

Case Study #1

Pseudoacromegaly

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The diagnosis of acromegaly or gigantism due to GH excess is usually straightforward, and these cases are often diagnosed and managed by the adult or paediatric endocrinologist. However, occasionally patients display facial appearance resembling acromegaly with characteristic soft tissue thickening, skin changes and bone widening. These group of heterogeneous conditions are broadly recognized as "acromegaloidism" or "pseudoacromegaly". In addition, patient may suffer from overgrowth or extreme tall stature/gigantism but without GH and IGF-1 abnormalities.

Case Study #2

Thyroid – to be “free”, or not to be?

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Case

Mr IO was 48 years old when he was referred to an endocrinology outpatient clinic with a five year history of sweats, tremors, increased bowel motions and intermittent palpitations. He had lost 6-8kg three years prior which was thought due to stress. He occasionally experienced migraines but had no other past medical history and was on no regular medications. He had been screened previously for hyperthyroidism with TSH in June 2013, which was measured at 3.30mU/L (0.4-4.0). As his TSH was within the reference range, free T4 was not tested.

IO is a mechanic who lives with his wife in Esperance, a small town 720km south-east of Perth. There was no relevant family history. He is a non-smoker.

On examination in clinic, he had BMI 23kg/m² and heart rate 90 beats/minute in suspected atrial fibrillation. He had a fine tremor and sweaty palms. He had a smooth, diffuse goitre which was non-tender. Eye examination and visual fields were normal. ECG confirmed atrial fibrillation with borderline left ventricular hypertrophy.

Biochemistry showed TSH 5.2mU/L (0.4-4.0), free T4 34pmol/L (9-19) and free T3 23pmol/L (3-5.5). These results were confirmed on both the Architect and Centaur platforms. TSH receptor antibodies and TPO antibodies were negative. Prolactin was 1100mU/L (<340), SHBG 216nmol/L (10-70), total testosterone 37nmol/L (10-35), free testosterone 182pmol/L (260-750), FSH 5U/L (1-8), LH 3.9U/L (1-8), morning cortisol 330nmol/L (150-700) and IGF-1 182ug/L (94-252). HCG alpha subunit was 38.2IU/L (<0.9). Molar ratio of alpha-subunit to TSH was 73.4 (<3).

TRH stimulation test was performed with results as shown in the table.

Minutes Prolactin (mU/L) TSH (mU/L) FT4 (pmol/L) FT3 (pmol/L)

| | | | | |
|----|------|------|----|----|
| 0 | 1090 | 5.20 | 37 | 29 |
| 20 | 1690 | 4.70 | | |
| 40 | 1690 | 4.80 | | |
| 60 | 1540 | 5.00 | | |

Cranial MRI confirmed a suspected pituitary macroadenoma, measuring 40 mm craniocaudal x 26mm transverse. There was suprasellar and inferior intra-sinus extension, right cavernous sinus invasion, and suspicion of possible left cavernous sinus invasion. There was elevation and mass effect on the optic chiasm and prechiasmatic optic nerves, without associated signal abnormality.

IO was started on carbimazole 10mg twice daily and atenolol 25mg daily. Five days prior to pituitary surgery, he started prednisolone 50mg daily. Thyroid function pre-operatively showed TSH 7.4mU/L, free T4 24pmol/L and free T3 7.4 pmol/L. He underwent trans-sphenoidal resection of pituitary macroadenoma on 29/9/16. Post-operatively he was admitted to the high dependency unit where he became agitated, pyrexial, tachycardic and hypertensive with Burch-Wartofsky Point Scale 60, suggestive of thyroid storm. He was managed with esmolol, hydrocortisone, intravenous fluids and 200mg propylthiouracil three times daily, with gradual improvement in clinical state. His propylthiouracil was weaned over the following four days, and glucocorticoids were reduced to maintenance dose. He had a high urine output consistent with diabetes insipidus and required desmopressin. He was discharged home on 7/10/16 on cortisone acetate 25mg BD and desmopressin 200mcg nocte.

One week post discharge, IO presented to his local hospital with meningism and fevers; subsequent lumbar puncture confirmed *Pseudomonas aeruginosa* meningitis. He was treated with intravenous meropenem for three weeks, with step down to oral ciprofloxacin. IO's recovery was further complicated by pneumocephalus requiring a left frontal burr hole procedure. He underwent rehabilitation for cognitive issues and memory impairment, and returned home with his wife on 9/11/16.

Histopathology confirmed pituitary adenoma with patchy immunoreactivity for TSH and FSH, and possibly minor immunoreactivity for prolactin and growth hormone evident in a proportion of the adenomatous cells. Post-operative pituitary biochemistry has shown a persistence of central hyperthyroidism, with bloods from 9/11/16 showing TSH 0.94mU/L, free T4 29pmol/L and free T3 7.1pmol/L. Short synacthen test showed an adequate serum cortisol concentration of 730nmol/L 30 minutes after tetracosactrin, therefore glucocorticoid replacement was ceased. IO's SHBG remains elevated at 88nmol/L, testosterone 14nmol/L, total testosterone 143pmol/L, LH 2.8U/L, FSH 3U/L, prolactin 130mU/L, IGF-1 194ug/L and GH 0.7mU/L.

At last review, IO has regained some weight and his cognitive function is improving, although he continues to have subtle short term memory issues. He remains in rate-controlled AF. Further follow up in clinic will involve a discussion of second-line treatment options, including somatostatin analogues or radiotherapy.

Review

Thyrotropin-secreting pituitary adenomas (TSHomas) are a rare cause of hyperthyroidism and account for <1% of all pituitary adenomas (1). The majority of TSHomas are macroadenomas, although in more recent studies, there have been an increased proportion of microadenomas, postulated to be due to earlier detection (2). Our case highlights a recognised but often overlooked pitfall of TSH screening, with a probable long delay in diagnosis of central hyperthyroidism due to a "normal" TSH. Presenting symptoms of TSHoma include those of hyperthyroidism, although often milder than expected on the basis of circulating thyroid hormone levels, and goitre due to TSH hyperstimulation (2). Partial or total hypopituitarism can occur, and patients may present with symptoms or signs of expanding tumour mass, such as headache and visual field defects (2).

Typically, biochemical testing shows elevated concentrations of circulating free thyroid hormones with a non-suppressed TSH. Hypersecretion of the alpha glycoprotein subunit and raised molar ratio of alpha subunit to TSH favours a TSHoma over thyroid hormone resistance. A raised SHBG is common, as a marker of peripheral thyroid hormone action (3).

Dynamic tests include the T3 suppression test and the TRH stimulation test. The T3 suppression test is both sensitive and specific for diagnosing TSHoma, although it is contraindicated in the elderly or those with ischaemic heart disease. The TRH stimulation test can also be used, and in the majority of patients with TSHoma, there is no increase in TSH after TRH administration (2).

The mainstay of treatment for TSHoma is pituitary surgery, aiming to remove neoplastic tissue and restore normal pituitary function (4). Achieving a euthyroid state pre-operatively is preferred, using antithyroid drugs, beta blockers and occasionally iopanoic acid. Thyroid storm post pituitary surgery is rare, but cases have been published (5, 6). Pituitary surgery is reported to be successful at restoring euthyroidism in 75-84% of patients (7, 8). In cases of surgical failure, pituitary radiotherapy and/or medical treatment with somatostatin analogues can be used (2). Medical or surgical treatments against the thyroid gland have traditionally been avoided, for fears that this may predispose to aggressive transformation of the tumour, as is seen with Nelson's syndrome after adrenalectomy for Cushing's disease. However, some recent cases reported in patients with TSHoma who underwent radioactive iodine treatment or thyroidectomy showed restoration of normal thyroid function and no rapid enlargement of TSHoma (9). In other cases thyroidectomy was performed after pituitary surgery failure, as the patients were at risk of thyroid storm (1).

Long term follow-up of patients with TSHoma is recommended, with recurrence reported in up to 31% of cases (2).

Learning points:

- A TSH within the reference range does not exclude central thyroid disease.
- In cases with elevated thyroid hormone concentrations and non-suppressed TSH, initial diagnostic assessment should include exclusion of assay interference, measurement of alpha subunit, SHBG, and full pituitary profile, with option to progress to dynamic tests such as T3 suppression and/or TRH stimulation.
- Surgery is the first-line treatment for confirmed TSHoma.
- Thyroid storm after pituitary surgery for TSHoma is rare, but has been reported.
- Management options for TSHoma after non-curative pituitary surgery include somatostatin analogues or radiotherapy.

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Case Study #3

Midnight craving for ice - for better or worse: a diagnostic enigma

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A 28-year-old woman was referred to Endocrinologist with excessive thirst and polyuria of 3 months' duration with a fluid intake and urine output of 12 litres a day. This was accompanied by mild headache and fatigue. She had no contributory past medical history apart from being 10 months postpartum. She reported an uncomplicated pregnancy and breastfed uneventfully for 4 months before weaning. She had 2 menstrual cycles since followed by development of amenorrhoea, which coincided with the onset of polyuria and polydipsia.

Physical examination was unremarkable. Initial biochemistry and pituitary function (Table 1) revealed mild hyperprolactinaemia and hypogonadotrophic hypogonadism. The rest of anterior pituitary function is intact. During water deprivation test, diabetes insipidus (DI) criteria was met after 2 hours of fasting (serum osmolality of 310 mmol/kg, sodium of 148 mmol/L, and urine osmolality of 107 mmol/kg). This normalised completely with low dose DDAVP (Table 2), thereby confirming the diagnosis of central DI.

Table 1. Initial biochemistry and anterior pituitary function results

| Test | Result | Normal reference range |
|-----------------------------|----------|------------------------|
| Prolactin (mIU/L) | 526 -750 | < 500 |
| IGF-1 (nmol/L) | 26 | 13 -44 |
| Growth hormone (mIU/L) | 2 | |
| TSH (mIU/L) | 1.1 | 0.3 - 3.5 |
| T4 (pmol/L) | 13.3 | 9 - 19 |
| Cortisol (nmol/L) | 408 | 160 - 650 |
| ACTH (ng/L) | 15 | 9 - 51 |
| FSH (IU/L) | 6 | 2 - 20 |
| LH (IU/L) | 6 | 2 - 70 |
| Oestradiol (pmol/L) | 91 | 110 - 180 |
| Sodium (mmol/L) | 145 | 135 - 145 |
| Potassium (mmol/L) | 4 | 3.5 - 5.5 |
| Corrected calcium (mmol/L) | 2.39 | 2.1 - 2.6 |
| Random glucose (mmol/L) | 4.8 | 3.6 - 7.7 |
| Serum Osmolality (mmol/kg) | 304 | 275 - 297 |

Table 2. Water deprivation test

| Test | 2-hour fast | DDAVP given | Reference range |
|------------------|-------------|-------------|---------------------|
| Serum Na | 148 | 142 | 135 - 145 (mmol/L) |
| Serum Osmolality | 310 | 295 | 275 - 295 (mmol/kg) |
| Urine Osmolality | 107 | 535 | 50 - 1450 (mmol/kg) |

MRI pituitary with gadolinium revealed markedly thickened pituitary stalk and a diffusely enlarged pituitary gland (Figure 1). Causative markers for secondary hypophysitis, including b-HCG, AFP, ACE and IgG4, were all negative (Table 3), supporting the likely diagnosis of autoimmune postpartum hypophysitis. The patient was treated with desmopressin and, after extensive discussion, she declined glucocorticosteroid therapy with an expectant approach to recovery of anterior pituitary (gonadal) function. She was keen to extend her family, and understood that assisted fertility may be required if the pituitary function did not recover.

Figure 1. MRI pituitary

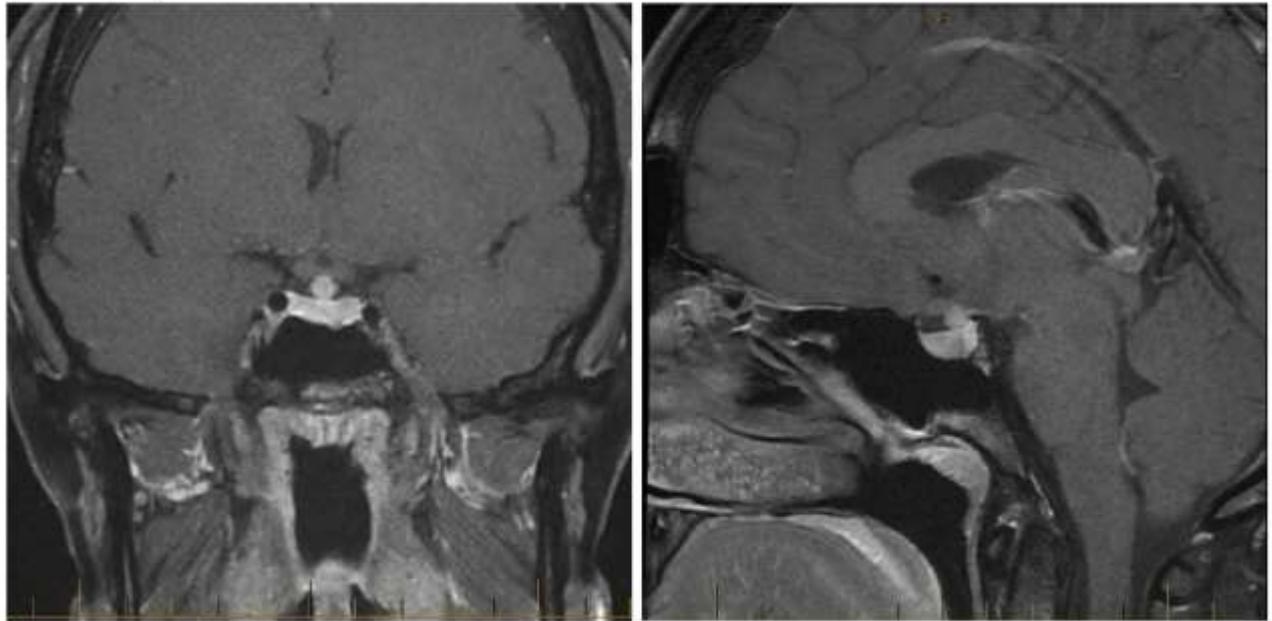
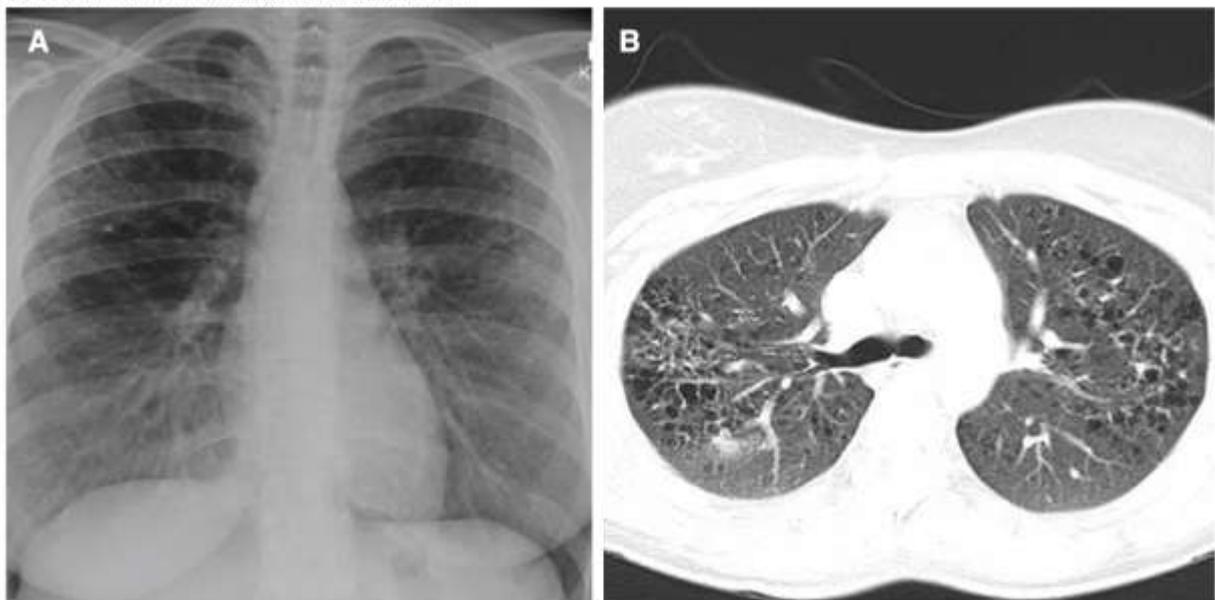


