Molecular Syndromology

Novel Insights from Clinical Practice

Mol Syndromol 2023;14:71–79
DOI: 10.1159/000526320

Received: September 14, 2021
Accepted: July 31, 2022
Published online: October 11, 2022

A Novel Mutation in Melanocortin Receptor 2 and a Reported Mutation in Melanocortin Receptor 2 Accessory Protein: Three Chinese Cases with Familial Glucocorticoid Deficiency

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Established Facts

- Mutations in *MC2R* and *MRAP* cause familial glucocorticoid deficiency (FGD) type 1 and type 2, respectively.
- Skin hyperpigmentation and episodic hypoglycemia are common symptoms in FGD.
- Most patients with FGD type 1 present later and are taller compared with FGD type 2 patients.

Novel Insights

- Our patients presented with hyponatremia at birth due to variants in *MRAP* (c.106+1delG).
- One patient carried a novel missense mutation in *MC2R* and displayed milder symptoms.
- The patient with a mutation in *MC2R* presented with a taller stature, and her height gradually approached the midparental height by supplementation with glucocorticoids.

Keywords
Familial glucocorticoid deficiency · *MC2R* · *MRAP* · Mutation · Case report

Abstract

Background: Familial glucocorticoid deficiency (FGD) is a rare autosomal recessive disease characterized by glucocorticoid deficiency without mineralocorticoid deficiency. We report 3 Chinese patients with *MRAP* or *MC2R* mutations.

Case Reports: Patient 1 presented with hyperpigmentation. Endocrine investigations revealed low serum cortisol levels and elevated adrenocorticotropic hormone (ACTH) levels. Furthermore, low serum sodium was evident. She was diagnosed with FGD type 2 due to a homozygous mutation in *MRAP* (c.106+1delG), revealed through exome sequencing (ES). After 2-year treatment with hydrocortisone, skin hyper-

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pigmentation was improved. Patient 2 initially presented with hyponatremia. Low cortisol levels and high levels of ACTH were subsequently detected; he was subjected to a hydrocortisone treatment during which he experienced repeated hypoglycemic attacks and pigmentation. ES revealed the same mutation as in patient 1 in *MRAP* (c.106+1delG), thus he was diagnosed with FGD type 2. After 6 years of age, his symptoms remarkably improved, and there was no episode of hypoglycemia. Patient 3 mainly presented with hyperpigmentation, hypoglycemic attack, and tall stature. Laboratory findings were normal except for low serum cortisol levels and high ACTH levels. She was diagnosed with FGD type 1 as ES revealed a novel homozygous mutation in *MC2R* (c.712C>A, p.His238Tyr). After nearly 2 years of hydrocortisone replacement therapy, the excessive growth was reduced to near normal, and the skin color returned to normal. ***Conclusions:*** Three patients were diagnosed with FGD (one with FGD type 1 and two with FGD type 2). They all presented with hyperpigmentation and hypoglycemia; however, compared with patient 1, the clinical manifestations of patient 2 were more complicated. Patient 3 had later onset and taller stature than patients 1 and 2. A novel mutation in patient 3 expands the mutation spectrum of *MC2R*.

Introduction

Familial glucocorticoid deficiency (FGD) is a rare autosomal recessive disorder characterized by isolated glucocorticoid deficiency with normal mineralocorticoid function. This disease is caused by adrenal resistance to adrenocorticotropic hormone (ACTH), which is characterized by low or undetectable serum cortisol concentrations in the presence of markedly elevated plasma ACTH levels. Patients diagnosed with FGD typically present with hyperpigmentation, hypoglycemic attacks, jaundice, failure to thrive, and infections during the neonatal period to late childhood [Cooray et al., 2008]. Mutations in multiple genes have been reported to cause FGD, the most common mutations occurring in melanocortin 2 receptor (*MC2R*). In 1993, mutations in *MC2R* were first identified as the cause of FGD [Tsigos et al., 1993] and now account for 25% of all FGD cases, designated as FGD type 1. In 2005, mutations in the MC2R accessory protein (MRAP) were also demonstrated to be responsible for FGD [Metherell et al., 2005]. MRAP is a protein with a single transmembrane domain that is essential for the trafficking of MC2R from the endoplasmic reticulum to the cell surface and subsequent signaling in response to ACTH [Metherell et al., 2005]. This form of FGD, designated FGD type 2, accounts for nearly 8% of cases [Guran et al., 2016; Amano et al., 2017; Buonocore et al., 2021]. Mutations in the mini chromosome maintenance-deficient 4 homolog (*MCM4*) and nicotinamide nucleotide transhydrogenase (*NNT*) have been reported in patients with FGD [Meimaridou et al., 2013], accounting for further 10% of FGD cases.

Here, we describe 2 Chinese patients with FGD type 2, which is associated with a mutation in *MRAP* (c.106+1delG), and 1 Chinese patient with FGD type 1, who carries a novel mutation in *MC2R* (c.712C>A, p.His238Tyr). The 3 patients' clinical presentations varied.

