

CLINICAL STUDY

Isolated Addison's disease is unlikely to be caused by mutations in MC2R, MRAP or STAR, three genes responsible for familial glucocorticoid deficiency

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Abstract

Background: Familial glucocorticoid deficiency (FGD) is a rare autosomal recessive disease caused by ACTH resistance and leads to isolated glucocorticoid deficiency. Although FGD patients typically have normal mineralocorticoid secretion, subtle alterations in the renin–angiotensin–aldosterone axis have been reported in a subset of patients at presentation. Anecdotally, some patients with FGD have been initially diagnosed as having Addison's disease (AD), with implications for treatment and genetic counselling. Currently, mutations in three genes: the ACTH receptor (*MC2R*); the melanocortin 2 receptor accessory protein (*MRAP*); and the steroidogenic acute regulatory protein (*STAR*) are known to give rise to FGD types 1–3. We investigated a cohort of autoantibody-negative AD patients for mutations in these genes. **Methods:** Forty patients with known AD without evidence of autoimmune disease were screened for mutations in *MC2R*, *MRAP* and *STAR*. In addition, patients were genotyped for the *MC2R* promoter polymorphism previously associated with reduced responsiveness to ACTH.

Results: No mutations in *MC2R*, *MRAP* or *STAR* were identified in any patient. The frequencies of the *MC2R* promoter polymorphism were similar to those reported in healthy controls.

Conclusions: FGD does not appear to be underdiagnosed in the AD population. However, in ~50% of patients with FGD, no genetic cause has yet been identified and it is possible that the other, as yet unidentified, genes giving rise to FGD may be implicated in AD.

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Introduction

Primary adrenal failure is relatively rare with a prevalence of approximately five cases per 100 000 in Northern Europe (1, 2). The initial cases described by Addison in 1855 involved tuberculosis, adrenal malignancy and idiopathic adrenal fibrosis, which is now thought to be the first report of autoimmune adrenal disease (3). Currently, autoimmune Addison's disease (AAD) accounts for the majority of cases of primary adrenal failure in western countries (up to 94%) (1). AAD can occur either in conjunction with other autoimmune conditions (autoimmune polyendocrine syndromes), or in isolation. Isolated AAD with no other autoimmune clinical manifestations occurs in about 40% of cases, but positive autoantibodies have been shown to be present in up to 80% of these (4).

Primary adrenal insufficiency (or antibody-negative AD) covers a broad spectrum of pathologies including malignant, infectious and genetic causes. Genetic causes range from autosomal or X-linked recessive conditions such as familial ACTH resistance syndromes

(familial glucocorticoid deficiency (FGD), Triple A), congenital adrenal hyperplasia and adrenoleukodystrophy to mitochondrial disorders such as Kearns-Sayres syndrome.

FGD (OMIM 202200) is an autosomal recessive condition characterised by ACTH resistance leading to isolated glucocorticoid deficiency with normal mineralocorticoid secretion. The syndrome was first described by Shepard *et al.* in 1959 in two siblings who had initially been diagnosed with AD (5). However, although patients with FGD have normal renin and aldosterone levels, subtle abnormalities of the renin–angiotensin axis are sometimes seen in this condition at diagnosis, most notably with nonsense mutations of the ACTH receptor (*MC2R*) (6, 7). In contrast, the recently described *MC2R* knockout mouse model demonstrates classical adrenal failure with both glucocorticoid and mineralocorticoid deficiency (8).

The first inactivating mutation of the *MC2R* (also known as the melanocortin type 2 receptor) in a patient with FGD was reported in 1993 (9), and subsequently more than 30 mutations in the *MC2R* have been

found (10). In 2004, a polymorphism in the *MC2R* promoter at position 2 was described as having decreased response to ACTH both *in vitro* and in normal human subjects following i.v. ACTH infusion (11). More recently, mutations in a novel gene, the melanocortin 2 receptor accessory protein (*MRAP*), which is involved in trafficking *MC2R* to the plasma membrane, have been described in FGD (12). In 2009, Metherell *et al.* reported that a proportion of patients with FGD (5–10%) have mutations in *STAR* (13). *STAR* mutations usually give rise to a severe form of adrenal insufficiency known as lipoid congenital adrenal hyperplasia (LCAH; OMIM 201701), leading to both mineralocorticoid and glucocorticoid deficiency. Mutations in these three genes lead to FGD types 1, 2 and 3 respectively and account for just over 50% of all known clinical cases of FGD. Furthermore, there are reports of patients initially diagnosed with AD subsequently having their diagnosis refined to FGD, allowing them to omit their mineralocorticoid replacement therapy (5). Of note, two families found to have *STAR* mutations leading to adrenal failure were initially diagnosed with non-AAD. Having noted that there appear to be certain mutations in *MC2R* and *STAR* causing adrenal failure with both mineralocorticoid and glucocorticoid abnormalities, we investigated here the possibility that a subset of patients with isolated AD without evidence of autoimmune disease may have mutations in *MC2R*, *MRAP* or *STAR*. In addition, we screened the AD cohort for possible increased frequency of the *MC2R* promoter polymorphism associated with reduced responsiveness to ACTH.

