CASE REPORT

Carbonic Anhydrase II Deficiency: A Novel Mutation

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Carbonic anhydrase II (CA II) deficiency is an extremely rare autosomal recessive disorder, characterised by a triad of osteopetrosis, renal tubular acidosis and cerebral calcifications. A 12-year-old boy with classical features of CA II deficiency is reported who was found to be homozygous for the mutation in CA II gene and parents were heterozygous for the same mutation .To the best of our knowledge this is the first case report of mutation proven CA II deficiency from India.

Key words: *Carbonic anhydrase II deficiency, Intracranial calcifications, Osteopetrosis, Renal tubular acidosis.*

Carbonic anhydrase II (CA II) deficiency is a rare autosomal recessive disorder leading to the development of osteopetrosis, renal tubular acidosis (RTA) and intracranial calcification.

CASE REPORT

A 12-year-old boy who was diagnosed as having osteopetrosis at 8 months of age was brought for evaluation. The child was receiving low dose corticosteroid therapy from 8 months of age. At the age of 8 ½ years he was found to have short stature. Steroids were stopped and detailed evaluation revealed renal tubular acidosis. Other hormonal causes of short stature were ruled out. He was started on oral potassium citrate and was periodically evaluated.

Presently the parents had brought him to the Genetics department as they were planning another baby. The couple had a younger girl child who had increased bone density and died at the age of 5 years following drowning. On evaluation at 12 year, he was studying in 5th standard and has scholastic backwardness. His weight was 19.5 kg, height 116.5 cm and head circumference 49 cm (all were below third centile). He did not have any dysmorphic features apart from overcrowded teeth (***Fig*.1**).

Skeletal survey showed hyperdensity of bones compatible with osteopetrosis with flared metaphyses of femurs (***Fig.* 2a**). Ophthalmologic evaluation showed bilateral posterior subcapsular cataracts, secondary to prolonged steroid therapy. Fundus evaluation and audiogram were normal. His laboratory results showed metabolic acidosis (pH 7.32, bicarbonate 16.3 mmol/L, sodium 138 mmol/L, potassium 3.9 mmol/L, chloride 110 mmol/L, urine pH 6.3, anion gap 11.7, creatinine 0.7 mg/dL). All features were consistent with classical renal tubular acidosis. Ammonium chloride loading test failed to reduce the urine pH to less than 6 suggesting distal RTA. Hematological work up showed hemoglobin of 12.9 g/dL, WBC 8650/cc, platelet count of 2,79000/cc with a normocytic normochromic picture in the peripheral smear.

A combination of osteopetrosis with distal renal tubular acidosis raised the possibility of carbonic anhydrase II deficiency. Acid phosphatase was found to be elevated 7 U/L (normal up to 5.1). Abdominal ultrasound scan was normal whereas brain CT scan showed calcification of both caudate nuclei and left

putamen (***Fig.2b***). With these evaluations the diagnosis of Carbonic anhydrase II deficiency was confirmed. DNA was extracted from peripheral blood samples of the proband and parents. Genomic DNA of the patient was then PCR amplified for each of seven exons of the CA2 gene and analysed by direct nucleotide sequencing. A novel T85C substitution that causes the S29P (serine to proline) amino acid change was identified (***Fig.3***). This missense mutation in exon 2 had destroyed a Bsr1 restriction site in the mutant amplicon. Using this restriction enzyme, the parents were checked for this novel mutation and they were found to be heterozygous. Treatment is being continued with potassium citrate and the biochemical parameters and growth is being monitored periodically. Mother is now 8 weeks pregnant and we are planning to offer prenatal diagnosis for this couple.

