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Monogenic Diabetes in Overweight and Obese Youth Diagnosed with Type 2 Diabetes: The TODAY Clinical Trial

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Abstract

Purpose—Monogenic diabetes accounts for 1–2% of diabetes cases. It is often undiagnosed, which may lead to inappropriate treatment. This study was performed to estimate the prevalence of monogenic diabetes in a cohort of overweight/obese adolescents diagnosed with type 2 diabetes (T2D).

Methods—Sequencing using a custom monogenic diabetes gene panel was performed on a racially/ethnically diverse cohort of 488 overweight/obese adolescents with T2D in the TODAY clinical trial. Associations between having a monogenic diabetes variant and clinical characteristics and time to treatment failure were analyzed.

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†A listing of the TODAY Study Group can be found in the online appendix

Conflict of Interest

The authors of this study have no conflicts of interest to disclose, including consultancies, stock ownership, or other equity interests, patent licensing arrangements and payments for conducting or publicizing a study described in the manuscript.

Results—Over four percent (22/488) had genetic variants causing monogenic diabetes (7 *GCK*, 7 *HNF4A*, 5 *HNF1A*, 2 *INS*, and 1 *KLF11*). Patients with monogenic diabetes had a statistically, but not clinically, significant lower BMI Z-score, lower fasting insulin, and higher fasting glucose. Most (6/7) patients with *HNF4A* variants rapidly failed TODAY treatment across study arms (HR=5.03, p=0.0002), while none with *GCK* variants failed treatment.

Conclusions—Discovery of 4.5% of patients with monogenic diabetes in an overweight/obese cohort of children and adolescents with T2D suggests monogenic diabetes diagnosis should be considered in children and adolescents without diabetes-associated autoantibodies and maintained C-peptide, regardless of BMI, as it may direct appropriate clinical management.

Keywords

monogenic diabetes; obesity; youth; type 2 diabetes; clinical trial

INTRODUCTION

Maturity-onset diabetes of the young (MODY), the most common category of monogenic diabetes, results from a single variant in an individual patient in one of 14 known genes. In the 1970s, the term MODY was created to characterize patients described by Dr. Stefan Fajans as having a non-insulin-dependent form of diabetes at a young age.1,[1] Epidemiological studies across Europe determined that MODY accounts for approximately 1–2% of all diabetes.[3] *GCK*-MODY, *HNF1A*-MODY, and *HNF4A*-MODY account for at least 85% of MODY cases.[4–6] MODY generally presents in an autosomal dominant pattern of inheritance early in life as non-insulin requiring hyperglycemia. Correct characterization of monogenic diabetes is important for optimal patient treatment since the most common etiologies can be effectively treated with methods different from first-line treatments for type 1 diabetes (T1D) (insulin) or T2D (metformin). Patients with *HNF1A*-MODY and *HNF4A*-MODY are effectively treated with oral sulfonylurea therapy.[7,8] Patients with *GCK*-MODY have mildly elevated baseline blood glucose concentrations that commonly do not require treatment and do not lead to diabetic complications.[9] Therefore, proper diagnosis of monogenic diabetes can lead to more effective, less invasive, and less expensive treatment for patients and potentially family members with the same variant.

American Diabetes Association (ADA) guidelines suggest a diagnosis of monogenic diabetes be considered when diabetes is diagnosed in the first 6 months of life, when the patient does not have features of T1D (negative for diabetes-associated antibodies) or T2D (nonobese, lacking other metabolic features) especially when there is a strong family history of diabetes, or when there is stable, mildly elevated fasting blood glucose.[10] However, studies indicate these guidelines are either not utilized or fail to detect many cases of monogenic diabetes. The SEARCH study for diabetes in youth discovered that greater than 90% of patients with *GCK*, *HNF1A*, or *HNF4A* variants were misdiagnosed as T1D (36%)

[1]Since then, it has been suggested MODY be changed to “familial young-onset diabetes” because of the discovery of multiple causative genes and better understanding of the gene-specific patient characteristics combined with the increased prevalence of type 2 diabetes (T2D) in childhood.2 While this nomenclature change is appropriate, the term MODY will be used in this article to differentiate from other forms of monogenic diabetes, such a neonatal or syndromic forms of monogenic diabetes, and because the term is well-recognized by the general population.

or T2D (51%), and only 19% of patients with MODY variants had treatment appropriate to their etiology.[11] Many factors contribute to the underdiagnosis of monogenic diabetes, including: heterogeneity of monogenic diabetes patient characteristics, similarity between monogenic diabetes and the more common forms of diabetes (especially with increasing prevalence of T2D in children and adolescents), cost of genetic testing, lack of insurance reimbursement, and lack of awareness among healthcare providers. With such high rates of overweight and obesity in young people, one might expect common co-occurrence of a T2D phenotype with monogenic diabetes, making currently suggested algorithms for diagnosis of monogenic diabetes even less sensitive.

