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Clinical characteristics, molecular features and long-term follow up of 15 patients with neonatal diabetes: a single center experience

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Abstract

Background—Diabetes diagnosed within the first six months of life is defined as neonatal diabetes mellitus (NDM). Mutations in the *KCNJ11, ABCC8* and *INS* genes are the most common cause of permanent NDM (PNDM). In populations with a high rate of consanguinity, Wolcott-Rallison syndrome caused by biallelic *EIF2AK3* mutations is common.

Methods—We studied the clinical characteristics and underlying genetic cause of disease in 15 individuals with diabetes onset before 6 months of age as defined by sustained hyperglycaemia requiring insulin treatment. Patients who had a remission of the diabetes, defined by a normal blood glucose and HbA1c value without insulin or sulphonylurea (SU) treatment within the first 18 months of life were classified as having transient NDM (TNDM).

Results—We report 15 patients with NDM from 14 unrelated families, including ten with reported parental consanguinity. 1/15 patients had a remission of diabetes, leading to a diagnosis of TNDM. Mutations were detected in 80% (n = 12/15) of the cohort (*ABCC8* n=4, *PTF1A-distal* enhancer n=3, *KCNJ11* n=2, *EIF2AK3* n=1, *INS* n=1, *SLC19A2* n=1). All cases were initially treated with multiple dose insulin injections. One patient with an *ABCC8* mutation transitioned from insulin to SU resulting in improved metabolic control at the age of 20 years.

Conclusion—Although the number of individuals born to consanguineous parents was considerably high in this cohort, KATP channel mutations (*ABCC8*/*KCNJ11*) were more common

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Statement of Ethics

All the parents in the study gave their written informed consent for the genetic testing of their children. Written informed consent was obtained from the parents for publication of this study and any accompanying images.This study was approved by the Local Ethical Committee of Istanbul Faculty of Medicine (2019/1212).

Conflict of interest statement

The authors have no conflict of interest to declare.

Author contributions

Z.Y.A., E.K.O., S.P., R.B., F.B. and F.D. designed the research study and analysed the data. Z.Y.A., E.D.F., S.E.F., S.P., F.B. and F.D. did the research and wrote the paper. E.D.F and S.E.F., performed the molecular analyses. All authors read and approved the final manuscript for publication.

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than *EIF2AK3* mutations (n=6 vs n=1). Genetic analyses should be performed in all NDM cases due to the potential impact on treatment and prognosis.

Keywords

Neonatal diabetes; monogenic diabetes of infancy; *ABCC8*; *KCNJ11*; *PTF1A*; *EIF2AK3*; *INS*; *SLC19A2*

Introduction

Diabetes diagnosed within the first 6 months of life is defined as neonatal diabetes mellitus (NDM). In approximately 82% of cases a monogenic aetiology is identified [1,2]. NDM can be subgrouped according to whether the diabetes is transient (TNDM) or permanent (PNDM). TNDM occurs in approximately half of the cases; in these patients the diabetes usually resolves within a few months after onset but may relapse later in life [3]. Rare syndromes which feature NDM also exist; these constitute a third subgroup, syndromic NDM.

The incidence of NDM varies regionally. In European populations it affects approximately 1 in 90,000–160,000 live births [2–4]. A higher incidence of disease is reported in populations with high consanguinity rates. For example, in the South-East Anatolian region of Turkey the incidence is approximately 1 in 48,000 live births [5] whilst in the Middle East PNDM affects 1 in 21,000 live births [6].

Mutations in the *KCNJ11* and *ABCC8* genes are the most common cause of PNDM accounting for approximately 50% of cases in outbred populations [1,7–9]. *KCNJ11* and *ABCC8* encode the ATP-sensitive (KATP) channel and for approximately 90% of patients with a mutation in these genes there is a marked improvement in glycaemic control following transfer from insulin injections to sulfonlyurea (SU) therapy [7, 9–11]. This provides one of the best examples of precision medicine whereby understanding the underlying molecular mechanism of disease results in disease-specific therapy and improved outcome.

Biallelic mutations in the *EIF2AK3* gene, encoding the eukaryotic translation initiation factor 2-α kinase 3 (also known as PERK), cause the rare and often fatal Wolcott-Rallison syndrome (WRS). This is the most common cause of PNDM in countries with high consanguinity rate [12]. PERK expression in multiple tissues explains the range of clinical manifestations, typically including infancy-onset diabetes, skeletal dysplasia and liver dysfunction [13,14].

Monogenic diabetes is clinically and genetically heterogeneous, with mutations in at least 36 genes reported [15]. Early comprehensive testing of all the causative genes using targeted next generation sequencing provides an early genetic diagnosis which will guide the clinical management strategies [2].

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In this study, we describe the clinical features, long term follow up and underlying molecular aetiologies in a cohort of 15 NDM patients. We analyse the phenotypic characteristics, treatment decisions and the impact of understanding the molecular aetiology on prognosis.

