Introduction

Diabetes presenting in the first six months of life is classified as neonatal diabetes mellitus (NDM) (1,2,3,4). Its incidence in Europe is reported to be 1:90,000 (5). NDM may be transient or permanent with about 50-60% of NDM being transient (2,3). Although most cases remit within three months after diagnosis, about 50% of the patients relapse later in life, and most frequently during adolescence (5). Insulin treatment is usually required during the first few days following initial diagnosis, but it is life-long after relapse (3,4).

The incidence of permanent NDM in the Middle East is more than in Europe at 1:21,000 (6). To date, mutations in more than 50 different genes are associated with NDM (7). NDM can present with severe hyperglycemia and ketoacidosis and may have significant short- and long-term complications (8). The diagnosis of NDM is usually made by the detection of high blood glucose levels in the first six months of life. Early diagnosis is essential to select the patients who will respond to oral treatment. In this investigation, we aimed to present the phenotype and genotype of our patients with NDM and share our experience in a single tertiary center.

Objectives and Methods

The present study included 16 NDM patients from 12 unrelated families. The clinical presentation, age at diagnosis, perinatal and family history, consanguinity, gender, hemoglobin A1c, C-peptide, insulin, insulin autoantibodies, genetic mutations, and response to treatment were retrospectively evaluated. The median age at diagnosis of diabetes was five months (4 days-18 months) although six patients with a confirmed genetic diagnosis were diagnosed >6 months. Three patients had KCNJ11 mutations, six had ABCC8 mutations, three had EIF2AK3 mutations, and one had a de novo INS mutation. All the permanent NDM patients with KCNJ11 and ABCC8 mutations were started on sulfonylurea treatment resulting in a significant increase in C-peptide level, better glycemic control, and discontinuation of insulin.

Conclusion

Although NDM is defined as diabetes diagnosed during the first six months of life, and a diagnosis of type 1 diabetes is more common between the ages of 6 and 24 months, in rare cases NDM may present as late as 12 or even 24 months of age. Molecular diagnosis in NDM is important for planning treatment and predicting prognosis. Therefore, genetic testing is essential in these patients.

Keywords:
Neonatal diabetes, genetic, sulfonylurea, monogenic diabetes, potassium channel, syndromic neonatal diabetes

Abstract

Objective: Neonatal diabetes mellitus (NDM) may be transient or permanent, and the majority is caused by genetic mutations. Early diagnosis is essential to select the patients who will respond to oral treatment. In this investigation, we aimed to present the phenotype and genotype of our patients with NDM and share our experience in a single tertiary center.

Methods: A total of 16 NDM patients from 12 unrelated families are included in the study. The clinical presentation, age at diagnosis, perinatal and family history, consanguinity, gender, hemoglobin A1c, C-peptide, insulin, insulin autoantibodies, genetic mutations, and response to treatment are retrospectively evaluated.

Results: The median age at diagnosis of diabetes was five months (4 days-18 months) although six patients with a confirmed genetic diagnosis were diagnosed >6 months. Three patients had KCNJ11 mutations, six had ABCC8 mutations, three had EIF2AK3 mutations, and one had a de novo INS mutation. All the permanent NDM patients with KCNJ11 and ABCC8 mutations were started on sulfonylurea treatment resulting in a significant increase in C-peptide level, better glycemic control, and discontinuation of insulin.

Conclusion: Although NDM is defined as diabetes diagnosed during the first six months of life, and a diagnosis of type 1 diabetes is more common between the ages of 6 and 24 months, in rare cases NDM may present as late as 12 or even 24 months of age. Molecular diagnosis in NDM is important for planning treatment and predicting prognosis. Therefore, genetic testing is essential in these patients.

Keywords:
Neonatal diabetes, genetic, sulfonylurea, monogenic diabetes, potassium channel, syndromic neonatal diabetes

Introduction

Diabetes presenting in the first six months of life is classified as neonatal diabetes mellitus (NDM) (1,2,3,4). Its incidence in Europe is reported to be 1:90,000 (5). NDM may be transient or permanent with about 50-60% of NDM being transient (2,3). Although most cases remit within three months after diagnosis, about 50% of the patients relapse later in life, and most frequently during adolescence (5). Insulin treatment is usually required during the first few days following initial diagnosis, but it is life-long after relapse (3,4).

