Familial Glucocorticoid Deficiency: Advances in the Molecular Understanding of ACTH Action

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Key Words

Familial glucocorticoid deficiency • MC2R/ACTHR • *MRAP* • ACTH resistance

Abstract

Familial glucocorticoid deficiency (FGD), otherwise known as hereditary unresponsiveness to ACTH, is a rare autosomal recessive disease characterized by glucocorticoid deficiency in the absence of mineralocorticoid deficiency. Mutations of the ACTH receptor, also known as the melanocortin-2 receptor (MC2R), account for approximately 25% of FGD cases. More recently a second gene, *MRAP* (melanocortin-2 receptor accessory protein), was identified and found to account for a further 15–20%. *MRAP* encodes a small single transmembrane domain protein, which is essential in the trafficking of the MC2R to the cell surface. In this review, we will firstly summarize the clinical presentation and genetic aetiology of this condition. Secondly, we will discuss how the discovery of *MRAP* has enhanced our understanding of the mechanisms of ACTH/MC2R action. Finally, we will explore future developments in this field.

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Introduction

Over the past 15 years significant advances have been made in the understanding of the genetic basis of adrenal disease with regard to primary and secondary causes of adrenal insufficiency. The discovery of individual gene defects has contributed to understanding the mechanisms controlling adrenal gland development and steroidogenesis pathways (some examples are shown in table 1). The study of adrenal resistance syndromes, namely triple A syndrome and in particular familial glucocorticoid deficiency (FGD), has provided insight into the mechanism of ACTH action.

We searched PubMed for articles published between 1970 and 2006 using the search terms 'familial glucocorticoid deficiency', 'ACTH resistance' and 'adrenal unresponsiveness'. We also searched the reference lists of identified articles for further papers.

The triple A syndrome is a complex multisystem disorder characterized by adrenal failure, alacrima and achalasia. Not all patients have adrenal involvement, but of those with adrenal insufficiency approximately 80% will have isolated glucocorticoid deficiency. This condition has been reviewed elsewhere and will not be covered here [1].

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0301–0163/08/0692–0075\$24.50/0

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Fig. 1. Schematic diagram of the human MC2R showing the locations of all the known polymorphisms and mutations. Each circle represents an amino acid residue. The *F278C mutation is the only known activating MC2R mutation [reprinted from 62, with permission].

Table 1. Examples of single gene defects in inherited forms of primary adrenal insufficiency

Disease	OMIM	Gene
Congenital adrenal hyperplasia		
21-Hydroxylase deficiency	201910	Cyp21
11 β -Hydroxylase deficiency	202010	Cyp11B1
3 β -Hydroxysteroid dehydrogenase deficiency	109715	HSD3B2
17 α -Hydroxylase deficiency	202110	Cyp17
Lipoid adrenal hyperplasia	201710	STAR
Congenital adrenal hypoplasia	300200	NR0B1 (DAX-1)
	300473	NR5A1 (SF-1)
Familial glucocorticoid deficiency		
Type 1	202200	MC2R
Type 2	609196	MRAP
Type 3	609197	unknown
Triple A syndrome	202110	AAAS
X-linked adrenoleucodystrophy	300371	ABCD1
Autoimmune polyglandular syndrome type 1	240300	AIRE

FGD (OMIM 202200) was first described by Shepard et al. [2] in 1959. The report of two siblings with what was called ‘familial Addison’s disease’ prompted subsequent papers describing patients with isolated glucocorticoid deficiency [3–5]. It took a further 30 years before the identification of the first inactivating ACTH receptor mutation in a patient with FGD [6]. Since then a large number of mutations have been found which are summarized in figure 1. The ACTH receptor was the second melanocortin receptor to be cloned hence the alternative nomenclature of MC2R [7].

FGD is characterized by glucocorticoid deficiency in the presence of normal plasma renin and aldosterone levels. Nevertheless, mild derangements of the renin-angiotensin system at the time of diagnosis are sometimes seen with this condition [8], and it has been suggested that a partial mineralocorticoid-deficient state exists in those patients with ‘severe genotypes’ [9]. This may mean that in some cases a diagnosis cannot be made using clinical and biochemical parameters alone. The finding of mutations in the MC2R gene and the more recently described MRAP gene would provide a definitive diagnosis. How-

ever, genetic defects in *MC2R* and *MRAP* only account for 40–45% of cases, leaving the remaining 55–60% of patients with no identifiable gene defect.

Clinical and Biochemical Features of FGD

Presentation

Patients with FGD are usually diagnosed during the neonatal period or by early childhood. They may present with hypoglycaemic seizures, hyperpigmentation, recurrent infections, failure to thrive, collapse and coma. Transient neonatal hepatitis has also been described as a presenting feature [10]. All the symptoms and signs seen in FGD are the result of either hypocortisolaemia or elevated ACTH levels. The long-term neurological consequence of FGD can vary from learning difficulties to spastic quadriplegia, which may reflect the severity and number of hypoglycaemic episodes during childhood [11]. There may be a family history of unexplained neonatal deaths, the presence of another affected family member and/or consanguinity.

