# LETTERS TO THE EDITOR

## Letter in response to Chitale *et al.*: determining the utility of the 60-minute cortisol measurement in the short synacthen test

Dear Editors,

We read with interest the paper by Chitale *et al.*[1] and agree that the 60-minute sample is essential to the standard 250-mcg short synacthen test (SSST), in contrast to the low-dose short synacthen tests (LDSSTs), and welcome any research helping to clarify the optimal use of the short synacthen test in terms of dose, sampling times and diagnostic cut-offs.

A recent survey of UK paediatric endocrinologists demonstrated 82% using a form of LDSST and 87% the SSST. All centres ($N = 39$) sampled at 0 and 30 minutes but 18% did not perform a 60-minute sample. There were a number of additional samples taken at different time points, with a mean of 3·8 samples per SSST for all centres.[2]

In keeping with national practice, our tertiary paediatric endocrinology service is predominantly performing LDSST; however, on analysis of the SSSTs undertaken between January 2011 and May 2012 ($N = 20$), we have found similar results to Chitale *et al.* in our paediatric cohort. Of the 20 tests analysed, the 60-minute cortisol value was higher than the 30-minute sample in 90%, on average 84·1 nmol/l higher (SD 70·6 nmol/l) supporting the use of a 60-minute sample to guard against false-positive results.

Discrepant results, that is, those where the patient would be felt to have adrenal insufficiency based on the 30-minute cortisol value but not on the 60-minute result or *vice versa* depend on the cut-off value applied. Our national survey revealed that 54% of responding centres use 500 nmol/l and 44% 550 nmol/l.[2] Employing a cut-off of 500 nmol/l, 32% of our patients undergoing an SSST would have been misdiagnosed with adrenal insufficiency without the 60-minute sample and 10% using 550 nmol/l. In conjunction with our laboratory colleagues, we have recently revised our diagnostic cut-off to 450 nmol/l following the introduction of a new cortisol assay and welcome the authors' view that local reference ranges need to be determined rather than historically set values that may not reflect laboratory advances and variability.

### Conflict of interest

There are no conflict of interests to declare.

**C. J. Elder*, L. M. Somerset†, V. Ram†, N. P. Wright‡, J. K. Wales* and P. J. Dimitri‡**
**Academic Unit of Child Health, University of Sheffield,*
*†School of Medicine, University of Sheffield and*
*‡Sheffield Children's NHS Foundation Trust, Western Bank, Sheffield, UK*
*E-mail: c.j.elder@sheffield.ac.uk*

doi: 10.1111/cen.12285

### References

1 Dhatariya, K., Chitale, A., Musonda, P. *et al.* (2013) Determining the utility of the 60 minute cortisol measurement in the short synacthen test. *Clinical Endocrinology*, **79**, 14–19.
2 Elder, C.J., Sachdev, P. & Wright, N.P. (2012) The short Synacthen test: a questionnaire survey of current usage. *Archives of Disease in Childhood*, **97**(10), 870–873.

## An oncocytic adrenal tumour in a patient with Birt-Hogg-Dubé syndrome

A 62-year-old female patient was initially diagnosed with a histologically confirmed trichodiscoma. Due to the association of trichodiscomas with Birt-Hogg-Dubé Syndrome (BHD), the patient underwent screening for renal tumours, which revealed a heterogeneous right adrenal mass (Fig. 1a). The patient did not have any clinical hormone excess. Adrenalectomy revealed a tumour measuring 6·2 cm and weighing 104 g with features diagnostic for an adrenal oncocytoma (Fig. 1b). While the tumour did not fulfil the established criteria for malignancy in oncocytic adrenal neoplasms, the borderline Ki67 labelling of 5% suggested the diagnosis of at least uncertain malignant behaviour rather than a benign lesion.[1] The traditional Weiss scoring system to differentiate benign and malignant lesion fails in oncocytomas as they almost invariably are positive for at least two of the criteria, leading to overdiagnosis of malignant lesions.[1] The patient remains disease-free 24 months following surgery. Review of a chest CT imaging revealed two small cysts. The patient's family history was suggestive of BHD, with a maternal cousin with a history of spontaneous pneumothorax (Fig. 1c). The patient's mother had a diagnosis of basal cell carcinoma and one maternal aunt died of lung cancer. The patient's father died of pancreatic cancer. The patient underwent germline genetic testing, revealing a deleterious mutation in exon 11 of *FLCN* (c.1252delC; p.Leu418TrpfsX50) leading to a premature stop codon and confirming the diagnosis of BHD. Unfortunately, insufficient tumour tissue precluded loss of heterozygosity analysis.

