

Clinical and Molecular Genetic Analysis of Iranian Neonatal Diabetic Cases Demonstrating Mutations in KCNJ11 gene

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Abstract

Introduction:

We screened the KCNJ11 gene from 35 individuals clinically diagnosed with type 1 diabetes mellitus under the age of 6 months in 3 years duration. Six different heterozygous missense mutations were found in 7 of the 35 probands, which accounted for 20% of all individuals. A novel mutation W68R (No Locus, GU170814; 2009) was identified in the kir6.2, the pore-forming subunit of the KATP channels from pancreatic β -cells.

Our results demonstrated that activating mutations in KCNJ11 gene could cause Permanent Neonatal Diabetes Mellitus (PNDM) with onset prior to six months.

Keywords:

Genetic Analysis, Mutations, Neonatal Diabetic, KCNJ11 gene.

Introduction

Neonatal diabetes mellitus (diabetes mellitus occurring prior to 6 months of age) is a rare appearance of diabetes, reportedly affecting 1 in 400,000 infants worldwide (1,2). In about 50% of the patients, the diabetes is transient (TNDM; MIM#601410), resolving within a median of 3 months, and 50% of the patients have a permanent form of diabetes (PNDM; MIM# 606176) (3). In this type of diabetes, auto antibodies are rare and human leukocyte antigen (HLA) genotyping reveals HLA haplotypes actually protective for type 1 diabetes mellitus (2). Recent

studies demonstrated that the majority of patients with neonatal diabetes mellitus were caused by a mutation in the heterozygous or homozygous state in one of possible genes (3). Mutations in the KCNJ11 (MIM# 600937), and ABCC8 (MIM# 600509) genes encoding the two protein subunits (kir6.2 and SUR1) of the ATP-sensitive potassium channel are one of the most common causes of both permanent and transient neonatal diabetes mellitus (3,4,9-12). Other genetic causes of PNDM are mutation in the insulin promoter factor-1, forehead box-P3 and glucokinase (3).

Over expression of chromosome 6q24 accounting for more than 70% of the transient neonatal diabetes mellitus, three types of abnormality involving 6q24 have been described as a cause of (TNDM).

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The beta-cell k-ATP channel consists of two essential subunits: two subunits of kir6.2, which is the pore forming unit, belongs to the inwardly rectifying potassium channel family, and 2 subunits of sulfonylurea receptor 1 (SUR1), which belongs to the ATP-binding cassette (ABC) transporter family (5).

The determination of the underlying genetic cause has led to improved treatment, and it is necessary for genetic counseling in family planning. Although insulin is necessary for initial management of all patients with neonatal diabetes, there have been several reports of successful transition from insulin to sulfonylurea agents in patients with PNDM caused by mutation in the KCNJ11 gene (6). Glyburide has been the most widely used sulfonylurea in the treatment of (NDM).

In this study, we examined the KCNJ11 gene in 28 Iranian patients with (NDM). Molecular genetic analysis of the patients revealed various heterozygous missense mutations in 7 cases of unrelated families including c.770 T>G (p.W68R) as a novel mutation, c.1169 C>A (p.R201S), c.1169 C>T (p.R201C), c.1170 G>A (R201H) and c.1247G>A (p.E227K) which has been detected in two probands.

Materials and Methods

Subjects: This study was conducted for detection of KCNJ11 mutations in neonatal diabetes mellitus in Mashhad University of Medical Sciences, Mashhad, Iran. Patients were referred by their local pediatric endocrinologist or recalled by phone if a hospital records were found. Inclusion criteria were insulin-treated diabetes diagnosed within the first 6 months. Clinical data were recorded in a questionnaire such as family history, anthropometric measurement from birth to latest visit, physical examination and neurologic finding. The signs and symptoms at presentation, blood glucose level, biochemical and immunological tests were obtained.

Genetic studies

DNA Extraction: Genomic DNA was extracted from the peripheral blood leucocytes of all 28 patients, and 100 unrelated healthy controls using standard salting out method (8). The study was approved by the Mashhad University of Medical Sciences Ethics Committee and informed consent was obtained from all subjects' parents. Moreover, the research was conducted in accordance with ethical standards outlined in the Helsinki Declaration.