Assessment

FGD can usually be distinguished from other causes of primary adrenal insufficiency by the absence of mineralocorticoid deficiency and associated features seen in such conditions. This would include progressive neurological manifestations (triple A, adrenoleucodystrophy), ambiguous genitalia (congenital adrenal hyperplasia, CAH), other dysmorphic features (IMAGe), hypogonadism, delayed puberty (congenital adrenal hypoplasia), alacrima/achalasia (triple A) and presence of other autoimmune deficiencies (polyglandular autoimmune syndromes). These differential diagnoses should always be considered prior to the diagnosis of FGD and in some instances specific investigations may be required. For example, adrenal antibodies, very-long-chain fatty acid measurement, 17-OHP, Schirmer test of tear production together with a barium swallow could help to differentiate autoimmune cause of adrenal insufficiency, adrenoleucodystrophy, CAH and triple A syndrome, respectively. Monitoring of blood pressure is an essential part of assessment and may diagnose some forms of CAH presenting with hypertension.

Specific Features of FGD

Tall Stature

Tall stature is observed in some FGD patients with *MC2R* mutations [12, 13]. In addition, a proportion of

these patients have been shown to have advanced or dissociated bone age, e.g. advanced bone maturation in the radius and phalanges with delay seen in the carpal bones. This excessive growth is more noticeable prior to the initiation of treatment, and although hydrocortisone replacement appears to bring the height back towards the mid-parental target, children remain tall as adults [8]. The rather limited data available suggests that the insulin-like growth factor I-growth hormone axis is normal in these patients [12]. It has been proposed that the excessive growth is due to high plasma ACTH levels [13]. The presence of all five melanocortin receptors in bone and the ability of ACTH to stimulate cAMP production and gene expression in bone cells provide a plausible mechanism [12, 14, 15]. However, not all FGD patients with inactivating mutations of the *MC2R* are tall, short stature has also been described [16].

Absent Adrenarche

ACTH is required in the regulation of adrenarche in normal children and absent adrenarche is a feature seen in children with FGD. Clinically this presents as delayed or absent pubic hair development associated with extremely low/undetectable adrenal androgen levels in children >7 years of age and those in puberty [17]. Ishii et al. [18] report a FGD patient with sparse pubic hair in adulthood despite normal progression in puberty and regular menses, demonstrating the importance of ACTH in the induction and subsequent maintenance of adrenarche and pubic hair attainment. In contrast, true puberty defined as breast development and subsequent ovarian development, controlled by the hypothalamic-pituitary-gonadal axis, is unaffected in FGD.

Markedly High Plasma ACTH

Most FGD patients have markedly raised plasma ACTH levels reflecting the extent of resistance. However, suppression of plasma ACTH levels in FGD can be very difficult despite treatment with high doses of hydrocortisone. The reason for this apparent lack of suppression is unclear. There is some suggestion of the existence of a short ACTH negative feedback loop at the level of the pituitary/hypothalamus. Hence, patients with mutations which render the *MC2R* inactive would lack negative inhibition of ACTH release at this level [19–22].

Hyperpigmentation

Hyperpigmentation is almost always observed in FGD. The child often develops pigmentation by the 1st month of life and usually remains pigmented despite treatment.

Pigmentation is generally thought to be due to the high ACTH levels which act on the melanocortin-1 receptors in melanocytes.

Investigations

Biochemistry

The typical biochemical results in FGD are a combination of low cortisol levels paired with extremely high plasma ACTH levels, in the presence of normal plasma renin and aldosterone. ACTH levels of above 1,000 pg/ml (normal range <80 pg/ml by RIA; <50 pg/ml by IRMA) are commonly found [8, 23]. Occasionally, children may have minor impairment of the renin-angiotensin-aldosterone axis at the time of initial presentation [8, 24]. The physiological explanation of this observation is unclear. Two factors may play a part. Firstly, there is evidence that ACTH directly activates the production of aldosterone in the zona glomerulosa (ZG). It has been found that MC2R mRNA localizes to the ZG and administration of synthetic ACTH to normal human subjects results in a rise in plasma aldosterone levels [5, 24–27]. The physiological importance of ACTH-dependent regulation of aldosterone in normal physiology and disease is undetermined. Interestingly, the ability of ACTH to stimulate aldosterone production may stem from the role of ACTH in the normal organization of the ZG during development of the adrenal gland, as suggested by the abnormal ZG histology seen in patients with FGD (see later). Secondly, during periods of illness and acute stress, changes in the renin-angiotensin-aldosterone axis are seen [8, 28]. The difficulties in dissecting the biochemical results in a sick child and the potential of misdiagnosing patients presenting with adrenal insufficiency and mild renin-angiotensin-aldosterone disturbance were exemplified by Lin et al. [9]. They identified 7 children diagnosed with probable primary adrenal hypoplasia who on genetic evaluation were found to have FGD with MC2R mutations. This enabled the withdrawal of fludrocortisone treatment in a number of patients. However, there was a suggestion that a partial mineralocorticoid-deficient state might exist in some 'MC2R severe loss of function mutations'. Patients with minor changes in renin/aldosterone levels would benefit from re-evaluation as this would have significant implications on their long-term management and genetic counselling [8, 9].

