

A Novel Mutation *KCNJ11* R136C Caused *KCNJ11*-MODY

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Research Article

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Abstract

A young female patient, diagnosed with diabetes mellitus at the age of 28 years old in 2009, carries *KCNJ11* R136C by whole-exome sequencing and her daughter doesn't carry this mutation. Bioinformatics software predicted that the 136th amino acid is highly conservative and deleterious. And *KCNJ11* R136C can result in the change of channel port structure of K_{ATP} channel. So she was diagnosed as *KCNJ11*-MODY.

1. Introduction

Maturity-onset diabetes of the young (MODY) is a group of clinically heterogeneous and hereditary diabetes, caused by a single-gene mutation involving in the development and function of β -cells.^[1] According to the involved genes and clinical phenotypes, 14 subtypes of MODY have been reported.^[2] *KCNJ11*-MODY (known as MODY13) is an autosomal dominant diabetes caused by *KCNJ11* gene mutations, which codes inward rectifying potassium channel (Kir)6.2. Four Kir6.2 subunits and four sulphonylurea receptor 1 (SUR1) subunits constitute K_{ATP} channel. K_{ATP} channel plays an important role in insulin secretion. We report a young female patient was misdiagnosed with type 2 diabetes and carried *KCNJ11* p.R136C (c. 406C>T), a new mutation causing *KCNJ11*-MODY.

2. Patient And Methods

2.1 Subjects

The informed consent was obtained from the participants (or the child, from her parents). The study complied with Declaration of Helsinki. Regrettably, the proband is an orphan and blood samples of her parents can't be obtained.

In 2009, the proband of 28 years old was diagnosed with diabetes mellitus during pregnancy. Although she received insulin treatment, blood glucose was controlled poor. Caesarean section was performed at 38 weeks of gestation because of a large fetus and the newborn weighed 5500mg. She had taken metformin 500mg tid and acarbose 50mg tid since 2011. Fasting blood glucose (FBG) was about 12mmol/L and postprandial 2h blood glucose was 16mmol/L or so. She was admitted to our department in April 2017. Her height, weight, waist circumference and BMI were 172cm, 73kg, 95cm and 24.68kg/m², respectively. Glycosylated Hemoglobin was 10.3% and Anti-glutamic acid decarboxylase, islet-cell, and insulin antibody were all negative. When FBG was 13.06mmol/L, the values of fasting C-peptide and insulin were 1.49(0.8–3.85)ng/ml and 15.06mIU/L(11.7–17.3). She was diagnosed as type 2 diabetes, and 52IU of glargine was added. FBG still fluctuated above 10mmol/L. In September 2020, retinal examinations by an ophthalmologist, urinary albumin/creatinine and electrophysiological testing of peripheral neuropathy were assessed to be normal.

2.2 Mutation detection

5ml venous blood was taken from the subjects, and genomic DNA was extracted by standard process (MagPure Buffy Coat DNA Midi KF Kit). Whole-exome sequencing and high throughput genotyping was carried. Sanger sequencing verified all discovered suspicious pathogenic mutations.

2.3 Functional prediction

PhyloP and GERP++_RS softwares were used to analyze the conservation of the mutation site. Condel and Mutation-taster software can predict the pathogenicity of the missense mutation. The effect of the mutation on protein function was evaluated by SIFT and PolyPhen2 software.

3. Results

3.1 Mutation analysis

The whole-exome sequencing of the proband showed that she carried *KCNJ11* c.406C > T(p.R136C). Her daughter didn't carry this mutation by Sanger Sequencing.(see Fig. 1)

3.2 Search for R136C mutation of *KCNJ11* gene

Referring to 1000 Genomes database, ESP6500si_all database, ExAC database and Genome Aggregation Database(GnomAD), the frequency of this mutation in public population databases is extremely low (ESP6500:-, 1000 Genomes:-, EXAC: 0.000008, GnomAD: 0.000004).

3.3 Conservative property of R136C mutation in *KCNJ11* gene

The mutation site of *KCNJ11* R136C was analyzed for conservative property among multiple species: PhyloP: conservative, GERP++_RS: conservative. In all 28 different species, the 136th amino acids of Kir 6.2 subunit are arginine, which is highly conserved (see Fig. 2) and suggests the functional importance of the residue.

3.4 Prediction of pathogenicity of *KCNJ11* R136C

According to SIFT:D, Condel:deleterious, MutationTaster:D and Polyphen2:D, this mutation can result in changes in protein function. The results of SIFT and Polyphen-2 software showed that the score of SIFT was 0 and that of Polyphen-2 was 1.

