Published in final edited form as:
J Pediatr Endocrinol Metab. 2018 January 26; 31(2): 195–204. doi:10.1515/jpem-2017-0284.

Genetic Mutations Associated with Neonatal Diabetes Mellitus in Omani Patients

Aisha Al Senani, MD[1,*], **Nishath Hamza, PhD**[2,*], **Hanan AlAzkawi, MD**[1], **Manal Al Kharusi, PhD**[2], **Nashat Al Sukaiti, MD**[3], **Maryam Al Badi, MD**[1], **Moza Al Yahyai, MD**[1], **Matthew Johnson, B.Sc.**[4], **Elisa De Franco, PhD**[4], **Sarah Flanagan, PhD**[4], **Andrew Hattersley, FRCP FMedSci FRS**[4], **Sian Ellard, PhD FRCPath**[4,5], **Waad-Allah Mula-Abed, MBChB MSc FRCPath**[2]

[1]National Diabetes and Endocrine Center, Royal Hospital, Ministry of Health, Oman

[2]National Genetic Center, Royal Hospital, Ministry of Health, Oman

[3]Department of Pediatrics, Allergy and Clinical Immunology Unit,Royal Hospital, Ministry of Health, Oman

[4]University of Exeter Medical School,Institute of Biomedical and Clinical Science, Exeter, UK EX2 5DW

[5]Royal Devon & Exeter Hospital, Molecular Genetics Laboratory, Exeter, UK EX2 5DW

Abstract

Objectives—Neonatal Diabetes Mellitus (NDM) is a rare disorder worldwide where diabetes is diagnosed in the first 6 months of life. However, Oman has a relatively high incidence of NDM. In this study, we investigated the genetic etiologies underlying NDM and their prevalence in Oman in order to strategize the provision of medical genetic services to NDM patients and their families.

Research Design and Methods—We collected a cohort of 24 NDM patients, with and without genetic diagnosis, referred to our Center from 2007 to 2015. All patients without a genetic diagnosis were tested for mutations in 23 NDM-associated genes using a custom-targeted next generation sequencing panel and methylation analysis of the 6q24 locus.

Results—A genetic abnormality was detected in 15/24 (62.5%) of our Omani NDM patients. We report the detection of 6q24 methylation abnormalities and KCNJ11 mutations for the first time in Omani NDM patients. Unlike Western populations where NDM is predominantly due to mutations in the KCNJ11, ABCC8 and INS genes, NDM due to recessive GCK gene mutations were most

Corresponding address: Dr. Aisha Al Senani, National Diabetes and Endocrine Center, Royal Hospital, Ministry of Health, P.O. Box 1331, PC-111, Seeb, Muscat, Oman, Tel: +96824211296, Fax: +96824211270, amsalsenani@gmail.com.
[*] *Co-first Authors*

Author Contributions:
A.S. initiated the study, collected patient samples and clinical data and edited the manuscript. N.H. designed the study, compiled data and wrote the manuscript. Both A.S. and N.H. are co-first authors of this article. H.A., N.S., M.B. and M.Y. collected patient samples. M.K. analyzed pedigree data and created pedigree chart. M.J., E.F., S.F. A.H. and S.E. provided the results of genetic testing, edited and reviewed the manuscript. W.M. reviewed the study and manuscript. A.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

All the authors of this study have no relevant conflicts of interest to disclose.

Europe PMC Funders Author Manuscripts

prevalent in Oman, having been observed in 7/15 NDM patients in whom we established the genetic etiology.

Conclusions—Homozygous mutations in GCK are the commonest genetic etiology underlying NDM in Oman. This reflects the high degree of consanguinity which makes recessive conditions more likely. On comparing the spectrum of NDM-associated mutations in Oman withother countries, we observed that unlike Western populations where GCK mutations are rare in NDM patients, NDM due to homozygous mutations in the GCK gene are most prevalent in Oman. The results of this study are likely to impact any future strategy to introduce genetic testing for NDM disorders within the national healthcare system in Oman.

Oman has a relatively high incidence of Neonatal diabetes mellitus (NDM). In 1995, the mean incidence was 2.2 per 100,000 live births/year and the prevalence among <5 year olds during 1995 was 2.0/100,000 (1). NDM usually presents as hyperglycemia, failure to thrive and, in some cases, dehydration and ketoacidosis that can lead to coma in affected children within the first months of life. NDM can be classified into two types; namely, Transient (TNDM) and Permanent (PNDM) neonatal diabetes. Both TNDM and PNDM are rare conditions which are estimated to occur in 1:90,000-1:260,000 (2, 3) live births worldwide.

Patients with TNDM generally develop diabetes in the first weeks of life. The diabetes usually remits within the first year. Relapse to a permanent diabetic state most often occurs in adolescence or in young adulthood, likely precipitated by the physical stresses and increased insulin requirement of puberty or pregnancy. These individuals are typically younger at presentation than PNDM patients and have lower initial insulin requirements (4). TNDM patients are also more likely to have intrauterine growth retardation than PNDM patients, but display decreased tendency for ketoacidosis. One of the common causes of TNDM is aberrant methylation pattern at the 6q24 locus. As relapse into diabetes with progress in age or due to pregnancy is common in TNDM patients, long-term monitoring is imperative for such patients.

In PNDM, insulin secretory failure generally occurs in the late fetal or early post-natal period and does not enter remission (5). Despite the differences between TNDM and PNDM patients, considerable overlap occurs between the two groups, so that TNDM is sometimes indistinguishable from PNDM based on clinical features at presentation.

