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Short Bones, Renal Stones, and Diagnostic Moans: Hypercalcemia in a Girl Found to Have Coffin-Lowry Syndrome

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Abstract

Pathogenic variants in *RPS6KA3* are associated with Coffin-Lowry syndrome (CLS), an X-linked semidominant disorder characterized by intellectual disability, stimulus-induced drop attacks, distinctive facial features, progressive kyphoscoliosis, and digit anomalies in hemizygous males. Heterozygous females may also have features of CLS; however, there can be considerable phenotypic variation, often attributed to ratios of X-inactivation in various tissue types. Although skeletal anomalies and short stature are hallmarks of CLS, hypercalcemia has not been reported. Here we describe a 30-month-old girl with gross motor delays, short stature, dysmorphic features, bilateral duplicated renal collecting systems, and no family history of hypercalcemia who required multiple admissions for idiopathic hypercalcemia necessitating bisphosphonate infusions at 12.5 and 15 months of age. A maternally inherited likely-pathogenic variant in *RPS6KA3* was identified by trio exome sequencing, consistent with the diagnosis of CLS in the proband and her mother. Maternal history was notable only for decreased height compared to first-degree relatives, bilateral genu valgum, and a bicornuate uterus; she was later found to also have a partially duplicated left renal collecting system. Subsequent X-inactivation studies in blood aligned with the phenotypic variation between mother and daughter. Although hypercalcemia is not a reported feature in CLS, there is evidence of interrupted osteoblast differentiation, providing a potential mechanism for hypercalcemia in this genetic condition. The hypercalcemia in this case may represent a severe presentation of an unrecognized clinical feature in CLS that resolves with age. This case further highlights the intrafamilial phenotypic variation of CLS among females, suggesting X-inactivation as the underlying mechanism, and demonstrates the value of exome sequencing in patients for whom a genetic disorder is highly suspected but not identified despite thorough evaluation.

Keywords

genetic and molecular medicine, endocrinology, pediatrics

Introduction

Coffin-Lowry syndrome (CLS) is an X-linked semidominant genetic disorder caused by pathogenic loss-of-function variants in *RPS6KA3*, a gene located on the X chromosome at Xp22.2 that encodes the ribosomal S6 kinase, RSK2.[1] In hemizygous males, CLS is characterized by intellectual disability, short stature, progressive kyphoscoliosis, tapered fingers, stimulus-induced drop episodes, and distinctive facial features. Facial features include a prominent forehead with supraorbital ridges, widely spaced eyes with downslanted palpebral fissures, depressed nasal bridge, thick nasal alae, wide mouth, and an everted vermilion of lower lip.[2] The true prevalence of CLS is not known; however, the estimated incidence is between 1:50 000 to 1:100 000 with approximately 70% to 80% of affected individuals representing sporadic cases secondary to a de novo pathogenic variant in *RPS6KA3*.[1,3] The remaining 20% to 30% of cases are maternally inherited, as males with CLS do not typically reproduce.[2]

There is considerable phenotypic variation among females with CLS who are heterozygous for a pathogenic variant in *RPS6KA3*, ranging from normal appearance and intelligence with short stature +/− digit abnormalities, to characteristic facial features with moderate developmental and intellectual

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delay/disability.[1,2,4,5] The wide range of clinical severity reported among females with CLS is thought to be secondary to ratios of X-inactivation in various tissue types.[2,4] Thus, in inherited cases of CLS, a mildly affected woman may have more severely affected daughters in addition to having a 50% chance of conceiving a son with CLS. Of note, a study by Simensen et al[5] demonstrated that females heterozygous for a pathogenic variant in *RPS6KA3* are more likely to have skewed X-inactivation patterns in blood (favorable skewing) and decreased IQ compared with female family members without the pathogenic variant; there was no significant correlation between IQ and X-inactivation among heterozygous females.

