Case report

Stress Induced Hyperglycemia in Early Childhood as a Clue for the Diagnosis of NEUROD1-MODY

Celik NB et al. Stress Induced Hyperglycemia and NEUROD1-MODY

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What is already known on this topic?
The clinical features of NEUROD1-MODY vary in a large clinical spectrum in terms of age and BMI at diagnosis and response to oral hypoglycemic agents.

What this study adds?
Here, to the best of our knowledge, we reported the youngest patient with a heterozygous *NEUROD1* variant in whom stress-induced hyperglycemia during a febrile illness led to the diagnosis. Obtaining a careful and detailed family history for diabetes could help to identify children who are at risk of monogenic diabetes.

Abstract
Maturity-onset diabetes of young 'MODY' Type 6 is a rare form of monogenic diabetes caused by mutations in *Neuronal differentiation 1* (*NEUROD1*). Clinical features vary in a large spectrum in terms of age and BMI at diagnosis. Here, we reported the youngest patient with a *NEUROD1* variant to the best of our knowledge. A 2.1-year-old girl was referred to pediatric endocrinology clinic for elevated fasting blood glucose (BG) (104 mg/dL) which was detected at another center where she had been evaluated for loss of appetite. Her maternal aunt and uncle had been diagnosed with type 2 diabetes mellitus (DM) at the age of 40 and 45 years; they were obese (BMI: 30.2 and 30.6 kg/m^2). At the age of 3.7 years old, she was hospitalized for buccal cellulitis and plasma glucose concentration was 239 mg/dL at admission. Targeted next-generation sequencing (NGS) was performed considering the stress induced hyperglycemia without serious illness, negative islet cell antibodies and insulin autoantibodies, age at the presentation, and family history of DM. NGS analysis revealed a previously reported heterozygous missense variant in *NEUROD1*. Segregation studies showed that the identified variant was inherited from her 44-year-old mother with a BMI of 27.2 kg/m^2 and a normal oral glucose tolerance test (OGTT). Heterozygous *NEUROD1* mutations cause low-penetrant diabetes that is heterogeneous in terms of clinical features as some patients fulfill the classic MODY definition and others are mimicking type 2 DM. Clinical manifestations and family history should be carefully evaluated in patients with stress induced hyperglycemia to identify candidate cases for molecular testing, and proper follow-up should be initiated in affected individuals.
Keywords: MODY, NEUROD1, stress induced hyperglycemia, early childhood

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Introduction
Maturity-onset diabetes of the young (MODY) is an inherited disorder of non-autoimmune diabetes mellitus (DM) with a young age of onset. It accounts for 1-5% of all patients with diabetes (1), and 1-6% of pediatric patients with diabetes (2-4). However due to existence of overlapping features with other types of diabetes, its prevalence could be higher than estimated (5). As the correct diagnosis prevents unnecessary therapies such as insulin in some types of MODY like *HNF1A*-MODY, clinical manifestations and family history should be evaluated properly. Nevertheless, most cases need confirmatory genetic testing for the exact diagnosis. Mutations in *GCK*, *HNF1A*, *HNF4A*, and *HNF1B* are the most common causes of MODY (6). With the recent identification of novel genes, there are a total of 14 genes including *Neuronal differentiation 1* (*NEUROD1*) that cause MODY (7,8). NEUROD1 is a helix-loop-helix (bHLH) transcription factor that is expressed in pancreatic islet cells, intestine, and neurons in the central and peripheral nervous system (9). NEUROD1 dimerizes with E47, a ubiquitous bHLH transcription factor, and regulates insulin gene expression (9). Autosomal dominantly inherited *NEUROD1* mutations were first reported by Malecki et al. in two families including both obese and non-obese individuals with type 2 DM whose

aged 17 to 59 at the time of diagnosis (10). Afterward, *NEUROD1* mutations were classified as MODY6, considering the clinical features (11). Since then, patients with *NEUROD1* mutations whose clinical features vary in a large clinical spectrum in terms of age and BMI at diagnosis, and response to oral hypoglycemic agents have been reported (12,13).

Stress induced hyperglycemia is a transient condition associated with insulin resistance and relative insulin deficiency (14). It is often considered as a physiologic response to stress. However, stress induced hyperglycemia could be important in terms of uncovering the underlying islet cell dysfunction. Previous studies demonstrated a higher risk of future insulin-dependent diabetes in the case of positive islet cell antibodies, insulin autoantibodies and stress induced hyperglycemia without serious illnesses (15). Also, positive family history of diabetes could enable the patients with stress induced hyperglycemia to be diagnosed with monogenic diabetes (16).

Here, to the best of our knowledge, we reported the youngest patient with a heterozygous *NEUROD1* variant in whom stress induced hyperglycemia during a febrile illness led to the clinical diagnosis. Obtaining a careful and detailed family history for diabetes could help to identify children who are at risk of monogenic diabetes.