Conclusive distinction between PNDM and TNDM is made possible by genetic testing. To date 23 genetic causes accounting for 82% of patients with neonatal diabetes have been identified (6). It was reported that 37% of all NDM cases are caused by mutations in the KCNJ11 and ABCC8 genes, encoding the Kir6.2 and SUR1 proteins, which are both subunits of the pancreatic KATP channel involved in the regulation of insulin secretion (6). Mutations in the INS gene, which provides instructions for making insulin, have been identified in about 20% of individuals with PNDM (6). Another gene commonly implicated in PNDM is EIF2AK3 which causes Wolcott-Rallison syndrome (6, 7). Homozygous mutations in the GCK gene are also reported as one of the rarer causes of PNDM (8). Uncovering the molecular etiology of NDM has potentially important therapeutic consequences, since patientswith KCNJ11 and ABCC8 gene mutations can transfer from insulin therapy to sulfonylureas (9).

Europe PMC Funders Author Manuscripts

Given that the rate of consanguineous marriages is quite high (52%) in Oman (10), genetic testing for monogenic diabetes also has significant implications for the provision of pre-marital and pre-natal counseling of family members of NDM patients. In this study, we present new mutation results and data compiled from previously reported genetic studies on Omani NDM patients to understand the prevalence of NDM-associated mutations in Oman. It is hoped that the results of this study will provide the basis for a future strategy to introduce genetic testing for NDM disorders within the national healthcare system in Oman.

Research Design and Methods

Patients

We collected a cohort of 24 patients from 2007 to 2015 with a diagnosis of NDM, referred to our National Diabetes and Endocrine Center (NDEC), where nearly all Omani NDM patients requiring specialist care are referred. None of the patients had dysmorphic features or pancreatic aplasia. Twenty patients were negative for anti-GAD & anti-islet cell antibodies. Two of the four patients who were positive for other auto-antibodies manifested later with clinical signs such as immunodeficiency and autoimmunity (11).

Of the 24 patients, 21 presented with hyperglycemia in the first week of life and three patients presented with skeletal abnormalities and proximal tubulopathies. One patient (Patient 15) was diagnosed at the age of 6 months with hyperglycemia and proceeded to develop thrombocytopenia (Evans syndrome) which was later treated with Rituximab. Another patient (Patient 14) who presented at birth with autoimmune diabetes and autoimmune hypothyroidism, went on to develop autoimmune hemolytic anemia and hepatitis at the age of 5 months. This was then followed by alveolitis and pulmonary hemorrhage at the age of 9 months.

Patients 3 and 4 are first cousins (Figure 1) and are offspring of double consanguineous unions, where even their grandparents were first cousins. Patients 5, 6 and 7 belong to families distantly related to Patients 3 and 4. Patients 8 and 9; as well as 11 and 12 are siblings from two different families.

Methods

Previous publications reporting mutations in six patients amongst the 24 patients in our NDM cohort were reviewed (11,12,13). All available patient clinical details were collected from our hospital records. Of the remaining 18 patients included in this study, one patient (Patient 15) was tested using whole-exome sequencing (WES) in a separate, parallel study (manuscript under preparation).

The other 17 patients were sent to the Exeter laboratory, where the proband was first tested for the ABCC8, KCNJ11, INS and EIF2AK3 gene mutations using Sanger sequencing. This was then followed up by targeted next generation sequencing of the 22 known genes implicated in NDM and where appropriate, analyzing for TNDM due to abnormalities of the 6q24 locus by methylation analysis. Family members were tested only for the mutation detected in the probands. The DNA of all patients tested in this study was extracted using standard commercial kits.

The custom gene panel for NDM at the University of Exeter Medical School laboratory was used to analyze the coding regions and conserved splice sites of the KCNJ11, ABCC8, INS, EIF2AK3, FOXP3, GATA4, GATA6, GCK, GLIS3, HNF1B, IER3IP1, IL2RA, LRBA, PDX1, PTF1A, NEUROD1, NEUROG3, NKX2-2, RFX6, SLC2A2, SLC19A2, STAT3, WFS1 and ZFP57 genes using targeted next generation sequencing (NGS) with the Agilent custom capture kit v5 on the IlluminaHiSeq platform as previously reported (14). This assay can also detect partial or whole gene deletions and duplications. All mutations detected by NGS were confirmed using Sanger sequencing analysis of the mutated exon in the affected gene.

Methylation analysis of the 6q24 locus was carried out using Methylation-specific PCR (6). To determine whether any loss of maternal methylation at the 6q24 locus was caused by paternal uniparental disomy (UPD) of chromosome 6, microsatellites were analyzed at polymorphic loci throughout chromosome 6.

The pedigree diagram shown in Figure 1 was created using the program Genial Pedigree Draw.

Results

Of the 24 patients in our NDM cohort, six patients (Patients 8, 9, 11, 12, 13 and 14) had been previously investigated and their genetic test results were curated. Patients 8 and 9 were reported to carry the p.Gly261Arg mutation in the GCK gene, which encodes for glucokinase, a key enzyme responsible for regulating insulin secretion in pancreatic beta cells (12). Patients 11, 12 and 13 were previously diagnosed as having Fanconi-Bickel syndrome due to homozygous mutations in the SLC2A2 gene (13); which encodes for the glucose transporter type-2 protein mediating bidirectional glucose transport.

Patient 14 had presented quite early at 3 days after birth with insulin-dependent diabetes mellitus (IDDM) and subsequently developed autoimmune cytopenia and pulmonary hemorrhage. Since Patient 14 exhibited CD25 (IL2RA) deficiency, Sanger sequencing of the IL2RA gene was undertaken at an external laboratory and Patient 14 wasfound to harbor a novel homozygous mutation in the IL2RA gene (11).

The 18/24 patients who had yet to receive a genetic diagnosis were analyzed in this study; which resulted in the detection of genetic abnormalities in nine patients. Hence, this study and previous studies (11,12,13) combined, provided a genetic diagnosis for 15/24 (62.5%) patients in our NDM cohort. The clinical picture and mutation details of these 15 patients are reported in Table 1 and 2, respectively.