Despite the well-observed impact of CLS on a variety of skeletal tissues in affected males and females, including the axial skeleton (progressive kyphoscoliosis and ligamenta flava calcification), long bones (short stature with disproportionately short lower limbs),[2,6-8] and distal phalanges (tapered fingers), the underlying pathophysiology of this phenotype in CLS remains unclear. Here, we describe a 30-month-old girl with idiopathic hypercalcemia necessitating multiple hospital admissions and bisphosphonate (pamidronate and zoledronate) infusions at 12.5 and 15 months of age who was found to have CLS via trio exome sequencing.[9] Although her calcium levels normalized around 22 months of age and have remained stable, she continues to have secondary nephrocalcinosis with normal renal function. Hypercalcemia is not a reported feature in individuals with CLS; however, this individual's presentation and calcium dysregulation may provide insight into the underlying mechanism of skeletal anomalies associated with CLS.[9]

Case Presentation

A 12-month-old girl with history of mild motor delay was admitted to the pediatric intensive care unit for electrolyte derangements in the setting of 2 weeks of fatigue and decreased oral intake, and a 1-day history of persistent nausea and vomiting. Comprehensive metabolic panel (Table 1) was most notable for profound hypercalcemia (14.7 mg/dL), hyponatremia (129 mmol/L), hyperkalemia (6.2 mmol/L), hypochloremia (89 mmol/L), decreased bicarbonate (16 mmol/L), anion gap of 24, elevated BUN (48 mg/dL), and elevated creatinine (0.96 mg/dL). Additionally, hyperuricemia (10.3 mg/dL) was noted. Renal ultrasound on admission was significant for bilateral grade 3-4 medullary calcinosis. Nephrology evaluation, pertinent for an appropriately and acutely elevated urine Ca/Cr ratio which later resolved, provided evidence against a renal etiology with no evidence of primary renal disease. Endocrinology evaluation at the time of hypercalcemia was notable for appropriately suppressed parathyroid hormone (3.3 pg/mL; Table 1) with normal albumin, phosphorus, alkaline phosphatase, magnesium, thyroid stimulating hormone, free-T4, Vitamin A, 25-hydroxy and 1,25-dihydroxy vitamin D levels. Inappropriately elevated parathyroid hormone-related peptide (PTHrP, 9.7 pmol/L; Table 1) was noted without identifiable source despite laboratory studies and extensive imaging in consultation with Oncology; lactate dehydrogenase, alpha-fetoprotein, beta-human chorionic gonadotropin, and C-reactive protein were all within normal limits. Abdominal magnetic resonance imaging (MRI) revealed bilateral duplicated renal collecting systems, redemonstration of bilateral nephrocalcinosis, and no suspicious abdominal or pelvic mass to suggest malignancy. Additionally, brain and neck MRI were normal. Testing for lead toxicity and human immunodeficiency virus (HIV) were both negative. The proband was treated with intravenous fluids with resolution of her hypercalcemia (calcium 10.3 mg/dL) and other electrolyte abnormalities, and discharged on day 11 of admission. Ten days later, the proband was admitted a second time for profound hypercalcemia (14.3 mg/dL), at which time she was treated unsuccessfully with calcitonin (4 doses of 4 units/kg q12 hours), followed by intravenous bisphosphonate therapy (1 dose 0.5 mg/kg of pamidronate) with good response (Figure 2). The proband was discharged on day 7 of admission with a serum calcium level of 8.5 mg/dL.

Medical Genetics was consulted on both admissions due to concern for an underlying genetic etiology of her hypercalcemia. Perinatal history was notable for high-risk noninvasive prenatal screening for Turner syndrome which was further evaluated by karyotype via amniocentesis and revealed a normal female karyotype. The proband was born at 39 weeks gestation to a 32-year-old G1P0 woman via cesarean section for breech presentation. Pregnancy was complicated by fetal growth restriction thought to be secondary to the mother's bicornuate uterus. Birth weight was 2390 g (fourth percentile) with a birth length of 46 cm (seventh percentile) and head circumference of 32.5 cm (seventh percentile). The proband's mother's medical history was notable for decreased height (61 inches, seventeenth percentile for US adult women) compared to first-degree relatives (mid-parental height 67 inches), bilateral genu valgum, and a bicornuate uterus; there was no maternal history of hypercalcemia.