Although T2D has been historically found mainly in overweight adults over age 40, recent increases in overweight or obese adolescents have led to an increased occurrence of T2D in young populations. Because of the increasing prevalence of T2D in adolescents and the lack of data regarding adolescent-specific T2D treatment methods, the Treatment Options for type 2 Diabetes in Adolescents and Youth (TODAY) study analyzed the effectiveness of metformin alone or in combination with rosiglitazone or lifestyle changes in adolescents with recently-diagnosed T2D.[12] Approximately half (48.3%) of the 699 participants treated with metformin alone maintained glycemic control, while the combination of metformin and rosiglitazone showed a small but significant improvement in the durability of glycemic control (61.4% maintained control, 25.3% decrease in primary outcome occurrence compared to metformin alone, p=0.006) over a relatively short duration of treatment (patients were followed for an average of 3.86 years).[13] In our current study, we analyze the prevalence of monogenic diabetes in the TODAY study participants and their outcomes.

MATERIALS AND METHODS

Patient Characteristics

The TODAY study participants were adolescents age 10–17 diagnosed with T2D according to ADA criteria within 2 years of study enrollment. The protocol for the TODAY study was approved by the Institutional Review Board at each participating institution (ClinicalTrials.gov: NCT00081328), and informed consent was obtained from all subjects. Eligibility criteria also included: body-mass index (BMI) above the 85th percentile while accounting for age and sex, negative for diabetes-associated autoantibodies (GAD65 and ICA512), and fasting C-peptide ≥ 0.20 nmol/L. Following a run-in period in which glycemic control (HbA1c <8%) on metformin alone was attained, metabolic, glycemic, anthropometric, and lifestyle attributes were collected longitudinally from all TODAY study participants. Patients were followed longitudinally until they lost glycemic control and reached the primary outcome of the study, defined as a glycated hemoglobin value of at least 8.0% for 6 consecutive months or the inability to wean the participant from insulin within 3 months after treatment for acute metabolic decompensation. Further descriptions of study protocol, design, methods, and results have been previously reported.[12,13]

This study analyzed DNA from a subset of 488 adolescents (177 males and 311 females) from the total TODAY cohort of 699 participants. Some TODAY participants had no DNA available to analyze because they did not attend a study visit during the DNA collection period or because they refused to consent to provide genetic data. In addition, all participants

of undefined race/ethnicity or of a race/ethnicity with a small sample size were excluded from this analysis. Subject data included in this analysis were Hispanic (n=217), non-Hispanic black (NHB, n=166), or non-Hispanic white (NHW, n=105) and showed no obvious differences from the entire TODAY cohort with those race/ethnicities (Tables S1, S2).

Sequencing methods

Coding and flanking regions of 40 autosomal genes with variants known or predicted to cause monogenic diabetes, including 13 genes identified to cause MODY at the time of study design (*APPL1* was published as a cause of MODY after design of our study) as well as genes causing neonatal diabetes, diabetes syndromes, lipodystrophy, severe obesity, and hyperinsulinemia (based on the theory that gain of function mutations could cause diabetes), were analyzed by next-generation sequencing using a customized gene panel (Table S3). DNA amplification, barcoding, and purification were performed using the Ion AmpliSeq Library Kit 2.0, Ion Xpress Barcode Adapter Kit, and Ion Library Equalizer Kit (Life Technologies). Emulsion PCR was performed on the Ion One Touch 2 using the Ion PGM Template OT2 200 Kit, and isolation of Ion Sphere Particles with clonally amplified DNA was performed with the Ion Torrent OneTouch ES (Life Technologies). Sequencing was performed using the Ion Torrent Personalized Genome Machine with the Ion PGM Sequencing 200 Kit version 2 and Ion 316 Chip version 2 (Life Technologies). Alignment was performed using TMAP version 4.2.14 software. Variant Calling was performed using the Torrent Suite variantCaller plugin version 4.2–14 and coverage analysis was performed using the Torrent Suite coverageAnalysis plugin version 4.2. Only samples with ≥20x mean base coverage depth of ≥80% of the target region (139,491bp) were used for analysis. Single-nucleotide variants with genotype quality scores <20 and coverage depth <10x were filtered from analysis, as were insertions/deletions with genotype quality scores <50 and coverage depth <50x. Samples had a mean of 186,170±67,019 (s.d.) mapped reads, and the single nucleotide variant transition to transversion ratio was 2.31. Quality metrics of monogenic diabetes variants are included in Table S4. Variants were annotated using a customized pipeline using multiple large population datasets, *in silico* prediction tools, and conservation metrics. All variants in non-coding or non-canonical splice regions, variants with a minor allele frequency (MAF) > 5.0%, or synonymous variants were filtered from further analysis.