Patients 1 and 2 had methylation abnormalities at the 6q24 locus and were therefore diagnosed with 6q24 TNDM. To determine the underlying cause of the loss of maternal methylation at the 6q24 locus, microsatellite analysis was carried out at polymorphic loci throughout chromosome 6 (6). Patient 1 had complete loss of methylation at the maternal 6q24 locus, whilst Patient 2 displayed homozygosity of a single paternal allele with no maternal contribution at three different non-consecutive loci on chromosome 6. This

indicated that the TNDM in Patient 2 was most likely due to partial paternal UPD of the 6q24 locus.

Targeted next generation sequencing on the remaining 16 patients identified mutations in six patients (Patient nos. 3-7 and 10) with PNDM (Table 2). Patients 3-7 were homozygous for the p.Glu98Ter mutation in the GCK gene. In Patient 10, we detected a heterozygous mutation in the KCNJ11 gene for the first time in Oman.

Even after targeted next generation sequencing, nine patients were still negative for any pathogenic mutations in all known NDM-associated genes. Among these nine, Patient 15 was later classified as having autoimmune disease and recruited for whole-exome sequencing in a separate, parallel study (manuscript under preparation). As a result, Patient 15 was reported to be homozygous for the nonsense mutation p.Arg1271Ter (c.3188C>T) in exon 23 of theLRBAgene.

Discussion

Major progress has been made in understanding the genetic etiology behind NDM syndromes which present in the first year of life with 22 genes having been identified to date (6). The most common genetic defects accounting for the majority of NDM cases worldwide are mutations in the genes encoding the two subunits of the ATP-sensitive potassium channel (K_{ATP}), KCNJ11 and ABCC8, and the INS gene (5,6).

Characterizing the associated genetic defects is crucial to improving the clinical management of NDM patients. For example, patients with activating mutations inKCNJ11 and ABCC8 can besuccessfully transferred from insulin therapy to sulfonylureas (9). Thus, adoption of a personalized genetic medicine approach for the management of monogenic diabetes patients will likely provide better glucose regulation and quality of life in these individuals.

In our cohort of Omani NDM patients, 15/24 individuals were found to carry genetic variants associated with NDM. This study is the first to report the detection of NDM cases due to (epi)genetic abnormalities of the 6q24 locus in Omani patients. The TNDM patients 1 and 2 exhibited a typical clinical picture as they presented with failure to thrive and hyperglycemia. Patient 1 had complete loss of methylation at the maternal 6q24 locus, while Patient 2 exhibited aberrant methylation due to paternal UPD of the 6q24 locus. Genetic imprinting defects of the 6q24 locus is associated with TNDM and usually presents as severe intrauterine growth retardation, neonatal hyperglycemia in a term infant which may resolve by the age of 18 months, dehydration and absence of ketoacidosis. Macroglossia and umbilical hernia are also often present (7). The TNDM Patients 1 and 2 were treated with small doses of intermediate insulin (NPH) at a rate of 0.3 iu/kg/day. Neither of these patients developed diabetic ketoacidosis. Both Patients 1 and 2 are at present clinically well and have been in remission since the age of one year and four months, respectively.

We also observed two different homozygous GCK gene mutations in Oman. Patients 3 and 4 are first cousins (Figure 1), while Patients 5, 6 and 7 belong to families distantly related to Patients 3 and 4, but from the same tribe and they carried the p.Glu98Ter in exon 3 of the

GCK gene. Patients 5, 6 and 7 were referred to our clinic separately, without knowledge of the familial ties between them or with Patients 3 and 4. Indeed, in an example of genetic disease testing leading to relationship discovery, the family ties of patients 5, 6 and 7 with patients 3 and 4 were discovered, even by the parents of the patients, during genetic counseling sessions held after genetic test results were reported. However, the parents of 5, 6 and 7 declined to provide more information necessary to create elaborate pedigrees. In Patients 8 and 9 the p.Gly261Arg mutation in the GCK gene was previously reported (12). Since homozygous mutations in the GCK gene are generally quite rare worldwide (7, 8), it is evident that the high rate of consanguinity in Oman has contributed to the persistence of GCK-associated NDM in this population. This phenomenon is reflected in the highly consanguineous pedigree shown in Figure 1.

The GCK gene encodes glucokinase, a key regulatory enzyme in the pancreatic beta-cell. Glucokinase plays a crucial role in the regulation of insulin secretion and has been termed the glucose sensor in pancreatic beta-cells(15). Given its central role in the regulation of insulin release, it is understandable that homozygous and heterozygous mutations in the gene encoding glucokinase (GCK) can cause both hyper- and hypoglycemia respectively (16).

This study is the first report of KCNJ11 mutations in an Omani NDM patient with Asian sub-continental Balushi ethnicity. Patient 10 in this study carries a known (17) heterozygous p.V59M mutation in the *KCNJ11* gene. *KCNJ11* encodes Kir6.2, which serves as the pore-forming subunit of the ATP-sensitive K+ (K_{ATP}) channel in multiple tissues. This channel is a hetero-octameric structure comprising four Kir6.2 subunits and four regulatory Sulfonylurea receptor (SUR) subunits (18,19), of which SUR1 is expressed in β-cells andneurons(18).

Published functional studies of the p.V59M mutationin the KCNJ11 gene have indicated that the severe neurological phenotype associated with this mutation is due to the effect of the mutation on K_{ATP} channels in neuronal tissue (17,20). Functional studies in mice (21) have shown this mutation also affects the potassium channels in neuronal tissue, which probably affects brain function adversely.

Patients with the p.V59M mutation were reported to develop intermediate DEND syndrome,with developmental delay and PNDM, but no epilepsy (17,20,21). A similar phenotype was noted in Patient 10. It is unclear whether hypoxic insult on the brain during severe diabetic ketoacidosis episodes may have also contributed to the patient's severe phenotype.

