Adrenal insufficiency in phytosterolaemia
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Abstract
Background: Phytosterolaemia (sitosterolaemia) is a rare autosomal recessive condition caused by mutations on the ABCG5 and ABCG8 gut transporter proteins. This leads to accumulation of plant-derived cholesterol-like molecules in blood and tissues.

Case: We describe a family of Bangladesh origin, where three siblings (two males and one female) have homozygous mutations for phytosterolaemia, and exhibit short stature and adrenal failure with the female having ovarian failure.

Findings: The index case (18-year-old female) and her sibling (16 years) have adrenal insufficiency with hyperpigmentation and raised levels of ACTH, at 367 and 690 ng/l respectively. The youngest child at 7 years has normal adrenal function. In addition, the index case has ovarian failure and sibling 2 has partial growth hormone deficiency.

Conclusion: Although short stature is a recognised phenomenon, no previous association has been made between phytosterolaemia and other endocrine abnormalities. We postulate that the elevated plant sterol levels in phytosterolaemia may interfere with endocrine hormone synthesis; in particular, we present evidence that adrenal cholesterol metabolism may be preferentially affected, accounting for the adrenal insufficiency.

Introduction
Phytosterolaemia (sitosterolaemia) is a rare condition that causes the accumulation of plant cholesterol-like molecules in the blood and tissues. These plant sterols, such as β-sitosterol, campesterol and stigmasterol are structurally similar to cholesterol and many laboratory assays cannot distinguish between cholesterol and plant sterols, thus leading to an erroneous diagnosis of hypercholesterolaemia (1). This condition was first described in two affected sisters with tendon xanthomas and haemolytic anaemia by Bhattacharya & Connor in 1974 (2) and is characterised by the presence of xanthomata, premature coronary artery disease, atherosclerotic disease, haemolytic episodes and arthritis. It is inherited as an autosomal recessive trait and elevation of sitosterol levels may also be marginally elevated but without any of the clinical abnormalities (3).

Plants do not make cholesterol, but use similar molecules, such as sitosterol, which are abundant in vegetable oils, nuts and fruits, such as olive, avocado and soya beans. Similarly, phytosterols are not synthesised in the body but are derived entirely from the diet. In humans, a precise mechanism exists which strictly limits the amount of plant sterols that are absorbed. The ATP-binding cassette (ABC) transporters, ABCG5 and ABCG8, which are expressed almost exclusively on the luminal surface of the enterocyte and the liver, efficiently and more specifically pump the sterols back into the intestinal lumen so that only traces remain in the body. Any absorbed phytosterols are rapidly taken up by the liver and excreted into bile. Phytosterolaemia is attributable to mutations in the closely related ABCG5 and ABCG8 genes, which code for the gut transporter proteins, located on chromosome 2p21. Thus, in phytosterolaemia there is an unrestricted absorption of plant sterols from the gut and inefficient secretion of sterols into bile by the liver, causing a net accumulation of sterols (4–6).

We report a family where three offspring had phytosterolaemia, short stature, hyperpigmentation, ovarian failure and markedly elevated levels of adrenocorticotrophin (ACTH). We hypothesise that the elevated sterol levels interfere with cholesterol metabolism, thus leading to adrenal insufficiency.

Patients
Genetic analysis and biochemistry
The family originate from the Sylet region of Bangladesh. The affected siblings (two males and one female)
all had short stature (<0.4th centile), elevated plant sterols and a blood film that showed stomatocytes and macrothrombocytopenia. The parents are consanguinous and the family tree is consistent with autosomal recessive inheritance (Fig. 1). This family had mutations in ABCG5 at E77X with affected members being homozygous. This was reported as part of a larger analysis of the haematological abnormalities, such as macrothrombocytopenia and stomatocytic haemolysis present in phytosterolaemia (7). Plasma sterols were measured by gas chromatographic mass spectrometry as described by Clayton et al. (8). The results of the phytosterol levels are shown in the Table 1.

