Q1: Please supply first full name of the author "I. Karen Temple" unless this is the name by which the author is commonly known

Evgenia Globa*, Nataliya Zelinska, Deborah J.G. Mackay, I. Karen Temple, Jayne A.L. Houghton, Andrew T. Hattersley, Sarah E. Flanagan and Sian Ellard

Neonatal diabetes in Ukraine: incidence, genetics, clinical phenotype, and treatment

DOI 10.1515/jpem-2015-0170
Received April 21, 2015; accepted June 8, 2015

Q2: Please check and confirm the minor edit made to the article title

Abstract

Background: Neonatal diabetes has not been previously studied in Ukraine. We investigated the genetic etiology in patients with onset of diabetes during the first 9 months of life.

Methods: We established a Pediatric Diabetes Register to identify patients diagnosed with diabetes before 9 months of age. Genetic testing was undertaken for 42 patients with permanent or transient diabetes diagnosed within the first 6 months of life (n=22) or permanent diabetes diagnosed between 6 and 9 months (n=20).

Results: We determined the genetic etiology in 23 of 42 (55%) patients; 86% of the patients diagnosed before 6 months and 20% diagnosed between 6 and 9 months. The incidence of neonatal diabetes in Ukraine was calculated to be 1 in 126,397 live births.

Conclusions: Genetic testing for patients identified through the Ukrainian Pediatric Diabetes Register identified *KCNJ11* and *ABCC8* mutations as the most common cause (52%) of neonatal diabetes. Transfer to sulfonylureas improved glycemic control in all 11 patients.

Keywords: neonatal diabetes; sulfonylurea; treatment; Ukraine.

Introduction

The incidence of diabetes mellitus (DM) in pediatric population of Ukraine has greatly increased in recent years, especially in children aged from 0 to 6 years (1, 2). The Ukrainian Pediatric Diabetes Register was established in 2002 and includes 8629 children with type 1 diabetes, a prevalence of 1 in 925 for the pediatric population in 2012.

Over the last decade, it has become clear that not all cases of childhood-onset diabetes are autoimmune type 1 diabetes and patients diagnosed with neonatal diabetes in the first 6 months of life are very likely to have a monogenic form of diabetes (3, 4). The key breakthrough was the discovery that activating mutations in the *KCNJ11* and *ABCC8* genes [encoding the ATP-sensitive potassium channel subunit Kir6.2 and the regulatory subunit sulfonylurea receptor 1 (SUR1)] are the most common cause of neonatal diabetes (5–7). Most of these patients respond to sulfonylurea treatment with an improvement in glycemic control (5, 8–12) without hypoglycemia (13–15). Approximately, 20% of patients have more severe mutations that also cause developmental delay. Improvements in motor tone and cognition have been reported after transfer to sulfonylureas (16).

We established a new neonatal section of the Ukrainian Pediatric Diabetes Registry to identify cases of neonatal diabetes for genetic testing. The aim of the study was to determine the genetic cause of neonatal diabetes in Ukraine and investigate treatment change in patients with *KCNJ11* or *ABCC8* mutations.

Q5: Please check and confirm that section headings have been applied with the level of heading you intended

Materials and methods

Subjects

A neonatal diabetes section of the Ukrainian Pediatric Diabetes Registry was created in 2012 to include children diagnosed with diabetes before 9 months of age identified by regional Ukrainian pediatric endocrinologists. A pediatric neurologist confirmed the presence of any abnormal neurological features.

***Corresponding author: Dr. Evgenia Globa,** Ukrainian Center of Endocrine Surgery, Pediatric Endocrinology Department, Kyiv, Ukraine, Phone: +380-68-530-40-41, E-mail: ie.globa@i.ua
Nataliya Zelinska: Ukrainian Center of Endocrine Surgery, Pediatric Endocrinology Department, Kyiv, Ukraine
Deborah J.G. Mackay and I. Karen Temple: Faculty of Medicine, University of Southampton, UK
Jayne A.L. Houghton, Andrew T. Hattersley, Sarah E. Flanagan and Sian Ellard: Institute of Biomedical and Clinical Science, University of Exeter Medical School, Exeter, EX2 5DW, UK

Q3: Should I be Kiev in English?

