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Abstract

Insulin gene mutation (INS) is the second most common cause of permanent neonatal diabetes (PNDM). We present the 1st cases of Saudi monozygotic twins with permanent neonatal diabetes mellitus who had simultaneous onset of disease due to p.C109Y (p.Cys109Tyr, c.326G>A) heterozygous missense mutation in exon 3 of the insulin (INS) gene. The twin patients had the same mutation while their parents are unaffected with normal genetic testing suggesting that this mutation had raised de novo. This p.C109Y mutation affects a highly conserved cysteine residue which is crucial for protein folding. Subjects with this form of diabetes will need lifelong insulin therapy.

Keywords: Permanent neonatal Diabetes; Monozygotic; Twins; INS gene; Saudi Arabia

Introduction

Neonatal diabetes mellitus (NDM) was previously defined as diabetes with onset within 6 weeks from birth that requires insulin therapy for at least 2 weeks. The new definition recently adopted, includes patients with diabetes onset within 6 months of age [1]. NDM was considered exceedingly rare condition but actually its incidence varies from country to another [1]. High incidence of 1 in 21,196 live births was reported from Saudi Arabia [2].

NDM can be classified according to the duration of insulin dependence in the first months/years of life and according to the molecular mechanisms responsible for the severe insulin secretion deficiency into 2 types; transient or permanent [3]. The number of cases of neonatal diabetes continues to accumulate, but the available, combined data indicate that somewhat over half (~57%) of NDM cases are transient [4].

Permanent neonatal diabetes mellitus (PNDM) /monogenic diabetes of infancy (MDI) is a rare condition associated with defects in genes that play major roles in pancreatic beta cell development and function; mutations in the genes encoding the ATP-sensitive potassium channel (K-ATP) subunits, KCNJ11 (encoding KIR 6.2 subunit), ABCC8 (encoding SUR1 subunit) and insulin (INS), account for most cases of PNDM/MDI [4-10]. Moreover, individuals with some exceedingly rare forms of syndromic PNDM may bear recessive mutations in several genes, including PDX1, EIF2AK3, PTF1A, GLIS3, RFX6 or FOXP3 [11-17], while recessive mutations in glucokinase give rise to isolated neonatal diabetes [18].

In fact undoubtedly the discovery of INS mutations on chromosome 11p15 [8] was the most important discovery in PNDM from the clinical perspective in the last few years [8]. It accounts for about 10-15% of NDM cases [3,9,19,20]. Both heterozygous and homozygous INS mutations can cause NDM. The heterozygous coding mutations in the INS gene are considered the second most common cause of isolated PNDM after the activating mutations in the K-ATP channel genes [3,8,9,20]. Recently Raile et al. reported INS deletions in homozygous and heterozygous individuals [21].

Case Report

We here in report the first cases of PNDM due to INS heterozygous mutation from Saudi Arabia in 15 years-old identical twin females, born to non-consanguineous parents. They were delivered at term by normal spontaneous vertex vaginal delivery with uneventful antenatal and postnatal periods. Family history was negative for DM. At birth, their general condition was unremarkable with birth weight 2.3 kg (twin 1) and 2.1 kg (twin 2). At the age of 3 months, their mother noticed irritability and fussiness with failure to gain weight for both of her daughters simultaneously. Random blood glucose was checked and found to be 340 mg/dl (twin 1) and 500 mg/dl (twin 2) with no ketosis. Thyroid function tests and lipid profile were normal. Structural diseases of the pancreas were excluded by ultrasonography of the abdomen. They were started on NPH insulin with dose of about 1 unit/kg/day. Glutamic acid decarboxylase-65 antibody (GAD-65) and anti-islet cell antibodies were negative. Celiac profile was also negative. Basal and glucagon stimulated C-peptide levels were undetectable (reference range: 1.1-4.4 ng/mL). Since diagnosis, the patients are being followed up and they are now 15 years old with average weight and height and normal motor, mental and pubertal development with no extrapancreatic disease. They are currently on insulin glargine only with a dose of about 1 unit per kg and refusing the short acting analogues with poor glycemic control (HbA1C between 10-14%). As previously reported by Kamal Alanani and Alsulaimani, low awareness and persistent denial of the parents about the fact that their children are diabetic is the greatest obstacle Pediatricians meet in Saudi Arabia [22]. We faced that in our patients whose parents are very poorly compliant to give proper insulin regimens to their daughters. Their parents were
Genetic testing was carried for the patients and their parents. Sequence analysis of coding and flanking intronic of the KCNJ11 gene (NM_000525.3), ABCC8 gene (U63421and L78208), EIK2AK3 gene (AF110146.1) and INS gene (NM_000207.2) were done. Genetic tests for mutations in the gene subunits of the ATP-sensitive potassium channel, which are commonly associated with NDM, were negative. Both the twin sisters had a heterozygous missense mutation, (p.Cys109Tyr, c.326G>A) in exon 3 of the INS gene (Figure 1). This mutation results in the substitution of tyrosine for cysteine at codon 109. The mutation was not detected in the parental samples and it is therefore likely that the mutation has raised de novo (Figure 1) (Parents tested by Sanger sequencing). The twins tested using a set of microsatellite markers (powerplex 16) and results are consistent with them being monozygous. Figure 2, illustrates the monozygosity of the twin patients.

