sciendo

ENDOCRINE REGULATIONS, Vol. 54, No. 4, 260–265, 2020

doi:10.2478/enr-2020-0029

Comparison of diabetes phenotype in children and their mothers with permanent neonatal diabetes mellitus carrying the same *KCNJ11* variants

Juraj Stanik [1,2], Lubomir Barak [2], Adriana Dankovcikova [3], Terezia Valkovicova [1], Martina Skopkova [1], Daniela Gasperikova [1]

[1]DIABGENE Laboratory, Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia; [2]Children Diabetes Centre of the Slovak Republic at the Department of Paediatrics, Medical Faculty of Comenius University and National Institute for Children´s Diseases, Bratislava, Slovakia; [3]Department of Pediatrics, Children Faculty Hospital, Kosice, Slovakia
E-mail: juraj.stanik@savba.sk

Objective. Mutations of the *KCNJ11* gene are the most common cause of the permanent neonatal diabetes mellitus (PNDM). Majority of people with KNCJ11-PNDM have a de-novo mutation. We aimed to compare diabetes phenotype in two children and their mothers with PNDM carrying the same sulfonylurea-sensitive *KCNJ11* variants.

Methods. We have compared glibenclamide (sulfonylurea) dose, C-peptide, and HbA1c serum levels in two children and their mothers with PNDM up to 5.5-year follow-up. All of them were carrying a heterozygous activating *KCNJ11* pathogenic variant (p.R201H in Family 1 or p.H46Y in Family 2). The mothers were initially treated with insulin and successfully switched to sulfonylurea at the age of 24 and 11 years, respectively. Both children were treated with sulfonylurea since the diagnosis of PNDM.

Results. Glibenclamide dose was similar in both children (0.02–0.03 mg/kg/day), but lower compared to their mothers (0.1–0.4 mg/kg/day) ($p<0.002$). Fasting serum C-peptide levels were also lower in children (70–210 pmol/l) than in their mothers (263–720 pmol/l) ($p<0.002$), but no significant differences were observed in postprandial C-peptide levels. HbA1c was lower only in the son of SVK4 (Family 2) compared to his mother, as she had poor adherence to the sulfonylurea therapy during the first years after the sulfonylurea switch.

Conclusions. Evaluation of the treatment in people with sulfonylurea-sensitive KNCJ11-PNDM should respect the age of patients together with the type of mutation and duration of diabetes at therapy start and may differ within one family.

Key words: permanent neonatal diabetes mellitus, KCNJ11, children, mothers, diabetes control, sulfonylurea, C-peptide

Permanent neonatal diabetes mellitus (PNDM) is a rare condition (Stanik et al. 2007), mostly with a monogenic origin. Heterozygous activating mutations in the *KCNJ11* gene are the most common cause of PNDM and majority of the people with KCNJ11-PNDM could be successfully treated with sulfonylureas (Pearson et al. 2006; Bowman et al. 2018). Until 2005, all people with KCNJ11-PNDM were treated with insulin. Currently, insulin treatment is mainly used before the confirmation of a (likely) pathogenic sulfonylurea-sensitive *KCNJ11* variant. Only children with PNDM, who inherited a mutation from their parents, could be treated with sulfonylurea since the diabetes onset.

However, not all of the people with KCNJ11-PNDM treated with sulfonylureas could maintain

Corresponding author: Juraj Stanik, MD, Ph.D., Department of Paediatrics, Medical Faculty of the Comenius University, Limbova 1, 833 40 Bratislava, Slovakia; phone: +421 259 371 201; e-mail: juraj.stanik@savba.sk.

good glycemic control during the long-term follow-up (Bowman et al. 2018; Stanik et al. 2018). Some of them have very high sulfonylurea dose or have a combination therapy with insulin (Myngheer et al. 2014; Thurber et al. 2015; Misra et al. 2018). According to some studies, the age at sulfonylurea switch (i.e. time of DM duration) is important for the success of the switch. Most of the people with KCNJ11-PNDM have a *de-novo* mutation. Only few repots on the PNDM course within one family have previously been published (Pearson et al. 2006; Thurber et al. 2015). According to the knowledge from other types of monogenic diabetes (e.g. MODY), diabetes phenotype and treatment within one family is often similar. Therefore, we have compared diabetes phenotype and treatment up to 5.5 years after the onset of sulfonylurea therapy in two children and their mothers with KCNJ11-PNDM.

