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Original Research

Genetic Confirmation Rate in Clinically Suspected Maturity-Onset Diabetes of the Young

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ABSTRACT

Objectives: Maturity-onset diabetes of the young (MODY) is the most common form of monogenic diabetes, reportedly accounting for 2% to 5% of all cases of diabetes. In samples from Canadian patients referred for molecular genetic confirmation of a clinically suspected MODY, we determined the prevalence of likely disease-causing DNA variants in known MODY genes.
Methods: Between 1999 and 2015, our centre received requests from colleagues for DNA sequencing of 96 samples from unrelated Canadian patients with clinically suspected MODY. Prior to 2012, we used Sanger sequencing, and since 2012 we have used targeted next-generation sequencing.
Results: Of 96 samples received, 39 (40.6%) had a likely rare causal variant in 1 of 8 known MODY genes. Of these, 20 (51.3%) and 19 (48.7%) were diagnosed by Sanger and targeted next-generation sequencing, respectively. The 39 mutation-positive samples had 1 of 39 rare variants, of which the majority were in genes encoding either glucokinase (*GCK*, or MODY2) or hepatocyte nuclear factor 1-alpha (*HNF1A*, or MODY3). Furthermore, 12 (30.8%) of the detected rare variants had been unreported previously but were likely to have been clinically significant according to standard bioinformatic methods. An additional 6 samples had rare variants in MODY genes that were of uncertain clinical significance.
Conclusions: The findings suggest that clinical suspicion for MODY has a diagnostic yield of ~40% at the molecular level. Confirmatory genetic testing in patients suspected to have MODY allows for definitive diagnoses which, in turn, may guide management and provide rationales for screening other family members presymptomatically.

Mots clés :
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RÉSUMÉ

Objectifs : Le diabète de la maturité apparaissant chez le jeune (MODY) est la forme la plus fréquente de diabète monogénique, qui représenterait 2 % à 5 % de tous les cas de diabète. Dans les échantillons de patients canadiens orientés pour la confirmation en génétique moléculaire d'un MODY cliniquement suspecté, nous avons déterminé la prévalence de variants de l'ADN possiblement pathogènes dans les gènes connus du MODY.
Méthodes : Entre 1999 et 2015, notre centre a reçu des requêtes de confrères pour effectuer le séquençage de l'ADN de 96 échantillons de patients canadiens non apparentés chez qui l'on suspectait cliniquement un MODY. Avant 2012, nous avons utilisé le séquençage de Sanger et, à partir de 2012, nous avons utilisé le séquençage ciblé de nouvelle génération.
Résultats : Parmi les 96 échantillons reçus, 39 (40,6 %) avaient des variants causals vraisemblablement rares dans 1 des 8 gènes connus de MODY, dont 20 (51,3 %) et 19 (48,7 %) ont été respectivement diagnostiqués par le séquençage de Sanger et le séquençage ciblé de nouvelle génération. Les 39 échantillons positifs pour les mutations avaient 1 des 39 variants rares, dont la majorité était dans les gènes codant soit la glucokinase (*GCK*, ou MODY2) ou le facteur nucléaire hépatocytaire 1–alpha (*HNF1A*, ou MODY3). De plus, 12 (30,8 %) des variants rares détectés n'avaient pas été rapportés précédemment, mais étaient susceptibles d'avoir été cliniquement significatifs selon les méthodes bio-informatiques standards. Six autres échantillons avaient des variants rares dans les gènes de MODY dont l'importance clinique demeurait incertaine.

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Conclusions : Les résultats suggèrent que la suspicion de MODY a un rendement diagnostique de ~40 % au niveau moléculaire. Les épreuves génétiques de confirmation chez les patients chez qui l'on suspecte un MODY permettent des diagnostics définitifs qui, successivement, peuvent orienter la prise en charge et fournir des éléments qui justifient le dépistage présymptomatique d'autres membres de la famille.

