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Original Article

Genetic Determinants of Myocardial Infarction Risk in Familial Hypercholesterolemia

Pei Jun Zhao, MD,[a] Matthew R. Ban, BSc,[b] Michael A. Iacocca, MSc,[b] Adam D. McIntyre, BSc,[b] Jian Wang, MD,[b] and Robert A. Hegele, MD[a,b,c]

[a] *Department of Medicine, Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada*

[b] *Robarts Research Institute, Western University, London, Ontario, Canada*

[c] *Department of Biochemistry, Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada*

ABSTRACT

Background: Familial hypercholesterolemia (FH) is an inherited condition of elevated serum low-density lipoprotein (LDL) cholesterol leading to premature coronary heart disease. We evaluated whether FH mutations are independently associated with the development of myocardial infarction (MI), after adjusting for LDL cholesterol level and clinical risk factors.

Methods: In 182 unrelated patients from different families referred with clinically suspected FH, targeted next-generation DNA sequencing was performed on 73 lipid-related genes and 178 single nucleotide polymorphisms, at 300-times mean read depth, to identify monogenic mutations and high-risk single nucleotide polymorphisms.

Results: Pathogenic FH mutations were identified in 27% of patients. Patients with mutations, compared with those without, were 12 years younger when referred to the lipid clinic ($P < 0.001$) and had higher baseline and post-treatment LDL cholesterol by 1.11 mmol/L ($P < 0.001$) and 0.62 mmol/L ($P = 0.01$), respectively. The hazard ratio for premature MI with respect to having an FH mutation, controlling for sex, hypertension, body mass index, diabetes, LDL cholesterol, and smoking, was 4.51 ($P = 0.002$).

RÉSUMÉ

Introduction : L'hypercholestérolémie familiale (HF) est une maladie héréditaire caractérisée par des concentrations élevées de lipoprotéines de faible densité (LDL) qui entraîne de manière précoce la maladie coronarienne. Nous avons évalué si les mutations propres à la HF sont indépendamment associées au développement de l'infarctus du myocarde (IM) après l'ajustement des concentrations de cholestérol LDL et des facteurs de risque cliniques.

Méthodes : Chez les 182 patients non apparentés de différentes familles envoyés en consultation en raison d'une suspicion clinique de HF, nous avons réalisé le séquençage ciblé de l'ADN de nouvelle génération de 73 gènes du métabolisme des lipides et 178 polymorphismes mononucléotidiques, à une profondeur de lecture moyenne de 300 fois, pour déterminer les mutations monogènes et les polymorphismes mononucléotidiques à haut risque.

Résultats : Nous avons déterminé les mutations pathogènes à l'origine de la HF de 27 % des patients. Comparativement aux patients qui ne présentaient pas de mutations, les patients qui en présentaient étaient 12 ans plus jeunes lorsqu'ils étaient envoyés en consultation à la clinique des lipides ($P < 0,001$) et avaient des concentrations

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.[1] 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.[2,3] Globally, FH affects approximately 1 in 250 people,[4,5] with a higher prevalence in Quebec.[6] However, FH remains underdiagnosed and undertreated in the general population.[7]

With advances in genetic testing, the yield of finding a genetic cause in patients referred with suspected FH is up to 67%[8] depending on the patient cohort. There is also considerable genetic diversity within FH that is associated with variable clinical outcomes.[9] 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.[10,11] Moreover, the degree of atherosclerosis is higher in patients with monogenic FH compared with others.[12] But without a comprehensive FH database, previous studies could not

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Ethics Statement: This research has adhered to relevant ethical guidelines (Western University Research Ethics Board reference number 07920E).

Corresponding author: Dr Robert A. Hegele, Robarts Research Institute, 4288A-1151 Richmond Street North, London, Ontario N6A 5B7, Canada. Tel.: +1-519-931-5271; fax: +1-519-931-5218.

