

Primary Adrenocortical Insufficiency Case Series: Genetic Etiologies More Common than Expected

Sarah L. Tsai^{f, g} Jane Green^{b–d} Lou A. Metherell^e Fiona Curtis^b
Bridget Fernandez^{b–d} Ara Healey^{a, d} Joseph Curtis^{a, d}

Disciplines of ^aPediatrics (Division of Endocrinology), ^bGenetics, and ^cMedicine, and ^dFaculty of Medicine, Memorial University of Newfoundland, St. John's, Nfld., Canada; ^eCentre for Endocrinology, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK; ^fDivision of Pediatric Endocrinology, Department of Pediatrics, UMKC School of Medicine, and ^gDivision of Pediatric Endocrinology, Department of Pediatrics, Children's Mercy Hospital, Kansas City, Mo., USA

Key Words

Adrenal insufficiency · Addison's disease · Lipoid congenital adrenal hyperplasia · Autoimmune polyglandular syndrome · Familial glucocorticoid deficiency

Abstract

Background/Aims: Primary adrenal insufficiency (AI) is an important cause of morbidity in children. Our objectives were: (1) to describe the clinical presentation of children with new-onset primary AI, and (2) to identify monogenic causes of primary AI in children. **Methods:** Chart review and mutation detection in candidate genes were conducted for 11 patients with primary AI. **Results:** The likely cause of AI was determined in 9 patients. One had a homozygous *MC2R* mutation associated with familial glucocorticoid deficiency. Two had the same homozygous mutation in the *AIRE* gene which is associated with type 1 autoimmune polyglandular syndrome. One patient had a heterozygous change in this gene of undetermined significance. Five were homozygous for the previously reported p.R188C *STAR* mutation causing nonclassic lipoid congenital adrenal hyperplasia, represent-

ing the largest cohort of such patients from a single geographic area. In the remaining 2 patients, no clear etiology was identified. **Conclusions:** We recommend genetic testing for patients who have negative anti-adrenal antibodies or suggestive family history. Diagnosing a genetic etiology can provide information about prognosis and treatment, and is therefore beneficial for patients. Our high proportion of patients with nonclassic lipoid congenital adrenal hyperplasia likely represents a founder effect.

© 2015 S. Karger AG, Basel

Introduction

The most common cause of primary adrenocortical insufficiency (AI) is autoimmune destruction of the adrenal glands [1]. The incidence in adults is 120 per million [2]. Autoimmune AI most often affects young and middle-aged individuals and is more common in females [3]. Anti-adrenal antibodies are detectable in about 55–90% of adult patients [4]. The pathological mechanism leading to destruction of the adrenal gland remains un-

known [3]; however, there are associations with certain HLA types (HLA-DR3, CTLA-4) [5]. Like type 1 diabetes, the incidence of AI may be increasing [6], indicating that environmental factors may play a role in the pathogenesis of AI.

Familial glucocorticoid deficiency (FGD), also known as hereditary unresponsiveness to ACTH (OMIM No. 202200), is a rare condition in which there is lack of response to ACTH. This leads to adrenal insufficiency with subnormal glucocorticoid levels, preserved aldosterone secretion and significantly elevated plasma ACTH levels. Of note, patients may have mild derangements of the renin-angiotensin-aldosterone axis at presentation, which may be transient [7]. In studies conducted by Meimari-dou et al. [8], mutations in the ACTH receptor gene (*MC2R*) accounted for 25% of cases, mutations in the melanocortin receptor accessory protein gene (*MRAP*) accounted for 20%, and mutations in the steroidogenic acute regulatory protein (*STAR*) gene accounted for 2.5% of cases. Mutations in the mini-chromosome maintenance-deficient 4 homologue (*MCM4*) and nicotinamide nucleotide transhydrogenase (*NNT*) genes, which are involved in DNA replication and antioxidant defense, have been recognized in other patients with FGD and may be involved in the pathophysiology of adrenal malfunction [9, 10]. Mutations in *TXNRD2*, which encodes the mitochondrial selenoprotein thioredoxin reductase 2, have also recently been implicated in FGD [11].