PCR amplification and Sequencing of the KCNJ11 Gene: The small intronless KCNJ11 gene was amplified by PCR in three overlapping fragments of 476, 542, and 463 bp using three primer pairs as reported elsewhere (7). PCR products were cleaned-up using a DNA Gel Extraction Kit (Invitex, Berlin, Germany) and bidirectional sequencing was performed using the Applied Biosystem, ABI 3730 XL automated DNA sequencer. DNA sequences were compared to the human GenBank reference for the KCNJ11 gene (OMIM * 600937) using the sequencer sequence alignment software (version 4.10.1).

Results

Clinical presentation: The patients participating in this study were from different part of Iran including: (Khorasan, Fars, Isfahan, Kerman, Semnan province), and all of them presented with diabetes mellitus from birth to 6 months of age. The median age at diagnosis was 52 days (range 14 -128), and 54.5 percent of the patients were male. At diagnosis, the patients had severe hyperglycemia with a median blood glucose 630 mg/dl (range 280-940), and all of them were treated with insulin from the time of diagnosis. Median birth weight was 2160gr (range 1500-3700). The family history was positive for adult onset diabetes mellitus in seven cases. Brief History of 7 cases with KCNJ11gene mutations has been presented as below:

Case 1, E227K Mutation

A term female, born from a 28 year old healthy mother (G4, L4). She was born at 38 weeks of gestation with a birth weight of 2700 gr and NVD. There was no gestational diabetes and the father was not diabetic. There was third degree consanguinity in the family.

She was referred to our center at the age of 2 months because she developed respiratory distress, fever and hyperglycemia (Hi with Glucometer = over 650 mg/dl). At the time of admission she was febrile (temperature rectal =38.7° C), heart rate was 128 beats per minute, and respiratory rate was 70 breaths per minute. Her weight was 4350 gr. Initial laboratory evaluations revealed a blood glucose level of 465 mg/dl, a venous PH ed 7.169 and HCO₃ 5.3 mmol/L the Hb was 9 g/dl, and the ketones and glucose were positive in urine analysis.

Initial treatment with intravenous fluid and regular insulin, and Empiric antibiotics (vancomycine and cerftriaxone) were started. Eighteen hours following treatment the metabolic acidosis was resolved. Bacterial cultures of blood, and urine were negative, ESR was 14 mm/h, CRP was negative m and antibiotics were discounted. One week later, she was discharged on 2 units of NPH insulin at 8 AM and 1 unit of NPH insulin at 8 PM.

In the outpatients follow-up, she tested for ICA and GAD - 65 antibodies that were 0.8 IU/ml (negative<0.95), and 1.5 IU/ml (negative<5), respectively.

Molecular testing revealed the heterozygous missense mutation E227K (c.1247G>A) in KCNJ11 gene.

The parent discontinued his medication at the age of 9 months and in the last visit she was 3.75 years old with the weight of 16.5 (z-score=0.4) kg and a height of 101cm (z-score=-0.7) and of normal development. HbA_{1C} was 6.8%, Insulin level Of 3.4IU/ml (normal 2.1-22) and a FBS of 95 mg/dl. Annual blood glucose with glucometer was measured within acceptable limits.

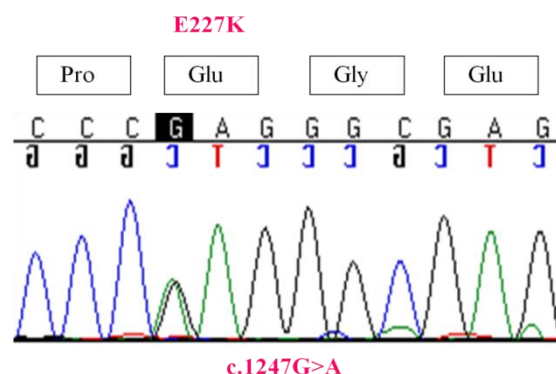


Fig 1: E227K Mutation

Case 2, E227K Mutation

A term female, born from a 33 years old healthy mother (GH, L4) with the weigh of 2700gr and normal vaginal delivery had been presented. The parent was first degree relative and family histories for diabetes were negative. At age of 2 months while she was receiving routine immunizations she developed fever and lethargy and severe respiratory distress, one attack of seizure, and was referred to our center. At the time of admission she was febrile (Rectal temperature =38.9° C), heart rate was 130 beats per minute, respiratory rate was 62 breaths per minute, and weight of 3.8 kg. At the time of admission in hospital, laboratory investigation revealed a blood glucose level of 789 mg/dl, a venous PH of 7.064 and HCO₃ 4.6 mmol/L, a BUN of 68 mg/dl, a creatinine of 1.0 mg/dl, and 2 plus sugar and ketones in urine. After an initial intravenous fluid and insulin therapy, NPH insulin was started and she was discharged on 3 units NPH insulin. Blood sample for ICA and GAD-65 antibodies were 0.4 (<0.95 negative) and 5.8 (<5negative) respectively. Subsequent testing for the KCNJ11gene demonstrated the same mutation E227K (c.1247G>A), similar to case 1, in KCNJ11 gene.

