier FH patients are diagnosed and treated, the better the outcomes(1, 85). Accordingly, within our FH cohort we also found that women had higher LDL-C and ApoB levels at registration compared to men. Therefore, further investigation looking into delayed diagnosis of FH in women and potential causes of this disparity should be considered. The finding of these sex differences in our FH cohort stresses the

importance of reported data separated by sex, which has often been lacking in clinical studies historically. Only more recently have many research organizations been working to promote the inclusion of sex and gender components within health research.

The new genetic testing for FH presented in our study is accessible across Canada through the FH Canada Registry network (www.FHCanada.net). Since the creation of the FH Canada Registry in 2014, participants of the FH Canada network have worked together to publish new guidelines for the treatment of FH in Canada (102, 130), a new validated definition of FH (7), and created clinical tools for accurate diagnosis (7). Despite these efforts, the need to improve access to genetic testing for FH still exists. Throughout all of Canada, full genetic screening is mostly accessible through research testing and is still not regularly available as part of clinical care which may play a part in low diagnosis rates(8). Therefore, even more efforts are needed to improve the accessibility of genetic sequencing to allow for majority of physicians to use the gold standard method of diagnosis.

Our study of genetic testing in Quebec is not without some limitations. The molecular diagnosis of FH was limited to exome sequencing of the *LDLR*, *APOB* and *PCSK9* genes and acknowledge that rare variants in *APOE* (e.g., p.Leu167del), LDL-R adapter protein 1 (*LDLRAP1*), lysosomal acid lipase (*LIPA*), and the ATP binding cassette G5 and G8 (*ABCG5, ABCG8*) that can cause a phenocopy of FH, will be missed. Additionally, our genetic sequencing technique does not cover deep intronic portions of the *LDLR*, *APOB*, or *PSK9* genes, thus these intronic variants will be missed. Secondly, it is known that some patients with elevated LDL-C do not have a monogenic variant in the genes known to cause FH, but rather, exhibit a cumulative sum of genetic polymorphisms in different genes increasing LDL-C in Mendelian randomization studies (125). Based on this, a "genetic LDL score" has been derived in order to distinguish

patients with a polygenic form of FH (125). While our study did not focus on obtaining an LDL-C score, our findings are consistent with data showing that 20% of patients with a presumed diagnosis of FH may have a polygenic form of the disease. Lastly, our results are limited to a single-centre cohort from the McGill University Health Centre, and therefore needs subsequent validation in other centres or multicentre cohorts as well.

5.2 Preliminary results presented in the Transition

Through our preliminary analysis of sex differences of FH patients at the McGill University Health Centre, we revealed more important sex differences in our FH cohort, particularly in regard to treatment. Within our FH cohort, we found that only 35% of women were on high-intensity statins compared to 74% of men and that 40% of women reported statin intolerance compared to just 20% of men (**Table 10**). Additionally, we found that only 32% of women were reaching a target LDL-C level of ≤ 2.5 mmol/L compared to 55% of men. Other LDL-C targets were also less achieved by women compared to men (**Table 11**). Therefore, we revealed a clear sex disparity in the treatment of FH in favour of men present. Women were diagnosed later, treated less aggressively, and do not reach target LDL-C levels as much as men. All of which warrant further investigation.

Our finding of less intensive treatment in FH women has similarly been reported by another FH Canada member Dr. Brunham and his student Ryzhaya in British Columbia. Through use of data from the BC FH Registry, their study reports that women were less likely to be prescribed high-potency statins (45.0% vs. 55.0%, P=0.03) or ezetimibe (41.0% vs. 51.5%, P=0.02) compared to men, quite comparable to our results (121). Additionally, a CASCADE-FH registry study reported that women were less likely than men to receive a high-intensity statin (OR, 0.60,

95% CI, 0.49-0.72) or even any statin therapy (OR, 0.60, 95% CI, 0.5-0.73), further confirming our finding (129). Our analysis also found that less women were reaching guideline-recommended LDL-C targets. This was also found in the Ryzhaya study where they reported a lower reduction of LDL-C and apoB in women compared to men and that less women reached an LDL-C <2.00 mmol/L (16.4% vs. 33.3%, *P*<0.001) (121). The CASCADE-FH registry study also showed that women had reduced odds of attaining LDL-C targets, further supporting our preliminary findings (129). Importantly, a recent publication from the European Atherosclerosis (EAS) Familial Hypercholesterolemia Studies Collaboration (FHSC) initiative, reported comparable data of women being less likely to a achieve LDL-C thresholds. FH women were less likely to attain LDL-C levels of <1.8mmol/L (OR, 0.63, 95% CI, 0.48-0.82) and <1.4 mmol/L (OR, 0.65, 95% CI, 0.44-0.96), including an adjustment for age, baseline comorbidities, and index case status (99). Thus, all of these findings are significant and merit further investigation.

Our preliminary sex differences registry analysis of FH patients at the McGill University Health Centre does have some limitations. Firstly, our analysis did not go into rigorous statistical detail to investigate potential confounders present in our cohort that may affect results. For example, we did not adjust for patients' prior cardiovascular events and thus primary vs. secondary status. We acknowledge that this could possibly contribute to treatment intensity and target LDL-C attainment differences observed, and future association analyses are needed to investigate further. Additionally, sex differences were not adjusted for the proportion of women within child-bearing age which could have an influence on results, as women who are pregnant or planning to conceive are advised not to take any lipid-lowering medications to protect the health of the fetus. Also of note, results indicated that more women reported statin intolerance compared to men, and thus may serve as a partial explanation for reduced use of high-intensity statins among women.

Lastly, the analysis was a single centre study with a limited cohort size, therefore confirming our findings within other larger cohorts is necessary.

Importantly, although in our FH cohort more women than men reported statin intolerance, which may account for less intensive statin use among women compared to men. This does not account for our finding of less women taking Ezetimibe or PCSK9i numerically compared to men, which has been demonstrated in other studies as well. The study by Vellejo-Vaz et al reported that Ezetimibe was used among 23.4% of FH women compared to 25.9% of FH men (P<0.0001) and PCSK9i was used among only 2.5% of women compared to 3.5% of men (P=0.0002)(99). If other studies report high statin-intolerance in women, than one may expect the use of other lipid lowering medications to be higher in women accordingly, however this is not the case. Perhaps one reason for such limited PCSK9i use among all FH patients, but especially FH women is the number of obstacles currently in place to prevent wide-spread use of the costly new medication. Although, one may argue that if women are more susceptible to statin-intolerance and are left to no choice but to take lower potency statins leaving them at increased risk, PCSK9is should be considered rapidly. Ultimately, the incidence of statin-intolerance among men and women should continue to be investigated.

The question of how to treat women who are attempting to conceive or are already pregnant who are hypercholesterolemic is a grave one. For many female patients, the inability to treat their dangerously high level of cholesterol while childbearing means they may go untreated for multiple years at a time, increasing their risk excessively. Currently, it is believed that since a healthy pregnancy is characterized by physiological hyperlipidemia, that taking lipid-lowering medications, such as statins, is considered an alteration of maternal metabolism. Therefore, it is thought that this has the ability to impair early fetal development(131). The safety of statins during

pregnancy needs to be investigated further, to truly understand the risk, as currently it is not concretely known how they affect fetal development(132). Additionally, further efforts to develop lipid-lowering medications that are safe to take during pregnancy, if at all possible, would drastically improve the quality of care of FH women during these child-bearing years, and thus should be prioritized.

5.3 Systematic review

To look into the sex disparities we identified within our FH cohort further, we performed a systematic review to investigate sex-specific differences in treatment of FH. To date, we have successfully employed a database-specific, librarian designed search strategy, which initially identified 5,218 records(**Table 12**). We then generated a detailed set of inclusion/exclusion criteria to only keep English language records published in 1987 or that report a sex comparison in treatment of HeFH adults (**Table 13**). After multiple rounds of screening titles, abstracts, and full texts in duplicate, we narrowed down the records to 50 studies that fit our inclusion criteria, of which 12 are clinical drug trials and 11 are registry studies (**Figure 6)**. We also performed an in-depth data extraction to collect all relevant sex separated data for future analyses. Our current study selection and aggregated data is promising that we will be able to pool together data from studies around the world, in order to rigorously investigate sex differences in treatment between men and women in FH.

We will be able to answer many questions surrounding sex disparities in treatment of FH by comparing and contrasting results from various studies. Importantly, we would like to see whether there is a discordance between clinical trial data and what is actually observed in a real-world setting, which can often be the case. In fact, blinded randomized clinical drug trials often

have very strict inclusion and exclusion criteria, and often times men and women are matched for age or for baseline LDL-C. Studies with this design are useful when studying specific drug effects, such as determining the lipid-lowering effect of statins or PCSK9is, however may mask a difference between men and women observed in real-world settings. Based on our own preliminary data and results from our FH Canada Colleagues in BC, our hypothesis is that there is a systematic bias in the treatment of women in FH.

