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Applications of Genetic Testing for Endocrine and Metabolic Disorders

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A thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in Biochemistry
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Abstract:

Knowledge of inherited diseases and the ability to rapidly, efficiently and comprehensively perform genetic testing are advancing steadily. However, the ideal approach to translate this ability into clinical applications for endocrine disorders has yet to be determined. This work focuses on aspects of clinically translating knowledge of select heritable endocrine and metabolic conditions.

For maturity onset diabetes of the young (MODY), a monogenic disorder with no current consensus guidelines governing testing procedures, this work addresses methods to improve detection by validating the use of next generation sequencing-based techniques to identify MODY cases and to detect copy number variations.

For very severe hypertriglyceridemia, a largely polygenic trait, this work explores clinical differences associated with the underlying genotype, assesses treatment of pancreatitis, the most severe acute complication of hypertriglyceridemia, and presents a population-based study of Ontario adults to identify the most important modifiable risk factors associated with expression of hypertriglyceridemia, and to identify any gaps in appropriate care for this population.

For heterozygous familial hypercholesterolemia, a condition for which universal genetic screening has been recommended, this work explores the personal impact of this diagnosis on the patient in terms of quality of life, lifestyle and self-care habits.

The ultimate goal of this project is to expand the available knowledge on how best to translate the laboratory ability and findings into the clinical realm for these select endocrine and metabolic conditions.

Keywords:

Genetics, Maturity onset diabetes of the young (MODY), Hypertriglyceridemia, Familial chylomicronemia, Familial hypercholesterolemia, Endocrinology, Copy number variations, Polygenic risk scores, Personalized medicine, Genetic testing, Dyslipidemia, pancreatitis

Summary for lay audience

Understanding of the role that genetics plays in human diseases and the technology available to investigate this influence is increasing. With this knowledge comes the opportunity to improve the care of clinic patients by incorporating this new information into their care and treatment plan. In this work, I explore several aspects related to the care of endocrine and metabolic conditions that have genetic components.

I aimed to improve the ability to detect and diagnose individuals suspected of having an inherited form of diabetes called maturity onset diabetes of the young (MODY). I did this by examining ways to improve the testing process for these conditions by using newer technology and software to avoid missing cases.

I also aimed to improve care and counselling for patients with very high levels of a blood lipid called triglycerides. To accomplish this, I examined three separate populations. I looked at a group of patients with a rare condition called familial chylomicronemia syndrome (FCS), caused by different DNA changes, to assess if there are differences in their physical symptoms or bloodwork. Secondly, I looked at adults with very elevated triglyceride levels in Ontario, Canada to evaluate factors that can contribute to these severe triglyceride elevations. Lastly, I looked at management of pancreatitis, a condition that can be caused by very high levels of triglycerides to assess what happens naturally to patients with this complication with no active interventions to lower triglycerides.

Finally, I aimed to assess the positive and negative impacts of genetic testing on the patient. To do this, individuals with elevated cholesterol who underwent genetic testing were asked to participate in a survey assessing their experience and perceptions.

Ultimately, the goal of this project is to improve the ability of doctors to use genetic information in the clinic to provide more personalized and optimized care.

Co-Authorship statement:

Chapter 2.2: I was responsible for reviewing and interpreting the findings for this manuscript. I was responsible for the majority of the manuscript preparation (>90%). My supervisor, Dr. Robert A. Hegle provided supervision and guidance throughout, funding support, and assisted in data interpretation, manuscript preparation and revisions. Technical support was provided by core members of the Hegele Lab including Dr. Jian Wang for validating the variant calls for the study subjects, Adam D. McIntyre for extracting and isolating DNA from patient samples, Dr. Henian Cao, for preparing DNA samples for sequencing; John F. Robinson for project management and Matthew R. Ban for lab management.

Chapter 2.3: I was responsible for reviewing and interpreting the findings for these manuscripts. I was responsible for the majority of the manuscript preparation (>90%). My supervisor, Dr. Robert A. Hegle provided supervision and guidance throughout, funding support, and assisted in data interpretation, manuscript preparation and revisions. Clinical information, patient samples and manuscript input were provided by Dr. Tamara Spaic, Dr. David B Miller, Dr. Susan Stock, Dr. Celine Huot, Dr. Robert Stein, and Dr. Arati Mokashi. Technical support was provided by core members of the Hegele Lab including Dr. Jian Wang for validating the CNvs for the study subjects, Adam D. McIntyre for extracting and isolating DNA from patient samples, Dr. Henian Cao, for preparing DNA samples for sequencing; John F. Robinson for project management, Matthew R. Ban for lab management, Ericka Simon for providing clinical information, Brooke Kennedy for maintaining ethical protocols, approval and managing patient consents and Drs. Joan H Knoll and Ping Yang for cytogenetic analysis.

Chapter 3.2: I was responsible for reviewing and interpreting the data for this project. I was responsible for the majority of manuscript preparation (>60%). My supervisor, Dr. Robert A. Hegele contributed to manuscript preparation, provided supervision and guidance throughout, and assisted in data interpretation and manuscript revisions. Study samples and laboratory information were obtained and provided as part of an ongoing clinical trial with the involvement of Dr. Andres Digenio, Dr. Veronica J Alexander, Dr. Laura D;Erasmo,

Dr. Marcello Arca, Dr. Alan Jones, Dr. Eric Bruckert, Dr. Erik S Stroes, Dr. Jean Bergeron, Dr. Fernando Civeira, Dr. Joseph l Witztum and Dr. Daniel Gaudet. These authors additionally provided critical feedback and helped shape the research, analysis and manuscript. Technical support was provided by core members of the Hegele Lab including Dr. Jian Wang for validating the variant calls for the study subjects, Adam D. McIntyre for extracting and isolating DNA from patient samples, Dr. Henian Cao, for preparing DNA samples for sequencing; John F. Robinson for project management and Matthew R. Ban for lab management.

Chapter 3.3: I conceived this project, and helped develop the protocol. I conducted the majority of the data interpretation and manuscript preparation (>90%). My supervisor, Dr. Robert A. Hegle provided supervision and guidance throughout, funding support, and assisted in study design, manuscript preparation and revisions. Dr. Kristin K Clemens planned the protocol through ICES, with input and supervision from Salimah Z. Shariff. Alexandra M. Ouedaogo conducted the analysis and statistical summary with support from Salimah Z. Shariff.. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Chapter 3.4: I was responsible for obtaining and verifying data from chart review, interpretation of the data and manuscript preparation (>85%). My supervisor, Dr. Robert A. Hegle provided supervision and guidance throughout, and assisted in study design, manuscript preparation and revisions. Dr. Ahmed Ziada assisted in chart review,subject identification and data collection. Statistical support was provided by Dr. Guangyong Zou.

Chapter 4.2: I conceived of this study and was responsible for drafting and submission of protocol and obtaining ethics approval. I conducted the majority of the data analysis and manuscript preparation (>90%). My supervisor, Dr. Robert A. Hegele provided supervision and guidance throughout, provided access to the patient population for the study, and assisted with revisions to the study design and manuscript. Brooke Kennedy and Colin Chan assisted with patient recruitment and data input. Colin Chan additionally assisted with data entry, analysis and chart review. Statistical support was provided by Steve H. Lee.

Epigraph:

"There are only two ways to live your life. One is as though nothing is a miracle. The other is as though everything is a miracle."

-Albert Einstein

Dedication:

To my family, with sincere gratitude

Acknowledgments:

I would like to take the opportunity to recognize the many people who have helped me reach this final goal in my graduate studies. This accomplishment would not have been possible without the support, guidance and encouragement of my family, mentors, colleagues and friends.

Firstly, Dr. Hegele, your mentorship has been invaluable. It has truly been an honour to have the opportunity to work with you and learn from your example. You have been a constant source of guidance and support from the first time I met with you as a first year internal medicine resident. Your ability to balance a world-class research career while also being an excellent, dedicated and compassionate clinician is inspiring. But beyond that, it is your patience, kindness and ability to see and seek the best in people that I most strive to emulate.

Thank you to my graduate committee members, Dr. Murray Huff and Dr. Richard Kim, your constructive guidance has been instrumental in helping me to reach this point. I am grateful for your feedback and support throughout the last few years.

To all the Hegele Lab members, especially Jian Wang, Adam McIntyre, Henian Cao, Matthew Ban, John Robinson, Ericka Simon and Brooke Kennedy, your help and assistance throughout my time at Robarts was substantial and greatly appreciated. You were all consistently generous with your time and willing to help whenever possible. I could not have gotten to this point without your efforts and contributions.

Dr. Stan Van Uum and my other clinical mentors at in the SJHC Endocrine group, I would like to thank you all for everything I learned through your mentorship. Especially to Dr. Van Uum, your support as my clinical supervisor, and ongoing support and advocacy as Division Chair has been invaluable, and I am very grateful for the opportunity to learn from your excellent example as a clinician and teacher.

I would also like to thank and acknowledge my clinical collaborators from SJHC and other centers, Dr. Tamara Spaic, Dr. David Miller, Dr. Susan Stock, Dr. Celine Huot, Dr. Robert Stein, and Dr. Arati Mokashi, for providing clinical context as well as valued input on the findings reported in Chapter 2.

While I spent less time with many of you than I would have liked, I would also like to thank my fellow graduate students, especially Jaqueline, Allison, Julieta, Rosettia and Michael for all your help, kindness and contributions to my work. I would also like to extend a special thank you to Colin Chan for all his help and hard work in patient recruitment and data collection for the work presented in Chapter 4.

Mom and Dad, I would like to thank you to you both for being an unfailing rock of support and encouragement for me and for always being there whenever I needed an ear, a shoulder or an extra set of hands. Jenn and Laura, thank you for being the best sisters and friends that I could ask for. Michael, thank you for your encouragement, kindness, support and for always picking up the slack when the going got tough.

To Ian, Alex and Cassie thank you for your patience when 'mommy had to work'. More importantly, thank you for filling my days with laughter, love and joy, and making every day worthwhile.

Funding Acknowledgements:

Part of this work was funded through the support of the Dr. Fernand Labrie Fellowship Research Grant from the Canadian Society for Endocrinology and Metabolism

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List of Abbreviations, Symbols, Nomenclature:

***ABCC8**:* gene encoding ATP-binding cassette, subfamily c, member 8
ACMG: American College of Medical Genetics and Genomics
AD: autosomal dominant
ACE: Angiotensin-converting enzyme
AHA: American Heart Association
AIDS: Acquired immunodeficiency syndrome
ALT: alanine aminotransferase
***ANGPTL3*:** gene encoding angiopoietin-like protein 3
ANOVA: Analysis of variance
Apo: apolipoprotein
***APOA5*:** gene encoding apolipoprotein A-V;
APOB, gene encoding apolipoprotein B
APOC2: gene encoding apolipoprotein C-II
APOE: gene encoding apolipoprotein e
***APPL1*:** gene encoding adaptor protein, phosphotyrosine interaction, ph domain and leucine zipper-containing protein 1
AR:autosomal recessive
ARB: Angiotensin receptor blockers
ASCVD: atherosclerotic cardiovascular disease
ASSEDA: Automated Splice Site and Exon Definition Analyses
BAM: Binary alignment map
***BLK*:** gene encoding blk protooncogene, src family tyrosine kinase
BMI: body mass index
bp: base pair
BRCA: breast cancer
C: cholesterol
Ca+: Calcium
CADD: Combined Annotation Dependent Depletion
CAPE: Client Agency Program Enrolment
***CASR*:** gene encoding calcium-sensing receptor
***CCDC22*:** gene encoding coiled-coil domain-containing protein 22
CCI: Canadian Classification of Health Interventions
CDA: Canadian Diabetes Association
CE: cholesterol ester
***CEL*:** gene encoding carboxyl-ester lipase
CESD: cholesteryl ester storage disease
CETP: cholesterol ester transport protein
CHR: chromosome;
CI: confidence intervals
CIHI-DAD: Canadian Institutes for Health Information's Discharge Abstract Database
***CILP2*:** gene encoding cartilage intermediate layer protein 2;
CKD: chronic kidney disease

CNV: Copy number variation
CYP26A1**:** gene encoding cytochrome P450 26A1;
ddNTPs: dideoxyribonucelotides
DGAT: diacylglycerol O-acyltransferase;
DHA: docosahexaenoic acid
DIN: Drug Identification Number
DKA: diabetic ketoacidosis
DLCN: Dutch Lipid Clinic Network
DNA: deoxyribonucleic acid
dNTPs: deoxyribonucelotides
DOC: depth of coverage
DPP-4: Dipeptidyl peptidase-4
EPA: Eicosapentaenoic acid
ER: endoplasmic reticulum
ERN: extended-release preparations of niacin
EU: European Union
EVS: Exome Variant Server
ExAC: Exome Aggregation Consortium.
FABP: fatty acid-binding protein;
FAS: fatty acid synthase
FCS: Familial chylomicronemia
FFA: free fatty acids;
FH: familial hypercholesterolemia
FHH: familial hypocalciuric hypercalcemia
FP: family physician
GAD-7:Generalized anxiety disorder-7
GCK: gene encoding glucokinase
GCKR*:* gene encoding glucokinase regulatory protein
GDM: gestational diabetes
GI: gastrointestinal
GLP-1: glucagon-like peptide 1
GLUT2: glucose transporter 2
GP: General practitioner
GPIHBP1*:* gene encoding glycosylphosphatidylinositol-anchored high density lipoprotein binding protein 1
GWAS: genome-wide association studies
HbA1c: Glycated hemoglobin
HDL: high density lipoprotein
HeFH: heterozygous FH
HGMD: Human Genome Mutation Database
HIPPA: Health Information Patient Protection Act
HIV: Human immunodeficiency virus
HLA: human leukocyte antigen
HLP: hyperlipoproteinemia

HMG-CoA: hydroxyl-methyl-glutaryl coenzyme A
***HNF1A*:** gene encoding hepatocyte nuclear factor-1-alpha
***HNF1B*:** gene encoding hepatocyte nuclear factor-1-beta
***HNF4A**:* gene encoding hepatocyte nuclear factor 4-alpha
HoFH: homozygous FH
HTG: Hypertriglyceridemia
HTN: hypertension
HYPER: hypertension database
ICD-10: International Classification of Diseases 10th Revision (ICD-10)
IDL: intermediate density lipoproteins
IKG: 1000 Genomes Project
Indel: insertion/deletion
INS IV: Insulin infusion
***INS**:* gene encoding insulin
IQR: interquartile range
***KCNJ11*:** gene encoding potassium channel, inwardly rectifying, subfamily j, member 11
***KLF11*:** gene encoding kruppel-like factor 1
***KLHL8*:** gene encoding Kelch like protein 8;
LDL: low density lipoprotein
***LDLR**:* gene encoding low density lipoprotein receptor
***LDLRAP1*:** gene encoding low density lipoprotein receptor adaptor protein 1
***LIPA**:* gene encoding lysosomal acid lipase
***LMF1**:* gene encoding lipase maturation factor 1
***LPL*-FCS**: FCS due to homozygous *LPL* mutations
***LPL*,** gene encoding lipoprotein lipase;
LRGC: London Regional Genomics Centre
LRP1: gene encoding LDL receptor related receptor 1
MAF: minor allele frequencies
MAQ: simple medication adherence questionnaire
MASS-8: Eight-Item Morisky Medication Adherence Scale
MCTG: medium chain triglycerides
MED-PED: Make Early Diagnosis-Prevent Early Death
MI: myocardial infarction
MLPA: multiplex dependent primer amplification
MLXIPL, gene encoding MLX interacting protein-like 1;
MODY: maturity onset diabetes of the young
MTTP: microsomal triglyceride transfer protein
MutNeg: mutation negative
***MYLIP**:* gene encoding myosin regulatory light chain-interacting protein
n: number of individuals
NACRS: National Ambulatory Care Reporting System
***NEUROD1*:** gene encoding neurogenic differentiation 1
NGS: Next generation sequencing
Non-*LPL*-FCS: FCS due to homozygousity or compound heterozygousity of a non-LPL gene

***NPC*:** gene encoding NPC intracellular cholesterol transporter 1 and 2
NPC1L1: Niemann-Pick C1 like 1
NPO: withholding of all oral intake
ODB: Ontario Drug Benefit
ODD: Ontario Diabetes Database
OGTT: oral glucose tolerance test
OHA: oral antihyperglycemic agent
OHIP: Ontario Health Insurance Program
OLIS: Ontario Laboratories Information System
OMIM: Online Mendelian Inheritance in Man
ON: Ontario
OR: odds ratio
***PAX4*:** gene encoding paired box gene 4
PCR: Polymerase Chain Reaction
PCSK9: proprotein convertase subtilisin kexin 9
***PDX1*:** gene encoding pancreatic and duodenal homeobox 1
PIPEDA: Personal Information Protection and Electronic Documents Act
PolyPhen2: Polymorphism Phenotype Version 2
Pop.: population
PPAR: peroxisome proliferator-activated receptor
PRS: polygenic risk score
PTH: parathyroid hormone
PUFA: omega-3 fatty acids
PVD: peripheral vascular disease
QALY: quality adjusted life years
RAB5: ras-associated protein 5
RECORD: REporting of studies Conducted using Observational Routinely-collected Data
REDCap: Research Electronic Data Capture
RPDB: Registered Persons Database
rsID: Reference SNP identification number
SAS: statistical analysis software
S-HTG: severe hypertriglyceridemia
SBR: Simon Broome Register
SD: standard deviation
SF-12: Health Related Quality of Life measure, short form 12
SIFT: sorting intolerant from tolerant
SLIQ: simple lifestyle indicator questionnaire
SNP: single nucleotide polymorphisms
SNV: single nucleotide variant
SPANR: Splicing Based Analysis of Variants
SREBP2: gene encoding sterol regulatory element binding protein 2
STAP1: gene encoding signal transducing adaptor family member 1
StDiff: standardized differences
STROBE: STrengthening the Reporting of OBservational studies in Epidemiology

SUs: sulfonylureas
TC: total cholesterol
TG: triglyceride
***TRIB1*:** gene encoding Tribbles homolog 1
TSH*: thyroid stimulating hormone*
TZD: thiazolidinediones
UCL: University College London
USA: United States of America
VCF: variant call format
VLDL: very-low-density lipoprotein;
VS-HTG: very severe hypertriglyceridemia
VUS: variants of unknown significance
WES: whole exome sequencing
WGS: whole genome sequencing
WHO: World Health Organization
Yrs: years

Chapter 1: Introduction

Some of the work incorporated in Chapter 1 has been edited from original manuscripts listed below for brevity and consistency throughout this dissertation:

Brahm A, Hegele RA. Hypertriglyceridemia. Nutrients. 2013 Mar 22;5(3):981-1001. doi: 10.3390/nu5030981. PMID: 23525082

Brahm AJ, Hegele RA. Chylomicronaemia--current diagnosis and future therapies. Nat Rev Endocrinol. 2015 Jun;11(6):352-62. doi: 10.1038/nrendo.2015.26. Epub 2015 Mar 3. PMID: 25732519.

Brahm AJ, Hegele RA. Combined hyperlipidemia: familial but not (usually) monogenic. Curr Opin Lipidol. 2016 Apr;27(2):131-40. doi: 10.1097/MOL.0000000000000270. PMID: 26709473.

Brahm AJ, Wang G, Wang J, McIntyre AD, Cao H, Ban MR, Hegele RA. Genetic Confirmation Rate in Clinically Suspected Maturity-Onset Diabetes of the Young. Can J Diabetes. 2016 Dec;40(6):555-560. doi: 10.1016/j.jcjd.2016.05.010. Epub 2016 Sep 12. PMID: 27634015.

Berberich AJ, Hegele RA. The complex molecular genetics of familial hypercholesterolaemia. Nat Rev Cardiol. 2019 Jan;16(1):9-20. doi: 10.1038/s41569-018-0052-6. PMID: 29973710.

Berberich AJ, Hegele RA. The role of genetic testing in dyslipidaemia. Pathology. 2019 Feb;51(2):184-192. doi: 10.1016/j.pathol.2018.10.014. Epub 2018 Dec 14. PMID: 30558903.

Berberich AJ, Wang J, Cao H, McIntyre AD, Spaic T, Miller DB, Stock S, Huot C, Stein R, Knoll J, Yang P, Robinson JF, Hegele RA. Simplifying Detection of Copy-Number Variations in Maturity-Onset Diabetes of the Young. Can J Diabetes. 2021 Feb;45(1):71-77. doi: 10.1016/j.jcjd.2020.06.001. Epub 2020 Jun 8. PMID: 33011132.

Berberich AJ, Huot C, Cao H, McIntyre AD, Robinson JF, Wang J, Hegele RA. Copy Number Variation in GCK in Patients With Maturity-Onset Diabetes of the Young. J Clin Endocrinol Metab. 2019 Aug 1;104(8):3428-3436. doi: 10.1210/jc.2018-02574. PMID: 30912798; PMCID: PMC6594302.

Hegele RA, **Berberich AJ**, Ban MR, Wang J, Digenio A, Alexander VJ, D'Erasmo L, Arca M, Jones A, Bruckert E, Stroes ES, Bergeron J, Civeira F, Witztum JL, Gaudet D. Clinical and biochemical features of different molecular etiologies of familial chylomicronemia. J Clin Lipidol. 2018 Jul-Aug;12(4):920-927.e4. doi: 10.1016/j.jacl.2018.03.093. Epub 2018 Apr 4. PMID: 29748148.

Berberich AJ, Ouédraogo AM, Shariff SZ, Hegele RA, Clemens KK. Incidence, predictors and patterns of care of patients with very severe hypertriglyceridemia in Ontario, Canada: a population-based cohort study. Lipids Health Dis. 2021 Sep 3;20(1):98. doi: 10.1186/s12944-021-01517-6. PMID: 34479547;

Berberich AJ, Mokashi A, McIntyre AD, Robinson JF, Cao H, Wang J, Hegele RA. Bioinformatic detection of copy number variation in HNF4A causing maturity onset diabetes of the young. Clin Genet. 2019 Oct;96(4):376-377. doi: 10.1111/cge.13599. Epub 2019 Jul 15. PMID: 31309534;

Berberich AJ, Ziada A, Zou GY, Hegele RA. Conservative management in hypertriglyceridemia-associated pancreatitis. J Intern Med. 2019 Dec;286(6):644-650. doi: 10.1111/joim.12925. Epub 2019 Jun 6. PMID: 31077464.

1.1 Overview:

Increased appreciation of genetics among clinicians, researchers and the general public parallels the recognition of its role in the development of many diseases. This enhanced understanding, along with the advent of new genetic testing technologies, and rapidly decreasing costs, allows for consideration of genetic testing for a number of clinical conditions with a suspected genetic etiology. However, the exact role and extent of genetic testing in these cases has not yet been clearly established. This work seeks to establish how knowledge of patient and disease genetics can be applied effectively in the adult endocrine clinic to impact patient management and improve patient satisfaction and outcomes.

This is explored here using three separate endocrine conditions as illustrative examples of how this can be effectively achieved. The first of these conditions is maturity onset diabetes of the young (MODY), a monogenic form of diabetes that is often misclassified as type 1 or type 2 diabetes. This work aims to improve detection rates by validating the use of next generation sequencing-based techniques and provider clinical suspicion of MODY to detect new cases and identify copy number variations.

As a second aim, this work seeks to improve the counselling and management of genetic triglyceride disorders by evaluating the differences in phenotypic expression for different molecular etiologies of familial chylomicronemia, the contributing secondary risk factors associated with expression of hypertriglyceridemia, and the conservative management of pancreatitis, the most severe acute complication of extreme hypertriglyceridemia.

Finally, this work aims to assess the personal impact of genetic testing results on individuals with high cholesterol who receive a diagnosis of familial hypercholesterolema in terms of levels of anxiety, health-related quality of life, perceived cardiovascular risk and healthy lifestyle changes.

1.2 Basics of genetic change:

1.2.1 Variant Types:

Several different forms of genetic variants, or mutations, exist and are abundant throughout the genome. These include single nucleotide variants (SNVs) or single nucleotide polymorphisms (SNPs), which are changes from the consensus DNA sequence at a single nucleotide, and may or may not be clinically relevant. Most commonly, an SNV will occur in a non-coding region of DNA and therefore be clinically silent. An SNV that occurs within or near a gene may be synonymous, coding for the same amino acid as the original nucleotide sequence, and would also be expected to be clinically silent. A change may lead to an amino acid substitution, which may or may not affect the functioning of the resultant protein. Several features of the substitution can influence the likely effect. Observable factors that make it more likely the change will have a significant clinical impact include: 1) the less similar an amino acid is to the one it replaces, 2) the more critical the region of the substitution is to the overall structure and function of the resultant protein, and 3) the less frequently a change is seen, or tolerated, at a given site in the genome. In some cases, the nucleotide change may result in the generation of a premature stop codon, effectively truncating the protein production early. This change is much more likely to be clinically significant. The SNV could also occur in a region involved in gene regulation or translation, such as a promotor region, or splice-site, that could affect protein production processes, leading to clinical effects.

A change that leads to either an insertion or deletion of a few contiguous nucleotides (generally between 2 and 50) is known as a small indel (insertion/deletion). These may be more likely to disrupt protein production, and therefore have a clinical effect, especially if they lead to a frameshift change (a change in the codon reading frame) of the nucleotide, causing every subsequent codon to be mis-read. Even if the indel does not shift the reading frame, insertion of new amino acids, or deletion of amino acids that normally appear in the mature protein, may affect protein structure and function, leading to clinical impact.

Copy number variations (CNVs) are large duplications or deletions, generally between 50 base pairs (bps) and 1 million bps. If affected regions include a gene, deletions may lead to loss of gene function. In the case of a duplication, extra copies of the gene product may also be seen. A duplication event may also lead to an insertion of the duplicated fragment of DNA within another gene, affecting the function of that gene product as well. Historically, CNVs have been difficult to detect on standard genetic tests or chromosomal analysis and have only recently become more recognized as an important cause of genetic variation and possibly genetic disease. In fact, between 5-10% of the entire human genome may represent CNVs (1).

1.2.2 Determining Pathogenicity:

Even when sequencing methods can detect rare single base-pair changes in a gene of interest that has been definitively linked to a disease, it does not automatically follow that this base-pair change is causally linked to the disease. Such DNA changes may be synonymous, meaning that they encode for the same amino acid as the base pair that was replaced, or they may be benign, in that the altered amino acid has no biological or clinically relevant effect on the structure or the function of the translated protein.

The highest level of evidence for the pathogenicity of a detected mutation is a functional study, usually performed in a research laboratory, where the mutation has been generated in an in vivo model and shown to result in some objective or quantitative change in an assay or model that is consistent with the observed clinical phenotype (2). However, this is time and labour intensive and is not feasible to do for most identified SNVs. Familial segregation analysis can also provide convincing evidence if the nucleotide change shows complete phenotype concordance; i.e. each family member who carries the variant also has the disorder (this type of evidence becomes statistically stronger as more family members are tested). Conversely, the presence of the mutation in healthy, phenotype-free individuals argues strongly against pathogenicity (2).

A second tier of evidence for potential clinical relevance is derived from "bioinformatics" or analysis using computational models. Here, well established prediction software tools employ complex but validated mathematical algorithms to rank the effect of the amino acid change based on the degree of evolutionary conservation of the wild-type amino acid, and the predicted 3-D structure of the resultant protein, and other circumstantial information to gauge pathogenicity (2). These also include the rarity of the mutation. Generally any change that is seen in at least 1% of the population is considered common enough that it is unlikely to be pathogenic. However, these methods are not clear-cut and sometimes give inconsistent results for the same mutation. Even when these contentious variants are manually adjudicated by human genetic experts, there can be dissention regarding the pathogenicity of a given base pair change. This underscores the perhaps surprising subjectivity that underlies some genetic testing, and emphasizes the importance of having highly trained, knowledgeable and skilled individuals available to help interpret non-definitive genetic testing results, both to avoid incorrectly assigning pathogenicity to a benign change or conversely ascribing neutral functionality to a change that is actually pathogenic.

1.2.3 Classifying a variant:

In an attempt to standardize variant calling, the American College of Medical Genetics and Genomics (ACMG) created a consensus method for determining pathogenicity of variants, with 5 classifications possible: “pathogenic”, “likely pathogenic”, “likely benign”, “benign”, and “uncertain significance” (3). However, even variants classified using this scoring system could be re-classified at a later date if new information comes to light through research, new discovery or the incorporation of data from additional family members or probands. This is especially true for variants of uncertain significance (VUSs). There can also be an element of subjectivity when using the scoring system that could lead two different investigators to assign different pathogenicity ratings to the same variant.

1.3 Genetic Sequencing:

1.3.1 Polymerase Chain Reaction:

Most currently available sequencing technologies depend first upon achieving amplification of available DNA to create multiple copies of the starting DNA sequence that can then be used to determine the genetic sequence.   The most common way this is achieved is through use of the polymerase chain reaction (PCR). The principle behind PCR is to take advantage of the natural replicative ability of DNA through the production of complementary strands. This involves separating the DNA into two single strands, and creating a complementary stand for each of the two separated DNA chains. Repeating this process with the newly produced double-stranded DNA fragments will copy the sample exponentially to produce millions of copies of the target fragment.

1.3.2 Sanger sequencing:

The first sequencing technology that was capable of determining the genetic sequence for a large chain of DNA was Sanger sequencing, developed in the mid-1970's by Frederick Sanger (4). In Sanger sequencing, PCR is conducted with a mixture of normal deoxyribonucelotides (dNTPs), along with a small proportion of chain-terminating dideoxyribonucelotides (ddNTPs). As ddNTPS lack the 3'-OH group necessary to form a phosphodiester bond with the next dNTP in sequence, when these ddNTPS are incorporated at random in a PCR chain elongation cycle, the extension of the chain will prematurely terminate, leading to a shortened strand, which will then go on to be amplified in its shortened state during the next cycle.

This process is repeated at random enough times that a library of oligonucleotide chain lengths is produced, with representation of every chain length possible between a single nucleotide length up to the full length of the target region of DNA. The oligonucleotides are then physically separated by length using gel electrophoresis and each terminal nucleotide identified. In automatic Sanger sequencing, this process will generate an output with

different colour peaks in order corresponding to a colour-coded nucleotide sequence, known as a chromatogram.

This technique is best used to examine small segments of DNA at a time, usually a single gene or single exon. It allows for a high degree of DNA amplification. It is the most precise method to look for a single candidate mutation, typically a rare large-effect variant in a known gene causing a monogenic disorder. This method could be helpful in familial cascade screening for focussed assessment of the inheritance of a single, previously identified mutation. It has the added benefit of providing only the desired genetic result with a lower chance of incidental findings. This method is used both clinically and in research. For the latter application, Sanger sequencing is often used for 'gold standard' confirmation when other methods initially detect a potentially causative variant (5, 6).

1.3.3 Next generation sequencing (NGS):

Next-generation sequencing (NGS) refers to a variety of different methods, each of which use a massively parallel sequencing design to amplify and examine multiple segments of DNA concurrently (7). This technique is typically applied to detect a rare causative variant for a monogenic condition when many possible mutations exist, and can be used in a variety of ways: to sequence 1) a targeted selection of pre-specified genes, accomplished using a designed panel; 2) the complete collection of all expressed protein-coding sequences (‘whole exome sequencing’ or WES), which represents approximately 2% of the entire genome; or 3) whole genome sequencing (WGS) (8), which simultaneously amplifies all DNA sequences from an individual, including both coding and non-coding regions (7). Because most common SNPs reside within non-coding regions, WGS allows for both common and rare variants to be assayed, while WES is optimal for detecting rare coding variants. Targeted panels can be designed to both screen for rare coding variants and to concurrently evaluate non-coding regions containing selected common SNPs as part of a polygenic risk score. The number of these tests that have been clinically validated is on the rise, and the costs decreasing steadily (9). The current cost of this technology varies depending on how

many genes are concurrently assessed, with approximate costs of $300 for a targeted panel, $800-$1200 for a whole exome panel and $5000-$10,000 for a whole genome sequence (8).

1.3.4 Microarray Genotyping:

Genotyping refers to a range of dedicated types of inexpensive genetic testing methods that directly assay specific known rare variants or common SNVs across individuals. Several different technical and chemical platforms can be used to determine genotypes, from a few to a large set of pre-defined SNVs that have been previously associated with certain conditions of interest. High density genome-wide microarrays can be used in unbiased genome-wide association studies (GWAS) to discover associations among millions of SNV markers of chromosome regions and either quantitative or qualitative complex clinical traits (7).

A microarray chip contains up to hundreds of thousands of oligonucleotide probes arranged and immobilized in certain positions on the chip. Upon addition of fragmented, single-stranded DNA to the microarray and binding of target DNA sequence, florescence tags are released that can be detected by photosensors. The intensity and colour of the signal emitted helps identify which SNV is present. Because this technology is dependent on probes that are designed to look for and identify known SNVs, it cannot be used to detect novel or unknown genetic changes. However, it can be used to detect CNVs by looking for an abnormally high number or low number of SNP signals in contiguous SNPs. For example, in a deletion, the number of detected SNP fragments will be reduced by approximately half over the span of the deletion, whereas SNP fragment detection will be roughly 1.5 times the baseline SNV detection rate (or multiples thereof) for a duplication.

SNVs detected are often common variants that can be associated with genetic conditions, but may or may not actually be at the site of the pathogenic variant. Microarrays can also be used to detect known disease-causing variants at specific loci if the mutations are established as disease-causing and the microarray is designed to look for that variant. Once certain SNV genotypes have been definitely associated with clinical phenotypes, a

smaller, cost-effective panel of SNVs can be constructed using dedicated chemistry (e.g. TaqMan) or custom-made microarrays permitting high throughput cost-effective focused testing in clinical samples. Most commercially available direct-to-consumer genetic testing uses this technique, and thus provides information primarily on common SNVs that may determine susceptibility but are not directly causative for diseases.

1.4 Sequencing options for clinical applications:

1.4.1 Single gene sequencing:

This is the most selective genetic sequencing test. It is highly sensitive in detecting changes for the gene tested. Cost for this testing will vary based on type of test performed and the size of the gene. Single gene testing may be useful in a number of clinical settings, such as when there is a single candidate gene for the condition of interest, or to verify the presence or absence of a known familial mutation. Disadvantages include the lack of information on any other gene, which may result in higher costs if sequential single-gene tests are eventually required (Table 1.1).

1.4.2 Gene panel sequencing:

For this type of sequencing, the coding and surrounding regions of several selected genes are sequenced simultaneously. These can be designed to capture a number of genes associated with a particular condition of interest, for example dyslipidemias, epilepsy, developmental delay etc., or genes grouped together for another reason (ie commonly tested genes, genes of interest in a particular study protocol etc). Panels can be useful clinically when they can be focussed on a particular clinical question of interest, and they allow for selective information to be obtained without the risk for extraneous results. By focusing on only a few genes, panels also allow for more copies of the DNA sequence to be generated at each site, increasing the accuracy of variant calls. Panels are also generally less expensive than other multi-gene sequencing methods (Table 1.1).

1.4.3 Whole Exome Sequencing (WES);

This type of sequencing captures data from all exomes and surrounding regions (~2% of the entire genome). It is generally considerably more expensive than testing a gene panel and may also be less sensitive. It will also be more likely to generate incidental findings compared to targeted panel sequencing. Some advantages of this type of sequencing is that it allows researchers to evaluate multiple sequential gene hypotheses without needing to repeat a sequencing step. The data is also available if a new clinical concern arises in the future without the need for re-sequencing. Clinically, this sequencing may be useful when the genetic basis for a condition is poorly or incompletely understood, if multiple gene testing is desired but no panel is available, or if it is anticipated that the genetic basis for other conditions may be sought in the future (Table 1.1).

1.4.4 Whole Genome Sequencing (WGS):

This sequencing provides data on most of the entire genome (>99%), including coding and non-coding regions. Generally, this is an expensive and labour-intensive undertaking and may not provide much more clinically useful information compared to whole exome sequencing, as most clinically significant DNA changes will be seen within coding regions. The addition of non-coding sequence may allow for better examination of regulatory elements. This type of genetic sequencing is likely best for research endeavors rather than for regular clinical practices (Table 1.1).

1.4.5 DNA Microarray Genotyping:

Genotyping may be useful clinically as it can provide information of targeted select changes of interest and can be helpful to detect known genetic variants throughout the genome. Genotyping is also clinically validated to detect large duplications or deletions known as copy number variations (CNVs). This is done predominantly by looking for SNVs throughout the genome. Each genotyping array is designed to detect a pre-specified number of SNVs with known clinical consequences. It is important to note that this type of genetic testing

will not provide data on the exact DNA sequence and will be unable to detect novel changes, or any nucleotide changes that are not designed to be identified in the specific panel (Table 1.1).

1.4.6 Risk scores for polygenic traits:

Many inherited conditions are not due to a single genetic variant but instead are inherited through accumulation of several small-effect changes. These common allelic variants each contribute only fractionally to the development of the condition, but when present in sufficient numbers in the genome of a single individual they can underlie a phenotype resembling that of an individual who has a single, rare, large-effect mutation (10-12).

For example, a number of identified independent common SNPs from GWAS on various chromosomes have been reproducibly shown to be associated with alleles that slightly raise or lower a lipoprotein fraction, e.g. high density lipoprotein (HDL) cholesterol [C], low density lipoprotein (LDL)-C or triglyceride (TG) (13). Most people have some alleles that raise and others that lower lipoproteins, with the usual net result being an average serum level. However, some individuals, by unlucky chance, will inherit an overburden of alleles that act to alter the lipid trait in the same direction (e.g. to raise LDL-C) (14). High cholesterol due to the accumulation of a high burden of small-effect SNPs can be indistinguishable clinically from a single gene rare variant cause. However, this genetic mechanism of disease will not be detected by standard genetic testing, e.g. WES or targeted exon sequencing that is not designed to concurrently detect common non-coding SNPs associated with the trait of interest (14, 15). Methods to evaluate these lipid trait-altering SNP genotypes include TaqMan-based amplification assays, SNP microarrays, or targeted NGS panels that have been consciously designed to detect non-coding SNPs.

Once these key SNPs have been genotyped, they can be entered into a scoring system to estimate their cumulative effect on the respective clinical trait. These systems sum the expected effect of each individual SNP in a given patient to derive a score that is then

compared to a healthy reference population. These scores use a simple weighted sum of the identified risk alleles to generate a risk score, normalized to the average population risk:

$$(\text{score} = \sum_{i=1}^{n} (number\ of\ risk\ alleles) * (effect\ size\ of\ allele\ on\ trait\ of\ interest)\ )$$

with the number of risk alleles ranging between 0 to 2 for each trait.

If an individual has a high SNP score, such as $\geq 75^{th}$ percentile compared to the reference population (meaning that only 25% of individuals would have such a high burden of trait-altering alleles), this would be expected to predispose to the development of clinically apparent trait.

Since targeted NGS is the method of choice to detect rare large-effect disease-causing variants, in most diagnostic laboratories it would be most efficient to expend some excess capacity on such targeted panels to also sequence the non-coding regions harboring the key SNPs comprising a polygenic risk score (PRS). This means that both rare and common DNA variation underlying a clinical condition can be concurrently evaluated using a single laboratory method, which saves time, effort and expense. The use of NGS data to generate a PRS for certain conditions can add valuable information regarding the underlying genetic contribution to a phenotype, and can help fill in the missing genetic gap for many disorders. This is especially important in conditions such as in FH, where up to one-third of clinically-ascertained cases without a discrete rare large-effect mutation will actually have a high polygenic score (15); a similar pattern is seen in patients with extremely low or high levels of HDL-C (16).

SNP scores have been generated using a small number of SNPs, several hundred SNPs and recently several million SNPs (12). While there is no consensus yet for the most clinically useful or practical method of generating a polygenic risk score, the numerical predictive value of the score tends to increase with the greater number of SNPs incorporated (Table 1.1).

Table 1.1: Genetic Testing Methods

Method:	Description:	Strengths:	Weaknesses:	Detection Possible:				Best Use:
				CNV:	Rare Variants:	Common Variants:	PRS:	
Sanger Sequencing	Amplification using individual primer pairs in separate amplification and sequencing reactions	-Gold standard for diagnosis of small DNA changes -Highest depth of coverage	-Labour intensive -Highest cost when done in high volumes	No	Yes	No	No	-Single gene tests -Confirmation of presence/absence of a known mutation in familial cascade testing
Targeted NG Sequencing:	Simultaneous screen of a pre-selected subset of genes	- Maximize diagnostic yield and minimize off-target results -High depth of coverage	-will not find mutations in genes outside the panel design	Yes*	Yes	Yes~	Yes~	-Investigating a condition with multiple causal/contributing genes
Whole Exome Sequencing:	Simultaneous screen of all exons in an individual (2% of total DNA)	-Can identify new or unexpected genes -Could be re-examined for other conditions at a later date	-potential for off-target results -higher probability of uncertain results	Yes*	Yes	No	No	-Conditions with an unclear genetic basis -Conditions expected to be genetic but without a cause identified on other testing

Whole Genome Sequencing:	Simultaneous screen of all DNA in an individual, including mitochondrial	-Includes all genetic material, including regulatory regions -Could be re-examined for other conditions at a later date	-Lower depth of coverage -higher probably of uncertain and off target results -Labour intensive	Yes	Yes	Yes	Yes	-Same as for whole exome but provides more comprehensive data
Genotyping:	Rapid screen of entire genome using an array of common variant SNPs	-Rapid -Lower cost -Entire genome examined	-Cannot detect novel variants -No sequence-level data	Yes	Yes~	Yes~	Yes	-Can be used to look for disease risk, ethnicity and to determine familial relationships
Polygenic Trait Score:	Derived by summing the minor effects of several common variants to generate an overall estimate of risk	-Can provide information on risk that will not be apparent with other forms of sequencing	-Ethnic variations can limit use -Predictive value varies with each score	N/A	N/A	N/A	N/A	-Used as a complement to traditional sequencing to help provide an additional clinical prediction of risk

PRS: Polygenic Risk Score; *With specialized bioinformatics tools; ~If designed to target

1.5 Benefits, Drawbacks and Indications for Genetic Testing:

1.5.1 Potential Benefits of Genetic Testing:

The promise of genetic testing for the individual is securing a definitive diagnosis. This has several possible benefits, including the ability to alter management to better suit the individual patient, depending on the condition. The genetic diagnosis in such instances could reduce the delay in selecting an appropriate treatment (17). Additionally, a genetic diagnosis is required in order to procure funding or eligibility for targeted therapies in some conditions. This is the case in certain jurisdictions for obtaining third party private coverage for injectable inhibitors of proprotein convertase subtilisin kexin 9 (PCSK9) for the treatment of familial hypercholesterolemia (FH).

Some genetic syndromes may have other associated features, or carry risks, that are not readily apparent early in the condition, such as genitourinary or hepatic manifestations in maturity onset diabetes of the young (MODY) diabetes caused by mutations in *HNF1B*, hepatic cirrhosis or other organ infiltration in cholesteryl ester storage disease (CESD) or Wolman syndrome due to bi-allelic *LIPA* variation (18). Confirmation of these genetic syndromes can allow for these complications to be monitored for and potentially prevented or their consequences ameliorated. Additionally, some conditions can mimic a presentation of a classic clinical condition, but have a different etiology, such as sitosterolemia, which can present like FH, but require very different management strategies (19). Knowing the underlying genetics can help tailor therapy most effectively to the individual patient.

Furthermore, many genetic conditions are picked up later in life, as they often fail to present with overt symptoms, such as atherosclerosis in the case of FH, until well into the disease course (20). Because there are effective therapies for early prevention and management that have been shown to prevent or delay onset of complications, there is a large potential benefit to identifying and treating these individuals aggressively as early as possible.

Beyond the individual patient, there may be a significant window of opportunity for early detection and intervention for children or other family members of identified individuals who have a discrete molecular cause for their condition. For instance, heterozygous FH (HeFH) and MODY both follow an autosomal dominant transmission pattern, meaning that approximately half of all first-degree relatives would also have the condition (5). Many of these individuals may be in the pre-clinical stage of cardiovascular disease and would benefit from an early diagnosis. Having a definite DNA diagnosis could also have implications for family planning, especially for the parents of children presenting with severe homozygous conditions which would be expected to be manifest in approximately 25% of their future offspring, or for adults who are considering starting a family and wish to consider the potential risks. Often a genetic counsellor can help explain these risks to families and allow them to make more informed decisions about their future plans.

There are also other non-clinical benefits of genetic testing that come in the form of patient empowerment. Knowing a diagnosis, even if there are no interventions or prevention strategies, can allow an individual an awareness of the expected natural course and provide the opportunity for advanced planning and more emotional and mental control over their healthcare (21). There can also be a significant sense of relief for at-risk individuals who test negative for a genetic condition, and reduction in costs of surveillance (21).

On a societal level, genetic testing can contribute to a better understanding of the pathophysiology of a condition. This in turn can lead to the development of new, more effective pharmacological treatments or management strategies. A prime example of this is the development of PCSK9 inhibitors, which owe their inception and development to studies of patients and later families identified as having low LDL-C and low rates of cardiovascular disease.

1.5.2 Potential drawbacks of Genetic Testing:

A major limitation to the widespread use of genetic testing is the cost, which can be prohibitive, especially for larger analyses such as WES or WGS. However, the cost is

dropping rapidly following upon Moore's law of the economics of new technologies (9). From the first completed whole DNA sequence in 2000 which cost $2.7 billion, the costs are now down to a minuscule fraction of that, at approximately $5-10,000 for a whole genome and much less for a whole exome. With the costs expected to continue to decrease, this barrier is increasingly overcome.

There are, however, other ethical and societal considerations to the widespread use of genetic testing. Ethical dilemmas arise when researchers obtain unexpected off-target results, for instance incidental findings unrelated to the disease of interest that are present in WES or WGS. There is some debate surrounding whether these need to be looked for and communicated to tested patients, or if genetic researchers or technicians should report only on the specific genes or diseases for which the test was conducted. For example, if a test in a patient with dyslipidemia picked up a mutation in a known breast cancer (*BRCA*) gene, what is the obligation to look for this finding and report it to the patient. Similarly, there are hundreds to thousands of incidental variants of unknown significance (VUSs) that are picked up during the sequencing process (2). There is significant debate regarding the most appropriate way for these to be evaluated and communicated. There is also a conflict of interest that can arise between the researcher or physician ‘duty to inform’, especially in the case of at-risk family members, and the individuals’ ‘right not to know’ (22, 23). Furthermore, genetic testing could be seen as infringing on the privacy of other family members, as a genetic result will indirectly provide information about them as well (22). The ACMG has recommended that incidental findings related to several severe medical conditions should be communicated to patients if detected (24), although this is an active area of debate.

There is a prevalent concern that genetic information may be used to determine eligibility for work or for insurance, or otherwise lead to forms of ‘genetic discrimination’ (22, 25). Legislation in many countries, including the United States and Canada is intended to protect against genetic discrimination in the workplace, and by tentative consensus for the

insurance providers, but the issues regarding who would be able to access genetic information, and for what purpose, remain unresolved.

There is also often a commonly held misbelief that genetic testing is an absolute certainly, where the results are either positive or negative. However, genetic testing is prone to the same limitation of false positives and negatives as any other diagnostic test, though the exact sensitivity and specificity of genetic testing is often impossible to determine due to the lack of a reference standard (25-27). This misconception can potentially lead to both unnecessary anxiety in the case of a false positive (i.e. when a VUS in a known causal gene is deemed pathologic when it is in fact benign, or when two causal mutations for a homozygous trait are present on the same allele but are reported to be disease-causing as a compound heterozygote), or inappropriate reassurance in the case of a false negative (i.e. a mutation exists in a gene not yet associated with the disease) (27).

There are also financial limitations to consider. While the number of clinically validated tests available is increasing, the guidelines surrounding indications for their use or guidance for interpretation is slower to emerge, as is insurance and government reimbursement (9, 21).

1.5.3 Testing Indications:

In general, the argument to proceed with genetic testing is strengthened when there is strong suspicion (no secondary causes are apparent, a strong family history, values are far outside standard reference ranges, there are other possibly syndromic features, or the patient is young) AND when there might be a change in management, monitoring or intervention that could affect outcomes for the patient or family members (i.e. will affect eligibility for new drugs, would potentially lead to a different choice of therapy) OR if there a strong patient desire to have a definitive diagnosis (Table 1.2) (21, 27, 28).

Table 1.2: Indications for Genetic Testing in Inherited Endocrine Conditions:

Indications
Testing might change management
Strong clinical suspicion
Patient preference
Family planning
Early interventions available
Eligibility for new drugs
Strong family history
Other, related syndromic features

1.6 Maturity Onset Diabetes of the Young:

1.6.1 What is MODY?

Maturity-onset diabetes of the young (MODY) is an umbrella term for a genetically heterogeneous collection of monogenic (single gene) diabetes syndromes, inherited in an autosomal dominant manner (29). The term had its origin in the now outdated classification of diabetes as either juvenile-onset (type 1) diabetes, or maturity-onset (type 2) diabetes. The Canadian Diabetes Association (CDA) categorizes MODY under "genetic defects of beta-cell function", with sub-classification according to the gene defect (30). Many subtypes of MODY are characterized by deficiencies in the signaling pathways for insulin production, release or responsiveness, that are often linked to insufficient glucose-mediated insulin release from the pancreatic beta cells. Unlike type 1 diabetes, which is an autoimmune condition resulting in immune-mediated destruction of the pancreatic beta cells, the complement of beta cells in MODY is usually normal. In MODY, deficiencies in insulin production are due to inherited defects that are often sufficient on their own to lead to hyperglycemia and a diagnosis of diabetes without any additional trigger. MODY subtypes generally follow an autosomal dominant inheritance pattern. Penetrance and expressivity can vary considerably in different kindreds depending on the mutation involved and even amongst family members with the same mutations. In some milder forms of MODY, environmental or other metabolic factors may influence the expression of the MODY phenotype, by either exacerbating or ameliorating the effect of the inherited defect. Exacerbating factors could include a hypercaloric diet high in carbohydrates, development of overweight or obesity, or a sedentary lifestyle. Conversely, ameliorating factors could include higher fiber, lower simple carbohydrate diet, regular physical activity and maintenance of ideal body weight. Many of the exacerbating factors can be similar to those that predispose to insulin resistance, which contributes to the development of type 2 diabetes, but individuals with MODY mutations may develop a diabetes phenotype more readily in the presence of these influences. However, in the majority of MODY cases, the inherited deficits are sufficient on their own to manifest a diabetic phenotype irrespective

of other influencing factors, and will occur at a young age even in the absence of any other identifiable risk factors.

1.6.2 MODY Subtypes:

The prevalence of MODY has been estimated at 0.5-6.5% of new-onset diabetes cases, depending on the population studied (29), with MODY2 (glucokinase or *GCK* gene) and MODY3 (hepatocyte nuclear factor 1-alpha or *HNF1A* gene) being the most prevalent forms, involving about three-quarters of all MODY patients (Table 1.3). To date, 14 different MODY subtypes (MODY1 – 14) have been described (Table 1.3). While initially these were described numerically, there has been a shift in recent years to classify the MODY subtypes according to the underlying gene defect. Both naming terminologies are used here, with preference for classifying by genetic defect.

GCK-MODY (alias MODY2) is caused by mutations in the *GCK* gene, encoding glucokinase, a hexokinase enzyme that catalyzes the phosphorylation of glucose to glucose-6-phosphate, the first step in glycolysis (31-37) (Figure 1). Heterozygous mutations in this gene have been linked to a mild, non-progressive form of diabetes that is usually asymptomatic with mild elevations in fasting blood sugar and mildly increased post-prandial glucose excursions. In contrast, MODY1 and MODY 3-7, 9 and 11 each result from rare mutations in transcription factors, which regulate the embryonic development of beta-cells, in addition to their proliferation and programmed cell death (38) (Figure 1). These transcription factors also govern expression of insulin, glucose transporters, and related beta-cell factors (38, 39). MODY1 and MODY 3-7, 9 and 11 are collectively termed “transcription factor MODY” (38, 39).

1.6.2.1 HNF4A MODY (MODY 1) and HNF1A MODY (MODY 3):

HNF1A-MODY (MODY 3) is the most common form of transcription factor MODY, and accounts for 30-60% of all MODY cases. It is caused by mutations in the *HNF1A* gene encoding hepatocyte nuclear factor (HNF) 1-alpha (40). *HNF1A* is expressed in the

pancreas, liver, kidney and intestine and is involved in glucose transport and metabolism, as well as mitochondrial metabolism in pancreatic beta cell (41).

HNF1A-MODY patients generally have normal fasting glucose levels, but exaggerated post-prandial hyperglycemia, i.e. > 5 mmol/L blood glucose excursion, that worsens with time (38-40) due to progressively reduced beta-cell proliferation and increased apoptosis (38). *HNF1A*-MODY patients are often very responsive to sulfonylureas (SUs) or meglitinides, with glycated hemoglobin (HbA1c) improvement of 4-5% in absolute terms compared to metformin (40). These agents bind to receptors on the beta cell surface, leading to an influx of calcium which induces the direct release of insulin from storage vesicles within the beta cell, bypassing the normal glucose trigger for insulin release, which is defective in this type of MODY (41). Consequently, low-dose SUs are first line therapy in *HNF1A*-MODY and may be able to be used effectively as monotherapy for decades (40). However, since *HNF1A*-MODY is progressive, patients often eventually progress to requiring other therapies, including insulin.

Patients with *HNF1A*-MODY are notable for an usually low renal threshold for glucose (41). They can also be more susceptible to the development of microvascular complications over time, especially retinopathy, with inadequate glycemic control (39, 40).

HNF4A-MODY (MODY 1) is caused by mutations in the *HNF4A* gene, encoding for hepatocyte nuclear factor 4-alpha (HNF4-alpha), which is an upstream regulator of HNF1-alpha, the transcription factor involved in the pathogenesis of *HNF1A*-MODY (MODY 3). Thus *HNF1A*-MODY (MODY 3) and *HNF4A*-MODY (MODY 1) patients have similar clinical features, course and recommended treatment (38, 42). Individuals with *HNF4A*-MODY may have a history of higher birthweight or macrosomia, and a history of transient neonatal hypoglycemia (41). Mutations in *HNF4A* account for approximately 5-10% of all identified MODY mutations (41).

1.6.2.2.GCK-MODY (MODY 2):

The first causative gene found for MODY, *GCK,* encoding glucokinase (MODY2), was isolated using a candidate gene approach (40). Glucokinase (GCK) is expressed in the liver and pancreas and catalyzes the first reaction in the glycolytic pathway, namely the conversion of glucose to glucose-6-phosphate (40). In pancreatic beta cells, the rate of glucose phosphorylation by GCK is directly proportional to serum glucose concentration, allowing it to function as a glucose sensor (38, 40). In *GCK*-MODY patients with GCK deficiency, the threshold glucose level required to stimulate insulin release is higher than in normal subjects, but the overall secretion curve and response are similar. This results in a higher, but stable, overall set point for plasma glucose, essentially shifting the dose-response curve to the right (40, 42). This explains the generally benign course of *GCK*-MODY, in which affected individuals have mild elevations in fasting glucose, generally between 6 to 8 mmol/L, that deteriorates minimally over time, and rarely leads to microvascular complications (39, 40). Oral glucose tolerance testing shows mildly increased 2-hour increments in glucose from baseline, usually < 3.0 mmol/L and off-treatment HbA1c rarely exceeds 7.5-8% (38, 40, 43, 44). Mutations in this gene are a common form of MODY, with an overall prevalence of ~1 in 1000 individuals (31-37, 44), and accounts for 30-60% of all MODY cases.

1.6.2.3 PDX1-MODY (MODY 4):

PDX1-MODY is a rare transcription factor MODY subtype, resulting from mutations in the *PDX1* gene encoding for pancreatic and duodenal homeobox 1, which is involved in pancreatic development and beta cell maturation, as well as regulating insulin gene expression (36, 41).

1.6.2.4 HNF1B-MODY (MODY 5):

Mutations in *HNF1B* lead to MODY5, and are responsible for 5-10% of all MODY cases (45, 46). This condition is also known as renal cysts and diabetes syndrome. Whole gene deletions of one copy of *HNF1B* are responsible for up to 50% of cases of MODY5, with virtually all of these being chromosome 17q12 deletions of varying magnitude (45-48). The

majority (~70%) of these deletions arise *de novo* (48). The "17q12 deletion syndrome" is a term that refers to more extensive deletions of ~1.4 Mbps that encompass *HNF1B* and several surrounding genes (47-49). Clinical manifestations of 17q12 deletion syndrome include a mild to moderate, progressive diabetes with a mean onset of age 24, but which can present from the neonatal period up to middle age (46, 48).

Individuals with the condition can have congenital or later-onset urogenital malformations, and also have frequent renal, pancreatic or hepatic complications, including cysts, hypomagnesemia, hyperuricemia, or exocrine pancreatic deficiencies (46-48). Primary hyperparathyroidism also appears with higher prevalence (46). Cognitive impairment and/or developmental delay is common, presenting in 50% of those with 17q12 deletion syndrome (46-48). Individuals with impaired function of HNF1B may respond initially to low-dose oral sulfonylurea therapy but insulin is often required (46, 50). Progressive loss of renal function that is distinct from development of diabetic nephropathy can also be seen in these individuals (41). Due to these extra-pancreatic manifestations, these individuals may warrant periodic monitoring for genitourinary, parathyroid, hepatic or other complications.

1.6.2.5 NEUROD1-MODY (MODY 6):

Mutations in *NEUROD1,* encoding for a basic helix-loop-helix protein transcription factor known as neurogenic differentiation 1, is responsible for *NEUROD1*-MODY, a rare MODY subtype. This transcription factor is thought to be involved in the development of endocrine cell lineages as well as regulates expression of insulin (*INS*), *GCK* and *GLUT2* genes (36). Minimal clinical data is available on this form of MODY, but most individuals with this type of MODY seem to require insulin (36).

1.6.2.6 KLF11-MODY (MODY 7):

KLF11-MODY is a rare form of MODY caused by mutations in *KLF11*, encoding for kruppel-like factor 11, an SP1-like zinc finger transcription factor that is thought to be involved in activating the insulin promoter in response to hyperglycemia (51). In has been described in

3 kindreds with impaired glucose tolerance and early onset apparent type 2 diabetes (51). Minimal data is available on optimal treatment strategies or clinical course.

1.6.2.7 CEL-MODY (MODY 8):

CEL-MODY, also known as diabetes and pancreatic exocrine dysfunction syndrome, is a rare MODY subtype caused by mutations in the *CEL* gene, encoding for carboxyl-ester lipase, an enzyme produced by the adult pancreas that aids in the digestion of fats (52-55). In addition to early-onset diabetes, this form of MODY presents with progressive decline in pancreatic exocrine function, lipomatosis, or fatty replacement, of pancreatic tissue, and the development of pancreatic cysts (52-55). While the clinical course of individuals carrying these mutations is not well-described, insulin is likely be required due to the progressive pancreatic destruction seen in these cases.

1.6.2.8 PAX4-MODY (MODY 9):

PAX4-MODY is caused by mutations in the *PAX4* gene, encoding for paired box 4, a member of the paired box family of transcription factors. Along with PAX6, PAX4 is thought to be involved in differentiation of the endocrine pancreas and pancreatic islet and beta cell development (56).

1.6.2.9 INS-MODY (MODY 10):

INS-MODY is caused by mutations in the *INS* gene, encoding for the peptide hormone insulin that is expressed in the pancreatic beta cells in response to rising glucose levels and is the main hormone responsible for regulating metabolism of carbohydrates. Mutations in *INS* that affect the post-translational processing of insulin have been linked to the development of this rare form of MODY (57, 58). Other mutations in this gene have been linked to permanent neonatal diabetes and hyperproinsulinemia. Limited data is available with respect to clinical course and optimal management, but case reports suggest effective management may range from diet alone to small doses of insulin.

1.6.2.10 BLK-MODY (MODY 11):

BLK-MODY is a rare subtype of MODY caused by mutations in the *BLK* gene, encoding for B lymphocyte kinase, a tyrosine kinase expressed mainly in human lymphatic organs, but also shows expression in pancreatic islet cells, where it is thought to modulate insulin synthesis and secretion, possibly through upregulation of *PDX1* transcription (59, 60). While data on this form of MODY, as well as clinical course, are lacking, available data suggests that additional factors, such as elevated body mass index (BMI) or otherwise diabetogenic environment may be required to fully express this phenotype (59).

1.6.2.11 ABCC8-MODY (MODY 12) and KCNJ11-MODY (MODY 13):

ABCC8-MODY is a rare form of MODY caused by mutations in the *ABCC8* gene, which encodes for ATP-binding cassette C8, also known as the sulfonylurea receptor. This protein encodes for the binding domain for ATP in ATP-sensitive K+ channels in pancreatic beta cells (61).

KCNJ11-MODY is caused by mutations in the *KCNJ11* gene, that encodes for potassium channel, inwardly rectifying, subfamily J, member 11 (62, 63). This potassium channel forms an octameric complex of KCNJ11 along with inclusion of ATP-binding cassette C8 (ABCC8), to form an ATP-responsive potassium channel that is an essential component in the pathway for glucose-induced insulin secretion from the pancreatic beta cells.

Binding of SUs to the ABCC8 receptor, or interaction with glucose-derived ATP, blocks the outflux of potassium from the pancreatic beta cell, subsequently leading to depolarization and the opening of voltage-gated calcium channels, leading to the fusion of insulin-containing intracellular vesicles to the cell membrane and release of insulin from the beta cell (61).

Mutations in *ABCC8* and *KCNJ11* are most often associated with transient or permanent neonatal diabetes, or neonatal hypoglycemia (61, 63). In cases of transient neonatal hypoglycemia or diabetes, these patients will often re-present later in life with diabetes (63). It is unclear if mutations in *ABCC8* are truly associated with MODY, or more appropriately a cause of neonatal conditions that may have been missed until they re-

present at a later age.   Mutations in *KCNJ11* have been associated in two separate kindreds with MODY phenotypes (62). Individuals with these mutations who develop diabetes later in life generally respond well to SUs.

1.6.2.12 APPL1-MODY (MODY 14):

APPL1-MODY is caused by mutations in the APPL1 gene, which encodes for adaptor protein, phosphotyrosine interaction, PH domain, and leucine zipper-containing Protein 1, which interacts in its role as an adaptor protein with several critical proteins in the insulin-signaling pathway (64). One of these key interactions is with the serine/threonine kinase AKT, allowing AKT to translocate to the cell membrane, become phosphorylated and propagate the insulin signal (64). While only limited data is available, this form of MODY has shown later onset (average age onset ~38) and incomplete penetrance, suggesting onset may be partially influenced by environmental factors (64).