Subjects and Methods

We studied 15 patients with NDM (1 female) from 14 unrelated families followed in the Pediatric Endocrinology and Diabetes Unit, Istanbul Faculty of Medicine. Inclusion criteria was diabetes onset below 6 months of age (defined by hyperglycaemia and insulin requirement for at least two weeks). Individuals with stress-related or drug-induced hyperglycaemia were excluded. Patients who initially presented with hyperglycaemia followed by normal blood glucose and normal HbA1c values without insulin or sulphonylurea (SU) requirement under 18 months of age were defined as having TNDM.

Data on the diagnosis and treatment of diabetes, family history and other clinical features were obtained from the medical records. Autoantibodies including anti-glutamic acid decarboxylase (Anti-GAD), islet cell antibody (ICA), anti-insulin antibody (AIA) were recorded.

The transfer to SU was carried out using a standard protocol similar to that described previously [7] (available at https://www.diabetesgenes.org/about-neonatal-diabetes/).

Molecular analyses

Genomic DNA was extracted from peripheral leukocytes of patients using standard procedures. Genetic testing was performed at the Exeter Genomics laboratory as previously described [1]. For 12 patients, the KATP channel genes (*ABCC8* and *KCNJ11*), *INS* and *EIF2AK3* were initially tested using Sanger sequencing to analyse the coding regions and conserved splice sites. In patients without a pathogenic variant, analysis of all the other known genetic causes of neonatal diabetes (*EIF2AK3*, *FOXP3*, *GATA4*, *GATA6*, *GCK*, *GLIS3*, *HNF1B*, *IER3IP1*, *IL2RA*, *LRBA*, *PDX1*, *PTF1A* including the distal enhancer, *NEUROD1*, *NEUROG3*, *NKX2-2*, *RFX6*, *SLC2A2*, *SLC19A2*, *STAT3*, *WFS1*) was performed using a targeted next generation sequencing assay as previously described [16]. This assay can also detect partial or whole gene deletions and duplications. All mutations detected by this method were confirmed using Sanger sequencing analysis of the mutated exon in the affected gene. Family members were tested only for the mutation detected in the probands. In one patient Sanger sequencing of only the *ABCC8*, *KCNJ11*, *INS* and *GCK* genes was undertaken as the family did not consent to additional genetic testing. In two patients without a genetic diagnosis, genetic testing was not performed.

Statistical Analysis

Statistical analysis was performed using SPSS 21.0 for Windows statistical software. Data were expressed as mean ± SD. A p value ≤ 0.05 was considered to be statistically significant.

Results

Clinical characteristics of patients with NDM

Clinical, biochemical and molecular characteristics of patients at admission and at last evaluation are provided in Table 1 and 2.

Fifteen patients (two siblings) were diagnosed with NDM requiring multiple dose insulin (MDI) injection. Mean age at diagnosis was 2.4±1.5 months (median 2.0, range 0.2-6.0 months). Gestational ages ranged between 35-40 gestational weeks (GW). Birth weight (BW) was between 1400-3680g and BW-SDS was -1.7±1.7 (median -1.1). Small for gestational age (SGA, BW <-2.0 SDS) ratio was 40%. Mean serum glucose level at diagnosis was 529.2±160.0 mg/dL (29.4±8.9 mmol/L). Three patients (20%) had diabetic ketoacidosis (DKA) at diagnosis. Seizures were the presenting complaint in two (13.3%) patients.

Ten patients were born to consanguineous parents (9/14 families, 64.2%). Mutations were identified in 6 different genes in 80% of the cohort (12/15) (*ABCC8* (n=4, two siblings), *PTF1A-distal* enhancer (n=3), *KCNJ11* (n=2), *EIF2AK3* (n=1), *INS* (n=1), and *SLC19A2* (n=1)). In 3 patients comprehensive genetic testing of all known NDM genes was not possible. The distribution of NDM cases according to molecular aetiology is illustrated in Figure 1.

NDM due to *ABCC8* mutations

Two siblings (P#1 and P#2) in our cohort were diagnosed with diabetes at the age of 5 months and 2.5 months, respectively. After the genetic diagnosis of *ABCC8*-NDM was established in these cases, transfer from insulin therapy to SU was attempted at 15.2 years of age in P#1 and 10.7 years of age in P#2. Insulin requirements decreased by 50% within a week and C-peptide levels increased from 0.07 to 2.2 (N:1.4-4.4 ng/mL) and from 0.17 to 2.0 ng/mL in P#1 and P#2, respectively. Insulin therapy was ceased at the end of two months in both patients. These two patients were previously described by Aydin *et al* [17]. Currently, P#1 has been on SU only therapy for four years and his last HbA1c level was 6.7%. However, due to hyperglycaemia and increased HbA1c levels in P#2, MDI treatment (insulin aspart and glargine) was added at the age of 16 years. It was stated that compliance to SU treatment was appropriate. At last evaluation at 19 years old, he was using both glibenclamide and insulin treatment. Both of the siblings had no diabetic complications. Neurodevelopment is normal.