5.4 Future directions:

Our results demonstrate a clear benefit of a full unbiased genetic screening for FH patients for them to have a more definitive diagnosis and have improved access to effective medications. However, we must also consider how a genetic diagnosis affects a patient's perception about their own condition. Additionally, based on our preliminary analysis, we suspect that important sex differences in diagnosis, treatment, and outcomes within FH may exist, yet the reasons behind these disparities remain unclear. Many have suggested that the less than optimal care that women receive may be due to lack of physician knowledge in regards to women's health(111, 112). Another aspect that may contribute could be implicit biases engrained in physicians. As well, existing patient biases and perceptions towards their condition or medication use. Therefore, we have begun to distribute an online FH patient questionnaire to identify the role of sex and gender in perception of disease (FH), medication tolerance and adherence, and quality of treatment. It will evaluate their perception of disease, impact of pharmacological treatment on health, and statin use. We will distribute the questionnaire to 400 patients across Canada, making it the largest FH patient questionnaire in the country. Using the results of the survey, we will look at sex- and gender-based differences in perceptions, based on the 5 point-Likert scale of 6 themes (risk assessment; perceived personal control of health; disease identity; family influence; informed decision-making;

and incorporating treatment into daily life). Additionally, we will analyze how a genetic diagnosis impacts a patient's perception of their diagnosis and disease. Through these analyses we hope to reveal some insight for observed sex differences in treatment of FH and determine how a genetic diagnosis affects a patient's perceptions of their own diagnosis.

6. CONCLUSION

In summary, our results suggest that a sequencing of *LDLR*, *APOB*, and *PCSK9* genes in patients suspected of having FH provides diagnostic certainty and valuable diagnostic re-classification. Ultimately, genetic diagnosis would allow for improved cascade screening of family members. These results also have implications on health policies, such as the use of genetic panels offered by Québec's MSSS. As such, a full unbiased genetic screening will allow for increased identification of FH patients and help reduce the burden of CVD and death in Canadians with FH. Additionally, our preliminary analysis of sex differences in treatment and lipid level target achievement revealed a clear sex disparity in FH care, in accordance with other recent studies. The preliminary results of our systematic review confirm we will be able to investigate these disparities in care between men and women in rigorous detail to produce a thorough review of available literature. Investigating these important imbalances in care will allow us to further improve quality of patient care, treatment, and outcomes of FH for whom it is lacking.

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APPENDIX

Table A1. MEDPED Criteria for Diagnosis of Probable FH

Total Cholesterol and (low-density lipoprotein cholesterol) Criteria for Diagnosis of Probable FH				
	Degree of Relatedness to Closes FH Relative			
Age Group	**First**	**Second**	**Third**	**General Population**
Age < 18	220 (155)	230 (165)	240 (170)	270 (200)
Age 20	240 (170)	250 (180)	260 (185)	290 (22)
Age 30	270 (190)	280 (200)	290 (210)	340 (240)
Age 40+	290 (205)	300 (215)	310 (225)	360 (260)
Adapted from reference (6). Total cholesterol and low-density lipoprotein cholesterol levels in mg/dL, expected to diagnose heterozygous FH with 98% specificity. FH= familial hypercholesterolemia, First= parents, offspring, brothers, and sisters; Second = aunts, uncles, grandparents, nieces, nephews; Third = first cousins, siblings or grandparents				

Table A1. 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 over 55 yr, women over 60 yr)	**1 point**
or	
• First-degree relative known with LDL-C > 95th percentile	
• First-degree relative with tendon xanthomata and/or arcus cornealis	**2 points**
or	
• Children under 18 yr with LDL-C > 95th percentile	
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**
• Arcus cornealis 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**

Per group, only one score, the highest applicable can be chosen.

Adapted from Reference (5) : World Health Organization. Familial Hypercholesterolemia – Report of a Second WHO Consultation. Geneva, Switzerland 1999.

Table A3. Embase (Ovid) Search Strategy (July 21, 2020)

#	Searches	Results
1	familial hypercholesterolemia/	9624
2	((familia* or type* 2 or type* 2s or type* ii or type iis or type* iia* or type* iib* or essential* or autosomal dominant or genetic*) adj3 (hypercholesterolemi* or hypercholesterolaemi* or hyperlipoproteinemi* or hyperlipoproteinaemi* or hyper-cholesterolemi* or hyper-cholesterolaemi* or hyper-lipoproteinemi* or hyper-lipoproteinaemi* or apolipoprotein-b* or dyslipidemi*)).tw,kw.	14268
3	(HoFH or HFH or HzFH or HeFH or HHF).tw,kw.	1943
4	((extreme* or rare* or severe* or homozyg* or homo-zygo* or heterozygo* or hetero-zygo*) adj3 (hypercholesterolemi* or hypercholesterolaemi* or hyperlipoproteinemi* or hyperlipoproteinaemi* or hyper-cholesterolemi* or hyper-cholesterolaemi* or hyper-lipoproteinemi* or hyper-lipoproteinaemi* or apolipoprotein-b*)).tw,kw.	4286
5	lipoid gout*.tw,kw.	0
6	(tendon* adj2 (xanthoma* or xanthogranulomatos*)).tw,kw.	604
7	((heterozygo* or hetero-zygo*) adj2 FH).tw,kw.	865
8	1 or 2 or 3 or 4 or 5 or 6 or 7	18974
9	exp sexual characteristics/	1706
10	sex/ or sex differentiation/	43123
11	sex ratio/	70111
12	sex factor/	7412
13	((sex* or gender* or man or men or male* or woman or women or female*) adj3 (difference* or different or characteristic* or ratio* or factor* or imbalanc* or issue* or both or specific* or disparit* or dependen* or gap or gaps or influenc* or discrepan* or distribut* or composition* or variability or comparison* or accept* or barrier* or perception* or perceiv* or between* or treat* or alirocumab or evolocumab or statin or	832945