Subjects and methods

Subjects. Children and their mothers from two families with KCNJ-PNDM were included to the study. All patients or their parents signed the informed consent. All steps of this study were approved by the Faculty Hospital Ethics Committees in Bratislava and Kosice.

Family 1 (*KCNJ11* pathogenic variant c.602G>A, p.R201H). Mother (SVK1) and her daughter (SVK10) have a heterozygous activating *KCNJ11* pathogenic variant (c.602G>A, p.R201H) and developed PNDM at the age of 18 (SVK1) and 3 (SVK10) weeks, respectively. The mother was initially treated with insulin and successfully switched to sulfonylurea derivative glibenclamide (Maninil, Berlin-Chemie, Germany) aged 24 years (Stanik et al. 2007). Her daughter (SVK10) was born at the 38th gestational week by caesarean section without signs of diabetic fetopathy [birth weight 3 230g (–0.1 SD), length 49 cm (–0.3 SD)] (Stanik et al. 2020). During the first weeks of postnatal life, the mother glycemia was regularly monitored and described only mild decrease of appetite at the time of diabetes onset at three weeks. Diabetic ketoacidosis was not present. Despite not checking the *KCNJ11* mutation in the child prior the diabetes onset, we decided for the sulfonylurea therapy (glibenclamide), as the PNDM inherited from her mother was the most probable diagnosis. The diagnosis of KCNJ11-PNDM was later confirmed in SVK10 by DNA analysis.

Family 2 (*KCNJ11* pathogenic variant c.136C>T, p.H46Y). Mother (SVK4) and her son (SVK11) have a heterozygous activating *KCNJ11* pathogenic variant (c.136C>T, p.H46Y) and developed PNDM at the age of 15 (SVK1) and 2 (SVK11) weeks, respectively. The mother was successfully switched from insulin to glibenclamide (Maninil, Berlin-Chemie, Germany) aged 11 years (Stanik et al. 2007). Her son (SVK11) was born during the 39th gestational week by a spontaneous delivery, without any complications or signs of diabetic fetopathy [birth weight 3 230 g (–0.5 SD), length 50 cm (–0.3 SD)] (Stanik et al. 2020). He become diabetic at 2 weeks of age and similarly to SVK10, we decided to start the therapy with sulfonylurea (glibenclamide). The diagnosis of KCNJ11-PNDM was later confirmed by DNA analysis.

Methods. We have compared data from the first 5 years (in SVK1 and SVK10) and 4 years (in SVK4 and SVK11) on sulfonylurea treatment in two mothers and their children with KCNJ11-PNDM.

Genetic analysis of the *KCNJ11* gene (NM_000525.3) was performed in children SVK10 and SVK11 by Sanger sequencing using previously described primers (Flanagan et al. 2006). In all individuals, the glucose and serum C-peptide levels were measured in local laboratories by standardized methods. HbA1c was evaluated from whole blood by HPLC analyzer (Bio-Rad, Hercules, CA) in SVK4 and SVK11, and by LPLC DiaSTAT analyzer (Bio-Rad) in SVK1 and SVK10. Anthropometric data (i.e. height and weight) were taken by specialized nurses in the pediatric endocrinology outpatient clinics. The weight, height, and BMI percentiles and standard deviation scores were calculated using international reference data (Fenton et al. 2013) and standardized to sex and gestational age for values at birth and using local anthropometric references for the follow-up values (Kobzova et al. 2004).

Statistical analysis. Comparison of two groups metric data was performed using t-test after checking for normality. The data of sulfonylurea dose, HbA1c, and C-peptide in mothers were taken prior pregnancies. Therefore, we consider them as independent from the values in their children. Statistical analyses and graphs were performed with GraphPad Prism v7.0 software (GraphPad Software, San Diego, USA).

