J Korean Med Sci 2009; 24: 979-81
ISSN 1011-8934
DOI: 10.3346/jkms.2009.24.5.979

Copyright © The Korean Academy of Medical Sciences

Familial Glucocorticoid Deficiency with a Point Mutation in the ACTH Receptor: A Case Report

Familial glucocorticoid deficiency (FGD) is a rare autosomal recessive disorder characterized by severe glucocorticoid deficiency associated with failure of adrenal responsiveness to ACTH but no mineralocorticoid deficiency. We report a 2 month-old boy of nonconsanguineous parents, presented with hyperpigmentation. Physical examination showed diffuse dark skin of body including, oral mucosa, gum, hands, nails and scrotum. Laboratory evaluation revealed low serum cortisol (0.3 μg/dL), with very high plasma ACTH level (18,000 pg/mL), and serum cortisol level did not increase after ACTH stimulation test. Serum sodium, potassium, plasma renin activity, aldosterone and 17-hydroxyprogesterone were normal. Sequence analysis of the ACTH receptor (MC2R) gene showed a homozygous mutation of D103N. Diagnosis of FGD was made and treatment started with oral hydrocortisone.

Key Words : *Familial Glucocorticoid Deficiency; Adrenocorticotropic Hormone; Receptor, Melanocortin, Type 2*

Chan Jong Kim[1], Young Jong Woo[1], Gu Hwan Kim[2], and Han Wook Yoo[2]

Department of Pediatrics[1], Chonnam National University Medical School, Gwangju; Department of Pediatrics[2], Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

Received : 5 December 2007
Accepted : 28 April 2008

Address for correspondence
Chan Jong Kim, M.D.
Department of Pediatrics, Chonnam National University Medical School, 8 Hak-dong, Dong-gu, Gwangju 501-757, Korea
Tel : +82.62-220-6645, Fax : +82.62-222-6103
E-mail : cjkim@jnu.ac.kr

INTRODUCTION

Familial glucocorticoid deficiency (FGD), also known as isolated glucocorticoid deficiency or hereditary unresponsiveness to ACTH, is an autosomal recessive disorder (1-4). Patients suffer from recurrent hypoglycemia, convulsions, hyperpigmentation, recurrent infections and failure to thrive (3). Investigation shows severe cortisol deficiency despite a high plasma ACTH levels, which leading to increased skin pigmentation as a result of activation of α-melanocyte stimulating hormone receptors (melanocortin-1 receptors) on cutaneous melanocytes (3, 4). There is no mineralocorticoid deficiency and the renin-angiotensin system is not affected. The molecular basis of this condition was first described in 1992 (5), and approximately 20 different ACTH receptor (MC2R) mutations have been reported in individuals or families with FGD (4, 6). Here, we report a boy with clinical and biochemical features of FGD who is homozygous for a mutation (D103N) in the MC2R gene. This is the first Korean report of FGD associated with a mutation of the MC2R gene.

CASE REPORT

The patient was born to nonconsanguineous Korean parents at 40 weeks of gestation by Caesarean section for induction failure, with a birth weight of 3.7 kg. He was the only baby and there was no neonatal or infant death on family history. At first week after birth, he showed jaundice and treated with phototherapy for 3 days at local hospital. At 2 months of age, the patient was referred to our hospital for generalized hyperpigmentation. His weight was 7.1 kg (75th percentile) and length was 62.7 cm (75th percentile). Physical examination showed diffuse pigmentation of body including, oral mucosa, gum, hands, nails and scrotum. The external genitalia showed normal development. Routine laboratory tests including CBC, Na, K, Cl, Bun, Cr and glucose were normal, but AST (139 IU/L, normal range 15-55 IU/L), ALT (60 IU/L, normal range 5-45 IU/L), total bilirubin (6.7 mg/dL, normal range 0.2-1.2 mg/dL) and direct bilirublin (2.6 mg/dL, normal range 0-0.4 mg/dL) levels were elevated. A 99mTc-DISIDA (Diisopropyl Iminodiacetic Acid) hepatobiliary scan was done and revealed neonatal hepatitis. Endocrinological evaluation showed very low serum cortisol level (0.3 μg/dL, normal range 5-23 μg/dL, 8:00 A.M.), with very high plasma ACTH level (18,000 pg/mL, normal range 10-60 pg/mL, 8:00 A.M.) at 09:00 hours. In response to a rapid ACTH stimulation test (15 μg/kg bolus i.v.), his cortisol rose minimally from 1.0 to 2.6 μg/dL. Plasma renin activity, aldosterone, 17-hydroxyprogesterone and very long-chain fatty acids were all normal. Abdominal ultrasonography showed normal adrenal glands. On the basis of the above findings, he was diagnosed with FGD, and was treated with oral hydrocortisone (15 mg/m^2/day). Six months later, hyperpigmentation was markedly reduced, and the plasma ACTH level was reduced to 195.7 pg/mL. The patient has done well, but the