P#3 presented with seizures and hyperglycaemia without acidosis at the age of 1.5 months. He was treated with MDI (insulin lispro and glargine) and dose was increased up to 1.0 U/kg/day. A homozygous ABCC8 mutation (p.R826W) was detected and treatment was switched to SU at the age of 16.3 years. HbA1c was 7.5% before SU. After glibenclamide treatment, insulin requirement decreased by 50% on both SU and MDI. Between 17.2-19.2 years of age, SU and insulin requirements were 0.8 mg/kg/day and 0.2 U/kg/day (insulin glargine only), respectively. Diabetic complications were not observed.

P#4 was diagnosed with NDM at 2 months of age and compound heterozygous *ABCC8* mutation (p.(I557R/R1145L) was detected. Follow up data for this patient was not available, and it is not known if SU transfer has been attempted.

NDM due to *PTF1A* mutations

Three patients in our cohort had NDM due to a recessive mutation in the *PTF1A* distal enhancer (P#5, 6 and 7). All three patients had severe SGA. P#5 was diagnosed with NDM at 2.2 months of age. He had a triangular face with small chin and his head circumference was 10-25th centile. His basal serum insulin and C-peptide levels were 0.4 μu/ml and 0.12 ng/ml, respectively. Following the diagnosis of NDM, NPH insulin (0.5 U/kg/day) was commenced. Ultrasonography revealed hypoplastic pancreas (5mm pancreas corpus) and faecal elastase was low confirming exocrine pancreatic insufficiency. An atrial septal defect of secundum type was also observed. The patient had a history of seizures during follow up and phenobarbital was added. The previously reported (18) homozygous g.23508437A>G mutation was detected in the *PTF1A* distal enhancer. His unaffected parents were heterozygous for the mutation.

P#6 was diagnosed with diabetes at 6 days old, in Iraq. The patient was admitted to our emergency unit at 36 days old at which point plasma glucose was 600 mg/dl (33.3 mmol/L) with extremely low C-peptide concentration (0.01 ng/ml, N:1.4-4.4). He had fatty stools suggesting exocrine pancreatic insufficiency. Abdominal ultrasonography revealed pancreatic hypoplasia. Echocardiography was normal. Initial insulin requirement was 0.2U/kg/day. Genetic testing identified the same homozygous g.23508437A>G mutation in the *PTF1A* distal enhancer detected in P#5.

P#7 was referred at 15 days old due to swelling of the face and legs. She had severe hyperglycaemia without metabolic acidosis requiring full replacement insulin treatment (1.0 U/kg/day). Her unaffected parents were from the Eastern region of Turkey and were non-consanguineous. Hypoalbuminemia (1.7 g/dl) and anaemia were observed and she was transfused. Coarse face with micrognathism was also observed. Head circumference was -2.8 SDS. Echocardiography was normal. Chymotryptic activity was low and pancreatic hypoplasia was detected on ultrasonography. Pancreatic enzyme replacement treatment was commenced. Genetic testing identified the homozygous g.23508437A>G mutation in the *PTF1A*-distal enhancer, her parents were heterozygous carriers. The family was non-compliant and HbA1c levels of the subject were often >10.0% despite intensive education.

NDM due to *KCNJ11* mutations

Two patients in our cohort had NDM caused by activating *KCNJ11* mutations (P#8 and 9).

P#8 was admitted with fever at 3 months of age. He was treated for DKA and after recovery MDI treatment at a dose of 0.3 U/kg/day was continued. During the first year of treatment, insulin requirement was decreased and he only used insulin in stress conditions such as infections. A heterozygous *KCNJ11* mutation (p.E322K) was detected. He had been followed in our institution until the age of three years. His developmental milestones were normal for age and transfer to SU was not attempted during this period (no further follow up available).

P#9 was referred for fever and irritability at 1 month of age. Hyperglycaemia was detected without DKA. MDI regimen (regular short acting and NPH) was commenced. A heterozygous p.R201C *KCNJ11* mutation was detected. Transfer to SU was not attempted whilst this patient was under the care of our centre. Follow up data after the age of 2 years of age is not available.

NDM due to *EIF2AK3, INS* and *SLC19A2* mutations

P#10 was diagnosed with NDM at 6 months of age. Parents of P#10 were second degree cousins without any family history of diabetes. P#10 did not have any skeletal findings or liver abnormalities at admission. Molecular genetic testing identified a homozygous *EIF2AK3* (p.L742*) mutation, confirming a diagnosis of Wolcott Rallison syndrome. He died of acute liver failure in a different center (age not known).