Index case

This girl initially presented at 3 years of age with poor growth. Extensive investigations excluded inflammatory diseases, metabolic and mitochondrial disorders. Abnormal results included macrocytosis, giant platelets and thrombocytopenia, with a platelet count of 97 × 10⁹/l and elevated triglycerides of 9 mmol/l (0.5–2.1). The cholesterol levels remained within the normal range, whereas the triglyceride level fluctuated. Her height and weight was consistently below the third centile (Fig. 2). Pituitary function tests undertaken at 11 years of age showed a normal thyrotrophin and growth hormone (GH) level (peak GH 27 μg/l (83 mIU/l)), in response to thyrotrophin-releasing hormone (200 μg) and Clonidine (150 μg/m²) respectively. The basal cortisol was 428 nmol/l but no ACTH result was available at the time. Basal follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels were 15.1 and 0.5 IU/l respectively. Following stimulation (LH-releasing hormone: LHRH 100 μg) at 60 min, the peak FSH was 47.8 IU/l and LH was 18.5 IU/l. The oestrogen levels were undetectable. Although she was prepubertal, the exuberant rise in FSH indicated possible ovarian failure. Subsequently, streak ovaries with a prepubertal uterus were confirmed on ultrasound, and bilateral laparoscopic gonadal biopsies were undertaken which, like the peripheral blood, demonstrated a normal female karyotype of 46, XX with no evidence of mosaicism. Gonadal histology showed scant stroma containing primordial and several early Graffian follicles, overall the features were consistent with follicular ovarian failure. Oestrogen replacement therapy was commenced at 12.7 years to induce pubertal changes. Compliance has been intermittent and basal gonadotrophin levels at age 19 years of age demonstrated her LH was 40.2 IU/l and FSH was 44.6 IU/l.

Phytosterolaemia was diagnosed at 17 years of age. At 18 years of age, hyperpigmentation was noted and she had a baseline ACTH of 367 ng/l and a cortisol level of 581 nmol/l. This was followed by a standard dose synacthen test (250 μg) which revealed cortisol levels of 477 and 464 nmol/l at 30 and 60 min respectively. Repeat ultrasound scanning did not reveal any adrenal abnormalities, and no autoantibodies were detected against the adrenals or ovaries. The combination of dietary phytosterol restriction and ezetimibe (which inhibits intestinal absorption of cholesterol and sterols) reduced her levels of circulating plant sterol levels.

Sibling 2

Pituitary function tests were undertaken for short stature at 11 years age in sibling 2. Following clonidine stimulation (150 μg/m²), partial GH deficiency was indicated by a peak GH level of 3.7 μg/l (11.6 mIU/l). The baseline cortisol level was 294 nmol/l, with no corresponding ACTH. LHRH (100 μg) stimulation testing was normal: at 0, 20 and 60 min, the LH was <0.5, 2.4 and 5.8 IU/l and FSH 3.4, 4.7 and 9.9 IU/l respectively. Institution of GH (0.7 mg/m² per day) at 12.5 years appeared to result in catch-up growth, although patient compliance with therapy remained an issue (Fig. 3). It is possible that part of the acceleration in growth could have been due to puberty, which he underwent normally. His diagnosis of phytosterolaemia was confirmed at 14 years of age. At 16 years, he was also noted to have hyperpigmentation with elevated ACTH confirmed on separate occasions, 399 and 690 ng/l, with corresponding baseline cortisol values of 293 and 237 nmol/l respectively.

Sibling 3

Sibling 3 has similar haematological abnormalities and short stature like his elder siblings. Clonidine provocation testing at 7 years of age revealed a maximum GH level of 5.7 μg/l (17.1 mIU/l); a repeat stimulation test using Glucagon (1 mg) revealed a peak GH of 8.5 μg/l (25 mIU/l). He continues to grow below but parallel to the 0.4th centile. Adrenal function at 7 years is normal with an ACTH level of 21.1 ng/l and cortisol levels of 285, 646 and 400 at 0, 30 and 60 min respectively following a low dose Synacthen test (1 μg).
Discussion

The combination of increased absorption and decreased secretion of plant sterols accounts for phytosterolaemia. There have been at least 45 reported cases of phytosterolaemia in the literature but the true prevalence is unknown (9). The treatment consists of restriction of dietary cholesterol and plant sterols (10), or pharmacological therapy with cholestyramine and ezetimibe. The latter selectively inhibits plant sterol and cholesterol absorption from the jejunum and is the only drug with an indication for treatment of homozygous phytosterolaemia and familial hypercholesterolaemia (11–13).

Animal models provide an insight into the potential mechanisms linking phytosterolaemia to adrenal insufficiency, as plant sterols have been shown to accumulate in the liver, adrenals, ovaries and testis (14, 15). Thus, this accumulation of sterols may interfere with cholesterol metabolism in the adrenal gland and lead to adrenal insufficiency. ABCG5 and ABCG8 mRNAs are present exclusively in the intestine and liver but not in the adrenal glands of normal mice (4). Mice with disruptions of these ABCG5 and ABCG8 transporters have comparable plant sterol levels to humans with phytosterolaemia and they appear phenotypically normal (16). However, the adrenal glands of the G5G8−/− mice show significantly less lipid accumulation, which is visibly different from wild type mice adrenals (17). Thus, although ABCG5 and ABCG8 are not expressed in the adrenal gland (4), the cholesterol levels in the adrenal glands are dramatically reduced by the inactivation of these two half-transporters. Additionally, a paradoxical increase in sterol efflux is also implicated as a contributing factor to sterol depletion in the adrenal gland (17). Despite this reduction in cholesterol levels, there is no compensatory increase in enzymes involved in the cholesterol biosynthetic pathway (18). Treatment with ezetimibe in mice results in a marked reduction in plant sterols and increase in cholesterol content respectively of the adrenal glands, supporting the view that the cholesterol depletion of the adrenal glands in the G5G8−/− mice results from the accumulation of plant sterols that inhibit cholesterol synthesis (17).