3.5 Structure prediction of Kir6.2 subunit and K_{ATP} channel carrying *KCNJ11* R136C

KCNJ11 c.406C > T(p.Arg136Cys) changed the 136th amino acid from hydrophilic basic amino acid arginine with a positive charge to hydrophilic neutral amino acid cysteine, which can change the charge distribution of the channel port. The three-dimensional structure of wild-type K_{ATP} channel shows that Arg136 is located at the port of the channel. R136 is located in a β turn, so R136C may result in the change

of β turn and further change the tertiary structure of pore portal. The real analytical structure of Kir6.2 subunit and K_{ATP} channel carrying *KCNJ11* R136C predicted by computer software is shown in Fig. 3.

4. Discussion

This paper reports a young female patient who carried *KCNJ11* R136C mutation. She was considered as *KCNJ11*-MODY and prescribed by metformin, acarbose and glimepiride. Blood sugar control improved significantly.

KCNJ11-MODY is an autosomal dominant diabetes mellitus caused by mutations in *KCNJ11* gene, first reported by Bonnefond *et al.* in 2012. *KCNJ11* gene is located at 11p15.1, contains only one exon and encodes Kir6.2 subunit. Kir6.2 contains the binding sites of ATP and phosphatidylinositol 4,5-diphosphate, which can inhibit and activate the channel, respectively.^[3, 4] K_{ATP} channel on pancreatic β cells couples energy metabolism and electrical activity and plays an important role in the process of insulin secretion.^[5] Under sub-stimulus glucose concentration, the membrane potential of β cell is affected by the conductance of the K_{ATP} channel, maintaining the cell member potential at a hyperpolarized level. When blood glucose rises, glucose is quickly taken up and metabolized into ATP. ATP binds to K_{ATP} channel, closing the channel, depolarizing cell membrane, opening voltage-gated calcium channels, calcium ion influx and triggering insulin vesicles release.

Mutations in *KCNJ11* gene affect the activity of K_{ATP} channels, causing abnormal insulin secretion in pancreatic β -cells. Activating mutation can cause a decrease in the affinity of ATP to the channel in pancreatic β cells. K_{ATP} channel can't be closed normally under the stimulation of glucose and cell membrane continues to be in a hyperpolarized state. Extracellular Ca^{2+} can't inflow and insulin can't be secreted normally, which leads to a series of continuous and varying degrees of glucose metabolism abnormalities, including neonatal diabetes mellitus, impaired fasting glucose, impaired glucose tolerance and *KCNJ11*-MODY. Inactivation mutation in *KCNJ11* gene can lead to continuous closure of K_{ATP} channel, continuous depolarization of β cell membrane, continuous inflow of extracellular Ca^{2+} , excessive secretion and release of insulin, resulting in congenital hyperinsulinism hypoglycemia.

Bonnefond *et al.* ^[6] reported among a four-generation family of thirty-seven members, twelve members carried *KCNJ11* E227K mutation: three members (aged 11–40 years old) with normal glucose metabolism, and nine members with abnormal glucose metabolism. They were diagnosed with diabetes at the age of 13–59 years old. This mutation can cause a decrease in the sensitivity of ATP to K_{ATP} channel. Before that, there were two reports about *KCNJ11* mutation causing diabetes. Some patients might also be diagnosed as *KCNJ11*-MODY. Four members in a three-generation Japanese family carried *KCNJ11* C42R and were diagnosed as diabetes, three cases of which were 3, 22 and 26 years old. Functional identification showed that the mutation channel is less sensitive to ATP.^[7] An Italian family carrying *KCNJ11* c.679G > C and c.680A > T(p.E227L) was reported, two of which can be considered as *KCNJ11*-MODY.^[8]

Ang *et al.*^[9], Ren *et al.*^[10], Li *et al.*^[11] and He *et al.*^[12] reported MODY13 family trees in Chinese, *KCNJ11* c.392T>C (p.I137T) c.679G>A>E227K c.602G>A>p.R201H and c.142A>G>p.N48D but none of mutation channels have been identified functionally. Liu *et al.*^[13] reported three new *KCNJ11* heterozygous mutations in three MODY diabetic families: two activating mutations R27H and R192H, one inactivating mutation S116F117del. In vitro studies showed that the sensitivity of K_{ATP} channel to ATP carrying R27H or R129H is significantly reduced. The authors also pointed out that *KCNJ11* mutation was measured in 3.2% of 96 Chinese families with early-onset type 2 diabetes mellitus.