Table 1.3: Molecular and clinical features of MODY subtypes

Type/ OMIM	Prev.	Gene	Gene Location	Gene Product Description	Mechanism of Deficiency	Age of Onset	Hyperglycemia Severity	Microvascular Complications	Treatment	Other features
MODY1 **125850** **(29, 36, 38-40, 42, 65, 66)**	5-10%	Hepatocyte nuclear factor 4-α (*HNF4α*)	20q13.12	Transcription factor regulating transcription of many genes, involved in β-cell development and function, including HNF1α	Impaired glucose-dependent insulin release; decreased B-cell mass over time	Adolescence/ early adulthood	Severe, progressive	Frequent	OHA (SU) >> insulin	Low TG, HDL, high LDL.
MODY2 **125851** **(29, 36, 38-40, 42, 66, 67)**	30-70%	Glucokinase (*GCK*)	7p13	Phosphorylates glucose into glucose-6-phosphate; regulates carbohydrate catabolism	Defective glucose sensing	Childhood (birth)	Mild	Rare	None often; monitor in pregnancy	
MODY3 **600496** **(29, 36, 38-40, 42, 65-67)**	30-70%	Hepatocyte nuclear factor 1-α (*HNF1α*)	12q24.31	Transcription factor regulating transcription of many genes, involved in β-cell development and function	Impaired glucose-dependent insulin release; decreased B-cell mass over time	Adolescence/ early adulthood	Severe progressive	Frequent	OHA (SU) >> insulin	Low renal glucose threshold; high HDL
MODY4 **606392** **(29, 36, 38-40, 42, 65)**	<<1%	Pancreatic and duodenal homeobox 1 (*PDX1*)	13q12.2	Transcription factor necessary for pancreatic development and β-cell maturation. Role in insulin gene expression	Impaired pancreatic development; impaired expression of islet hormones	Early adulthood	Mild, progressive	Minimal data	diet, OHA, insulin	Pancreatic agenesis if homozygous

MODY5 **137920** **(29, 36, 38-40, 42, 65)**	5-10%	Hepatocyte nuclear factor 1β (*HNF1β*)	17q12	Transcription factor involved in embryonic development and ongoing function of pancreas, liver, kidneys, genitals and gut	Impaired glucose-dependent insulin release; insulin resistance	Adolescence/ early adulthood	Severe, progressive	Retinopathy seen; Prone to early ESRD	insulin > OHA	Renal cysts/failure; genital malformations; pancreatic atrophy; high uric and transaminases; low Mg
MODY6 **606394** **(29, 36, 38, 42, 65)**	<1%	Neurogenic differentiation 1 (*NEUROD1*)	2q31.3	Transcription factor involved in development of endocrine cell lineages. Regulates expression of insulin, *GCK* and *GLUT2* genes	Impaired insulin gene expression; abnormal islet morphology	Adolescence/ early adulthood	Minimal data.	Frequent nephropathy	insulin > OHA	
MODY7 **610508** **(38, 51)**	<<1%	Kruppel-like factor 11 (*KLF11*)	2p25.1	Activates the insulin promoter in response to hyperglycemia	Impaired glucose-dependent insulin release	Minimal data	Minimal data	Minimal data	Minimal data	
MODY8 **609812** **(38, 65)**	<<1%	carboxyl-ester lipase (*CEL*)	9q34.13	Enzyme produced by the adult pancreas and aids in the digestion of fats	Likely destruction of beta cells over time due to pancreatic infiltration	Minimal data	Minimal data	Minimal data	Minimal data	Pancreatic atrophy; Exocrine pancreatic dysfunction
MODY9 **612225** **(56)**	<<1%	Paired box gene 4 (*PAX4*)	7q32.1	Member of the paired box (PAX) family of transcription factors. Involved in pancreatic islet development	Defective beta-cell development; impaired beta-cell proliferation	Minimal data	Minimal data	Minimal data	Minimal data	

MODY10 **613370** **(38, 57, 65)**	<1%	Insulin (*INS*)	11p15.5	Peptide hormone produced by β cells; regulates metabolism of carbohydrates and fats	Abnormal post-translational processing of insulin	Childhood	Minimal data	Rare	diet alone, OHA (SU) > insulin	Expression may be triggered by increasing weight
MODY11 **613375** **(59)**	<<1%	B lymphocyte kinase (*BLK*)	8p23.1	Tyrosine-protein kinase that modulates insulin synthesis and secretion; upregulates PDX-1	Impaired glucose-dependent insulin release	Adolescence/early adulthood	Minimal data	Minimal data	Minimal data	Expression may be triggered by increasing weight
MODY12 **(61)**	<<1%	ATP-binding cassette C8 (ABCC8)	11p15.1	Target for SUs. Binding domain for ATP in ATP-sensitive K+ channels	Impaired glucose-dependent insulin release	Childhood	Minimal data	Minimal data	OHA (SU*) > insulin	Not clearly causal for MODY; associated with permanent neonatal diabetes
MODY13 **616329** **(38, 62, 65)**	<<1%	potassium channel subfamily J, member 11 (*KCNJ11*)	11p15.1	ATP-sensitive K+ channel; couples cell metabolism to membrane excitability	Impaired glucose-dependent insulin release	Minimal data	Minimal data	Minimal data	OHA (SU*) > insulin	Often associated with permanent neonatal diabetes
MODY 14 **616511** **(64)**	<<1%	Adaptor Protein, Phosphotyrosine Interaction, PH domain, and Leucine Zipper-containing Protein 1 (*APPL1)*	3p14.3	Involved in regulation of endocytosis, signal transduction and mitogenesis through RAB5 pathway	Impaired glucose-dependent insulin release	Early to mid adulthood	Mild to moderate	Minimal data	Diet/OHA/ insulin	Shows later onset (average age onset ~38), incomplete penetrance; onset may be influenced by environmental factors

abbreviations: OMIM, Online Mendelian Inheritance in Man; Prev., prevalence; Tx, Treatment; OHA, oral hyperglycemic agent; SU, sulfonylurea; TG, triglycerides; *high dose

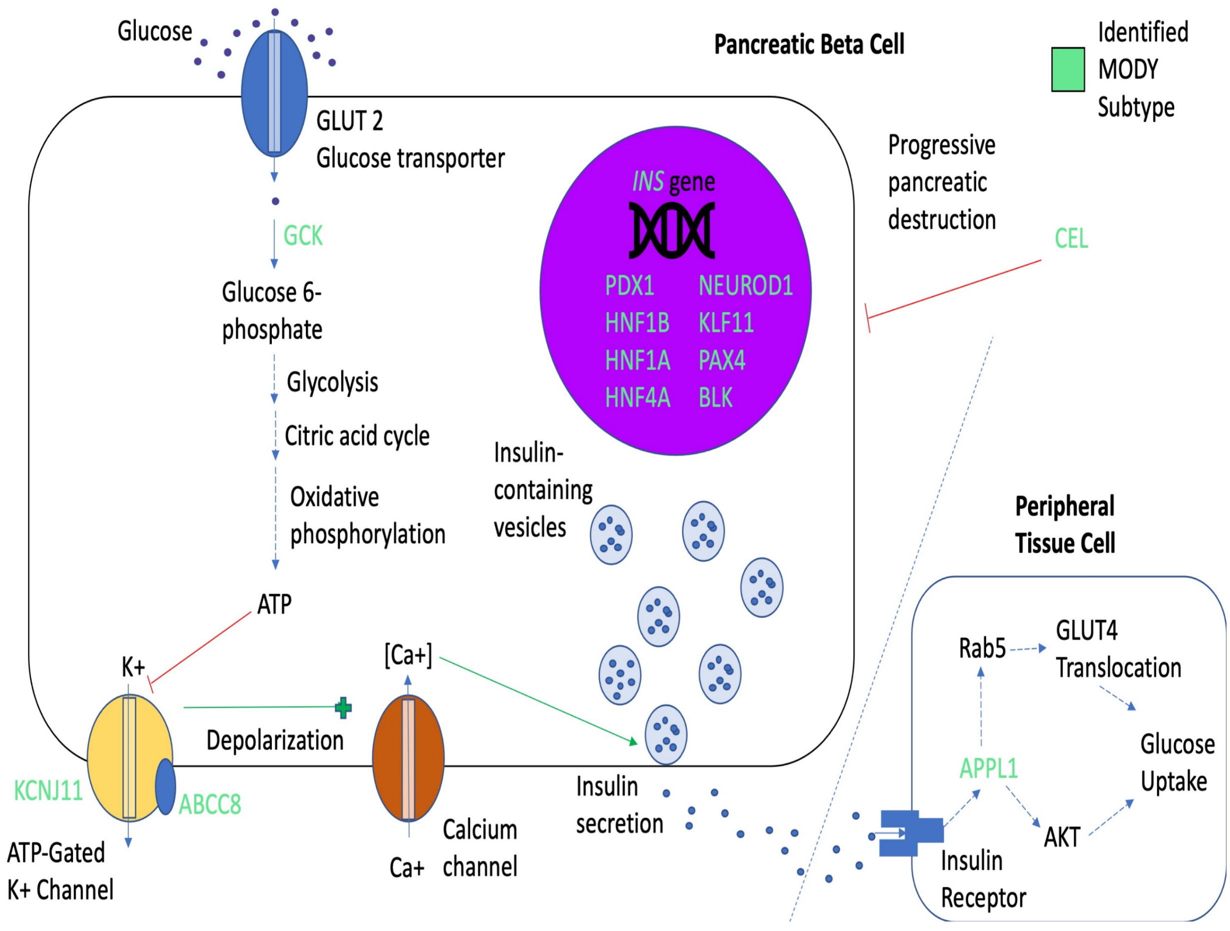

Figure 1.1: Pancreatic beta cell signalling pathway with MODY gene products identified

Within the pancreatic beta cell, insulin is synthesized from the *INS* gene under the control of number of transcription factors. Insulin is subsequently packaged into vesicles for release into the extracellular space and transport to target organ tissue receptors throughout the body. Circulating blood glucose enters the beta cell through the GLUT2 glucose transporters on the beta cell surface. Glucokinase (GCK) then catalyzes the first step in the breakdown of glucose through glycolysis. The ATP generated from glucose catabolism leads to closure of the ATP-gated potassium channel. This leads to beta cell depolarization and the opening of voltage-gated calcium channels. The subsequent influx of calcium leads to the expulsion of vesicle-stored insulin from the beta cell into circulation. Insulin binds to its' receptors on target tissues, allowing for the expression of GLUT4 receptors on the cellular surface and glucose uptake. Genetic alterations that affect several points in this pathway have been linked to MODY and to other forms of genetic glucose dysregulation. The class of medications known as the sulfonylureas (SUs) act by binding to the ATP-gated potassium channels, promoting the subsequent steps leading to insulin release in the absence of a glucose signal.

1.6.3 Suspecting MODY clinically:

Before the causative genes were defined, MODY was recognized to follow vertical transmission in families (36, 39, 40). Characteristic features of MODY are shown in (Table 1.4) and include onset before age 25 with a strong multi-generational family history of diabetes and an autosomal dominant pattern of inheritance (29, 38-40, 66-68). Other features include non-insulin dependence with detectable circulating C-peptide levels for five years following diagnosis, absence of autoantibodies, no episodes of diabetic ketoacidosis and prolonged survival after onset of symptoms (38-40). MODY patients are also generally not overweight and lack other physical features associated with insulin resistance such as acanthosis nigricans (39, 40).

Suggestive features for MODY include: 1) age of diagnosis <25 years; 2) normal BMI; 3) non-insulin dependence or no episodes of diabetic ketoacidosis (DKA); 4) a strong family history following an autosomal dominant (AD) inheritance pattern, although *de novo* mutations are possible (69); and 5) robust response to SU treatment in some forms.

Clinical prediction calculators, and other diagnostic tools, are also available to aid clinicians in selecting patients who may benefit from genetic testing (70, 71). Strategies that incorporate the use of fasting blood sugars and response to an oral glucose tolerance test to predict a potential MODY diagnosis may also be effective as a screening tool (72).

Table 1.4: Clinical factors that raise suspicion for maturity onset diabetes of the young (MODY)

Key Features	Age at diagnosis <25 years
	Family history of diabetes (>2 generations)
	Autosomal dominant inheritance pattern
Supporting Evidence	Non-obese or non-overweight patient
	Negative autoantibodies
	Non-ketotic in absence of insulin therapy
	Non-insulin dependence or significant C-peptide levels while on insulin for at least 5 years after diagnosis

1.6.4 Health and management implications of MODY diagnosis:

A diagnosis of MODY can significantly alter the management and expected clinical course of diabetes (34). Consequently, suspecting and confirming a diagnosis can be important for optimal patient care.

As outlined above, specific treatment recommendations depend on the MODY subtype identified (50, 73-75). For example, MODY2, caused by mutations in *GCK,* is a benign, non-progressive form of mild hyperglycemia that does not require monitoring or management (34, 73, 76). MODY1 and MODY3 are caused by mutations in *HNF4A* and *HNF1A*, respectively, and can often be managed with low-dose sulfonylurea (SU) monotherapy for many years, with significantly improved glycemic control compared to insulin or other oral agents (77). MODY5, caused by mutations in *HNF1B,* is associated with other manifestations that warrant regular screening, such as renal or hepatic cysts, hypomagnesemia, hyperparathyroidism or exocrine pancreatic insufficiency (46, 47, 73).

Recommendations for optimal management of hyperglycemia in pregnancy are also different for individuals with MODY, who may be misdiagnosed as having gestational diabetes (GDM), or type 1 or type 2 diabetes during routine pregnancy screening, and can depend on the genotype of the offspring. Pregnancy may also be a time when many women are found to have previously unidentified hyperglycemia, and the prevalence of MODY amongst women presumptively diagnosed with GDM has been estimated to be approximately 5% (78).

1.6.5 Cost-Benefit of genetic testing for MODY:

There can be a significant financial benefit to establishing a diagnosis of MODY, especially if the individual was misdiagnosed with type 1 diabetes, which may allow for discontinuation of insulin and switch to a low dose oral SU with improved glycemic control. Similarly a diagnosis of *GCK*-MODY would prompt discontinuation of surveillance, treatment or follow up for diabetes, which could result in significant cost-savings.

One Australian study conducted a cost-benefit analysis of universal MODY screening using a multi-gene sequencing panel (cost estimated at $500 Australian dollars (AUD)/test) in all patients with presumed type 1 diabetes (79). In this model, incremental costs and quality adjusted life years gained from universal MODY screening were modelled over a 30 year period. The model estimated a MODY detection prevalence of 2.14% with universal screening, compared to 0.7% in standard practice, and the estimated rates of successful conversion to SU, improvements in HbA1c and proportion of *GCK*-MODY patients in whom

treatment could be discontinued (79). The model reported multi-gene panel sequencing for MODY was less costly than standard care, with 26 quality adjusted life years (QALYs) gained and $1,016,000 AUD saved per 1000 patients screened. Cost of screening was fully offset within 10 years. Universal screening remained cost-effective until prevalence rates fell below 1.1% (79). Further selecting presumed type 1 patients based on absence of autoantibodies and preserved c-peptide levels could further improve the cost effectiveness, with these selection criteria yielding a detection rate of 1 case of MODY detected for every 5 patients tested (80).

1.6.6 Suspecting MODY:

Despite the benefits of optimized management seen when individuals are correctly identified as having MODY diabetes, several studies have suggested that MODY is under-suspected and under-recognized clinically, and therefore under-diagnosed by genetic confirmation. In a UK cohort of 74 children diagnosed with presumptive type 1 diabetes, who had persistent insulin reserve, as demonstrated by detectable c-peptide levels and negative autoantibodies, 20 (27%) were ultimately re-classified as MODY following genetic testing (81). Similarly, in a US cohort of 586 pediatric patients with diabetes, insulin reserve and negative autoantibodies, 47 (8%) were re-classified as MODY following genetic testing for causal variants in *GCK, HNF1A* and *HNF4A* (82). In another US study that assessed individuals presumptively diagnosed with type 2 diabetes under the age of 30, or under the age of 45 with no features of metabolic syndrome, testing for *GCK, HNF1A* and *HNF4A* reclassified 13 (4%) of subjects as having MODY diabetes (83).

The barriers to recognition of these cases may include a bias towards diagnoses of the more common diabetes subtypes (type 1 or type 2 diabetes). Suspecting a diagnosis can also be more challenging given the fact that the features of MODY can also overlap with other diabetes classifications. Furthermore, a significant barrier may be the lack of available and/or affordable genetic testing to confirm these diagnoses. This work examines the role of provider clinical suspicion coupled with available NGS testing in ultimately making a MODY diagnosis in chapter 2.2.

1.6.7 Confirming a genetic diagnosis:

Confirmation of MODY is via genetic testing to rule out mutations in one of the 14 known causative genes (29, 31, 33, 39, 44, 66, 67, 84) (Table 1.3).

Early genetic testing for MODY was often accomplished by Sanger sequencing, using a step-wise candidate gene approach. More recently, however, there has been a shift to primarily

using NGS techniques, which allow for the identification of SNVs, small-scale insertions or deletions, as well as small-scale frameshift and null mutations for multiple MODY genes simultaneously (44, 66, 85, 86). However, neither of these sequencing methods are optimized to detect large-scale CNVs (87).

1.6.8 Copy number variations in MODY:

CNVs are large-scale deletions or duplications of DNA that may encompass part of a gene, a whole gene, or several contiguous genes (88-90). Traditionally CNVs were difficult or impossible to detect using sequencing technology. This was due to the fact that the affected sequence of duplicated or deleted genomic DNA in a CNV appears qualitatively normal, but there is instead a quantitative change affecting the dosage of genetic material, CNVs can be difficult to detect and confirm with traditional sequencing methods that are optimized to detect small qualitative changes in the genetic code. Without a robust quantitative analytical tool, even when there appears to be an increase or decrease in the amount of genetic material replicated in certain DNA sections, it is impossible to distinguish a true deletion or duplication from the natural variability in chemical amplification of DNA that is used in most sequencing platforms (Figure 1.2, Figure 1.3)

In the past, a separate methodology, such as high definition cytogenetic analysis, or comparative genomic hybridization with DNA microarrays or multiplex dependent primer amplification (MLPA) were required. Secondary non-sequencing-based dedicated targeted DNA analytical methods using specific probes to assess for CNVs are costly and of uncertain value in MODY, although a few gene deletions have been detected this way (33, 91).

Recently, new bioinformatic techniques have been developed to provide the robustness needed to assess for CNVs using NGS output data, without requiring additional testing. These tools may offer a cost-effective strategy to increase diagnostic accuracy for MODY.

These methods take advantage of the fact that current NGS protocols generate large numbers of short partially overlapping DNA fragments that are assembled computationally to seamlessly reflect the genomic sequence of the source material (87). In addition, the total number of these synthetically-generated DNA fragments reflects the amount of starting material in the genome. This has enabled the development of new algorithms that, through tallying the numbers of chemically-generated DNA fragments, can impute deviations in the amount of starting material from the normal diploid two copies (i.e. maternal and paternal) for any particular chromosomal region. This approach has successfully been applied to detect CNVs using NGS data for several genes causing

dyslipidemias (6, 89, 92, 93). This work attempts to validate this method of CNV detection for MODY and clarify the value of seeking CNVs for this condition in chapter 2.3.

1.6.9 Case finding:

Given the potential positive impact on both quality of life and optimization of management that results from making a MODY diagnosis, ensuring that optimal methods of identifying individuals that are likely to benefit from testing, and ensuring that the genetic tests used are comprehensive and cost effective are important goals.

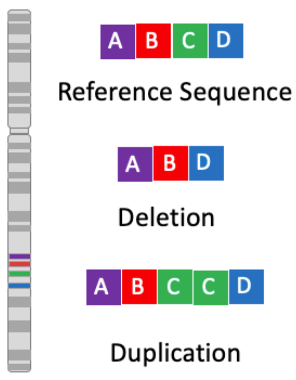

Figure 1.2: Copy number variations

In large-scale deletion and duplication events, or copy number variations, amplified sequences appear normal in standard NGS analysis.

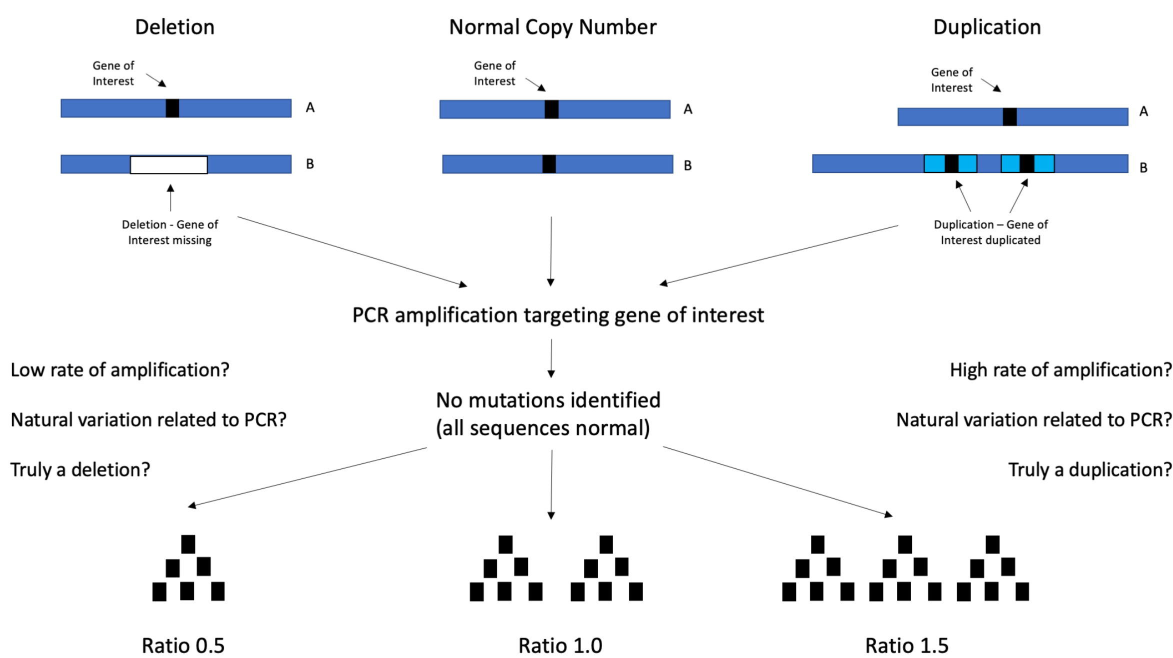

Figure 1.3: Using Depth of Coverage ratios to identify suspected deletions or duplications

Following polymerase chain reaction (PCR) amplification of a region of interest, amplified sequences from starting material containing either a deletion or duplication appear qualitatively normal. There is a natural degree of variation in the number of copies of each section of DNA that is amplified. It can therefore be difficult to ascertain if the number of copies amplified by PCR represents a normal complement state or a duplication or deletion state, making determination of copy number variation (CNV) challenging. A statistically significant difference in the ratio of depth of coverage (DOC; i.e. the number of copies of amplified DNA containing the region of interest) in the affected individual when compared to a reference population sequenced using the same panel and conditions is suggestive of a possible CNV. A ratio of approximately 0.5 would be suggestive of a deletion. A ratio of 1.5 or higher would be suggestive of duplication.

1.7 Hypertriglyceridemia:

Hypertriglyceridemia (HTG) is a common clinical diagnosis, sometimes defined when plasma triglyceride (TG) concentration rises above a threshold value, such as the 90th or 95th percentile for age and sex. HTG frequently co-exists with secondary conditions, including poor diet, alcohol use, obesity, metabolic syndrome, and type 2 diabetes (94, 95). HTG is sometimes classified as primary, when a familial or inherited basis is suspected, or secondary, when one or more secondary factors contribute to the clinical presentation (94). Genetic factors can also influence the severity of the plasma TG elevation in the presence of a secondary factor (95).

1.7.1 Clinical Diagnosis of HTG

HTG is usually a biochemical diagnosis, based on fasting plasma TG concentration above a certain cut point. For instance, the 95th percentile for plasma TG is ~3.0–3.4 mmol/L for North American adults. Severe HTG is sometimes diagnosed as fasting plasma TG concentration >10 mmol/L (885mg/dL) or >1000mg/dL (11.2 mmol/L) (94-98). Proposed HTG definitions vary (Table 1.5). For instance, the Adult Treatment Panel III guidelines of the National Cholesterol Education Program has suggested four discrete categories: normal fasting TG is <1.7 mmol/L (<150 mg/dL), borderline high TG is 1.7–2.3 mmol/L (150–199 mg/dL), high TG is 2.3–5.6 mmol/L (200–499 mg/dL) and very high TG is >5.6 mmol/L (>500 mg/dL) (99). The Endocrine Society has proposed another system with five clinical strata: normal TG is <1.7 mmol/L (<150 mg/dL), mild HTG is 1.7–2.3 mmol/L (150–199 mg/dL), moderate HTG is 2.3–11.2 mmol/L (200–999 mg/dL), severe HTG is 11.2–22.4 mmol/L (1000–1999 mg/dL) and very severe HTG is >22.4 mmol/L (>2000 mg/dL)(100). Other systems have been proposed, but no single scheme has become predominant in the clinic. A well-established classification system—known as the Fredrickson or World Health Organization (WHO) International Classification of Diseases (ICD) hyperlipoproteinemia (HLP) phenotypes—is based on patterns of lipoprotein fractions (Table 1.6) (95, 96, 101), though this classification system is largely being replaced due to improved understanding of the molecular etiology of triglyceride states.

Table 1.5: Hypertriglyceridemia: some proposed clinical definitions.

General clinical definition		NCEP Guidelines		Endocrine Society	
Category	Serum TG (mmol/L)	Category	Serum TG (mmol/L)	Category	Serum TG (mmol/L)
Normal	<2.3	Normal	<1.7	Normal	<1.7
Hypertriglyceridemia	2.3-10	Borderline High	1.7-2.3	Mild	1.7-2.3
				Moderate	2.3-11.2
Severe hypertriglyceridemia	>10	High	2.3-5.6	Severe	11.2-22.4
Very Severe hypertriglyceridemia	>20	Very high	>5.6	Very severe	>22.4

Abbreviations: TG, triglyceride; NCEP: National Cholesterol Education Program

Table 1.6 Classification of hypertriglyceridemia (modified Fredrickson)

Name	Primary Lipoprotein Abnormality	Lipid Profile	Clinical manifestations	Population Prevalence
Familial chylomicronemia (HLP type 1)	Elevated chylomicrons	↑↑↑ TG ↑TC	- Cutaneous eruptive xanthomata, lipemia retinalis, failure to thrive, recurrent epigastric pain, hepatosplenomegaly, pancreatitis, focal neurologic symptoms	1 in 1 million
Combined hyperlipidemia (HLP type 2B)	Elevated VLDL, Elevated LDL	↑↑TG ↑↑TC	- Physical stigmata such as xanthomas or xanthelasmas are uncommon;	1 in 40
Dysbetalipoproteinemia (HLP type 3)	Elevated IDL, Elevated chylomicron remnants	↑↑TG ↑↑TC	Tuberous and palmar xanthomata Elevations in atherogenic IDL results in increased risk for CVD	1 in 10,000
Primary simple hypertriglyceridemia (HLP type 4)	Elevated VLDL	↑↑TG ↑TC	Associated with increased risk of CVD, obesity, DM2, hypertension, hyperuricemia, insulin resistance	1 in 20
Primary mixed hyperlipidemia (HLP type 5)	Elevated chylomicrons, Elevated VLDL	↑↑↑ TG ↑↑↑ TC	Similar clinical manifestations as Type I but develops in adulthood Frequently exacerbated by secondary factors	1 in 600

Abbreviations: as in Table 1.5, plus: HLP, hyperlipoproteinemia; TC, total cholesterol; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; IDL, intermediate density lipoprotein; TG, triglyceride.

1.7.2 Chylomicronemia:

Chylomicronemia is characterized by the pathological persistence of chylomicrons in the serum, usually manifest with TG levels >10 mmol/L, after a fasting period of 12–14 h (94-96). In individuals with normal metabolism (Figure 1.4), chylomicrons are cleared from plasma within 3–4 h of eating (94-96, 102, 103).

1.7.3 Primary Hypertriglyceridemia:

The genetic basis for primary chylomicronaemia is heterogeneous. Before the human genome era, all primary HTG was thought to be monogenic, by analogy with other monogenic lipid disorders, namely FH. But while FH results from mutations of strong effect in genes that perturb low-density lipoprotein (LDL) receptor function and visibly segregate with high LDL cholesterol concentrations in family pedigrees, most cases of familial HTG are polygenic rather than monogenic disorders (95-97, 104). This critical distinction is necessary for any current review of this topic.

While cases of HTG cluster in families, HTG within a family does not typically follow classical Mendelian patterns of inheritance. HTG does not consistently show vertical transmission in family pedigrees. But the idea that most HTG states are monogenic has persisted in the literature and textbooks over decades, likely because the term familial is included in the names of several classical primary HTG disorders. However, a familial disorder should not be confused with a monogenic disorder: while many HTG cases are familial, they are usually not monogenic (95-97, 104).

The term 'familial chylomicronaemia' (formerly known as type 1 hyperlipoproteinaemia (HLP) has traditionally referred to the subgroup that has a monogenic basis. Monogenic chylomicronaemia results from loss-of-function mutations within genes that encode key check-point molecules in lipolysis (105). These disorders typically show autosomal recessive inheritance, with onset in childhood or young adulthood, often associated with failure to thrive and pancreatitis together with relatively low levels of all other classes of lipoprotein fractions. The estimated population prevalence of this rare phenotype is ~1 in 1 million (94-96). In contrast, the overall population prevalence of individuals with fasting triglyceride levels >10 mmol/L from polygenic or secondary causes is ~1:600 adults in the general population.

1.7.4 Clinical Features of Chylomicronemia:

Development of physical findings in HTG is less common today that in the past, likely due to earlier diagnosis and treatment. Presence of physical findings is generally related to the degree of TG elevation.

Familial chylomicronemia or familial chylomicronemia syndrome (FCS) often presents during infancy or childhood, and generally becomes manifest by adolescence (94, 102). Clinical features include failure to thrive, eruptive xanthomas, lipemia retinalis, hepatosplenomegaly, recurrent abdominal pain, nausea and vomiting, and risk of acute pancreatitis (102, 106). Less common clinical features include intestinal bleeding, pallor, anemia, irritability, diarrhea, seizures and encephalopathy (102, 106).

Plasma from individuals with chylomicronemia appears lipemic: turbid and milky (106). If allowed to settle overnight, it develops a cream-like supernatant above a virtually clear infranatant (94). Fasting serum TG concentration is generally >10 mmol/L), and sometimes can exceed 100 mmol/L (107). Concomitant lipid abnormalities include a modest elevation in serum total cholesterol, with decreases in LDL and HDL cholesterol (94).

Eruptive xanthomas appear on extensor surfaces of the extremities, the buttocks and the shoulders as raised crops of small yellowish papules encircled by erythematous halos (108). Xanthomas erupt when plasma TG is severely elevated, and gradually disappear over weeks to months as TG levels improve (109). Microscopically, xanthomas contain lipid-laden macrophages (foam cells) within the superficial reticular dermis, as well as infiltrations of lymphocytes and neutrophils (108).

Lipemia retinalis refers to a whitish-pink appearance of retinal vessels on fundoscopic examination, and is due to the presence of chylomicron-rich serum. This finding is more likely to be present when TG is > 30 mmol/L) (94). Vision is unaffected (109).

Hepatosplenomegaly is also related to the degree of TG elevation and results from lipid accumulation within cells of the reticuloendothelial system. Hepatosplenomegaly is rapidly reversible with correction of plasma TG levels (94).

Patients with familial chylomicronemia have increased lifelong risk of recurrent pancreatitis (110). Pancreatitis due to HTG can be serious and sometimes fatal, (mortality 5-6% overall) (111-115). Retrospective studies have shown that at least 15% of patients with severe hypertriglyceridaemia have a history of pancreatitis (116) with five-year rates of pancreatitis of at least 3.5% (117). Although most patients present with low-risk clinical features, mortality can approach 30% in select subgroups with severe clinical symptoms (111-115).

The highest mortality has been linked to the development of pancreatic necrosis in patients who also develop infected abscesses or have persistent multiple organ failure (113-115). The absolute and relative risks of developing pancreatitis increase when triglyceride levels are >10 mmol/L (94, 106, 118) and sharply increase when triglyceride levels are >20 mmol/L (116).

Pancreatitis in patients with HTG is hypothesized to be a consequence of the pathological release of normally exocrine pancreatic lipase into local pancreatic capillaries, which results in partial lipolysis of lipoproteins and generates free fatty acids that prematurely activate trypsinogen and lead to autodigestion of the pancreas (94, 112, 118). Increased chylomicrons might further worsen the process by causing capillary plugging and local ischemia (94, 118). Risk of pancreatitis in patients with chylomicronaemia is markedly reduced after lower triglyceride levels are achieved (119).

The relationship between chylomicronaemia and atherosclerotic cardiovascular end points is less well understood. In monogenic chylomicronaemia (ie FCS), the occasional reports of premature atherosclerosis seem to be the exception and corroborate the clinical rule that elevated chylomicrons in isolation are not atherogenic (120). Younger patients with chylomicronemia are less prone to develop ASCVD than patients with other lipid disorders (121). Autopsies of some familial chylomicronemia patients showed no significant burden of atherosclerosis (107), possibly because chylomicrons are too large to penetrate the endothelial surface (107). In addition, LDL cholesterol is relatively low in patients with chylomicronemia (107). Small case studies suggest that some patients with chylomicronemia can still develop premature atherosclerosis (107). However, the presence of atherosclerosis in this situation could have been due to pro-atherogenic effects of modified chylomicron remnants, or to the impact of low HDL cholesterol in these patients (107).

However, in polygenic chylomicronaemia there is a much broader range of associated lipid disturbances, including increased levels of apolipoprotein B-48 (apoB-48)-containing chylomicron remnants, as well as increased levels of apoplipoprotein B-100 (apoB-100)-containing VLDL, VLDL remnants and intermediate density lipoproteins (IDL), together with reduced levels of HDL (122). Chylomicronaemia in this instance is a marker for postprandial lipaemia, which is increasingly being appreciated as a proatherogenic metabolic state (122). In particular, triglyceride-rich lipoprotein remnants that accumulate postprandially, both of intestinal origin (apoB-48-containing chylomicron remnants) and of hepatic origin (apoB-100-containing VLDL remnants) are considered to be proatherogenic (109), although standardized procedures for measuring these entities in the clinic have proven to be elusive.

While large triglyceride-rich particles such as chylomicrons and VLDL cannot cross the endothelial membrane, smaller VLDL or other triglyceride-rich remnant particles may be able to enter the artery walls, and lead to atherosclerotic change (123, 124). Furthermore, when triglyceride levels are elevated, cholesterol ester transport protein (CETP) mediates the transfer of triglycerides from chylomicrons and VLDL to LDL and HDL in exchange for cholesterol ester (CE) from LDL and HDL, leading to CE-enriched remnant particles and a shift towards smaller, denser LDL particles, which may both contribute to an atherogenic environment (123, 125, 126).

1.7.5 Molecular Basis of Monogenic Chylomicronemia:

Patients with fasting triglyceride levels >10 mmol/L probably have a component of chylomicronaemia and should be investigated following a step-wise approach. First, evidence of clinical features of chylomicronaemia syndrome should be sought and secondary causes such as uncontrolled type 1 or type 2 diabetes mellitus, hypothyroidism, poor diet, alcohol use, nephrotic syndrome or use of associated medications should be ruled out (94). For younger patients with few or no secondary factors a monogenic cause can be searched for, of which LPL deficiency is the most probable cause. In the past, biochemical studies were used to determine whether LPL or apoC-II activities were depressed or deficient (107, 120, 121), but today gene sequencing has become the diagnostic method of choice. At the DNA level, patients with monogenic hyperchylomicronaemia have homozygosity or compound heterozygosity for rare loss-of-function mutations in causative genes, such as *LPL*, *APOC2*, *APOA5*, *LMF1* or *GPIHBP1* (127, 128). These genes can be included as part of a targeted next-generation sequencing diagnostic panel for monogenic dyslipidaemias (129). Having a molecular diagnosis could aid in the early identification of at-risk family members and also in establishing candidacy for emerging therapies targeting primary LPL deficiency, especially when patients present at a young age. On the basis of the current standard of genetic investigation in these patients, a new diagnostic classification of primary chylomicronaemia that is founded on a molecular diagnosis might be warranted.

Monogenic chylomicronaemia typically presents in infancy or childhood, and by adolescence at the latest (102, 103). The most common gene affected in these individuals is *LPL* (encoding lipoprotein lipase; also known as LPL), in which loss-of-function mutations account for >90% of cases (105) (Table 1.7). More than 114 mutations in *LPL* have been described as leading to chylomicronaemia, including frameshift, missense and nonsense mutations; however, no single mutation in *LPL* predominates (103, 105, 130-137). LPL localizes to muscle and adipose tissue and catalyzes the hydrolysis and uptake of

triglycerides into peripheral tissues (Figure 1.4) (138); in the absence of LPL, chylomicrons accumulate in the plasma (102). Biallelic *LPL* deficiency has an estimated prevalence of 1 in 10^6, but has a carrier frequency of 1 in 40 persons in some founder populations (95).

Mutations in *APOC2* are the second most frequently reported cause of monogenic chylomicronaemia (103, 139-141). Mutations in other genes causing monogenic chylomicronaemia are even rarer, with only a handful of kindreds described in the literature for each. These other mutations include those in *APOA5* (encoding the LPL cofactor apolipoprotein A-V; commonly known as apoA-V), *LMF1* (encoding the LPL chaperone lipase maturation factor 1; also known as LMF1) and *GPIHBP1* (encoding glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1; also known as GPIHBP1. ApoA-V is believed to stabilize the lipoprotein–enzyme complex and to enhance lipolysis; thus, when apoA-V is defective or absent, the efficiency of LPL-mediated lipolysis is decreased (142, 143). Mutations in *APOA5* have been described in 3 families (142, 144-146). GPI-HBP1 directs the transendothelial transport of LPL and helps anchor chylomicrons near LPL on the endothelial surface, thus supporting lipolysis. Mutations in *GPIHBP1* have been reported in 10 families (142, 144, 147-156). LMF1 is a chaperone molecule required for the proper folding and expression of LPL on the endothelial cell surface; mutations in *LMF1* lead to reduced LPL expression and have been reported in two families (96, 144, 157). It seems that apoA-V, LMF1 and GPIHBP1 are enhancers or modifiers of chylomicron hydrolysis; carriers of recessive mutations in the genes encoding these proteins tend to present later and with less severe phenotypes than individuals with deficiencies in LPL and apoC-II (Table 1.7)(96, 144). However, given the rarity of monogenic chylomicronemia, not much is known about the potentially different presentations associated with these different molecular etiologies. This work attempts to expand on current knowledge of these rare phenotypes in chapter 3.2.

Table 1.7: Genetic basis of primary monogenic chylomicronaemia

Gene (gene product)	Homozygote prevalence	Gene product role	Clinical features	Molecular features	% of monogenic mutations	References
LPL **(LPL)**	~1 per million(109)	Hydrolysis of triglycerides and peripheral uptake [	Severe chylomicronaemia in infancy or childhood	Severely reduced or absent LPL enzyme activity	95	(109, 130, 134, 138)
APOC2 **(apoC-II)**	10 families reported	Required cofactor of LPL	Severe chylomicronaemia in childhood or adolescence	Absent or non-functional apoC-II	2	(109, 139)
GPIHBP1 **(GPI-HBP1)**	10 families reported	Stabilizes binding of chylomicrons near LPL Supports lipolysis	Chylomicronaemia in late adulthood	Absent or defective GPI-HBP1	2	(149, 153)
APOA5 **(apoA-V)**	3 families reported	Enhancer of LPL activity	Chylomicronaemia in late adulthood	Absent or defective apoA-V	0.6	(142, 143)
LMF1 **(LMF1)**	2 families reported	Chaperone molecule required for proper LPL folding and/or expression	Chylomicronaemia in late adulthood	Absent or defective LMF1	0.4	(157)

Abbreviations: apoA-V, apolipoprotein A-V; apoc-II, apolipoprotein C-II; GPI-HBP1, glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1; LMF1, lipase maturation factor 1; LPL, lipoprotein lipase

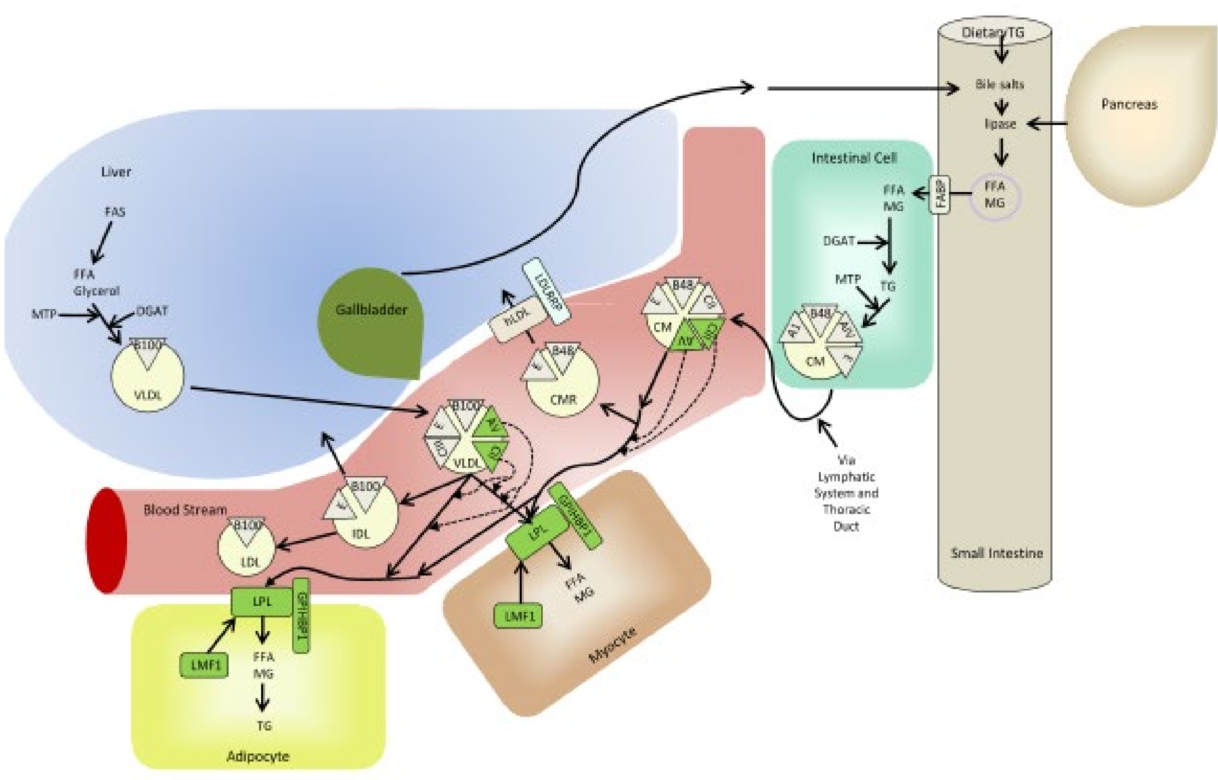

Figure 1.4: Triglyceride-rich lipoprotein metabolism.

Shaded molecules indicate those implicated in monogenic chylomicronaemia. Dotted lines indicate a key functional role of the apolipoprotein in the indicated process. In normal individuals, dietary fat is hydrolysed by pancreatic lipase, which requires emulsification with bile salts. Fatty acids enter intestinal cells via fatty acid binding proteins. Triglyceride-rich lipoproteins of intestinal origin are assembled in a multistep process requiring DGAT and MTP, and through the lymphatics enter the circulation as chylomicrons, which are ~90% triglycerides, with a small amount (1–3%) of cholesterol ester and surrounded by a phospholipid envelope containing several apolipoprotein molecules, including the chylomicron-specific apoB-48 as well as apoA-I, apoA-V, apoC-II, apoC-III and apoE.(102) By contrast, endogenously derived triglyceride-rich lipoproteins of hepatic origin are assembled de novo, also requiring MTP and DGAT, and circulating plasma within apoB-100-containing VLDL particles. Chylomicrons are usually cleared from the circulation within minutes by LPL-mediated hydrolysis, which is assisted by the essential cofactor apoC-II and enhanced and stabilized by apoA-V (dashed lines indicate the facilitatory role of apolipoproteins).(102, 158) Kinetic studies indicate that chylomicrons compete with VLDL for saturable catabolism by LPL. GPIHBP1 directs the transendothelial transport of LPL, helps anchor chylomicrons to the endothelial surface and enhances lipolysis.(102) Fatty acids liberated by lipolysis are taken up by peripheral cells, where they can be oxidized for energy or stored as triglycerides, depending on the cell type. After lipolysis, chylomicron remnants are removed by the liver, likely through LRP1 receptor, which contrasts with postlipolytic

VLDL remnants, most of which undergo further processing, ultimately resulting in LDL. Abbreviations: A-I, apolipoprotein A-I (apoA-I); A-IV, apolipoprotein A-IV (apoA-IV); A-V, apolipoprotein A-V (apoA-V); B-48, apolipoprotein B-48 (apoB-48); B-100, apolipoprotein B-100 (apoB-100); C-II, apolipoprotein C-II (apoC-II); C-III, apolipoprotein C-III (apoC-III); DGAT, diacylglycerol O-acyltransferase; E, apolipoprotein E (apoE); FABP, fatty acid-binding protein; FAS, fatty acid synthase; FFAs, free fatty acids; GPI-HBP1, glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1; LRP1, LDL receptor related receptor 1 ; LMF1, lipase maturation factor 1; LPL, lipoprotein lipase; MTTP, microsomal triglyceride transfer protein.

1.7.6 Polygenic or Complex HTG

Polygenic HTG has a complex genetic etiology. First, certain common small effect variants (SNPs) are consistently overrepresented in the genomes of adult patients with all subtypes of HTG (96, 97, 104, 159). Second, the genetic pool of adult HTG patients is enriched for rare heterozygous large-effect mutations within genes that are associated with elevated plasma TG levels (96, 97, 104). Finally, secondary factors can push a genetically susceptible individual over the edge metabolically, resulting in clinical presentation (94).

Polygenic chylomicronaemia is also 'familial' in the sense that multiple predisposing genetic variants cluster within families, although the disease trait does not show sharp vertical transmission across generations. Instead, susceptibility results from the accumulation of multiple genetic variants, which include both heterozygous rare variants with large metabolic effects and common variants with small effects (that is, single nucleotide polymorphisms (SNPs))(95-98, 104, 159-162). These variants all reside on different chromosomes and are co-inherited stochastically. Individual variants are insufficient to cause a clinical phenotype, instead each variant incrementally increases the risk of developing chylomicronaemia. When a sufficient number of these genetic variants are simultaneously inherited, they cumulatively create a state of predisposition, which is further modulated by secondary factors such as poor diet, obesity, alcohol intake and uncontrolled type 1 or 2 diabetes mellitus. The metabolic phenotype in patients with polygenic chylomicronaemia tends to be less severe than those with monogenic chylomicronaemia. Individuals with polygenic chylomicronaemia tend to present later in life, usually as adults and often not until middle age, and have lower levels of triglycerides, less severe physical manifestations and fewer complications (102, 144) (Table 1.8).

The same SNPs identified in genome-wide association studies as being associated with subtle variations in triglyceride levels in the healthy general population are also associated with increased risk of severe hypertriglyceridaemia and chylomicronaemia (95-98, 103, 104). Heterozygous rare variants that in the homozygous state cause autosomal recessive chylomicronaemia are also markedly over-represented in patients with polygenic HTG. The proportions of patients with these heterozygous rare variants are ~15% and ~6% for HTG patients and normolipidemic individuals, respectively (160, 163)}. Furthermore, in patients with polygenic chylomicronaemia, the number of rare heterozygous variants found within hypertriglyceridaemia-associated genes (discovered from SNP genotypes in genome-wide association studies and from animal model studies) is increased (97). HTG patients as a group have significantly higher polygenic risk scores (PRS) than normolipidemic patients (159-161). A very high or very low genetic risk score can discriminate between HTG and

normolipidemic subjects at the extremes of the distribution; however there is substantial overlap of scores between patients and healthy subjects in the middle of the distribution (96, 97, 104, 159).

Despite advances in our understanding of the genetic basis of both monogenic and polygenic chylomicronaemia (Table 1.9), ~30% of patients with chylomicronaemia neither have any recessive rare variants identified nor have an increased number of heterozygous rare variants or common SNPs in known HTG-associated genes (105). This information suggests that additional, as yet unidentified, genes or factors are be involved in the development of chylomicronaemia.

Table 1.8: Common DNA polymorphisms associated with hypertriglyceridemia.

CHR	Gene	SNP	Risk allele	OR (95% CI)	*P*-value
11	*APOA5*	rs964184	G	3.43 (2.72–4.31)	1.12×10^{-25}
2	*GCKR*	rs1260326	T	1.64 (1.36–1.97)	1.97×10^{-7}
8	*LPL*	rs12678919	A	2.21 (1.52–3.22)	3.5×10^{-5}
8	*TRIB1*	rs2954029	A	1.50 (1.24–1.81)	3.8×10^{-5}
1	*ANGPTL3*	rs2131925	T	1.51 (1.23–1.85)	1.0×10^{-4}
7	*MLXIPL*	rs7811265	A	1.63 (1.25–2.13)	3.3×10^{-4}
4	*KLHL8*	rs442177	T	1.36 (1.13–1.64)	1.5×10^{-3}
10	*CYP26A1*	rs2068888	G	1.29 (1.08–1.55)	5.9×10^{-3}
19	*CILP2*	rs10401969	T	1.72 (1.16–2.54)	6.8×10^{-3}
2	*APOB*	rs1042034	T	1.28 (1.02–1.61)	0.032

Abbreviations: CHR, chromosome; SNP, single nucleotide polymorphism; OR, odds ratio for hypertriglyceridemia per risk allele; CI, confidence interval; *APOA5*, gene encoding apolipoprotein A-V; *LPL*, gene encoding lipoprotein lipase; *TRIB1*, gene encoding Tribbles homolog 1; *ANGPTL3*, gene encoding angiopoietin-like protein 3; *MLXIPL*, gene encoding MLX interacting protein-like 1; *KLHL8*, gene encoding Kelch like protein 8; *CYP26A1*, gene encoding cytochrome P450 26A1; *CILP1*, gene encoding cartilage intermediate layer protein 2; *APOB*, gene encoding apolipoprotein B (159-162).

Table 1.9: Primary chylomicronemia: monogenic and polygenic forms

Features	Monogenic chylomicronaemia	Polygenic chylomicronaemia
Former designations	Familial chylomicronaemia Type 1 hyperlipoproteinaemia (WHO)(164)[	Mixed dyslipidaemia Type 5 hyperlipoproteinaemia(164) (WHO)
Main lipoprotein disturbances	Increased number of chylomicron particles only(94, 165)	Transient increase in levels of triglyceride-rich lipoproteins Increased number of chylomicron particles, Increased levels of VLDL Increased number of chylomicron remnants Increased levels of VLDL remnants(109)
Associated lipoprotein disturbances	Reduced levels of VLDL, LDL and HDL	Usually reduced levels of HDL, sometimes reduced levels of LDL
Typical onset	Paediatric or adolescent	Adulthood
Clinical features	Failure to thrive Abdominal pain Nausea Vomiting Eruptive xanthomas Lipaemia retinalis Pancreatitis Hepatosplenomegaly(94)	Abdominal pain Nausea Vomiting Eruptive xanthomas (rare) Lipaemia retinalis (rare) Pancreatitis (~1% risk per year)(109)
Association with ASCVD	Minimal	Some evidence of increased risk (121, 122)
Prevalence	~1:100,000 to ~1:1,000,000(109)	~1:600 (102)
Contribution of secondary factors	Minimal	Major
Inheritence pattern	Autosomal recessive	Familiar clustering, but no discrete classical pattern
Genetic causes	Mutation in *LPL(109)*, *APOC2(109)*, *APOA5(145)*, *GPIHBP1(153)* and *LMF1(157)*	Genetic pool of affected individuals has increased prevalence of: - heterozygous rare variants in *LPL*, *APOC2*, *APOB*, *GCKR*, *APOA5*, *LMF1*, *GPIHBP1* and *CREBH* with large effect(159, 161) - common variants (SNPs) with small effects in ~40 genes identified in genome-wide association studies(161)
Current treatment	Dietary control: restriction of fat intake ± increased consumption of MCTGs Pharmacologic control: minimal effect of fibrates, niacin, ω-3 fatty acids, statins.	Dietary control: reduced intake of calories, fats, simple sugars and alcohol Control of secondary factors Pharmacologic control: ω-3 fatty acids and niacin (both have variable efficacy)

Abbreviations: CVD, cardiovascular disease; MCTGs, medium chain triglycerides; SNPs, single nucleotide polymorphisms.

1.7.7 Secondary Factors Contributing to Polygenic HTG

Most cases of adult-onset hypertriglyceridemia result, at least in part, from secondary causes, often in conjunction with inherited partial impairment in TG metabolism (96, 166-169), and are manifested only under conditions that increase TG production or impair clearance. Previous studies have suggested that secondary causes of VS-HTG, include obesity and metabolic syndrome, poorly controlled diabetes (170-172), diet with high positive energy-intake balance and high fat or high glycemic index, excessive alcohol consumption (165, 173, 174), pregnancy (particularly in the third trimester) (165, 173, 174), nephrotic syndrome, severe hypothyroidism, oral estrogen or tamoxifen, glucocorticoids, non-cardioselective beta blockers, bile acid sequestrants, cyclophosphamide, retinoids and HIV antiretroviral regimens and second generation antipsychotic agents (94, 175, 176).

The actual mechanisms whereby these factors increase chylomicronemia risk are complex and include increased production of triglyceride-rich lipoproteins, which could saturate genetically compromised lipolytic machinery (165, 177). Alternatively, some factors may directly down-regulate lipolysis, which could magnify partial impairment due to inherited factors. While some work has been done to determine which of these factors are most important in leading to the expression of severe triglyceride phenotypes, this is incompletely understood. This work attempts to further this understanding in chapter 3.3.

1.7.8 Non-Pharmacologic Management

Non-pharmacologic therapy is the only therapy required in patients with borderline-high TG levels (150–199 mg/dL). However, non-pharmacologic interventions (178) must be optimized, since HTG is often exacerbated by modifiable factors. Non-pharmacologic management includes: 1) strict glycemic control in patients with diabetes or impaired glucose metabolism; 2) treatment with levothyroxine in patients with hypothyroidism; 3) avoidance (if possible) of medications that increase TG (such as beta-blockers or thiazide diuretics); 4) limitation or abstinence of alcohol; 5) avoidance of simple carbohydrates; 6)

low fat diet (<30% of total daily caloric intake) and when TG level >10mmol/L, a very low fat diet (<15% of total daily caloric intake); and 7) weight loss in patients who are overweight or obese (94).

For patients with monogenic chylomicronaemia, the mainstay of therapy is a diet very low in fat (15–25% of daily caloric intake), which equates to ~30–50 g of fat daily (102, 103). Adherence to this diet can markedly improve the clinical manifestations of primary chylomicronaemia, including resolution of hepatosplenomegaly, abdominal pain and xanthomas, and a greatly reduced risk of pancreatitis. Unfortunately, such extreme diets are difficult to maintain and long-term compliance, especially in younger patients, is poor. Given this strict dietary regimen, supplementation with essential fatty acids (such as walnut oil or sunflower oil topically) (179) and fat soluble vitamins must be considered. The support of a dietician or nutritionist is also generally required for patients to achieve and maintain low-fat intake targets. Introducing oils high in medium-chain triglycerides, such as coconut oil, has had some anecdotal success in patients with chylomicronaemia, particularly in young, growing individuals with a high-energy demand and also possibly in pregnant women; the incorporation of medium-chain triglycerides into chylomicron particles is lower than that of longer-chain fatty acids (157, 180).

1.7.9 Standard Pharmacologic Management

In addition to non-pharmacologic therapy, pharmacologic intervention using fibrates, statins, niacin, ezetimibe, or fish oil may be required if TG ≥ 2.3 mmol/L. Note that bile acid sequestrants should be avoided in patients with moderate to severe HTG due to their potential for further increasing TG levels.

1.7.9.1 Fibrates

In patients with TG ≥ 5 mmol/L, fibrates such as gemfibrozil, bezafibrate, and fenofibrate may be the preferred pharmacologic therapy. Fibrates may also be used in patients with TG ≥ 2.3 mmol/L to help attain non-HDL cholesterol targets after LDL cholesterol target have

been met. Fibrates are weak agonists of peroxisome proliferator-activated receptor (PPAR)-α and lower TG by up to ~40-60% by: 1) inhibiting hepatic synthesis and secretion of TG; and 2) stimulating degradation of TG-rich lipoproteins (181). However, while randomized clinical trials clearly demonstrate the TG-lowering efficacy of fibrates, they have shown inconsistent impact on reduction of ASCVD (182-191).

1.7.9.2 Niacin

Niacin (nicotinic acid or vitamin B3, in high doses) is a therapeutic option for patients with TG ≥ 2.3 mmol/L who are unable to attain their non-HDL cholesterol goals, and also for patients with TG ≥ 5 mmol/L. It acts in the liver to decrease VLDL production via Diacylglycerol acyltransferase-1 (DGAT-1) inhibition and peripherally by increasing LPL sensitivity (192). Given crystalline niacin's main side effect of flushing and vasodilation, extended-release preparations of niacin (ERN) are preferred for use compared to niacin or nicotinic acid. Doses of 500–2000 mg of ERN can lower TG by 5%–35% (193).

1.7.9.3 Omega-3 Fatty Acids

The exact mechanism of action of omega-3 fatty acids (PUFAs) is unknown but is proposed to be related to a number of effects including inhibition of hepatic TG synthesis and VLDL secretion, decreased TG content of VLDL and increased FFA oxidation, with some of this action mediated through Apo CIII inhibition (194, 195). PUFAs are available in two forms, docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) and when consumed in quantities of 2-4g/day have been shown to reduce TG by 20-50% (194, 196). The TG-lowering efficacy is related to baseline TG values (197). They are generally well tolerated, with minimal side effects other than a concern for increased LDL with DHA use, thought to be due to the increased conversion of VLDL to IDL (194). Occasional mild gastrointestinal side effects of nausea and diarrhea may also occur in up to 27% of individuals taking a dose of 4g/day (198).

There are three prescription formulations of omega 3 fatty acids available in the United States: 1) omega 3 fatty acid ethyl esters (EPA + DHA, ie Lovaza (Glaxo-Smith-Kline), Omacor

(Reliant Pharmaceuticals)); 2) icosapent ethyl (EPA, ie Vascepa (Amarin Pharma)); and 3) omega 3 carboxylic acids (EPA, DHA and docosapentaenoic acid (DPA), ie Epanova (AstraZeneca))(198, 199). Vascepa has also been approved for use by Health Canada.

1.7.9.4 Statins

Statins act through inhibition of hydroxyl-methyl-glutaryl coenzyme A (HMG-CoA) reductase to prevent de novo cholesterol synthesis in the liver, thereby causing increased expression of the LDL receptor and increased removal of cholesterol from the bloodstream (192, 200-202). Their effect on plasma triglyceride levels may be due in part to increased large TG-rich lipoprotein lipolysis and increased plasma clearance of remnant particles (192, 201-203). In addition, statins may have pleiotropic effects, including anti-inflammatory, anti-thrombotic, and anti-proliferative properties that may prevent plaque growth and rupture (204). Statins also have proven cardiovascular morbidity and mortality benefit.

Individual statins have varying efficacy and potency but generally reduce TG in a dose dependent manner by 10-30%, with greater efficacy in patients with higher baseline TGs and with rosuvastatin and atorvastatin having the most robust effect (200, 205-209). Statins are relatively ineffective in lowering TG among patients with severe HTG, but can help simultaneously achieve LDL cholesterol targets among patients with TG in the 2.3-5 mmol/L range.

Although statins are generally well-tolerated drugs they do occasionally have adverse effects. These range from disruptive side effects such as gastrointestinal (GI) disturbances and myalgias, to modest to severe elevations in liver transaminases and life threatening events such as rhadomyolysis (200).

Combination therapy with a statin and fibrate can help normalize several components of the lipid profile (210, 211). In combination therapy with statins, fenofibrate is preferred to other fibrates, particular gemfibrozil, which has a higher rate of rhabdomyolysis when combined with a statin (212).

1.7.9.5 Ezetimibe

Ezetimibe inhibits intestinal absorption of dietary and biliary cholesterol through its effect on the Niemann-Pick C1 like 1 (NPC1L1) cholesterol transporter protein (200, 207). It is primarily indicated as an adjunct to statin therapy, or in patients with statin intolerance, to reduce LDL cholesterol, and has only minimal TG-lowering efficacy, generally 5-10% from baseline (207). While some patients may experience nausea or bloating with ezetimibe use, it is generally well-tolerated (194, 200).

1.7.9.6 Incretin-based therapies

Incretin-based therapies, including glucagon-like peptide 1 (GLP-1) agonists, such as exenatide and liraglutide, and Dipeptidyl peptidase-4 (DPP-4) inhibitors, such as sitagliptin, are employed as adjunct agents to improve glycemic control in patients with type 2 diabetes mellitus by increasing post-prandial insulin secretion (213). Their role in TG lowering is unclear but may be attributed to decreased intestinal TG absorption and decreased hepatic VLDL production (194). Since the incretin-based therapies have been primarily tested for glycemic control outcomes, not all trials report the effects on TG, but reductions range between 12-25%, with greater effects seen in those with high baseline TG levels (194, 214-217). The main observed adverse effects of incretin therapies are nausea and GI disturbance, with some hypoglycemia in the exenatide group (215, 216).

1.7.10 Approach to acute pancreatitis

Severe hypertriglyceridemia (S-HTG) is implicated in ~9% of acute pancreatitis cases (218-223). Cohort studies suggest that HTG-associated pancreatitis may have greater risk of complications and mortality than pancreatitis from other etiologies (224-226). As with non-HTG-related pancreatitis, conservative treatment consists of withholding oral intake plus supportive measures such as intravenous hydration. Insulin and/or heparin infusions and, more rarely, plasmapheresis have also been recommended in the past (223, 227). Heparin

and/or insulin infusions have been successfully used in cases of HTG-related pancreatitis, especially when hyperglycemia was present (219, 223, 228-238).

