Mosaic *PRKACA* duplication causing a novel and distinct phenotype of early-onset Cushing syndrome and acral cutaneous mucinosis

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Abstract

Genetic alterations within the cAMP/PKA pathway result in a spectrum of adrenocortical disorders. Implicated genes include *GNAS, PDE8, PDE11A, PRKAR1A/B,* and *PRKACA.* To date, somatic *PRKACA* mutations and germline *PRKACA* copy number gain have been associated with the development of cortisol-secreting adrenocortical adenomas and bilateral adrenal hyperplasia, respectively. While perturbations within the *PRKAR1A* gene are known to cause Carney complex, *PKRACA* mutations are rarely associated with an extra-adrenal phenotype. We describe a mosaic *PRKACA* duplication in an infant who presented with a Carney-like complex at the age of three months with bilateral non-pigmented micronodular adrenal hyperplasia, severe early-onset Cushing syndrome, and distinct acral soft tissue overgrowth due to cutaneous mucinosis. This represents a novel manifestation of *PRKACA* disruption and broadens its extra-adrenal phenotype. It suggests that the Cushing syndrome phenotypes arising from somatic and germline *PRKACA* abnormalities likely exist on a spectrum. We emphasise the importance of ascertaining a genetic diagnosis for *PRKACA*-mediated disease.

Significance statement

We describe a mosaic PRKACA duplication in a young infant who presented with a Carney-like complex: bilateral non-pigmented micronodular adrenal hyperplasia, severe early-onset Cushing syndrome, and distinct acral soft tissue overgrowth due to cutaneous mucinosis. This represents a novel manifestation of PRKACA disruption and broadens the extra-adrenal phenotype of PRKACA-associated Cushing syndrome. Our data suggest that Cushing syndrome phenotypes arising from somatic and germline PRKACA abnormalities can exist on a spectrum. We emphasise the value of ascertaining a genetic diagnosis for PRKACA-mediated adrenal and extra-adrenal disease to guide individualised and targeted care.

Introduction

The cAMP/PKA intracellular signalling pathway mediates the action of several endocrine processes. Cyclic AMP (cAMP) and protein kinase A (PKA) are key regulators of cell growth and development, which are in turn regulated by the opposing effects of adenylyl cyclase and phosphodiesterases (PDEs). In the adrenal gland, ACTH controls adrenocortical cell proliferation by binding to the melanocortin 2 receptor (MC2R) on the surface of adrenocortical cells to activate adenylyl cyclase and increase intracellular cAMP[1]. This stimulates PKA, drives cellular proliferation[1,2], and increases glucocorticoid production. *PRKAR1A* and *PRKAR1B* encode for the main regulatory subunits (R1α and R1β) of the PKA holoenzyme, and *PRKACA* and *PRKACB* encode for its catalytic subunits (Cα and Cβ)

Genetic alterations within the cAMP/PKA pathway have been implicated in the pathogenesis of ACTH-independent hypercortisolism[3]. For example, activating somatic mutations in *GNAS,* encoding for the G-protein alpha subunit, result in McCune-Albright syndrome, adrenal hyperplasia, cortisol-secreting adrenocortical adenomas (ACAs), and Cushing syndrome[4,5]. Inactivating germline mutations in *PDE8B* and *PDE11A,* encoding for PDEs, predispose to adrenal hyperplasia[6–9]. Mutations affecting the regulatory subunits similarly drive adrenal disease. Germline inactivating *PRKAR1A* mutations, which lead to constitutive PKA activation, have been implicated in primary pigmented nodular adrenocortical disease (PPNAD) and cortisol-secreting ACAs[10,11]. *PRKAR1B* somatic missense variants and germline amplification have been associated with ACAs and BAH (bilateral adrenal hyperplasia), respectively[12]. Recently, somatic pathogenic variants in *GNA11* and *GNAQ* have been shown to result in aldosterone-producing adenomas[13].