Results

Family 1 (*KCNJ11* pathogenic variant c.602G>A, p.R201H). Mother SVK1 was switched to glibenclamide at 24 years of age (Stanik et al. 2007). The maximum sulfonylurea dose of 0.5 mg/kg/day was given during the first week and reached minimum of 0.1 mg/kg/day 6 months after the switch. During the next years, the glibenclamide dose increased up to

0.54 mg/kg/day. After the therapy switch from insulin to glibenclamide monotherapy, HbA1c decreased in SVK1 from 9.3% (78.1 mmol/mol) to minimum 5.8% (39.9 mmol/mol) during the 5th year of the follow-up (Figure 1). However, later HbA1c worsened during the follow-up (Stanik et al. 2018). As expected, C-peptide increased on glibenclamide compared to the values on insulin treatment (C-peptide mean on glibenclamide

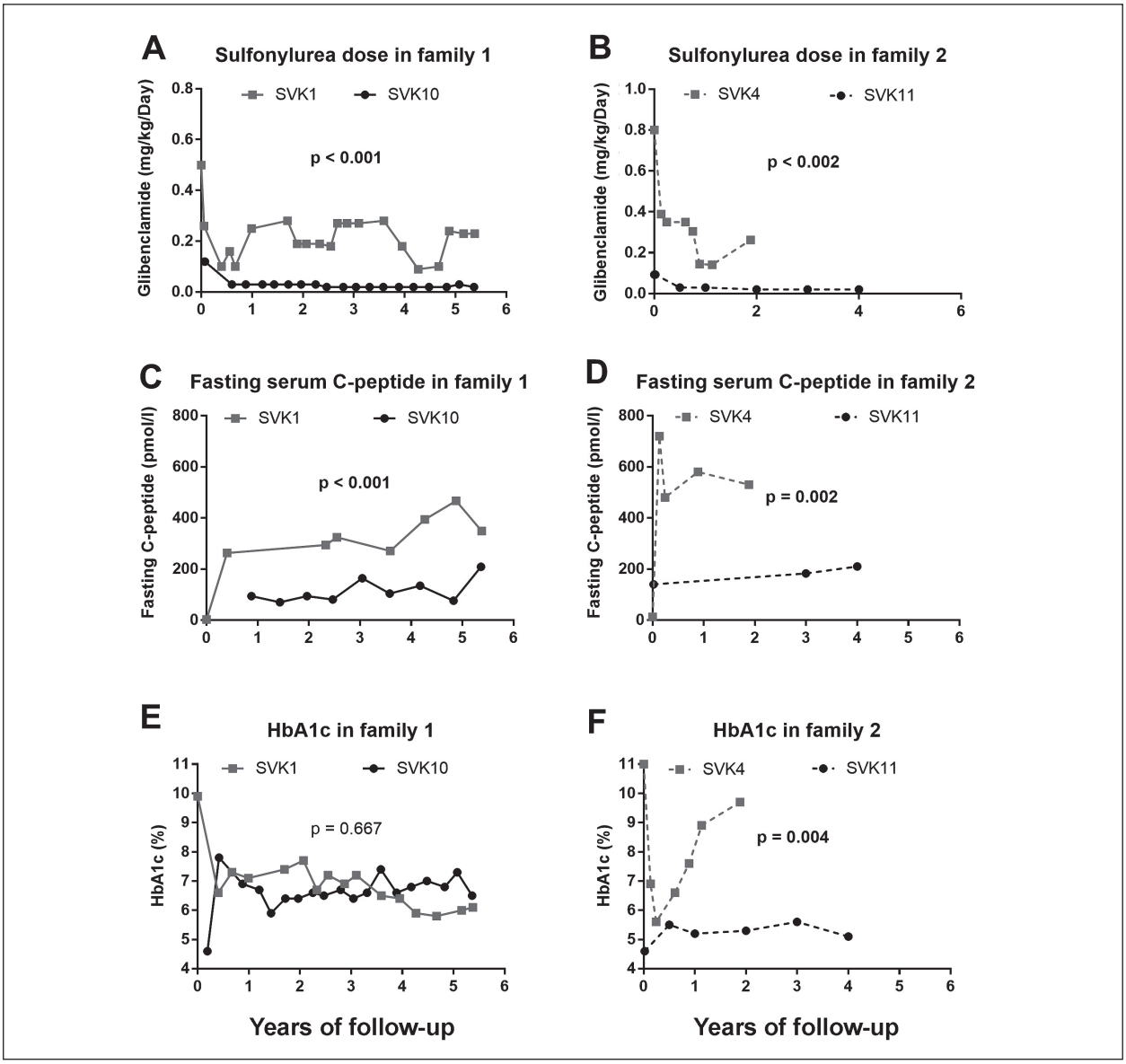

Figure 1. Comparison of the sulfonylurea dose and C-peptide and HbA1c levels in children and their mothers with PNDM due to the mutation in the *KCNJ11* gene. The figure shows follow-up to 5.5-year after starting the sulfonylurea treatment. Values for sulfonylurea dose (mg/kg/day) (**A** for Family 1 and **B** for Family 2), C-peptide (pmol/l) (**C** for Family 1 and **D** for Family 2), and HbA1c (%, DCCT) (**E** for Family 1 and **F** for Family 2) are shown by grey lines for SVK1 (mother), black lines for SVK10 (daughter of SVK1), grey dotted lines for SVK4 (mother), and dotted black lines for SVK11 (son of SVK4). SVK4 omitted her sulfonylurea therapy twice for two weeks during the 2nd year of follow-up leading to diabetic ketoacidosis. The dose of glibenclamide was calculated as the sum of the glibenclamide doses (in mg) during a day divided by the weight in kg. The first values (X=0) of C-peptide and HbA1c for the SVK1 and SVK4 represent the data on insulin treatment and were not included to the comparison between mothers and their children on sulfonylurea treatment. Differences between the child and the mother within a family was calculated with unpaired t-test. The p-values <0.05 were considered as significant. Abbreviations: DM – diabetes mellitus; PNDM – permanent neonatal diabetes mellitus; KCNJ11-PNDM – permanent neonatal diabetes mellitus due to a heterozygous activating mutation of the *KCNJ11* gene; HbA1c – glycated hemoglobin.