Autoimmune Addison's disease may occur as a component of several polyglandular syndromes. Type 1 autoimmune polyglandular syndrome (APS-1, APECED, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy; OMIM No. 240300) usually presents in childhood. This condition is traditionally characterized by chronic mucocutaneous candidiasis, hypoparathyroidism and adrenal insufficiency, in addition to other autoimmune disorders. It is inherited in an autosomal recessive manner, and is due to mutations in the *AIRE* gene that has been mapped to chromosome 21q22.3 [12]. Type 2 autoimmune polyglandular syndrome (APS-2) consists of primary AI associated with autoimmune thyroid disease (Schmidt syndrome) or type 1 diabetes (Carpenter syndrome) and other autoimmune disorders. The underlying pathophysiology of APS-2 is not fully understood.

Classic lipid congenital adrenal hyperplasia (LCAH; OMIM No. 201710) due to mutations in the *STAR* protein gene is a very severe form of adrenal hyperplasia. Adrenal and gonadal steroidogenesis is severely impaired due to a defect in the conversion of cholesterol to pregnenolone [13]. Patients present in infancy with signs

and symptoms of mineralocorticoid and glucocorticoid deficiency. In XY infants, the external genitalia may appear female due to failure of the testes to produce testosterone [14]. Baker et al. [14] first described a nonclassic form of LCAH which presents like Addison's disease rather than congenital adrenal hyperplasia (CAH). The patients present with primary adrenal insufficiency in childhood, and males may have near-normal genital development [15].

Subjects and Methods

The patients in this study are from the island portion of Newfoundland and Labrador (NL), which is the easternmost province in Canada (current estimated population: 527,000) [16]. The population of NL mainly consists of the descendants of about 20,000 English and Irish immigrants who settled in the province in the mid-18th century [17]. The original immigrants were attracted to NL because of the rich fish stocks, so they settled in small communities around the coastline, called outports. Many outports have remained isolated from each other to the present day. Founder effects have been described for several diseases in NL [18, 19]. Newfoundland also has one of the highest incidences of type 1 diabetes worldwide [20].

We identified 11 patients who had been diagnosed with non-CAH primary AI at the Janeway Children's Health and Rehabilitation Centre from 1984–2008. Patients were included if (1) they had documented primary AI, and (2) diagnosis was at 18 years of age or younger. Patients with classic CAH (21-hydroxylase deficiency) were excluded from this series as the clinical presentation differs considerably from autoimmune AI. Patients with CAH present in the newborn period, and if not picked up by a newborn screening program, are commonly identified by failure to thrive and salt-wasting in males and ambiguous genitalia in females.

This study was approved by the institutional Human Investigations Committee. Written informed consent was obtained from subjects over 16 years old. For subjects under age 16, written informed consent was obtained from the legal guardian of the subject, and the child provided assent. The work was conducted at Memorial University of Newfoundland (St. John's, Nfld., Canada) and William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London (London, UK).

A retrospective chart review was conducted for all patients. The following data were abstracted from the charts: clinical and biochemical features at initial presentation and/or diagnosis, history of adrenal crisis (if present), information on growth and sexual development, additional clinical features (i.e. neurological abnormalities, other autoimmune diseases), adrenal imaging, anti-adrenal antibody status, treatment and response.

Primary adrenal insufficiency was diagnosed based on short synacthen (Cortrosyn®) stimulation testing as per institution protocol if possible. Two patients did not have ACTH stimulation testing; however, all of their additional biochemical investigations were consistent with primary autoimmune adrenal insufficiency (tables 1 and 2). All patients had high ACTH levels at diagnosis with abnormally low cortisol response to stimulation testing.

Table 1. Clinical features at diagnosis

Pa-tient	Age, years	Sex	Lethargy	Hypo-tension	Hyper-pigmen-tation	BMI z score	Diagnosis (genetic change if applicable)	Treatment required GC/MC
1	4	F	+	–	+	–0.95	<i>MC2R</i> mutation g.30239G>T; p.Ser74Ile	GC
2	12	F	–	+	+	–1.31	<i>AIRE</i> mutation (homozygous) p.R471C	GC/MC
3	9	F	–	–	+	–0.25	<i>AIRE</i> mutation (homozygous) p.R471C	GC/MC
4	14	M	+	+	+	–1.83	<i>AIRE</i> mutation (heterozygous) p.R356W (rs376901046)	GC/MC
5	3	F	–	–	+	n.a.	<i>STAR</i> mutation (homozygous) p.R188C	GC
6	6	M	+	+	+	–1.15	<i>STAR</i> mutation (homozygous) p.R188C	GC
7	9	M	–	–	+	n.a.	<i>STAR</i> mutation (homozygous) p.R188C	GC/MC
8	4	M	+	+	+	–1.64	<i>STAR</i> mutation (homozygous) p.R188C	GC
9	17	F	–	–	–	–2	<i>STAR</i> mutation (homozygous) p.R188C	GC
10	10	M	–	+	+	–1.35	unknown	GC/MC
11	15	F	+	+	+	–0.9	unknown	GC/MC

n.a. = No result available; GC = glucocorticoid; MC = mineralocorticoid.