Subjects and methods

Patients

Forty patients (17 males and 23 females) with isolated AD were identified from a previously described cohort of patients studied in the north-east of England (14). The mean age of diagnosis was 36.5 years (range 8–79 years). All patients demonstrated a peak cortisol response of <550 nmol/l following a short synacthen test (250 µg synthetic ACTH administered i.m). All patients had a diagnosis of primary adrenal failure (secondary adrenal failure, infiltrative and infective causes were excluded). All patients with evidence of autoimmune disease or polyendocrinopathy (positive adrenal autoantibody screen by immunofluorescence or other organ system affected, e.g. thyroid dysfunction, vitiligo, type 1 diabetes mellitus and pernicious anaemia) were excluded. Patients with any family history of autoimmune disease were excluded. No patient had formal investigation of the renin–angiotensin axis, although none were known to have maintained normal mineralocorticoid secretion. There was one familial case of AD in the cohort (mother diagnosed at the age of 28 years and her son diagnosed at the age of 8).

DNA extraction

Genomic DNA was extracted from whole blood using the Nucleon BACC DNA extraction kit according to the manufacturer's instructions (GE Healthcare, Buckinghamshire, UK).

Sequencing

MC2R has a single-coding exon. It has previously been reported that the majority of the mutations in *MRAP* in FGD patients are in exon 3. Exon 5 of *STAR* is where all mutations in non-classical LCAH and/or FGD have been described (6, 13, 15), whereas mutations found in other exons in this gene cause the classical LCAH phenotype.

PCR was carried out using published primers directed to intronic sequences to cover the coding exon of *MC2R*, exon 3 of *MRAP* and exons 5 and 6 of *STAR* in the entire AD cohort (12, 13). Cycling conditions were 95 °C for 5 min (1 cycle); 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s (30 cycles); and 72 °C for 5 min. PCR products were visualized on 1% agarose gel, and PCR fragments were sequenced using the ABI Prism Big Dye Sequencing kit and an ABI 3700 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA) in accordance with the manufacturer's instructions.

Genotyping

PCR was carried using previously published primers around the –2 *MC2R* promoter polymorphism (11). PCR products were digested with *SacI* giving three possible genotypes (CTC/CTC homozygote, CTC/CCC heterozygote and CCC/CCC homozygote), when run on a 2% agarose gel as previously reported. To confirm the accuracy of restriction enzyme digestion, conventional sequencing of three apparent homozygotes for both alleles and three heterozygotes was carried out as above.

Results

No mutations in *MC2R*, *MRAP* or *STAR* were identified in any patient. There were no single nucleotide polymorphisms (SNPs) identified in the *MC2R* coding exon, but a number of individuals had a previously reported intronic SNP (rs2254251) downstream of exon 3 of *MRAP*.

For the promoter polymorphism in *MC2R*, the C allele frequency in our patient population was 0.112 (9/80), which is comparable with the HapMap CEPH SNP database C allele frequency of 0.117. In the AD cohort studied, frequencies of *MC2R* promoter polymorphism were not significantly different to previously reported frequencies in normal controls using χ^2 test with 2 d.f.: CTC/CTC 71.0% (80.2%); CTC/CCC 26.4% (19.0%) and

CCC/CCC 2.6% (0.8%); figures given in parentheses describe reported frequencies in healthy controls (11). The population studied was in Hardy–Weinberg equilibrium.

Discussion

AD is a common cause of adrenal failure in adults. In our cohort of FGD patients and other reported patients with non-classical LCAH, there are several reports of family members diagnosed initially with Addison's disease or with late onset of symptoms of glucocorticoid deficiency who have subsequently been found to have mutations in *MC2R* or *STAR* with alterations in their renin–angiotensin–aldosterone axis. However, our results indicate that FGD is not being underdiagnosed within the wider AD population in the UK. Furthermore, there does not appear to be any significant increase in the *MC2R* promoter polymorphism consistent with reduced responsiveness to ACTH, consistent with other Caucasian populations previously reported (11, 16, 17), although we accept that a detailed analysis of a local control population would be desirable. However, it may be that in other regions, mutation screening for FGD is not considered perhaps because of an atypical biochemical profile or resource considerations, and consequently such patients are being diagnosed as having antibody negative AD. Nevertheless, mutations in these genes account for only just over 50% of cases of FGD. It is known that there are several further loci involved in FGD and there is phenotypic variability in patients with FGD of unknown cause (18). It is possible that as yet unidentified gene(s) for FGD could account for familial cases of non-AAD.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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