Q4: Please check and confirm the affiliation 3

Q6: Please check and cor the running head

Genetic testing

Sequencing of the *KCNJ11*, *ABCC8*, and *INS* genes was carried out according to previously described methods (5, 17–18). Additional testing for chromosome 6q24 methylation and *GLIS3* (19) was performed according to the clinical phenotype of the patients. Analysis of the chromosome 6q24 locus was undertaken using previously described methods to detect duplications, uniparental disomy, and methylation abnormalities (20, 21). Next-generation sequencing of all known neonatal diabetes genes (22) was performed in any child diagnosed in the first 6 months of life where a mutation had not been identified.

Statistical analysis

Clinical characteristics are presented as median (range) and comparative statistics predominantly used the Friedman's test and Fisher's exact test.

Results

Incidence of neonatal diabetes in Ukraine

We identified 46 cases with diabetes diagnosed before 9 months of age (including 24 cases diagnosed in the first 6 months of life) from the Ukrainian Pediatric Diabetes Register. DNA samples were obtained from 42 patients for genetic testing (91.3%). Between January 2012 and December 2014 there were 12 new registered cases with neonatal diabetes (diagnosed before 6 months of age) and 1,516,760 live births, giving an incidence of neonatal diabetes of 1 in 126,397.

Patients with a genetic diagnosis

Mutations were identified in 19/22 patients (86.4%) diagnosed with diabetes in the first 6 months of life (see Table 1). Only 4/20 patients (20%) diagnosed between 6 and 9 months had a mutation. The clinical characteristics of all patients with a mutation causing neonatal diabetes are shown in Table 2.

KCNJ11 mutations (n=8 patients)

All eight patients have isolated diabetes: seven were diagnosed before 6 months and one at 7.5 months. One patient heterozygous for the transient diabetes p.E229K mutation is aged 1.5 years but has not entered diabetes remission yet. The patients with the p.G53D and p.V59M mutations are currently aged 3 and 8 years and show no evidence of developmental delay. One patient, who was diagnosed with diabetes at 3 months of age, inherited the p.R201H *KCNJ11* mutation from his/her 29-year-old mother. Analysis of parental samples confirmed that the remaining seven probands had de novo mutations. Testing of one patient's unaffected twin brother identified mosaicism in his leukocyte DNA at a level of ~10%.

Q7: Please check the edit made to the sentence "One patient, who...29- -old mother." retained its intended meaning

ABCC8 mutations (n=4 patients)

One patient with transient diabetes was heterozygous for a novel p.I585T *ABCC8* mutation which was inherited from his 23-year-old mother who was diagnosed with "type 2" diabetes at 21 years. The proband's maternal grandfather and uncle are also affected (genetic testing was not undertaken in any relatives).

Three patients had severe developmental delay (two siblings with a previously reported heterozygous p.I49F mutation) (11) and one patient had compound heterozygous for the previously reported p.V324M mutation (23) and a novel p.R1394L mutation.

Table 1: Genetic causes of neonatal diabetes in patients from the Ukraine Pediatric Diabetes Register.

Gene	Mutation(s)	Onset before 6 months, n=22 (52.4%)	Onset between 6 and 9 months, n=20 (47.6%)
KCNJ11	p.R201C (n=3), p.R201H (n=2), p.G53D (n=1), p.V59M (n=1), p.E229K (n=1)	7 (31.8%)	1 (5%)
ABCC8	p.V324M/p.R1394L (compound heterozygous), p.I585T, p.I49F (n=2)	4 (18.2%)	–
INS	p.C96Y, p.G32S (n=2), p.L41P	1 (4.5%)	3 (15%)
EIF2AK3	p.E419fs/p.G1010V (compound heterozygous), p.G1010V/p.G1010V (homozygous)	2 (9.1%)	–
GLIS3	p.P444fs/p.H647R	1 (4.5%)	–
Chr6q24	Paternal uniparental isodisomy	3 (13.7%)	–
GCK	p.E395X	1 (4.5%)	–
No mutation		3 (13.7%)	16 (80%)

Table 2: Clinical characteristics of patients with mutations causing neonatal diabetes[a].