K_{ATP} channels can be regulated by Sulfonylurea and Glinide drugs, which stimulate insulin secretion through binding of the SUR subunit of the K_{ATP} channel (22,23,24,25,26). As a result, these drugs are widely used to treat Type 2 diabetes (22) and are alsoeffective in treating PNDM and TNDM caused by mutations in the potassium channel genes (23,24).

Accordingly, in Patient 10, we observed significant improvement of glycemic control after introduction of sulfonylurea therapy. At diagnosis in 2014, the HbA1C value in Patient 10 was 7.9% (63 mmol/mol). After treatment with Sulfonylurea (Glibenclamide) at an initial

dose of 20mg/BID (twice a day) for three months, the fasting HbA1C value in Patient 10 was in the range of 4.1-4.7% (21-28 mmol/mol). Due to hypoglycemic episodes, the sulfonylurea dose was gradually reduced to 10mg/BID. Since 2015, the HbA1C values have consistently been ≤5%(31 mmol/mol). Also, before treatment with sulfonylurea, Patient 10 was completely wheelchair-bound. However, after treatment, Patient 10 displayed clinical improvement in neurological features as she was able to get up from her wheelchair and walk slowly with support.

Based on the findings of this study and previous studies on our NDM patients, we analyzed the prevalence of genetically diagnosed NDM cases in our cohort. The published report on over 1000 patients with NDM by De Franco et al shows that activating mutations in KCNJ11 or ABCC8 account for 46% of NDM cases in patients born to non-consanguineous unions; with mutations in the INS gene being the next most common etiology (6). On comparing the spectrum of NDM-associated mutations in Oman with other countries we observed that unlike Western populations (5,6,27,28) where GCK mutations are rare in NDM patients, NDM due to homozygous mutations in the GCK gene are most prevalent in Oman. Worldwide, homozygous mutations in the EIF2AK3 gene are known to be the most common cause of neonatal diabetes in patients born to consanguineous parents (7). It is therefore striking that mutations in this gene have not been identified in our cohort.

A previous study conducted in Saudi Arabia also reported that the incidence of genes commonly mutated in NDM patients were different from that seen in Western populations (29). Since consanguinity has been widely practiced in both Oman and Saudi Arabia for centuries, it is possible that founder effect has a role to play in contributing to relatively high numbers of individuals with rare pathogenic mutations within certain tribes in both these countries.

In this study, we chose to evaluate the prevalence as opposed to incidence of genetic etiology underlying NDM (30). It may be argued that the sampling of multiple affected individuals from the same family or large consanguineous tribal pedigrees contributes to an over-estimation of certain genetic etiology;as seen in the case of NDM due to GCK mutations in this study. However, by focusing on the prevalence or the total number of genetically-affected individuals with NDM, we obtained a better perception of the burden of genetic testing, counseling services and clinical management that NDM disorders are likely to add to Oman’s healthcare services. On the other hand, studying the incidence of genetic etiology (or different genes involved in NDM),which requires the counting of individual mutation events (or sampling a single mutation per family) would have resulted in an under-representation of the healthcare burden due to NDM in Oman. This is a significant point to consider because the total number of NDM-affected individuals being born each year is a critical parameter used toassess whether the introduction of genetic testing, genetic counseling and proactive pre-marital and prenatal screening within affected families of NDM patients is effective in reducing numbers of NDM patients over time. Our results also indicate the need for a genetic testing strategy that takes into account the ethnicity and tribal affiliations of the patients. For example, it may be more cost-effective to test for homozygous GCK mutations using whole-gene Sanger sequencing in future Omani patients with Arab-African ancestry, rather than using next-generation sequencing.

It is to be noted that only 62.5% (15/24) of our NDM patients had a genetic defect known to be associated with NDM. The genetic disorder underlying NDM in the remaining nine patients is still unknown despite being analyzed using the targeted sequencing panel and/or exome sequencing. These nine patients were all negative for autoantibodies and other physical or mental abnormalities. All nine patients will be recruited for a future study to look for novel causes of NDM.

Since the NDEC is the national center for specialist diabetic care to which nearly all Omani NDM patients are referred to since 2007, estimating and monitoring NDM prevalence among the population has significant implications not just for treatment of NDM patients, but also in adjudicating sufficient resources for genetic counseling and the provision of pre-marital and pre-natal screeningin family members of NDM patients in Oman. However, given the large genetic heterogeneity underlying NDM and the possibility of yet more genes being associated with NDM in the future, a strategic approach to genetic testing for NDM disorders is essential. It is hoped that this study will influence and guide the adoption of an effective protocol for NDM genetic testing in Oman with the aim of faster genetic diagnosis for NDM patients and timely clinical intervention.

Acknowledgements

Funding:

Genetic testing performed at the University of Exeter Medical School was funded through a Wellcome Trust Senior Investigator Award to Professors Andrew Hattersley and Sian Ellard.

References

1. Bappal B, Raghupathy P, de Silva V, Khusaiby SM. Permanent neonatal diabetes mellitus: clinical presentation and epidemiology in Oman. Archives of Disease in Childhood. Fetal and Neonatal Edition. 1999; 80:209–12.
2. Iafusco D, Massa O, Pasquino B, et al. Minimal incidence of neonatal/infancy onset diabetes in Italy is 1:90,000 livebirths. ActaDiabetol. 2012; 49:405–8.
3. Slingerland AS, Shields BM, Flanagan SE, 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. [PubMed: 19499210]
4. Polak M, Cavé H. Neonatal diabetes mellitus: a disease linked to multiple mechanisms. Orphanet J Rare Dis. 2007; 9:2–12.
5. Naylor RN, Greeley SA, Bell GI, Philipson LH. Genetics and pathophysiology of neonatal diabetes mellitus. J Diabetes Investig. 2011; 2:158–169.
6. De Franco E, Flanagan SE, Houghton JA, et al. The effect of early, comprehensive genomic testing on clinical care in neonatal diabetes: an international cohort study. Lancet. 2015; 386:957–963. [PubMed: 26231457]
7. Rubio-Cabezas O, Patch AM, Minton JA, et al. Wolcott-Rallison syndrome is the most common genetic cause of permanent neonatal diabetes in consanguineous families. J ClinEndocrinolMetab. 2009; 94:4162–70.
8. Esquiaveto-Aun AM, De Mello MP, Paulino MF, Minicucci WJ, Guerra-Júnior G, De Lemos-Marini SH. A new compound heterozygosis for inactivating mutations in the glucokinase gene as cause of permanent neonatal diabetes mellitus (PNDM) in double-first cousins. DiabetolMetabSyndr. 2015; 7:101.