Variant Analysis

Non-common (<5% MAF) coding or splice-site variants were analyzed for pathogenicity according to American College of Medical Genetics/Association for Molecular Pathology (ACMG/AMP) guidelines for variant interpretation.[14] These guidelines were created to standardize the complex process of classifying variants into categories (“pathogenic,” “likely pathogenic,” “uncertain significance,” “likely benign,” or “benign”) based on population data, computational data, functional data, and segregation data. Criteria (Table S5) were assessed for each variant and pathogenicity was determined based on the total evidence according to the algorithm of the ACMG/AMP guidelines.[14]

Statistical Analysis

Patients were grouped for statistical analysis. The unaffected group (n=426) did not have any pathogenic, likely pathogenic, or previously-cited monogenic diabetes variants. Other groups included patients with pathogenic or likely pathogenic variants in any monogenic diabetes gene (n=22), *HNF1A* (n=5), *HNF4A* (n=7), or *GCK* (n=7). Participants with previously-cited monogenic diabetes variants that were not classified as pathogenic or likely pathogenic (n=40) were not included in analysis due to uncertainty over their monogenic diabetes status. Associations between monogenic diabetes subcategories and patient characteristics, including BMI Z-score, HbA1c, fasting glucose, fasting insulin, insulinogenic index, dual energy x-ray absorptiometry (DXA), lipid measures, and blood pressure, were evaluated using linear models accounting for sex, age, race, and BMI Z-score (where indicated). These measures were collected from the earliest available time-point for the trait (screening data for those traits where it was available; otherwise from baseline measures). Log transformation was used to normalize datasets with skewed distributions. Because these analyses were hypothesis-driven, a p-value of <0.05 was considered nominally significant and no adjustment for multiple comparison testing was performed.[15] Treatment failure was defined as the primary outcome of the TODAY Study (loss of glycemic control, defined as a glycated hemoglobin values of at least 8.0% for 6 consecutive months or the inability to wean the participant from insulin within 3 months after treatment for acute metabolic decompensation). Treatment failure analyses were performed using a Cox proportional hazards model using the patient outcomes specified in the original TODAY study.[13] The treatment failure analysis accounted for participant sex, age, race/ethnicity, and TODAY treatment group. The proportional hazards assumption was met by each variable, except for sex, which was treated as a stratifying variable in the model.

RESULTS

Monogenic Diabetes Gene Variants

The thirteen genes reported to cause MODY at the time of study design were analyzed for non-common coding and splice site variants in this cohort (Table S6). Twenty-six previously-cited MODY variants were assessed according to ACMG/AMP criteria, and 18 were classified pathogenic or likely pathogenic (Table 1), while eight were classified as benign, likely benign, or variants of uncertain significance (Table S7). Thirty novel variants were discovered, and four of those variants were classified as pathogenic or likely pathogenic, while 26 were variants of uncertain significance (Table S8). Patients with pathogenic or likely pathogenic variants were considered to have monogenic diabetes. In sum, 22 individuals with 22 different pathogenic or likely pathogenic variants were discovered; i.e. 22/488 of those analyzed, or 4.5% of this sample of TODAY participants, had monogenic diabetes. Patients with monogenic diabetes were found across each of the three categories of race/ethnicity and across all treatment arms of the TODAY clinical trial (Table 1, S2). While variants were found in non-MODY genes in this study, none were determined to be pathogenic or likely pathogenic for monogenic diabetes or monogenic forms of obesity (Table S9).

Characteristics of Patients with Monogenic Diabetes Gene Variants and Study Outcomes

Characteristics at the earliest available time-point (BMI Z-score, HbA1c, blood pressure, triglycerides, and lipid measures were from the screening visit, while fasting glucose, fasting insulin, insulinogenic index, and DXA measures were from the baseline visit) of subjects with pathogenic or likely pathogenic monogenic diabetes variants (n=22) were compared to those without monogenic diabetes (n=426) (Table 2, S10). Individuals with monogenic diabetes showed lower BMI Z-scores (2.05 vs. 2.32 p=0.004), higher fasting glucose (6.65 vs. 6.08 mmol/L p=0.02), lower fasting insulin (152.1 vs. 213.91 pmol/L p=0.03) and higher total cholesterol (4.50 vs. 4.00 mmol/L p=0.003) compared to individuals without monogenic diabetes. These associations were still significant when adjusted for BMI Z-score (Table S10). Separated by gene etiology, monogenic diabetes subgroups showed similar trends in patient characteristics, although they were generally underpowered to detect significant differences.