Table 2. Biochemical features at presentation

Patient	BG (3.7–5.6 mmol/l)	Na (135–145 mEq/l)	K (3.5–5.0 mEq/l)	ACTH (0–10 pmol/l)	Plasma renin (3–28 ng/l)	Adrenal antibodies	Stimulated cortisols 0 and 30 min, nmol/l ^b
1	1.9↓	N	N	410	–	negative	<50, <50
2	4.0	117↓	6.2↑	425	–	positive	<11, <11
3	4.4	137	4.1	625	81.3	negative	<50, <50
4	5.6	127↓	4.4	377	–	n.a.	<50 (no stim. value)
5	2.0↓	114↓	5.1↑	1,380	162	negative	346, 344
6	<2↓	127	4.3	454	18.0 ^a	negative	120, 120
7	–	–	–	57	20.16 ^a	negative	340, 340
8	‘low’	‘high’	–	>385	–	negative	<50, <50
9	–	–	–	253	–	negative	<11, 11.82
10	5.6	128↓	6.1↑	310	–	positive	82, 79
11	2.8↓	113 ↓	5.9↑	>275	1,148	n.a.	60 (no stim. value)

BG = Blood glucose; n.a. = no result available; N = normal value. ^a Plasma renin activity, run at different laboratory, normal range 0.5–2.8 ng/ml/h. ^b Cortisol peak with stimulation testing >500 nmol/l is normal.

Based on clinical details of individual patients, candidate genes (including *MC2R*, *MRAP*, *NROB1*, *STAR*, *AIRE*, *NNT*, *CYP11A1*, *AAAS* and *ABCD1*) were sequenced to identify mutations. *TXNRD2* and *MCM4* are very rare and each specific to one particular population; therefore, they were not sequenced for our patients.

Results

Summary of Findings

We reviewed the records of 11 children diagnosed with chronic adrenal insufficiency between 1984 and 2008

who had been under the care of the endocrinology service at the Janeway Children’s Hospital and Rehabilitation Centre in St. John’s, Nfld., Canada. The average age at presentation was 10.3 years (range 3–17 years). Two of the patients (6 and 7) were brothers, but there were no known familial relationships among any of the other patients. The patients were Caucasian and of English or Irish ancestry. All patients had normal intellectual development, and there was no history of neurological regression. There were no patients with ambiguous genitalia or intersex conditions. All male patients had normal very-long-chain fatty acids. All patients had normal growth