9. Sagen JV, Raeder H, Hathout E, et al. Permanent neonatal diabetes due to mutations in KCNJ11 encoding Kir6.2: patient characteristics and initial response to sulfonylurea therapy. Diabetes. 2004; 53:2713–2718. [PubMed: 15448106]
10. Islam MM. The practice of consanguineous marriage in Oman: prevalence, trends and determinants. J BiosocSci. 2012; 44:571–94.
11. Al Sukaiti N, Al Sinani A, Al Ismaily, Shaikh S, Al Abrawi S. Pulmonary hemorrhage in a case of CD25 deficiency. LymphoSign Journal. 2014; 01:39–43.
12. Bennett K, James C, Mutair A, Al-Shaikh H, Sinani A, Hussain K. Four novel cases of permanent neonatal diabetes mellitus caused by homozygous mutations in the glucokinase gene. Pediatr Diabetes. 2011; 12:192–6. [PubMed: 21518409]
13. Sansbury FH, Flanagan SE, Houghton JA, et al. SLC2A2 mutations can cause neonatal diabetes, suggesting GLUT2 may have a role in human insulin secretion. Diabetologia. 2012; 55:2381–5. [PubMed: 22660720]
14. Ellard S, Lango Allen H, De Franco E, et al. Improved genetic testing for monogenic diabetes using targeted next-generation sequencing. Diabetologia. 2013; 6:1958–63.
15. Matschinsky FM. A lesson in metabolic regulation inspired by the glucokinase glucose sensor paradigm. Diabetes. 1996; 45:223–241. [PubMed: 8549869]
16. Gloyn AL. Glucokinase (GCK) mutations in hyper- and hypoglycemia: maturity-onset diabetes of the young, permanent neonatal diabetes, and hyperinsulinemia of infancy. Hum Mutat. 2003; 22:353–62. [PubMed: 14517946]
17. Sang Y, Ni G, Gu Y, Liu M. AV59M KCNJ11 gene mutation leading to intermediate DEND syndrome in a Chinese child. J PediatrEndocrinolMetab. 2011; 24:763–6.
18. Seino S, Miki T. Physiological and pathophysiological roles of ATP-sensitive K+ channels. ProgBiophysMolBiol. 2003; 81:133–76.
19. Proks P, Girard C, Ashcroft FM. Functional effects of KCNJ11 mutations causing neonatal diabetes: enhanced activation by Mg-ATP. Hum Mol Genet. 2005; 14:2717–26. [PubMed: 16087682]
20. Clark RH, McTaggart JS, Webster R, et al. Muscle dysfunction caused by a KATP channel mutation in neonatal diabetes is neuronal in origin. Science. 2010; 329:458–61. [PubMed: 20595581]
21. Gloyn AL, Diatloff-Zito C, Edghill EL, et al. KCNJ11 activating mutations are associated with developmental delay, epilepsy and neonatal diabetes syndrome and other neurological features. Eur J Hum Genet. 2006; 14:824–30. [PubMed: 16670688]
22. Inagaki N, Gonoi T, Clement JP, et al. A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K+ channels. Neuron. 1996; 16:1011–7. [PubMed: 8630239]
23. Gribble FM, Reimann F. Sulfonylurea action revisited: the post-cloning era. Diabetologia. 2003; 46:875–91. [PubMed: 12819907]
24. Zung A, Glaser B, Nimri R, Zadik Z. Glibenclamide treatment in permanent neonatal diabetes mellitus due to an activating mutation in Kir6.2. J ClinEndocrinolMetab. 2004; 89:5504–7.
25. Pearson ER, Flechtner I, Njølstad PR, et al. Switching from insulin to oral sulfonylureas in patients with diabetes due to Kir6.2 mutations. N Engl J Med. 2006; 355:467–77. [PubMed: 16885550]
26. Rafiq M, Flanagan SE, Patch AM, et al. Effective treatment with oral sulfonylureas in patients with diabetes due to sulfonylurea receptor 1 (SUR1) mutations. Diabetes Care. 2008; 31:204–9. [PubMed: 18025408]
27. Støy J, Edghill EL, Flanagan SE, et al. Insulin gene mutations as a cause of permanent neonatal diabetes. ProcNatlAcadSci U S A. 2007; 104:15040–4.
28. Greeley SA, Naylor RN, Philipson LH, Bell GI. Neonatal diabetes: an expanding list of genes allows for improved diagnosis and treatment. CurrDiab Rep. 2011; 11:519–32.
29. Habeb AM, Al-Magamsi MS, Eid IM, et al. Incidence, genetics, and clinical phenotype of permanent neonatal diabetes mellitus in northwest Saudi Arabia. Pediatr Diabetes. 2012; 13:499–505. [PubMed: 22060631]
30. Fu YX, Huai H. Estimating mutation rate: how to count mutations? Genetics. 2003; 164:797–805. [PubMed: 12807798]

