Genetics of ACTH insensitivity syndromes

J.L.A. Clark(1), L.A. Metherell(1), D. Naville(2), M. Begeot(2), A. Huebner(3)

(1) Department of Endocrinology, William Harvey Research Institute, Barts & the London, Queen Mary, University of London, West Smithfield, London EC1A 7BE, UK,
(2) INSERM U449, INRA UMR 1235, IFR Laennec, UCB Lyon, Faculty of Medicine RTH Laennec, 69372 Lyon, France,
(3) Children’s Hospital, Technical University Dresden, Dresden, Germany.

Reprint request: J.L. A Clark, address above.
e-mail: a.j.clark@qmul.ac.uk

Although ACTH insensitivity may be a feature of certain acquired conditions (such as septic shock), it is in certain inherited disorders that its impact is most obvious. All forms of inherited ACTH insensitivity described to date are autosomal recessive in origin. (Note this does not include developmental or degenerative disorders of the adrenal such as DAX1 defects, adrenoleucodystrophy, etc). Two main clinical syndromes are described.

CLINICAL SYNDROMES

Familial glucocorticoid deficiency (FGD) also known as isolated glucocorticoid deficiency or hereditary unresponsiveness to ACTH was originally described in 1959 by Shepard et al. [13]. It may present in the neonate with hypoglycaemia, or at a later stage with malaise, excessively frequent or severe infections and hypoglycaemia accompanied by skin pigmentation. Diagnosis depends on demonstrating complete or partial cortisol deficiency in the presence of markedly elevated plasma ACTH, and normal or near-normal renin, aldosterone and electrolytes. The cortisol deficiency may only be fully apparent during a short ACTH stimulation test. The differential diagnosis requires exclusion of the commoner acquired Addison’s disease which is likely to show marked disturbance of the renin-aldosterone...
system and electrolytes balance in a child with a past history of good health.

Triple A syndrome is so named because of the coexistence of Adrenal failure, Achalasia of the cardia and Alacrima (absence of tears). These features are often accompanied by a variety of motor, sensory and autonomic neurological disorders and other occasional features such as palmar-plantar keratopathy. Other than alacrima, features of this disorder are not usually apparent at birth, but tend to develop over the ensuing years in an unpredictable manner. Consequently considerable heterogeneity of symptoms exists between patients and even between affected siblings. There are several documented cases of individuals who once had demonstrably normal adrenal function progressing to adrenal failure. The clinical features of this disorder are not usually apparent at birth, but tend to develop over the ensuing years in an unpredictable manner. Consequently considerable heterogeneity of symptoms exists between patients and even between affected siblings. There are several documented cases of individuals who once had demonstrably normal adrenal function progressing to adrenal failure. The clinical features of this disorder are reviewed in Clark & Weber, 1998 [3].

Pathogenesis of FGD type 1

Since the time of the initial clinical characterization of FGD it had been proposed that the inherited defect may lie in the receptor for ACTH, or the cellular response to stimulation of that receptor. Thus in 1993 we and others were able to show that homozygous or compound heterozygous mutations in this receptor were present in some cases of FGD [2, 14]. The ACTH receptor is the smallest of the seven transmembrane domain G protein-coupled receptors and a member of the melanocortin receptor family — amongst which it is known as the melanocortin 2 receptor (MC2R). This nomenclature will be used henceforth. We have now identified or become aware of over 35 mutations within the intronless coding region of this receptor. The majority of these are missense mutations, and very few patients with a nonsense mutation on both alleles of the receptor have been identified. Numbers are currently too few to determine whether there is any greater severity of the phenotype in these rare “null receptor” cases. This subject has been reviewed [3, 4].

FGD type 2

Only about 25% of FGD patients have mutations of the MC2R, and we have shown genetically that many of these other cases must result from genetic disorders at other loci — i.e. there is at least one other gene for FGD. Those cases resulting from MC2R mutations we have called FGD type 1 [12]. Attempts to identify alternative genetic causes for FGD have included analysis of a number of candidate genes in affected patients and gene linkage and homozygosity mapping approaches. This led to the identification of a locus on chromosome 8 in one family [9], although other cases mapping to this locus have yet to be identified. Gene mapping techniques have become significantly more powerful with the advent of the Affymetrix 10K SNP chips which enable one to characterize over 10,000 single nucleotide polymorphisms in informative families in a few days.

In this way we identified a new locus on chromosome 21 in one family with three affected members. Further genotyping using microsatellite markers in this and other families allowed us to focus on a 2.2 Mbp region containing only 30 known or predicted genes. We reasoned that the FGD gene would be expressed in the adrenal cortex, but in few other tissues, and so screened all 30 genes for tissue distribution. A single gene was expressed in only the adrenal in this screen [11]. This was a gene previously known as C21orf61 and more recently described as fat tissue-specific low molecular weight protein or FALP, as it was identified as a protein that appeared on 2-D gel electrophoresis in differentiating adipocytes [19].

We found a number of mutations (missense, splice site and nonsense mutations) in the first and second coding exons of this gene encoding a 19kDa single transmembrane domain protein in several patients with FGD. Our current data suggests that about 20% of FGD results from defects in this gene. We have been able to demonstrate that the protein encoded by C21orf61 is localized in the endoplasmic reticulum and plasma membrane of the cell, and co-localises with MC2R when present. Furthermore, co-precipitation studies show that it interacts directly with MC2R, and co-transfection studies show that in some cell lines in which transfected MC2R does not reach the cell surface, the C21orf61 gene product enables the MC2R to traffic to the plasma membrane and respond to ACTH. For these reasons this protein has now been named the melanocortin 2 receptor accessory protein (MRAP) [11]. Further work will be required to fully elucidate the mechanism of action of MRAP.

Triple a syndrome

Understanding the pathogenic mechanisms underlying the triple A syndrome is clearly likely to be more complex in view of its heterogeneous and progressive nature. We were able to identify its genetic locus following a microsatellite based genome search — initially analyzing a single very large family with multiple affected members, followed by extension of those findings to other informative families [17]. This revealed a locus on chromosome 12. Further work involving extensive fine mapping in a large number of affected families enabled two groups to independently identify the same gene — now known as the AAAS gene [10,
A large number of homozygous and heterozygous mutations in \textit{AAAS} have been identified in patients with the syndrome. The majority of cases are associated with mutations in this gene. \textit{AAAS} encoded a novel 60 kDa protein called ALADIN (\textit{ALacrima A}chalasia \textit{a}Drenal \textit{Insufficiency N}eurologic disorder) which included four WD repeat elements which predict that it would assume a four-bladed beta propeller structure. A limited number of WD repeat proteins have been described which have a variety of functional roles. In most cases they are involved in important protein-protein interactions.

The next step in this puzzle was the serendipitous discovery that ALADIN was a component of the mammalian nuclear pore complex, being located on the cytoplasmic face of this key structure in import and export of large molecules into and out of the nucleus \[6, 8\]. Mutant ALADIN in most cases did not localize to the nuclear membrane \[7\]. These findings raise the possibility that the role of ALADIN is to transport a key molecule into the nucleus. In \textit{in situ} hybridization studies in the rat show that AAAS mRNA is widely distributed, and that although affected tissues often express greater quantities of this mRNA, this is not a perfect association \[15\]. Complete understanding of this syndrome and progress in its treatment will require identification of the transported molecule.

\textbf{RÉFÉRENCES}