Case Presentations

Case 1

Case 1 was a 37-week-old female patient born to nonconsanguineous parents. Birth weight and length were 2.7 kg and 49 cm, respectively. Cesarean section was performed because of oligohydramnios. At birth, she had an Apgar score of 10 with no signs of asphyxia, hypoxia, or hypoxic-ischemic encephalopathy. There was a family history of 2 previous miscarriages for which the cause could not be identified. One of her sisters died 3 days after birth due to dyspnea. Another 8-year-old sister was healthy. The patient was the third child in the family. Hyperpigmentation of the skin was observed after birth. At 10 days of age, she was admitted to the hospital and was diagnosed with mild neonatal pneumonia. Endocrine investigations revealed low cortisol levels (<1.0 μg/dL, normal range: 5–25) with extremely elevated ACTH levels (>1,250 pg/mL, normal range: 6.4–40). Dehydroisoandrosterone sulfate concentration was <15 μg/dL (normal range: 9.11–47.9) [de Peretti and Mappus, 1983], testosterone concentration was 0.72 nmol/L (normal range: 0.21–2.7) [Kulle et al., 2010], 17α-hydroxyprogesterone concentration was 0.2 nmol/L (normal range:1.7–4.1), renin activity was 3.84 μg/L/h (normal range: 4–24), and aldosterone (ALD) concentration was 157.49 ng/L (normal range: 20–1,100). Hyponatremia was evident (119 mmol/L, normal range:135–145). Levels of serum potassium (4.3 mmol/L, normal range:3.5–5.5) and blood glucose (4.7 mmol/L, normal range: 3.9–5.8) were normal. Pituitary and adrenal gland magnetic resonance imaging findings were normal. The high urinary output (6.9 mL/kg/h) and low urine specific gravity (1.005) suggested that a diagnosis of SIADH was unlikely. The presence of tears and the absence of achalasia and neuropathy made triple A or Allgrove syndrome unlikely. Additionally, 3β-hydroxysteroid dehydrogenase deficiency and 46,XY StAR deficiency were ruled out due to the 46,XX karyotype and normal female genitalia. These results led to a presumed diagnosis of FGD or 46,XX StAR deficiency.

Because she was initially under stress due to neonatal pneumonia, she was treated for preventing the development of adrenal crisis with hydrocortisone (25 mg/m^2/day) and 9-fludrocortisone (9-FHC; 0.083 mg/day). The hydrocortisone dosages were reduced to 12 mg/m^2/day when she was 2 months old. During the subsequent treatment, the dose of hydrocortisone was adjusted (10.5–12 mg/m^2/day) based on clinical assessment including growth, weight

gain, and general well-being. At 6 months, 9α-FHC treatment was stopped because of 2 episodes of hypokalemia. At her last visit at 2 years of age, her skin had mostly returned to normal, and ACTH level was 215 pg/mL. Her head circumference was normal; however, Gesell developmental schedules at 24 months revealed that the developmental quotients (DQs) of 5 domains (adaption 70, gross motor 90, fine motor 86, language function 70, and personal/social function 86) were below normal levels (84 < DQ < 100), with a mildly defective DQ score in the domain of language functioning and adaption (54 < DQ < 75), indicating slight global developmental delay.

Genomic DNA was extracted from peripheral whole blood using a QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) following standard procedures. Exome sequencing (ES) was performed as previously described [Sun et al., 2017]. The capture kit used for the patient was the xGen Exome Research Panel (Integrated DNA Technologies, Coralville, IA, USA). ES identified a homozygous deletion of one nucleotide at the canonical 5α donor splice site (c.106+1delG) in intron 3 of *MRAP*. This mutation was confirmed by Sanger sequencing. The parents were heterozygous carriers of the same mutation. This variant has been reported in several FGD type 2 patients [Akın et al., 2010; Chen et al., 2016]. It could be classified as pathogenic (PVS1+PM2+PM3_Very Strong) according to the American College of Medical Genetics (ACMG) guidelines for variant interpretation [Richards et al., 2015].

Case 2

Case 2 was a male patient, who was the first child born to healthy nonconsanguineous parents after 40 weeks of gestation by cesarean section. The birth weight was 3.1 kg. The newborn was admitted to the hospital due to neonatal pneumonia. He received an Apgar score of 10 and showed no signs of asphyxia, hypoxia, or hypoxic-ischemic encephalopathy. The blood pressure was 77/48 mm Hg, and neither oliguria nor edema was observed. Endocrine investigations revealed an ALD level of 121.13 pg/mL. Serum sodium levels were slightly decreased (133 mmol/L); an increased serum potassium level (6.55 mmol/L) was observed only once, which could not be excluded as an artefactual result of lysed sample. Hyperpigmentation of the skin was noted after birth. Therefore, the patient was clinically diagnosed with adrenal hypoplasia and treated with hydrocortisone (11–14 mg/m^2/day) and 9α-FHC (0.05 mg/day).

Although the patient had good compliance with a stable frequency of medication, severe repeated hypoglycemic attacks (once to twice per year), fatigue, sweating, and hypoglycemia occurred in the morning until 6 years of age. However, after 6 years of age, his symptoms remarkably improved, and there was no episode of hypoglycemia. At 7 years of age, the patient was suggested to stop 9α-FHC administration due to the genetic diagnosis of mutations in *MRAP*. At the time of his most recent visit, he did not experience hypoglycemic attacks or hyperpigmentation, and his serum glucose level was 4.97 mmol/L.

Genomic DNA was extracted from peripheral blood leukocytes from the patient and his mother after obtaining informed consent. PCR and Sanger sequencing for the *NR0B1* gene were performed, but the result was negative. ES was then performed, and a homozygous deletion of one nucleotide at the canonical 5α donor splice site (c.106+1delG) in intron 3 of *MRAP* was found, which was the same as in patient 1, and his parents were heterozygous carriers for the same mutation. This variant has been reported in several FGD type 2 patients in the literature and could be classified as pathogenic (PVS1+PM2+PM3_Very Strong).