Blood glucose monitoring is essential in any child who is unwell and in FGD hypoglycaemia may result in a number of symptoms ranging from failure to thrive to coma. Dynamic testing with the short ACTH stimulation

test (Synacthen® = 1–24 ACTH) will confirm a diagnosis of primary adrenal insufficiency. A normal response is defined as a peak plasma cortisol level of more than 550 nmol/l. There is evidence that some patients with FGD have a normal response to a short Synacthen test initially which on re-testing in later life becomes abnormal (unpubl. observation). Additional investigations may help with differentiating FGD from other causes of primary adrenal insufficiency. For example, detectable adrenal autoantibodies can indicate autoimmune Addison's disease. Elevated levels of very-long-chain fatty acids would suggest adrenoleucodystrophy. Adrenal androgen levels including 17-OHP and urinary steroid chromatography would help to differentiate CAH.

Despite careful clinical and biochemical evaluation some patients may not easily fall into a diagnostic category. In such patients diagnosed with 'primary adrenal insufficiency', mutational analysis of genes such as those listed in table 1 has proved successful [29].

Adrenal Imaging

Adrenal imaging in the form of MRI/CT scanning can be helpful. In FGD the glands are usually small in size [8]. This is in contrast to tuberculosis (calcified adrenal glands), CAH (enlargement), storage disorders and infiltrative disorders (enlargement of the gland).

Histopathology

There are several histopathology studies from cases where a sibling has died prior to the diagnosis being made on the index child. These report the absence of fasciculated or reticularis cells together with disorganization of granulosa cells [2, 8, 30, 31].

Treatment

The treatment is by replacement with hydrocortisone. An oral dose of 10–12 mg/m²/day in three divided doses in children and 20–30 mg/day in adults is usually sufficient. The suppression of plasma ACTH levels in FGD can be very difficult and should not be used as the goal of treatment. Educating parents and patients on the need to increase hydrocortisone dosages during illness and the emergency management with intramuscular hydrocortisone is vital. Recently, Crown et al. [32] reviewed the management of glucocorticoid deficiency, highlighting controversies that still exist in the 'optimal' treatment regime of such patients. Medic alert bracelet, 'steroid card' and handheld summaries should be given to all patients.

Fig. 2. Diagram illustrating the transcription and translation of MRAP. Exons 5 and 6 are alternatively spliced to generate the MRAP- α and MRAP- β isoforms, respectively. Protein prediction programmes suggest that the N-terminus of these proteins lies on the intracellular cytoplasmic surface of the membrane.

FGD and ACTH Receptor Action

*FGD Type 1 (OMIM*202200) and MC2R Mutations*

The cloning of the MC2R by Mountjoy et al. [33] in 1992 enabled researchers to identify point mutations in the ACTH receptor in patients with FGD [34–39]. The MC2R gene, located on chromosome 18q11, encodes a 297-amino acid G-protein-coupled receptor. More than 30 mutations have been described in the MC2R (fig. 1), the majority of which are homozygous missense or compound heterozygous mutations. In comparison, homozygous nonsense mutations are rare. The functional consequence of various MC2R mutations has been demonstrated by a number of groups [36, 40–44]. To date there is no strong evidence to suggest that heterozygous carriers, i.e. parents or siblings of FGD patients, have abnormal cortisol secretion or response. On detailed investigation, Tsigos et al. [34] found that the mother and grandmother of an affected child with the R201X (substitution of an arginine residue at amino acid position 201 with a stop codon) and S120R (serine → arginine at position 120) mutations had exaggerated and prolonged ACTH responses to administration of corticotropin-releasing hormone (CRH). This suggests subclinical resistance to ACTH in heterozygous carriers; however, an investigation into 4 patients heterozygous for 4 different MC2R mutations revealed normal responses to CRH in 3/4 carriers. The 4th carrier heterozygous for the L192fs (frameshift at the leucine residue, position 192) mutation had an exaggerated

increase in plasma ACTH combined with an elevated serum cortisol response to CRH. The findings in this patient were believed to be a stress response to human CRH administration as a previous ovine CRH test was reportedly normal [36]. CRH responses have been shown to vary according to ethnic background [45, 46]. The family studied by Tsigos et al. [35] were of black ethnicity which may account for the elevated ACTH response after CRH.