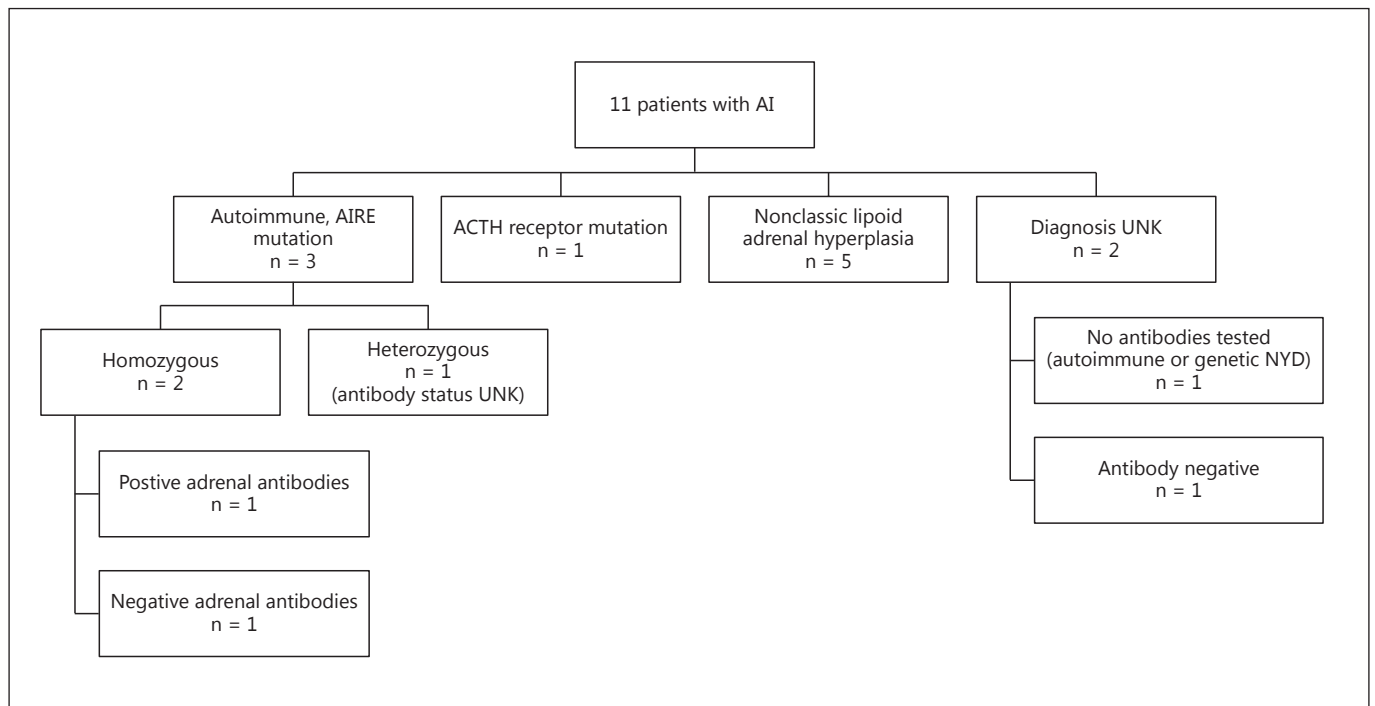


Fig. 1. Patients by diagnosis. UNK = Unknown; NYD = not yet diagnosed.

and normal sexual development, unless otherwise noted. Adrenal imaging, if available, was unremarkable in all cases. Refer to figure 1 and tables 1 and 2 for a summary of clinical and biochemical features.

Case Descriptions

Patient with ACTH Receptor Mutation

Patient 1 presented at age 4 with fever, vomiting, diarrhea, hypoglycemic seizure and typical features of AI in the context of viral upper respiratory tract infection. This patient, who is of Irish descent, has a homozygous missense mutation in the ACTH receptor gene (*MC2R*, g.30239G>T; p.Ser74Ile), previously reported by Clark et al. [21] particularly in patients of Scottish/Irish ancestry. There is no known consanguinity between her parents.

Patients with APS-1

Patient 2 presented at 12 years of age with a 2-week history of dizziness, abdominal pain, headaches, vomiting and blurry vision. She had extreme salt craving, which was precipitated by the family adopting a salt free cooking regime. Patient 3 presented at age 9 with bronzed-looking skin and no other signs or symptoms of adrenal insufficiency. After being treated with hydrocortisone alone, she later developed mild hyponatremia (Na 130 mmol/l). Hy-

ponatremia resolved with fludrocortisone. Patients 2 and 3 had a homozygous change p.R471C of the *AIRE* gene, which is known to cause adrenal insufficiency in humans [22].

Patient 4 presented with lethargy, salt-craving, nausea, vomiting and postural hypotension at age 14. He was heterozygous for *AIRE* gene change at codon p.R356W (rs376901046; minor allele frequency in European Americans = 0.0354%, African-Americans = 0.0). This may represent a dominant negative mutation, similar to those previously described [23, 24]. Also of note, patients 2–4 have only manifested adrenal insufficiency and have no other autoimmune glandular disorders to date.

Patients with Nonclassic LCAH

Patient 5 presented at 3 years of age with typical biochemical features in the context of an upper respiratory tract infection, vomiting and diarrhea. Patient 6 presented at age 6 with vomiting and diarrhea that led to coma. Patient 7 (who is patient 6's older brother) was 9 years old at diagnosis. He was well and was investigated due to uniform tanning and family history. He had been 'unresponsive' after a previous episode of 'flu' several years prior to diagnosis. These siblings and their mutation were previously described by Metherell et al. [15].

