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The Polymorphism of *OCRL* Gene in Kidney Stones and Kidney Failure Patients

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Abstract

Kidney disease is a kidney injury or disease that affects many people globally. The study aims to evaluate the effect of Phosphatidyl Inositol (4,5) Bisphosphate, 5-Phosphatase (PIP2) enzyme on calcium (Ca^{2+}) levels and its relationship to oculocerebrorenal gene variations in kidney stone and kidney failure patients. The enzyme-produced amount was measured by enzyme-linked immunosorbent assay (ELISA). Genetic variations were studied through the polymerase chain reaction (PCR) technique, followed by the sequencing of fragments of the exons (9, 13, 15). Blood samples were collected from eighty patients (40 with kidney stones and 40 with kidney failure), and 40 were healthy individuals. Results showed a significant difference between the levels of PIP2 in patients with kidney stones (6.63±0.22) and kidney failure patients (9.13±0.27), at a *p*-value of 0.004, compared with the control group (1.87±0.14), at a *p*-value of 0.02. Also, the differences in the serum Ca^{2+} levels were highly significant differences (9.55±0.25, 8.19±0.17) and the control group (9.46±0.04) at *p*-values of 0.004 and 0.001, respectively. In addition to the results of urea, creatinine, and uric acid, there were highly significant differences between kidney failure 140.35±6.55, 8.69±0.50, and 8.42±0.23 versus the control group 27.07±1.13, 0.69±0.04, and 4.53±0.14, respectively. The *p*-value was 0.001. In patients with kidney stones, the uric acid level was significant at 6.30±0.21, with a *p*-value of 0.001. Sequencing revealed a variant A/G in exon 15 in a female patient suffering from kidney failure and two intronic substitutions (c.1359+262G>A) and (c.1359+251G>C) before exon 13 in a 55-year-old male patient with kidney failure.

Keywords: Kidney stone; Lowe syndrome; *OCRL* gene; PIP2; kidney failure.

العلاقة بين تعدد أشكال جين المتلازمة العينية الدماغية الكلوية مع حصى الكلى ومرضى الفشل الكلوي

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الخلاصة:

"مرض الكلى" يشير إلى إصابة الكلى أو المرض الذي يؤثر على العديد من الناس على مستوى العالم، تهدف الدراسة إلى تقييم تأثير إنزيم فوسفاتيديل إينوسيتول (4،5) ثنائي الفوسفات، 5-فوسفاتيز (PIP2) على مستويات الكالسيوم (Ca^{+2}) وعلاقته باختلافات جين المتلازمة العينية الدماغية الكلوية في مرضى حصوات

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الكلى والفشل الكلوي. تم قياس الكمية التي ينتجها الإنزيم بواسطة اختبار الامتزاز المناعي المرتبط بالإنزيم (ELISA).تمت دراسة الاختلافات الجينية من خلال تقانة تفاعل انزيم البلمرة المتسلسل (PCR)، تليها تسلسل اجزاء من الإكسونات (9،13،15). تم جمع عينات الدم من ثمانين مريضًا (40 مصابًا بحصوات الكلى و 40 مصابًا بالفشل الكلوي) و 40 فردًا سليمًا. أظهرت النتائج وجود فرق معنوي بين مستويات PIP2 لدى مرضى حصوات الكلى (0.22±6.63) ومرضى الفشل الكلوي (0.27±9.13)، عند قيمة 0.004 p، مقارنة بالمجموعة السليمة (0.14±1.87)، عند قيمة 0.02. p. كما كانت الاختلافات في مستويات Ca+2 في المصل ذات دلالة إحصائية عالية (0.25±9.55، 0.17±8.19) والمجموعة السليمة (0.04±9.46) عند قيم 0.004 p و0.001، على التوالي. بالإضافة إلى نتائج اليوريا والكرياتينين وحمض البوليك، كانت هناك فروق ذات دلالة إحصائية عالية بين الفشل الكلوي 140.35±6.55، 8.69±0.50، و8.42±0.23 مقابل المجموعة السليمة 27.07±1.13، 0.69±0.04، و4.53±0.14، على التوالي. وكانت القيمة الاحتمالية 0.001. وفي المرضى الذين يعانون من حصوات الكلى، كان مستوى حمض البوليك ذا دلالة إحصائية عند 6.30±0.21، بقيمة احتمالية 0.001. كشف تتابع التسلسلات عن وجود متغير A/G في الإكسون 15 في مريضة تعاني من الفشل الكلوي واستبدال قاعدة واحدة في الانترون (c.1359+262G>A)و(c.1359+251G>C)، قبل الإكسون 13 في مريض ذكر يبلغ من العمر 55 عامًا يعاني من الفشل الكلوي.

1. Introduction:

Chronic kidney diseases (CKDs) are defined as abnormalities of kidney structure or function, present for a minimum of 3 months, with health implications according to The Kidney Disease: Improving Global Outcomes Organization (KDIGO) guideline updated in 2024. These chronic diseases are classified according to the cause, glomerular filtration rate (GFR) into (G1-G5), and presence of albumin in the urine (A1-A3). These three categories determine the risk and severity of the disease [1], which is characterized by irreversible kidney damage resulting from gradual and progressive damage in the tubular epithelial cells inflecting loss of approximately 95% of kidney functions. CKD may eventually progress into end-stage kidney disease (ESKD), a condition that requires interventions like dialysis or a kidney transplant for survival. Patients with GFR less than 15 mL/min per 1.73 m^2 have poor prognosis and are classified as the end stage of chronic kidney diseases that have lethal complications [2]. Recurrent kidney stones, considered a primary cause of CKD, may lead to ESKD. Several risk factors are associated with the development of chronic kidney disease. Metabolic disorders, in addition to cardiovascular diseases, hypertension, and diabetes mellitus, are the most relevant causes of CKD, as well as patient sex, race, and lifestyle habits [3]. The essential kidney functions include the elimination of toxins and metabolic wastes, such as urea and creatinine, from renal blood flow and allow effective waste excretion via urine production. Also, the kidneys reabsorb necessary metabolites and maintain homeostatic fluid and electrolyte balance in the body. Calcium absorption and transforming inactive vitamin D3 into its active form (calcitriol) are important to maintain balance and bone formation [4]. This homeostasis can be significantly disrupted by inflammatory status and oxidative stress that contributes to renal damage by causing renal cell injury and glomerular lesions that ultimately, end with renal cell apoptosis and/or necrosis, leading to acute and chronic kidney disease. Moreover, a notable association has been found among insufficient serum vitamin D3, low blood calcium levels, and supersaturation of urine with calcium salts, which may result in calculi formation and elevated parathyroid hormone (PTH) levels, which in turn lead to bone demineralization, and worsen the kidney disease condition [5].

