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Genetic counseling in monogenic diabetes *GCK* MODY

Rola poradnictwa genetycznego w cukrzycy monogenowej *GCK* MODY

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Abstract

Introduction. Genetic testing in families with monogenic *GCK* MODY has predictive, diagnostic, and preventive utility. Predictive tests relate to people who have no features of the disorder themselves at the time of testing. Diagnostic tests relate to family members who have been previously diagnosed with diabetes mellitus or glucose metabolism disturbances. The preventive value of genetic testing for families is to raise awareness of the circumstances leading to glucose metabolism disorders. **Aim.** The detection of mutation carriers among family members of patients with *GCK* MODY and the determination of the clinical significance of the genetic test result. **Methods.** The study group included 27 families of adolescent patients with *GCK* MODY (39 (75%) of parents and 19 (73.08%) of siblings) monitored in the Department of Pediatrics, Endocrinology and Diabetes and in the Diabetes Clinic of John Paul II Upper Silesian Child Health Centre in Katowice in the years 2007-2012. Subjects underwent a blood sample drawing for genetic and biochemical testing. **Results.** Through the genetic diagnostics we diagnosed *GCK* MODY in 14 (63.64%) mothers, 6 (35.29%) fathers and in 7 (36,84%) siblings. Genetic testing has contributed to the detection of 7 (26.92%) asymptomatic carriers of GCK gene mutation among parents and 3 (15,79%) asymptomatic carriers among siblings declaring no carbohydrate metabolism disturbances (before genetic testing there were no indications suggesting carbohydrate metabolism disturbances; OGTT were performed after positive genetic testing). **Conclusions.** Each case of mutation detection, which is the cause of monogenic diabetes in a patient, justifies the genetic testing in other members of his/her family. Awareness of the genetic status may allow sick family member to confirm the diagnosis, while asymptomatic mutation carriers could benefit from an early clinical observation. Consequently, in each case it gives an opportunity to take diagnostic and therapeutic measures in accordance with the current state of knowledge.

Key words:
GCK MODY, monogenic diabetes, glucokinase gene, genetic testing

Streszczenie

Wstęp. Badania genetyczne w rodzinie, w której występuje cukrzyca monogenowa *GCK* MODY, mają z założenia charakter prognostyczny, diagnostyczny oraz prewencyjny. Testy prognostyczne odnoszą się do osób, które w chwili badania są zdrowe, ale ze względu na autosomalny dominujący sposób dziedziczenia objęte są dużym ryzykiem zachorowania. Badania diagnostyczne odnoszą się do członków rodziny, którzy mają wcześniej postawione rozpoznanie cukrzycy lub zaburzeń gospodarki węglowodanowej. Rola prewencyjna w przypadku rodzin, w których przeprowadzone zostały badania genetyczne, polega na uświadomieniu, w jakich sytuacjach dochodzić może do zaburzeń gospodarki węglowodanowej. **Cel pracy.** Wykrycie nosicieli mutacji wśród członków rodziny pacjentów z *GCK* MODY i określenie znaczenia klinicznego wyniku badania genetycznego. **Materiał i metodyka.** Badana grupa obejmowała rodziny 27 pacjentów z cukrzycą *GCK* MODY (39 <75%> rodziców oraz 19 <73,08%> rodzeństwa) pozostających

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pod opieką diabetologiczną w Oddziale Pediatrii, Endokrynologii i Diabetologii Dziecięcej z Pododdziałem Diabetologii Dziecięcej oraz Poradni Diabetologicznej GCZD w Katowicach w latach 2007–2012. U członków rodziny pacjenta z rozpoznaną cukrzycą typu *GCK* MODY pobrano próbkę krwi celem wykonania badań genetycznych i biochemicznych. **Wyniki.** W wyniku przeprowadzonej diagnostyki genetycznej cukrzycę *GCK* MODY zdiagnozowano u 14 (63,64%) matek, u 6 (35,29%) ojców oraz u 7 (36,84%) rodzeństwa. Badania genetyczne przyczyniły się do wykrycia 7 (26,92%) bezobjawowych nosicieli mutacji w genie GCK wśród rodziców oraz 3 (15,79%) u rodzeństwa, deklarujących brak zaburzeń ze strony gospodarki węglowodanowej (u tych członków rodzin przed badaniami genetycznymi nie zaistniały wskazania do przeprowadzenia testów OGTT; zostały one wykonane po uzyskaniu pozytywnych wyników badań genetycznych). **Wnioski.** Podsumowując, w każdym przypadku identyfikacji mutacji, która jest przyczyną cukrzycy monogenowej, istnieje potencjalnie sens wykonania badań genetycznych u innych członków rodziny (43,52). Wiedza na temat statusu genetycznego może pozwolić chorym członkom rodziny na zweryfikowanie rozpoznania, bezobjawowych nosicieli mutacji pozwoli objąć wczesną obserwacją kliniczną, a w rezultacie w każdym z tych przypadków daje szansę na prowadzenie postępowania diagnostyczno-terapeutycznego zgodnego z bieżącym stanem wiedzy.

