Genetic analysis of two Iranian patients affected with cystinosis identified a novel CTNS mutation: case report

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Abstract

Two Iranian patients presented in this study was suffering from cystinosis diagnosed based on their clinical symptoms and laboratory tests. The variations c.257_258delCT and c.323delA in the CTNS gene found in them are frameshifts and truncating mutations that affect product function and result in the signs and symptoms of cystinosis.

INTRODUCTION

Cystinosis (OMIM # 219800), an autosomal recessive disease, is caused by the CTNS gene mutation. This gene encodes cystinosin, which transports the lysosomal free cystine. In cases where cystinosin is deficient, lysosomal free cystine accumulates throughout the body[1–3].

Cystinosin has additional roles, such as trafficking of the vesicles in the epithelial cells lining the proximal tubules, biogenesis of lysosomes, autophagy regulation, and mTOR signaling[4].

Systemic complications associated with cystinosis include the Fanconi syndrome, reduced functional activity of gonads, diabetes mellitus, hypothyroidism, exocrine pancreatic enzymes shortage, calcification of blood vessels, crystalline keratopathy, retinal blindness, involvement of the central nervous system, benign increased intracranial pressure[5], and occasionally gastrointestinal symptoms[6].

Based on the disease severity, three different clinical types of cystinosis are dependent on the cysteine accumulation degree and the age at presentation. In the most sever and common case, in terms of severity, nephropathic cystinosis occurs in infancy. The second type is the late-onset adolescent form (intermediate), also known as the nephropathic juvenile cystinosis. The third type is the benign adult form, also known as the ocular non-nephropathic form[7].

The CTNS gene, situated on chromosome 17p13, normally has 12 exons. Ever since the gene was discovered in 1998, more than 148 different CTNS pathogenic and deleterious mutations have been reported in the Ensemble database and various articles[1,8].

In this study, we reviewed the genetic basis of cystinosis and investigated two Iranian cases affected by cystinosis one of which revealed a novel mutation in the CTNS gene.

CASE PRESENTATION

Clinical information was collected by reviewing the medical records. Evaluation of history, clinical manifestations, and laboratory findings of symptomatic patients A and B were performed. Patients A and B were 9, and 11-year-old females with renal insufficiency living in the Iranian capital city of Tehran diagnosed with suffering from cystinosis on the basis of their clinical symptoms and laboratory tests. Data were reviewed from birth until December 2020. After genetic counseling and assessing the familial pedigree (Fig.

1), both patients' parents gave their informed consent before being included in this investigation. Extraction of genomic DNA from whole blood was done using standard extraction methods. Mutation analysis and sequencing of the 10 CTNS gene exons were performed. The exon amplification was done via PCR. The products were purified on an agarose gel and directly sequenced with similar PCR primers.

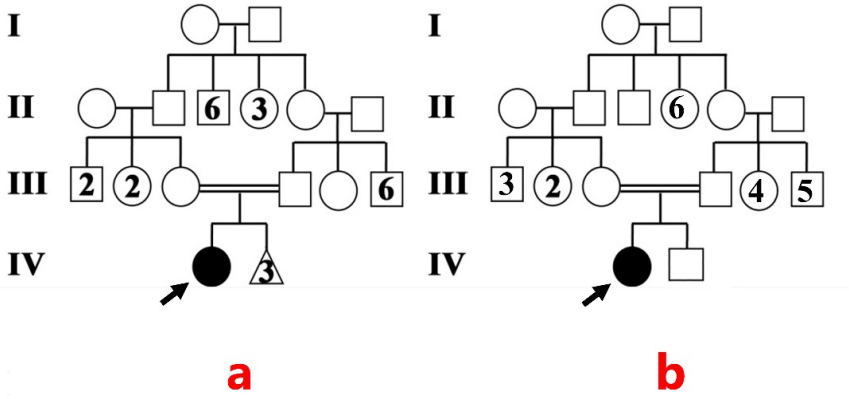

Fig. 1. (a) Family pedigree of patient A, (b) Family pedigree of patient B.