The incidence of permanent NDM in the Middle East is more than in Europe at 1:21,000 (6). To date, mutations in more
than 25 genes have been reported to cause NDM (7,8). The most frequent mutations in Europe are reported to affect the pancreatic ATP-dependent potassium channel genes (\textit{KCNJ11} and \textit{ABCC8}), and most of them are spontaneous mutations (9). Early diagnosis is essential because NDM due to these mutations is responsive to sulphonylurea (SU) treatment, and early treatment improves neurocognitive development (10,11,12,13,14,15).

In this investigation, we present the genotypic and phenotypic characteristics of patients with NDM, followed at the pediatric endocrinology clinic of Bursa Uludağ University Hospital.

**Methods**

**Patients**
A total of 16 NDM patients from 12 unrelated families were included in the study. Clinical data were obtained from medical records, and a consent form for genetic analysis was filled out by all parents and participants. Patients diagnosed with diabetes below the age of 12 months and/or those with infantile diabetes with syndromic features and/or those with a family history of NDM were included in the study. The clinical presentation, age at diagnosis, perinatal and family history, consanguinity, gender, glycated hemoglobin (HbA1c), C-peptide, insulin, and insulin autoantibodies, genetic mutations, and response to treatment were retrospectively evaluated. Informed consent for genetic testing was obtained from the parents. The study was approved by the Ethical Committee of Bursa Uludağ University (approval number: 2020-8/23).

**Laboratory Analysis**

Serum glucose was analyzed by spectrophotometric methods (C16000 Architect System, Abbott, USA). C-peptide and insulin were assessed with chemiluminescent microparticle immunoassay (i2000 Architect System, Abbott, USA). HbA1c was measured by high-pressure liquid chromatography (Hb9210 Trinity Biotech Premier, USA). Glutamic acid decarboxylase antibody (GAD-65) and anti-insulin antibody were performed by enzyme immunoassay (DiaSarin ETI-MAX 3000, Italy). Pancreatic islet cell antibody was studied by indirect fluorescent antibody method.

**Genetic Analysis**

Analysis of all coding regions and exon/intron boundaries of the \textit{KCNJ11}, \textit{ABCC8}, \textit{INS} and \textit{EIF2AK3} genes was performed by Sanger sequencing. Genetic testing for all known genetic causes of NDM for eight of the patients was performed by the Exeter genomic laboratory, as previously described (16). The clinical significance of the variant was assessed using the Association for Clinical Genomic Science Best Practice Guidelines for Variant Classification 2019 (17).

**Statistical Analysis**

Descriptive analysis was performed using SPSS, version 21.0 (IBM Inc., Armonk, NY, USA). Data were expressed as median (minimum-maximum range) or mean±standard deviation (range).

**Results**

The median age at diagnosis of diabetes for the whole cohort (n = 16) was five months (4 days to 18 months), and the female to male ratio was 1.3:1. The mean glucose level at diagnosis was 475±137 mg/dL. Nine patients presented with diabetic ketoacidosis (DKA), two patients with ketosis, and four with hyperglycemia. One patient was diagnosed elsewhere, and the initial presentation was not known (patient 12.15). The median HbA1c at the time of diagnosis was 10.2% (5.8-17.1%), and the median C-peptide was 0.085 ng/mL (0.01-1.22 ng/mL) (reference range 0.78-5.19 ng/mL). Eleven patients were born full-term, three of them with low birth weight (<2,500 g), and five with a birth weight of 2,500-3,500 g. The gestational age and birth weight of four patients were not available. Multiple insulin regimens such as intermediate-acting (NPH), rapid-acting (insulin lispro) and short-acting insulin (regular), were started in 15/16 of the patients. Only one patient was treated with an insulin pump. Pancreatic imaging (sonographic examination) was performed in all of the patients, and none of them showed pancreatic abnormality. A genetic test was performed in 15 patients (Table 1).

A mutation in a gene known to cause NDM was identified in thirteen (86.7%) patients, but for two patients testing for all the known NDM genes did not detect a likely causative mutation. These patients without a mutation identified were diagnosed at the age of seven months and four days, respectively, and were both positive for anti-GAD antibodies (concentrations were 26.5 and 53.95 IU/mL, normal level <5 IU/mL). Although anti-GAD antibodies were positive, anti-insulin antibodies were in the normal range (concentrations were 0.2 and 2.5 IU/mL, normal level 0-10 IU/mL). Their birth weights were 3,700 g and 2,300 g, and they were not significantly different from the rest of the cohort.