Calcium homeostasis is maintained by several hormones and metabolic pathways. One crucial metabolic mechanism that regulates cellular calcium levels in the kidneys is the phosphoinositide 3-kinase PI3K/Akt pathway. This pathway comprises the Phosphatidyl

Inositol (4,5) Bisphosphate,5_Phosphatase (PI (4,5) P2), also known as PIP2, is the most prevalent phosphoinositide (PI) in the glycerophospholipid family, which is derived from phosphatidyl inositol. It has a special inositol head group that can be phosphorylated and dephosphorylated reversibly to form seven different phosphorylated species. The PIP2 is a key player in several processes, including membrane fluidity, glucose metabolism, cell adhesion, motility, apoptosis, and transport of ions through channels controlled by particular intracellular chemicals, such as calcium signaling [6, 7]. Thus, the formation of kidney stones can be influenced by the actions of phospholipase C (PLC) and phosphoinositide 3-kinase (PI3K), which convert signaling molecules into second messengers. PLC hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) to produce diacylglycerol (DAG) and inositol triphosphate (IP3). The IP3 then stimulates the release of calcium ions from the endoplasmic reticulum, leading to increased calcium levels in the cytosol [8, 9]. Elevated calcium can saturate urine with calcium salts, contributing to kidney stone formation if not regulated. Furthermore, the dysregulation of this signaling pathway can result in cellular dysfunction and potentially kidney failure [10]. This enzyme is encoded by the gene known as the Oculocerebrorenal of Lowe (*OCRL*) gene. It has been found to affect the eyes, brain, and kidneys, hence the gene's name. the *ORCL* gene is located at Xq26.1, and covers 24 exons in the entire *OCRL* mRNA transcript, taking up around 58 kb. It's found in the three splice isoforms a, b, and c expressed differentially in a tissue-specific manner. More than 250 pathogenic mutations were reported on the *OCRL* gene, and the majority of these mutations are exonic, splicing, missense, nonsense, deletion, and insertional mutations [11]. Some of these mutations in *the OCRL* gene cause kidney stone formation and kidney failure as a phenotypic feature of monogenic X-linked multisystemic syndrome such as Lowe and Dent syndromes [12, 13], as well as the less frequent Fanconi syndrome with phenotypic expression of hypercalciuria, nephrocalcinosis, low molecular weight proteinuria, and progressive to CKD, as well as those related to proximal tubular dysfunction, such as aminoaciduria, glycosuria[14, 15]. To identify if CKD is caused by mutations in the *OCRL* gene, therefore, this study aimed to evaluate the PIP2 enzyme, calcium, and Vitamin D levels, as well as the detection of *OCRL* gene mutation or polymorphisms in some Iraqi patients with recurrent kidney stones and end-stage kidney failure.

2. Subjects and Methods

2.1 Sampling

In this study, which lasted from November 2023 to March 2024, 80 patients, 40 were diagnosed with recurrent kidney stones, distributed into 24 males and 16 females (20-66) years old, and 40 patients were diagnosed with end-stage kidney failure, also distributed into 28 males and 12 females between (5-66) years old, and 40 healthy individuals (20 males and 20 females) between (5-67) years old. These samples were taken from patients who visit the following hospitals in Baghdad: Baghdad Teaching Hospital-Iraqi Dialysis Center, Abu Ghraib General Hospital/Al-Mustafa Kidney Dialysis Center, Al Karama Teaching Hospital, Al-Imam Al-Kazemin Medical City Hospital/ Al-Jawadin Dialysis Center, Yarmouk Teaching Hospital/ Al-Shifa Dialysis Center.

2.2 Included and Excluded Criteria

To demonstrate the connection between the hereditary influence of a gene (*OCRL*) with kidney disease, we concentrated on cases with hereditary disorders like kidney disease when gathering samples from adult individuals suffering from kidney failure, as for patients who are between the ages (5 and 20) who had phenotypic and genetic conditions such as kidney symmetry, major and minor pleurodesis, congenital cataracts, mental retardation, Fanconi syndrome, Prader-Willi syndrome, focal segmental glomerulosclerosis, Cakut, kidney cysts,

neurotic bladder, congenital kidney malformation, retinal and nerve dysfunction, Mental distraction, Joint diseases and congenital dislocation and kidney symmetry and who, in the opinion of their physician, was suspected of having Lowe Syndrome. The exclusion criteria were patients who were diagnosed with autoimmune disorders, diabetes, hypertension, thalassemia, hemophilia, HIV, hepatitis B and C, or coronavirus, and none of the participants had cancer or underwent radiation therapy or chemotherapy. Individuals who experienced severe bleeding, kidney-related accidents, or kidney impairment as a result of receiving lengthy treatment were also excluded. The participants provided consent through a questionnaire and were registered with the Iraqi Ministry of Health. The questionnaire included age, sex, diet, smoking, and family history.

2.3 Collection of Blood Samples

Peripheral whole blood samples were collected in a gel tube (2.5 mL) and an ethylene diamine tetraacetic acid (EDTA) tube (2.5 mL) from each individual's vein using disposable syringes in an aseptic procedure. The gel tube was kept for 15–20 minutes until a clot formed. Then, it was centrifuged for ten minutes to extract serum at 4000 rpm. After that, the sera were transferred into a clean, sterile Eppendorf tube and kept at -20 ° until use. Each serum sample underwent evaluation of phosphatidyl inositol (4,5) bisphosphate and 5-phosphatase enzyme concentration. Biochemical tests were also conducted for kidney function, such as serum uric acid, urea, and creatinine, which were performed at the hospital laboratories for both the patients and the control group, together with Vitamin D3 and calcium. The blood within the tubes containing EDTA was used for DNA extraction.

2.4 ELISA for (PIP2)

The concentration of the human Phosphatidyl Inositol (4,5) Bisphosphate,5-Phosphatase (PIP2) enzyme was measured in the sera of both patients and healthy by sandwich enzyme-linked immunosorbent assay according to the manufacturer's instructions (Sun Long/China). The absorbance (O.D.) was read at 450nm using a Microtiter Plate Reader (Human, USA). With a sensitivity of 0.06 ng/mL and a detection range of 0.3-20 ng/mL.