Case 3

Case 3 was a female patient who was the first child of nonconsanguineous parents and born at full term with a birth weight of 3.1 kg and birth length of 50 cm. She was referred to our hospital at the age of 2 years and 11 months for generalized hyperpigmentation of the skin after birth. By the age of 2, she had experienced one episode of hypoglycemia. She was tall, with a height of 100 cm (1.32 height standard deviation score [Ht SDS]). The midparental height was −1.17 Ht SDS based on parental heights (father 167 cm, mother 155 cm), indicating that her actual height was obviously greater than her target height. Physical examination revealed no craniofacial dysmorphism. She had normal female genitalia and no signs of alacrimia and achalasia. Serum electrolyte levels were normal, but blood glucose level was obviously low (1.54 mmol/L). Urine tests were positive for ketones. Endocrine investigations revealed low serum cortisol levels (3.21 μg/dL) with extremely elevated ACTH levels (>2,000 pg/mL), suggesting ACTH resistance. Testosterone, 17-OH progesterone, estradiol, and progesterone levels were normal. The diagnosis was primary adrenal insufficiency. The patient was then treated with hydrocortisone (16 mg/m^2/day). The bone age was 4 years and 2 months at her age of 3 years and 9 months. Clinical follow-up of the patient showed that the skin color was light and Ht SDS decreased. However, ACTH was maintained at an elevated level. On her last visit at the age of 5 years, the ACTH level was 1,843 pg/mL and her height was 110.5 cm (0.07 SDS). The value of actual Ht SDS minus midparental height Ht SDS decreased from initially 2.49 Ht SDS to 1.24 Ht SDS at the last follow-up. The growth data (Fig. 1) indicated the reduction of excessive growth to near normal levels following the introduction of glucocorticoid replacement. The heights of the 3 patients (pre-treatment, post-treatment, and midparental) are shown in Table 1. No hypoglycemic attacks occurred after a follow-up for 5.3 years. Skin color had returned to near normal.

ES revealed a homozygous mutation (NM_000529.2: c.712C>A, p.His238Tyr) in *MC2R*. The parents were heterozygous for the mutation. This mutation was not present in the 1000 Genomes Project, gnomAD, EVS, or in-house databases and has not previously been reported in the literature. According to the ACMG guidelines for variant interpretation [Richards et al., 2015], this mutation could be classified as likely pathogenic (PM1+PM2+PM3_Supporting+PP3+PP4). The clinical features and identified mutations observed in this case were submitted to the *MC2R* variant database (https://databases.lovd.nl/shared/individuals/MC2R) in the Leiden Open Variant Database (LOVD) [Fokkema et al., 2011].

Discussion

FGD is a rare autosomal recessive disease. Mutations in *MC2R* and *MRAP* account for approximately 30% of all FGD cases. Primary adrenal insufficiency (PAI) patients, such as FGD patients, are classified as having biochemically uncharacterized PAI if they cannot be diagnosed based on specific biochemical findings or urine/serum