Introduction

Maturity-onset diabetes of the young (MODY) is an umbrella term for a genetically heterogeneous collection of monogenic (single-gene) diabetes syndromes (1). 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 clinical presentation in MODY patients can resemble type 2 diabetes, but often at <25 years of age. The Canadian Diabetes Association (CDA) categorizes MODY under "genetic defects of beta-cell function," with subclassifications according to the gene defect (2).

MODY subtypes generally follow an autosomal dominant inheritance pattern and result predominantly from rare variants in genes that cause compromised beta-cell function (Appendix 1). The prevalence of MODY has been estimated to be 2% to 5% of all cases of diabetes (1). Characteristic features of MODY include onset before age 25, with a strong multigenerational family history of diabetes and an autosomal dominant pattern of inheritance (1,3–8). Other features include non-insulin dependence with detectable circulating C-peptide levels for 5 years following diagnosis, absence of autoantibodies, no episodes of diabetic ketoacidosis and prolonged survival after onset of symptoms (3,4,6).

Individual MODY subtypes are distinct entities that are defined by their underlying mutations. MODY caused by mutations in glucokinase (GCK-MODY, formerly MODY2) and hepatocyte nuclear factor 1-alpha (HNF1A-MODY, formerly MODY3) are the most prevalent forms, involving about three-quarters of all patients with MODY. Mutations can affect glucose sensing, as in GCK-MODY resulting in a mild diabetes phenotype (3). They may also affect transcription factors, collectively termed *transcription factor MODY* (MODY1 and MODY3-7 in older nomenclature) (4,6). These MODY subtypes tend to follow a progressive course (3,4,6) but are often initially highly responsive to sulfonylureas (SUs) (3). Clinical and molecular features of the MODY subtypes are shown in the Supplementary Table (Appendix 1).

The role of widespread genetic testing to screen for MODY is controversial, given the associated cost, although that has declined dramatically. Furthermore, the impact of a definitive MODY diagnosis can be significant, especially for young individuals misdiagnosed with type 1 diabetes; these patients are often able to stop insulin and transition to SU therapy, with improved glycemic control (4,6). Similarly, patients with SU-sensitive MODY who have been misdiagnosed with "garden–variety" type 2 diabetes may also benefit from molecular diagnoses because they are commonly well maintained on SU monotherapy for decades prior to advancing to additional treatment (4,6). Also, patients with GCK-MODY generally require less intensive treatment and have less risk for microvascular complications, which may be reassuring to patients and families and may reduce long-term monitoring and treatment costs (4). 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 presymptomatic screening for early intervention and counselling.

Who should be screened genetically for MODY? 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. Over the past 16 years, we have received 96 samples 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 molecular diagnoses and to assess whether high provider clinical suspicion is a sufficient criterion for proceeding with genetic testing.

Methods

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, on the basis of clinical assessment, have referred patient samples for DNA analysis, appreciating 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 physicians. There was no cost to either referring physicians or patients, 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).

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 through 6, using the former nomenclature. If a causal mutation was detected, it was reported to the patients and referring physicians, 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 (9), which has greatly enhanced our ability to detect clinically relevant mutations. LipidSeq is high-throughput platform that has been designed to screen simultaneously for DNA variants in dozens of genes linked to metabolic and dyslipidemia disorders, including all 13 MODY subtypes (9). The coding region of each gene is sequenced, together with all intron-exon boundaries, at least 150 base pairs of flanking intronic sequence and at least 500 base pairs of the promoter and 3'-untranslated region. Samples in which no MODY mutation was found by Sanger sequencing were reanalyzed using the LipidSeq NGS panel. Samples received after 2012 underwent processing directly on LipidSeq. When multiple samples from affected individuals with the same kindred were received, the first tested affected individual was considered to be 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 (9). Variant frequencies in control populations were determined from the 1000 Genomes and Exome Variant Server databases. A variant was considered to be causative if it had been previously reported as such in the literature or in the Human Genome Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk/). Rare variants detected in MODY genes that had not been reported previously in HGMD were