Time-to-treatment failure analyses, the primary outcome of the TODAY Study, compared unaffected individuals (n=426) with individuals with *HNF1A*-MODY, *HNF4A*-MODY, and *GCK*-MODY (Figure 1). No patients with *GCK*-MODY (0/7) failed treatment in the TODAY study. Across all three study arms, 6 of 7 of the subjects with *HNF4A*-MODY failed treatment over the first 2 years of study, with a hazard ratio of 5.03 (2.18–11.58 95%CI) (p=0.0002) compared to subjects without monogenic diabetes. Three patients with *HNF4A*-MODY lost glycemic control by their first post-baseline study visit. There was no significant difference in treatment response of individuals with *HNF1A*-MODY compared to individuals without monogenic diabetes.

DISCUSSION

Based on our findings, an appreciable number of youth diagnosed with T2D may, in fact, have undiagnosed monogenic diabetes. Individuals with monogenic diabetes participating in TODAY displayed subtle, but significant, differences in select characteristics compared to unaffected TODAY participants. However, in this adolescent population sample selected for being overweight or obese and having non-autoimmune, C-peptide positive diabetes, it was not possible to reliably distinguish between T2D and monogenic diabetes based on clinical features at baseline in this study. Importantly, patients with monogenic diabetes were found in each race/ethnicity examined, emphasizing that race/ethnicity should not be used to discriminate between those with and without monogenic diabetes. We also confirmed the importance of a genetic diagnosis of monogenic diabetes to inform treatment protocols since metformin, while the first line treatment of T2D, was ineffective in those who turned out to have *HNF4*-MODY. Further emphasizing the known relationship between genetic diagnosis and established treatment protocols, those with *GCK*-MODY did not fail treatment, since patients with *GCK*-MODY do not generally have highly elevated HbA1c and typically do not require drug therapy. Both of these findings have strong implications for clinical practice.

At least 4.5% of TODAY participants (22/488) had pathogenic or likely pathogenic monogenic diabetes variants (Table 1). It is estimated that there are approximately 3,700 new diagnoses of T2D in youth each year in the United States, and our findings suggest as

many as 160 of those cases could be misdiagnosed cases of monogenic diabetes.[16] The SEARCH study for diabetes in youth previously found 8% of participants with diabetes-associated autoantibody-negative, C-peptide positive diabetes had monogenic diabetes variants in *GCK*, *HNF1A*, and *HNF4A*.[11] Similar to the SEARCH study, we found no differences in family history of diabetes, as measured by reported maternal and paternal diabetes status, between TODAY patients with or without monogenic diabetes, and we found comparable racial/ethnic distributions of patients with monogenic diabetes between TODAY and SEARCH (Table S2). We probably found a lower percentage of patients with monogenic diabetes because the TODAY study eligibility criteria required adolescents with diabetes to be overweight or obese. Additionally, it is possible for individuals to have monogenic diabetes and coincident insulin resistance or T2D, which can further complicate both diagnosis and treatment. While likely to only account for a minority of cases, patients with authentic T1D or T2D harboring monogenic diabetes variants have been described before.[17,18]

By following the ACMG/AMP guidelines for variant annotation, our study has potentially limited our estimation of the prevalence of monogenic diabetes in the cohort. While the ACMG/AMP guidelines are important for correctly classifying clinical implications of variants before being returned to patients, the need for evidence across multiple categories (functional studies, family co-segregation data, *de novo* status, etc.) can be restrictive, especially for recently-discovered variants. As shown in previous studies, this standardized variant classification process often reclassifies variants previously assumed to be pathogenic as benign, likely benign, or variants of uncertain significance (VUS) due to increases in genetic and phenotypic database information.[19] Patients with “reclassified” variants were excluded from our statistical analysis, but separate analysis of clinical characteristics of carriers of the *BLK* p.A71T and *KLF11* p.T220M variants showed no differences from those without monogenic diabetes (data not shown). Analysis of 18 VUS discovered (11 novel variants) in the 3 most common MODY genes showed patients with VUS were intermediate or close to the monogenic diabetes group in terms of clinical characteristics (BMI Z-score =2.27±0.48, fasting glucose =6.72±1.57 mmol/L, fasting insulin =173±123 pmol/L, mean ±s.d.), but differences from those without monogenic diabetes (n=412 in this analysis) were not statistically significant. This finding supports the hypothesis that at least some of the VUS may cause monogenic diabetes. Since the ACMG/AMP guidelines often classify novel variants as VUS due to the lack of evidence to suggest the variant is either pathogenic or benign, further study of the novel variants found in this study is a potentially fruitful topic for future research and could increase the estimate of monogenic diabetes prevalence in this study.