E-mail: hegele@robarts.ca

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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.

respectives plus élevées de cholestérol LDL de 1,11 mmol/l ($P < 0,001$) et de 0,62 mmol/l ($P = 0,01$), au début et après le traitement. Le rapport de risque d'IM prématuré par rapport à la présence d'une mutation à l'origine de la HF, qui tient compte du sexe, de l'hypertension, de l'indice de masse corporelle, du diabète, du cholestérol LDL et de la consommation du tabac, était de 4,51 ($P = 0,002$).
Conclusion : La HF est une maladie diversifiée sur le plan génétique. Les mutations à l'origine de la HF sont indépendamment associées à un risque plus élevé d'IM prématuré chez les patients envoyés en consultation en raison d'une hypercholestérolémie. Par conséquent, le génotypage pourrait être utile à la stratification du risque cardiovasculaire dans le traitement personnalisé de la HF.

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).[13,14] 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 barcoded 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, ABCG5/8, NPC1L1, STAP1, SORT1, MYLIP*), and 178 SNPs associated with lipid traits.[14,15] 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.[16] 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.[17]

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.[18] When conflicting evidence was present, the variant was manually reviewed, and American College of Medical Genetics guidelines were enforced.[19]

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.[6] 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.[20] 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, $P < 0.001$

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

	Female (N = 102)	Male (N = 80)	*P* value
Age (y)	50.7 ± 17.0	44.9 ± 14.5	0.015
Total cholesterol (mmol/L)	8.66 ± 1.63	8.15 ± 1.68	0.041
Triglycerides (mmol/L)	1.93 ± 1.00	1.89 ± 0.77	0.79
HDL cholesterol (mmol/L)	1.43 ± 0.36	1.25 ± 0.35	< 0.001
LDL cholesterol (mmol/L)	6.35 ± 1.53	6.04 ± 1.62	0.18
Cholesterol-lowering medication:			0.72
None	95 (93.1%)	72 (90.0%)	
Low Intensity	4 (3.9%)	4 (5.0%)	
High Intensity	3 (2.9%)	4 (5.0%)	
BMI (kg/m^2)	27.2 ± 5.5	27.5 ± 4.8	0.67
Hypertension	28 (27.5%)	23 (28.7%)	0.85
Diabetes mellitus	10 (9.8%)	7 (8.8%)	0.81
Smoking	10 (9.8%)	18 (22.5%)	0.018
Family history of premature MI	52 (51.0%)	43 (53.8%)	0.71
MI (nonfatal)	10 (9.8%)	18 (22.5%)	0.018
Stroke/TIA (nonfatal)	3 (2.9%)	4 (5.0%)	0.47
Other arterial diseases*	6 (5.9%)	4 (5.0%)	0.80
FH mutation found	24 (23.5%)	25 (31.3%)	0.24
LDL after treatment	3.30 ± 1.48	3.11 ± 1.41	0.38

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

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

*Includes documented coronary artery disease (excluding MI), carotid stenosis, abdominal aortic aneurysm, and peripheral vascular disease requiring arterial bypass.

(Pearson chi-square test). The yield of finding a mutation roughly doubles with every level increase in clinical FH certainty. Mutation details are shown in Supplemental Table S4.

The probability distribution of carrying an FH mutation, plotted against baseline LDL cholesterol level and age at referral are shown in Supplemental Figures S1 and S2. The probability of finding a mutation increased as serum LDL cholesterol increased. Approximately half of patients had a mutation when LDL cholesterol was > 7.0 mmol/L (Supplemental Fig. S1). In contrast, the probability of carrying an FH mutation decreased with increasing age at referral. For patients age 40 years or younger, approximately half carried a mutation (Supplemental Fig. S2).

FH mutation status was associated with a documented family history of premature MI. The risk of finding a mutation for patients with a positive family history was 2.1-fold

Table 2. FH mutations identified in this study

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 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.