	atorvastatin or rosuvastatin or simvastatin or ezetimibe or ezetimib or niacin or enduracin or nicamin or nicobid or nicocap or nicolar or nicotinate or nicotinic or bile acid sequestrant or bempedoic acid or lomitapide or mipomersen or apheresis or PCSK9 inhibitor or anticholesteremic* or hypocholesteremic* or hmg-coa or hydroxymethylglutaryl or hydroxymethylglutaryl-coa or hydroxymethylglutaryl-coenzyme or (cholesterol adj2 inhibitor*))).tw,kw.	
14	((men or men's) adj2 women*).tw,kw.	172190
15	(gender*-related or gender*-based).tw,kw.	10902
16	9 or 10 or 11 or 12 or 13 or 14 or 15	1000931
17	8 and 16	994
18	("31652114" or "32536129" or "32405159" or "32284160" or "31914797" or "32299761" or "32671484" or "32128483" or "30563368" or "31840162" or "31674218" or "31235401" or "32576364" or "32592555" or "32616509" or "32655404" or "31868931" or "30549443" or "30957178" or "30974472" or "30567480" or "31877157" or "30986362" or "31280039" or "31153370" or "31870448" or "31696945" or "30683281" or "31878353" or "31523650" or "31733217" or "31048275" or "30953107" or "30573307" or "31336553" or "31380502" or "30609272" or "31617858" or "31617858" or "30649025" or "31378630" or "31758975" or "30993573" or "31307727" or "31847331" or "31596376" or "31618540" or "31708406" or "32237625" or "31133496" or "31092766" or "30129670" or "31409451" or "31003151" or "31818452" or "30380048" or "31860991" or "29569117" or "29852873" or "31483296" or "30652328" or "31196897" or "31171318" or "30442422" or "30934611" or "30794474" or "30653535" or "30937890" or "30004840" or "30786876" or "32245299" or "31293133" or "29124388" or "30755550" or "31197927" or "30720747" or "31555747" or "31229021" or "30640363" or "30460370" or "31006811" or "30753598" or "31130525" or "31130525" or "30732662" or "30527766" or "30158971" or "29459263" or "30172432" or "29863817" or "29500790" or "29149237" or "29973570" or "30253291" or "30371190" or "29137927" or "29795368" or "30433876" or "29447778" or "30055622" or "29969922" or "30374235" or "30156607" or "29321515" or "27738812" or "30055758" or "29566018" or "29576254" or "29178257" or "29980385" or "29407882" or "30260983" or "30270087" or "29789037" or "29622598" or "30039844" or "30203672" or "30021394" or "29983553" or "29458704" or "29788966" or "29534385" or "29630642" or "29315217" or "30270070" or "30276217" or "29703506" or "29673349" or "29174032" or "29331793" or "30259501" or "29154680" or "29512172" or "30026278" or "29716848" or "29751283" or "30580708" or "29609857" or "30012291" or "30270058" or "29800656" or "29461386" or "29321397" or "30005909" or "29386415" or "29453308" or "29402233" or "29726288" or "29377473" or "29247152" or "29459468" or "30007775" or "29791657" or "30625075" or "29229197" or "29412322" or "29871648" or "28600126" or "27794107" or "28826564" or "30375557" or "26686841" or "28663044" or "28685504" or "28637586" or "29109861" or "28321063" or "28878106" or "28153993" or "29264879" or "29473516" or "28480675" or "27822850" or "27920219" or "28946037" or "28156256" or "29286319" or "28762908" or "29169169" or "28595498" or "28081939" or "28941610" or "28990939" or "28532484" or "27811231" or "28559401" or "28652530" or "28225859" or "28158877" or "28038989" or "28356271" or "28419274" or "28062275" or "28461020" or "28906356" or "27932355" or "27923207" or "28110940" or "28458923" or "28882818" or "28515188" or "28078997" or "28738470" or "28460769" or "28379035" or "29146640" or "28992466" or "29050004" or "27885059" or "28825891" or "28457799" or "28759039" or "28430795" or "28241992" or "28499375" or "27853041" or "28376458" or "28314542" or "28445539" or "27426616" or "28526006" or "28776358" or "28699974" or "28434814" or "28615375" or "28623742" or "28697983" or "28554703" or "29080546" or "27697065" or "28449836" or "28532431" or "26896267" or "27556703" or "27655594" or "26539650" or "27916084" or "27208892" or "26970227" or "26555221" or "27294152" or "26875131" or "26670865" or "27048383" or "26957235" or "26302681" or "26999119" or "27538393" or "27100157" or "26589821" or "26889924" or "26506085" or "26806045" or "27062405" or "27019267" or "27444203" or "26521021" or "27452484" or "27119267" or "27664850" or "27558979" or "27127013" or "27125949" or "26387811").pm.	233
19	("27486898" or "27559550" or "27532919" or "27559556" or "27206944" or "27307718" or "26466344" or "27678432" or "26586781" or "26810159" or "27678436" or "27595674" or "29537209" or "26807712" or "26932978" or "27225142" or "27021286" or "27525363" or "26910623" or "27221952" or "27316775" or "27389632" or "26669922" or "26975627" or "27429668" or "28197499" or "27919349" or "26976914" or "27543802" or "27998915" or "27098076" or "26795925" or "26820799" or "27671328" or "26733115" or "27462052" or "27698541" or "27055902" or "26608823" or "25810065" or "27070077" or "27620358" or "26900131" or "27919363" or "26577223" or "26496982" or "26892126" or "26589291" or "25580863" or "26652826" or "25799502" or "26227068" or "25649483" or "26704520" or "26520897" or "26255620" or "25514902" or "26458977" or "25891209" or "26474596" or "26178021" or "26714540" or "26195677" or "25857271" or "26178535" or "25904591" or "25681607" or "26109505" or "25809853" or "25274942" or "26516874" or "26437530" or "26121190" or "25532994" or "26135220" or "25935113" or "25944971" or "25572014" or "26411937" or "26623014" or "25978320" or "25819605" or "26788101" or "26633047" or "26656346" or "26402926" or "26228672" or "25670360" or "26436287" or "26044581" or "26112012" or "26116691" or "25034455" or "24882148" or "25931040" or "26146893" or "25658659" or "26011609" or "25953004" or "25815614" or "26066290" or "26161751" or "25273856" or "26203098" or "25936320" or "25952300" or "25687463" or "26687704" or "26423083" or "25559341" or "26603088" or "26112890" or "26027631" or "26135349" or "25936329" or "26564631" or "25862452" or "26648432" or "25856966" or "26319815" or "25997074" or "25787240" or "26160405" or "26297334" or "25906268" or "25952471" or "26079405" or "26047876" or "25443400" or "25716968" or "26174092" or "25330368" or "25989805" or "25760954" or "25907492" or "25905475" or "24681217" or "24831481" or "25126774" or "25333326" or "24630267" or "24906846" or "24611005" or "25623096" or "25216757" or "24085382" or "25120083" or "25264877" or "25156893" or "25709121" or "25516229" or "24531085" or "25195188" or "25122648" or "24430948" or "24993977" or "24645848" or "25152081" or "25468658" or "24936728" or "24287284" or "25074400" or "24572249" or "24652812" or "25139168" or "26316666" or "24192652" or "25056604" or "25347198" or "24350931" or "25082159" or "24742812" or "25034080" or "25163226" or "24600594" or "25190228" or "24800666" or "24504152" or "25536410" or "24781821" or "24668570" or "24327522" or "24676369" or "24449092" or "24476220" or "22739687" or "24667004" or "26982659" or "24263182" or "25176936" or "24720534" or "24806352" or "24206823" or "24728953" or "24743011" or "25210095" or "24452112" or "25600043" or "24325864" or "24944061" or "24853155" or "25341306" or "24529147" or "24662777" or "25335544" or "25097940" or "24721317" or "24632281" or "25425215" or "24712315" or "24300152" or "24378270" or "24321022" or "24025637" or "23246328" or "23070631" or "23805252" or "23560969" or "23196351" or "23294904" or "23510561" or "23919842" or "23018766" or "23866938" or "23677857" or "22345688" or "23058720" or "23458042" or "23517282" or "24296750" or "22664309" or "24217264" or "23551672" or "23565931" or "24028260" or "24244645" or "23623012" or "23302603" or "22975714" or "23463454" or "24099726" or "23332383" or "22971964" or "23947939" or "22770541" or "23541013" or "22954159" or "22144263" or "23758630" or "23841468" or "22633791" or "23480442" or "23647398" or "23357154" or "29805870" or "24024768" or "22842974" or "23137925" or "23628306" or "23357141" or "23991608" or "23506430" or "24094079" or "23459084").pm.	227
20	("23221025" or "24314361" or "23902756" or "23933695" or "23382331" or "23623643" or "23759268" or "23642324" or "24156549" or "23594919" or "23638783" or "23157464" or "23122768" or "23671433" or "23256179" or "22647482" or "24070336" or "24007717" or "23864194" or "24238499" or "22936204" or "23985183" or "24044467" or "23375686" or "23642926" or "23835301" or "24579506" or "23071140" or "24158101" or "23919812" or "23876348" or "23254630" or "23193114" or "23162007" or "23266621" or "23346951" or "22866937" or "22243772" or "22578720" or "21821844" or "21416117" or "23228012" or "22031213" or "22481692" or "22119890" or "22785138" or "22469073" or "22626876" or "22689050" or "23140032" or "22153777" or "22148868" or "22933212" or "22701100" or "25058963" or "22727798" or "22906083" or "22468143" or "22517421" or "22100028" or "22153979" or "22170216" or "22293316" or "22717466" or "22775419" or "22856164" or "22087585" or "22504754" or "22192156" or "23336188" or "22030117" or "21641813" or "22883727" or "22390808" or "22890973" or "22893402" or "23362564" or "22017319" or "21940276" or "23108199" or "22978800" or "22071413" or "22981166" or "22732744" or "22840347" or "22483404" or "22341486" or "20645024" or "22698766" or "23264238" or "22226369" or "22672914" or "22809114" or "22471945" or "22547221" or "22819352" or "22611827" or "22261195" or "22429228" or "22699246" or "21918828" or "22533665" or "22572422" or "22799293" or "21810288" or "23045823" or "22190137" or "22221517" or "22836151" or "23815022" or "22712740" or "22908087" or "22459551" or "21640350" or "20509032" or "21332946" or "20580254" or "21354724" or "22070582" or "19939652" or "20606469" or "22580728" or "22111468" or "20739064" or "21123766" or "21605293" or "21256834" or "21452966" or "21348923" or "21821527" or "22104509" or "21273557" or "20807166" or "21199964" or "21714932" or "21376325" or "22018443" or "21815708" or "21862153" or "21274756" or "22082357" or "22128042" or "20807459" or "21297494" or "21324888" or "20546164" or "21804350" or "20171845" or "21483226" or "21791073" or "21198993" or "21513480" or "21775565" or "21372492" or "21247526" or "21640762" or "22087679" or "21557673" or "21796141" or "21486801" or "21715698" or "21807618" or "22185846" or "21311603" or "21701079" or "21728907" or "21585569" or "21488754" or "21295847" or "21356148" or "21332996" or "21407165" or "21550577" or "22157149" or "21487128" or "21687917" or "20973765" or "21625818" or "21519634" or "20837371" or "21798967" or "21764497" or "21947005" or "21257349" or "21814848" or "21670376" or "20957811" or "19927160" or "21092333" or "21248454" or "20842602" or "19995791" or "19958209" or "20036859" or "20367964" or "21154329" or "20630099" or "19834104" or "20571286" or "19895880" or "20339287" or "19217673" or "21067805" or "20002875" or "20660987" or "20305563" or "20512225" or "20561029" or "19699070" or "19505972" or "20806200" or "19954956" or "20723257" or "20409259" or "20044742" or "20965319" or "21189169" or "20733267" or "19494836" or "20965889" or "19695854" or "20534947" or "20870177" or "20060988" or "20072809" or "20347450" or "20202105" or "19851300" or "20383691" or "21122701" or "21073069" or "20859896" or "20435353" or "20045568" or "20460242" or "20142527" or "20739726" or "20881421" or "20379119" or "20634651" or "20495954" or "20726197" or "20446878" or "20006530" or "20668250" or "20144596" or "21591389" or "20435231" or "20107198" or "20081323" or "20034957" or "20679142" or "20339369" or "20550064" or "20186243" or "22338441" or "20351074" or "20236527" or "19081646" or "19995404" or "20815157" or "20377162" or "20591696" or "20660284").pm.	253