The spectrum of adrenal disease caused by cAMP/PKA signalling pathway perturbations further extends to the catalytic PKA subunits. Somatic point mutations in *PRKACA* have been demonstrated to drive constitutive PKA activation and cortisol-secreting ACAs[14–18]. Cushing syndrome caused by *PRKACA* mutations tends to be severe[17–19]. Germline copy number gain of the chromosomal region encompassing *PRKACA* (19p) has been associated with bilateral adrenocortical hyperplasia and Cushing syndrome[17,20]. The specific adrenal histology resulting from germline *PRKACA* amplification

varies and includes PPNAD and non-pigmented bilateral macro- or micronodular hyperplasia[20,21]. Less commonly, somatic *PRKACB* mutations result in cortisol-secreting ACAs[22]. Genetic abnormalities within the cAMP/PKA pathway can also have extra-adrenal features. *PRKAR1A* mutations can cause Carney complex and Leydig/Sertoli cell tumours[11,23,24]. *PRKACA* and *PRKACB* mostly give rise to an adrenal-only phenotype[19]. However, somatic and germline missense mutations in *PRKACA* and *PRKACB* have been recently associated with a multiple congenital anomaly syndrome in the absence of adrenal pathology, including cardiac defects and postaxial polydactyly[25].

Taken together, existing data suggest that somatic *PRKACA* mutations lead to cortisol-secreting adrenocortical adenomas; germline *PRKACA* copy number gain results in bilateral adrenal hyperplasia; and germline *PRKACA* mutations are rarely associated with an extra-adrenal phenotype[26]. We report the case of a boy who presented with a Carney-like complex at the age of three months due to a mosaic *PRKACA* duplication resulting in bilateral non-pigmented micronodular adrenal hyperplasia, Cushing syndrome, and distinct acral soft tissue overgrowth due to cutaneous mucinosis.

Case

Clinical presentation

We report a patient born following an uncomplicated pregnancy at term by Caesarean section with a birth weight of 3450g. He is the first child of non-consanguineous parents. He was exclusively breastfed with initially appropriate weight gain. At the age of three months, parents sought medical attention for unusual multiple non-tender, non-pigmented, non-fluctuant, firm lumps on his feet *(Figure 1)*. Initially, these lesions were restricted to the soles, but by four months included the dorsum of his feet and the toes. Shortly after, his hands and fingers developed similar lesions *(Figure 1)*, resulting in hand architecture distortion and finger displacement. He had no other dermatological lesions; in particular, he had no pigmentation abnormalities. By four months of age, he became hyperphagic, rapidly gained excessive weight (from 50th to 98th centile) and became hypertensive (systolic blood pressure reaching 130mmHg). He was clearly Cushingoid on examination. His height was also affected: he measured 66cm at diagnosis (<0.4th centile, -3.2 standard deviation score (SDS)).

Laboratory and radiological investigations

Investigation followed best practice guidelines[27]. At presentation, midnight and 08.00h cortisol concentrations were 822nmol/L and 938nmol/L respectively with a suppressed ACTH (<5ng/L). Further testing demonstrated raised liver transaminases (ALT 89U/L), neutrophilia (12.2x10^9/L), elevated platelets (623x10^9/L), and elevated lipid concentrations (total cholesterol 8.37mmol/L; triglycerides 2.74mmol/L). Three urinary free cortisol:creatinine ratios were raised (1543, 1008, and 611nmol/mmol). 24-hour urine free cortisol was 656nmol/L (a second attempt at 24-hour collection failed, reflecting the practical difficulties inherent in performing this test in a young infant). Androgen concentrations were elevated (DHEA-sulfate 1.1umol/L; androstenedione 29.30nmol/L; testosterone 2.79nmol/L). Cortisol was not suppressed on a high-dose dexamethasone suppression test (pre-dexamethasone 1043nmol/L; 24 hours post-dexamethasone 897nmol/L). Plasma renin activity (PRA) was 8.0nmol/L/h and aldosterone 1570pmol/L. A skeletal survey (a series of bilateral anteroposterior (AP) and posteroanterior (PA) plain film X-rays) suggested diffuse osteopenia and previous fractures.