Three unrelated patients had the \textit{KCNJ11} mutations, six (including three from the same kinship) had \textit{ABCC8} mutations, three had \textit{EIF2AK3} mutations, and one had a \textit{de novo INS} mutation.
## Table 1. The age of diagnosis, genetic analysis, and treatment response of neonatal diabetes mellitus patients

| Family number and patient number | Age at diagnosis (d/m/y) | Current age (year) | Sex | Consanguinity | HbA1c at diagnosis | C-peptide at diagnosis | Gene | Location |
|----------------------------------|--------------------------|--------------------|-----|--------------|--------------------|------------------------|------|----------|
| 1.1                              | 3 m                      | 6.5                | F   | No           | 11.8               | 0.01                   | KCNJ11 | Exon 1   |
| 2.2                              | 2.5 m                    | 6                  | F   | No           | N/A                | N/A                    | KCNJ11 | Exon 1   |
| 3.3                              | 40 d                     | 6                  | F   | No           | 10.5               | 0.07                   | KCNJ11 | Exon 1   |
| 4.4                              | 4 m                      | 5                  | M   | No           | 9.9                | 0.75                   | INS   | Exon 3   |
| 5.5                              | 45 d                     | 9                  | M   | No           | N/A                | N/A                    | Unknown | -        |
| 6.6                              | 18 m                     | 23                 | F   | Yes (1st degree cousins) | 10.8               | 1.22                   | ABCC8 | Exon 7   |
| 6.7                              | 9 m                      | 27                 | M   | Yes (1st degree cousins) | N/A                | N/A                    | ABCC8 | Exon 7   |
| 6.8                              | 18 m                     | 36                 | M   | Yes (1st degree cousins) | N/A                | N/A                    | ABCC8 | Exon 7   |
| 7.9                              | 7 m                      | 5.5                | F   | Yes (1st degree cousins) | 6.7                | 0.02                   | No disease-causing variant identified (anti GAD65 ab positive) | - |
| 8.10                             | 12 d                     | 8                  | F   | No           | 6.5                | 0.06                   | ABCC8 | Exon 28  |
| 9.11                             | 4 d                      | Died               | M   | Yes (2nd degree cousins) | 5.8                | 0.9                    | No disease-causing variant identified (anti GAD65 ab positive) | - |
| 10.12                            | 6 m                      | 1                  | F   | Yes (1st degree cousins) | 17.1               | 0.01                   | ABCC8 | Exon 29  |
| 10.13                            | 8 m                      | 3                  | F   | Yes (1st degree cousins) | N/A                | 0.1                    | ABCC8 | Exon 29  |
| 11.14                            | 15 m                     | 14.5               | M   | No           | 13.1               | N/A                    | EIF2AK3 | Exon 13  |
| 12.15                            | 17 m                     | 15.5               | M   | Yes (2nd degree cousins) | N/A                | N/A                    | EIF2AK3 | Exons 11-13 |
| 12.16                            | 3.5 m                    | 4.5                | F   | Yes (2nd degree cousins) | 9.7                | 0.48                   | EIF2AK3 | Exons 11-13 |

**Novel mutations.
N/A: not applicable, SU: sulphonylurea, M: male, F: female, m: month, d: day, y: year, HbA1c: hemoglobin A1c, NDM: neonatal diabetes mellitus

### Patients with ATP-Dependent Potassium Channel Mutations

Patient 6.6 was diagnosed with ketosis at 18 months of age and was on insulin treatment until she was 17 years old when she was found to be homozygous for a previously reported ABCC8 mutation classified as pathogenic (p.Glu382Lys) and switched to SU treatment. She had two cousins with diabetes on insulin treatment at 18 and 24 years of age who were also diagnosed during infancy (patients number 6.7.
These patients were also found to be homozygous for the ABCC8 pathogenic variant and switched to SU. These three patients all responded well to oral treatment, and insulin was successfully discontinued. One patient, diagnosed at twelve days of age with a previously reported ABCC8 heterozygous mutation (p.Arg1183Gln), was off-treatment at four months of age, confirming transient NDM (patient 8.10).