At 12.5 months of age, physical exam was notable for length of 68 cm (<third percentile, Z-score = −2.05) with relative macrocephaly (44.5 cm, 33rd percentile, Z-score = −0.45) and frontal bossing, depressed nasal bridge with anteverted nares and bulbous nasal tip, and everted lower vermillion border (Figure 1). Initial Medical Genetics evaluation included a skeletal survey and microarray, both of which were reported as normal. A custom calcium homeostasis gene panel with 37 genes was ordered and nondiagnostic. After multiple failed attempts in obtaining insurance authorization for additional genetic testing, trio exome sequencing was funded by the nonprofit organization, Little Zebra Fund.[10]

Exome sequencing revealed a maternally inherited likely pathogenic variant (c.325+1G>T) in *RPS6KA3*, located within intron 4 at a canonical splice site and predicted to result in a null allele. This finding is consistent with the

Table 1. Laboratory Results at Various Time Points, Including 3 Separate Hospital Admissions for Hypercalcemia (Ages 12, 12.5, and 15 months) and Most Recent Laboratory Evaluations (Ages 24 and 29 months).

Laboratory test	First admission (12 months old)	Second admission (12.5 months old)	Third admission (15 months old)	Most recent (24 months old* or 29 months old†)
Calcium (reference: 8.8-10.8 mg/dL)	14.7 mg/dL (3.67 mmol/L)	14.3 mg/dL (3.58 mmol/L)	12.5 mg/dL (3.13 mmol/L)	10.4 mg/dL† (2.60 mmol/L)
Ionized calcium (reference: 1.12-1.32 mmol/L)	1.45 mmol/L	1.62 mmol/L	n/a	n/a
Phosphorus (reference: 3.4-6.0 mg/dL)	4.7 mg/dL (1.52 mmol/L)	3.9 mg/dL (1.26 mmol/L)	5.1 mg/dL (1.65 mmol/L)	5.2 mg/dL† (1.68 mmol/L)
Parathyroid hormone (reference: 15-65 pg/mL)	3.3 pg/mL (3.3 ng/L)	3.5 pg/mL (3.5 ng/L)	n/a	11.2 pg/mL* 11.2 ng/L
Parathyroid hormone-related peptide (reference: ≤4.2 pmol/L; amino-terminus assay)	9.7 pmol/L	4.6 pmol/L	4.6 pmol/L	7.4 pmol/L*
Magnesium (reference: 1.7-2.3 mg/dL)	2.2 mg/dL (0.91 mmol/L)	2.0 mg/dL (0.82 mmol/L)	2.0 mg/dL (0.82 mmol/L)	2.3 mg/dL† (0.95 mmol/L)
Sodium (reference: 135-145 mmol/L)	129 mmol/L	142 mmol/L	n/a	137 mmol/L†
Potassium (reference: 3.5-5.5 mmol/L)	6.2 mmol/L	4.3 mmol/L	n/a	5.0 mmol/ L†
Chloride (reference: 98-107 mmol/L)	89 mmol/L	105 mmol/L	n/a	99 mmol/L†
Bicarbonate (reference: 22-29 mmol/L)	16 mmol/L	22 mmol/L	n/a	25 mmol/L†
Blood urea nitrogen (reference: 5-18 mg/dL)	48 mg/dL (17 mmol/L)	16 mg/dL (5.7 mmol/L)	n/a	19 mg/dL† (6.8 mmol/L)
Creatinine (reference: 0.18-0.35 mg/dL)	0.96 mg/dL (73 μmol/L)	0.41 mg/dL (31 μmol/L)	0.33 mg/dL (25 μmol/L)	0.40 mg/dL† (31 μmol/L)

Results displayed in conventional units, followed by Système International (SI) units in parentheses, if applicable. For most recent laboratory evaluations: *24-months-old; †29-months-old

diagnosis of CLS in our proband, as well as her mother; no other variants were reported. Subsequent X-inactivation studies[11] performed on blood demonstrated complete favorable skewing (100:0) in the mother and random X-inactivation in the proband (57:43), consistent with the phenotypic variation between mother and proband. Familial variant testing in the proband's maternal grandmother and maternal half-aunt were negative, suggesting the pathogenic variant to be de novo in the proband's mother.

