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Chromosome 7 Deletion and Its Role in Nephrocalcinosis: A Study in an Iraqi Family

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Abstract

Background: Nephrocalcinosis refers to medulla calcium phosphate deposition in the kidney. It can occur as a result of some diseases, drugs, or physiological or genetic effects. Genetic effects can involve either genes or chromosomal disorders. Nephrocalcinosis was thought to be not inherited condition. Objective: A family of six members who were suffering from nephrocalcinosis were referred to the Iraqi Center for Cancer and Medical Genetics for chromosomal analysis. Methods: G-banding chromosomal analysis was performed according to the ICCMGR protocol, karyotype analysis was performed via CytoVision (Leica Microsystems Crop), and chromosomal nomenclature was designated according to ISCN 2020. The aim of this study was to report the role of the deletion of region 7p22 associated with nephrocalcinosis. Results: The relatives exhibited breaks at the common fragile site 7p22, some of which were chromatid breaks and others chromosomal deletions. Few cells within adult samples presented chromosomal abnormalities extending to the 7p21 band. To our knowledge, the correlation between fragile site 7p22 and nephrocalcinosis has not been previously reported, as we have shown in our present study. Only two previous studies mentioned the role of the association between aldosterone and 7p22, and the other study mentioned the association between aldosterone and nephrocalcinosis; thus, our current study clarifies a new relationship between 7p22 and nephrocalcinosis, especially when we know that the region holds some genes involved in renal genesis and kidney function. Our conclusion is that the region 7p22-ter holds effective genes involved in kidney function, and its deletion may lead to the appearance of nephrocalcinosis, and the fragile site 7p22 may play a role in nephrocalcinosis.

Keywords: *Chromosomal aberration, deletion7p22, nephrocalcinosis, Iraqi patients*

Introduction

The term “nephrocalcinosis” refers to the buildup of calcium salts caused by hyperparathyroidism in the renal parenchyma [1]. The phrase was subsequently adopted as a radiologic concept to characterize the tiny, widespread renal parenchymal calcification that was evident during radiation screening. Nephrocalcinosis can be divided into two types according to the calcification site: the cortical type and the medullary type. The cortical type results in acute tubular necrosis; however, the medulla type can occur alone with other metabolic diseases or as a continuation of cortical nephrocalcinosis [2]. Nephrocalcinosis is a term used exclusively to refer to the type of medulla in nephrology. Hyperparathyroidism, medullary sponge kidney, renal tubular acidosis, renal papillary necrosis, immobilization, milk-alkali syndrome, and hyperoxaluria are among the conditions that might result in medullary nephrocalcinosis. [3-9]. Psychological factors may lead to an increase in the level of serum creatinine, causing nephrocalcinosis [10]. Drugs and chemicals, such as furosemide, may induce nephrocalcinosis [11]. On the other hand, a genetic role in nephrocalcinosis has been mentioned in some studies. Genetic factors involved in metabolic disorders may lead to nephrocalcinosis. For example, at the beginning of childhood acute lymphoblastic leukemia, elevated parathyroid hormone secretion leads to renal impairment and hypercalcemia with nephrocalcinosis. [12]. Hereditary nephrolithiasis (X-linked recessive) is accompanied by renal failure, including NC [13]. Mutations in genes such as Npt2 at 5q35.3 and Cldn16 at 3q28 have been identified in patients with familial hypercalciuria and nephrocalcinosis [14, 15]. Nephrocalcinosis itself is not inherited. However, a person’s nephrocalcinosis may be inherited because of an underlying medical problem. A form of congenital adrenal hyperplasia (CAH), Bartter syndrome, primary hyperaldosteronism, Liddle syndrome, multiple endocrine neoplasia type 1 (MEN1) on 11q13, chronic granulomatous disease, primary hyperoxaluria, and 11-beta hydroxylase deficiency are a few hereditary conditions that may be linked to nephrocalcinosis in affected individuals. [2], hypomag-

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nesemia-hypercalciuria syndrome, X-linked hypophosphatemic rickets, Bartter syndrome, and X-linked hypercalciuric nephrolithiasis. X-linked hypercalciuric nephrolithiasis, also known as Dent disease, is associated with certain mutations that impact the X chromosome's CLCN5 gene, which results in the inactivation of CLC-5 voltage-gated chloride channels. Young boys experience a clinical syndrome, which typically includes rickets, low-molecular-weight proteinuria, hematuria, glycosuria, aminoaciduria, hypophosphatemia, nephrocalcinosis, and nephrolithiasis[16].