Most of the reported *KCNJ11*-MODY patients had successfully converted from insulin to sulfonylureas, which can not only improve blood glucose, reduce medical costs, but also improve the quality of life. The key point lies in the accurate screening and effective identification of *KCNJ11*-MODY.

It was reported that the mutation of *KCNJ11* R136 to other amino acids, such as Arg136His, Arg136Leu, can cause congenital hyperinsulinemia,^[14, 15] and the authors didn't conduct functional studies on related mutations. More surprisingly, Bellanné-Chantelot *et al.*^[16] reported that an infant with congenital hyperinsulinemia carried *KCNJ11* R136C mutation. Park *et al.*^[17] also reported a Korean infant with congenital hyperinsulinemia carried Arg136Cys and Ala187Val compound heterozygous mutations, but the authors didn't describe the above two cases in detail. The function of the mutation channel wasn't been studied. The same mutation in the same site of the same gene cause contrary clinical phenotype, which also reflects the clinical heterogeneity of *KCNJ11* gene mutation.

Further studies are required to carry out the functional identification and related research of *KCNJ11* R136C to gain a deeper understanding of the clinical heterogeneity.

Declarations

Disclosure

Yaning Chen, Xiaodong Hu, Jia Cui, Mingwei Zhao, and Hebin Yao declares no conflict of interest.

Ethics approval

This research was approved by 1EC, the sixth medical center of PLA General Hospital(Former Navy General Hospital). Permitted NO: HZKY-PJ-2021-20.

Data availability statements

All data generated during this study are included in this published article.

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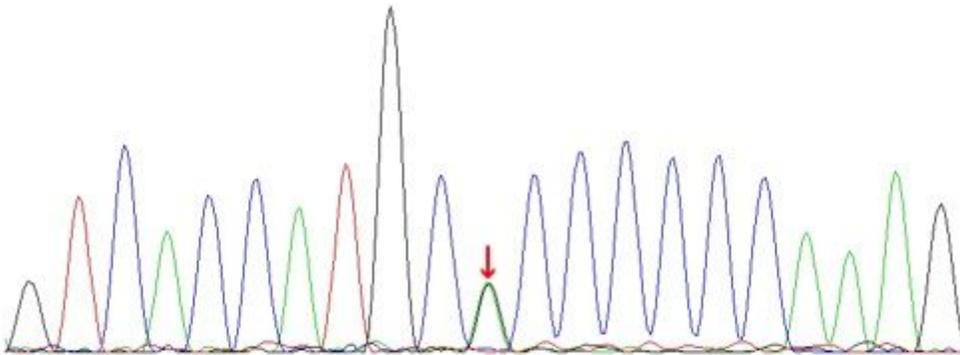
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Figures

