Neonatal severe hyperparathyroidism secondary to a novel homozygous CASR gene mutation

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Summary

Neonatal severe hyperparathyroidism (NSHPT) is a rare autosomal recessive disease. Children present within the first 6 months of life and more commonly in the first few weeks. Common presentation is poor feeding, polyuria, dehydration, lethargy, failure to thrive, hypotonia, gastrointestinal dysmotility, osteopenia and symptoms of respiratory distress due to a poorly developed chest cage. We present a case of a 2-month-old girl with severe hypercalcemia and hyperparathyroidism. She was found to have a novel homozygous mutation in the acceptor splicing site of intron 4 (c.1378-2A>G) of the calcium sensing receptor gene (CASR). This mutation causes a frame shift deletion of exon 5 and insensitivity of CASR to calcium. The patient was treated with intravenous fluids, frusemide, calcitonin, intravenous pamidronate and oral cinacalcet. She did not respond to medical treatment. Parathyroid gland imaging including ultrasound, MRI and sestamibi nuclear scan were not helpful in localizing the glands. Her symptoms resolved following total parathyroidectomy. She is being treated with alfalcacidol and calcium supplements to maintain normal serum calcium and phosphate. She achieved her normal developmental milestones.

KEY WORDS: neonatal severe hyperparathyroidism; calcium sensing receptor; parathyroidectomy.

Introduction

Calcium sensing receptors (CASR) are the major sensors of serum calcium level and have a critical role in maintaining normal calcium homeostasis. CASR are present in various tissues of the body but they are largely expressed with well-defined function in parathyroid chief cells and in the epithelial lining of renal tubules. It is a plasma membrane G coupled receptor with seven transmembrane domains. The serum calcium set point causes activation or inactivation of CASR. Through this receptor, serum calcium higher than the set point would inhibit the parathyroid hormone release from chief cells and prevents renal tubular reabsorption of calcium; serum calcium lower than the set point would trigger parathyroid hormone release and reabsorption of calcium in renal tubules (1).

Various genetic mutations in CASR cause different phenotypes. Mutations with loss of function as well as gain of function have been described. CASR gene (OMIM#601199) is located at chromosome 3q13.3-q21.1. Loss of function mutations may result in any of two recognized phenotypes; familial hypocalciuric hypercalcemia (FHH) or severe neonatal hyperparathyroidism (NSHPT).

Neonatal hyperparathyroidism presents early in infancy, usually within the first six months of life although the majority present in the first few weeks. The young babies present with signs of hypercalcemia and hyperparathyroidism which include poor feeding, polyuria, dehydration, lethargy, failure to thrive, hypotonia, gastrointestinal dysmotility, osteopenia and symptoms of respiratory distress due to a poorly developed chest cage (2).

Early diagnosis and treatment for NSHPT is critical; delay in diagnosis and treatment is associated with high morbidity and mortality and devastating neurodevelopmental outcome. General treatment strategies for hypercalcemia like intravenous hydration with forced diuresis by frusemide and use of calcitonin may provide some transient improvement but this effect is usually minimal in homozygous cases. Other medical treatment options are intravenous bisphosphonate therapy and calcimimetics e.g. cinacalcet which are reported to be effective in heterozygous cases. Children not responding to medical treatment require an early parathyroidectomy (3, 4).

In this report, we present a case of a Saudi female baby born to consanguineous parents with neonatal severe hyperparathyroidism presenting in the first month of life; DNA analysis revealed a novel homozygous mutation in the CASR gene.