| Family number and patient number | Age at diagnosis (d/m/y) | Current age (year) | Sex | Consanguinity | HbA1c at diagnosis | C-peptide at diagnosis | Gene Location | DNA-protein description | Variant classification according to ACMD guidelines | Consequence | Zygosity | NDM subtype | Treatment | SU response |
|----------------------------------|--------------------------|--------------------|-----|---------------|-------------------|------------------------|----------------|------------------------|-----------------------------------------------|------------|----------|-------------|-----------|-------------|
| 1.1                             | 3 m                      | 6.5                | F   | No            | 1                 | 1.8                    | 0.01           | KCNJ11 Exon 1          | c.175G>A                                      | pathogenic | missense | heterozygous | SU        | yes         |
| 2.2                             | 2.5 m                    | 6                  | F   | No            | N/A               | N/A                    | KCNJ11 Exon 1          | c.175G>A                                      | pathogenic | missense | heterozygous | SU+insulin | yes         |
| 3.3                             | 40 d                     | 6                  | F   | No            | 10.5              | 0.07                   | KCNJ11 Exon 1          | c.175G>A                                      | pathogenic | missense | heterozygous | SU        | yes         |
| 4.4                             | 4 m                      | 5                  | M   | No            | 9.9               | 0.75                   | INS Exon 3            | c.285C>G                                      | likely pathogenic | missense | heterozygous | SU       | yes         |
| 5.5                             | 45 d                     | 9                  | M   | No            | N/A               | N/A                    | Unknown              | -                                      | pathogenic | missense | homozygous | SU        | yes         |
| 6.6                             | 18 m                     | 23                 | F   | Yes (1st degree cousins) | N/A               | 10.8                   | ABCC8 Exon 7             | c.1144G>A                                      | pathogenic | missense | homozygous | SU        | yes         |
| 6.7                             | 9 m                      | 27                 | M   | Yes (1st degree cousins) | N/A               | N/A                    | ABCC8 Exon 7             | c.1144G>A                                      | pathogenic | missense | homozygous | SU        | yes         |
| 6.8                             | 18 m                     | 36                 | M   | Yes (1st degree cousins) | N/A               | N/A                    | ABCC8 Exon 7             | c.1144G>A                                      | pathogenic | missense | homozygous | SU        | yes         |
| 7.9                             | 7 m                      | 5.5                | F   | Yes (1st degree cousins) | N/A               | 6.7                   | no disease-causing variant (anti-GAD65 ab positive) | -                                      | -            | -        | -           | -         | -           |
| 8.10                            | 12 d                     | 8                  | F   | No            | 6.5               | 0.06                   | ABCC8 Exon 28            | c.3548G>A                                      | likely pathogenic | missense | heterozygous | insulin- | -           |
| 9.1                             | 1 4 d                    | Died               | M   | Yes (2nd degree cousins) | 5.8               | 0.9                    | no disease-causing variant (anti-GAD65 ab positive) | -                                      | -            | -        | -           | -         | -           |
| 10.12                           | 6 m                      | 1                 | F   | Yes (1st degree cousins) | 17.1              | 0.01                   | EIF2AK3 Exons 1-13       | c.1886_ (c.2817+1_c.2818-1)_del p.? | likely pathogenic | missense | homozygous | SU        | yes         |
| 10.13                           | 8 m                      | 3                 | F   | Yes (1st degree cousins) | N/A               | 9.7                   | EIF2AK3 Exons 1-13       | c.1886_ (c.2817+1_c.2818-1)_del p.? | likely pathogenic | missense | homozygous | SU        | yes         |
Two sisters, diagnosed with NDM at six and eight months of age, were homozygous for the p.Trp231Leu mutation in the ABCC8 gene (patients 10.12 and 10.13). Although, this variant was not previously reported in the literature and initially classified as a variant of uncertain significance, a trial switch from insulin treatment to SU was successful and the variant could therefore be re-classified as likely pathogenic.

Three unrelated patients were found to be heterozygous for the previously reported pathogenic KCNJ11 p.Val59Met mutation. This variant has been previously reported in patients with iDEND (18,19). However, none of our patients was reported to have neurological features at the ages of seven, six and a half and six years.

All the permanent NDM patients with KCNJ11 and ABCC8 mutations were successfully transferred to SU treatment, resulting in a significant increase in C-peptide level after three months, better glycemic regulation, and discontinuation of insulin (Table 2). SU was started at a dose of 0.2 mg/kg/day, twice a day. Later, doses were adjusted with blood glucose levels. The doses of SU were in the range 0.2-1.2 mg/kg/day. Only one patient required a single dose of long-acting insulin four years after the diagnosis (patient 2.2).