21	("19726160" or "20956490" or "20482882" or "20375102" or "19798074" or "20200337" or "20670729" or "20719839" or "20648735" or "20964105" or "20005542" or "20063111" or "20170916" or "19528346" or "18325138" or "19092705" or "19204599" or "19285607" or "18824493" or "19231603" or "19436781" or "20209716" or "19423132" or "19331683" or "19672745" or "18270526" or "25998591" or "18845302" or "19761211" or "19273871" or "20139652" or "19380898" or "19656039" or "18608549" or "19196895" or "19165168" or "20129372" or "20065615" or "19873941" or "18932049" or "19252236" or "19008872" or "19154954" or "19671400" or "19439299" or "19535749" or "19114224" or "19493634" or "19627212" or "19247187" or "19398913" or "19254121" or "19827646" or "19827648" or "19219403" or "19209184" or "20172409" or "19096366" or "19253714" or "18479686" or "19286092" or "20514896" or "19835569" or "19286194" or "19605566" or "19026412" or "20131928" or "19393847" or "19469439" or "19380709" or "19443470" or "19702122" or "19017774" or "20020602" or "19429836" or "19922832" or "20183988" or "19273719" or "19552291" or "19725447" or "19675201" or "18715284" or "19159492" or "19402225" or "19070521" or "19122174" or "19537224" or "19476583" or "20583681" or "19628659" or "19520994" or "24683220" or "19331437" or "19825213" or "19558660" or "19251275" or "19399742" or "18649056" or "18372576" or "18472352" or "18379204" or "18461008" or "18799983" or "19186337" or "18728169" or "17714881" or "18258533" or "19009746" or "17714716" or "18240193" or "18689364" or "18460478" or "19167588" or "18601600" or "18838533" or "18317846" or "19301716" or "18469202" or "18332151" or "17399968" or "18782143" or "18502227" or "18609063" or "18054295" or "18173546" or "18727282" or "21291723" or "18654948" or "18164018" or "18385045" or "19169496" or "18481232" or "18714150" or "18446311" or "18377643" or "18616850" or "18388034" or "18191050" or "18670365" or "18385536" or "19166165" or "18974708" or "18926611" or "17901467" or "18719340" or "18442505" or "18172039" or "18309236" or "18426997" or "18806522" or "18219179" or "18397584" or "18274806" or "18070819" or "19064527" or "17981917" or "19000490" or "18681780" or "18714375" or "18277138" or "18684751" or "18506369" or "18241302" or "18762168" or "18548846" or "18346648" or "18495951" or "19161079" or "18376349" or "18700439" or "18309111" or "18806630" or "18302312" or "18378244" or "18479282" or "18826453" or "18356332" or "18791341" or "18603596" or "18700238" or "18727833" or "18564121" or "18350202" or "18223624" or "18334776" or "19157268" or "18684164" or "18364261" or "18718484" or "18720274" or "17953518" or "18727989" or "17123536" or "18334815" or "18160469" or "19011318" or "18430270" or "18803993" or "18796541" or "18243212" or "18420951" or "18246242" or "17517690" or "17526655" or "17510467" or "17986900" or "17160750" or "17209184" or "17557999" or "17641228" or "17718788" or "17490923" or "17513208" or "17186134" or "17311944" or "17324737" or "17435857" or "17199722" or "17972053" or "17380167" or "17161236" or "16616756" or "17526978" or "17988409" or "17105840" or "17264848" or "17985810" or "17664376" or "17217555" or "17233690" or "17563556" or "17546618" or "18217459" or "17985033" or "17386092" or "17050011" or "17826698" or "18260936" or "18260968" or "17394870" or "17353585" or "17468091" or "17935245" or "18156097" or "17721029" or "17299378" or "17167279" or "17961529" or "17143557" or "18074758" or "17919399" or "17762454" or "17908002" or "17379491" or "17803342" or "18080553" or "18082090" or "23074505" or "23074505" or "17306735" or "17645871" or "17625723" or "18038584" or "17536607").pm.	261
22	("17705891" or "17884344" or "18158077" or "17673701" or "18180216" or "17431185" or "18000290" or "17719720" or "17445284" or "17123537" or "17334091" or "17215383" or "17824470" or "17911781" or "18154231" or "17996658" or "17606543" or "17341535" or "17578886" or "17705568" or "17383264" or "17889493" or "18095777" or "17576866" or "17644340" or "17081219" or "17903646" or "17227806" or "17900516" or "17629824" or "16970954" or "16540111" or "17623813" or "17903577" or "18019216" or "17407633" or "17725268" or "17124393" or "17927933" or "17366357" or "17569881" or "17317371" or "17265193" or "17996508" or "17552140" or "17275490" or "17935545" or "17143134" or "16855199" or "17016617" or "17101888" or "16648620" or "16988848" or "16465756" or "16802576" or "16549264" or "16979399" or "16716095" or "16808132" or "16356567" or "16574890" or "16722972" or "16528005" or "16873790" or "17001216" or "16443564" or "16713434" or "16858752" or "16881992" or "16009526" or "16458974" or "16516587" or "16961668" or "17085984" or "16945743" or "16799230" or "16990079" or "17027563" or "16509450" or "17784544" or "16736386" or "17173797" or "17203827" or "17191025" or "17633399" or "16368469" or "17019381" or "16709304" or "16931783" or "17144426" or "16821269" or "16864925" or "16095600" or "16703925" or "16978137" or "16704556" or "16529663" or "16532085" or "17323605" or "16882279" or "17007658" or "17130276" or "16603852" or "17109362" or "16575282" or "16631436" or "17333747" or "16433936" or "16705668" or "16849409" or "16412525" or "16910905" or "16636482" or "16728551" or "17145421" or "16556855" or "16741320" or "16721398" or "16740849" or "17046559" or "16935763" or "16964311" or "16796076" or "16860258" or "16482073" or "16860175" or "16735520" or "16464813" or "16875509" or "16990155" or "16466730" or "16269257" or "16271780" or "16510091" or "16375582" or "16639078" or "16858759" or "16246347" or "17274457" or "16293256" or "16963350" or "16783272" or "17001217" or "16879111" or "16750103" or "16520721" or "17001218" or "16719843" or "30000416" or "30000420" or "30000421" or "17179595" or "15699227" or "16123850" or "15857272" or "16389851" or "15774702" or "16285998" or "16106049" or "16030523" or "16092050" or "15333620" or "15975790" or "16157786" or "16583932" or "15986112" or "15766689" or "15891275" or "16309370" or "15920065" or "16030068" or "16555576" or "16014090" or "15656878" or "16176168" or "16009795" or "15687443" or "16144549" or "15777550" or "15836544" or "15887425" or "16154486" or "15703509" or "20527433" or "16003294" or "16602438" or "15827865" or "15798121" or "15864114" or "15769763" or "16207260" or "15955465" or "16034448" or "16093405" or "16305587" or "15309416" or "16541719" or "15703504" or "15741835" or "15826890" or "16227145" or "15846260" or "15942550" or "15910869" or "16123315" or "16020749" or "16014208" or "15691040" or "16204427" or "15988705" or "15658097" or "15746228" or "16141286" or "15935359" or "16279505" or "16036545" or "16142019" or "15984375" or "15781755" or "15508014" or "15932697" or "15749623" or "15619173" or "16220697" or "15864113" or "15830122" or "15982505" or "15536473" or "16477767" or "16141779" or "15817854" or "15547298" or "15824910" or "15776996" or "15904881" or "15674310" or "15671032" or "15823288" or "15907855" or "15777557" or "16020744" or "16041450" or "15876802" or "16407605" or "15797163" or "15990755" or "16400780" or "15857334" or "15703508" or "16602445" or "16061941" or "16344385" or "15687137" or "15514264" or "15110745" or "14746413" or "15135857" or "15375596" or "15226625" or "15199439" or "14968337" or "17516697" or "14722362" or "15055240").pm.	260
23	("15186959" or "15611671" or "15184285" or "14604830" or "15687407" or "14709677" or "15313964" or "15353416" or "15628647" or "15540545" or "15111542" or "15177701" or "15639694" or "15096336" or "14709372" or "15164320" or "15462690" or "15317046" or "15177301" or "15005509" or "15103514" or "15282667" or "15717141" or "15623679" or "15037535" or "15300004" or "15073652" or "15177127" or "15327691" or "15565928" or "15446673" or "15064110" or "15687405" or "15111372" or "15466496" or "15547022" or "15648805" or "15340676" or "15119832" or "15012019" or "15327591" or "15128051" or "15077569" or "14972073" or "14693979" or "15649295" or "15631531" or "14999031" or "14757692" or "15291694" or "15214960" or "15242063" or "15119835" or "15475176" or "15184689" or "14693778" or "15212768" or "15066061" or "15195867" or "15257723" or "15554949" or "15564707" or "14996476" or "15238453" or "15455181" or "15853118" or "15765037" or "15458693" or "15059707" or "15199349" or "15174739" or "15211364" or "15158296" or "15532130" or "15334371" or "15630635" or "15194017" or "15247960" or "15277400" or "14988449" or "15630632" or "15326068" or "14766115" or "15223989" or "15633853" or "14985690" or "15598092" or "14962437" or "14967166" or "15199031" or "15256790" or "14724558" or "15617195" or "15884199" or "15225529" or "14740240" or "15477786" or "15136072" or "15650343" or "15220952" or "15531664" or "15639688" or "15682353" or "15639697" or "14994781" or "15566253" or "14656730" or "15530458" or "12889988" or "15222635" or "14564313" or "14975168" or "14639019" or "14699858" or "12878452" or "12646733" or "12966126" or "12832311" or "15116471" or "12707402" or "12939356" or "14624049" or "12872711" or "12829221" or "12776271" or "12714863" or "12877676" or "14578611" or "14625131" or "12745196" or "14593381" or "12671581" or "14501025" or "14735020" or "12648031" or "14663597" or "12756407" or "12706470" or "14618093" or "14564085" or "12600905" or "12791613" or "14629322" or "14644397" or "12886133" or "12737782" or "14506545" or "14671811" or "12755140" or "14725205" or "12923015" or "12967690" or "12769254" or "12637115" or "12601531" or "12806724" or "14709849" or "12687649" or "12689687" or "12915428" or "12975599" or "12824094" or "15190632" or "12801604" or "12963571" or "12535745" or "14608054" or "12614415" or "12754449" or "12708250" or "12887727" or "12802899" or "12885701" or "24944394" or "12939864" or "12511745" or "14522571" or "12819993" or "12524233" or "12933534" or "14704504" or "12859032" or "14704511" or "12727230" or "15562735" or "12818409" or "12840178" or "12932630" or "14671463" or "12719276" or "14638534" or "12700893" or "14624727" or "12588950" or "14704508" or "12756561" or "12492446" or "12750110" or "12735892" or "12544724" or "12639974" or "14693301" or "12548313" or "12668913" or "14679272" or "12705620" or "12805251" or "12600847" or "12887719" or "14597936" or "12488246" or "12938249" or "12681161" or "12742284" or "12208481" or "11928067" or "11947911" or "12503475" or "12196085" or "11897443" or "12436707" or "12356004" or "11834528" or "12473547" or "12081819" or "11922642" or "12324284" or "12149041" or "12494016" or "12494221" or "12417832" or "12480773" or "12223326" or "11910049" or "12011578" or "12241128" or "12356387" or "12044587" or "12141531" or "12116743" or "12082364" or "12364448" or "11840368" or "12118856" or "12182255" or "12534449" or "11910299" or "12237025" or "12118909" or "12063444" or "12187397" or "11982953" or "12138367" or "12817199" or "12417279" or "11858478" or "12357133" or "11966345" or "11901049" or "12208171" or "12470198" or "12450888" or "11833047" or "12356389").pm.	263
24	("11923123" or "12001831" or "12473254" or "12219187" or "12073409" or "12164469" or "12366606" or "12409747" or "11985406" or "12474722" or "12052483" or "12077742" or "12126462" or "12122545" or "12029990" or "12401533" or "12218765" or "12217263" or "11782586" or "12119208" or "12165052" or "12464375" or "19667596" or "12394687" or "11790693" or "11888526" or "12045169" or "12423709" or "11821719" or "12427649" or "11834531" or "12626213" or "12027776" or "11833826" or "11900713" or "12137985" or "12435337" or "12202481" or "12046033" or "12390056" or "12195798" or "12123487" or "12419484" or "11815311" or "11879241" or "12172393" or "12048142" or "12403804" or "12087575" or "11434800" or "11324369" or "11481566" or "11711272" or "11213091" or "11571834" or "11293949" or "11248748" or "11434192" or "12063772" or "11328302" or "11325764" or "11243306" or "11253736" or "11255880" or "11181283" or "11320358" or "11213168" or "11230787" or "11412677" or "11500190" or "11794823" or "11688949" or "11412807" or "11358159" or "11933923" or "11721164" or "11928592" or "11182189" or "11338344" or "11376309" or "11730829" or "11297296" or "11556929" or "11480153" or "11900393" or "11860839" or "12439378" or "11246583" or "11501710" or "12063773" or "11257254" or "11374884" or "11498127" or "11474894" or "11427213" or "11675946" or "11678968" or "11583720" or "11230837" or "11438195" or "11324372" or "11368750" or "11520486" or "11136703" or "11136952" or "11828923" or "11791398" or "11347120" or "11570118" or "11735685" or "11772284" or "11738396" or "11507971" or "11573752" or "11304891" or "11488557" or "11383131" or "11683775" or "11586494" or "11298777" or "11773670" or "11775412" or "11483625" or "11572515" or "11379378" or "11405386" or "11383320" or "11449080" or "11704319" or "11253155" or "11582971" or "11520484" or "12596954" or "10744590" or "10949188" or "10785876" or "11082147" or "10980587" or "10729389" or "10845875" or "10909990" or "11128726" or "10911769" or "11031214" or "10947889" or "11044482" or "11252539" or "10607765" or "11798745" or "10641021" or "10849014" or "10953872" or "10729393" or "10998931" or "10781644" or "10751747" or "10962844" or	263