Patient	Relative	Gene	Mutation (protein description)	Mutation (DNA description)	Birth weight, g	Gestation, weeks	Age at diagnosis of diabetes, days	Age at remission of diabetes	Age at relapse of diabetes	Current age, years	C-peptide	HbA_{1c}, mmol/mol at Investigation	Antibodies	Developmental delay (mild/ moderate/ severe)	Epilepsy	Other clinical features
1		*KCNJ11*	p.R201C	c.601C>T	2500	40	93	NA	NA	6	0.05	54	GAD 92,6 IE/ mL (n<10)	No	No	
2		*KCNJ11*	p.R201C	c.601C>T	2600	41–42	81	NA	NA	1.5	0.35	81	NA	No	No	
3		*KCNJ11*	p.R201C	c.601C>T	2800	39	121	NA	NA	0.5	0.17	148 (at diagnosis)	NA	No	No	
4		*KCNJ11*	p.R201H	c.602G>A	2300	37	1	NA	NA	1	0.09	128 (at diagnosis)	NA	No	No	
5	Mother of patient 4	*KCNJ11*	p.R201H	c.602G>A	3600	37	90	NA	NA	29	NA	65	NA	No	No	
6		*KCNJ11*	p.G53D	c.158G>A	2650	40	224	NA	NA	3	0.005	47	ICA – neg.	No	No	
7		*KCNJ11*	p.V59M	c.175G>A	2280	39	33	NA	NA	7.5	0.06	54	NA	No	No	Multiply lipoatrophy
8		*KCNJ11*	p.E229K	c.685G>A	2920	40	167	NA	NA	1.5	0.77	49	NA	No	No	
9		*ABCC8*	p.I49F	c.145A>T	2880	38	90	1 y.	2 y. 3m	3.5	0.88	41	NA	Severe	Yes	Severe physical delay
10	Sibling of patient 9	*ABCC8*	p.I49F	c.145A>T	2750	39	180	NA	NA	1.5	0.64	57	NA	Severe	No	Severe physical delay
11		*ABCC8*	p.V324M p.R1394L	c.970G>A c.4181G>T	3100	40	74	NA	NA	6	0.07	61	NA	Severe	No	
12		*ABCC8*	p.I585T	c.1754T>C	2780	34–35	4	10 days	NA	0.2	0.6	100	NA	No	No	
13		*INS*	p.G32S	c.94G>A	3580	41	244	NA	NA	7.5	0.19	90	NA	No	No	
14		*INS*	p.L41P	c.122T>C	2800	40	244	NA	NA	2.5	0.14	77	GAD<5 IE/ mL, ICA neg.	No	No	
15		*INS*	p.C96Y	c.287G>A	3000	40	130	NA	NA	5.5	NA	81	NA	No	No	
16		*INS*	p.G32S	c.94G>A	3000	40	222	8 months	10 months	7	NA	77	NA	No	No	
17		6q24	UPD	–	2010	37	35	5 months	NA	4.5	1.31	37	ICA neg.	No	No	Macroglossia, anemia, patent foramen ovale, intrauterine growth retardation
18		6q24	UPD	–	2000	43	10	8 months	NA	5	0.87	33	NA	No	No	Macroglossia, anemia, patent foramen ovale, intrauterine growth retardation, speech development delay
19		6q24	UPD	–	2350	39	2	16 days	NA	0.5	0.5	22	NA	No	No	Anemia
20		*GLIS3*	p.P444fs p.H647R		1900	39	7	NA	NA	5	Undetectable	76	NA	Mild	No	Congenital hypothyroidism, patent foremen ovale, polycystic kidneys
21		*EIF2AK3*	p.E419fs/ p.G1010V	c.1254_1257 delinsCGCAA TCTGTCACTAA CGCAATTGGT/ c.3029G>T	2800	39	105	NA	NA	3	71.5 pmol/L	68	NA	No	No	
22		*EIF2AK3*	p.G1010V	c.3029G>T	2500	39	70	NA	NA	0.5	0.27	81	NA	No	No	
23		*GCK*	p.E395X	c.1183G>T	3080	37	150	NA	NA	6	0.77	50	NA	No	No	

[a]All mutations are heterozygous except *EIF2*AK3 p.G1010V in patient 22 which is homozygous.

The parents of the two siblings with the p.I49F mutation are unaffected and the mutation was not detected in either parent's leukocyte DNA in keeping with germline mosaicism. Both children have a severe form of developmental delay, epilepsy, and neonatal diabetes (DEND) syndrome. The proband was diagnosed with diabetes at 3 months which remitted at the age of 1 year and relapsed at 2 years 3 months after starting hypothyroidism treatment. At age 2 years, the proband had a severe generalized hypotonia, with no visual contact, no babbling, psychomotor retardation, epilepsy, and severe physical delay [height: 75 cm (<3rd centile), weight: 6000 g (<3rd centile)]. His sister at age 5 months developed convulsions and hypoglycemic coma, and at age 6 months developed neonatal diabetes and received insulin for only few days. At 18 months, she was noted to have psychomotor retardation (severely hypotonic, no babbling, poor visual contact) and growth delay (height: 75 cm [<3rd centile], weight: 8100 g [<3rd centile]).