1.7.10.1 Therapeutic plasmapheresis or plasma exchange

In patients with chylomicronaemia with acute pancreatitis, direct removal of triglyceride-rich lipoproteins by plasmapheresis or plasma exchange has been used and reported in a few case reports and case series (158, 239-246). However, no controlled data indicate whether plasmapheresis is associated with better clinical outcomes or even superior lipid profile trajectories than supportive measures that combine fasting, hydration, pain relief and control of secondary factors. In the acute situation, when oral intake is halted, supportive measures applied and secondary causes managed, plasma levels of triglycerides decrease precipitously (50% reductions from baseline within 48–72 h) without plasmapheresis (221) . Small clinical trials comparing plasmapheresis to conservative management showed that plasmapheresis had no overall benefit (112, 247, 248). Also, plasmapheresis is costly, requires a specialized centre and staff, involves exposure to blood products and only temporarily improves triglyceride levels without addressing the original cause (249). A case series published in 2014 suggests that when chylomicronaemia occurs in association with uncontrolled diabetes mellitus, fasting plus insulin infusion leads to more rapid and effective triglyceride lowering than plasmapheresis (250). Given the lack of data, optimal management of HTG-associated pancreatitis is not yet determined. This work attempts to address some of these unresolved issues in chapter 3.4.

1.8 Familial Hypercholesterolemia:

The term familial hypercholesterolemia (FH) refers to a autosomal semi- or co-dominant genetic form of hypercholesterolemia generally associated with elevated total cholesterol and LDL-C levels >95% percentile compared to the general population. This disorder predisposes to silent and premature accumulation of cholesterol plaques in the coronary, central and peripheral vasculature that, without timely intervention, can lead to early-onset atherosclerotic cardiovascular disease (ASCVD), including coronary heart disease, stroke and peripheral limb ischemia.

1.8.1 Epidemiology

Heterozygous FH (HeFH) was traditionally taught to have a prevalence of 1 in 500; however, data now suggest a higher frequency (251, 252). For instance, a survey of 69,106 individuals in Denmark using the Dutch Lipid Clinic Network (DLCN) diagnostic criteria found a frequency of 1 in 219 for HeFH (253). Prevalence estimates from studies conducted in Australia (254) (*n* = 18,322), China (255) (*n* = 9,324), and the USA (256) (National Health and Nutrition Examination Survey; *n* = 36,949) were 1 in 229 to 350, 1 in 322, and 1 in 250, respectively. Pooled data from 19 studies totalling 2,458,456 individuals similarly estimated the overall prevalence of HeFH to be ~ 1 in 250 (257). The prevalence of homozygous FH (HoFH) has also been revised upwards to ~1 in 300,000 (range 1 in 160,000 to 1,000,000) (258, 259).

1.8.2 Diagnosis

Agreement exists on the elements required to diagnose FH, but the weighting of these elements varies between algorithms (260). Two clinical scoring systems are in general use: the Simon Broome Register (SBR) criteria (261) and the DLCN criteria (262) (Table 1.10). The US Make Early Diagnosis-Prevent Early Death (MED-PED) system(263) is less widely used (Table 1.10). Other proposed algorithms include that used by the American Heart

Association (AHA) (264) and the Canadian simplified FH definition (265) (Table 1.10). Concordance between these various algorithms is inconsistent (251, 266).

Most FH diagnostic algorithms score and assign weights to: lipid values (total cholesterol and/or LDL-C levels); presence of physical stigmata considered pathognomonic for FH, such as tendon xanthomas, xanthelasmas, or arcus cornealis; and personal or family history of premature ASCVD; or pathogenic DNA variants (Table 1.10). Secondary causes of elevated LDL-C level, such as obstructive liver disease, hypothyroidism, and nephrotic syndrome, must first be ruled out (265). Physical stigmata were observed in >50% of patients reported to have FH as recently as the 1970s, but these physical findings are found in only 5–20% of contemporary, well-characterized FH cohorts, owing possibly to early diagnosis and treatment or less careful ascertainment; nonetheless, physical stigmata are a highly specific diagnostic feature when present (265). An algorithm from Wales includes normal triglyceride levels to increase specificity of diagnosis (267); this modification excludes possible cases of combined hyperlipidaemia, a related but distinct and essentially polygenic phenotype (268).

A generally accepted diagnostic criterion is the presence of a pathogenic variant in one of three main genes associated with HeFH: *APOB*, *LDLR*, or *PCSK9* (15, 269, 270). However, many patients meet clinical criteria without a detected pathogenic variant; these patients also carry substantial ASCVD risk compared with the general population (271).

Genetic confirmation rate in patients with suspected FH varies depending on patient ascertainment. In cardiology cohorts, pathogenic variants are seen in ~2% of patients with an LDL-C level >5 mmol/L and early ASCVD (271). By contrast, in tertiary care lipid clinics, a genetic basis is found in up to two-thirds of patients referred with suspected FH (15). Untreated LDL-C levels >8 mmol/L were associated with ~90% genetic confirmation (that is, presence of a pathogenic variant) among patients with suspected FH (15, 269). Other predictors of a positive DNA test result included a personal or family history of tendon xanthomas, a personal history of CVD, or imaging evidence of increased atheroma burden (272, 273).

1.8.3 Pathophysiology

The chronically excessive levels of LDL-C in FH cause ASCVD (274). LDL has manifold deleterious effects on vascular function, including corruption of the normal arterial response to vasodilatory stimuli, promotion of vascular inflammation through multiple mechanisms, and pathological internalization by arterial wall macrophages when LDL particles become oxidized or are otherwise modified (95, 275). When overloaded with cholesterol, arterial wall macrophages become foam cells, which are components of atherogenic plaques that can eventually occlude arteries, leading to tissue ischaemia (275).

About two-thirds of plasma cholesterol is transported within LDL particles (274); the majority of these particles are removed by LDL receptors, which reside on most cell surfaces but are especially concentrated on hepatocytes (276). Increased LDL-C levels in FH results from impaired LDL-receptor activity, which is often caused by different classes of mutations that directly affect the receptor (276). The functional domains of the LDL receptor include the ligand or apoB-binding domain, epidermal growth factor-like domain, *O*-linked sugar domain, and transmembrane domain, as well as the anchoring cytoplasmic tail domain (276). Pathogenic DNA variants have been observed in all domains. Because of the central role of the LDL receptor in FH, its life cycle is briefly summarized (Figure 1.5), including the roles of several interacting proteins (276-282).

Given the plethora of interacting proteins, it is perhaps remarkable that genes encoding most of these proteins have not been reported to harbour FH-associated DNA variants. Mutations in three genes acting in receptor-associated pathways cause HeFH: the *LDLR* gene itself (259, 283); receptor-binding defects in *APOB* (259, 283), and *PCSK9* gain-of-function mutations (284). Mutations in the *LDLRAP1* gene (also known as *ARH*) cause a severe, recessive phenotype (285). Although no variants in the *MYLIP* gene (also known as *IDOL*) cause FH, a common polymorphism is associated with mildly increased LDL-C level (286). Rare variants in particular genes encoding proteins involved in LDL-receptor trafficking cause multisystem disorders, such as X-linked intellectual disability owing to rare *CCDC22* variants; elevated LDL-C level is a component sub-phenotype (278). The absence of

mutations in other genes encoding receptor-associated proteins suggests that dysfunctional variants are embryonically lethal.

1.8.4 Inheritance of FH

For five FH-causing genes (*APOB*, *APOE*, *LDLR*, *PCSK9*, and *STAP1*) one copy of a mutant allele acts dominantly to produce the disease phenotype, adhering to the conventional idea of HeFH (259, 283). For the *APOB*, *LDLR*, and *PCSK9* genes, many patients who inherit two mutant alleles have a more severe phenotype, consistent with HoFH, which some groups designate as autosomal dominant homozygous FH (287). However, the inheritance pattern is more accurately viewed as autosomal semi-dominant, given that both variant alleles from each affected parent contribute to the phenotype, additively raising the LDL-C level.

For *LDLRAP1*, *LIPA*, *ABCG5*, and probably *ABCG8*, two mutant alleles act recessively, producing a severe phenotype consistent with HoFH (258, 259, 283, 287). Here, the recessive label is appropriate, because carrier parents have normal lipid levels. Before next-generation DNA sequencing was used to identify pathogenic variants in *ABCG5*, *ABCG8*, *APOE*, and *LIPA* in a few patients with FH, these four genes were known to cause distinctive non-FH dyslipidaemia syndromes: sitosterolaemia (*ABCG5* and *ABCG8*), dysbetalipoproteinaemia (*APOE*), and cholesterol ester storage disease (*LIPA*) (288). The reason why FH is expressed instead of the classical disease phenotypes in these patients is unclear.

1.8.4.1 LDLR gene

Most cases of monogenic FH are caused by *LDLR* variants, with >2,000 rare variants reported (15, 264) and ~3,000 deposited in the ClinVar database. Variant types include: large-scale DNA CNVs (289); nonsense mutations within the coding region; small insertions or deletions (insertion–deletion variants or ‘indels’) within or near the coding sequence, some of which might shift the reading frame; splicing mutations, typically non-coding and

occurring at intron–exon boundaries; and missense mutations altering a single amino acid residue (15, 270).

These *LDLR* variants affect all stages of receptor-mediated endocytosis, but the mutation types and classes can be reduced to two categories: those resulting in synthesis of either no protein or a completely nonfunctional receptor (that is, receptor-negative or receptor-null mutations) and those resulting in synthesis of an ineffective receptor (that is, receptor-defective mutations). About 10% of *LDLR* variants that have been deemed pathogenic have actually been studied functionally in cell biology experiments in vitro (290).

1.8.4.2 LDLR DNA copy number variation in FH

The *LDLR* locus is particularly prone to CNVs because of an abundance of *Alu* repeat sequences mostly within introns (289). These underlie predominantly in-frame, whole-exon events: at least 56 unique deletions and 27 unique duplications of *LDLR* have been reported in patients with FH (289). More than 90% of *LDLR* CNVs are heterozygous deletions spanning multiple exons (289).

Given that CNVs account for $\geq$10% of pathogenic *LDLR* variants, especially in some founder populations (291), diagnostic laboratories must be able to detect CNVs in addition to single nucleotide variants. But because dedicated laboratory methods were required to detect CNVs, many laboratories decided to forego the expense and accepted the compromised ability to detect variants (292). However, next-generation DNA sequence data can now be bioinformatically processed to detect *LDLR* CNVs with complete concordance with previous methods (92). CNVs in other FH-related genes have not yet been reported.

1.8.4.3 APOB, PCSK9 and LDLRAP1 genes

Genetic mapping and next-generation sequencing studies in families with FH and no *LDLR* mutations revealed additional causative loci. Mutations in the region of *APOB* encoding the receptor-binding domain of apoB, the structural protein for LDL and an essential ligand for the receptor, cause an FH phenotype referred to as familial defective apoB, which accounts

for 5–10% of patients with FH (15, 266, 283, 285). Rare gain-of-function mutations in *PCKS9* account for ~1% of patients with FH (15, 266, 283, 285). Additionally, rare mutations in *LDLRAP1*, a gene that encodes LDL receptor adaptor protein 1, can cause an autosomal recessive form of FH (251, 259, 269). Dysfunction in any of these gene products impairs LDL clearance via receptor-mediated endocytosis, leading to the elevated LDL-C levels common to all definitions of FH.

1.8.4.4 Minor genes

FH-like phenotypes have also been seen in families with rare heterozygous variants in *APOE* (293), encoding apoE, and *STAP1* (294), encoding signal-transducing adaptor protein 1(15). Next-generation DNA sequencing of patients with severe, recessive hypercholesterolaemia have found rare bi-allelic mutations of other dyslipidaemia genes: *ABCG5* (295), encoding ATP-binding cassette sub-family G member 5, and *LIPA*, encoding lysosomal acid lipase (296), showing that mutations in these genes can cause an FH-like phenotype (15). Comprehensive exome-wide and genome-wide sequencing efforts have not identified other FH-related genes (297).

1.8.4.5 Polygenic influences

A polygenic trait is influenced simultaneously by functional alleles of many different genomic loci (298). Plasma lipid levels, including LDL-C levels, are polygenic traits (298). Furthermore, polygenic predisposition is recognized to cause high LDL-C levels in up to half of patients referred to lipid clinics who have possible or probable HeFH (15, 299-301). Instead of a single large-effect variant, many of these patients have inherited numerous small-effect alleles of single nucleotide polymorphisms (SNPs) that are common in the population (298). Each SNP genotype is associated in GWAS studies with subtle but reproducible and significant increases in LDL-C level (162, 302). More than 50 genomic loci have been associated with increased LDL-C levels (302); some examples are shown in Table 1.11. These small-effect loci can cumulatively raise LDL-C level into the same range as with a single HeFH-causing rare variant in *APOB*, *LDLR*, or *PCSK9* (299-301). Also, these small-

effect polygenic loci encompass some gene products that result in LDL overproduction, which is distinct from the monogenic forms that are predominantly catabolic defects.

This polygenic basis of FH was clearly demonstrated by Talmud and Humphries, who showed that many patients with FH and no monogenic mutation had a high score based on 12 common LDL-C-associated SNPs from GWAS (301). Using a reduced six-SNP score, they claimed that 88% of mutation-negative patients with FH had a polygenic basis (299). However, the proportion of patients with a polygenic basis depends on the cut-off point of the score distribution. From our lipid clinic, ~30%, ~50%, and ~80% of mutation-negative patients with possible FH have a polygenic score in the top 10th, 25th, and 50th percentiles, respectively (15). When the median polygenic score is chosen to represent high risk, a large proportion of mutation-negative patients are captured. However, half of the normal population also has a score this high, weakening its discriminatory power in an individual patient with FH.

Some of the top small-effect loci associated with LDL-C level overlap with large-effect loci such as *APOB*, *LDLR*, and *PCSK9* (162, 302), whereas others have no connection to lipid metabolism (Table 1.12). Determination and distribution of polygenic scores for LDL-C level are shown in Figure 1.6.

A high polygenic score can worsen the biochemical phenotype when a heterozygous large-effect FH variant is present (303), although not always (304). Also, patients with FH owing to polygenic risk seem to have less severe preclinical atherosclerosis in non-invasive imaging studies compared with individuals with large effect FH-causing mutations (305).

1.8.5 Phenotypic variability

Among carriers of the identical FH-causing variant, a wide range of LDL-C levels exists (306). Mean untreated LDL-C levels vary more predictably when individuals are grouped according to causative gene and mutation. For instance, *LDLR* variants are associated with higher LDL-C levels than *APOB* variants (15). Among *LDLR* variants, CNVs and splicing and nonsense

variants are associated with the highest mean LDL-C levels, with the lowest LDL-C levels seen with missense variants (15, 307). Patients with null variants have a more severe phenotype than patients with defective variants, presenting with significantly higher plasma levels of total cholesterol, LDL-C, and apoB (308). A further complication is that LDL-C levels in monogenic FH are higher in the branch of the family that presented to medical attention; mean LDL-C levels in affected relatives in more distant branches can be less extreme (304). This observation suggests the modulatory influence of background genetic effects, gene–environment interactions, or other biological factors in addition to simple Mendelian inheritance, even in the most apparently straightforward families with FH.

Variations in lipid levels among individuals with similar FH-causing mutations can also be caused by interacting genetic effects, either large-effect variants (309-311), polygenic effects (303), gene–environment interactions (including the effects of diet and lifestyle) (312-314), or non-Mendelian mechanisms (315). The non-Mendelian mechanisms include environmentally induced epigenetic effects (316), mitochondrial influences (317), or somatically acquired DNA variation in the liver or other tissues.

1.8.6 Cascade versus universal screening

The best screening method to find new cases of FH has been debated (272, 318-323). Universal population-wide screening has been proposed in the adult, adolescent, child, or infant populations, using lipid values, DNA testing, or both (324, 325). A pilot screening project obtained capillary blood samples from 10,095 children aged 1–2 years during routine immunization visits and tested for both cholesterol levels and known genetic mutations; family members of positive cases were then also screened (324). This programme had an overall case-finding utility of eight FH cases identified for every 1,000 children screened (four children and four parents), allowing for early monitoring and intervention (324). Other childhood universal screening programmes using lipid levels and prediction scores have shown success (326). Universal screening programmes minimize missed cases, but can be costly especially if genetic testing is included (264, 327). However,

preventing morbidity and mortality makes universal screening potentially cost-effective (264, 327, 328), especially with declining costs for DNA analysis.

By contrast, cascade screening tests all first-degree relatives of patients identified with FH, followed by all first-degree relatives of further identified cases, and so on (301, 320, 329, 330). The target population is enriched for positive cases because first-degree, second-degree, and third-degree relatives will have a 50%, 25%, and 12.5% likelihood, respectively, of carrying the causative mutation, which maximizes cost-effectiveness (320, 327, 330). Both lipid-only and genetic-only screening of relatives has been proposed, as has a combination approach, whereby relatives are screened with lipid levels followed by genetic testing when values exceed diagnostic thresholds (330).

Current Canadian Cardiovascular Society and National Institute for Health and Care Excellence guidelines support cascade screening (265, 331). The US Preventive Services Task Force cites insufficient evidence for or against universal screening in childhood or adolescence (318). The National Heart, Lung, and Blood Institute (332), endorsed by the US National Lipid Association (333), the AHA (264), and the American Academy of Pediatrics (332), recommends universal lipid screening in paediatric and adolescent patients aged between 9–11 years and 17–21 years and referral for genetic testing if thresholds are met (332), although adherence among providers to this testing strategy was found to be low (16–18%) (334).

1.8.7 Genotype-guided management

Determining the causative gene could theoretically guide tailored management in FH; for example, patients with *PCSK9* gain-of-function mutations might respond well to PCSK9 inhibitors. Some studies suggest pharmacogenomics could identify patients with HeFH who are less likely to respond to traditional therapies, allowing for earlier use of costly second-line agents (335-337). Testing for the underlying mutations might be more important in predicting drug response in HoFH (338).

The mean LDL-C response to statin treatment might vary by genotype in patients with HeFH (339). In Brazilian patients with HeFH, achieving target LDL-C levels was greatest in those with no mutation detected (that is, putative polygenic FH), intermediate in those with a receptor-defective allele, and worst in those with a receptor-negative allele, who coincidentally had the highest baseline LDL-C levels (340). The Spanish SAFEHEART registry of 2,752 adult patients with genotyped HeFH showed that those with a receptor-defective allele were more likely to reach their LDL-C goal than those with a receptor-negative allele (341). Several other unvalidated genetic determinants of response to statins have been reported (342).

Genetic factors have been studied with respect to response to PCSK9 inhibitors. Subgroup analyses of trials in patients with HeFH showed that baseline LDL-C level was related to the type of *LDLR* mutation, but no between-genotype differences were found in relative reductions in LDL-C level (343, 344). Clinical response seemed to be related to the ability of the wild-type allele to be upregulated. Furthermore, patients with heterozygous *APOB*-binding defective mutations (343) and those with heterozygous *PCSK9* gain-of-function mutations (345) responded equally well to PCSK9 inhibitors, and no differently from patients with heterozygous *LDLR* mutations.

In patients with HoFH, genotype seems to determine response to PCSK9 inhibitor treatment. For instance, in two studies, evolocumab given to patients with HoFH reduced LDL-C level by 31% and 21% compared with placebo (346, 347). Subgroup analyses showed that response was restricted to individuals with one or no receptor-defective alleles, whereas those with two receptor-negative alleles had no response (346, 347). In vitro studies of cells from patients with HoFH indicate that response to PCSK9 inhibition depends on having one or more receptor-defective allele whose residual function can be upregulated (348, 349).

Genetic prediction of treatment-related adverse effects has not been studied specifically in patients with FH. In individuals with FH, pharmacogenetic algorithm-based statin dose

adjustment might reduce statin-associated muscle symptoms or elevated muscle enzymes (350).

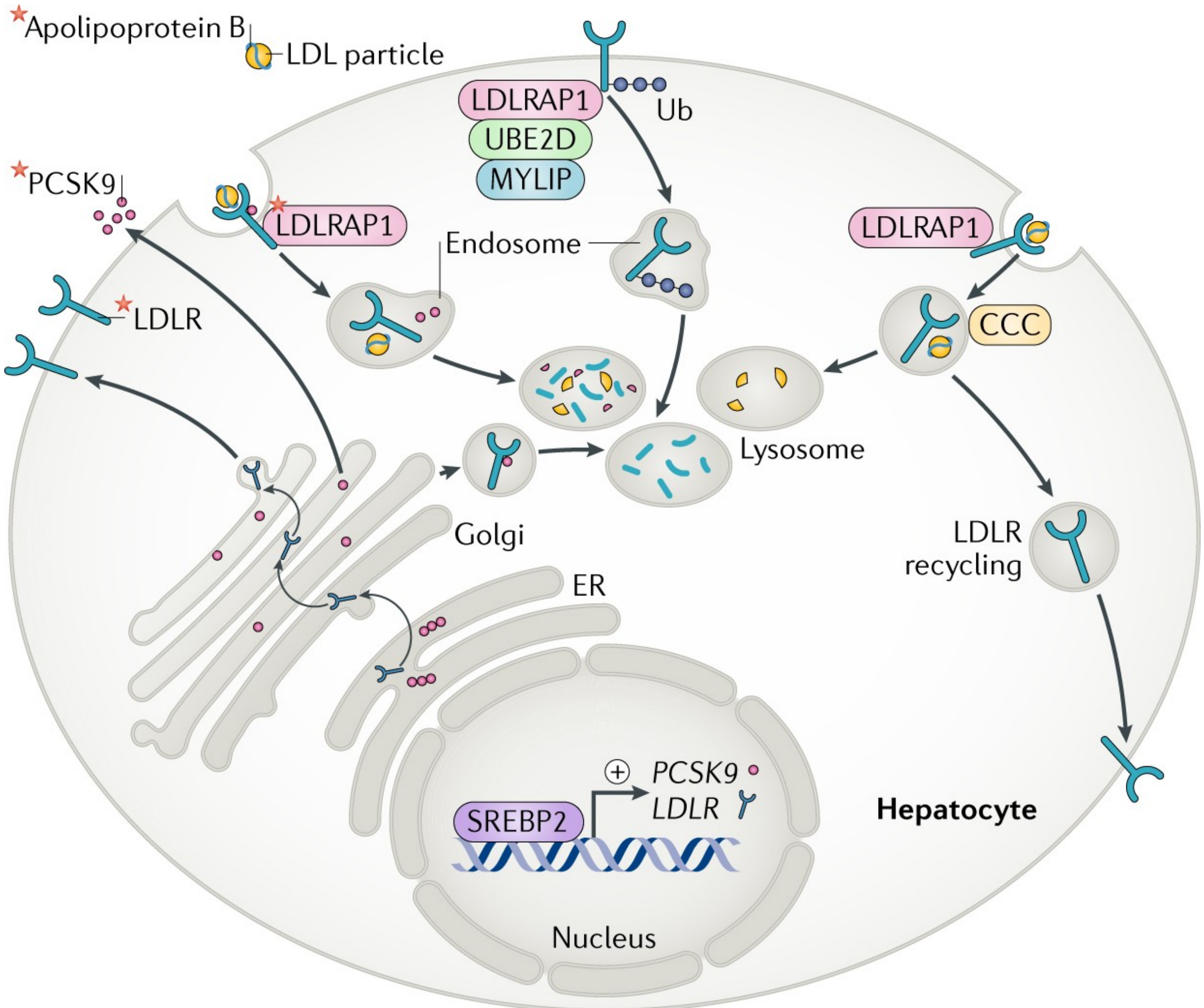

Figure 1.5: LDL-receptor lifecycle.

Normally, low intracellular cholesterol levels cause upregulation of the *LDLR* gene via the transcription factor sterol regulatory element binding protein 2 (SREBP2) (277, 278). LDL receptors (LDLRs) are synthesized in the endoplasmic reticulum (ER) and glycosylated in the Golgi; mature receptors reach the cell surface and cluster within clathrin-coated pits. The apolipoprotein B-binding domain of the receptor binds to circulating LDL particles and the receptor–ligand complex is internalized through clathrin-coated vesicles, which fuse with

early endosomes (277, 278). Internalization depends on the NPxY motif within the cytoplasmic tail of the receptor, which is the binding site for several proteins including the LDL receptor adaptor protein 1 (LDLRAP1, encoded by *LDLRAP1*) and a multiprotein complex called CCC that includes coiled-coil domain-containing proteins and COMM domain-containing protein (MURR1); collectively these proteins determine whether the receptor proceeds through endocytosis or is recycled (277-279). As the receptor–LDL complex is exposed to lower pH in the endosome, the receptor dissociates and is recycled to the cell surface; a single receptor can be recycled ≥100 times (277, 278). The retained LDL particles proceed from late endosomes through to lysosomes (277-279). After particle degradation, cholesterol is released into the cell, facilitated by NPC intracellular cholesterol transporter 1 and 2 (280). In response to cellular cholesterol content, the LDLR is degraded either by PCSK9 or by the E3 ubiquitin-protein ligase MYLIP (also known as IDOL). PCSK9 mediates receptor degradation both intracellularly and extracellularly (281, 282). In both instances, the LDLR is chaperoned to endosomes. PCSK9 perturbs the normal pH-dependent conformational switch, causing the receptor to remain within the endosome, leading to its degradation within lysosomes (281, 282). By contrast, internalization and degradation of the LDLR by MYLIP in response to increased intracellular cholesterol levels occurs through ubiquitination, endocytosis that is independent of clathrin and LDLRAP1, and an alternate pathway leading to the lysosome (277-279). The stars indicate proteins that when mutated cause FH.

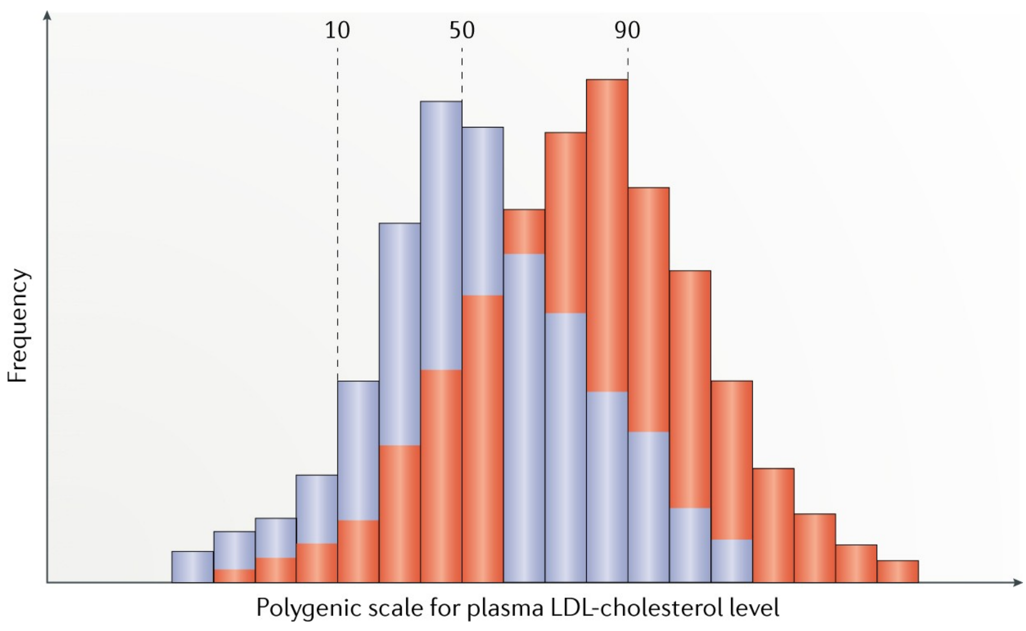

Figure 1.6: Polygenic influences on plasma LDL-cholesterol concentrations.

Illustrative distributions for polygenic risk scores for LDL-cholesterol (LDL-C) levels in the general normolipidaemic population (blue) and in clinically ascertained patients with suspected familial hypercholesterolaemia (FH) but no monogenic mutation (red). Scores are calculated from single nucleotide polymorphism genotypes such as those shown in Table 1.12. Scores are comprised either by simply tallying trait-raising alleles, or they can be further weighted according to effect sizes for the alleles reported in genome-wide association studies (162, 302). Because polygenic LDL-C loci are scattered throughout the genome and segregate independently during meiosis, most individuals have an overall balance between LDL-C-raising and LDL-C-lowering alleles. Rare individuals at the high extreme of polygenic scores have inherited a preponderance of LDL-C-raising alleles; they comprise a substantial proportion of individuals with suspected FH but no detected mutation. Cut-off points for 10th, 50th, and 90th percentiles for the general population are shown. No standard definition exists for a high polygenic score: choosing the median for the general population captures the majority of patients with FH but no detected mutation; however, it also captures half the normal population. By contrast, the more stringent 90th percentile is much more specific for patients with FH, but leaves about two-thirds of these patients with an unexplained genetic basis.

Table 1.10: Comparison between clinical scoring systems for FH

Criteria	Simon Broome Register(261)	Dutch Lipid Clinic Network(262)	MED-PED[a,](263)	AHA(264)	Canadian Criteria[22]
Lipids					
Total cholesterol (mmol/l)	>7.5 (adult) (a) >6.7 (child) (a)	NA	NA	NA	NA
LDL cholesterol (mmol/l)	>4.9 (adult) (a) >4.0 (child) (a)	>8.5 (8) 6.5–8.4 (5) 5.0–6.4 (3) 4.0–4.9 (1)	>5.7–9.3[b]	>5.0 (adult) (a) >4.0 (child) (a)	>4.0 (child) (a) >4.5 (18–39 years) (a) >5.0 (>40 years) (a) >8.5 (b)
Physical stigmata					
Personal	Tendon xanthoma (b)	Tendon xanthoma (6) Arcus cornealis[c] (4)	NA	NA	Tendon xanthoma (c)
Family	Tendon xanthoma in one relative (b)	Tendon xanthoma or arcus cornealis (2)	NA	NA	NA
Family history					
CAD	MI aged <50 years in two relatives or aged <60 years in one relative (d)	Premature CAD[d] (2) Premature CVD or PVD[d] (1)	NA	Premature CAD in one relative (b)	Premature CAD in one relative[d] (d)
LDL cholesterol (mmol/l)	>7.5 in one or two relatives (e)	Child with LDL-cholesterol >95th percentile (2)	NA	One affected relative (c)	One relative with high LDL-cholesterol level (d)
Genetics	NA	NA	Known FH in family member	NA	FH mutation in one family member (c)
Genetics					
Genetic mutations	*APOB*, *LDLR*, or *PCSK9* gene mutation (c)	*APOB*, *LDLR*, or *PCSK9* gene mutation (8)	NA	*APOB*, *LDLR*, or *PCSK9* gene mutation (d)	*APOB*, *LDLR*, or *PCSK9* gene mutation (c)
Diagnosis					
Diagnosis of FH	Definite: a + b or c Probable: a + d OR a + e	Definite: >8 Probable: 6–8 Possible: 3–5	Meets adjusted LDL-cholesterol cut-off point	a + (b or c) OR d	Definite: (a + c) OR b Probable: a + d

[a]Requires a diagnosis of FH in a family member. [b]Cut-off based on year and degree of separation from affected relative. [c]Arcus cornealis when aged <45 years. [d]Aged <55 years in men and aged <60 years in women. CAD, coronary artery disease; CVD, cerebrovascular disease; FH, familial hypercholesterolaemia; ICD, International Classification of Diseases; MED-PED, Make Early Diagnosis – Prevent Early Death; MI, myocardial infarction; NA, not applicable; PVD, peripheral vascular disease.

Table 1.11: Major and minor monogenic determinants of FH

Gene	Inheritance pattern	OMIM number	Proportion of patients with FH (%)	Mutation types	Refs
Major determinants					
LDLR	Autosomal co-dominant	606945	80–85	Splicing, frameshift, copy number variation, nonsense, and missense	(259,283)
APOB	Autosomal co-dominant	107730	5–10	Frameshift, missense, nonsense, and splicing	(259,283)
PCSK9	Autosomal co-dominant	607786	<1	Frameshift and missense	(259,283)
LDLRAP1	Autosomal recessive	605747	<1	Frameshift, missense, and nonsense	(258, 259)
Minor determinants					
APOE	Autosomal dominant	107741	<<1	Missense	(293)
STAP1	Autosomal dominant	604298	<<1	Missense	(294)
LIPA	Autosomal recessive	613497	<<1	Frameshift	(296)
ABCG5	Autosomal recessive	605459	<1	Nonsense	(295)
ABCG8	Autosomal recessive	605460	<<1	Unproven (only by analogy with *ABCG5*)	(295)

FH, familial hypercholesterolaemia; OMIM, Online Mendelian Inheritance in Man.

Table 1.12: SNPs used to calculate polygenic genetic risk scores

SNP number	Location	Gene	LDL-C-raising allele	Effect on LDL-C level (mmol/l)
rs6511720	19:11202306	*LDLR*	G	0.26
rs12740374	1:109817590	*CELSR2*	G	0.23
rs515135	2:21286057	*APOB*	C	0.16
rs6544713	2:44073881	*ABCG8*	T	0.15
rs11206510	1:55496039	*PCSK9*	T	0.09
rs3846663	5:74655726	*HMGCR*	T	0.07
rs1501908	5:156398169	*TIMD4*	C	0.07
rs2650000	12:121388962	*HNF1A*	A	0.07
rs6102059	20:39228784	*MAFB*	C	0.06
rs10401969	19:19407718	*NCAN*	T	0.05

LDL-C, LDL cholesterol; SNP, single nucleotide polymorphism.

1.8.8 Patient Impact of Genetic Testing for FH:

1.8.8.1 Potential Benefits of Genetic Testing for FH:

As outlined above (chapter 1.8.7), knowing the underlying genetics of hypercholesterolemia can help tailor therapy most effectively to the individual patient   It may also be necessary to obtain a genetic diagnosis in order to procure funding or eligibility for newer therapies, such as PCSK9 inhibitors. Some conditions can mimic HeFH, such as sitosterolemia, but require very different management strategies (19, 351). . There is also a large potential benefit to identifying and treating at-risk individuals aggressively as early as possible; therefore in addition to allowing initiation of this treatment in the proband, genetic testing may provide an opportunity for early detection and intervention for children or other family members of identified individuals. There is therefore a compelling reason from the provider perspective to offering genetic testing for suspected HeFH cases.

1.8.8.2 Past studies on patient impact of genetic testing in other conditions:

From the patient perspective, some studies have found a lack of retention or true understanding when it comes to understanding genetic risk. For example, a study of Alzheimer's susceptibility testing found that only 27% of patients tested could accurately recall their results a year later, whereas 23% were unable to recall any of the information conveyed to them at the time of testing (352). Qualitative studies suggest a wide range of individual responses to genetic information depending on a number of factors. These include the strength of the genetic findings with penetrance of the disease, whether the individual has witnessed family members express the disease in question, or whether active disease symptoms are present (351, 353).

1.8.8.3 Past Studies on Provider Impact of Genetic Testing for FH:

The degree to which a genetic test alters management in the clinic can be variable. Some studies have found that providing genetic test results to clinicians failed to result in significant changes in clinical practice (28, 351, 354).   A consistent belief was that the

clinical diagnosis of FH was more important than the DNA testing results, and that a negative genetic test did not rule out the disorder in patients with a strong clinical suspicion (351). Many felt that monitoring and basing management on lipid levels alone was sufficient and management was not often changed on the basis of a genetic test results (351). Some responders suggested that the real impact is on the family members, especially children, of the affected individuals, who could be diagnosed prior to developing any clinical concerns (351). It is important to note that this study was conducted prior to the introduction of PCSK9 inhibitors to the market for treatment of FH, and it is possible that a genetic diagnosis of FH may now have more clinical impact.

1.8.8.4 Impact of genetic testing for FH:

A number of past studies have attempted to look at patient impact or attitudes towards genetic testing, but often address only one aspect of testing impact, with small numbers of responders, and no incorporation of polygenic hypercholesterolemia. Results of these studies have also been mixed, highlighting the need for further investigation into these areas. Some prior studies have suggested that undergoing genetic testing for FH when offered is undertaken by a majority of individuals. One study showed that when approached in a cascade screening program for FH, only 2% of individuals did not participate, though 20% of respondents reported feeling social pressure to agree (355). Impact of genetic testing on patients seems to be variable and dependent on a number of factors, including those that are disease-related, past personal experiences, the method of information delivery, and many individual patient factors (356). In FH testing in particular, one study of newborn FH screening found that if the diagnosis was explained as made based on an elevated cholesterol, it was interpreted as controllable and caused less distress in parents than when the diagnosis was stated to have been made based on the discovery of a mutation (357). However, when disclosure of FH status was delivered to an adult population, it tended to be considered no more or less important than any other cardiovascular risk factor (357), or had very little impact on beliefs and behaviors overall (358, 359). One study found that patients with a diagnosis of FH felt less sense of

responsibility for their high cholesterol, and made a distinction between themselves and other individuals who had high cholesterol associated with what they perceived to be lifestyle (351, 359, 360). Some work has also been done on the potential negative impact of a negative test for FH, when the individual was expecting a positive test. One qualitative study suggested that failing to receive the expected diagnosis left patients feeling uncertain about their risk and the risks to their offspring and left them confused about their own contribution to their elevated cholesterol (361). Some felt that a genetic diagnosis was the only explanation for their elevated cholesterol because their diet and lifestyle habits were good, consequently these patients found the negative test difficult to accept (361). More promising results were seen in a cohort of patients undergoing cardiovascular risk stratification randomized to receive information on either their conventional risk factors alone, or their conventional risk factors as well as their genetic risk profile. Those with high genetic risk were more likely to remain on their statins than those at low genetic risk or those who were not informed of their genetic profile (362). This mirrors the findings of another study that suggested that those individuals with a genetic diagnosis of FH were less likely to rely on the efficacy of diet in improving their cholesterol, and more likely to believe in the efficacy of cholesterol-lowering medications (363). In a small qualitative study of 23 individuals, those with familial hypercholesterolemia reported increased guilt when not compliant with their medication or treatment recommendations, and reported being more attentive to their food choices but otherwise did not feel that the diagnosis significantly impacted their lives (364). None of those questioned indicated that they would have preferred to remain ignorant of their diagnosis (364). Further insight may also be derived from the GenTLe-FH study (Trial registration number UMIN000029375). Recruitment is underway for this study, in which patients will be randomized to either standard FH counselling, without disclosure of genetic information, or to disclosure of genetic information combined with genetic counselling regarding their diagnosis and will look at the impact on LDL-C levels, smoking status, and medication use in individuals at 24 and 48 months following the intervention (365). Given the overall conflicting and inconsistent results, it is clear that further study is needed in this area to clarify understanding. The

GenTLe-FH study may complement the results of the study presented in this work (chapter 4.2), which aims to look at which factors may be helpful to address during genetic counselling sessions about FH, and which behaviours may be impacted and to what degree.

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Chapter 2: Improving detection of Maturity Onset Diabetes of the Young (MODY)

2.1 Overview:

Monogenic disorders that can be fully explained by the presence of a rare pathologic variant are some of the most important clinical conditions to identify for a number of reasons. Firstly, for many of these conditions optimal management may depend on establishing an underlying genetic cause. Treatments or early interventions may be available that may significantly improve outcomes or quality of life by making an early definitive diagnosis. Secondly, these conditions will follow a consistent inheritance pattern within families so identifying one family member may have benefits for other related individuals. By tracing the mutation through the family it may allow for enhanced surveillance, early intervention, and may assist in family planning discussions. Furthermore, providing a definitive diagnosis for the patient often provides clarity and relief from uncertainty. It can also allow the affected individual and his or her physician to make more informed health-related decisions and have a better understanding of the expected disease course and prognosis. Patient advocacy and support groups may be available for these conditions, allowing affected individuals to seek information and access peer and community support.

One monogenic endocrine condition for which genetic testing may play a significant role is in a group of monogenic diabetes syndromes, collectively termed Maturity onset Diabetes of the Young (MODY) (see chapter 1.6 for background). MODY is inherited in an autosomal dominant manner with the expressed phenotype largely dependent on the underlying genotype. Optimal management of MODY depends on making the correct diagnosis and tailoring management based on the specific subtype. As MODY can often be difficult to clinically distinguish from more common forms of diabetes such as type 1 and type 2 diabetes, the ability to accurately identify mutations in those clinically suspected of MODY is critical for establishing the correct diagnosis, and also for optimizing patient management.

2.2 Genetic confirmation rate in clinically suspected maturity-onset diabetes of the young.

The work presented in Chapter 2.2 has been edited from this original manuscript for brevity and consistency throughout this dissertation.

Brahm AJ, Wang G, Wang J, Cao H, McIntyre A, Hegele RA. Genetic confirmation rate in clinically suspected maturity-onset diabetes of the young. Canadian Journal of Diabetes. 2016 Dec; 40(6): 555-560. PMID: 27634015.

2.2.1 Background:

The best approach to genetic testing in MODY is controversial. Some argue for universal testing in young individuals (≤25 years of age) with diabetes and without evidence of diabetes-associated antibodies; others suggest that it does not affect management or patient outcomes sufficiently to justify the cost and inconvenience of widespread testing. However, the cost of DNA sequencing has declined dramatically. Furthermore, the impact of a definitive MODY diagnosis can be significant, especially for young individuals misdiagnosed with type 1 diabetes. Many of these patients are able to stop insulin and transition to SU therapy, with improved glycemic control (1, 2). Similarly, patients with SU-sensitive MODY misdiagnosed with "garden variety" type 2 diabetes may also benefit from a molecular diagnosis, since they are frequently well-maintained on SU monotherapy for decades prior to advancing to additional treatment (1, 2). Also, GCK-MODY (MODY 2) patients generally require less intensive treatment and have less risk of microvascular complications, which may be reassuring to patients and families, and may reduce long term monitoring and treatment costs (1). Finally, each first-degree relative of a mutation-positive patient has a 50% chance of carrying the mutation, which opens the possibility of predictive or pre-symptomatic screening for early intervention and counselling.

Who should be screened genetically for MODY? What type of testing should be conducted? To answer this question in a Canadian context, we have informally offered research-grade MODY genetic testing to colleagues since 1999, taking advantage of excess capacity on our Sanger and next-generation sequencing platforms. Between 1999 and 2015, 96 samples were received from unrelated patients for whom the referring endocrinologist had a high index of clinical suspicion for MODY. The aim of this study was to determine the proportion of suspected MODY cases submitted for analysis that resulted in a molecular diagnosis and to assess whether high provider clinical suspicion is a sufficient criterion to proceed with genetic testing.

2.2.2 Methods

2.2.2.1 Patient samples

In 1999, research-based MODY gene sequencing was instituted at the Robarts Research Institute in London, Ontario. Since that time, 25 Canadian physicians who suspected MODY in patients based on clinical assessment have referred patient samples for DNA analysis, with the caveat that the method was research-based and not clinically accredited. Referred samples arrived in an ad hoc unsolicited manner, initiated at the discretion of the referring physician. There was no cost to either referring physician or patient, except for the cost of sample shipping. There were no specific inclusion or exclusion criteria. Informed consent was obtained from patients prior to proceeding with DNA collection and analysis using a protocol approved by the University of Western Ontario Ethics Review Board (#07920E) (Appendix B).

2.2.2.2 DNA sequencing

DNA was extracted from whole blood. Samples received before 2012 were analyzed using traditional Sanger sequencing to detect mutations in genes associated with MODY subtypes 1-6 (Table 1.3). If a causal mutation was detected, this was reported to the patient and referring physician, with no further testing. All samples were retained for potential future analysis.

More recently, we developed a targeted next generation sequencing (NGS) panel and custom bioinformatic pipeline for metabolic disorders, known as LipidSeq (3) (Appendix C), which has greatly enhanced our ability to detect clinically relevant mutations. LipidSeq is high-throughput platform which has been designed to simultaneously screen for DNA variants in dozens of genes linked to metabolic and dyslipidemia disorders, including 13 MODY subtypes (3). The coding region of each gene is sequenced, together with all intron-exon boundaries, at least 150 bp of flanking intronic sequence and at least 500 bp of the

promoter and 3'-untranslated region. Samples in which no MODY mutation was initially found by Sanger sequencing were re-analyzed using the LipidSeq NGS panel. Samples received after 2012 underwent processing directly on LipidSeq. When multiple samples from affected individuals in the same kindred were received, the first tested affected individual was considered as the index case and samples from other family members were excluded from subsequent data analysis here.

Conventional prioritization criteria were applied to impute causality or potential clinical relevance to a DNA variant (3). A variant was considered to be causative if it had been previously reported in the literature and Human Genome Mutation Database (HGMD; http://www.hgmd.cf.ac.uk/) as being causative for MODY. Rare variants detected in MODY genes that had not previously been reported in HGMD were considered causal if: 1) their reported frequency was < 1% in the general population; and 2) they was classified as having a strong likelihood of being deleterious based on *in silico* prediction tools, such as sorting intolerant from tolerant (SIFT) (4) and PolyPhen version 2.0 (5).

2.2.3 Results

In total, 96 index samples were received between 1999 and 2015. From 87 samples received prior to 2012 that underwent Sanger sequencing, 20 had likely causative MODY rare variants and 2 had non-MODY diabetes-related rare variants (Figure 2.1). The 65 mutation-negative samples were added to 9 samples received since 2012: 19 had likely causative MODY rare variants and 6 had variants of uncertain significance in genes associated with MODY (Figure 2.1). Thus, out of 96 samples, 39 had probable causative variants in MODY genes, 6 had variants of uncertain significance in MODY genes, and 51 had no likely causative variant detected in any MODY or other diabetes-related genes.

The overall genetic confirmation rate for patients with high clinical suspicion for MODY was thus 40.6% in our sample set (39/96 patients). Of the 39 unique likely causative MODY variants detected, 27 (71.1%) had been previously reported as being causative (Table 2.1) whereas 12 (30.8%) were novel and had not previously been reported in the HGMD

database (Table 2.2). The novel variants were all likely to be deleterious based on their very low frequency in the general population and in silico bioinformatic prediction software (Table 2.2). Furthermore, rare variants of uncertain significance for MODY or non-MODY related diabetes were seen in 6.3% of samples (6/96 patients); the clinical relevance of these will require additional genetic, clinical and biochemical studies to evaluate (Table 2.3)

Rare variants most frequently involved *GCK* (MODY2) and *HNF1A* (MODY3), with 14 and 15 mutations in each, accounting for a combined 74.4% of mutation-positive samples. Mutations in other MODY genes were seen less frequently, including *HNF4A, HNF1B, PDX1, PAX4, BLK* and INS. No mutations were seen in *CEL, NEUROD, KLF11* or *KCNJ11*.

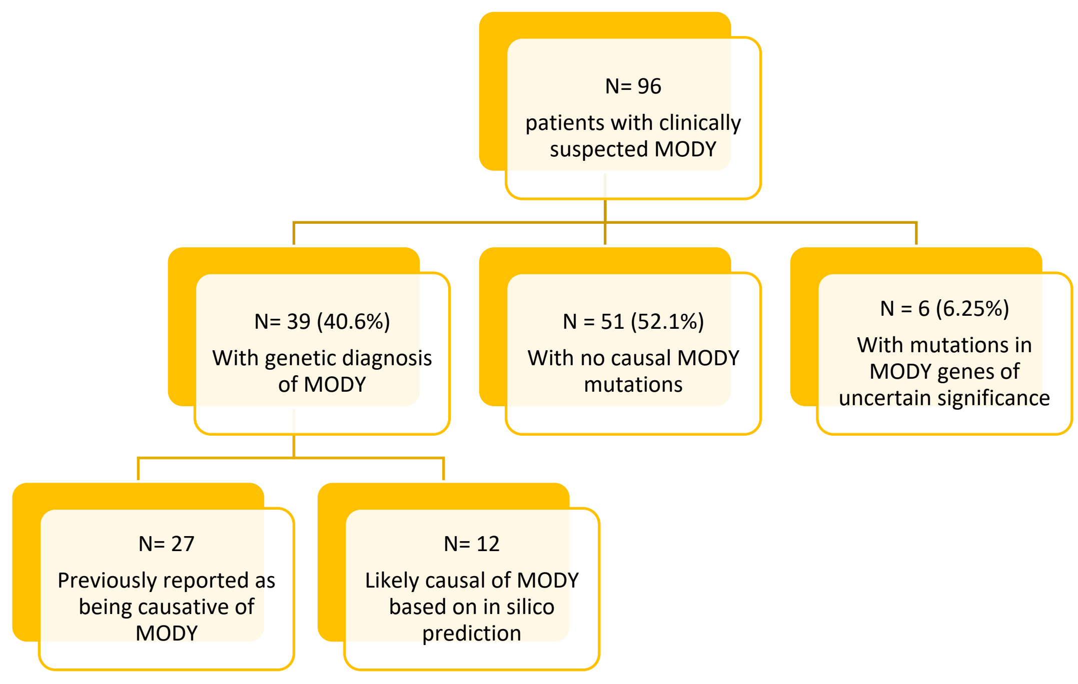

Figure 2.1: Suspected MODY cases

A total of 96 index samples were received between 1999 and 2015. 87 samples underwent Sanger sequencing, which identified 20 MODY mutations and 2 Non-MODY diabetes-related mutations. The remaining 65 samples, along with 9 additional samples received after 2012, were sequenced using targeted next generation sequencing (LipidSeq). An additional 19 MODY mutations (10 previously described, 9 novel but likely deleterious) were detected. 6 were found to have mutations of uncertain significance in MODY genes. MODY-related mutations were not identified in 51 individuals.

Table 2.1: Previously reported rare MODY variants identified in referred Canadian samples

Gene	Type	Nucleotide change	Mutation name	In silico prediction		Detection method	Previously reported in:
				SIFT	PolyPhen		
GCK	SNV	c.T59C	p.L20P	Damaging	Probably damaging	LipidSeq	MODY2
	SNV	c.G128C	p.R43P	Damaging	Probably damaging	LipidSeq	
	SNV	c.C175T	p.P59S	Damaging	Probably damaging	Sanger	
	SNV	c.G214A	p.G72R	Damaging	Probably damaging	Sanger	
	SNV	c.G386A	p.C129Y	Damaging	Probably damaging	LipidSeq	
	SNV	c.C617G	p.T206R	Damaging	Probably damaging	Sanger	
	SNV	c.G676A	p.V226M	Damaging	Probably damaging	Sanger	
	SNV	c.G706A	p.E236K	Damaging	Possibly damaging	Sanger	
	SNV	c.G766A	p.E256K	Damaging	Probably damaging	Sanger	
	SNV	c.T787C	p.S263P	damaging	Benign	Sanger/LipidSeq	
	SNV	c.C834G	p.D278E	damaging	Probably damaging	LipidSeq	
	SNV	c.T971C	p.L324P	Damaging	Probably damaging	Sanger	
	SNV	c.C1148T	p.S383L	Damaging		LipidSeq	
HNF1A	FS del	c.130delC	p.L44W fs X110	NA	NA	Sanger	MODY3
	SNV	c.G392A	p.R131Q	Damaging	Probably damaging	Sanger	
	SNV	c.C748T	p.Q250X	Damaging	NA	Sanger	
	SNV	c.T803C	p.F268S	Damaging	Probably damaging	Sanger	
	FS del	c.823_826 del GAAG	p.E275P fs X65	NA	NA	Sanger	
	FS ins	c.872_873 ins C	p.P291P fs X25	NA	NA	Sanger	
	FS del	c.1028_1029 del CA	p.T343S fs X74	NA	NA	Sanger	

	FS del	c.1054 del T	p.S352P fs X11	NA	NA	Sanger	
	FS del	c.1136_1137 del CT	p.P379R fs X38	NA	NA	Sanger	
	FS del	c.1268 del G	p.G423V fs X33	NA	NA	LipidSeq	
	SNV	c.C1298T	p.T433I	Tolerated	Probably damaging	LipidSeq	
HNF1B	SNV	c.G244A	p.D82N	Tolerated	Possibly damaging	LipidSeq	MODY5
PAX4	SNV	c.G575A	p.R192H	Damaging	Probably damaging	LipidSeq	MODY9
INS	SNV	c.G94A	p.G32S	Damaging	Probably damaging	LipidSeq	MODY10

FS del, frameshift mutation due to deletion; FS ins, frameshift mutation due to insertion; LipidSeq, targeted next-generation sequencing; NA, not applicable; SIFT, sorting intolerant from tolerant software; SNV, single nucleotide variant.

Note: Accession numbers for specific genes (National Center for Biotechnology Information, www.ncbi.nlm.nih.gov/) as follows: HNF4A: NM_000457; GCK: NM_000162.3; HNF1A: NM_000545.5; HNF1B: NM_000458.2; CEL: NM_001807.3; PAX4: NM_006193.2; BLK: NM001715.2.

Table 2.2: Novel heterozygous rare variants in MODY genes likely to be clinically relevant in study cohort

Gene	Mutation type	Nucleotide change	Mutation name	In silico prediction		Detection method
				SIFT	PolyPhen	
HNF4A	SNV	c.G25A	p.D9N	Damaging	Probably damaging	LipidSeq
GCK	Frameshift deletion	c.1226 del A	p.D409V fs X21	Damaging	Probably damaging	Sanger
HNF1A	SNV	c.G707A	p.C236Y	Damaging	Probably damaging	Sanger
	Frameshift insertion	c.137_138 ins G	p.K46K fs X13	NA	NA	LipidSeq
	Frameshift insertion	c.243_244 ins AG	p.T82R fs X73	NA	NA	LipidSeq
	Splicing	c.1108 -3 del TAG	IVS5 -3delTAG	NA	NA	Sanger
PDX1	In-frame insertion	c.713_714 ins GCC	L238 ins P	NA	NA	LipidSeq
	SNV	c.A571C	p.K191Q	Damaging	Probably damaging	LipidSeq
PAX4	SNV	c.G92T	p.R31L	Damaging	Probably damaging	LipidSeq
	SNV	c.G290A	p.R97H	Damaging	Benign	LipidSeq
INS	SNV	c.T89G	p.L30R	Damaging	Probably damaging	LipidSeq
BLK	SNV	c.C809T	p.T270M	Damaging	Probably damaging	LipidSeq

LipidSeq, targeted next generation sequencing; NA, not applicable; SIFT, sorting intolerant from tolerant software; SNV, single nucleotide variant.

Note: Accession numbers for specific genes (National Center for Biotechnology Information, www.ncbi.nlm.nih.gov/) as follows: HNF4A: NM_000457; GCK: NM_000162.3; HNF1A: NM_000545.5; PDX1: NM_000209.3; PAX4: NM_006193.2; INS: NM_000207.2; BLK: NM001715.2.

Table 2.3: Known and novel heterozygous rare variants in MODY genes of uncertain clinical significance found in study cohort

Gene	Mutation type	Mutation	In silico prediction		Reported in HGMD	Previously associated with:
Known rare variants in MODY genes, but not associated with MODY						
			SIFT	PolyPhen		
HNF4A	SNV	p.T117I	tolerated	benign	Yes	Type 2 diabetes (not MODY)
GCK	SNV	p.R36W	damaging	probably damaging	Yes	Type 2 diabetes (not MODY)
	SNV	p.F150S	damaging	probably damaging	Yes	Hyperglycemia
INS	SNV	p.G32S	damaging	probably damaging	Yes	Permanent neonatal diabetes
Novel rare variants of uncertain clinical significance						
Gene	Mutation Type	Mutation	In Silico Prediction		Reported in HGMD	Previously associated with:
			SIFT	PolyPhen		
ABCC8	SNV	p.G505S	damaging	probably damaging	No	possibly MODY12; familial hyperinsulinemic hypoglycemia; non-MODY type 2 diabetes
	SNV	p.V560M	tolerated	benign	No	possibly MODY12; familial hyperinsulinemic hypoglycemia; non-MODY type 2 diabetes

Abbreviations: SIFT, sorting intolerant from tolerant software; SNV, single nucleotide variant; prob., probably; HGMD, Human Genetic Mutation Database

2.2.4 Discussion

The relatively high genetic confirmation rate we observed (40.6% of samples with likely causative variants in MODY-related genes suggests that among Canadian endocrinologists, a high clinical suspicion for MODY provides a reasonable yield of positive genetic testing. We did not systematically evaluate the clinical criteria considered by each referring physician prior to requesting genetic testing. Similarly, we did not systematically collect the clinical features of the patients. Nonetheless, while we performed the analyses as a collegial no-cost service, with the caveats inherent to a research-based method, the relatively high detection rate of known or likely disease-causing MODY variants is a testament to the clinical acumen of the referring Canadian endocrinologists. But even without standardized inclusion criteria, the nonspecific gestalt of "high suspicion for MODY" seems to be a reasonable determinant of who should undergo genetic confirmatory testing. If such testing was to be developed into a clinical service with formal laboratory accreditation and proficiency testing, formal pre-test clinical criteria for MODY suspicion might further increase the diagnostic yield.

MODY could be a consideration in all patients with new-onset diabetes, especially those presenting under age 25. A key supporting feature is the presence of a strong family history: autosomal dominant inheritance with vertical transmission from affected parent to 50% of offspring, with similar clinical course in affected individuals. Other factors that would enhance suspicion for MODY include non-insulin requiring diabetes in a non-overweight or obese young person, or a patient diagnosed with type 1 diabetes but with no episodes of ketoacidosis, even years after diagnosis, and when insulin is withheld. Observation of a robust response to SUs and minimal response to metformin, may further increase suspicion of MODY. Evidence of preserved beta-cell function and negative autoantibodies would also support the diagnosis. Factors that may prompt genetic screening for MODY are summarized in Table 1.4. The use of a clinical prediction tool such as that used by Shields et al (6) may also be useful; it is readily available online and demonstrates a sensitivity of 91% and a specificity of 94% for predicting genetic confirmation of MODY (6).

Genetic confirmation of MODY may be valuable in clearly defining the primary mechanism of hyperglycemia. This could permit tailored and possibly more effective management, especially in patients with mutations in the most common causal genes, i.e. *HNF4A*-, GCK- and *HNF1A*-MODYs which, together, constitute 70% to 90% of all patients with MODY. Positive diagnoses can also allow for presymptomatic identification of at-risk family members who may benefit from increased monitoring, early intervention and counselling or reassurance if they are mutation negative.

As discussed above (chapter 1.6.4), management of *HNF4A*-MODY and *HNF1A*-MODY includes consideration of low-dose SUs as a first-line medication, as response is often robust, achieving 4-5% lowering of glycated hemoglobin (HbA1c) with monotherapy; patients can be commonly maintained on this inexpensive and generally well-tolerated monotherapy for decades (1, 7). Patients with *GCK*-MODY can generally be managed with reassurance and lifestyle modifications only, and may benefit from reduced cost and effort stemming from ongoing surveillance or monitoring (1, 7). Having a specific MODY diagnosis may also permit more appropriate management during pregnancy, with frequent fetal surveillance to guide therapy.

Assuming that genetic testing is appropriate when suspicion of MODY is high, what is the best technology or system to use? The recent global experience for most diseases, also reflected in our lab's study of patients with diabetes and dyslipidemia, is that next-generation sequencing technology (NGS) should be the standard diagnostic platform. NGS has reduced costs and expanded the range of gene loci and sequences that can be screened. For instance, cost recovery for DNA preparation and Sanger sequencing for only MODY2 (*GCK*) and MODY3 (*HNF1A*), is ~ $600 CDN at our centre. The cost for Sanger sequencing each additional MODY gene ranges from ~ $100 to ~1000 CDN, depending on the size of the gene. Thus, comprehensive screening of all 14 MODY loci using Sanger sequencing could total several thousand dollars per sample. This contrasts with the total cost recovery of ~ $500 for targeted NGS sequencing of 13 MODY genes using LipidSeq, which also captures several other inherited diabetes and dyslipidemia loci (3).

Screening all MODY loci simultaneously with targeted NGS also improves diagnostic yield. Although most MODY variants in our samples were found in *GCK* or *HNF1A* genes, about 30% of variants were scattered among other MODY genes. This emphasizes that NGS allows for simultaneous screening of all known MODY loci. Including the minor MODY loci in comprehensive screening process from the outset reduces the false negative detection rate. LipidSeq was a valuable and cost-saving tool in identifying MODY mutations compared to traditional Sanger sequencing, and was able to identify an additional 19 mutations in our sample set (Figure 2.1).

Factors that may result in false negative results from standard NGS include the presence of mutation types that escape detection by sequencing, such as large-scale copy number variations or chromosomal rearrangements. Also, some mutations may lie deep within non-coding regions outside the range of the NGS capture reagents. We designed LipidSeq to capture at least 150 base pairs at each intron-exon junction and up to 1000 base pairs in the 5' and 3' untranslated regions, so detection of potentially causative non-coding variants should be reasonably good. However, we cannot exclude the presence of potential causative variants deep within introns or within remote non-coding regions that may be important in emerging pathogenic mechanisms, such as micro RNAs or long non-coding RNAs, among others. Furthermore, there may still be unknown and undiscovered MODY loci that may be relevant in some currently mutation-negative patients. Finally, we cannot exclude undetected non-mendelian, mitochondrial, epigenetic, gene-gene interactions or gene-environment interactions as being potentially causative for MODY in some mutation-negative patients studied here.

Our false positive rate is constrained in part because of our stringent criteria for defining a detected variant as "likely causative" or clinically relevant, which include cross-checking past publications and existing reports of variants in global disease databases. Because of our overall caution when reporting findings, we are less concerned with reporting potentially false positive results, although this remains possible, especially for rare variants detected for the first time in this sample set. Sorting the wheat from the chaff is becoming

a general concern for high-throughput NGS analysis of human disease samples. We use state-of-the-art criteria, including stringent bioinformatic algorithms, to impute potential causality or clinical relevance of newly detected variants reported here. However, we realize that the ultimate proof of causality for many newly detected variants means further studies, including expanded family studies showing co-segregation of the variant with the MODY phenotype across generations. Also, each new variant can be studied functionally at the laboratory bench in a range of experimental model systems to acquire more confidence in their causative nature.