Adrenocortical tumours, benign and malignant, are well known to occur as part of hereditary cancer susceptibility syndromes.[2] Adrenocortical carcinoma (ACC) is a core malignancy of Li Fraumeni syndrome. ACC and adrenocortical adenomas are observed in Beckwith Wiedemann syndrome, Multiple Endocrine Neoplasia type 1 and Familial Adenomatous Polyposis. Oncocytic adrenal tumours are rare, have biological behaviour, which can be difficult to predict and have not been reported in association with hereditary syndromes.[1]

Birt-Hogg-Dubé Syndrome was first described in 1977 with the classical manifestations of benign hair follicle-associated tumours. In the decades to follow, BHD has been defined as an autosomal

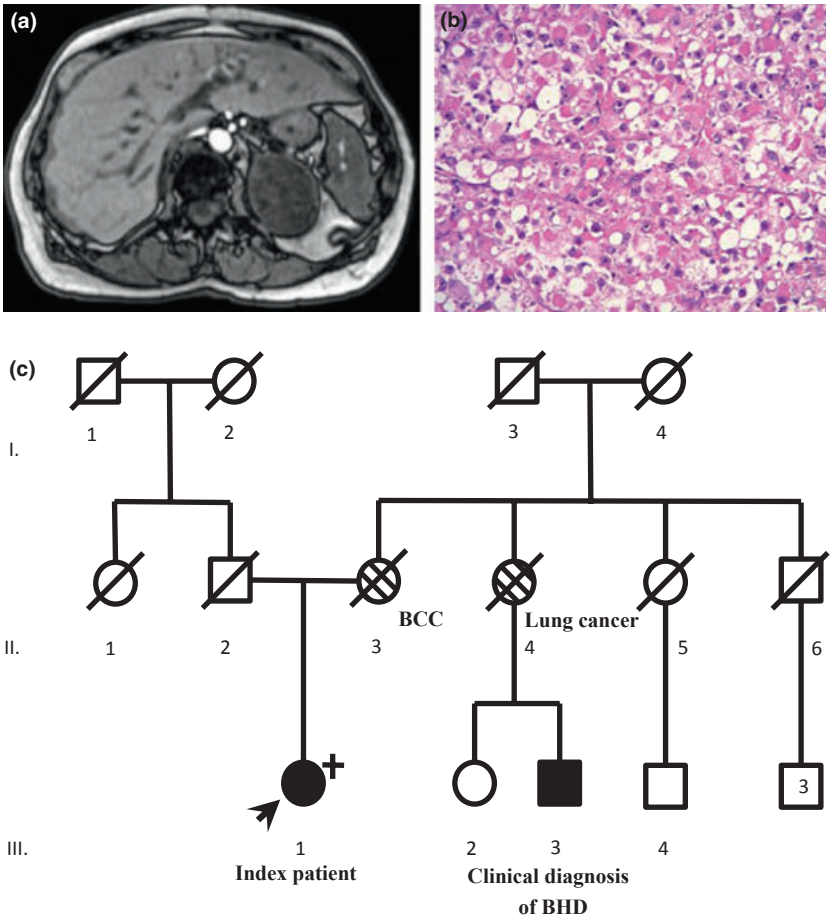


**Fig. 1** (a) heterogeneous adrenal mass on T1 weighted MRI. (b) oncocytic adrenal neoplasm with diffuse growth pattern. (c) Pedigree. Proband (III.1) indicated with the arrow. A maternal cousin fulfilled clinical criteria for BHD (III.3), making the patient's mother (II.3) and aunt (II.4) obligate carriers.

