Report of Another Mutation Proven Case of Carbonic Anhydrase II Deficiency

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Abstract

Carbonic anhydrase (CA) II deficiency results in an uncommon type of autosomal recessive sclerosing bone dysplasia with renal tubular acidosis and intracerebral calcification. We report a classic case of CA II-associated osteopetrosis with a previously reported homozygous frameshift mutation. Child was evaluated for short stature and failure to thrive. He was diagnosed as osteopetrosis in view of the presence of hepatosplenomegaly and increased bone density though hematological parameters were normal. Further evaluation showed presence of associated distal renal tubular acidosis raising a possibility of CA II deficiency. Mutation analysis revealed a previously reported homozygous frameshift mutation c.143-146delCTGT (p.Ser48Phefs*9) in *CA2*.Child has normal growth after initiation of alkali therapy.

Keywords
- osteopetrosis
- renal tubular acidosis
- CA II deficiency

Introduction

Carbonic anhydrase II (CA II; MIM No.259730) deficiency is a rare autosomal recessive disorder due to pathogenic variants in the CA gene, *CA2*, at chromosome locus 8q22. It manifests with osteopetrosis, renal tubular acidosis (RTA), and intracranial calcification with mild hematological involvement. Unlike infantile malignant osteopetrosis, most patients have indolent course and are diagnosed after infancy. There are other milder autosomal dominant forms of osteopetrosis which are usually asymptomatic or present with multiple fractures. We report the third molecularly confirmed case of autosomal recessive form of osteopetrosis from India.

Case

A 2.5-year-old boy born full term to a third-degree consanguineous couple with birth weight of 1.75 kg without any adverse perinatal events presented with complaints of failure to thrive with motor delay. He attained head control at around 5 months, sitting at 10 months, and walking at 18 months. There was no history of any seizure, blood transfusion, and dystonia. Child had failure to thrive with weight 10.6 kg (at −2 standard deviation [SD]) and height 86 cm (at −2 SD). Physical examination revealed mild pallor with hepatosplenomegaly and bilateral nystagmoid movements. Fundus examination revealed bilateral disc pallor. Rest of the examination was unremarkable. Laboratory investigation revealed hemoglobin of 10.2 g/dL with normal total leukocyte (11.3×10^3/µL) and platelet count (293×10^3/µL). Peripheral smear was suggestive of normocytic normochromic anemia without any evidence of any atypical cells. Visual evoked potential (VER) showed bilaterally extinguished response. Skeletal survey showed increased bone density of long bones and vertebrae suggestive of osteopetrosis (►**Fig. 1A**). Bone marrow biopsy revealed histological features consistent with osteopetrosis. He was detected to have concomitant hypothyroidism and initiated on thyroxin therapy.

On follow-up at 2.5 years of age, child developed normal anion gap metabolic acidosis though there was no history of polyuria or any features of rickets (►**Table 1**). Urine pH was > 5.5 without any evidence of glucosuria or proteinuria, suggesting distal renal tubular acidosis. Urine to blood PCO_2 gradient also favored the diagnosis of distal RTA which was calculated after normalization of serum bicarbonate with sodaBicarb loading. Child had persistent hyperphosphatemia

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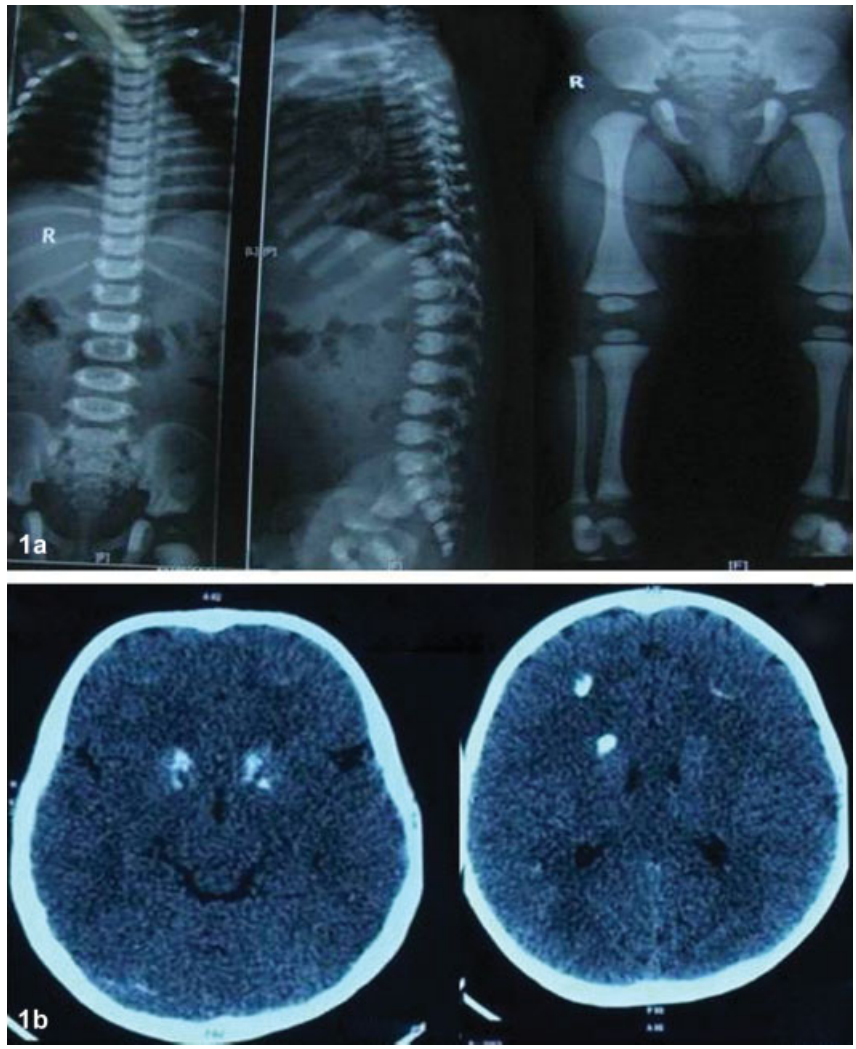