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25	("10406451" or "10084016" or "10551207" or "10611909" or "10636448" or "10081033" or "10024863" or "10381159" or "10447222" or "10570905" or "10487500" or "10075320" or "10208479" or "10426702" or "10372308" or "10376199" or "9927392" or "10448768" or "10368792" or "9973073" or "10199475" or "10482153" or "10606208" or "11122694" or "10580395" or "10529757" or "10439497" or "10547136" or "10565602" or "10690329" or "10395624" or "10386509" or "10493319" or "10498131" or "10735582" or "11279918" or "10584419" or "9880433" or "10522984" or "10408999" or "11122686" or "10529129" or "10475870" or "10094580" or "10322565" or "10503616" or "10572218" or "10394436" or "9920511" or "11503831" or "10500014" or "10445837" or "9578244" or "9603795" or "9642116" or "10933428" or "9893006" or "9656651" or "9819102" or "9554107" or "9647874" or "9651456" or "9688439" or "9856223" or "9756426" or "9646096" or "9484771" or "9713078" or "9766706" or "9465706" or "9619005" or "9434647" or "9519020" or "9588433" or "10085714" or "9701246" or "9805562" or "9611906" or "9424571" or "9684746" or "9800230" or "9830842" or "9709409" or "9645827" or "9598575" or "9808796" or "9699905" or "9789853" or "9882961" or "9622280" or "9596233" or "9533233" or "9457050" or "9839103" or "10335028" or "9740341" or "9531256" or "9551392" or "9465989" or "9678774" or "9711171" or "9738161" or "9861590" or "9915661" or "9845140" or "9809137" or "9504445" or "9651560" or "9571357" or "9550499" or "9580388" or "9791635" or "9497178" or "9808644" or "9622289" or "9792666" or "9799908" or "9706584" or "9424570" or "9699897" or "9714133" or "9573756" or "9484425" or "9729642" or "9688236" or "9781976" or "9614611" or "11907931" or "9571339" or "9419190" or "9082037" or "9105570" or "9262494" or "9505937" or "9117913" or "9081694" or "9409936" or "9050775" or "9303663" or "9340086" or "9358426" or "9098596" or "9211063" or "9297170" or "9183227" or "9372993" or "9476817" or "8994481" or "9174230" or "9499282" or "9192911" or "9033222" or "9103094" or "9409324" or "9476683" or "9157944" or "9110123" or "9280882" or "9252956" or "9409937" or "9043971" or "9245545" or "9101108" or "9603697" or "9183304" or "9379730" or "9048115" or "9217585" or "9267993" or "9012636" or "9354440" or "9355889" or "9189647" or "9340188" or "9411422" or "9062840" or "9409318" or "11517479" or "9011785" or "9024732" or "9091527" or "9395808" or "9358017" or "9519126" or "9108789" or "9192227" or "9426354" or "9392426" or "9178790" or "9039882" or "9217583" or "9182655" or "9271481" or "9029226" or "9217584" or "9135930" or "9377752" or "9372997" or "9100140" or "9380384" or "9334463" or "9152737" or "8970509" or "8650967" or "8974213" or "8979120" or "8696963" or "8686314" or "8839657" or "8792826" or "8847874" or "8740948" or "8807702" or "8998799" or "8880894" or "8783032" or "8678927" or "8561065" or "9006811" or "9083865" or "8903864" or "8679872" or "8784361" or "8917325" or "8664620" or "9010744" or "9015672" or "9091946" or "8724114" or "8889490" or "8843050" or "8814524" or "9003110" or "9008925" or "8851938" or "8999461" or "8635262" or "8681371" or "8729584" or "8759095" or "8722158" or "8685519" or "8620340" or "8864962" or "8974552" or "8867362" or "9302828" or "8693169" or "9395578" or "8964318" or "8992854" or "8722744" or "8762687" or "8759943" or "8768633" or "8957200" or "8774269" or "9269097" or "8734860" or "8609958" or "8572841" or "8656540" or "8721863" or "8596497" or "8824022" or "8676819" or "8683643" or "8843892" or "8769353" or "8548413" or "8902157" or "8644746" or "8726633" or "8647378" or "8929255" or "8656142" or "8677767" or "8681156" or "8949979" or "8930228" or "9225220" or "9226461" or "8787369" or "8642233" or "7503003" or "7561980" or "7652481" or "7616853" or "7604783" or "7478472" or "7614520" or "7867176" or "7752281" or "7676216" or "7491685" or "7702230" or "7585299" or "7474191" or "7627699" or "7485220").pm.	302
26	("7711988" or "7723899" or "7606845" or "7476293" or "7572747" or "7573090" or "7738190" or "8770321" or "7858682" or "8620056" or "8610084" or "8520892" or "7597541" or "7583549" or "7604798" or "8838332" or "7772062" or "7866933" or "8522729" or "7484265" or "7642011" or "8579620" or "7828385" or "7494939" or "7575771" or "7778537" or "7769402" or "7642176" or "7781495" or "9827075" or "7801966" or "7606643" or "7571764" or "7604799" or "7585284" or "7578834" or "7489031" or "7625348" or "7625364" or "7852377" or "7490996" or "8520275" or "7897787" or "7840078" or "7672076" or "7604805" or "7699185" or "8549923" or "7634440" or "7662446" or "7604800" or "7650079" or "7595099" or "7744541" or "8871266" or "8561652" or "7893737" or "7676762" or "7579744" or "7548643" or "8867781" or "8296711" or "7966894" or "7933263" or "7797219" or "8012881" or "7860175" or "8163645" or "7730737" or "7979101" or "8282821" or "7954676" or "7809768" or "8186654" or "7802728" or "7919081" or "8017448" or "7960472" or "8143006" or "10150300" or "7886290" or "7979498" or "7517836" or "8012882" or "7726691" or "8048085" or "7910281" or "8270757" or "8274473" or "7517834" or "7710201" or "8122946" or "8181150" or "8016034" or "19489169" or "7882658" or "8017469" or "8113822" or "8066704" or "8002848" or "8047526" or "8082956" or "7517829" or "8004802" or "8185885" or "7988779" or "7866081" or "7863486" or "8017474" or "8044949" or "8060385" or "8208397" or "7918304" or "7816977" or "8202188" or "8066357" or "8297199" or "8147544" or "7857367" or "7914265" or "8106688" or "7997692" or "7918305" or "8064900" or "7931039" or "7980716" or "7917813" or "7968073" or "7803063" or "8172843" or "7989900" or "8334949" or "8518573" or "8425468" or "8328379" or "8317435" or "8519205" or "8410106" or "8011962" or "8378736" or "8348061" or "8513315" or "8363164" or "8297546" or "7681515" or "8342189" or "8254266" or "8289097" or "8337460" or "8144284" or "8405029" or "8450292" or "8463785" or "8440848" or "8409092" or "8502905" or "8361860" or "8492598" or "8486007" or "8292095" or "8320981" or "8399083" or "8036577" or "8512044" or "8503516" or "8518354" or "8457250" or "8506890" or "8235557" or "7941761" or "8257617" or "8366183" or "8334804" or "24921113" or "8465806" or "8213502" or "7732293" or "8240474" or "8482062" or "8370576" or "8480942" or "8503586" or "8460835" or "8460836" or "8439103" or "8480943" or "8423226" or "8287156" or "8506889" or "8363165" or "8495121" or "8345800" or "8268084" or "8446869" or "8144283" or "8480959" or "8501483" or "8239396" or "8443740" or "8274921" or "8111502" or "8114261" or "8403359" or "8430625" or "8336217" or "1516292" or "1536350" or "1731860" or "1603147" or "1407133" or "10148720" or "10146977" or "1626496" or "1738814" or "1516875" or "1291621" or "1635494" or "1736560" or "1425090" or "1302897" or "1475701" or "1348666" or "1466918" or "1449569" or "1296541" or "1509220" or "1632851" or "1482576" or "1405284" or "1534257" or "1420786" or "1479313" or "1390323" or "1360061" or "1341866" or "1619198" or "1334574" or "1587400" or "1546864" or "1327742" or "1596851" or "1483414" or "1597213" or "1546702" or "1477994" or "1315570" or "1591376" or "1734550" or "1296550" or "1352930" or "1727908" or "1576582" or "1310566" or "1583818" or "1542261" or "1341147" or "1506163" or "1414915" or "1534286" or "1359022" or "1609919" or "1550087" or "1499466" or "1468089" or "1424049" or "1306590" or "1296548" or "1610539" or "1466665" or "1285697" or "1576628" or "1518504" or "1734676" or "1360559" or "1488408" or "1581939" or "1429490" or "1477992" or "1375692" or "1342268" or "1480808" or "1627020" or "1329482" or "1513124" or "1308800" or "2011075" or "1790033" or "1880840" or "1899938" or "2004412" or "1790627" or "2040869" or "2040207" or "1751710" or "1880861" or "1785285" or "1655015" or "1750429" or "1992960" or "1754864" or "1915205" or "2044648" or "1775703" or "1832729" or "1879732" or "2025730" or "1991249" or "1813637").pm.	306
27	("2044257" or "1927944" or "1903849" or "1858187" or "1659171" or "1854109" or "1750045" or "1660530" or "1911720" or "1747553" or "1680391" or "1998646" or "1763862" or "2071804" or "1771957" or "1911722" or "1767696" or "2029780" or "1899896" or "1861922" or "1650421" or "1936105" or "1842523" or "1685207" or "1809892" or "1910254" or "1988005" or "2016863" or "2071797" or "1819418" or "2002818" or "1941853" or "1880482" or "1898717" or "1746296" or "1933004" or "1872664" or "2073672" or "2320553" or "1965418" or "2296124" or "2181692" or "2073668" or "2301164" or "2371635" or "2371640" or "2374043" or "2218778" or "2240916" or "2262252" or "1670530" or "2218650" or "2308515" or "2391728" or "2353352" or "2106919" or "2363952" or "2271263" or "2254766" or "2372967" or "2082621" or "2188241" or "2332817" or "2267362" or "2083935" or "2286856" or "2268697" or "2343674" or "2402946" or "2206030" or "2243428" or "2380678" or "2073969" or "2243435" or "2360291" or "2229942" or "2332214" or "2275284" or "2271215" or "2184712" or "2154513" or "2390138" or "2184006" or "2344298" or "2186451" or "2109783" or "2205799" or "2206036" or "2212879" or "2359503" or "2405605" or "2919540" or "2912434" or "2784236" or "2798366" or "2607722" or "2733124" or "2784930" or "2787564" or "2709545" or "2606449" or "2736571" or "2677611" or "2709886" or "2585960" or "2773118" or "2495023" or "2600493" or "2650524" or "2600495" or "2925333" or "2676653" or "2734659" or "2807707" or "2628954" or "2712708" or "2725596" or "2809554" or "2638436" or "2815034" or "2689125" or "2648923" or "2802415" or "2531881" or "2711758" or "2692571" or "2551157" or "2712858" or "2736820" or "2812486" or "2690048" or "2715556" or "2811031" or "3180583" or "3141689" or "3185048" or "3188982" or "3231844" or "3202019" or "3288476" or "3041982" or "3224001" or "3226223" or "3414733" or "3267710" or "3348758" or "3247710" or "3042197" or "3178382" or "3378557" or "3363513" or "3264450" or "3389027" or "3188664" or "3202023" or "3042201" or "3348841" or "3810359" or "3494320" or "3827979" or "3501638" or "3310570" or "3824155" or "3118060" or "3695108" or "3661536" or "2961916" or "2951547" or "2885572" or "3812209" or "3678235" or "3826039" or "3632751" or "3687114" or "3120215" or "3680913" or "3628256" or "3110726" or "2886738" or "3941501" or "3940678" or "2873641" or "3535128" or "3543817" or "3714568" or "3457291" or "2871933" or "3955643" or "3946460" or "3535814" or "3728290" or "3961687" or "3820578" or "3524520" or "3526270" or "3940684" or "3951912" or "3513134" or "2952074" or "3718994" or "3534334" or "2955619" or "3711914" or "3953435" or "2873741" or "4062130" or "2934849" or "3874756" or "4008771" or "4068979" or "4072526" or "4058254" or "4021807" or "4015511" or "3835855" or "4035101" or "4023047" or "3974449" or "3891266" or "4046317" or "3907645" or "6521201" or "6730738" or "6590003" or "6323545" or "6491620" or "6531358" or "6731433" or "6697533" or "6531453" or "6723318" or "6368715" or "6331021" or "6492568" or "6465119" or "6469537" or "6422891" or "6521204" or "6374831" or "6373280" or "6710113" or "6588552" or "6361300" or "6731432" or "6480065" or "6573160" or "6860476" or "6414238" or "6645197" or "6685165" or "6659567" or "6228091" or "6680537" or "6873209" or "6665222" or "6844393" or "6579340" or "6396733" or "6648889" or "7046692" or "7123589" or "7183053" or "6125861" or "7136019" or "6132433" or "7058778" or "6752528" or "7092575" or "7138620" or "7079619" or "7042218" or "7154491" or "7120357" or "6818977" or "7170492" or "7068811" or "7175379" or "7225167" or "6112635" or "7259072" or "6945753" or "7303665" or "6118533" or "7301182" or "7339867" or "7322621" or "7033903" or "7289368" or "7293252" or "7329510" or "7052358" or "7464557" or "7298021" or "7225568" or "7263187" or "7240364" or "6781999" or "7247985" or "7314922" or "6118924" or "7355782" or "7395296" or "7353267" or "6992525" or "7428376" or "7392357" or "7365983" or "7245576").pm.	308