Recently, United States data were used to analyse cost-effectiveness of genetic screening for MODY (8). The model estimated that indiscriminately testing all patients diagnosed with type 2 diabetes only modestly improved quality adjusted life years (QALYs) by 0.012, compared to no screening, at a cost for one QALY of > $200,000 US (8). The total cost difference between testing and no testing scenarios on an individual patient basis was $2400 US, accounted for by the costs of screening and treatment (8). Raising prevalence (i.e. pre-test likelihood) of MODY to 6%, or reducing the cost of genetic testing to $700 UD in the model, which is consistent with our cost, suggested a cost-effective strategy, with $50,000 US per QALY. Actual cost savings were achieved at a MODY prevalence (i.e. pre-test likelihood) of > 30% (9). This suggests that while indiscriminate testing of all type 2 diabetic patients is not reasonable, cost-effectiveness is improved as the cost of genetic analysis decreases, or in subgroups with high suspicion who have an expected pre-test prevalence > 6%, such as those patients meeting the criteria in Table 1.4. Prevalence of mutation-positive samples in our data set – 40.6% - far exceeded the cost-saving threshold of 30%, suggesting that testing patients with high pre-test clinical suspicion may actually result in an overall cost benefit. As a result, identification of each MODY case that led to initiation of SU treatment resulted in improved glycemic control, with reduced HbA1c by ~ 1.5% (8).

The use of high-throughput targeted NGS techniques may further tip the cost-benefit equation in favor of testing. LipidSeq also has the additional benefit of testing for mutations

in genes causing non-MODY diabetes, dyslipidemias and related conditions such as lipodystrophy (3). Communicating these results back to the referring physician may perhaps prompt reassessment of the patient's clinical situation and adjustment of the short and long-term treatment plans.

Another potential benefit of genetic testing is improved quality of life and cost reductions when a patient misdiagnosed with type 1 diabetes is reclassified as having MODY. For instance, in the SEARCH for Diabetes in Youth study, 586 patients with diabetes onset before age 20, negative autoantibodies and C-peptide levels ≥ 0.8 ng/mL were genetically tested for mutations *HNF1A* (MODY3), *GCK* (MODY2),and *HNF4A* (MODY1) (10). MODY mutations were identified in 47 patients, or 8% of the participants, representing 1.2% of the overall pediatric diabetes population studied (10). Of the 47 newly identified MODY patients, all but three received insulin prior to reclassification (10); insulin was stopped or postponed in most re-classified patients.

Our study has some important limitations. First, we have minimal data for patients receiving genetic analysis, including the clinical factors that prompted genetic testing, and no follow-up data on the impact of the diagnosis on their subsequent management or outcomes. There were no defined inclusion or exclusion criteria applied to samples before proceeding with genetic testing, and no data regarding those factors that prompted physicians to send samples for analysis. Furthermore, we did not collect data related to ethnicity, which may have influenced the prevalence of specific MODY mutations. Finally, patients tended to be referred from tertiary care facilities, from providers with more experience with MODY (and perhaps with genetic testing in general), and from providers who may have believed that a positive diagnosis would change their management approach.

2.2.5 Conclusion

MODY generally presents in young individuals with a family history of diabetes. It might be underdiagnosed based on the low volume of requests for confirmatory genetic testing. A confirmatory molecular genetic diagnosis of MODY in patients who meet clinical criteria for high suspicion can have significant benefits in terms of improving diabetes management and control. It might even be cost-saving if diagnostic testing is confined to high risk groups (i.e. high pre-test likelihood) with an expected confirmation rate of > 30% (8). Further study is required to determine the precise criteria to select individuals for genetic testing. Newly discovered MODY mutations can be studied functionally in basic research laboratories to confirm their causal nature. However, even in the absence of such data, our findings suggest that high provider suspicion of MODY among Canadian endocrinologists is a reasonable first screen and may be sufficient in some cases to warrant consideration of confirmatory genetic testing.

2.3 Copy Number Variations in Patients With Maturity-Onset Diabetes of the Young

The work presented in Chapter 2.3 has been edited from these original manuscripts for brevity and consistency throughout this dissertation.

Berberich AJ, Huot C, Cao H, McIntyre AD, Robinson JF, Wang J, Hegele RA. Copy Number Variation in GCK in Patients With Maturity-Onset Diabetes of the Young. J Clin Endocrinol Metab. 2019 Aug 1;104(8):3428-3436. PMID: 30912798.

Berberich AJ, Mokashi A, McIntyre AD, Robinson JF, Cao H, Wang J, Hegele RA. Bioinformatic detection of copy number variation in HNF4A causing maturity onset diabetes of the young. Clin Genet. 2019 Oct;96(4):376-377. PMID: 31309534;

Berberich AJ, Wang J, Cao H, McIntyre AD, Spaic T, Miller DB, Stock S, Huot C, Stein R, Knoll J, Yang P, Robinson JF, Hegele RA. Simplifying Detection of Copy-Number Variations in Maturity-Onset Diabetes of the Young. Can J Diabetes. 2021 Feb;45(1):71-77. PMID: 33011132.

2.3.1 Background:

Genetic testing for MODY is currently accomplished primarily using next generation sequencing (NGS) techniques, which allow for the identification of single nucleotide variants (SNVs), small-scale insertions or deletions, as well as small-scale frameshift and null mutations (11-14) (see chapter 2.2). However, copy number variations (CNVs), defined as large-scale deletions or duplications in a genomic DNA region that can involve part of a gene, a whole gene or multiple genes, have historically been missed using sequencing techniques, while simultaneously being too small to be detected by traditional cytogenetic methods. Specialized targeted probe-based analysis or genotyping techniques can be used to assess for CNVs, but these add additional cost, and have not been incorporated into routine diagnostic procedures (15). They are currently of uncertain value in MODY, although a few gene deletions have detected this way (15, 16).

Affected sequences of duplicated or deleted genomic DNA in a CNV appears qualitatively normal. Therefore without a robust quantitative analytical tool, even when there appears to be an increase or decrease in the amount of genetic material replicated in certain DNA sections, it is impossible to distinguish a true deletion or duplication from the natural variability in chemical amplification of DNA that is used in most sequencing platforms (Figure 1.2, Figure 1.3), which are optimized to detect small qualitative changes in the genetic code.

Recently, new bioinformatic techniques have been developed to provide the robustness needed to assess for CNVs using NGS output data, without requiring additional sequencing. These methods take advantage of the fact that current NGS protocols generate large numbers of short partially overlapping DNA fragments that are assembled computationally to seamlessly reflect the genomic sequence of the source material (17). In addition, the total number of these synthetically-generated DNA fragments reflects the amount of starting material in the genome. This enabled the development of new algorithms that, through tallying the numbers of chemically-generated DNA fragments, impute deviations of

the amount of starting material from the normal diploid two copies (i.e. maternal and paternal) for any particular chromosomal region using a depth of coverage (DOC) ratio.

This approach has successfully been applied to detect CNVs using NGS data for several genes causing dyslipidemias (18-21). Here we describe the application of this technique to identify novel heterozygous large-scale deletions in individuals with suspected MODY that was not identified using traditional Sanger or targeted NGS-based sequencing.

2.3.2 Materials and Methods:

2.3.2.1 Subjects:

96 unselected individuals were suspected of having MODY by their individual clinicians and were referred for research-based MODY genetic testing at our center (see chapter 2.2). No specific inclusion or exclusion criteria were applied to individuals tested. The original testing results from this cohort found likely causal variants found in 39 of the original 96 patient samples (see chapter 2.2). The original NGS output data for the remaining undiagnosed 51 samples was re-analyzed for copy number variations using the CNV caller tool in VarSeq v1.4.3.

We focused on these 51 individuals in whom our initial targeted NGS screening failed to detect likely or definitely causative DNA variants in MODY genes (chapter 2.2). Patients and family members provided informed consent for genetic testing and analysis and under a protocol approved by the University of Western Ontario Ethics Review Board (#07920E) (Appendix B).

2.3.2.2 Targeted NGS:

All individuals were assessed for mutations in known MODY-associated genes using the targeted NGS panel and bioinformatics pipeline known as LipidSeq (3), designed to test for clinically relevant mutations in 73 specific genes associated with metabolic disorders, including those associated with MODY (Appendix C). Targeted NGS was performed using

standard operating procedures of the London Regional Genomics Centre (LRGC; www.lrgc.ca). Sequencing reactions were designed to include all coding regions, as well as the flanking ~150 base pairs (bp) of intronic DNA for each exon and ~500 bp at the promotor and 3' untranslated regions. The average depth of coverage (DOC) generated using this method is ~300-fold for each base, meaning that there are ~300 partially overlapping, non-identical small generated DNA fragments covering all coding regions of MODY-related genes; these can be quantified using bioinformatic analysis for CNVs.

2.3.2.3 Original variant calling:

A variant was considered causal if it had previously been reported as pathogenic in the Human Genome Mutation Database (HGMD). A previously uncharacterized rare variant in a MODY gene was considered pathogenic if the reported frequency was <1% in the general population and if it was predicted to be deleterious using in silico prediction tools, i.e. Sorting Intolerant from Tolerant (SIFT) (4), PolyPhen2 (5) and Combined Annotation Dependent Depletion (CADD) (see chapter 2.2)(22).

2.3.2.4 Bioinformatic analysis:

Following library preparation and enrichment, .FASTQ files of sequence data were generated using the MiSeq personal sequencer platform (Illumina) and sequence alignments, variant calling (.VCF files) , and target region coverage statistics (.BAM files) were generated using a custom automated workflow in CLC Genomics Workbench (CLC Bio, Aarhus, Denmark). Using this method, a variant is considered causative if it had been previously reported as causative in the Human Genome Mutation Database (HGMD) or if present in <1% of general population and predicted to be pathologic using in-silico prediction models. None of the individuals we report here were found to have causative mutations for MODY using this method.

2.3.2.5 CNV detection using NGS data:

LipidSeq data (in the form of .VCF and .BAM output files) was analyzed using the CNV caller function in VarSeq v1.4.3 (Golden Helix, Bozeman MT). A .BED file defining the target region and probes used in the NGS panel is also required. The algorithm uses a ratio of DOC in each region compared to a healthy reference population of 73 samples that were subjected to the same NGS sequencing panel as the sample being analyzed to identify potential CNVs.

The CNV caller tool makes use of "depth of coverage" (DOC), which refers to the number of amplification copies at a particular genomic location, to determine likely deletions or duplications. This technique takes small segments of DNA of approximately 100 base pairs (bps) in length, and determines the DOC at that location for that sample (23, 24). The DOC in the region of interest is compared to the surrounding regions and to a population of control genomes to determine if there in an unusual increase or decrease in DOC at that location (23, 24). If there is a statistically significant change in DOC, it is considered suspicious for a copy number change. This technique is based on the principle that the number of amplification copies present will be directly related to the amount of starting material, such that if there are fewer than expected DNA strands to begin with, there will be fewer copies when it is amplified, with the opposite being true for a duplication (23, 24). Using the CNV caller tool, a ratio is calculated for each segment of DNA by dividing the DOC in the target sample by the mean DOC in a reference population as follows:

$$\frac{\text{DOC target sample}}{\text{DOC reference sample}}$$

The reference sample is derived from individuals who do not express the condition of interest who were sequenced using the same targeted sequencing panel, chemistry and conditions as the target sample. The reference sample used in our study was obtained from a population of 73 individuals who did not have features of MODY. A deletion may be suspected if the ratio is 0.75 or lower and duplication if the ratio is 1.25 or higher. A Z-score is used to determine statistical significance, with a score 5 standard deviations away from

the mean reference population score considered significant. This corresponds to a Z-score of 5 or -5.

2.3.2.6 Confirmation of CNV state using WES:

We confirmed each CNV call by next performing WES on samples of interest, using standard operating methods and procedures at LRGC (www.lrgc.ca). The average DOC for WES in our facility is ~100. We determined the extent of the deletions using the same CNV caller analysis tool and procedure applied to WES-generated data. The ratio used for CNV suspicion is the same as for the LipidSeq targeted NGS data, however since the reference population for this analysis was derived using only 15 WES samples, the Z-score significance threshold was decreased to 3 (or -3). CNVs were also considered significant for samples with Z-scores between 2 to 3 (-2 to -3), if the p-value was strongly significant.

2.3.2.7 Cytoscan analysis:

Confirmation of the CNV state was conducted using a clinically validated single nucleotide polymorphism (SNP) microarray to assess for CNVs utilizing 2.6 million probes to determine gene dosage through the genome (CytoScan HD array analysis, Affymetrix/ThermoFisher Scientific, Waltham, MA, USA).

2.3.2.8 Breakpoint analysis:

The CNV caller analysis results allow for strong suspicion of a CNV deletion using probe-level data. These methods allow determination of the approximate size of the deletion and the approximate breakpoints on either end of the deletion. To determine the exact breakpoint, Sanger polymerase chain reaction (PCR)-based probe analysis is required. Probes were designed to target either side of the suspected deletion in an attempt to identify the exact start and stop position of the deletion. The span of the wild-type strand is too great to amplify under the same conditions as the mutant allele that contains a deletion between the primers; therefore if amplification occurs in the candidate sequence but not in the control, it suggests the primers hybridize to DNA that is relatively close to each side of the

breakpoint (Figure 2.2). PCR amplification and sequencing of the abnormal amplified fragment using the identified primer pair is then completed. Sequence alignment between the amplification product and the known reference sequence then allows for exact identification of the start and stop points for the deletion.

2.3.2.9 Family member analysis:

Following breakpoint determination in the two probands, the designed primer pair was used to conduct targeted assessment of available DNA for first-degree relatives of each proband to detect either the presence or absence of the deletion.

2.3.3 Results:

Likely CNVs were seen in *GCK* in two male individuals with clinically suspected MODY using CNV caller analysis of LipidSeq output data. These were confirmed by WES-generated data in one of the two individuals (Table 2.4)

The two patients with CNVs affecting *GCK* (MODY2) were confirmed to have deletions at precisely the same breakpoint, suggesting this may have been a single ancestral event. As far as we are aware, there was no direct familial relationship between the two individuals. The *GCK* deletions spanned the 5'UTR – altExon 1 in both individuals, affecting probes 7:44228257-44229272. In the first individual, the CNV average ratio for this deletion was calculated at 0.575312 with Z-score of -7.69375 for the LipidSeq-generated data and an average ratio of 0.581204 with a Z-score of -2.94078 for the whole exome sequencing data. In the second individual, the CNV average ratio for this deletion was calculated at 0.57517 with Z-score -7.02283 for the LipidSeq-generated data. WES was not conducted in this individual due to low volume of DNA. Breakpoint analysis confirmed the presence of a 4,763 bp deletion in both individuals spanning chr7: 44224750 – 44229512 (Figure 2.3). Because the deletion encompasses the promoter region, initiation codon and all of exon 1, neither transcription of RNA nor translation of mature enzyme are predicted from this allele.

Family analysis revealed the presence of the identical deletion in an affected mother of *GCK* proband 1, while no deletion was detected in the unaffected father. Similarly, analysis from the family of *GCK* proband 2 revealed the presence of the deletion in the affected mother and sister, but not in the unaffected father (Figure 2.2)

The clinical presentation in the two *GCK*-MODY probands was similar (Table 2.5). Both were of French Canadian descent and each presented in the ninth year of life. Both had fasting hyperglycemia, mild elevations in glycated hemoglobin and negative auto-antibodies; proband 1 was being treated with metformin and proband 2 was following a diabetic diet. Both had an affected mother and genotypically and phenotypically normal father. Both individuals presented with polyuria, which is atypical for *GCK*-MODY. The sister of proband 1 carried the same mutation but presented with a milder phenotype.

Disclosure of the *GCK* genetic results to the sending physician resulted in a significant change to the management approach and recommendations were made to the affected probands and family members that they could safely discontinue all therapeutic interventions.

In an additional individual, a deletion was also detected affecting *HNF4A* (MODY1) that appeared to span the entire gene from the 3' to the 5' untranslated regions, involving consecutive probes 20:42835510 -43115348 from reference Human Genome build hg19 (Figure 2.4). The CNV average ratio for this deletion was calculated at 0.531123 with Z-score of -6.6691 for the targeted LipidSeq-generated data and an average ratio of 0.577031 with a Z-score of -2.94078 and a p-value of $<1x10^{-30}$ for the WES data. CytoScan results also demonstrated a large-scale deletion spanning this region (Figure 2.5). Breakpoint analysis by Sanger sequencing confirmed the presence of a heterozygous 242,258 bp deletion spanning chr20:42871409-43113666 (Figure 2.4, Table 2.6)

The proband carrying this mutation was a Caucasian female, who was diagnosed with impaired glucose tolerance (2-hour oral glucose tolerance test (OGTT) blood sugar of 8.6mmol/L (N<7.8mmol/L)) in 2002 at age 13, after an incidental finding of elevated fasting

blood glucose prompted testing and yearly follow-up. She developed symptoms of polyuria and thirst 3 years later and was diagnosed with diabetes at that time with an HbA1c of 7.6% and a random blood sugar of 12.9mmol/L. She was 50.3kg (normal BMI) at the time of diagnosis with negative diabetes associated autoantibodies. She had a significant family history of diabetes, with her brother diagnosed at age 14, a paternal aunt diagnosed with diabetes at age 19, both paternal grandparents diagnosed >60 years of age, a mother diagnosed with GDM with both pregnancies, maternal grandfather diagnosed with diabetes in his sixties and several maternal and paternal aunts and uncles diagnosed with diabetes in childhood or adolescence. Initial genetic testing for MODY done at that time (2005) did not identify any mutations. She was initially managed with gliclazide 30mg daily, but experienced hypoglycemia with increase to 60mg with an HbA1c of 5.1%. She was trialed on a long acting insulin analogue. Her HbA1c rose to 7.5% on Levemir and a rapid acting insulin analogue was added without significant HbA1c benefit (7.2%). She was then switched to glimiperide (a SU) at 2mg daily with significant improvement in HbA1c to 5.6%. She was managed effectively with SU therapy for several years, but this eventually became ineffective and she was switched to multiple daily injections of insulin and then a continuous insulin infusion pump.

Three more CNVs, namely three distinct chromosome 17q12 deletions, ranging in size from 1.4-1.9 million base pairs, and all spanning *HNF1B* (MODY5) were also detected (Table 2.7, Figure 2.5).

To confirm the three CNV calls for the chromosome 17q12 deletions, which all extended into regions not covered by the targeted NGS panel, WES sequencing was performed. Results of WES were concordant with those of targeted NGS (Table 2.7, Figure 2.5).

Clinical genotyping using CytoScan HD array analysis (Affymetrix/ThermoFisher Scientific, Waltham, MA, USA) confirmed the presence of a chromosome 17q12 deletion at the predicted location for all three individuals tested (Table 2.7, Figure 2.5). Family studies of two probands found to have chromosome 17q12 *HNF1B* deletions suggested *de novo* mutations in both, as neither parent was found to carry the mutation. Adding this new

information to the findings of the original MODY cohort (chapter 2.2), CNVs accounted for 3 out of 4 (75%) of the *HNF1B* mutations found.

Following confirmation of the validity of CNV calling added to variant calling for MODY, this analysis was incorporated routinely for all new samples received with clinical suspicion for MODY. This allowed for the detection of two additional CNVs causing whole-gene deletions of *HNF1B* in two further individuals, unrelated to each other or the original probands.

Clinical information for the five probands found to have heterozygous *HNF1B* gene deletions on chromosome 17q12 is summarized in Table 2.8. All five probands initially presented with symptoms in adolescence, between ages 12 and 18 years. All were of Caucasian ethnicity. Four had a normal body-mass index (BMI), while the fifth had a BMI that just met the threshold for overweight (25.1 kg/m^2). Four of these five subjects were negative for anti-GAD antibodies (Table 2.8). Glycosylated hemoglobin (HbA1c) during available follow-up period ranged between 5.0-8.1% (Table 2.8). Four of these five subjects were known to have additional manifestations related to the *HNF1B* deletion, including congenital uterine or renal malformations, renal cysts, hypomagnesemia and hyperparathyroidism (Table 2.8).

The positive antibody status in proband 2 is unusual for MODY. It is possible these are elevated due to the presence of other autoimmune conditions (ie thyroid autoimmunity (25)) or other conditions (ie cerebellar ataxia (26), which have both been linked to anti-GAD positivity. Furthermore, previous studies (27) have also shown anti-GAD positivity in 1.7% of non-diabetic adults, so it may be an incidental finding. Additionally, the HLA-DQA1/DQB1 risk haplotype for autoimmunity is also associated with anti-GAD positivity, even in the absence of diabetes (27, 28), and she may carry this risk haplotype given that she has had multiple family members diagnosed with T1DM. It is also possible that she may have a combined type 1 and MODY phenotype.

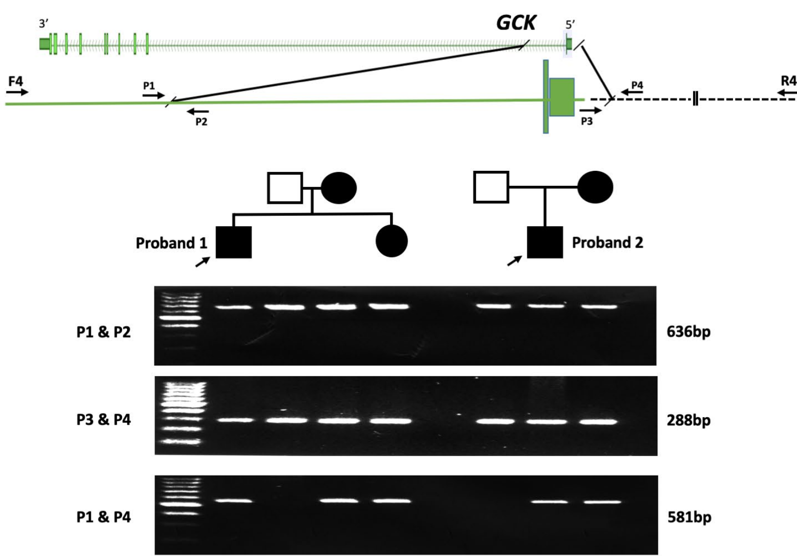

Figure 2.2: PCR amplification of DNA subjected to primers designed on either side of suspected breakpoint in both probands and family members.

The top section shows the GCK gene and location of primers used to confirm and sequence across the breakpoint. The normal sequence distance between primer pair F4 and R4 is 10,655 base pairs (bp), however PCR amplification in probands using primer pair F4 and R4 generated a product size of 5,893 bp, suggesting a 4,763 bp deletion**.**

In the pedigree charts for proband 1 and 2, affected status is indicated by solid color and probands are indicated by arrows as shown. Gels show PCR amplified fragments aligned beneath each individual.

The top gel shows amplification products generated using primers P1 located on the proximal side of the suspected breakpoint, and P2, within the deleted fragment. The middle gel contains amplification products generated using primers P4, located on the distal side of the suspected breakpoint, and P3 within the deleted fragment. All subjects demonstrated amplification for both the proximal (636 bp) and distal (288 bp) primer pairs.

The bottom gel shows amplification products generated using primers P1 and P4 (581 bp). In individuals without the deletion, the span between these two primers would be too great to amplify under standard conditions; therefore if amplification occurs, it confirms the presence of a large deletion between the two primers. Amplification between P1 and P4 is seen in both probands and all affected family members, but not in unaffected family members, confirming the presence of a deletion in these individuals.

The normal amplification products generated in individuals carrying the deletion with both the proximal (P1 and P2) and distal (P3 and P4) primer pairs confirms these individuals carry one normal copy of the gene and are heterozygous for the deletion. Thus, the mother and both children in Family 1, and the mother and proband in Family 2 are all heterozygous for the normal and deleted *GCK* alleles.

Primer (P) design: F4 (GTTCAGCCTCAGGTGTAGAAGCAG); R4 (AGGAACAGGACAGGAGTATACGTGG); P1 (TGAGTCAGTGGCTCCTGGAAAGG); P2 (CTGTCATTCCTCAGCTGAGCCAG); P3 (CTAGGGCTGTAAACTCTCCAGAG); P4 (AGGCTGAAGCTTCCTGAGCAGG). All PCR reactions used an annealing temperature of 60 degrees Celsius and a 5% DMSO solution. bp= base pair;

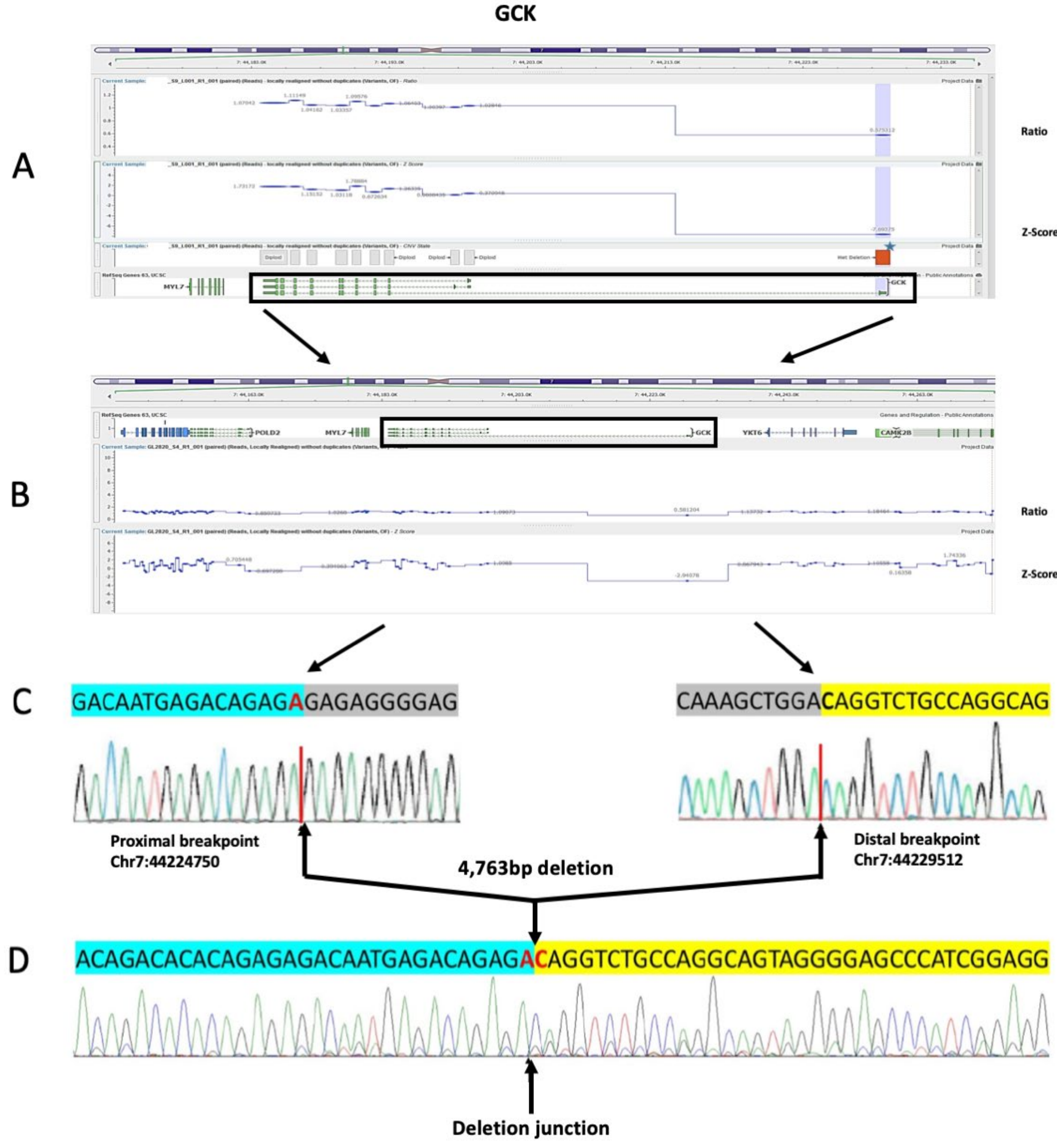

Figure 2.3 Determination of deletion breakpoint for GCK

A) Screen capture of targeted NGS-generated data from Proband 1 processed by the CNV caller tool identifies a potential deletion (shown by the star), as indicated by a significant drop in DOC ratio to less than 0.75 and a Z-score less than -5, when compared to DOC in a reference population. The bottom section maps the extent of involvement of *GCK* coding

sequence, which is oriented 5' to 3' left-to-right, indicating that exon 1 is involved; B) WES-generated data from the same sample using the same tool to confirm and also determine the approximate extent of the deletion involving exon 1 and the 5'-flanking region of *GCK* but not neighboring genes; C) Sanger sequencing electropherogram tracings showing normal DNA sequences in the vicinity of the 3' (left side; letter codes shaded blue) and 5' (right side; letter codes shaded yellow) breakpoints of the deletion. Internal sequence that is missing in the deleted allele is shaded grey; D) Sanger sequencing electropherogram tracings mutated DNA sequence in which the 3' (shaded blue) and 5' (shaded yellow) regions flanking the deletion breakpoint, with absence of the intervening 4763 nucleotides.

Table 2.4: Suspected MODY patients with Confirmed *GCK* CNVs

MODY Subtype	Gene	Affected Exons	Probes Affected Chr:positionrange	CNV State	VarSeq Avg Ratio	VarSeq Avg Z-score	WES Avg Ratio	WES Avg Z-Score	Deletion Size	Breakpoint
MODY 2	*GCK*	5UTR – altExon 1	7:44228257 -44229272	Del, het	0.575312	-7.69375	0.581204	-2.94078	4,763 bp	chr7: 44224750 – 44229512
MODY 2	*GCK*	5UTR – altExon 1	7:44228257-44229272	Del, het	0.57517	-7.02283	N/A	N/A	4,763 bp	chr7: 44224750 – 44229512

Table 2.5: Clinical Features of GCK-MODY Probands

	GCK Proband 1:	Sister of GCK Proband 1:	GCK Proband 2:
Gender	Male	Female	Male
Age at diagnosis	8.5 years	11.8 years	8.9 years
Duration of Symptoms Pre-Diagnosis	Longstanding polydipsia	Asymptomatic	9-12 months of polyuria, polydipsia
Presentation	Fasting hyperglycemia and abnormal OGTT	Elevated fasting BG (6-7 mmol/L)	Polyuria, polydipsia Fasting BG 7 mmol/L
Family history	Mother: impaired glucose tolerance Paternal grandmother: T2DM	Mother: impaired glucose tolerance Paternal grandmother: T2DM	Mother: impaired glucose tolerance Maternal aunts: DM early adulthood
Ethnic background	French Canadian	French Canadian	French Canadian
Weight at diagnosis	Weight 95th percentile Height 75th percentile	Weight 95th percentile Height 50th percentile	23 kg (25th percentile)
Anti-GAD antibodies	<1	N/A	<
Evolution	Fasting BG 6-9 mmol/L	Fasting BG 5-7 mmol/L	No decompensation
OGTT (at 120 mins)	BG 11.1 mmol/L	BG 10.0 mmol/L	BG 7.4 mmol/L Peak insulin 149 pmol/L
Treatment	Metformin	Metformin	Diet only
Autoimmunity	No thyroid antibodies	No thyroid antibodies	No thyroid antibodies
HbA1c	6.4-6.9%	6.0-6.2%	5.9-7.0
Family	M: positive for mutation F: negative for mutation S: positive for mutation	M: positive for mutation. F: negative for mutation B: positive for mutation	M: positive for mutation. F: negative for mutation

M: mother; F: Father; S: Sister; B: Brother; OGTT: oral glucose tolerance test; BG: Blood glucose; DM, diabetes; T2DM: type 2 diabetes; GAD, glutamic acid decarboxylase; HbA1c, glycosylated hemoglobin

Table 2.6: Summary of findings for *HNF4A* deletion

MODY Subtype	Gene	Affected Exons	Probes Affected Chr:position	CNV State	VarSeq Avg Ratio	VarSeq Avg Z-score	WES Avg Ratio	WES Avg Z-Score	P-Value	CytoScan Results	Deletion Size	Breakpoint
MODY 1	*HNF4A*	5UTR – 3UTR	20:42835510 -43115348	Del, het	0.531123	-6.6691	0.577031	-2.5639	$<1x10^{-30}$	Positive	242,258 bp	chr20:42871409-43113666

Del: deletion; het: heterozygote; UTR: untranslated region; NA: not available; WES: Whole exome sequencing; CNV: copy number variation; bp: base pair; Avg: average;

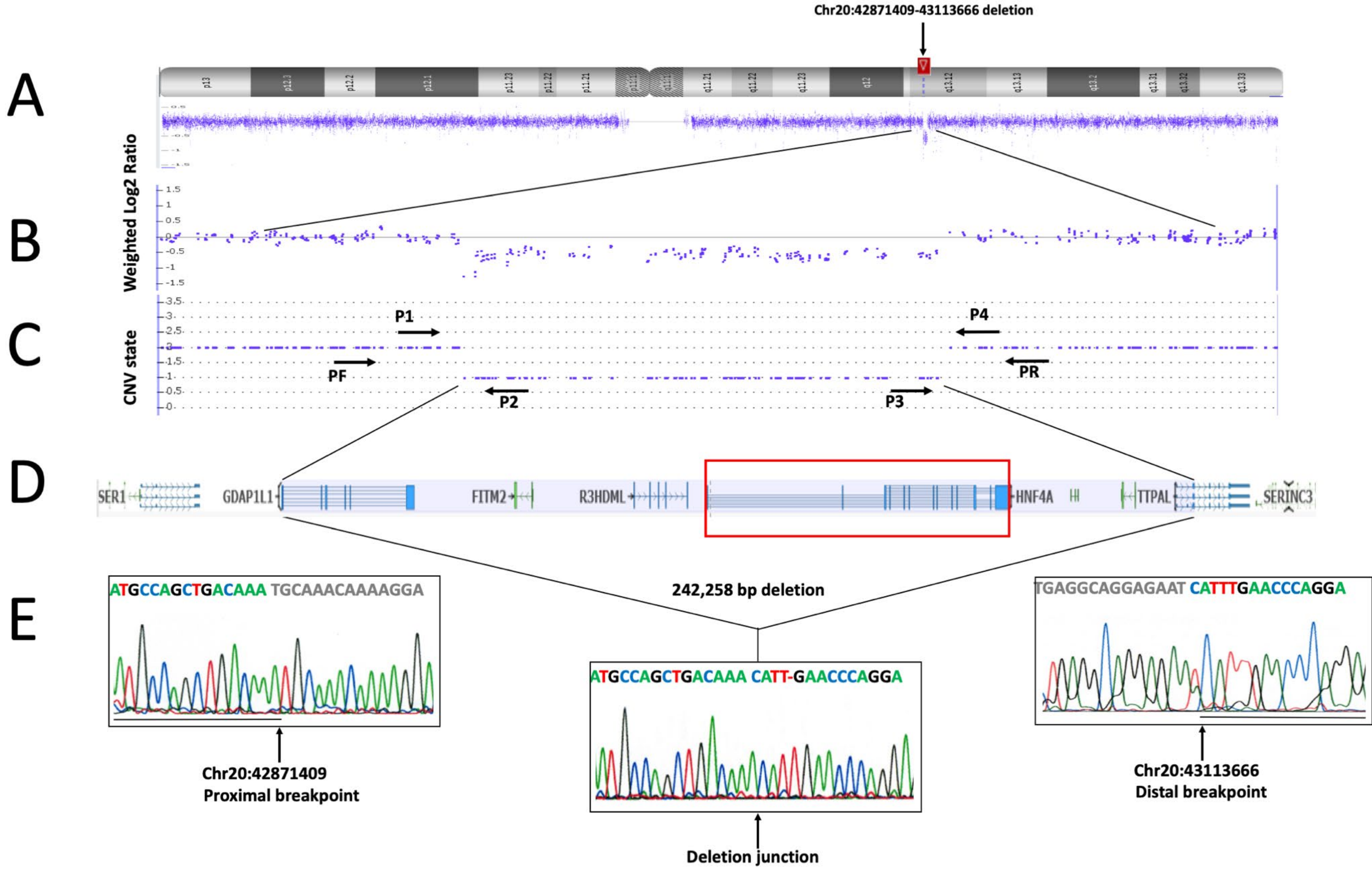
Chr20:42871409-43113666 deletion
A
B
C
D
E
Weighted Log2 Ratio
CNV state
P1
P4
PF
PR
P2
P3
SER1
GDAP1L1
FITM2
R3HDML
HNF4A
TTPAL
SERINC3
242,258 bp deletion
ATGCCAGCTGACAAA TGCAAACAAAAGGA
Chr20:42871409
Proximal breakpoint
ATGCCAGCTGACAAA CATT-GAACCCAGGA
Deletion junction
TGAGGCAGGAGAAT CATTTGAACCCAGGA
Chr20:43113666
Distal breakpoint

Figure 2.4: Confirmation of CNV detection using NGS output data in HNF4A

A CytoScan genotyping output showing loss of zygosity on chromosome 20q13 spanning the *HNF4A* gene, with reduced intensity of probe signals, shown on an expanded scale in panel B. Panel C shows the corresponding region with the same deletion detected using depth-of-coverage analysis from NGS and WES output data. Panel D shows the genes that map within the deleted region, which includes the entire *HNF4A* gene. Panel E shows Sanger sequencing electropherogram tracings that demonstrate normal DNA sequences in the vicinity of the proximal 3' (left side) and distal 5' (right side) breakpoints of the deletion. Internal sequence that is missing in the deleted allele is shaded grey. In the bottom center is the Sanger sequencing electropherogram tracing from the proband, showing the deletion junction, with absence of the intervening 242,258 nucleotides. Amplification primer locations relative to the deletion are indicated on the CNV state output in panel C. The normal sequence distance between primer pair PF and PR is 246,593 base pairs (bp), and PCR amplification between them would not be possible, however PCR amplification in the proband, but not the normal control, using primer pair PF and PR generated a product size of 4,335 bp, suggesting a 242,258 bp deletion. Amplification is also seen in the proband using primer pair P1 and P2 on the proximal end of the breakpoint, and primer pair P3 and P4 on the distal end of the breakpoint, confirming the presence of at least one normal gene copy. Thus a heterozygous deletion spanning the entire *HNF4A* gene is confirmed in the proband.

Primer (P) design: PF (GTCAGCCATGTGTCCTAGCCATGTTCAGG); PR (CTGAGTAGTGGAGACTACAGACATGTG); P1 (TCCAGAAGTGCTGGGATTACAGGTG); P2 (GATCTGTCACGTTGCTTCACTGTGG); P3 (CACTTGAGGTCAGGAGTTTGAGACCAG); P4 (AGTTTTGCTGTTCTCACCCAGGCTG); PCR reactions for P1-P4 used an annealing temperature of 60 degrees Celsius and reactions for PF/PR used annealing temperature of 63 degrees Celsius. All PCR reactions used a 5% DMSO solution. bp= base pair.

Table 2.7: CNV Caller Summary HNF1B deletions

Type	TNGS Probes Affected	TNGS Ratio	TNGS Z-score	WES Ratio	WES Z-score	Cytoscan	Probes Affected	Size
Del, het	17:36046183-36105346	0.581791	-5.5768	0.579920	-2.13712	17q12 Deletion	17:34455782-36307773	1.852 million bp
Del, het	17:36046183-36105346	0.540671	-9.32017	0.521851	-3.89335	17q12 Deletion	17:34822465-36404138	1.582 million bp
Del, het	17:36046183-36105346	0.539208	-12.4028	0.535156	-2.27315	17q12 Deletion	17:34822465-36283612	1.461 million bp

Abbreviations: Del, deletion; Het, heterozygous; TNGS, Targeted Next Generation Sequencing, WES, Whole Exome Sequencing; bp, base pairs

Table 2.8: Clinical Characteristics of Probands carrying HNF1B mutations

	HNF1B Proband 1	HNF1B Proband 2	HNF1B Proband 3	HNF1B Proband 4	HNF1B Proband 5
Gender	Female	Female	Female	Male	Female
Age at diagnosis	12.2 years	15 years	17.6 years	18 years	15 years
Duration and Symptoms Pre-Diagnosis	Polyuria, polydipsia for few months	Polyuria, polydipsia	1 month of fatigue, anorexia, slight weight loss	Weight loss	8 months of fatigue, trouble focusing
Presentation	BG 19 mmol/L; urinary ketones 1+; no acidosis	BG 32; No ketones	2 days nausea + vomiting BG 24 without acidosis	Elevated BG; no ketones	IGT on OGTT
Family history	Mother: GDM Mat GM: T2DM	Mat GM: T2DM Siblings: T1DM Uncle T1DM	None	Father: T2DM Sister: GDM	Mother: GDM x3 → T2DM Mat GM: T2DM Mat uncle: T2DM
Ethnic background	Caucasian, French Canadian	Caucasian	Caucasian	Caucasian	Italian
BMI	18.7	23.3	21.6	25.1	Normal
Anti-GAD antibodies	<1	>250	Negative	Negative	Negative
Treatment	Insulin 0.87 U/kg/day	Insulin Failed Metformin	Metformin Insulin 0.1U/kg/day	Metformin Gliclazide	Insulin → metformin → insulin + metformin
Autoimmunity	No thyroid antibodies	-	TTG neg TSH normal TPO AB negative	No other Autoimmune disease	No other Autoimmune disease
HbA1c	6.1-6.7%	5.0-6.9%	<6%	6.2-8.6%	max 8.1%
Other manifestations	Uterine agenesis; Renal cysts	Left partial UPJ obstruction	Hypomag / Gittelman's Normocalcemic hyperPTH	Congenital renal malformation	None Known
Family	M: Negative for mutation F: Negative for mutation	ND	M: negative for mutation	ND	ND

Abbreviations: BG: blood glucose in mmol/L; F: father; GDM: gestational diabetes; GM: grandmother; HbA1c: hemoglobin A1c; hyperPTH: hyperparathyroidism; IGT: impaired glucose tolerance; M: mother; Mat: maternal; Max: maximum; ND: not done; OGTT: oral glucose tolerance test; T2DM: type 2 diabetes; TPO AB: thyroid peroxidase antibody; TSH: thyroid stimulating hormone; TTG: tissue transglutaminase; UPJ: ureteropelvic junction

Figure 2.5*:* Identification and confirmation of 17q12 deletion

Figure 2.5A: Screen capture showing data generated using the CNV caller tool applied to targeted NGS-generated output. Ratio is approximately 0.5 throughout with an average Z-score of -9.32017. The potential deletion spanning the entire *HNF1B* gene is shown by the large red box.

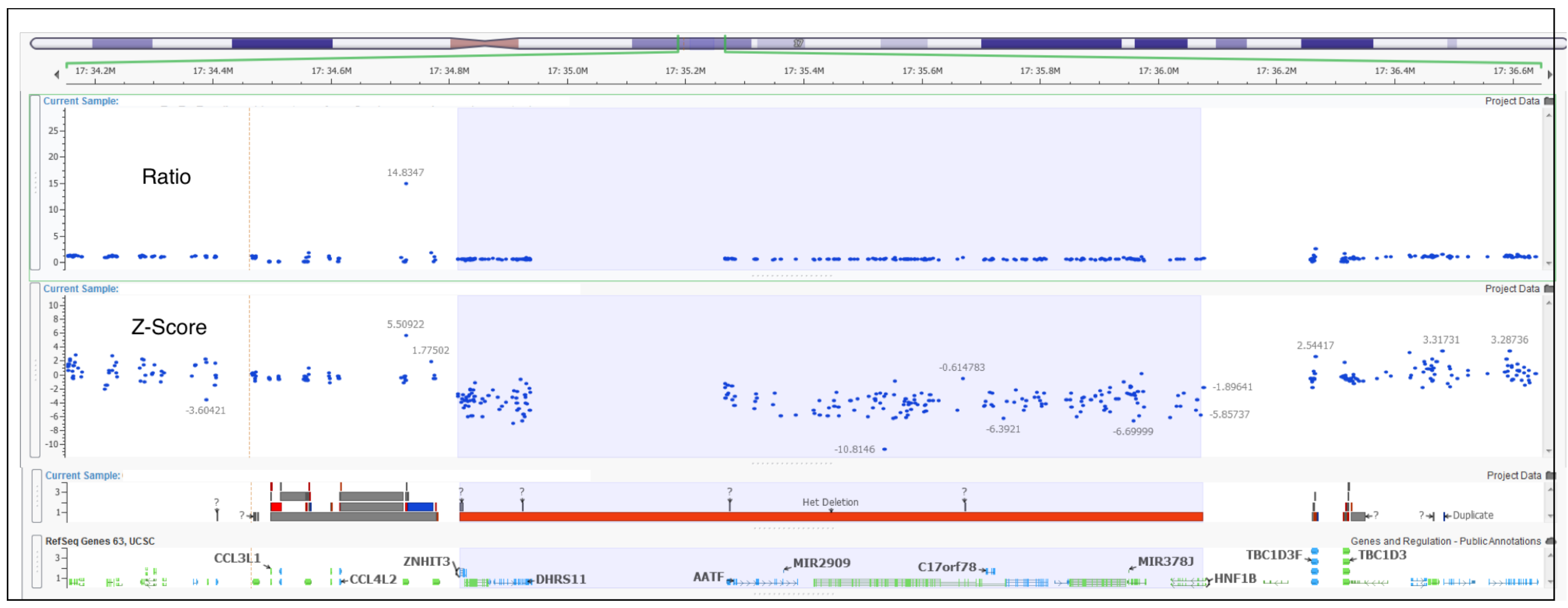

Figure 2.5B: The deletion was predicted to span approximately 1.6 million base pairs by the CNV caller tool using WES-generated data. The span of the deletion is shown by the large red box, with an average ratio of approximately 0.5 throughout and an average Z-score of -3.89335.

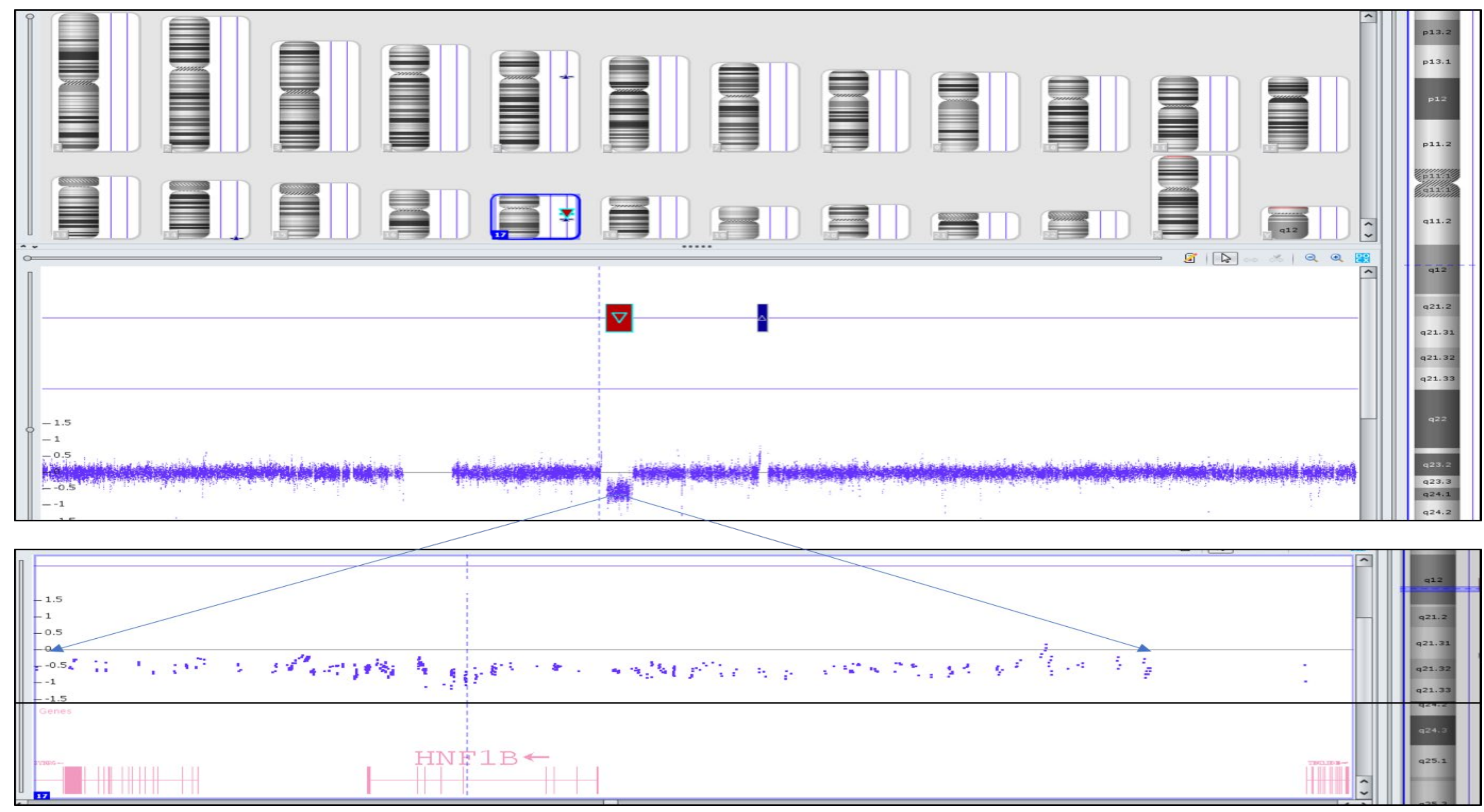

Figure 2.5C: Results of a CytoScan^{TM} HD Array, visualized using Chromosome Analysis Suite identifies the region containing the CNV, on chromosome 17 as shown in the top panel. The middle panel shows a drop in signal, indicating a decrease in copy number at that position. The bottom panels shows an expanded view of the deleted region, which includes the HNF1B gene, as indicated

2.3.4 Discussion:

The work outlined above has expanded the available knowledge on MODY in a number of important ways. Firstly, novel mutations, including CNVs, were identified as likely causal for MODY through investigation into clinically suspected MODY cases. Additionally, while further investigation is needed, this work established that clinical suspicion of MODY can be a reasonable screening tool on its own to help select appropriate candidates for genetic testing.

These investigations also demonstrated that the use of gene panels incorporating all MODY genes, along with routine CNV testing is a beneficial and cost effective way to screen for MODY in suspected cases. This work suggests that incorporating these methods into routine screening may significantly improve genetic confirmation rate and maximize genetic testing utility, and would be recommended

Furthermore, these studies also highlight the benefits of reclassifying individuals who have been presumptively diagnosed with other diabetes phenotypes as having MODY. Changes in management were recommended not only for the probands, but also for other affected family members in the majority of cases.

As previously discussed, optimal management of MODY diabetes is highly dependent on the underlying genotype. For example, as seen in the proband diagnosed with *HNF4A* -MODY (MODY1), this form of MODY may be optimally treated with low-dose oral sulfonylurea therapy for many years, although insulin therapy may eventually be required (2, 12, 29).

Three individuals in this series were diagnosed with *HNF1B* deletions. Mutations in *HNF1B* lead to MODY5, also known as renal cysts and diabetes syndrome (30, 31). Whole gene deletions of one copy of *HNF1B* are responsible for up to 50% of cases of MODY5, with virtually all of these being chromosome 17q12 deletions of varying magnitude (30-33). The majority (~70%) of these deletions arise *de novo (33)*. The "17q12 deletion syndrome" is a term that refers to more extensive deletions of ~1.4 Mbps that encompass *HNF1B* and

several surrounding genes (32-34). Clinical manifestations of 17q12 deletion syndrome include a mild to moderate, progressive diabetes with a mean onset of age 24, but which can present from the neonatal period up to middle age (31, 33). Individuals with the condition can have congenital or later-onset urogenital malformations, and also have frequent renal, pancreatic or hepatic complications, including cysts, hypomagnesemia, hyperuricemia, or exocrine pancreatic deficiencies (31-33). Primary hyperparathyroidism also appears with higher prevalence (31). Cognitive impairment and/or developmental delay is common, presenting in 50% of those with 17q12 deletion syndrome (31-33). Individuals with impaired function of HNF1B may respond to low-dose oral sulfonylurea therapy but insulin is often required (31, 35). They may also warrant periodic monitoring for genitourinary, parathyroid, hepatic or other manifestations.

Generally, *GCK*-MODY (MODY2) is benign and non-progressive and can be managed without medication, or other therapeutic measures, including diet, with the exception of special monitoring during pregnancy (12, 36, 37). However, it is important to distinguish it from other forms of MODY and from type 1 or type 2 diabetes in order to optimize management decisions (38). A recent study from the United States found that 49% of patients with *GCK*-MODY were unnecessarily being treated with glucose-lowering agents (38). As patients with MODY often present at a young age and with a normal BMI they are frequently misdiagnosed as having type 1 diabetes and are started on multiple daily injections of insulin (6, 10, 39). For example, two recent large cohort studies identified MODY, mutations in up to 6.5% of children with diabetes, many of whom had not previously been identified (40, 41). Re-classifying some of these patients as *GCK*-MODY could allow for safe discontinuation of insulin, and generally all other treatments, which can have a profound impact on quality of life as well as healthcare costs and burden.

Clinically, there is also some suggestion that large-scale deletions may present with a slightly more severe phenotype than in other forms of MODY. For example, both probands with *GCK* deletions experienced polyuria, a symptom not generally associated with *GCK*-MODY, which is usually asymptomatic. *GCK* proband 1 also had higher than expected

fasting blood glucose, up to 9 mmol/L. However, both affected mothers and the sister of *GCK* proband 1 appeared to have only mild phenotypes, with the mother of *GCK* proband 1 was diagnosed with only impaired glucose tolerance and the mother of *GCK* proband 2 with isolated gestational diabetes. The sister of *GCK* proband 1 presented with features more classically consistent with *GCK*-MODY, being asymptomatic, with lower fasting BG (5-7). Both families were also French Canadian, and may have shared a common ancestor. The milder phenotype in the females harboring the deletion raises the possibility that there may be gender differences associated with phenotype expression, or this difference could be related to other factors, such as variable penetrance, environmental or lifestyle influence or simple chance. Further work to investigate whether other CNVs support a distinctive clinical phenotype may help clarify the importance of these observations.

While CNVs have not traditionally been screened for or reported as causal mutations for MODY, our findings here, as well as other emerging work, suggests CNVs may be a more significant contributor to MODY than previously thought (6, 12, 38, 39). One report identified deletions causing MODY in *GCK* and *HNF1A* using a dedicated multiplex ligation-dependent probe amplification (MLPA) assay (16). Another reported large-scale CNVs in *HNF1B* make up a significant proportion of causative mutations for MODY5 (15). Case reports of partial gene deletions in *GCK* have also been reported in the literature (42, 43). Our data provide further support the presence of CNVs in at least 3-4% of previously undiagnosed MODY patients, while demonstrating a simpler, more cost-effective approach to make this molecular diagnosis. The overall proportion of MODY cases that can be attributed to CNVs may become more apparent as CNV analysis is incorporated routinely when assessing for MODY via NGS.

In total, we now have identified heterozygous CNVs in 8 individuals with MODY, representing ~13% of those with a clear molecular diagnosis. For those who could be re-classified from "mutation-negative" to "CNV positive", quality of life and management decisions were impacted, allowing for more personalized and effective treatment. Our approach can effectively detect CNVs in MODY genes from NGS data. It seems to be

important to seek CNVs when performing molecular testing for MODY using NGS to avoid false negative results, which occurred in ~6% of our original cohort using NGS alone without CNV detection. This is accomplished by incorporating CNV detection routinely during NGS variant calling, without significantly adding to the cost of sequencing. As shown, the routine use of this method helped to diagnose two additional cases subsequent to our initial cohort, which would otherwise have been missed using standard NGS sequencing.

Seeking CNVs may be of particular importance when *HNF1B* mutations are suspected. This was supported by our data, which showed that five of the 8 detected cases of CNVs causing MODY in our series were caused by deletions in *HNF1B.* Furthermore, prior work suggests that deletions comprise ~50% of all *HNF1B* mutations (32). At a minimum, if MODY is suspected and there are any signs of urogenital malformations, exocrine pancreatic dysfunction, hypomagnesaemia, hypercalcemia, or other features associated with *HNF1B*-MODY or 17q12 deletion syndrome, and no variants are detected using bioinformatic calling algorithms for small mutations in NGS output, effort should be made to extend the investigation to include CNVs to avoid missing cases and making false negative diagnoses.

Detected deletions of *HNF1B* appear to be distinct in different families and vary notably in size, ranging from 1.4-1.9 million base pairs. Exact breakpoint determination was attempted in the individuals studied here but this proved to be unsuccessful due to technical challenges related to the high degree of DNA sequence homology involving the surrounding genomic region. It may be that sequence homology, which made primer-based breakpoint analysis impossible, causes a tendency for misreads during DNA duplication *in vivo*, which may account for the relatively high frequency of deletions and duplications that occur in this region. This is also supported by the fact that most detected cases of deletions in this region arise *de novo* (32-34, 44). This also suggests that a lack of family history should not be a reason to refrain from genetic testing for *HNF1B*-MODY if clinical suspicion is otherwise present.

Overall, by reclassifying these new mutation-positive cases, the genetic confirmation rate we originally reported in <u>chapter 2.2</u> would increase from ~40% to close to 50% (45/96 =

47%), which strengthens the argument that clinical suspicion for MODY by a specialist may be sufficient on its own, without additional selection criteria, to recommend referral for genetic testing.

Our results have some limitations. While these large deletions would be expected to have deleterious effects, and they seem to segregate with phenotype in family members, no functional studies were conducted to directly confirm lack of *in vivo* activity. Also, while the CNV caller tool was validated using our specific NGS panel and reference population, this may not be universally applicable. Also, given our small sample size, generalizations about any phenotype differences with this large-scale deletion compared to causes related to pathogenic SNPs or small inserts/deletions is limited.

Future work is still needed to better refine the strategies for suspecting and identifying MODY clinically and selecting the most appropriate candidates for genetic testing. Establishing an estimate of the unrecognized MODY burden may also provide valuable information, and may be obtained by systematically assessing the local diabetes database to identify those who may be most likely to benefit from genetic testing. Additionally, the standard incorporation of NGS-based CNV testing for MODY may help to more accurately determine the prevalence of CNVs amongst MODY genes. Furthermore, systematically screening populations with higher potential for MODY burden, such as newly diagnosed patients under the age of 35 with negative autoantibodies or atypical features, or those diagnosed with hyperglycemia in pregnancy, may also help establish the best methods for case detection and more accurately determine the prevalence of MODY subtypes in these populations.

2.3.5 Conclusion:

These findings confirm the utility of applying the CNV caller tool to screen for CNVs in MODY genes from NGS data. In doing so, we have identified novel deletions in two MODY genes and confirmed the presence of CNVs causal for MODY in 8 individuals with consistent

clinical features. Our data suggests that routinely incorporating CNV analysis of NGS data may increase diagnostic yield when investigating a suspected MODY case.

2.4 Chapter Conclusions:

Establishing a MODY diagnosis can have a significant impact on optimal management, quality of life and disease course in individuals misclassified with other diabetes subtypes, as well as for their family members. CNVs may be an under-recognized cause of MODY, and may be overlooked with standard genetic testing. The use of panel-bases NGS sequencing with incorporated CNV analysis improved genetic confirmation rates in a cost-effective manner, and should be considered as a standard testing method when evaluating an individual clinically suspected of MODY.

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Chapter 3: Improve counselling and management of genetic triglyceride disorders

3.1 Overview:

Unlike MODY, which has monogenic origins, many endocrine conditions have more complex genetic determinants, and are largely polygenic in nature. For most of these conditions there may be a very small fraction of the population that expresses the phenotype due to large effect mutations, but the majority of individuals will have a high burden of smaller effect changes that lead to expression of the phenotype.

The expression of these conditions tends to be more variable than in monogenic conditions, and, while there is often a family history present, there is not a clearly predictable pattern of inheritance like that seen in monogenic traits. Clinical assessment, therefore, plays an important role in the evaluation of these disorders.

For most polygenic conditions, the genetic background of an individual may predispose to a certain phenotype but environmental factors may also play a significant role. For many of these, it may be possible to modify expression and ameliorate risk by adjusting these environmental influences. It is therefore important to identify which factors are most likely to lead to expression of these conditions to achieve best possible control and prevention.

One endocrine condition with complex molecular genetics is hypertriglyceridemia. There are very rare, autosomal recessive monogenic forms of hypertriglyceridemia that are important to identify and treat accordingly. Understanding how to differentiate these individuals from the more common individual with polygenic basis is important clinically for optimal management.

The first study in this section (chapter 3.2) investigates the phenotypes seen in the rare monogenic forms of hypertriglyceridemia that lead to familial chylomicronemia syndrome

(FCS) to identify the clinical features that may help predict the underlying genotype, and may distinguish them from other more common HTG phenotypes.

While understanding these rare monogenic forms is important, most individuals with hypertriglyceridemia will have polygenic susceptibility. Therefore, counselling and management for most individuals with HTG will focus on addressing modifiable risk factors and optimally managing complications, which are the focus of the second (chapter 3.3) and third (chapter 3.4) study presented here.

3.2 Clinical and biochemical features of different molecular etiologies of familial chylomicronemia

The work presented in Chapter 3.2 has been edited from this original manuscript for brevity and consistency throughout this dissertation:

Hegele RA, **Berberich AJ**, Ban MR, Wang J, Digenio A, Alexander VJ, D'Erasmo L, Arca M, Jones A, Bruckert E, Stroes ES, Bergeron J, Civeira F, Witztum JL, Gaudet D. Clinical and biochemical features of different molecular etiologies of familial chylomicronemia. J Clin Lipidol. 2018 Jul-Aug;12(4):920-927.e4. doi: 10.1016/j.jacl.2018.03.093. Epub 2018 Apr 4. PMID: 29748148.

3.2.1 Background:

Familial chylomicronemia syndrome (FCS) is the rare monogenic form of hypertriglyceridemia, affecting between 1 and 10 per million individuals (1-3). While severe hypertriglyceridemia (TG > 10 mmol/L or 880 mg/dL) is relatively common with a prevalence of ~ 1 in 600 in North America, only a small fraction of these individuals have FCS (1, 4, 5) (see chapter 1.7 for additional background).

FCS follows an autosomal recessive pattern of inheritance and results predominantly (>90%) from bi-allelic mutations in the *LPL* gene encoding lipoprotein lipase (LPL) (2, 3, 6-15); this form we abbreviate herein as *LPL*-FCS. The remainder are caused by bi-allelic mutations in four additional genes involved in supporting or enabling LPL function, namely *APOC2*, *APOA5*, *LMF1* and *GPIHBP1*, which encode, respectively, apolipoprotein (apo) C-II and A-V, lipase maturation factor 1 (LMF1) and glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1 (GPIHBP1) (16-18), and we abbreviate this group of patients as non-*LPL*-FCS. The role of each of these gene products in lipolysis of TG is discussed in chapter 1.7.5 (4, 5).

Given the extreme rarity of these conditions, especially of non-*LPL*-FCS, no prior studies have directly compared clinical and biochemical features of the different monogenic causes of FCS. Here, we evaluate phenotypic differences between *LPL*-FCS and non-*LPL*-FCS patients.