Fig. 1 (**A**) Limited skeletal survey showing increased bone density at long bones and vertebra. (**B**) NCCT scan of the brain showing intracranial calcification. NCCT, Nnoncontrast computer tomography.

(5.8 mg/dL) with normal serum parathyroid hormone level (27.7 pg/mL) and (25-OH) Vitamin D (54.9 ng/mL); however, there was no evidence of nephrocalcinosis on kidney ultrasound. Noncontrast computer tomography (NCCT) of brain revealed the presence of intracerebral calcification (►**Fig. 1B**).The details of various biochemical parameters, pre and post-alkali therapy are given in► **Table 1**.

Table 1 Parameters at baseline and after treatment

Parameters	Baseline	After treatment
Blood pH	7.27	7.36
S. bicarbonate (meq/L)	14.8	19.9
S. sodium (meq/L)	131	139
S. potassium (meq/L)	3.2	4.0
S. calcium (mg/dl)	8.9	9.2
S. chloride (meq/L)	106	104
S. alkalinephosphatase (IU)	364	460
S. creatinine (mg/dL)	0.3	0.3
Urine pH	7.2	7.6

In presence of osteopetrosis associated with renal tubular acidosis, a clinical possibility of CA II deficiency was presumed. After obtaining informed consent, a mutation analysis was performed to confirm the diagnosis. Child was initiated on bicarbonate supplement at a dose 2 meq/kg/day. Targeted next generation sequencing showed the presence of a previously reported homozygous four base pair deletion, c.143_146delCTGT, resulting in a frameshift mutation (p.Ser48Phefs*9) in exon 2 (ENST00000520127.5) of the *CA2* gene. Both the parents were found to be heterozygous for the same deletion in Sanger's sequencing (►**Fig. 2**). A confirmed molecular diagnosis in the proband helped in providing prenatal diagnosis in the subsequent pregnancy.

Currently, the child is 4 years old with weight 14.2 kg (0–2 SD) and height of 98 cm (0–2 SD) and has serum bicarbonate of 19.9 meq/L.

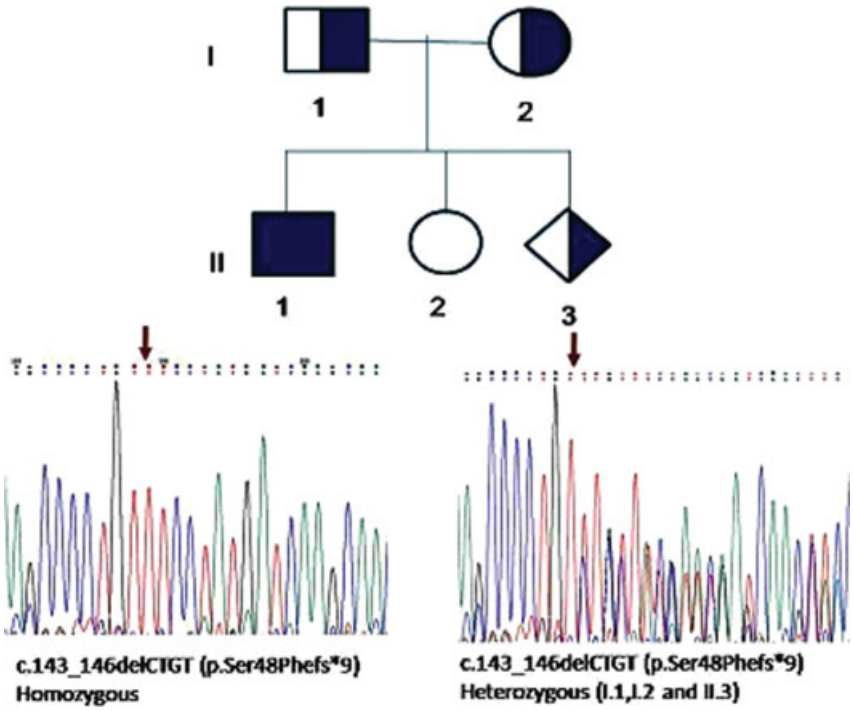

Fig. 2 Pedigree with electropherogram showing the 4-bp homozygous deletion (c.143_146delCTGT) in the proband and heterozygous deletion in father(I-1), mother (I-2), and fetus (II-3).