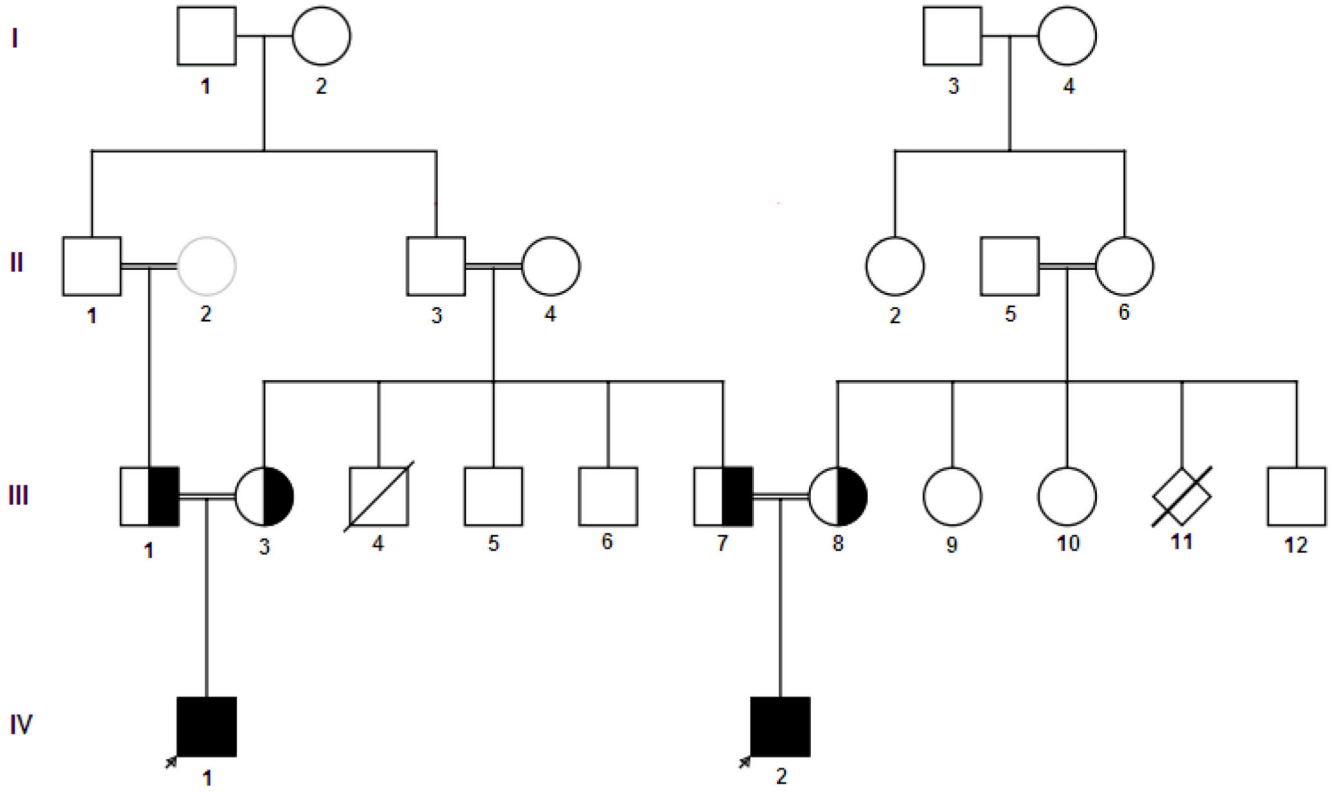

Figure 1. Consanguineous pedigrees of Patients 3 and 4

This pedigree represents the high degree of consanguinity practiced in many tribes of Oman. The probands, Patient 3 and 4 are represented by IV.1 and IV.2 respectively (indicated by arrows). The diamond symbol in III.11 represents five or six infants of unknown gender who died of unknown causes. The individual II.2 (grey circle) is married to II.1 and is also a sister of II.6, who is the maternal grandparent of proband IV.2 (Patient 4). Hence, the individual II.2 has been represented twice in this pedigree and both representations are linked by a dotted line.