Fragile sites are chromosomal loci prone to breakage and rearrangement [17]. The human genome can be thought of as a mosaic composed of fragile, reorganization-prone areas that have been preserved during evolution in several lineages [18]. The causes of fragile sites can be either genetic or epigenetic modifications. An increased rate of mutations that might result in base alterations, chromosomal abnormalities, translocations, large or tiny insertions or deletions, and other modifications is referred to as genomic instability. The term "epigenetic instability" describes aberrant gene regulatory responses to changes in the environment. High levels of genetic and epigenetic instability are found in genomic areas identified as fragile sites. [19]. At particular locations on chromosomes from cells subjected to partial restriction of DNA replication, DNA replication can present as constrictions, gaps, or breaks [20]. Scanning electron microscopy has revealed several fragile patches that appear as an isochromatid gap connecting two parts of the chromosome via individual chromatin fibers. The diameter of these fibers is consistent with the measurement of a single chromatin fiber. This discovery indicates that at weak points, chromatin fibers are unable to fold into higher-order structures. [21].

Fragile sites are classified as common or rare depending on their frequency within the population and their specific mode of induction[20]. Large chromosomal areas known as "common fragile sites" (CFSs), which are susceptible to breaking under replication stress, are thought to be key factors in the development of cancer. It has long been thought that CFSs contain sequences that prevent fork progression, which prevents replication from finishing and results in DNA breaks during chromosomal condensation. On the other hand, a previous study indicated that a lack of initiation events in a particular area is what causes delayed DNA replication completion. Because the timing and location of these events change depending on the type of cell, various chromosomal regions can be committed to fragility in different types of cells. These new discoveries highlight the epigenetic nature of CFSs and open the door to reconsidering the role these sites play in the chromosomal rearrangements that arise in cancers that start in different organs. [22]. Chromosome 7 has some fragile sites along both arms, ranging between common and rare. FRA7A is rare on chromosome 7p11.2, FRA7B is rare on chromosome 7p21.3-22.3, FRA7E is rare on chromosome 7q21.2, FRA7F is rare on chromosome 7q22, FRA7G is rare on chromosome 7q31.2, FRA7H is rare on chromosome 7q32.3, FRA7I is common on chromosome 7q36, and FRA7J is rare on chromosome 7q11. Our region of interest in the current study is 7p22–ter, where ter means terminal or the end; this region contains FRA7B.

The aim of this study was to report the de novo role of FRA7B on 7p21.3-22.3 and the deletion of region 7p22-ter, which is associated with familial nephrocalcinosis.

Materials and methods

Patients

Six relative patients who suffered from medullary nephrocalcinosis were referred to ICCMGR, Medical Genetic lab for chromosomal analysis. The first patient was a 30-year-old man, and the second patient was a 29-year-old wife who was cousing at the same time. Kids: A 10-year-old boy (the third patient), a 9-year-old boy (the fourth patient) and a fifth patient were 8-year-old girls. Finally, the sixth patient was a 26-year-old aunt. The patient's relatives were suffering from nephrocalcinosis, but only the third patient reported hyperparathyroidism, while the little girl had a normal range of PTH. The reports included ultrasonic reports and revealed that both kidneys were normal in size and position, had smooth outlines, had normal parenchymal thickness and that all the renal pyramids were filled with linear coarse dots of calcification (largest in the right kidney, 11.6 mm long, and left kidney). of 11.7 mm), with no stone or foacal lesions, and only a few crystals were present in both. The families were referred to our center for chromosomal analysis to determine whether their nephrocalcinosis was due to genetic causes. Unfortunately, chromosomal analysis was subsequently performed. Owing to the displacement that the country experienced, we lost contact with the members of the study, which limited our knowledge of their hormonal state and the remaining biochemical parameters and ultrasonic reports. The family reported that there was a member of this family who died and was suffering from nephrocalcinosis. This man was the father's brother, and he did not undergo chromosomal analysis. Additionally, the mothers of patients 1 and 2 were sisters, both of whom had nephrocalcinosis but did not undergo chromosomal analysis.

Preparation of blood samples and cytogenetic analysis:

Peripheral blood cells that were stimulated with phytohemagglutinin (PHA) were cultured at 37°C for 72 hours via the short-term culture technique (processed at the Iraqi Center for Cancer and Medical Genetics, Iraq). Penicillin and streptomycin were added as antibiotics to RPMI 1640. Standard procedures for culture, harvesting, and slide preparation were performed according to Verma and Babu[23]. The cells were harvested for chromosome analysis of G-banded metaphases after being exposed to colcemid (0.2 µg/ml) during the final 30 min of culture at 37 °C. Karyotype designation adhered to the International System for Human Cytogenetic Nomenclatur2020 (24).