P#11 was referred for severe hyperglycaemia and diagnosed with NDM at the age of 20 days. Parents were related and his mother had diabetes diagnosed at 41 years requiring oral antidiabetic initially and afterwards insulin. Systemic examination was unremarkable. MDI treatment (regular short acting and NPH) was initiated (0.5U/kg/day). At his last evaluation at 9.5 years of age, his weight and height were 21.5 kg (-2.3 SDS) and 119.0 cm (-2.8 SDS), respectively. Total insulin dose was 0.8 U/kg/day with HbA1c level of 10.0%. A homozygous *INS* promoter mutation, c.-331C>G, was detected. He had no regular follow up visits after 9.5 years of age. He was recalled and evaluated at 18 years of age. Neurodevelopment was normal. Total insulin requirement on MDI regimen (short acting insulin and NPH) was 1.8 U/kg/day.

P#12 was evaluated for being pale and weak at 3 months of age at which point biochemical studies identified a low haemoglobin (Hb:6.9 g/dL) and high plasma glucose (400 mg/dL, 22.2 mmol/L). Insulin and C-peptide levels were low. MDI treatment (insulin lispro and insulin detemir) was commenced (0.5-0.7U/kg/day). Anaemia with poikilocytosis was detected. Mean corpuscular volume (MCV) was 89.0 fL and vitamin B12 concentration was 378 ng/L (N:191-663) at admission. Due to severe anaemia, blood transfusion was performed. Echocardiography was normal. Sensorineural deafness was detected at 14 months of age. He had been also followed for 1° atrioventricular block. Genetic testing identified a homozygous frameshift mutation in *SLC19A2* gene (p.S214fs*14) confirming a diagnosis of thiamine responsive megaloblastic anaemia (TRMA, Roger's syndrome). His unaffected parents were heterozygous carriers. Thiamine was added to the treatment and the anaemia resolved.

In three cases comprehensive molecular genetic testing could not be performed

P#13 presented at 3 months of age with fever and vomiting after vaccination. Severe hyperglycaemia, ketonuria and metabolic acidosis (bicarbonate:4.7mmol/L) were detected. Insulin and C-peptide levels at diagnosis were low and diabetes autoantibodies were negative. Cranial, abdominal ultrasonography and fundoscopic examination were normal. Low free T4 (FT4) and inappropriately normal TSH concentrations in two different measurements [FT4:11.3 pmol/L (12-22), TSH: 5.9 mIU/L (0.27-4.9); FT4:11.0 pmol/L (12-22), TSH: 3.42 mIU/L (0.27-4.9)] were detected, at the age of 4 months. Differential

diagnosis was nonthyroidal illness (euthyroid sick syndrome) or central hypothyroidism. Since these measurements were performed one month after the initial diagnosis of diabetes with no any other confounding disease, the possibility of nonthyroidal illness was low. However, central hypothyroidism could not be excluded. Serum basal cortisol concentration was normal (16.0 μg/dl) and TSH was increased (peak TSH level 53 mIU/L) after TRH stimulation test. In this young infant, l-thyroxine replacement was commenced to prevent any complication of hypothyroidism. During follow up, l-thyroxine dose requirement per weight decreased gradually and treatment was stopped at 3 years of age suggesting a transient hypothyroidism. Haemoglobin level was 8.4 g/dl (lower limit for age: 9.4g/dl) with MCV 78.9 fL (lower limit for age 84 fL) and oral iron replacement was also added. Parental consent for genetic testing could not be obtained.

P#14 presented with fever and vomiting at the age of 30 days. Metabolic acidosis and hyperglycaemia (792 mg/dl) were detected. After recovery of DKA, MDI (NPH and insulin aspart) was started (0.5-0.6U/kg/day). Thyroid function and tests for pancreatic exocrine function were normal. Neurodevelopmental milestones were normal. His last evaluation was at 13 months of age and HbA1c was 10.9% with insulin dose of 0.3 U/kg/day (insulin detemir only, rarely insulin aspart). Molecular analysis was not performed.

P#15 was diagnosed at 5.9 months of age with extremely high plasma glucose. There was no family history of diabetes. Autoantibodies were negative and C-peptide was low. He had no dysmorphic features or evidence of exocrine pancreatic insufficiency. MDI regimen (regular short acting and NPH) was started at a dose of 0.6U/kg/day. Sequencing analysis of the *ABCC8, KCNJ11, INS*, and *GCK* genes were negative. Parental consent was not obtained for further genetic testing.

A pipeline for the diagnosis and follow up of patients with NDM is suggested in Figure 2.

Pedigrees of the cases with the family member with diabetes (Patient 1, 2, 3,7 and 11) are illustrated in Supplementary Figure.

Discussion

Major advances in molecular techniques have led to better understanding of the genetic basis of NDM over the last two decades. In this cohort, patients were followed from the date of diagnosis of diabetes and categorized as permanent (PNDM) or transient (TNDM) during follow up. Only one subject (P#8) with the *KCNJ11* mutation had TNDM and 93.3% of subjects had PNDM.

Infants with NDM may present with vague signs that overlap with other conditions such as infections. Clinical presentation may range from asymptomatic hyperglycaemia to polydipsia, failure to thrive, vomiting and irritability or acute symptoms with severe dehydration, seizure, ketoacidosis. In our cohort two patients with *ABCC8* mutations (P#1 and P#3) presented with seizures and DKA was present in one case with a *KCNJ11* mutation (P#8) and two cases without a genetic diagnosis.