Słowa kluczowe:
GCK MODY, cukrzyca monogenowa, gen glukokinazy, badania genetyczne

Introduction

GCK MODY develops following heterozygous inactivating mutation in the glucokinase gene. The disease is inherited as an autosomal dominant disorder, but it also occurs as a de novo mutation [1,2]. Thus, if the parents are healthy and the mutated gene has a full penetration, it must be assumed that the child's illness occurred as a result of a new mutation. In contrast, a person suffering from an autosomal dominant disease has a 50% risk of the disease in the offspring, if the penetrance of the mutated gene is full, regardless of the gender of the parent and the gender or the number of the offspring. Autosomal dominant diseases are characterized by a vertical pattern of inheritance. This means that, in typical cases of inheritance, each patient has a sick parent who transmits the abnormal gene. However, there are phenomena in the autosomal dominant inheritance pattern which hinder the determination of genetic risk. These include incomplete penetrance, variable expressivity, and delayed manifestation. Incomplete penetrance is when the disease is not disclosed in all pathological gene carriers. Variable expressivity refers to the range of signs and symptoms that occur in family members with the same genetic condition. Delayed manifestation is characterized by the disclosure of the disease at the age where the patient has already his own children, who could have received the abnormal gene.

Genetic counseling is therefore extremely important for families with *GCK* MODY and has a predictive, diagnostic, and preventive value.

Aim

Detection of mutation carriers among family members of patients with *GCK* MODY and determination of the clinical significance of the genetic test result.

Material and Methods

The study group included 27 families of adolescent patients with *GCK* MODY (39 <75%> of parents and 19 <73.08%> of siblings) monitored in the Department of Pediatrics, Endocrinology and Diabetes and in the Diabetes Clinic of John Paul II Upper Silesian Child Health Centre in Katowice in the years 2007–2012.

The family members of patients with *GCK* MODY and, if necessary their parents/legal guardians, gave informed written consent to participate in the study. Subjects underwent a blood sample drawing for: 1. Genetic testing. 2. Biochemical testing. Genetic analysis DNA was extracted from peripheral blood cells drawn into EDTA-coated vials. Isolation was performed according to the manufacturer's protocol using QiaAmp DNA mini kits from Qiagen (Qiagen, Hilden, Germany). PCR amplification and DNA purification were carried out using standard methods. DNA sequencing was performed using BigDye Seq kit v3.1 (Applied Biosystems, Foster City, CA, USA) and pairs of specific oligonucleotide primers spanning the coding and promoter sequence of the GCK gene. The identification of mutations was performed by direct sequencing using the ABI 3130 genetic analyzer and DNA Sequencing Analysis Software (Applied Biosystems, Foster City, CA, USA). For the comparative analysis of evaluated sequences, human genome reference using Sequencher software v4.1.4 (GeneCodes, Ann Arbor, MI, USA) was used. DNA was also subjected to multiplex ligation-dependent probe amplification (MLPA) testing according to the manufacturer's protocol using a commercially available set MODY-P241 (MRC-Holland, Netherlands) to search for deletions in known monogenic diabetes genes including GCK. All genetic analyses were performed in the Immunopathology and Genetics Laboratory at the Department of Pediatrics, Oncology, Hematology, and Diabetology in Lodz, which is reference ISO9001 certified laboratory with European Molecular Genetics Quality Network (EMQN) collaboration)

Biochemical tests of serum - the oral glucose tolerance test (OGTT) according to WHO recommendations performed in patients not meeting the diabetes criteria in casual plasma glucose sample. This test involves administering 1.75 g of glucose per kilogram of body weight, but a maximum of 75 g glucose to a patient, and blood glucose measurements at

two time points (before the administration of the glucose and after 2 hours).