was 337.4±27.7 pmol/l versus 3.3 pmol/l on insulin). Since the start of the sulfonylurea treatment, there were no apparent changes in the fasting C-peptide values. However, they were below the normal range (370–1470 pmol/l) (Figure 1).

SVK10 (daughter of SVK1) was treated since diabetes diagnosis with glibenclamide with the initial dose of 0.12 mg/kg/day divided into three doses. Since the 6th month of therapy, the dose decreased to 0.02–0.03 mg/kg/day during the next years of follow-up. HbA1c was highest during the first half of year on glibenclamide treatment (7.8%; 61.7 mmol/mol) and fluctuated between 5.9% and 7.4% (41–57.4 mmol/mol) later. Fasting serum C-peptide levels were between 94 and 209 pmol/l during the 5.5-year follow-up. All of the fasting C-peptide values were below the normal range (370–1470 pmol/l).

Family 2 (KCNJ11 pathogenic variant c.136C>T, p.H46Y). Mother (SVK4) had the maximum sulfonylurea dose (0.8 mg/kg/day) during the first week and reached minimum at the end of the first year after the switch (0.14 mg/kg/day). During the next years, glibenclamide dose was stable. However, her compliance was poor during the 2nd to 5th year of follow-up (age 15 to 18 years), when she did not attend any diabetologist, and she omitted her sulfonylurea therapy twice for two weeks leading to diabetic ketoacidosis. During the adulthood, she had better compliance and also better diabetes control prior the first pregnancy (HbA1c 5.6–6.2%; 37.7–44.3 mmol/mol) on glibenclamide dose 0.11–0.2 mg/kg/day. After the switch from insulin to sulphonylurea monotherapy, the HbA1c decreased in SVK4 from 11% (96.7 mmol/mol) to minimum of 5.6% (38 mmol/mol) during the 1st and 7th year of follow-up. Her fasting C-peptide levels were higher on sulphonylurea treatment compared to the values on insulin (C-peptide mean on glibenclamide was 508.3±104.4 versus 11.5 pmol/l on insulin). Since the start of sulfonylurea treatment, there were no apparent changes in the fasting C-peptide values (Figure 1).

SVK11 (son of SVK4) was treated with glibenclamide from the diabetes diagnosis with the initial dose of 0.09 mg/kg/day divided into three doses. Since the 5th month of the therapy, glibenclamide dose decreased to 0.02–0.03 mg/kg/day during the next years of follow-up. HbA1c was in the range of 5.1–5.6% (32.2–37.7 mmol/mol) during the follow-up. Fasting serum C-peptide levels were 183 and 210 pmol/l during the 3rd and 4th year of follow-up, which was below the normal range (370–1470 pmol/l).