The probands described in this study had clinical manifestations, which conform to the diagnostic cystinosis such as atrophy of the proximal convoluted tubules and renal stones, severe thirst and dehydration, failure to thrive, hematuria, proteinuria, glycosuria, phosphaturia, decreased serum Na, K, Ca, P, Mg and urea, metabolic acidosis, and decreased skin and hair pigmentation. Their physical examination showed growth retardation with a history of teething delay.

Furthermore, both cases unusually did not show photophobia. Slit-lamp evidenced cystine crystals in the cornea of patients. Skeletal surveys showed mild rickets in case A. They both attended a normal school at a normal age level, so their intelligence was normal. Exon analysis in our patients detected a novel homozygous DNA variation c.257_258delCT (p.Ser86PhefsTer38) in exon 6 of the CTNS gene in patient A, and another homozygous DNA variation, c.323delA (p.Q108RfsTer10), in the same exon in patient B. These variations were detected in their parents in heterozygous states (Fig. 2 and 3).

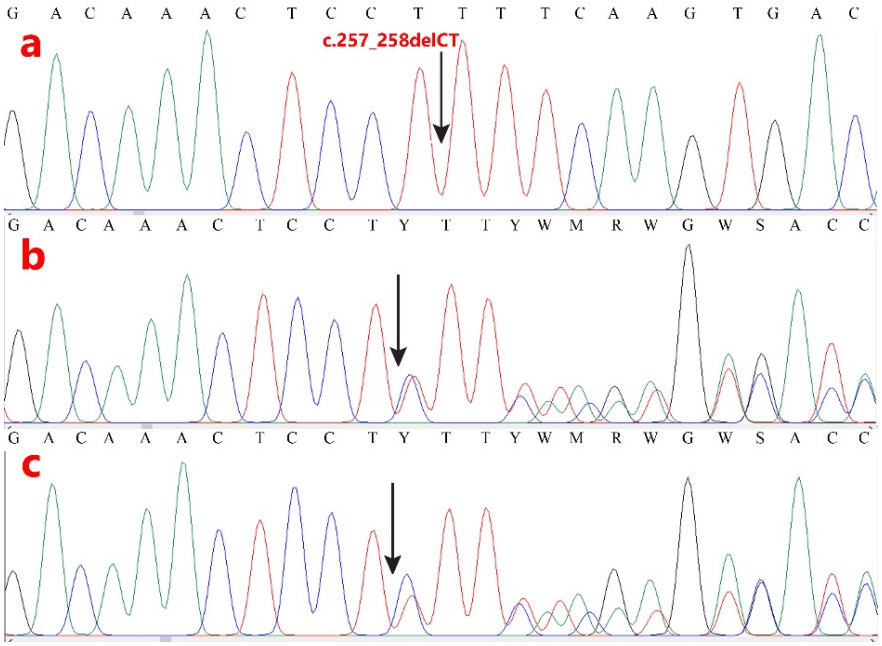

Fig. 2. The chromatogram shows the mutation c.257_258delCT in the CTNS gene; (a) a homozygous state in patient A, (b and c) heterozygous states in her parents.