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

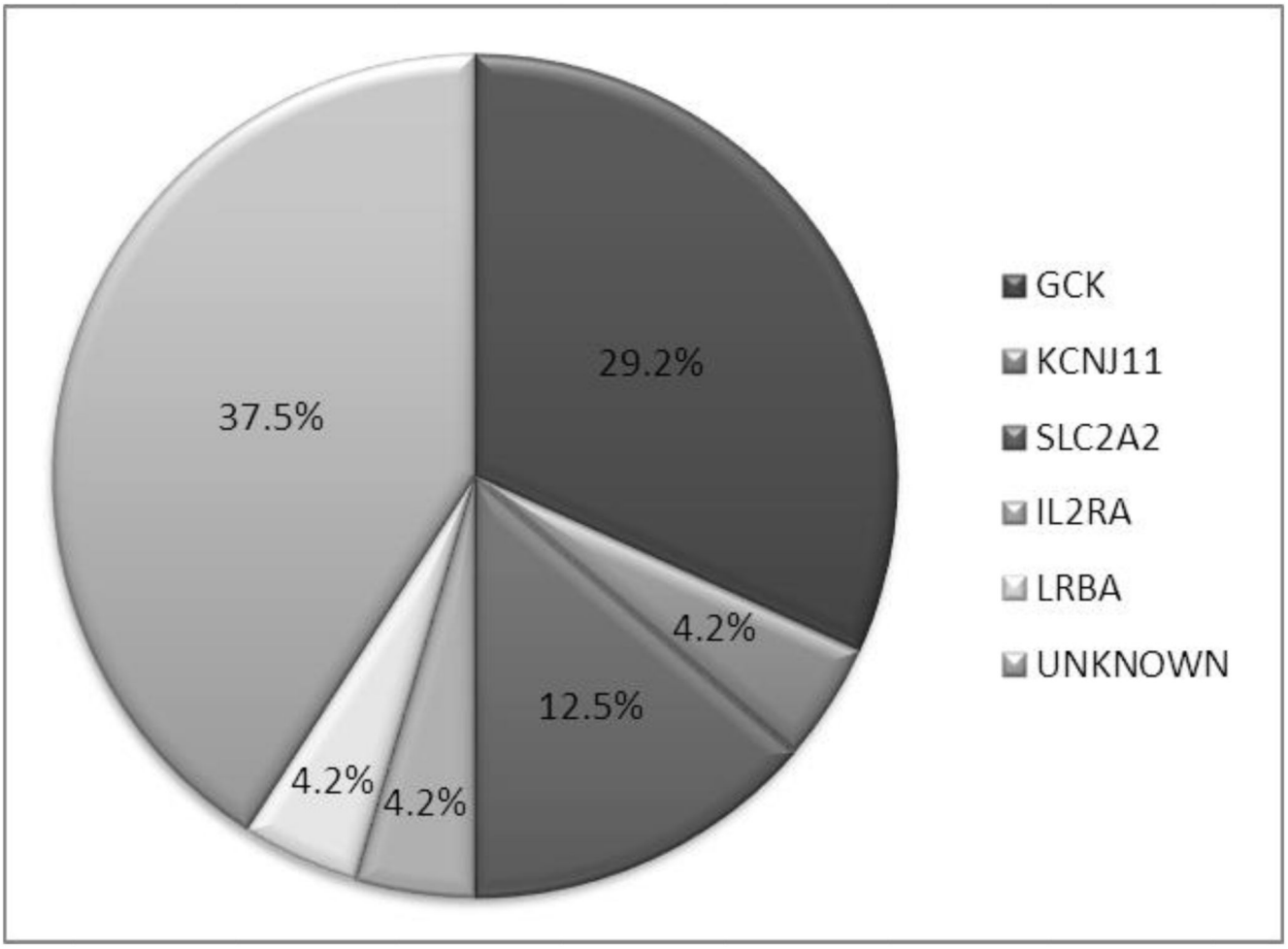

Figure 2. Prevalence of genetic abnormalities in Omani NDM patients

Mutations in the GCK gene are the most prevalent genetic cause of NDM in Oman.

Europe PMC Funders Author Manuscripts

Table 1

Clinical features of NDM patients with defined mutations

Patient No. (Sex)	Clinical details	Biochemical data				Molecular genetic data		Patient's current status
		Blood sugar or HBA1C at presentation	C-peptide	HBA1C after treatment	Auto-antibodies	Type	Gene	
1 (F)	• Presented on day 1 of life • Normal abdominal ultrasound • Started on insulin at 19 days of life (Glargine& Glipizide) • Sulfonylurea stopped at 5 month and insulin stopped at 1 yr. of age	12.9 mmol/L	N/A	5.0% (31 mmol/mol)	• Anti islet negative • Anti GAD = 22 (positive)	Complete loss of maternal methylation at the TND differentially methylated region on chromosome 6q24		Off medications since one year of age. Last visit was in 2009
2 (M)	• Presented on day 1 of life • Normal abdominal ultrasound and KUB • Normal TF and cortisol • Started on NPH , changed to Glargine and Lispro, then suphonylurea at 3 months of age • Stopped treatment at 4 months of age due to good glycemic control	16.8 mmol/L	<33 pmol/L	5.0% (31 mmol/mol)	• Anti islet negative • Anti GAD = 35 (positive)	Complete loss of maternal methylation at the TND differentially methylated region on chromosome 6q25 due to UPD		Off medication since 4 month of age
3 (M)	• Presented with PNDM on day 1 of life • Normal abdominal ultrasound • On insulin pump since diagnosis • Normal RF, LF , TF & CBC • Vitamin D25 =139nmol/l	37.2 mmol/L	4 pmol/L	9.0% (75 mmol/mol)	Negative for antibodies	HO	GCK	Doing well, gaining weight: 6.4kg, Ht: 62.2cm both are below 3rd centile
4 (M)	• Presented with PNDM on day 1 of life. • Normal abdominal ultrasound • On insulin pump since 21 days of age • Normal RF, LF , TF & CBC	>30 mmol/L	28 pmol/L	6.8% (51 mmol/mol)	Negative for antibodies	HO	GCK	Doing well, gaining weight, looks well, active, playing weight 10.6 kg on 10thrd centile height: 78.3 on 50th centile
5 (M)	• Presented with PNDM on day 1 of life. • Normal abdominal ultrasound • On insulin pump since day 2 of life • Normal RF, LF , TF & CBC	18 mmol/L	Not done	8%	Negative for antibodies	HO	GCK	Doing well, thriving with normal development.
6 (M)	• Presented with hyperglycemia on day 3 of life. • Normal abdominal ultrasound • On insulin pump since day 4 of life (insulin NPH and regular) • Normal RF, LF , TF & CBC	BG 18 mmol/L	<30 pmol/L	N/A	Negative for antibodies	HO	GCK	Doing well, thriving with normal development.
7 (F)	• Presented with PNDM on day 1 of life. • Normal abdominal ultrasound • On insulin pump since day 2 of life • Chronic renal impairment with profound proteinuria , normal LF & TF	21.8 mmol/L	<30 pmol/L	6.7% (50 mmol/mol)	Negative for antibodies	HO	GCK	Developed renal impairment and hypertension with profound proteinuria, short stature (height 133.7cm), regular normal menstruation, no retinopathies, developed Murriac syndrome