Table 3. Causative markers screen for secondary hypophysitis

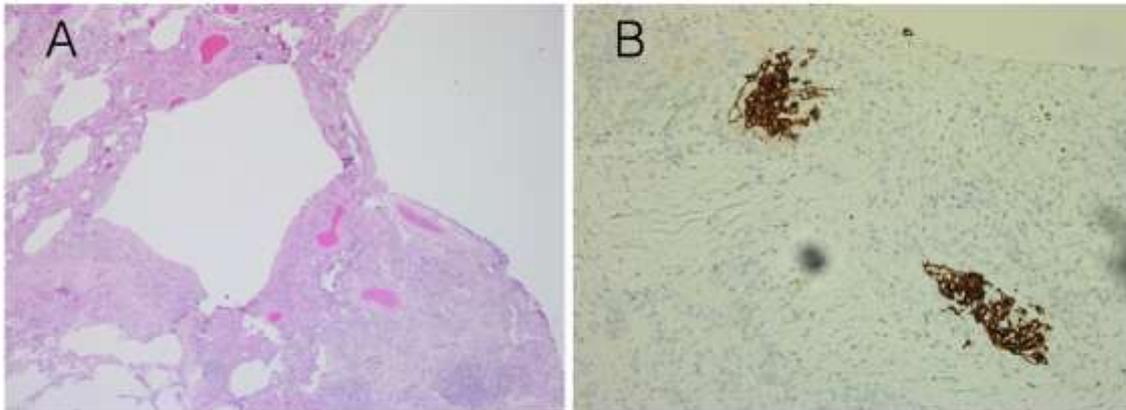
| Test | Result | Reference |
|-------------------------------|--------|----------------------|
| AFP | 2 | < 12 (ug/L) |
| β -HCG | < 2 | < 2 (IU/L) |
| ACE | 69 | 20 - 70 (U/L) |
| 1,25 Vitamin D | 122 | 60 - 208 (pmol/L) |
| Ionised calcium | 1.29 | 1.15 - 1.30 (mmol/L) |
| IgG4 | 0.69 | 0.030 - 2.010 (g/L) |
| Free α glycoprotein SU | 0.13 | < 0.6 (IU/L) |
| p-ANCA, c-ANCA | < 40 | < 40 |

Figure 2. (A) Plain chest radiograph. (B) High resolution CT chest.



Two months later, she reported to her GP with a prolonged episode of respiratory tract infection. She had been smoking intermittently in the past. Chest radiograph revealed interstitial changes, and subsequently a high resolution CT confirmed bilateral upper zone cystic lung disease (Figure 2A, 2B). Respiratory opinion was sought, and the clinical impression was in line with pulmonary lymphangioleiomyomatosis (PLAM). At this stage, given the rarity of both hypophysitis and PLAM, an alternative unifying diagnosis such as sarcoidosis or Langerhan cell histiocytosis (LCH) was sought. However, skeletal survey for bony lesions, calcium metabolism, bronchoscopic lavage and trans-bronchial biopsy failed to achieve a conclusive diagnosis. Since PLAM exacerbates with pregnancy, and the patient was keen to extend the family, a definitive diagnosis was felt all the more necessary. We proceeded with an open-lung biopsy for definitive diagnosis. The initial histopathology was equivocal for histiocytosis as well as for PLAM. After two further specialist opinions, pulmonary pathologists were finally able to identify CD1a-positive histiocytes in a biopsy specimen from the middle lobe (see Figure 3B), favouring the working diagnosis of burnt-out histiocytosis.

Figure 3. (A) H&E slides of the cystic lesion with the chronic inflammatory cells. (B) CD1a positive Histiocytes



DISCUSSION:

New onset central diabetes insipidus (DI) and hypopituitarism encompasses a broad list of differential diagnoses which include trauma, inflammatory, infective and neoplastic lesions that involves the pituitary gland and the stalk. In this case, the initial presentation with central DI and thickened stalk on pituitary MRI in a postpartum setting was typical of autoimmune infundibulohypophysitis.

Autoimmune hypophysitis (AH) is a chronic inflammatory condition characterised by lymphocytic infiltration of the pituitary, usually affecting young women with a striking temporal association to pregnancy¹. Due to potential hazards associated with pituitary biopsy, most of the diagnosis in clinical practice is presumptive based on consistent clinical, hormonal and radiological profile. The natural history and management of AH is not well defined, as most of the existing literature is based on case reports. Reported outcome are variable, ranging from permanent hypopituitarism to usually spontaneous recovery of pituitary function. The use of corticosteroids has been mainly to attenuate the inflammatory process and reduce mass effect, but the role of immunosuppression in functional pituitary axis recovery remains controversial^{2,3}.

Systemic diseases such as sarcoidosis, granulomatous vasculitis, tuberculosis and Langerhan cell histiocytosis (LCH) may manifest in pituitary infiltration and hypopituitarism, and should be considered in the differential diagnosis of hypophysitis. The presence of sellar mass and other systemic manifestations of the primary pathology usually provide clues to such diseases. However, as is seen in the current case of LCH, such differentiation may not be obvious, and the pursuit of diagnosis with an appropriate management can be challenging. LCH is a rare disorder affecting (2 per million) characterised by the abnormal proliferation and infiltration of pathological Langerhan cells. The clinical course is highly variable, ranging from indolent disease limited to single skeletal lesion, to disseminated disease which can result in fatality⁴. Interestingly, LCH has a predilection to involve the hypothalamus-pituitary system⁵. Infiltration of the pituitary stalk with resultant central DI is amongst the most common endocrine manifestations in adults with LCH⁶. Anterior pituitary deficiencies are reported in 20% of patients with LCH, growth hormone deficiency being the most common, followed by gonadotrophins, TSH and ACTH^{5,6}. Other commonly involved systems in adult LCH are bone, lungs and skin, where pulmonary involvement carries the highest risk of mortality⁴.

Pulmonary lymphangioleiomyomatosis (PLAM) was considered as a likely cause of the pulmonary cysts in our case based on the radiological appearance of cystic lung changes and patient demographics. This is a rare lung disease characterised by abnormal smooth muscle proliferation within pulmonary lymphatics and airways, with resultant cyst formation and destruction of normal pulmonary parenchyma. True prevalence and underlying pathogenesis of PLAM is unknown due to its rarity, but in overwhelming majority of cases it affects women of child-bearing age^{7,8}. The diagnosis of PLAM is associated with variable prognosis, sometimes with progression to end-stage lung disease and mortality at 10-20 years after diagnosis^{7,8}.

To the best of our knowledge, there is no association between PLAM and pituitary disease, making it unlikely for two rare conditions to co-exist, although not impossible. Ascertaining correct diagnosis in this patient has profound implication with regard to prognosis, future follow-up and perhaps most importantly, decision making with regard to assisted fertility options. PLAM is reported to accelerate in pregnancy with loss of lung function, and disease onset is rare outside of reproductive years, implicating that oestrogen is central in the progression of PLAM. On the other hand, similar gender preference and pregnancy related morbidity is not observed in mild forms of LCH, and patient with LCH have been described to undergo pregnancies safely without adverse impacts⁹. Pre-existing DI in women with LCH may worsen during pregnancy and require increased

supervision^{9,10}. Assisted fertility and management of diabetes insipidus as well as monitoring for possible pituitary insufficiency and progression of lung disease in the context of pregnancy in our case would be a challenge.

This case demonstrates the difficulties in differentiating underlying causes of hypophysitis. Presumptive diagnosis of autoimmune hypophysitis and 'premature closure' in a young female may lead to missed diagnosis of important systemic pathology. However, this needs to be weighed against the significant morbidity associated with a pituitary stalk biopsy. Furthermore, the detection and diagnosis of secondary causes such as LCH can pose further diagnostic and management dilemmas.

Take home messages & discussion:

1. Differential diagnosis of postpartum hypophysitis and other granulomatous diseases
 2. Monitoring and treatment of hypophysitis and DI particularly in reference to cortisol axis and future assisted fertility planning
 3. Diagnostic challenges with LCH and its endocrine manifestations and follow-up
 4. Effect of oestrogen on respiratory disease in particular exacerbation of PLAM.
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Case Study #4

A curious case of acromegaly

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Case: A 59 yo ex-smoker presented to hospital in 2013, two weeks post-haemorrhoidectomy with abdominal pain and constipation. Abdominal and chest X-rays were performed; the latter revealed a 7 cm parahilar mass. He was noted to be acromegalic on review by the thoracic surgeon. Further history confirmed a 5-year history of the development of coarse facial features, enlargement of his hands and feet, and enlarged nose. Elevated serum growth hormone (GH) and insulin like growth factor 1 (IGF-1) levels prompted referral to an endocrinologist and neurosurgeon.

Past history: Type 2 diabetes well controlled on sitagliptin/metformin 50/1000mg mane; hypertension treated with perindopril/amlodipine 10/5mg mane; hepatitis B.

Investigations:

1. **Biochemistry.** Glucose Tolerance Test demonstrated failure of suppression of GH with corresponding rise in glucose (Table 1) IGF-1 147 nmol/L (normal range (NR) 7-24 nmol/L), Chromogranin A 113 U/L (NR 0-21.8 U/L). Total testosterone 7.0 nmol/L (NR 8.3-30.2 nmol/L), FSH 5 IU/L (NR 1-10 IU/L), LH 3 IU/L (NR 1-10 IU/L). Levels of serum prolactin, thyroid stimulating hormone, thyroxine, cortisol, adrenocorticotropic hormone, parathyroid hormone, serum calcium, and metanephrines were in the normal range.

Table 1. Pre and postoperative glucose tolerance test for growth hormone suppression

| Glucose Tolerance Test | 0 minutes | 60 minutes | 120 minutes |
|--|-----------|------------|-------------|
| 2013 Growth Hormone levels (NR 0-7 mIU/L) | 85 mIU/L | >120 mIU/L | 94 mIU/L |
| 2017 Growth Hormone levels (NR 0-7 mIU/L) | 8 mIU/L | 15 mIU/L | 7 mIU/L |

1. **Imaging.** MRI pituitary (Figure 1): enlarged pituitary gland measuring 12 mm craniocaudal height, likely macroadenoma centred within the anteroinferior pituitary, mildly heterogeneous post contrast, without suprasellar extension. CT chest: a solitary well circumscribed left intraparenchymal perihilar 5.4 x 7.1 x 6.2 cm heterogeneous enhancing mass with flecks of internal calcium (Figure 2). FDG PET/CT (Figure 3): 7 cm diameter left upper lobe parahilar lung mass with moderately intense heterogeneous FDG avidity, intense metabolic activity in the pituitary fossa and focal intense FDG activity in (R) thyroid lower pole. Features were thought to be consistent with a bronchial carcinoid and a Ga-68 DOTATE PET/CT was performed (Figure 2) – DOTATE positivity indicating somatostatin receptor activity was demonstrated within the lung mass and the pituitary. Thyroid ultrasound: small multinodular goitre, final needle aspirate biopsy of dominant nodule demonstrated colloid only.

Figure 1: MRI pituitary (A) preoperatively, (B) postoperatively

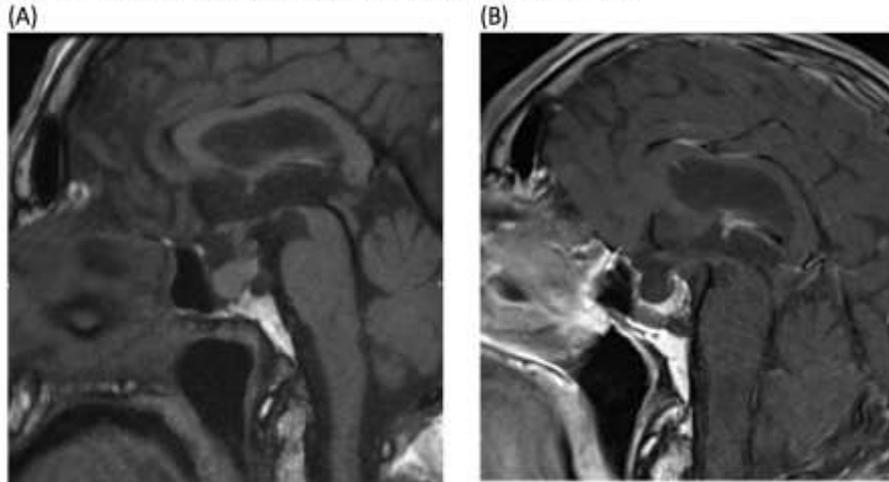
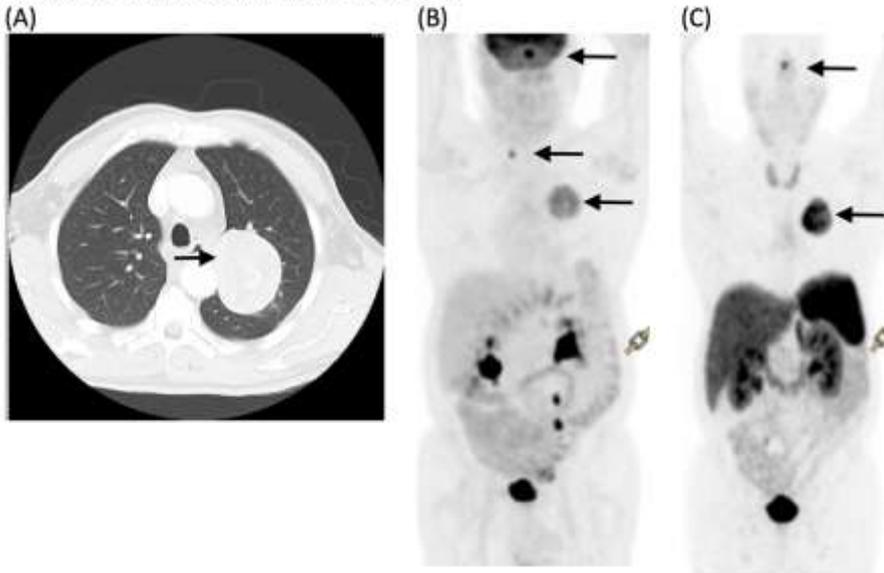


Figure 2: Imaging A: CT chest demonstrating heterogeneous lung mass with flecks of internal calcium (arrow). **B:** FDG PET/CT demonstrating FDG avidity in the perihilar lung mass, lower pole of the right lobe of the thyroid, and pituitary fossa (arrows). **C:** Ga-68 DOTATATE PET/CT demonstrating inverse heterogeneity in somatostatin receptor uptake to the FDG tumour distribution, with increased pituitary uptake (arrows).



Progress:

In 2014, the patient underwent left pneumonectomy with complete resection of the lung tumour. Histology: 7.2cm left upper lobe well differentiated carcinoid, with no evidence of vascular or pleural invasion. All 18 sampled lymph nodes were negative for malignancy. Tumour staining was positive for chromogranin and synaptophysin consistent with neuroendocrine tumour. Further immunohistochemistry performed in Toronto, Canada confirmed strong positivity for Growth Hormone Releasing Hormone (GHRH); and focal positivity for serotonin.

Post operatively, the patient had dramatic clinical improvement in his acromegalic features. There was biochemical improvement but not normalisation of IGF-1 levels, with failure to suppress GH following glucose load (Table 2). Serum chromogranin-A had normalised to 88 ug/L (NR 27-94 ug/L). 24 hour urinary 5-HIAA was mildly elevated at 52 umol/24h (NR 10-40 umol/24 h). Repeat MRI pituitary three months post-pneumonectomy demonstrated decreased size of the previously hypoenhancing inferior aspect of the pituitary to measure 8 x 7 x 7 mm. Subsequent annual MRI imaging has demonstrated size stability of the hypoenhancing nodule within the pituitary gland (Figure 2).

