Case Report

Missense mutations in the pancreatic beta-cell ATP-sensitive potassium channel Kir6.2: A case study of Pakistani patient of neonatal diabetes

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ABSTRACT

This case study describes clinical and molecular genetic data of a 45 days old male patient of neonatal diabetes mellitus. PCR amplification followed by DNA sequencing revealed two point mutations at positions 67A>G and 1009G>A in *KCNJ11* gene encoding Kir6.2 protein, a component of the beta-cell ATP-sensitive potassium (KATP) channel which is a key component involved in insulin secretion.

KEY WORDS: Single Nucleotide Polymorphism; Nonsynonymous mutation; Genetic variation.

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INTRODUCTION

Neonatal diabetes mellitus (NDM) is a rare condition with an estimated incidence of 1:400,000 and 1:500,000 live births in England and Germany respectively.[1,2] It is defined as persistent hyperglycemia occurring within the first 6 months of life, lasting for more than two weeks.[3] Patients diagnosed with diabetes in the first 6 months of life are more likely to have monogenic neonatal diabetes rather than type 1 diabetes which is of autoimmune origin.[4]

There are two main types of NDM, i.e. transient (TNDM) or permanent (PNDM).[5] The TNDM is a clinically defined group affecting approximately 50% of children with NDM. It remits in infancy or early childhood. Relapse in childhood or adolescence occurs in up to 50% of cases.[6]

The PNDM is a group affecting 40-50% cases with no remission.[7] Defining genetic etiology of this rare condition has not only given insights into clinical classification and disease mechanism, but has also influenced treatment.[8] Mutations of the *KCNJ11* gene have been identified as the most common genetic etiology in patients with PNDM.[9] The *KCNJ11* gene (present on chromosome 11) is comprised of 3409 base pairs and has a single exon flanked by two introns.[10] There are 5 splice variants of this gene (ENSEMBL ID: ENSG00000187486).

Identification of genetic polymorphism in *KCNJ11* gene is important in the diagnosis of

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NDM.[11] Mutations in *KCNJ11* and *ABCC8* genes which encode the Kir6.2 and SUR1 subunits of the pancreatic ATP-sensitive potassium channel respectively have been implicated in the genesis of PNDM.[9]

Here, we report a case of 45 days old male child having neonatal diabetes mellitus. Genetic studies revealed two point mutations in *KCNJ11* gene of the patient.

CASE REPORT

Forty five days old child presented with generalized seizures for four days with no history of fever, vomiting or diarrhea. He was the first child to his mother, born through normal vaginal delivery by traditional birth attendant without any complications in the Khyber Pakhtoonkhwa province of Pakistan. Birth weight was not recorded. The parents were having non-consanguineous marriage and there was no family history of diabetes. The newborn was well before he presented with fits to a general practitioner. There was no previous history of seizures.

At the time of presentation his weight was 3.8 kg. Fits were controlled by giving Inj Valium (0.3 mg/kg IV stat) and Inj Phenobarbitone 15 mg/kg IV stat. On investigations, his random blood sugar was found to be 530 mg/dl with no evidence of ketones on urine analysis. Blood sugar was brought down to 230 mg/dl by giving repeated boluses of regular insulin. Blood sample of the subject was collected for molecular genetic analysis to detect genetic variation. He was discharged home on one unit of regular insulin once a day. The patient then lost to follow up for a month, and had severe gastroenteritis which ultimately lead to his death as he remained untreated for circumstantial reasons.

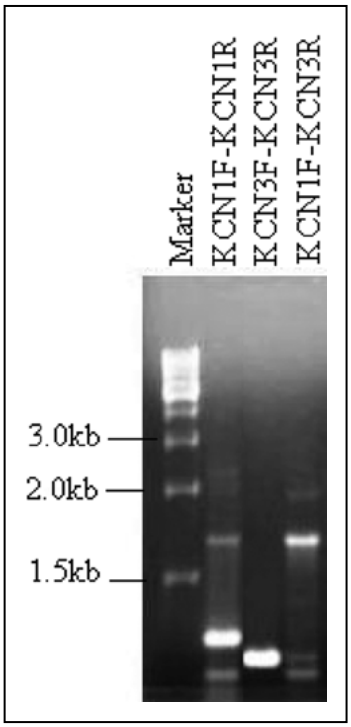

Fig.1: DNA electrophoresis of PCR products of amplified KCNJ11 DNA. Lane 'Marker': 1kb DNA ladder. Lanes 'KCN1F-KCN1R', 'KCN3F-KCN3R' and 'KCN1F-KCN3R' represent PCR products generated by these primer pairs. Details of primer are given in Table-I.