Children versus mothers. Both children with inherited KCNJ11-PNDM had lower sulfonylurea doses compared to their mothers ($p<0.001$ in Family 1; $p=0.002$ in Family 2) (Figure 1). Fasting serum C-peptide levels were also lower in children ($p<0.001$ in Family 1; $p=0.002$ in Family 2) (Figure 1), while postprandial C-peptide levels were similar compared to their mothers (462–491 pmol/l in SVK10 vs. 383–890 pmol/l in SVK1, $p=0.640$; 456–1790 pmol/l in SVK11 vs. 800–1920 pmol/l in SVK4, $p=0.916$). Differences in HbA1c levels were not significant in family 1 ($p=0.667$). In family 2, HbA1c levels were lower in the son ($p=0.004$), as his mother had poor compliance during the first several years of the follow-up after the sulfonylurea switch (Figure 1).

Discussion

In the present report, we are describing two children with KCNJ11-PNDM who had good glycemic control with lower sulfonylurea doses and lower fasting serum C-peptide levels compared to their mothers carrying the same *KCNJ11* pathogenic variant.

Sulfonylurea is a therapy of choice for the majority of the patients with PNDM caused by mutations activation in the *KCNJ11* gene (Pearson et al. 2006). However, there are differences among people with KCNJ11-PNDM in sulfonylurea dose and diabetes control (Bowman et al. 2018; Stanik et al. 2018). We have found some similarities in diabetes phenotype and treatment in 2 families with a pathogenic heterozygous activating *KCNJ11* variant (p.R201H in Family 1 and p.H46Y in Family 2). They had similar pattern in sulfonylurea dose and HbA1c (decrease after the start of sulfonylurea treatment and subsequent stabilization of values) during the first years of follow-up. After 6 months of treatment, glibenclamide dose was similar in both children (0.02–0.03 mg/kg/day) and lower compared to their mothers (0.1–0.4 mg/kg/day).

Pearson et al. (2006) have described two families with sulfonylurea-sensitive children, but their mothers aged 27 and 43 years failed to be successfully switched to sulfonylurea. In our report, both mothers were successfully switched to sulfonylurea (Stanik et al. 2007), but their higher age at sulfonylurea switch could be one of the factors causing that their sulfonylurea dose was higher compared to children. Thurber et al. (2015) have shown that the age of the initiation of sulfonylurea correlates with the dose of sulfonylurea required at follow-up in probands with various mutations. However, this phenomenon has not been previously described within one family or in children who were not previously treated with insulin. Prenatal or early postnatal identification of

a causal *KCNJ11* mutation in offspring of a parent with KCNJ11-PNDM could help to use sulfonylurea since diabetes diagnosis in the future (Myngheer et al. 2014).

We have also found lower C-peptide serum levels in children (70–210 pmol/l) than in their mothers (263–720 pmol/l), but no significant differences in postprandial C-peptide levels. Low fasting C-peptide levels in people with KCNJ11-PNDM have also been described in our previous study (Stanik et al. 2007). This could be partially explained by the physiology of sulfonylurea action, as the drug enhances particularly glucose/food stimulated insulin secretion. C-peptide levels could be also influenced by the sulfonylurea dose, as lower fasting C-peptide levels were in children with lower glibenclamide dose. On the other hand, we have also previously shown that increasing of sulfonylurea dose over normal therapeutical dose does not lead to an increase in the endogenous insulin secretion nor better glycemic control (Stanik et al. 2018). Insulin sensitivity could be another factor of different fasting C-peptide levels in children and their mothers. However, pre-pubertal children and adults with T1D require similar dose of insulin substitution (units/kg/day) after the end of postinitial remission (honey-moon period). This points to similar insulin sensitivity. Therefore, insulin sensitivity probably could not explain differences in fasting C-peptide levels in children compared to their mothers.

From previous studies (Pearson et al. 2006; Stanik et al. 2007) and our data, it is also apparent that neither sulfonylurea dose nor C-peptide levels correlate with HbA1c levels.

The main limitation of our study is the small number of the participants determined by the low prevalence of PNDM (Stanik et al. 2007). Another limitation is that the children included in this study could benefit from their mothers' experience with PNDM. The mothers have a *de novo KCNJ11* mutation and their parents are not diabetic. However, previous experience could influence the diabetes control in children, but not sulfonylurea dose or C-peptide serum levels.