Case report:

A 2.1-year-old girl was referred to pediatric endocrinology clinic for elevated fasting blood glucose (BG) (104 mg/dL) which was detected at another center where she had been evaluated for loss of appetite. There was no history of weight loss or polyuria-polydipsia. She was born at term to healthy nonconsanguineous parents with a birth weight of 2800 g (-0.7 SDS). Her mother's pregnancy was uncomplicated including a normal oral glucose tolerance test (OGTT). Developmental milestones were normal and the mental and psychomotor developmental index of Bayley Scales of Infant Development-II, which was performed at the age of 13 months, was compatible with 14 months and 13 months of age, respectively. Her maternal aunt and uncle had been diagnosed with type 2 DM. The age at diabetes diagnosis in these subjects was 40 and 45 year; they were obese (BMI:30.2 and 30.6 kg/m^2) and on metformin therapy (Fig. 1). Maternal grandmother and grandfather were died of unknown causes. Index patient presented with a height of 87.7 cm (-0.17 SDS), a weight of 12.7 kg (0.25 SDS), a BMI of 16.47 kg/m^2 (0.41 SDS) at the age of 2.1 years old. She was prepubertal, and other systemic examination was unremarkable. Biochemical analysis revealed normal fasting BG concentration at the time of admission (plasma glucose 95 mg/dL, insulin 25.1 pmol/L, C-peptide 0.92 ng/mL, HbA1c 5.7%). There was no glucosuria. Anti-glutamic acid decarboxylase, islet cell and insulin antibody were all negative. Dietary interventions were performed maintaining age appropriate calories and reducing simple carbohydrates. At the age of 3.7 years old, she was hospitalized for buccal cellulitis. She was hemodynamically stable and had low grade fever. She had elevated acute phase reactant (c-reactive protein 0.86 mg/dL [N: 0-0.5]), leukocytosis 28,700/µL [N: 4,000-12,000] and plasma glucose concentration was 239 mg/dL at admission. She had glucosuria and no ketonemia. Other biochemical parameters were all normal, and blood culture test was negative. Normoglycemia was provided spontaneously without insulin treatment while she was administered intravenous antibiotics. An OGTT was performed later to evaluate the insulin secretory capacity (fasting BG 87 mg/dL, insulin 71.7 pmol/L; 2-hour BG 98 mg/dL, insulin 276.9 pmol/L).

Considering the stress induced hyperglycemia without serious illness, negative islet cell antibodies and insulin autoantibodies, age at the presentation, and family history of DM, genetic studies were conducted after obtaining written informed consent from the patient's parents. Genomic DNA of peripheral blood leukocytes was extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Targeted next-generation sequencing (NGS) was performed with VariFindTM Diabetes assay (Parseq Lab, Saint Petersburg, Russia) on a MiSeq platform (Illumina, San Diego, CA, USA) and then analyzed by VariFind software. This targeted assay covers a total of 24 genes (Supplementary Table 1) associated with various types of sugar intolerance. Target regions include exon-intron boundaries and coding sequences for 12 genes and only hot spots regions for the remaining genes (Supplementary Table 1). After the filtering steps, targeted NGS analysis revealed a previously reported heterozygous missense variant c.723C>G (p.His241Gln) in *NEUROD1* (NM_002500). Parental segregation studies were performed by Sanger sequencing using an ABI 3500 Genetic Analyzer (Thermofisher Scientific, Waltham, MA, USA). Identified variant was found to be inherited from her mother (Fig. 1). Her mother was 44 years old, her BMI was 27.2 kg/m^2 and OGTT revealed no abnormalities of glucose metabolism (fasting BG 100 mg/dL, insulin 51.6 pmol/L, C-peptide 1.66 ng/mL; 2-hour BG 128 mg/dL, insulin 669.4 pmol/L, C-peptide 12.4 ng/mL, HbA1c 5.7%). Other family members including the two individuals with DM did not accept the genetic testing.

Discussion:

Maturity-onset diabetes of the young is an autosomal dominantly inherited non-autoimmune diabetes classically presenting before the age of 25 years (a more liberal definition is before the age of 35 years). Based on this definition of MODY, the frequency of *NEUROD1* mutations were reported to be between 0.64% and 7.14% (13). However, segregation analysis of families revealed a wide range of phenotypic features of *NEUROD1* mutations. Contrary to the classical MODY definition of presentation in adolescence or young adulthood, the age of diagnosis can vary in a wide range up to the seventh decade (8). Here we reported the youngest patient who presented with stress hyperglycemia, and underwent genetic analysis based on the family history of diabetes and a previously reported *NEUROD1* variant was detected.