After discharge from her second admission, the proband's calcium levels were monitored weekly by Endocrinology and continued to be elevated but stable (range 10.5-12.1 mg/dL). At 15 months of age, the proband was admitted for a third time for severe hypercalcemia (12.5 mg/dL; Table 1) and treated again with intravenous bisphosphonate therapy (1 dose of 0.0125 mg/kg of zoledronate, in hope of a longer duration of effect). Her calcium level on 5 days post-zoledronate infusion was 10.9 mg/dL (Figure 2). Monitoring of the proband's calcium level at least monthly from age 15 to 22 months demonstrated consistent levels below 11.5 mg/dL (range 10.9-11.4 mg/dL). Monitoring of her calcium level at least every 3 months from age 22 to 30 months demonstrated consistent levels below 11 mg/dL (range 10.4-10.8 mg/dL).

At Medical Genetics outpatient follow-up visits at 21 and 30 months of age, many of the facial features previously noted had become more pronounced and tapered fingers were also noted (Figure 1). At 30 months of age, growth parameters included length of 85 cm (fifth percentile), weight of 11.2 kg (eighth percentile), and head circumference of 48 cm (45th percentile). Parental reports and evaluations by Developmental and Behavior Pediatrics suggest evidence of global delays that are improving with therapies including occupational therapy for gross and fine motor (unsteady gait with frequent falls and now able to manipulate small items at 30 months), speech-language therapy for delayed speech (>100 words and able to form 2-word sentences at 30 months), and poor feeding by mouth. The proband continues to gain new skills and is socially engaged. Follow-up evaluation with Nephrology continues to provide evidence against a renal etiology for her prior hypercalcemia and has demonstrated stable bilateral nephrocalcinosis with normal kidney function; she is being monitored for hypertension and proteinuria. She continues to follow with Endocrinology for monitoring of calcium levels. Overall, the proband's clinical features are consistent with her diagnosis of CLS with the exception of her history of profound hypercalcemia. The cause and/or mechanism of her hypercalcemia are unclear despite thorough evaluation by Endocrinology, Nephrology, Oncology, and Medical Genetics.

Figure 1. Clinical features of a mother and daughter with Coffin-Lowry syndrome.

Discussion

Although various degrees of cognitive disability, short stature, skeletal anomalies, and facial features have been reported in females with CLS, hypercalcemia has not been reported in affected females or males. The underlying etiology of the proband's hypercalcemia remains unclear despite thorough evaluation by multiple subspecialists. The persistent mild elevation of PTHrP may be contributing to her unexplained hypercalcemia; however, her PTHrP levels have remained elevated while calcium levels have decreased over time. The cause of her elevated PTHrP remains unclear despite thorough evaluation by Oncology and close follow-up with Endocrinology; no additional imaging has been performed in the interim. Given our proband's diagnosis of CLS and considerable clinical, laboratory, radiological, and genetic evidence against another cause and/or second genetic condition, we hypothesize at this time that her transient, yet profound, hypercalcemia of early childhood is related to her diagnosis of CLS.

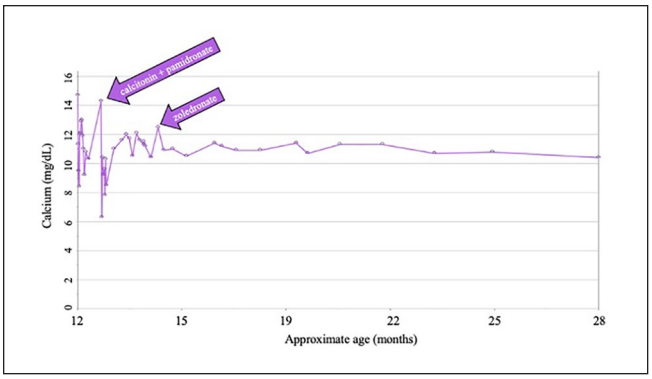

Figure 2. Serum calcium levels (mg/dL) from age 12 to 28 months in a girl with Coffin-Lowry syndrome.
Calcium levels are illustrated by the purple line. Purple arrows indicate time points at which intravenous bisphosphonate infusions were administered during the proband's second and third hospital admission at 12.5 and 15 months of age, respectively.