At the age of 4 months, we attempted to convert her treatment from insulin to Glibenclamide, glyburide which was initiated as a dose 0.2 mg/kg/day. At age 7 months, she had blood glucose 134 mg/dl, HbA_{1C} 5.8 % and insulin level 13.2 IU/ml

(2.1-22), and a C-peptide 0.8 ng/ml (0.7-109). Annual blood Glucose with Glucometer was acceptable and in the latest visit at the age of 20-months she was a well developed and well nourished (W z score=0.9 H z score=1.2) and HbA1C was 5.1%.

Case 3, W68R Mutation

A term male, born from an addicted mother (G6, D2), weighing 3250 gr and normal vaginal delivery. There was no known consanguinity in the family. Family history was notable for a maternal grandfather and aunt of adult onset diabetes. Socioeconomic status of parent was very low.

He was referred at age 1month old to our center because of fever, severe hyperglycemia, and metabolic acidosis.

One week later, the patient was discharged on 2 units of NPH insulin. He had a second admission at age of 3months, because of poor control and an unreliable parent. In this admission, the laboratory study revealed blood glucose of 975 mg/dl, a creatinine of 0.9 mg/dl, a urea level of 64 mg/dl, and a PH of 7.25. After initial intravenous fluid and regular insulin treatment, he was discharged with 3 units of NPH insulin in the morning and 1.5 units of NPH insulin in the evening. Thereafter the parent discontinued follow-up and we lost track of the case because there was no clear home address or contact details. By directly sequencing of KCNJ11 gene, a novel missense mutation identified.

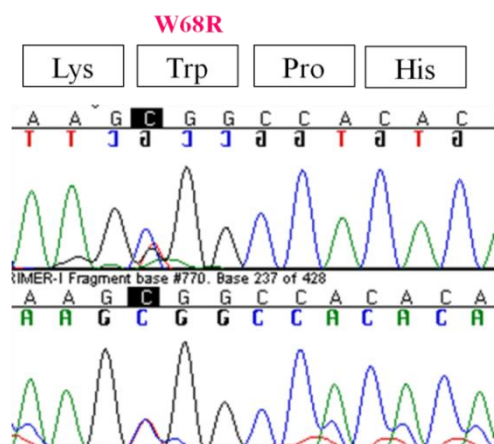


Fig 2: W68R Mutation

T to C transition mutation at bp 770 (c.770T>C) that resulted in Arginine to Tryptophan substitution at residue 68 (W68R) (GenBank accession number_GU170814; 2009) for KCNJ11 gene. PCR sequencing demonstrated that this novel mutation was not found in 100 healthy controls, which suggest that the variation is not a single nucleotide polymorphism (SNP).

Case 4, R201S mutation

A term male that referred to our center for genetic study from Semnan province. The parent was first degree relatives and positive history of adult onset of diabetes in the family. He was diabetic from 2 months of age and received 4 units of NPH insulin per day. Mutational analysis for the KCNJ11 gene of this case revealed R201S (c.1169C>A).

According to the genetic study, we recommended a change to treatment glyburide. In the last visit he was 3 years old and a body weight of 10.300 kg (Z-Score=-3.8), a height of 88 cm (z-Score=-2.5), HbA1C of 7.8 % and an average blood glucose of 122 mg/dl. He received 2.5 mg glyburide BID.

Case 5, R201C Mutation

A 30 day old infant was admitted with a two days history of diarrhea, fever and respiratory distress with the impression of sepsis. Her initial assessment revealed tachypnea, subcostal & subxiphoid retraction, mild abdominal distention without noticeable organomegally and relatively severe dehydration. The patient was the second child of the family born by cesarean section with a birth weight of 2900gr and height of 47cm, from relative parents (cousin).