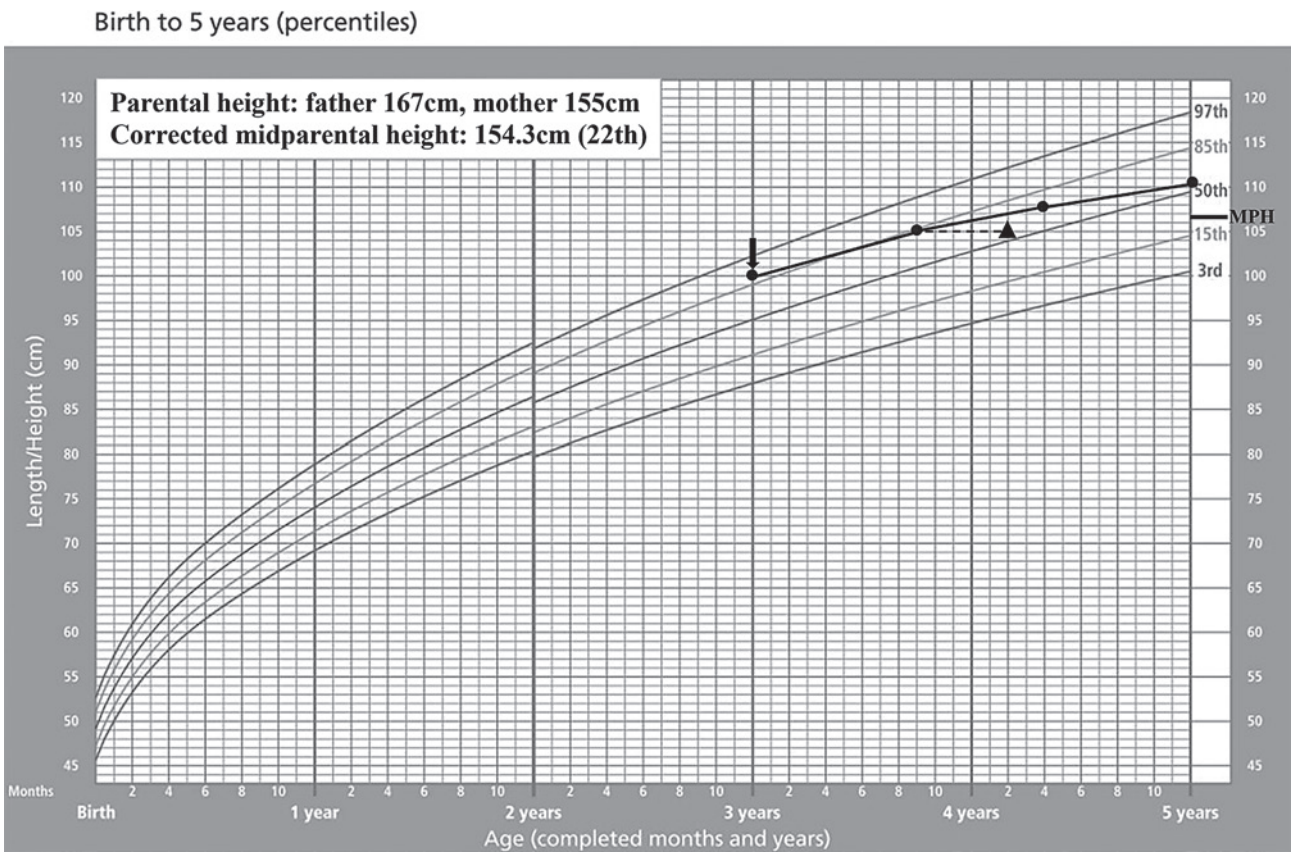

Fig. 1. Growth chart of patient 3. Each black dot represents a growth measurement from an office visit at the pediatrician or endocrinologist. The arrow represents treatment initiation with hydrocortisone; the triangle represents bone age at the time point. Parental heights and the corrected midparental height (MPH) are shown. MPH is marked on the right side of the growth chart.

Table 1. Comparison of pre- and post-treatment heights of the 3 cases

Patients	Sex	Height, cm		Midparental height, cm (SDS)	Pre-treatment			Post-treatment		
		father	mother		age	height, cm (SDS)	Δheight SDS[a]	age	height, cm (SDS)	Δheight SDS[a]
Case 1	Female	172	157	157.6 (−0.56)	At birth	49 (−0.41)	0.15	3 y 2 m	92.2 (−1.23)	−0.67
Case 2	Male	168	158	176 (0.54)	1 m	57 (1.23)	0.69	9 y 2 m	132 (−0.72)	−1.26
Case 3	Female	167	155	154.3 (−1.17)	2 y 11 m	100 (1.32)	2.49	5 y	110.5 (0.07)	1.24

[a] Δheight SDS = height SDS – midparental height SDS. m, months; y, years.

steroid metabolites (elevated 17α-hydroxyprogesterone [17-OHP], elevated very long chain fatty acids, etc.) [Amano et al., 2017]. In our hospital, of the 80 patients with biochemically uncharacterized PAI, 3 were diagnosed with FGD (1 with mutations in *MC2R*, 2 with mutations in *MRAP*).

We compared the clinical features and genetic etiology of the 3 Chinese patients who were admitted with FGD based on their medical records. Episodic hypoglycemia and skin hyperpigmentation were the most common symptoms in the 3 patients. Cortisol deficiency may result in episodic hypoglycemia; additionally, it contributes

Table 2. FGD type 2 patients with detailed phenotypic data reported in published cases

Cases	Gender	Onset age	Clinical features	Biochemical and endocrine test data							Variation (homozygous)
				cortisol, μg/dL	ACTH, pg/mL	renin, ng/mL	glucose, mmol/L	electrolytes, mmol/L	17-OHP, nmol/L	ALD, ng/dL	
Patient 1	Female	At birth	Hyperpigmentation, fatigue	<1	>1250	7	4.5	Sodium (119)	0.2	15.75	c.106+1delG
Patient 2	Male	At birth	Hyperpigmentation, fatigue, hypoglycemia, infection	1.84	1,250	4.14	4.6	Sodium (133), potassium (6.55)	N	121.13	c.106+1delG
Akin et al., 2010	Male	6 months	Hyperpigmentation, hypoglycemic convulsions	0.6	708	57	3.6	N	0.01	80.1	c.106+1delG
Chen et al., 2016	Female	At birth	Hyperpigmentation	<1	1,250	–	N	N	N	N	c.106+1delG
Modan-Moses et al., 2006	Male	19 months	Neurological disability	<0.1	>1,250	3.4	–	N	–	–	c.17-23del p.L31X
Rumié et al., 2007	Male	9 hours	Hypoglycemia, hypotension, sepsis, jaundice, hyperpigmentation, eczema	<10.18	>730	4	0.74	N	–	–	c.130delG p.V44X
Habeb et al., 2013	Male	4.5 years	Hypoglycemia, hypotension, hyperpigmentation	<0.9	1,050	2.1	2.8	N	–	–	c.175T>G p.Y59D
Hughes et al., 2010	Male	4 years	Hypoglycemia, hypotension, hyperpigmentation	<9.09	1,050	2.1	–	N	6.1	–	c.175T>G p.Y59D
Hughes et al., 2010	Male	18 years	Fatigue, weight loss, depression, hyperpigmentation	125	1,000	1.32	–	N	3.4	8.88	c.76T>C p.V26A
Selva et al., 2004	Male	At birth	Hyperpigmentation, hypoglycemic seizures	0.22	170	5.72	1.14	N	N	9.79	c.106+ 2-3dupTA

N, normal.

to diminished steroidal feedback on the anterior pituitary and hypothalamus and promotes ACTH secretion, which overstimulates MC1R in melanocytes and causes hyperpigmentation [Flück et al., 2002]. As a result, all 3 patients were born with hyperpigmentation. This has also been reported in other cases, revealing that fetal corticotropes can produce excessive ACTH in response to low fetal cortisol levels, which then acts on melanocytes to promote eumelanin synthesis before birth [Ramachandran et al., 2003; Jain et al., 2011; Chen et al., 2016]. The skin hyperpigmentation in these patients faded with hydrocortisone replacement therapy; however, they demonstrated different ACTH levels. The suppression of plasma ACTH levels with physiological glucocorticoid replacement is difficult to achieve [Metherell et al., 2006]. Suppressing the ACTH levels to the normal range easily results in overtreatment, giving rise to iatrogenic Cushing's syndrome and growth failure [Metherell et al., 2006; Kirkgoz and Guran, 2018].