Case report

A two-month-old girl presented with a history of failure to thrive, poor feeding, and lethargy. She was born to first degree consanguineous parents at term via caesarean section with a birth weight of 2.4 kg (-5.6 SDS). She was presented to local hospital with tachycardia, tachypnea, prolonged capillary refill time and hypotonia. The full septic screen including cerebral spinal fluid examination was negative. Laboratory workup revealed an incidental finding of hypercalcemia;
5.6 mmol/L (2.10-2.55), hypophosphatemia; 0.9 mmol/L (1.00-2.00) and elevated alkaline phosphatase; 940 U/L (142-335). Parathyroid hormone was elevated; 1451.2 ng/L (10-65), and 25 hydroxy vitamin D was within the normal range 82 nmol/L (75-200). She was treated with intravenous fluids, furosemide and subcutaneous calcitonin; her calcium level improved transiently but then remained significantly high on above treatment. At the age of two months she was transferred to our hospital; the laboratory workup on admission showed calcium; >7 mmol/L (2.10-2.55), phosphate; 0.76 mmol/L (1.00-2.00), alkaline phosphatase; 955 U/L(142-335), parathyroid hormone; 1414 ng/L (10-65), 25 hydroxy vitamin D; 78 nmol/L (75-200), and 1.25 hydroxy vitamin D;133 pg/ml (24-86). Urinary calcium in 24 hours collected sample was 0.68 mmol/L (2.5-8.00). She had a clinical diagnosis of severe neonatal hyperparathyroidism.

She was started on medical treatment to achieve normal calcium level. Intravenous fluid of double maintenance volume along with furosemide 1 mg/kg twice a day for forced diuresis and calcitonin 10 units every eight hours by subcutaneous injections. She was also started on calcimimetic drug cinacalcet 5 mg twice a day (4 mg/kg/day); the serum calcium improved but remained > 3 mmol/L (2.10-2.55). Medical treatment was intensified by the addition of intravenous pamidronate therapy initially at a dose of 0.5 mg/kg then increased to 1 mg/kg. Despite the addition of pamidronate serum calcium was 2.7-3.0 mmol/L.

The radiological workup including neck ultrasound, MRI and sestamibi nuclear scan were performed to localize parathyroid glands. Sestamibi nuclear scan showed increased uptake at the base of tongue and partial washout suggestive of ectopic parathyroid gland (Figure 1); these findings were not present on US and MRI and both examinations could not localize the parathyroid gland.

The patient had first exploratory surgery during which multiple frozen sections per-operatively were taken but parathyroid gland could not be identified. She had total thymectomy assuming parathyroid glands are embedded in thymus. Parathyroid hormone level dropped from 1250 ng/L pre-operatively to 840 per-operatively. There was transient hypocalcemia on the first post-operative day but then calcium level climbed back to > 3 mmol/L. She was recommenced on medical treatment but hypercalcemia was not controlled despite parathyroid hormone level remained lower than the pre-operative level (500-600 ng/L). She had second surgery and parathyroid glands were identified per-operatively within thyroid gland (normal anatomy contrast to sublingual as suggested by sestamibi scan) and verified with frozen section. Total parathyroidectomy was carried out without any auto-transplantation. Parathyroid hormone dropped from 598 to 18.4 on first post-operative day and then to 9.6 ng/L. There was significant hypocalcemia (1.7 mmol/L) after surgery which was managed with intravenous calcium initially and then with oral alfacalcidol.

At the age of 18 months she is maintaining a normal serum calcium, moderately high phosphate and normal alkaline phosphatase with alfacalcidol and calcium supplements. Parathyroid hormone, alkaline phosphatase, serum calcium and phosphate levels during the course of treatment are shown in Figures 2, 3. She achieved appropriate motor milestones at one year of age. Genetic analysis

Genomic DNA was extracted from 5 cc blood collected in EDTA containing tubes using the Gentra Puregene blood kit (Catalog#158389, Qaigen, Valencia, CA, USA) according to the manufacturer’s instructions. All exons and exon-intron boundaries of the underlying genes were amplified by polymerase chain reaction (PCR) using primers that have been previously published. Successfully amplified products were resolved on 2% agarose gel and directly sequenced using the Dideoxy Termination Method (ABI PRISM Big-Dye Terminator V3.1 Cycle sequencing Reaction kit; Catalogue # 4337455, Applied Biosystems, Foster City, CA 94404, USA).
A homozygous mutation was detected at the splicing acceptor site in intron 4 (c.1378-2A>G). This mutation has not been reported in the literature. This mutation causes a frame shift deletion of exon 5 (Figure 4) and insensitivity of CASR to calcium (5).