Magnetic resonance imaging (MRI) and fine-slice contrast computed tomography (CT) imaging of the adrenal glands did not reveal any adrenal gland abnormality. Testicular ultrasound demonstrated a single small calcification in the left testis. An echocardiogram revealed mild left ventricular hypertrophy. Both kidneys appeared structurally normal. A clinical and biochemical diagnosis of aggressive ACTH-independent Cushing syndrome was made.

Histopathological findings

Histopathological examination of skin biopsy from the acral tissue demonstrated a non-pigmented, poorly circumscribed lesion comprising fibrous tissue with background myxoid stroma *(Figure 2)*. Immunostaining was negative for desmin, lysozyme, D240, S100, NSE, PGM1 and SOX-10. Expression of H3K27me3 was retained, with non-specific staining of CD34, CD31, vimentin and CD10. These findings are consistent with a histological diagnosis of cutaneous mucinosis.

Genetic testing

The patient and his parents underwent trio whole exome sequencing with no relevant findings. Array comparative genomic hybridisation (aCGH) analysis was performed on DNA extracted from the affected skin biopsy, and from blood. This demonstrated a duplication (three copies) on the short arm of chromosome 19, spanning a 900kB region inclusive of the *PRKACA* gene (breakpoints within 19p13.13 and p13.12) in the skin, which was absent from blood. Other genes within this duplicated region included *RFX1, DCAF15, LPHN1, SAMD1, PALM3, DDX39A, CC2D1A, PODNL1, RLN3, IL27RA, ASF1B, CD97, PKN1, GIPC1, TECR, DNAJB1, PTGER1, and C19orf67.*

Clinical course

Given the severity of his presentation, this patient underwent retroperitoneoscopic bilateral adrenalectomy at 11 months of age and was commenced on lifelong hydrocortisone and fludrocortisone replacement therapy. Most Cushing syndrome features have subsequently resolved and a pre-hydrocortisone cortisol concentration was 203nmol/L. There has also been improvement in

the appearance of his skin lesions, suggesting that circulating cortisol may have contributed to the underlying pathological process *(Figure 1E, 1F)*. He remains on amlodipine treatment for persistent hypertension. Histopathology of the resected adrenal tissue showed bilateral micronodular cortical hyperplasia characterised by variably-sized nodules and compact nests comprising polygonal cells with vacuolated eosinophilic cytoplasm and large round nuclei with small nucleoli *(Figure 2)*. Immunohistochemistry for synaptophysin and inhibin highlight the hyperplastic nodules. aCGH analysis of resected adrenal tissue confirmed the same *PRKACA* copy number gain (duplication, three copies) as identified in the skin. This confirmed the hypothesis that the clinical features in this case were due to a post-zygotic mosaic duplication of *PRKACA*. While the adrenal features may not be unexpected, to date only *germline PRKACA* duplication has been associated with Cushing syndrome secondary to adrenal hyperplasia. Furthermore, the skin features have not been described previously in association with either germline or somatic mutations affecting the pathway in question. These could be due to contiguous gene involvement within the duplication; however, no convincing candidates within the duplicated region were identified. Alternatively, the *PRKACA* duplication may be driving mucin deposition in the acral skin, potentially as a result of a specific cell type affected by the post-zygotic mutation during development.

Several multidisciplinary specialists remain involved in this patient's care, including a paediatric endocrinologist, surgeon, dermatologist, clinical geneticist, cardiologist, and pathologist. He will remain on lifelong adrenal hormone replacement. He will also undergo annual testicular ultrasound imaging to monitor for Leydig or Sertoli cell tumour development. Management of the skin is extremely challenging, as the lesions may interfere with hand and foot function. Surgical excision is likely to be needed but regrowth is a distinct possibility. Targeted personalised medical therapy using repurposed oncology treatments may be required on a trial basis.