2.5 Genomic DNA Extraction

The extraction of DNA was performed according to the instructions provided by a Reliaprep^{TM} kit (Promega, USA), and the concentration and purity of extracted genomic DNA were measured using a NanoDrop^{TM} spectrophotometer (Thermo Science, USA). The purity of DNA samples ranged from 1.6 to 2. The quality of DNA samples was examined by electrophoresis in 1% agarose gel in TAE (Tris-acetate-EDTA) buffer and stained by red safe dye.

2.6 Primer design

The oligonucleotide primers were designed using Genious Prime software (version 24.0.7, New Zealand) (www.geneious.com) for the studied exons 9, 13, and 15, provided by Macrogen/ Korea. As shown in Table 1.

Table 1: Primers sequences and fragment size for (OCRL) gene.

The exon	Primers sequences	Amplicon size
Exon 9	F=5'TGGAAGCGAAAAGAAAGAACT3' R=5'ATTATCTCTGGCTACCTCCTG3'	667 bp
Exon 13	F=5'AATCCATTGTCTCTCTCAGGT3' R=5'CTCAGCTTGCCAAGTAATCAT3'	696 bp
Exon 15	F=5'GCTAGGTTCTAGGAGTTCAGT3' R=5'CTCAAGTTACCACCTAACCAC3'	600 bp

An amount of 90 µl of nuclease-free water was added to 10 µl of stock solution, and then it was mixed by the vortex to obtain 10 picomol/µl, and then the stock solution was kept in the freezer until needed.
The working solution was stored in the freezer at -20°C until the time of use in the PCR reaction.

2.7 Polymerase chain reaction (PCR)

A thermal cycler amplified The target DNA fragments through a conventional polymerase chain reaction (PCR) (Applied Biosystems, USA). For each single reaction, the PCR's components were master mix (12.5 µl) and forward primer (1 µl), reverse primer (1 µl), and DNA (2 µl). The final volume was adjacent to 25 µl by adding nuclease-free water.

Table 2: PCR Programming for the exons (9, 13, 15).

Step	Temperature (C°)	Time	Cycle number
Initial denaturation	95	1 minute	1 cycle
Denaturation	95	15 seconds	35 cycle
Annealing	60	30 seconds	
Extension	72	1 minute	
Final extension	72	7 minutes	1 cycle

Then, the PCR product was electrophoresed using 2% agarose gel to analyze DNA according to its molecular weight/electromobility, with the addition of the red safe dye. Electrophoresis was performed at 110 volts for 65 minutes. The 100bp ladder was provided by TransGen Biotech Company/ China was used.

2.8 Sequencing

Twenty-two microliters of PCR product were sent to Macrogene/Korea for Sanger sequencing. After trimming each sequence, the results of the trimmed sequence were blasted in NCBI (NG_008638.1) to check the similarities and differences with the database. Genious Prime software (version 24.0.7, New Zealand) was used to check the similarities and differences. The 3D structure of the protein shows the position of the mutant amino acid predicted by the homology model, which is available at https://swissmodel.expasy.org/templates/ [16].

2.9 Statistical analysis:

The SPSS statistics software (IBM SPSS) (version 26.0 for Windows, USA) was used to detect the effect of different factors on study parameters. T-test and least significant difference (LSD) test (Analysis of Variation, ANOVA) were used to significantly compare between means. Study of genotypes with Hardy Weinberg's equilibrium (free online). The chi-square test was used to significantly compare between percentages (0.05 and 0.01 probability) in this study.

3. Results:

The distribution of samples according to sex was studied in the control and patient groups. The recurrent kidney stone patients group included 24(60%) males and 16(40%) females, and the ESKF patients group also included 28(70%) males and 12(30%). The healthy group registered in this study was 20 (50%) male and 20 (50%) female, as shown in Figure 1.

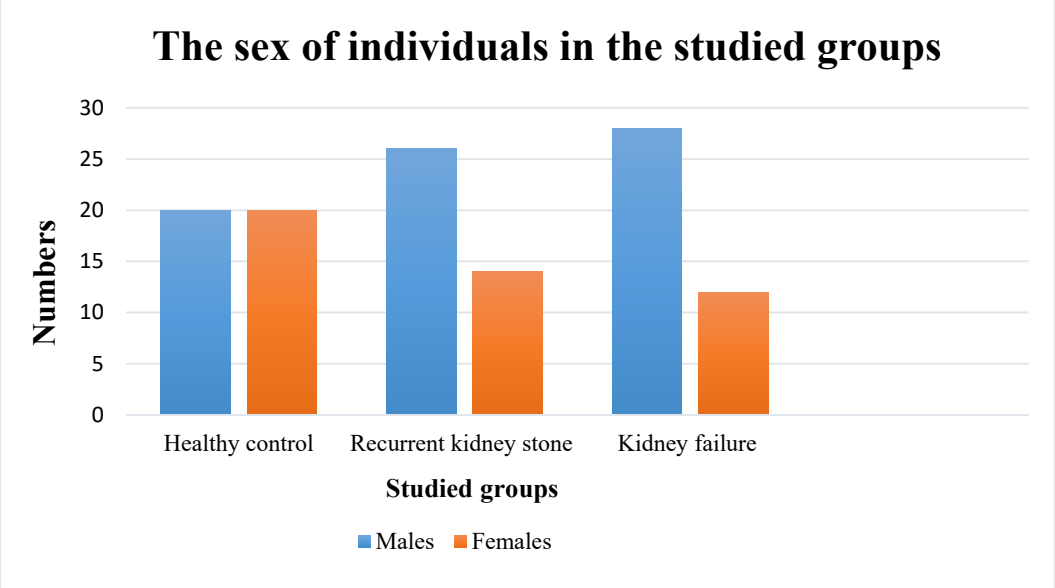

Figure 1: The sex of individuals in the studied groups.

The distribution of samples according to age was divided into 4 age groups: 1-19, 20-39, 40-59, and 60-79 years old. The highest prevalence of recurrent kidney stones was 19 patients (47.5%) in the age group (40-59) years old while the highest prevalence of ESKF was 21 (52.5%) in the age group (1-19) years old compared with ages of the controls group, as explained in Figure 2.