Results

The fragile site on chromosome 7 was the break in region 7p22, which recurrently appeared in the six members of the family who suffered from nephrocalsinosis, as shown in Figure 1. The members of the study were case 1, the father, case 2, the mother, case 3, the daughter, case 4, and case 5, the sons, case 6, the aunt. Two grandmas and one brother (who died during the study) were not included in the study despite their suffering from nephrocalcinosis. Six members of this family carried the same defect at chromosome 7p22. Young members (cases 3 and 4, as shown in Figure 2 and 5, as shown in Figure 3) and their aunts (case 2, as shown in Figure 4E)

carried the break at 7p22 only. While patients 1 and 6 carried breaks at chromosome 7p21-22 in a few cells in addition to deletion at chromosome 7p22, the region presented with chromatid breakage once and deletion in both chromatids, as shown in Figures 4B, 4C, and 4D. The chromosomal aberration frequencies are illustrated in Table 1.

Table 1 Frequency of chromosomal aberrations in spread metaphase cells among patients.

Chromosomal aberration	Frequency in metaphase cells	Case number
Chromatid break7p22	32%	All cases
Chromosome deletion 7p22-ter	66%	All cases
Chromosome deletion 7p21-ter	Less than 1%	Case1,case6
Chromatid break 7p21-ter	Less than 1%	Case 1,case6

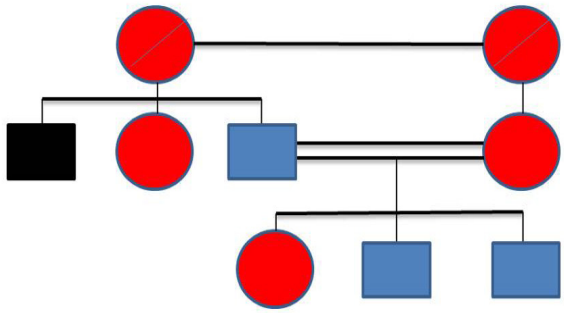

Figure 1: Pedigree of the studied family with nephrocalcinosis. Red circle indicates females with nephrocalcinosis. The blue square represents males with nephrocalcinosis, and the black square died male with nephrocalcinosis (not included in the study). The red circle with line females with nephrocalcinosis is not included in the study.

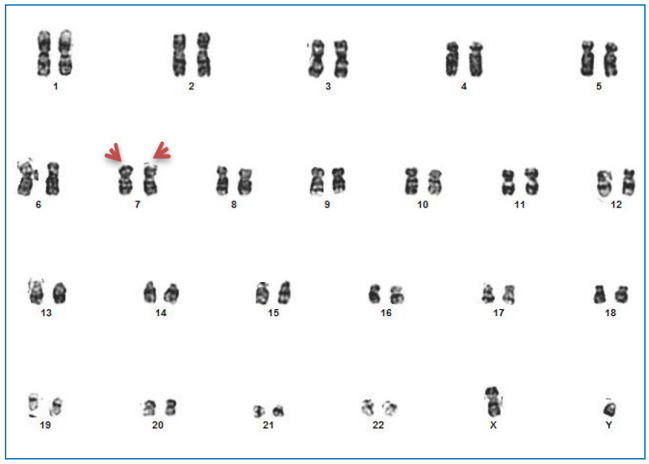

Figure 2- G-banding male karyotype : 46,XY,del(7)(p22)

