

Characterization of the ABCC8 gene mutation and phenotype in patients with congenital hyperinsulinism in western Saudi Arabia

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ABSTRACT

الأهداف: دراسة فرط الانسولين الخلقي السبب الأكثر شيوعاً والمسبب لنقص السكر في الدم في حديثي الولادة والأطفال الأقل من عام في العمر والطفرة التي تعطل الجينات هي السبب الأكثر شيوعاً.

الطريقة: أجريت دراسة رجعية على 11 طفل مصاب بفرط الانسولين الخلقي في مستشفى جامعة الملك عبد العزيز، جدة، المملكة العربية السعودية خلال الفترة من مارس 2007م حتى فبراير 2012م. تم إجراء تحليل للطفرة المسببة لفرط الانسولين الخلقي (ABCC8, KCN11) لتحديد النمط الظاهري والوراثي.

النتائج: تم الكشف عن تحول جيني في 9 مريض 81.8% بينما لم يتم وجود تحول جيني في 2 مريض. ظهرت طفرة متماثلة على جميع المرضى لدى جين ABCC8، ومريض في أكسين 29، و2 مريض في أكسين 1-22، 2 في أكسين 28، و4 في انترون 36. وظهرت طفرة متماثلة لدى مريض واحد. تم الاستجابة لعقار ديازوكسيد في 5 مريض بينما تتطلب استئصال البنكرياس في 6 مريض 54.6% توفي منهم 3 مريض 33.3% أثناء انتظار إجراء العملية بسبب تسمم الدم وتجلطه. تحسنت حالة 2 مريض 18.1% وذلك بعد استئصال البنكرياس.

خاتمة: أن طفرة هي الأكثر شيوعاً في المرضى السعوديين والتشخيص المبكر أمر ضروري لمنع تأخير النمو العصبي.

Objectives: To understand the genetic etiologies of congenital hyperinsulinism (CHI) in a population of Saudi patients, and to explore genotype-phenotype characteristics.

Methods: We retrospectively reviewed a cohort of 11 children with CHI presenting to King Abdulaziz University Hospital, Jeddah, Kingdom of Saudi Arabia between March 2007 and February 2012. Mutational analysis (ABCC8 and KCN11) was

performed retrospectively to identify phenotype and genotype characteristics.

Results: Analysis revealed ABCC8 mutations in 81.8% (9/11) of patients, with 2 patients not revealing any gene mutation. All positive patients showed a homozygous mutation in the ABCC8 gene, one in exon 29, 2 in exon 1-22, 2 in exon 28, and 4 in intron 36; one patient had a heterozygous mutation. Five patients (45.4%) responded well to treatment with diazoxide not requiring subtotal pancreatectomy, while 6 patients (54.6%) required subtotal pancreatectomy despite treatment with diazoxide and octreotide. Three patients (33.3%) died while waiting for surgery due to sepsis and thrombosis. Two patients (18.1%) showed remission, one of them after subtotal pancreatectomy.

Conclusion: Homozygous mutations in ABCC8 are the most common causes of CHI in Saudi patients. Early diagnosis and therapy for persistent hyperinsulinemic hypoglycemia of infancy are essential to prevent neurodevelopmental delay.

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Congenital hyperinsulinism (CHI) is a genetic disorder characterized by inadequate suppression of insulin secretion in the presence of hypoglycemia. The incidence is approximately 1/27,000-1/50,000 live births; however, in parts of Finland, the incidence is much higher.¹ A very high incidence of 1/2,675 has been reported from Saudi Arabia due to the high rate of consanguinity. Congenital hyperinsulinism, a rare cause of hypoglycemia, is one of the most common causes of persistent hypoglycemia in infancy. It is a heterogeneous condition with a significant genetic component.² The adenosine triphosphate (ATP)-sensitive KC (KATP) channel gene mutations represent a major cause for this disease.³ Mutation testing of the ABCC8/KCNJ11 genes can be carried out within 10 days. Rapid genetic analysis of the KATP channel genes has several benefits in CHI therapy, from genetic counseling to the differential diagnosis of focal and diffuse disease, and the likelihood of remission.^{3,4} Recessive mutations in the ABCC8 and KCNJ11 genes, which encode the sulfonylurea receptor 1 (SUR1) and Kir6.2 (potassium inwardly rectifying channel) subunits of the KATP channel, which control insulin secretion, are the most common genetic etiology. However, autosomal dominant mutations in the ABCC8 and KCNJ11 genes have also been reported.⁵ Early diagnosis and treatment are important to prevent permanent brain damage.⁶ Surgery is often necessary to control hypoglycemia in both diffuse hyperinsulinism (HI) and focal HI, but is only curative in cases of focal HI. There are variable presentations ranging from mild hypoglycemia that responds to drugs, and severe CHI requiring pancreatectomy. Infants with CHI are usually large for gestational age and commonly present with seizures. Patients who fail to respond to therapy often require pancreatectomy. The histological diagnosis is important in deciding whether a near-total pancreatectomy or a lesionectomy is required. Little is known of CHI in the Saudi population. Therefore, the objectives of this study were to understand the genetic etiologies of CHI in a population of Saudi patients, and to explore genotype-phenotype characteristics.

