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A Dominantly Inherited KCNJ11 Q235E Mutation Leading to Diazoxide-Unresponsive Congenital Hyperinsulinism in a Chinese Child

Yanmei Sang*, Zidi Xu, Jie Yan and Min Liu

National Key Discipline of Pediatrics (Capital Medical University), Ministry of Education, Department of Endocrinology, Beijing Children's Hospital, Capital Medical University, Beijing, China

Abstract

The ATP-sensitive Potassium (K^+) channel (K_{ATP}) controls insulin secretion from the pancreatic islet cells. Genetic mutations causing loss of function in potassium channel subunits are an underlying cause of human congenital hyperinsulinism (CHI). To date, more than twenty KCNJ11 mutations have been revealed, most of which are recessively inherited and refractory to diazoxide treatment. Several dominantly inherited KCNJ11 mutations have been reported recently, all of which are responsive to diazoxide treatment. In this study, we sequenced the KCNJ11 gene in both a Chinese boy diagnosed with congenital hyperinsulinism and in his parents. A dominantly inherited heterozygous missense 703 C > G [p, Q235E] mutation was identified in the patient and in his father. The patient was refractory to diazoxide treatment. This is the first report of a dominantly inherited Q235E KCNJ11 mutation leading to the onset of diazoxide-unresponsive K_{ATP}-CHI.

Keywords: Congenital hyperinsulinism; KCNJ11; Diazoxide; KATP channel

Introduction

Congenital Hyperinsulinism (CHI) manifests as the inappropriate secretion of insulin by the pancreatic beta-cells secondary to various genetic disorders [1]. At least nine genes have been found to be related to the genetic mechanism of CHI, leading to 8 genetic types of CHI [2-4]. K_{ATP}-CHI, which is caused by ABCC8 and KCNJ11 mutations, is the most common type of CHI. Most of the ABCC8 and KCNJ11 mutations are recessively inherited, while a minority of the ABCC8 and KCNJ11 mutations are dominantly inherited. Recently, a few cases have been reported in which mutations of the K_{ATP} channel are associated with dominantly inherited hyperinsulinism. Children with dominant K_{ATP} hyperinsulinism mutations seem to have a milder hypoglycemia phenotype than that seen in children with hyperinsulinism resulting from recessive K_{ATP} mutations [5]. Diazoxide is the first line of treatment for patients with CHI [6]. Most of the recessively inherited ABCC8 and KCNJ11 mutations are refractory to diazoxide. During the dominantly inherited mutations, the majority of the ABCC8 mutations and all the KCNJ11 mutations found so far are responsive to diazoxide [7,8]. In this study, we sequenced the KCNJ11 gene of a patient with CHI and, for the first time, reported a dominantly inherited Q235E mutation which led to the onset of diazoxide-unresponsive K_{ATP}-HI.

Patient Report

Clinical data

A boy with congenital hyperinsulinism was chosen as the research subject. The patient's birth weight was 4.2 kg (macrosomia) and CHI began at the age of 1.5 months. When the patient was diagnosed with CHI, his blood glucose was 1.7 mmol/L, his insulin level was 17.4 U/L, his C-peptide was 3.3 ng/ml, and his β-hydroxybutyric acid was 0.04 mmol/L. His blood ammonia level was normal. Results of urine screening and tandem mass spectrometry were normal. The parents of the patient are non-consanguineous and there is no family history of diabetes mellitus or hypoglycemia. After being hospitalized, the patient was diagnosed with HI and a trial of diazoxide was administered for 10 days. The initial dosage of diazoxide was 5 mg/kg/day and the dose was increased gradually according to the results of blood glucose monitor-

ing. At a diazoxide dose of 15 mg/kg/day, the patient still suffered from obvious hypoglycemia, indicating that he was refractory to treatment with diazoxide.