**Patients with Mutations in Other Genes**

One patient with a novel heterozygous de novo mutation in the INS gene (p.Cys95Trp) was diagnosed at the age of four months. He remains insulin-treated (patient 4.4). One patient diagnosed at 15 months of age developed elevated transaminase levels, persistent hyperkalemia, thrombocytopenia, and skeletal dysplasia after two years and was also found to be homozygous for an EIF2AK3 mutation (patient 12.15). His sister, diagnosed with NDM at four months of age, was homozygous for the same mutation (patient 12.16). Both patients are still on insulin and supportive treatment (for orthopedic and renal complications) at the age of 15.5 and 4.5 years.

**Table 2. Values of C-peptide and hemoglobin A1c levels of neonatal diabetes mellitus patients with ATP-dependent potassium channel mutations before and after sulphonylurea treatment**

| Family and patient number | HbA1c at diagnosis and after SU treatment | C-peptide at diagnosis and after SU treatment |
|---------------------------|------------------------------------------|---------------------------------------------|
| 1.1                       | 11.8, 5.9                                | 0.01, 2.31                                  |
| 2.2                       | N/A, 6.8                                 | N/A, 2.89                                   |
| 3.3                       | 10.5, 6.3                                | 0.07, 1.11                                  |
| 5.5                       | N/A, 7.1                                 | N/A, 1.12                                   |
| 6.6                       | 10.8, 9.3                                | 1.22, 5.8                                   |
| 6.7                       | N/A, 8.1                                 | N/A, 3.18                                   |
| 6.8                       | N/A, 7.4                                 | N/A, 2.5                                    |
| 10.12                     | 17.1, 9.6                                | 0.01, 3                                     |
| 10.13                     | N/A, 8.1                                 | 0.1, 3                                      |

N/A: not applicable.
SU: sulphonylurea, HbA1c: hemoglobin A1c, SU: sulphonylurea

Discussion

Although NDM is defined as diabetes diagnosed during the first six months of life, recent research has shown that, rarely, it may present as late as 12 or even 24 months of age (1-4) although between the ages of six and 24 months a diagnosis of type 1 diabetes is much more common. The median age of diagnosis in our study was five months (four days-18 months). The most striking finding in this investigation was the presentation of diabetes in a patient with genetically confirmed NDM at 18 months of age. This patient and his two cousins were found to have a homozygous pathogenic ABCC8 mutation, and after many years on insulin treatment, they were successfully switched to SU therapy. NDM genes must therefore be considered when carefully collected family history suggests a possible genetic cause.

There was no statistical difference in terms of gender in our patients. Iafusco et al (20) similarly reported no gender difference in their cohort.

In a study reported by Russo et al (21), 75% of the patients with NDM diagnosed during the first six months of life had a mutation in KCNJ11, ABCC8, or INS gene. This ratio dropped to 12% in patients diagnosed between 7-12 months. The same study also reported that the patients diagnosed with permanent NDM before six months of age but without mutations in KCNJ11, ABCC8, or INS had higher birth weight than those with the mutations. In our smaller cohort, we did not observe a similar difference between patients with and without a causative mutation. Similarly to Besser et al (22), more than 50% of our patients with NDM had low birth weight despite term delivery, likely due to in utero hypoinsulinemia. Letourneau et al (23) reported that 66.2% of the patients with monogenic diabetes presented with DKA, similar to our patients, with 60% having DKA at the time of diagnosis.
Previous reports have suggested that autoantibodies are usually negative in NDM patients diagnosed before six months of age, except for maternal autoantibodies, which may have crossed the placenta (24) and patients with monogenic autoimmunity such as IPEX syndrome (25). GAD-65 were positive, but anti-insulin antibodies were negative, in two of our patients diagnosed at seven months and four days (the patients’ number 7.9 and 9.11). These patients did not have mutations in the known NDM genes (including monogenic autoimmunity genes such as FOXP3, IL2RA, and LRBA), however more causal genes remain to be discovered and a monogenic etiology is therefore possible. Whilst the antibody positivity in the patient diagnosed at seven months suggests a diagnosis of type 1 diabetes is likely, further investigations are needed to define the genetic etiology of the patient diagnosed at four days, since antibody positivity is common in patients with NDM caused by monogenic autoimmunity (26).