Treatment and clinical management of NDM still represent a challenge. Insulin treatment has traditionally been the first and only choice for NDM therapy. After stabilization via intravenous insulin, patients are usually switched to subcutaneous forms using either regular insulin or rapid acting analogue plus basal insulin. All of the patients in our cohort were managed as described. Basal insulin coverage can be regulated using the intermediate-acting (NPH) or insulin analogues (glargine or detemir). Insulin treatment in infants must be carefully designed. Continuous subcutaneous insulin infusion (CSII) was successfully included in the management of NDM, providing more flexibility in time and insulin amounts [18–20]. CSII was discussed with some of the families in our cohort, and they felt they would prefer to continue with MDI. None of the patients in our cohort used CSII in infancy.*KCNJ11* mutations most commonly cause PNDM whereas *ABCC8* mutations are more likely to lead transient diabetes [21]. Interestingly, *ABCC8* mutations were more frequent than *KCNJ11* mutations in our PNDM cohort. This is likely due to the high-rate of consanguinity in our cohort as recessively-acting mutations are more common in *ABCC8* than *KCNJ11*. Varying degrees of neurologic impairment are often found in patients with NDM due to *ABCC8* and *KCNJ11* mutations [22,23]. None of the subjects in our cohort had neurodevelopmental delay or features of DEND (developmental delay, epilepsy, neonatal diabetes) syndrome, according to initial neurological assessments at diagnosis. However, follow up data was limited for the patient with the *KCNJ11* p.R201C mutation which has been previously reported to be associated with mild developmental delay.

Most patients with activating mutations in *KCNJ11* or *ABCC8* can be transitioned from insulin to SU with improvement in metabolic control [8,24]. Due to KATP channels also being expressed in the brain, SU treatment is able to improve neurological impairment in some cases [25] in addition to leading to better metabolic control. Importantly, it is reported that those who transfer to SU at a younger age require a lower dose and are more likely to remain well controlled on monotherapy, while those who transition later (>13 years) are more likely to need additional treatment [8,10,25]. In contrast, although P#1 in our study has been transitioned to SU at a later age, he had no insulin requirement later on. His sibling P#2, was transitioned to SU in a relatively earlier age than his brother, however he had requirement for both SU and MDI. This is likely to be due to non-compliance to treatment regimens. P#3 was also transitioned at a later age (16.3 years of age) and required both SU and MDI but insulin dose decreased significantly requiring only small doses of basal insulin. Severe hypoglycaemic episodes were not reported in any of the 3 patients treated with SU even at high doses (~ 2.0 mg/kg/day), in keeping with previous reports [26].

PTF1A is a transcription factor important for brain and pancreas development (18). In keeping with these homozygous null mutations in the gene have been shown to cause pancreatic and cerebellar agenesis [27]. More recently biallelic mutations in a novel enhancer 25 kb downstream from the *PTF1A* gene have been identified [18]. These mutations cause isolated pancreatic agenesis without cerebellar involvement, suggesting the enhancer is specific to the pancreas (18,28). Three patients in our cohort (20%) had *PTF1A*-distal enhancer mutations, this is more common than reported in other cohorts. Given that all three patients had the same mutation, it is possible that this represents a founder effect in patients in our region. Similar to previous reports, all three patients had pancreatic hypoplasia without cerebellar agenesis and birth weight SDS were significantly

low, as observed in the previously reported *PTF1A* cases. Evidence of phenotypic variability has been reported in patients with *PTF1A* enhancer mutations [28]. Although the majority are diagnosed in the first month of life, diabetes with onset later in life was also reported, suggesting that *PTF1A* enhancer mutations may also be a cause for late-onset monogenic diabetes [29].

Our cohort included only one female patient (P#7 with PTF1A enhancer mutation). Although, it would not be expected to have a specific sex predominance in NDM cohort since the majority of genetic causes have autosomal inheritance, an enrichment for males in PNDM cohorts has been observed previously. In the study of Hussain et al., describing the clinical and genetic characteristics of nine PNDM cases from the state of Qatar, male to female ratio was 2:1 [30]. In another study, female to male ratio was reported as 5/0 in TNDM and 4/13 in PNDM cases (p=0.006) [5]. Finally, in a large study of 1020 patients with NDM published by De Franco et al., there were 571 males and 449 females (p=0.006) [1]. Further studies are needed to assess the possible causes of this apparent sex bias.