Table-I: Names and sequences of the primers used for the PCR amplification of the KCNJ11 gene.

No.	*Primer name*	*Sequence*
1	KCN1F	GTGCCCACCGAGAGGACT
2	KCN1R	GAGCCCCACGATGTTCTG
3	KCN3F	CTACCATGTCATTGATGC
4	KCN3R	CCACATGGTCCGTGTGTA

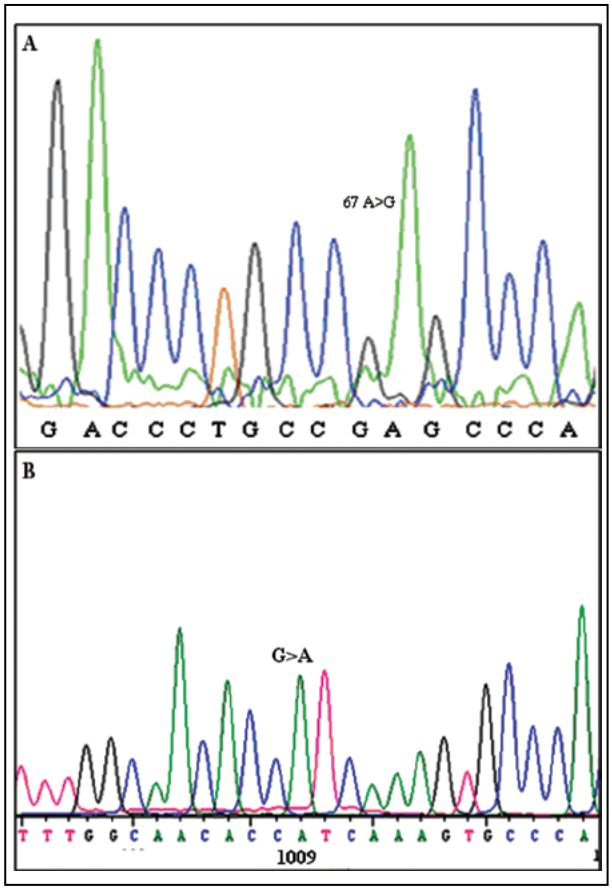

Fig.2: DNA sequencing trace of KCNJ11 gene of showing mutation at (A) positions 67A>G and (B) 1009 G>A. The genomic DNA of the subject was isolated followed by PCR, sequencing and bioinformatics analysis.

To characterize the *KCNJ11* gene polymorphism, the genomic DNA of the patient was purified from the whole blood followed by DNA amplification by PCR using primers mentioned in Table-I. The PCR was performed using 0.5 µM primers, 100 ng template DNA, 2.5 µl of high fidelity DNA polymerase mix (Fermentas Inc. Lithuania) and 0.2mM of dNTPs. The PCR conditions were as follows; initial denaturation at 94°C for 2 min, denaturation at 94°C for 45 sec, annealing at 51°C for 45 sec and extention at 68°C for 2 min (35 cycles). The primer pair KCN1F-KCN3R was used to amplify 1.8 kb region while other two primer pairs (Table-I and Fig.1) were used for internal amplification. The primer pairs KCN1F-KCN1R and KCN3F-KCN3R produced PCR products of ~500 bp while primer pair KCN1F-KCN3R produced an amplicon of 1.8 kb. The obtained amplicons were subjected to DNA sequencing using the same primers. Autamated DNA sequencing was done by Genetic Analysis System CEQ 8000 (Beckman Coulter Inc. USA). The obtained sequence data was analyzed using Lasergene software package (DNA Star Inc., USA), Staden R[12] and Clustal W.[13]

Fig.1 shows amplified band of *KCNJ11* gene (1.8 kilo bases). DNA sequencing was performed by using automated Genetic Analysis system (Beckman Coulter Inc. USA). Analysis of sequencing results revealed single nucleotide polymorphism at two positions i.e. 67A>G and 1009G>A.