3.2.2 Methods

3.2.2.1 Participants

The study included data from clinically identified FCS participants who were screened and included in a phase 3 randomized placebo-controlled trial of volanesorsen (NCT02211209). Briefly, the trial duration was 52 weeks, and subjects were randomized 1:1 to receive either volanesorsen 300 mg SC or placebo injections. The primary outcome was percent change in triglyceride levels at 13 weeks. Inclusion criteria for the clinical trial included a personal

history of pancreatitis and with documented chylomicronemia or triglyceride values exceeding 10 mmol/L and genetic testing or post-heparin lipolytic assay consistent with FCS. Patients without pancreatitis could be eligible up to a maximum of 28% of all patients. The study enrolled adult patients (age ≥18 years) with history of chylomicronemia as evidenced by: 1) documentation of lactescent serum in the fasting state; or fasting triglyceride measurement ≥ 10 mmol/L (880 mg/dL). The diagnosis of FCS required documentation of at least one of the following: 1) known bi-allelic loss-of-function mutations in *LPL*, *APOC2*, *APOA5*, *GPIHBP1* or *LMF1* genes; 2) post-heparin plasma LPL activity of ≤20% of normal; plus 3) fasting TG ≥ 8.4 mmol/L (750 mg/dL) at screening. Patients were willing to follow a restrictive diet (≤20 g fat per day). The patients included for analysis here were derived from screening the confirmatory testing on referred samples from study subjects in the clinical trial. Subjects gave informed consent for DNA sequencing, which was performed under Western University Research Ethics protocol 07920E (Appendix B).

3.2.2.2 Clinical and biochemical assessments

Baseline lipids, lipoproteins, apolipoproteins and metabolic assays were performed on fasting plasma as described in the trial protocol (19, 20). Fasting venous blood samples were taken 10 minutes pre- and post-intravenous infusion of heparin (50 U/kg), and total post-heparin lipolytic activity was determined as described (21, 22). A subset of markers was also examined 4 hours post liquid-formulated high fat-test meal (4800 kJ, 130 g of fat, 17 g of protein and 21 g of carbohydrate).

3.2.2.3 Molecular analysis

Genomic DNA was isolated from whole blood as described (23). Genomic libraries of indexed and pooled patient samples were generated for target candidate genes in lipid metabolism. These included the coding regions, > 150 base pairs (bp) at intron-exon boundaries and > 1000 bp of the 5' untranslated region of the known causative genes for monogenic chylomicronemia, including *LPL, APOC2*, *APOA5*, *LMF1* and *GPIHBP1.* The targeted next-generation DNA sequencing and custom bioinformatic pipeline are known

collectively as "LipidSeq" (23, 24) (Appendix C). Prepared DNA libraries from each patient sample were assayed using the MiSeq personal sequencer (Illumina, San Diego CA). The method has mean > 300-fold coverage for each base. Sanger sequencing was used to confirm all variants.

3.2.2.4 Annotation and evaluation of observed variants

FASTQ files derived from the MiSeq output were processed individually using a custom automated workflow in CLC Genomics Workbench version 8.5.1 (CLCbio, Aarhus, Denmark) for sequence alignment, variant calling, producing a variant call format (vcf) file, and target region coverage statistics. Variant annotation was performed using ANNOVAR (25, 26) using customized scripts (http://annovar.openbioinformatics.org/en/latest/).

There is no consensus on the procedure to attribute causality or pathogenicity to FCS variants detected by NGS. Fortunately, many variants detected in monogenic chylomicronemia genes have had a long history of archiving and annotation, as well as abundant publications of functional consequences. For instance, > 150 individual variants previously reported as being causative in LPL deficiency are reported in the Human Gene Mutation Database (HGMD; HGMD; http://www.biobase-international.com/product/hgmd) (27-29).

Annotated coding and noncoding (± 10 base pairs from adjacent exon) variants in vcfs were first filtered to select the rare variants according to minor allele frequencies (MAF) <1% in 1000 Genomes Project (1KG) (30), Exome Variant Server (EVS) (31) or Exome Aggregation Consortium (ExAC) (32) databases. Polymorphism Phenotype Version 2 (PolyPhen2) (33), Sorting Intolerant from Tolerant (SIFT) (34, 35) and Combined Annotation Dependent Depletion (CADD) (36) scores were used to evaluate the deleteriousness of the filtered coding variants. Splicing Based Analysis of Variants (SPANR) (37) and Automated Splice Site and Exon Definition Analyses (ASSEDA) (38) were used to identify rare deleterious splicing variants.

Novel variants found in this study were determined to be likely causative when: 1) they had no listed allele frequencies in 1KG, EVS or ExAC databases, no reference SNP indentification number (rsID) in the dbSNP database, and/or were not reported in the HGMD database; 2) for coding variants, a deleterious score for > 2 in silico algorithms; and 3) for non-coding variants, a deleterious score for > 1 in silico algorithm. Copy number variants (CNVs), sometimes referred to as "del-dup" mutations, were determined using a custom bioinformatics approach.

As controls for our annotation pipeline, we used sequence data from the 1KG database. Standard criteria based on standards of the American College of Medical Genetics, and also on published functional studies where available, were used to assess the potential pathogenicity of identified mutations. Individuals with bi-allelic pathogenic mutations in *LPL* were classified as "*LPL*-FCS" while those with pathogenic mutations in the other genes were classified as "non-*LPL*-FCS". Simple heterozygotes for loss-of-function mutations were excluded from the analysis.

3.2.2.5 Statistical analysis

Statistical analyses were conducted using SAS v9.3 (SAS Institute, Cary NC). Between-group differences for mean quantitative traits were evaluated with Wilcoxon signed-rank test for non-parametric sample distribution, while differences in discrete traits were evaluated using chi-square analysis with Fisher's exact test. Statistical significance was defined as $P<0.05$ for all comparisons. Our study sample afforded 84% power to detect a 50% difference in a quantitative trait whose standard deviation was 20% of the mean with 2-sided alpha = 0.05.

3.2.3 Results

3.2.3.1 Classification and mutation distribution

Of 67 individuals with phenotypic data who underwent targeted next generation sequencing, 41 had likely or definitely pathogenic bi-allelic *LPL* gene mutations: of these

82%, 7% and 11% were missense, nonsense and splicing variants, respectively (Table 3.1) DNA quality for one patient from Québec was inadequate for LipidSeq; in this case we made an exception and accepted results from the certified provincial DNA testing facility, which diagnosed homozygosity for the founder *LPL* p.P234L loss of function variant. An additional 11 individuals had non-*LPL* FCS, of whom 2 (22%) had bi-allelic mutations in *APOA5*, 5 (45%) had bi-allelic mutations in *GPIHBP1*, 1 (11%) had bi-allelic mutations in *LMF1*, 1 (11%) had bi-allelic mutations in *APOC2* and 2 were compound heterozygotes for mutations in *LPL* and either *APOA5* (11%) or *LMF1* (11%). The remainder of individuals, with a clinical FCS phenotype, but in whom genetic testing was not fully confirmatory, were excluded from this analysis. The mutations detected in study subjects are shown in Tables 3.1 and 3.2

3.2.3.2 Clinical and biochemical features

Numerous clinical, demographic and biochemical features were determined from studied individuals (Table 3.3). Non-*LPL*-FCS individuals were younger at screening than *LPL*-FCS cases (38.3 ± 12.3 vs. 47.1± 12.8 years, P=0.075). As expected compared to *LPL*-FCS individuals, non-*LPL*-FCS individuals had significantly higher post-heparin LPL activity (39.7±29.8 vs. 6.3±5.8 U, P=0.0028). Also, low density lipoprotein (LDL) cholesterol, determined directly by ultracentrifugation, was significantly higher in individuals with non-*LPL*-FCS compared to *LPL*-FCS (0.57 ±0.27 vs 0.75 ±0.21 mmol/L (28.9 ± 8.1 vs. 22.1 ± 10.4 mg/dl) P=0.027), although levels in both subgroups were very low. Apo B levels were not significantly different. Finally, compared to *LPL*-FCS individuals, non-*LPL*-FCS individuals had higher postprandial insulin (302.7±159.3 vs 97.0 ±137.1 u/L; P= 0.0089) and higher postprandial C-peptide levels (32.5 ± 12.3 vs 20.7 ± 6.2, P=0.0492).

Compared to *LPL*-FCS individuals, non-*LPL*-FCS individuals had a non-significant trend towards lower total triglycerides (TG) (20.79 ±10.6 vs 29.5 +14.8 mmol/L (1840± 938 vs 2613 ± 1307 mg/dL) P=0.091), chylomicron TG (16.52 ± 10.1 vs 25.94 ±14.7 mmol/L (1462 ± 894 vs 2296±1298 mg/dL) P=0.060), 4-hour post prandial chylomicron triglycerides (68.3±51.1 vs 104.3 ± 56.1 mmol/L (6045 ± 4525 vs 9229 ± 4973 mg/dL) P=0.082) and higher

VLDL triglycerides (3.68 ± 1.65 vs 2.76 ± 1.63 mmol/L (325.9 ± 145.7 vs 244.4 ± 144.2 mg/dL) P= 0.059).

There were no significant differences in other measured biochemical variables, including traditional and non-traditional lipid, lipoprotein and apolipoprotein variables. Furthermore, most variables from advanced lipoprotein analysis and from post-prandial studies were similar between groups. There was no significant difference in acute pancreatitis history between non-*LPL*-FCS and *LPL*-FCS groups (72.7% vs. 85.4%, respectively) or age of first episode of pancreatitis (27.8±8.5 vs. 25.6±16.5, respectively). We repeated these analyses post hoc, excluding the *LPL* p.P234L homozygote whose diagnosis was determined by traditional sequencing only; all statistical comparisons and interpretation of results remained the same.

Table 3.1: Lipoprotein lipase gene (*LPL*) mutation status and LPL activity

Patient mutation status	Type	Mutation name and zygosity	Number of patients	LPL activity pre-treatment* (umol/L/min)	LPL activity post-treatment*	CNV state	Previously reported (reference numbers)
Homozygous	SNV	p.P234L homozygote	8	7.4	8.7	-	(39, 40)
		p.G215E homozygote	3	7.4	8.7	-	(41-43)
		p.D277N homozygote	2	3.6	3	-	(44)
		p.Y329X homozygote	2	6.45	3.85	-	(37, 45)
		p.I221T homozygote	2	18.9	11.8	-	(46, 47)
		p.G166S homozygote	2	3.1	3.2	-	(48)
		p.W113R homozygote	1	1.8	1.8	-	(44)
		p.V206A homozygote	1	7.4	4.8	-	(49)
		p.R270H homozygote	1	4.9	1.8	-	(50, 51)
		p.L330P homozygote	1	5.9	9.6	-	(52)
		p.H210R homozygote	1	2.6	ND	-	No
	nonsense/ frameshift	p.D277D fs X4 homozygote	2	ND	11.2	-	No
		p.A61A fs X28 homozygote	1	6.1	5.2	-	No
	intronic SNV	intron 6 -2A>T homozygote	2	2.9	3.6	-	(53)
		intron 7 +7A>G homozygote	1	6.9	1.8	-	No
Double heterozygote (*LPL*	SNV/ intronic SNV	p.P234L heterozygote; *GPIHBP1*, intron 1 +4C>T heterozygote	1	7.5	8	-	(39, 40)

and *GPIHBP1* genes)							
Homozygous and CNV in additional geneb(*GPIHBP1*)	SNV/CNV	p.A185T homozygote	1	19.2	ND	*GPIHBP1*, exon 3-4 deletion, heterozygote	(52)
Compound heterozygous	SNV/ SNV	p.G215E heterozygote and p.R270C heterozygote	2	3.15	2.6	-	(41-43)
		p.W113R heterozygote and p.H163R heterozygote	1	3.6	3.4	-	(44) (54)
		p.G81D heterozygote and p.F212L heterozygote	1	2.3	ND	-	(9) No
		p.G215E heterozygote and p.P234L heterozygote	1	2.1	ND	-	(41-43)
		p.G215E heterozygote and p.V96L heterozygote	1	14.8	11.6	-	(41-43) (55)
		p.G215E heterozygote and p.I221T heterozygote	1	3.4	3.5	-	(41-43) (46, 47)
	SNV/ intronic SNV	p.A61V and intron 2 -1G>C homozygote	1	6.2	1.8	-	(41-43)
		p.G215E heterozygote, and intron 1 +5G>C	1	3.5	2.4	-	(41-43)

*average taken if data available for more than one patient with mutation

abbreviations: LPL, lipoprotein lipase; SNV, single nucleotide variant; CNV, copy number variant; ND, not determined; "-" absent

Table 3.2: Non-Lipoprotein lipase gene (non-*LPL*) mutation status and LPL activity

Gene	Mutation	CNV state	LPL activity pre-treatment* (umol/L/min)	LPL activity post-treatment*	Previously reported?
APOA5	p.Q330Q fs X6 homozygote	-	37	4	No
	p.L253P homozygote	-	56.4	-	(56, 57)
APOC2	intron 3 +1G>C homozygote	*APOC2*, exon 1 deletion (noncoding), homozygote	80	.	No
LMF1	p.P248S homozygote	-	31.7	13.5	No
GPIHBP1	p.A6D homozygote	-	16.1	14.6	No
	p.Q132X homozygote	-	-	4.6	No
Double heterozygote *LPL* + other	*LPL* p.G215E heterozygote; *APOA5* p.A315V heterozygote	-	80	-	(41-43) No
	LPL p.R116Q heterozygote; *LMF1* p.R233X heterozygote	-	-	-	(53) No
CNV	-	*GPIHBP1*, exon 1-4 deletion, homozygote	1.8	.	No
	-	*GPIHBP1*, exon 3-4 deletion, homozygote	14.9	17.3	(58)
	-	*GPIHBP1*, exon 3-4 deletion, homozygote	.	3.9	(58)

abbreviations: as in Table 3.2

Table 3.3: Characteristics of molecularly characterized familial chylomicronemia subjects LPL vs non-LPL FCS

	LPL-FCS	non-LPL-FCS	p-value (Wilcoxon)
Number of individuals	41	11	
Sex (% female)	61.0	72.7	NS
Age at screening	47.1 ± 12.8	38.3 ± 12.3	NS (0.075)
Body mass index (kg/m²)	24.0 ± 5.6	24.6 ± 6.7	NS
History of acute pancreatitis (%)	85.4	72.7	NS
Age of onset of pancreatitis (years)	25.6 ± 16.5	27.8 ± 8.5	NS
Post heparin LPL activity at baseline (□mol/L/min)	6.3 ± 5.8	39.7 ± 29.8	0.0028
Total cholesterol (mg/dL) **(mmol/L)**	300.9 ± 125.4 7.78 ± 3.24	242.5 ± 86.2 6.27 ± 2.23	NS
Triglycerides (mg/dL) **(mmol/L)**	2613 ± 1307 29.5 ± 14.8	1840 ± 938 20.79 ± 10.6	NS (0.091)
LDL cholesterol (mg/dL; by ultracentrifuge) **(mmol/L)**	22.1 ± 10.4 0.57 ± 0.27	28.9 ± 8.1 0.75 ± 0.21	0.027
HDL cholesterol (mg/dL; by precipitation) **(mmol/L)**	15.2 ± 4.1 0.39 ± 0.11	15.7 ± 3.3 0.41 ± 0.09	NS
Non-HDL cholesterol (mg/dL) **(mmol/L)**	284.9 ± 124.5 7.36 ± 3.22	226.8 ± 87.1 5.86 ± 2.25	NS
Apo A-I (mg/dL)	92.6 ±13.8	99.2 ± 26.0	NS
Apo B-100 (mg/dL)	59.5 ± 18.0	60.3 ± 13.6	NS
Apo B-48 (mg/dL)	11.4 ± 7.11	10.1 ± 7.97	NS
Apo C-III (mg/dL)	27.4 ± 12.1	28.4 ± 12.7	NS
Chylomicron triglycerides (mg/dL) **(mmol/L)**	2296 ± 1298 25.94 ± 14.7	1462 ± 894 16.52 ± 10.1	NS (0.060)
VLDL cholesterol (mg/dL) **(mmol/L)**	31.3 ± 26.0 0.81 ± 0.67	40.4 ± 23.2 1.04 ± 0.60	NS
VLDL triglycerides (mg/dL) **(mmol/L)**	244.4 ± 144.2 2.76 ± 1.63	325.9 ± 145.7 3.68 ± 1.65	NS (0.059)
Chylomicron plus VLDL apo C-III content (mg/dL)	26.7 ± 12.6	25.6 ± 14.2	NS
HDL apo C-III content (mg/dL)	1.26 ± 0.56	1.11 ± 0.39	NS
Glucose (mg/dL) **(mmol/L)**	93.7 ± 13.8 5.21 ± 0.77	99.4 ± 23.9 5.52 ± 1.33	NS
Triglycerides 4 h post-prandial (mg/dL) **(mmol/L)**	10031 ± 5125 113.3 ± 57.9	7467 ± 4844 84.4 ± 54.7	NS
Apo B-48 4 h post-prandial (mg/dL)	41.08 ± 23.09	36.51 ± 31.79	NS
Chylomicron triglycerides 4 h post-prandial (mg/dL)	9229 ± 4973	6045 ± 4525	NS (0.082)

(mmol/L)	104.3 ± 56.1	68.3 ± 51.1	
Glucose 4 h post-prandial (mg/dL)	408.7 ± 92.8	531.6 ± 244.1	NS
(mmol/L)	22.7 ± 5.16	29.5 ± 13.6	
Insulin 4 h post-prandial (U/L)	97.0 ± 137.1	302.7 ± 159.3	0.0089
C-peptide 4 h post-prandial (U/L)	20.7 ± 6.2	32.5 ± 12.3	0.0492

Abbreviations: apo, apolipoprotein; HDL, high density lipoprotein; LDL, low density lipoprotein; LPL-FCS, familial chylomicronemia syndrome due to bi-allelic mutations in the *LPL* gene encoding lipoprotein lipase; non-LPL-FCS, familial chylomicronemia syndrome due to bi-allelic mutations in the either the *APOC2*, *APOA5*, *LMF1* or *GPIHBP1*, which encode, respectively, apo C-II, apo A-V, lipase maturation factor 1, or glycosylphosphatidylinositol-anchored HDL-binding protein 1; NS, not significant; VLDL, very-low density lipoprotein

Table 3.4: Characteristics of molecularly characterized familial chylomicronemia subjects by gene

	LPL	*APOC2*	*APOA5*	*LMF1*	*GPIHBP1*	Compound Heterozygote	P-value (overall)
Number of individuals	41	1	2	1	5	2	-
Sex (% female)	61.0	100.0	100.0	100.0	60.0	50.0	NS
Age at screening	47.1 ± 12.8	56	46.5 ± 6.4	39	33.8 ± 11.9	32.0 ± 17.0	NS
Body mass index (kg/m^2)	24.0 ± 5.6	20.5	23.6 ± 1.98	17.9	22.4 ± 4.21	36.2 ± 3.39	0.045
History of acute pancreatitis (%)	85.4	100.0	50.0	100.0	60.0	100.0	NS
Age of onset of pancreatitis (years)	25.6 ± 16.5	41	26.0	24.0	24.3 ± 6.03	29.0 ± 14.1	NS
Post heparin LPL activity at baseline (μmol/L/min)	6.3 ± 5.8	80.0	46.7 ± 13.7	31.7	10.9 ± 7.93	80.0	<.0001
Total cholesterol (mg/dL)	300.9 ± 125.4	188	246.5 ± 143.5	362	254.8 ± 53.9	175.5 ± 122.3	NS
(mmol/L)	7.78 ± 3.24	4.86	6.37 ± 3.71	9.36	6.59 ± 1.39	4.53 ± 3.16	
Triglycerides (mg/dL)	2613 ± 1307	1254	1969 ± 1790	2683	2096 ± 627.3	944 ± 1001	NS
(mmol/L)	29.5 ± 14.8	14.2	22.2 ± 20.2	30.3	23.7 ± 7.08	10.7 ± 11.3	
LDL cholesterol (mg/dL; by ultracentrifuge)	22.1 ± 10.2	28	30.5 ± 10.6	20	27 ± 7.81	37 ± 8.49	NS
(mmol/L)	0.57 ± 0.26	0.72	0.79 ± 0.27	0.52	0.70 ± 0.20	0.96 ± 0.22	
HDL cholesterol (mg/dL; by precipitation)	15.2 ± 4.1	14	18	18	13.6 ± 1.67	18.5 ± 6.36	NS
(mmol/L)	0.39 ± 0.11	0.36	0.47	0.47	0.35 ± 0.043	0.48 ± 0.16	
Non-HDL cholesterol (mg/dL)	284.9 ± 124.6	174	228.5 ± 143.5	344	241.2 ± 53.4	157 ± 128.7	NS
(mmol/L)	7.36 ± 3.22	4.50	5.91 ± 3.71	8.90	6.24 ± 1.38	4.06 ± 3.33	
Apo A-I (mg/dL)	92.6 ±13.8	79	136 ± 11.3	140	85.8 ± 12.3	85.5 ± 0.71	<0.0001
Apo B-100 (mg/dL)	59.5 ± 18.0	55	69.15 ± 2.62	66.3	52.6 ± 13.2	70.6 ± 20.3	NS
Apo B-48 (mg/dL)	11.4 ± 7.11	4.88	3.62 ± 2.73	18.1	14.3 ± 8.21	4.5 ± 5.62	NS
Apo C-III (mg/dL)	27.4 ± 12.1	19.39	31.9 ± 11.8	38.6	25.9 ± 11.1	30.6 ± 27.1	NS
Chylomicron triglycerides (mg/dL)	2296 ± 1298	959	1551 ± 1730	2123	1780 ± 624.2	501.5 ± 623	NS
(mmol/L)	25.94 ± 14.7	10.8	17.5 ± 19.5	24.0	20.1 ± 7.05	5.66 ± 7.03	
VLDL cholesterol (mg/dL)	31.3 ± 26.0	28	36.5 ± 3.54	67	34.2 ± 10.4	49.5 ± 57.3	NS
(mmol/L)	0.81 ± 0.67	0.72	0.94 ± 0.092	0.76	0.88 ± 0.27	1.28 ± 1.48	

VLDL triglycerides (mg/dL)	244.4 ± 144.2	238	312 ± 1.41	449	290 ± 95.5	394 ± 355	NS
(mmol/L)	2.76 ± 1.63	2.69	3.52 ± 0.016	5.07	3.27 ± 1.08	4.45 ± 4.01	
Chylomicron plus VLDL apo C-III content (mg/dL)	26.7 ± 12.6	.	.	.	24.4 ± 11.05	28.5 ± 26.5	NS
HDL apo C-III content (mg/dL)	1.26 ± 0.56	0.92	.	.	1.15 ± 0.50	1.09 ± 0.071	NS
Glucose (mg/dL)	93.7 ± 13.8	79.0	93.5 ± 3.54	167	90.0 ± 5.5	105 ± 4.24	<0.0001
(mmol/L)	5.21 ± 0.77	4.39	5.19 ± 0.20	9.28	5 ± 0.31	5.83 ± 0.24	
Triglycerides 4 h post-prandial (mg/dL)	10031 ± 5125	7706	6124 ± 4019	18173	7042 ± 3090	1331	NS
(mmol/L)	113.3 ± 57.9	87.0	69.1 ± 45.4	205.2	79.5 ± 34.9	15.0	
Apo B-48 4 h post-prandial (mg/dL)	41.08 ± 23.09	42.1	29.4 ± 28.1	114.52	29.2 ± 13.7	3.87	0.021
Chylomicron triglycerides 4 h post-prandial (mg/dL)	9229 ± 4973	6473.5	4396 ± 3650	15685	5784 ± 3250	583.5	NS
(mmol/L)	104.3 ± 56.1	73.09	49.6 ± 41.2	177.1	65.3 ± 36.7	6.59	
Glucose 4 h post-prandial (mg/dL)	408.7 ± 92.8	.	420.5	943	365 ± 15.6	564.5	0.0003
(mmol/L)	22.7 ± 5.16		23.4	52.4	20.3 ± 0.87	31.4	
Insulin 4 h post-prandial (U/L)	97.0 ± 137.1	.	413.2	527.4	177.9 ± 16.1	216.8	0.022
C-peptide 4 h post-prandial (U/L)	20.7 ± 6.1	.	38.1	49.1	27.7 ± 9.95	20.0	0.0015

Abbreviations: apo, apolipoprotein; HDL, high density lipoprotein; LDL, low density lipoprotein; LPL-FCS, familial chylomicronemia syndrome due to bi-allelic mutations in the *LPL* gene encoding lipoprotein lipase; non-LPL-FCS, familial chylomicronemia syndrome due to bi-allelic mutations in the either the *APOC2*, *APOA5*, *LMF1* or *GPIHBP1*, which encode, respectively, apo C-II, apo A-V, lipase maturation factor 1, or glycosylphosphatidylinositol-anchored HDL-binding protein 1; NS, not significant; VLDL, very-low density lipoprotein; P-values calculated from analysis of variance and indicates an overall difference between classes. Pairwise comparisons between individual classes were not performed

3.2.4 Discussion

While most patients with monogenic chylomicronemia have bi-allelic, loss-of-function variants in *LPL*, a substantial minority has the phenotype due to mutations in the four remaining minor genes. A direct head-to-head comparison of clinical and biochemical features among individuals with *LPL*-related and non-*LPL* related monogenic chylomicronemia has not yet been reported, generally because these patents are so rare and consistent harmonized methods have not been used in a larger cohort of individuals. Here, because of the comprehensive assessments of these individuals due to their involvement in a clinical trial, we had a unique collection of phenotypically and genotypically well-characterized individuals with monogenic chylomicronemia to allow for a detailed assessment of phenotypic differences according to the main underlying molecular classes.

Our findings primarily indicate that for most fasting and dynamic metabolic measures, individuals with monogenic chylomicronemia are phenotypically similar whether the underlying cause is bi-allelic mutations in *LPL* or one of the other four genes. The difference in post-heparin LPL activity was expected based on our understanding of the biochemistry and genetic basis of the different forms of the condition. Individuals with mutations in non-*LPL* genes, by definition, had normal lipoprotein lipase enzyme, and thus there would be less expected lipolytic compromise under the conditions of the *ex vivo* lipolytic assay. We note in Table 3.4 that *ex vivo* lipolysis is lower in patients with *LMF1* and *GPIHBP1* mutations than in the other non-*LPL*-FCS genetic subgroups, but not as low as for *LPL*-FCS patients. Under *in vitro* conditions, the deficiencies resulting from *LMF1* and *GPIHBP1* mutations that would otherwise impair *in vivo* function become less relevant. Similarly, the deficiencies resulting from mutations in *APOA5* and *APOC2* can be rescued since the *in vitro* test substrate contains some normal apolipoproteins. Thus, on balance, non-*LPL* mutation patients had higher LPL activity. The trend towards significantly higher levels of chylomicrons and triglycerides seen in *LPL*-mutation positive patients suggests that LPL

deficiency may manifest with somewhat more severe chylomicronemia than FCS resulting from mutations in non-*LPL* genes.

Of particular note, total cholesterol values were high in both groups, while LDL-C, HDL-C, VLDL-C levels were all low in both *LPL*-FCS and non-*LPL*-FCS groups. This highlights a fundamental feature of the metabolic defect in FCS. With absent or minimal LPL activity, there is a significant impairment in conversion of triglyceride-rich particles to their remnant lipoproteins, which generates a cascading impairment in production of subsequent smaller lipoprotein sub-fractions, for which these remnants are the initial substrate. Each step of the lipoprotein metabolism pathway involves lipolysis of triglyceride via LPL for conversion to a smaller lipoprotein species (i.e. from chylomicrons to chylomicron remnants to VLDL to VLDL remnants and IDL to LDL). Though LPL deficient individuals commonly exhibit elevated total cholesterol values, the cholesterol measured is predominantly present within chylomicrons, with a triglyceride to cholesterol ratio of ~20:1, with very-low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and low-density lipoprotein (LDL) cholesterol all typically at very low levels. HDL cholesterol is similarly low due to a decrease in available cholesterol from its primary sources: VLDL, LDL and peripheral tissues. This does not hold true, however, for individuals with other secondary or polygenic causes of elevated triglycerides, in which LPL is generally functional, and all lipoproteins and their remnants can be present and elevated to varying degrees, along with an increase in cardiovascular risk. This key concept has significant implications when selecting appropriate therapy. While secondary and polygenic (type V) hypertriglyceridemia patients who have some residual LPL activity can often be effectively managed with fibrates or other oral agents, this is not expected to be effective in patients with LPL deficiency, who require more targeted triglyceride-specific therapy.

Further subdividing the non-*LPL*-FCS subgroup by genotype allows for some additional anecdotal observations (Table 3.4). For instance, in the *LMF1* deficient patient, plasma apo A-I is very high, consistent with a concomitant low hepatic lipase activity affecting apo A-I-containing particles, as would be expected given the role of LMF1 in maturation of both

lipases. Although in this patient, there is no obvious difference in baseline LDL and HDL cholesterol levels, it is notable that total and especially post prandial triglycerides are very high. Furthermore, the triglyceride levels in the *APOC2* and *APOA5* deficient patients appear somewhat lower than in the *LPL*-FCS group, as might be expected. Finally, the double heterozygotes who each have one normal functioning *LPL* allele have, as expected, the lowest triglyceride levels at baseline and especially post-prandially.

The significantly higher level of LDL cholesterol seen in patients with non-*LPL*-FCS compared to the LPL-FCS population has not been previously described. It may reflect a less severe impairment of LPL activity in these patients, or the presence of concomitant secondary causes, compared to the *LPL*-FCS group. It may also suggest a minor or indirect role of *APOC2*, *APOA5*, *LMF1* or *GPIHBP1* in LDL processing or clearance, or potentially the clustering of other unmeasured risk genes in this subgroup compared to those with *LPL*-FCS. Some support for this may come from studies that have shown an association between mutations in *APOA5* and *APOC2* and hypercholesterolemia (59). Environmental, or gene-environment interactions, may also be playing a role. Interestingly apo B levels were not significantly different between the two groups, although specific B-100 and B-48 assays were not performed. Further investigation is required to fully mechanistically define such potential differences.

The tendency of non-*LPL* mutation carriers to have somewhat worse indices of insulin resistance suggests that this secondary factor may be playing an underlying role in this subgroup. Alternatively, perhaps the *LPL* defect leads to greater requirements for glucose in peripheral tissues with improved insulin sensitivity and lower glucose levels, assuming the islets are functioning normally. Also, some of the non-*LPL* gene product mechanisms may be predisposing to somewhat compromised insulin sensitivity, in addition to their major effects on chylomicron and lipoprotein metabolism. However, the similarity of the remainder of the phenotypes suggests that regardless of etiology, large effect mutations directly affecting *LPL* or one of its critical interacting factors results in a severe phenotype that is clinically and biochemically similar irrespective of the molecular etiology. It would be

important to rigorously evaluate insulin sensitivity in individuals subdivided according to FCS genotype class to confirm our observations here.

The pancreatitis rates were high in both groups (75% and 85% respectively), however it is important to note that this was as a result of the clinical trial selection criteria, which included a personal history of pancreatitis, and should not be misinterpreted as prevalence rates for pancreatitis in either genotype.

3.2.4.1 Limitations

Even though we have collected a unique and relatively large group of these extremely rare individuals, the sample size is small, and it is possible that more subtle differences in some of these variables would be apparent with a larger number of subjects, particularly those with non-*LPL* monogenic chylomicronemia. Furthermore, we did not have sufficient numbers of individuals to further subdivide according to minor gene etiology or mutation type. Because these individuals were studied in a trial of an inhibitor of apo C-III, it would have been informative to have collected not just apo C-III content in subfractions but also apo E and apo A-V, however these were not available. We note that apo C-III content was available in TG-rich particles (chylomicrons plus VLDL) and also in HDL: overall, these were not statistically different between the genotypic classes.

Additionally, it is important to note that the trial inclusion criteria limited this data analysis to relatively severe cases of chylomicronemia. This has the potential to miss important differences between *LPL* and non-*LPL* FCS that may have been seen if those with milder phenotypes were included. Furthermore, different mutations in both *LPL* and non-*LPL* genes could present with a wide phenotypic spectrum based on the severity of functional compromise that results from a given mutation. We also excluded heterozygous *LPL* mutation-positive patients from analysis, however it is possible that heterozygous null mutation carriers may present with a phenotype that overlaps with milder homozygous or compound heterozygous mutation-positive individuals and further analysis based on degree of functional compromise could be informative. Finally, it might also have been

informative to examine the individuals with a clinical FCS phenotype but in whom genetic testing or LPL activity were not fully confirmatory, although such patients would not be easily classified in this experimental design.

3.2.4.2 Future directions

This study compared phenotypes of the individuals tested who presented with a clinical FCS phenotype and had a confirmed genetic basis for their severe chylomicronemia. It is possible that the remaining individuals have as-yet unidentified mutations causing true FCS, or they may simply have severe secondary or polygenic (type V) chylomicronemia. The features of these 15 individuals were not compared to those who had confirmatory genetic testing in our study. For some lipidologists, the diagnosis of FCS should include genetic testing, although the ability to make a diagnosis based on predictive clinical features alone is highly attractive. It is beyond the scope of this study to recommend the necessity for genetic testing to diagnose patients with true bi-allelic FCS from among the multitude of those with severe HTG. However, once genetic testing has been performed, and individuals with FCS are subdivided between bi-allelic *LPL* and non-*LPL* subgroups, the between-genotype clinical and biochemical differences are subtle. A comparison between individuals with a clinical FCS phenotype but no genetic diagnosis to those who have identified mutations could be informative. Some preliminary work suggests that some predictive features may include a personal history of pancreatitis, low BMI and low apo B levels.

3.2.5 Conclusions

In summary, we found that patients with *LPL*-related and non-*LPL*-related monogenic chylomicronemia are largely phenotypically similar, but that *LPL*-FCS has lower post-heparin LPL activity and a trend towards somewhat higher chylomicrons and triglycerides, while LDL cholesterol and markers of insulin resistance were higher in non-*LPL*-FCS. It remains to be determined whether these two subgroups of patients differ with respect to other attributes, including the response to interventions.

3.3 Incidence, Predictors and Patterns of Care of Patients with Very Severe Hypertriglyceridemia in Ontario, Canada: A Population-Based Cohort Study

The work presented in Chapter 3.3 has been edited from this original manuscript for brevity and consistency throughout this dissertation

Berberich AJ, Ouédraogo AM, Shariff SZ, Hegele RA, Clemens KK. Incidence, predictors and patterns of care of patients with very severe hypertriglyceridemia in Ontario, Canada: a population-based cohort study. Lipids Health Dis. 2021 Sep 3;20(1):98. doi: 10.1186/s12944-021-01517-6. PMID: 34479547.

3.3.1 Background:

Triglyceride (TG) values >20 mmol/L can have significant consequences. One of the most detrimental outcomes is pancreatitis, which often requires hospitalization or admission to intensive care, and associated mortality. In clinical practice, very severe hypertriglyceridemia (VS-HTG), defined in this study as triglyceride (TG) levels that exceed 20 mmol/L, is captured through routine lipid testing and commonly misattributed to an exclusive genetic etiology. A minority of cases of VS-HTG (~1 in 1 million) arise from a primary TG disorder, as reviewed in chapter 3.2. Most cases result from secondary causes, often in conjunction with inherited partial impairment in TG metabolism (1, 60-63). These cases may only manifest under conditions that increase TG production or impair clearance. Previous studies conducted elsewhere have suggested that secondary causes of VS-HTG, include obesity and metabolic syndrome, poorly controlled diabetes, nephrotic syndrome, severe hypothyroidism, oral estrogen or tamoxifen, glucocorticoids, beta blockers, retinoids and HIV antiretroviral regimens (64-66). Irrespective of the underlying cause, VS-HTG is of clinical concern due to the risk of HTG-associated pancreatitis. Thus, it remains important to understand and manage the secondary risk factors associated with this condition (67).

Studies conducted in other regions, such as Norway and the United States, have suggested the prevalence of S-HTG (TG > 10mmol/L) to be between 0.13-0.4% (68, 69) and VS-HTG to be 0.05-0.1% (70, 71), there is minimal documentation of the epidemiology of S-HTG or VS-HTG in Canada. In this study we examined the incidence of S-HTG and VS-HTG in Canada's most populous province (Ontario), which is ethnically and socially distinct from populations studied previously (68, 70). We also determined the demographic distribution, laboratory features and co-morbidities associated with VS-HTG in Ontario. Further, as studies conducted in regions without universal health care coverage (70) suggest a significant care gap for patients with VS-HTG, we examined the care patterns of Ontario residents with VS-HTG to determine whether a similar care gaps exist.

3.3.2 Methods:

3.3.2.1 Design and setting:

This was a population-based retrospective cohort study of adults ≥18 years in Ontario, Canada between 2010 and 2015. Ontario's approximately 13.5 million residents have universal healthcare through the Ontario Health Insurance Program (OHIP), with comprehensive medication coverage provided to those ≥65 years or using social assistance. Information on the use of these health services is maintained at ICES (formerly The Institute for Clinical Evaluative Sciences). ICES is an independent, non-profit research institute whose legal status under Ontario's health information privacy law allows it to collect and analyze health care and demographic data, for health system evaluation and improvement.

3.3.2.2 Participants:

All individuals aged ≥18 years with at least one TG value in the Ontario Laboratories Information System (OLIS) between September 1, 2010 and September 1, 2015 were included. This study period was selected to allow for a 2-year follow up for care patterns and outcomes of interest following identification of an incident case (OLIS laboratory data available until the end of 2017). Standard data cleaning excluded those with invalid health card numbers, age ≥105 years, missing sex, and non-Ontario residents. Three patient cohorts were then created. Individuals who had TG levels >20 mmol/L were included in the VS-HTG cohort (cohort 1) and individuals who had TG levels >10-20 mmol/L were included in the S-HTG cohort (cohort 2). Those with prior evidence of TG >20 or >10 mmol/L between September 1 2007-August 31, 2010 were excluded to define new evidence of VS-HTG or S-HTG respectively.

A third cohort (cohort 3) was used to contrast characteristics of those with VS-HTG and with no HTG and establish predictors for VS-HTG. This comparator group included all Ontario residents ages ≥18 years with at least one TG value in OLIS during the study period, no evidence of TG value >3 mmol/L and who were not already in the S-HTG or VS-HTG cohort. In all cohorts, those who died within two years after a TG test were excluded to limit to

those in whom we could establish follow up care patterns. If individuals had more than one TG test >20mmol/L during the study period, the first TG test was selected (Figure 3.1).

3.3.2.3 Sources of data:

Data were drawn from a number of ICES databases (Appendix D). Datasets were linked using unique encoded identifiers and analyzed at ICES. Demographic data were obtained from the Registered Persons Database (RPDB), which includes all individuals who have been issued an Ontario health card. The Client Agency Program Enrolment (CAPE) database was used to determine patients who were rostered to a family physician. The Canadian Institutes for Health Information's Discharge Abstract Database (CIHI-DAD) and the National Ambulatory Care Reporting System (NACRS) contain diagnostic and procedural information captured during hospitalizations and emergency department visits, respectively, using International Classification of Diseases 10th Revision (ICD-10) (72) and Canadian Classification of Health Interventions (CCI) (73) codes. Additional comorbidities and healthcare services use was captured through OHIP, which contains billing and diagnostic codes (Appendix D). Hypertension and diabetes status were determined from the hypertension database (HYPER) (74) and Ontario Diabetes Database (ODD) (75) , respectively. For individuals ≥65 years, use of medications that can be associated with or used to treat HTG was captured using the Ontario Drug Benefit (ODB) and Drug Identification Number (DIN) database. Laboratory data were obtained through OLIS. The OLIS database holds laboratory data from both community and hospital laboratories with good catchment across Ontario (76). As of 2016, 95% of community lab volume and over 80% of total provincial lab volume were recorded in OLIS (77). Appendix D includes full details on variable definitions.

3.3.2.4 Incidence of VS-HTG:

The primary aim was to calculate the incidence of S-HTG and VS-HTG over the 5 year period. This was done using two denominators: the mean number of Ontarians ≥18 years between

2010-2015, and the number of adults ≥18 years who had a measured TG level over the study period.

3.3.2.5 Outcomes:

The 2-year patterns of care of those with VS-HTG were examined, including contacts with family physicians, internists and endocrinologists, repeat TG testing, new prescriptions for fibrates, statins and niacin, and minimum and last follow-up TG values. The secondary exploratory outcomes of interest included hospital encounters for pancreatitis, acute myocardial infarction or ischemic stroke over the 2-year follow up period.

3.3.2.6 Statistical analysis:

The incidence of S-HTG and VS-HTG was provided as a rate per 100,000. Descriptive statistics were used to capture baseline differences between the VS-HTG cohort 1 and the comparator cohort 3. Continuous variables were reported as mean +/- standard deviation (SD) and median with interquartile range (IQR). Binary variables were reported as percentages. Differences between cohorts were evaluated using standardized differences (StDiff), with a value of >0.1 considered significant (78). One-way ANOVA or Chi Square tests were also used to compare the means and medians of continuous variables and the proportions of categorical variables, respectively. *P*-values < 0.05 were considered statistically significant. Variables that were hypothesized to affect TG levels, co-exist with HTG or related complications from HTG were included in the analysis. To identify predictors of VS-HTG, both univariable and multivariable logistic regression were used (nominal *P*-value for significance <0.05) and reported results as odds ratios (OR) and 95% confidence intervals (CI). Descriptive statistics were used to report crude rates of secondary outcomes of interest.

3.3.2.7 Ethics Approval:

The use of data in this project was authorized under section 45 of Ontario's Personal Health Information Protection Act, which does not require review by a Research Ethics Board.

Guidelines for reporting of studies outlined by routine collected healthcare data (RECORD) were used (Addendix E) (79), as well as STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) cohort reporting guidelines for observational studies (80).

3.3.3 Results:

There were 22,745,387 TG tests performed in Ontario between 2010-2015 in those ≥18 years (7,047,586 unique individuals). A total of 17,615 and 2,869 people had S-HTG and VS-HTG respectively. The comparison cohort included 6,742,506 individuals (Figure 3.1).

Baseline differences considered between the VS-HTG cohort 1 and the comparison cohort 3 are shown in Table 3.5. In general, those with VS-HTG were more often male (78.0% vs 45.6%; StDiff 0.71, *P*-value <0.001), had diabetes (44.0% vs 13.9%; StDiff 0.7, *P*-value <0.001), chronic liver disease (7.9% vs 3.4%; StDiff 0.19, *P*-value <0.001), alcohol abuse (2.3% vs 0.4%; StDiff 0.17; P-value <0.001), obesity (1.8% vs 0.6%; StDiff 0.12; *P*-value <0.001) and HbA1c >8.5% (13.8% vs 1.5%; StDiff 0.48; *P*-value <0.001). Significantly more individuals in the VS-HTG cohort had a baseline history of pancreatitis (6.2% vs 0.3%; StDiff 0.34; *P*-value <0.001) (Table 3.5). All measured components of metabolic syndrome appeared higher in the VS-HTG cohort: (HTN (43.4% vs 33.1%; StDiff 0.21; *P*-value <0.001), low HDL cholesterol (C) (mean 0.97 vs 1.27 mmol/L; StDiff 0.79; *P*-value <0.001), elevated HbA1c (mean 7.88% vs 6.63%; StDiff 0.67; *P*-value <0.001) and obesity (1.8% vs 0.6%; StDiff 0.12; *P*-value <0.001).

Incidence of S-HTG and VS-HTG in Ontario adults between 2010 and 2015 was 163.61 and 26.65 per 100,000 (~1 in 615 and 1 in 3750) adults overall, and 250.18 and 40.75 per 100,000 (~1 in 400 and 1 in 2500) adults who had at least one TG test (Table 3.6). Combined incidence rate of TG >10 mmol/L was ~1 in 344 adults who had at least one TG value and ~1 in 526 adults overall (Table 3.6, Figure 3.2). The highest incidence of S-HTG and VS-HTG appeared between ages 31-45 (315.17 and 63.07/100,000 with a TG test; 197.12 and 39.45/100,000 population) and 46-65 (324.86 and 48.21/100,000 with a TG test;

266.40 and 39.54/100,000 population) (Table 3.6). Overall, incidence was higher in men (Table 3.7).

Medication use was only available for individuals ≥65 years (N=136 in the VS-HTG cohort) (Table 3.8) At baseline, a higher proportion of VS-HTG patients used a fibrate (18.4% vs 1.6%; SD 0.58) and a lower proportion used statins (40.4% vs 45.7%; StDiff 0.11) compared to the comparison cohort (N=1,392,795). A higher proportion of VS-HTG patients also appeared to use other lipid-lowering agents (11.8% vs 4.2%; StDiff 0.28), oral furosemide (16.2% vs 6.3%; StDiff 0.32) chlorthalidone (<5% vs 0.6%; StDiff 0.14) and salicylates (5.9% vs 3.5%; StDiff 0.11) (Table 3.8).

Results of univariable and multivariable analysis are presented in Table 3.9. In multivariable analysis, significant predictors of VS-HTG were diabetes (OR 5.38, CI 4.93-5.88), male sex (OR 3.83, CI 3.50-4.18), alcohol abuse (OR 2.47, CI 1.90-3.19), chronic liver disease (OR 1.71, CI 1.48-1.97), hypertension (OR 1.69, CI 1.54-1.86), obesity (OR 1.49, CI 1.13-1.98), and chronic kidney disease (OR 1.39, CI 1.19-1.63). Older age was associated with reduced risk of VS-HTG (OR 0.64/decade, CI 0.62-0.66). Higher income quintiles (4 and 5) were associated with lower odds of having VS-HTG in both univariable and multivariable analysis (0.78, CI 0.70-0.88 for quintile 4 and 0.72, CI 0.63-0.81 for quintile 5). In univariable analysis**,** lowest income quintile was associated with VS-HTG (OR 1.21, CI 1.09- 1.35) but this did not remain significant in the multivariate analysis. Likewise, higher Charlson comorbidity scores (moderate to high) were associated with VS-HTG in the univariate analysis. A Charlson index of 3+ was no longer significantly associated with VS-HTG in multivariable analysis (Table 3.9).

3.3.3.1 Outcome measures:

The majority of individuals with VS-HTG received follow-up healthcare within two years of their TG test (Table 3.10); 98.4% had at least one follow up visit to a family physician (FP), 32.8% had at least one visit to an endocrinologist and 56.7% had at least one visit to a general internist. The majority achieved TG reduction below the high-risk pancreatitis

threshold of 10 mmol/L, with 89.3% having a repeat TG test. The last TG measured had a median TG value of 4.7 mmol/L (IQR 2.7-9.1) and lowest TG value had a median TG value of 3.3 mmol/L (IQR 2.0-5.9). For individuals >65 years of age, new prescriptions for statins were provided in 32.4% and for fibrates, in 19.9% (Table 3.8).

Following detection of VS-HTG, pancreatitis occurred in 4.1% and 6.0% of individuals in the VS-HTG cohort within one and two years, respectively (Table 3.10). Within two years, 1.4% and 0.8% of individuals in the VS-HTG cohort had at least one hospital encounter for acute myocardial infarction and ischemic stroke, respectively (Table 3.10).

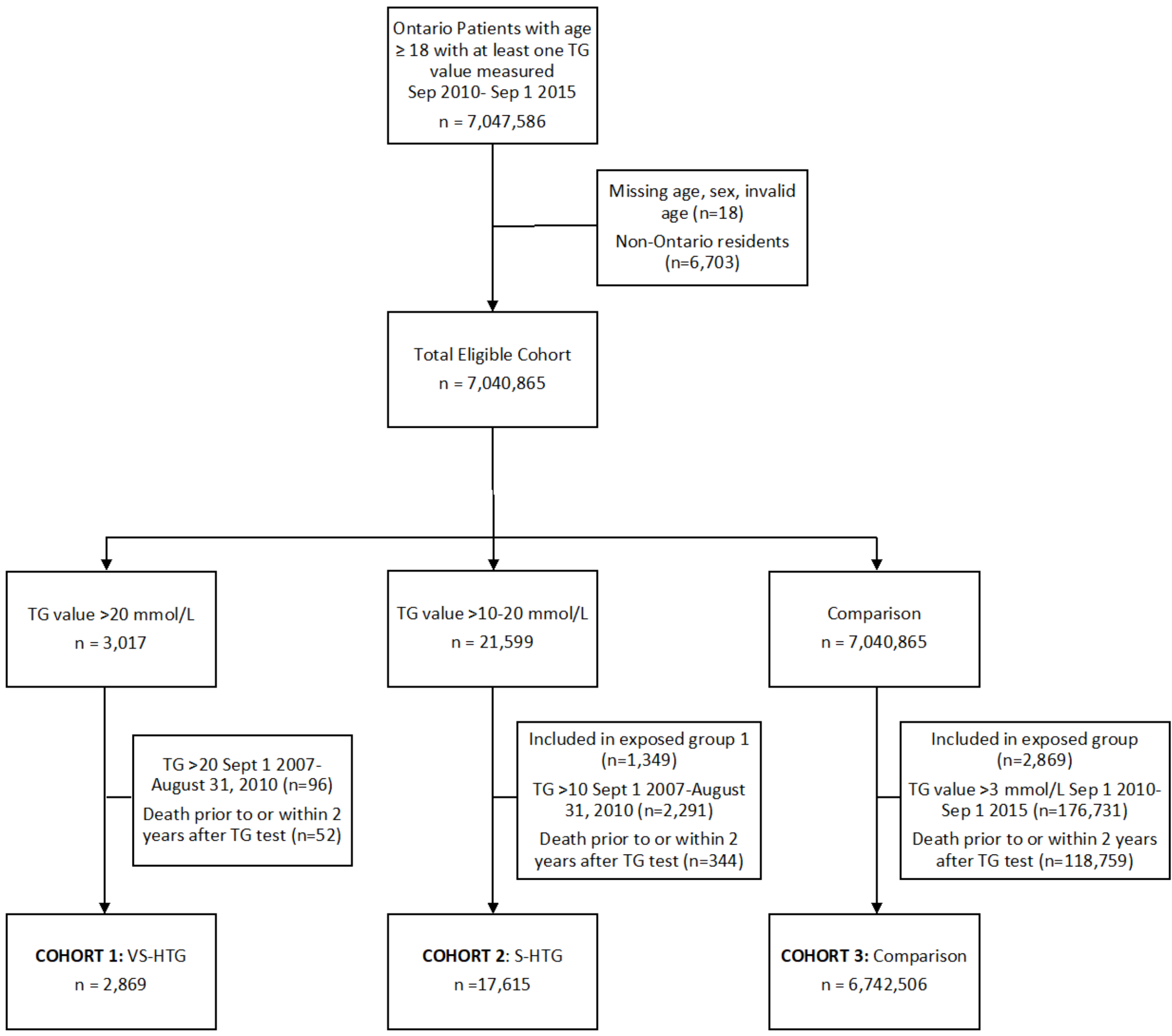

Figure 3.1: Flowchart of study participants.

After removal of duplicate and invalid data, the final cohorts included 2,869 individuals in VS-HTG cohort 1 (TG >20mmol/L), 17,615 in S-HTG cohort 2 (TG >10-20mmol/L) and 6,742,506 in the comparison cohort 3 (no TG >3.0mmol/L).

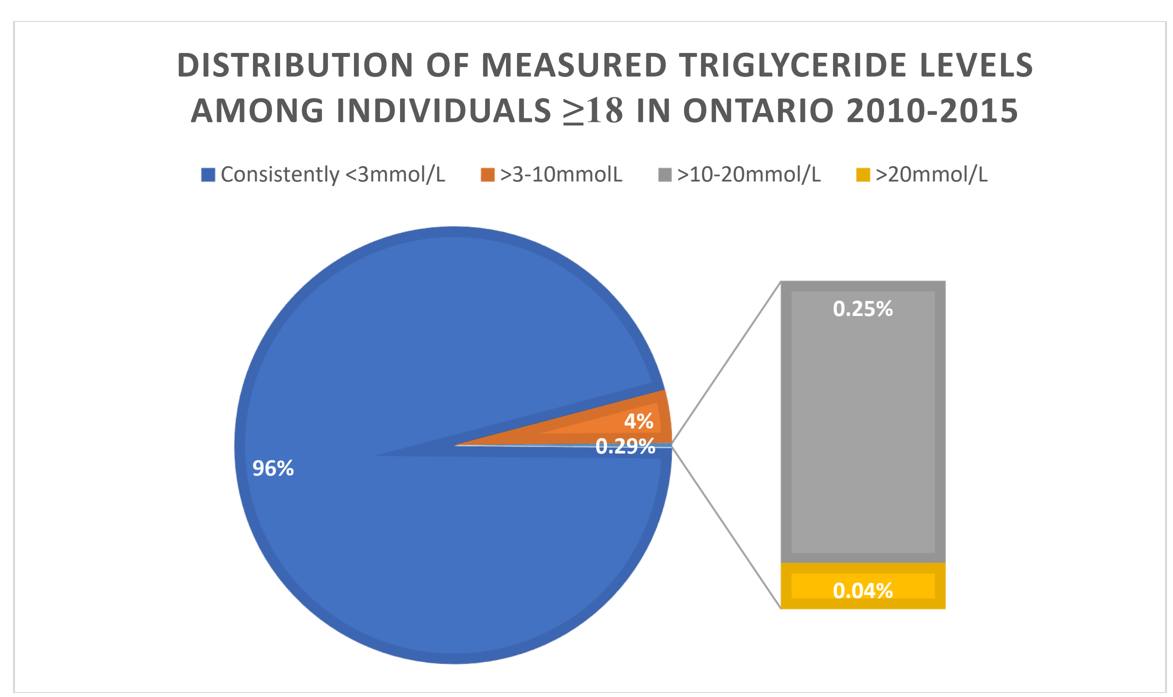

Figure 3.2: Summary of measured triglyceride (TG) values in Ontario from 2010-2015.

Among 7,040,865 individuals ≥18 years with measured TG in Ontario between 2010 and 2015: 6,742,506 (96%) had no TG value measured above 3 mmol/L; 21,484 individuals in Ontario (0.29%) had at least one TG value >10 mmol/L, with 0.25% (n=17,615) having severe hypertriglyceridemia (TG>10-20 mmol/L) and 0.04% (n=2,869) having very severe hypertriglyceridemia (TG>10 mmol/L). 277,875 (4%) individuals were not included in the other three cohorts and were assumed to have at least one measured TG value within the range of >3-10 mmol/L.

Table 3.5: Baseline characteristics of VS-HTG and comparison cohorts

	VS-HTG Cohort N=2,869	Comparison Cohort N=6,742,506	Standardized differences	*P*-value
Age at index date, years				
Mean (SD)	47.28 ± 10.87	51.74 ± 16.21	0.32	<0.001
Median (IQR)	47.0 (40.00-54.00)	52.0 (40.00-63.00)	0.35	<0.001
18-30 yrs	174 (6.1%)	744,062 (11.0%)	0.18	<0.001
31-45 yrs	1,078 (37.6%)	1,642,193 (24.4%)	0.29	
46-65 yrs	1,481 (51.6%)	2,963,456 (44.0%)	0.15	
66+ yrs	136 (4.7%)	1,392,795 (20.7%)	0.49	
Sex, female	630 (22.0%)	3,667,595 (54.4%)	0.71	<0.001
Income quintile*				
1 - lowest	690 (24.1%)	1,231,411 (18.3%)	0.14	<0.001
2	649 (22.6%)	1,323,360 (19.6%)	0.07	
3	635 (22.1%)	1,371,761 (20.3%)	0.04	
4	493 (17.2%)	1,432,535 (21.2%)	0.1	
5 - highest	402 (14.0%)	1,383,439 (20.5%)	0.17	
Rostered to a GP	2,346 (81.8%)	5,619,709 (83.3%)	0.04	0.023
Comorbidities				
Diabetes (Type 1 and Type 2)	1,262 (44.0%)	940,151 (13.9%)	0.70	<0.001
Hypertension	1,244 (43.4%)	2,232,885 (33.1%)	0.21	<0.001
Coronary artery disease (excluding angina)	339 (11.8%)	600,499 (8.9%)	0.10	<0.001
Cardiovascular disease	87 (3.0%)	218,670 (3.2%)	0.01	0.524
Peripheral vascular disease	18 (0.6%)	21,539 (0.3%)	0.04	0.003
Chronic liver disease	226 (7.9%)	230,046 (3.4%)	0.19	<0.001
Alcohol	66 (2.3%)	24,957 (0.4%)	0.17	<0.001
Obesity	53 (1.8%)	38,645 (0.6%)	0.12	<0.001
Hypothyroidism	15 (0.5%)	24,618 (0.4%)	0.02	0.161
Multiple myeloma	0	1,060 (0.0%)	0.02	0.502
Nephrotic syndrome	29 (1.0%)	14,392 (0.2%)	0.10	<0.001
Chronic kidney disease	199 (6.9%)	209,348 (3.1%)	0.18	<0.001
Acute Pancreatitis	177 (6.2%)	19,602 (0.3%)	0.34	<0.001
Gallstone disease	212 (7.4%)	114,035 (1.7%)	0.28	<0.001
Current pregnancy in women	47 (1.6%)	385,120 (5.7%)	0.22	<0.001
Charlson comorbidity index				
Mean (SD)	0.92 ± 1.38	0.44 ± 1.05	0.39	<0.001
Median (IQR)	0.00 (0.00-1.00)	0.00 (0.00-0.00)	0.52	<0.001
0	2,241 (78.1%)	6,104,946 (90.5%)	0.35	<0.001
1	331 (11.5%)	302,652 (4.5%)	0.26	

2	142 (4.9%)	193,468 (2.9%)	0.11	
3+	155 (5.4%)	141,440 (2.1%)	0.17	
Health care utilization				
Primary care (FP/GP) visits				
Mean (SD)	6.87 ± 8.85	5.24 ± 6.45	0.21	<0.001
Median (IQR)	5.00 (2.00-8.00)	4.00 (2.00-7.00)	0.21	<0.001
Endocrinologist visits				
Mean (SD)	0.23 ± 0.93	0.06 ± 0.48	0.22	<0.001
Internal medicine visits				
Mean (SD)	1.03 ± 2.90	0.62 ± 2.36	0.15	<0.001
Laboratory measurements				
HbA1c	1,083 (37.7%)	929,242 (13.8%)	0.57	<0.001
HbA1c >8.5%	397 (13.8%)	100,258 (1.5%)	0.48	<0.001
Mean (SD)	7.88 ± 2.29	6.63 ± 1.37	0.67	<0.001
Median (IQR)	7.30 (6.00-9.40)	6.20 (5.80-7.10)	0.53	<0.001
LDL cholesterol	485 (16.9%)	955,143 (14.2%)	0.08	<0.001
Mean (SD) mmol/L	2.52 ± 1.49	2.71 ± 1.02	0.15	<0.001
Median (IQR) mmol/L	2.31 (1.49-3.26)	2.58 (1.93-3.38)	0.25	<0.001
HDL cholesterol	1,202 (41.9%)	986,111 (14.6%)	0.64	<0.001
Mean (SD) mmol/L	0.97 ± 0.38	1.27 ± 0.38	0.79	<0.001
Median (IQR) mmol/L	0.91 (0.76-1.11)	1.21 (1.00-1.47)	0.93	<0.001
non-HDL cholesterol	327 (11.4%)	18,595 (0.3%)	0.49	<0.001
Mean (SD) mmol/L	5.14 ± 2.27	4.58 ± 1.38	0.30	<0.001
Median (IQR) mmol/L	4.68 (3.75-5.98)	4.52 (3.64-5.40)	0.17	0.001
Total cholesterol	1,206 (42.0%)	994,409 (14.7%)	0.63	<0.001
Mean (SD) mmol/L	6.41 ± 2.75	4.78 ± 1.25	0.76	<0.001
Median (IQR) mmol/L	5.79 (4.71-7.33)	4.66 (3.85-5.57)	0.74	<0.001
ALT	1,282 (44.7%)	1,384,012 (20.5%)	0.53	<0.001
Mean (SD) U/L	40.99 ± 39.83	27.36 ± 29.50	0.39	<0.001
Median (IQR)	31.00 (21.00-46.00)	22.00 (17.00-31.00)	0.58	<0.001
Corrected calcium	210 (7.3%)	166,005 (2.5%)	0.23	<0.001
Mean (SD) mmol/L	2.30 ± 0.15	2.31 ± 0.11	0.12	0.045
Median (IQR) mmol/L	2.29 (2.23-2.37)	2.31 (2.24-2.37)	0.10	0.127

Standardized difference > 10% are considered statistically significant; <1% of income quintiles were missing and were re-coded as ‘3’; Small cells (<6) are suppressed as per ICES privacy policy; Comorbidities were obtained in the 5 years prior to the index date; Health care utilization measures were obtained in the 1 year prior to index date; Health care utilization measures were obtained in the 1 year prior to index date; Laboratory measurements were obtained in the 1 year prior to index prescription date; Evidence of pregnancy in women was obtained in the 1 year prior and within 9 month after to index date. Please refer to supplementary table 1 for further information on coded data included for each variable. * Missing income (<1%) was recoded as '3'. Missing Charlson (~50%) was due to patients having no hospitalizations for relevant

comorbidities found during the 5 year lookback period; they were recoded as '0'; ^P-value for variable as a whole.

Abbreviations: VS-HTG: very severe hypertriglyceridemia (TG>20mmol/L); yrs: years; SD: Standard deviation; IQR: Interquartile range; GP: General Practitioner; HTN: hypertension; CAD: coronary artery disease; PVD: peripheral vascular disease; CVD: cerebrovascular disease; CKD: chronic kidney disease; FP: family practitioner; HbA1c: hemoglobin A1c; LDL: low-density lipoprotein; HDL: high density lipoprotein; ALT: alanine aminotransferase.