Sepsis workup was performed and the patient was treated with ampicillin and cefotaxime. In the primary investigations, the high serum glucose level was noted. The results of investigation were as below BS=1192 mg/dL, VBG: PH=7, Pco2= 14.1, BE=

-26.1, HCO₃⁻= 3.5, Urine analysis: Acetone 2+, glucose 2+, Na=154mmol/L, K= 6.8mmol/L, Ca⁺⁺= 4.8 mg/dL, Urea= 81 mg/dL, Cr=2mg/dL, and normal CBC.

Admission vital signs were as pulse rate 190, respiratory rate of 60, auxiliary temperature of 38°C, and blood pressure of 50/pulse.

The patient was treated for DKA in the ICU, and the diabetic ketoacidosis resolved over 11 hours. Blood, urine and CSF cultures yielded negative findings. The patient was discharged in a good general condition, with serum blood glucose of 200 mg/dL and the insulin regimen of 4 units NPH in the morning and 2 units at night. Her parents were advised to refer to a well-equipped molecular genetics investigation center.

On the follow-up, the patient's general condition was good and had an appropriate weight gain. Mutational analysis for the KCNJ11 gene of this case revealed a C to T transition at bp 1169 (c.1169C>T) in heterozygous state, leading to the substitution of arginin by cystine at codon 201, R201C.

Case 6, R201H

A preterm male with a birth weight of 2400 gr that was diagnosed at the age of 2 months was referred to us from Zabol, eastern part of Iran, for genetic investigation. He received 8 unites NPH insulin daily from 2 to 18 months. Laboratory investigation revealed HbA_{1c}=6.5%, ICA of 0.4 IU/MI (<0.95 negative), GAD-65 of 23 IU/MI(>5 positive). At this time, the treatment switched to glibenclamide. In last visit, he was a 3.5 years old and had a weight of 13kg (z-score = -1.5), height of 95 cm (z-score=-0.9), and there was no sign or symptom of developmental delay.

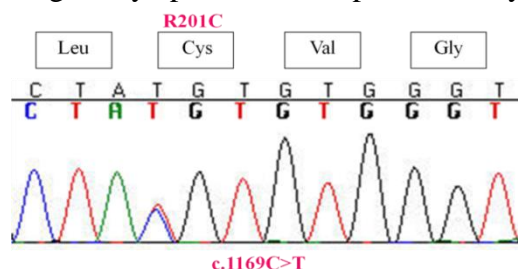


Fig 3: R201H

Case 7, A174G

A term male born from a mother with gestational diabetes (G₂, L₂). He was born at 38 weeks of gestation with a birth weight of 3100 gr and normal vaginal delivery. He was referred to our center at the age of 5 months since he developed dehydration, gastroenteritis, and hyperglycemia. At the time of admission, he was febrile (rectal temperature =38.8 °C), heart rate was 125 beats per minute, and respiratory rate was 40 breaths per minute. Her weight was 6500 gr and an initial laboratory study revealed a blood glucose level of 445 mg/dl, a venous PH ed 7.22, HCO₃⁻ 15.2 m mol/L, Hb 9.7 g/dL, and HbA_{1c} 15.1%. Anti TTG was 3.3 u/ml (<12=negative), thyroid function tests were normal, ICA antibodies were 0.84 IU/mL (negative <0.95), and GAD-65 were 1.4 IU/mL (negative <5).

Initial treatment with intravenous fluid and regular insulin were started and following 5 days after he was discharged on 3 units of NPH Insulin daily.

The parent discontinued her follow-up and changed treatment to glibenclamide which was impossible since there were no reliable contact details.

Discussion

Onset of classic autoimmune Type 1 diabetes before the age of 6 months is most unusual and most cases of diabetes in this age are caused by genetic mutations. (13) In the present study, we evaluated the genetic basis of diabetes in infants with diabetes mellitus for mutations in KCNJ11 which is the most common cause and mutation of neonatal diabetes. (4) The analysis of these genes in the Iranian population with neonatal diabetes shows that 20% our patients have an identified alteration at KCNJ11 gene. Stay and coworker's reported that of 43.8% of this mutation occurs in the US (4).

Patients with kir 6.2 mutations may present with different clinical features from diabetes diagnosed in the infancy or

later in life (11). In our cases at the time of diagnosis, pancreatic auto antibodies were negative in 3 of our cases, but in patients with long-standing diabetes caused by KCNJ11 mutations may be changed by KCNJ11 mutations and may be changed to positive due to beta cells dysfunction and increased apoptosis (14).

One case in this study that referred to us at the age of 18 month has a GAD-65 antibody 23 IU/mL (>positive).

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