Discussion

The chronic hypercortisolism associated with paediatric Cushing syndrome, possibly lasting decades if not diagnosed, carries a significant burden of morbidity and mortality if not managed promptly and appropriately[28,29]. This case expands our knowledge of cAMP signalling defects, specifically *PRKACA*-mediated, in adrenal disease. Cushing syndrome in this patient was of very early-onset and initially treatment refractory, emphasising that the adrenal phenotype associated with *PRKACA* aberrations tends to be severe. aCGH revealed the patient to be mosaic for a *PRKACA* copy number gain on chromosome 19 and bilateral nodular adrenal hyperplasia was histologically confirmed. Classically, somatic *PRKACA* missense mutations are associated with cortisol-secreting ACAs and germline copy number gain with bilateral nodular hyperplasia, with the latter associated with a more severe phenotype. Given the involvement of multiple tissue types, this case likely involves mosaicism – where the mutation arises prenatally[30]. Clinically and histologically, it resembles previously described cases resulting from *PRKACA* germline amplification – at least from an endocrine perspective. This suggests that Cushing syndrome phenotypes arising from somatic compared to germline *PRKACA* abnormalities are not dichotomous as previously thought and more likely exist on a spectrum, with overlap occurring particularly if the mosaicism occurred early in embryogenesis.

We also expand the phenotype of the extra-adrenal manifestations arising from genetic abnormalities within the cAMP/PKA pathway. Germline *PRKAR1A* mutations are associated with Carney complex manifestations, including PPNAD, cardiac myxomas, skin myxomas, lentiginoses, and testicular tumours. *PRKACA* abnormalities mostly result in an adrenal-specific phenotype. However, germline and mosaic *PRKACA* mutations have recently been associated with extra-adrenal features, such as cardiac septal defects and polydactyly[25]. We associate a novel extra-adrenal manifestation with *PRKACA* copy number gain – soft tissue overgrowth of the palms and soles with cutaneous mucinous deposition. Although this is redolent of the Carney complex spectrum, it does not fit within its criteria and likely represents a new distinct entity of *PRKACA*-mediated disease. *In vitro* studies interrogating this mechanistically would strengthen this association and would be further corroborated by reports of the same cutaneous presentation in other patients with *PRKACA* abnormalities. The mosaic nature of the genetic variant possibly causes this phenotypic feature, a phenomenon known from other mosaic

disorders, demonstrating features undescribed in germline versions of the same genetic abnormality[30]. Interestingly, somatic *PRKACA* in-frame microinsertions within exons 7 and 8 have been associated with cardiac myxomas[31]. This suggests that the extra-adrenal phenotype of *PRKACA* aberrations in general may have some overlap with that of classic *PRKAR1A*-mediated Carney complex.

The functional consequences of *PRKACA* variants have been investigated previously. PKA is a key regulator of cell growth and development. *PRKACA* variants associated with cortisol-secreting ACAs cluster on exon 7 (e.g., p.L206R *(Figure 3)*. L206 is located within the highly conserved PKA enzyme core and is part of the catalytic subunit to which the inhibitory element of the regulatory subunit binds[17]. The p.L206R somatic mutation abolishes PRKACA binding to PRKAR1A, which encodes the regulatory PKA subunit, resulting in constitutive cAMP-independent PKA activation and the development of ACAs[16]. The role of the cAMP/PKA pathway in endocrine regulation is well-acknowledged. In addition, PKA regulates hedgehog (Hh) signalling via phosphorylation of the transcription factor GLI3 which transcriptionally represses Hh-target genes such as the Hh signal transducer SMO[32]. SMO is recruited to primary cilia, increasing cAMP concentrations[33]. The *PRKACA* p.G137R mutation has been shown to inhibit Hh signalling in mutant fibroblasts, resulting in increased cAMP concentrations and yielding a PKA holoenzyme more sensitive to cAMP than wild-type. p.G137R has been recently associated with an extra-adrenal multiple congenital malformation syndrome, including polydactyly and atrioventricular canal defects, which has been attributed to aberrant Hh signalling[34]. *PRKACA* germline duplication (or triplication) has previously been associated with CS secondary to adrenal hyperplasia. We demonstrate for the first time that this phenotype can be associated with mosaic *PRKACA* duplication and associate this with a novel extra-adrenal manifestation of *PRKACA* disease. Taken together, these data suggest that the proposed novel clinical phenotype of *PRKACA*-mediated disease may depend both on tissue-specific genetic dosage as well as the genetic region affected and may result from regulatory perturbations of both cAMP/PKA and Hh signalling pathways.