Although pathogenic and likely pathogenic monogenic diabetes genetic variants were found in each race/ethnicity, a higher proportion of NHW participants carried monogenic diabetes variants (Table S2). Similarly, more NHB participants carried previously-cited monogenic diabetes variants reclassified as VUS, likely benign, or benign variants. The odds ratio for NHB participants carrying reclassified variants was 2.15 compared to NHW, 3.84 compared to HIS, and 3.10 compared to the combined NHW and HIS populations. We hypothesize this trend is due to the reliance of the ACMG/AMP standards and guidelines on previously published data on genetic variants. Previous monogenic diabetes studies have mainly

focused on European populations, which could cause bias in classifying variants. Underrepresentation of minority populations in genetic studies is a well-known concern that must be addressed in order for precision genetic medicine to be effective across all races/ethnicities. At this point, it is important to recognize that monogenic diabetes variants can and have been found across multiple different races/ethnicities.

Through the design of the gene panel, we have potentially limited discovery of patients with monogenic diabetes in this cohort. Since there have been relatively few reported pathogenic variants in the 27 non-MODY genes compared to the number of reported pathogenic MODY gene variants, coding variants in non-MODY genes are less likely to be classified as pathogenic or likely pathogenic. Although the discovery of more pathogenic or likely pathogenic variants in less common monogenic diabetes genes may increase in the future, because we were unable to define any likely pathogenic or pathogenic variants in non-MODY monogenic diabetes genes, MODY-specific gene panels may be a more appropriate approach for future studies screening for monogenic diabetes at this time. Also, there are potentially other unknown causative monogenic diabetes genes or non-exonic/splicing variants not assessed by using our gene panel.

We found a larger proportion of individuals with *HNF4A*-MODY than expected in our cohort (Table 1). While *HNF1A*-MODY accounts for approximately 30–50% of MODY diagnoses and *HNF4A*-MODY accounts for less than 10%, we observed more patients with *HNF4A*-MODY than with *HNF1A*-MODY.[20] Interestingly, *HNF4A*-MODY has been associated with increased birth weight and macrosomia in the neonatal stage, regardless of maternal genotype but exacerbated by the mother having the same mutation and the associated hyperglycemic intrauterine environment.[21] Macrosomia has been correlated with overweight or obese status through adolescence and adulthood.[22] Thus there could be an association between *HNF4A*-MODY with higher BMI that could cause *HNF4A*-MODY to be misdiagnosed as T2D when using BMI as a criterion for monogenic diabetes. We hypothesize that the TODAY study inclusion criteria (BMI $\geq 85^{th}$ percentile for age and sex) may have created a selection bias toward *HNF4A*-MODY compared to the other gene-specific subgroups; however, this remains to be demonstrated. Further studies incorporating birthweight and prevalence of T2D misdiagnosis of patients with *HNF4A*-MODY are necessary to test this hypothesis.

We did not discover any clinical criteria to differentiate overweight or obese adolescents with monogenic diabetes from those with T2D in this cohort selected for overweight or obese status and non-autoimmune, C-peptide positive diabetes. Individuals with monogenic diabetes had lower BMI Z-score (p=0.004), but all 22 adolescents with monogenic diabetes were still overweight (>85^{th} percentile by age) per the TODAY study design. For each of the clinical characteristics measured in the TODAY study, values of patients with monogenic diabetes could be found throughout the range of values for patients with T2D (Table 2). The functional effects of genetic defects leading to an insulin secretion deficit were demonstrated as adolescents with monogenic diabetes had lower fasting plasma insulin (p=0.03) compared to those without monogenic diabetes. They also had higher fasting blood glucose (p=0.02) concentrations. Similar to published studies on populations with broader patient demographics, our patients with monogenic diabetes have greater insulin sensitivity (defined

as the inverse of the fasting insulin) compared to those in our cohort with apparent T2D, but characteristics such as HDL cholesterol and beta cell function (insulinogenic index) were not different between the groups in this specific cohort selected by overweight/obese status and non-autoimmune diabetes.[23–25] In summary, within this cohort of overweight/obese autoantibody-negative, C-peptide positive diabetic cohort, there were differences in the means of some metabolic characteristics, but it was not possible to identify any characteristics to reliably distinguish between those with and without monogenic etiologies. Other markers shown to help identify patients with monogenic diabetes, such as high-sensitivity C-reactive protein and plasma glycan profile, were not collected in the TODAY study.