The study protocol was approved by the Bioethics Committee of the Medical University of Silesia in Katowice KNW/0022/KB1/93/I/11 and the Bioethics Committee of the Medical University of Lodz RNN/62/08/KE of 19 February 2008. The study was conducted in due diligence in accordance with "The Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine" of 4 April 1997 and according to "the

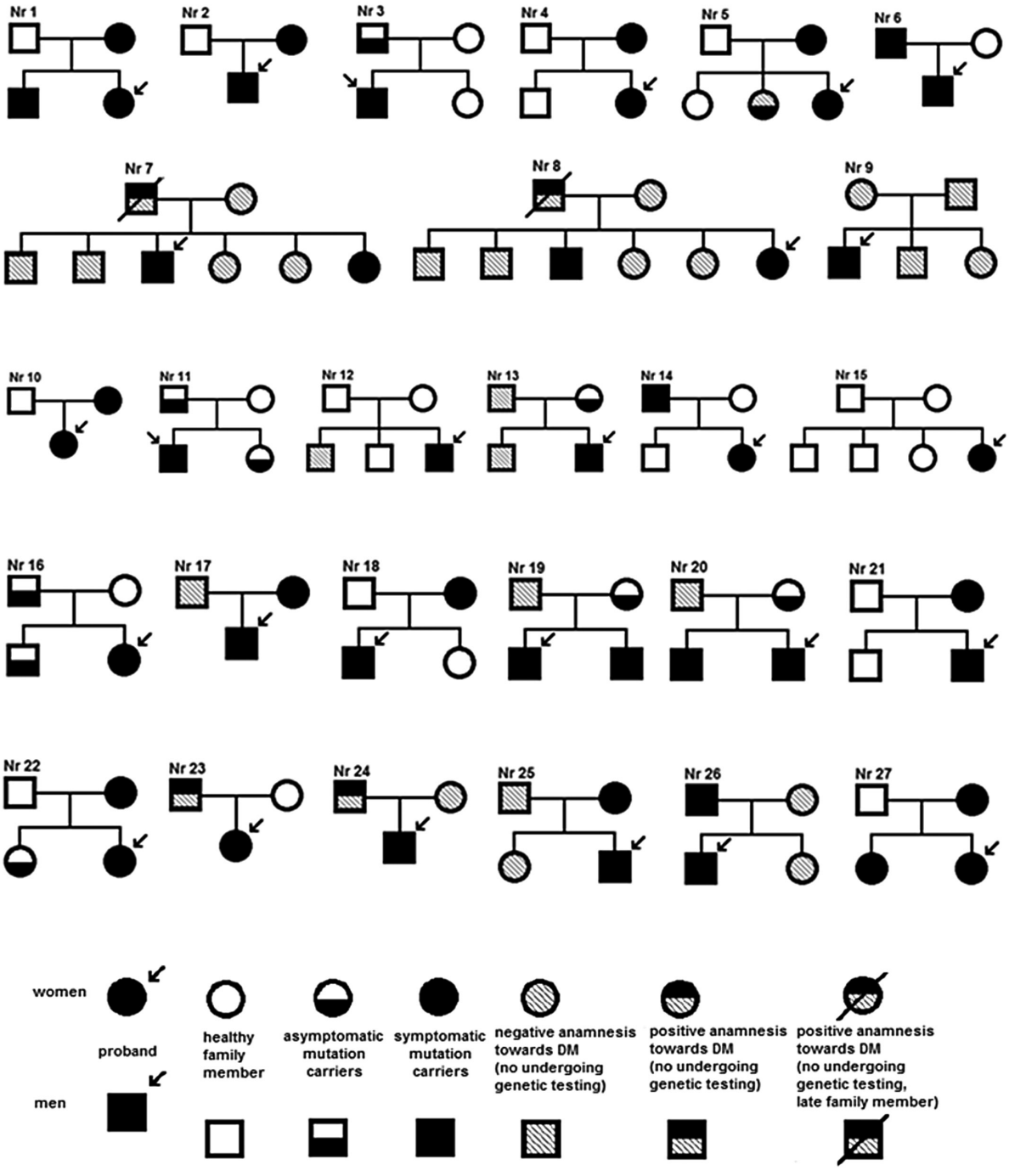


Fig. 1. The glucokinase gene mutation inheritance pattern
Ryc. 1. *Schemat dziedziczenia mutacji w genie glukokinazy*

Genetic testing for health purposes" guidelines (Report of the Genetic Research and the Biological Storage Working Group) of 2012.