In conclusion, low sulfonylurea dose and low C-peptide levels may be specific features in young children with KCNJ11-PNDM treated with sulfonylureas since diagnosis of DM. Therefore, evaluation of the treatment in people with sulfonylurea-sensitive KNCJ11-PNDM should respect not only the type of mutation and duration of diabetes at the therapy start but also current age. However, if these factors are not similar, they also could differ within one family.

Acknowledgements

We are grateful for help to physicians of the Children Diabetes Center in Bratislava (Dr. Jancova and Dr. Podolakova), and Pediatric Departments of Safarik University in Kosice (Dr. Paskova and Dr. Petrasova). We also like to thank Alica Mitkova and Miroslava Huckova for their technical support. This work was supported by research grants supported by the Slovak Ministry for Health 2019/20-LFUK-8, Slovak Research and Development Agency APVV 0107-12, and VEGA 1/0211/18.

References

Bowman P, Sulen A, Barbetti F, et al. Effectiveness and safety of long-term treatment with sulfonylureas in patients with neonatal diabetes due to KCNJ11 mutations: an international cohort study. Lancet Diabetes Endocrinol 6, 637–646, 2018.

Fenton TR, Kim JH. A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. BMC Pediatr 13, 59, 2013.

Flanagan SE, Edghill EL, Gloyn AL, Ellard S, Hattersley AT. Mutations in KCNJ11, which encodes Kir6.2, are a common cause of diabetes diagnosed in the first 6 months of life with the phenotype determined by genotype. Diabetologia 49, 1190–1197, 2006.

Kobzova J, Vignerova J, Blaha P, Krejcovsky L, Riedlova J. The 6th nationwide anthropological survey of children and adolescents in the Czech Republic in 2001. Cent Eur J Public Health 12, 126–130, 2004.

Misra S, Vedovato N, Cliff E, De Franco E, Hattersley AT, Ashcroft FM, Oliver NS. Permanent neonatal diabetes: combining sulfonylureas with insulin may be an effective treatment. Diabet Med 35, 1291–1296, 2018.

Myngheer N, Allegaert K, Hattersley AT, McDonald T, Kramer H, Ashcroft FM, Verhaeghe J, Mathieu C, Casteels K. Fetal macrosomia and neonatal hyperinsulinemic hypoglycemia associated with transplacental transfer of sulfonylurea in a mother with KCNJ11-related neonatal diabetes. Diabetes Care 37, 3333–3335, 2014.

Pearson ER, Flechtner I, Njolstad PR, Malecki MT, Flanagan SE, Larkin B, Ashcroft FM, Klimes I, Codner E, Iotova V, Slingerland AS, Shield J, Robert JJ, Holst JJ, Clark PM, Ellard S, Sovik O, Polak M, Hattersley AT, Neonatal Diabetes International Collaborative Group. Switching from insulin to oral sulfonylureas in patients with diabetes due to Kir6.2 mutations. N Engl J Med 355, 467–477, 2006.

Stanik J, Gasperikova D, Paskova M, Barak L, Javorkova J, Jancova E, Ciljakova M, Hlava P, Michalek J, Flanagan SE, Pearson E, Hattersley AT, Ellard S, Klimes I. Prevalence of permanent neonatal diabetes in Slovakia and successful replacement of insulin with sulfonylurea therapy in KCNJ11 and ABCC8 mutation carriers. J Clin Endocrinol Metab 92, 1276–1282, 2007.

Stanik J, Dankovcikova A, Barak L, Skopkova M, Palko M, Divinec J, Klimes I, Gasperikova D. Sulfonylurea vs insulin therapy in individuals with sulfonylurea-sensitive permanent neonatal diabetes mellitus, attributable to a KCNJ11 mutation and poor glycaemic control. Diabet Med 35, 386–391, 2018.

Stanik J, Barak L, Dankovcikova A, Valkovicova T, Skopkova M, Gasperikova D: Diabetes treatment in two pregnant women with permanent neonatal diabetes mellitus due to a KCNJ11 mutation. Diabet Med 2020. Online ahead of print.

Thurber BW, Carmody D, Tadie EC, Pastore AN, Dickens JT, Wroblewski KE, Naylor RN, Philipson LH, Greeley SA, US Neonatal Diabetes Working Group. Age at the time of sulfonylurea initiation influences treatment outcomes in KCNJ11-related neonatal diabetes. Diabetologia 58, 1430–1435, 2015,