Familial hypercholesterolemia (FH) is a heritable condition that leads to significantly elevated serum low-density lipoprotein (LDL) cholesterol, generally > 5 mmol/L (>190 mg/dL), resulting in increased risk of premature coronary artery disease. In patients with FH compared with normolipidemic individuals, atherosclerotic cardiovascular disease (ASCVD) incidence is 4.1 times higher, and the age of onset is accelerated by 10 to 20 years in men and 20 to 30 years in women. Globally, FH affects approximately 1 in 250 people, with a higher prevalence in Quebec. However, FH remains underdiagnosed and undertreated in the general population.

With advances in genetic testing, the yield of finding a genetic cause in patients referred with suspected FH is up to 67% depending on the patient cohort. There is also considerable genetic diversity within FH that is associated with variable clinical outcomes. Recent studies have found that hypercholesterolemic individuals with FH mutations have higher ASCVD risk than patients with similar levels of hypercholesterolemia but without a mutation. Moreover, the degree of atherosclerosis is higher in patients with monogenic FH compared with others. But without a comprehensive FH database, previous studies could not...
Conclusion: FH is a genetically diverse condition. FH mutations are independently associated with higher risk of premature MI in patients referred for hypercholesterolemia. Therefore, genotyping could guide cardiovascular risk stratification in the personalized treatment of FH.

control for confounders from genetic, biochemical, and clinical risk factors simultaneously. Moreover, genotyping efforts traditionally have been limited to a few select genes, or in some cases, a microarray panel.

This study aims to assess whether FH genotype is an independent risk factor for ASCVD, after adjusting for LDL cholesterol level and common clinical risk factors. Comprehensive medical histories were obtained for all study participants. Genotyping was performed via targeted next-generation DNA sequencing (NGS) of 73 lipid metabolism genes and 178 single nucleotide polymorphisms (SNPs). We found that FH genotype is independently associated with myocardial infarction (MI) risk, suggesting that genetic diagnosis could help with risk stratification.

Methods

Study subjects

This project was designed as a bidirectional cohort study that examined clinical outcomes and FH genotypes. A total of 182 unrelated patients with clinically suspected FH were recruited from the Lipid Clinic at University Hospital, London Health Sciences Center, in Southwestern Ontario. They were then followed for up to 1 year to assess response to cholesterol-lowering treatment (Supplemental Material).

Genetic characterization

Genomic DNA was extracted from whole blood, fragmented, enriched for target candidate genes, and then molecularly barcorded and pooled into genomic libraries, according to the Illumina Nextera Custom Enrichment protocol (San Diego, CA) as implemented at the London Regional Genomics Centre (LRGC; www.lrgc.ca). The LipidSeq genetic panel contains 73 lipid metabolism-related genes, including FH genes (LDLR, APOB, PCSK9), other hypercholesterolemia-associated genes (APOE, LDLRAP1, LIPA, ABCGS5/8, NPC1L1, STAP1, SORT1, MYLIP), and 178 SNPs associated with lipid traits. Genomic libraries were sequenced at LRGC using the Illumina MiSeq, with 300-times mean read depth of coverage for all exons, intron-exon boundaries (10-20 base pair [bp]), and 5’ untranslated regions (250 bp upstream). Copy number variation for the LDLR gene was further assessed as described. In case of ambiguity, Sanger sequencing was used to confirm variants detected by NGS. FASTQ sequencing files were processed by the CLC Bio Genomics Workbench v 8.5.1 (Aarhus, Denmark) leading to binary alignment (bam) files, and variant call format (vcf) files, that were annotated by ANNOVAR.

Annotated genetic variants were classified as “mutation-positive” (pathogenic or likely pathogenic) or “mutation-negative” (benign, likely benign, or variant of uncertain significance) using the ClinVar database. When conflicting evidence was present, the variant was manually reviewed, and American College of Medical Genetics guidelines were enforced.

Statistical analysis

Statistical analysis and graphs were produced in Microsoft Office Excel 2016 (Redmond, WA), Stata 15.1 (StataCorp LP, College Station, TX), and SAS 9.3 (SAS Institute Inc, Cary, NC).