Table 3. Summary of pediatric AI case series by identified etiology

Etiology	This study	Hsieh et al. [36]	Perry et al. [37]	Simm et al. [25]
Autoimmune AI	2 APS homozygotes 1 APS heterozygote	18 isolated AI 4 APS 1 confirmed APS-1	8 non-APS-1 5 confirmed APS-1	5
FGD	1	4	0	0
Nonclassic LCAH	5	0	0	0
ALD	0	3	4	5
IMAGe syndrome ^a	0	1	0	1
Wolman syndrome	0	0	3	0
Zellweger syndrome	0	0	1	0
Triple A ^b	0	0	1	0
AHC	0	2	1	5
Unknown	2	2	6	n.a.
Total number of patients with non-CAH primary AI	11	35	29	16

ALD = Adrenoleukodystrophy; AHC = adrenal hypoplasia congenita; n.a. = no result available. ^a Intrauterine growth restriction, metaphyseal dysplasia, adrenal hypoplasia congenita, and genital anomalies. ^b Achalasia, adrenocortical insufficiency, and alacrima.

Patient 8 was diagnosed at age 4 following two hospital admissions for severe hyponatremic dehydration and hypotension precipitated by a viral illness. He had difficulty recovering from anesthesia for circumcision at age 3. Patient 9 was diagnosed at age 17 after having been followed for abnormal thyroid function tests for 3 years (low free T4/mildly increased TSH). Symptoms of fatigue did not improve with administration of L-thyroxine. She had taken a long time to rouse after orthopedic surgery prior to diagnosis.

All 5 patients had negative anti-adrenal antibodies. Patients 5–9 were homozygous for the p.R188C mutation in the *STAR* gene, but did not manifest the full phenotype of LCAH (OMIM 201701). These findings are similar to previous descriptions of patients with the p.R188C mutation [15].

Patients with No Identifiable Etiology

Patient 10 was diagnosed at age 10 in the context of acute gastroenteritis accompanied by electrolyte disturbances and hypotension. Patient 11 presented at age 15 and had hyponatremia and hypotension in the context of a 3–4 week history of weakness, anorexia, weight loss, vomiting and increased thirst. Patients 10 and 11 have had testing for mutations in all genes listed in the Methods section in addition to whole-exome sequencing. No clear etiology for their AI has been determined.

Discussion

We present the first case series of primary AI for NL, an island province on the east coast of Canada. This is the first Canadian case series that has focused exclusively on primary AI and excluded CAH. The strengths of this study are that we were able to obtain genetic testing on all patients. The main weakness of this study is that the chart review was retrospective; therefore, some data are not available. Our findings are unique compared to other case series of AI because we have described the largest cohort to date of patients with nonclassic LCAH from a single geographic area.

Upon review of our cases, it is noteworthy that a few patients had normal electrolytes at presentation. This suggests that serum electrolytes would not be an adequate clinical screen for such patients. Three patients who were non-salt-wasters had quite a lengthy prodrome prior to diagnosis and had no record of adrenal crisis; furthermore, 2 individuals in this series with nonclassic LCAH experienced difficulty recovering from anesthesia prior to diagnosis (patients 8 and 9). Of note, all but one of our patients with LCAH had been treated with glucocorticoid replacement alone. The patient who was prescribed fludrocortisone was treated for empiric reasons, had no biochemical evidence of salt wasting, and was treated with glucocorticoid alone for several years.

There was some variability in clinical presentation among our patients with AI. Documented hypotension was present in 6/11 (55%), and hyperpigmentation was present in 10/11 (90%); this is similar to 43 and 75%, respectively, described in Simm et al. [25]. Six of 11 (55%) patients required mineralocorticoid replacement; however, only 3 were specifically noted to have salt craving upon chart review. Two patients had loss of consciousness likely associated with primary AI (15%; patients 6 and 7) and 1 had a seizure (patient 1) associated with primary AI. There have been several case series looking at primary AI; see table 3 for comparison of etiologies with this series.