The ongoing issues are of continued growth hormone excess. The patient is currently being evaluated for potential carcinoid tumour recurrence and the possibility of a GH secreting pituitary adenoma. Although he has no family history suggestive of multiple endocrine neoplasia syndromes, the potential presence of two tumours has prompted a referral to genetic services.

Table 2. Biochemistry and pituitary hormone panel results

| | Preoperative (2013) | 5 months postoperative (2014) | 3 years postoperative (2017) |
|---|------------------------|----------------------------------|---------------------------------|
| IGF-1 (NR 6-29 nmol/L) | 147 nmol/L | 44 nmol/L | 43 nmol/L |
| TSH (NR 0.50-4.00 mIU/L) | 1.83 mIU/L | 1.23 mIU/L | 1.55 mIU/L |
| FT4 (NR 10.0-19.0 pmol/L) | 17.1 pmol/L | 14.0 pmol/L | 16.0 pmol/L |
| FT3 (NR 3.5-6.5 pmol/L) | 4.8 pmol/L | - | 4.6 pmol/L |
| Cortisol (NR 145-619 nmol/L) | 289 nmol/L | *222 nmol/L | 366 |
| ACTH (NR <20 pmol/L) | 3.4 pmol/L | *2.7 pmol/L | pending |
| Prolactin (NR 45-375 mIU/L) | 30 mIU/L | - | 177 mIU/L |
| Testosterone (total) (NR 10.0-25.0 nmol/L) | 7.0 nmol/L | 8.8 nmol/L | 11.8 nmol/L |
| LH (NR 1-10 IU/L) | 3 IU/L | 4 IU/L | 3 IU/L |
| FSH (NR 1-10 IU/L) | 5 IU/L | 7 IU/L | 6 IU/L |
| Chromogranin A (NR 27-94 ug/L) (NR 0-21.8 U/L) | 113 U/L | 88 ug/L | pending |
| 24h Urine 5-HIAA (NR 10-40 umol/24h) | - | 52 umol/24h | pending |
| PTH (NR 1.5-7.6 pmol/L) | 5.4 pmol/L | - | 10.1 pmol/L |
| Calcium (NR 2.15-2.65 mmol/L) | 2.40 mmol/L | 2.20 mmol/L | 2.46 |
| Plasma normetanephrine (NR <500 pmol/L) | 424 pmol/L | - | pending |
| Plasma metanephrine (NR <500 pmol/L) | 131 pmol/L | - | pending |
| HbA1c % | - | 5.9 % | 6.0 % |

Discussion: Ectopic secretion of GHRH from a neuroendocrine tumour comprises <1% of cases of acromegaly(1). It was first described in 1959, and by 2013, 98 cases had been reported. Greater than 90% of GHRH secreting tumours arise from bronchial carcinoid tumours and pancreatic neuroendocrine tumours (1-3), with the latter associated with MEN-1 (2).

As a result of GHRH hypersecretion, pituitary hyperplasia develops in up to 60% of cases. This can be difficult to distinguish from a pituitary adenoma on MRI imaging alone. MRI in patients with ectopic acromegaly was interpreted as pituitary adenoma in approximately 20% of cases (1). In the French case series, histology demonstrated pituitary hyperplasia in all 4 patients with ectopic acromegaly who underwent trans-sphenoidal resection for suspicion of adenoma on MRI (2). In contrast, the development of adenoma has been reported in a mouse model (4), but is likely much rarer in humans.

The underlying pathogenesis of pituitary adenoma formation in the setting of hypersecretion of ectopic GHRH continues to be subject of unresolved debate. Pituitary adenoma formation, in general, is likely to occur from loss of function in tumour suppressor genes and/or oncogene activation leading to monoclonal expansion of a cell (5). In GH-secreting pituitary adenomas the *gsp* gain of function mutation is the most frequent genetic change observed, present in up to 40% of cases (5). Familial pituitary adenoma syndromes are present in approximately 5% of patients with pituitary adenomas (5).

Garby *et al.* in 2012 described a French nationwide series of 21 cases of ectopic GHRH secreting tumours between 1983-2008. GHRH levels are undetectable in normal individuals (<30 ng/L) and low in patients with typical acromegaly arising from pituitary or patients with GHRH secretion arising from a hypothalamic tumour (6). In this French series, serum GHRH was elevated in all patients with ectopic acromegaly with a range from 100ng/L to 145,000ng/L, and median level of 860ng/L (2). Serum GHRH may thus be useful as a surveillance tool post operatively (2).

Complete tumour resection is the only curative treatment for ectopic acromegaly. Where complete surgical resection was unsuccessful or unable to be performed, treatment with a somatostatin analog is recommended to decrease mortality risk associated with hypersecretion of GH (7). Somatostatin analog therapy has been shown to reduce levels of IGF-1 and GH, but has not been shown to significantly decrease tumour size (2, 8).

Long-term overall survival from ectopic acromegaly is still unknown, however prognosis is thought to be favourable and comparable to other neuroendocrine tumours, despite 50% of cases of ectopic acromegaly having metastatic disease at diagnosis (1). In the French series, there were 7 bronchial carcinoids of which 5 did not have metastases at diagnosis –these remained in remission after median follow-up of 9.5 years after complete resection (2). In a further study of 23 operated cases, 87% were in remission, after median follow-up of 2 years (9).

Take home messages:

- Ectopic acromegaly arising from GHRH secreting tumours accounts for <1% of all cases of acromegaly.
 - The majority of cases of ectopic acromegaly arise from GHRH hypersecretion from bronchial carcinoid tumours, and pancreatic neuroendocrine tumours.
 - Patients with ectopic acromegaly from pancreatic tumours should be evaluated for MEN-1
 - Suspicion for ectopic acromegaly may arise when there is discordance between extremely high GH/IGF-1 levels and non-enlarged/minimally enlarged pituitary gland, though degree of granulation also affects hormone production.
 - Surgical resection remains the primary treatment for ectopic acromegaly, and if not feasible or incomplete, somatostatin analog therapy should be considered.
 - Multi-disciplinary approach with attentive surveillance is fundamental to provide optimal care for these complex patients
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Case Study #5

Alpha cell hyperplasia due to a glucagon receptor mutation complicated by hypercalcaemia

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Introduction: This unusual case describes a 47 year old male with alpha cell hyperplasia and a germline glucagon receptor mutation who presented with hypercalcaemia which resolved post subtotal pancreatectomy. It highlights alpha cell hyperplasia as a premalignant condition and further study is required to understand the physiology of the unexplained hypercalcaemia.

Case report: A 47yo man presented with PTH independent hypercalcaemia (3.48mmol/L). The presenting symptoms were abdominal pain and intermittent vomiting. Significant past medical history was epilepsy. Extensive investigations yielded no cause. Persistently low 1,25-Vit D, 25OH Vitamin D and PTH-rP were observed. Further investigations with CT of his abdomen demonstrated multiple punctate foci of calcification in keeping with chronic pancreatitis (figure 3). FDG-PET showed no evidence of significant FDG-avid disease or lymphoma. The hypercalcaemia was attributed to a septic state in combination with immobility contributing to increased bone resorption.

Subsequent pancreatic investigations yielded high pancreatic polypeptide 2890 pmol/L [< 55] and an exceptionally high glucagon level of 43 547 pg/mL [40-140]. Fasting insulin was low 2.3 mIU/L [< 10] and there was a raised Chromogranin A 17.8 nmol/L [< 3] (on PPI). Throughout these investigations, Mr PM's glucose had remained in the normal range.

Further imaging confirmed a slightly bulky pancreas with no pancreatic duct dilatation. MRCP showed no solid neoplasms in pancreas. Ga-68 DOTATATE-PET showed diffusely intense increased tracer uptake throughout the pancreas, with no abnormal uptake elsewhere suggesting a diffuse neuroendocrine cell hyperplastic process. Endoscopic ultrasound showed lobulation and stranding heterogeneity of entire pancreas, without a single dominant lesion.

Mr PM remained normoglycaemic and there was no skin rash to suggest necrolytic migratory erythema. Mr PM described episodes of feeling 'hypoglycaemic' and had neuroglycopenic symptoms which resolved with food intake. Consideration was given to the possibility of germline glucagon receptor mutation, in association with reactive alpha cell hyperplasia.

Hypercalcaemia was investigated with a Tc99m sestamibi and SPECT CT which showed no abnormal accumulation of tracer on either phase of the study. At one point a high calcium and inappropriately normal PTH was seen so exploratory neck surgery was performed and 2 parathyroid glands were excised with pathology confirming no adenoma. Mr PM's calcium remained labile.

Repeat Ga-68 DOTATATE-PET was performed and there was a suggestion of increased tracer uptake in pancreatic head with an increased SUVmax. Mr PM was commenced on Sandostatin LAR with the diagnosis of alpha cell hyperplasia.

Glucagon sampling was performed from the portal, splenic and superior mesenteric vein to assess for a localised area of glucagon secretion. A focal area of production was found around the pancreatic neck consistent with alpha cell hyperplasia. Splenectomy and subtotal pancreatectomy was performed (65% removed). Pathology confirmed diffuse alpha cell hyperplasia (figures 1 and 2) and multiple pancreatic neuroendocrine tumours were identified with micrometastases in 11/30 lymph nodes. IHC was positive for glucagon and pancreatic polypeptide and he was staged as Stage p2B (mT3N1). Calcium normalised post operatively and has remained stable despite ongoing abdominal pain. Post operative OGTT was normal and glucagon levels remained high, but reduced at 14,498 pg/mL. DOTATATE PET showed no new metastatic disease and he was recommenced on lanreotide 120mg monthly.

Genomic analysis confirmed a homogenous missense variant was detected in the glucagon receptor gene (Asp63Asn). This is a previously described pathologic variant and has a known association with Mahvash syndrome.

Figure 1: Pathology of pancreas: Immunohistochemistry for Glucagon

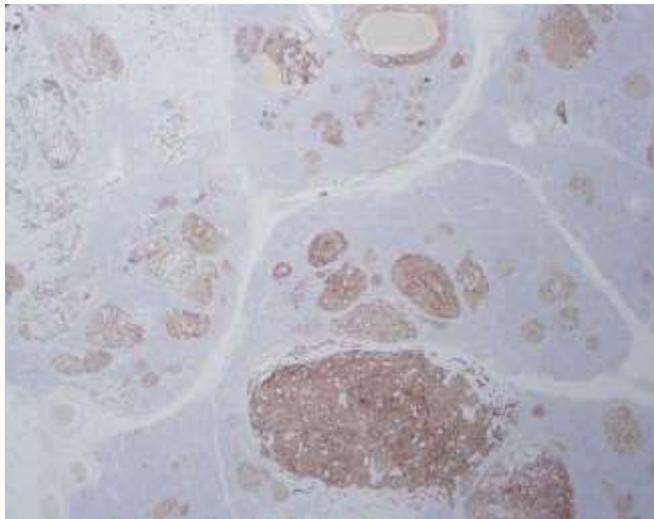


Figure 2: H/E Stain with grossly abnormal Islets of Langerhans

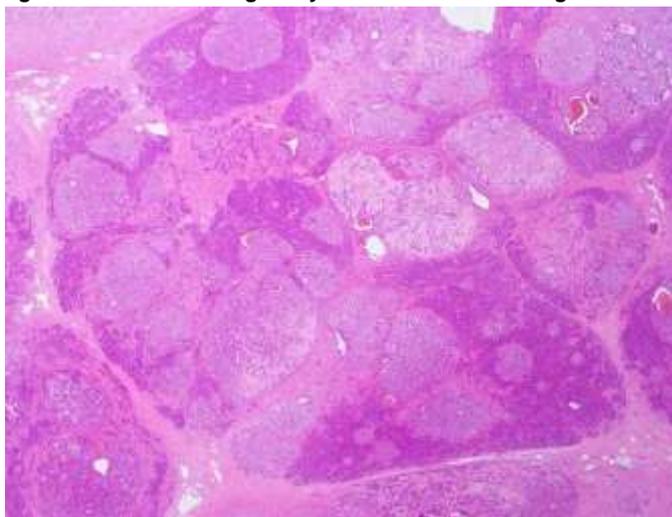


Figure 3: CT of pancreas showing diffuse enlargement and coarse calcifications:



Discussion:

Hyperglucagonaemia in the absence of glucagonomas is rare. Inactivating mutations in the glucagon receptor (GCGR) cause reactive alpha cell hyperplasia and have been associated with Mahvash syndrome [1]. This autosomal recessive phenotype is characterised by alpha cell hyperplasia with non functioning hyperglucagonemia without other features of glucagonoma syndromes. Beta cells have glucagon receptors to inhibit insulin release and ensure normoglycaemia. Dysregulation of beta cells causes mild fasting hypoglycaemia, reported both by our patient, and in the literature. Physiological consequences of hyperglucagonaemia are not well understood but include dysfunction in hepatic lipid metabolism and fatty acid oxidation [2].

The glucagon receptor is a G protein coupled receptor that signals through adenylate cyclase and stimulates intracellular calcium release [3]. Glucagon's precursor Proglucagon, is cleaved into glucagon, GLP-1, GLP-2 and others following post translational processing. GCGR contains 485 amino acids and shares 42% sequence identity with the GLP-1 receptor [4]. High levels of Glucagon affect levels of GLP-1 but it is unclear to what degree and whether there are significant physiological consequences.

Glucagon cell adenomatosis was recently classified as a new pathological entity constituting alpha cell hyperplasia and hyperglucagonaemia [5]. Reactive alpha cell hyperplasia but has been shown to be preneoplastic in both murine models and human subjects [6]. ACH eventually evolves into slow growing pancreatic neuroendocrine tumours and these contribute to the demise of affected mice.

GCGR mutations are also associated with high aminoacidaemia. In the GCGR knock out mice, amino acid catabolism genes are downregulated [6]. Glucagon affects amino acid production in the liver and patients with glucagonomas are hypoaminoacidaemic. A lack of glucagon leads to increased production of amino acids causing alpha cell hyperplasia and an attempt to reinstitute normal levels of glucagon [7]. In these patients with GCGR mutations, the high levels of glucagon are non functional hence amino acids are raised to compensate.

The GCGR mutation identified in Mr PM has been described in a family with hyperglucagonaemia and diffuse pancreatic neuroendocrine tumours [8]. Functional studies of variants affecting this amino acid residue are suggestive of loss of function which is in keeping with Mr PM's phenotype.

The normalisation of hypercalcaemia post subtotal pancreatectomy is highly suggestive of a causal relationship between hypercalcaemia and hyperglucagonaemia but the mechanism is not well understood. Variations in protein intake through diet are known to affect calcium levels by unknown mechanisms. The calcium sensing receptor is activated not only by extracellular calcium ions but also by aromatic amino acids [9]. Further complicating calcium homeostasis, glucagon is known to stimulate the parathyroid glands [10].

Key points:

- Inactivating mutations of the glucagon receptor leads to non functional hyperglucagonaemia and are associated with Mahvash syndrome.
- Minor modification of glucose homeostasis occurs due to insulin dysregulation but clinically it is limited to fasting hypoglycaemia
- GCGR mutations cause alpha cell hyperplasia
- Alpha cell hyperplasia is a precursor to pancreatic neuroendocrine tumours

- Amino acid levels are exquisitely sensitive to glucagon levels
- Hypercalcaemia is an unreported consequence of Mahvash disease with an unclear mechanism

Case Study #6

Medullary thyroid carcinoma: a rare cause of Cushing's syndrome

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A 34-year-old man was diagnosed with sporadic (RET negative) metastatic medullary thyroid carcinoma (MTC) in July 2013 after presenting with persistent left-sided cervical lymphadenopathy. His serum calcitonin was elevated at 492 pmol/L. Initial staging did not reveal detectable distant metastases. Despite several neck surgeries, his serum calcitonin remained detectable rising from 38-100 pmol/L. In March 2015, restaging FDG-PET demonstrated evidence of metastatic disease involving the neck, pulmonary hila and mediastinum. Given asymptomatic status and slow progression, no systemic treatment was given.