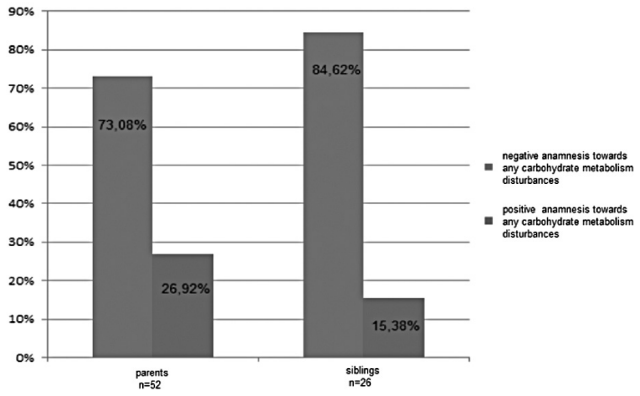
Results

The analysis of the type of inheritance of 27 patients with *GCK* MODY revealed that 14 patients (51.85%) inherited the disease from their mothers, 6 patients (22.22%) from their fathers. In two patients (7.41%) the glucokinase gene mutation occurred *de novo*. In 6 cases (18.52%) the nature of inheritance has not been determined for objective reasons (lack of one of the parents, single parenthood, lack of cooperation). Figure 1 presents the *GCK* MODY inheritance pattern.

Based on the medical history and medical data of 54 parents and 26 siblings, 17 (62.96%) mothers, 21 (77.78%) fathers and 22 (84.62%) siblings had not declared any carbohydrate metabolism disturbances prior to the genetic testing (parents and siblings with no diabetes history were qualified to the healthy group). While 10 (37.04%) of mothers, 4 (14.81%) of fathers and 4 (15.38%) of siblings had a history of such disturbances. The genetic research involved a total of 39 (72.22%) parents, 22 (81.48%) mothers, 17 (62.96%) fathers and 19 (73.08%) siblings. 12 of the mothers (54.55%) under genetic diagnostics were healthy, 10 (45.45%) had a carbohydrate metabolism disturbances. Among fathers: 14 (82.35%) were healthy, 3 (17,65%) had carbohydrate metabolism disturbances; among siblings 15 (78.95%) were healthy and 4 (21.05%) had carbohydrate metabolism disturbances. Through the genetic diagnostics we diagnosed *GCK* MODY in 14 (63.64%) mothers, 4 of whom (18.18%) were initially included in the group of healthy parents; 6 (35.29%) fathers, 3 of whom (21.43%) were initially included in the group of healthy parents and in 7 (36.84%) siblings, 3 of whom (15.79%) at first belonged to the group of healthy siblings. Genetic testing has contributed to the detection of 7 (26.92%) asymptomatic carriers of GCK gene mutation among parents and 3 (15.79%) asymptomatic carriers among siblings declaring no carbohydrate metabolism disturbances. The detailed characteristics of the health status of family members is presented in graphs 1-2.

The parents and siblings newly diagnosed with *GCK* MODY were invited to continue the diagnostics. The 16 (84.21%) of parents, and the 6 (75.0%) of siblings underwent the oral glucose tolerance test, or in the case of family members diagnosed with diabetes in advance we used the OGTT result from their medical data. The diagnostic criteria for diabetes in the OGTT were met by 8 (50.0%) of the parents and 2 (33.33%) of the siblings. Impaired fasting glucose and/or impaired glucose tolerance were detected in 7 (46.67%) of the parents and 2 (33.33%) of the siblings. Only 1 (6.67%) parent and 2 (33.33%) siblings had normal glucose levels in the OGTT.