We describe 2 patients with homozygous mutations of the *AIRE* gene p.R471C. It is possible that they are distantly related given that the parents of both came from the same region of NL. Interestingly, the change is more common in Americans of European rather than African extraction (minor allele frequency 1.153 vs. 0.248%). *AIRE* mutations associated with autoimmune AI have been described in both homozygous and heterozygous form [1, 22, 26]. This particular change has previously been described in patients with primary autoimmune hypoparathyroidism; however, neither of these individuals has manifested that condition to date [22]. Autoimmune thyroid disease is quite common in patients with APS-1 (68.4%) as is autoimmunity towards pancreatic islet cells [26]. It will be important to follow these individuals for signs and symptoms of other autoimmune conditions. Patient 4 had a change in *AIRE* as well, which is of unknown clinical significance. It is possible that this could represent a dominant negative mutation, similar to what has been described by Oftedal et al. [24] and Ilmarinen et al. [23]. Interestingly, the dominant negative *AIRE* mutations have been noted to cause more variability in clinical presentation [24].

The high number of patients in our series with nonclassic LCAH is unique compared to other case series. Several mutations in *STAR* have been shown to result in this phenotypic syndrome [15]. All of the patients in our series had homozygous mutations causing p.R188C, a protein change which was originally described in 2006 [14]. More than 40 different mutations in *STAR* protein have been described [27–31], many causing classic LCAH. The features of nonclassic LCAH differ considerably from the classic form, in which patients present within the first few months of life with hyperpigmentation, both glucocorticoid and mineralocorticoid deficiency, and ambiguous genitalia in males due to the ab-

sence of fetal testosterone/dihydrotestosterone which results in a failure of virilization [32]. In contrast, most patients with nonclassic LCAH do not have evidence of mineralocorticoid deficiency, and males have normal genitalia at birth. Patients also typically present at an older age, likely due to partial functionality of the protein. Functional analysis of the p.R188C mutants has revealed that >20% of normal cholesterol binding activity is retained [14, 33–34].

Based on the findings of this case series, it is our opinion that the unexpectedly high number of patients with nonclassic LCAH with a specific genotype is a reflection of the genetic structure of the population of NL rather than an under-recognized cause of primary AI in pediatric patients. Upon review of our patients with LCAH, there is good evidence of a founder effect. Five members of 4 families in this study were identified to have the same homozygous mutation in the *STAR* gene. Two brothers in 1 of these families, as well as 3 members of another family were previously reported by Metherell et al. [15] in 2009. It is likely that this represents a founder effect in a geographic isolate with all affected individuals descending from a common ancestor. Because of the large family size in previous generations, there are likely to be many carriers of this mutation in this region with the potential for other affected individuals in the future. Genetic testing is recommended for relatives of those known to be affected in order to identify carrier couples who would then have the option of genetic testing for their children at birth to identify and provide early diagnosis and treatment for other homozygous individuals.

A significant number of antibody-negative presumed primary AI patients may in fact have single-gene mutations for a condition that was previously thought to be extremely rare. Further research directions could include performing genetic testing, such as whole-exome sequencing, for patients with suggestive family histories and/or negative anti-adrenal antibodies to identify mutations in other genes.

Conclusions

Primary AI is an important condition to identify due to the risk of acute adrenal crisis. The presenting features of AI are often nonspecific, i.e. lethargy, weight loss, nausea and vomiting, and this may result in delayed diagnosis. ‘Classic’ features including hyperpigmentation and electrolyte abnormalities were identified in

many of our patients; however, the hyperpigmentation was occasionally very subtle and electrolyte abnormalities were mild or not present in some cases. Salt wasting was not biochemically evident in the patients with LCAH, and only 1 required mineralocorticoid replacement. Furthermore, hyperpigmentation, which is often considered a clinical hallmark of AI, may not always be present [35]. We recommend genetic testing for single-gene mutation etiologies in particular for patients who have negative anti-adrenal antibodies, suggestive family histories, and/or do not have strong evidence of mineralocorticoid deficiency.

Acknowledgements

We would particularly like to thank the patients who agreed to participate in this study and who provided specimens for genetic analysis. We would also like to acknowledge Dr. Tracy Bridger, MD, and Dr. Albert Davis, MD, for clinical care of a number of these patients, and Dr. Ed Randell, PhD, for his expertise in interpretation of biochemical data.

Funding for this study was provided by a Janeway Foundation Grant and the Medical Research Council UK (Project Grant MR/K020455/1 to L.A.M.).

Disclosure Statement

There is no conflict of interest on part of any of the authors that could prejudice the impartiality of this research.