In August 2015, despite stability of his metastatic MTC, within 3 months, he deteriorated from an active mountain biker to ECOG 3 functional status. He reported fatigue, insomnia and 8 kg weight gain. Exam showed marked plethora with evidence of marked proximal myopathy and fragile skin with easy bruising, but no striae or hyperpigmentation. A clinical diagnosis of rapidly progressing Cushing's syndrome was made.

Biochemistry was in keeping with ACTH-dependent Cushing's syndrome with a random cortisol of 810 nmol/L with corresponding ACTH 104 ng/L (7.2-63.3). 24-hour urinary free cortisol (UFC), measured by immunoassay was markedly elevated up to 3270 nmol/day (<150). Serum potassium was normal. Pituitary MRI was unremarkable. Inferior petrosal sinus sampling (IPSS) was consistent with ectopic Cushing's syndrome (Table 1).

Table 1: Inferior petrosal sinus sampling results

| Time (min)* | Right | Left | Periphery | PPR† (right) | PPR (left) |
|-------------|-------|------|-----------|--------------|------------|
| -2 | 172 | 174 | 185 | 0.93 | 0.94 |
| 0 | 197 | 169 | 159 | 1.24 | 1.06 |
| 2 | 209 | 185 | 156 | 1.34 | 1.19 |
| 5 | 212 | 186 | 180 | 1.18 | 1.03 |
| 10 | 226 | 197 | 173 | 1.31 | 1.14 |

*Time since CRH injection (1 µg/kg)

†PPR: petrosal sinus to peripheral gradient

Given the rapidly progressive ectopic Cushing's syndrome, and relative stability of his MTC, in December 2015 he underwent laparoscopic bilateral adrenalectomy, with postoperative hydrocortisone and fludrocortisone replacement. Despite DVT prophylaxis the perioperative period was complicated by pulmonary embolus requiring therapeutic enoxaparin. Postoperatively, his functional status improved markedly, to ECOG 0-1.

In February 2016 FDG-PET imaging revealed progressive metastatic MTC involving cervical lymph nodes, thoracic spine, and mediastinum despite a stable serum calcitonin of 1495 pmol/L. Absent Ga-68 DOTATATE uptake in the mediastinum suggested of poorly differentiated disease.

Following palliative radiotherapy to the thoracic spine, he enrolled in a clinical trial of cabozantinib. However, this was ceased after 7 months due to disease progression with a rising calcitonin (to 2158 pmol/L) and new bony metastases.

He is currently pursuing options for vandetanib or pembrolizumab.

Discussion

Ectopic CS in MTC

MTC-associated ectopic Cushing's syndrome (CS) almost always occurs in the setting of metastatic MTC, with an estimated prevalence of 0.6%. Less than 100 cases have been reported in the literature.¹ MTC accounts for 1-3% of cases of ectopic CS² and is associated with a poor prognosis.^{2,3} Interestingly, case series consistently demonstrate a male predominance but the reasons for this are unknown. Clinical presentation is usually florid, and commonly accompanied by hypokalaemic metabolic alkalosis. However, the clinical picture may be less specific in a patient with metastatic cancer, and ectopic CS must be considered

in otherwise unexplained deterioration in a patient with slowly progressive MTC. While immunohistochemistry of the tumour (if resected) with anti-ACTH and/or anti- CRF antibodies can be confirmative, this is positive only in the minority of ectopic CS associated with MTC. Reasons for this are unknown, but de-differentiation, continuous secretion without cellular storage of ACTH, or abnormal processing of proopiomelanocortin (POMC) have been proposed.¹

The molecular defects that lead to ectopic ACTH secretion in neuroendocrine tumours are largely unknown.⁴ ACTH-producing neuroendocrine tumours in MEN-1 or MEN-4 can harbour germline menin or other somatic mutations. ACTH-producing MTC has been described in in MEN-2 syndrome with oncogenic RET gene mutations, but can also occur with RET negative sporadic MTC.⁴ A summary of the genetic and molecular mechanisms implicated in CS is shown in Figure 1.

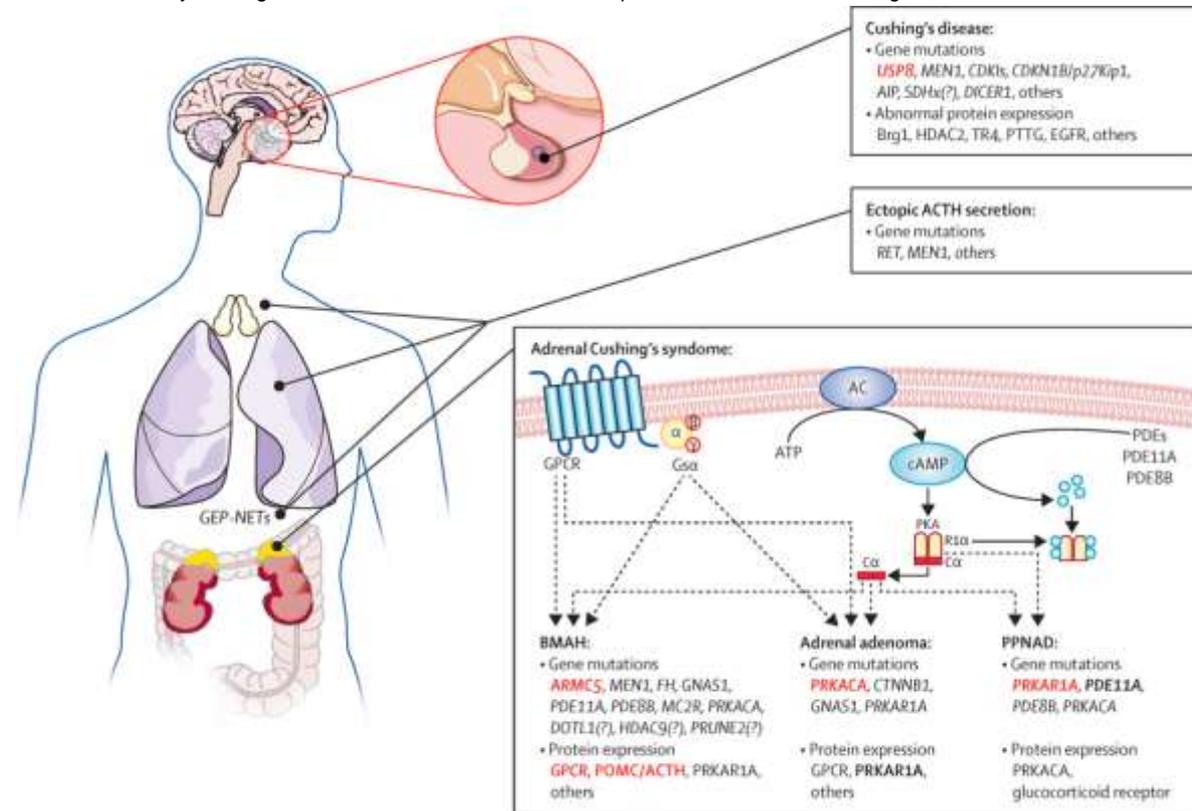


Figure 1: Summary of genetic and molecular mechanisms implicated in Cushing's syndrome. From: Lacroix *et al.* (2015) Cushing's syndrome. *Lancet*, **386**, pg 915.

Investigation of ACTH-dependent CS: IPSS pitfalls

In the differential diagnosis ACTH-dependent CS, non-invasive dynamic testing (corticotrophin-releasing hormone (CRH) test or high dose dexamethasone suppression test (HDDST)) and pituitary imaging may be misleading. Indeed, in a case series of 10 patients, in the one patient with a pituitary lesion, this proved to be a metastasis of MTC.¹ In general, IPSS is considered the gold-standard test for the differential diagnosis of ACTH-dependent CS and is required in almost all patients. A petrosal sinus to peripheral ACTH gradient of at least 2.0 at baseline or at least 3.0 after CRH administration suggests a pituitary source of ACTH.⁴ However, false-negative results (i.e. the absence of a gradient falsely suggestive of ectopic ACTH syndrome) have been reported in 1–10% of cases, most commonly due to unsuccessful inferior petrosal sinus catheterization.⁵ Conversely, if IPSS is performed in the absence of sustained hypercortisolism (e.g. mild, cyclical or medically treated CS), false positive results can occur. This is because the normal corticotrophs are not suppressed and will respond to CRH suggesting Cushing's disease, regardless of the aetiology of CS. An algorithm for the aetiological investigation of confirmed CS is shown in Figure 2.

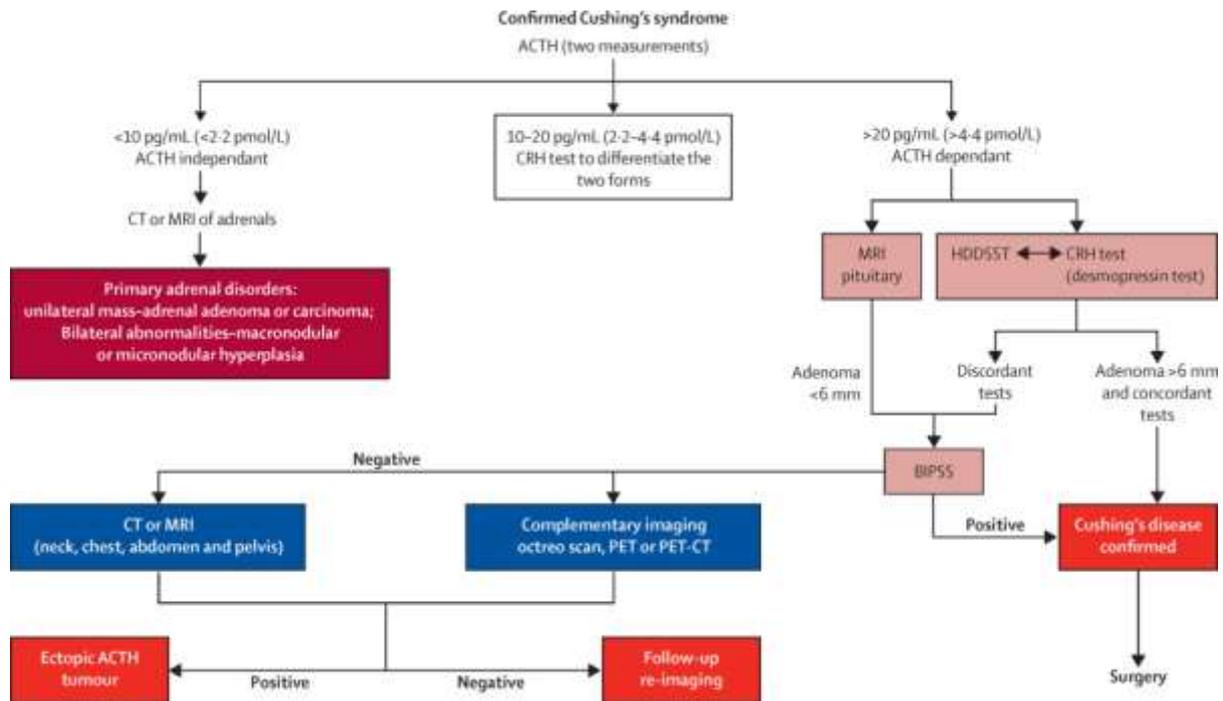


Figure 2: Algorithm for the differential diagnosis of the causes of Cushing's syndrome. From: Lacroix *et al.* (2015) Cushing's syndrome. *Lancet*, **386**, pg 918.

Prognosis and management of MTC-associated ectopic CS

Uncontrolled ectopic CS was responsible for 50% of the mortality associated with metastatic MTC in the largest case series ($n=10$).¹ Complications of peritonitis due to bowel perforation has been reported to account for 30% of the mortality among patients with MTC-associated ectopic CS, and this should be considered in any patient presenting with sepsis and/or unexplained abdominal pain.^{1, 6} Resection of the ACTH-producing tumour is generally not possible in the context of disseminated MTC, and conventional medical adrenolytic treatment is usually ineffective. Therefore, bilateral adrenalectomy, preferentially laparoscopic in a timely fashion is recommended, before surgical risks become prohibitive. Indeed, given that MTC is often slowly progressive, adrenalectomy should be considered even in the context of metastatic disease. Perioperatively, hypercoagulability and immunosuppression are concerns, but high level evidence for prophylactic management is not available. Venous thromboembolism prophylaxis should be considered for four weeks post-operatively.⁷ Consideration should also be given to prophylaxis against invasive fungal infections, such as *Pneumocystis carinii*.⁸

In the setting of ectopic CS associated with MTC, there have been several recent case reports of reversal of CS following the administration of the tyrosine kinase inhibitor (TKI), vandetanib, to patients with advanced MTC.⁹⁻¹³ A decision was made for our patient to undergo bilateral adrenalectomy prior to commencement of TKI therapy due to the rapidly progressive nature of CS.

Key learning points

- MTC is a rare cause of ectopic ACTH-dependent Cushing's syndrome.
- The gold standard investigation for the differential diagnosis of ACTH-dependent CS is IPSS but false positive and false negative results can occur.
- Ectopic CS in the context of MTC is usually aggressive with substantial mortality if untreated. It is often refractory to medical management, and timely bilateral adrenalectomy needs consideration on an individualised basis, even in the setting of metastatic MTC.
- The utility of TKIs such as vandetanib for control of ectopic CS requires further study.

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Case Study #7

A case of low trauma fracture associated with Adult Hypophosphatasia

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Mrs. B.G, a 72 year old woman, was referred to the Metabolic Bone Disorders Clinic in 2016 with a history of a low trauma fracture.

She had sustained a right Colle's fracture in 2009, at age 65, after slipping on ice from a standing height. There was no history of arthralgias or myalgias. Her top teeth had been removed in her 30s. Her bottom teeth were in good condition. She underwent menopause in her late 40s, did not smoke or consume alcohol regularly. There was no personal history of chronic liver, kidney or lung disease, celiac disease or diabetes. Mrs B.G had a history of Autoimmune Hypothyroidism (anti-TPO >1300 U/ml, anti-TG 83 U/ml) with Thyroxine replacement since September 2016. Hypertension was controlled with Amlodipine/Valsartan/HCT 10/160/12.5mg.

Her 8 year old granddaughter had a history of recurrent fractures and Odontohypophosphatasia and had been found to be heterozygous for an Alkaline Phosphatase gene mutation.

Pathology results revealed a consistently low Alkaline Phosphatase (ALP) of 14 - 17 U/L (ref: 30-115). Bone Mineral Density at the Left Femoral Neck was 0.760g/cm² with a T-score of -1.4 in 2014, and had decreased to 0.670g/cm² with a T-score of -1.6 in 2016. Lumbar Spine BMD was 0.940g/cm² with a T score of -1.0 in 2014 and 0.912g/cm² with a T score of -1.2 in 2016. Vitamin D was at 111 nmol/L (ref >50). Calcium concentration was 2.37nmol/L(ref: 2.15 - 2.65) and PTH was 2.1 pmol/L (ref: 3.5 - 7.1). Phosphate was 1.51 nmol/L (ref: 0.8 - 1.4). Bone turnover markers were P1NP of 34 mcg/L (ref: 15 - 115) and CTX of 79ng/L (ref: 100 - 1000). Qualitative analysis of urine phosphoethanolamine (PEA) excretion was normal. Screening for causes of a low ALP revealed no abnormalities (Table 1). No causes for secondary osteoporosis were found.

Genetic testing by the Department of Bone and Mineral Research in Osaka Prefectural Hospital Organization detected a heterozygous missense mutation (c.1132G>T), resulting in Aspartic Acid to Tyrosine conversion at codon 378 of TNSALP protein in exon 10.