The patient with the compound heterozygous *ABCC8* mutation had inherited the p.V324M mutation from the father and the novel p.R1394L mutation from the mother. This child has severe iDEND syndrome but no epilepsy. At 7 years of age, he has a severe generalized hypotonia and is unable to sit, hold his head upright, walk or talk.

INS mutations (n=4 patients)

These included one patient diagnosed with diabetes at 4 months with a de novo p.C96Y *INS* mutation and three patients diagnosed between 6 and 9 months (p.L41P and p.G32S n=2). In one case, p.G32S was inherited from the unaffected father whose mutation level in leukocyte DNA is ~15%. Interestingly, the child's diabetes had remitted for 2 months from the age of 8 months. Another child diagnosed at 8 months with a de novo p.G32S mutation is obese (BMI 19.8 kg/m², >95 p.c.) at the age of 6 years. Adding metformin (850 mg/day) to insulin treatment has improved glycemic control with HbA_{1c} reduction from 90 to 70 mmol/mol (10.4%–8.6%), respectively, after 3 months and without any change in insulin dose.

EIF2AK3 (n=2 patients)

One patient was diagnosed with diabetes at the age of 3 months. Sanger sequencing of the *ABCC8* gene identified a novel, maternally inherited, heterozygous variant of uncertain significance, p.P201L (c.602C>T). A trial with sulfonylurea was started, but no durable response was achieved. Further genetic testing of all known neonatal genes by next-generation sequencing identified compound heterozygous *EIF2AK3* mutations, p.E419fs and p.G1010V. At age 3 years the child does not have additional features of Wolcott-Rallison syndrome besides mild growth retardation. Targeted next-generation sequencing in a 2nd patient born to consanguineous parents demonstrated homozygosity for the same novel *EIF2AK3* p.G1010V mutation. The child is currently 8 months of age and does not have other features of Wolcott-Rallison syndrome. Identification of the same novel mutation in two probands raises the possibility that p.G1010V is a founder mutation.

GLIS3 mutations (n=1 patient)

This patient presented in the 1st week of life with diabetic ketoacidosis (glucose >20 mmol/L) and has congenital hypothyroidism, patent foramen ovale with left ventricle distention and polycystic kidneys. The patient is compound heterozygous for two *GLIS3* mutations, p.P444fsdelG and p.H647R (19).

Chromosome 6q24 abnormalities (n=3 patients)

All three patients have paternal uniparental isodisomy. Two had intrauterine growth retardation, macroglossia, anemia, and patent foramen ovale.

GCK (n=1 patient)

The child had mild fasting hyperglycemia (FBG at 5 months 6.1 mmol/L, at 8 months 7.4 mmol/L). Her mother was diagnosed with gestational diabetes at 19 years of age and there is a family history of diabetes affecting the maternal grandparents. A heterozygous *GCK* mutation, p.E395X, was identified in both the mother and child. Neither are receiving pharmaceutical treatment.

Transfer to sulfonylurea therapy for patients with *KCNJ11/ABCC8* mutations

The transfer was performed according to the protocol devised by Hattersley et al. (24) but with three to five daily doses (later reducing to two to three doses) and omission of fast-acting insulin from the outset.

Infants generally needed more frequent doses than those who transferred later. A total of 11 patients with *KCNJ11* and *ABCC8* mutations were able to stop insulin and achieve glycemic control with sulfonylurea (glibenclamide). After 3 months of sulfonylurea treatment, the median HbA_{1c} level decreased from 57 (49–81) to 42 (37–47) mmol/mol [7.4 (6.6–9.6)–6.0 (5.5–6.5)%). After 1 year of sulfonylurea treatment all children had HbA_{1c} level <48 mmol/mol (<6.5%), the median HbA_{1c} level was 38 (36–41) mmol/mol [5.6 (5.4–5.9)%], p=0.016 (see Table 3, Figure 1).

The patient with severe DEND syndrome caused by the compound heterozygous *ABCC8* mutations p.V324M and p.R1394L showed no neurological improvement after 2 years on sulfonylurea treatment (current dose 0.15 mg/kg/day).