Methods. We retrospectively reviewed a cohort of 11 children with CHI presenting to King Abdulaziz University Hospital, Jeddah, Kingdom of Saudi Arabia between March 2007 and February 2012. Local ethical approval was obtained, and the study was conducted according to the principles of the Helsinki Declaration.

Diagnostic criteria. The diagnosis of persistent hyperinsulinemic hypoglycemia of infancy (PHHI) was confirmed if serum insulin was detectable (>2 mU/l)

at the same time as hypoglycemia (blood glucose <2.6 mmol/l), along with concomitant evidence of elevated glucose requirement (>12 mg/kg/minute) at least twice in 24 hours and lack of urinary ketosis.

Exclusion criteria. Patients with intrauterine growth retardation, asphyxia at birth, or presenting with congenital syndromes were excluded. Children with birth weight <2nd centile for gestational age were also excluded, unless diazoxide treatment was continued for 4 weeks or more, indicating persistent hyperinsulinism.⁷

Rapid KATP channel mutation analysis for the ABCC8 and KCNJ11 genes was carried out for all patients and their parents. If the mutation analysis was normal, the hepatic nuclear factor (HNF4A) and/or glucokinase (GCK) genes were analysed. The GCK mutation analysis was undertaken if there was a dominant family history of hypoglycemia and presentation was later than the neonatal period. The HNF4A mutation testing was performed if there was a history of large for gestational age at birth or dominant family history of hyperglycemia or diabetes. Standard procedures were used to extract DNA from peripheral leukocytes. All coding exons and exon/intron boundaries of ABCC8, KCNJ11, GCK, and HNF4A genes and exons 6, 7, 10, 11, and 12 were PCR amplified, purified and sequenced using universal M13 primers and a Big Dye Terminator Cycler Sequencing Kit v3.1 (Applied Biosystems, Warrington, UK) according to the manufacturer's instructions. Reactions were analysed on an ABI 3730 Capillary sequencer (Applied Biosystems, Warrington, UK), and sequences were compared with the reference sequences (NM_000525 for KCNJ11, NM_000352.3 for ABCC8, and NM_000162.3 for GCK) using Mutation Survey v3.24 (SoftGenetics, State College, PA, USA). Parents were tested to establish the mode of inheritance if a mutation was detected, and microsatellite analysis (PowerPlex 16 System, Promega, Madison, WI) was undertaken to confirm de novo mutations. Children with PHHI were considered to be diazoxide-responsive if satisfactory glycemic control was achieved with doses of oral diazoxide not exceeding 20 mg/kg/day and oral hydrochlorothiazide not exceeding 10 mg/kg/day. Children were considered to be in remission either if no medication was required, or if medication was stopped and no further medication or surgery was required.

Results. Clinical characteristics. Consanguinity was reported in 7 families. Patients reported a family history of hypoglycemia in 4 families. The median follow-up

observation period for all patients was 5 years and 4 months (range: 4 months to 14 years). The time of symptom manifestation ranged from immediately following birth to one year, with 72.7% (8/11) of the patients presenting within the first 3 days of life. The median age at diagnosis was one month (range: 3 days to 24 months; Table 1). The weight of 5 patients was large for gestational age (average birth weight: 4.2 Kg; range: 3.500-4.500 Kg), although the average gestational age was 39 weeks and 4 days (6 females and 5 males). Six patients required subtotal pancreatectomy despite treatment with diazoxide and octreotide, 3 of them died while waiting for surgery due to sepsis and thrombosis. After pancreatectomy, most patients showed, at least for a period of 15 months, less severe and less frequent episodes of hypoglycemia still requiring further medication, and 2 showed complete remission. None of the patients undergoing near-total pancreatectomy developed any signs of malabsorption. Five patients responded well to treatment with diazoxide (up to 20 mg/kg/day) not requiring subtotal pancreatectomy. In most patients treated with diazoxide, moderate hypertrichosis was noted and hyponatremia required adding hydrochlorothiazide 3.5 mg/kg/BID, but no further side effects were observed. Psychomotor retardation was present in 5 patients (45.4%), and 3 more could not be assessed for development due to death in the early neonatal period, while 3 (27.2%) showed no developmental delay. We failed to detect any mutations in 2 of the patients, one of them presented at the age of 4 months with hemihypertrophy with a

history of omphalocele giving the probable diagnosis of Beckwith-Wiedemann syndrome. Seven patients showed a homozygous mutation in the ABCC8 gene, one in exon 29, 2 in exon 1-22, 2 in exon 28, and 4 in intron 36 (Table 1).

Discussion. Persistent hyperinsulinemic hypoglycemia of infancy is a rare autosomal recessive disorder characterized by deregulated insulin secretion.⁸ It usually presents soon after birth with severe hypoglycemia associated with continued insulin secretion despite a low blood glucose level.⁹ Subtotal pancreatectomy is necessary in many cases due to poor response to therapy with diazoxide or somatostatin. A delay in diagnosis results in irreversible brain damage due to prolonged hypoglycemia.⁹

In this study, we evaluated clinical findings, mutational analysis, and therapeutic strategies in 11 patients with CHI. Most important for the clinical outcome of these patients was an early diagnosis. Five patients were diagnosed with developmental delay. In these patients, psychomotor retardation was already present when the diagnosis of PHHI was made. Neurological deficits are probably caused by untreated repeated and prolonged episodes of hypoglycemia, regardless of whether they responded to medical treatment or required surgery.