Hypophosphatasia is a rare metabolic bone disease involving loss of function in the gene that encodes Tissue Nonspecific isoenzyme of Alkaline Phosphatase (TNSALP). It can be inherited in an Autosomal Dominant or Recessive pattern [1]. The clinical phenotype is variable with over 200 mutations and 12 polymorphisms described to date. The estimated prevalence is between 1/100,000 to 1/300,000 in severe forms and 1/6370 in milder forms [2].

Hypophosphatasia can be broadly categorised into five forms of increasing severity:

- 1) Odontohypophosphatasia is hallmarked by premature loss of deciduous teeth in children without evidence of rickets or osteomalacia.
- 2) Adult hypophosphatasia usually presents during middle age with osteomalacia, osteopenia, recurrent or poorly healing metatarsal fractures, chondrocalcinosis or calcific peri-arthritis causing joint or bone pain. Although in some cases, the condition can be asymptomatic.
- 3) Childhood form of the disease is characterized by skeletal deformities that may result in short stature. Fractures and muscle weakness can lead to bone pain and gait issues. Premature loss of deciduous teeth is common.
- 4) Infantile hypophosphatasia presents before 6 months of age with Failure to thrive, delayed motor milestones and signs of rickets.
- 5) Perinatal hypophosphatasia is the most extreme form and usually fatal soon after birth. [2-4].

Diagnosis is suspected on clinical grounds combined with biochemical abnormalities, and can be confirmed with genetic testing. A low serum ALP is usually associated with elevated enzymatic substrates of PEA and pyridoxal 5'-phosphate (PLP). Inorganic pyrophosphate can also be elevated, which acts as potent inhibitor of mineralisation. The degree of hypophosphatasemia and substrate accumulation appears to reflect severity of the condition [4].

Distinguishing Adult Hypophosphatasia from Osteoporosis is paramount in that treatment with bisphosphonates, which are structural analogs of pyrophosphate, may increase fracture risk in this condition[1]. Vitamin D and calcium supplements are best avoided unless deficiencies are detected [4]. Although Teriparatide has been shown to reduce bone pain and accelerate fracture healing, its efficacy evaluation is limited to small number of case reports. Its mechanism of action may involve stimulating ALP production by osteoblasts and increasing ALP catalytic activity by reducing extracellular inorganic phosphate [3]. The improvement in biochemical markers of ALP, PEA, PLP and bone turn over markers was inconsistent in both duration and magnitude in case reports.

Administration of Asfotase Alfa, a bone targeted recombinant human TNSALP, has become available for treatment in children with this disorder. Thirty-seven children with perinatal and infantile hypophosphatasia treated with Asfotase Alfa for median duration of 2.7 years demonstrated improved survival of 84% compared to 27% in historical controls at age 5 years [5]. In another study involving 12 children with Childhood Hypophosphatasia treated for 5 years, the Radiographic Global Impression of Change score significantly increased by +2.2. The median ALP activity increased from 49 IU/L to 5747 IU/L. PLP

concentration normalised from 218ng/ml to 10.6ng/ml by 6 weeks, as did pyrophosphate, from 4.9µM to 2.0µM . Height Z-scores significantly increased after 1 year from -1.26 to -0.87. The most common adverse effect was injection site reactions consisting of erythema or lipohypertrophy [6]. Studies with Asfotase Alfa in adolescents and adults are currently underway. Preliminary results of a phase II randomised control study of 19 patients aged 12 to 17 years who were treated for 2-4 years showed significant decrease in PLP levels and non-significant decrease of pyrophosphate levels after 6 months. The effect was sustained through 4 years of treatment. There was also increase in 6-minute walk test and Bruininks-Oseretsky Test of Motor Proficiency [7].

Anti-sclerostin therapy with a monoclonal antibody BPS804 could serve as a novel treatment for Adult Hypophosphatasia. A phase II trial involved 8 adults (mean age 47.8 years) given three ascending doses of BPS804 15 days apart and monitored for 16 weeks. Bone formation markers transiently increased with maximum mean increase of 101% for P1NP and 92% for osteocalcin 6 weeks after treatment. Bone resorption marker of CTX decreased to 35% at 5 weeks post treatment. Lumbar Spine BMD increased by 3.9% at week 16. The most common adverse effects were arthralgias, bone pain, headache, myalgias, nasopharyngitis and toothache [8].

Summary

- The diagnosis of Adult Hypophosphatasia should be considered in patients with low ALP presenting for management of bone fragility.
- Clinical manifestations do not necessarily correlate with genotype. The severity of disease corresponds to the magnitude of the biochemical abnormality.
- Bisphosphonates can increase fracture risk in this disorder.
- Asfotase Alfa, a recombinant TNSALP, has been approved for treatment in the paediatric population.
- Phase II trials with anti-sclerostin antibody are promising.

| | March, 2016 | Sept, 2016 | Feb, 2017 |
|----------------------------------|-------------|------------|-----------|
| ALP (U/L) | 16 | 14 | 14 |
| Calcium (nmol/L) | 2.37 | 2.29 | 2.39 |
| Phosphate (nmol/L) | | 1.1 | 1.4 |
| Folate (nmol/L) | | 17.2 | |
| Vitamin D (nmol/L) | 111 | 101 | |
| Intrinsic factor Ab | | negative | |
| PTH (pmol/L) | 2.1 | | |
| Parietal cell ab | | negative | |
| B12 (pmol/L) | | 306 | |
| Zinc (umol/L) | | 11.6 | |
| Ceruloplasmin (g/L) | | 0.30 | |
| P1NP (ug/L) | 34 | 25 | |
| CTX (ng/L) | 70 | 201 | |
| Urine PEA (mg/mol-Cr) | | 2.8 | |
| IgA (g/L) | 2.0 | | |
| Gladin Ab (CU) | <20 | | |
| Tissue Transglutaminase IgA (CU) | <20 | | |

Table 1. Investigation results

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Case Study #8

CAH and fertility – both male and female

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Coming soon!

Case Study #9

Unravelling an unusual case of amenorrhoea, hirsutism and virilisation

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Case Presentation

A 33-year-old lady was referred for opinion and management of hirsutism, absent breast development and clitoromegaly.

She had normal female genitalia at birth. During late childhood, she gained approximately 30 kg in weight and described a tall stature for her age. Spontaneous puberty did not occur during her teenage years. Menarche occurred at the age of 27 years by which time she weighed 165 kg. Menses were irregular over the next five years, with worsening hirsutism. She was prescribed the oral contraceptive pill at the age of 32.

Her past medical history includes osteoarthritis, she does not drink excessive alcohol and smokes cigarettes infrequently. In terms of family history, her mother developed hirsutism during pregnancy, and has treated hypothyroidism. Her two sisters do not have symptoms of androgen excess or infertility.

At the time of referral, she had managed significant weight loss through major lifestyle changes, and weighed 82.2 kg (BMI 25.9 kg/m²). Her height was 178 cm, well above her mid-parental predicted height of 168 cm (mother 163 cm, father 186 cm). Her blood pressure was 118/70 mmHg. She had no significant breast tissue (Tanner stage I) and a clitoral diameter of 10 mm. She had treated hirsutism (patient estimated Ferriman-Gallwey score 10), mild acne and muscular upper body.

The patient kindly provided some photos to illustrate some pertinent features of her case (**Figures 1-4**).



Figure 1- The patient aged around 18 months.



Figure 2- The patient (centre) and two of her peers aged around 13 years, illustrating the significant height difference.



Figure 3- The patient's pertinent clinical examination findings at the time of Endocrine review, aged 33 years. These photographs illustrate absent breast development, loose skin and striae from her significant weight loss, and removed hirsutism in the centre of her chest.



Figure 4- The patient's pertinent clinical examination findings at the time of Endocrine review, aged 33 years. This photograph illustrates absent breast development and muscular upper body.

Investigations are shown in Table 1.

Table 1- Laboratory results:

| <u>Test</u> | <u>Prior to commencing OCP</u> | | <u>After commencing OCP</u> | <u>Reference Range (follicular phase)</u> |
|----------------------|--------------------------------|------------------------------|------------------------------|---|
| | <u>Result 1 (July 2003)</u> | <u>Result 2 (March 2004)</u> | <u>Result 3 (March 2016)</u> | |
| Testosterone (Total) | 4.4 nmol/L | 4.2 nmol/L | 1.5 nmol/L | 0.2- 2.0 nmol/L |
| SHBG | 10 nmol/L | 37 nmol/L | 58 nmol/L | 25-90 nmol/L |
| FAI | 44 % | 11.4 % | 2.6 % | 1.5- 8 % |
| Oestradiol | 83 pmol/L | 94 pmol/L | 248 pmol/L | 150-600 pmol/L |
| FSH | 7 IU/L | 13 IU/L | | 0-12 IU/L |
| LH | 8 IU/L | 9 IU/L | | 0-12 IU/L |
| Progesterone | | 7 nmol/L | | < 6 nmol/L |
| DHEA-S | | 7.3 umol/L | | 1.0-8.5 umol/L |

Serum 17-OH progesterone Synacthen test was normal, excluding non-classical congenital adrenal hyperplasia (CAH). Pituitary hormones were otherwise unremarkable. OGTT was normal.

Urinary steroid profile showed elevated androgens, but was not suggestive of any form of virilising CAH. Twenty-four-hour urine free cortisol was normal, excluding Cushing's syndrome.

CT abdomen and pelvic U/S revealed no adrenal masses and normal-appearing uterus. She had a single right ovarian cyst (28 x 20 x 19 mm diameter) which resolved on further follow-up.

Her karyotype was 46 XX, with SRY gene testing negative.

She underwent breast augmentation uneventfully. She is now awaiting clitoral reduction surgery. She was referred for a Genetics opinion regarding testing for the suspected underlying genetic cause of hyperandrogenism.

Discussion

In reproductive-aged women, testosterone is produced from direct ovarian production (one third), and from androstenedione produced by the ovaries and adrenal glands which is converted to testosterone in the peripheral tissues (two thirds).¹ LH from the pituitary stimulates androgen formation within the theca cells of the ovary. FSH stimulates the synthesis of oestradiol from androgen via the enzyme aromatase in the granulosa cells.² Conversion of testosterone to DHT is catalysed by the 5 α -reductase in the liver and within target cells such as sex skin fibroblasts.³ Androgenic effects of testosterone are determined by

local 5 α -reductase activity and androgen receptor content. Other enzymes at target tissues such as aromatase and 17 β -HSD also regulate hormone action by metabolizing testosterone to the androgenically-inactive androstenedione or to oestradiol, a potent oestrogen.¹

Evaluation of Androgen Excess in Women

Hirsutism is defined as the presence of terminal hair in locations at which hair is not commonly found in women.

Virilisation is a more severe form of androgen excess and implies significantly higher rates of testosterone production. Manifestations include temporal balding, voice deepening, decreased breast size, increased muscle mass and loss of female body contours. Clitoral length greater than 10 mm is considered abnormal.¹

The most useful initial test in women presenting with hirsutism and virilisation is serum total testosterone. Causes of elevated testosterone levels are summarised in **Table 2**.

Table 2- Causes of Androgen Excess in Women of Reproductive Age

| |
|---|
| <p>Ovarian</p> <p>Polycystic ovary syndrome (PCOS)</p> <p><u>Hyperthecosis (a severe PCOS variant)</u></p> <p><u>Ovarian tumour (e.g., Sertoli-Leydig cell tumor)</u></p> |
| <p>Adrenal</p> <p>Non-classical adrenal hyperplasia</p> <p>Cushing’s syndrome</p> <p>Glucocorticoid resistance</p> <p>Adrenal tumour (e.g., adenoma, carcinoma)</p> |
| <p>Specific Conditions of Pregnancy</p> <p>Luteoma of pregnancy</p> <p><u>Hyperreactio luteinalis</u></p> <p><u>Aromatase deficiency in fetus</u></p> |
| <p>Other Causes</p> <p>Hyperprolactinemia, hypothyroidism</p> <p>Medications (danazol, testosterone, anabolizing agents)</p> <p>Idiopathic hirsutism (normal serum testosterone in an ovulatory woman)</p> <p><u>Idiopathic hyperandrogenism (patients who do not fall into any of the other categories)</u></p> |

Adapted from: Melmed S et al. Williams Textbook of Endocrinology (13th ed.) Elsevier 2016.

If androgen excess is associated with primary amenorrhoea, abnormal *in utero* sexual differentiation should be suspected. Disorders of sex development (DSDs) represent a broad range of conditions that can present at different stages of life. Reproductive system development begins at 4 to 5 weeks’ gestation and is complete with the achievement of secondary sexual characteristics and production of viable gametes. Ambiguous genitalia occur in approximately 1 in 4500 births. DSDs can present in many other ways, including virilisation, absent puberty, primary amenorrhoea or infertility.¹

Sex development is a dynamic process that requires the interaction of many genes, proteins, signalling molecules and endocrine stimuli (**Figure 5**).

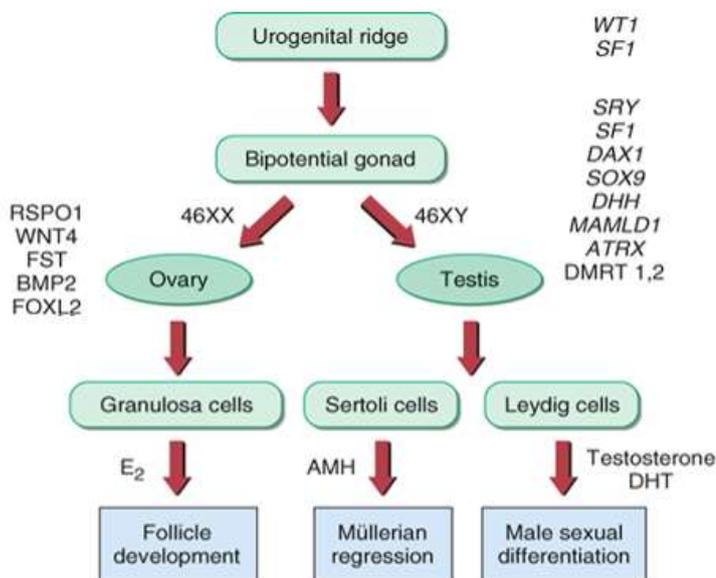


Figure 5- The development of the reproductive system. Adapted from Harrison's Principles of Internal Medicine, 18th Edition.

Disorders of sex development can be sub-classified and worked up according to karyotype as shown in **Table 3** below.

Table 3- Disorders of Sex Development

| Sex Chromosome DSD | 46 XY DSD | | 46 XX DSD | | |
|--------------------------------------|-------------------------------------|---|--------------------------------------|-----------------------|----------------------|
| | Disorders of Testicular Development | Disorders of Androgen Synthesis/ Action | Disorders of Ovarian Development | Fetal Androgen Excess | |
| | | | | CAH | Non CAH |
| 45 XO Turner and variants | Complete gonadal dysgenesis | Androgen synthesis defect | Ovotesticular DSD | 21-OH deficiency | Aromatase deficiency |
| 47 XXY Klinefelter and variants | Partial gonadal dysgenesis | LH receptor defect | Testicular DSD (eg. SRY +, dup SOX9) | 11-OH deficiency | POR gene defect |
| 45 X/ 46 XY mixed gonadal dysgenesis | Gonadal regression | Androgen insensitivity | Gonadal dysgenesis | | Maternal |
| Chromosomal ovotesticular DSD | Ovotesticular DSD | 5 α -reductase deficiency | | | Luteoma |
| | | Disordered AMH | | | Iatrogenic |
| | | Timing defect | | | |
| | | Endocrine disruptors | | | |
| | | Cloacal extrophy | | | |

Adapted from: Ocal G. J Clin Res Ped Endo 2011;3(3):105-114.

Our patient fits into the 46 XX DSD category and has normal ovaries. CAH was excluded with 17-OH progesterone Synacthen test. She does not have ambiguous genitalia. Our working diagnosis is a partial aromatase deficiency.