References

- 1 Betterle C, Morlin L: Autoimmune Addison's disease. *Endocr Dev* 2011;20:161–172.
- 2 Ten S, New M, MacLaren N: Clinical Review 130: Addison's disease 2001. *J Clin Endocrinol Metab* 2001;86:2909–2922.
- 3 Husebye E, Løvås K: Pathogenesis of primary adrenal insufficiency. *Best Pract Res Clin Endocrinol Metab* 2009;23:147–157.
- 4 Kong M, Jeffcoate W: Eighty-six cases of Addison's disease. *Clin Endocrinol (Oxf)* 1994; 41:757–761.
- 5 Arlt W, Allolio B: Adrenal insufficiency. *Lancet* 2003;361:1881–1893.
- 6 Løvås K, Husebye E: High prevalence and increasing incidence of Addison's disease in western Norway. *Clin Endocrinol (Oxf)* 2002; 56:787–791.
- 7 Lin L, Hindmarsh P, Metherell L, Alzyoud M, Al-Ali M, Brain C, Clark A, Dattani M, Achermann J: 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;66:205–210.
- 8 Meimaridou E, Hughes CR, Kowalczyk J, Chan L, Clark A, Metherell L: ACTH resistance: genes and mechanisms. *Endocr Dev* 2013;24:57–66.
- 9 Meimaridou E, Hughes C, Kowalczyk J, Gausi L, Chapple J, King P, Chan L, Clark A, Metherell L: Familial glucocorticoid deficiency: new genes and mechanisms. *Mol Cell Endocrinol* 2013;371:195–200.
- 10 Meimaridou E, Kowalczyk J, Guasti L, Hughes C, Wagner F, Frommolt P, Nurnberg P, Mann N, Banerjee R, Saka H, Chapple J, King P, Clark A, Metherell L: Mutations in NNT encoding nicotinamide nucleotide transhydrogenase cause familial glucocorticoid deficiency. *Nat Genet* 2012;44:740–742.
- 11 Prasad R, Chan L, Hughes C, Kaski J, Kowalczyk J, Savage M, Peters C, Nathwani N, Clark A, Storr H, Metherell L: Thioredoxin reductase 2 (TXNRD2) mutation associated with familial glucocorticoid deficiency (FGD). *J Clin Endocrinol Metab* 2014;99:E1556–E1563.
- 12 Nagamine K, Peterson P, Scott H, et al: Positional cloning of the APECED gene. *Nat Genet* 1997;17:393–398.
- 13 Miller W: Congenital lipid adrenal hyperplasia: the human gene knock-out of the steroidogenic acute regulatory protein. *J Mol Endocrinol* 1997;19:227–240.
- 14 Baker B, Lin L, Kim C, Raza J, Smith C, Miller W, Achermann J: Nonclassic congenital lipid adrenal hyperplasia: a new disorder of the steroidogenic acute regulatory protein with very late presentation and normal male genitalia. *J Clin Endocrinol Metab* 2006;91: 4781–4785.
- 15 Metherell L, Naville D, Halaby G, Begeot M, et al: Nonclassic lipid congenital adrenal hyperplasia masquerading as familial glucocorticoid deficiency. *J Clin Endocrinol Metab* 2009;94:3865–3871.
- 16 Statistics Canada: Estimates of Population, Canada, Provinces and Territories. Ottawa, CANSIM Database, 2013.
- 17 Senior Citizens of Burin: The History of Burin. Marystown, South Coast Printers, 1977.
- 18 Spirio L, Green J, Robertson J, Robertson M, Otterud B, Sheldon J, Howse E, Green R, Groden J, White R, Leppert M: The identical 5' splice-site acceptor mutation in five attenuated APC families from Newfoundland demonstrates a founder effect. *Hum Genet* 1999; 105:388–398.
- 19 Olufemi S, Green J, Manickam P, Guru S, Agarwal S, Kester M, Dong Q, Burns A, Spiegel A, Marx S, Collins F, Chandrasekharappa S: Common ancestral mutation in the MEN-1 gene is likely responsible for the prolactinoma variant of MEN1 (MEN1Burin) in four kindreds from Newfoundland. *Hum Mutat* 1998; 11:264–269.
- 20 Newhook L, Grant M, Sloka S, Hoque M, Paterson A, Hagerty D, Curtis J: Very high and increasing incidence of type 1 diabetes mellitus in Newfoundland and Labrador, Canada. *Pediatr Diabetes* 2008;9:62–68.
- 21 Clark A, McLoughlin L, Grossman A: Familial glucocorticoid deficiency associated with point mutation in the adrenocorticotropin receptor. *Lancet* 1993;341:461–462.
- 22 Cervato S, Morlin L, Albergoni MP, et al: AIRE gene mutations and autoantibodies to interferon omega in patients with chronic hypoparathyroidism without APECED. *Clin Endocrinol (Oxf)* 2010;73:630–636.
- 23 Ilmarinen T, Eskelin P, Halonen M, Ruppel T, Kilpikari R, Torres GD, Kangas H, Ullmanen I: Functional analysis of SAND mutations in AIRE supports dominant inheritance of the G228W mutation. *Hum Mutat* 2005;26: 322–331.
- 24 Oftedal B, Hellesen A, Erichsen M, et al: Dominant mutations in the autoimmune regulator AIRE are associated with common organ-specific autoimmune disease. *Immunity* 2015;42:1185–1196.
- 25 Simm P, McDonnell C, Zacharin M: Primary adrenal insufficiency in childhood and adolescence: advances in diagnosis and management. *J Paediatr Child Health* 2004;40:596–599.
- 26 Betterle C, Scarpa R, Garelli S, et al: Addison's disease: a survey of 633 patients in Padova. *Eur J Endocrinol* 2013;169:773–784.

