A novel homozygous mutation in the CASR gene in a neonate with severe primary hyperparathyroidism: A case report.

King Fahd Armed Forces Hospital, Jeddah – Saudi Arabia
Adress for correspondence : Ali Alqadi, E-mail : alqadi_1425@yahoo.com

OBJECTIVE
Is to report clinical and genetic findings in Saudi baby boy with NSHPT due to a novel homozygous mutation in the CASR transmitted as an autosomal recessive trait that has never been described and to run over the monitoring of his biochemical profile throughout the clinical course.

INTRODUCTION
Neonatal severe primary hyperparathyroidism (NSHPT, MIM239200) is a potentially lethal autosomal recessive disorder characterized by severe hypercalcemia, markedly elevated serum PTH levels and skeletal abnormalities that include multiple fractures, demineralization and erosions[1]. Children present with failure to thrive, poor feeding, lethargy and respiratory distress at any time in the first six months of life, however, symptoms often manifest in the first few days after birth. NSHPT is often refractory to medical therapy and requires surgical treatment with subtotal parathyroidectomy or total parathyroidectomy with autotransplantation[1,2,3,11]. Prompt diagnosis and early appropriate intervention for NSHPT is of utmost importance in order to avoid long term consequences caused by bone loss and function impairment in the CASR gene that encodes the calcium sensing receptor (CaSR)[4,5,6,7]. It is located at chromosome 3q13.3-q21.1 as it spans over 50 kb of genomic DNA and has a coding region of 3234 bp, which is contained within 6 exons (7,8). About 200 Mutations have been previously described in the CASR gene can result in gain or loss of function receptor. Gain of function mutations are associated with autosomal dominant hypocalciuric hyperparathyroidism while loss of function mutations are associated to two recognized phenotypes; familial hypocalciuric hyperparathyroidism (FHH) and neonatal severe primary hyperparathyroidism (NSHPT)[6,9,13,15]. The calcium-sensing receptor (Casr) is a G-protein coupled receptor (GPCR) family member that adjusts the extracellular calcium set point regulating PTH secretion and renal calcium excretion. CASR is expressed in various tissues but mostly in the parathyroid chief cells and in epithelial lining of renal tubules (5,6).

SUBJECTS AND METHODS
A 3200 grams–male newborn with 39 weeks gestation was born to first degree consanguineous Saudi parents by normal vaginal delivery after eventless pregnancy. On the postnatal 10th day, he was hospitalized via our pediatric emergency room for poor feeding, vomiting and failure to thrive, requiring hospitalization for poor feeding, respiratory distress, and electrolyte disturbance. The newborn was delivered at term by spontaneous labor rupture of membranes, with a birth weight of 3100 g, length of 49 cm, and Apgar score 10. He was intubated and ventilated for apnea of respiration. He Fed normally, had an initial serum calcium of 6.9 mg/dl, phosphate of 3.3mg/dl, and alkaline phosphatase (ALP) of 131 U/L. Neonatal primary hyperparathyroidism (hypercalcemia, polyuria, polydipsia, hypocalciuria, and failure to thrive) was the clinical suspicion. Total parathyroid hormone (PTH) level was >600 pg/ml, alkaline phosphatase was 1300 U/L, with a total serum calcium of 11.1 mg/dl. He suspected the diagnosis of NSHPT. The newborn was put on a diet rich in calcium, and parenteral calcium infusion with frequent monitoring of lab tests. He was initially on 250mg of calcium daily, with gradual weaning to 200mg/day. The serum calcium level was still high. We, hereby, report the identification of a novel homozygous loss of function mutation in the CASR gene G652SV in this Saudi neonate with severe hypercalcemia which has never been described before. Unaffected parents were heterozygous carrier. Functional studies are needed to examine the role of this mutation in CASR activity.

RESULTS
A sequence variant in the CASR was identified (G>T) point mutation at nucleotide c. 2084 in exon 7 (c. 2084 G>T) resulting in the replacement of Glycine at codon 685. The newborn was not consanguineous, and the parents were not on treatment for hyperparathyroidism. Both parents have signed informed consent and protocol was approved by the research ethics committee at King Fahd armed forces hospital. Genomic DNA was extracted from peripheral white blood cells for the patient and his parents, 5 cc of blood collected from each one in EDTA tubes using the standard methods and sent to Germany. The gene regions of interest were captured with truSight (illumina) kit. High throughput sequencing analysis restricted to CASR (NM_00137805.1) through Illumina测序仪 of reads: 102/-3, 33 coverage, 100% PCR amplification and bidirectional sequencing analysis of the exon 7 of CASR gene was undertaken. This analysis technique can not detect mutations in the promoter repeats and large deletions or duplications.

Table 1

<table>
<thead>
<tr>
<th>Test</th>
<th>Reference values</th>
<th>On admission</th>
<th>2 weeks post medical treatment (Day of D0)</th>
<th>2 months post parathyroidectomy</th>
<th>6 months post parathyroidectomy</th>
<th>18 months post parathyroidectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>2.1 - 2.7 mmol/L</td>
<td>5.44</td>
<td>3.11</td>
<td>2.6</td>
<td>2.33</td>
<td>2.34</td>
</tr>
<tr>
<td>PnO4</td>
<td>1.45 - 2.1 mmol/L</td>
<td>0.76</td>
<td>1.28</td>
<td>2.1</td>
<td>1.98</td>
<td></td>
</tr>
<tr>
<td>PTH</td>
<td>1.6 - 6.5 mmol/L</td>
<td>55.78</td>
<td>161.2</td>
<td>0.72</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>ALP</td>
<td>70 - 250 U/L</td>
<td>279</td>
<td>125</td>
<td>198</td>
<td>131</td>
<td></td>
</tr>
<tr>
<td>25- OH vitamin D</td>
<td>75 - 250 mmol/L</td>
<td>59.3</td>
<td>74.2</td>
<td>79.2</td>
<td>79.1</td>
<td>90.6</td>
</tr>
</tbody>
</table>

CONCLUSION
We, hereby, report the identification of a novel homozygous loss of function mutation in the CASR gene G652SV in this Saudi neonate with severe hypercalcemia which has never been described before. Unaffected parents were heterozygous carrier. Functional studies are needed to examine the role of this mutation in CASR activity.

References

A sequence variant in the CASR was identified (G>T) point mutation at nucleotide c. 2084 in exon 7 (c. 2084 G>T) resulting in the replacement of Glycine at codon 685. The newborn was not consanguineous, and the parents were not on treatment for hyperparathyroidism. Both parents have signed informed consent and protocol was approved by the research ethics committee at King Fahd armed forces hospital. Genomic DNA was extracted from peripheral white blood cells for the patient and his parents, 5 cc of blood collected from each one in EDTA tubes using the standard methods and sent to Germany. The gene regions of interest were captured with truSight (illumina) kit. High throughput sequencing analysis restricted to CASR (NM_00137805.1) through Illumina测序仪 of reads: 102/-3, 33 coverage, 100% PCR amplification and bidirectional sequencing analysis of the exon 7 of CASR gene was undertaken. This analysis technique can not detect mutations in the promoter repeats and large deletions or duplications.