Discovery of MRAP and FGD Type 2

Mutational screening of the MC2R revealed that MC2R defects could only account for approximately 25% of cases [47, 48]. Linkage studies provided evidence of a second FGD locus [49] and, in parallel with this, evidence accumulated of the existence of an ‘MC2R’ accessory factor [50, 51]. Homozygosity mapping, utilizing SNP (single nucleotide polymorphisms) microarrays across the whole genome, identified a third locus on chromosome 21 in some families with FGD. Fine mapping of the region together with mRNA expression studies of genes within this region revealed a candidate gene, now known as MRAP (melanocortin-2 receptor accessory protein). This gene was found to encode a single transmembrane protein with 2 isoforms of 19 and 11.5 kDa in size derived from alternative splicing of exons 5 and 6 (fig. 2). Functional analysis of the protein revealed four key findings: (1) direct interaction with MC2R, (2) requirement for MC2R function, (3) co-localization with the MC2R and

Fig. 3. Schematic representation of the human MRAP α protein showing the locations of the mutations identified so far. All 9 mutations result in either an absent or significantly truncated protein of both isoforms.

(4) requirement for MC2R cell surface expression [52]. So far our group has identified 8 different mutations of *MRAP* in FGD patients (publ. and unpubl. data). Modan-Moses et al. [11] identified a further mutation consisting of a 7-nucleotide deletion in exon 3, resulting in a frame shift and a premature stop codon, L31X. These mutations are summarized in figure 3. In contrast to the MC2R mutations, these mutations would all result in either the complete absence of the protein or a severely truncated product.

Future for FGD Type 3, 4 and Beyond

FGD type 1 and type 2 refer to patients with MC2R and *MRAP* mutations, respectively. In clinical samples over 50% of FGD patients have no identifiable mutation in either gene – this group of patients are referred to as having FGD type 3. Linkage to chromosome 8 has been found in a minority of families with FGD type 3, although a candidate gene has yet to be identified [49], but not all families segregate to chromosome 8 suggesting the involvement of additional genes.

Receptor Accessory Proteins

The discovery of MRAP has provided a new mechanism of melanocortin receptor regulation which was previously unknown. Small single transmembrane proteins such as the RAMPs, RTPs and REEPs are a growing number of receptor accessory proteins which interact with G

protein-coupled receptors [53–55]. These accessory proteins assist receptor trafficking to the cell surface and may be able to determine ligand specificity. MRAP may also have functions in the folding and translocation of the MC2R through the endoplasmic reticulum hence acting as a molecular chaperone. The molecular properties of MRAP are being investigated by a number of groups including our own. Roy et al. [56] recently reported their work looking at the differential regulation of the human MC2R by MRAP isoforms.

Future

Single gene defects in rare diseases have been crucial to the understanding of a variety of signalling pathways. At the molecular level, *CUL-7* in 3-M syndrome [57], P450 oxidoreductase deficiency in Antley-Bixler syndrome [58], and *POMC* in the syndrome of adrenal insufficiency and red hair [59] are just some of the examples in which the study of rare diseases has provided insight into the respective signalling mechanisms. Homozygosity mapping in consanguineous families has proved to be a highly successful strategy in the mapping of rare recessive traits. One may argue that the identification of such genes in rare diseases may be of limited benefit to the population at large. However, it is difficult to quantify the benefit from improvement in the understanding of the

molecular biology of the cell. Moreover, it may be that aetiological genes in rare disorders can also play a role in conditions of high relevance to the population at large. An example of this success is the study of the melanocortin-4 receptor (MC4R) in obesity. MC4R mutations, identified initially as a rare cause of severe obesity in childhood, are now believed to be the most common monogenic cause of obesity in the general population [60, 61].

The discovery of mutations in genes that cause FGD now enables a definitive genetic diagnosis of FGD in some cases. Such a diagnostic test not only has relevance for diagnosing new cases of FGD but also revisiting of patients thought to have adrenal failure of unknown cause. Isolated glucocorticoid deficiency should still be considered the norm. However, in cases where there may have been derangements of renin-aldosterone levels at

the time of diagnosis and those who may have slight derangements, these children may benefit from review of their mineralocorticoid status and genotyping of the MC2R/MRAP genes.

At present a genetic diagnosis is not possible for a large proportion of patients and the aetiology of FGD type 3 patients needs to be elucidated. It is hoped that such discoveries will shed more light on the ACTH signalling pathway and the diseases which arise when regulation of this pathway fails in underactive and overactive states.

Acknowledgments

L.F.C. is supported by a Clinical Research Training Fellowship from the Medical Research Centre, and L.A.M. by the Wellcome Trust.

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