KCNJ11 gene analysis

Informed consent was obtained from the patient's parents on his behalf. Genomic DNA was extracted from peripheral leukocytes using standard procedures. The primers were designed by the primer 5.0 software according to the KCNJ11 gene sequence. The patient was screened for untranslated regions and for mutations in the 5′ exon of the KCNJ11 gene. PCR was performed using a MultiGene TM Gradient Thermal Cycler (Labnet, Edison, Middlesex, NJ, USA). Reactions were performed in a total volume of 50 μL containing 0.5 ng of genomic DNA, 1 μM of each primer, 0.2 mM dNTPs, 2 mM MgCl2, 5x GoTaq buffer 10 μL and 1.25 U Platinum Taq DNA Polymerase (GoTaq® Hot Start Polymerase, Promega, WI, USA). Following a 5-min denaturation step at 94°C, samples were processed through 38 cycles of denaturation (94°C for 30 s), annealing (62-64°C for 30s), and elongation (72°C for 45s). After 38 cycles were completed, a 72°C elongation for 7 min was performed. PCR products were purified and sent to Invitrogen (Beijing, China) for direct sequence analysis (DNA Analyzer 3730, ABI, USA).
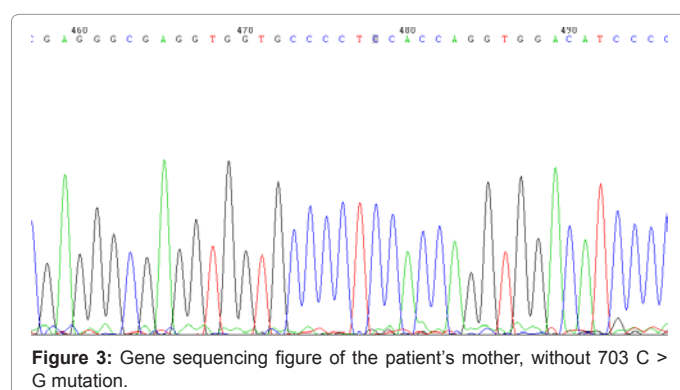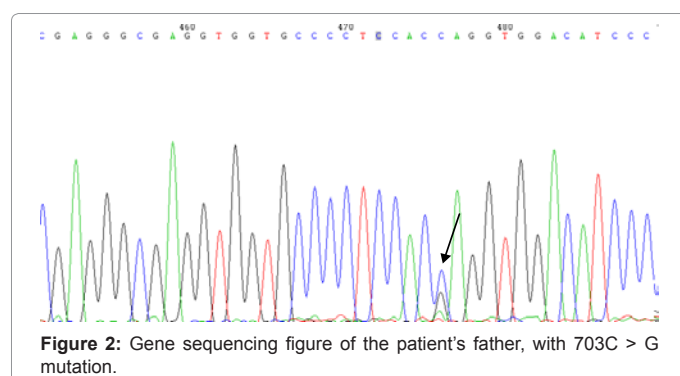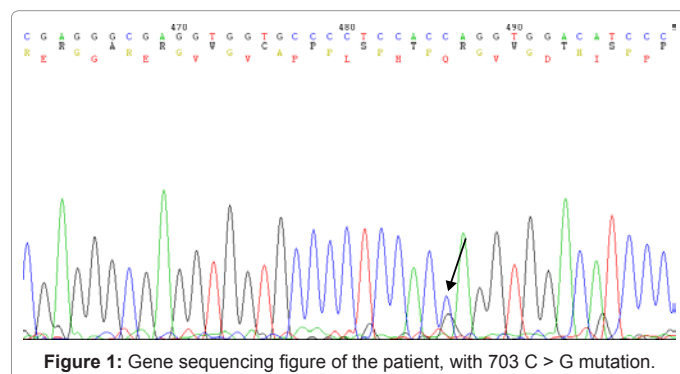
KCNJ11 gene mutation analysis results

Gene mutation analysis revealed a heterozygous inactivating Q235E (703C > G) mutation in the exon of the KCNJ11 gene in the patient and in the patient's father (Figure 1,2). The genotype of the patient's mother was normal (Figure 3), indicating that this mutation was dominantly inherited. The patient's father is an asymptomatic carrier. The mutation

*Corresponding author: Yanmei Sang, Department of Endocrinology, Beijing Children's Hospital, Capital Medical University, Beijing, China, E-mail: sangyanmei@yahoo.com

Figure 1: Gene sequencing figure of the patient, with 703 C > G mutation.

Figure 2: Gene sequencing figure of the patient's father, with 703C > G mutation.

Figure 3: Gene sequencing figure of the patient's mother, without 703 C > G mutation.

was not found in the 50 control individuals who were screened to determine whether the change was a common polymorphism.

Discussion

Congenital hyperinsulinism (CHI, OMIM 256450) is a rare genetic disorder characterised by hyperinsulinemic hypoglycemia which is caused by unpredictable levels of excessive insulin secretion. It may be caused by a range of biochemical disturbances and molecular defects. To date, at least 9 genes have been found to be related to the onset of the disease. These genes encode for the following proteins: glucokinase(GK), glutamate dehydrogenase (GDH), short-chain-3-hydroxyacyl-CoA dehydrogenase (SCHAD), hepatocyte factor 4α (HNF-4A), mitochondrial uncoupling protein 2(UCP2), monocarboxylate transporter 1 (MCT-1), the insulin receptor, sulfonylurea receptor1(SUR1), and potassium inward rectifying channel (Kir6.2), etc. Genetic research has revealed that about 50% of cases of CHI in patients are associated with one of above 8 types of gene mutations [9]. Even so, in approximately 40-50% of all CHI cases, no mutation has been found in any of these known genes, suggesting the existence of other disease-associated genes.