In countries with high rates of consanguinity, WRS is the most common cause of NDM. Clinical features such as skeletal dysplasia (epiphyseal and spondyloepiphyseal dysplasia), cerebellar cortical dysplasia, hepatic and renal dysfunction, cardiomegaly, and mental retardation often develop after infancy [31,32]. *EIF2AK3* should be included in all NDM genetic testing panels, especially when used to test patients born to consanguineous parents, even if skeletal dysplasia and liver dysfunction are not present [33]. Although WRS represents a major cause of NDM in the Middle East [31], only one *EIF2AK3* mutation was found in in our patient cohort. Until now data on fewer than 100 children with WRS have been published [34,35] suggesting careful evaluation of glycaemic control, as well as renal and pancreatic function and growth, is fundamental. These patients are prone to repeated episodes of acute liver failure which may increase in severity with time with the risk of life-threatening fulminant liver failure. Liver transplantation is important, both as rescue treatment and as elective treatment, for patients with WRS [35]. Unfortunately, our case had no regular visits to our unit and died due to acute liver failure.

Patients with *INS* homozygous mutations have low birth weight due to reduced insulin secretion *in utero*. Postnatal growth failure is also frequent. Our patient with a homozygous *INS* promoter mutation had significantly low birth weight SDS and postnatal growth failure with a final height of -3.2 SDS. Insulin levels were undetectable in our case, demonstrating the virtual absence of proper insulin production [36,37].

TRMA is a recessively inherited disorder characterized by early onset diabetes, sensorineural hearing loss, and megaloblastic anaemia. Other rare clinical features include congenital heart disease, arrhythmias, retinal degeneration, optic atrophy, aminoaciduria, short stature, and situs inversus. 1° atrioventricular block was detected also in our patient with TRMA. The gene responsible for TRMA, *SLC19A2* codes for a high affinity thiamine transporter protein (THTR-1) [38,39] and treatment with thiamine (vitamin B1) can improve haematological and endocrine function, but neurological manifestations and deafness (which is progressive and irreversible) do not respond as well. Our patient with TRMA was treated with thiamine which improved the anaemia.

In a previous study reporting 22 patients from Turkey, biallelic *GCK* mutations were found to be a common cause of PNDM [5]; however, the most frequent cause in our cohort were KATP channel mutations (predominantly *ABCC8*), and *PTF1A* enhancer mutations. Another difference from this study was that none of the patients in our cohort in whom comprehensive genetic testing could be performed had a chromosome 6q24 methylation abnormality. These results highlight the genetic heterogeneity of NDM even within the same country.

Most of the studies and reported cases of NDM describes the clinical and biochemical characteristics of patients at diagnosis. This is one of the few studies reporting the anthropometric characteristics (10 patients) and the final height (*ABCC8* mutation:3, *INS* mutation:1) data of patients with NDM. Indeed, further studies describing follow up data of large NDM patient cohorts would be extremely valuable to help improve treatment protocols and highlight the importance of compliance to treatment.

In conclusion, although the consanguinity ratio was high, KATP channel and *PTF1A* enhancer mutations were the most frequent aetiology in our cohort, not the recessively inherited WRS. In the cases with KATP channel mutations and TRMA we report, treatment was modified after genetic testing. These results highlight the importance of early and accurate molecular testing for all cases with NDM due to potential consequences for the treatment and prognosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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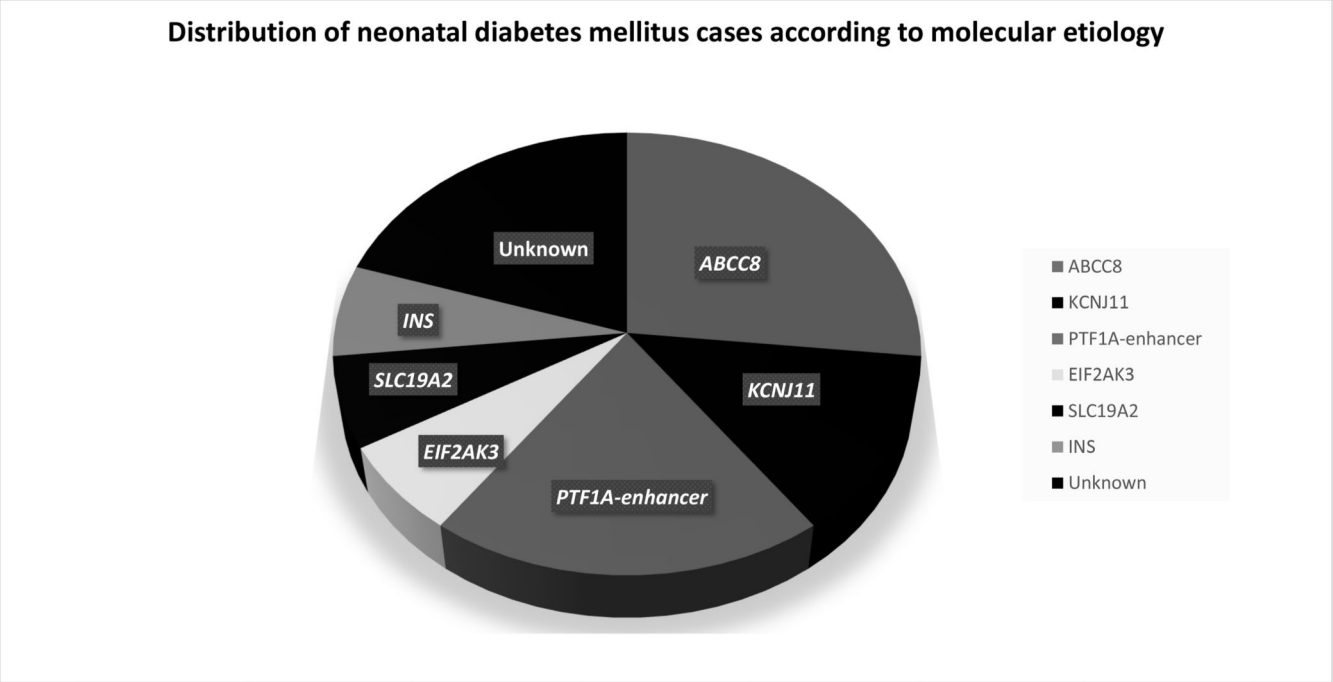