Discussion

CA is a zinc containing metalloproteinase involved in the reversible conversion of carbon dioxide and bicarbonate with release of H^+ ions into blood and other tissues in the body.[1] CA II is one of the most enzymatically active forms with a single α-CA domain (http://bioinf.umbc.edu/dmdm/) and distributed widely in red blood cells (RBCs), bone, kidney, and brain. It helps in maintaining the acid–base neutrality in blood and tissues as well. It provides the acidic environment in the bone matrix by producing H^+ ions by osteoclasts and thus helps in bone resorption. Deficiency of CA II impairs bone resorption, causing disruption of the balance between osteoblast and osteoclast function resulting in osteopetrosis.[2] CA II also plays a major role in acidification of urine in the distal collecting duct, lack of which results in distal RTA though both proximal and distal can occur.[3]

Cranial nerve involvement is rare and occurs secondary to entrapment of nerves resulting in vision loss and sensorineural hearing loss. Developmental delay is variable in these children. Arabic and Japanese populations show severe developmental delay, whereas normal or only mildly abnormal development has been reported in Americans.[4,5] Severe hematologic involvement including anemia, leucopenia, and thrombocytopenia is rare in osteopetrosis with CA II deficiency.[6] Intracranial calcifications are reported to be an uncommon manifestation of CA II deficiency and usually appear between 2 and 5 years of age. Basal ganglia, precisely caudate and putamen, is the common site of calcification; however, an involvement of cortex is not unusual.[7] The cause of calcification is still unclear. The three important differential diagnoses for osteopetrosis and intracranial calcifications include Sanjad–Sakati syndrome, Kenny–Caffey syndrome, and Kirk–Richardson syndrome. ►**Table 2** shows the important differentiating features between these disorders. Our patient had an initial diagnosis of a milder form of osteopetrosis in view of failure to thrive, hepatosplenomegaly, classical radiological findings but without significant hematological manifestations. Development of renal tubular acidosis in follow-up and presence of intracranial calcification helped in arriving at a final diagnosis.

A total of 29 mutations have been identified yet in the *CA2* gene involving all the seven exons and four introns.[8] Patients with missense, splice site, nonsense, and frameshift mutations, resulting in loss of function of CAII have been described.[8,9] In contrast to other Indian studies,[10,11] our case had a previously reported, four base pair deletion, resulting in a frameshift. The frameshift mutations are known to be associated with severe phenotypes.[12]

Alkali therapy is the mainstay of treatment in children with RTA. Early correction of acidosis ensures normal growth and attainment of final height in children with osteopetrosis and RTA.[6]Our patient was put on alkali therapy and showed improvement in acidosis and growth. Corticosteroids have a limited role as hematological involvement is rare. Bone marrow transplantation has a role in attenuation of osteopetrosis and cerebral calcification but it has no effect of renal tubular acidosis.[13]

This case highlights the importance of excluding RTA in a toddler presenting with osteopetrosis to exclude CA deficiency. Molecular testing helps in confirmation of diagnosis but also helps in the management and prenatal counseling in subsequent pregnancy.

Table 2 Differentials for osteopetrosis with intracerebral calcification

Disease	Sanjad–Sakati syndrome	Kenny–Caffey syndrome	CA II
Dysmorphism	• Deep-set eyes • Depressed nasal bridge • Beaked nose • Micrognathia • Long philtrum • Microcephaly • Thin lips • Large floppy ears • Teeth anomalies • Medullary stenosis • Short stature	• Growth retardation (postnatal) • Normal intelligence • Small hands and feet • Microphthalmia • Corneal opacity • Myopia/hyperopia • Optic atrophy • Macular clouding, • Macrocephaly • Cortical thickening and medullary stenosis of the long bones	• Increased bone density, diffuse and focal sclerosis of varying severity • Modeling defects at metaphyses • Increased frequency of fractures • Short stature • Dental abnormalities • Cranial nerve compression • Developmental delay • Bone marrow impairment rare
Biochemical Abnormality	• Hypocalcemia hyperphosphatemia • Low PTH levels	• Hypocalcaemia • Hyperphosphatemia • Low PTH	• Normal calcium • Metabolic acidosis ± Hypokalemia
Molecular abnormality	*TBCE* gene	*FAM111A* gene	*CA II* gene
Inheritance	AR	AD	AR

Abbreviations: AD, autosomal dominant; AR, autosomal recessive. CA, carbonic anhydrase; PTH, parathyroid.

Authors' Contribution

S.P. and A.K.S. managed the case. A.K.S., S.P., and N.G. prepared the manuscript. K.U. helped in molecular diagnosis and analysis. M.R.C. and N.G. were involved in prenatal diagnosis. M.K., N.G., and A.B. critically revised the manuscript and N.G. will act as guarantor.

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Conflict of Interest

None declared.

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