Table 3.6:

	Ages 18-30	Ages 31-45	Ages 46-65	Ages 66+	Total
Number of individuals with at least one TG test	761,224	1,709,237	3,071,797	1,498,607	7,040,865
Mean Ontario population between 2010-2015	2,412,041	2,732,915	3,745,940	1,875,874	10,766,770
S-HTG					
n	835	5,387	9,979	1,414	17,615
Incidence in those with at least one TG test (per 100,000)	109.69	315.17	324.86	94.35	250.18
Incidence in Ontario population (per 100,000)	34.62	197.12	266.40	75.38	163.61
VS-HTG					
n	174	1,078	1,481	136	2,869
Incidence in those with at least one TG test (per 100,000)	22.86	63.07	48.21	9.08	40.75
Incidence in Ontario population (per 100,000)	7.21	39.45	39.54	7.25	26.65

Abbreviations: S-HTG: severe hypertriglyceridemia (TG >10-20 mmol/L); VS-HTG: very severe hypertriglyceridemia (TG >20mmol/L); ON: Ontario; pop: population; n: number of individuals;

Table 3.7: Incidence of severe (S-HTG; TG >10-20mmol/L) and very severe (VS-HTG; TG>20mmol/L) hypertriglyceridemia in Ontario by age group and gender

	Ages 18-30	Ages 31-45	Ages 46-65	Ages 66+	Total
		Women			
Number of individuals with at least one TG test	424,465	933,287	1,604,830	817,872	3,780,454
Mean Ontario population between 2010-2015	1,201,563	1,392,309	1,895,645	1,047,061	5,536,578
S-HTG					
n	206	1043	2387	564	4,200
Incidence in those with at least one TG test (per 100,000)	49	112	149	69	111
Incidence in Ontario population (per 100,000)	17	75	126	54	76
VS-HTG					
n	39	211	322	58	630
Incidence in those with at least one TG test (per 100,000)	9.19	22.61	20.06	7.09	16.66
Incidence in Ontario population (per 100,000)	3.25	15.15	16.99	5.54	11.38
		Men			
Number of individuals with at least one TG test	336,759	775,950	1,466,967	680,735	3,260,411
Mean Ontario population between 2010-2015	1,210,478	1,340,606	1,850,295	828,813	5,230,192
S-HTG					
n	629	4,344	7,592	850	13,415
Incidence in those with at least one TG test (per 100,000)	187	560	518	125	411
Incidence in Ontario population (per 100,000)	52	324	410	103	256
VS-HTG					
n	135	867	1,159	78	2,239
Incidence in those with at least one TG test (per 100,000)	40.09	111.73	79.01	11.46	68.67
Incidence in Ontario population (per 100,000)	11.15	64.67	62.64	9.41	42.81

Abbreviations: S-HTG: severe hypertriglyceridemia (TG >10-20 mmol/L); VS-HTG: very severe hypertriglyceridemia (TG >20mmol/L); ON: Ontario; pop: population; n: number of individuals;

Table 3.8: Prescription characteristics of VS-HTG and comparison cohorts

Characteristics in ages 66+	VS-HTG cohor N=136	Comparison cohort N=1,392,795	
Oral glucocorticoid	<6	8,741 (0.6%)	0.01
Statin	55 (40.4%)	636,487 (45.7%)	0.11
Fibrate	25 (18.4%)	22,422 (1.6%)	0.58
Niacin	0 (0.0%)	147 (0.0%)	0.01
Other lipid	16 (11.8%)	57,839 (4.2%)	0.28
Lipid combination (e.g. with blood pressure medication)	0	13,984 (1.0%)	0.14
Metronidazole	<6	15,899 (1.1%)	0.03
Tetracycline	0 (0.0%)	2,287 (0.2%)	0.06
Oral furosemide	22 (16.2%)	87,477 (6.3%)	0.32
Hydrochlorothiazide	32 (23.5%)	315,376 (22.6%)	0.02
Chlorthalidone	<6	8,525 (0.6%)	0.14
Indapamide	<6	47,567 (3.4%)	0.01
Sulphasalazine	0	13 (0.0%)	0
Azathioprine	0	1,912 (0.1%)	0.05
Valproic acid	0	908 (0.1%)	0.04
Sulindac	0	2,665 (0.2%)	0.06
Salicylates	8 (5.9%)	48,599 (3.5%)	0.11
HIV/AIDS specific meds	0	128 (0.0%)	0.01
ACE/ARB	38 (27.9%)	396,107 (28.4%)	0.01
Beta blocker	54 (39.7%)	320,828 (23.0%)	0.37

Standardized difference > 10% are considered statistically significant; <1% of income quintiles were missing and were re-coded as '3'; Small cells (<6) are suppressed as per ICES privacy policy; Medication use were obtained in the 1 year prior to index date; A few other medications of interest (e.g. oral estrogen, pentamidine, 5-ASA, L-asparaginase, didanosine) were not found.

Table 3.9 Predictors of VS-HTG, ranked by odds ratio

	Univariable analysis				Multivariable analysis			
	Odds Ratio	**LCL**	**UCL**	***P*-value**	**Odds Ratio**	**LCL**	**UCL**	***P*-value**
Diabetes	4.85	4.50	5.22	<0.0001	5.38	4.93	5.88	<0.0001
Sex								
Male	4.24	3.88	4.63	<0.0001	3.83	3.50	4.18	<0.0001
Female	REF	REF	REF		REF	REF	REF	
Alcohol	6.34	4.96	8.09	<0.0001	2.47	1.90	3.19	<0.0001
Chronic liver disease	2.42	2.11	2.77	<0.0001	1.71	1.48	1.97	<0.0001
HTN	1.55	1.44	1.67	<0.0001	1.69	1.54	1.86	<0.0001
Obesity	3.27	2.49	4.29	<0.0001	1.49	1.13	1.98	0.0049
CKD	2.33	2.01	2.69	<0.0001	1.39	1.19	1.63	<0.0001
Income quintile								
1 - lowest	1.21	1.09	1.35	0.0005	1.09	0.98	1.22	0.1134
2	1.06	0.95	1.18	0.3011	1.02	0.92	1.14	0.6747
3	REF	REF	REF		REF	REF	REF	
4	0.74	0.66	0.84	<0.0001	0.78	0.70	0.88	<0.0001
5 - highest	0.63	0.55	0.71	<0.0001	0.72	0.63	0.81	<0.0001
Age (per decade)	0.84	0.82	0.86	<0.0001	0.64	0.62	0.66	<0.0001
Charlson comorbidity index*								
0	REF	REF	REF		REF	REF	REF	
1	2.98	2.66	3.34	<0.0001	1.61	1.42	1.82	<0.0001
2	2.00	1.69	2.37	<0.0001	1.26	1.05	1.50	0.0137
3+	2.99	2.54	3.51	<0.0001	1.15	0.95	1.38	0.1504

Sample size is the full cohort (N= 6,745,375); Exposed (n= 2,869); Unexposed (n= 6,742,506)All predictors were associated with the odds of having hypertriglyceridemia. Diabetes includes both type 1 and type 2.

Abbreviations: LCL: lower confidence limit; UCL: upper confidence limit; HTN: hypertension; CKD: chronic kidney disease; REF: used as reference

* Missing income (<1%) was recoded as '3'. Missing charlson (~50%) was due to patients having no hospitalizations for relevant comorbidities found during the 5 year lookback period; they were recoded as '0'

Table 3.10: Healthcare Patterns and Events of Interest in patients with VS-HTG

	Within 1 year	**Within 2 years**
Healthcare patterns (N= 2,869)		
General practitioner visit	2,786 (97.1%)	2,822 (98.4%)
Endocrinologist visit	775 (27.0%)	941 (32.8%)
Internist visit*	1,279 (44.6%)	1,628 (56.7%)
TG test	2,218 (77.3%)	2,561 (89.3%)
Last TG value		
Mean (SD) (mmol/L)	7.66 ± 8.29	7.68 ± 8.35
Median (IQR) (mmol/L)	4.7 (2.7-9.1)	4.7 (2.7-9.1)
Lowest TG value		
Mean (SD) (mmol/L)	6.09 ± 6.74	5.30 ± 6.05
Median (IQR) (mmol/L)	3.8 (2.2-7.1)	3.3 (2.0-5.9)
New prescriptions (restricted to ages 66+, N=136)		
Statin	35 (25.7%)	44 (32.4%)
Fibrate	25 (18.4%)	27 (19.9%)
Niacin	0	0
Events	Within 1 year	Within 2 years
At least one hospital encounter for pancreatitis	118 (4.1%)	171 (6.0%)
At least one hospital encounter for acute myocardial infarction	22 (0.8%)	39 (1.4%)
At least one hospital encounter for Ischemic stroke	12 (0.4%)	24 (0.8%)

***** *For the purposes of this data 'internal medicine' refers specifically to the subspecialty of general internal medicine and does not include other internal medicine subspecialties.*

3.3.4 Discussion:

The incidence of TG >10-20 (S-HTG) and >20 mmol/L (VS-HTG) between 2010 and 2015 was approximately 1 in 400 (0.25%) and 1 in 2500 (0.04%) among adults in Ontario with measured TG, and 1 in 613 (0.16%) and 1 in 3750 (0.27%) of the adult population, respectively. These numbers align with prevalence rates reported in other studies (68, 70, 71). Peak age of incidence was 31-65 years, which may correspond to the age at which a screening lipid profile may first be conducted, or the age at which related chronic conditions start to manifest. While the Endocrine Society defines severe hypertriglyceridemia as a serum TG level >1000 mg/dL (11.3 mmol/L) and very severe as >3000 mg/dL (22.6 mmol/L) (81), the approximations of 10 and 20 mmol/L, respectively, are more practical for a Canadian population.

Significant predictors of VS-HTG in Ontario included male sex, as well as known risk factors for HTG, including diabetes, chronic liver disease, alcohol abuse, obesity and chronic kidney disease. Hypertension was likely a predictor given its association with metabolic syndrome. The two most significant controllable predictors were diabetes and alcohol abuse. The definition used for alcohol abuse in this study was broad to allow for increased sensitivity, but included any complication that stemmed from chronic or acute alcohol ingestion (see Appendix D). It is thus difficult to determine which specific alcohol-related behaviors or patterns of intake may be most contributory.

The VS-HTG cohort had more contacts with healthcare providers, which may relate to more associated chronic diseases. This may have predisposed individuals to having had a screening lipid test. Patients in the VS-HTG group were also more likely to have had HbA1c testing, and to have an HbA1c >8.5%, likely relating to the higher prevalence of metabolic syndrome and diabetes in the VS-HTG cohort. Mean LDL-C was minimally but significantly higher in the VS-HTG. Mean HDL-C was significantly lower in the VS-HTG cohort, reflecting the inverse relationship between TG and HDL-C. Non-HDL and total cholesterol were higher in the VS-HTG cohort, likely driven by elevated TG-rich lipoproteins and remnant particles.

While other studies showed poor follow up care following identification of HTG, in this Ontario cohort, no significant gaps in appropriate care were identified. This may be due to universal healthcare access in Ontario, which was not a feature of other populations studied (70). Within the 2 year follow-up period, 98.4% of individuals in the VS-HTG cohort were seen by a FP and 89.3% had a repeat TG test. One-third were seen by an endocrinologist and over half by an internist. Follow-up TG tests showed significant reductions in TG levels, with the median TG level falling below the threshold for pancreatitis risk (4.7 mmol/L; IQR 2.7-9.1). The median of the lowest recorded TG values remained elevated at 3.3 mmol/L (IQR 2.0-5.9) (normal <1.7 mmol/L), likely reflecting the limitations of currently available pharmacological and lifestyle interventions to fully correct TG to a normal range, particularly in those with underlying inherited metabolic defects. Discussions of current management recommendations for HTG and options are beyond the scope of this work, but are discussed in detail elsewhere (67). In the subset of the VS-HTG cohort that were over the age of 65, most were on, or were placed on, a statin (40.4% at baseline, 32.4% with new prescriptions following identification of VS-HTG) and a third were on a fibrate (18.4% at baseline, 19.9% with new prescriptions). The substantial reduction in TG levels, despite only a third of patients with medication information available being placed on a fibrate, suggests that control of contributory secondary factors, such as diabetes, obesity or lifestyle may have played a considerable role in the improvements in TG levels.

Pancreatitis rates in individuals with VS-HTG (4.1% within 1 year, 6% within 2 years) were within the range of expectation based on other reports (82). There was no apparent excess risk of ASCVD within the VS-HTG cohort at baseline or within the year follow up period. However, this study was not designed to examine these outcomes. A separate study focusing on the risk of ASCVD and ischemic stroke in the VS-HTG population that accounts for potential confounders may provide further support for the findings observed.

Future studies that evaluate TG as a continuous variable would help define the risk of acute pancreatitis associated with a given degree of HTG. Further investigations that focus on outcomes in VS-HTG patients is another avenue of investigation, including prevalence and

predictors of pancreatitis and its complications. Genetic investigation into a subset of this cohort may also help define the spectrum of genetic variation that may underlie a presentation of VS-HTG.

3.3.4.1 Comparison with other provinces in Canada and European Union (EU) countries:

All Canadian provinces have universal healthcare coverage, however there are differences across the Canadian provinces in terms of risk factors for VS-HTG (e.g. obesity and type 2 diabetes). For example, provinces such as Saskatchewan, New Brunswick, Nova Scotia and Newfoundland and Labrador have higher rates of obesity (35-38%) compared to Ontario (26%), which is just below the national average of 27% (range of all provinces 22-38%) (28). Approximately 30% of Ontarians live with diabetes or prediabetes, which is slightly higher than the national average of 29% (range of all province 25 - 35%) (29). There is also ethnic variation between the provinces, with Ontario and British Columbia having the highest rates of ethnic diversity (29.3 and 30.3% of adult population identifying as visible minorities, respectively, national average 22.3%, range for all provinces 2.3%-30.3%) (30).

Most EU countries are similar to Ontario in that they have universal access to healthcare, but may differ in other ways that could affect prevalence and predictors of VS-HTG. Obesity rates are overall lower in the EU compared to Ontario (17%; range 10.7-30.6%) (31), as are rates of type 2 diabetes (~10%) (32). There may also be differences in ethnic diversity.

3.3.4.2 Study strengths and limitations:

Strengths of this study include access to province-wide laboratory data, the large study cohort with access to universal health services and the use of well-defined coding algorithms used to investigate patient characteristics. It is also, to our knowledge, the first study to systematically assess incidence and characteristics associated with HTG in a Canadian population.

While this study provides useful province-wide information on incidence of S- and VS-HTG, there are some limitations. Prevalence was not examined and information was only gathered over a 5-year time frame with the study collection period ending in 2015, limiting capture of more recent trends. Furthermore, not all laboratory data were available for all individuals (e.g. HbA1c), potentially creating bias. Additionally, not all laboratories submitted data to OLIS simultaneously and data may be less complete for earlier time points (76). Inclusion in the VS-HTG or S-HTG cohort were also based upon a single TG measurement, which could have allowed for inclusion of individuals in these cohorts who had only transiently elevated TG levels. However, TG levels in this range, even transiently can expose an individual to the risk of pancreatitis. Furthermore, literature supports day-to-day variability in TG of approximately 20% (83), therefore even accounting for maximal variability, TG levels are likely to remain elevated on subsequent tests. Information on pharmacological treatment was limited to a subset of the VS-HTG cohort, limiting the usefulness and generalizability of this data. The use of over the counter supplements could not be captured with our data sources. Certain susceptibility states, such as obesity and alcohol abuse, may be underestimated in the administrative data due to low coding sensitivity, which may have underestimated their contribution to HTG. Individuals with poor access to healthcare may be under-represented in the study sample as they would be less likely to obtain a lipid profile. Similarly, it is possible that including these patients with undetected VS-HTG may have resulted in lower follow-up rates than seen in this VS-HTG cohort. Additionally, data regarding visits to some specialists that may be involved in the management of VS-HTG, such as cardiologists or gastroenterologists, was not captured. While it was concluded that there was no significant care gap, it could be argued that specialist referral would be most appropriate for anyone with a history of VS-HTG, given it is a rare condition with potentially serious side effects, suggesting that there may still be a gap in appropriate care within Ontario. There were overall low numbers in the VS-HTG cohort, but only 10 predictors were chosen and there should have had sufficient statistical power. Additionally, the baseline rates of pancreatitis and use of fibrates were high in those with VS-HTG, suggesting that these may not have all been incident cases. Finally, given the

observational nature of the data set, causality cannot be determined, and the findings may not be generalizable outside Ontario.

3.3.5 Conclusions:

In conclusion, this study shows that ~1/400 adults in Ontario have S-HTG and ~1/2500 had new evidence of VS-HTG from 2010-2015. Peak incidence occurs between the ages of 31 and 65 years. Conditions that are most strongly associated with VS-HTG include diabetes, male sex, alcohol, chronic liver disease, hypertension, obesity and chronic kidney disease. No significant care gap was identified for individuals in Ontario with identified VS-HTG and the majority had repeat TG below the threshold for pancreatitis risk.

These findings may assist clinicians in recognizing individuals at heightened risk for VS-HTG, who may benefit from increased surveillance. Male patients with diabetes, obesity and alcohol abuse are at the highest risk; early attention to these patients may assist in developing an individualized treatment plan to monitor for HTG and prevent associated adverse outcomes.

3.4 Conservative management in hypertriglyceridemia-associated pancreatitis

The work presented in Chapter 3.4 has been edited from this original manuscript for brevity and consistency throughout this dissertation.

Berberich AJ, Ziada A, Zou GY, Hegele RA. Conservative management in hypertriglyceridemia-associated pancreatitis. J Intern Med. 2019 Dec;286(6):644-650. doi: 10.1111/joim.12925. Epub 2019 Jun 6. PMID: 31077464.

3.4.1 Background:

Acute pancreatitis can result from severe elevations in TG in both monogenic and polygenic forms of hypertriglyceridemia. Despite absence of high-quality evidence, acute pancreatitis with severe HTG (TG >20 mmol/L) has been listed as a category 3 indication for plasmapheresis (84). However, plasmapheresis risks include those associated with central line placement, potential allergic or transfusion reactions, bleeding and possible infection. Plasmapheresis is also costly, requires specialized staff and infrastructure, and only temporarily lowers TG levels without addressing underlying causes (85). We followed TG levels in patients with HTG-associated pancreatitis managed conservatively with discontinuation of oral intake and without plasmapheresis in order to determine the safety and effectiveness of this approach.

3.4.2 Methods

We conducted an observational, retrospective review of the medical records for patients with HTG-associated pancreatitis admitted to our institution between 2002 and 2018, approved by the University of Western Ontario Ethics Review Board (#07920E) (Appendix B). A diagnosis of pancreatitis was based on at least two of: 1) medical history and clinical examination confirming the presence of abdominal pain; 2) serum lipase elevations greater than three times the upper limit of normal; and 3) CT abdominal imaging studies consistent with pancreatitis.

Demographic and clinical data were extracted from retrospective chart review, including identified secondary factors for HTG such as alcohol intake, diabetes, related medications and elevated body-mass index (BMI), history of past pancreatitis or dyslipidemia, treatment with cessation of oral intake (NPO) and/or insulin acutely and TG levels measured over the course of hospital admission (Table 3.11, Figure 3.3). All patients were managed with supportive measures, including withholding of all oral intake (NPO), intravenous fluid replacement, pain management, frequent monitoring of TG and pancreatic lipase levels and surveillance for complications such as pancreatic necrosis and pseudocyst. Insulin infusion

(INS IV) was used in 12 patients for the purpose of treating hyperglycemia; heparin was not used in any patient. Duration of NPO status and implementation of other supportive measures was determined at the discretion of the treating physician.

All statistical analyses were performed using Stata Statistical Software: Release 15 (StataCorp LLC, College Station, TX). Data were analyzed using a mixed effects model approach to account for repeated measurements and inconsistent time points. Any TG value above the detection limit of the laboratory assay was assigned the maximum measured value of 62.2 mmol/L. Model selection was done by a backward procedure. Specifically, a model with a quadratic effect of time and interactions with treatment modality (NPO versus INS IV/NPO) was first considered, followed by a model omitting the interaction terms if p-values > 0.05, and finally by a model with linear effect of time if the p-value for the quadratic term > 0.05. Normality of residues for the final model was assessed. The Kenward-Rogers method was applied to adjust degree-of-freedom in the analyses. The mixed statistical model procedure permitted use of all TG values from all time points in all patients to estimate the mean absolute value and mean percentage decline at 48 hours post-admission. We also estimated the half-life for TG in this conservatively managed cohort.

3.4.3 Results

Our sample included 20 patients who sustained 22 separate episodes of HTG-associated pancreatitis. Mean patient age was 37 (range 22 to 60 years) and 13 patients (65%) were male. All but two cases had at least one identified secondary cause for HTG, with 9 having more than one identified secondary factor (Table 3.11). Nine individuals had type 2 diabetes, two had type 1 diabetes; 9 had a history of chronic alcohol misuse; 10 had obesity (defined as BMI > 30 kg/m^2); two were taking oral contraceptive pills and one was taking glucocorticoids. Eight individuals had a history of recurrent pancreatitis. Seven patients had severe pancreatitis, defined as requiring admission to the intensive care unit. One patient died as a result of complications from severe necrotizing pancreatitis, despite his TG

levels decreasing by 87%, from 31.4 to 4.10 mmol/L, within 49 hours. Two additional patients developed pancreatic necrosis and abscess formation. One patient developed a pseudocyst, which did not require drainage and one patient developed a splenic vein thrombosis.

Among 14 individuals who consented to genetic testing, we found similar complex genetic influences as we observed previously in other severe HTG cohorts (86): 0/14 had bi-allelic large-effect mutations in *LPL* or related genes (87) (see chapter 3.2) while 2/14 had heterozygous mutations in these genes (*APOA5* p.G185C and *LPL* p.G300R) and 7/14 had a polygenic risk score (PRS) for HTG in the top 75th percentile or higher (Table 3.12). An additional 2/14 had PRS scores at the 65th percentile, suggesting a possible contribution to the HTG phenotype. A high PRS for HTG is the most common genetic profile seen in adults with TG >10 mmol/L (88).

The mean baseline TG level was 45.4 mmol/L (range 11.2 to 91.1 mmol/L). This mean value is certainly an underestimate, since four patients had TG levels exceeding the laboratory's limit of detection for TG. Nonetheless, we took the value of this upper detection limit (i.e. 62.2 mmol/L) as the baseline TG measurement for these four patients. Because there was no standardized protocol for blood sampling, we tabulated the TG value obtained closest to the 48 hour mark from admission (Table 3.11).

Individual plasma TG profiles during admission are shown in Figure 3.3. The mixed effects statistical model determined that mean TG level at 48 hours was 13.3 mmol/L, which represented a mean decrease of 67.8%. Regression analysis using the mixed effects model was used to generate a predicted rate of TG decline based on all available data. The final model of fixed effects that fits the data is given by the equation: $TG = 43.0097 – 0.8367\ hours + 0.0044\ hours^2$. Using this equation, the predicted estimated TG half-life was 30.6 hours in patients managed supportively, with predicted TG reductions of 40.8%, 69.8% and 87.0% at 24, 48 and 72 hours respectively (Figure 3.4).

To account for a possible confounding effect of insulin infusion on the decline in TG levels, data were also analyzed separately for the supportively managed patients only ("NPO"; N=10) and those who concurrently received insulin infusion for management of their hyperglycemia ("NPO/INS IV"; N=12). Results for these sub-groups were similar to the overall group; treatment modality was not associated with rate of TG decline. The average baseline TG level in the NPO group was 45.7 mmol/L (range 13.8 - 87.9 mmol/L). At 48 hours, this had decreased to 13.1 mmol/L (range 1.75 - 28.0 mmol/L), which represented a mean decrease of 71.4%. For the NPO/INS IV group, the average baseline TG level was 40.9 mmol/L (range 11.2 - 91.1 mmol/L), which declined to 13.5 mmol/L (range 1.03 - 28.7 mmol/L) at 48 hours, which represented a mean decrease of 67.8%. These values did not differ between groups.

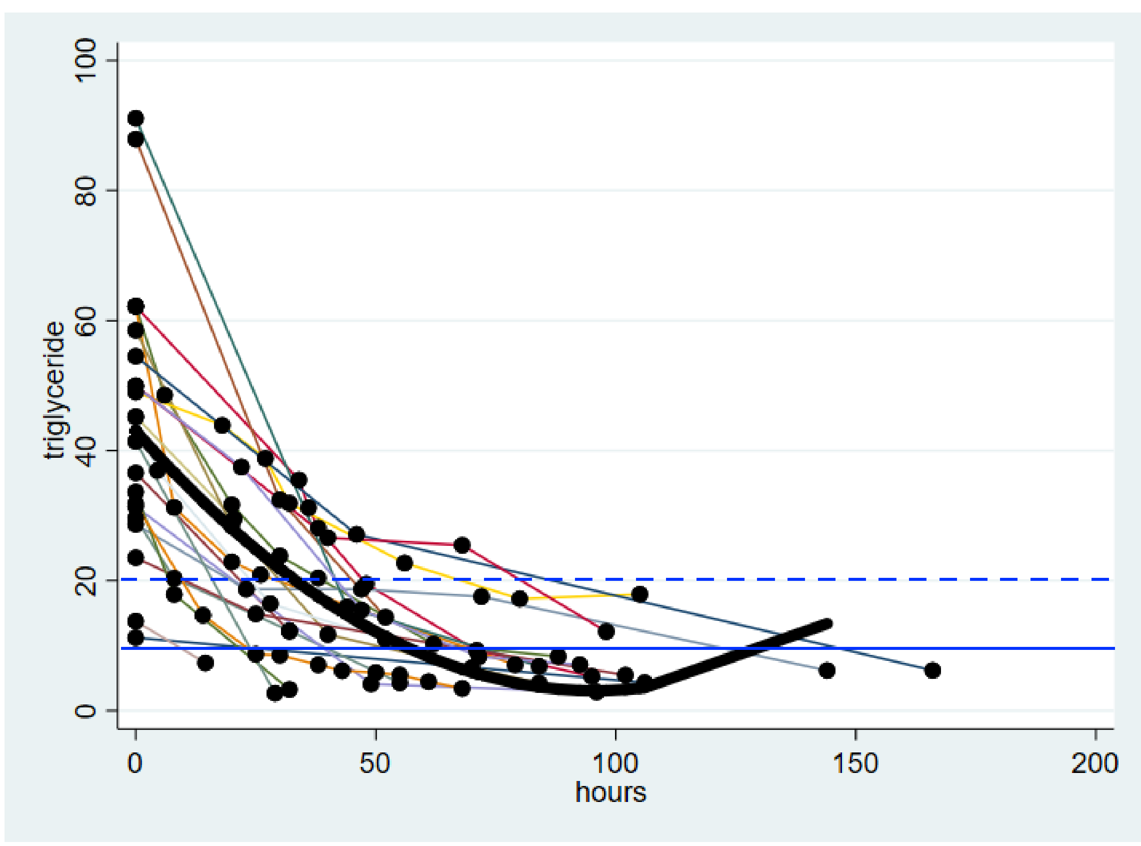

Figure 3.3: Predicted individual trajectories of triglyceride levels (in mmol/L) over time for patients presenting with severe hypertriglyceridemia and pancreatitis.

Dark black line shows mean trend curve incorporating all available data. Dotted blue line indicates highest risk threshold for pancreatitis; solid blue line indicates triglyceride threshold below which there is minimal risk of pancreatitis.

Table 3.11: Demographic and clinical data on admission of patients with severe HTG and pancreatitis

	Sex	Age (years)	Initial TG (mmol/L)	Follow-up TG (mmol/L)/ elapsed time (hours)	% decrease at 48 hours‡	DM	EtOH	Obesity	Peak serum lipase	Other Risk Factors	Recurrent Pancreatitis	ICU	Complications:
NPO1	M	27	36.6	12.2 / 32	80.5%	N	Y	N	1492	-	Y	N	-
NPO2	M	40	>62.2	20.4 / 38	66.9%	N	Y	Y	1702	-	N	Y	-
NPO3	M	45	>62.2	23.8 / 30	68.4%	N	Y	N	2407	-	Y	N	-
NPO4	F	26	29.6	4.28 / 55	79.5%	N	N	N	771	OCP, GC	Y	N	-
NPO5	M	39	45.3	29.5 / 20.5	63.5%	N	Y	Y	2015	-	N	N	-
NPO6	M	46	87.9	14.4 / 52	81.4%	N	Y	N	2558	-	N	N	-
NPO7	F	38	58.5	11.7 / 40	77.4%	N	N	N	2186	-	N	N	-
NPO8	F	26	13.8	7.35 / 14.5	87.3%	N	Y	Y	>600	-	N	N	-
NPO9	F	38	45.2	16.5 / 28	72.6%	Y	N	Y	232	-	N	N	-
NPO10	M	50	54.5	27.1 /46	46.4%	Y	Y	Y	265	-	N	N	Pseudocyst~
NPO /INS IV1	M	49	11.2	4.31 /106	35.1%	Y	N	N	492	-	Y	Y	-
NPO/ INS IV2	M	39	>62.2	19.5 / 48	73.9%	Y	N	N	>600	-	Y	N	-
NPO/ INS IV3	M	36	31.4	4.10 / 49	86.9%	Y	N	Y	14447	-	N	Y	Necrosis/ Death
NPO/ INS IV4	F	43	28.7	18.7 / 47	38.1%	Y (1)	N	N	N/A	-	Y	Y	-
NPO/ INS IV5	F	38	91.1	15.9 / 44	77.4%	Y (1)	N	N	1039	-	Y	Y	-
NPO/ INS IV6	M	52	49.0	31.9 /3)	41.0%	N	N	N/A	196	-	N	N	Necrosis/ Abscess

NPO/ INS IV7	F	27	23.5	14.9 / 25	48.9%	Y	N	Y	491	-	N	Y	Splenic vein thrombosis
NPO/ INS IV8	M	22	33.6	3.26 / 32	96.8%	Y	N	Y	218	-	Y	N	-
NPO/ INS iV9	M	23	31.8	6.15 / 43	75.1%	Y	N/A	Y	>599	-	Y	N	-
NPO/ INS IV10	M	60	41.4	2.68 / 29	98.9%	N	Y	Y	290†	-	N	N	-
NPO/ INS IV11	F	43	50.0	26.5 / 40	46.4%	Y	N	N	>599	OCP	Y	N	-
NPO/ INS IV12	M	26	>49.9	15.58 / 47	73.3%	Y	Y	Y	>599		N	Y	Necrosis/ Abscess

Abbreviations: TG: serum triglycerides (mmol/L), DM: diabetes mellitus; (1) type 1; EtOH: alcohol intake> 3/day; ICU: Required ICU admission; NPO: managed by withholding oral intake only; NPO/INS IV: managed by withholding oral intake in addition to insulin infusion; M: Male; F: Female; Y: Yes; N: No; OCP: Oral contraceptive pill; GC: glucocorticoids; ~ no drainage required, *died,†pancreatic amylase; ‡When actual measurement of 48 hour triglycerides were unavailable, percentage decrease was based on projected 48 hour triglycerides calculated using a line of best fit for individual triglyceride trajectory. NPO/INS IV4 and NPO/INSIV5 were the same individual; NPO/INS IV8 and NPO/INS IV9 were the same individual.

Table 3:12 Contributing genetic profiles in HTG pancreatitis patients

	Monogenic mutation	Polygenic risk score	Percentile	Phenotype contribution*
NPO2	APOA5 G185C	16/28	82nd	Likely
NPO3	None	15/28	65th	Possible
NPO4	None	14/28	50th	Not Identified
NPO5	None	18/28	99th	Likely
NPO6	None	13/28	35th	Not Identified
NPO7	LPL G300R	11/28	5th	Likely
NPO10	None	15/28	65th	Possible
NPO/INS IV1	None	18/28	99th	Likely
NPO/INS IV2	None	16/28	82nd	Likely
NPO/INS IV4/5	None	19/28	$>99^{th}$	Likely
NPO/INS IV6	None	19/28	$>99^{th}$	Likely
NPO/INS IV7	None	18/28	99th	Likely
NPO/INS IV10	None	13/28	35th	Not Identified
NPO/INS IV12	None	11/28	5th	Not Identified

All monogenic mutations are heterozygous unless otherwise stated; ~unlikely causal mutation but possibly contributory; `predicted benign; *Phenotype contribution of genetic findings was considered likely if there was a) presence of homozygous rare, predicted or known pathogenic mutations in a gene known to be associated with hypertriglyceridemia; b) a polygenic risk score $>75^{th}$ percentile or c) presence of a single, rare, predicted or known pathogenic mutations in a gene known to be associated with hypertriglyceridemia AND a polygenic risk score greater than or equal to the 65th percentile; phenotype contribution was considered possible if 1) there was there is a polygenic risk score greater than or equal to the 65th percentile.

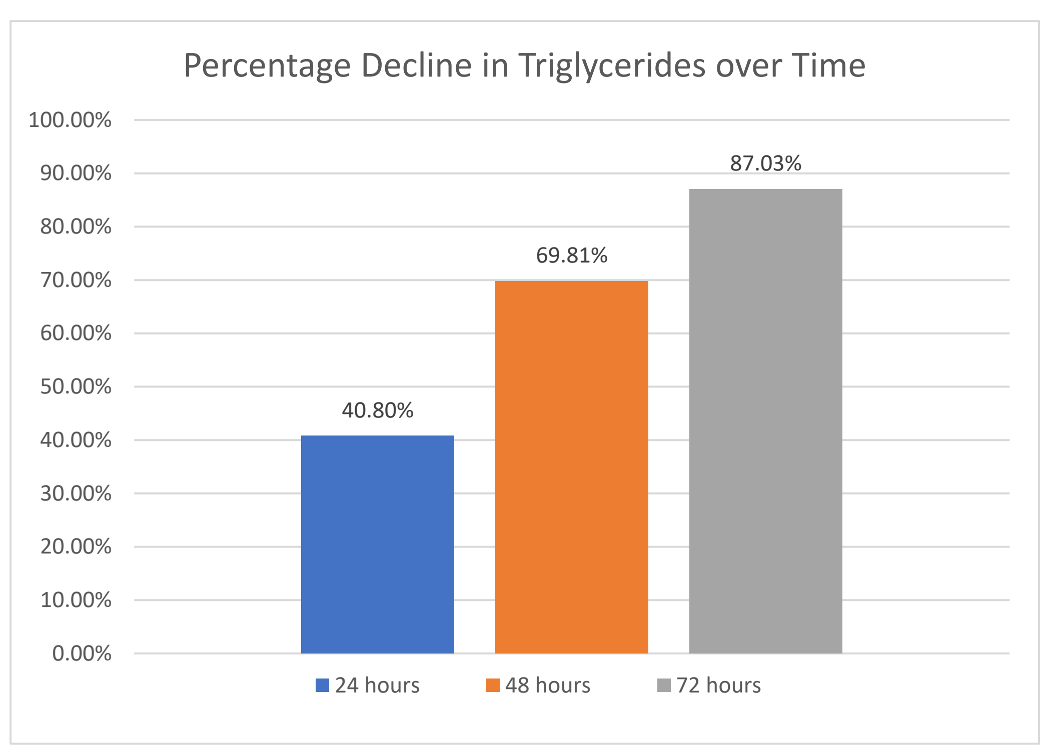

Figure 3.4 Predicted decrease in triglycerides at 24, 48 and 72 hours in patients managed supportively

Predicted percentages of triglyceride decline were determined using the equation derived from regression analysis of available data. These compare favourably with reports of triglyceride decline from a single plasmapheresis session (49-80%) (85, 89-102).

3.4.4 Discussion

This case series highlights the natural trajectory of serum TG in patients managed conservatively with fasting, hydration, pain management and alleviation of underlying contributing factors. The 22 episodes of HTG-associated pancreatitis in our series had a calculated fall in serum TG of 69.8% by 48 hours. This rate of decline is similar to those reported in plasmapheresis case series, which demonstrated 49-80% reductions in serum TG after a single session (85, 89-102). These findings are also consistent with other observational reports that showed no difference in the rate of TG decline between patients managed with or without plasmapheresis (103). Furthermore, our observed plasma TG half-life of 30.6 hours is an expected physiologic response to the elimination of oral fat in patients who do not have complete LPL deficiency. By removing the metabolic source, i.e. dietary fat or alcohol, the persisting chylomicrons can be cleared through residual LPL activity.

There has been no randomized, controlled head-to-head comparison of plasmapheresis versus supportive measures alone in severe HTG evaluating clinical outcomes, or comparing TG trajectories. One study compared use of plasmapheresis in 10 individuals versus conservative management in 19 retrospective controls and showed no differences in morbidity and mortality (104). Another retrospective study that evaluated 30 patients with HTG-associated pancreatitis, of whom 10 and 20 were managed, respectively, with plasmapheresis and conservatively, found no differences in either TG trajectory or clinical outcomes (103). However, there is no evidence for incremental short or long term clinical benefit with plasmapheresis.

Interestingly, the 8 patients with positive genetic findings were found to have polygenic susceptibility rather than monogenic HTG. 90% of patients also had secondary factors. Thus, supporting the assertion that polygenic susceptibility plus non-genetic stressors is required to express the severe HTG phenotype in most affected adults. Proposed mechanisms underlying HTG-associated pancreatitis include obstruction of the local capillary bed by circulating chylomicrons, leading to ischemia (85, 89, 93, 99, 105, 106).

Further, pancreatic lipase that mislocalizes to plasma might partially hydrolyze TG in chylomicrons to free fatty acids, leading to cytotoxic injury and release of inflammatory cytokines (85, 89, 93, 99, 105, 106).

Prophylaxis of future pancreatitis episodes requires managing secondary factors (see chapter 3.3), including eliminating alcohol, attaining good glycemic control, weight reduction, improved physical activity, dietary reduction of simple sugars and high fat foods, and review of any contributing medications. Pharmacotherapies include fibrates, high dose omega-3 fatty acids and possibly niacin (5). Experimental biological agents, such as volanesorsen (Akcea Therapeutics) an antisense oligonucleotide inhibitor of apolipoprotein C-III (APOC3) or evinacumab (Regeneron Therapeutics), a monoclonal antibody against angiopoietin-like 3 (ANGPTL3), may also find future utility in patients with recurrent HTG-associated pancreatitis (107-110).

Our report has some limitations. First, we could not control for all possible confounding factors. Data were obtained from chart review and not all relevant information was available for all patients included in the series, including TG values at all time points. Additionally, we had no corresponding plasmapheresis-treated group to provide direct comparison. Nonetheless, we show that supportive measures alone excluding plasmapheresis are effective in a relatively large cohort of patients with HTG-associated pancreatitis.

3.4.5 Conclusion:

Plasmapheresis has been suggested as a consideration for the management of HTG-associated pancreatitis, despite the lack of well-designed trials confirming its benefit. While plasmapheresis may hasten the TG decline, this was not observed in a recent comparison series (103). Furthermore, TG levels fall rapidly without any intervention other than supportive management and withholding oral intake. Finally, there is no evidence that a more rapid decline in TG levels, or that plasmapheresis itself, results in superior clinical outcomes in HTG-associated pancreatitis. Given the lack of evidence to suggest benefit of

plasmapheresis, we feel there is insufficient justification for plasmapheresis in the treatment of HTG-associated pancreatitis, except perhaps during pregnancy (111) . We suggest that supportive management is relatively easy, safe and effective, and further that outcomes appear no worse compared to literature references of patients who undergo plasmapheresis for severe HTG-associated pancreatitis.

3.5 Chapter Conclusions:

The findings of these three studies collectively will help inform the clinical evaluation, assessment, counselling and management of individuals who present with severe hypertriglyceridemia.

For those who present young, with a strong family history, low BMI and a severe phenotype, FCS may be suspected. The study in chapter 3.2 suggests that there is no clear way to distinguish the molecular etiologies of FCS based on clinical presentation, highlighting the importance of genetic confirmation in suspected FCS cases in order to establish a clear diagnosis.

For those with a polygenic pattern of inheritance, risk of expression was most strongly associated with the modifiable risk factors of diabetes, obesity and alcohol abuse, suggesting that these may be important to address when counselling individuals with hypertriglyceridemia.

Furthermore, of those who present with hypertriglyceridemia of either monogenic or polygenic etiology who present with pancreatitis, conservative management seems safe and appropriate for most individuals.

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Chapter 4: Assessing the personal impact of genetic testing results on individuals with hypercholesterolemia

4.1 Overview:

An important consideration when attempting to establish the role of genetic testing in the clinical setting is to evaluate the indirect effects it may have on the individuals tested.

While there can be many potential benefits to establishing a genetic diagnosis, as seen in chapter 2 above, there is also the potential for unintended harm. Benefits to patients could include the ability to receive personalized care, allow for early intervention, for more informed decision-making, as well as provide the opportunity for screening at-risk family members. A genetic diagnosis may also alleviate a sense of responsibility for a certain health condition, or motivate an individual to attain a healthy lifestyle. However, there can also be valid concerns about the potential negative impacts. Some individuals may be concerned about the possibility of genetic discrimination from insurance agencies or employers, a genetic diagnosis may also cause undue worry about health and long-term risk, or may lead to guilt about passing the condition to offspring.

One of the most common inherited endocrine disorders to be confirmed genetically is familial hypercholesterolemia (FH) (see chapter 1.8 for additional background). The heterozygous form (HeFH) affects 1 in ~250 individuals, and establishing a genetic diagnosis is becoming more common as a clinical tool. Genetic screening for FH is currently recommended by many clinical guideline committees when it is suspected clinically. Establishing the impact of this testing on patients may help to better guide the use of genetic testing in the clinic and improve counselling around testing by increasing awareness of the potential benefits or harms.

4.2 Patient impact of genetic testing for familial hypercholesterolemia

4.2.1 Background:

Heterozygous familial hypercholesterolemia (HeFH) can have significant health implications, with patients harbouring the mutation at greatly increased risk of premature ASCVD and death (1-3). Compared to patients with a similar LDL-C level, patients with a genetic diagnosis of FH have a significantly greater risk and often warrant earlier treatment and closer monitoring to prevent these possible complications (4, 5). Early intervention with effective medications, such as statins, has been shown to greatly attenuate the risk of cardiovascular events in these individuals, highlighting the need to identify these patients and begin treatment as early as possible (5).

Genetic testing for familial hypercholesterolemia (FH) is becoming more common as a clinical tool and is currently used to help guide eligibility for funding for certain medications, such as PCSK9 inhibitors. Clinicians may find the genetic diagnosis of HeFH helpful in recommending treatments and management strategies, selecting the most suitable pharmacological agent and providing a better estimate of prognosis (see chapter 1.8.7). The impact of this diagnosis on the patients themselves is less certain.

Results of studies addressing this issue in the past have been mixed, with some studies suggesting a genetic diagnosis had very little impact on patient behavior or sense of health burden, but perhaps contributed to a decreased sense of responsibility for their health concerns (6, 7), and others suggesting improved medication compliance in those with a genetic diagnosis (8) (see chapter 1.8.8.3). This study aims to address the question of whether a genetically confirmed diagnosis of HeFH, or high polygenic risk for hypercholesterolemia (PHC), rather than just elevated cholesterol, changes perceptions of health, motivation to achieve a healthy lifestyle or adhere to prescribed medications, or perception of disease burden and level of anxiety.

This was done using a survey-based method of assessing these outcomes in patients who have been diagnosed with HeFH based on a genetic test positive for a pathologic mutation, compared to those with high polygenic risk for hypercholesterolemia based on a 10-SNP score (polygenic hypercholesterolemia (PHC)), and compared to responses from patients with high levels of LDL-C ($\geq$5.0mmol/L) who underwent genetic testing but were not found to have a pathogenic mutation (mutation negative (Mut Neg)).

It is hoped that this information may help to better guide the use of genetic testing in the clinic by increasing awareness of the potential patient benefits or harms. Additionally, this study aims to identify any potential areas of concern to address with individuals during pre-test and post-test counselling sessions.

4.2.2 Methods:

4.2.2.1 Study subjects:

Study subjects were recruited from general endocrine and lipid specialty clinics. Eligible individuals were greater than 18 years of age, found to have an off-treatment LDL-C above 4.9 mmol/L and subsequently underwent genetic testing to look for a genetic cause for their elevated cholesterol. Results must have been disclosed to the individual at least one year prior to the date of screening for inclusion eligibility. Those unable to provide consent or unable to complete English-language surveys were excluded. Attempts were made to contact all patients attending the specialty lipid clinic at our center who met eligibility criteria for study inclusion through in-person, email or telephone recruitment, to assess for willingness to participate. All interested individuals who provided informed consent were invited to complete the surveys. The protocol was approved by the University of Western Ontario Ethics Review Board (#113228) (<u>Appendix B</u>).

4.2.2.2 Comparison Groups:

Participating individuals were divided into three groups for analysis, and compared independently for each aim.

Group 1 Familial hypercholesterolemia (HeFH): This group consists of individuals found to be positive for a pathogenic heterozygous mutation in one of the canonical HeFH genes: *LDLR, ApoB* or *PCSK9.* They may or may not have additional high polygenic risk.

Group 2 Polygenic hypercholesterolemia (PHC): This group consists of individuals ascertained to be at high polygenic risk, defined as a 10-SNP polygenic risk score that falls above the 75th percentile for the general population.

Group 3 Mutation negative hypercholesterolemia (Mut Neg): This group consists of individuals with untreated LDL-C levels 5.0 mmol/L or above who are not found to have a genetic cause for their hypercholesterolemia.

4.2.2.3 Polygenic Risk Score:

For determination of polygenic hypercholesterolemia, a set of 10 genetic markers (SNPs) most strongly associated with raising plasma LDL cholesterol were used to generate a weighted and unweighted polygenic risk score (PRS). The 75th percentile for the unweighted PRS corresponds to an unweighted PRS of 14/20 risk alleles present. A weighted score for each SNP is generated by multiplying the number of alleles associated with the risk trait levels at each SNP locus (0, 1, or 2) by the reported effect size of the risk allele (beta coefficient) (see chapter 1.4.6). The weighted scores for each SNP locus are then totaled to generate the overall weighted PRS for an individual. Higher scores indicate that individuals carry a greater number of risk alleles, with presumed additive effect on raising cholesterol levels, while lower scores indicate that individuals carry fewer risk alleles. Included SNPs and beta coefficients for weighted scores are listed in Table 1.12.

4.2.2.4 Chart Review:

Chart review was conducted on all consenting patients who agreed to participate in the study and completed the surveys. Extracted data included information on personal demographics, genetic statis and results of genetic testing, relevant laboratory tests, physical exam finding, family history and cardiovascular medical history.

4.2.2.5 Surveys:

Survey Completion: Participants were offered the opportunity to complete surveys in one of four ways, selected based on patient preference: a) in-person paper-based survey conducted in clinic; b) in-person online-based survey conducted in clinic; c) mailed out paper-based survey, completed at home, returned by pre-paid mail; d) online survey conducted at home, with a link provided by mail, telephone or email. Participants were provided with as much time as they required to complete the survey and completed the survey on their own outside the presence of any study personnel.

Paper-Based Survey*:* The paper based survey is attached in the supplemental material (Appendix F) and consists of 9 pages of 46 multiple-choice style questions, and also offers an opportunity to provide qualitative statements at the end. Paper surveys were labelled with an anonymized patient study reference number in order to be linked to chart review data but did not contain participant name. Data from the paper-based surveys was then entered manually into the REDCap database to aid in generation of a complete data set.

Online Survey: The online survey followed the same structure as the paper-based survey, displaying the same questions in the same order as the paper-based version. Participants were provided with a anonymized study reference number to complete the online surveys. The electronic survey was hosted on the Personal Information Protection and Electronic Documents Act (PIPEDA) and Health Information Patient Protection Act (HIPPA)-compliant online version of REDCap (Research Electronic Data Capture) (9) hosted at the Lawson Health Research Institute in London, Ontario.

Survey Content: Survey questions are formatted according to the original validated surveys.

Health Related Quality of Life Measure (12 questions) (10, 11):

The Health Related Quality of Life measure, short form 12 (SF-12), is a 12-item questionnaire designed to assess quality of life for individuals with chronic conditions. It is

subdivided into a Physical Component and Mental Component summary, and has been validated in a number of populations and for a number of chronic diseases (10, 11).

Medication Adherence Measure (8 questions) (12, 13):

The Eight-Item Morisky Medication Adherence Scale (MMAS-8) is an 8-question version of the more simple medication adherence questionnaire (MAQ) that has been validated in a broad range of diseases and patient populations (13). It allows for disclosures of non-adherence by using a "yes-saying" bias and explores some of the contributors to medication non-compliance (13). Previous studies have shown good concordance of the MMAS-8 with pill counting for patients taking statins (14). Results are reported as an overall score and divided into low, intermediate and high adherence. License is required for use and scoring in proprietary (Appendix A).

Simple Lifestyle Indicator Measure (10 questions)(15, 16):

The simple lifestyle indicator questionnaire (SLIQ) is an 8 question survey consisting of questions designed to ascertain patterns of behavior in various lifestyle components that affect cardiovascular disease, including diet, activity level, stress, smoking and alcohol consumption. Each category is scored separately and the results are combined to generate the overall finding. It has been validated previously in a Canadian population at elevated cardiovascular risk administered in a clinical research setting (15, 16). Scores from 0-4 are considered 'unhealthy', 5-7 are 'intermediate', and 8-10 are 'healthy'.

Generalized Anxiety Measure (8 questions) (17):

Receiving a genetic diagnoses of FH may contribute to heightened levels of anxiety. This has been seen in other conditions that receive a genetic diagnosis, such as in inherited cancer syndromes, along with feelings of guilt about potentially having passed the condition to offspring (18). The generalized anxiety disorder screener (GAD-7) is a 7 item questionnaire validated in the general population to assess for anxiety (17). The GAD-7 has

been used in other cardiovascular disease populations to clinically assess for anxiety and been shown to perform well (19).

Perceived Cardiovascular Risk (1 question):

This was addressed by a single item question in which the respondent was asked to rate his or her own risk of having a heart attack or stroke as low, moderate, high or very high over the next ten years.

Perceived Impact (7 questions)*:*

These 7 items directly asked the respondent to address their own perceptions of their experience with genetic testing. Using a 5-point Likert scale ranging from strongly negative to strongly positive they rated their own perceived impact of genetic testing for FH. They were also asked how likely they would be to recommend testing to family members. Additionally, they were asked to respond to questions commenting on any lifestyle or medication adherence changes they may have made in response to the genetic testing results, as well as perceptions of health and level of worry about health.

4.2.2.6 REDCap Database:

REDCap (Research Electronic Data Capture) (9) is a PIPEDA and HIPPA compliant secure server that is encrypted, has user authentication, data logging with mechanisms in place to ensure confidentiality. All data entered online or in the study database was de-identified. Data was available from the online REDCap system to study personnel only through secure log-in, user authentication and site encryption.

4.2.2.7 Statistical Analysis:

For each survey outcome, differences between any two groups were evaluated with two-tailed unpaired t-tests and are reported as mean difference $\pm$ standard deviation. With the three groups (Group1: patients who diagnosed with FH by a single genetic mutation, Group 2: those who have polygenic scores greater than 75 percentile, and Group 3: those who

have no pathogenic mutations), comparisons were made between Group1 vs. Group2, Group 1 vs. Group3, Group 2 vs. Group 3 and pooled results from Group 1 and 2 (genetic hypercholesterolemia) vs. Group 3). For outcome variables measured by categories, or Likert scale, such as perceived cardiovascular risk by very high, high, mod, low, Kruskal-Wallis test was used. Alpha level for significance was set at $P < .05$.

4.2.3 Results:

4.2.3.1 Responders:

A total of 139 individuals completed the survey. Of the respondents, 63 (45.3%) were diagnosed with heterozygous FH (HeFH - group 1), 48 (34.5%) were diagnosed with polygenic hypercholesterolemia (PHC – group 2) and 28 (20.1%) had mutation negative hypercholesterolemia (Mut Neg – group 3) (Table 4.1).

4.2.3.2 The Health Related Quality of Life:

There were no significant differences in health related quality of life, as assessed by the SF-12 score between the study groups, and none differed significantly from the literature reported average (50 ± 10) , for either physical or mental quality of life scores (Table 4.2). Average physical scores were 51.58 ± 8.54, 48.91 ± 7.92 and 47.73 ± 10.44 and average mental scores were 52.14 ± 7.92, 51.51 ± 9.27 and 50.30 ± 8.48 for HeFH, PHC and Mut Neg groups respectively (Table 4.2).

4.2.3.3 Medication Adherence Measure:

Respondents not taking medications did not complete this section of the survey. Average scores for the MASS-8 were 6.41 ± 1.84, 7.20 ± 0.84 and 6.36 ± 1.88 for HeFH (n=60), PHC (n= 46) and Mut Neg (n=26) groups respectively (Table 4.3). Average scores were significantly higher for PHC compared to the other two groups ($p = .0105$ (v HeFH) and .00841 (v Mut Neg)) by paired T-test. Low adherence was found in 32% of the HeFH group, 9% in the PHC group and 31% in the Mut Neg group, compared to the literature average of

32%. Medium adherence was seen in 32% of the HeFH group, 54% of the PHC group and 35% of the Mut Neg group, with a literature average of 52%. High adherence was seen in 37% of the HeFH group, 37% of the PHC group and 35% of the Mut Neg group, compared to the literature average of 16% (Table 4.3).

In the analysis of categorical data, the mean ranks were 63.21, 73.01 and 62.56, for HeFH, PHC and Mut Neg groups respectively. From Kruskal-Wallis rank-sum test, the difference in rank was not found to be statistically significant (Kruskal-Wallis chi-squared = 2.0512, degrees of freedom = 2, p-value = 0.35858) (Table 4.3). For the pooled HeFH/PHC vs Mut Neg comparison, mean ranks were 67.47 and 62.56, the difference in rank was not found to be statistically significant (Kruskal-Wallis chi-squared = 0.344, degrees of freedom = 1, p-value = 0.55756) (Table 4.3).

4.2.3.4 Simple Lifestyle Indicator Measure:

No significant differences were noted between groups in terms of the SLIQ mean scores (7.36 ± 1.38, 6.98 ± 1.4 and 6.79 ± 1.99 for HeFH, PHC and Mut Neg groups respectively) and all were similar to the reported literature average (6.73 ± 0.713) (Table 4.4).

For categorical assessment, unhealthy lifestyle was seen in 11%, 17% and 29%, intermediate in 70%, 73% and 57%, and healthy in 19%, 10% and 14% for HeFH, PHC and Mut Neg groups respectively (Table 4.4). From Kruskal-Wallis rank-sum test, the mean ranks were 75.49, 67.21 and 62.43 for HeFH, PHC and Mut Neg groups respectively; difference in rank was not found to be statistically significant (Kruskal-Wallis chi-squared = 2.3923, degrees of freedom = 2, p-value = .30236); difference in rank was also not statistically significant for the pooled HeFH/PHC analysis (Kruskal-Wallis chi-squared= 1.2395, degrees of freedom = 1, p-value .266) (Table 4.4).

4.2.3.5 Generalized Anxiety Measure:

There were no statistically significant differences in mean GAD-7 score between the respective groups (5.69 ± 3.70, 4.54 ± 5.09 and 5.61 ± 5.09 for HeFH, PHC and Mut Neg

groups respectively (Table 4.5). Scores were also similar to the literature average of 4.9 ± 4.8.

For categorical assessment, anxiety scores were scored as minimal in 60%, 71% and 57%, mild in 23%, 21% and 36%, moderate in 13%, 8% and 4% and severe in 5%, 0% and 4% in HeFH, PHC and Mut Neg groups respectively. From Kruskal-Wallis rank-sum test, the mean ranks were 73.0, 63.6 and 71.9 for HeFH, PHC and Mut Neg groups respectively; difference in rank was not found to be statistically significant (Kruskal-Wallis chi-squared = 1.6142, degrees of freedom = 2, p-value = .44615); difference in rank was also not statistically significant for the pooled HeFH/PHC analysis (Kruskal-Wallis chi-squared= 0.1277, degrees of freedom = 1, p-value .721) (Table 4.5).

4.2.3.6 Perceived Cardiovascular Risk:

Cardiovascular risk was self-reported as low in 52.38%, 45.83% and 46.42%, moderate in 33.33%, 35.42% and 35.71%, high in 7.94%, 14.58% and 14.29%, and very high in 6.35%, 2.08% and 3.57% of individuals in HeFH, PHC and Mut Neg groups respectively (Figure 4.1).

The mean ranks were 71.7, 67.4 and 71.0 for HeFH, PHC and Mut Neg groups respectively. From Kruskal-Wallis rank-sum test, the difference in rank was not found to be statistically significant (Kruskal-Wallis chi-squared = 0.3278, degrees of freedom = 2, p-value = 0.84883).

In the pooled genetic data, 49.55%, 34.23%, 10.81% and 4.50% of the combined HeFH/PHC group self-reported ASCVD risk as low, moderate, high and very high respectively. The mean ranks were 71.7, and 68.9 for Mut Neg, and pooled HeFH/PHC groups respectively. From Kruskal-Wallis rank-sum test, the difference in rank was not found to be statistically significant (Kruskal-Wallis chi-squared = 0.1043, degrees of freedom = 1, p-value = 0.74673).

4.2.3.7 Perceived Impact:

Question 1: How do you feel having a genetic test for familial hypercholesterolemia has impacted you?”

Impact was reported as strongly negative in 4.84%, 0.00%, and 0.00%, as mildly negative in 1.61%, 2.13% and 3.57%, no impact in 27.42%, 36.17% and 28.57%, mildly positive in 19.35%, 27.66% and 46.43%, and strongly positive in 46.77%, 34.04% and 21.43% in HeFH, PHC and Mut Neg groups respectively (Figure 4.2).

The mean ranks were 74.6, 64.8, and 71.1 for no mutation, heterozygous, and polygenic groups respectively. From Kruskal-Wallis rank-sum test, the difference in rank was not found to be statistically significant (Kruskal-Wallis chi-squared = 1.5409, degrees of freedom = 2, p-value = 0.4628).

Pooled results from HeFH/PHC showed similar results with 2.75%, 1.83%, 31.19%, 22.94% and 41.28% responding with strongly negative, mildly negative, no impact, mildly positive and strongly positive impact respectively. The mean ranks were 74.6, and 67.6 for Mut Neg and HeFH/PHC groups respectively. From Kruskal-Wallis rank-sum test, the difference in rank was not found to be statistically significant (Kruskal-Wallis chi-squared = 0.7868, degrees of freedom = 1, p-value = 0.3751).

Question 2: How likely are you to recommend genetic testing to your family members?

Responses indicated not at all likely in 1.61%, 4.26%, and 0%, somewhat unlikely in 1.61%, 2.13%, and 10.71%, neither likely or unlikely in 11.29%, 10.64% and 28.57%, somewhat likely in 22.58%, 23.40% and 21.43% and very likely in 62.90%, 59.57% and 32.29% in HeFH, PHC and Mut Neg groups respectively (Figure 4.3).

The mean ranks were 63.9, 66.8 and 83.9 in HeFH, PHC and Mut Neg groups respectively. Compared to Mut Neg, HeFH and PHC groups were both statistically more likely to answer higher (Kruskal-Wallis chi-squared = 6.4167, degrees of freedom = 2, p-value = 0.04042).

The man ranks for Mut Neg and HeFH/PHC groups in the pooled analysis were 83.9, and 65.2. Compared to Mut Neg, the pooled HeFH/PHC group was more likely to answer higher (Kruskal-Wallis chi-squared = 6.2319, degrees of freedom = 1, p-value = 0.01255).

Question 3: Did you make changes to your dietary habits after learning your genetic testing results?

Diet was reported to have significantly worsened in 0% of all respondents, mildly worsened in 0%, 4.26% and 7.14%, stayed the same in 22.58%, 23.40%, and 46.43%, mildly improved in 45.16%, 57.45% and 28.57%, and significantly improved in 32.26%, 14.89% and 17.86% in HeFH, PHC and Mut Neg groups respectively (Figure 4.4).

The mean ranks were 60.1, 71.8 and 84.1 in HeFH, PHC and Mut Neg groups respectively. The answers were more likely to be higher for the HeFH group followed by PHC then Mut Neg (Kruskal-Wallis chi-squared = 8.5178, degrees of freedom = 2, p-value = 0.01414).

For the pooled analysis, the mean ranks were 84.1, and 65 for Mut Neg and HeFH/PHC respectively. The answers were more likely to be higher for the pooled heterozygous/polygenic group than the no mutation group (Kruskal-Wallis chi-squared = 5.8408, degrees of freedom = 1, p-value = 0.01566).

Question 4: Did you make changes to your physical activity habits after learning your genetic testing results?

Physical activity was reported as significantly deceased in 1.61%, 2.13% and 0%, mildly decreased in 3.23%, 2.13% and 0%, 45.16%, 42.55% and 60.71%, mildly increased in 37.10%, 36.17% and 28.57% and significantly increased in 12.9%, 17.02% and 10.71% in HeFH, PHC and Mut Neg groups respectively (Figure 4.5).

The mean ranks were 69.1, 65.7 and 74.2 in HeFH, PHC and Mut Neg groups respectively and were not found to be significantly different (Kruskal-Wallis chi-squared = 0.9437, degrees of freedom = 2, p-value = 0.6238).

In the pooled analysis, mean ranks were 74.2, and 67.7 for Mut Neg and HeFH/PHC, which were not found to be statistically different (Kruskal-Wallis chi-squared = 0.71657, degrees of freedom = 1, p-value = 0.3973).

Question 5: Did you make changes to taking all medications as prescribed after learning your genetic testing results?

Medication adherence was reported to have significantly worsened in 0%, 2.13% and 3.57%, mildly worsened in 1.61%, 2.13% and 0%, stayed the same in 54.84%, 61.70% and 64.29%, mildly improved in 9.68%, 6.38% and 25% and significantly improved in 33.87%, 27.66% and 7.14% in HeFH, PHC and Mut Neg groups respectively (Figure 4.6).

The mean ranks were 63.7, 71.1 and 77.1 in HeFH, PHC and Mut Neg groups respectively, which were not found to be statistically different (Kruskal-Wallis chi-squared = 3.0874, degrees of freedom = 2, p-value = 0.2136).

For the pooled HeFH/PHC analysis, the mean ranks were 77.1, and 66.9 for Mut Neg and HeFH/PHC respectively, which were also not found to be statistically different (Kruskal-Wallis chi-squared = 1.8978, degrees of freedom = 1, p-value = 0.1683).

Question 6: Compared to before you underwent genetic testing, how do you feel about your state of overall health?

In response to this question, perceptions of overall health were reported as significantly worse in 1.61%, 2.13% and 0%, mildly worse in 12.9%, 8.51% and 10.71%, stayed the same in 30.65%, 34.04% and 64.29%, mildly improved in 33.8%, 42.55% and 14.29%, and significantly improved in 20.97%, 12.77% and 10.71% in HeFH, PHC and Mut Neg groups respectively (Figure 4.7).

The mean ranks were 65.2, 66.4 and 81.8 in HeFH, PHC and Mut Neg groups respectively, which were not found to be statistically different (Kruskal-Wallis chi-squared = 4.0918, degrees of freedom = 2, p-value = 0.1293).

Pooling the HeFH and PHC groups resulted in mean ranks of 81.8, and 65.7 for Mut Neg and HeFH/PHC respectively. Compared to the Mut Neg group, the pooled HeFH/PHC group were statistically more likely to have a higher score (Kruskal-Wallis chi-squared = 4.063, degrees of freedom = 1, p-value = 0.04383).