Sulfonylurea treatment was initiated in the boy with the p.I49F *ABCC8* mutation when his diabetes relapsed aged 2 years 3 months. The initial dose of 0.07 mg/kg/day was increased to 0.2 mg/kg/day after starting treatment with growth hormone (0.025 mg/kg/day). His sister has been treated with low-dose sulfonylurea (0.06 mg/kg/day) for a year.

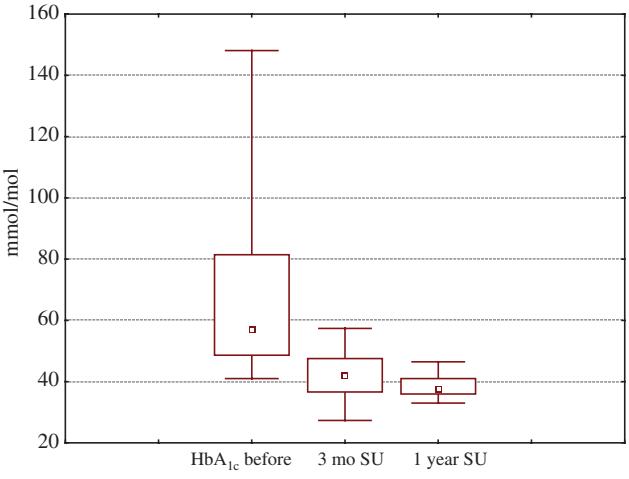

Figure 1: HbA_{1c} (mmol/mol) before 3 months and 1 year after sulfonylurea treatment (p=0.016). The daily dose of sulfonylurea decreased from 0.48 (0.09–0.75) to 0.32 (0.1–0.59) and 0.16 (0.12–0.28) mg/kg/day after 3 months and 1 year of treatment accordingly, p>0.05. No side effects were reported.

Discussion

Establishing a neonatal diabetes section of the Ukrainian Pediatric Diabetes Register has facilitated the identification of cases for genetic testing and provided an estimate of the incidence.

Incidence of neonatal diabetes in Ukraine

The incidence of neonatal diabetes in Ukraine over a 36-month period (2012–2014) was 1 in 126,397. This figure is similar to studies from Germany [1 in 89,000 (25)] and Italy [1:90,000 (26)]. As permanent diabetes (PNDM) accounts for ~50% of neonatal diabetes cases, our incidence is also consistent with reports of PNDM in the UK, the Netherlands, and Poland, of at least 1 in 260,000 live births (27), 1 in 210,000 in Italy (28), and 1 in 214,000 live births from the Slovakian diabetes register (29). The highest incidence

Table 3: Sulfonylurea transfer in 11 patients with *KCNJ11/ABCC8* gene mutations[a].

Patient	Gene	Mutation	Age at transfer	Starting dose, mg/kg/day	Maximum dose, mg/kg/day	Dose after 3 months, mg/kg/day	HbA_{1c} pre-transfer, mmol/mol	HbA_{1c} 3 months later, mmol/mol	Side effects	Neurological improvement (N/A if no features)
1	*KCNJ11*	p.R201C	62 months	0.1	1.1	0.58	54	45	No	NA
2	*KCNJ11*	p.R201C	6 months	0.1	0.7	0.32	81	48	No	NA
3	*KCNJ11*	p.R201C	4 months	0.1	0.2	0.15	148	37	No	NA
4	*KCNJ11*	p.R201H	1 month	0.1	0.25	0.18	128	27	No	NA
5	*KCNJ11*	p.R201H	28 years	5 mg/day	15 mg/day	15 mg/day	65	57	No	NA
6	*KCNJ11*	p.G53D	20 months	0.1	0.75	0.75	47	42	No	NA
7	*KCNJ11*	p.V59M	72 months	0.1	1.0	0.68	54	41	No	NA
8	*KCNJ11*	p.E229K	16 months	0.05	0.1	0.075	49	43	No	NA
9	*ABCC8*	p.I49F	25 months	0.05	0.1	0.1	41	42	No	No
10	*ABCC8*	p.I49F	6 months	0.05	0.07	0.07	57	48	No	No
11	*ABCC8*	p.V324M/ p.R1394L	57 months	0.1	1.0	0.54	61	32	No	No

[a]Patient 12 with the p.I585T *ABCC8* mutation entered remission before initiation of sulphonylurea therapy.

Q8: Please check the unit for "Age at transfer" column in Table 3. Also, please check the footnote for the patient 12

of permanent neonatal diabetes is observed in countries with high rates of consanguinity [1 in 21,196 (30)].

