The molecular pathogenesis of ACTH insensitivity syndromes

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BACKGROUND

Rare, congenital forms of ACTH insensitivity were first formally described in the 1960s [10, 17, 18, 27]. These patients characteristically had glucocorticoid, but not mineralocorticoid deficiency, and high circulating ACTH. In time it became apparent that this finding was associated with at least two distinct clinical syndromes. Some patients exhibited an absence of tears (alacrima), achalasia of the oesophagus and a variable range of neurological defects in addition to the adrenal disorder. This syndrome, which accounts very approximately for about half of all ACTH insensitive cases, became known as Allgrove’s syndrome or the Triple A syndrome [1, 5, 15, 26]. In the remainder of the patients the adrenal disorder was the only primary clinical feature, and this syndrome is known by several equally adequate descriptive names including familial glucocorticoid deficiency, isolated glucocorticoid deficiency and hereditary unresponsiveness to ACTH. We prefer the term familial glucocorticoid deficiency (FGD) and will use this term subsequently.
CLINICAL FEATURES AND DIFFERENTIAL DIAGNOSIS

Predictably, the adrenal component of both syndromes may present in the neonatal period or later. In the neonatal period, the presentation is usually with hypoglycaemia, (sometimes leading to convulsions), jaundice, (as a result of glucocorticoid deficiency) and failure to thrive. Children in whom the diagnosis is not made at this time and in whom appropriate therapy is not instituted will usually present over the first few years of childhood with malaise and increased susceptibility to infection as well as a continuing risk of hypoglycaemia.

Increased cutaneous pigmentation is usually noted, resulting from the high circulating ACTH which stimulates melanocortin 1 receptors (α-MSH receptors) on melanocytes. Some children seem to survive early childhood relatively well and can present later with signs of mild glucocorticoid deficiency including asthma and excema. At any age there is a high risk of overwhelming infection which, if not recognised and treated early, can result in significant morbidity and mortality. Occasionally, patients with FGD are noted to be unusually tall and this can be a reason for the initial referral to a paediatric endocrinologist.

In the triple A syndrome the adrenal features may not be apparent until later, and normal adrenal function has clearly been documented in some cases at this early stage [11]. This implies a distinct pathogenic mechanism in these two clinical syndromes. Triple A syndrome patients very often present with achalasia or neurological disorders and alacrima, particularly when parents are alerted to the syndrome as a result of another affected sibling or relative.

The main differential diagnosis of the adrenal disorder in both syndromes is Addison’s disease, followed by adrenoleucodystrophy, congenital adrenal hyperplasia and congenital adrenal hypoplasia. The most useful differentiating factor is the function of the renin aldosterone system, which should be normal or near normal in ACTH insensitivity. Adrenal autoantibodies should be negative, and very long chain fatty acids and 17 α-hydroxyprogesterone should be within normal limits. Adrenal imaging will usually reveal small shrunken adrenals.

MOLECULAR PATHOGENESIS

From the time of the earliest descriptions of these syndromes, it has been postulated that a genetic defect in the ACTH receptor [7, 10], its intracellular signaling components, the development of the adrenal cortex [9, 20] or its degeneration [12] may underlie these disor-

Table I
Summary of published and some unpublished ACTH receptor mutations and their probable functional consequences.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Functional consequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro 27 Arg</td>
<td>Benign polymorphism</td>
<td>Weber &amp; Clark, 1994 [23]</td>
</tr>
<tr>
<td>Val45ile</td>
<td>Benign polymorphism</td>
<td>unpublished</td>
</tr>
<tr>
<td>Ser 74 Ile</td>
<td>Intramolecular bond/signal transduction defect</td>
<td>Clark et al, 1993 [2]</td>
</tr>
<tr>
<td>Asp 103 Asn</td>
<td>Loss of ligand binding</td>
<td>Elias et al, 1999 [6]</td>
</tr>
<tr>
<td>Ile 118 frameshift</td>
<td>Truncated receptor</td>
<td>(unpublished)</td>
</tr>
<tr>
<td>Ser 120 Arg</td>
<td>Structural disruption of transmembrane domain 3</td>
<td>Tsigos et al, 1993 [21]</td>
</tr>
<tr>
<td>Thr 159 Lys</td>
<td>Structural disruption of transmembrane domain 4</td>
<td>Elias et al, 1999 [6]</td>
</tr>
<tr>
<td>Arg 201 X</td>
<td>Truncated receptor</td>
<td>Tsigos et al, 1993 [21]</td>
</tr>
<tr>
<td>Tyr 254 Cys</td>
<td>Interference with disulphide bonds</td>
<td>Tsigos et al, 1995 [22]</td>
</tr>
<tr>
<td>Pro 273 His</td>
<td>Disruption of conserved region of transmembrane domain 7</td>
<td>Wu et al, 1998 [28]</td>
</tr>
</tbody>
</table>
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ders. As the triple A syndrome became more clearly defined the concept of a contiguous genetic defect underlying this disease was proposed. This was supported by the realisation that the alacrima-achalasia syndrome (without adrenal failure) may be related [8]. However, observations of heterogeneity within a single family argued against this and in favour of a single gene disorder with variable penetrance [11].