Table 1 Phytosterol levels in the affected individuals taken at first presentation are all raised. Total sterol, equivalent to ‘routine cholesterol’ measurement was determined by a cholesterol oxidase technique.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>β-sitosterol (umol/l)</th>
<th>Stigmasterol (umol/l)</th>
<th>Campesterol (umol/l)</th>
<th>Total sterol (mmol/l)</th>
<th>TG (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Index case</td>
<td>17</td>
<td>F</td>
<td>1471</td>
<td>167</td>
<td>1340</td>
<td>5.1</td>
<td>17.5</td>
</tr>
<tr>
<td>Sibling 2</td>
<td>14</td>
<td>M</td>
<td>970</td>
<td>119</td>
<td>1040</td>
<td>3.7</td>
<td>3</td>
</tr>
<tr>
<td>Sibling 3</td>
<td>5</td>
<td>M</td>
<td>625</td>
<td>26</td>
<td>472</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Normal range</td>
<td></td>
<td></td>
<td>4–46</td>
<td>0.17–0.58</td>
<td>2–40</td>
<td>2.3–5.0</td>
<td>0.23–1.7</td>
</tr>
</tbody>
</table>

TG, triglyceride.

Figure 2 Height chart for index case. Closed circle, height; open circle, bone age.
In our family, both older children had hyperpigmentation and markedly elevated ACTH levels. Although the baseline cortisol levels were satisfactory, the index case had a very poor increment in her cortisol levels after stimulation. This attenuated cortisol response following ACTH stimulation is at variance with the G5G8−/− mice, where the ability of the glands to respond acutely to ACTH was unimpaired. However, it is possible that some of the plant sterols could be used as a substrate for steroid synthesis in the adrenal glands of humans (17). Sibling 3 at 8 years of age has no clinical indicators of adrenal insufficiency and remains under surveillance.

To date, there are no reports of adrenal insufficiency in patients with phytosterolaemia, and only a single patient with this condition has undergone a post-mortem examination, which unfortunately did not examine the adrenal glands (19). All affected individuals in our family have short stature; the family has mutations in ABCG5 at E77X, whereas the most common mutation described is in ABCG8 (4, 6) and it is possible that differences in gene mutations could account for the adrenal abnormalities, but this remains speculative. However, the increased knowledge of cholesterol metabolism makes it probable that the adrenal glands are a likely organ for interference with the cholesterol regulatory pathways by the plant sterols. It is also plausible that patients with phytosterolaemia who have died of cardiovascular events could have succumbed to adrenal insufficiency.

The cause of the additional hormone problems in the family remains uncertain. Both male siblings initially had an abnormal GH stimulation test. Sibling 2 appeared to respond to GH treatment, but a repeat test has not been performed, and some of the response may have been pubertal acceleration in growth. Sibling 3 had a borderline GH stimulation test on repeat testing, but maintains satisfactory growth without treatment. However, the issue of GH deficiency as a consequence of phytosterolaemia remains inconclusive as the index case continued to demonstrate poor growth despite a normal GH stimulation test. We plan to retest both males after they have completed normal linear growth.

While we are unable to postulate a link between phytosterolaemia and disruption of GH, there are some potential mechanisms by which ovarian function may be disrupted. Animal studies where rats have been exposed to high concentrations of phytosterols have shown reduced fertility in males (20) and altered fertility indices in both sexes (21), but again no humans with infertility from ovarian failure have been reported. As the ovaries are a source of steroidogenesis, the elevated plant sterols may interfere with cholesterol metabolism. Thus, it is possible that the biochemical and histological evidence of ovarian failure in the index case could possibly be ascribed to phytosterolaemia. In another human model, females with a StAR (steroidogenic acute regulatory protein) mutation encounter infertility from premature ovarian failure as they are also unable to synthesise steroids from cholesterol.

This report would have been strengthened by having a full synacthen test for all the siblings, as well as urine steroid analysis and detailed studies of adrenal precursors following stimulation. However, follow-up for further investigation has been inconsistent and the family is reluctant to undergo further testing. In summary, we present a family with phytosterolaemia, short stature and endocrine abnormalities. We
hypothesise that elevated plant sterol levels in phytosterolaemia may interfere with adrenal hormone synthesis and this is the first description in humans to highlight that cholesterol metabolism is disrupted in the adrenal gland and leads to adrenal insufficiency, which needs to be monitored and treated.

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