Almost all *MRAP* mutations are homozygous nonsense or splice site mutations that result in severe truncation or ablation of the protein [Berruien and Smith, 2020]. We analyzed 8 cases from the literature with detailed clinical information regarding mutations in *MRAP* (Table 2). Hyperpigmentation and hypoglycemia symptoms were the most common clinical manifestations. As shown in Table 2, two patients with frameshift mutations and three with splice site mutations had earlier onset. The clinical manifestations of one patient with a frameshift mutation were more severe, including global developmental delay and microcephaly. Our patient 1 presented with mild global developmental delay and one sibling died in infancy. In contrast, patients with a missense mutation had a later onset. A homozygous deletion of one nucleotide at the canonical 5α donor splice site (c.106+1delG) in intron 3 was found in 4 of these patients, including the 2 patients in this study. A recent study described 24 cases with this mutation, accounting for 30% of the 78 reported cases with *MRAP* mutation [Heshmatzad et al., 2020]. Metherell et al. [2005] highlighted that several of these mutations disrupt or substitute essential residues at the intron 3 donor splice site and are likely to result in a transcript with a foreshortened open reading frame encoding a prematurely terminated translation product. This mutation leads to the skipping of exon 3 (no protein or lack of a transmembrane domain) [Jain et al., 2011], resulting in MC2R remaining within the endoplasmic reticulum and failing to

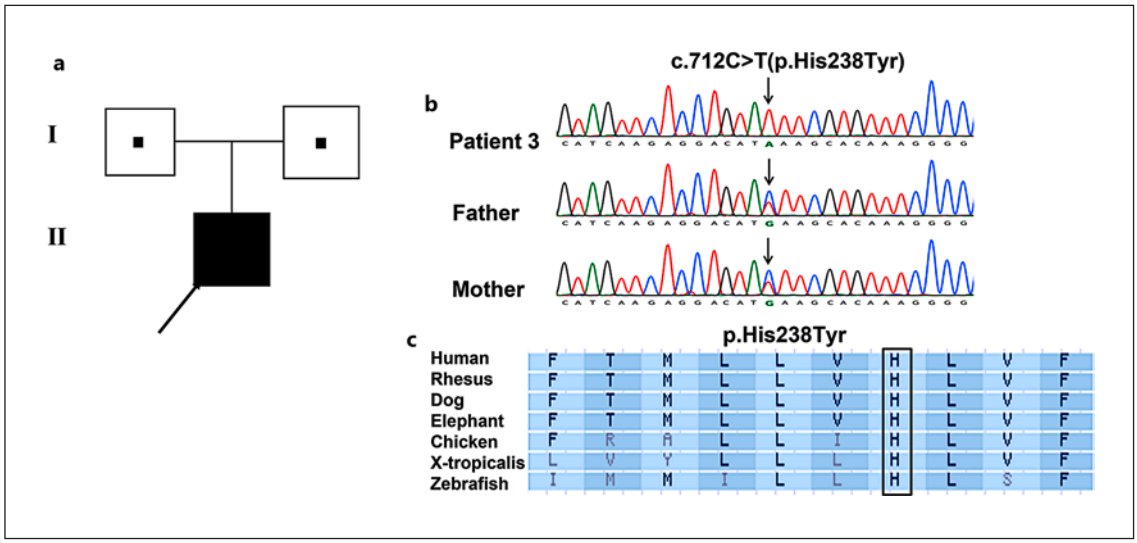

Fig. 2. Mutations in *MC2R* (c.712C>A, p.His238Tyr). **a** Family pedigree of patient 3. **b** Sanger sequencing of patient 3 and her parents. **c** Multiple sequence alignment of selected MC2R orthologues. Changes of the amino acid sequences due to *MC2R* mutation are framed.

reach the cell surface. Our patients, like the majority of splicing variant patients, had an early onset, with hyperpigmentation and hypoglycemia symptoms at birth. Interestingly, laboratory findings at birth revealed hyponatremia and/or hyperkalemia in patients 1 and 2, which differ from the currently accepted opinion that FGD patients have a normal mineralocorticoid function. Nevertheless, minor impairment of the renin-angiotensin-aldosterone axis is sometimes seen at the time of initial presentation [Clark and Weber, 1998]. It has been suggested that a partial mineralocorticoid-deficient state exists in patients with "severe genotypes" [Lin et al., 2007; Chan et al., 2008]. Re-evaluation would be beneficial for patients with minor changes in renin/aldosterone levels, as this would have a significant impact on their long-term management and genetic counseling. The 2 patients with *MRAP* splicing mutations described here were treated with 9α-FHC due to transient hyponatremia. The administration of 9α-FHC was stopped as mineralocorticoid function was found to be normal after re-evaluation. Guran et al. [2016] have also reported that fewer children with *NNT* (2 out of 7; 28%), *MRAP* (2 of 9; 22%), and *MC2R* (2 of 25; 8%) defects required mineralocorticoid replacement because of hyponatremia. Although children with FGD do not typically have a salt loss, transient hyponatremia has been reported in several children, sometimes leading to a misdiagnosis of adrenal hypoplasia. Transient hyponatremia complicates the diagnosis of FGD patients and emphasizes the importance of genetic testing.