Results

Baseline clinical data, grouped by gender, are presented in Table 1. Compared with the male group, the female group was 6 years older ($P = 0.015$), had higher high-density lipoprotein cholesterol by 0.18 mmol/L ($P < 0.001$), had 56% fewer smokers ($P = 0.018$), and had a 56% lower incidence of coronary events ($P = 0.018$). Other cardiovascular risk factors and outcomes were similar.

To provide consistency in clinical FH diagnosis, we reevaluated each case strictly using the Canadian FH definition. At the time of referral, DNA information was not yet known; thus, mutation status was not used in making any diagnoses. Some 9% of patients had definite FH, 42% had probable FH, 42% had severe hypercholesterolemia, and 7% did not fit criteria because their baseline lipid panel was measured while on cholesterol-lowering medications.

DNA sequencing results are summarized in Table 2. A total of 49 of 182 patients had mutations, of which 43 involved the LDLR gene and 6 involved APOB. The most common mutation was the French-Canadian 5’ 15 kb deletion of LDLR promoter and first exon. Nonsense and copy number variation mutations were associated with the highest LDL cholesterol levels. A total of 34 of the 49 mutations were unique. Table 3 shows the diagnostic yield of DNA sequencing stratified by the strength of clinical FH diagnosis, $P < 0.001$
LDL-C, low-density lipoprotein cholesterol; FH, familial hypercholesterolemia; indel, insertion or deletion mutation; referral. For patients age 40 years or younger, approximately (Supplemental Fig. S1). In contrast, the probability of mutation when LDL cholesterol was cholesterol increased. Approximately half of patients had a cholesterol level and clinical risk factors. Patients with a positive family history was 2.1-fold increased (P = 0.005). Patients with positive family history were also 6.7 years younger at the time of referral (P = 0.005). As for comorbidities, patients without a positive family history had higher body mass index by 2.1 kg/m² (P = 0.006) and 2.6 times higher prevalence of diabetes (P = 0.048) (Supplemental Table S1).

Clinical characteristics of participants categorized by FH mutation status are shown in Table 4. Patients with mutations compared with those without were 11.8 years younger when referred to the lipid clinic (P < 0.001), had higher baseline LDL cholesterol by 1.11 mmol/L (P < 0.001), and had higher post-treatment LDL cholesterol by 0.62 mmol/L (P = 0.024). Event curves for nonfatal premature MI by FH mutation status (Fig. 1) were statistically different (P = 0.002, log-rank test). Data for the Canadian population were obtained from Statistics Canada.21

Cox proportional hazard ratios (HRs) of clinical predictors with respect to developing premature MI are displayed in Table 5. After adjusting for sex, hypertension, body mass index, diabetes, smoking, LDL cholesterol, and use of cholesterol-lowering medications, the HR of developing premature MI with respect to having an FH mutation was 4.51 (95% confidence interval, 1.74-11.7, P = 0.002). Other significant factors in the multivariable model were male sex (HR, 5.35, P = 0.001) and diabetes (HR, 3.16, P = 0.031). In comparison, the HR of premature MI with respect to positive family history was 2.03 (P = 0.109) after adjusting for LDL cholesterol and the same clinical risk factors (Supplemental Tables S2 and S3). In case the association between FH mutation and MI was largely driven by higher LDL level, we also included LDL cholesterol as a categorical variable in the multivariate model. The effect estimates remain similar (Supplemental Tables S2 and S3).

Subgroup analysis comparing mutation-positive and mutation-negative patients is shown in Figure 2. The effect of having a FH mutation was similar across subgroups.

**Discussion**

Despite being thought of as a single clinical entity, FH is genetically diverse.22 Mutation status is an independent predictor of premature MI with an HR of 4.51 (95% confidence interval, 1.74-11.7) after adjusting for LDL cholesterol level and clinical risk factors. Patients with a mutation were also 11.8 years younger when referred to a lipid specialist, likely due to a combination of disease severity and positive family history. Compared with family history, mutation status was a stronger predictor of premature MI.

### Table 1. Baseline data for patients at their initial consultation appointment

| Gene | N | Number of distinct mutations | Mean LDL-C (mmol/L) |
|------|---|-----------------------------|---------------------|
| LDLR | 43 (88%) | 33 | 7.18 ± 2.19 |
| Missense | 21 | 18 | 6.90 ± 2.05 |
| Nonsense | 4 | 4 | 7.86 ± 3.20 |
| Frameshift | 4 | 4 | 6.24 ± 0.89 |
| Splicing | 8 | 5 | 7.63 ± 2.58 |
| CNV* | 6 | 2 | 7.76 ± 2.35 |
| APOB | 6 (12%) | 1 | 5.86 ± 0.66 |
| Total | 49 | 34 | 7.02 ± 2.11 |

APOB, gene encoding apolipoprotein B; CNV, copy number variation; FH, familial hypercholesterolemia; indel, insertion or deletion mutation; LDL-C, low-density lipoprotein cholesterol; LDLR, gene encoding the LDL receptor.