In our study, 9 (81.8% of cohort) patients were positive for ABCC8 mutations, and in 2 (19.2%) patients we failed to detect any mutations. This rate

Table 1 - Characteristics and gene mutation of the 11 CHI Saudi patients.

Age	Gender	Age at diagnosis	Consanguinity	Required surgery	Response to diazoxide	Gene	Mutation
11 y	Female	2 y	Negative	No	Yes	ABCC8 exon 29	c.3643C>T
4 y	Female	1 m	Positive	Yes	No	ABCC8 exon 1-22	c.1-?_2697+?del
3 y	Male	1 m	Positive	Yes	No	ABCC8 exon 1-22	c.1-?_2697+?del
3.5 y	Male	9 m	Negative	No	Yes	Negative	Negative
2 y	Female	2 y	Positive	No	Yes	ABCC8 intron 36	c.4415-13G>A
5 m	Male	2 w	Negative	No	Yes	Negative	Negative
2 m	Female	2 d	Positive	Yes	No	ABCC8 intron 36	c.4415-13G>A
1 m	Female	2 d	Positive	Yes	No	ABCC8 intron 36	c.4415-13G>A
1 m	Male	2 d	Positive	Yes	No	ABCC8 intron 36	c.4415-13G>A
6 y	Male	3 d	Positive	No	Yes	ABCC8 intron 28	c.3410dupC
9 y	Female	4 d	Positive	Yes	No	ABCC8 intron 28	c.3410dupC

CHI - congenital hyperinsulinism, y - years, m - months, w - weeks, d - days

of mutation detection is higher than that in previous reports, which describe ABCC8 or KCNJ11 gene mutations in 30-60% of patients with CHI.¹⁰ Despite the small number of patients in this study, it is possible that the high detection rate of mutations is due to the high sensitivity of direct sequence analysis.¹¹ The mutation-detection rate in a Japanese study with mutations in either the ABCC8 or the KCNJ11 genes was 24% (4/17) by single strand conformation polymorphism analysis,¹² and 79% (11/14) via the direct sequence method.¹³ In addition, the hereditary differences between races, the high degree of consanguineous marriage, and the limited selection of patients cannot be overlooked.

In patients positive for ABCC8 mutations, all of them were homozygous, except for one who was heterozygous. These mutations affected amino acid residues conserved throughout evolution, or resulted in a different amino acid substitution at a known mutation site. We also found that 4 patients (36.3%) of children who tested positive for ABCC8 mutations achieved euglycemia with medical treatment. One of these was a heterozygous mutation. Response to diazoxide was noted in those without identified genetic mutations.

Clinical outcome. In patients with ABCC8 or KCNJ11 gene mutations diazoxide is affectless, also 90% of neonates do not respond to diazoxide. Diazoxide was originally started in all patients with a satisfactory response to medication observed in 6 patients. In 5 patients who were not responsive to diazoxide, subcutaneous octreotide injections were administered.

In approximately 50% of CHI-cases, no gene mutation is found, suggesting the existence of other disease-associated genes.¹⁴ Positive mutation testing in patients and parents confirmed recessive inheritance. Mutations in ABCC8, KCNJ11, or GCK were not found in 2 of our patients. The GLUD1 gene was not sequenced because hyperammonemia had not been reported in any of these patients. In a previous study reporting that 16-29% of neonates,¹⁵ and 62-69% of infants¹⁶ with CHI responded to medication. In our study, the response rate was 33.3% (3/9) in neonates, and 100% (2/2) in infants. This high drug response rate in infants remains unclear and may include ethnic differences in genetic background and may also be due to the small study sample number. Two operated patients had the diffuse form of the disease and one had focal hyperinsulinemia, which is in contrast to previous studies reporting focal disease in 30-65% of surgically treated patients.¹⁷ We found one patient with a heterozygous mutation ABCC8 in exon 29 with negative consanguinity who responded well to diazoxide. This is in concordance with a study from Korea,⁷ which

revealed that paternal heterozygous KATP channel mutations in the medically treated group respond well to diazoxide, as in our patient.

While, we could integrate the clinical findings and genetic results in our study, it was not possible to predict the response to diazoxide or histological findings through ABCC8 or KCNJ11 mutation analysis as reported due to the lack of positron emission tomography scan (PET-Scan) in our hospital.¹⁸ This makes it difficult to predict the drug responsiveness and histology through genetic analysis.⁷ A further limitation was the small study sample size.

We conclude that a genetic diagnosis is important for patients with CHI as it may provide important information regarding the histology of the disease, and will provide information regarding the recurrence risk for future generations. Further work is required to identify patients who may benefit from a genetic diagnosis, to correlate the type of mutation and specific management and prediction of remission.

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