Patients diagnosed with FGD type 1 typically present with prolonged jaundice, hyperpigmentation, hypoglycemia or hypoglycemic convulsion, failure to thrive, and sepsis. More than 100 patients with *MC2R* mutations have been reported so far. Missense mutations, nonsense mutations, frameshift mutations, and regulatory variants were among the 59 variants reported [Heshmatzad et al., 2020]. The clinical manifestations of nonsense mutations and insertion mutations were more severe in heterozygous mutations, including repeated hypoglycemic convulsions, infections, and hepatitis. Nonsense or splice site mutations result in the abolition of functional proteins (with a severe phenotype), whereas missense mutations give rise to proteins with some residual function (with a milder phenotype) [Chung et al., 2010]. Patient 3 was referred to the hospital at 3 years of age because she had a homozygous missense mutation with a milder phenotype. In vitro studies have revealed that missense mutations in *MC2R* cause varying degrees of impaired trafficking in reduced receptor expression. The missense variant c.712C>A (p.His238Tyr) in *MC2R* of our patient 3 resides in residues that are highly conserved, indicating its functional importance (Fig. 2). This was identified as a novel missense variant. The same amino acid change was seen in a patient with c.712C>T (p.H238Y) in *MC2R* [Abuduxikuer et al., 2019]. H238 is located in transmembrane domain 6, which is one of 7 sites binding with ACTH. A mutation at the same amino acid residue (H238A) resulted in a 1.4-fold decrease in MC2R membrane expression

and significantly reduced ACTH binding affinity and signaling in an in vitro functional study. According to the findings, the H238Y variant may influence cortisol production by affecting MC2R localization and ACTH binding [Chen et al., 2007]. These findings also suggest that the H238Y mutation in patient 3 might have the same effect on MC2R localization and ACTH binding, leading to cortisol deficiency.

A study on a series of patients with FGD resulting from various *MC2R* mutations found that the longitudinal height of these patients was approximately two standard deviations greater than normal [Elias et al., 2000]. Tall stature was observed in 8 of 22 cases with *MC2R* mutations [Abuduxikuer et al., 2019]. Patient 3 was taller than her target height at the time of diagnosis. Because the relationship between the length of exposure to high ACTH or low glucocorticoid levels and tall stature has not been statistically confirmed, the cause of this inappropriate height is unknown [Chung et al., 2010]. Several studies have described some reasonable possible causes of tall stature. An in vitro study reported those high levels of ACTH promoted chondrogenesis in multipotential progenitor cells and matrix production in chondrocytes [Evans et al., 2004]. Another study showed that the synthesis of insulin-like growth factor-binding protein-5 (IGFBP-5) is blocked by cortisol in bone cell cultures [Gabbitas et al., 1996]. Therefore, cortisol deficiency may fail to inhibit the growth of IGFBP-5, resulting in a tall stature. Elias et al. [2000] did not find elevated levels of growth hormone and insulin-like growth factor-1 among patients with *MC2R* mutations, implying that there are no abnormalities in the growth hormone/insulin-like growth factor-1 axis in these patients. Although growth hormone testing was not performed in our study, no manifestations of acromegaly were observed. This observation may, to some extent, suggest that *MC2R* does not affect height through the activation of growth hormone/insulin-like growth factor-1 axis. A mouse model of MC2R-deficient ($MC2R^{-/-}$) mice displayed abnormal bone metabolism, but no growth abnormality was observed [Sato et al., 2020]. B6N5 generations of $MC2R^{-/-}$ mice showed normal development and function of reproductive organs but did not grow abnormally [Chida et al., 2009]. Because mouse models are unable to fully simulate complex phenotypes in humans, they are unable to explain changes in human growth. To date, no clear mechanism has been identified as the cause of FGD type 1 patients' tall stature.

In FGD type 2 patients, tall stature has not been reported. We observed that the growth and development of patients 1 and 2 were normal (Table 1). It is conceivable that MC2R may restrain height via an unknown pathway. Mutations in *MC2R* eliminate inhibition of growth and result in growth acceleration. This pathway may be different from androgen-stimulated growth due to normal bone age of patient 3, and her height can return to the normal hereditary levels after glucocorticoid supplementation (16 $mg/m^2/day$). During hydrocortisone treatment, patient 3 had a normal BMI and did not show signs of Cushing's syndrome. Meanwhile, she did not exhibit delayed bone age, so we tend to believe that the normalization of height was related to adequate replacement of hydrocortisone rather than the overtreatment. However, since we did not assess bone age prior to treatment, it is difficult to determine whether the pretreatment bone age of children is normal or delayed. As a result, further follow-up is required.

We observed significant distinctions between FGD type 1 and FGD type 2 concerning the age of presentation and height. Most patients with FGD type 1 are prone to present later and are taller compared with FGD type 2 patients [Chung et al., 2010]. Similarly, patients 1 and 2, who were diagnosed with FGD type 2, had earlier onset than patient 3. Patient 3 was taller than both patients 1 and 2.

