The Case | Epistasis and urolithiasis

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The case: make your diagnosis

Epistasis and Urolithiasis

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The case

A 26-year-old woman was referred for recurrent urolithiasis. The first manifestation occurred 3 years before, followed by recurrent acute episodes. She underwent one ureteral stent placement, one shock wave lithotripsy, and at last a flexible ureteroscopy that allowed to retrieve all the remaining stones. She had no other medical history with the exception of paroxysmal tachycardia, poor dairy intake in her diet and did not take any medication interfering with calcium or phosphate homeostasis. Regarding her family history, her mother had also recurrent renal colic due to urolithiasis. Morphoconstitutional analysis revealed a stone made of calcium phosphate and to a lesser extent calcium oxalate, in concentric layers. According to infrared spectra analysis, the stone consisted mainly of calcium phosphate: 56% carbonated apatite, 20% octacalcium phosphate pentahydrate (OCP), 12% calcium oxalate monohydrate, 10% calcium oxalate dihydrate and 2% proteins.
Her physical examination was normal. Biochemistry revealed increased serum calcium level at 2.59 mmol/l (N 2.16-2.52 mmol/l), low serum phosphate level at 0.7 mmol/l (N 0.8-1.4) mmol/l, parathyroid hormone (PTH) level in normal range at 46 pg/ml (N 8-76 pg/ml) and elevated calcitriol serum level at 87 pg/ml (N<67 pg/ml). Urine calcium excretion was increased at 8.2 mmol/24-hr (N<6 mmol/24-hr). Fasting urine pH was 5.9. An oral calcium load test (Pak test) was performed, the results of which are shown in Table 1. The test revealed increased serum calcium levels induced by the calcium load, with an adequately suppressed PTH level. A 99mTc Sestamibi parathyroid scintigraphy did not reveal any adenoma.

What is your diagnosis?

Nephrolithiasis revealing heterozygous mutation of *SLC34A*3 and *CYP24A1* (respectively encoding for NPT2c and 25-(OH) vit D_3-24-hydroxylase).

The diagnosis

In this case, the first suspected diagnosis was primary hyperparathyroidism, according to hypercalcemia, hypercalciuria, renal phosphate leak and intermediate PTH levels. However, calcium load test evidenced appropriate PTH suppression. Moreover, parathyroid scintigraphy did not disclose a hyperfunctioning parathyroid tissue. The elevated level of calcitriol subsequently led us to suspect either a cause of elevated 1-alpha hydroxylase activity (granulomatous disease), which increases the production of calcitriol, or a decreased 25-(OH) vit D_3-24-hydroxylase activity, the enzyme promoting calcitriol degradation.[1] There were no symptoms consistent with granulomatous disease. Furthermore, the more striking outlier was renal phosphate wasting, inconsistent with elevated calcitriol.
Kidneys stones consisting of OCP are uncommon since OCP is an unstable calcium phosphate crystalline phase. These stones are hallmarks of massive and recent calcium phosphate supersaturation. This situation is mainly encountered in pregnancy, which leads to rapid onset of hyperphosphaturia and hypercalciuria.[2]
The patient had a condition responsible for hypercalcemia, hypercalciuria and renal phosphate loss that did not prove to be primary hyperparathyroidism. We subsequently suspected a genetic disease underlying the clinical presentation. The patient was tested for mutations of genes coding for enzymes involved in vitamin D metabolism, and for proteins involved in renal phosphate handling.
Gene sequencing revealed heterozygous mutation and variant of *CYP24A1* and *SLC34A3* genes (respectively encoding for 25-(OH) vit D_3-24-hydroxylase and NPT2c).[3] The *CYP24A1* mutation identified in patient DNA (c.1226T>C; p.Leu409Ser) has been previously described. Heterozygous *CYP24A1* mutation carriers, while usually asymptomatic, may present an increased risk of kidney stones. The heterozygous variant of *SLC34A3* (c.1357_1359del; p.DelPhe453; http://exac.broadinstitute.org/allele frequency<1/10000) is predicted to be deleterious (UMD-

Predictor®, Alamut®) and kidney stones are more frequent in carriers of heterozygous *SLC34A3* mutations than in the general population.

Epistasis, defined as the interaction between different genes, has become a topic of major interest in recent years. In this case, the patient is affected by negative epistasis, the combined deleterious effect of two harmful genetic variants, resulting in a phenotype greater than expected from individual mutations or variants. It gives new insights in interpreting phenotypes of complex diseases that are not fully explained by a monogenic disease. Thus we could hypothesize that the patient had a primary renal phosphate wasting caused by defective NPT2c function, inducing "appropriate" but moderate increase in calcitriol production.[3] The second genetic defect affecting calcitriol degradation led subsequently to inappropriately high level of calcitriol, resulting in hypercalcemia, hypercalciuria and nephrolithiasis.[1] The finding of a "normal" PTH was quite surprising since *CYP24A1* mutation and high calcitriol serum levels are associated with suppressed PTH. One may hypothesize that heterozygous mutation would result in a less severe phenotype.

Another diagnostic feature of *CYP24A1* mutation is low 24,25-(OH)2 vitamin D levels. Although we did not perform this test, it may be useful to screen appropriate phenotypes.

Overall, this observation may explain why single mutant allele carriers (of SLC34A3 gene for instance) may become symptomatic, when affected by a second gene mutation or variant.

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Table 1

	Before Calcium Load	After Calcium Load	Normal Range
Serum PO_4^{3-} (mmol/l)	**0.73** ↓		0.85-1.31
Phosphate FE	**33.3%**		<20%
Serum calcium (mmol/l)	**2.58** ↑	**2.97** ↑	2.16-2.52
Ionized calcium (mmol/l)	**1.3** ↑	**1.41** ↑	1.14-1.31
PTH (pg/ml)	37	24	8-76
25 (OH) vitamin D_3 (ng/ml)	**18** ↓		30-100

$1,25(OH)_2D_3$ (pg/ml)	**87** ↑	17-67
C-terminal FGF23 (RU/ml)	62	20.9-91.1
Serum creatinine (µmol/l)	61	45-80

Abbreviations: Phosphate FE: Phosphate Fractional excretion calculated as following: (urine phosphate*serum creatinine)/(urine creatinine*serum phosphate) in standardized conditions; PTH, parathyroid hormone; FGF23, fibroblast growth factor 23. Values out of normal range are shown in bold.