Sulfonylurea transfer in patients with *KCNJ11* and *ABCC8* mutations

K_{ATP} channel gene mutations were the leading cause of neonatal diabetes (52.2%). Transfer from insulin to sulfonylurea was successful in all 11 patients with these mutations with improved glycemic control within 3 months and HbA_{1c} levels below <48 mmol/mol (<6.5%) after 1 year.

The patients with the p.G53D and p.V59M *KCNJ11* mutations are currently aged 3 and 8 years. They have been treated with high-dose sulfonylureas (>0.5 mg/kg/day) and show no evidence of the mild to moderate developmental delay observed in other patients with these mutations (10, 31–34). In contrast, three patients with *ABCC8* mutations (p.I49F in two siblings and one case compound heterozygous for p.V324M and p.R1394L) have severe developmental delay. The siblings with the p.I49F mutation were transferred to low-dose sulfonylurea therapy (≤0.1 mg/kg/day) because of the transient nature of their diabetes. A spike of blood glucose 1 h after eating was observed in two patients (with *KCNJ11* p.R201C and the *ABCC8* mutations p.V324M and p.R1394L). This was solved by increasing the interval of sulfonylurea uptake up to 50 minutes pre meals.

Other genetic causes of neonatal diabetes in Ukraine

We identified three cases of transient neonatal diabetes due to chromosome 6q24 uniparental isodisomy and eight patients with mutations in non-K_{ATP} channel genes. These included four patients with heterozygous *INS* mutations and one patient where sequence analysis of the *GLIS3* gene was indicated by his hypothyroidism and polycystic kidneys. For three patients, the genetic analysis was achieved through testing all the known neonatal diabetes genes by targeted next-generation sequencing (22). Two probands were diagnosed with Wolcott-Rallison syndrome but have not yet developed the extra-pancreatic features. One patient with mild fasting hyperglycemia has a heterozygous *GCK* mutation.

Cut-off age for genetic testing to identify patients with neonatal diabetes

In this study, we performed genetic testing for any patient diagnosed with diabetes before 9 months and identified a genetic etiology in four cases diagnosed after 6 months (7.3–8.0 months), the usual definition of neonatal diabetes. Three probands had an *INS* mutation and one individual was heterozygous for a *KCNJ11* mutation. This patient was able to transfer from daily insulin injections to sulfonylurea tablets resulting in improved glycemic control [HbA_{1c}: 47 mmol/mol (pre-transfer) vs. 42 mmol/mol (3 months post-transfer)]. Consistent with our earlier study, the pick-up rate for mutations was significantly lower in patients diagnosed after 6 months compared to those diagnosed before 6 months (20% vs. 86%; $p \leq 0.0001$) (35). The benefits of a genetic diagnosis, in terms of improving medical management and quality of life for those with K_{ATP} channel mutations as well as providing an accurate recurrence risk, highlights the utility of testing all patients diagnosed up to 9 months of age.

In conclusion, Ukraine has a similar incidence of neonatal diabetes as other European countries and K_{ATP} channel gene mutations are the most common cause. All patients with *KCNJ11* and *ABCC8* mutations were successfully switched from insulin injections to oral sulfonylurea therapy with an improvement in their glycemic control. Targeted next-generation sequencing technology increased the number of patients with a confirmed genetic etiology of neonatal diabetes and provides an accurate recurrence risk for the families. The identification of a *KCNJ11* mutation in a patient diagnosed at 7.5 months highlights the importance of providing genetic testing to those diagnosed between 6 and 9 months.

Acknowledgments: We would like to thank all Ukrainian regional endocrinologists for referring patients. We are grateful to M. Borowiec, K. Antosik, W. Fendler, and W. Mlynarsky from Medical University of Lodz, Poland for the molecular genetic analysis of *GLIS3* in one patient. SE and ATH are supported by a Wellcome Trust Senior Investigator award. DJGM is supported by Diabetes UK project grant 12/0004501. SF has a Sir Henry Dale Fellowship jointly funded by the Wellcome Trust and the Royal Society (Grant Number 105636/Z/14/Z).

References

1. Globa Y, Zelinskaya N. Diabetes complications at pediatric patients of different age groups in Ukraine (results from 3 years follow up data based on Ukrainian Pediatric Diabetes Register). Abstract book of IDF 2011;248.
2. Globa Y, Zelinska N. Trends in incidence and prevalence of DM type 1 in children in Ukraine during 2002–2012. Abstracts for the ESPE Meeting of Pediatric Endocrinology, 18–20 September 2014, Dublin, Ireland.