Aromatase Deficiency

Aromatase, expressed in the ovary, testis, placenta, adipose tissue, skin, and brain, is the key enzyme for oestrogen biosynthesis encoded by the CYP19A1 gene. Oestrogen levels in the circulation are primarily maintained by aromatase activity in the ovaries of ovulatory women, and adipose tissue of men and postmenopausal women.⁶

Aromatase deficiency exhibits autosomal recessive inheritance and results in varying degrees of oestrogen deficiency, depending on the degree of enzyme defect. Several CYP19A1 gene alterations associated with aromatase deficiency have been described in both sexes⁷. It is a rare cause of oestrogen deficiency and virilisation in females. Its precise incidence is unknown. There are few case reports in the literature.^{7,8}

In females, oestrogen deficiency can induce disorders of sex development including ambiguous genitalia. At puberty, affected girls have hypergonadotropic hypogonadism, lack of secondary sexual characteristics, and progressive virilisation.⁶ In both genders, it can result in delayed epiphyseal closure, eunuchoid body habitus and osteoporosis⁹. Abdominal obesity, insulin resistance and other features of the metabolic syndrome develop at early ages.

Aromatase deficiency in the fetus can be associated with transient maternal virilisation during pregnancy.⁶ DHEA produced by the fetal adrenal glands cannot be converted to oestrogen due to placental aromatase deficiency, allowing conversion to testosterone peripherally and resulting in virilisation of both fetus and mother.¹⁰

Treatment

Treatment of aromatase deficiency consists of oestrogen replacement, which has been shown to normalize bone maturation, gonadotropin secretion and ameliorate the metabolic syndrome.⁹

Treatment can be commenced as early as 2 years of age at the lowest possible dose. Bone age and signs of premature breast development should be monitored closely. Oral conjugated oestrogens (0.15 mg/day, or every other day) may be used, and the dose may be titrated to maintain suppression of FSH and LH. The dose should be raised to 0.3 mg/day at the chronological age of 10–12 (or bone age of 11) to initiate secondary sexual characteristics. The dose may be gradually increased over the next 2 years. From the second year on, a cyclical progestin should be given to imitate menses and prevent endometrial hyperplasia. By age 14, all aromatase-deficient girls may be switched to a combination oral contraceptive containing ethinyl oestradiol (20–35 µg).^{6,10}

Case Conclusion

Our patient has many features of aromatase deficiency, without ambiguous genitalia at birth. We believe a diagnosis of partial aromatase deficiency is likely to be the underlying cause of her amenorrhoea, hirsutism, tall stature and progressive virilisation with clitoromegaly after puberty. She has been referred for genetic analysis of the CYP19A1 gene, and results are awaited.

Take-Home Messages

- Aromatase deficiency is a rare cause of androgen excess resulting from mutations in the *CYP19A1* gene
- Women with complete aromatase deficiency have ambiguous genitalia, hypergonadotrophic hypogonadism, lack of secondary sexual characteristics, progressive virilisation and features of the metabolic syndrome
- Women with partial aromatase deficiency have a milder form of these features
- Treatment involves oestrogen replacement

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Case Study #10

Abiraterone treatment in congenital adrenal hyperplasia (salt-wasting)

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Abiraterone treatment in congenital adrenal hyperplasia (salt-wasting)

Deila Dedic and Bronwyn Stuckey

Sir Charles Gairdner Hospital, Nedlands, Western Australia

Introduction

This case documents the use of oral abiraterone acetate with replacement doses of adrenal steroids in order to minimise glucocorticoid excess in the treatment of congenital adrenal hyperplasia due to 21-hydroxylase deficiency.

Case:

We present KS, a 26-year-old woman diagnosed at birth with congenital adrenal hyperplasia (salt wasting) secondary to 21-hydroxylase deficiency. Her long-term management has been complicated by excessive adrenal androgen production despite high doses of glucocorticoids, leading to multiple treatment side effects including obesity, cushingoid features and recurrent infections.

Her other medical history includes Arnold-Chiari I malformation with a ventriculoperitoneal (VP) shunt for obstructive hydrocephalus inserted in 2008, posterior fossa decompression in 2012 and thoracolumbar scoliosis requiring spinal fusion in 2015.

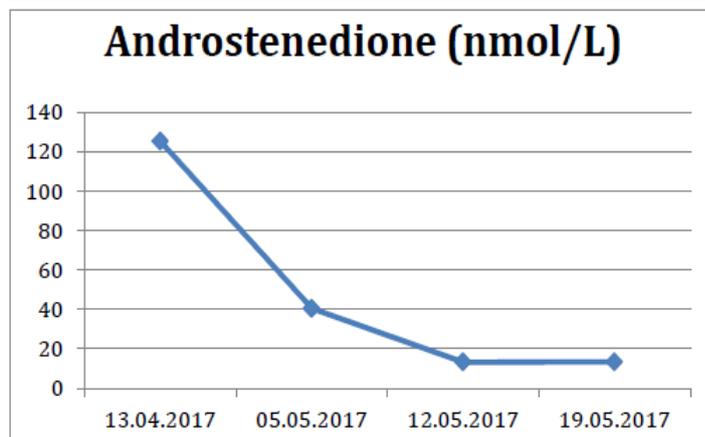
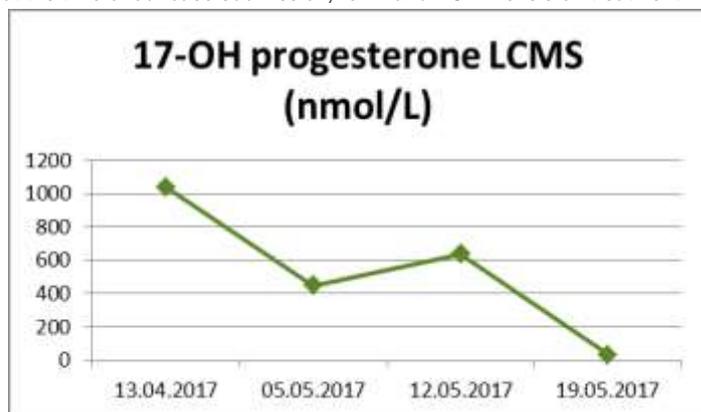
Analysis of the CYP21A2 gene revealed KS is a compound heterozygote for the Q318X mutation [c.952C>T p.Gln318X] and has a deletion of the entire gene on the other allele. Clinically, she had menarche at 11 following the VP shunt insertion, however her periods disappeared and returned for only 3 months at age 13. KS has not had any periods since. Her other symptoms include ongoing fatigue and hirsutism over the face and abdomen. KS has also undergone clitoral reduction, labioplasty and more recently a vaginoplasty (December 2013)

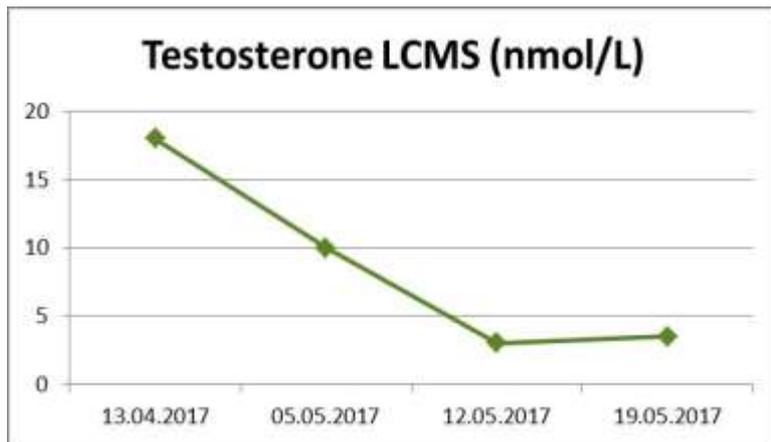
Reduction of adrenal androgen production during her childhood was difficult without supraphysiological glucocorticoid doses, including dexamethasone 500mg bd, which lead to weight gain and cushingoid features. Better control was attempted with continuous subcutaneous hydrocortisone delivery 30 – 40 mg/day infusion via a Medtronic Solu-Cortel Pump (commenced in May 2009) with diurnal variation to anticipate normal circadian rhythm. Pump therapy was complicated by localised infections at site of pump and needle insertion, thus triggering a change to prednisolone in our clinic. On transfer to our care, KS was also on fludrocortisone 150mcg bd for salt wasting with ongoing craving for salt on her food.

On examination, patient's height was 152.8m, weight 94.65kg, giving her a BMI 41. She was normotensive (blood pressure 116/80 sitting, 117/80 standing). She has a Cushingoid appearance with abdominal striae as well as facial and abdominal hirsutism.

The patient's mother requested that we consider bilateral adrenalectomy with physiological adrenal hormone replacement to alleviate weight gain. On consultation with colleagues and a review of the literature, the possibility of a trial of abiraterone therapy was discussed with the patient and was agreed to. This was to be a "medical adrenalectomy" and proof of concept that proceeding to surgery would deliver the therapeutic outcome we hoped for – i.e. control of the hyperandrogenism without supraphysiological doses of glucocorticoids. The patient was already taking the combined oral contraceptive pill Yaz.

Abiraterone was commenced on 1 May 2017 in a dose of 125mg daily and increased on 8 May to a dose of 250mg daily. Steroid regimen at the time consisted of hydrocortisone 10mg mane and 4mg afternoon, fludrocortisone 150mcg mane and 100mcg nocte. Patient's renal and liver functions remained stable on treatment. Pre-treatment renin level on was 156 mu/L and at the time of our case submission, renin and ACTH levels on treatment were pending.





Discussion

Congenital adrenal hyperplasias (CAH) are autosomal recessive disorders, 95% of which are caused by 21-hydroxylase deficiency due to mutations in the 21-hydroxylase (*CYP21A2*) gene which leads to defective steroidogenesis and glucocorticoid deficiency (7). The worldwide incidence of classic or severe 21OHD is 1:16 000 and 1:1600 for non-classic or mild 21OHD, however incidence is more common in certain populations (2, 6).

The goals of treatment in adults with CAH are exogenous glucocorticoid and mineralocorticoid administration as adrenal replacement therapies whilst preventing their long-term consequences, restoring fertility in those wishing to have a family, and finally preventing adrenal and gonadal hyperplasia and neoplasia. 17-OH progesterone within or below the reference range and a low level of androstenedione are thought to indicate excess glucocorticoid exposure (1, 6). It is also important to note treatment regimens when interpreting serum levels of androgens, with 17-OH progesterone being highest pre-morning hydrocortisone dose and low or normal in those on nocturnal prednisolone or dexamethasone (1). Unfortunately, only a third of CAH patients achieve biochemical control, and poor health outcomes are common due to increased incidence of obesity, hypertension, osteoporosis and impaired fertility (1).

Abiraterone is a potent specific inhibitor of cytochrome P450 17A1 and blocks androgen biosynthesis in adrenal glands by decreasing formation of dehydroepiandrosterone and androstenedione, and shunting steroid production to the mineralocorticoid pathway via *CYP21A2* (2,3). Due to abiraterone's ability to reduce circulating levels of adrenal androgens, this medication is already in use for testosterone reduction in patients with castration-resistant prostate cancer, improving their survival (3-5).

Since individuals with 21-OH deficiency do not have *CYP21A2*, the blocking effect of abiraterone on *CYP17A1* will not cause shunting to the mineralocorticoid pathway. Hence, the role of abiraterone acetate in patients with congenital adrenal hyperplasia due to 21-OH deficiency would be as a glucocorticoid-sparing agent to reduce the complications of CAH and treatment with supraphysiological glucocorticoid doses without causing hypertension or hypokalaemia, as demonstrated in a 2014 pilot study by Auchus et al (2).

Indications for surgical bilateral adrenalectomy in congenital adrenal hyperplasia include minimisation of supraphysiological glucocorticoid doses (6), weight loss and reduction in virilisation (7), improvement in fertility (7), and as treatment for large adrenal myelolipomas that might develop in adults (8). This procedure should not be attempted in those with a history of poor compliance, as this issue in the post-operative setting could prove to be fatal (6). Thus, attempting a medical adrenalectomy with abiraterone can be a safer proof of concept in assessing individual patient outcomes before a permanent adrenalectomy.

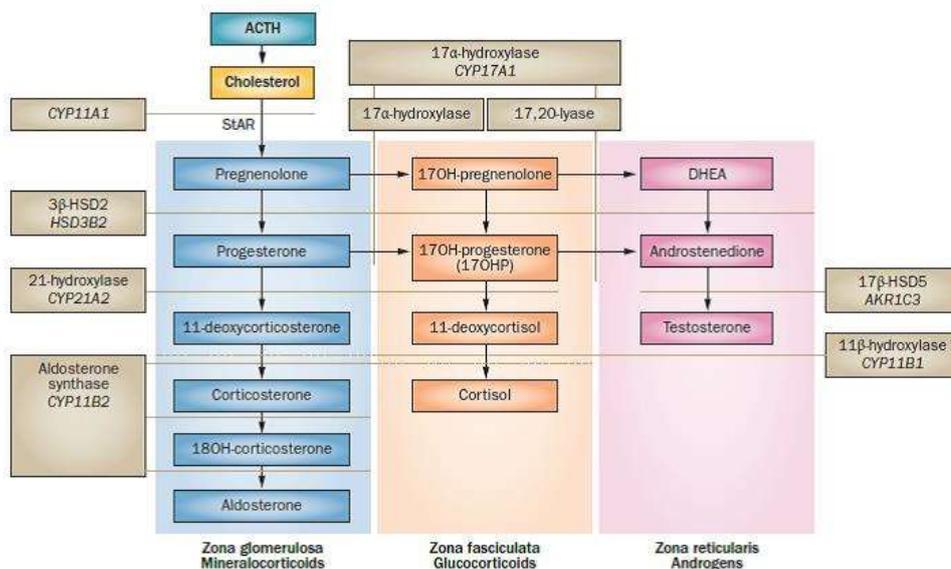


Figure 1. The adrenal steroidogenesis pathway (1)

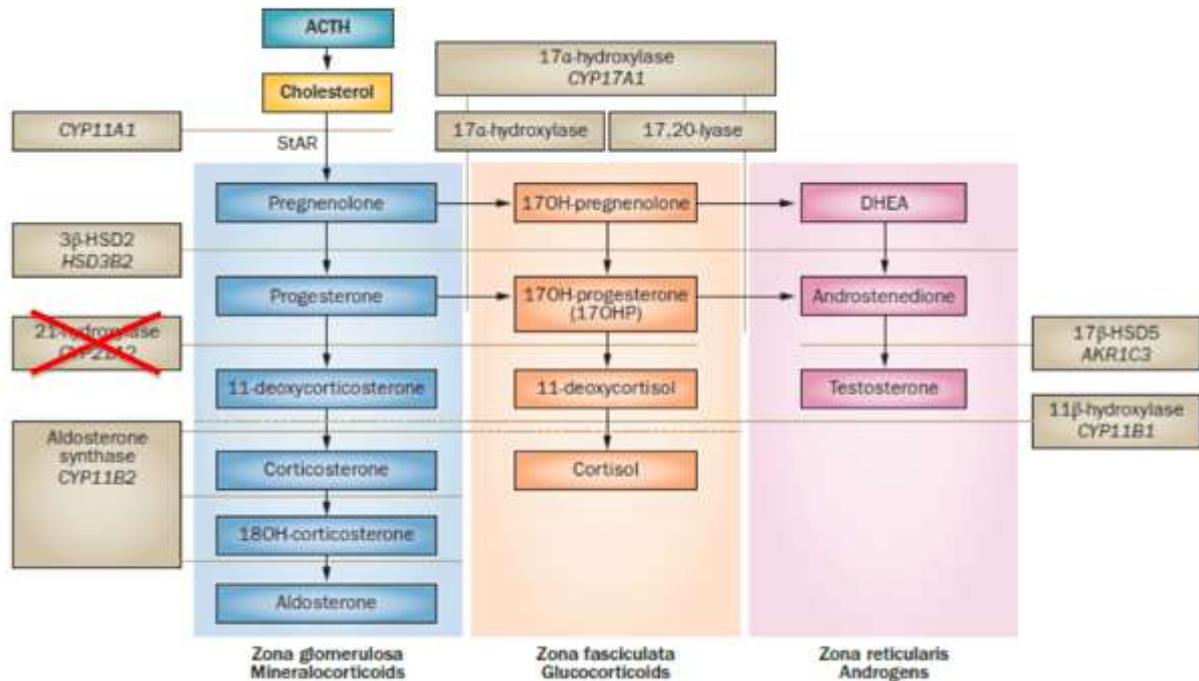


Figure 2. 21-hydroxylase deficiency in Congenital Adrenal Hyperplasia, resulting in an inability to convert 17-hydroxyprogesterone (17-OHP) to 11-deoxycortisol (cortisol precursor) and progesterone to deoxycorticosterone (aldosterone precursor), subsequent glucocorticoid and mineralocorticoid deficiencies, and shunting to testosterone pathway.