Although the TODAY study provided unique information about the effectiveness of metformin alone or in combination with rosiglitazone or lifestyle changes, the small numbers of patients with each gene subtype of monogenic diabetes reduced our ability to draw conclusions regarding response to each of the treatment arms. However, none of the patients with *GCK*-MODY failed the treatment regimens in the TODAY study. This is consistent with the *GCK*-MODY phenotype of mildly elevated fasting blood glucose (fasting glucose of 5.49–8.66 mmol/L and HbA1c of 5.6–7.6%) that usually needs no treatment to avoid chronic complications of diabetes. In contrast, 6 of 7 patients with *HNF4A*-MODY failed treatment across study arms (HR=5.03 p=0.0002), indicating poor response regardless of therapies offered in the TODAY study. Similarly, though not statistically significant, 3/5 patients with *HNF1A*-MODY failed the TODAY study treatments. These results would be expected since the established treatment for *HNF1A*- and *HNF4A*-MODY are sulfonylurea drugs, rather than metformin and/or rosiglitazone.[7,8] Metformin is an insulin-sensitizing agent, while sulfonylureas are insulin secretagogues that improve the insulin secretion deficit found in *HNF1A*- and *HNF4A*-MODY patients. Therefore the finding that *HNF4A*-MODY patients failed metformin treatments in the TODAY trial is a demonstration of the consequences of not attaining a genetic diagnosis of MODY. We note that it is possible that in addition to sulfonylureas, metformin and/or thiazolidinediones may be appropriate for some monogenic diabetes patients with concomitant obesity and insulin resistance.

Genetic testing is not commonly implemented in diabetes clinical care due to current costs of testing, uncertainty over insurance reimbursement, and difficulty of interpretation of sequencing results. However, clinical care is moving into an era of genomic medicine, and monogenic diabetes provides a unique opportunity for immediate implementation of personalized genomic medicine. Under specific conditions of cost and discovery rate, genetic testing for MODY has been modeled to be as cost-effective as current medical practices and potentially cost-saving.[6] As the cost and throughput of genetic testing continues to improve, the knowledge base of rare genetic variation will continue to grow to inform clinical practice. Although current impediments to genetic testing such as cost, availability, and reimbursement may limit genetic testing for monogenic diabetes in large populations, this study has shown monogenic diabetes should be considered as a possible diagnosis in young people with antibody-negative and C-peptide positive diabetes. However, it is also important to recognize that T2D is the most common form of diabetes in the world, and most overweight/obese individuals with diabetes will have T2D, especially those not diagnosed at a young age (>30 years old).

The findings from this study have strong implications for informing the practice of managing diabetes in youth. We discovered individuals with monogenic diabetes across all races/ethnicities in a cohort of overweight and obese adolescents diagnosed with T2D, raising concerns about the currently recommended use of BMI and previously recommended use of race/ethnicity to select patients for genetic testing. We suggest that with secular trends of increasing obesity in children and adolescents, monogenic diabetes be considered as a potential etiology in diabetes-associated autoantibody-negative and C-peptide-positive adolescents regardless of BMI. Despite the small sample size of our cohort, treatment response based on monogenic diabetes diagnosis was consistent with predicted results, indicating the importance of monogenic diabetes genetic testing and proper genetic interpretation for providing optimal treatment to youth with diabetes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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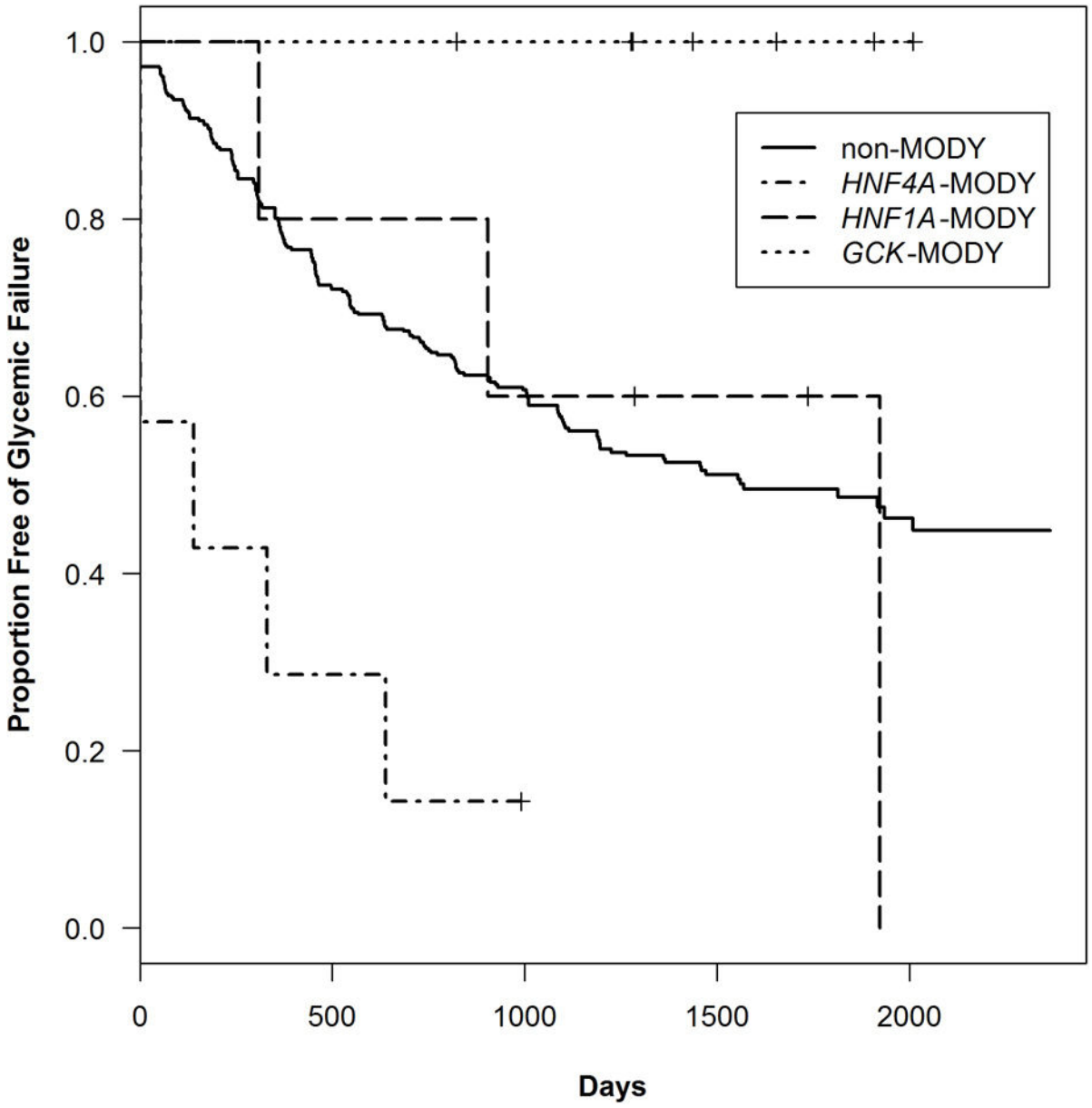