Lastly, this case illustrates the importance of ascertaining a genetic diagnosis for *PRKACA*-mediated disease when planning management. The severity and young age of onset in this case prompted

rapid genetic analysis. A confirmed mosaic *PRKACA* duplication linked the patient's adrenal disease to his extra-adrenal features. This broadened his care plan to include a tumour surveillance programme and multidisciplinary input going forward. In the future, personalised treatments targeting the cAMP/PKA pathway, currently trialled in the oncology setting, may become options for Cushing syndrome (for example, antisense oligonucleotide targeting PKA expression[35]; PDE4 activator compounds[36]).

Declaration of interest

The authors declare no conflict of interest.

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Consent

Written informed consent for publication of their clinical details and images was obtained from the parent of the patient.

Figure 1

Multiple non-tender, non-pigmented, non-fluctuant, firm lesions at the time of initial presentation on the patient's index finger (A) and on the dorsal and ventral aspects of the patient's foot (B-D). (E-F) Improvement of lesions on feet and hands at one month after adrenalectomy. Note: Image backgrounds have been blurred for clarity.

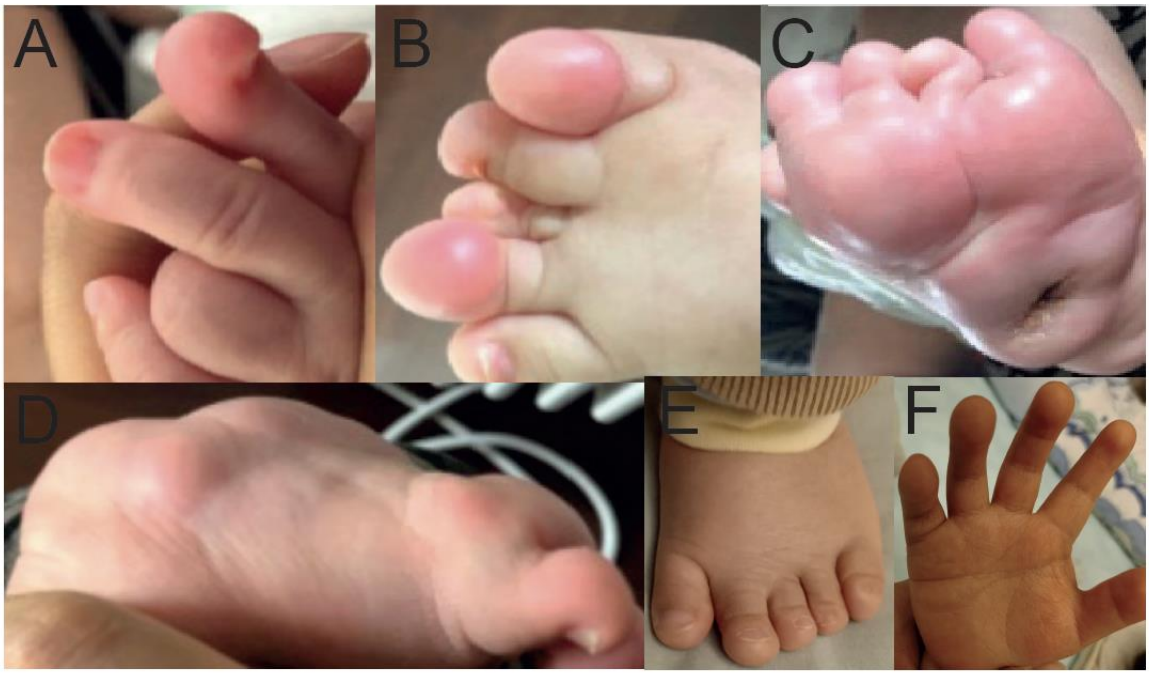


Figure 2

(A) Left panel: Skin showing degenerate collagenous and background myxoid stroma (H&E stain, x4 magnification). Right panel: Skin with mucin in the background highlighted by Alcian blue staining (Alcian blue stain, x4 magnification). (B) Left panel: Adrenal gland showing nodular architecture (H&E stain, x2 magnification). Middle panel: Adrenal gland showing nodules and nests of polygonal cells with vacuolated to eosinophilic cytoplasm (H&E stain, x4 magnification). Right panel: Inhibin immunostain highlighting the nodules in the adrenal gland (Inhibin immunohistochemistry, x4 magnification)

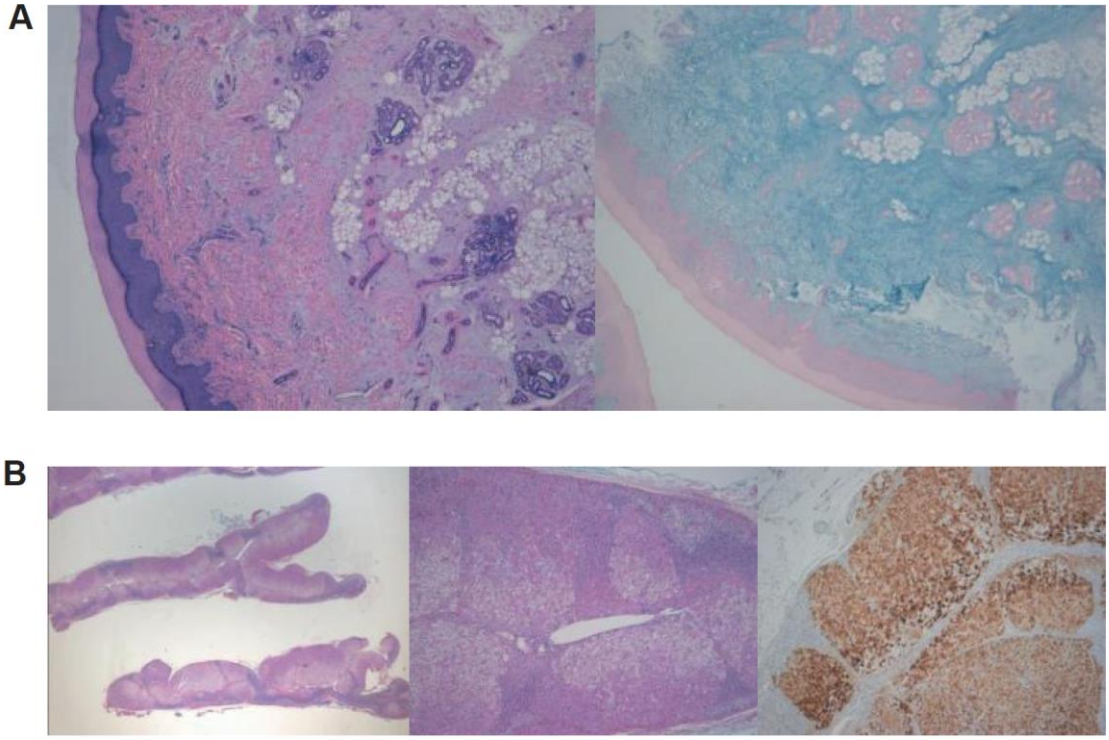
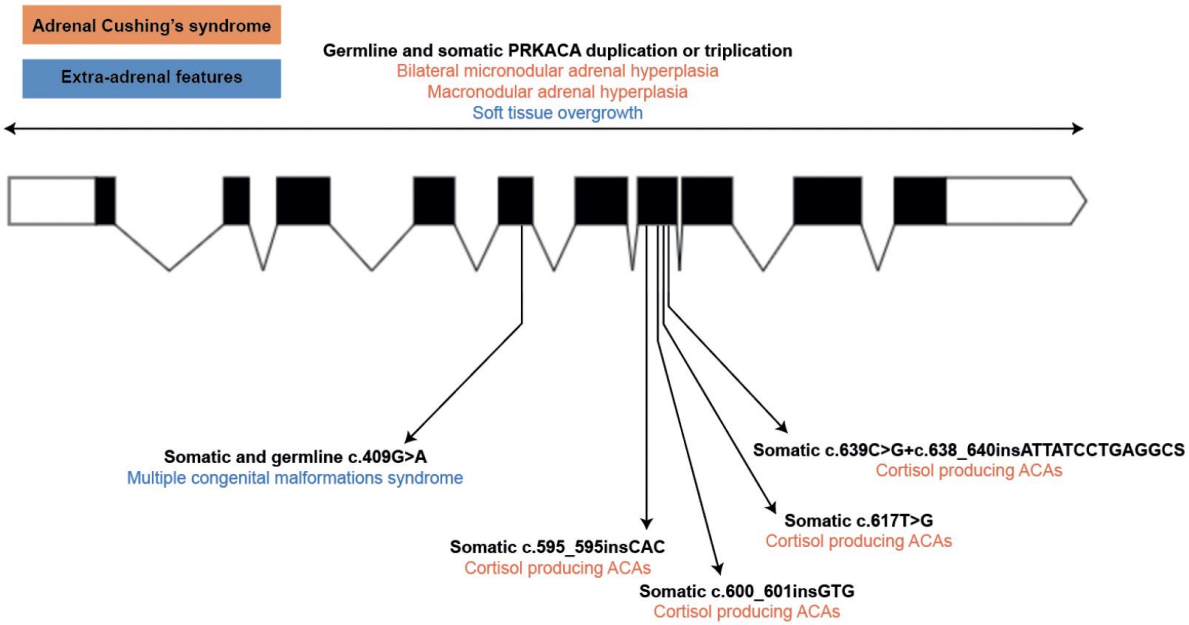