Question 7: Compared to before genetic testing, how would you rate your level of worry or concern over your health?

Responses from this question reported that respondents were much less concerned in 9.68%, 4.26% and 0%, slightly less concerned in 11.29%, 19.15% and 14.29%, no change was reported in 41.94%, 34.04% and 50.00%, slightly more concerned in 27.42%, 36.17% and 28.57% and significantly more concerned in 9.68%, 6.38% and 7.14% in HeFH, PHC and Mut Neg groups respectively (Figure 4.8).

The mean ranks were 70.1, 68.4 and 67.5 for HeFH, PHC and Mut Neg groups respectively, which were not found to be statistically different (Kruskal-Wallis chi-squared = 0.1037, degrees of freedom = 2, p-value = 0.9495).

Pooling the HeFH/PHC data generated mean ranks of 67.5, and 69.4 for Mut Neg and HeFH/PHC respectively. This difference was not found to be statistically significant (Kruskal-Wallis chi-squared = 0.053269, degrees of freedom = 1, p-value = 0.8175).

Table 4.1: Survey Respondents

Study Group:	Number of Responders:
Heterozygous FH	63
Polygenic FH	48
Mutation-negative HC	28
TOTAL:	139

HC: hypercholesterolemia

Table 4.2: SF-12 Health Related Quality of Life Scores

	Physical Score ± SD	p-value	Mental Score ± SD	P-value
HeFH n=63	51.58 ± 8.54	-	52.14 ± 7.92	-
PHC n =48	48.91 ± 7.92	-	51.51 ± 9.27	-
Mut Neg HC N= 28	47.73 ± 10.44	-	50.30 ± 8.48	-
Literature Average	50 ± 10	-	50 ± 10	-
Mut Neg HC vs HeFH		.093		.336
Mut Neg HC vs PHC		.609		.565
HeFH vs PHC		.091		.709
Genetic FH (HeFH + PHC) vs Mut Neg		.213		.388

HeFH: heterozygous familial hypercholesterolemia; PHC: polygenic hypercholesterolemia; Mut Neg: mutation negative hypercholesterolemia; SD: standard deviation.

Table 4.3: MASS-8 Scores

	Average Score +/- SD	p-Value	Low (%)	Medium (%)	High (%)	P-Value
HeFH n= 60	6.41 +/- 1.84	-	32%	32%	37%	
PHC n= 46	7.20 +/- 0.84	-	9%	54%	37%	
Mut Neg HC n= 26	6.36 +/- 1.88	-	31%	35%	35%	
Literature Average		-	32%	52%	16%	-
Mut Neg HC vs HeFH		.889				.951
Mut Neg HC vs PHC		**.0105**				.260
HeFH vs PHC		**.00841**				.194
Genetic FH (HeFH + PHC) vs Mut Neg		.255				0.558

HeFH: heterozygous familial hypercholesterolemia; PHC: polygenic hypercholesterolemia; Mut Neg: mutation negative hypercholesterolemia; SD: standard deviation; **bold:** statistically significant

Table 4.4: Simple Lifestyle Indicator:

	Score ± SD	p-value	Unhealthy (%)	Intermediate (%)	Healthy (%)	p-Value
HeFH n=63	7.36 ± 1.38	-	11%	70%	19%	-
PHC n=48	6.98 ± 1.4	-	17%	73%	10%	-
Mut Neg HC n=28	6.79 ± 1.99	-	29%	57%	14%	-
Literature Average	6.73 ± 0.713	-	-	-	-	-
Mut Neg HC vs HeFH		.169				.164
Mut Neg HC vs PHC		.65				.599
HeFH vs PHC		.15				.273
Genetic FH (HeFH + PHC) vs Mut Neg		.3079				.266

HeFH: heterozygous familial hypercholesterolemia; PHC: polygenic hypercholesterolemia; Mut Neg: mutation negative hypercholesterolemia

Table 4.5: GAD-7 Score

	Score ± SD	p-Value	Minimal (%)	Mild (%)	Moderate (%)	Severe (%)	p-value
Mut Neg HC n=28	5.61 ± 5.09	-	57%	36%	4%	4%	-
HeFH n=63	5.69 ± 3.70	-	60%	23%	13%	5%	-
PHC n=48	4.54 ± 5.09	-	71%	21%	8%	0%	-
Literature Average	4.9 ± 4.8	-	-	-	-	-	-
Mut Neg HC vs HeFH		.35					.886
Mut Neg HC vs PHC		.30					.366
HeFH vs PHC		.93					.230
Genetic FH (HeFH + PHC) vs Mut Neg		.294					.721

HeFH: heterozygous familial hypercholesterolemia; PHC: polygenic hypercholesterolemia:Mut Neg: mutation negative hypercholesterolemia

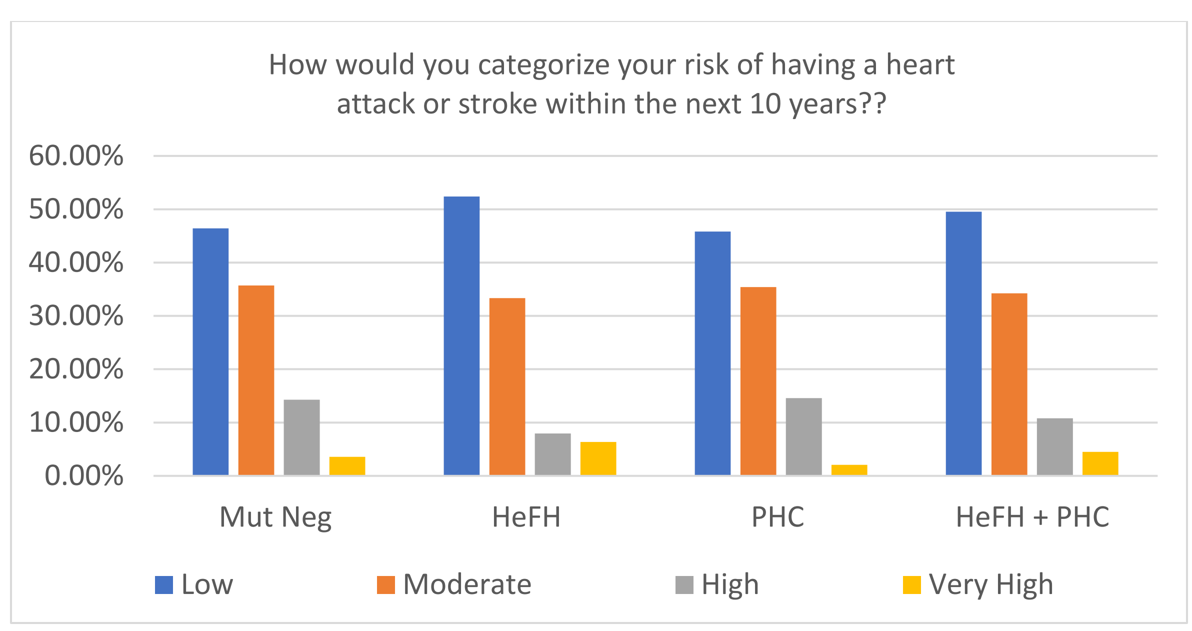

Figure 4.1: Perceived cardiovascular risk

Cardiovascular risk was self-reported as low in 52.38%, 45.83% and 46.42%, moderate in 33.33%, 35.42% and 35.71%, high in 7.94%, 14.58% and 14.29%, and very high in 6.35%, 2.08% and 3.57% of individuals in HeFH, PHC and Mut Neg groups respectively. No statistically significant differences were seen between groups in terms of perceived cardiovascular risk (Kruskal-Wallis chi-squared = 0.3278, degrees of freedom = 2, p-value = 0.84883).

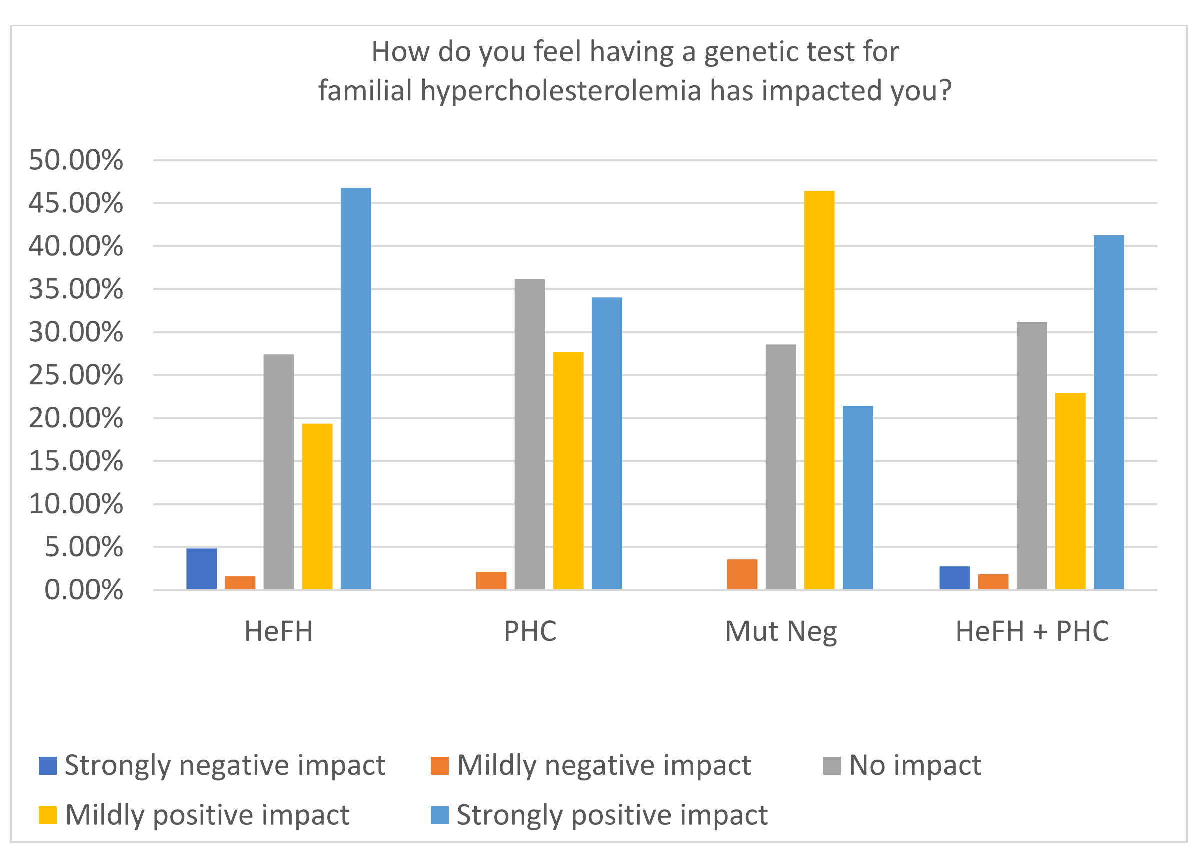

Figure 4.2: Perceived impact of genetic testing

Impact was reported as strongly negative in 4.84%, 0.00%, and 0.00%, as mildly negative in 1.61%, 2.13% and 3.57%, no impact in 27.42%, 36.17% and 28.57%, mildly positive in 19.35%, 27.66% and 46.43%, and strongly positive in 46.77%, 34.04% and 21.43% in HeFH, PHC and Mut Neg groups respectively. There were no statistically significant differences between groups in terms of perceived impact (Kruskal-Wallis chi-squared = 1.5409, degrees of freedom = 2, p-value = 0.4628).

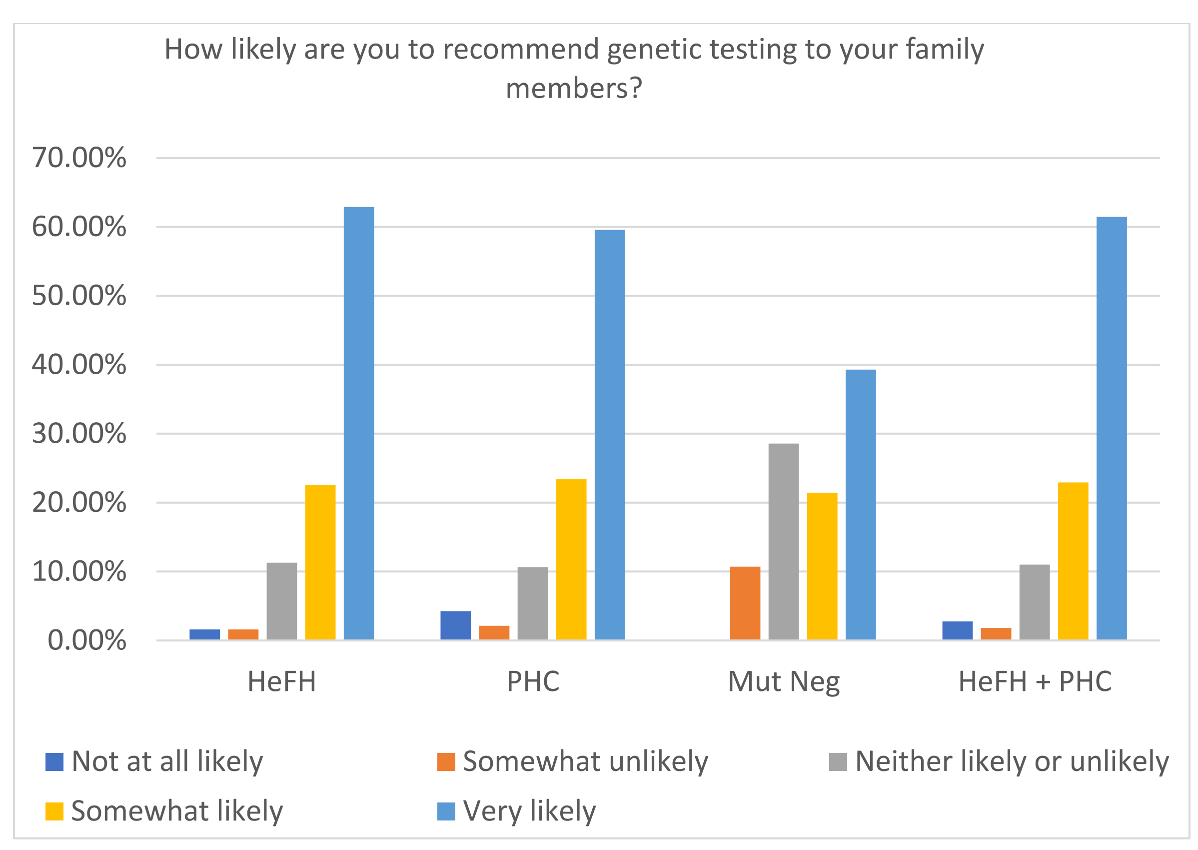

Figure 4.3: Recommendation to family members

Responses indicated not at all likely in 1.61%, 4.26%, and 0%, somewhat unlikely in 1.61%, 2.13%, and 10.71%, neither likely or unlikely in 11.29%, 10.64% and 28.57%, somewhat likely in 22.58%, 23.40% and 21.43% and very likely in 62.90%, 59.57% and 32.29% in HeFH, PHC and Mut Neg groups respectively. **Compared to no mutation, heterozygous and polygenic groups are more likely to answer higher** (Kruskal-Wallis chi-squared = 6.4167, degrees of freedom = 2, p-value = 0.04042). **Compared to no mutation, the pooled heterozygous/polygenic group was more likely to answer higher** (Kruskal-Wallis chi-squared = 6.2319, degrees of freedom = 1, p-value = 0.01255).

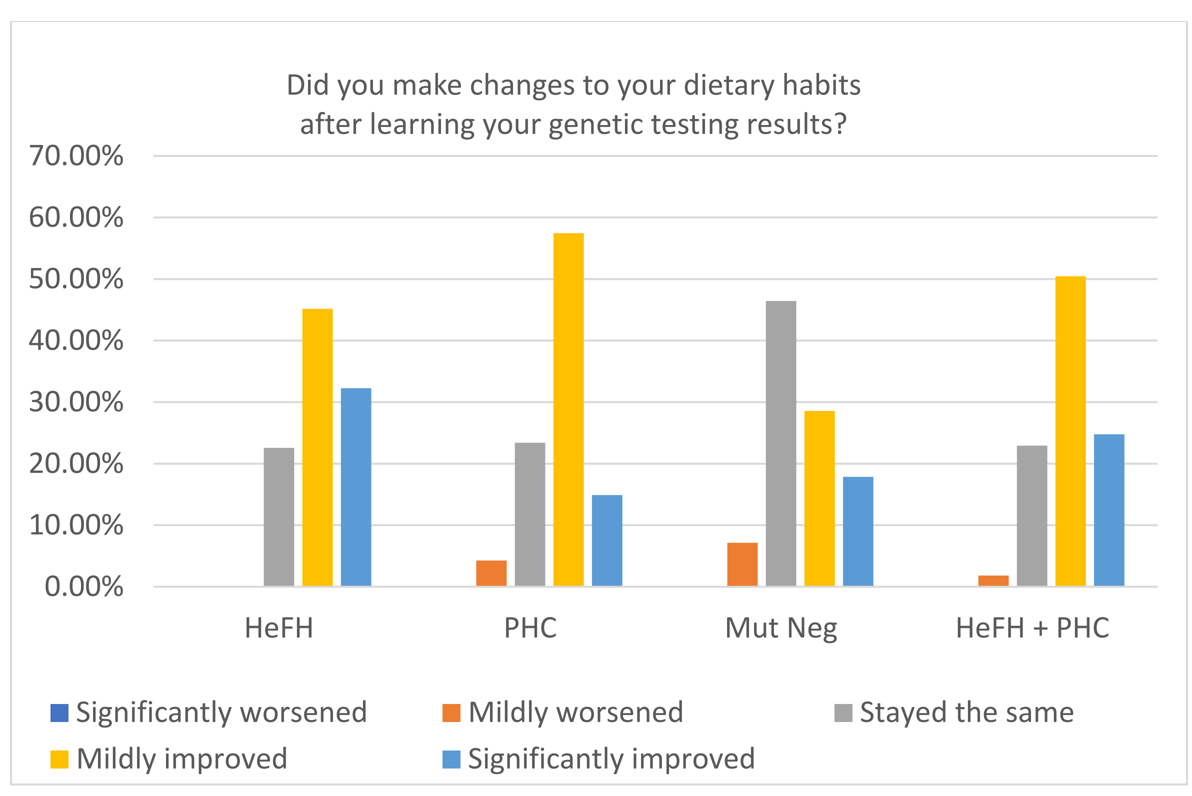

Figure 4.4: Perceived dietary changes

Diet was reported to have significantly worsened in 0% of all respondents, mildly worsened in 0%, 4.26% and 7.14%, stayed the same in 22.58%, 23.40%, and 46.43%, mildly improved in 45.16%, 57.45% and 28.57%, and significantly improved in 32.26%, 14.89% and 17.86% in HeFH, PHC and Mut Neg groups respectively. **The answers were more likely to be higher for the heterozygous group followed by polygenic then no mutation (**Kruskal-Wallis chi-squared = 8.5178, degrees of freedom = 2, p-value = 0.01414)**. Additionally, the answers were more likely to be higher for the pooled heterozygous/polygenic group than the no mutation group (**Kruskal-Wallis chi-squared = 5.8408, degrees of freedom = 1, p-value = 0.01566).

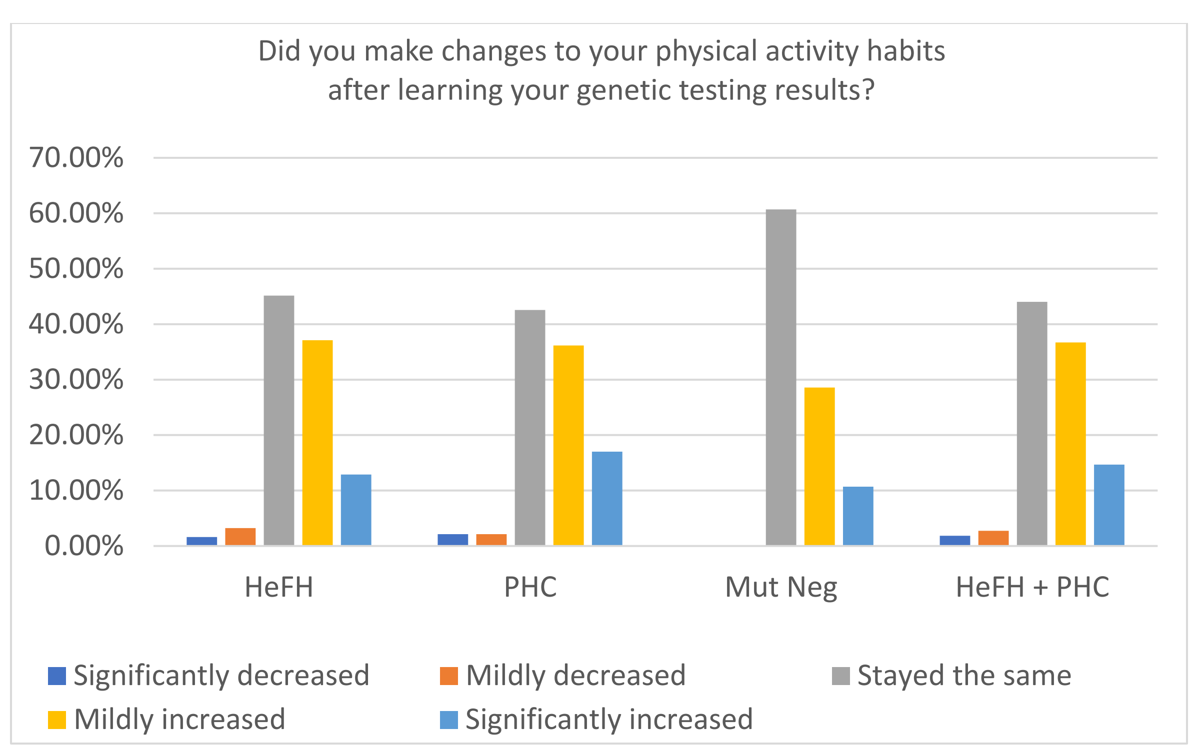

Figure 4.5: Physical activity changes

Physical activity was reported as significantly deceased in 1.61%, 2.13% and 0%, mildly decreased in 3.23%, 2.13% and 0%, 45.16%, 42.55% and 60.71%, mildly increased in 37.10%, 36.17% and 28.57% and significantly increased in 12.9%, 17.02% and 10.71% in HeFH, PHC and Mut Neg groups respectively. There were no statically significantly differences between groups in terms of reported changes in physical activity habits (Kruskal-Wallis chi-squared = 0.9437, degrees of freedom = 2, p-value = 0.6238).

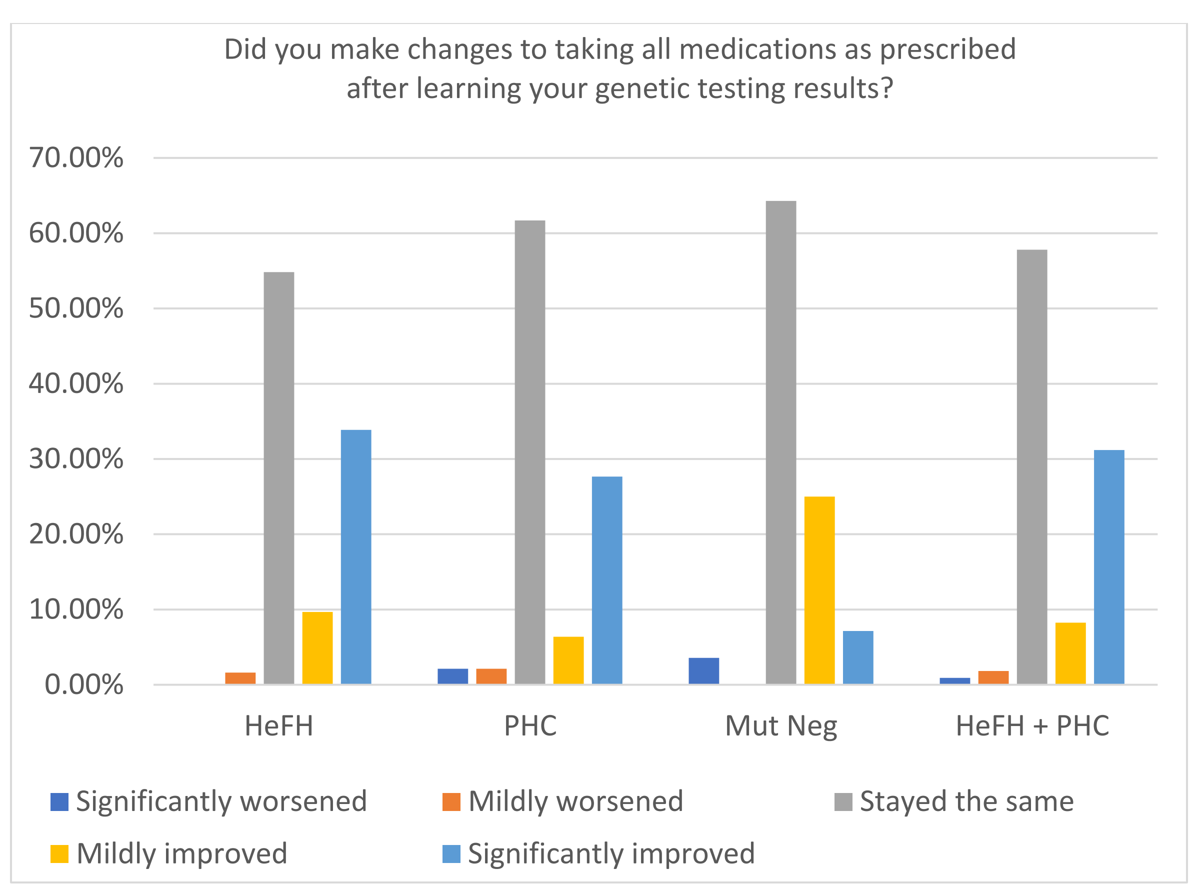

Figure 4.6: Perceived medication adherence:

Medication adherence was reported to have significantly worsened in 0%, 2.13% and 3.57%, mildly worsened in 1.61%, 2.13% and 0%, stayed the same in 54.84%, 61.70% and 64.29%, mildly improved in 9.68%, 6.38% and 25% and significantly improved in 33.87%, 27.66% and 7.14% in HeFH, PHC and Mut Neg groups respectively. No statistically significant results were found between groups in terms of perceived improvements in medication adherence (Kruskal-Wallis chi-squared = 3.0874, degrees of freedom = 2, p-value = 0.2136).

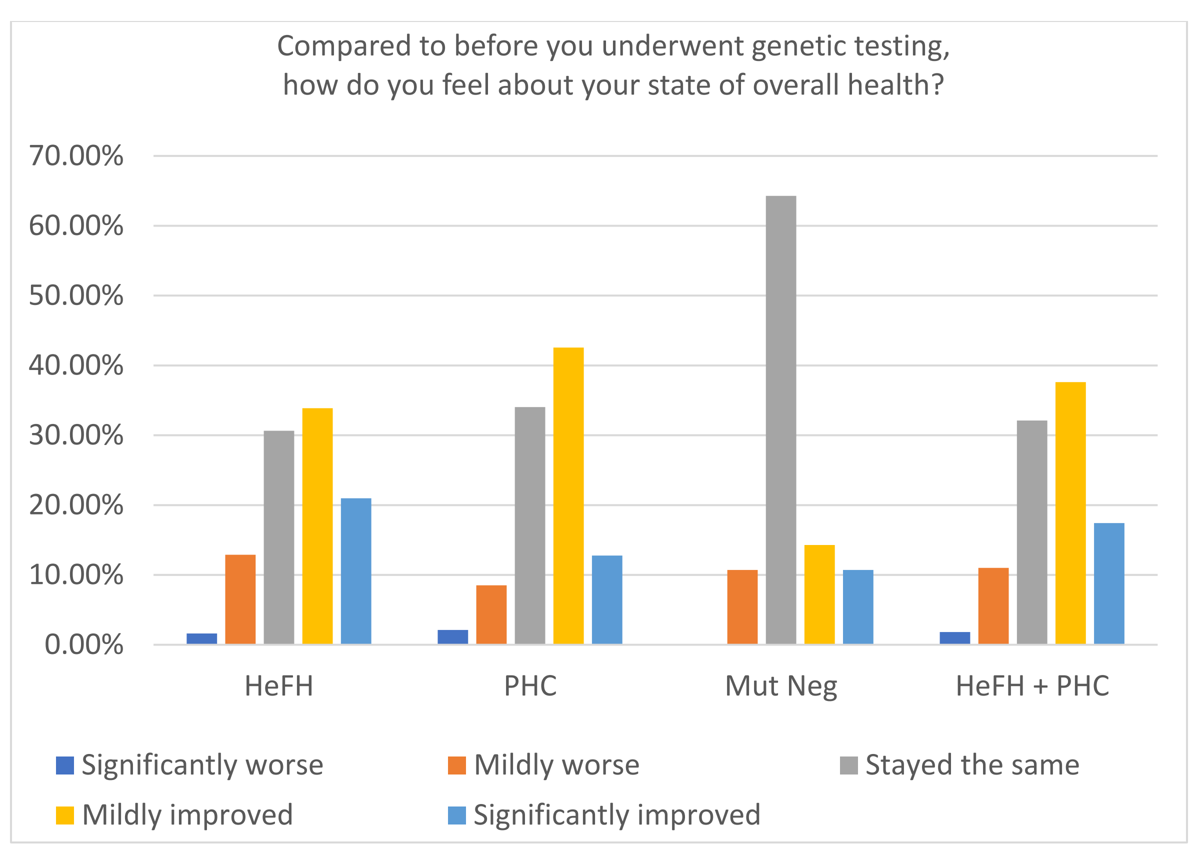

Figure 4.7: Perceived state of health

Perceptions of overall health were reported as significantly worse in 1.61%, 2.13% and 0%, mildly worse in 12.9%, 8.51% and 10.71%, stayed the same in 30.65%, 34.04% and 64.29%, mildly improved in 33.8%, 42.55% and 14.29%, and significantly improved in 20.97%, 12.77% and 10.71% in HeFH, PHC and Mut Neg groups respectively. **For the pooled analysis, the difference was found to be statistically significant with individuals with in the pooled HeFH/PHC having higher scores compared to those with no mutation identified** (Kruskal-Wallis chi-squared = 4.0918, degrees of freedom = 2, p-value = 0.1293).

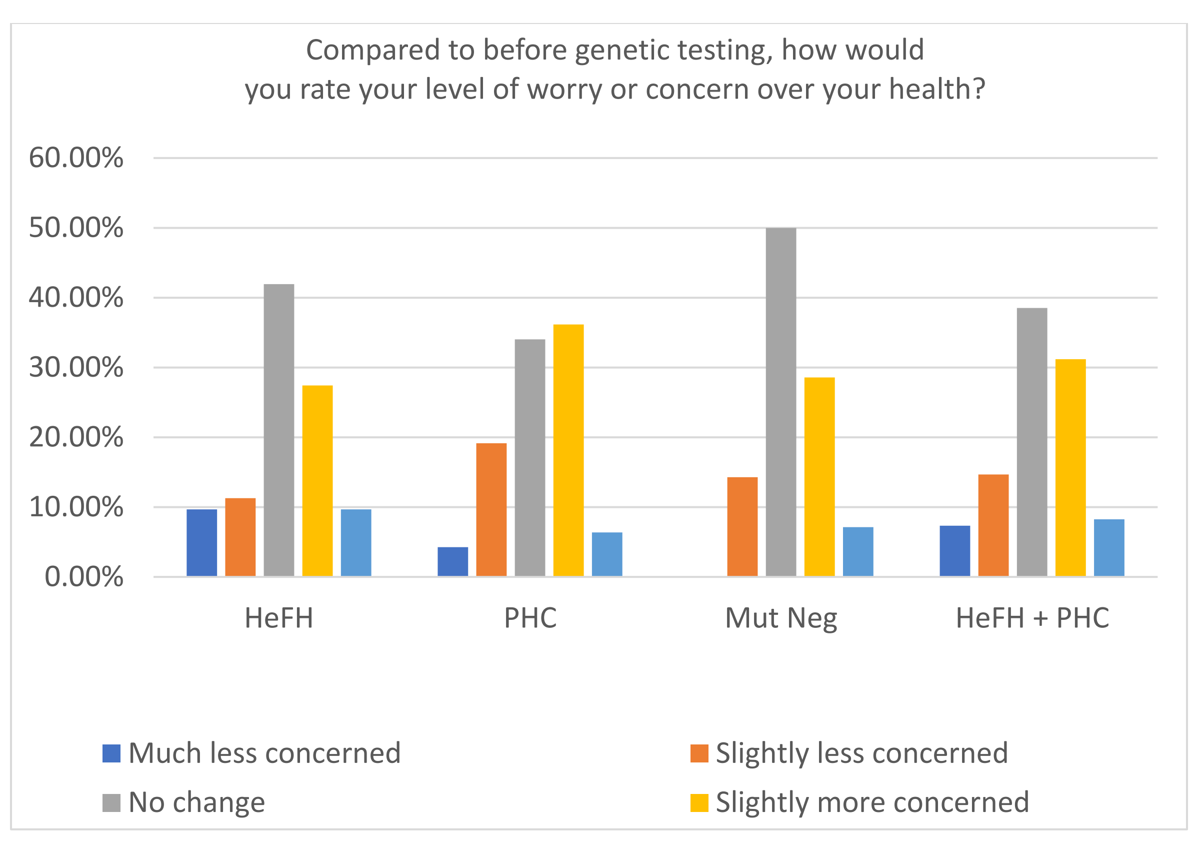

Figure 4.8: Perceived worry or concern

Responses from this question reported that respondents were much less concerned in 9.68%, 4.26% and 0%, slightly less concerned in 11.29%, 19.15% and 14.29%, no change was reported in 41.94%, 34.04% and 50.00%, slightly more concerned in 27.42%, 36.17% and 28.57% and significantly more concerned in 9.68%, 6.38% and 7.14% in HeFH, PHC and Mut Neg groups respectively. There were no statistically significant differences between the groups (Kruskal-Wallis chi-squared = 0.1037, degrees of freedom = 2, p-value = 0.9495).

4.2.4 Discussion:

The overall findings suggest that in the population studied, genetic testing for FH had either neutral or positive impacts on most individuals undergoing testing.

In terms of health related quality of life, both the validated survey as well as the patient perceptions of overall health suggested no negative impact from genetic testing or from the underlying hypercholesterolemia, and perhaps a feeling of improved overall health in those with a genetic diagnosis . The results of the SF-12 survey showed that all three study groups appeared similar to the population reported literature average, with no differences noted between groups (Table 4.2). This is consistent with prior use of this survey to assess quality of life in individuals undergoing cascade genetic screening for FH, which did not find an association between quality of life by this measure and affected or non-affected status (20).

Patient perceptions of their overall health after genetic testing were reported to be neutral or improved in the majority of individuals in all three study groups (85.49%, 89.36% and 89.29% in HeFH, PHC and Mut Neg respectively). In the pooled analysis, individuals with a genetic diagnosis were significantly more likely to report higher scores than those without a genetic cause identified. Potential explanations for this difference include the relief from diagnostic uncertainty that comes with a genetic diagnosis that could potentially improve individuals understanding of their medical condition. It may also be that once they were diagnosed, individuals took active steps to improve their health. There may also be a sense of relief among the individuals with a genetic diagnosis that their elevated cholesterol levels are predominantly hereditary and not due to lifestyle choices.

In terms of medication adherence, MASS-8 scores were similar to reported literature average, with individuals with PHC reporting significantly higher rates of adherence than those in the other two groups. Patient perceptions of medication adherence were not significantly different between the three study groups, but there was a higher percentage of individuals in the HeFH and PHC groups that reported “significantly improved” medication adherence following their genetic testing results compared to those who were not found to

have a mutation (33.87%/27.66% vs 7.14% for HeFH/PHC and Mut Neg groups respectively). The reasons for this difference are unclear, but could be due to improved appreciation for the role of medications over lifestyle changes in controlling hypercholesterolemia in those with an underlying genetic cause.

There was also a higher percentage of individuals with high adherence on the MASS-8 in all three study groups compared to the literature reported average (35-37% vs 16%), which may suggest that patients attending the lipid clinic may be more likely in general to take prescribed medications than the average population. This may be in line with other studies examining lifestyle patterns and medication adherence, where one study found that in women with hypercholesterolemia, medication adherence was generally high and did not differ between those with a diagnosis of definite or probable FH and those with no FH diagnosis (21). While we did not examine an association with these factors here, in other prior studies of FH, non-adherence with medications has been associated with younger age and lower untreated cholesterol values (22).

Assessment of lifestyle with the SLIQ did not reveal any statistical differences between groups, with average scores for all groups falling close to the literature average. Patient perceived physical activity habits also showed no differences between the three groups. However, patient perceived changes to diet showed that scores were highest for the HeFH group, followed by the PHC group then the Mut Neg group. This may suggest that those with a genetic diagnosis are, at minimum, making more of an effort to address any dietary contribution to their elevated cholesterol, though further investigation to clarify this finding would be required.

Individuals undergoing genetic testing were not found to have higher levels of anxiety when study groups were compared to each other or with the literature average. Similarly, patient perceived worry or concern over health was not statistically different between the three study groups. This suggests that the genetic diagnosis does not appear in this study to be associated with higher rates of health-related concerns or anxiety. As higher levels of

generalized anxiety have also been associated with higher ASCVD event rates (23), this is a reassuring finding in this population.

Perceived cardiovascular risk was also similar between the three study groups, and overall was perceived to be low or moderate by the majority in all groups (85.71%, 81.25% and 82.13% in HeFH, PHC and Mut Neg respectively), further suggesting that the genetic diagnosis did not lead to increased perceptions of having a high-risk condition.

Perhaps one of the most important questions to gauge the patient perspective on genetic testing was the responses to the question "How do you feel having a genetic test for familial hypercholesterolemia has impacted you?". Responses were not statistically different between the three study groups, but all reported high rates of neutral or positive impacts (93.54%, 97.87% and 96.43% for HeFH, PHC and Mut Neg groups respectively). Importantly, 46.77% of individuals diagnosed with HeFH reported a "strongly positive impact".

When asked if they would recommend genetic testing to family members, both the HeFH and PHC study groups were statistically more likely to recommend testing, with the majority of individuals in all three study groups reporting neutral or affirmative responses (96.77% 93.61% and 89.29% in HeFH, PHC and Mut Neg groups respectively). The fact that those who ultimately received a genetic diagnosis are more likely to recommend testing to other family members makes intuitive sense, as relatives of these individuals would be at higher risk of carrying the same pathologic variants, whereas family members of those without identified genetic risk would be less likely to have informative findings. The high overall rates of test recommendation is further support of the overall positive testing experience.

However, despite the overall findings of neutral or positive impact, it is important to note that 6.45% of patients with a diagnosis of HeFH reported that they felt that genetic testing had a mildly (1.61%) or strongly (4.84%) negative impact on them. While this is a minority of the patients tested, it highlights the need to properly counsel patients before and after the genetic testing process to explain the diagnosis, prepare them for the possible outcomes and support them in managing the condition if diagnosed.

4.2.4.1 Strengths and limitations

The strengths of this study include the use of a range of validated surveys as well as patient perceptions to assess several aspects of genetic testing impact. Some limitations include the relatively low numbers of respondents, which may have led to a lack of power to detect differences between the groups. However, since the data captured trended towards neutral or positive impact when not statistically different, the likelihood that a significant negative impact was missed is low. Given that the patients included in this study were all followed in a speciality lipid clinic, which may be different in terms of the patient population (ie more motivated or concerned patients), or healthcare delivery (ie administered by experts in the field), the results of this study may not be generalizable to patients in other settings, such as general medicine clinics or family doctor's offices. Furthermore, these results may be specific for FH/hypercholesterolemia, and may not be generalizable to other genetic conditions.

4.2.5 Conclusions:

Perceived experiences with genetic testing in this study were neutral or positive across all groups and most would recommend testing to other family members. Ultimately, it does not seem that a genetic diagnosis of HeFH or polygenic hypercholesterolemia negatively affected the mental health or well-being of individuals who underwent testing. Furthermore, a genetic diagnosis may have led to greater efforts to improve lifestyle factors such as diet and possibly medication adherence in PHC, and improved feelings of overall health in some patients, though more investigation is needed to confirm these observations. While the results of this study may not be generalizable to all inherited conditions, the lack of significant negative impact in this study is reassuring. Ultimately, this study suggests that genetic testing for HeFH or polygenic hypercholesterolemia can be used by physicians to help guide management, more accurately assess cardiovascular risk and identify at-risk family members without adversely affecting the patient.

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Chapter 5: Discussion, Conclusion and next steps

5.1 Overview:

This work has explored several ways in which genetic knowledge and laboratory techniques can be translated into the clinical sphere for select endocrine and metabolic conditions. To accomplish this goal, this work has examined laboratory and bioinformatic techniques that can help improve diagnosis of MODY (chapter 2), has looked for phenotypic features of extreme monogenic and polygenic triglyceride phenotypes (chapter 3), and assessed the patient impact associated with genetic testing for familial hypercholesterolemia (chapter 4). Collectively, the findings presented here have advanced knowledge of select translational aspects of genetics and how they may be optimally applied within the endocrine clinic.

5.2 Summary of Findings:

5.2.1 Use of NGS and provider clinical suspicion as criteria to screen for MODY

In chapter 2.2, this work showed that gestalt provider clinical suspicion has a reasonable genetic confirmation rate for MODY of approximately 40%. The addition of CNV detection explored in chapter 2.3 further improved this confirmation rate to approximately 47%. The use of NGS examining all MODY genes simultaneously was found to improve diagnostic yield compared to sequential single gene testing for the most common MODY subtypes.

5.2.2 CNVs in the diagnosis of MODY:

Prior to the work presented in chapter 2.3, CNVs were not actively sought during the investigation of potential MODY cases due to the need for separate testing procedures that were of uncertain value. The bioinformatic reassessment of the NGS data for 51 suspected

MODY patients in whom initial genetic testing via NGS was negative found that 6 of these individuals had pathogenic CNVs underlying their MODY phenotype.

Taken together, the findings from chapter 2.2 and 2.3 suggest that to optimize diagnosis of MODY, the use of NGS testing that incorporates CNV detection is preferred.

5.2.3 Distinguishing FCS phenotypes from different molecular etiologies

In chapter 3.2, the features of a relatively large cohort of rare FCS patients were compared by molecular etiology to determine if clinical phenotypic differences were present. While a few minor differences were seen (in post-heparin LPL activity, insulin resistance markers, LDL-C and possibly minor differences in other lipid levels), FCS caused by mutations in *LPL* were largely similar to FCS from other molecular etiologies, highlighting the role of genetic testing in establishing a definitive diagnosis.

5.2.4 Incidence, predictors and care patterns for extreme hypertriglyceridemia

In chapter 3.3, a 5-year retrospective cohort study of all adults within Ontario with VS-HTG was conducted to investigate the incidence rate of HTG, the strongest predictors for expression of VS-HTG and the patterns of care for these individuals. This study showed that diabetes, alcohol abuse and obesity were the most significant modifiable predictors of VS-HTG expression. This study provides valuable information to aid in counselling those with polygenic risk for VS-HTG regarding which risk factors can be addressed to minimize the expression of VS-HTG and its complications.

5.2.5 Triglyceride rate of fall in the conservative management of hypertriglyceridemia-associated pancreatitis

The most severe complication of VS-HTG from monogenic, polygenic or other causes is pancreatitis. The optimal method of treatment for HTG-associated pancreatitis has not yet

been established. In chapter 3.4, retrospective analysis of triglyceride rates of decline in 22 cases of HTG-associated pancreatitis managed supportively, demonstrates the validity and safety of the conservative approach to management and also approximates the serum half-life of triglycerides in the fasting state at 30.6 hours. This study also demonstrated the dual role of polygenic susceptibility coupled with secondary risk factors in the expression of this severe presentation of HTG.

5.2.6 Patient impact of genetic testing for familial hypercholesterolemia

In chapter 4.2, survey results for 139 individuals who underwent genetic testing for FH and were found to carry a heterozygous rare variant (HeFH), have high polygenic risk scores for hypercholesterolemia (PHC) or who had elevated cholesterol (LDL-C $\geq$5mmol/L) without a mutation detected were assessed for the impact of this diagnosis on quality of life, motivation to adhere to lifestyle and medication advice, levels of anxiety and cardiovascular risk. Overall findings suggest that the impact of genetic testing was neutral or positive for most individuals for the measures assessed. This is a reassuring finding and supports the continued use of appropriate genetic testing in the clinic.

5.3 Discussion:

While the findings presented here are only stepping stones in the application of genetic testing for the clinical management of inherited endocrine conditions, there are several important findings from each chapter that collectively inform various aspects of patient care.

5.3.1 Identification of Monogenic Conditions:

One of the most potentially impactful uses of genetic testing within the endocrine clinic is for the investigation and confirmation of monogenic conditions, especially those that can result in improved management or outcomes for the proband tested.

This was demonstrated through the confirmation of MODY in chapter 2 of this work, which resulted in management changes for most of the individuals identified. This work also demonstrated that incorporating NGS and CNV detection are important additions to standard Sanger sequencing that allowed for the identification of additional cases in an efficient and cost-effective manner.

This was also seen in chapter 3.2 when examining FCS patients, who were clinically similar despite different molecular origins. This study demonstrated that genetic confirmation was the most effectual method of clearly determining the etiology of this rare condition. Determining the underlying genotype in these cases may have an impact of responsiveness to various treatments or to long term outcomes.

Chapter 4 of this work demonstrated that genetic confirmation of monogenic hypercholesterolemia was a neutral to positive experience for patients, and most would recommend testing to family members, suggesting that this type of testing is also worthwhile from a patient perspective.

5.3.2 Improved counselling:

An understanding of the genetic origins of an endocrine disorders can also improve the evaluation and counselling delivered surrounding the diagnosis, and allow for an explanation about what can be done to prevent, modify or manage the risks associated with the condition.

This was seen in chapter 2.3 of this work where by establishing the correct diagnosis of MODY subtype providers were able to more accurately predict disease course and prognosis, allowing for a more informed discussion of treatment options and their risks and benefits.

The predictors for the expression of severe hypertriglyceridemia identified in chapter 3.3 allows for more informed discussions regarding susceptibility and how to overcome genetic risk.

The results of the surveys in chapter 4.2 raise awareness about the potential psychological impacts of receiving a genetic diagnosis. While no adverse effects were noted in this work, it is important to appropriately counsel patients during the genetic testing process to avoid causing undue anxiety and to ensure understanding of any results obtained. This was highlighted in this work by the fact that 6.45% of respondents diagnosed with HeFH described the impact of genetic testing to have had a strongly (4.84%) or mildly (1.61%) negative impact. While this was clearly a minority of individuals, these concerns and experiences should not be discounted.

5.3.3 Personalized Management:

One of the most tangible outcomes of genetic testing within the endocrine and metabolic clinic context is the ability to personalize management based on individual needs and findings. This is one of the major benefits of identifying a patient with a monogenic condition such as MODY, FCS or FH.

In many cases, this results in improved quality of life, was seen in chapter 2.3 of this work where in *HNF1A* and *HNF4A* MODY, sulfonylureas can be used to optimize control of hyperglycemia, allowing discontinuation of insulin in some cases, or de-escalation of treatment in others. In *GCK*-MODY, patients can be reassured that no special monitoring or treatment are required, and no long term risks are expects.

Similarly, chapter 3.2 describes some of the features of FCS, a very rare condition that has not previously been systematically studied in a large group of patients. The descriptions of FCS provided in this work may help clinicians more appropriately distinguish FCS patients from the more common polygenic HTG patients.

Similarly, being able to recognize those individuals that have major modifiable risk factors contributing to their extreme phenotype, as identified in chapter 3.3, and convey this information to patients can potentially lead to a sense of empowerment to control and improve their condition.

The results of the work describing the fall of triglycerides during conservative management of pancreatitis in chapter 3.4 can also be used to optimize management of this condition as well as illustrate to patients the influence of diet and other secondary factors on their serum lipid profile.

In chapter 4.2 of this work, a diagnosis of HeFH was demonstrated to be acceptable for patients, and most felt satisfied that they had undergone the testing. It may also have led to improved motivation to adhere to recommended diet, prescribed medication, and increase overall sense of health, but this needs further validation. Irrespective of the personal patient impact, a diagnosis of FH can change recommended management and potentially improve access to medications such as PCSK9 inhibitors.

5.3.4 Judicious use of genetic testing:

An understanding of the genetic landscape of endocrine and metabolic conditions can also allow for more informed discussions with patients regarding their own risk, as well as the

risk that their family members might carry, and to discuss why and when genetic testing might be helpful.

For example, when considering MODY testing (chapter 2.2 and 2.3), if pancreas autoantibodies are present, or if there are clear physical stigmata of insulin resistance, genetic testing may not be indicated as it would be unlikely to yield positive findings.

Similarly, genetic testing for triglyceride disorders might be best for those who have features of FCS discussed in chapter 3.2 of this work. Those with likely polygenic HTG may not derive benefit from genetic study, but may find a discussion of the modifiable nature of their condition, as evidenced in chapter 3.3 and 3.4, helpful.

The findings from chapter 4.2 suggest that if genetic testing is deemed to be important or helpful for clinical indications, it does not seem to adversely affect the patient, at least in the context of FH.

5.3.5 Improve diagnostic accuracy:

An increased awareness that some monogenic disorders that would benefit from detection may be under-recognized is also an important finding in this work. While approximately 50% of patients referred for clinical suspicion of MODY in chapter 2.2 and 2.3 were ultimately diagnosed with MODY, this high confirmation rate conversely suggests that many MODY patients are going unrecognized.

Furthermore, for triglyceride disorders in chapter 3, it's important to realize that most are not monogenic, and to offer testing only to those who seem to fit the rare clinical picture for FCS (chapter 3.2).

FH is more common, and more commonly considered; it can also be missed in younger patients who have not had a lipid profile. It is important to consider family history of premature ASCVD and/or elevated cholesterol, and to consider screening patients with this history at an earlier age to lead to improved ASCVD outcomes. Anyone with a known family

history should be screened early, ideally upon entering adulthood if not before, and timely treatment initiated. Chapter 4.2 suggest most patients who undergo this testing will experience a largely neutral or positive impact.

5.3.6 Test selection:

In terms of which tests are most appropriate, the findings in chapter 2.2 and 2.3 suggest that NGS testing, either by gene panel or whole exome, that incorporates CNV analysis as a single test may provide the highest yield when testing for monogenic conditions such as MODY or FH. The use of validated testing methods is important due to the potential for incorrect variant calls.

Genetic testing should be used judiciously when assessing polygenic traits, such as most cases of hypertriglyceridemia (chapter 3.3, chapter 3.4). Caution should also be exercised when considering the use of polygenic risk scores, as these are largely unstandardized and their prognostic value still uncertain.

However, chapter 4.2 of this work suggests that testing for polygenic hypercholesterolemia (PHC) was associated with neutral or positive impact in most patients, with PHC patients in particular showing a significant increase in medication adherence following their diagnosis.

5.3.7 Cascade testing:

Many individuals found to carry genetic risk for an endocrine or metabolic condition will be concerned about the possibility that it may also be present in other family members. For monogenic dominant traits, such as HeFH (chapter 4) and MODY (chapter 2), 50% of all first degree relatives of identified probands will also carry the pathologic variant. Therefore, effort should be made to convey this information through family groups and facilitate testing in a cascading manner. For polygenic conditions, such as most cases of hypertriglyceridemia (chapter 3), familial testing is less helpful. These conditions can cluster within families but the inheritance pattern is variable and phenotypes can vary significantly,

therefore cascade testing is not often warranted, or useful, for polygenic traits. For recessive traits such as FCS (chapter 3.2), both parents are usually carriers of the pathologic variant. Therefore, genetic counselling for the parents of the affected child may be appropriate, as there is a 25% chance of a subsequent child born to the same parents expressing the condition. Testing other relatives to determine their carrier status in the context of genetic counselling for family planning purposes may also be considered. As chapter 4.2 indicates, most patient with HeFH would recommend genetic confirmatory testing to their family members, suggesting cascade testing would be reasonable and acceptable to most patients.

5.3.8 Optimize Care:

Another finding highlighted by this work is the importance of modifying what can be modified to optimize care for the patient. For example, in MODY, medications and screening programs can be modified to align with the genetic diagnosis and specific risk factors (chapter 2). Important secondary risk factors can be addressed in patients with hypertriglyceridemia (chapter 3). The identification of HeFH can modify the risk assessment and treatment targets for cholesterol (chapter 4). By understanding the most important genetic and non-genetic factors influencing expression of these traits, an informed discussion and personalized care plan are possible.

5.3.9 Future Advances:

Another important take-away from this work is that knowledge is always evolving, especially when it comes to genetics. Variants may be re-classified from being of undetermined significance to being pathogenic with the incorporation of new information; pathogenic changes may be detected with the use of refined testing techniques, as was seen with the detection of CNVs in chapter 2 of this work; or new causal genes may be detected. Ongoing work is being done on the utility of polygenic risk scores. These may become standardized, and may be useful in refining risk stratification in polygenic conditions such as hypertriglyceridemia (chapter 3) and other complex traits. It is important

to leave the door open, in both the clinician and patient's minds to allow for the incorporation of potential new discoveries.

5.4 Study strengths and limitations

Individual study strengths and limitations of included studies are discussed within each relevant section. Here, the strengths and limitations of the data as a whole are discussed.

5.4.1 Strengths:

Strengths of the study include the availability and use of LipidSeq, a targeted NGS panel that was used in the genetic testing for chapters 2.2, 2.3, 3.2, 3.4 and 4.2. LipidSeq is designed to capture all MODY related genes as well as genes related to other lipid and metabolic phenotypes (Appendix C). It has also been designed to capture the SNPs used to generate the TG and FH PRS in chapter 3.4 and chapter 4.2, respectively. Due to the high depth of coverage (approximately 300-times), output data from LipidSeq was also able to be used for CNV analysis in the diagnosis of MODY, FH and FCS in chapters 2.3, 3.2 and 4.2. This allowed for a single test to be conducted rather than using separate methodologies to capture SNPs, CNVs and single candidate genes.

Another major strength of this work is the large population available for chapter 3.3 through the ICES network, which draws on data from multiple databases, with a wealth of information available for the majority of residents within Ontario, providing a large and robust data set. Access to this powerful tool allowed for a population-wide assessment of the incidence, predictors, care patterns and outcomes for the hypertriglyceridemia phenotype of interest.

Additionally, through collaboration with multiple clinical sources, this work had access to genetic and clinical information from patients with relatively rare clinical conditions such as MODY in chapter 2.2 and 2.3 and FCS in chapter 3.2, FH in chapter 4.2, and HTG-associated pancreatitis in chapter 3.4.

5.4.2 Limitations:

A notable limitation of this study is the potential lack of generalizability in many of the findings. The CNV analysis in chapter 2.3 was conducted using the LipidSeq NGS testing and bioinformatic software pipeline to identify CNVs from NGS data. The ability to accurately detect the CNVs is dependent on the depth of coverage for the sample assessed. Other panels may be less accurate in detecting CNVs.

The data obtained in chapter 3.3 may also suffer from lack of generalizability outside the population of Ontario studied due to differences in ethnicity, genetic backgrounds, environmental influences, healthcare systems and population demographics.

Generalizability of the results in chapter 4.2 may also be a concern given that the patient population studied was drawn from a specialized lipid clinic and may not reflective of the average patient presenting with severe hypercholesterolemia.

Polygenic risk scores (PRS) for triglycerides (chapter 3.4) and hypercholesterolemia (chapter 4.2) used in this study were generated using only 16 and 10 SNPs respectively. Some data suggests that incorporation of more SNPs may provide greater prognostic information. However, the SNPs chosen are ones that have been found to have the largest effect sizes, minimizing any loss of discernability.

Furthermore, the data used to generate and validate the PRSs used in this study were derived largely from subjects of European background and may not be generalizable to populations from other ethnic backgrounds. Similarly, most of the patient data presented here was from a population of predominantly European descent, which may further impact generalizability.

Additionally, while CNVs were found in 6 initially negative suspected MODY patients, the true population frequency of these mutations amongst MODY patients is unavailable as the provided samples may have been enriched for these cases. More extensive case finding study may be required to gain a better understanding of the role of CNVs in MODY cases.

Furthermore, the findings regarding clinical suspicion for MODY being a reasonable selection criteria for MODY genetic testing may not hold true for other populations of providers.

Finally, given the breadth of the topics studied, this work leaves many unanswered questions that will require additional study to adequately address. This is particularly true for the findings favouring conservative management of HTG-associated pancreatitis in chapter 3.4 and the patient impact of genetic testing in chapter 4.2.

5.5 Future Directions:

5.5.1 MODY case finding:

Given the importance of identifying MODY to achieve optimal management, and the overall high rates of missed MODY diagnoses, future research plans include studies aimed at actively seeking potential candidates who may benefit from genetic testing for MODY. Initial efforts will address two distinct populations: 1) pregnant individuals who have screening positive for GDM; 2) patients with an established diagnosis of either type 1 or type 2 diabetes followed in the pediatric or adult endocrine clinics who are assessed at having >50% probability of MODY based on a validated clinical prediction tool.

5.5.1.1 Identifying pregnant patients with MODY:

MODY can be picked up during routine glucose tolerance tests conducted during pregnancy and is often misdiagnosed as gestational diabetes (GDM). Studies have estimated that up to 5-6% of women diagnosed with GDM actually have MODY (1, 2). Optimal care of women with MODY and their unborn children can depend on distinguishing MODY from GDM. Confirming a diagnosis will also have long-term management implications for both mother and baby. A definitive diagnosis of MODY in the mother can be made by performing a genetic test on DNA extracted from a routine blood sample. For this project, all women evaluated in our Endocrine and Pregnancy Clinic would be clinically screened for MODY at

their initial consult visit. Any individual deemed to be at high risk of MODY would be appropriately counselled and offered confirmatory genetic testing.

5.5.1.2 Identifying MODY amongst established diabetes patients:

MODY continues to be underrecognized clinically, but affects up to 5% of individuals diagnosed with diabetes under the age of 35.   There are 14 genes associated with MODY subtypes, which are usually inherited in an autosomal dominant pattern. Many patients with MODY are misdiagnosed as having either type 1 or type 2 diabetes. Each subtype of MODY is unique, and establishing the correct diagnosis can help with selecting the most appropriate treatment options, lead to improvements in glycemic control and provide a clearer picture of the expected course of the diabetes over time. As a start to this project, our clinic practice has been screened using the local online diabetes database to identify those individuals diagnosed with diabetes under the age of 35 and have identified up to 359 individuals who may benefit from additional screening and potentially genetic testing for this condition (Table 5.1). For this project, this additional screening would be conducted and genetic testing and counselling offered to any individual who has a >50% probability of having MODY based on a validated clinical prediction tool.

Table 5.1: Preliminary data for MODY case finding from online database query

	MODY Probability <50%	MODY Probability Potentially >50%	Combined Population
N	262	359	621
Age at Diagnosis			
-mean	28.96	13.59	20.2
-median	30	13	20
-range	6-35	1-34	1-35
Sex			
-male	123 (46.9%)	147 (40.9%)	270 (43.5%)
-female	139 (53.1%)	212 (59.1%)	351 (56.5%)
BMI			
-mean	33.75	26.05	29.31
-median	32.7	25.1	27.7
-range	19.3-64.9	14.4-46.3	14.4-64.9
HbA1c			
-mean	8.78%	8.16%	8.42%
-median	8.4%	8%	8.2%
-range	5.4-15.1%	4.4-13.4%	4.4-15.1%
Current Age			
-mean	51.01	34.4	41.43
-median	51	31	41
-range	15-85	8-82	8-85
Min Prob Score:			
-mean	N/A	3.41	N/A
-median		0.7	
-range		0.7-75.5	
Max Prob Score:			
-mean	15.79	72.81	48.33
-median	4.6	75.5	62.4
-range	4.5-45.5	58-75.5	4.5-75.5

5.5.2 Assessing the genetic confirmation rate of genetic testing for other monogenic conditions:

As seen for MODY in chapter 2, confirming the presence of a monogenic condition may provide a powerful clinical tool to provide more personalized and effective care for patients and their family members. Investigating how this tool may be best applied clinically will help guide the incorporation of genetic tests into clinical practice. This study will help establish how to select patient who are most likely to benefit from testing and identify specifically how they may benefit in terms of measurable clinical outcomes as well as patient and provider satisfaction.

Following the principles discussed above, testing for conditions for which genetic testing results may materially affect patient management or outcomes, have the highest potential benefit. The three conditions below meet this criteria and will be studied in an attempt to assess those clinical features that are predictive of a positive or negative genetic confirmation test. The main goals of this assessment would be to develop more discriminating selection criteria for testing, as well as to assess for the presence of meaningful changes to management or outcomes that could be attributed to the genetic diagnosis.

5.5.2.1 Familial Hypocalciuric Hypercalcemia (FHH):

FHH is a benign condition caused by mutations in *CASR*, encoding for the calcium sensing receptor, that leads to a higher setpoint for calcium mediated suppression of parathyroid hormone (PTH) release and renal excretion of calcium. Distinguishing this condition from alternative diagnoses that can mimic the presentation of FHH, mainly primary hyperparathyroidism, is important as the management of each condition is drastically different. The management of primary hyperparathyroidism would usually be surgical removal of an autonomously functioning and hypertrophied parathyroid gland, whereas treatment is not usually required for FHH. FHH tends to follow a benign course, with mild biochemical abnormalities seen and no end organ damage or dysfunction. Therefore

management of FHH is reassurance in most cases. While there are urinary tests that can be helpful in distinguishing FHH from primary hyperparathyroidism there can be significant overlap in findings between these two conditions. In cases of ambiguity, genetic testing to look for a pathologic variant in *CASR* may help guide appropriate management.

5.5.2.2 Thyroid Hormone Resistance:

Thyroid hormone resistance is caused by defective receptors to thyroid stimulating hormone (TSH) on target tissues throughout the body. There are multiple subtypes of thyroid hormone resistance, with the most common caused by mutations affecting the beta type receptors. Thyroid hormone alpha receptors can also be affected, though less commonly. These receptors are variably distributed throughout the body with different tissues having higher or lower concentration ratios of beta to alpha receptors. This condition can manifest with symptoms of hypothyroidism, hyperthyroidism, or patients may be asymptomatic. A mosaic pattern of symptoms, with some tissues displaying features of hyperthyroidism and others features of hypothyroidism are also possible based on inconsistent receptor distribution among different body tissues. Variable patterns of thyroid function tests can be seen with this condition and it is possible that this condition could be mistaken for either central hyperthyroidism, or hypothyroidism if an isolated TSH is assessed. Incorrect treatment can therefore be initiated in some cases. Confirming this condition with genetic testing can help guide optimal management as well as help identify this condition in other family members, avoiding potentially unnecessary or incorrect treatments such as thyroidectomy, thyroid ablation, or inappropriate use of thyroid hormone replacement.