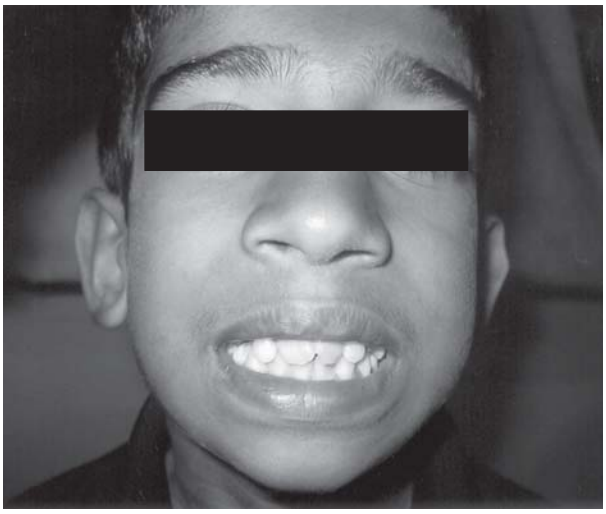

FIG. 1 *Proband with over crowded teeth.*

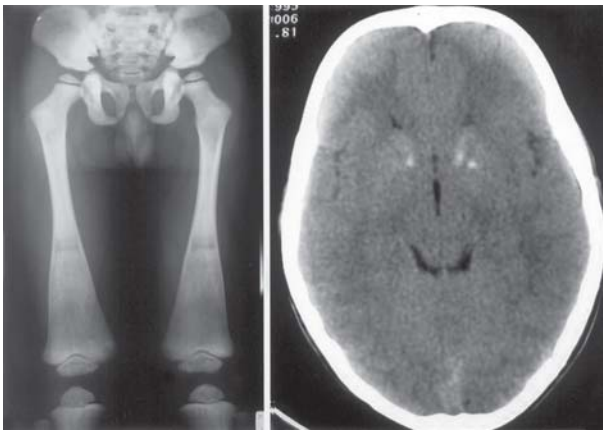

FIG. 2*a* *Osteopetrosis of the femur with flared metaphysis at the lower end.*

FIG. 2*b* *CT scan of brain showing bilateral basal ganglia calcifications.*

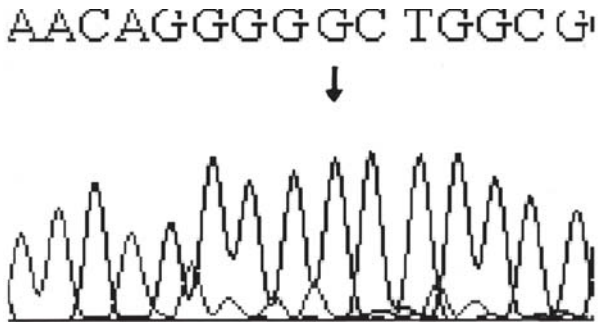

FIG. 3 *Reverse sequencing showing T>C substitution at position 85 in the exon 2 in genomic DNA.*

DISCUSSION

The clinical course of CA II deficiency is benign and this condition is compatible with long-term survival and hematological abnormalities associated with lethal form of osteopetrosis are absent in CA II deficiency(1). This condition is called as marble brain disease as these patients present with osteopetrosis and cerebral calcifications(2). Carbonic anhydrase gene has been mapped on chromosome 8q 22. Carbonic anhydrase is a zinc metalloenzyme and catalyses reversible hydration of CO_2. CA II has a wide tissue distribution and is found in bone, kidney (proximal tubule and collecting duct), erythrocytes, glial cells and osteoclasts(3). The facial features of CAII deficient patients include broad forehead, small mouth, delayed dentition and dental malocclusion(1). These patients have failure to thrive and growth retardation as universal features. Around 50% of the patients have mild mental retardation.

CAII is involved in the proximal renal tubular bicarbonate reabsorption and distal renal tubular acidification. Hence the patients with CA II deficiency have characteristics of both proximal and distal renal tubular acidosis(4). There is increased tendency for nephrocalcinosis in patients who have predominant distal RTA. Deficiency of CA II impairs the production of H^+ ions by osteoclasts thereby

blocking the resorption of bones and leads to development of osteopetrosis(3). As the balance between osteoclast and osteoblast activity is disrupted the excessive accumulation of brittle bone leads to osteopetrosis and increased risk for fractures. All patients have increased density of axial skeleton, long bones and skull along with widening of metaphysis of long bones. These patients have normal levels of calcium and phosphorus, mildly elevated alkaline phosphatase and acid phosphatase.

Intracranial calcifications in CAII deficiency are not usually present at birth and they usually develop by 2-5 years of age. Calcifications are seen in basal ganglia and also in cortex. Basal ganglia calcifications primarily appear in putamen and caudate nucleus. Calcification of cortex initially involves frontal lobes(5). The reason for intracranial calcification is still unclear. Cranial nerve entrapments leading to blindness and deafness are rare in CA II deficiency(1). Restrictive lung disease has also been observed in few patients. Hence periodic pulmonary function tests can detect these complications early(6).

There is no role for corticosteroids in CAII deficiency due to relative lack of hematological complication. Allogenic bone marrow transplantation has a role in the attenuation of osteopetrosis and cerebral calcification but has no effect on renal tubular acidosis and mental retardation(1,3,7).

Early institution of alkali supplementation is the sheet anchor in the management of renal tubular acidosis as RTA is the most likely cause for growth retardation.The patients who were diagnosed and treated with sodium bicarbonate before the age of 4 years have attained normal adult height(1). CA II deficiency should be considered in children presenting with osteopetrosis without anemia and thrombocytopenia so that long-term steroid therapy can be avoided. Prenatal diagnosis could be offered either from cultured amniocytes or from chorionic villus biopsy by direct sequencing provided the mutation in the proband is identified beforehand(8, 9).

Contributors: SN diagnosed the condition in the index case, evaluated the family tree and drafted the article. She will act as the guarantor of the manuscript. YA has done the molecular genetic analysis for the family for confirmation of the diagnosis and has contributed to the drafting.

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