3. Iafusco D, Stazi MA, Cotichini R, Cotellessa M, Martinucci ME, et al. Permanent diabetes mellitus in the first year of life. Diabetologia 2002;45:798–804.
4. Edghill EL, Dix RJ, Flanagan SE, Bingley PJ, Hattersley AT, et al. HLA genotyping supports a nonautoimmune etiology in patients diagnosed with diabetes under the age of 6 months. Diabetes 2006;55:1895–8.
5. Gloyn AL, Pearson ER, Antcliff JF, Proks P, Bruining GJ, et al. Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. N Engl J Med 2004;350:1838–49.
6. Hattersley AT, Ashcroft FM. Activating mutations in Kir6.2 and neonatal diabetes: new clinical syndromes, new scientific insights, and new therapy. Diabetes 2005;54:2503–13.
7. Ashcroft FM, Harrison DE, Ashcroft SJ. Glucose induces closure of single potassium channels in isolated rat pancreatic beta-cells. Nature 1984;312:446–8.
8. Klupa T, Edghill EL, Nazim J, Sieradzki J, Ellard S, et al. The identification of a R201H mutation in KCNJ11, which encodes Kir6.2, and successful transfer to sustained-release sulphonylurea therapy in a subject with neonatal diabetes: evidence for heterogeneity of beta cell function among carriers of the R201H mutation. Diabetologia 2005;48:1029–31.
9. Zung A, Glaser B, Nimri R, Zadik Z. Glibenclamide treatment in permanent neonatal diabetes mellitus due to an activating mutation in Kir6.2. J Clin Endocrinol Metab 2004;89:5504–7.
10. Sagen JV, Raeder H, Hathout E, Shehadeh N, Gudmundsson K, et al. Permanent neonatal diabetes due to mutations in KCNJ11 encoding Kir6.2: patient characteristics and initial response to sulfonylurea therapy. Diabetes 2004;53:2713–8.
11. Zwaveling-Soonawala N, Hagebeuk EE, Slingerland AS, Ris-Stalpers C, Vulsma T, et al. Successful transfer to sulfonylurea therapy in an infant with developmental delay, epilepsy and neonatal diabetes (DEND) syndrome and a novel ABCC8 gene mutation. Diabetologia 2011;54:469–71.
12. Zhang M, Chen X, Shen S, Li T, Chen L, et al. Sulfonylurea in the treatment of neonatal diabetes mellitus children with heterogeneous genetic backgrounds. J Pediatr Endocrinol Metab 2015. [Epub ahead of print].
13. Pearson ER, Flechtner I, Njølstad PR, Malecki MT, Flanagan SE, et al. Neonatal Diabetes International Collaborative Group: switching from insulin to oral sulfonylureas in patients with diabetes due to Kir6.2 mutations. N Engl J Med 2006;355:467–77.
14. Rafiq M, Flanagan SE, Patch AM, Shields BM, Ellard S, et al. Neonatal Diabetes International Collaborative Group: effective treatment with oral sulfonylureas in patients with diabetes due to sulfonylurea receptor 1 (SUR1) mutations. Diabetes Care 2008;31:204–9.
15. Klupa T, Skupien J, Mirkiewicz-Sieradzka B, Gach A, Noczynska A, et al. Efficacy and safety of sulfonylurea use in permanent neonatal diabetes due to KCNJ11 gene mutations: 34-month median follow-up. Diabetes Technol Ther 2010;12:387–91.
16. Slingerland AS, Nuboer R, Hadders-Algra M, Hattersley AT, Bruining GJ. Improved motor development and good long-term glycaemic control with sulfonylurea treatment in a patient with the syndrome of intermediate developmental delay, early-onset generalised epilepsy and neonatal diabetes associated with the V59M mutation in the KCNJ11 gene. Diabetologia 2006;49:2559–63.
17. Stoy J, Edghill EL, Flanagan SE, Ye H, Paz VP, et al. Insulin gene mutations as a cause of permanent neonatal diabetes. Proc Natl Acad Sci USA 2007;104:15040–4.
18. Proks P, Arnold AL, Bruining J, Girard C, Flanagan SE, et al. A heterozygous activating mutation in the sulphonylurea receptor SUR1 (ABCC8) causes neonatal diabetes. Hum Mol Genet 2006;1:1793–800.
19. Borowiec M, Hrytsuik I, Antosik K, et al. Novel complex mutations in *GLIS3* gene cause neonatal diabetes and congenital hypothyroidism. Pediatr Diabetes 2012;13:66.
20. Temple IK, Gardner RJ, Mackay DJ, Barber JC, Robinson DO, et al. Transient neonatal diabetes: widening the understanding of the etiopathogenesis of diabetes. Diabetes 2000;49:1359–66.
21. Mackay DJ, Temple IK, Shield JP, Robinson DO. Bisulphite sequencing of the transient neonatal diabetes mellitus DMR facilitates a novel diagnostic test but reveals no methylation anomalies in patients of unknown aetiology. Hum Genet 2005;116:255–61.
22. Ellard S, Lango Allen H, De Franco E, Flanagan S, Hysenaj G, et al. Improved genetic testing for monogenic diabetes using targeted next-generation sequencing. Diabetologia 2013;56:1958–63.
23. Flanagan SE, Clauin S, Bellané-Chantelot C, de Lonlay P, Harries LW, et al. Update of mutations in the genes encoding the pancreatic beta-cell KATP channel subunits Kir6.2 (KCNJ11) and sulfonylurea receptor 1 (ABCC8) in diabetes mellitus and hyperinsulinism. Hum Mutat 2009;30:170–80.
24. Hattersley A, Pearson E. Transferring patients with diabetes due to Kir6.2 mutation from insulin to suphonylureas. Diabetes Genes [online]. Available at: http://www.diabetesgenes.org/content/transferring-patients-diabetes-due-Kir6.2-mutation-insulin-sulphonylureas (2011).
25. Grulich-Henn J, Wagner V, Thon A, Schober E, Marg W, et al. Entities and frequency of neonatal diabetes: data from the diabetes documentation and quality management system (DPV). Diabet Med 2010;27:709–12.
26. Iafusco D, Massa O, Pasquino B, Colombo C, Iughetti L, et al. Early Diabetes Study Group of ISPED. Minimal incidence of neonatal/infancy onset diabetes in Italy is 1:90,000 live births. Acta Diabetol 2012;49:405–8.
27. Slingerland AS, Shields BM, Flanagan SE, Bruining GJ, Noordam K, et al. Referral rates for diagnostic testing support an incidence of permanent neonatal diabetes in three European countries of at least 1 in 260,000 live births. Diabetologia 2009;52:1683–5.
28. Russo L, Iafusco D, Brescianini S, Nocerino V, Bizzarri C, et al. Permanent diabetes during the first year of life: multiple gene screening in 54 patients. Diabetologia 2011;54:1693–701.
29. Stanik J, Gasperikova D, Paskova M, Barak L, Javorkova J, et al. 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 2007;92:1276–82.
30. Habeb AM, Al-Magamsi MS, Eid IM, Ali MI, Hattersley AT, et al. Incidence, genetics, and clinical phenotype of permanent neonatal diabetes mellitus in northwest Saudi Arabia. Pediatric Diabetes 2012;13:499–505.
31. Flanagan SE, Edghill EL, Gloyn AL, Ellard S, Hattersley AT. Mutations in KCNJ11, which encodes Kir6.2, are a common