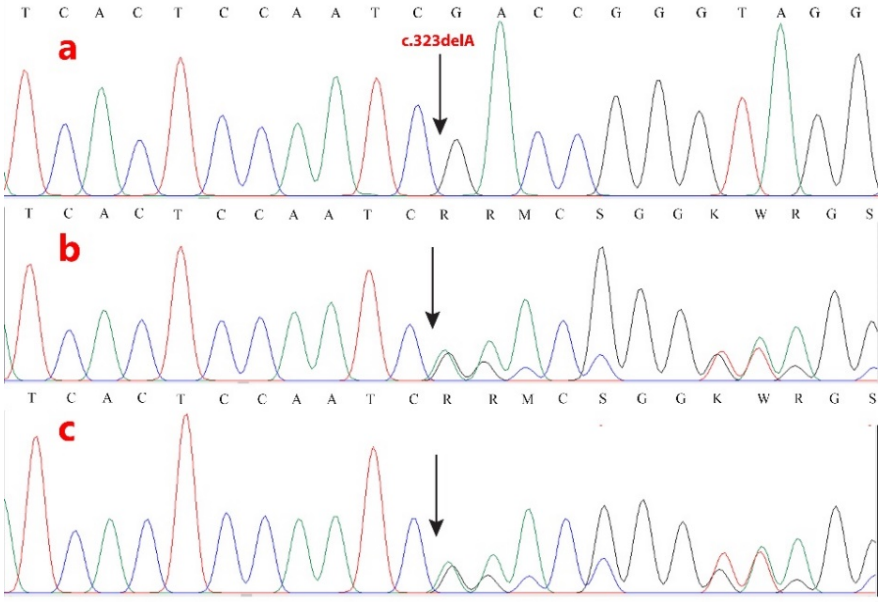

Fig. 3. The chromatogram shows the mutation c.323delA in the CTNS gene; (a) a homozygous state in patient B, (b and c) heterozygous states in her parents.

DISCUSSION

The incidence of cystinosis is approximately 1 in 100,000 to 200,000 newborns[3]; however, this frequency is expected to be higher in populations in which consanguinity is prevalent, such as North Africa, Pakistan,

the Middle East, Turkey[9,10], and Iran[11].

The most prevalent causative mutation in cystinosis in northern Europe and North America is a large 57-kb deletion[12–15]. In contrast, this deletion is absent in cases reported from Turkish[10,16,17], Egyptian[18], and Saudi Arabian populations[19]. In Iran, a patient was reported with this mutation. Hence this 57-kb deletion is extremely rare in cases reported from in the Middle East[20].

The c.681G>A (p.E227E) mutation is the most frequent mutation in Iran[21] and also in the Middle East[18].

In Canada's French population, a nonsense mutation, p.W138*, accounts for cystinosis's most common mutation[22].

The CTNS gene mutations were studied in 28 Iranian patients aged 1–17, affected by nephropathic cystinosis. In 50% of patients (14 individuals), the mutations were identified in exon 6, among which 7.18% (5persons) were detected to have novel homozygous deletions, all of which cause the cystinosin protein to be truncated prematurely. The c.323delA (p.Q108RfsX10) mutation was also detected in 3 cases[23].

In 2018 Bastug et al. reported a novel CTNS homozygous splice mutation, c.853-1G>A, in a rare nephropathic cystinosis case, initially presenting with Bartter syndrome features[24].

CONCLUSIONS

The variations c.257_258delCT and c.323delA in the CTNS gene found in this study are frameshifts and truncating mutations that affect product function and result in the signs and symptoms of cystinosis. As far as we know, the identified mutation in our case A has not been reported before and can be considered a novel mutation. The detected mutation in our case B has been only repoeted in 3 other Iranian patients, showing that this mutation can be considered a specific one related to the Iranian population. The present finding will be useful for carrier detection in the family and other patients with similar disease manifestations and genetic diagnosis.

ACKNOWLEDGMENTS

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

SM drafted the work, provided approval for publication of the content, and collected the detailed information. FS is responsible for designing this study, acquisition, analysis, and interpretation of data for the work. FS, and SH: revised the draft critically. All authors read and approved the final manuscript.

ETHICS APPROVAL

Written consent for participating in this study was obtained from the parents of both patients. The study was performed in accordance with the principles of the Helsinki Declaration.

INFORMED CONSENT

Informed consent was obtained from all individual participants included in the study.

DATA AVAILABILITY STATEMENT

Some data regarding the above case are present within this manuscript and authors have access to all data for this case report.

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