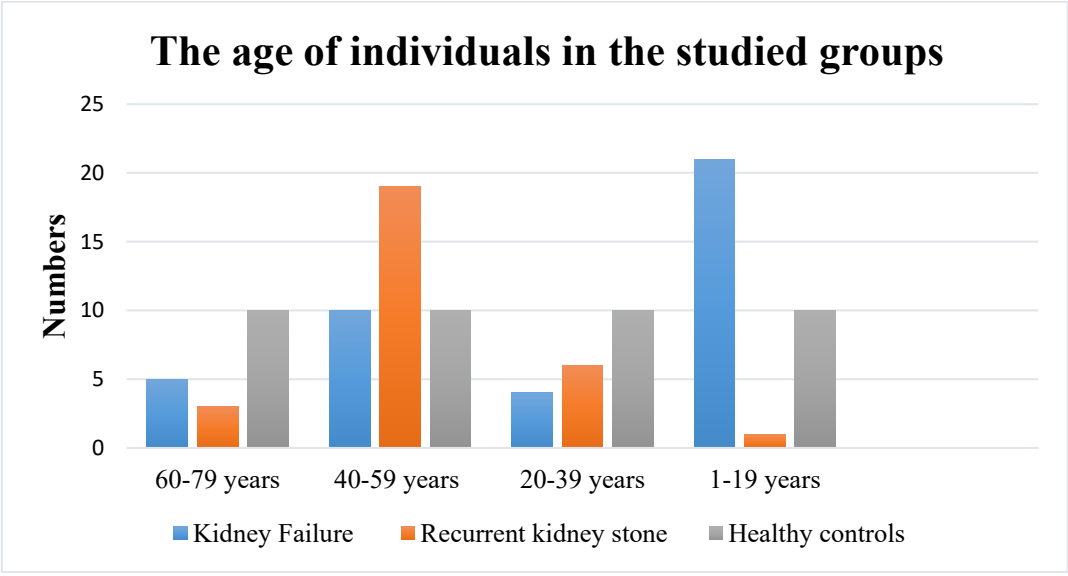

Figure 2: The age of the individuals in the studied groups.

3.1 Kidney function tests

The results show no significant difference in serum urea and creatinine (p = 0.16 and 0.51, respectively, between kidney stone patients and the control group. Still, there was a substantial increase in uric acid (p = 0.001), as shown in Table 3.

Significant increases in serum urea, creatinine, and uric acid (p = 0.001) in the kidney failure patients group compared to the control group, as shown in Table 3.

Table 3: kidney function tests of the study groups

Kidney Function Tests(mg/dL)			
Groups (Mean± SE)	Urea	Creatinine	**Uric Acid**
Control	27.07±1.13[b]	0.69±0.04[b]	**4.53±0.14[c]**
Recurrent Kidney Stone Patients	33.04±1.80[b]	0.98±.095[b]	**6.30±0.21[b]**
ESKF patients	140.35±6.55[a]	8.69±0.50[a]	**8.42±0.23[a]**
***P*-value**	0.001	0.001	**0.001**

Different letters indicate significant differences, and similar letters indicate no significance, p < 0.001: Significant.

3.2 Measurement of calcium, vitamin D3, and PIP2 enzyme concentration in patients' serum

There was a significant increase in serum Ca^{+2} in the recurrent kidney (9.55±0.25 mg/dl) compared with the control group (9.46±0.04 mg/dl). The Ca^{2+} concentrations were significantly decreased in ESKF patients (8.19±0.17 mg/dl) compared with the control group and recurrent kidney stone patients (p = 0.004) and (p = 0.001), respectively**.**

It has been found that serum vitamin D3 was decreased dramatically in ESKF patients (12.65±0.87mg/dl) compared to recurrent kidney stone patients (16.87±0.81mg/dl) and the control group(19.68±0.90mg/dl), but these differences were not statistically significant, as shown in Table 4.

ELISA measured a significant increase in serum enzyme PIP2 in recurrent kidney stone patients (6.63±0.22 mg/dl) and ESKF patients (9.13±0.27 mg/dl) compared to the control group (1.87±0.14 mg/dl) at (p = 0.02) and (p = 0.004), respectively**,** also shown in Table 4

Table 4 : Serum of calcium, vitamin D3, and (PIP2) enzyme of the study groups**.**

Parameters (mg/dl)			
Groups (Mean ±SE)	Calcium (Ca^{+})	Vitamin D3	PIP2 enzyme
Control	9.46±0.04[c]	19.68±0.90[a]	1.87±0.14c
Recurrent Kidney Stone Patients	9.55±0.25[b]	16.87±0.81[a]	6.63±0.22[b]
ESKF Patients	8.19±0.17[a]	12.65±0.87[a]	9.13±0.27[a]
***P*-value**	0.001	0.001	0.001

Different letters indicate significant differences, and similar letters indicate no significant, p < 0.001: Significant.

3.3 The genetics study

Genomic DNA was extracted, and amplicons were analyzed using electrophoresis, which was carried out using 2% agarose gel using a 100-bp DNA ladder in TAE (Tris-acetate-EDTA) for 65 minutes at 110 Volts; the PCR product was 667,696 and 600 bp for exons 9,13 and 15, respectively, as shown in Figures 3,4 and 5.

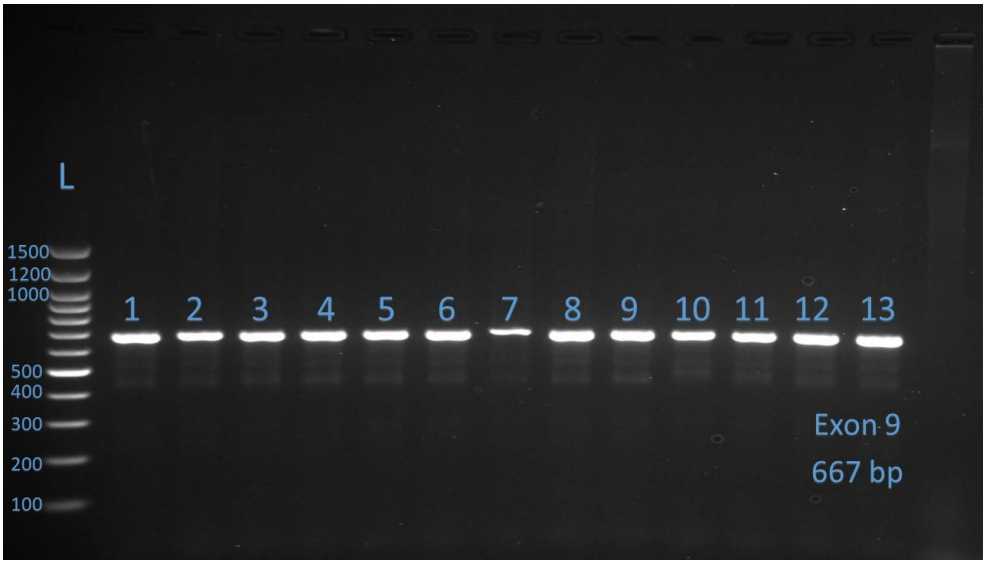

Figure 3. PCR product gel electrophoresis at 2% Agarose using 100 bp DNA ladder in TAE for 65 minutes at 110 Volt for exon 9, band size 667 bp.