Molecular diagnosis in NDM is important for planning treatment and predicting prognosis. Therefore, genetic testing is essential in these patients. Carmody et al (13) have discussed the pros and cons of trying SU treatment awaiting the results of genetic tests. The advantages are a neurologic improvement, shorter hospital stay, lower cost, easier than insulin injections, and safety. On the other hand, increased expectance and disappointment of the family in case of treatment failure, risk of hypoglycemia in transient NDM, unknown long-term risks, and lack of FDA approval for SU in infants are the disadvantages. One of our patients was started on SU and responded well before the genetic test result was obtained, and insulin was successfully discontinued (patient 5.5). After receiving the test results, all of the patients were switched to SU, and better glycemic control was achieved along with significant elevation in C-peptide. Other family members with diabetes were also tested and switched to SU, which markedly improved their quality of life. Bowman et al (15) published a cohort of 90 patients with KCNJ11 mutations causing permanent NDM and followed for ten years. SU response was excellent in 93%, and neurologic development was improved by 47%. Similarly, we observed an excellent response to SU in 7/8 (87.5%) patients with mutations affecting the pancreatic potassium channel. Only one patient required the addition of long-acting insulin to the treatment.

Despite the importance of genetic diagnosis, it may not be possible in all patients as some etiological genes still remain to be discovered. De Franco et al (16) reported that a genetic mutation was detected in 82% of patients in an international cohort of 1200 probands. Similarly, we found a causative mutation in 87% of our patients.

The most common syndromic form of NDM in countries with high consanguinity rate is Wolcott Rallison syndrome (16). We had three patients with this syndrome, including two siblings born to consanguineous parents. All three patients had hepatic dysfunction and skeletal dysplasia, which are known features of the syndrome (27). They are on insulin treatment, and their diabetes is well controlled. Demirbilek et al (28) investigated the genetic profile of the patients with NDM in Southeastern Turkey and found that mutations in potassium channel were less common in consanguineous families, while syndromic diabetes was more common. In our cohort, potassium channel mutations were more common, similarly to what is reported from Western countries.

Study Limitations
There were some difficulties in obtaining complete data because of the retrospective nature of the study. The age range of the patients was wide, and some patients had antibody positivity, which rendered patient selection for the study difficult. The relatively small number of patients in this cohort is limited, and further, ideally prospective, research with larger numbers of patients is warranted.

Conclusion
The recognition of NDM has increased with the identification of new genetic causes and the wider availability of genetic testing. Early diagnosis is essential to identify the patients who may respond to SU treatment. NDM has been defined as diabetes diagnosed during the first six months of life, but it is now increasingly recognized that the presentation of NDM may be delayed. In rare cases, it may present as late as 12 or even 24 months of age. Therefore very careful investigation of family history is essential. However, most patients still present before six months of age, and rapid genetic diagnosis must be obtained to plan the treatment. Syndromic diabetes must be considered in those with additional findings.

Ethics
Ethics Committee Approval: The study was approved by the Ethical Committee of Bursa Uludağ University (approval number: 2020-8/23).

Informed Consent: Consent form for genetic analysis was filled out by all parents and participants.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions
Surgical and Medical Practices: Elif Sobu, Özgecan Demirbaş, Yasemin Denkboy Öngen, Concept: Yasemin
Denkboy Öngen, Erdal Eren, Ömer Tamir, Design: Elif Sobu, Özgecan Demirbaş, Data Collection or Processing: Elisa De Franco, Elif Sobu, Analysis or Interpretation: Yasemin Denkboy Öngen, Erdal Eren, Sian Ellard, Literature Search: Ömer Tamir, Sian Ellard, Elisa De Franco, Writing: Yasemin Denkboy Öngen, Erdal Eren, Elisa De Franco, Ömer Tamir.

Financial Disclosure: Genetic testing at the Exeter genetic laboratory was funded by a Wellcome Trust senior investigator grant to Sian Ellard and Andrew Hattersley. EDF is a Diabetes UK RD Lawrence fellow.