In 2004, Yang et al[12] proposed that lack of phosphorylation of the transcription factor, ATF4, by inactive RKS2 (due to loss-of-function variants in *RPS6KA3*) may interrupt the normal regulatory role of ATF4 in osteoblast differentiation, accounting for some of the skeletal features seen in CLS. This hypothesis is further supported by the Rsk2-null mouse model created and characterized by Marques Pereira et al with phenotypic features including mild reduction of bone

mass, mild teeth anomalies, and development of progressive osteopenia. These findings were reportedly due to impaired osteoblast function with the lack of phosphorylation of the transcription factor ATF4 by RSK2 found to be a cause of the skeletal abnormalities.[1] The features seen in the Rsk2-null mouse model appear to be consistent with the delayed bone development seen on radiological studies of individuals with CLS, which also include cranial hyperostosis, ligamental flava calcification, and tufting of the distal phalanges.[1,6,13] Whether or not these radiologic features seen in individuals with CLS are associated with transient or continuous calcium dysregulation is unclear at this time.

Both the proband and her mother were diagnosed with CLS through trio exome sequencing, and except for the proband's hypercalcemia, the clinical features of both individuals are consistent with the diagnosis. The likely pathogenic variant in *RPS6KA3* in the proband was maternally inherited, highlighting the intrafamilial phenotypic variation of CLS among heterozygous females. The results of X-inactivation studies were consistent the discordant phenotype between mother and daughter, supporting X-inactivation as the underlying determinant of phenotype severity in females with heterozygous pathogenic variants in *RPS6KA3*. Interestingly, our proband's mother was also recently found to have a partially duplicated left renal collecting system upon evaluation for a kidney stone. Renal anomalies are not a commonly reported feature of CLS, with only 1 individual reported to have unilateral renal agenesis.[6,14] It is unclear at this time if the finding of a duplicated renal collecting system in both the proband and her mother is related to CLS, hereditary, or coincidental, but is unlikely to have contributed to the finding of hypercalcemia in the proband given evidence against a renal etiology.

After extensive multidisciplinary clinical evaluation and continued absence of a unifying diagnosis to explain our proband's constellation of features, trio exome sequencing was warranted to further evaluate for an etiology of her hypercalcemia for which medical intervention may be beneficial. Although it remains unclear if the finding of CLS explains her hypercalcemia, the result of trio exome sequencing in this case provided 2 members of this family a clinically actionable diagnosis with associated medical management, screening, and surveillance guidelines that would not have been provided to the family without this genetic testing result. In addition, this testing result has provided multiple family members with valuable reproductive information. A second medical or genetic condition is possible, although exome sequencing did not report any other variants. Exome reanalysis will be of value in the future as the field of Medical Genetics continues to expand and our understanding of human disease improves.

To conclude, our proband was found to have hypercalcemia after presenting acutely with symptoms of vomiting, dehydration, and failure to thrive. To our knowledge, there is no maternal history of hypercalcemia in infancy or childhood, and recent calcium levels in the proband's mother were within normal limits. It is possible that the proband represents a severe presentation of an unrecognized phenotype that resolves with age, and likely prior to most patients' diagnosis. As exome sequencing and perinatal genetic testing become more routinely offered to individuals with overlapping features or a history of CLS, respectively, more individuals with CLS will be diagnosed in infancy. Earlier diagnosis and phenotyping of individuals with CLS will not only aid in the medical management of infants and children with CLS but will also help to clarify if hypercalcemia, as seen in this case, represents an expansion of the phenotype or a truly unrelated feature.

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Ethics Approval

Our institution does not require ethical approval for reporting individual cases or case series.

Informed Consent

Written informed consent was obtained from a legally authorized representative (parent) and the patient for photographs and anonymized health information to be published in this article.

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