Figure 4. PCR product gel electrophoresis at 2% Agarose using 100 bp DNA ladder in TAE for 65 minutes at 110 Volt for exon 13, band size 696 bp.

Figure 5. PCR product gel electrophoresis at 2% Agarose using 100 bp DNA ladder in TAE for 65 minutes at 110 Volt for exon 15, band size 600 bp.

The Sanger sequencing method was applied to the amplified fragments to find potential alterations in both the patients and control groups.

The sequencing results showed that exons 9 and 13 did not carry any pathogenic variants in patients with recurrent kidney stones and ESKF. Still, it appeared to be a missense mutation in a female patient with ESKF who was in exon 15, as shown in Figures 6,7 and 8, respectively.

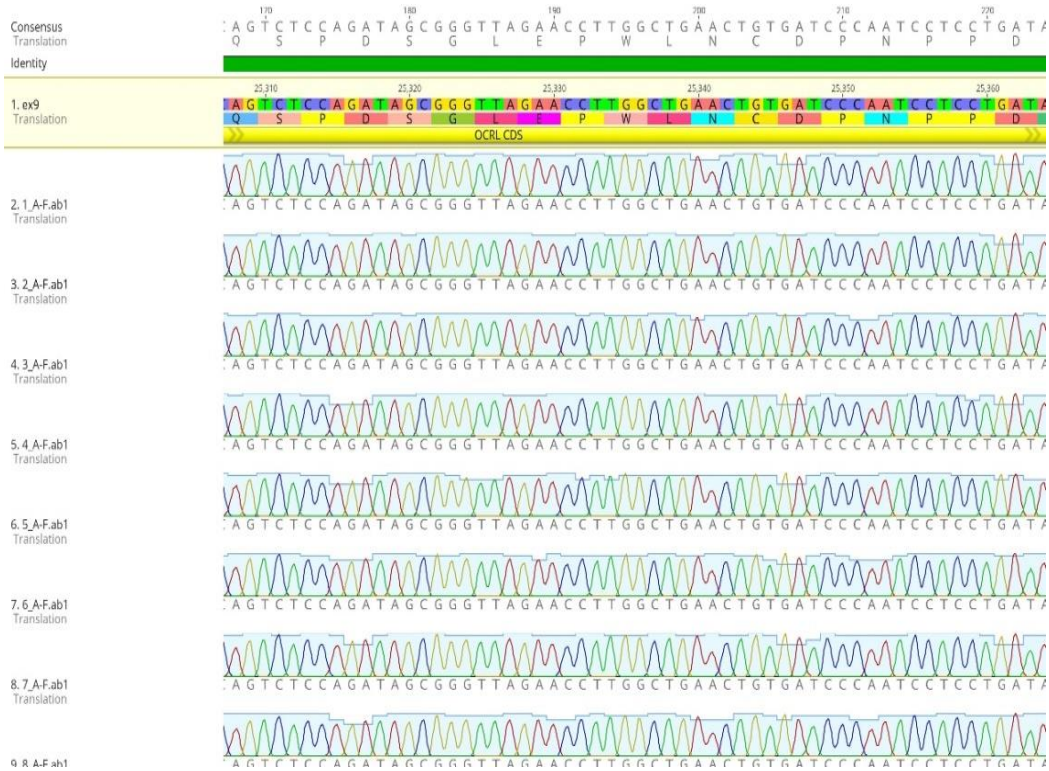

Figure 6: The chromatograms show the results of the sequencing of the *OCRL* gene, and the results showed no variation in exon 9.

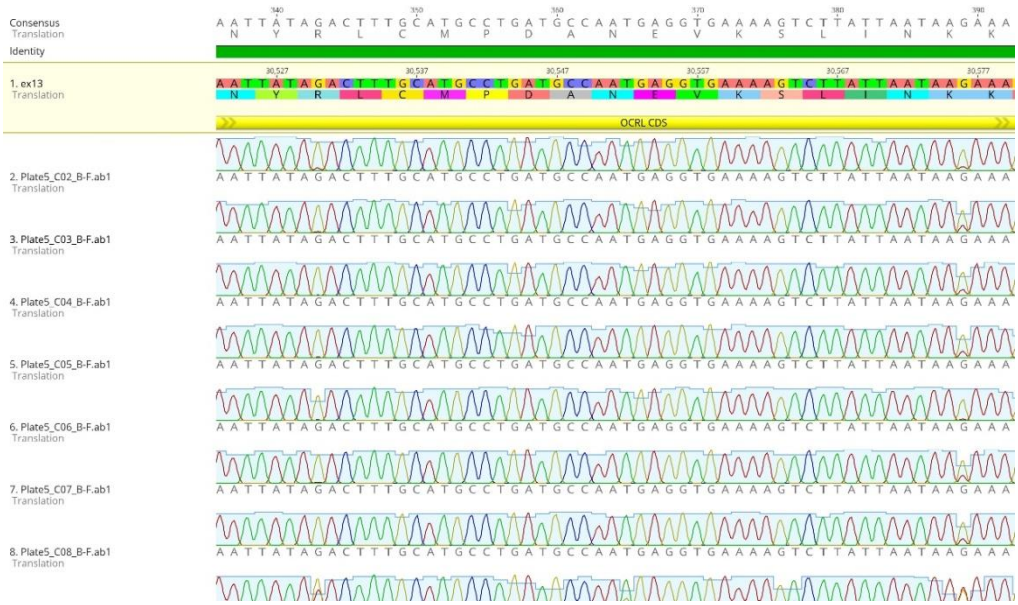

Figure 7: The chromatograms show the results of the sequencing of the *OCRL* gene, and the results showed no variation in exon 13.