Mother

C12_S052613_17C009553_KCNJ11-chr11-17409233_F229-B7_F.ab1

G T C A C C A T G ²⁰⁰ C A C C C C C C A A ³⁰⁰ A G



Daughter

A09_SG0922_19C026407_KCNJ11-chr11-17409233_F812-J7_F.ab1

G T C A ¹¹⁰ C C A T G C G C C C ¹²⁰ C C A A A G

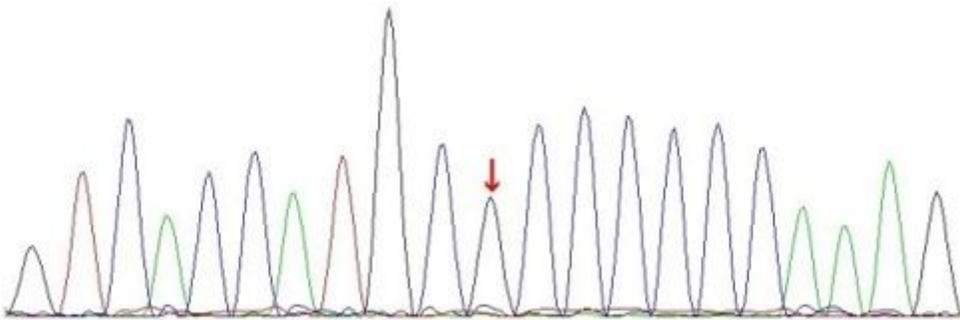


Figure 1

The genetic test report of the proband(mother) and her daughter

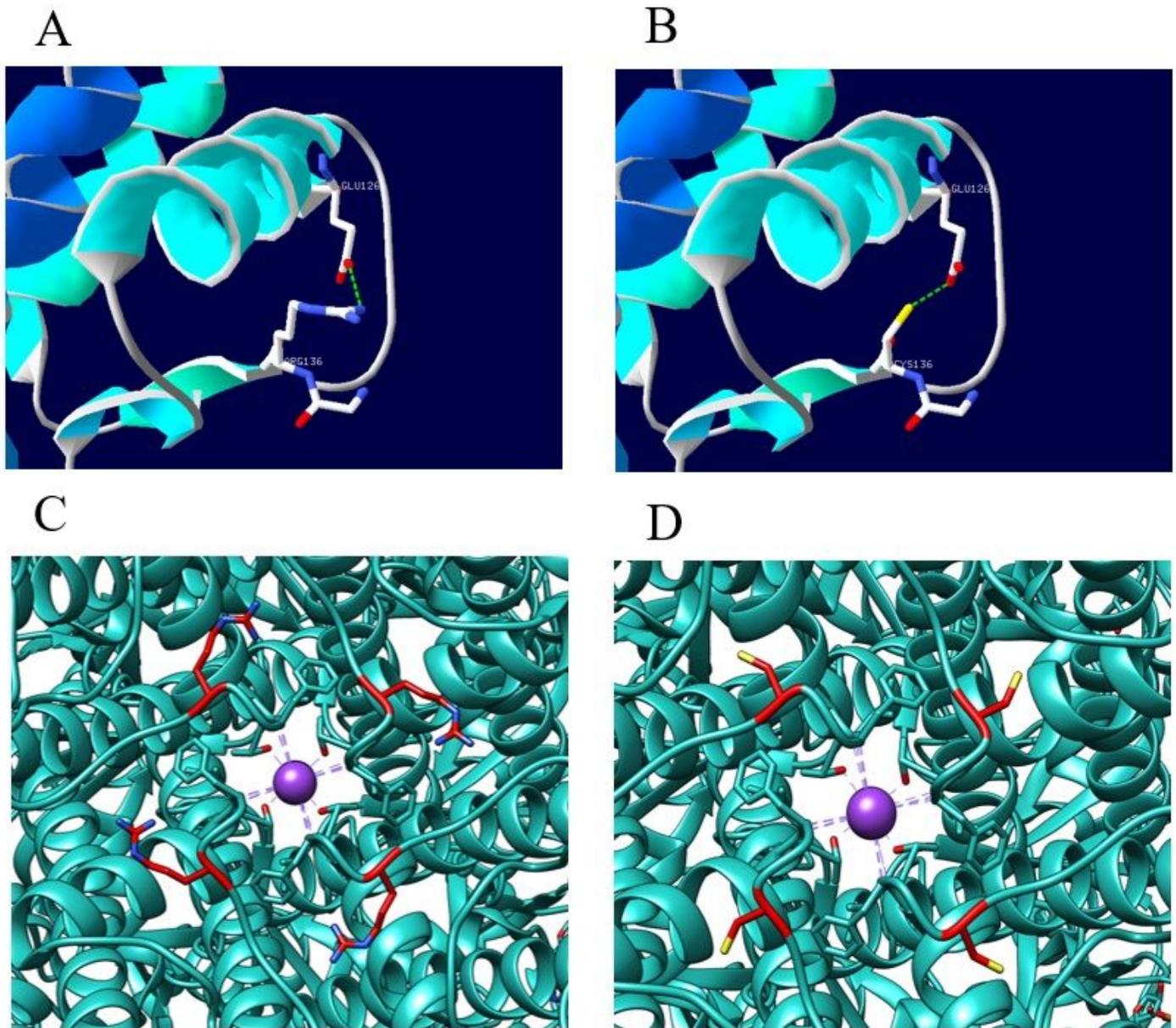


Figure 3

The real analytical structure of wide-type Kir6.2 subunit and KATP channel and Kir6.2 subunit and KATP channel carrying KCNJ11 R136C predicted by computer software. (A) A hydrogen bond is formed between R136 and E126 in wide-type Kir6.2. (B) The hydrogen bond still exists between R136C and E126 in Kir6.2 subunit carrying KCNJ11 R136C. And the real analytical structure predicted by computer software changes, marked by color yellow. (C) In wide-type KATP channel, color blue in the middle is potassium ions, which is in the channel port. (D) In KATP channel carrying KCNJ11 R136C, the amino acid changes from hydrophilic basic amino acid with a positive charge to hydrophilic neutral amino acid, which can change the charge distribution of the channel port and thus affect the function of KATP channel.