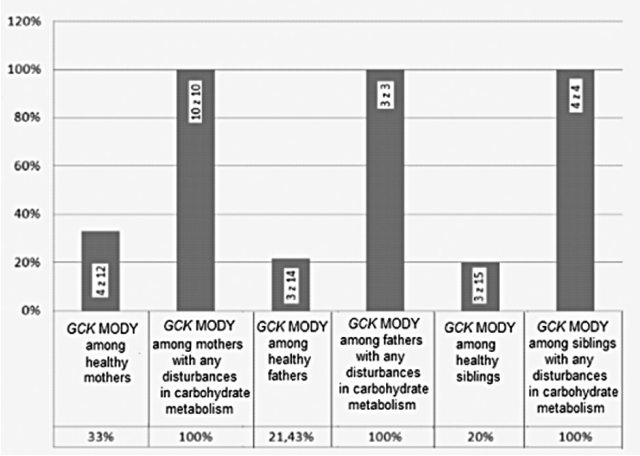
Analyzing the therapy of the parents diagnosed with *GCK* MODY we found out that 2 (11.76%) out of the 17 parents with *GCK* MODY used insulin, 1 parent (5.88%) used insulin associated with the oral hypoglycemic agents, 3 parents



Graph 1. Family members health status prior to the genetic diagnostics

Wykres 1. *Status zdrowia członków rodziny przed badaniami genetycznymi*

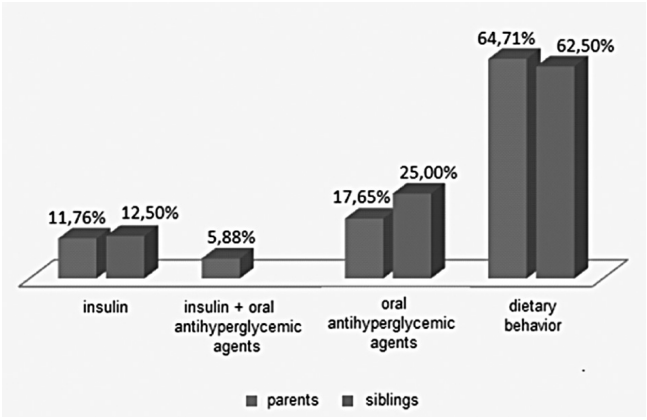
(parents – rodzice, siblings – rodzeństwo, negative anamnesis towards any carbohydrate metabolism disturbances – ujemny wywiad w kierunku zaburzeń gospodarki węglowodanowej, positive anamnesis towards any carbohydrate metabolism disturbances – dodatni wywiad w kierunku zaburzeń gospodarki węglowodanowej)



Graph 2. Family members health status after the genetic diagnostics

Wykres 1. *Status zdrowia członków rodziny po badaniach genetycznych*

(GCK MODY among healthy mothers – GCK MODY wśród zdrowych matek, GCK MODY among mothers with any disturbances in cabohydrate metabolism – GCK MODY wśród matek z zaburzeniami gospodarki węglowodanowej, GCK MODY among healthy fathers – GCK MODY wśród zdrowych ojców, GCK MODY among fathers with any disturbances in cabohydrate metabolism, GCK MODY wśród ojców z zaburzeniami gospodarki węglowodanowej, GCK MODY among healthy siblings – GCK MODY wśród zdrowego rodzeństwa, GCK MODY among siblings with any disturbances in cabohydrate metabolism – GCK MODY wśród rodzeństwa z zaburzeniami gospodarki węglowodanowej)



Graph 3. The therapies of the parents and siblings with GCK MODY

Wykres 3. *Model terapii wśród rodziców i rodzeństwa z cukrzycą GCK MODY*

(parents – rodzice, siblings – rodzeństwo, insulin – insulina, oral antihyperglicemic agents – doustne leki hipoglikemizujące, dietary behavior – postępowanie dietetyczne)

(17.65%) and 2 siblings (25.0%) used oral hypoglycemic agents only, 11 parents (64.71%) and 5 siblings (62.5%) used dietary behavior. A detailed analysis of the therapies is presented in the graph 3.

Discussion

Recently, one of the most important achievement is the growing availability of genetic testing. It allows to extend the diagnosis not only in patients with atypical clinical course of diabetes, but also in members of their families. Thus, it provides us with earlier diagnosis verification and allows appropriate treatment initiation.