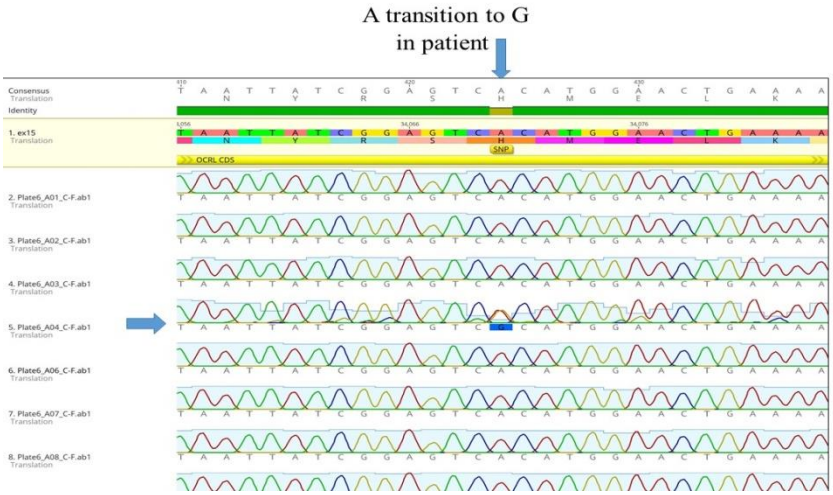

Figure 8: The chromatograms show the results of the sequencing of the *OCRL* gene (exon 15). The genotype was A/A. Also, chromatograms show p.516A>G. The amino acid histidine (His) was changed to arginine (Arg) at position 516.

The frequency of alleles that appeared in a sequence of patients and control groups in the exons 9, 13, and 15 in Table 5.

Table 5 : Genotype and allele frequency of *OCRL* gene, exon (9,13,15) in control and patient groups**.**

Genotype	Control	Kidney stone patients	Kidney failure patients
A/A	40(100%)	40(100%)	39(39.01%)
A/G	0(0%)	0(0%)	1(0.99%)
G/G	0(0%)	0(0%)	0(0.01%)
Total	40	40	40
P-value	---	---	0.93
Allele	**Frequency**		
A	239(99.58%)		
G	1(0.42%)		

DNA sequencing showed that the patient had heterozygote alleles (A/G) compared with normal individuals that had (A/A), as shown in Figure 9.

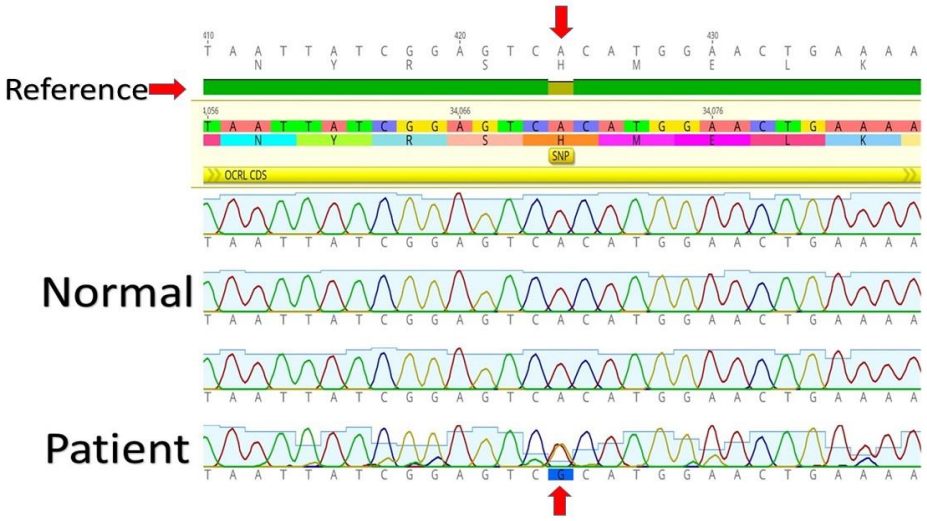

Figure 9: DNA sequencing of the *OCRL* gene (Exon 15) indicated a missense mutation (p.516 A>G) in patient 4, suffering from ESKF, and this SNP is not registered in the NCBI.

The mutation caused alteration in the resulting protein in the patient, leading to alteration of base number 516 from His to Arg.

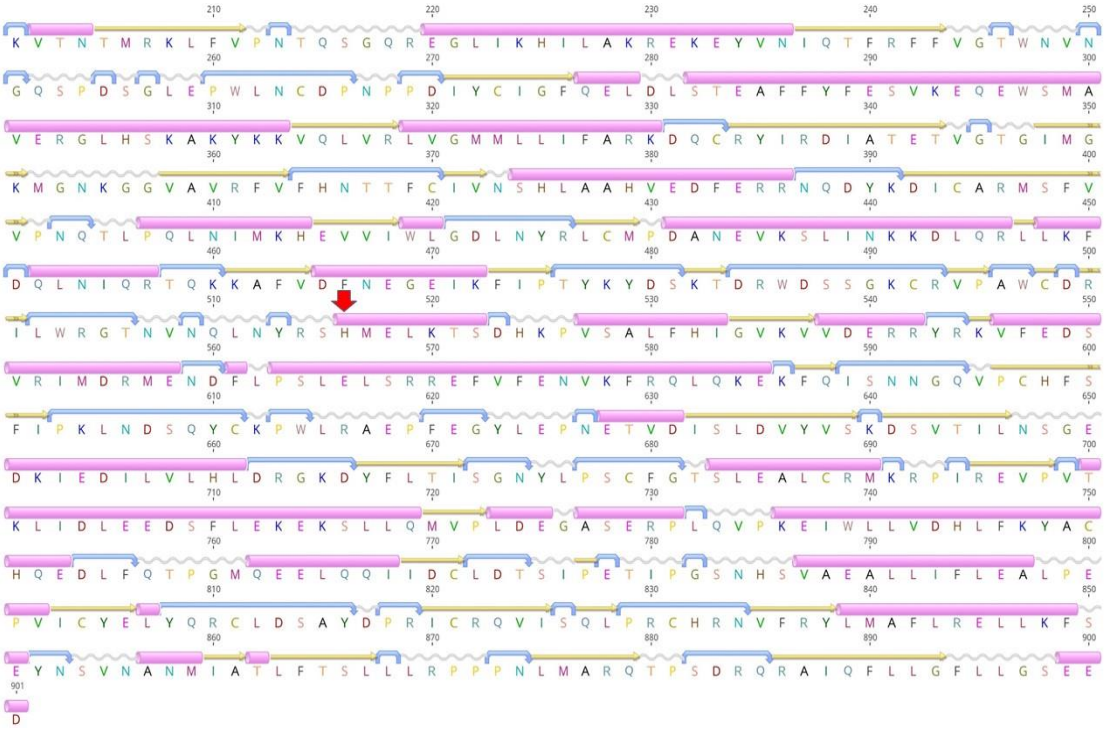

Figure 10: The tertiary protein structure by Protein Data Bank (PDB) shows the normal protein sequence, and the red arrow indicates the amino acid (histidine) in the normal protein.