We and others have been able to identify a variety of point mutations in the ACTH receptor gene in many patients with FGD (table I). These include nonsense mutations which result in major truncations of the receptor and missense mutations. As is seen from the Table, mutations are scattered throughout the receptor. Some of these mutations have been identified in a number of different families – most notably the S74I mutation that we have now found, usually in homozygous form, in ten apparently unrelated families, and which have been identified by at least one other group [28]. In this case, haplotype analysis of the patients we have studied suggests the existence of a single common S74I founder, probably arising in the Scottish lowlands. In other cases – e.g. the R146H mutation – both haplotype analysis and ethnic considerations imply that the same mutation has arisen independently twice over [24].

The functional significance of the missense mutations has only become apparent more recently with the advent of effective cell expression systems for this receptor, which has been extremely difficult to express using conventional methods. Effective expression methods in-

Figure 1: Diagrammatic representation of ACTH signalling in the adrenal gland as an indication of possible candidates for genetic defects in FGD. In addition to ACTH (1) and its receptor (2), it can be conceived that the receptor requires a co-factor for normal function (3), or that in parallel with other melanocortin receptors, a naturally occurring receptor antagonist might exist (4). ACTH insensitivity would require overexpression of a putative antagonist which is likely to be dominantly inherited. Furthermore, the factors involved in ACTH-R expression have not been well defined, and conceivably a defect in a tissue specific transcription (5), translation (6) or processing (7) factor could cause FGD. Defects in the signalling components (8-10) are probably less likely candidates because of their ubiquitous roles. Equally good possibilities not illustrated in this figure include defects in development or survival of zona fasciculata/reticularis cells in the adrenal gland.

Figure 1: Représentation schématique des signaux de l’ACTH au niveau de la surrénale donnant une indication des anomalies génétiques possibles dans le déficit familial en glucocorticoïdes. Outre le gène de l’ACTH (1) et de son récepteur (2), on peut concevoir que le fonctionnement du récepteur nécessite un co-facteur (3) ou qu’existent d’autres récepteurs antagonistes parallèles à la mélanocortine (4). L’insensibilité à l’ACTH nécessiterait une expression excessive d’un antagoniste à transmission dominante. D’autres facteurs jouant un rôle dans l’expression des récepteurs de l’ACTH ne sont pas bien connus. On peut concevoir un défaut de transcription tissu spécifique (5) ou un défaut de translation (6) ou de traitement (7). Les défauts de messagerie (8-10) sont des candidats moins probables en raison de leurs rôles ubiquitaires. D’autres possibilités, comme un défaut de développement ou de survie de la zone fasciculaire-réticulaire des cellules corticosurrénales, ne sont pas présentées.
clude the Cloudman M3 cell line [13] and the mouse Y6 cell line [16]. In our own studies with the Y6 cell line we have demonstrated that these missense mutations can interfere with ligand binding or signal transduction [6]. Further support for the pathogenic role of these mutations comes from segregation studies demonstrating that the mutation co-segregates with the disease [23].

**FGD WITH NORMAL ACTH RECEPTORS**

Many patients with FGD do not have mutations in the ACTH receptor, and indeed genetic studies can demonstrate in some of these cases that the ACTH receptor locus could not account for the disease [14, 23]. There are no obvious differences in the clinical syndrome exhibited by those with and without mutations, other than an apparent segregation of tall stature with ACTH receptor defects [4].

Thus there must be at least one other genetic cause for FGD, and few, if any, clinical clues as to its identity. No other very obvious candidates for this role present themselves, and some of these are illustrated diagrammatically in (Fig. 1). Clearly, defects in some of the signalling components might result in ACTH resistance, but because these factors have multiple other roles one would expect that disease would not be limited to the adrenal gland. One plausible candidate is the ACTH molecule itself. Precedents exist for a biologically inactive, yet immunologically detectable peptide hormone (e.g. growth hormone), and this is theoretically possible for ACTH. However, we have sequenced the proopiomelanocortin gene in several of these patients with FGD without receptor mutations, but failed to identify any defect [3]. Current studies are focusing on families with one or more affected members with FGD and trying to identify a genetic locus for this syndrome as well as studying a number of other candidate genes.

**TRIPLE A SYNDROME**

The triple A syndrome is not associated with mutations in the ACTH receptor and is not linked to this locus [4]. Previously we conducted a whole genome scan in a number of large families with one or more affected members, and were able to identify a genetic locus for triple A syndrome on chromosome 12q13 [25]. All affected families studied, now numbering over 50, segregate with this locus, and this finding has been reproduced by other groups [19]. Thus it appears that the triple A syndrome, despite being phenotypically heterogeneous, is genetically homogeneous. A number of candidate genes for the triple A syndrome at this locus have been excluded, and current efforts are aimed at narrowing this interval.

**CONCLUSION**

In summary, at least three genetic loci for ACTH insensitivity syndromes exist. One of these is the ACTH receptor gene, whilst another lies on chromosome 12. Identification of these unknown genes will be of both clinical and biological interest.

Note added in proof : Since submission of this manuscript identification of the triple A syndrome gene has been reported by two groups, revealing it to be a WD-repeat protein of unknown function (Tullio-Pelet et al. Nature Genetics 2000 ; 26 : 332-335 ; Handshug et al. Human Molecular Genetics 2001 ; 10 : 283-290).

**REFERENCES**

13. Naville D, Barjhoux L, Jaillard C et al. Demonstration by transfection studies that mutations in the adrenocorticotropic receptor gene are one cause of the hereditary syndrome of