References
1. Flechner I, Vaxillaire M, Cavé H, Scharffmann R, Fuguel P, Polak M. Neonatal hyperglycemia and abnormal development of the pancreas. Best Pract Res Clin Endocrinol Metab 2008;22:17-40.
2. Polak M, Cavé H. Neonatal diabetes mellitus: a disease linked to multiple mechanisms. Orphanet J Rare Dis 2007;2:12.
3. Aguilar-Bryan L, Byran J. Neonatal diabetes mellitus. Endocr Rev 2008;29:265-291. Epub 2008 Apr 24.
4. Murphy R, Ellard S, Hattersley AT. Clinical implications of a molecular genetic classification of monogenic beta cell diabetes. Nat Clin Pract End Met 2008;4:200-213. Epub 2008 Feb 26.
5. Iafusco D, Massa O, Pasquinio D, Colombo C, Iughetti L, Bizzarri C, Mammi C, Lo Presti D, Supranii T, Schiaffini R, Nichols CG, Russo L, Grassi V, Meschi F, Bonfanti R, Brescianini S, Barbetti F; Early Diabetes Study Group of ISPED. Minimal incidence of neonatal/infancy onset diabetes in Italy is 1:90,000 live births. Acta Diabetol 2012;49:405-408. Epub 2011 Sep 28.
6. Habe AM, Al-Magamsi MS, Eid IM, Ali MI, Hattersley AT, Hussain K, Ellard S. Incidence, genetics and clinical phenotype of permanent neonatal diabetes mellitus in northwest Saudi Arabia. Pediatri Diabetes 2012;13:499-505. Epub 2011 Nov 8.
7. Flanagan SE, De Franco E, Lango Allen H, Zerah M, Abdul-Rasoul MM, Edge JA, Stewart H, Alami E, Hussain K, Wallis S, de Vries L, Rubio-Cabezás O, Houghton JA, Edghill EL, Patch AM, Ellard S, Hattersley AT. Analysis of transcription factors key for mouse pancreatic development establishes NKX2-2 and MXN1 mutations as causes of neonatal diabetes in man. Cell Metab 2014;19:146-154.
8. Rubio-Cabezás O, Ellard S. Diabetes mellitus in neonates and infants: genetic heterogeneity, clinical approach to diagnosis, and therapeutic options. Horm Res Paediatr 2013;80:137-146. Epub 2013 Sep 18.
9. Gloyon AL, Pearson ER, Antcliff JF, Proks P, Bruining GJ, Slingerland AS, Howard N, Srinivasan S, Silva JM, Molines J, Edghill EL, Frayling TM, Temple IK, Mackay D, Shield JP, Summick Z, van Rhijn A, Wales JK, Clark P, Gorman S, Aisenberg J, Ellard S, Njølstad PR, Ashcroft FM, Hattersley AT. Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. N Engl J Med 2004;350:1838-1849.
10. Pearson ER, Flechner I, Njølstad 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, Sevik 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 2006;355:467-477.
11. Tonini G, Bizzarri C, Bonfanti R, Vaneli M, Cerutti F, Faleschini E, Meschi F, Prisco F, Ciacco E, Cappa M, Torelli C, Cauvin V, Tumini S, Iafusco D, Barbetti F; Early-Onset Diabetes Study Group of the Italian Society of Paediatric Endocrinology and Diabetology. Sulfonylurea treatment outweighs insulin therapy in short-term metabolic control of patients with permanent neonatal diabetes mellitus due to activating mutations of the KCNJ11 (Kir6.2) gene. Diabetologia 2006;49:2210-2213. Epub 2006 Jul 1.
12. Zhang H, Zhong X, Huang Z, Huang C, Liu T, Qiu Y. Sulfonylurea for the treatment of neonatal diabetes owing to KATP-channel mutations: a systematic review and meta-analysis. Oncotarget 2017;8:108274-108285.
13. Carmody D, Bell CD, Hwang JL, Dickinson JT, Sima D, Felipe DL, Zimmer CA, Davis AO, Kotlyarevskaya K, Naylor RN, Philipson LH, Greeley SA. Sulfonylurea treatment before genetic testing in neonatal diabetes: pros and cons. J Clin Endocrinol Metab 2014;99:2709-2714.
14. Babiker T, Vedovato N, Patel R, Thomas N, Finn R, Männikö R, Chakera AJ, Flanagan SE, Shepherd MH, Ellard S, Ashcroft FM, Hattersley AT. Successful transfer to sulfonylureas in KCNJ11 neonatal diabetes is determined by the mutation and duration of diabetes. Diabetes 2016;59:1162-1166. Epub 2016 Mar 31.