Figure 1.

Failure-free survival curve of MODY gene subtypes and unaffected patients. X-axis is defined as post-baseline-visit days until start of failure interval. Events are defined as elevated glycated hemoglobin (>8.0%) over a period of 6 months or the inability to wean the participant from insulin within 3 months after treatment initiation in the TODAY study. Hazard ratio for each subtype: *GCK*-MODY HR= undefined (no events), *HNF1A*-MODY HR=1.26 (0.40–4.02 95%CI) p=0.7, and *HNF4A*-MODY HR=5.03 (2.18–11.58 95%CI) p=0.0002.

Table 1

Pathogenic or likely pathogenic MODY gene variants and TODAY study patient data

Gene	Sex (age)	Race/ethnicity[a]	Treatment arm[b]	TODAY primary outcome[c]	Amino acid change / Site change[d]	ACMG pathogenicity	Prev. Studies
HNF4A	M (10)	His.	Met.+Ros.	−	p.R64Q	Likely pathogenic	26
HNF4A	F (12)	NHW	Met.+Ros.	+	p.R64fs	Likely pathogenic	Novel
HNF4A	F (13)	NHB	Met.+Life	+	p.Q86X	Pathogenic	Novel
HNF4A	F (13)	His.	Met.+Life	+	p.V105I	Pathogenic	27
HNF4A	F (14)	His.	Met.+Life	+	Splice-site (c.573+1G>A)	Pathogenic	8
HNF4A	M (16)	His.	Metformin	+	p.R308H	Likely pathogenic	5
HNF4A	F (14)	NHW	Met.+Life	+	p.H365fs	Likely pathogenic	Novel
GCK	M (10)	His.	Met.+Life	−	p.V62M	Pathogenic	28
GCK	F (13)	NHW	Met.+Ros.	−	p.R191W	Likely pathogenic	29
GCK	F (17)	NHW	Met.+Ros.	−	p.T206M	Pathogenic	30
GCK	M (13)	NHW	Met.+Life	−	p.N254H	Likely pathogenic	31
GCK	F (12)	NHW	Metformin	−	p.E265K	Pathogenic	32
GCK	F (13)	NHW	Met.+Life	−	p.R392C	Likely pathogenic	33
GCK	M (13)	NHW	Met.+Life	−	p.S396fs	Likely pathogenic	Novel
HNF1A	M (12)	His.	Metformin	+	p.P112L	Pathogenic	34
HNF1A	F (11)	NHB	Metformin	+	p.R131W	Pathogenic	35
HNF1A	F (12)	NHW	Metformin	−	p.R271Q	Pathogenic	36
HNF1A	M (14)	His.	Met.+Life	+	p.P379A	Pathogenic	37
HNF1A	M (10)	NHW	Met.+Life	−	p.P519L	Pathogenic	35
KLF11	M (16)	His.	Met.+Ros.	+	p.A347S	Pathogenic	38
INS	F (12)	NHB	Metformin	−	p.R6H	Pathogenic	39
INS	M (15)	NHW	Met.+Ros.	−	p.R46Q	Pathogenic	40

Quality metrics for each variant can be found in Table S4.