- 27 Fujieda K, Okuhara K, Abe S, Tajima T, Mukai T, Nakae J: Molecular pathogenesis of lipoid adrenal hyperplasia and adrenal hypoplasia congenita. *J Steroid Biochem Mol Biol* 2003;85:483–489.
- 28 Stocco D: Clinical disorders associated with abnormal cholesterol transport: mutations in the steroidogenic acute regulatory protein. *Mol Cell Endocrinol* 2002;191:19–25.
- 29 Bhargoo A, Gu W, Pavlakis S, Anhalt H, Heier L, Ten S, Jameson J: Phenotypic features associated with mutations in steroidogenic acute regulatory protein. *J Clin Endocrinol Metab* 2005;90:6303–6309.
- 30 Chen X, Baker B, Abduljabbar M, Miller W: A genetic isolate of congenital lipoid adrenal hyperplasia with atypical clinical findings. *J Clin Endocrinol Metab* 2005;90:835–840.
- 31 Flück C, Maret A, Mallet D, Portrat-Doyen S, Achermann J, Leheup B, Theintz G, Mullis P, Morel Y: A novel mutation L260P of the steroidogenic acute regulatory protein gene in three unrelated patients of Swiss ancestry with congenital lipoid adrenal regulatory protein. *J Clin Endocrinol Metab* 2005;90:5304–5308.
- 32 Sahakitrungruang T, Soccio R, Lang-Muritano M, Walker J, Achermann J, Miller W: Clinical, genetic and functional characterization of four patients carrying partial loss-of-function mutations in the steroidogenic acute regulatory protein (STAR). *J Clin Endocrinol Metab* 2010;95:3352–3359.
- 33 Baker B, Epand R, Epand R, Miller W: Cholesterol binding does not predict activity of the steroidogenic acute regulatory protein, STAR. *J Biol Chem* 2007;282:10223–10232.
- 34 Flück C, Pandey A, Dick B, Camats N, Fernández-Cancio M, Clemente M, Gussinyé M, Carrascosa A, Mullis P, Audi L: Characterization of novel star (steroidogenic acute regulator protein) mutations causing non-classic lipoid adrenal hyperplasia. *PLoS One* 2011; 6:e20178.
- 35 Turan S, Hughes C, Atay Z, Guran T, Haliloglu B, Clark A, Bereket A, Metherell L: An atypical case of familial glucocorticoid deficiency without pigmentation caused by co-existent homozygous mutations in MC2R (T152K) and MC1R (R160W). *J Clin Endocrinol Metab* 2012;97:E771–E774.
- 36 Hsieh S, White PC: Presentation of primary adrenal insufficiency in childhood. *J Clin Endocrinol Metab* 2011; 96:E925–E928.
- 37 Perry R, Kecha O, Paquette J, Huot C, Van Vliet G, Deal C: Primary adrenal insufficiency in children: twenty years of experience at the Sainte-Justine Hospital, Montreal. *J Clin Endocrinol Metab* 2005;90:3243–3250.