ABCC8 **n=4 (26.6%);** ***PTF1A*****-enhancer n=3 (20%);** ***KCNJ11*** **n=2 (13.3%);** ***EIF2AK3*** **n=1 (6.7%);** ***INS*** **n=1 (6.7%);** ***SLC19A2*** **n=1 (6.7%); unknown n=3 (20%)**

Figure 1. Distribution of Neonatal Diabetes Mellitus cases according to molecular aetiology

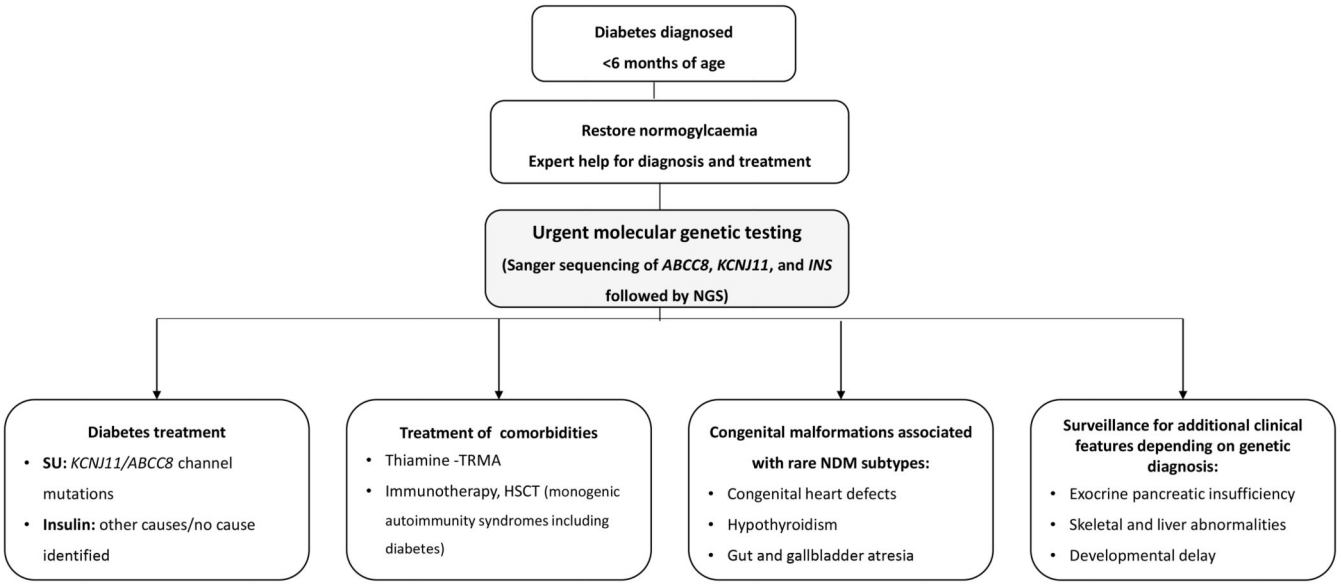

Figure 2. A pipeline for the diagnosis and follow up of NDM cases.