28	("7456148" or "7461569" or "7003789" or "7363194" or "7404060" or "7395212" or "7419983" or "7360532" or "6105293" or "7433204" or "6106825" or "7000350" or "7454136" or "6261774" or "7382258" or "7380228" or "525299" or "472597" or "758999" or "523488" or "453047" or "499929" or "217273" or "486513" or "161872" or "218071" or "227426" or "549252" or "461210" or "515458" or "86108" or "425874" or "513825" or "642859" or "207905" or "705116" or "214000" or "756589" or "738551" or "359229" or "717136" or "744681" or "622025" or "276566" or "366234" or "217108" or "215033" or "919596" or "417264" or "928817" or "928814" or "198911" or "402755" or "414113" or "884862" or "909482" or "610428" or "920421" or "871667" or "835511" or "412058" or "850311" or "597353" or "580920" or "856227" or "579623" or "958089" or "1269099" or "1106167" or "1244378" or "979847" or "946691" or "183321" or "768657" or "932167" or "179295" or "1257681" or "1017536" or "992559" or "958648" or "956689" or "1261061" or "1013392" or "132862" or "188434" or "1251811" or "1261427" or "951135" or "174850" or "183322" or "966076" or "1014624" or "770089" or "947672" or "1024628" or "187428" or "959629" or "178813" or "813212" or "947671" or "971346" or "792498" or "975971" or "1022601" or "178981" or "1144181" or "1096564" or "803123" or "803015" or "172533" or "1144817" or "164351" or "168737" or "1221379" or "1133650" or "171564" or "1180126" or "1107622" or "1105982" or "1157505" or "1057908" or "1104153" or "46958" or "1218738" or "1055951" or "1132094" or "1109485" or "47120" or "4377199" or "4810415" or "4819522" or "4548792" or "4813182" or "4548616" or "4421665" or "4810110" or "4427862" or "4828565" or "4424231" or "4548621" or "4425852" or "4548623" or "4603103" or "4370795" or "4824624" or "4281944" or "4371219" or "4422442" or "4366521" or "4456570" or "4373491" or "4442440" or "4445779" or "4821152" or "4366188" or "4424128" or "4598837" or "4369519" or "4377403" or "4821138" or "4449718" or "4600585" or "4371664" or "4691568" or "4763632" or "4801348" or "4581362" or "4719577" or "4726137" or "4121135" or "4693217" or "4695649" or "4585638" or "4715262" or "4578640" or "4357674" or "4763484" or "4605045" or "4351694" or "4742851" or "4771864" or "4766416" or "4760474" or "4741607" or "4755806" or "4550038" or "5019626" or "4665817" or "5048273" or "4635921" or "4642637" or "4145246" or "4110639" or "4564745" or "4551212" or "4657673" or "5049870" or "5073094" or "4678573" or "4335955" or "4340015" or "4649059" or "5051656" or "4344330" or "4563228" or "4565986" or "5009984" or "4112223" or "5072454" or "5081824" or "4542788" or "4563491" or "4665218" or "4649021" or "5539637" or "5567019" or "5132448" or "5556474" or "5094655" or "4257675" or "5121121" or "5563708" or "5574509" or "4945162" or "5546857" or "5132453" or "5095238" or "4937603" or "5132459" or "5568147" or "5108055" or "5097214" or "5132445" or "5443911" or "5465463" or "5493461" or "5420082" or "5004789" or "5508171" or "5450912" or "4914806" or "5452617" or "4246109" or "5782537" or "5774257" or "5823719" or "5396178" or "4987837" or "5819156" or "5385981" or "5822873" or "4306948" or "4386628" or "5304406" or "5640427" or "4888159" or "5682336" or "4241117" or "6052854" or "6040635" or "6031907" or "6019837" or "6018544" or "5988709" or "5957695" or "14328911" or "14217037" or "14232613" or "14235883" or "14119979" or "14074817" or "14047397" or "14104298" or "13606150" or "14911391" or "20722144").pm.	164
29	or/18-28	2840
30	limit 17 to (conference abstract or conference paper or "conference review")	288
31	limit 30 to yr="2018 -Current"	70
32	31 not 29	70
33	17 not (29 or 30)	205
34	from 32 keep 1-70	70