Europe PMC Funders Author Manuscripts

Patient No. (Sex)	Clinical details	Biochemical data				Molecular genetic data		Patient's current status
		Blood sugar or HBA1C at presentation	C-peptide	HBA1C after treatment	Auto-antibodies	Type	Gene	
8 (F)	• Presented with PNDM on day 16 of life. • Normal abdominal ultrasound • On insulin Glargine and Lisproto-date • Normal RF, LF & TF	HbA1C 8% , BG …20 mmol/l	<30 pmol/L	11% , 98 mmol/mol	Negative for antibodies	HO	GCK	Doing well with normal growth height 128.9 cm in 10th centile, weight 25.5 kg in 25th centile, but having difficulties in school with poor performance, difficult to have proper metabolic control as the compliance to dietary plan and insulin dose modifications is very poor
9 (F)	• Presented with hyperglycemia on day 4 of life. • Normal abdominal ultrasound • On insulin pump since day 4 of life (insulin NPH and regular) • Normal RF, LF , TF & CBC presented at	Not available	< 30 pmol/l	13%	Negative for antibodies	HO	GCK	Doing well clinically, but has poor metabolic control as family socio-economic status is poor, has normal growth with regular menstruation, her f inal height 151 cm at 3rd centile and compatible with mid parental height; weight is 51 Kg, no retinopathies , nephropathies or peripheral neuropathies
10 (F)	• Presented with severe diabetic ketoacidosis at 2 months of age • Patient was hyperventilated for 48 hours due to recurrent convulsions • Development and cognition has been affected secondary to the hypoxic insult on presentation • Started on insulin NPH at birth 2001, then changed to Glargine and Lispro in 2010, changed to Sulfonylurea since Sept 2014 (after 13 years with insulin injection, patient was successfully shifted to oral hypoglycemic agent)	79 mmol/L	261 pmol/L	5.0% (31 mmol/mol)	Negative for antibodies	HR	KCNJ11	Has developmental delay, failure to thrive, on regular medication (insulin, regular IVIG, AED, thyroxine, Cotrimoxazole, MMF), CD25 deficiency (primary immunodeficiency) on regular immunoglobulin, but no manifestation of immunodeficiency
11 (F)	• Presented at 9 months of age with hyperphosphatasia, extreme osteopenia, epiphyseal dysplasia, oval-shaped vertebra with post prandial hyperglycemia	HbAIC 5.7%	Not done	5.50%	Negative for antibodies	HO	SLC2A2	Has skeletal dysplasia and renal tubulopathies including glycosuria& nephrocalcinosis with preserved renal function, height 98 cm at < 3rd centile, weight 15.7 kg at < 3rd centile for her age
12 (M)	• Presented at day 7 of life with hyperphosphatasic rickets • Dysplasia ,with post prandial hyperglycemia	HbA1C 5.4%	Not done	5.40%	Negative for antibodies	HO	SLC2A2	Has skeletal dysplasia and renal tubulopathies including glycosuria&nephrocalcinosis with preserved renal function, height 99.8 cm on 3rd centile ,weight 13.5 kg on 10th centile for age.
13 (F)	• Presented at day 14 of life with hyperphosphatasia • ALP was high since birth • Normal Calcium, normal PTH (Parathyroid hormone)	N/A	N/A	5.8% (40 mmol/mol)	N/A	HO	SLC2A2	Lost to follow-up

Europe PMC Funders Author Manuscripts

Patient No. (Sex)	Clinical details	Biochemical data				Molecular genetic data		Patient's current status
		Blood sugar or HBA1C at presentation	C-peptide	HBA1C after treatment	Auto-antibodies	Type	Gene	
14 (M)	• Presented at day 3 of life with irritability and hyperglycemia • Has global developmental delay and multiple disease phenotypes • Neonatal diabetes on insulin pump • Autoimmune thyroid disease on thyroxine • Seizure disorder on antiepileptic medications • Had refractory pulmonary hemorrhage.	28 mmol/L	<3 pmol/L	5.2% (33 mmol/mol)	• Anti-GAD positive • Anti-islet positive • Anti-GBM positive • Anti-ANCA-MPO positive • Anti-PR3 positive	HO	IL2RA	Has developmental delay, failure to thrive, on regular medication (Insulin, regular IVIG, AED, thyroxine, Cotrimoxazole, MMF), CD25 deficiency (primary immunodeficiency) on regular immunoglobulin, but no manifestation of immunodeficiency
15 (M)	• Presented at age 7 months with hyperglycemia and hypothyroidism with positive thyroid peroxidase titer • At the age of one year developed thrombocytopenia and then developed interstitial pneumonia • Developed hepatosplenomegaly and generalized lymphadenopathy with no evidence of malignancy • Low immunoglobins	High	Low	9% (75 mmol/mol)	• Anti-GAD positive • Anti-islet positive	HO	LRBA	Clinically sick with immunodeficiency and hypothyroidism, on methylprednisolone pulses to control his autoimmunity

Abbreviations: RF – Renal Function, LF – Liver Function , TF – Thyroid Function & CBC – Complete Blood Count, GAD - Glutamic Acid Decarboxylase, GBM – Glomerular Basement membrane, ANCA - Anti-neutrophil Cytoplasmic Antibodies, N/A-Not Available

Europe PMC Funders Author Manuscripts

Table 2

Mutation and ethnicity details of PNDM patients

Patient No.	Ethnicity	Type	Gene	Location	Nucleotide variant	Protein Effect
3	Arab/African	HO	GCK	Exon 3	c.292C>T	p.Glu98Ter
4	Arab/African	HO	GCK	Exon 3	c.292C>T	p.Glu98Ter
5	Arab/African	HO	GCK	Exon 3	c.292C>T	p.Glu98Ter
6	Arab/African	HO	GCK	Exon 3	c.292C>T	p.Glu98Ter
7	Arab/African	HO	GCK	Exon 3	c.292C>T	p.Glu98Ter
8	Arab/African	HO	GCK	Exon 7	c.781G>A	p.Gly261Arg
9	Arab/African	HO	GCK	Exon 7	c.781G>A	p.Gly261Arg
10	Balushi	HR	KCNJ11	Exon1	c.175G> A	p.Val59Met
11	Arab	HO	SLC2A2	Exon9	c.1127T>G	p.Met376Arg
12	Arab	HO	SLC2A2	Exon9	c.1127T>G	p.Met376Arg
13	Arab	HO	SLC2A2	Exon9	c.1127T>G	p.Met376Arg
14	African	HO	IL2RA	Exon5	c.418T>C	p.Tyr140His
15	Arab	HO	LRBA	Exon 23	c.3188C>T	p.Arg1271Ter