Stress induced hyperglycemia is a disorder of glucose metabolism that develops during an acute physiological stress. Combination of increased counterregulatory hormones and overproduction of cytokines cause insulin resistance and impairment of insulin secretion (17). Although it is a physiological response to stress and associated with greater illness severity, it could be the earliest clinical manifestation of islet cell dysfunction (15,18). Besides the presence of markers of autoimmunity and no serious illness, a family history of diabetes could help to identify patients who are at risk of development of any type of diabetes. Detection of hyperglycemia during a relatively mild infection and a positive family history of diabetes prompted us to the molecular testing. So far, impaired fasting glucose, impaired glucose tolerance, overt diabetes and gestational diabetes have been reported in patients with *NEUROD1* mutations (10,12,19). Also, Szopa et al reported a newborn with neonatal hypoglycemia and macrosomia born to a mother with well-controlled gestational diabetes, and suggested *NEUROD1* mutations as a cause of biphasic diabetes, like *HNF1A* and *HNF4A* mutations (12).

Diabetic and non-diabetic individuals within the same family represent the intra-familial variability of *NEUROD1* mutations (20). Despite carrying the same variant, index patient had stress induced hyperglycemia in childhood while mother had normal glucose metabolism and did not have gestational diabetes. More than half of the patients harboring *NEUROD1* variants with overt diabetes reported so far had obesity. The presence of obesity within the families irrespective of carrying a *NEUROD1* variant and a higher frequency of diabetes in individuals with a high BMI suggests that obesity is not a hallmark phenotypic feature of the *NEUROD1* mutations, however, it could be a facilitating factor for the development of diabetes. So, the normal glucose metabolism of the mother could be explained by her not being obese. Intriguingly, approximately three-quarters of the MODY6 families, manifested with diabetes at an earlier age compared to the previous generation, suggesting there may be other factors modifying the phenotypic expression. Also, a parent-of-origin effect was suggested based on the observation of higher proportion of individuals who inherited variants from the mother developed disorder of glucose metabolism compared to ones who inherited variants from the father, as also observed in the presented family (21). Horikawa et al reported four MODY6 families in whom the probands with normal BMI manifested with ketosis or ketoacidosis at the age of 10 to 14 years (21), and authors thought that more severe phenotype, regarding younger age of diagnosis with ketosis or ketoacidosis despite normal BMI, was related to less insulin secretory capacity of Japanese population (22). So, one of the factors affecting the manifestation of the clinical and laboratory findings could be the ethnic background as complexities of genetic background can affect their occurrence (23).

NEUROD1 protein consists of two domains; bHLH domain and transactivation domain (7). Most of the reported variants of *NEUROD1* affected transactivation domain of the protein as did the identified variant (7). p.His241Gln was reported previously in individuals diagnosed with MODY whose unaffected family members were also heterozygous for the variant (20,24). In one of these studies, this variant was detected after the sequencing of *NEUROD1* and *PDX1* in all individuals who had previously tested negative for *HNF1A*, *GCK* and *HNF4A* mutations (20), while, in the other after using a targeted next generation sequencing panel for known MODY genes (24). Clinical details of those previously reported individuals carrying the same variant were also recently summarized by Horikawa et al and Medeiros Abreu et al. in 2019 (25, 26). Furthermore, this variant was classified as a rare coding variant of potential interest in RaDio study which included DNA samples from 2,178 people with type 2 diabetes and 4,170 control individuals (27).

Additionally, this variant was interpreted as having conflicting interpretations of pathogenicity in ClinVar database (28). In silico bioinformatics analyses, including MutationTaster and MutationAssessor, supports a deleterious effect in addition to a CADD score of 23.1, whereas SIFT sets a benign computational verdict on the variant. Moreover, in the gnoMAD database (v2.1.1), while we expect individuals with severe early onset disease to be heavily depleted and conditions with reduced penetrance such as MODY6 are likely included, this variant is observed mostly in South Asians with an allele frequency (AF) of 0.006467, while allele frequencies in other populations such as Europeans were much more lower (AF< 0.0001) (29). There is no clear genotype-phenotype correlation considering the phenotypic heterogeneity of the patients who carry the same genetic variant in the same family (21). The median age at the time of diabetes diagnosis in patients with p.His241Gln was 25 years (range between 19 and 65). Except for one patient with a digenic inheritance (*NEUROD1* and *PDX1* variants) (23), all of the other patients with p.His241Gln were obese (20). Despite being rare this variant is observed in healthy appearing South Asian population and since obesity or a digenic inheritance is observed in all patients with this variant for the manifestation of diabetes (30,31), it could be speculated that the His241Gln variant causes a minor impairment in insulin secretion capacity. In addition, we could not rule out the presence of other genetic variants contributing to phenotype throughout the genome or within the whole coding region of *NEUROD1* since the utilized targeted panel did not cover all known MODY genes or all coding regions of some MODY genes including *NEUROD1*. Other limitations of the present study were that we did not evaluate copy number variants of the known 14 MODY genes and were not able to extend segregation studies since samples from maternal relatives with diabetes were not available for testing.