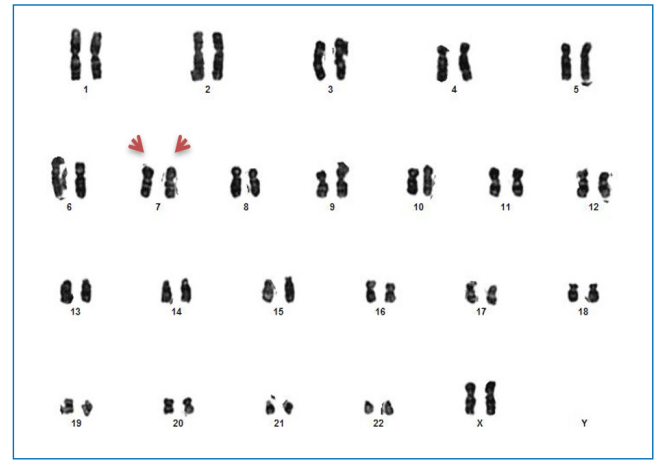

Figure-3- female karyotype: 46,XX,del (7)(p22) G-banding

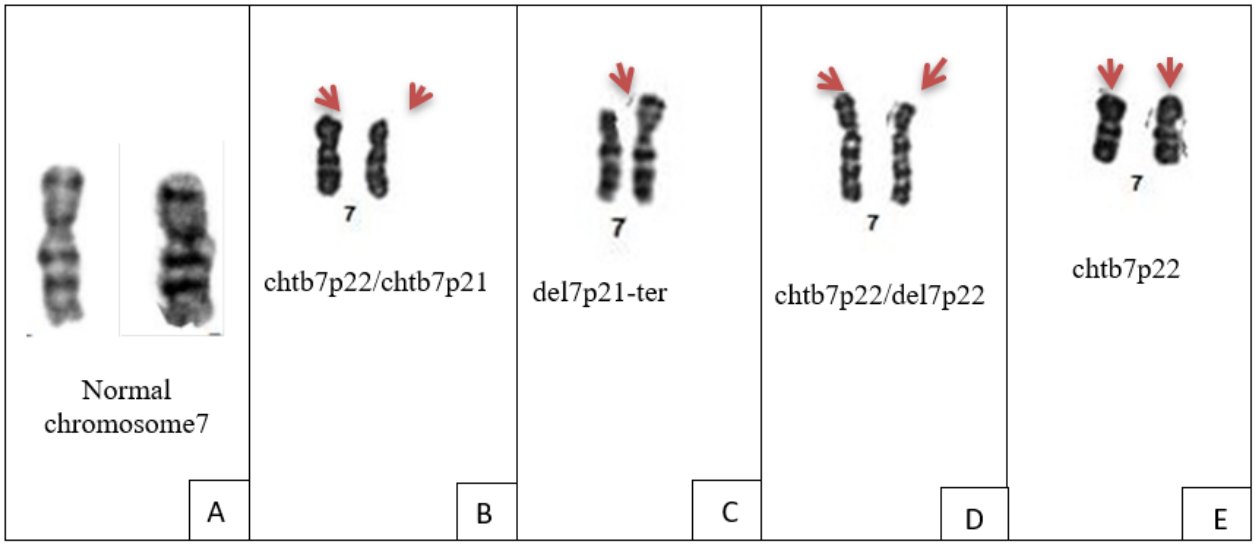

Figure-4-G-banding partial karyotypes; red arrows point to the deletion regions at chromosomes 7p22 and 7p21. A: Normal chromosome 7. B: The left chromosome with a chromatid break at 7p22, whereas the right chromosome with a chromatid break included region 7p21. C: Left chromosome 7 with a deletion from the 7p21- terminus, whereas the right chromosome is del7p22. D: The left chromosome shows chromatid break 7p22, whereas the right chromosome shows deletion 7p22. E: Both chromosomes with deletion of 7p22.
1*Chtb: chromatid break. 2*del: deletion. 3* ter: terminal region.

Discussion

The present study involved one of the most frequent fragile sites, 7p22, which is associated with the six relatives. They may have inherited the ability to break 7p22 because almost all the cells showed a break in chromatid at region 7p22, and the other cells showed a break in both chromatid (chromosome) regions. In two cases, few cells broke into region 7p21. All the relative included in this study were suffering from nephrocalcinosis. To our knowledge, the current study is the first to mention the relationship between nephrocalsinosis and the 7p22 fragile site and deletion of the region. Few previous studies have reported that this region is associated with several disorders, including acute myeloid leukemia, autism, craniosynostosis, and Baraitser-Winter syndrome [25-27]. However, none of them reported its association with nephrocalsinosis. Region 7p22 contains the UNCX gene. This gene has a role in kidney function, and polymorphisms in this gene are associated with renal dysfunction in the Asian population [28,29]. Another gene located at 7p22.1 that affects kidney function is the ACTB gene. Renal agenesis and impairment are caused by ACTB loss-of-function mutations [30], which are linked to an elevated risk of diabetic kidney disease [31]. As shown above, the region plays an important role in renal agenesis and kidney function, and its deletion explains its association with the appearance of nephrocalcinosis. To our knowledge, this is the first study to mention the direct association of 7p22 with nephrocalcinosis. Two previous studies reported closely related information: the first mentioned the role of the deletion of 7p22 associated with hyperaldosterone, and the second mentioned the role of hyperaldosterone associated with medulla nephrocalcinosis[32-38]. There may be a correlation among deletion of 7p22-aldosteron and neurocalcinosis. Unfortunately, a limitation of our study was that we could not estimate aldosterone levels for the members of the family because we lost contact with them because of the displacement that the country experienced. We recommend further studies on this correlation.

In conclusion: All the metaphase data revealed a deletion of the 7p22-ter , and that the region 7p22-ter holds effective genes which are involved in kidney function; thus, the deletion of this region led to the development of nephrocalcinosis, and the fragile site 7p22 plays a role in nephrocalcinosis.

Acknowledgment

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Ethical approval

The scientific committee of Mustansiriyah University, the Iraqi Center for Cancer and Medical Genetics, approved the study. The first (1) scientific committee meeting on January 26, 2014

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