Table 1. Sequences of oligonucleotide primers

1S	5′-AGAATCAATCAAGTTTTCCGT-3′
1A	5′-AGATAGCCCATGTTTCTCAAT-3′
2S	5′-AGAATAAGAATCTCCAGGCAC-3′
2A	5′-ACATGATGGGAGAAGATCAC-3′
3S	5′-GCTACATCACCATCTTCCAC-3′
3A	5′-TGTCATCAAGAGGACATGAA-3′
4S	5′-AGGAAGATCTCCACCCTCC-3′
4A	5′-GCATTTGTTGGAATGTTACAC-3′

1S, 2S, 3S and 4S are sense primiers, and 1A, 2A, 3A and 4A are antisense primers. Primers were designed with primer3 cgi v.2.0 served from Whitehead Institute (http://frodo.wi.mit.deu/cgi-bin/primer3/primer3_www.cgi) using sequences from GenBank accession number of NT_010859.14.

plasma ACTH level has been variable (range of 190 to 3,440 pg/mL), throughout the 2 yr of hydrocortisone replacement therapy (15-20 mg/m^2/day).

Mutational analysis of the MC2R gene

Genomic DNA was isolated from peripheral blood using PUREGENE DNA isolation kit (Gentra, Minneapolis, MN, U.S.A.). The entire coding sequence of the MC2R gene was amplified by PCR with 4 sets of primers (Table 1). The amplifications were performed in 30 cycles, each cycle consisting of denaturation at 95℃ for 30 sec, annealing at 55℃ for 30 sec, and extension at 72℃ for 45 sec. PCR was carried out in reaction volumes of 20 μL, containing 100 ng of genomic DNA template, 1 μM each primer, 200 μM each dNTPs, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), and one unit of Taq polymerase (Promega, Madison, WI, U.S.A.). After amplification, PCR mixtures were run on 1.2% agarose gel in the presence of ethidium bromide. PCR products were purified and subjected to direct sequencing from both directions on an ABI 3130xl Genetic analyzer (Applied Biosystems, Foster city, CA, U.S.A.). We identified a point mutation (D103N) in the MC2R gene. The patient was homozygous (Fig. 1), and both asymptomatic parents were heterozygous for this mutation.

DISCUSSION

The diagnosis of FGD is based on clinical findings, low serum cortisol in the presence of excessively elevated ACTH, proof of normal aldosteorne production, and the exclusion of other causes of adrenal failure (3, 4). The most common initial presenting sign is severe hyperpigmentation of the skin and mucosa membrane. Other presenting features include recurrent hypoglycemia, feeding problems, regurgitation, failure to thrive and severe infections (3, 4). In newborns, symptoms of hypoglycemia can be subtle, so a high index of suspicion is needed (3). Often these patients are significantly

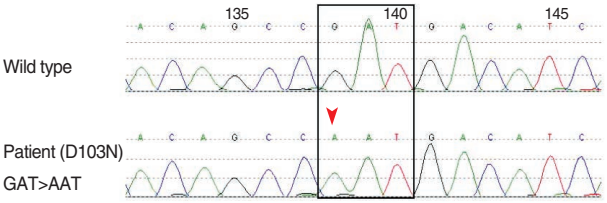

Fig. 1. Genetic analysis revealed a homozygous mutation in the coding region of the MC2R gene, consisting of a guanine to adenine transition at the first position of codon 103, resulting in substitution of aspartic acid for asparagine (D103N).

jaundiced and may require phototherapy (7). In some cases this appears to result from a transient hepatitis (8). Our case also showed jaundice and transient hepatitis. Although this disease is easily treatable when recognized, if left untreated it may be fatal or lead to severe mental disability as a result of recurrent hypoglycemia secondary to glucocorticoid insufficiency (3, 4). The exact incidence of FGD is not known. It is a rare disease, and only isolated case reports are documented. Three Korean patients of FGD were reported, but the gene studies were not done (9).