Figure 3

Schematic showing the 10 exons of the *PRKACA* gene with pathogenic variants and copy number variants known to cause disease annotated. Orange=adrenal manifestation. Blue=extra-adrenal manifestation.



Table

Clinical and biochemical characteristics

Clinical finding	**Result**	**Centile**
Weight	11.3kg	98th centile (+2 SDS)
Height	66cm	<0.4th centile (-3.2 SDS)
Systolic blood pressure	130mmHg	>>99.6th centile
Investigation	**Result**	**Reference range**
Sodium	137mmol/L	133-146
Potassium	4.4mmol/L	3.2-6.0
Cortisol 00:00	822nmol/L	<140
Cortisol 08:00	938nmol/L	110-560
ACTH 00:00	<5ng/L	10-50
ACTH 08:00	<5ng/L	10-50
Urinary free cortisol:cr		<20
1	1543 nmol/mmol	
2	1008 nmol/mmol	
3	611 nmol/mmol	
24 hour urine cortisol	656nmol/L	11-110
DHEAS	1.1umol/L	0.2-0.6
Androstenedione	29.30nmol/L	<1.0
Testosterone	2.79nmol/L	<0.8
Pre-DST cortisol	1043nmol/L	110-560
Post-DST cortisol	897nmol/L	<50nmol/L
PRA	8.0nmol/L/h	<8*
Aldosterone	1570pmol/L	<2000*
Total cholesterol	8.37mmol/L	2.3-5.4
Triglycerides	1.96mmol/L	0.5-1.8
Platelets	623x10^{9}/L	150-450
Neutrophils	12.2x10^{9}/L	1.0-8.5
ALT	89U/L	12-47
Free T4	15.7pmol/L	10.8-10.9
TSH	1.2	<6.0

TSH=thyroid stimulating hormone; DST=Dexamethasone suppression test; ACTH=adrenocorticotrophic hormone; DHEAS=dehydroepiandrosterone sulphate; ALT=alanine transaminase; cortisol:cr= cortisol:creatinine ratio; PRA=plasma renin activity

*Can be normal within this range in infancy within the context of normal electrolytes

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