In conclusion, we report 3 patients, two of whom harbored the same variation in *MRAP*. The clinical features of the 2 FGD type 2 patients were different. One of them suffered from repeated hypoglycemic attacks. All 3 patients had hyperpigmentation and hypoglycemia, both of which were linked to insufficient cortisol secretion. Of note, 2 patients presented with hyponatremia and/or hyperkalemia at birth due to severe *MRAP* genotypes of c.106+1delG. One patient (patient 3), who carried a novel missense mutation in *MC2R*, displayed milder symptoms. She initially presented with a taller stature. Her height gradually approached the midparental height by supplementation with glucocorticoids. Although the patients' clinical manifestations were different, hydrocortisone treatment was effective. Accurate diagnosis and appropriate therapy enable patients diagnosed with FGD to thrive normally. The mechanism underlying tall stature in some FGD type 1 patients needs to be investigated further.

Acknowledgements

We thank all the families and their physicians for their participation in this study.

Statement of Ethics

This study was approved by Xinhua Hospital Ethics Committee Affiliated to Shanghai Jiao tong University School of Medicine (XHEC-WJW-2019-045). Written informed consent for publication of clinical details was obtained from the patients' parents.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This work was financially supported by Shanghai Municipal Health Commission Project (201940226 to Wenjuan Qiu), Clinical Research Center For Primary Adrenal Insufficiency Pediatric College, Shanghai Jiao Tong University School of Medicine (ELYZX202106 to Wenjuan Qiu), and Shanghai Municipal Nature Science Foundation (19ZR1442100 to Bing Xiao). Funders had no role in the study design, data collection, data analysis, interpretation of results, and writing of the manuscript.

Author Contributions

Yi Yang, Kaichuang Zhang, Lili Liang, and Ruifang Wang evaluated the clinical features. Ying Duan and Yu Xia collected the clinical data of patients. Lili Liang annotated the WES data and called copy number variations through WES depth data. Huili Liu and Zhuwen Gong analyzed the ES data of the patients. Bing Xiao designed and performed the mutation verification assay. Ying Duan, Yu Xia, Bing Xiao, and Wenjuan Qiu wrote the manuscript. All authors have read and approved the final manuscript.

Data Availability Statement

The datasets used and analyzed during the current study are available from the corresponding author upon request. The phenotypes and mutations of patient 3 were submitted to Leiden Open Variation Database (https://databases.lovd.nl/shared/individuals) under accession number #00316036.

References

Abuduxikuer K, Li ZD, Xie XB, Li YC, Zhao J, Wang JS. Novel Melanocortin 2 Receptor Variant in a Chinese Infant With Familial Glucocorticoid Deficiency Type 1, Case Report and Review of Literature. Front Endocrinol (Lausanne). 2019;10:359.

Akın L, Kurtoğlu S, Kendirici M, Akın MA. Familial glucocorticoid deficiency type 2: a case report. J Clin Res Pediatr Endocrinol. 2010;2(3):122–5.

Amano N, Narumi S, Hayashi M, Takagi M, Imai K, Nakamura T, et al. Genetic defects in pediatric-onset adrenal insufficiency in Japan. Eur J Endocrinol. 2017;177(2):187–94.

Berruien NNA, Smith CL. Emerging roles of melanocortin receptor accessory proteins (MRAP and MRAP2) in physiology and pathophysiology. Gene. 2020;757:144949.

Buonocore F, Maharaj A, Qamar Y, Koehler K, Suntharalingham JP, Chan LF, et al. Genetic Analysis of Pediatric Primary Adrenal Insufficiency of Unknown Etiology: 25 Years' Experience in the UK. J Endocr Soc. 2021;5(8):bvab086.

Chan LF, Clark AJL, Metherell LA. Familial glucocorticoid deficiency: advances in the molecular understanding of ACTH action. Horm Res. 2008;69(2):75–82.

Chen C, Zhou R, Fang Y, Jiang L, Liang L, Wang C. Neonatal presentation of familial glucocorticoid deficiency with a MRAP mutation: A case report. Mol Genet Metab Rep. 2016;9:15–7.

Chen M, Aprahamian CJ, Kesterson RA, Harmon CM, Yang Y. Molecular identification of the human melanocortin-2 receptor responsible for ligand binding and signaling. Biochemistry. 2007;46(40):11389–97.

Chida D, Sato T, Sato Y, Kubo M, Yoda T, Suzuki H, et al. Characterization of mice deficient in melanocortin 2 receptor on a B6/Balbc mix background. Mol Cell Endocrinol. 2009;300(1-2):32–6.

Chung TTLL, Chan LF, Metherell LA, Clark AJL. Phenotypic characteristics of familial glucocorticoid deficiency (FGD) type 1 and 2. Clin Endocrinol (Oxf). 2010;72(5):589–94.

Clark AJ, Weber A. Adrenocorticotropin insensitivity syndromes. Endocr Rev. 1998;19(6):828–43.

Cooray SN, Chan L, Metherell L, Storr H, Clark AJL. Adrenocorticotropin resistance syndromes. Endocr Dev. 2008;13:99–116.

de Peretti E, Mappus E. Pattern of plasma pregnenolone sulfate levels in humans from birth to adulthood. J Clin Endocrinol Metab. 1983;57(3):550–6.

Elias LL, Huebner A, Metherell LA, Canas A, Warne GL, Bitti ML, et al. Tall stature in familial glucocorticoid deficiency. Clin Endocrinol (Oxf). 2000;53(4):423–30.