5.5.2.3 Familial Partial or Complete Lipodystrophy

There are several subtypes of familial partial or complete lipodystrophy (Table 5.2). Lipodystrophies are characterized by abnormal distribution of adipose tissue, with a paucity of subcutaneous fat and consequent pathological deposition of fat within other body regions such as within organs (ie the liver) or viscerally. Lipodystrophy is commonly

characterized by severe insulin resistance and severe hypertriglyceridemia. While management of lipodystrophy generally follows the same principles as managing these manifestations in non-lipodystrophy individuals, some medications, such as the thiazolidinediones (TZDs) may be more beneficial in patients this population and may be preferentially used. Additionally, making this diagnosis provides relief of uncertainty for both the patient and provider. In extreme cases characterized by refractory metabolic derangements, Metreleptin, a synthetic leptin analog, may also be used under research protocols in these patients, although long-term safety and efficacy of this treatment has yet to be established (3). Lipodystrophies are underrecognized clinically and active assessment for potential cases that may benefit from genetic testing may be warranted.

Table 5.2: Lipodystrophies

	Gene/chromosome	Inheritance	MIM reference numbers	Clinical features and comorbidities	Comments
Partial lipodystrophies	*LMNA*/1q22	AD	151660	-distinctive patterns of regional lipoatrophy associated with simultaneous lipohypertrophy in unaffected areas -insulin resistance -recurrent pancreatitis	-elevated TG which can be severe in 10-20% of cases
	*PPARG/*3p25.2	AD	604367		
	*PLIN1/*15p26.1	AD	613877		
	*CIDEC/*3p25.3	AR	615238		
Generalized lipodystrophies	*AGPAT2/*9q34.3	AR	608594	- absence of subcutaneous fat in subcutaneous tissues -insulin resistance -recurrent pancreatitis -hepatosplenomegaly	-elevated TG which can be severe in majority of cases -elevated liver enzymes
	*BSCL2/*11q12.3	AR	269700		
	*CAV1/*7q31.2	AR	612526		
	*CAVIN1/*17q21.2	AR	613327		

AD: autosomal dominant; AR: autosomal recessive; TG: triglyceride; MIM: mendelian inheritance in man

5.5.3 Creating an updated reference for the population distribution of lipid levels across residents of Ontario

Previous cross-sectional data on population-wide lipid distribution has been obtained in other populations. However since these studies were conducted, there has been significant changes in population characteristics such as age distribution, ethnic variability, rates of overweight and obesity, prevalence of diabetes and metabolic syndrome and a shift in dietary patterns and rates of physical activity, as well as increased prescribing of lipid-directed medications. Consequently, this older data may not reflect current trends.

This study will look at all individuals residing in Ontario with a valid OHIP card and a lipid profile obtained and available in OLIS for 2019. If more than one lipid profile is available for a single individual, data collection will be restricted to the first value over the accrual period. It will determine the lipid distribution for total, LDL, HDL and non-HDL cholesterol, as well as fasting and non-fasting triglyceride levels for the population, subdivided by sex and age by decade to generate the data necessary to determine mean, SD median, $1^{st,}$ 5th, 10th, 25th, 50th, 75th, 90th, 95th, 99th percentile for each lipid parameter.

5.5.4 ASCVD and pancreatitis among patients with HTG Ontario Cohort

This study will look at the HTG cohort population in Ontario obtained from the previous investigation into the incidence, predictors and care patterns in the population described in chapter 3.3 but will be optimized to assess the rates and predictors of outcomes, such as ASCVD events and pancreatitis risk based on degree of triglyceride elevation in order to further improve counselling and management of genetic triglyceride disorders.

5.5.5 Prospective Assessment of patients tested for FH for medication adherence

The initial investigation into the patient impact of genetic testing for familial hypercholesterolemia (FH) found that a genetic diagnosis of FH may improve adherence to

recommended medications in PHC individuals (chapter 4.2). To further investigate and confirm this preliminary signal, a dedicated prospective study will be done assessing medication adherence at baseline, prior to disclosure of genetic testing results, and then again at 6 months and one year following disclosure of a genetic confirmation of FH or PHC.

5.6 Conclusions:

The information gained from this work has allowed for several important advances and acts as a stepping stone to expand current understanding of how knowledge of disease genetics may be applied within the endocrine clinic. This work has helped to define genetic testing techniques that can improve case detection among patients with monogenic endocrine conditions such as MODY, and has helped to establish the importance of assessing for CNVs in suspected MODY cases to improve diagnostic yield. It has provided valuable insight into severe monogenic triglyceride phenotypes and highlighted the importance of seeking genetic confirmation in suspected cases of FCS. Data obtained from the identification of the most important predictors of severe hypertriglyceridemia, such as diabetes, obesity and alcohol, as well as safe and effective options for the management of HTG-associated pancreatitis will improve counselling and management of patients with genetic triglyceride disorders. Furthermore, this work has demonstrated that the patient impact of genetic testing for FH is largely neutral or positive, suggesting that genetic testing does not adversely affect individuals and may be appropriately used by clinicians to help optimize and personalize care for patients within the endocrine clinic.

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Appendices:

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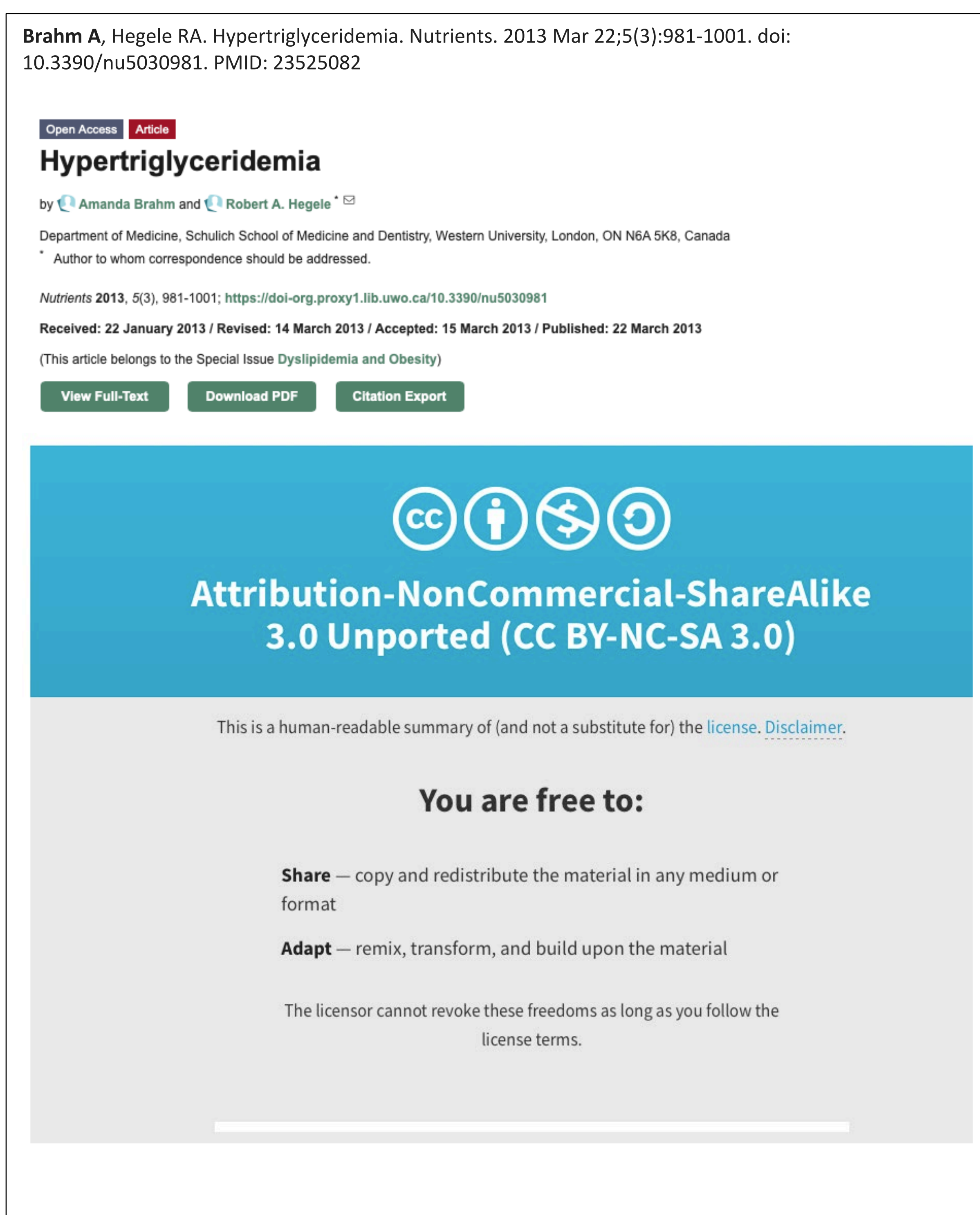

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Hypertriglyceridemia

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Nutrients **2013**, *5*(3), 981-1001; https://doi-org.proxy1.lib.uwo.ca/10.3390/nu5030981

Received: 22 January 2013 / Revised: 14 March 2013 / Accepted: 15 March 2013 / Published: 22 March 2013

(This article belongs to the Special Issue Dyslipidemia and Obesity)

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Author: Amanda J. Brahm et al
Publication: Nature Reviews Endocrinology
Publisher: Springer Nature
Date: Mar 3, 2015

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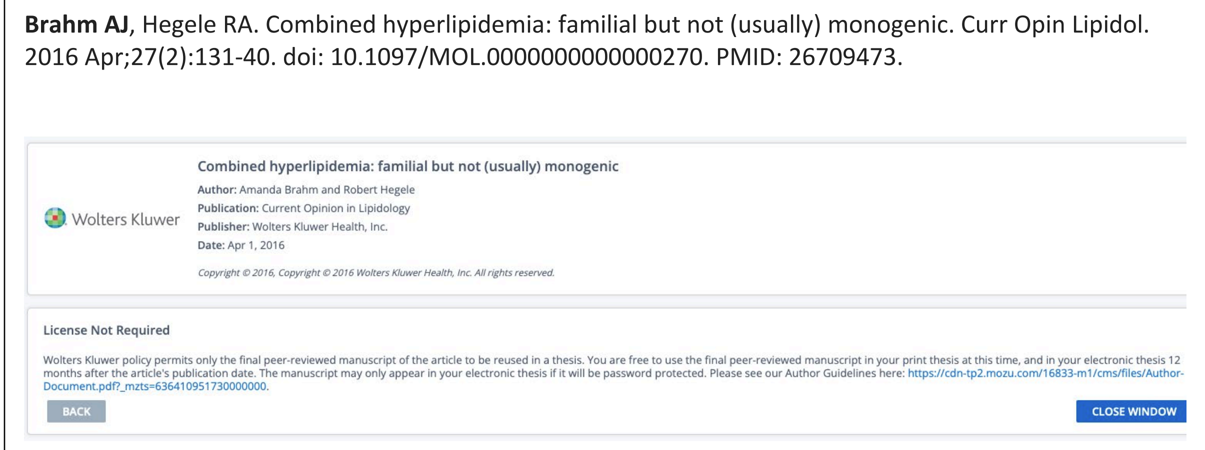
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Combined hyperlipidemia: familial but not (usually) monogenic

Author: Amanda Brahm and Robert Hegele
Publication: Current Opinion in Lipidology
Publisher: Wolters Kluwer Health, Inc.
Date: Apr 1, 2016

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Author: Amanda J. Brahm,Grace Wang,Jian Wang,Adam D. McIntyre,Henian Cao,Matthew R. Ban,Robert A. Hegele
Publication: Canadian Journal of Diabetes
Publisher: Elsevier
Date: December 2016

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The complex molecular genetics of familial hypercholesterolaemia

Author: Amanda J. Berberich et al
Publication: Nature Reviews Cardiology
Publisher: Springer Nature
Date: Jul 4, 2018

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The role of genetic testing in dyslipidaemia

Author: Amanda J. Berberich,Robert A. Hegele
Publication: Pathology
Publisher: Elsevier
Date: February 2019

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Berberich AJ, Wang J, Cao H, McIntyre AD, Spaic T, Miller DB, Stock S, Huot C, Stein R, Knoll J, Yang P, Robinson JF, Hegele RA. Simplifying Detection of Copy-Number Variations in Maturity-Onset Diabetes of the Young. Can J Diabetes. 2021 Feb;45(1):71-77. doi: 10.1016/j.jcjd.2020.06.001. Epub 2020 Jun 8. PMID: 33011132.

Simplifying Detection of Copy-Number Variations in Maturity-Onset Diabetes of the Young

Author:
Amanda J. Berberich,Jian Wang,Henian Cao,Adam D. McIntyre,Tamara Spaic,David B. Miller,Suzanne Stock,Celine Huot,Robert Stein,Joan Knoll,Ping Yang,John F. Robinson,Robert A. Hegele
Publication: Canadian Journal of Diabetes
Publisher: Elsevier
Date: February 2021

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Hegele RA, **Berberich AJ**, Ban MR, Wang J, Digenio A, Alexander VJ, D'Erasmo L, Arca M, Jones A, Bruckert E, Stroes ES, Bergeron J, Civeira F, Witztum JL, Gaudet D. Clinical and biochemical features of different molecular etiologies of familial chylomicronemia. J Clin Lipidol. 2018 Jul-Aug;12(4):920-927.e4. doi: 10.1016/j.jacl.2018.03.093. Epub 2018 Apr 4. PMID: 29748148.

Clinical and biochemical features of different molecular etiologies of familial chylomicronemia

Author:
Robert A. Hegele,Amanda J. Berberich,Matthew R. Ban,Jian Wang,Andres Digenio,Veronica J. Alexander,Laura D'Erasmo,Marcello Arca,Alan Jones,Eric Bruckert,Erik S. Stroes,Jean Bergeron,Fernando Civeira,Joseph L. Witztum,Daniel Gaudet

Publication: Journal of Clinical Lipidology

Publisher: Elsevier

Date: July–August 2018

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Berberich AJ, Ouédraogo AM, Shariff SZ, Hegele RA, Clemens KK. Incidence, predictors and patterns of care of patients with very severe hypertriglyceridemia in Ontario, Canada: a population-based cohort study. Lipids Health Dis. 2021 Sep 3;20(1):98. doi: 10.1186/s12944-021-01517-6. PMID: 34479547;

SPRINGER NATURE

Incidence, predictors and patterns of care of patients with very severe hypertriglyceridemia in Ontario, Canada: a population-based cohort study

Author: Amanda J. Berberich et al

Publication: Lipids in Health and Disease

Publisher: Springer Nature

Date: Sep 3, 2021

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Berberich AJ, Mokashi A, McIntyre AD, Robinson JF, Cao H, Wang J, Hegele RA. Bioinformatic detection of copy number variation in HNF4A causing maturity onset diabetes of the young. Clin Genet. 2019 Oct;96(4):376-377. doi: 10.1111/cge.13599. Epub 2019 Jul 15. PMID: 31309534;

Bioinformatic detection of copy number variation in HNF4A causing maturity onset diabetes of the young

Author: Amanda J. Berberich, Arati Mokashi, Adam D. McIntyre, et al

Publication: Clinical Genetics

Publisher: John Wiley and Sons

Date: Jul 15, 2019

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Berberich AJ, Ziada A, Zou GY, Hegele RA. Conservative management in hypertriglyceridemia-associated pancreatitis. J Intern Med. 2019 Dec;286(6):644-650. doi: 10.1111/joim.12925. Epub 2019 Jun 6. PMID: 31077464.

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Berberich AJ, Huot C, Cao H, McIntyre AD, Robinson JF, Wang J, Hegele RA. Copy Number Variation in GCK in Patients With Maturity-Onset Diabetes of the Young. J Clin Endocrinol Metab. 2019 Aug 1;104(8):3428-3436. doi: 10.1210/jc.2018-02574. PMID: 30912798; PMCID: PMC6594302.

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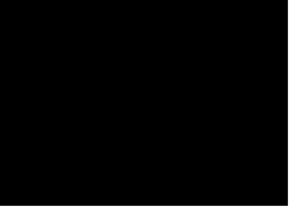
Requestor Location	Amanda Berberich 
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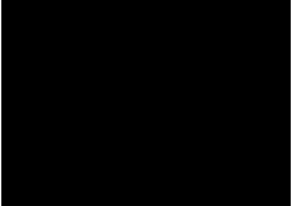
Requestor Location	Amanda Berberich 
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License Fee for single study: Patient Impact of genetic testing for familial hypercholesterolemia $2,500
Training: $1,500
Number of Assessments: $200 (200 estimated)

Total cost: $4,200

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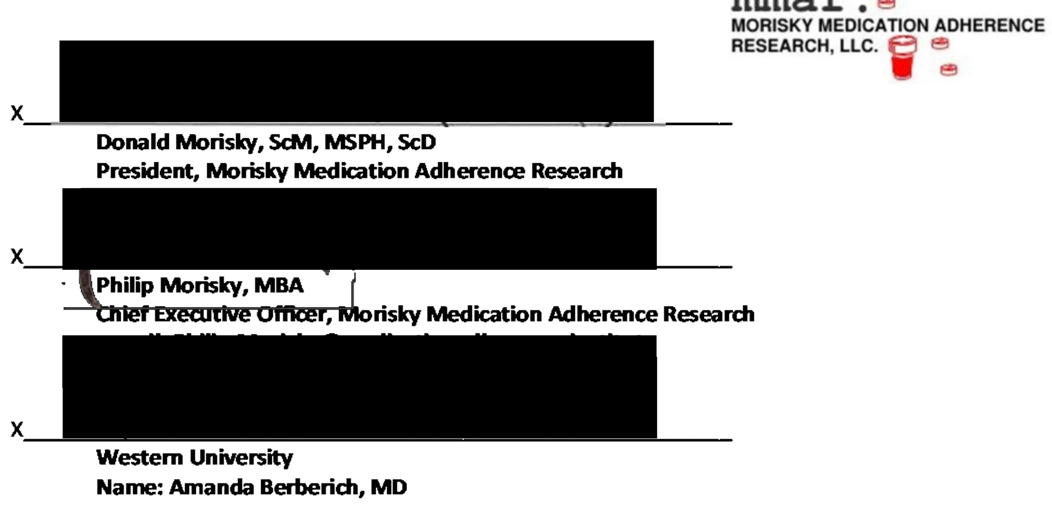

X____

Donald Morisky, ScM, MSPH, ScD
President, Morisky Medication Adherence Research

X____

Philip Morisky, MBA
Chief Executive Officer, Morisky Medication Adherence Research

X____

Western University
Name: Amanda Berberich, MD
Position: Assistant Professor

Appendix B: Ethics Approvals

Date: 18 May 2021

To: Dr. Robert A. Hegele

Project ID: 113228

Study Title: Patient Impact of Genetic Testing for Familial Hypercholesterolemia

Application Type: Continuing Ethics Review (CER) Form

Review Type: Delegated

REB Meeting Date: 08/June/2021

Date Approval Issued: 18/May/2021

REB Approval Expiry Date: 21/May/2022

Dear Dr. Robert A. Hegele,

The Western University Research Ethics Board has reviewed the application. This study, including all currently approved documents, has been re-approved until the expiry date noted above.

REB members involved in the research project do not participate in the review, discussion or decision.

Western University REB operates in compliance with, and is constituted in accordance with, the requirements of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The REB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

The Office of Human Research Ethics

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

Page 1 of 1

Date: 15 October 2020

To: Dr. Robert Hegele

Project ID: 0379

Study Title: Candidate gene sequencing, genetic and genomic analysis for identification of new genetic determinants of intermediate traits of atherosclerosis, dyslipidemia, diabetes, obesity, hypertension, lipodystrophy and other rare metabolic or cardiovascular disorders in the human population. 07920E

Application Type: Continuing Ethics Review (CER) Form

Review Type: Delegated

REB Meeting Date: November 3, 2020

Date Approval Issued: 15/Oct/2020 06:13

REB Approval Expiry Date: 03/Nov/2021

Dear Dr. Robert Hegele,

The Western University Research Ethics Board has reviewed the application. This study, including all currently approved documents, has been re-approved until the expiry date noted above.

REB members involved in the research project do not participate in the review, discussion or decision.

Western University REB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The REB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

The Office of Human Research Ethics

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Appendix C: Genes included on LipidSeq

Phenotype	Disorder	Gene	Chr	Start	Stop	RefSeq	Size (bp)	OMIM
MONOGENIC DYSLIPIDEMIAS								
LDL-C, High	ADH1, familial hypercholesterolemia (FH)	*LDLR*	19	11200037	11244506	NM_000527	14,387	#143890, *606945
	ADH2, familial defective apo B-100	*APOB*	2	21224301	21266945	NM_000384	26,888	#144010, #615558, +107730
	ADH3, familial hypercholesterolemia	*PCSK9*	1	55505149	55530526	NM_174936	10,358	#603776, *607786
	LDL-C cholesterol candidate	*STAP1*	4	68424446	68473055	NM_012108	6450	*604298
	ADH phenocopy; otherwise gene for dysbetalipoproteinemia	*APOE*	19	45409039	45412650	NM_000041	3,180	+107741
	ARH, autosomal recessive hypercholesterolemia	*LDLRAP1*	1	25870071	25895377	NM_015627	7,288	#603813, *605747
	ARH phenocopy; otherwise gene for CESD; Wolman syndrome	*LIPA*	10	90973326	91011660	NM_001127605	9,203	#278000, *613497
	Sitosterolemia	*ABCG5*	2	44039611	44066039	NM_022436	8,853	#210250, *605459
	Sitosterolemia	*ABCG8*	2	44066103	44105947	NM_022437	7,466	#210250, *605460
LDL-C, Low	Abetalipoproteinemia (ABL)	*MTTP*	4	100485240	100545154	NM_000253	13,649	#200100, *157147
	Homozygous hypobetalipoproteinemia (HHBL)	*APOB*	2	21224301	21266945	NM_000384	26,888	#144010, #615558, +107730
	Hypobetalipoproteinemia, PCSK9 deficiency	*PCSK9*	1	55505149	55530526	NM_174936	10,358	#605019, #613589, *607786
	Anderson disease, chylomicron retention disease	*SAR1B*	5	133936839	133968533	NM_001033503	10,651	#246700, *607690
	Familial combined hypolipidemia	*ANGPTL3*	1	63063158	63071976	NM_014495	6,250	#605019, *604774
HDL-C, High	Hyperalphalipoproteinemia	*CETP*	16	56995835	57017757	NM_000078	8,342	#143470, *118470
	Hepatic lipase deficiency	*LIPC*	15	58702953	58861073	NM_000236	7,957	#614025, *151670
	SR-B1 deficiency	*SCARB1*	12	125262174	125348519	NM_005505	11,327	#610762, *601040
	Hyperalphalipoproteinemia	*LIPG*	18	47087069	47119278	NM_006033	9,333	*603684
HDL-C, Low	Tangier disease, Primary familial hypoalphalipoproteinemia	*ABCA1*	9	107543283	107690527	NM_005502	35,253	#205400, *600046
	Primary hypoalphalipoproteinemia	*APOA1*	11	116706467	116708338	NM_000039	2,281	#604091, *107680
	Lecithin:cholesterol acyltransferase deficiency	*LCAT*	16	67973787	67978656	NM_000229	3,110	#245900, *606967
High TG	Chylomicronemia, LPL decficiency, severe hypertriglyceridemia (HTG),	*LPL*	8	19796582	19824770	NM_000237	8,747	+609708, #238600
	Chylomicronemia. apolipoprotein C-II deficiency, severe HTG	*APOC2*	19	45449239	45452822	NM_000483	1,611	#207750, *608083
	Severe HTG, hyperlipoproteinemia type V	*APOA5*	11	116660086	116663136	NM_052968	3,551	#145750, #144650, *606368
	Severe HTG. lipase maturation factor 1 deficiency,	*LMF1*	16	903634	1031318	NM_022773	15,351	#246650, *611761
	Severe HTG, hyperlipoproteinemia type V	*GPIHBP1*	8	144295068	144299044	NM_178172	3,913	*612757
	Infantile HTG,transient	*GPD1*	12	50497602	50505103	NM_005276	6,765	#614480, *138420
	HTG, hyperlipoproteinemia type III, dysbetalipoproteinemia, FH (rarely)	*APOE*	19	45409039	45412650	NM_000041	3,180	+107741
Low TG	Hypotriglyceridemia (with LDL-C and high HDL-C)	*APOC3*	11	116700624	116703787	NM_000040	2,168	#144250, *107720

OTHER DYSLIPIDEMIA CANDIDATES								
LDL-C, High	LDL-C candidate (GWAS)	*SORT1*	1	109852187	109940563	NM_002959	17,181	*602458
	LDL-C candidate (GWAS)	*MYLIP*	6	16129277	16148479	NM_013262	6,552	*610082
	LDL-C candidate (ezetimibe target)	*NPC1L1*	7	44552134	44580914	NM_013389	13,151	+608180
HDL-C, Low	HDL-C candidate gene (functional)	*PLTP*	20	44527259	44541003	NM_006227	7,770	+172425
	HDL-C candidate gene (functional)	*ABCG1*	21	43619799	43724497	NM_004915	13,722	*603076
High TG	TG candidate (GWAS)	*GALNT2*	1	230202956	230417875	NM_004481	13,187	*602274
	TG candidate (GWAS)	*MLXIPL*	7	73007524	73038903	NM_032951	8,890	*605678
	TG candidate (GWAS)	*TRIB1*	8	126442563	126450647	NM_025195	5,848	*609461
	TG candidate (GWAS and rare variant association)	*GCKR*	2	27719470	27746556	NM_001486	9,279	*600842
	TG candidate (rare variant association)	*CREB3L3*	19	4153598	4173051	NM_032607	7,001	*611998
	TG candidate gene (functional)	*APOA4*	11	116691418	116694011	NM_000482	2,817	*107690
	TG candidate gene (functional)	*PPARA*	22	46546458	46639653	NM_005036	17671	+170998
Misc	Lipid metabolism candidate (CAV gene family)	*CAV2*	7	116139655	116148595	NM_001233	15,288	*601048
	Neutral Lipid Storage Disease	*PNPLA2*	11	818901	825573	NM_020376	5,414	#610717, *609059

SECONDARY DYSLIPIDEMIAS								
Lipodystrophy	Partial lipodystrophy, FPLD2	*LMNA*	1	156052369	156109880	NM_170707	11,506	#151660, *150330
	Partial lipodystrophy, FPLD3	*PPARG*	3	12329349	12475855	NM_015869	9,036	#604367, *601487
	Partial lipodystrophy, FPLD4	*PLIN1*	15	90207598	90222648	NM_002666	7,191	#613877, *170290
	Partial lipodystrophy, FPLD2 (htz), FPLD5 (hmz)	*CIDEC*	3	9908394	9921938	NM_001199623	5,772	#151660, #615238, *612120
	Mandibuloacral dysplasia, with lipodystrophy	*ZMPSTE24*	1	40723722	40759856	NM_005857	8,165	*606480
	Late-onset lipodystrophy (AR)	*LIPE*	19	42905664	42931578	NM_005357	8,942	*151750
	Lipodystrophy (AR), recurrent acute myoglobinuria	*LPIN1*	2	11817705	11967535	NM_001261428	19,802	#268200, *605518
	Congenital generalized lipodystrophy 1 (CGL1) Berardinelli-Seip syndrome	*AGPAT2*	9	139567595	139581911	NM_006412	4,114	#608594, #269700, *603100
	Congenital generalized lipodystrophy 2 (CGL1) Berardinelli-Seip syndrome	*BSCL2*	11	62457734	62477091	NM_001122955	7,310	*606158
	Congenital generalized lipodystrophy 3 (CGL3)	*CAV1*	7	116164839	116201239	NM_001753	5,145	#612526, *601047
	Congenital generalized lipodystrophy (CGL4)	*PTRF*	17	40554467	40575506	NM_012232	4,634	#613327, *603198
	Acquired partial lipodystrophy (APL), Barraquer-Simons syndrome	*LMNB2*	19	2428163	2456966	NM_032737	8,902	#608709, *150341
	Lipodystrophy	*AKT2*	19	40736224	40791443	NM_001626	13,872	*164731
	Lipodystrophy, metabolic syndrome	*DYRK1B*	19	40315990	40324841	NM_004714	6,341	*604556
	Mandibular hypoplasia, deafness, progeroid and lipodystrophy syndrome (MDPL)	*POLD1*	19	50887461	50921273	NM_001256849.1	11736	#615381, *174761
Progeroid	Werner syndrome, aging and lipodystrophy	*WRN*	8	30890778	31031277	NM_000553	22,258	#277700
MODY	Maturity onset diabetes of the young subtype 1 (MODY1)	*HNF4A*	20	42984441	43061485	NM_000457	11,970	#125850, *600281
	MODY2	*GCK*	7	44183870	44229022	NM_033508	8,398	#125851, *138079
	MODY3	*HNF1A*	12	121415861	121440315	NM_000545	7,958	#600496, *142410
	MODY4	*PDX1*	13	28494168	28500451	NM_000209	3,573	#606392, *600733
	MODY5	*HNF1B*	17	36046434	36105096	NM_000458	8,441	#137920, *189907
	MODY6	*NEUROD1*	2	182540833	182545392	NM_002500	4,002	#606394, *601724
	MODY7	*KLF11*	2	10170776	10194963	NM_003597	6,723	#610508, *603301
	MODY8	*CEL*	9	135936741	135947250	NM_001807	6,092	#609812, *114840
	MODY9	*PAX4*	7	127250346	127255982	NM_006193	5,516	#612225, *167413
	MODY10	*INS*	11	2181009	2182439	NM_001185097	1,644	#613370, *176730
	MODY11	*BLK*	8	11351521	11422108	NM_001715	11,241	#613375, *191305
Neonatal DM	Permanent neonatal diabetes mellitus	*KCNJ11*	11	17406796	17410878	NM_000525	4,569	#606176, *600937
	Permanent neonatal diabetes mellitus	*ABCC8*	11	17414432	17498449	NM_000352	22,250	#606176, *600509,

Appendix D: Databases Utilized for Chapter 3.3

Variable	Database	Codes
Age	RPDB	
Sex	RPDB	
Income quintile	RPDB	
Rostered to family doctor	CAPE	
Charlson comorbidity status	CIHI-DAD	
Coronary artery disease (excluding angina)	CIHI-DAD NACRS OHIP	ICD10: "I21", "I22", "Z955", "T822", "I25" CCI: "1IJ50", "1IJ76" OHIP fee codes: "R741", "R742", "R743", "G298", "E646", "E651", "E652", "E654", "E655", "Z434", "Z448" OHIP Dx codes: "410", "412"
Cerebrovascular disease	CIHI-DAD NACRS	ICD10: "I60", "I600", "I601", "I602", "I603", "I604", "I605", "I606", "I607", "I608", "I609", "I61", "I610", "I611", "I612", "I613", "I614", "I615", "I616", "I618", "I619", "I630", "I631", "I632", "I633", "I634", "I635", "I638", "I639", "I64", "H341", "G450", "G451","G452","G453","G458","G459","H340" OHIP Dx codes: "436", "432", "435"
Diabetes	ODD (ICES validated cohort)	Lorraine L. Lipscombe, Jeremiah Hwee, Lauren Webster, Baiju R. Shah, Gillian L. Booth and Karen Tu. Identifying diabetes cases from administrative data: a population-based validation study. BMC Health Services

Peripheral vascular disease	CIHI-DAD OHIP	ICD10: "I700", "I702", "I708", "I709", "I731", "I738", "I739", "K551" CCI: "1KA76", "1KA50", "1KE76", "1KG50", "1KG57", "1KG76MI", "1KG87", "1IA87LA", "1IB87LA", "1IC87LA", "1ID87", "1KA87LA", "1KE57" OHIP fee codes: "R787", "R780", "R797", "R804", "R809", "R875", "R815", "R936", "R783", "R784","R785", "E626", "R814", "R786", "R937", "R860", "R861", "R855", "R856", "R933", "R934", "R791", "E672", "R794", "R813", "R867", "E649"
Chronic kidney disease	CIHI-DAD OHIP	ICD10: "E102", "E112", "E132", "E142", "I12", "I13", "N00", "N01", "N02", "N03", "N04", "N05", "N06", "N07", "N08", "N10", "N11", "N12", "N13", "N14", "N15", "N16", "N17", "N18", "N19", "N20", "N21", "N22", "N23" OHIP Dx codes: "403", "585"
Pancreatitis	CIHI-DAD	ICD10: "K85", "B252", "B263", "K860", "K861"
Hypertension	HYPER (ICES validated cohort)	Tu K, Chen Z, Lipscombe LL, Canadian Hypertension Education Program Outcomes Research Taskforce. Prevalence and incidence of hypertension from 1995 to 2005: a population-based study. Canadian Medical Association Journal. 2008 May 20;178(11):1429-35.
Chronic liver disease	CIHI-DAD NACRS OHIP	ICD10: "B16", "B17", "B18", "B19", "I85", "R17", "R18", "R160", "R162", "B942", "Z225", "E831", "E830", "K70", "K713", "K714", "K715", "K717", "K721", "K729", "K73", "K74", "K753", "K754", "K758", "K759", "K76", "K77"

		OHIP Dx codes: "571", "573", "070" OHIP fee codes: "Z551", "Z554"
Alcohol use	CIHI-DAD	ICD10: "E244", "E512", "E52", "F10", "G312", "G621", "G721", "I426", "K292", "K70", "K860", "T51", "X45", "X65", "Y15", "Y573", "Z502", "Z714", "Z721"
Hypothyroidism	CIHI-DAD	ICD10: "E00", "E01", "E02", "E03", "E890"
Multiple myeloma	CIHI-DAD	ICD10: "C900"
Obesity	CIHI-DAD	ICD10: "E66.25", "E66.26", "E66.27", "E66.28", "E66.29", "E66.0", "E66.1", "E66.2", "E66.8", "E66.9", "E66.2”,"E66.2"
Pregnancy	CIHI-DAD	ICD10: “Z34", P95", "Z371", "Z373", "Z374", "O00", "O021", "O03", "O04", "O08", "O60", "O42", "P072", "P073" CCI: "5CA88", "5CA20FK", "5CA24", "5MD5", "5MD6", "5MD4" OHIP FEE: "A922", "A920", "P001", "S752", "S785", "S756", "S768", "S784", "S770", "P006", "P007", "P008", "P009", "P010", "P011", "P013", "P014", "P014", "P015", "P016", "P016", "P018", "P020", "P022", "P023", "P027", "P028", "P029", "P030", "P031", "P032", "P034", "P036", "P038", "P039", "P041", "P042", "P045", "P046"

		OHIP DX: "632", "633", "634", "640"
Gallstone disease	CIHI-DAD	ICD10: "K80", "K81", "K82", "K83", "K85"
Acute myocardial infarction	CIHI-DAD	ICD10: "I21", "I22"
Nephrotic Syndrome	CIHI-DAD	ICD 10: "N044", "N022", "N043", "N040", "N08", "N048", "N049", "N033", "N052"
Ischemic Stroke:	CIHI-DAD	ICD-10: "I63", "I64", "I65", "I66", "I67", "I68
GP/FP visit	OHIP IPDB	OHIP spec: “00” IPDB Mainspecialty: “GP/FP”
Internist visit	OHIP IPDB	OHIP spec: “13” IPDB Mainspecialty: “INTERNAL MEDICINE”
Endocrinologist visit	OHIP IPDB	OHIP spec: “15” IPDB Mainspecialty: “ENDOCRINOLOGY”
HbA1c	OLIS	LOINC: “17855-8”, “17856-6“, “41995-2“, “4548-4”, “59261-8”, “71875-9”
LDL-C	OLIS	LOINC: “22748-8“, “39469-2“
HDL-C	OLIS	LOINC: “14646-4“, “32309-7
Non-HDL	OLIS	LOINC: “70204-3“
Total Cholesterol	OLIS	LOINC: “14647-2“
Corrected calcium	OLIS	LOINC: “29265-6”, “2000-8”, “1751-7”

ALT	OLIS	LOINC: “1742-6“, “1743-4“, “1744-2“
Lipid medication	ODB	
Triglyceride value	OLIS	LOINC: “14927-8“, “47210-0“

Abbreviations: RPDB**:** registered persons database; CAPE: Client Agency Program Enrolment database: CIHI-DAD: Canadian Institutes for Health Information’s Discharge Abstract Database; NARCRS: National Ambulatory Care Reporting System; OHIP: Ontario Health Insurance Program; ICD-10: the International Classification of Diseases 10th Revision; CCI: Canadian Classification of Health Interventions; HYPER: hypertension database; ODD: Ontario Diabetes Database; ODB: Ontario Drug Benefit (ODB) database. OLIS: Ontario Laboratories Information System (OLIS).

Appendix E: RECORD checklist for Chapter 3.3

	Item No	Recommendation	Reported
Title and abstract	1	1.1 The type of data used should be specified in the title or abstract. When possible, the name of the databases should be included.	Abstract
		1.2 If applicable, the geographic region and time frame within which the study took place should be reported in the title or abstract.	Abstract
		1.3 If linkage between databases was conducted for the study, this should be clearly stated in the title or abstract	Abstract
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Introduction
Objectives	3	State specific objectives, including any pre-specified hypotheses	Introduction
Methods			
Study design	4	Present key elements of study design early in the paper	Methods
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Methods
Participants	6	6.1 The methods of study population selection should be listed in detail. If this is not possible, an explanation should be provided.	Methods

		6.2 Any validation studies of the codes or algorithms used to select the population should be referenced. If validation was conducted for this study and not published elsewhere, detailed methods and results should be provided.	Methods
		6.3 If the study involved linkage of databases, consider use of a flow diagram or other graphical display to demonstrate the linkage process, including the number of individuals with linked data at each stage.	Figure 3.1
Variables	7	A complete list of codes and algorithms used to classify exposures, outcomes, confounders, and effect modifiers should be provided. If these cannot be reported, an explanation should be provided.	Appendix D
Data sources/ measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Methods, Appendix D
Bias	9	Describe any efforts to address potential sources of bias	Methods
Study size	10	Explain how the study size was arrived at	Methods
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Methods
Statistical methods	12	12.1 Describe all statistical methods, including those used to control for confounding	Methods
		12.2 Describe any methods used to examine subgroups and interactions	Methods

		12.3 Explain how missing data were addressed	Not applicable
		12.4 If applicable, explain how loss to follow-up was addressed	Not applicable
		12.5 Describe any sensitivity analyses	Not applicable
Data access and cleaning methods		12.6 Authors should describe the extent to which the investigators had access to the database population used to create the study population.	Methods
		12.7 Authors should provide information on the data cleaning methods used in the study	Methods
Linkage		12.8 State whether the study included person-level, institutional-level, or other data linkage across two or more databases. The methods of linkage and methods of linkage quality evaluation should be provided.	Methods
Results			
Participants	13	13.1 Describe in detail the selection of the persons included in the study (i.e. study population selection), including filtering based on data quality, data availability, and linkage. The selection of included persons can be described in the text and/or by means of the study flow diagram.	Results, Figure 3.1
Descriptive data	14	14.1 Give characteristics of study participants (e.g. demographic, clinical, social) and information on exposures and potential confounders	Results, Tables 3.5-3.8
		14.2 Indicate number of participants with missing data for each variable of interest	Results, Figure 3.1

		14.3 Summarize follow-up time (e.g. average and total amount)	Results, Table 3.10
Outcome data	15	Report numbers of outcome events or summary measures over time	Results, Figure 3.2 Table 3.6-3.9
Main results	16	16.1 Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g. 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Results, Table 3.8
		16.2 Report category boundaries when continuous variables were categorized	Tables 3.5 – 3.8
		16.3 If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable
Other analyses	17	Report other analyses done—e.g. analyses of subgroups and interactions, and sensitivity analyses	Results, Table 3.6, 3.7
Discussion			
Key results	18	Summarize key results with reference to study objectives	Discussion
Limitations	19	Discuss the implications of using data that were not created or collected to answer the specific research question(s). Include discussion of misclassification bias, unmeasured confounding, missing data and changing eligibility over time, as they pertain to the study being reported.	Discussion
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of	Discussion

		analyses, results from similar studies, and other relevant evidence	
Generalizability	21	Discuss the generalizability (external validity) of the study results	Discussion
Other information			
Funding	22	22.1 Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Declarations
Accessibility of protocol, raw data and programming code	22	22.2 Authors should provide information on how to access any supplemental information such as the study protocol, raw data, or programming code.	The dataset from this study is held securely in coded form at the Institute for Clinical Evaluative Sciences (ICES). While data sharing agreements prohibit ICES from making the dataset publicly available, access may be granted to those who meet pre-specified criteria for confidential access, available at www.ices.on.ca/DAS. The full dataset creation plan and underlying analytic code are available from the authors

			upon request, understanding that the programs may rely upon coding templates or macros that are unique to ICES.

*Reference: Benchimol EI, Smeeth L, Guttmann A, Harron K, Moher D, Petersen I, et al. The REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) statement. PLoS Med. 2015;12(10):e1001885.

*Checklist is protected under Creative Commons Attribution (CC BY) license.

Appendix F: Paper-based survey for chapter 4.2

PAPER-BASED SURVEY

STUDY TITLE: Patient Impact of Genetic Testing for Familial Hypercholesterolemia

PRINCIPAL INVESTIGATOR: Dr. Amanda Berberich, MD

Dr. Robert Hegele, MD;

Study Participant Number:

Date of Completion:

Part 1: These questions ask for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities. **Answer each question by choosing just one answer**. If you are unsure how to answer a question, please give the best answer you can.

---

1. In general, would you say your health is:

Excellent	Very good	Good	Fair	Poor

The following questions are about activities you might do during a typical day. Does <u>your health now limit you</u> in these activities? If so, how much?

	YES, limited a lot	**YES, limited a little**	**NO, not limited at all**
2. **Moderate activities** such as moving a table, pushing a vacuum cleaner, bowling, or playing golf.			
3. Climbing **several** flights of stairs.			

During the **<u>past 4 weeks</u>**, have you had any of the following problems with your work or other regular daily activities **<u>as a result of your physical health</u>**?

	YES	**NO**
4. **Accomplished less** than you would like		
5. Were limited in the **kind** of work or other activities.		

During the **<u>past 4 weeks</u>**, have you had any of the following problems with your work or other regular daily activities **<u>as a result of any emotional problems</u>** (such as feeling depressed or anxious)?

	YES	**NO**
6. **Accomplished less** than you would like		
7. Did work of activities **less carefully than usual.**		

8. During the **past 4 weeks**, how much **did pain interfere** with your normal work (including work outside the home and housework)?

Not at all	A little bit	Moderately	Quite a bit	Extremely

These questions are about how you have been feeling during the **past 4 weeks**.
For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the **past 4 weeks**…

	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
9. Have you felt calm & peaceful?						
10. Have you had a lot of energy?						
11. Have you felt down-hearted and blue?						

12. During the **past 4 weeks**, how much of the time has your **physical health or emotional problems** interfered with your social activities (like visiting friends, relatives, etc.)?

All of the time	Most of the time	Some of the time	A little of the time	None of the time

PART 2: You have been prescribed medication for your high cholesterol. Individuals have identified several issues regarding their medication-taking behavior and we are interested in your experiences. There is no right or wrong answer. Please answer each question based on your personal experience with your high cholesterol medication.

	YES	NO
1. Do you sometimes forget to take your high cholesterol pills?		
2. People sometimes miss taking their medications for reasons other than forgetting. Thinking over the past two weeks, were there any days when you did not take your cholesterol medication?		
3. Have you ever cut back or stopped taking your medication without telling your doctor because you felt worse when you took it?		
4. When you travel or leave home, do you sometimes forget to bring along your cholesterol medicine?		
5. Did you take your cholesterol medication yesterday?		
6. When you feel like your cholesterol is under control, do you sometimes stop taking your medicine?		
7. Taking medication everyday is a real inconvenience for some people. Do you ever feel hassled about sticking to you cholesterol treatment plan?		

8. How often do you have difficulty remembering to take all your medications?

Never/Rarely   Once in a while   Sometimes   Usually   All the time

PART 3:

DIET: To answer these questions, think about your eating habits during the past year. Indicate how often you eat the following foods. Please include all meals, snacks, and food eaten out.

---

1. Lettuce or green leafy salad, with or without other vegetables

   Less than 1/week   1/week   2-3 times/week   1/day   2 or more times/day

2. Fruit, including fresh, canned, or frozen, but not including juices

   Less than 1/week   1/week   2-3 times/week   1/day   2 or more times/day

3. High-fibre cereals, such as Raisin Bran or Fruit and Fibre, cooked oatmeal, or whole-grain breads such as whole wheat, rye or pumpernickel

   Less than 1/week   1/week   2-3 times/week   1/day   2 or more times/day

---

SMOKING: Please indicate your smoking habits below.

1. Are you a smoker?

   Yes   No

2. If no, did you ever smoke?

   Yes   No

ALCOHOL: Please indicate how many drinks of the following types of alcohol you consume in an average week.

Wine _______ drinks (3-5oz)   Beer _______ drinks (10-12 oz or 1 bottle)   Spirits ______ drinks (1-1.5 oz)

EXERCISE: To answer the following questions, please indicate how many times per week you take part in the following activities, for at least 30 minutes or more at a time.

---

1. Light exercise such as the following:
-light gardening and light housework (eg dusting, sweeping, vacuuming)
-leisurely walking (eg walking your dog)
-bowling, fishing, carpentry, playing a musical instrument
-volunteer work

0/week   1-3 times/week   4-7 times/week   8 or more times/week

2. Moderate exercise, such as the following:
-brisk walking
-bicycling, skating, swimming, curling
-gardening (eg raking, weeding, digging)
-dancing, Tai Chi, or moderate exercise classes

0/week   1-3 times/week   4-7 times/week   8 or more times/week

3. Vigorous exercise, such as the following:
-running, bicycling, cross-country skiing, lap swimming, aerobics
-heavy yard work
-weight training
-soccer, basketball or other league sports

0/week   1-3 times/week   4-7 times/week   8 or more times/week

---

LIFE STRESS: To answer this question, please indicate the number you feel best corresponds to the level of stress in your everyday life.

6   5   4   3   2   1

Not at all stressful   Very Stressful

PART 4:

Over the last 2 weeks, how often have you been bothered by the following problems?

	Not at all	**Several days**	**Over half the days**	**Nearly every day**
1. Feeling nervous, anxious, or on edge				
2. Not being able to stop or control worrying				
3. Worrying too much about different things				
4. Trouble relaxing				
5. Being so restless that it's hard to sit still				
6. Becoming easily annoyed or irritable				
7. Feeling afraid as if something awful might happen				

8. If you checked off any problems, how difficult have these made it for you to do your work, take care of things at home, or get along with other people?

Not difficult at all	Somewhat difficult	Very difficult	Extremely difficult

PART 5:

1. How would you categorize your risk of having a heart attack or stroke within the next 10 years?

Low	Moderate	High	Very High

2. How do you feel having a genetic test for familial hypercholesterolemia has impacted you?

Strongly negative impact	Mildly negative impact	No impact	Mildly positive impact	Strongly positive impact

3. How likely are you to recommend genetic testing to your family members?

Not at all likely	Somewhat unlikely	Neither likely or unlikely	Somewhat likely	Very likely

4. Did you make changes to your habits in any of the following areas after learning your genetic testing results?

a) Physical activity:

Significantly decreased	Mildly decreased	Stayed the same	Mildly increased	Significantly increased

b) Healthy dietary choices:

Significantly worsened	Mildly worsened	Stayed the same	Mildly improved	Significantly improved

c) Taking all medications as prescribed:

Significantly worsened	Mildly worsened	Stayed the same	Mildly improved	Significantly improved

Curriculum Vitae:

Amanda J Berberich

H-index: 12 | Citations: 757

EDUCATION:

Degrees and Diplomas

2017 - 2022	Doctor of Philosophy, Western University, Biochemistry, Doctor of Philosophy Supervisor: Dr. Robert Hegele, London, Ontario, Canada
2017 - 2022	Fellowship, Western University, Medicine, Postgraduate, Clinical Investigators Program, Supervisor: Dr. Robert Hegele, London, Ontario, Canada
2015 - 2017	Fellowship, Western University, Medicine, Postgraduate, Endocrinology, , Supervisor: Dr. Stan Van Uum, London, Ontario, Canada
2011 - 2015	Resident, Western University, Medicine, Postgraduate, Internal Medicine, Supervisor: Dr. Sherri Lynn Kane, London, Ontario, Canada
2007 - 2011	Doctor of Medicine, SUNY Upstate Medical University, Medicine, Doctor (Medical), Syracuse, New York, United States
2004 - 2007	Bachelor of Medical Sciences, Western University, Medicine, Undergraduate, London, Ontario, Canada

QUALIFICATIONS, CERTIFICATIONS AND LICENSES:

2017 - present	Certified Endocrinologist, Royal College of Physicians and Surgeons of Canada, License, Ontario, Canada
2016 - present	Fellow, Royal College of Physicians and Surgeons of Canada, License, Ontario, Canada

ACADEMIC APPOINTMENTS:

2019 - present	Assistant Professor, Department of Medicine, Endocrinology, Schulich School of Medicine & Dentistry, The University of Western Ontario

HONOURS AND AWARDS:

2017	Dr. Fernand Labrie Research Fellowship Award, A scholarship award granted to a 3rd- or 4th-year MD research fellow who is pursuing additional research training in the field of Endocrinology and Metabolism. Canadian Society of Endocrine and Metabolism, $30,000, Type: Research award
2015	Outstanding Abstract Award, Awarded to outstanding abstracts submitted to early career members who have been accepted to present their research during the annual Endocrine Society meeting, Endocrine Society
2013	Resident Research Day Best Oral Presentation, Award presented to a resident PGY1-PGY3 judged to have the best overall oral research presentation presented at resident research day, Department of Medicine, Western University
2011	Gold Humanism Honor Society, One of 20 graduating medical students nominated for this award presented for practicing integrity, excellence, compassion, altruism, respect and empathy in patient care. SUNY Upstate Medical University

PUBLICATIONS:

Research Articles:

1. **Berberich** AJ, Huot C, Cao H, McIntyre AD, Robinson JF, Wang J, Hegele RA. Copy Number Variation in GCK in Patients With Maturity-Onset Diabetes of the Young. J Clin Endocrinol Metab, 2019 Aug 1; 104 (8): 3428-3436, DOI: 10.1210/jc.2018-02574.
2. **Berberich** AJ, Ziada A, Zou GY, Hegele RA. Conservative management in hypertriglyceridemia-associated pancreatitis. J Intern Med, 2019 May 11, , DOI: 10.1111/joim.12925.
3. Dron JS, Wang J, **Berberich** AJ, Iacocca MA, Cao H, Yang P, Knoll J, Tremblay K, Brisson D, Netzer C, Gouni-Berthold I, Gaudet D, Hegele RA. Large-scale deletions of the ABCA1 gene in patients with hypoalphalipoproteinemia. J Lipid Res, 2018 Aug 1; 59 (8): 1529-1535, **Author**, DOI: 10.1194/jlr.P086280.
4. Hegele RA, **Berberich** AJ, Ban MR, Wang J, Digenio A, Alexander VJ, D’Erasmo L, Arca M, Jones A, Bruckert E, Stroes ES, Bergeron J, Civeira F, Witztum JL, Gaudet D. Clinical and biochemical features of different molecular etiologies of familial chylomicronemia. J Clin Lipidol, 2018 Jul 1; 12 (4): 920-927.e4, DOI: 10.1016/j.jacl.2018.03.093.

5. **Brahm AJ**, Wang G, Wang J, McIntyre AD, Cao H, Ban MR, Hegele RA. Genetic Confirmation Rate in Clinically Suspected Maturity-Onset Diabetes of the Young. Can J Diabetes, 2016 Dec 1; 40 (6): 555-560, DOI: 10.1016/j.jcjd.2016.05.010.
6. **Berberich AJ**, Wang J, Cao H, McIntyre AD, Spaic T, Miller DB, Stock S, Huot C, Stein R, Knoll J, Yang P, Robinson JF, Hegele RA. Simplifying Detection of Copy-Number Variations in Maturity-Onset Diabetes of the Young. Can J Diabetes. 2021 Feb;45(1):71-77. doi: 10.1016/j.jcjd.2020.06.001. Epub 2020 Jun 8. PMID: 33011132.
7. **Berberich AJ**, Mokashi A, McIntyre AD, Robinson JF, Cao H, Wang J, Hegele RA. Bioinformatic detection of copy number variation in HNF4A causing maturity onset diabetes of the young. Clin Genet. 2019 Oct 1, 96. (4): p.376-377, DOI: 10.1111/cge.13599
8. Lazarte J**, Berberich AJ**, Wang J, Hegele RA. A cautionary tale: Is this APOB whole-gene duplication actually pathogenic? J Clin Lipidol. 2020 Sep-Oct;14(5):631-635. doi: 10.1016/j.jacl.2020.06.007. Epub 2020 Jun 15. PMID: 32654994.
9. Gill PK, Dron JS, **Berberich AJ,** Wang J, McIntyre AD, Cao H, Hegele RA. Combined hyperlipidemia is genetically similar to isolated hypertriglyceridemia. J Clin Lipidol. 2021 Jan-Feb;15(1):79-87. doi: 10.1016/j.jacl.2020.11.006. Epub 2020 Nov 24. PMID: 33303402.
10. **Berberich AJ**, Ouédraogo AM, Shariff SZ, Hegele RA, Clemens KK. Incidence, predictors and patterns of care of patients with very severe hypertriglyceridemia in Ontario, Canada: a population-based cohort study. Lipids Health Dis. 2021 Sep 3;20(1):98. doi: 10.1186/s12944-021-01517-6. PMID: 34479547; PMCID: PMC8417954.

Book Chapters:

1. **Amanda Brahm**, Robert A. Hegele. Primary Hypertriglyceridemia. In:Abhimanyu Garg, editor(s). Dyslipidemias; 2015,

Case Reports:

1. **Berberich AJ**, Penava D, Sun D, MacDougall A, Lum A, Van Uum S. High aldosterone, hypertension and adrenal adenoma in a 36-year-old pregnant patient: Is this primary aldosteronism? Obstet Med. 2020 Jun;13(2):88-91. doi: 10.1177/1753495X18786422. Epub 2018 Nov 4. PMID: 32714441; PMCID: PMC7359658.
2. Khanna P, Khatami A, Swiha M, Rachinsky I, Kassam Z, **Berberich AJ**. Severe hypercalcemia secondary to paraffin oil injections in a bodybuilder with significant findings on scintigraphy. AACE Clin Case Rep. 2020 Jun 23;6(5):e234-e238. doi: 10.4158/ACCR-2020-0007. PMID: 32984528; PMCID: PMC7511097.

Review Articles:

1. **Brahm A**, Hegele RA. Hypertriglyceridemia. Nutrients, 2013 Mar 22; 5 (3): 981-1001, DOI: 10.3390/nu5030981.
2. **Berberich AJ**, Hegele RA. The role of genetic testing in dyslipidaemia. Pathology. 2019 Feb 1, 51. (2): p.184-192, DOI: 10.1016/j.pathol.2018.10.014.
3. **Berberich AJ**, Hegele RA. The complex molecular genetics of familial hypercholesterolaemia. Nat Rev Cardiol. 2019 Jan 1, 16. (1): p.9-20, DOI: 10.1038/s41569-018-0052-6.
4. **Berberich AJ**, Hegele RA. Nutraceuticals in 2017: Nutraceuticals in endocrine disorders. Nat Rev Endocrinol. 2018 Feb 1, 14. (2): p.68-70, DOI: 10.1038/nrendo.2017.169.
5. **Berberich AJ**, Hegele RA. Lomitapide for the treatment of hypercholesterolemia. Expert Opin Pharmacother. 2017 Aug 1, 18. (12): p.1261-1268, DOI: 10.1080/14656566.2017.1340941.
6. **Brahm AJ,** Hegele RA. Lomitapide for the treatment of hypertriglyceridemia. Expert Opin Investig Drugs. 2016 Dec 1, 25. (12): p.1457-1463, DOI: 10.1080/13543784.2016.1254187.
7. **Brahm AJ**, Hegele RA. Combined hyperlipidemia: familial but not (usually) monogenic. Curr Opin Lipidol. 2016 Apr 1, 27. (2): p.131-40, DOI: 10.1097/MOL.0000000000000270.
8. **Brahm AJ**, Hegele RA. Chylomicronaemia--current diagnosis and future therapies. Nat Rev Endocrinol. 2015 Jun 1, 11. (6): p.352-62, DOI: 10.1038/nrendo.2015.26
9. Jeraj N, Hegele RA, **Berberich AJ**. Apolipoprotein genetic variants and hereditary amyloidosis. Curr Opin Lipidol. 2021 Apr 1;32(2):132-140. doi: 10.1097/MOL.0000000000000736. PMID: 33395107.
10. **Berberich AJ**, Hegele RA. Lipid effects of glucagon-like peptide 1 receptor analogs. Curr Opin Lipidol. 2021 Jun 1;32(3):191-199. doi: 10.1097/MOL.0000000000000750. PMID: 33900276.
11. **Berberich, AJ**, Hegele RA. A modern approach to dyslipidemia. Endocrine Reviews. Accepted for publication Oct 2021.

Commentaries/Editorials:

1. **Berberich AJ**, Hegele RA. LDL cholesterol: lower, faster, younger? Lancet Diabetes Endocrinol, 2020 Jan 1; 8 (1): 5-7, DOI: 10.1016/S2213-8587(19)30389-4.
2. **Berberich AJ**, Hegele RA. Secondary causes of chylomicronemia: defining the underside of the iceberg. J Intern Med. 2018 Apr 1, 283. (4): p.405-407, DOI: 10.1111/joim.12727.
3. **Brahm AJ**, Hegele RA. Incident Diabetes With Statins: Biology, Artifact, or Both? Can J Cardiol. 2015 Aug 1, 31. (8): p.963-5, DOI: 10.1016/j.cjca.2015.06.010.

4. **Brahm A**, Hegele RA. Is plant-based cardioprotection evidence-based? Can J Cardiol. 2014 Oct 1, 30. (10): p.1142-4, DOI: 10.1016/j.cjca.2014.04.030.
5. **Berberich AJ**, Ho R, Hegele RA. Whole genome sequencing in the clinic: empowerment or too much information? CMAJ. 2018 Feb 5, 190. (5): p.E124-E125, DOI: 10.1503/cmaj.180076.
6. **Berberich AJ**, Hegele RA. Research digest: next-generation lipoprotein therapeutics. Lancet Diabetes Endocrinol. 2020 Mar;8(3):190. doi: 10.1016/S2213-8587(20)30032-2. PMID: 32085826.

PRESENTATIONS:

Conference Presentations

Local

1. **Presenter**, Copy Number Variation Analysis in the Diagnosis of MODY, London Health Research Day, 2018 May, London, Ontario, Canada
2. **Presenter**, Copy Number Variation Analysis in the Diagnosis of MODY, Biochemistry Research Day, 2018 Jan, London, Ontario, Canada
3. **Presenter**, High aldosterone levels, hypertension and adrenal adenoma in a 36 year-old pregnant patient: Is this primary hyperaldosteronism? Resident Research Day, 2017 May, London, Ontario, Canada
4. **Presenter**, Improving the Timing of Impatient Diabetes Education to Prevent Delayed Discharges, Resident Research Day, 2017 May, London, Ontario, Canada
5. **Presenter**, Genetic Confirmation Rate in Clinically Suspected MODY, Resident Research Day, 2016 May 1, London, Ontario, Canada
6. **Presenter**, Rare LMF1 Mutations in Patients with Hypertriglyceridemia. Resident Research Day, 2013 May, London, Ontario, Canada
7. **Presenter**, Rare LMF1 Mutations in Patients with Hypertriglyceridemia, London Health Sciences Research Day, 2013 Mar, London, Ontario, Canada

National

1. **Presenter**, High aldosterone levels, hypertension and adrenal adenoma in a 36 year-old pregnant patient: Is this primary hyperaldosteronism? Canadian Society of Endocrinology and Metabolism, 2016 Oct, Ottawa, Ontario, Canada
2. **Presenter**, Genetic Confirmation Rate in Clinically Suspected MODY. Canadian Society of Endocrinology and Metabolism, 2015 Nov, Vancouver, British Columbia, Canada

International

1. **Presenter**, Simplifying detection of copy number variations causing MODY 5, American Diabetes Association, 2019 Jun 7, San Francisco, California, United States
2. **Presenter**, Copy Number Variation Analysis in the Diagnosis of MODY, International Symposium on Atherosclerosis, 2018 Jun, Toronto, Ontario, Canada
3. **Presenter**, To PLEX or not to PLEX: Managing Hypertriglyceride Pancreatitis, International Symposium on Atherosclerosis, 2018 Jun, Toronto, Ontario, Canada
4. **Presenter**, High aldosterone levels, hypertension and adrenal adenoma in a 36 year-old pregnant patient: Is this primary hyperaldosteronism? Endocrine society, 2017 Apr, Orlando, Florida, United States

Supervised:

1. Parul Khanna, Determining the Undetermined: the role of tumor tissue staining for interpretation of inconclusive genetic testing results in patients with pheochromocytomas and paragangliomas. (cancelled), 2020 Mar 28, California, United States, **Supervisor**
2. Parul Khanna, Severe hypercalcemia secondary to paraffin oil injections in a bodybuilder with significant findings on scintigraphy. 2019 Oct 2, CSEM/Diabetes Canada, Winnipeg, Manitoba, Canada, **Supervisor**

Symposia:

Local

1. **Presenter**, Clinician Scientist Trainee Symposium, 2019 Aug 16, London, Ontario, Canada

Regional

1. **Invited Lecturer**, Discover Robarts (Cancelled), 2020 Mar 18, Community Outreach Activity

National

1. **Invited Lecturer**, I Am So Tired: Adrenal Insufficiency or Fatigue, Canadian Endocrine Review Course, 2019 Apr 27, London, Ontario, Canada
2. **Invited Lecturer**, Genetic Testing in the Endocrine Clinic: Ready for Prime Time? Canadian Endocrine Update (CEU) Scientific Meeting, 2019 Apr 26, London, Ontario, Canada