* Five CNV mutations are the French Canadian FH mutation.20

### Table 2. FH mutations identified in this study

### Table 3. Diagnostic yield of DNA sequencing stratified by strength of clinical FH diagnosis using the 2018 Canadian FH definition

| Clinical FH diagnosis | Mutation positive | Mutation negative | Total |
|-----------------------|-------------------|-------------------|-------|
| Definite              | 11 (69%)          | 5 (31%)           | 16    |
| Probable              | 25 (32%)          | 52 (68%)          | 77    |
| Severe hypercholesterolemia | 12 (16%)   | 64 (84%)          | 76    |
| Nondiagnostic        | 1 (8%)            | 12 (92%)          | 13    |
| Total                | 49 (27%)          | 133 (73%)         | 182   |

FH, familial hypercholesterolemia.
These results suggest clinical utility of having a genetic diagnosis in addition to a clinical diagnosis of FH. First, a genetic diagnosis allows improved cardiovascular risk stratification over clinical risk factors. Second, it can be performed at any age, before the onset of symptoms and complications. For example, the International Atherosclerosis Society recommended FH screening be extended to children, so that early cardiovascular prevention may be initiated.23

Study limitations

This study has several limitations. First, mutation classification is a work in progress. Therefore, genetic variants and mutations found in this study may be revised in the future.20 Second, FH can also result from an accumulation of common polygenic risk SNPs, rather than distinct mutations. But there is no consensus yet for the correct construction of polygenic risk scores, and using thousands of genome-wide markers to predict MI risk may become the norm in the future.24,25 Third, having a larger sample size will allow detailed risk stratification by mutation gene and type. The creation of FH databases and registries will be a foundational step in this direction.26,27 Finally, from a basic science perspective, the observation that having an FH mutation is independently associated with premature MI raises the possibility of additional pathways between mutation and cardiovascular disease outside of LDL cholesterol level and traditional risk factors.

Table 4. Clinical characteristics according to whether a FH mutation was identified on DNA sequencing

| Variable                        | Yes (N = 49) | No (N = 133) | P value |
|---------------------------------|--------------|--------------|---------|
| Age (y)                         | 39.5 ± 15.0  | 51.3 ± 15.4  | < 0.001 |
| Sex                             |              |              | 0.24    |
| Female                          | 24 (49.0%)   | 78 (58.6%)   |         |
| Male                            | 25 (51.0%)   | 55 (41.4%)   |         |
| Total cholesterol (mmol/L)      | 9.00 ± 2.18  | 8.22 ± 1.38  | 0.022   |
| Triglycerides (mmol/L)          | 1.55 ± 0.80  | 2.05 ± 0.91  | < 0.001 |
| HDL cholesterol (mmol/L)        | 1.28 ± 0.38  | 1.38 ± 0.36  | 0.11    |
| LDL cholesterol (mmol/L)        | 7.02 ± 2.11  | 5.91 ± 1.20  | < 0.001 |
| Cholesterol-lowering medication |              |              | 0.73    |
| None                            | 46 (93.9%)   | 121 (91.0%)  |         |
| Low Intensity                   | 2 (4.1%)     | 6 (4.5%)     |         |
| High Intensity                  | 1 (2.0%)     | 6 (4.5%)     |         |
| BMI (kg/m²)                     | 26.4 ± 5.8   | 27.7 ± 4.9   | 0.12    |
| Hypertension                    | 10 (20.4%)   | 41 (30.8%)   | 0.17    |
| Diabetes mellitus               | 2 (4.1%)     | 15 (11.3%)   | 0.14    |
| Smoking                         | 6 (12.2%)    | 22 (16.5%)   | 0.47    |
| MI (nonfatal)                   | 9 (18.4%)    | 16 (12.0%)   | 0.27    |
| Premature MI (nonfatal)*        | 9 (18.4%)    | 16 (12.0%)   | 0.024   |
| LDL cholesterol after treatment (mmol/L) | 3.67 ± 1.71 | 3.05 ± 1.31 |

For discrete variables, numbers are shown with percentages or proportions in parentheses.