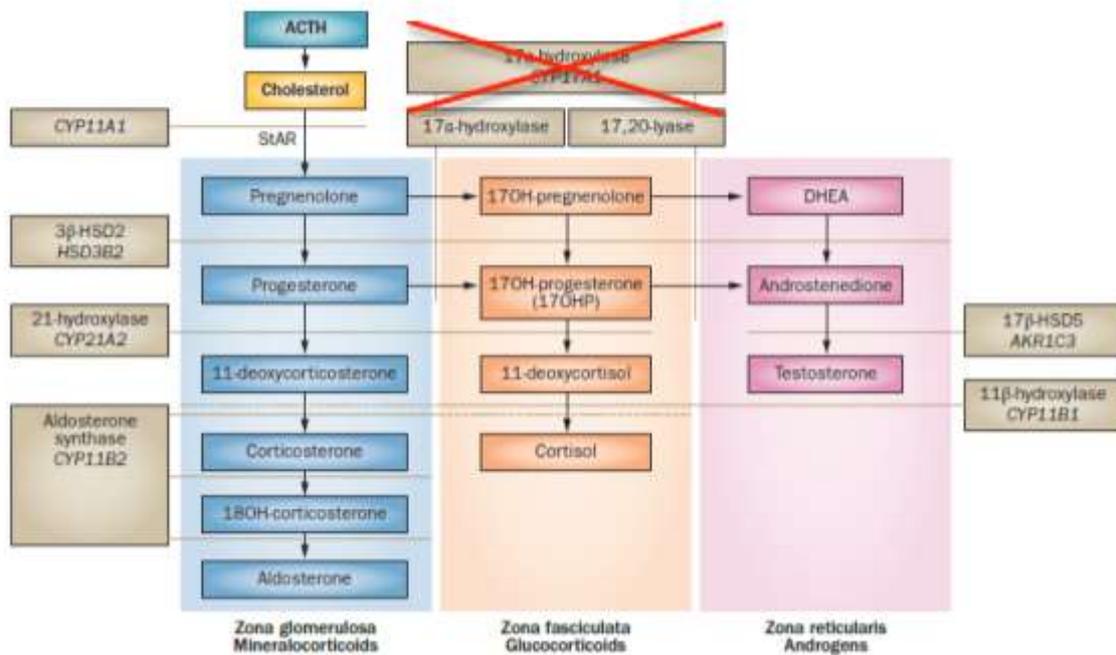


Figure 3. 17 α -hydroxylase (CYP17A1) is inhibited by abiraterone, with downstream effects of reduced testosterone and cortisol at expense of increasing ACTH.

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Case Study #11

Achondroplasia in a 46XX phenotypic male: a 'flying pig' phenomenon or is there a link?

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We present a novel case of a 40 year old phenotypic male with 2 rare conditions they may potentially be related – karyotype 46 XX disorder of sexual development (DSD) and achondroplasia.

Mr MH, a 40 year-old man was referred for assessment of painful left gynaecomastia, low libido, poor energy levels and irritable mood.

He was diagnosed with achondroplasia at birth to a non-consanguineous couple, in the absence of a family history of dwarfism. He underwent ventriculoperitoneal shunt insertion for hydrocephalus at age 4. Over the years he suffered from severe symptomatic spinal canal stenosis and cervicothoracic syringomyelia requiring multiple spinal interventions, with chronic back pain and neurological impairment particularly of his right leg affecting his mobility, balance and quality of life. He also has obstructive sleep apnoea treated with continuous positive airways pressure (CPAP) therapy, a sleeve gastrectomy for obesity in 2014 and an episode of renal colic in 2006.

His medications include Buprenorphine patch 10mg weekly, Pregabalin 300mg BD, Cholecalciferol 25ug daily and a multivitamin.

On examination, he was 127cm tall and weighed 70kg (BMI 43). He had macrocephaly with a prominent forehead, disproportionately normal-sized trunk and short limbs. He had a generalised paucity of body hair, and exhibited poor secondary sexual characteristics - Tanner's score was III with sparse pubic hair, bilaterally descended small testes (1 ml volume) and a penile length of 5 cm without hypospadias. His visual field examination was normal. There was tender left gynaecomastia with no masses on palpation.

Investigations revealed hypergonadotropic hypogonadism (LH 39U/L, FSH 36U/L, Testosterone 8.4nmol/L). There was no evidence of haemochromatosis, nor evidence of Müllerian structures on pelvic ultrasound. Cytogenetic analysis revealed 46,XX karyotype (see figure 1) and Fluorescent *In-Situ* Hybridisation (FISH) identified a SRY gene translocation onto one X chromosome (see figure 2).

The diagnosis of SRY-positive 46,XX testicular disorder of sex development (DSD) would explain his male phenotype and diminished secondary sexual characteristics. Testosterone replacement was commenced and he was advised about the need for surveillance for breast and germ cell cancer. Referral to the genetics clinic was made to discuss the implications of DSD on his fertility, but further testing was not pursued as reproduction was not desired.

Discussion:

Given the incidence of an SRY translocation is 1 in 20,000 and that of achondroplasia is 1 in 30,000 (1, 2), the odds of this man independently having both conditions is 1 in 600 million, making him perhaps one of 12 patients in the world to have both conditions. We hypothesize that the two conditions may be linked to the SOX9 transcription factor, which is critical to both sex determination and skeletal development, which would be statistically more likely than having the two conditions independently. The SRY gene is the sex determining region on the Y chromosome, encoding a DNA binding protein which acts to change DNA shape (3). This alteration of DNA shape is the mechanism in which transcription is upregulated or suppressed, depending on the target region (3). As a transcriptional factor, the SRY gene product has a large number of targets across the genome, with 71 being identified in study (1). Critically to our case, as a transcription factor, SRY gene products target genes beyond the chromosome that it is located (1, 4).

One of the key genes that SRY upregulates is SRY-like high mobility group box 9 (SOX9). SOX9, located on autosome 17, is another transcriptional factor with a higher number of known downstream gene targets than SRY (4). Of these 109 targets, correct functionality of the SOX9 gene has been linked to Sertoli cell differentiation and resultant testicular development (4). Loss of function of the SOX9 gene is noted to cause 46 XY male to female sex reversal (3, 4). Critically to the case of MH, SOX9 function is not limited to development of the male gonads, and is expressed in bone and cartilage (3, 4).

This additional function of SOX9 is highlighted in two cases. The first identified a linkage between SOX9 and chondrogenesis in the mouse fetus (6). A mutant activated Fibroblast Growth Factor Receptor 3 (FGFR3) was utilised in their study, leading to disrupted endochondral bone growth and a resultant phenotype of Thanatophoric Dysplasia Type II (TDII) (6). Downstream effects of this mutant upregulated FGFR3 were associated with a failure to downregulate SOX9, positively identified through increased levels of SOX9 RNA and protein (6). This association between upregulation of SOX9 and reduced endochondral bone growth was further confirmed through creation of mice with the upregulated mutant FGFR3 along with the deletion of a single copy of SOX9 (6). It was found these SOX9 haploinsufficient mice "exhibited little if any discernible skeletal abnormality" (6). In a second and converse example, a case has been noted where reduced dosage of the SOX9 gene has resulted in

skeletal abnormality (5). This case was reported over two decades ago, where autosomal sex reversal and associated campomelic dysplasia were caused by loss of function mutations within the SOX9 gene (5).

It is through these studies that a definitive link between dysregulated SOX9 and disrupted endochondral bone growth can be seen. A well-established upregulator of the SOX9 gene is noted to be the SRY gene (1,3). In addition, SRY gene products are noted to form a positive feedback loop, further upregulating SRY and the genes that it activates (1,3). We propose that a more likely outcome is that the SRY gene has translocated into a position that favours increased expression. This upregulation of SRY in both gonadal precursors and endochondral cells, and thus downstream upregulation of SOX9, would produce both the 46 XX male DSD and the achondroplastic phenotype (see figure 3).

Conclusions:

- Here, we present a patient with extreme odds of having two seemingly unrelated conditions occur independently
- SRY is a known upregulator of the SOX9 gene
- SOX9 is expressed in bone and increased SOX9 expression reduces endochondral bone growth
- Translocation of SRY into a position on the X chromosome increasing its expression in endochondral bone would reduce endochrondral bone growth
- Barring identification of an independent cause of the achondroplasia, this provides a possible link between these two rare conditions

Figure 1: Patient MH Karyotype

Figure 1 demonstrates a 46 XX karyotype of patient MH. An arrow has been added to mark the small amount of additional genetic material located on one of the X chromosomes.

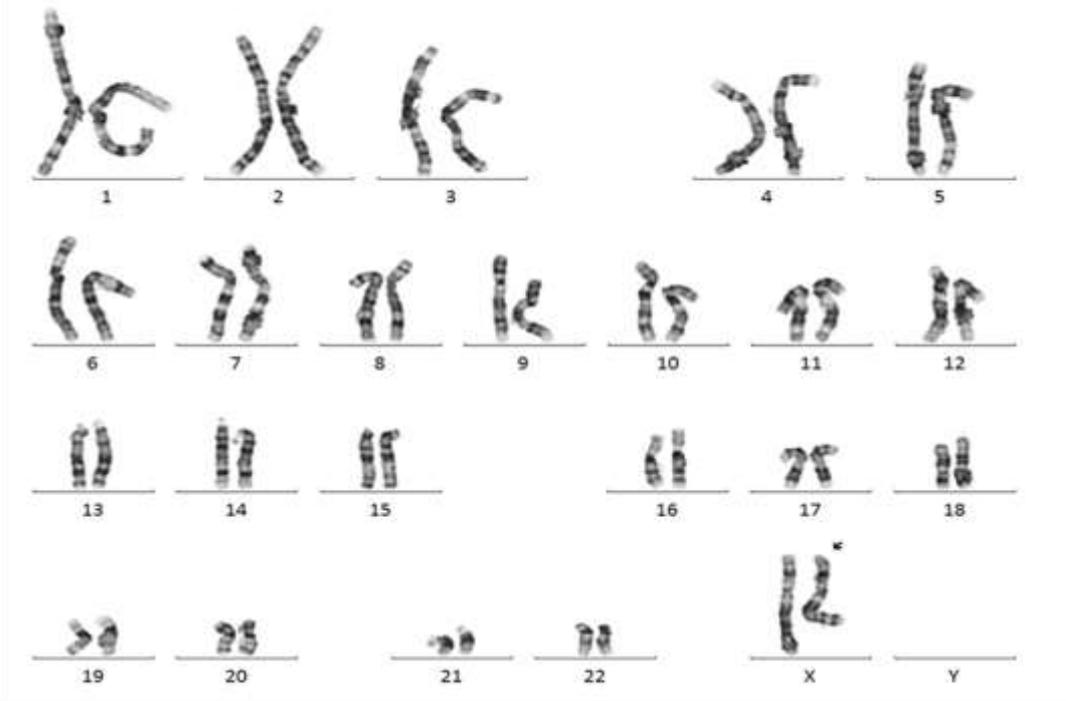


Figure 2: Patient MH Chromosomes with FISH targeting SRY gene and X Chromosome Centromere.
The probe coloured in green is targeted to the X chromosome centromere. The probe coloured in red is targeting the SRY gene. Both the red and green probes correlate to the same X chromosome, indicating a single translocation event of the SRY gene.

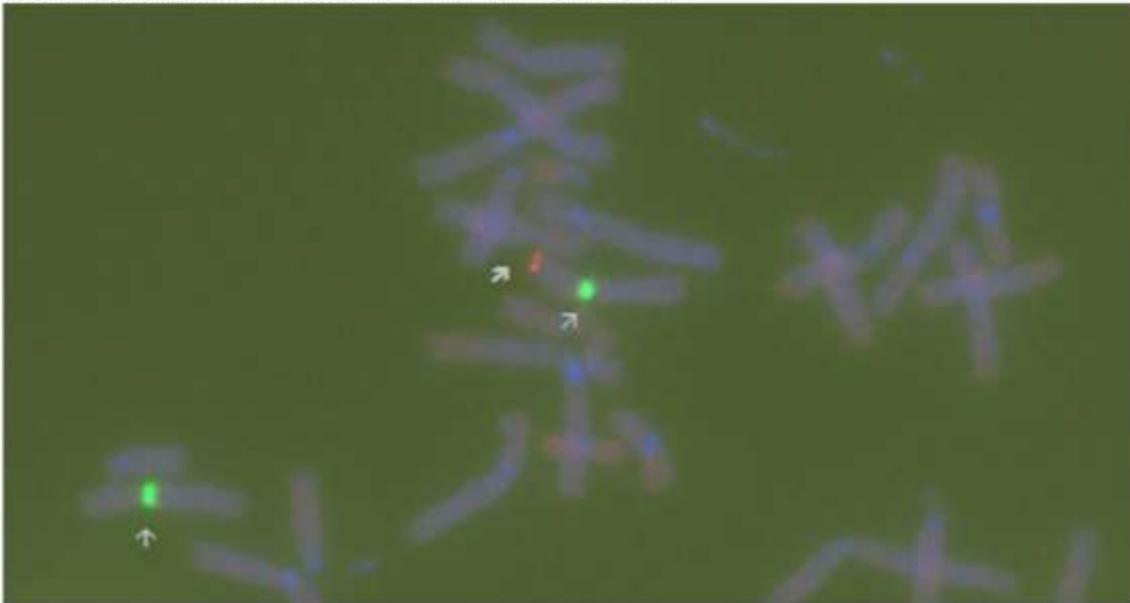


Figure 3: Hypothesised Genetic Mechanism for which SRY translocation results in concurrent Achondroplasia

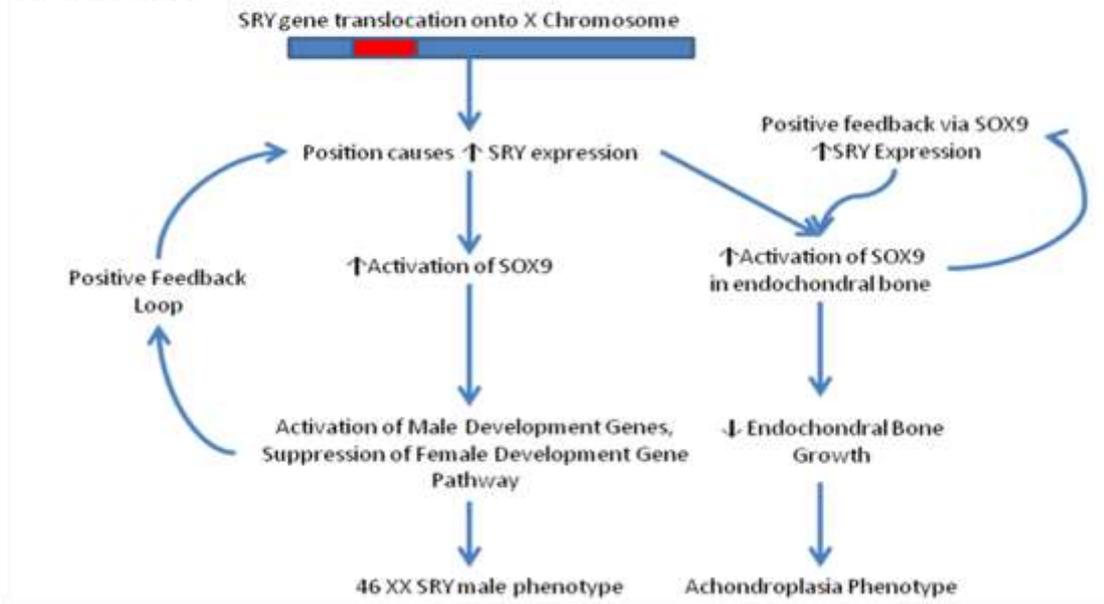


Table 1: Classification of DSDs

Table 1 is derived from the Consensus statement on Management of Intersex Disorders, generated in 2006 under the auspices of the Paediatrics Endocrine Society and European Society for Paediatric Endocrinology. Note the classification of the SOX9 duplication within the 46 XX DSD category.