**Discussion**

We presented a case of a three-months-old girl with severe neonatal hyperparathyroidism secondary to a novel homozygous mutation in CASR encoding gene. Calcium sensing receptor is comprised of 1078 amino acids protein. It is composed of three regions; a large extracellular N-terminal domain, central 7 transmembrane domains and intracellular C-terminal domain. Extracellular domain (ECD) consists of a signal peptide, Venus fly trap (VFT) and cysteine rich domain. A small linker region connects ECD to seven transmembrane domains and intracellular tail. CASR activation involves the activation of phospholipase C via G protein coupled with receptor. Seven transmembrane domains are mainly involved in the transaction of signal from ECD to its respective G protein. The human CASR gene maps to 3q13.3-21. Heterozygous and homozygous mutations in CASR gene causing loss of function result in familial hypocalciuric hypercalcemia or neonatal hyperparathyroidism (6).

Severe neonatal hyperparathyroidism is mostly treated with total parathyroidectomy as medical treatment is not effective. Medical management should start with restoration of extravascular volume; it can be followed by diuretics. Subcuta-
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Neonous calcitonin injections can also give some short term improvement in serum calcium. Bisphosphonates reduce osteoclast activity by limiting its recruitment and by shortening its life span. Bisphosphonates should be considered as second line treatment as halting the bone resorption would help in managing the milder case and to stabilize the severe cases for surgery. The main short term side effect is hypocalcemia but as it has long half-life in skeletal tissue it may have detrimental side effects on bone health by interference in bone turnover process (7). Calcimimetics increase the sensitivity of CASR; calcium binds to extracellular domain while calcimimetics bind to seven transmembrane domains increasing receptor sensitivity (8). Calcimimetics are reported to be effective in heterozygous mutation causing neonatal hyperparathyroidism (NHPT); 4 reported cases did not require parathyroidectomy (9, 10). Homozygous mutations causing NSHPT are mostly non-responsive to cinacalcet as mutation affecting the seven transmembrane domains region. Localization of parathyroid gland pre-operatively can influence the surgical approach and facilitate the work of surgeon to identify the glands per-operatively. Different imaging modalities are used including ultrasound, sestamibi nuclear scan and MRI. Radiological workup has shown good specificity and specificity in adults as well as in children with sporadic hyperparathyroidism in identifying solitary and multiple nodules. These imaging studies do not usually help in children with neonatal and familial hyperparathyroidism. Al-Shanafey et al. published series of five cases with NSHPT; all patients had ultrasound, CT scan and sestamibi nuclear scan but these studies could not identify the parathyroid glands in any of them (11).

There are different surgical options to treat NSHPT including total parathyroidectomy, sub-total parathyroidectomy and total parathyroidectomy with autotransplantation. Sub-total parathyroidectomy used to be procedure of choice but because of high recurrence rate it is not recommended any more. Autotransplantation in NSHPT cases carries a risk of persistent or recurrent hyperparathyroidism. There is also limited success reported for autotransplantation. Al-Shanafey et al. published five cases with total parathyroidectomy and autotransplantation in the anterior aspect of forearm; three required treatment for hypocalcemia immediately after surgery, one remained eucalcemic for two years then commenced on vitamin D analogue and calcium supplements and one remained eucalcemic after nine years follow-up (11). Alagaratnam et al. reported five cases with total parathyroidectomy requiring hypocalcemia treatment after surgery and one who had 3 ½ glands removed remain mildly hypercalcemic with elevated parathyroid hormone although doesn’t require treatment being asymptomatic (12).

In summary NSHPT is a rare and challenging disorder, which requires a dynamic management strategy. All cases should be treated medically initially to see the response of bisphosphonates and calcimimetics and to stabilize serum calcium for surgery. A homozygous mutation in the CASR gene would cause NSHPT which is usually non-responsive to medical treatment but heterozygous mutations causing NHPT may be managed with medical therapy. NSHPT which is non-responsive to medical treatment should be managed by total parathyroidectomy with or without autotransplantation. Post-operative hypocalcemia can be treated with vitamin D analogues and calcium supplements.

Figure 4 - Part of the sequence chromatogram of intron 4/exon 5 boundary showing a splicing site mutation (arrow) in intron 4 (c.1378 -2 A>G). This mutation causes failure of splicing of exon 4 to exon 5 with skipping of exon 5 and loss of CASR sensitivity to calcium.
References