dominant hereditary syndrome, characterized by the cutaneous triad of fibrofolliculomas, trichodiscomas and acrochordons, lung cysts and renal tumours. BHD is caused by mutations in *FLCN* (Online Mendelian Inheritance in Man #13510, http://omim.org/entry/135150, for photograph of typical skin lesions see Ref. 3). Renal oncocytomas, as well as chromophobe and mixed chromophobe oncocytic renal tumours, are typical for BHD.[3]

In order to explore an adrenal phenotype in BHD patients, we identified 14 patients from 11 unique families with genetically confirmed *FLCN* mutations in the University of Michigan Cancer Genetics Registry. A stable 1·2 cm adrenal nodule was observed in a 22-year-old male patient. Next, we retrospectively reviewed 359 patients diagnosed with ACC using the Michigan Endocrine Oncology Repository. None of the 359 patients had reported history of trichodiscoma or fibrofolliculoma. One patient reportedly had two lung cysts sized 1·2 cm. Three patients had histories of renal tumours, including one oncocytoma. Five patients reported first-degree relatives with kidney cancer. No patient fulfilled clinical criteria for BHD, had undergone germline genetic testing for *FLCN* mutations or had a reported family history of BHD. In summary, adrenal tumours may be more common in BHD patients, but BHD is not common amongst ACC patients.

Review of the literature yielded three reports of adrenal tumours in BHD patients. First, a study of 23 BHD patients with confirmed *FLCN* mutations presenting with lung cysts reported one patient with an adrenal nodule.[4] This adrenal nodule was small (1·5 × 2·0 cm) and nonhormone-secreting (Seyama K, pers. comm.). Second, Juszczak *et al.* reported a large oncocytic adrenal tumour in a 36-year-old female patient with BHD and confirmed *FLCN* mutation.[5] The third report of an adrenal tumour describes a presumed ectopic renal tumour in the adrenal gland of a BHD patient.[6]

Our case, together with the case described by Juszczak *et al.*, suggests that adrenal oncocytomas might be part of the BHD tumour spectrum. With the exception of trichodiscoma, fibrofolliculoma and typical kidney tumours, no specific association of other tumours with BHD has been proven, despite several reports of benign and malignant tumours in BHD patients.[3] As is often the case with rare cancer susceptibility syndromes, predisposition to tumours other than the core malignancies is not well defined. Furthermore, the phenotype of BHD can be subtle, leading to underdiagnosis. Suspicion of BHD should be raised in patients with adrenal oncocytomas. Dermatologic exam and review of chest imaging can reveal other hallmark clinical features of BHD.

## Acknowledgements

We appreciate Dr. Seyama's interest in sharing information on the patient with an adrenal tumour he had reported in a recent case series. Tobias Else is supported by NIH T32-DK007245.

## Disclosure statement

The authors have nothing to disclose.

**Victoria M. Raymond*, Jessica M. Long*, Jessica N. Everett*, Elaine M. Caoili†, Stephen B. Gruber‡, Elena M. Stoffel§, Thomas J. Giordano¶, Gary D. Hammer** and Tobias Else****

**Division of Molecular Medicine and Genetics, Department of Internal Medicine, University of Michigan, †Department of Radiology, University of Michigan, Ann Arbor, MI, ‡Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA, §Division of Gastroenterology, Department of Internal Medicine, University of Michigan, ¶Department of Pathology, University of Michigan and **Division of Metabolism, Endocrinology & Diabetes, Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA*

*E-mail: telse@umich.edu*

doi: 10.1111/cen.12292

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2 Else, T. (2012) Association of adrenocortical carcinoma with familial cancer susceptibility syndromes. *Molecular and Cellular Endocrinology*, **351**, 66–70.

3 Menko, F.H., van Steensel, M.A., Giraud, S. *et al.* (2009) Birt-Hogg-Dube syndrome: diagnosis and management. *The Lancet Oncology*, **10**, 1199–1206. (This publication contains clinical photographs of the typical skin lesions found in BHD Syndrome).

4 Juszczak, A., Halliday, D., Mihai, R. *et al.* (2011) A large adrenal tumour as a phenotypic manifestation of the Birt–Hogg–Dube syndrome. *European Congress of Endocrinology*. Endocrine Abstracts, Rotterdam, the Netherlands, P58.