| DSD Category | Examples | | Genes involved |
|--|---|--|--|
| Sex Chromosome DSD (karyotype is not the typical 46, XX or 46, XY) | Klinefelter syndrome | | 47,XXY and variants/mosaics |
| | Turner syndrome | | 45,XO and variants/mosaics |
| | Ovotesticular DSD | | 46,XX/46,XY chimerism |
| 46, XY DSD | Disorders of androgen action | Androgen insensitivity syndrome | AR |
| | | Drugs and environmental modulators | |
| | Disorders of androgen biosynthesis | LH receptor mutations, Smith-Lemli-Opitz syndrome, Steroidogenic acute regulatory protein mutations | CYP11A1, HSD3B2, CYP17A1, StAR, HSD17B3, SRD5A2, POR |
| | Disorders of gonadal (testicular) development | Complete or partial gonadal dysgenesis | DHH, SRY, SF1, WT1, SOX9 |
| | | Ovotesticular DSD | |
| | | Testis regression | |
| | Other | Syndromic associations of male genital development (e.g. cloacal anomalies, Robinow, Aarskog, Hand-Foot-Genital, popliteal pterygium), Persistent Mullerian duct syndrome, Vanishing testis syndrome, Isolated hypospadias, Congenital hypogonadotropic hypogonadism, Cryptorchidism, Environmental influences | CXorf6, INSL3, GREAT |
| 46, XX DSD | Disorders of ovarian development- | Gonadal dysgenesis | |
| | | Ovotesticular DSD | |
| | | Testicular DSD | SRY, RSPO1, dup SOX9* |
| | Disorders of androgen excess | Fetal | HSD3B2, CYP21A2, CYP11B1, POR |
| | | Feto-placental | CYP19, POR |
| | | Maternal-virilising tumours, leuteomas | |
| | Other | Syndromic associations (e.g. cloacal anomalies), Mullerian agenesis/hypoplasia (e.g. MURCS), Uterine abnormalities (e.g. MODY5), Vaginal atresia (e.g. KcKusicke-Kaufman), Labial adhesions | |

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Case Study #12

XX marks the spot

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Mr J initially presented in 1990, as a 28-year-old married store person. He presented for assessment and work-up of infertility. His medical history was significant for a traumatic tibial plateau fracture and asthma (on inhaled salbutamol PRN). He is a non-smoker, and drinks socially.

Clinical examination revealed a tall, thin, man with sparse body hair. He had small, firm testes with reduced testicular volume (2ml). Karyotyping confirmed a diagnosis of Klinefelter's syndrome. This was before reproductive techniques such as testicular sperm extraction existed and he was therefore commenced on testosterone undecanoate (Reandron) 1000mg IM 14 weekly. Clinically, he had an increase in body hair, increase in muscle mass and a deepening of his voice.

Klinefelter's syndrome was first described in 1942, and is the most common disorder of sexual development in males, with an estimated prevalence ranging between 1:400 and 1:1000. The majority of affected men carry a 47,XXY karyotype. Classic signs are small firm testes, primary testicular failure, and gynecomastia.

Mr J represented at the age of 36 years, this time with gender dysphoria. In retrospect he had identified as female since early childhood but he was not aware of the concept of gender dysphoria, or gender transition until more recently. Growing up, he remembers trying on his sister's clothing and was interested in her make-up.

The masculinising effects of testosterone coincided with a worsening of his gender dysphoria and prompted an internet search that made him realise that he was not the only person to feel they were 'born into the wrong body.' Many years later, from the age 36, she started dressing as female (female pronouns will be used from this point onwards). Initially this was done in private, but eventually she decided to live exclusively as female.

Gender dysphoria is the distress that occurs when a person's biological sex does not align with the deeply held sense of self. In the most recent iteration, DSM-5 reclassified the former *Gender Identity Disorder* to *Gender Dysphoria*. This shifts the focus towards the distress experienced, rather than gender incongruence, which is not necessarily pathological (1).

There is an association between disorders of sexual differentiation and gender dysphoria, although Klinefelter's syndrome appears to make up a small proportion. A Belgium study karyotyped 251 transfemales and found abnormal karyotypes in 3%, with Klinefelter's confirmed in only 3 patients (2). A similar study done in Germany in 2013 found that only 0.6% of transfemales (n=302) had chromosomal abnormalities, with Klinefelter's detected in only 1 patient (3). In addition there has been less than 20 cases reported in the literature. In 1968 Baker *et al* reported 11 cases, and in another historical review, nine of the 12 patients had chromosomal analysis and of these, two had Klinefelter's syndrome (4)

The majority of people with gender dysphoria have symptom onset in early childhood. Just over half of children with gender dysphoria have symptoms that persist beyond puberty. If gender dysphoria does persist into puberty, it is highly likely to endure. It is unclear what triggers a person to develop gender dysphoria and there has been some interest in identifying the brain region responsible for gender identity, and whether this is mediated sex hormone exposure.

The region of the brain that dictates gender identity remains elusive. Men and women do exhibit some structural dimorphism in brain structure and there is some evidence of structural similarities between people with gender dysphoria and their perceived/desired gender, however their gross morphology remains more similar to their natal sex (5,6). A functional MRI study in adolescents using androstenedione, an odorous hormone known to induce sex differences in hypothalamic activation in adults, investigated whether differences existed in transgender individuals. Their findings revealed sex differences in responsiveness to androstadienone were already present in pre-pubertal control children, and that adolescent males and females with gender dysphoria had functional brain characteristics more consistent with their desired gender (7). This suggests that an organic neurological difference exists, and is either congenital or present from a very early age, and that it is potentially confounded (or diminished) by changes at puberty, presumably mediated by sex-hormone exposure. This is of interest in our case, where there was a lack of sex hormone exposure due to primary hypogonadism and subsequent worsening of gender dysphoria following exogenous testosterone administration.

At age 54 she made the decision to commence hormone therapy. As per Endocrine Society and WPATH guidelines, a formal psychiatric review was undertaken prior to commencement, which confirmed the diagnosis of gender dysphoria and readiness for feminising hormone therapy. No psychiatric co-morbidities were present.

In order to alleviate gender dysphoria and promote feminisation, testosterone undecanoate was ceased and oestradiol valerate 2mg commenced. She was counselled about the potentially irreversible nature of hormone therapy, and the small but potential risks including thromboembolic disease, liver dysfunction, and breast malignancy. Spironolactone was later introduced and gradually up-titrated for its anti-androgen effect.

Although there is paucity of evidence in transgender medicine, guidelines suggest continuing cancer screening for relevant conditions concerning the natal as well as current gender. In transfemales this includes breast and prostate screening. While there have been no case reports of breast cancer in a transfemale patient with Klinefelter's syndrome, there is an increased theoretical risk given that both Klinefelter's and feminising hormones are independent risk factors. Breast cancer risk in Klinefelter's is estimated to be 20-30 fold higher than expected for men, it is still lower than in females. This is thought to be due an altered oestrogen:androgen ratio, as well as increased peripheral aromatisation of testosterone to oestradiol. Given that

gynecomastia is present from a young age, there is potential that this susceptible breast tissue has a longer duration of exposure to oestradiol and the presence of an additional X chromosome may confer an increased genetic risk (8)

The incidence of breast cancer in transfemales is unknown but there have been multiple cases reported. Interestingly 54% were oestrogen receptor negative, and therefore cannot solely be explained oestradiol exposure (9). A case of breast cancer was diagnosed in a transgender individual who was later found to carry the BRCA2 mutation after just 7 years of oestradiol therapy (10). Although no clear guidelines exist for a transgender person with Klinefelter's, it would be prudent to recommend monthly breast self-examination and screening mammography.

This case is rare presentation of Klinefelter's syndrome in combination with gender dysphoria and subsequent gender transition. This case provides a unique opportunity to examine aspects of gender dysphoria in a patient in the hypogonadal state, during testosterone exposure and after oestradiol exposure.

Ms J is still living with her wife. She reports a marked improvement gender dysphoria and currently has no intention of undergoing gender affirmation surgery. She attends regular mammography and prostate cancer screening.

Take Home Points

- The cause of gender dysphoria is unknown but neurological differences exist
 - There are clear links between disorders of sexual differentiation and gender dysphoria although the association with Klinefelter's is less clear
 - A patient with Klinefelter's syndrome on oestradiol therapy may have added risk of breast malignancy
 - There is a paucity of evidence to guide management in transgender medicine
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Case Study #14

"DIY" gender transition and a problem with clotting

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Case Presentation

A 39-year-old biological male was referred to the Endocrinology Outpatient Clinic after an admission for an extensive deep vein thrombus (DVT) involving the right peroneal, posterior tibial and popliteal veins.

During the admission, it transpired that he had gender dysphoria and was taking a herbal form of oestrogen in an attempt at male-to-female gender transition. He was advised to cease oestrogen in view of the DVT.

On review in clinic 3 months later, he was taking Oestradiol 6 mg and Spironolactone 100 mg daily, which he had self-initiated. He had stopped Warfarin after 3 months.

Since he had been taking oestrogen and Spironolactone, he had significant breast development but still had facial, axillary and chest hair that he shaved daily. He was no longer experiencing erections and had no sexual desire. His only other past medical history was depression, for which he was taking Mirtazepine 30mg daily. He did not drink excessive alcohol, but smoked 15 cigarettes and 2-3 cones of marijuana daily. He had a family history of Factor V Leiden in his mother, and was later confirmed to be heterozygous for Factor V Leiden.

Following extensive discussion, he was adamant he wanted to continue oestrogen for gender transition despite it being an absolute contraindication in the setting of recent venous thromboembolism (VTE). He stated he would obtain it over the Internet if it were not prescribed for him. A decision was made to continue with oestrogen and androgen blockade under the auspices of the Endocrine Clinic. He was persuaded to switch to trans-dermal oestrogen, and was changed to oestrogen patch 200 mcg every 4 days. He was commenced on Rivaroxaban 15 mg daily.

He continued to be followed up in the Endocrine Clinic. The oestrogen patch was increased gradually to 400 mcg every 4 days, aiming for serum oestrogens in the middle of the normal pre-menopausal female range. The Spironolactone was increased to

200 mg daily due to excess chest hair and intermittent morning erections. After 6 months he started living as a woman and officially changed his name. He subsequently attended the Clinic using his new female name, so I will now refer to her using the feminine pronoun.

Her mental state improved on subsequent visits as the transition progressed and she appeared more obviously female. Her family became more accepting of her gender reassignment. She was involved with the Trans-gender online community, and would often ask questions based on information received from her peers on the Trans-gender forums. She started taking progesterone cream purchased over the internet, with subjective improvement in breast development. She requested a prescription for progesterone and as she understood the risks and would continue to obtain it anyway, a prescription was provided for progesterone cream 100 mg/ml. Her testosterone level was suppressed but her oestrogen was over-replaced, so the oestrogen patch was reduced to 300 mcg every 4 days.

She underwent rigorous Psychological and Psychiatric assessment. She is awaiting gender reassignment surgery. She is certain she does not want fertility in the future and will not consider fertility preservation.

Questions

- What are the risks of oestrogen therapy for male-to-female transition in patients with a previous VTE?
- What is the evidence for the use of progesterone for breast development in this setting?
- How should this patient's gender transition hormone therapy be managed?

Discussion

Gender dysphoria is a discrepancy between psychological sex and phenotypic, biological, and social sex, which is often perceived as “non-self” and belonging to the opposite sex.¹ Gender identity disorder (GID) is a psychiatric diagnosis given when a strong and persistent cross-gender identification causes clinically significant distress.²

Gender dysphoria can be ameliorated by medical treatment with cross-sex hormone therapy and ultimately gender reassignment surgery to achieve physical characteristics more in keeping with the desired sex.

The World Professional Association of Transgender Health (WPATH) has published Standards of Care for the medical treatment of people with GID.³ The Endocrine Society Guidelines for the Endocrine Treatment of Transsexual persons² further expand on the topics of hormone therapy in gender transition. This discussion will focus mainly on male-to-female gender transition, and particularly the risk of venous thromboembolism with oestrogen treatment.

The guidelines list thromboembolic disease as a condition at very high risk of adverse outcomes if oestrogen therapy is used in male-to-female trans-gender hormone therapy.² Garib et al list previous venous thrombotic events related to an underlying hypercoagulable condition as an absolute contraindication for oestrogen use.⁴

Our patient had a history of DVT in the setting of oestrogen use, heterozygosity for Factor V Leiden and ongoing cigarette smoking, all of which would predispose to a high risk of VTE in the setting of oestrogen use. Trans-dermal oestrogen has been shown to confer less risk than oral, as shown in the largest observational study of 816 male-to-female transgender patients.⁵ In this study, a 20-fold increase in the risk of venous thromboembolism was observed in the oestrogen-treated patients compared to the historical controls. Most (21 of 36) cases occurred within the first year of hormone treatment (incidence 2.6%), but the risk was still significant after the first year: the average annual incidence of VTE from 1 to 12 years of hormone treatment was 0.4% (range 0.1- 0.7%). All cases of thromboembolism occurred in patients using oral oestrogens, with the exception of one patient who used trans-dermal 17 β -oestradiol but who had a previous VTE while taking oral oestrogen, and had unduly discontinued anticoagulant therapy. Since 1989, due to a high incidence of VTE in patients taking oral oestrogen for male-to female transition, the authors used trans-dermal oestrogen for patients over the age of 40, and subsequently observed a reduced rate of venous thromboembolism in their cohort.⁵

Our patient was adamant that she wanted to continue oestrogen therapy even at the cost of further thromboembolism and possibly death from thromboembolic complications, illustrating a ruthless self-advocacy not often encountered in clinical medicine. She was heavily influenced by her peers on the Trans-gender online community and often demanded to be prescribed different types of hormone treatment her peers were using.

One of the issues raised by our patient was that of topical progesterone to aid in breast development. This is not covered in the current guidelines, except to suppress pubertal gonadotropin production.² With the exception of cyproterone, the inclusion of progestins in feminizing hormone therapy is controversial.⁶ Because progestins play a role in mammary development on a cellular level, some authors believe that these agents are necessary for full breast development.^{6,7} However, a comparison of feminization regimens found that the addition of progestins neither enhanced breast growth nor lowered serum levels of free testosterone.⁸ There are concerns regarding potential adverse effects of progestins, including depression, weight gain, increased breast cancer and cardiovascular risk in women.^{8,9} Extrapolating from post-menopausal hormone therapy in biological females, progestins may also increase risk of VTE. In the Women's Health Initiative study, combined estrogen-progestin replacement therapy was associated with a significant increase in pulmonary embolism (8 more per 10,000 person-years; HR 2.13, 95% CI 1.39-3.25).⁹ These concerns were raised with our patient, but she insisted on continuing progesterone cream despite any possible risks, as she felt it had resulted in increased breast size.

Working in trans-gender endocrinology is a steep learning curve and requires a degree of compromise, open-mindedness and understanding of the complexities of the trans-gender community, as well as empathy and a compassionate approach to treating these patients at a typically challenging time in their lives.

Take-Home Messages

- Gender dysphoria is the distress experienced if gender identity and sex are not completely congruent. It may be alleviated by supporting changes in secondary sex characteristics through hormone therapy and surgery, along with a change in gender role.
- VTE is a strong contraindication to oestrogen therapy.

- A caring, compassionate attitude along with occasional compromise is required for treating patients in trans-gender endocrinology.

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