Table A4. Cochrane (Wiley) Search Strategy (July 21, 2020)

	Search	Results
1	((familia* or type* 2 or type* 2s or type* ii or type iis or type* iia* or type* iib* or essential* or autosomal dominant or genetic*) NEAR/3 (hypercholesterolemi* or hypercholesterolaemi* or hyperlipoproteinemi* or hyperlipoproteinaemi* or hyper-cholesterolemi* or hyper-cholesterolaemi* or hyper-lipoproteinemi* or hyper-lipoproteinaemi* or apolipoprotein-b* or dyslipidemi*)):ti,ab,kw	2152
2	(HoFH or HFH or HzFH or HeFH or HHF):ti,ab,kw	372
3	((extreme* or rare* or severe* or homozyg* or homo-zygo* or heterozygo* or hetero-zygo*) NEAR/3 (hypercholesterolemi* or hypercholesterolaemi* or hyperlipoproteinemi* or hyperlipoproteinaemi* or hyper-cholesterolemi* or hyper-cholesterolaemi* or hyper-lipoproteinemi* or hyper-lipoproteinaemi* or apolipoprotein-b*)):ti,ab,kw	545
4	((hyperbetalipoproteinemi* or hyperbetalipoproteinaemi* or hyper-beta-lipoproteinemi* or hyper-beta-lipoproteinaemi* or lipoproteinemi* or lipoproteinaemi*) NEAR/3 (hyper-low* or hyper-beta* or hyperlow* or hyperbeta* or ldl receptor disorder*)):ti,ab,kw	8
5	lipoid gout*:ti,ab,kw	0
6	(tendon* NEAR/2 (xanthoma* or xanthogranulomatos*)):ti,ab,kw	11
7	((heterozygo* or hetero-zygo*) NEAR/2 FH):ti,ab,kw	59
8	#1 or #2 or #3 or #4 or #5 or #6 or #7	2436
9	((sex* or gender* or man or men or male* or woman or women or female*) NEAR/3 (difference* or different or characteristic* or ratio* or factor* or imbalanc* or issue* or both or specific* or disparit* or dependen* or gap or gaps or influenc* or discrepan* or distribut* or composition* or variability or comparison* or accept* or barrier* or perception* or perceiv* or between* or treat* or alirocumab or evolocumab or statin or atorvastatin or rosuvastatin or simvastatin or ezetimibe or ezetimib or niacin or enduracin	86557

	or nicamin or nicobid or nicocap or nicolar or nicotinate or nicotinic or bile acid sequestrant or bempedoic acid or lomitapide or mipomersen or apheresis or PCSK9 inhibitor or anticholesteremic* or hypocholesteremic* or hmg-coa or hydroxymethylglutaryl or hydroxymethylglutaryl-coa or hydroxymethylglutaryl-coenzyme or (cholesterol NEAR/2 inhibitor*))):ti,ab,kw	
10	((men or men's) NEAR/2 women*):ti,ab,kw	17051
11	((gender* NEXT related) or (gender* Next based)):ti,ab,kw	415
12	#9 or #10 or #11	98335
13	#8 and #12	254
14	EMBASE:AN	552478
15	PUBMED:AN	673088
16	#14 or #15	1016113
17	#13 not #16	53

Table A5. PubMed (NLM) Search Strategy (July 21, 2020)

	Search	Results
1	(familia*[Text Word]) OR type* 2[Text Word]) OR type* 2s[Text Word]) OR type* ii[Text Word]) OR type iis[Text Word]) OR type* iia*[Text Word]) OR type* iib*[Text Word]) OR essential*[Text Word]) OR autosomal dominant[Text Word]) AND hypercholesterolemi*[Text Word] OR hypercholesterolaemi*[Text Word] OR hyperlipoproteinemi*[Text Word] OR hyperlipoproteinaemi*[Text Word] OR hyper-cholesterolemi*[Text Word] OR hyper-cholesterolaemi*[Text Word] OR hyper-lipoproteinemi*[Text Word] OR hyper-lipoproteinaemi*[Text Word] OR apolipoprotein-b*[Text Word] OR dyslipidemi*[Text Word]	67, 005
2	HoFH[Text Word] OR HFH[Text Word] OR HzFH[Text Word] OR HeFH[Text Word] OR HHF[Text Word]	1091
3	(extreme*[Text Word] OR rare*[Text Word] OR severe*[Text Word] OR homozyg*[Text Word] OR homo-zygo*[Text Word] OR heterozygo*[Text Word] OR hetero-zygo*[Text Word]) AND (hypercholesterolemi*[Text Word] OR hypercholesterolaemi*[Text Word] OR hyperlipoproteinemi*[Text Word] OR hyperlipoproteinaemi*[Text Word] OR [Text Word] OR apolipoprotein b*[Text Word])	5,160
4	((hyperbetalipoproteinemi*[Text Word] OR hyperbetalipoproteinaemi[Text Word]) OR (lipoproteinaemi*[Text Word] OR lipoproteinemi*[Text Word] OR lipoproteinaemi*[Text Word])) AND (hyperlow*[Text Word] OR hyperbeta*[Text Word] OR ldl receptor disorder*[Text Word])	123
5	lipoid gout*[Text Word]	4
6	tendon* xanthoma*[Text Word]) OR tendon* xanthogranulomatos*[Text Word]	383
7	**((heterozygo*[Text Word] or hetero-zygo*[Text Word]) AND FH[Text Word])**	1,399
8	#1 or #2 or #3 or #4 or #5 or #6 or #7	69,112
9	(sex[Text Word] OR sexes[Text Word] OR gender*[Text Word] OR man[Text Word] OR men[Text Word] OR male*[Text Word] OR woman[Text Word] OR women[Text Word] OR female*[Text Word]) AND (difference*[Text Word] OR different[Text Word] OR characteristic*[Text Word] OR ratio*[Text Word] OR factor*[Text Word] OR imbalanc*[Text Word] OR issue*[Text Word] OR both[Text Word] OR specific*[Text Word] OR disparit*[Text Word] OR dependen*[Text Word] OR gap[Text Word] OR gaps[Text Word] OR influenc*[Text Word] OR discrepan*[Text Word] OR distribut*[Text Word] OR	8,374,455

	composition*[Text Word] OR variability[Text Word] OR comparison*[Text Word] OR accept*[Text Word] OR barrier*[Text Word] OR perception*[Text Word] OR perceiv*[Text Word] OR between*[Text Word] OR treat*[Text Word] OR alirocumab[Text Word] OR evolocumab[Text Word] OR statin[Text Word] OR atorvastatin[Text Word] OR rosuvastatin[Text Word] OR simvastatin[Text Word] OR ezetimibe[Text Word] OR ezetimib[Text Word] OR niacin[Text Word] OR enduracin[Text Word] OR nicamin[Text Word] OR nicobid[Text Word] OR nicocap[Text Word] OR nicolar[Text Word] OR nicotinate[Text Word] OR nicotinic[Text Word] OR bile acid sequestrant[Text Word] OR bempedoic acid[Text Word] OR lomitapide[Text Word] OR mipomersen[Text Word] OR apheresis[Text Word] OR PCSK9 inhibitor[Text Word] OR anticholesteremic*[Text Word] OR hypocholesteremic*[Text Word] OR hmg-coa[Text Word] OR hydroxymethylglutaryl[Text Word] OR hydroxymethylglutaryl-coa[Text Word] OR hydroxymethylglutaryl-coenzyme[Text Word] OR cholesterol inhibitor*[Text Word])	
10	gender-related [Text Word] OR gender-based [Text Word]	8,318
11	#9 or #10	8,374,824
12	#8 and #11	39, 079
13	("2020/07/13"[Date - Create] : "3000"[Date - Create])	37,536
13	#12 and #13	41

