Brief Report

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Lack of Pathogenic Mutation in the Human CAII Gene in an Individual Suffering Renal Tubular Acidosis

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Renal tubular acidosis (RTA) is a rare genetic disorder. It has four clinical types, and type 3 demonstrates a mixed pattern of tubular dysfunction. The causative gene for type 3 RTA (CAII) is located on the 8q22 locus and encodes a protein called carbonic anhydrase II. In this study, we analyzed the entire exons and flanking regions of the CAII gene in a child suffering renal tubular acidosis with an autosomal recessive pattern that was diagnosed with type3 RTA. DNA was extracted from the blood sample of the patient and his parents by the salting out extraction method. The exons and flanking regions of the CAII gene were amplified using polymerase chain reaction (PCR). We performed exon direct sequencing by forward and reverse primers, which were designed by primer3 program. No mutation was found following the screening of the entire coding sequence of the CAII gene. It is likely that another gene might be involved in this case. In other words, other types of RTA have to be considered.

Keywords: Renal Tubular Acidosis, distal, type 3; Gene; Polymerase Chain Reaction; Sequence Analysis, DNA.

Running Title: Lack of pathogenic Mutation in CAII Gene in RTA

Introduction

The renal tubular acidosis (RTA) syndromes encompass a various group of tubular transportation flaws that have in common the disability to excrete hydrogen ions (H^+), a defect that is disproportionately large in relation to any decrease in the glomerular filtration rate [1]. As a result of this inability, acid excretion in the form of ammonium ($NH4^+$) ion and titratable acids or reabsorbption of some of the filtered bicarbonate ($HCO3^-$) load are disturbed. In both cases, the reduction of plasma bicarbonate leads to chronic metabolic acidosis [2]. Clinical and functional studies allow classification into four types, historically numbered in the order of discovery: classic distal (Type 1), proximal (Type 2), combined proximal and distal (Type 3) and hyperkalemic distal (Type 4) [3]. Type 3 RTA demonstrates a mixed pattern of tubular dysfunction. The attributed biochemical findings are bicarbonate wasting, incapability to lower urine pH below 5.5, a low urine-to-blood pCO2 difference in an alkaline urine, and decreased NH4 secretion [4]. This situation is characterized by fractures, short stature, mental retardation, dental malocclusion, and visual impairment from optic nerve compression [5]. The diagnosis of this disease requires a high degree of suspicion, since the symptoms are usually non particular and

highly changeable. The *CA II* gene is positioned at q22 on chromosome 8 [6]. It has 7 coding exons with a transcript length of 1,717 bps and its related protein has 260 residues. Mutations in *CA II* lead to CAII insufficiency, which is measurable in circulating erythrocytes [7]. Consanguinity is an ordinary characteristic in families with *CA II* mutations [3]. The present report describes a case of an Iranian patient whose clinical manifestations matched with Type 3 RTA. In this study, we analyzed all exons and flanking regions of the gene *CAII* in this patient.

Case Report

This study was carried out on a family from Khuzestan Province (where Bakhtiariz live). The parents were first cousins with an eight-year-old boy suffering from RTA Type 3. The proband was the first case of kidney disorder in at least the last 3 generations of the family. Diagnosis was made by the observation of perchloremic metabolic acidosis with a normal serum anion gap and hypokalemia, high urine pH during acidosis, and elevated fractional excretion of bicarbonate, leading to urinary acidification in both proximal and distal nephrons. In addition, the parents stated that the affected child's IQ was below the normal range in relation to his age, without clinical improvement.

Molecular genetic analysis: The child who was suspected of having RTA III was referred to us for genetic tests. Genomic DNA was extracted from blood cells of the patient and his parents by the salt precipitation method [8]. Through polymerase chain reaction (PCR), 7 coding exons, and exon–intron boundaries of the CAII gene were amplified by 8 pair primers designed by primer3out software (www.primer3out.com). The PCR products were separated on the 1% agarose gel by 1× Tris-acetate-EDTA running buffer electrophoresis. The PCR products were verified by their size and appropriate products were subjected to direct sequencing in two separate reactions with forward and reverse primers (ABI automated sequencer model 3130, genetic analyzer company, USA). The results were analyzed and proved by Chromas, FastPCR, and compared with reference gene (WWW.NCBI.org/nucleotide). Screening of the entire coding sequence of the *CAII* gene showed no pathogenic mutation in this gene. It is likely that clinical diagnosis on the basis of type 3 RTA was not correct.

Discussion

CA II insufficiency has been reported in several ethnic backgrounds including Arabic, Italian, German, French, Hispanic, and African American populations [1]. Venta et al. evaluated the *CA II* gene in a patient from a consanguineous Belgian family who suffered from renal tubular acidosis and osteopetrosis, and identified homozygosity for a missense mutation [9]. Roth et al. analyzed the CA II gene in an American family with 3 affected sisters and evaluated the association of CA II deficiency with a clinical syndrome. They identified compound heterozygosity for a paternally inherited splice site mutation and maternally H107Y mutation [10]. In this study, an 8-year-old boy with renal abnormalities was evaluated. Based on the involvement of the kidney tubules, early diagnosis was type 3 RTA. In this regard, exons and flanking regions of *CAII* gene were amplified using PCR with subsequent sequencing. The results did not show any mutation in this gene. Thus, molecular analysis identified that the gene which associated with type 3 disease is not involved. Beyond the difficulties inherent in delineating RTA, RTA can be subcategorized into different disorders with distinctly diverse prognoses. The diagnostic cataloguing of RTA is based on the underlying pathophysiology [11]. Regarding our negative results, it seems that either the type of the disease was not correctly diagnosed or other genes were involved in this case. In this context, we suggest audiometry for the affected child to evaluate his hearing status which could be used to differentiate RTA type 3 with other types. In conclusion, according to our findings, clinical diagnosis is not sufficient and the molecular tests along with clinical diagnosis are essential to proper treatment. Delayed diagnosis of this pathology may cause ionic balance, affect growth and development, and accelerate the progression of nephrocalcinosis and, eventually, chronic kidney disease in children [2]. In addition, genetic tests can help to analyze and correct the diagnosis in other pregnancies of the family. Since the symptoms are not specific and diagnosis requires a high degree of suspicion, it seems that other types of RTA should be also considered.

Conflict of Interest

None declared

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