Evans JF, Niu QT, Canas JA, Shen CL, Aloia JF, Yeh JK. ACTH enhances chondrogenesis in multipotential progenitor cells and matrix production in chondrocytes. Bone. 2004;35(1):96–107.

Flück CE, Martens JWM, Conte FA, Miller WL. Clinical, genetic, and functional characterization of adrenocorticotropin receptor mutations using a novel receptor assay. J Clin Endocrinol Metab. 2002;87(9):4318–23.

Fokkema IFAC, Taschner PEM, Schaafsma GCP, Celli J, Laros JFJ, den Dunnen JT. LOVD v.2.0: the next generation in gene variant databases. Hum Mutat. 2011;32(5):557–63.

Gabbitas B, Pash JM, Delany AM, Canalis E. Cortisol inhibits the synthesis of insulin-like growth factor-binding protein-5 in bone cell cultures by transcriptional mechanisms. J Biol Chem. 1996;271(15):9033–8.

Guran T, Buonocore F, Saka N, Ozbek MN, Aycan Z, Bereket A, et al. Rare Causes of Primary Adrenal Insufficiency: Genetic and Clinical Characterization of a Large Nationwide Cohort. J Clin Endocrinol Metab. 2016;101(1):284–92.

Habeb AM, Hughes CR, Al-Arabi R, Al-Muhamadi A, Clark AJL, Metherell LA. Familial glucocorticoid deficiency: a diagnostic challenge during acute illness. Eur J Pediatr. 2013;172:1407–10.

Heshmatzad K, Mahdieh N, Rabbani A, Didban A, Rabbani B. The Genetic Perspective of Familial Glucocorticoid Deficiency: In Silico Analysis of Two Novel Variants. Int J Endocrinol. 2020;2020:2190508.

Hughes CR, Chung TT, Habeb AM, Kelestimur F, Clark AJL, Metherell LA. Missense mutations in the melanocortin 2 receptor accessory protein that lead to late onset familial glucocorticoid deficiency type 2. J Clin Endocrinol Metab. 2010;95(7):3497–501.

Jain V, Metherell LA, David A, Sharma R, Sharma PK, Clark AJL, et al. Neonatal presentation of familial glucocorticoid deficiency resulting from a novel splice mutation in the melanocortin 2 receptor accessory protein. Eur J Endocrinol. 2011;165(6):987–91.

Kirkgoz T, Guran T. Primary adrenal insufficiency in children: Diagnosis and management. Best Pract Res Clin Endocrinol Metab. 2018;32(4):397–424.

Kulle AE, Riepe FG, Melchior D, Hiort O, Holterhus PM. A novel ultrapressure liquid chromatography tandem mass spectrometry method for the simultaneous determination of androstenedione, testosterone, and dihydrotestosterone in pediatric blood samples: age- and sex-specific reference data. J Clin Endocrinol Metab. 2010;95(5):2399–409.

Lin L, Hindmarsh PC, Metherell LA, Alzyoud M, Al-Ali M, Brain CE, et al. Severe loss-of-function mutations in the adrenocorticotropin receptor (ACTHR, MC2R) can be found in patients diagnosed with salt-losing adrenal hypoplasia. Clin Endocrinol (Oxf). 2007;66(2):205–10.

Meimaridou E, Hughes CR, Kowalczyk J, Guasti L, Chapple JP, King PJ, et al. Familial glucocorticoid deficiency: New genes and mechanisms. Mol Cell Endocrinol. 2013;371(1-2):195–200.

Metherell LA, Chapple JP, Cooray S, David A, Becker C, Rüschendorf F, et al. Mutations in MRAP, encoding a new interacting partner of the ACTH receptor, cause familial glucocorticoid deficiency type 2. Nat Genet. 2005;37:166–70.

Metherell LA, Chan LF, Clark AJL. The genetics of ACTH resistance syndromes. Best Pract Res Clin Endocrinol Metab. 2006;20(4):547–60.

Modan-Moses D, Ben-Zeev B, Hoffmann C, Falik-Zaccai TC, Bental YA, Pinhas-Hamiel O, et al. Unusual presentation of familial glucocorticoid deficiency with a novel MRAP mutation. J Clin Endocrinol Metab. 2006;91(10):3713–7.

Ramachandran P, Penhoat A, Naville D, Begeot M, Osama Abdel-Wareth L, Reza Sedaghatian M. Familial glucocorticoid deficiency type 2 in two neonates. J Perinatol. 2003;23(1):62–6.

Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405–24.

Rumié H, Metherell LA, Clark AJL, Beauloye V, Maes M. Clinical and biological phenotype of a patient with familial glucocorticoid deficiency type 2 caused by a mutation of melanocortin 2 receptor accessory protein. Eur J Endocrinol. 2007;157(4):539–42.

Sato T, Iwata T, Usui M, Kokabu S, Sugamori Y, Takaku Y, et al. Bone phenotype in melanocortin 2 receptor-deficient mice. Bone Rep. 2020;13:100713.

Selva KA, LaFranchi SH, Boston B. A novel presentation of familial glucocorticoid deficiency (FGD) and current literature review. J Pediatr Endocrinol Metab. 2004;17(1):85–92.

Sun Y, Hu G, Liu H, Zhang X, Huang Z, Yan H, et al. Further delineation of the phenotype of truncating KMT2A mutations: The extended Wiedemann-Steiner syndrome. Am J Med Genet A. 2017;173(2):510–4.

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(5):2458–61.