[a] Self-reported race/ethnicity: His.=Hispanic, NHW = Non-Hispanic White, NHB = Non-Hispanic Black

[b] TODAY clinical trial treatment arm: Met.+Ros. = Metformin and rosiglitazone, Met.+Life = Metformin and lifestyle intervention

[c] TODAY clinical trial outcome: (−) = Patient did not reach primary outcome (treatment failure), (+) = Patient reached primary outcome (treatment failure)

[d] Amino acid reported sites are according to the following reference sequences: *HNF4A*-NM_001287183; NP_001274112, *GCK*-NM_000162; NP_000153, *HNF1A*-NM_000545; NP_000536, *KLF11*-NM_003597; NP_003588, and *INS*-NM_000207; NP_000198.1

Table 2

Associations between all monogenic diabetes variants, gene subtypes and patient characteristics at earliest study timepoint (screening or baseline), adjusted for age, sex, and race/ethnicity

	Unaffected	All Monogenic Diabetes[b]	HNF4A -MODY	GCK-MODY	HNF1A- MODY	INS-MODY	KLF11-MODY
n	426	22	7	7	5	2	1
BMI Z-score Mean ± SD	2.32 ±0.42	2.05 ±0.42[a]	2.12 ±0.52	1.91 ±0.33 [a]	2.06 ±0.32	2.52 ±0.44	1.49
DXA fat %[c]	38.3 ±6.02	35.2 ±6.9	38.4 ±7.0	38.3 ±5.7	29.9 ±1.5 [a]	30.6	23.9
HbA1c (%)	7.51 ±1.97	7.45 ±1.93	7.27 ±2.35	6.99 ±2.23	7.78 ±1.03	9.40 ±0.57	6.4
Fasting glucose (mmol/L)	6.08 ±1.27	6.65 ±1.56[a]	7.15 ±1.09 [a]	6.69 ±0.28	5.46 ±1.2	5.22 ±0.24	11.6
Fasting insulin (pmol/L)	213.9 ±145.3	152.1 ±210.0 [a]	122.2 ±46.3	87.5 ±32.7 [a]	107.6 ±7.5[a]	588.9 ±679.7	164.6
Insulinogenic index ($Ins_{30\text{-}0}/Glu_{30\text{-}0}$)	1.63 ±2.45	0.81 ±0.84	0.56 ±0.41	1.28 ±1.24	0.49 ±0.17	1.29	0.08
Systolic blood pressure (mmHg)	113.8 ±11.4	107.9 ±13.6	113.1 ±16.5	99.4 ±9.4 [a]	107.6 ±7.5	119.8 ±22.3	109
Diastolic blood pressure (mmHg)	67.2 ±8.8	64.3 ±9.7	67.1 ±12.0	60.9 ±11.3	64.7 ±5.4	66.3 ±8.1	63.5
Total cholesterol (mmol/L)	3.98 ±0.86	4.50 ±1.06[a]	4.77 ±1.15 [a]	4.11 ±0.9	4.38 ±1.08	4.26 ±0.68	6.4
HDL cholesterol (mmol/L)	1.03 ±0.24	1.09 ±0.24	1.19 ±0.21	1.05 ±0.34	1.03 ±0.12	1.18 ±0.20	0.83
LDL cholesterol (mmol/L)	2.33 ±0.72	2.65 ±0.72[a]	2.82 ±0.96	2.48 ±0.68	2.56 ±0.47	2.51 ±0.84	3.34
Triglycerides (mmol/L)	1.38 ±0.91	1.71 ±1.30	1.66 ±0.77	1.27 ±0.79	1.72 ±1.32	1.23 ±0.07	6.09

Values are presented as mean ±SD. All measures were taken at screening in the TODAY study, except for fasting glucose, fasting insulin, insulinogenic index and DXA fat measures, which were taken at the baseline time-point.

[a] $p<0.05$ for the effect size of the classification (monogenic diabetes status in aggregate or separated by gene) in a linear model accounting for sex, age, and race/ethnicity (effect sizes, BMI Z-score adjusted results, and p-values found in Table S9)

[b] Monogenic diabetes Pathogenic or Likely Pathogenic Mutation

[c] DXA data was available for 303 unaffected individuals and 15 patients with monogenic diabetes (5 *HNF4A*-MODY, 5 *GCK*-MODY, 3 *HNF1A*-MODY, 1 *INS*-MODY, and 1 *KLF11*-MODY).