It has been shown that a considerable number of euglycemic individuals harbor pathogenic variants in monogenic diabetes genes (27). Pathogenic variants in *HNF1A*, for example, do not always cause MODY phenotype and may contribute to type 2 diabetes predisposition or may be found harmlessly in the genomes of healthy appearing individuals partially explained by reduced penetrance that may occur by the functional effects of regulatory variants (27, 32). These studies, which expose putative disease-causing alleles in the genome of healthy appearing individuals, complicate variant interpretation and precise pathogenicity assignments as in the present case (32).

Taking all of the abovementioned findings into consideration including the knowledge that NEUROD1 deficient diabetes appears to be low penetrant and possibly occur in combination with other environmental and genetic factors, it is currently unclear whether the identified *NEUROD1* heterozygous variant definitely contributes to the phenotypic manifestations in the proband. Therefore, further functional testing is required to elucidate the precise role of the identified missense variant on the development of diabetes.

The treatment modalities varied among patients that reported so far. The most common treatment is oral hypoglycemic agents (more than one third of patients), and combination of insulin and oral hypoglycemic agents. Some of the patients had to switch the treatment from oral hypoglycemic agents to insulin. We could not perform molecular analysis for maternal aunt and uncle whose diabetes had been regulated with oral hypoglycemic agents. Our case is not obese, however there are other factors that could modify the development of diabetes. Since most of the cases reported to date have been manifested with diabetes at an earlier age compared to the previous generations, we consider diabetes could develop at an earlier age compared to other family members in our case. Also, parent-of-origin effect could increase the probability of development of diabetes in our case. In this case, the treatments and treatment responses of other family members can be used as a guide to determine the use of oral antidiabetic drugs or insulin.

In conclusion, to the best of our knowledge, we reported the youngest patient with a heterozygous *NEUROD1* variant in whom stress induced hyperglycemia during a febrile illness led to the clinical diagnosis. Heterozygous *NEUROD1* mutations cause low-penetrant diabetes that is heterogeneous in terms of clinical features as some patients fulfill the classic MODY definition and others are mimicking type 2 diabetes mellitus. Hyperglycemia during a relatively mild infection with a family

history of diabetes should prompt clinicians to investigate monogenic diabetes with molecular test, and proper follow-up should be initiated in affected individuals.

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#	Gene	Target region
1	*GCK*	CDS
2	*HNF1A*	CDS
3	*HNF1B*	CDS
4	*INS*	CDS
5	*HNF4A*	CDS
6	*BLK*	CDS
7	*G6PC2*	CDS
8	*PDX1*	CDS
9	*INSR*	CDS
10	*KCNJ11*	CDS
11	*GLIS3*	CDS
12	*CEL*	CDS
13	*ABCC8*	exons 1-17, 19, 21-26, 28-30, 32-39, intron 8
14	*NEUROG3*	exon 2
15	*PAX4*	exons 1, 3-6, 8-9
16	*FOXP3*	exons 2-8, 10-12, intron 11, 3'UTR
17	*KLF11*	exons 2-3
18	*NEUROD1*	exon 2
19	*NKX2-2*	exon 2
20	*RFX6*	exons 1-2, 4, 6-7, 11, 15, 17-18
21	*ZFP57*	exons 1, 3-4
22	*HADH*	exons 1-3, 5-7, introns 5-6
23	*GLUD1*	exons 6-7, 11-13
24	*SLC16A1*	exons 1-2, 4-5

Supplementary Table 1. The list of genes and target regions.

CDS: Coding sequence

Figure 1. Pedigree, clinical characteristics, and genotype of the family. Filled symbols and empty symbols represent diabetic patients and healthy individuals with normal or unknown genotype, respectively. Dot filled symbol represents healthy individual with the alternate allele. The present age of the individuals is shown below the symbols, followed by the age at diagnosis, the most recent treatment, body mass index (kg/m2) and genotype interpretation. OHA, oral hypoglycemic agents; Genotypes are expressed by normal allele (wt) and alternate allele (at); NT, not tested. An arrow indicates the index case. *NEUROD1 (NM_002500.5)* is located on the reverse strand. IGV browser visualization of the identified heterozygous variant g.182542865G>C (c.723C>G;p.His241Gln) in the index patient is shown at the bottom of the figure. Sanger sequencing chromatograms of both parents, showing the heterozygous variant in the mother and the wild type sequence in the father.

I

II

III

63
40
OHA
30.2
NT

61
45
OHA
30.6
NT

3

2

44
wt/at

wt/wt

C T T A A C G/C T G G A A G

C T T A A C G T G G A A G

6.5
3.7
16.47
wt/at