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Table 1

Clinical, biochemical and molecular characteristics of the patients at diagnosis

Patient	Sex (M/F)	GW	BW kg (SDS)	Parental consanguinity	Family member with DM[β]	Age at diagnosis (mo)	Plasma glucose (mg/dL)	Urinary ketone	Serum insulin (μu/ml)	C-peptide (ng/ml)	Seizure at diagnosis	DKA at diagnosis	Molecular diagnosis
1 *	M	39	3.0 (-0.9)	Yes	(+)	5.0	426	(+)	5.8	0.12	(+)	(-)	*ABCC8* Homozygous p.E382K
2 *	M	39	2.7 (-1.8)	Yes	(+)	2.5	520	(-)	NA	NA	(-)	(-)	*ABCC8* Homozygous p.E382K
3	M	38	3.2 (0)	Yes	(+)	1.5	760	(+)	NA	NA	(+)	(-)	*ABCC8* Homozygous p.R826W
4	M	39	3.1 (-0.6)	Yes	NA	2.0	520	(-)	0.6	0.14	(-)	(-)	*ABCC8* Compound heterozygous p.I557R/ p.R1145L
5	M	38	1.8 (-5.2)	Yes	(-)	2.2	450	NA	0.4	0.01	(-)	(-)	*PTF1A*-distal enhancer Homozygous g.23508437A>G
6	M	35	1.5 (-3.1)	No	(-)	0.2	500	NA	NA	0.01	(-)	(-)	*PTF1A*-distal enhancer Homozygous g.23508437A>G
7	F	39	1.4 (-5.5)	No	(+)	0.5	522	(-)	NA	0.011	(-)	(-)	*PTF1A*-distal enhancer Homozygous g.23508437A>G
8	M	38	2.9 (-0.8)	No	(-)	3.0	900	(+)	NA	0.33	(-)	(+)	*KCNJ11* Heterozygous p.E322K
9	M	38	2.5 (-2.5)	No	(-)	1.0	695	(+)	NA	NA	(-)	(-)	*KCNJ11* Heterozygous p.R201C
10	M	40	3.25 (-0.5)	Yes	(-)	6.0	480	NA	NA	NA	(-)	(-)	*EIF2AK3* Homozygous p.L742X
11	M	40	1.5 (-7.4)	Yes	(+)	0.7	500	NA	0.02	NA	(-)	(-)	*INS* Homozygous c.-331C>G
12	M	39	3.01 (-0.9)	Yes	(-)	3.0	400	(-)	3.0	1.14	(-)	(-)	*SLC19A2* Homozygous p.S214fs *14
13	M	38	3.1 (-0.2)	Yes	(-)	3.0	564	(+)	0.2	0.22	(-)	(+)	NA[¥]
14	M	39	3.3 (-0.1)	1	(-)	1.0	792	NA	NA	NA	(-)	(+)	NA[¥]
15	M	40	3.68 (0.6)	No	(-)	5.9	477	NA	NA	0.3	(-)	(-)	NA[¥]

M, Male; F, Female; GW, Gestational week; BW, Birth weight; SDS, Standart deviation score; DKA, Diabetic ketoacidosis; NA, Not available

Conversion factor for glucose: mmol/L in mg/dL, conversion factor: 1 mmol/L = 18,018 mg/dL

[*] Patient 1 and 2 are siblings

[¥] Molecular genetic studies could not be performed in three patients (P#13, 14 and 15) because the family did not approve genetic testing. In P#15, sequencing analysis of the *ABCC8*, *KCNJ11*, *INS*, and *GCK* genes were negative, parental consent was not obtained for further genetic testing in this case.

[β] Family members with DM were diagnosed as type 2 diabetes and they were on oral antidiabetic therapy. Mother of P#11 had diabetes diagnosed at 41 years of age requiring oral antidiabetic initially and afterwards insulin. Segregation analyses of these family members (except parents) could not be performed at that time period.

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Table 2

Clinical, biochemical and molecular characteristics of 10 patients at last evaluation

Patient	Age (yrs)	Insulin requirement (dose U/kg/d)	Sulphonlyurea (dose mg/kg/d)	HbA1c (%)	Height at last evaluation (cm)/(SDS)	Final height (cm)/ (SDS)	Target height (cm)/ (SDS)	Molecular diagnosis
1*	20.0	-	0.4	6.0		174.0/ (-0.35)	174.0/ (-0.35)	*ABCC8* Homozygous p.E382K
2*	19.0	0.4	0.5	10.0		175.8/ (-0.1)	174.0/ (-0.35)	*ABCC8* Homozygous p.E382K
3	19.2	0.2	0.8	5.4		181.9/ (0.9)	171.0/ (-0.8)	*ABCC8* Homozygous p.R826W
5	4.8	0.55	-	7.3	107.8/(-0.35)		175.7/ (-0.1)	*PTF1A*-distal enhancer Homozygous g23508437A>G
7	11.8	1.5	-	11.6	136.9/(-1.2)		164.5/ (0.2)	*PTF1A*-distal enhancer Homozygous g23508437A>G
11	18.0	1.8	-	8.9		156.7/ (-3.2)	169.0/ (-1.0)	*INS* Homozygous c.-331C>G
12	10.0	1.4	-	11.0	148.9/(1.9)		176.7/ (0.02)	*SLC19A2* Homozygous p.S214fs*14
13	5.3	0.5	-	8.8	114.6/(0.5)		172.5/ (-0.5)	NA¥
14	1.0	0.3	-	10.9	70.0/(-2.4)		173.4/ (-0.4)	NA¥
15	4.8	0.9	-	6.1	113.4/(0.9)		178.3/ (0.25)	NA¥

*Patient 1 and 2 are siblings

¥Molecular genetic studies could not be performed in three patients (P#13, 14 and 15) because the family did not approve genetic testing. In P#15, sequencing analysis of the *ABCC8*, *KCNJ11*, *INS*, and *GCK* genes were negative, parental consent was not obtained for further genetic testing in this case.