Glial Cell Missing Homolog 2 Mutation Causing Severe Hypoparathyroidism: Report of Two Cases With Novel Mutations

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Abstract

Hypoparathyroidism is a common encounter in endocrinology practice. A thorough search for the etiology is generally futile, and most cases are labeled as idiopathic. Familial idiopathic hypoparathyroidism is a large chunk of these idiopathic cases. Here we present 2 cases who presented with features of hypocalcemia and were eventually diagnosed with hypoparathyroidism. Our first case is that of a middle-age woman who presented with spontaneous tetany and perioral numbness. She had very low serum calcium values, low serum magnesium, hypokalemia, hypercalcuria, and undetectable parathormone levels. She was initially managed with parental calcium, magnesium, and oral potassium chloride, which was shifted to oral replacements once stabilized. Focused exome sequencing for causes of hypoparathyroidism and hypocalcemia revealed a frameshift mutation in glial cell missing homolog 2 (GCM2) (NM_004752.4) on chromosome 6, c.737dupA variant (p. Asp246Glufs*25) located at exon 5. The second case presented is that of a 1-month-old infant presenting with hypocalcemic seizures, severe hypocalcemia, hyperphosphatemia, and low parathormone levels. The infant was stabilized with parenteral calcium and trial of subcutaneous teriparatide for further improvement. Oral calcium and calcitriol were instituted once stabilized, and teriparatide was tapered off. Focused exome sequencing revealed a homozygous mutation involving GCM2 (ENST0000379491.5) on chromosome 6, variant CM2 chr6:10876558_10877139insT located on exon1-2. Both of these mutations are novel and underscore the profound effect of GCM2 on parathyroid gland development in infants and maintenance in adults.

Key Words: parathyroid, GCM2, CaSR, hypocalcemia, idiopathic

Abstract

Calcium is essential for neurotransmission, cell signaling, and bone formation. Therefore, serum calcium must be maintained in a safe normal range. Otherwise, it can have severe consequences including death. The parathyroid hormone has a central role in calcium regulation acting at the level of the bone, kidney, or gastrointestinal tract [1]. Hypoparathyroidism is a condition of decreased production of parathyroid hormone. The clinical features of hypoparathyroidism thus revolve around the manifestations of hypocalcemia, low calcitriol, and hyperphosphatemia. Hypoparathyroidism can result from a variety of congenital or acquired causes of which genetic causes are an important contributor [2,3]. Glial cell missing homolog 2 (GCM2) located on chromosome 6p24.2, a zinc finger type transcription factor, is the master regulator of parathyroid gland development [4]. GCM2 encodes a 506-amino acid transcription factor that is expressed mainly in the developing and mature parathyroid glands. In mice in whom GCM2 is ablated, hypoparathyroidism developed because of aplasia of the parathyroid glands, which establishes GCM2 as critically important for calcium homeostasis. GCM2 is also known to regulate calcium sensing receptor (CaSR) expression, which is another mechanism of regulating calcium levels [5]. It has been shown that either dominant inhibitor mutations [5,6] or autosomal recessive amorphic mutations [7-11] in the GCM2 gene are the most common cause of isolated congenital hypoparathyroidism. Here we present 2 cases with hypoparathyroidism and clinical features of overt hypocalcemia, both due to GCM2 mutation and differential effects on the parathyroid gland and the kidney.

Case 1

Our first case is that of a 32-year-old female who presented to the emergency department with tetany and posturing of limbs for 3 to 4 days with perioral and acral paresthesia, tightness for the last 3 weeks. She had presented elsewhere twice in the last 5 years with similar complaints and had required hospitalization for resuscitation and management of acute hypocalcemic crises. On examination, she had spontaneous tetany. The rest of the physical examination was unremarkable...
except for a small soft goiter. She was found to have hypocalcemia and hypomagnesemia and was immediately treated with a continuous infusion of calcium gluconate (Table 1). Non-contrast computed tomography scan of brain showed bilateral basal ganglia calcification (Fig. 1). Ten ampoules of 10% calcium gluconate diluted in 500 mL 5% dextrose was infused over 12 hours. Each ampoule of calcium gluconate contains 92 mg of elemental calcium. This was continued for 48 hours until the hypocalcemic crisis abated. Oral calcium and calcitriol were added thereafter. Intravenous magnesium sulfate, 50% (5 g/10 mL), was used for correction of hypomagnesemia. One gram magnesium sulfate was diluted in 100 dextrose solution and infused over 30 minutes. This was followed by a continuous infusion at 1 g/hour in dextrose until serum magnesium was above 1.2 mg/dL. This was followed by oral magnesium hydroxide tablets, 200 mg thrice daily. The intact parathyroid hormone (iPTH) level was undetectable. She also had persistent hypokalemia with increased potassium excretion, increased renal loss of magnesium, and hypercalciuria. Investigating the small goiter, we arrived at a diagnosis of Graves’ disease (biochemical thyrotoxicosis with positive anti-thyrotropin receptor antibody and diffuse uptake in 99m technetium scan). Focused exome sequencing for causes of hypoparathyroidism and hypocalcemia revealed a heterozygous frameshift mutation in GCM2 (NM_004752.4) on chromosome 6, c737dupA variant (p. Asp246Glufs*25) located at exon 5, likely pathogenic in nature. The patient was discharged in stable condition on oral calcium carbonate at 40 mg/kg/day elemental calcium, 1 mcg calcitriol per day, 1600 mg magnesium oxide daily in