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## Functional consequences of a novel point mutation in the CYP21A2 gene identified in a Chinese Han patient with nonclassic 21-hydroxylase deficiency

Dear Sir,

Most of patients with 21-hydroxylase deficiency (21-OHD) are compound heterozygotes or homozygotes, but heterozygous carriers for CYP21A2 mutations and even no disease-causing mutations in the CYP21A2 allele have been detected in some patients with clinically and biochemically diagnosed 21-OHD. Recently, we indentified a novel point mutation c.446 G>C (p.R149P) in exon 4 in a female patient with hyperandrogenism and her father. The patient came from a family of consanguineous marriage and saw an endocrinologist at age 34 because of infertility. She had an uneventful history and her karyotype was 46 xx. Hormonal determinations demonstrated elevated levels of ACTH 22·42 (normal range, 1·6–13·9 pM), total testosterone 7·01 (normal range, 0·45-3·75 nM). Both basal and ACTH stimulated 17-OHP concentrations were 59·1 and 105·7, respectively (reference value in female, 1·3 ± 0·25 nM). The levels of plasma cortisol were normal. Her father has no clinical symptoms of endocrine and reproductive diseases, and his serum level of 17-OHP was 7·42 (reference value in male, 3·5 ± 1·2 nM).

Specific amplification of CYP21A2 by PCR was performed from the 5′regulatory region (c. −710) to the end of translation, followed by direct sequencing. The −4·6 to −5·6 kb and −2·6 to −2·8 kb upstream of CYP21A2, which was considered as a potential regulation region, were also investigated.[1] We did not detect any mutation except the p.R149P. This mutation was absent in 300 unrelated individuals (600 unrelated alleles) from a Chinese Han control cohort. Moreover, we sequenced CYP11B1 and 3$\beta$-HSD2 genes and found no mutations.

The human full-length CYP21A2-cDNA fragment was synthesized from the pGEM3Z-CYP21 that had been kindly provided by Dr Miller and was recloned into the HindIII/BamHI restriction site of the pcDNA3.1 eukaryotic expression vector, resulting in the pcDNA3.1-CYP21A2-WT construct. The mutagenesis were performed from the pcDNA3.1-CYP21A2-WT construct, using the fast-mutagenesis system (TransGen Biotech Co., Ltd, Beijing, China), and were checked by sequencing the entire constructs. Enzyme activity was analysed by taking the activity of wild-type as 100%, pcDNA3.1 alone as 0. The mutation p.P30L (c.39C>T) was analysed as a control under the same conditions. The mutant p.R149P enzyme showed a residual CYP21 activity of 16·9 ± 2·0% for the conversion of progesterone and 23·4 ± 1·7% for the conversion of 17-hydroxyprogesterone, much lower than the mutation p.P30L enzyme. Michaelis–Menten constant (Km) values revealed that both mutated proteins had a markedly decreased affinity for the two kinds of substrates compared with wild-type. The maximal velocity (Vmax) of p.P30L mutation was higher than p.R149P, although they both were slower than wild-type (Table 1). Analysis of

**Table 1.** Apparent kinetic constants for the CYP21 Wild Type and mutant proteins

| | Wild Type | P30L | R149P |
|---|---|---|---|
| 17-hydroxyprogesterone | | | |
| Km ($\mu$M) | 0·2 ± 0·1 | 0·4 ± 0·1 | 0·48 ± 0·06 |
| Vmax (pmol/min$^{-1}$/mg$^{-1}$) | 27·8 ± 1·3 | 17·8 ± 0·9 | 9·85 ± 0·25 |
| Vmax/Km | 132·3 | 40·5 | 20·5 |
| Progesterone | | | |
| Km ($\mu$M) | 0·4 ± 0·2 | 1·3 ± 0·3 | 1·2 ± 0·2 |
| Vmax (pmol/min$^{-1}$/mg$^{-1}$) | 34·6 ± 2·8 | 24·0 ± 1·5 | 9·3 ± 0·4 |
| Vmax/Km | 87·1 | 18·3 | 8·1 |