BMI, body mass index; FH, familial hypercholesterolemia; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MI, myocardial infarction; TIA, transient ischemic attack; FH, familial hypercholesterolemia.

* Premature MI: men age ≤ 55 y, women age ≤ 65 y. In comparison, according to the Canadian Chronic Disease Surveillance System,21 in 2015 the prevalence of ischemic heart disease is 1.65% for people age 35-49 y and 8.1% for people age 50-64 y.

Figure 1. Risk of nonfatal premature myocardial infarction (MI) vs age of event onset. This graph represents the proportion of patients with familial hypercholesterolemia (FH) referred for secondary prevention of cardiovascular disease. To avoid multiplicity, patients who experienced more than 1 event are only counted once at the earliest age of MI. Canadian population data were based on Statistics Canada’s self-reported health survey.7 The event curves for mutation positive and negative patients were statistically different (P = 0.002, log-rank test).

Table 5. Multivariable Cox proportional hazards model of premature MI with FH mutation and clinical risk factors as predictors

| Variable                        | Hazard ratio (95% CI) | P value |
|---------------------------------|-----------------------|---------|
| FH mutation                     | 4.51 (1.74-11.7)      | 0.002   |
| Male sex                        | 5.35 (2.01-14.2)      | 0.001   |
| Hypertension                    | 1.28 (0.53-3.11)      | 0.583   |
| Diabetes                        | 3.16 (1.11-8.99)      | 0.031   |
| BMI                             | 0.96 (0.87-1.06)      | 0.422   |
| Smoking                         | 2.14 (0.88-5.23)      | 0.094   |
| LDL cholesterol (mmol/L)        | 0.94 (0.73-1.20)      | 0.608   |
| Cholesterol medications*        | 3.00 (1.62-5.57)      | 0.001   |

BMI, body mass index; CI, confidence interval; FH, familial hypercholesterolemia; LDL, low-density lipoprotein.

* Some patients were already taking cholesterol medications, and we (including the referring physician) could not find a true “baseline” lipid panel.
Conclusions

Familial hypercholesterolemia is a genetically diverse condition. Mutations identified by targeted next generation DNA sequencing are typically distinct between families and many patients have a polygenic basis for their condition. FH patients with monogenic mutations, compared to those without, have a higher risk of premature myocardial infarction, even after adjusting for LDL cholesterol and clinical risk factors. Thus, genotyping in FH adds important information to the clinical picture that will enable more accurate cardiovascular risk prediction and personalized treatment.

Acknowledgements

The authors thank Ericka Simon for assistance during chart review and Dr Henian Cao for his expertise in LipidSeq.

Funding Sources

P.J.Z. was supported by a research grant from the Mach-Gaensslen Foundation. R.A.H. is supported by the Jacob J. Wolfe Distinguished Medical Research Chair, the Edith Schulich Vinet Canada Research Chair in Human Genetics, the Martha G. Blackburn Chair in Cardiovascular Research, and operating grants from the Canadian Institutes of Health Research (Foundation Grant) and the Heart and Stroke Foundation of Ontario (G-18-0022147).

Disclosures

R.A.H. has received honoraria for membership on advisory boards and speakers’ bureaus for Aegerion, Akcea, Amgen, Boston Heart, Gemphire, Regeneron, and Sanoﬁ, all unrelated to the content of this manuscript. The other authors have no conﬂicts of interest to disclose.