15. Bowman P, Sulen Å, Barbetti F, Beltrand J, Svalastoga P, Codner E, Tissmann EH, Juliusson PB, Skriverhaug T, Pearson ER, Flanagan SE, Babiker T, Thomas NJ, Shepherd MH, Ellard S, Klimes I, Szopa M, Polak M, Iafusco D, Hattersley AT, Njølstad PR; Neonatal Diabetes International Collaborative Group. 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 2018;6:637-646. Epub 2018 Jun 4.
16. De Franco E, Flanagan SE, Houghton JA, Lango Allen H, Mackay DJ, Temple IK, Ellard S, Hattersley AT. The effect of early, comprehensive genomic testing on clinical care in neonatal diabetes: an international cohort study. Lancet 2015;386:957-963. Epub 2015 Jul 28.
17. Ellard S, Babale EL, Berry I, Forrester N, Turnbull C, Owens M, Eccles DM, Abbas S, Scott R, Deans ZC, Lester T, Campbell J, Newman WG, McMullan DJ. 2019. ACGS Best Practice Guidelines for Variant Classification 2019. Retrieved from: https://www.acgs.org.uk/media/11285/uk-practice-guidelines-for-variant-classification-2019-v1-0-3.pdf.
18. Proks P, Girard C, Haider S, Gloyon AL, Hattersley AT, Sansom MS, Ashcroft FM. A gating mutation at the internal mouth of the Kir6.2 pore is associated with DEND syndrome. EMBO 2005;24:470-475.
19. Proks P, Antcliff JF, Lippiat J, Gloyon AL, Hattersley AT, Ashcroft FM. Molecular basis of Kir6.2 mutations associated with neonatal diabetes or neonatal diabetes plus neurological features. Proc Natl Acad Sci U S A 2004;101:17539-17544. Epub 2004 Dec 6.
20. Iafusco D, Stazi MA, Cotichini R, Cotelleessa M, Martinucci ME, Mazzella M, Cherubini V, Barbetti F, Martinioti C, Cerutti F, Prisco F; Early Onset Diabetes Study Group of the Italian Society of Paediatric Endocrinology and Diabetology. Permanent diabetes mellitus in the first year of life. Diabetologia 2002;45:798-804. Epub 2002 May 5.
21. Russo L, Iafusco D, Brescianini S, Nocerino V, Bizzarri C, Toni S, Cerutti F, Moncicti C, Pesavento R, Iughetti L, Bernardini L, Bonfante R, Gargantuini L, Vaneli M, Aguilar-Bryan L, Stazi MA, Grasso V, Colombo M, Barbetti F; ISPED Early Diabetes Study Group. Permanent diabetes during the first year of life: multiple gene screening in 54 patients. Diabetologia 2001;44:1753-1754. Epub 2004 Jan 2.
22. Besser RE, Flanagan SE, Mackay DJ, Temple IK, Shepherd MH, Shields BM, Ellard S, Hattersley AT. Prematurity and genetic testing for neonatal diabetes. Pediatrics 2016;138:138-140. Epub 2016 Aug 18.
23. Letourneau LR, Carmody D, Wroblewski K, Denson AM, Sanyoura M, Naylor RN, Philipson LH, Greeley SA. Diabetes Presentation in Infancy: High Risk of Diabetic Ketoacidosis. Diabetes Care 2017;40:147-148.
24. Proks P, Girard C, Haider S, Gloyon AL, Hattersley AT, Sansom MS, Ashcroft FM. A gating mutation at the internal mouth of the Kir6.2 pore is associated with DEND syndrome. EMBO 2005;24:470-475.
offspring of parents with type 1 diabetes: the 2-year analysis of the German BABYDIAB Study. Diabetes 1999;48:460-468.

25. Powell BR, Buist NR, Stenzel P. An X-linked syndrome of diarrhea, polyendocrinopathy, and fatal infection in infancy. J Pediatr 1982;100:731-737.

26. Johnson MB, Hattersley AT, Flanagan SE. Monogenic autoimmune diseases of the endocrine system. Lancet Diabetes Endocrinol 2016;4:862-872. Epub 2016 Jul 26

27. Delépine M, Nicolino M, Barrett T, Golamaully M, Lathrop GM, Julien C. EIF2AK3, encoding translation initiation factor 2-alpha kinase 3, is mutated in patients with Wolcott-Rallison syndrome. Nat Genet 2000;25:406-409.

28. Demirabilek H, Arya VB, Ozbek MN, Houghton JA, Baran RT, Akar M, Tekes S, Tuzun H, Mackay DJ, Flanagan SE, Hattersley AT, Ellard S, Hussain K. Clinical characteristics and molecular genetic analysis of 22 patients with neonatal diabetes from the South-Eastern region of Turkey: predominance of non-KATP channel mutations. Eur J Endocrinol 2015;172:697-705. Epub 2015 Mar 9