The ACTH receptor is a member of the melanocortin receptor family, consisting of five closely related genes that encode seven-transmembrane G protein-coupled receptors (5). All five of these receptors can bind ACTH to some extent, but MC2R binds ACTH at the highest affinity, is expressed almost exclusively in the adrenal cortex, and hence is the physiological ACTH receptor (3). After the MC2R gene was cloned (10), approximately 20 different MC2R mutations have been reported in patients with FGD (6, 7, 10). MC2R is a 297-amino acid protein, encoded by a gene on chromosome 18p11.2 (5). Our patient had a homozygous mutation (D103N) of MC2R gene, and it was previously reported (4). This missense mutation is in the first extracellular loop of the receptor and may impair ACTH binding (4, 6, 11). However, the etiology of FGD is heterogeneous, and not all patients with FGD have been found to have MC2R gene mutations (4, 7). Patients with FGD who have mutations in the MC2R gene are said to have FGD type 1, and patients in whom no such mutations are found have FGD type 2 (4). Recently, Metherall et al. (12) identified mutations in a gene encoding the melanocortin 2 receptor accessory protein (MRAP), which interacts with MC2R and may have a role in the trafficking of MC2R. They demonstrated that mutations in MRAP caused FGD in 19 of 104 kindreds with confirmed FGD and no MC2R mutations (12). On current evidence, inactivating mutations of MC2R gene account for about 25% of all FGD cases, and mutations in MRAP gene account for about 20% of FGD cases, implying that at least half of all FGD cases result from other genes yet to be identified (4). So further studies for identification of these genes will have implications for the detailed understanding of FGD.

REFERENCES

1. Shepard TH, Landing BH, Mason DG. *Familial Addison's disease; case reports of two sisters with corticoid deficiency unassociated with hypoaldosteronism. AMA J Dis Child 1959; 97: 154-62*.
2. Kelch RP, Kaplan SL, Biglieri EG, Daniels GH, Epstein CJ, Grumbach MM. *Hereditary adrenocortical unresponsiveness to adrenocorticotropic hormone. J Pediatr 1972; 81: 726-36*.
3. Clark AJ, Weber A. *Adrenocorticotropin insensitivity syndromes. Endocr Rev 1998; 19: 828-43*.
4. Clark AJ, Metherell LA, Cheetham ME, Huebner A. *Inherited ACTH insensitivity illuminates the mechanisms of ACTH action. Trends Endocrinol Metab 2005; 16: 451-7*.
5. Mountjoy KG, Robbins LS, Mortrud MT, Cone RD. *The cloning of a family of genes that encode the melanocortin receptors. Science 1992; 257: 1248-51*.
6. Flück CE, Martens JW, Conte FA, Miller WL. *Clinical, genetic, and functional characterization of adrenocorticotropin receptor mutations using a novel receptor assay. J Clin Endocrinol Metab 2002; 87: 4318-23*.
7. Naville D, Barjhoux L, Jaillard C, Faury D, Despert F, Esteva B, Durand P, Saez JM, Begeot M. *Demonstration by transfection studies that mutations in the adrenocorticotropin receptor gene are one cause of the hereditary syndrome of glucocorticoid deficiency. J Clin Endocrinol Metab 1996; 81: 1442-8*.
8. Lacy DE, Nathavitharana KA, Tarlow MJ. *Neonatal hepatitis and congenital insensitivity to adrenocorticotropin (ACTH). J Pediatr Gastroenterol Nutr 1993; 17: 438-40*.
9. Lim SH, Kim YW, Ahn BH, Moon HR. *Three cases of familial glucocorticoid deficiency. J Korean Pediatr Soc 1986; 29: 85-8*.
10. Tsigos C, Arai K, Hung W, Chrousos GP. *Hereditary isolated glucocorticoid deficiency is associated with abnormalities of the adrenocorticotropin receptor gene. J Clin Invest 1993; 92: 2458-61*.
11. Elias LL, Huebner A, Pullinger GD, Mirtella A, Clark AJ. *Functional characterization of naturally occurring mutations of the human adrenocorticotropin receptor: poor correlation of phenotype and genotype. J Clin Endocrinol Metab 1999; 84: 2766-70*.
12. Metherell LA, Chapple JP, Cooray S, David A, Becker C, Rüschendorf F, Naville D, Begeot M, Khoo B, Nürnberg P, Huebner A, Cheetham ME, Clark AJ. *Mutations in MRAP, encoding a new interacting partner of the ACTH receptor, cause familial glucocorticoid deficiency type 2. Nat Genet 2005; 37: 166-70*.