References

1. Defesche JC, Gidding SS, Harada-Shiba M, et al. Familial hypercholesterolaemia. Nat Rev Dis Primers 2017;7:17093.
2. Perak AM, Ning H, de Ferranti SD, et al. Long-term risk of atherosclerotic cardiovascular disease in US adults with the familial hypercholesterolemia phenotype. Circulation 2016;134:9-19.
3. Mundal L, Igland J, Ose L, et al. Cardiovascular disease mortality in patients with genetically verifed familial hypercholesterolemia in Norway during 1992-2013. Eur J Prev Cardiol 2017;24:137-44.
4. Santos RD, Gidding SS, Hegele RA, et al. Deﬁning severe familial hypercholesterolaemia and the implications for clinical management: a consensus statement from the International Atherosclerosis Society Severe Familial Hypercholesterolemia Panel. Lancet Diabetes Endocrinol 2016;4:850-61.
5. Akioyamen LE, Genest J, Shan SD, et al. Estimating the prevalence of heterozygous familial hypercholesterolaemia: a systematic review and meta-analysis. BMJ Open 2017;7:e016461.
6. Brunham LR, Ruel I, Aljenedil S, et al. Canadian Cardiovascular Society position statement on familial hypercholesterolemia: update 2018. Can J Cardiol 2018;34:1553-63.
7. Nordsetgaard BG, Chapman MJ, Humphries SE, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. Eur Heart J 2013;34:3478-90.
8. Wang J, Dron JS, Ban MR, et al. Polygenic versus monogenic causes of hypercholesterolemia ascertained clinically. Arterioscler Thromb Vasc Biol 2016;36:2439-45.
9. Iacocca MA, Hegele RA. Recent advances in genetic testing for familial hypercholesterolemia. Expert Rev Mol Diagn 2017;17:641-51.
10. Khera AV, Won HH, Peloso GM, et al. Diagnostic yield and clinical utility of sequencing familial hypercholesterolemia genes in patients with severe hypercholesterolemia. J Am Coll Cardiol 2016;67:2578-89.

11. Abul-Husn NS, Manickam K, Jones JK, et al. Genetic identification of familial hypercholesterolemia within a single U.S. health care system. Science 2016;354:aaaf7000.

12. Sharifi M, Higgenson E, Bos S, et al. Greater preclinical atherosclerosis in treated monogenic familial hypercholesterolemia vs. polygenic hypercholesterolemia. Atherosclerosis 2017;263:405-11.

13. Johansen CT, Dubé JB, Loyzer MN, et al. LipidSeq: a next-generation clinical resequencing panel for monogenic dyslipidemias. J Lipid Res 2014;55:765-72.

14. Hegele RA, Ban MR, Cao H, et al. Targeted next-generation sequencing in monogenic dyslipidemias. Curr Opin Lipidol 2015;26:103-13.

15. Teslovich TM, Musunuru K, Smith AV, et al. Biological, clinical and population relevance of 95 loci for blood lipids. Nature 2010;466:707-13.

16. Iacocca MA, Wang J, Dron JS, et al. Use of next-generation sequencing to detect LDLR gene copy number variation in familial hypercholesterolemia. J Lipid Res 2017;58:2202-9.

17. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from next-generation sequencing data. Nucleic Acids Res 2010;38:e164.

18. Iacocca MA, Chora JR, Carrié A, et al. ClinVar database of global familial hypercholesterolemia-associated DNA variants. Hum Mutat 2018;39:1631-40.

19. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405-24.

20. Davignon J, Roy M. Familial hypercholesterolemia in French-Canadians: taking advantage of the presence of a “founder effect”. Am J Cardiol 1993;72:6D-10D.

21. Lix LM, Ayles J, Bartholomew S, et al. The Canadian Chronic Disease Surveillance System: a model for collaborative surveillance. IJPDS 2018;3:3-5.

22. Berberich AJ, Hegele RA. The complex molecular genetics of familial hypercholesterolemia. Nat Rev Cardiol 2019;16:9-20.

23. Wiegman A, Gidding SS, Watts GF, et al. Familial hypercholesterolaemia in children and adolescents: gaining decades of life by optimizing detection and treatment. Eur Heart J 2015;36:2425-37.

24. Dron JS, Hegele RA. Polygenic influences on dyslipidemias. Curr Opin Lipidol 2018;29:133-43.

25. Dron JS, Hegele RA. The evolution of genetic-based risk scores for lipids and cardiovascular disease. Curr Opin Lipidol 2019;30:71-81.

26. Kindt I, Mata P, Knowles JW. The role of registries and genetic databases in familial hypercholesterolemia. Curr Opin Lipidol 2017;28:152-60.

27. Ng DM, Hooper AJ, Bellgard MI, Burnett JR. The role of patient registries for rare genetic lipid disorders. Curr Opin Lipidol 2018;29:156-62.

Supplementary Material

To access the supplementary material accompanying this article, visit CJC Open at https://www.cjcopen.ca/ and at https://doi.org/10.1016/j.cjco.2019.06.001.