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

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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-CHI.

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-
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 hypoglycaemia 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, each encoding for the following proteins: glucokinase (GK), Kir6.2 subunits and four sulfonylurea receptor subunits, which play an important role in insulin secretion.

Till date, more than 100 ABCC8 and 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 KATP 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 KATP-CHI. In general, diazoxide is not effective in controlling hypoglycemia in patients with recessive KATP-HI. On the contrary, essentially all of the dominantly inherited cases of KATP-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 KATP-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 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.

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 re-
search has revealed that different KCNJ11 mutations lead to $K_{ATP}$-CHI by different mechanisms. R301G, R301H, and R301P or R301C mu-
tations can not only reduce the efficiency of surface expression of the channel but also cause a gating defect characterized by rapid sponta-
neous 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.

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References
1. De Leon DD, Stanley CA (2007) Mechanisms of Disease: advances in diagnosis and treatment of hyperinsulinism in neonates. Nat Clin Pract Endocrinol Metab 3: 57-68.
2. Kapoor RR, James C, Flanagan SE, Ellard S, Eaton S, et al. (2009) 3-Hydroxy-
acyl-coenzyme A dehydrogenase deficiency and hyperinsulinemic hypoglyce-
emia: characterization of a novel mutation and severe dietary protein sensitivity. J Clin Endocrinol Metab 94: 2221-2225
3. Bellanné-Chantelot C, Saint-Martin C, Ribeiro MJ, Vaury C, Verkarre V, et al. (2010) ABCC8 and KCNJ11 molecular spectrum of 109 patients with diazoxide-unresponsive congenital hyperinsulinism. J Med Genet 47: 752-759.
4. Marquard J, Palladino AA, Stanley CA, Mayatepek E, Meissner T, et al. (2011) Rare forms of congenital hyperinsulinism. Semin Pediatr Surg 20: 38-44.
5. Pinney SE, MacMullen C, Becker S, Lin YW, Hanna C, et al. (2008) Clinical characteristics and biochemical mechanisms of congenital hyperinsulinism associated with dominant KATP channel mutations. J Clin Invest 118: 2877-2886.
6. Hussain K (2008) Diagnosis and management of hyperinsulinaemic hypoglycemia of infancy. Horm Res 69: 2-13.
7. Flanagan SE, Kapoor RR, Banerjee I, Hall C, Smith VV, et al. (2011) Domi-
antly acting ABCC8 mutations in patients with medically unresponsive hyper-
insulinaemic hyperglycaemia. Clin Genet 79: 582-587.
8. Lin YW, MacMullen C, Ganguly A, Stanley CA, Shyng SL (2006) A novel
KCNJ11 mutation associated with congenital hyperinsulinism reduces the in-
trinsic open probability of beta-cell ATP-sensitive potassium channels. J Biol Chem 281: 3006-3012.
9. Flanagan SE, Kapoor RR, Hussain K (2011) Genetics of congenital hyperinsu-
linemic hypoglycaemia. Semin Pediatr Surg 20: 13-17.
10. Park SE, Flanagan SE, Hussain K, Ellard S, Shin CH, et al. (2011) Charac-
terization of ABCC8 and KCNJ11 gene mutations and phenotypes in Korean patients with congenital hyperinsulinism. Eur J Endocrinol 164: 919-926.
11. Glaser B, Blech I, Krakinson V, Ekstein J, Gillis D, et al. (2011) ABCC8 muta-
tion allele frequency in the Ashkenazi Jewish population and risk of focal hyper-
insulinemic hypoglycemia. Genet Med 13: 891-894.
12. Pierro A, Nah SA (2011) Surgical management of congenital hyperinsulinism of infancy. Semin Pediatr Surg 20: 50-53.
13. Lin YW, Bushman JD, Yan FF, Haidar S, MacMullen C, et al. (2008) Destabil-
ization of ATP-sensitive potassium channel activity by novel KCNJ11 mutations identified in congenital hyperinsulinism. J Biol Chem 283: 9148-9156.
14. Amoux JB, Verkarre V, Saint-Martin C, Montravers F, Brassier A, et al. (2011) Congenital hyperinsulinism: current trends in diagnosis and therapy. Orphanet J Rare Dis 6: 63.
15. Saint-Martin C, Amoux JB, de Lonlay P, Bellanné-Chantelot C (2011) KATP channel mutations in congenital hyperinsulinism. Semin Pediatr Surg 20: 18-22.
16. Kapoor RR, Flanagan SE, James C, Shield J, Ellard S, et al. (2009) Hyperinsu-
linicaemic hypoglycaemia. Arch Dis Child 94: 450-457.
17. James C, Kapoor RR, Ismail D, Hussain K (2009) The genetic basis of congeni-
tal hyperinsulinism. J Med Genet 46: 289-299.
18. Loechner JK, Akrouh A, Kurata HT, Dionisi-Vici C, Maiorana A, et al. (2011) Congenital hyperinsulinism and glucose hypersensitivity in homozygous and heterozygous carriers of Kir6.2 (KCNJ11) mutation V290M mutation: K(ATP) channel inactivation mechanism and clinical management. Diabetes 60: 209-217.