Q9: Please update Ref. (12)

Q10: As per journal style 5 authors et al. only allowed. Please supply 5 authors et al. for Ref. [19]

Q11: Please check the URL in Ref. (24)

Q12: As per journal style 5 authors et al. only allowed. Please check and confirm list of authors name for Refs. [30–35]

cause of diabetes diagnosed in the first 6 months of life, with the phenotype determined by genotype. Diabetologia 2006;49:1190–7.
32. Massa O, Iafusco D, D'Amato E, Gloyn AL, Hattersley AT, et al. KCNJ11 activating mutations in Italian patients with permanent neonatal diabetes. Hum Mutat 2005;25:22–127.
33. Vaxillaire M, Populaire C, Busiah K, Cavé H, Gloyn AL, et al. Kir6.2 mutations are a common cause of permanent neonatal diabetes in a large cohort of French patients. Diabetes 2004;53:2719–22.
34. Naylor R, Greeley SA, Bell G, Philipson L. Genetics and pathophysiology of neonatal diabetes mellitus. J Diabetes Investig 2011;2:158–59.
35. Rubio-Cabezas O, Flanagan SE, Damhuis A, Hattersley AT, Ellard S. KATP channel mutations in infants with permanent diabetes diagnosed after 6months of life. Pediatr Diabetes 2012;13:322–5.