### Table 1. Biochemical and imaging investigations of both patients

| Parameters             | Results of case 1       | Results of case 2       | Reference range | Description                             |
|------------------------|-------------------------|-------------------------|-----------------|-----------------------------------------|
| iCa++                  | 0.699 mmol/L            | 0.536 mmol/L            | 1.15-1.33       |                                         |
| Serum corrected Ca     | 6.1 mg/dL               | 4.9 mg/dL               | 8.6-10.3        |                                         |
| Serum magnesium        | 0.9 mg/dL               | 1.8 mg/dL               | 1.6-2.6         |                                         |
| Serum potassium        | 3.2 mmol/L              | 4.6 mg/dL               | 3.6-5.2         |                                         |
| Serum inorganic phosphorus | 8.8 mg/dL             | 13 mg/dL                | 2.8-4.5 (4-7 in children) |                          |
| Serum albumin          | 3.7 mg/dL               | 3.2 mg/dL               | 3.5-5.5         |                                         |
| ECG qTc interval       | 484 ms                  | —                       | 360-460         |                                         |
| Serum iPTH             | <3 pg/mL                | 3.4 pg/mL               | 10-55           |                                         |
| Serum fT4              | 2.0 ng/dL               | 1.3 ng/dL               | 0.9-1.9         |                                         |
| Serum TSH              | <0.001 mIU/mL           | 2.02 mIU/mL             | 0.4-4           |                                         |
| Urine Ca/Cr (spot)     | 0.28                    | 0.48                    | 0.01-0.15 (Adult)| 0.03-0.78 (<1 yr of age) |
| FeMg                   | 6.4%                    | —                       | <2%             |                                         |
| ANA Profile            | negative                | —                       | —               |                                         |
| 25-hydroxy vitamin D   | 33.4 ng/mL              | 54.2 ng/mL              | >20             |                                         |
| USG KUB                | No apparent nephrolithiasis/ nephrocalcinosis | No apparent nephrolithiasis/ nephrocalcinosis |                     |                                         |
| CT brain               | Bilateral basal ganglia calcification | — Not done             | —               |                                         |
| BERA                   | —                       | WNL                     | —               |                                         |

**Abbreviations:** ANA, anti-nuclear antibody; BERA, brain stem evoked response audiometry; Ca, calcium; Cr, creatine; CT, computed tomography; ECG, electrocardiogram; FeMg, fractional excretion of magnesium; iCa++, ionized calcium; iPTH, parathyroid hormone; fT4, free thyroxine; TSH, thyrotropin; USG KUB, ultrasonography of kidney, ureter, and bladder.

**Figure 1.** Non-contrast computed tomography scan of brain of patient 1 showing hyperdensities bilaterally in the area of basal and periventricular ganglia suggestive of calcification. Few scattered areas of calcification are also seen bilaterally in the frontal cortex.
divided doses, 50 mg spironolactone once daily, and 20 mg carbimazole daily in 2 divided doses. Spironolactone was added for refractory hypokalemia. At 3-month follow-up, she had no symptoms of hypocalcemia. Trousseau and Chvostek sign were absent. Her corrected serum calcium was 8.8 mg/dL, magnesium was 1.7 mg/dL, inorganic phosphorus was 5.0 mg/dL, and serum potassium was 3.8 meq/L (Fig. 2). She continues to receive oral calcium, calcitriol, oral magnesium oxide, and carbimazole. The serial serum calcium and phosphorous data are depicted in Figure 2. At follow-up, the free thyroxine T4 level was 1.4 ng/dL and thyrotopin was 0.02 mcIU/mL.

Case 2

Our second case is that of a 1-month-old boy, born out of consanguineous marriage, who presented to the pediatrics emergency with sudden onset of abnormal involuntary jerky movement of all 4 limbs associated with rolling up of eyeballs followed by unresponsiveness. He had a normal birth history. Two of his siblings had developmental delay. While his seizures were controlled with antiepileptics, a detailed metabolic panel was ordered, which revealed very low serum total and ionized calcium, a raised serum inorganic phosphorus. Serum parathyroid hormone (PTH) was low (Table 1). Focused exome sequencing revealed a homozygous mutation involving GCM2 (ENST0000379491.5) on chromosome 6, variant CM2 chr6:10876558_10877139insT located on exon1-2. The variant was reported as likely pathogenic. Intravenous infusion of calcium gluconate was administered at the rate of 500 mg/kg/day of calcium gluconate as a continuous infusion but without any improvement of symptoms. Simultaneously oral calcium (75 mg/kg/day) and calcitriol (0.25 mcg/kg/day) were also added. However, optimum serum calcium could not be maintained mainly due to the failure of maintaining the intravenous line. Permission for the central line was declined. A trial of twice daily subcutaneous teriparatide (off label) (1 mcg/Kg/day) was started. By day 3, serum calcium was normalized and hyperphosphatemia was corrected. Teriparatide was tapered after 3 weeks and omitted at 4 weeks when the patient was able to maintain the serum calcium level with oral calcium and calcitriol. At follow-up, the child is seizure free with oral calcium (75 mg/kg/day) and calcitriol (0.25 mcg/day).