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/m^2 ($P = 0.006$) 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 4. Clinical characteristics according to whether a FH mutation was identified on DNA sequencing

FH mutation identified	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/m^2)	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%)	0.48
MI (nonfatal)	9 (18.4%)	19 (14.3%)	0.50
Premature MI (nonfatal)*	9 (18.4%)	16 (12.0%)	0.27
LDL cholesterol after treatment (mmol/L)	3.67 ± 1.71	3.05 ± 1.31	0.024

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 infraction; 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.

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]

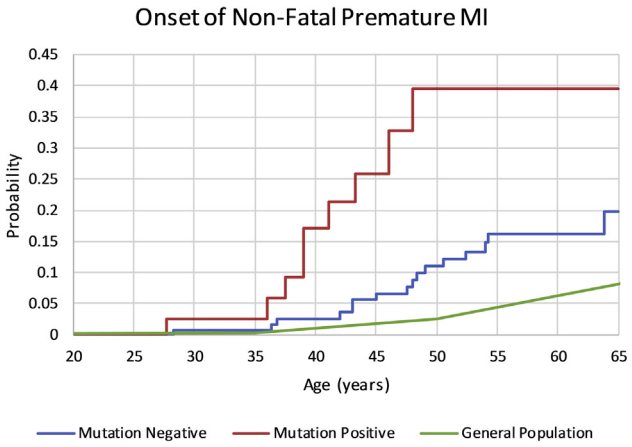

Figure 1. Risk of nonfatal premature myocardial infarction (MI) vs age of event onset. This graph represents the proportion of patients with familial hypercholesterolemia (FH) referred for secondary prevention of cardiovascular disease. To avoid multiplicity, patients who experienced more than 1 event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

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 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.

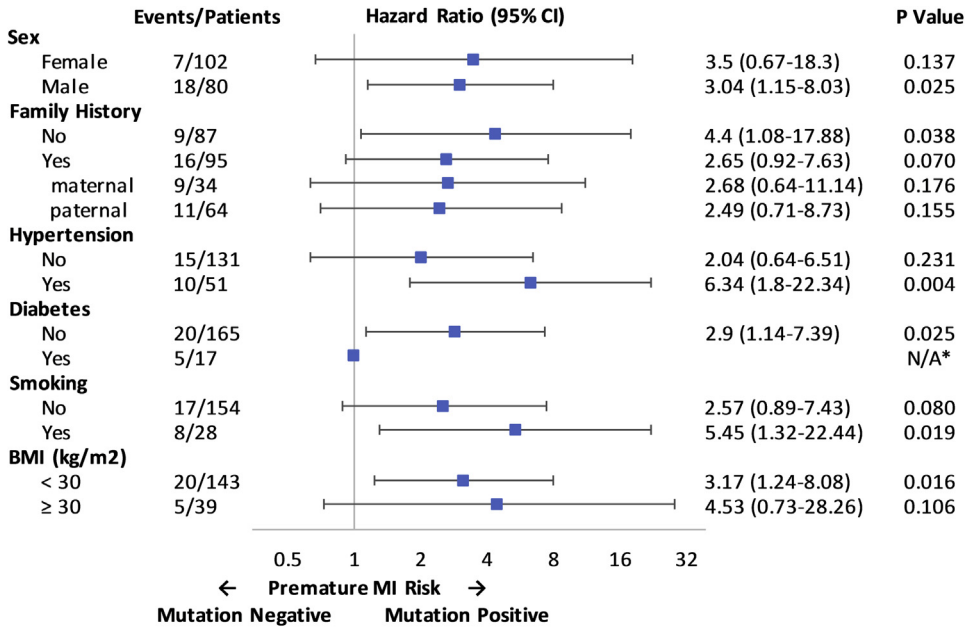

Figure 2. Hazard ratios (HRs) of premature MI, according to FH mutation status, for various subsets of the study population. Findings are based on univariable Cox proportional-hazards regression models. *Sample size for the diabetes group was too small and violated the positivity assumption. Also note that there were a few patients with both maternal and paternal family history of premature MI. BMI, body mass index; CI, confidence interval; MI, myocardial infarction; N/A, not available.

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.

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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.

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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.