DISCUSSION

The case we are reporting is not a very commonly encountered clinical condition. These patients usually present with hyperglycemia, failure to thrive or Diabetic Ketoacidosis (DKA) due to inadequate insulin production.[14] As defined in NDM, marked hyperglycemia in the first month of life, no ketosis despite marked hyperglycemia made this provisional diagnosis possible in our patient. Because the anthropometry was not recorded at birth, we cannot comment whether the baby was of normal birth weight or Small for Gestational Age as usually reported in such cases.

Studies have shown that approximately one-third to one-half of all cases of PNDM are due to mutations in *KCNJ11*.[15] Along with mutations in *KCNJ11*, the genes *GCK* and *GLUT2* are also related to NDM. The *GCK* is a glycolytic enzyme that acts as a glucose sensor in pancreatic β-cells and plays important role in the regulation of insulin secretion while *GLUT2* encodes glucose-transporter protein.[11] The *KCNJ11* gene encodes for Kir6.2 protein, a component of the beta-cell ATP-sensitive potassium (KATP) channel which is a key component involved in insulin secretion.[11]

The non-synonymous SNPs at two positions i.e. 67A>G and 1009G>A detected in this case would result in amino acid substitutions Glu23Lys and Ile337Val (Fig. 2 and 3). Activating mutations in *KCNJ11* cause permanent neonatal diabetes due to overactive Potassium ATP channels, resulting in reduced insulin secretion.[9]

Identification of a *KCNJ11* mutation has implications on clinical management. Many patients can be successfully treated with oral sulphonylureas rather than insulin.[16] Sulphonylureas have affinity to SUR1 and stimulate insulin secretion by an ATP-independent mechanism and can successfully replace insulin therapy to achieve a better metabolic control.[9] The effectiveness of oral sulfonylurea in improving glyceamic control of diabetic patients

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KCNJ11_Gene_from_emboss.seq     1 ATGCTGTCCCGCAAGGGCATCATCCCCGAGGAATACGTGCTGACACGCCT 50
KCNJ11_Gene_from_patient          ATGCTGTCCCGCAAGGGCATCATCCCCGAGGAATACGTGCTGACACGCCT
                                  **************************************************
                                                    67
KCNJ11_Gene_from_emboss.seq    51 GGCAGAGGACCCTGCCAAGCCCAGGTACCGTGCCCGCCAGCGGAGGGCCC 100
KCNJ11_Gene_from_patient          GGCAGAGGACCCTGCCGAGCCCAGGTACCGTGCCCGCCAGCGGAGGGCCC
                                  *************** **********************************



KCNJ11_Gene_from_emboss.seq   951 CATTGTAGCTGAGGAGGACGGACGTTACTCTGTGGACTACTCCAAGTTTG 1000
KCNJ11_Gene_from_patient          CATTGTAGCTGAGGAGGACGGACGTTACTCTGTGGACTACTCCAAGTTTG
                                  **************************************************
                                             1009
KCNJ11_Gene_from_emboss.seq  1001 GCAACACCGTCAAAGTGCCCACACCACTCTGCACGGCCCGCCAGCTTGAT 1050
KCNJ11_Gene_from_patient          GCAACACCATCAAAGTGCCCACACCACTCTGCACGGCCCGCCAGCTTGAT
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Fig.3: The pairwise alignment of the normal human KCNJ11 gene sequence (retrieved from Genbank database) with the sequence of the same gene from the subject under study. Two mutations at position 67 and 1009 are highlighted.

due to *KCNJ11* mutations after transfer from insulin therapy has been confirmed by many reports[16] but a molecular diagnosis is required before the use of sulfonylurea therapy in neonatal diabetes is considered.[17]

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