Genetic testing in families with monogenic *GCK* MODY has predictive, diagnostic and preventive utility [3,4]:

Predictive tests relate to people who have no features of the disorder themselves at the time of testing, but because of the autosomal dominant mode of inheritance are at high risk of developing the disorder in the future. The likelihood of inheritance of the mutated gene in each generation is statistically 50%. Detection of mutation carriers who have not yet become ill or remain unaware of the diagnosis may allow early detection of glucose metabolism disorders. It may allow the implementation of appropriate therapy to maintain a proper metabolic balance as a preventive action. In addition, a predictive test through exclusion of the inherited disease with such a high probability could reduce a feeling of insecurity, thus plays a psychological role. Negative testing result in patients without diabetes frees them from concerns for the development of this form of the disease in the future. Their risk of developing diabetes reduces to a value characterizing the general population [5,6].

Diagnostics tests relate to family members who are previously diagnosed with diabetes mellitus or glucose metabolism disturbances. Their main purpose is to determine the etiopathology and molecular basis of the disease, which is usually to confirm the diagnosis. As for the diagnostic tests, they allow an accurate diagnosis and implementation of adequate treatment [1,5,6]. It affects both the quality of life, and good metabolic control of hypoglycemia in particular [3].

The preventive value of genetic testing for families is to raise awareness of the circumstances leading to glucose metabolism disorders. Hyperglycemia occurs most frequently in the acute phase of diseases other than diabetes. It occurs in the course of infection, trauma, fever or a "metabolic stress" associated with a disease. In addition, a carbohydrate metabolism disorder can develop during the pregnancy. Therefore, careful observation of a pregnant woman should aim to protect the mother and the child from undesired consequences of diabetes.

In our study, family members of children with *GCK* MODY underwent the genetic testing for diagnostic, predictive and preventive reasons.

Diagnostic tests related to 17 family members who had been previously diagnosed with diabetes or with glucose metabolism disturbances. In this case, we managed to make the correct diagnosis and then take a proper care of juvenile siblings. In the case of adult mutation carriers we could discuss with their doctor and make suggestion for changing the therapy.

Predictive tests referred to the subjects who, at the time of the study, did not report any abnormalities in the carbohydrate metabolism or remained unaware of their occurrence. Through the genetic testing 10 asymptomatic mutation carriers have been detected. While in 19 of parents and in 12 of children the mutation in the glucokinase gene has not been found, which practically excludes the risk of developing this form of the disease for a lifetime.

Newly diagnosed carriers of the glucokinase gene mutation continued the diabetes observation as a preventive measure. It aims to early detect glucose metabolism disorders, to implement the appropriate therapy at the right time, thus to maintain the good metabolic control.

In conclusion, each case of mutation detection, which is the cause of monogenic diabetes in a patient, justifies the genetic testing in other members of his family [1,7]. Awareness of the genetic status may allow a sick family member to confirm the diagnosis, while asymptomatic mutation carriers could benefit from an early clinical observation. Consequently, in each case, it gives an opportunity to take diagnostic and therapeutic measures in accordance with the current state of knowledge.

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References

1. Hattersley A, Bruining J, Shield J, et al. *KC. ISPAD Clinical Practice Consensus Guidelines 2009 Compendium. The diagnosis and management of monogenic diabetes in children and adolescents.* Pediatr Diabetes. 2009; 10: 33-42.
2. Massa O, Meschi F, Cuesta-Munoz A et al. *High prevalence of glucokinase mutations in Italian children with MODY. Influence on glucose tolerance, first-phase insulin response, insulin sensitivity and BMI.* Diabetes Study Group of the Italian Society of Paediatric Endocrinology and Diabetes (SIEDP). Diabetologia. 2001; 44: 898-905.
3. Małecki M, Klupa T, Skupień J. *Poradnictwo genetyczne w cukrzycy typu MODY i w utrwalonej cukrzycy noworodków.* Diabet Prakt 2005; 6: 319-325.
4. Stein SA, Maloney KL, Pollin TI . *Genetic Counseling for Diabetes Mellitus.* Curr Genet Med Rep. 2014; 1; 2(2): 56-67.
5. Morel K et al. Genetic diabetes nurse. J Diabetes Nurse 2013; 7: 250-254.
6. Murphy R, Ellard S, Hattersley AT. *Clinical implications of a molecular genetic classification of monogenic beta-cell diabetes.* Nat Clin Pract Endocrinol Metabol 2008; 4: 200-213.
7. Ellard S, Bellanné-Chantelot C, Hattersley AT. *European Molecular Genetics Quality Network (EMQN) MODY group. Best practice guidelines for the molecular genetic diagnosis of maturity-onset diabetes of the young.* Diabetologia 2008; 51: 546-553.