Table A6. Scopus (Elsvier) Search Strategy (July 21, 2020)

	Search	Results
1	((TITLE-ABS-KEY ((familia* OR type*-2 OR type*-2s OR type-ii OR type-iis OR type-iia* OR type-iib* OR essential* OR autosomal-dominant) W/3 (hypercholesterolemi* OR hypercholesterolaemi* OR hyperlipoproteinemi* OR hyperlipoproteinaemi* OR hyper-cholesterolemi* OR hyper-cholesterolaemi* OR hyper-lipoproteinemi* OR hyper-lipoproteinaemi* OR apolipoprotein-b*))) OR (TITLE-ABS-KEY (HoFH or HFH or HzFH or HeFH or HHF)) OR (TITLE-ABS-KEY (extreme* OR rare* OR severe* OR homozyg* OR homo-zygo* OR heterozygo* OR hetero-zygo*) W/3 (hypercholesterolemi* OR hypercholesterolaemi* OR hyperlipoproteinemi* OR hyperlipoproteinaemi* OR hyper-cholesterolemi* OR hyper-cholesterolaemi* OR hyper-lipoproteinemi* OR hyper-lipoproteinaemi* OR apolipoprotein-b*)) OR (TITLE-ABS-KEY (hyperbetalipoproteinemi* OR hyperbetalipoproteinaemi* OR hyper-beta-lipoproteinemi* OR hyper-beta-lipoproteinaemi* OR ((lipoproteinemi* OR lipoproteinaemi*) W/3 (hyper-low* OR hyper-beta* OR hyperlow* OR hyperbeta*)) OR ldl AND receptor AND disorder*)) OR (TITLE-ABS-KEY (lipoid AND gout*)) OR (TITLE-ABS-KEY ((tendon*) W/2 (xanthoma* OR xanthogranulomatos*))) OR (TITLE-ABS-KEY ((heterozygo* OR hetero-zygo*) W/2 (fh)))) AND ((TITLE-ABS-KEY ((sex* OR gender* OR man OR men OR male* OR woman OR women OR female*) W/3 (difference* OR different OR characteristic* OR ratio* OR factor* OR imbalanc* OR issue* OR both OR specific* OR disparit* OR dependen* OR gap OR gaps OR influenc* OR discrepan* OR distribut* OR composition* OR variability OR comparison* OR accept* OR barrier* OR perception* OR perceiv* OR between* OR treat* OR alirocumab OR evolocumab OR statin OR atorvastatin OR rosuvastatin OR simvastatin OR ezetimibe OR ezetimib OR niacin OR enduracin OR nicamin OR nicobid OR nicocap OR nicolar OR nicotinate OR nicotinic OR bile-acid-sequestrant OR bempedoic-acid OR lomitapide OR mipomersen OR apheresis OR pcsk9-	1053

	inhibitor OR anticholesteremic* OR hypocholesteremic* OR hmg-coa OR hydroxymethylglutaryl OR hydroxymethylglutaryl-coa OR hydroxymethylglutaryl-coenzyme OR (cholesterol W/2 inhibitor*)))) OR (TITLE-ABS-KEY ((men OR men's) W/2 (women*))) OR (TITLE-ABS-KEY (gender*-related OR gender*-based)))	

Table A7. PsycInfo (OVID) Search Strategy (July 21, 2020)

#	Searches	Results
1	((familia* or type* 2 or type* 2s or type* ii or type iis or type* iia* or type* iib* or essential* or autosomal dominant or genetic*) adj3 (hypercholesterolemi* or hypercholesterolaemi* or hyperlipoproteinemi* or hyperlipoproteinaemi* or hyper-cholesterolemi* or hyper-cholesterolaemi* or hyper-lipoproteinemi* or hyper-lipoproteinaemi* or apolipoprotein-b* or dyslipidemi*)).tw.	149
2	(HoFH or HFH or HzFH or HeFH or HHF).tw.	10
3	((extreme* or rare* or severe* or homozyg* or homo-zygo* or heterozygo* or hetero-zygo*) adj3 (hypercholesterolemi* or hypercholesterolaemi* or hyperlipoproteinemi* or hyperlipoproteinaemi* or hyper-cholesterolemi* or hyper-cholesterolaemi* or hyper-lipoproteinemi* or hyper-lipoproteinaemi* or apolipoprotein-b*)).tw.	15
4	lipoid gout*.tw.	0
5	(tendon* adj2 (xanthoma* or xanthogranulomatos*)).tw.	14
6	((heterozygo* or hetero-zygo*) adj2 FH).tw.	3
7	1 or 2 or 3 or 4 or 5 or 6	177
8	((sex* or gender* or man or men or male* or woman or women or female*) adj3 (difference* or different or characteristic* or ratio* or factor* or imbalanc* or issue* or both or specific* or disparit* or dependen* or gap or gaps or influenc* or discrepan* or distribut* or composition* or variability or comparison* or accept* or barrier* or perception* or perceiv* or between* or treat* or alirocumab or evolocumab or statin or atorvastatin or rosuvastatin or simvastatin or ezetimibe or ezetimib or niacin or enduracin or nicamin or nicobid or nicocap or nicolar or nicotinate or nicotinic or bile acid sequestrant or bempedoic acid or lomitapide or mipomersen or apheresis or PCSK9 inhibitor or anticholesteremic* or hypocholesteremic* or hmg-coa or hydroxymethylglutaryl or hydroxymethylglutaryl-coa or hydroxymethylglutaryl-coenzyme or (cholesterol adj2 inhibitor*))).tw.	242471
9	((men or men's) adj2 women*).tw.	49897
10	(gender*-related or gender*-based).tw.	5947
11	8 or 9 or 10	264683
12	7 and 11	18

Table A 8. ClinicalTrials.gov Search Strategy (August 18, 2020)

	Search Terms	Results
1	Familial Hypercholesterolemia AND Gender	11
2	Familial Hypercholesterolemia AND Sex	12
3	Familial Hypercholesterolemia and (Male or Female)	4
4	Type-2 Hypercholesterolemia AND Gender	9
5	Type-2 Hypercholesterolemia AND Sex	6
6	Type-2 Hypercholesterolemia AND (Male or Female)	5
7	Familial hyperlipoproteinemia AND Gender	12
8	Familial hyperlipoproteinemia AND Sex	7
9	Familial hyperlipoproteinemia AND (Male or Female)	4
Total		**70**
After removal of duplicates		**18**

Table A9. International Standard Randomized Controlled Clinical Trial Number Registry Search Strategy (August 18, 2020)

	Search Terms	Results
1	Familial Hypercholesterolemia	12
2	Type-2 Hypercholesterolemia	13
3	Familial hyperlipoproteinemia	0
Total		**21**

Table A10. Health Canada Clinical Trials Database Search Strategy (August 18, 2020)

	Search Terms	Results
1	Familial Hypercholesterolemia	0
2	Type-2 Hypercholesterolemia	0
3	Familial hyperlipoproteinemia	0
4	Hypercholesterolemia	0
Total		**0**
After Duplicate Removal		**0**

Table A11. ProQuest Dissertations and Theses Search Strategy (August 18, 2020)

	Search Terms	Results
1	Familial Hypercholesterolemia and Gender.ti	0
2	Familial Hypercholesterolemia and sex.ti	0
3	Familial Hypercholesterolemia.ti	39
4	Type-2 Hypercholesterolemia.ti	0
5	Familial hyperlipoproteinemia	1
Total		**40**

Table A12. Google Scholar Search Strategy (August 18, 2020)

	Search Terms	Results
1	allintitle: gender OR or OR sex AND "Familial Hypercholesterolemia"	86
2	allintitle: gender OR or OR sex AND "Type-2 Hypercholesterolemia"	0
3	allintitle: gender OR or OR sex AND "Familial hyperlipoproteinemia"	3
Total		**89**
After Duplicate Removal		**85**

Table A13. Open Grey Search Strategy (August 18,2020)

	Search Terms	Results
1	Familial Hypercholesterolemia http://www.opengrey.eu/search/request?q=Familial+Hypercholesterolemia+	13
2	Type-2 Hypercholesterolemia http://www.opengrey.eu/search/request?q=Type-2+Hypercholesterolemia	4
3	Familial hyperlipoproteinemia"	0
Total		**17**

Table A14. American Heart Association Conferences Search Strategy (August 18, 2020)

	Search Terms
1	American Heart Association Conferences and Scientific Sessions https://professional.heart.org/en/meetings
2	Upcoming International Events https://www.heart.org/en/about-us/international-programs/upcoming-international-events

Table A15. Arteriosclerosis, Thrombosis and Vascular Biology Search Strategy (August 18, 2020)

	Search Terms	Link
1	Familial Hypercholesterolemia and Gender.ti	https://www.ahajournals.org/action/doSearch?field1=Title&text1=Familial+Hypercholesterolemia+and+Gender&ConceptID=&ConceptID=&publication=&Ppub=&access=on
2	Familial Hypercholesterolemia and sex.ti	https://www.ahajournals.org/action/doSearch?field1=Title&text1=Familial+Hypercholesterolemia+and+sex&ConceptID=&ConceptID=&publication=&Ppub=&access=on