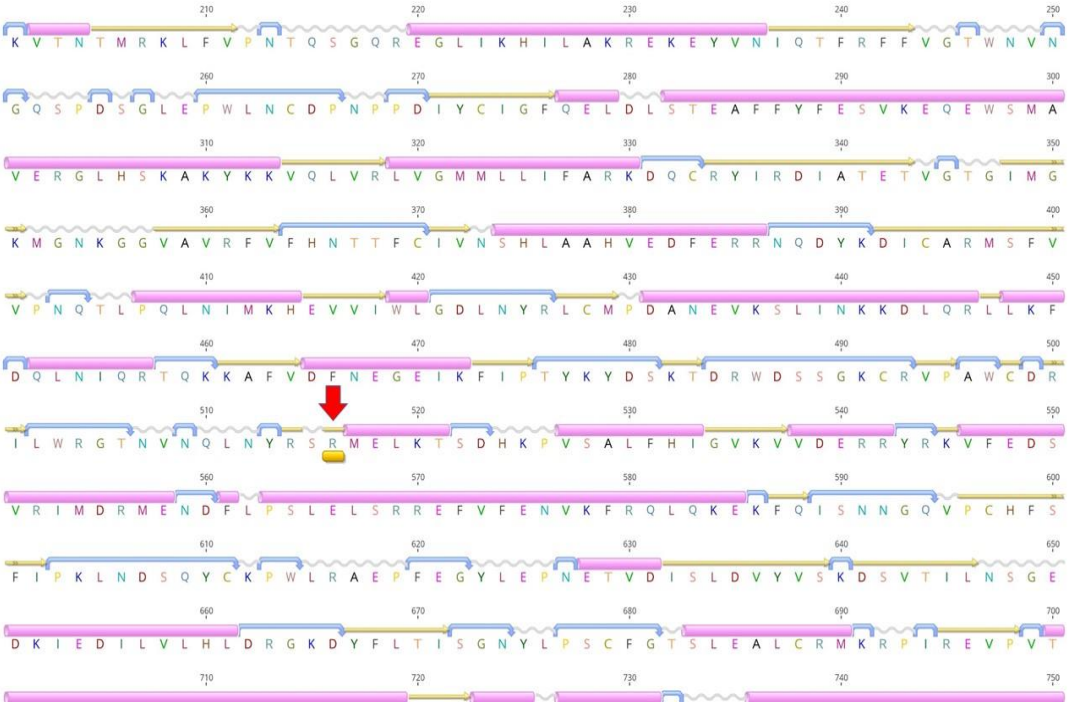

Figure 11: The tertiary protein structure by Protein Data Bank (PDB), the red arrow indicates the mutant amino acid, the histidine H in normal protein changed to arginine R in the patient.

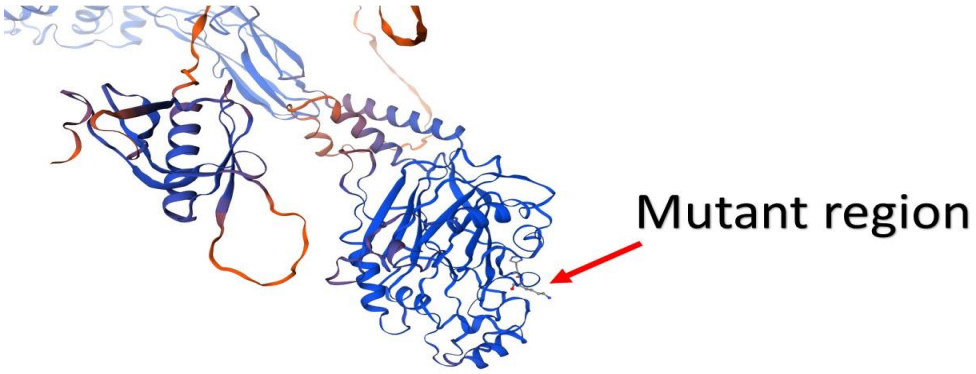

Figure 12: The 3D structure of the protein shows the position of the mutant amino acid, predicted by the SWISS- MODEL website [16].

Sequencing results also indicated two intronic single-nucleotide substitutions (c.1359+251G>C) and (c.1359+262G>A), and in the intron before exon 13 in a 55-year-old male patient with ESKF, all variants were denoted based on the NCBI reference sequence for *OCRL* (NM_001318784.2) and (NM_001318784.2), respectively. In addition to the appearance of another two unregistered variations.

4. Discussion:

This study highlights the association between polymorphisms in the *OCRL* gene and the prevalence of recurrent kidney stones, as well as their potential role in end-stage kidney failure among patients. Genetic variants, particularly single-nucleotide polymorphisms (SNPs), may significantly influence the risk of developing renal stone disease and related complications. This study included 120 participants, including 40 healthy individuals, 40 patients with end-stage kidney disease (ESKD), and 40 with recurrent kidney stones. According to sex, the males were the highest in both patients' groups; in recurrent kidney

stones were 24 (60%) males, and in ESKF were 28 (70%) compared with 14 (40%) and 12(30%) females in both groups, respectively. Globally, it was proved that CKD is more prevalent in females than males, but mortality rates are higher in males than women with kidney diseases [17]. In the Middle East, mortality rates are the highest in women for reasons related to socioeconomic, cultural norms, and comitative family essentialities [18].

In this study, it was found that the prevalence of ESKF in the age group 1-19 years was the highest compared with other age groups. In children and adolescents, kidney failure is related to congenital kidney disease and urinary tract, including large ranges of anomalies such as cystic/hereditary/congenital disorders, vasculitis, focal segmented glomerulosclerosis, renal hypoplasia/dysplasia, Wilms tumor, hematological anomalies, congenital obstructive uropathies, systemic lupus erythematosus and unspecified with renal failure [19].