Discussion

The GCM gene was originally identified in Drosophila melanogaster. The vertebrate glial cells missing transcription factors are a small family of unique proteins that are orthologous to those identified in drosophila [12]. Early in murine development, GCM2 is expressed in the pharyngeal endoderm of the third pouch, contributing to the formation of the parathyroid glands and the thymus [13]. GCM2 interacts with other transcription factors and is essential for parathyroid hormone production [14,15]. There was an association between GCM2 and the CASR and the development of the evolutionarily related parathyroid glands (in terrestrial vertebrates) and gills (in fish) [16]. GCM2 knockout mouse line studies have shown low or undetectable parathyroid hormone levels [17]. Investigators have established that GCM2 is not required for pouch patterning or to establish the parathyroid domain but is required for differentiation and subsequent survival of parathyroid cells [4].

Our case describing the neonate who presented with hypoparathyroidism soon after birth underscores the role of
GCM2 in early parathyroid gland development and differentiation. In this neonate, the deletion started at the 10876558th nucleotide position (p.M1; amino acid 1 to amino acid 115; p.115K) and the 10876559th nucleotide position, which is the splice acceptor region and is probably changing in the GCM2 protein at the junction of exon 1 and exon 2. The deletion is mapped up to 115th amino acid, (p.115K) amino acid. The splice acceptor site was deleted, which may lead to alternative splicing and loss of function in GCM2 protein. This case also demonstrates the utility of off-label short-term teriparatide to treat severe hypocalcaemia and hyperphosphatemia in a neonate with hypoparathyroidism.

GCM2 regulates CaSR and PTH gene expression in the mature glands [18,19]. These patients have no history of childhood hypocalcaemia or tetany and come to attention in adulthood for the first time. The disease has both autosomal recessive and dominant inheritance. GCM2 mutants are known to have a dominant negative effect on the CaSR, where they inhibit the transactivation of CaSR by the wild protein [5]. Canaff et al. identified functional glial cells missing response elements in each of the CASR promoter regions. In a study evaluating patients with idiopathic hypoparathyroidism for the prevalence of GCM2 mutations, only single nucleotide polymorphisms were found [7]. In a previous study, individuals with p.Asn502 variant showed a reduction in transactivation of CaSR. It was found in the heterozygous state in 1 patient who presented at the age of 5 years with hypocalcemia, hyperphosphatemia, hypomagnesemia, low 25-hydroxy vitamin D levels, and normal serum intact PTH levels and was then diagnosed as a case of GCM2 mutation.

Our adult case has all the classic presentations of hypoparathyroidism due to GCM2 mutation in the form of recurrent and refractory hypocalcaemia, hyperphosphatemia, hypomagnesemia, and tetany. CaSR at the distal renal tubules is responsible for calcium absorption and renin regulation. Bilateral basal ganglia calcification seen in this case due to hyperphosphatemia, which increases the deposition of calcium-phosphorous complex in the brain tissue. Another mechanism of basal ganglia calcification is the increased expression of osteogenic molecules like osteopontin, osteocalcin, and carbonic anhydrase-II in the caudate nucleus compared to grey matter in patients with hypoparathyroidism [20]. The frameshift duplication NM_004752.4 (GCM2): c.737dupA (p. Asp246Glufs*25) detected in this case has been reported previously neither as a pathogenic variant nor as a benign variant, to our knowledge. The p. Asp246Glufs*25 variant is novel (not in any individuals) in the Genome Aggregation Database. The p. Asp246Glufs*25 variant is novel (not in any individuals) in 1kG. This variant is predicted to cause loss of normal protein function through protein truncation caused by a frameshift mutation. The frame-shifted sequence continues through 25 residues until a stop codon is reached. This variant removes more than 10% of the protein. The p. Asp246Glufs*25 variant is a loss of function variant in the gene GCM2, which is intolerant of loss of function variants, as indicated by the presence of existing pathogenic loss of function variant NP_004743.1: p.Y136* [21]. For these reasons, this variant has been classified as likely pathogenic [22]. The association of Graves’ disease with this condition is also novel.

Conclusion

This report demonstrates the critical role of GCM2 activity in human parathyroid gland development through clinical and genetic analysis of 2 patients with hypoparathyroidism. These findings have more immediate relevance for the diagnosis and treatment of cases having isolated hypoparathyroidism.

Conflict of Interest

None declared.

Data Availability

The original contributions presented in the article are included. Further inquiries can be directed to the corresponding author.

Consent From Patient/Parents

Consent was granted.

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