The severity of CHI ranges from a mild form to a form so severe that it may require surgical removal of the pancreas in order to protect the brain from damage due to recurrent hypoglycemia [10-12]. ATP-sensitive potassium (K_{ATP}) channel-CHI, which is caused by ABCC8 and KCNJ11 mutations, is the most common and serious type of CHI. Till date, more than 100 ABCC8 and 20 KCNJ11 mutations have been found to be related to the development of K_{ATP}-HI. Of these mutations, most are recessively inherited, and only a few are dominantly inherited [13]. Recent research has revealed that the clinical features of the two types of K_{ATP}-HI are different. In patients with recessively inherited K_{ATP}-HI, the onset of CHI is almost always in the first week after birth, and presents with severe hypoglycemia. On the other hand, patients with CHI caused by dominantly inherited mutations usually have a relatively later onset of the disease with milder hypoglycemia. In these patients, only 37% develop CHI in the first week after birth, and, in many cases, the disease is unrecognized until much later in life [14]. There is an equal distribution of large for gestational age (LGA) infants between the two groups. The patient in our study was LGA, hypoglycemia occurred after the neonatal period and he suffered relatively mild hypoglycemia, all of which are consistent with the clinical features of dominantly inherited K_{ATP}-HI.

The KCNJ11 gene, the second most common gene to cause CHI, consists of only one exon. At present, more than 20 KCNJ11 mutations have been found, most of which are recessively inherited [15]. Recently, several dominantly inherited KCNJ11 mutations have been reported including F55L, G156R, D204E, and others. In our research, a dominantly inherited heterozygous missense mutation was found in the patient and in his father. This mutation, which led to an amino acid substitution of glutamine to glutamate (Q235E), has not previously been reported. The patient's father has no history of hypoglycemia, indicating that he is an asymptomatic carrier.

Diazoxide is the first line of treatment for patients with CHI. It works by binding to the SUR1 regulatory subunits responsible for keeping the K_{ATP} channels open, thereby preventing insulin secretion [16]. Loss-of-function mutations in the ABCC8 and KCNJ11 genes can lead to either a reduction in the number of channels within the β-cell membrane or a decrease in channel activity, and hence diazoxide treatment is often ineffective. Clinical research has shown that the responsiveness to diazoxide therapy is one of the most important differences in clinical phenotype between dominant and recessive K_{ATP}-CHI. In general, diazoxide is not effective in controlling hypoglycemia in patients with recessive K_{ATP}-HI. On the contrary, essentially all of the dominantly inherited cases of K_{ATP}-HI reported up until now have attained complete resolution of hypoglycemia using moderate doses of diazoxide. Thus, complete control of hyperinsulinism by diazoxide has been suggested to be a useful phenotypic marker for the dominant forms of K_{ATP} channel mutations [5]. In contrast to previously reported cases, the patient in our research who had a Q235E mutation was refractory to diazoxide treatment. The result suggests that the Q235E mutation is a rare KCNJ11 mutation which can lead to diazoxide-unresponsive K_{ATP}-CHI. The result has, to some degree, increased our knowledge of the KCNJ11 gene mutation.

Mature K_{ATP} channels are hetero-octomers of four pore-forming Kir6.2 subunits and four sulfonylurea receptor subunits, which play an important role in insulin secretion.

Mutations in the ABCC8 and KCNJ11 genes can abrogate the function of K_{ATP} channels and result in persistent β-cell depolarization and

insulin secretion despite severe hypoglycemia [17]. Mutagenesis research has revealed that different KCNJ11 mutations lead to K_{ATP}-CHI by different mechanisms. R301G, R301H, and R301P or R301C mutations can not only reduce the efficiency of surface expression of the channel but also cause a gating defect characterized by rapid spontaneous decay of channel activity in the absence of ATP and recovery of channel activity upon subsequent exposure to and removal of ATP [8]. F55L mutation greatly reduces the probability of open K_{ATP} channels in intact cells without affecting channel expression. The mutation V290M results in partial loss of K_{ATP} channel activity in heterozygous cases and more severe loss in homozygous cases [18],while R192A, E229R and R314A mutations can lead to the inactivation of K_{ATP} channels[8]. The Q235E mutation found in our study is the first of its type to have been reported. The mechanism by which it affects the K_{ATP} channel and leads to the onset of diazoxide-unresponsive CHI is still not clear.

In general, dominantly inherited KCNJ11 mutations have more complex clinical phenotypes. The dominantly inherited KCNJ11 Q235E mutation can lead to diazoxide-unresponsive K_{ATP}-HI. Large scale genetic research on CHI is necessary to elucidate the genetic mechanism of K_{ATP}-HI.

Acknowledgements

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