Discussion

Neonatal diabetes mellitus due to insulin gene mutation constitutes around 10-15% of all PNDM in some reports [3,9,19,20], while in others it reached up to 20% as in the ISPAD cohort compared with 4% for ABCC8 and 35% for KCNJ11 mutations [3]. Compared to K_ATP channel mutations, cases with INS mutations have a later presentation of diabetes (our twins presented at the age of 3 months) [3]. Several studies using linkage and candidate gene-based approaches have reported heterozygous missense mutations in the insulin gene.

This cysteine residue at codon 109 is highly conserved across species and the mutation abolishes a cysteine residue that is crucial for insulin folding [8] results in the formation of abnormally folded proinsulin molecules which are consequently degraded in the endoplasmic reticulum. This leads to severe endoplasmic reticulum stress followed by β cell death from apoptosis. Disulfide bonding seems to be crucial for proinsulin folding in the endoplasmic reticulum, and about 60% of the insulin gene mutations disrupt disulfide bridge formation within this protein either by substitution of a cysteine residue or by the creation of an additional cysteine [20].

Germ line mosaicism was reported involving either KCNJ11 mutation [26] or insulin gene [23,25]. Although we shared Bee et al. [25,26] same mutation but in their report the father had somatic mosaicism leading to transmission of PNDM to his children. Low level mutation was detected in the father's sequencing trace. Real-time polymerase chain reaction analysis showed that he had approximately 73% of the mutant allele relative to his affected son. This was in contrary to our report where the parent’s genetic testing was completely normal. Age of onset in our reported patients (3 months) was comparable to that in the siblings reported by Bee et al. [25] (3 and 7 months).

Clinically, patients with an insulin gene mutation have severe hyperglycemia, and ketoacidosis [3,20,27], however, presentation is variable and of, these patients, only 40% had marked hyperglycemia and about 39% had ketoacidosis [20]. The presented patients had marked hyperglycemia and no previous history of diabetic ketoacidosis. Birth weight is variable may be low or high depending on the rate of reduction of β cell in utero or in the first year of life [8]. The birth weight of our twins was normal, which is consistent with the birth weight of most patients with insulin gene mutation in the study of Colombo et al. [9].

Since INS is mostly expressed in pancreatic β-cells, the affected patients do not show any pancreatiregenerative features which are consistent with our findings. In addition to causing NDM, INS mutations may present acutely after the first 6 months of age and even beyond the age of one year, when monogenic diabetes becomes exceedingly rare [28]. Furthermore, up to 70% of the mutations are de novo, so that family history of early-onset diabetes is lacking which is in accordance with our patients. It is therefore likely that some patients with an INS mutation are clinically indistinguishable from early-onset type-1 DM. In these cases, routine measurement of pancreatic autoantibodies in young children with diabetes may be a helpful tool to identify candidates for genetic testing [29]. In our patients, both GAD-65 and ICA antibodies were negative.

Based on the study carried by Habe et al. [2] regarding the etiology of neonatal diabetes in KSA, where neither KCN11 nor ABCC8 nor INS mutation were detected, our twins are considered the first reported cases of PNDM with insulin gene mutation from Saudi Arabia.

A better understanding of the mechanisms leading to β-cell dysfunction in the patients with diabetes caused by an INS mutation will be crucial to define new treatments. As in the Akita mouse model, it may relate to the general concept of protein toxicity [30]. Strategies to increase insulin secretion by upregulating the normal allele of the INS gene may be applicable [3] and siRNA therapy for INS mutation is to be considered. Since INS mutations usually results in accumulation of a misfolded proinsulin molecule in the endoplasmic reticulum (ER), leading to ER stress and β-cell apoptosis [9], insulin is considered the only treatment currently available [31].

In summary, our case report highlighted the role of insulin gene mutation as a cause of neonatal diabetes in Saudi Arabia which was not noted as a common cause of neonatal diabetes before. To the best of our knowledge, this is the first reported case worldwide monozygotic twins presented with PNDM with INS gene mutation. The only reported monozygotic twins with PNDM are due to KCNJ11 mutation of the K-ATP channel [32].

We recommend that genetic testing for both the insulin gene as well as the channel mutations be carried out in all cases of neonatal diabetes and their parents, to identify the risk of recurrence in other siblings and meanwhile, this will help in premarital counseling in a community like Saudi Arabia with very high incidence of consanguineous marriage. It will also uncover cases which present late and misdiagnosed as type 1 DM in absence of positive autoantibodies.

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References