In adults, it was believed that testosterone worsens CKD in men for its effect on eGFR outcomes. Moreover, high serum testosterone induces cellular apoptosis and proinflammation via the production of reactive oxygen species, which may cause cell damage [20]. On the other hand, estrogen may have a protective role against the deterioration of kidney parenchyma in females at reproductive age through several mechanisms, including vasodilation of kidney tubes, production of nitric acid, mitigation of inflammatory responses, and ischemia mediators. Also, estrogen protects women of reproductive age from kidney stones by increasing citrate levels and reducing urinary calcium excretion [21]. Lifestyle factors such as diet, physical activity, and substance use (e.g., smoking and alcohol) may further account for these differences, as well as, genetic factors influencing kidney disease may also be differentially expressed in males and females [22]. The highest prevalence of recurrent kidney stones was detected in (47.5%) of 25 of the patients aged 40-59 years, potentially due to increased metabolic activities that lead to elevated calcium and oxalate excretion. Age is a risk factor for recurrent kidney stones in stone-former individuals, as it was previously recognized that the incidence of recurrent kidney stones is higher in older men than in women. It's often associated with cardiovascular, bone fractures, diabetes, and chronic kidney diseases and vice versa, and these systemic health conditions lead to the recurrence of kidney stones as well as metabolic disorders that affect calcium metabolism and thyroid hormone balance [23].
Routinely, serum levels of urea, creatinine, and uric acid were used to monitor impaired kidney function. Creatinine levels are the most significant biomarker used to assess the glomerular filtration rate (GFR) [24]. Urea and creatinine are the end product of endogenous tissue protein turnover and dietary portions. While uric acid is the end product of purine metabolism. In this study, the serum levels of urea, creatinine, and uric acid were significantly high in the end-stage kidney failure disease 140.35±6.55, 8.69±0.5, 8.42±0.23 ml/dl respectively compared with 33.04±1.8, 0.98±.095 and 6.30±0.21 mg/dl in recurrent kidney stone former and 27.07±1.13, 0.69±0.04 and 4.53±0.14 mg/dl in healthy controls as shown in Table 3. The breakdown in kidney function in CKD is a reflection of the severe damage in the renal tubulointerstitial cells caused by the long toxic effect of low molecular weight portions [25, 26], during the prolonged inflammation, high reactive oxygen species (ROS), lysosomal dysfunction, and endoplasmic reticulum stress [27].

The study indicates that recurrent kidney stone formers exhibit higher serum calcium levels than healthy controls and ESKF patients, which is associated with a significant decrease in Vit. D deficiency in patients with recurrent kidney stones and end-stage kidney failure (ESKF). Vitamin D_3 plays a crucial role in calcium/ phosphorus balance and bone minerals context by regulating the intestinal absorption of Ca^{2+} and its urinary excretion. It

was reported that the deficiency of 1,25-dihydroxy D3 in association with normal to mild elevated parathyroid hormones may cause hypercalcemia and disrupt Ca^{2+} regulation in patients with recurrent kidney stones due to bone resorption [28, 29] compared to healthy individuals. In ESKF patients, the decrease of Ca^{2+} is highly associated with high phosphate levels in the bloodstream, reducing free calcium levels [30].
Also, in this study, the enzyme phosphatidylinositol 4,5-bisphosphate (PIP2) levels were found to be significantly elevated in patients with recurrent kidney stones and ESKF than in control sera. These results may be associated with the proviral role of PIP2 in the pathophysiology of renal tubular cells and their recovery process [31]. This elevation in PIP2 levels in patients' sera may be a reflection of its protective role in the restoration of renal tubular epithelial functions post-injury to maintain renal tubular hemostasis and enhance cellular survival during hypoxia conditions in prolonged inflammation to reduce both inflammatory cell infiltrations and proinflammatory cytokine expression [32]. Furthermore, elevated levels of PIP2 could be linked to the upregulation of gene expression of kidney tissue repair post-injury [31].

This study highlights the possible role of mutations or SNPs in *OCRL* gene exons with elevated enzyme levels in renal dysfunction. Exons 9,13, and 15 were amplified and the PCR product was directly sequenced. No mutations or polymorphism were found in exons 9 and 13 in both patients and control groups. Two intronic single-nucleotide substitutions (c.1359+262G>A) and (c.1359+251G>C) were found in the intronic region of 13 in a 55-year-old male patient with kidney failure. These variants were identified by NCBI reference sequence for *OCRL* (NM_001318784.2) and (NM_001318784.2), respectively.

The impact of intronic variations in the *OCRL* gene is related to their effect on the splicing and editing of the transcripts, which leads to diverse phenotypic manifestations of Lowe syndrome. It was found that the splicing site at c.939+3A>C and c.2469+1G>A, disrupt the normal splicing, leading to exon skipping and the production of truncated protein. The splicing site at c2257-5G>A creates a cryptic splicing site, resulting in incomplete splicing and generating a premature termination codon [31,32].

A missense variant in exon 15, c.1548A>G, p. (His516 Arg), the frequency of an allele (G) in all participants was 1 (0.42%). This exonic mutation was detected in a female patient, 18-year-old, show the phenotypes of Lowe Syndrome as she exhibited kidney failure related to polycystic kidney disease, mental retardation, slow growth, and retinal problems that affected vision, in addition to a family history of kidney diseases including kidney stones, kidney cysts, and finally kidney failure. she may be inheriting the defective alleles from one of the parents as she has a family history of kidney disease. The detected exonic mutation p. Mutation of His 516Arg was not reported previously, and its effect on the phenotype is unclear. More than 250 exonic and mutations represented as frameshifts, nonsense, missense, insertion, duplication, and deletion were reported in 1-7 and 8-23 exons in Dents and Lowe syndromes, respectively. Lowe syndrome patients mainly exhibit mutation along exons 9-15 that encode for the 5-phosphatase catalytic domain of the protein, which may affect the enzyme activity in the patient [11, 33]. Nephrolithiasis and progressive renal insufficiency were the most common phenotypes associated with exon 15 mutations [34].

In addition, further investigations and exome sequencing are required for better molecular diagnosis of genetic variations in exons other than the targeted exons in this study.

5. Conclusions:

This study showed significant differences in the PIP2 levels in patients with kidney stones and kidney failure compared to healthy people, as the enzyme showed an indirect effect in patients with kidney stones by affecting calcium balance. As for genetic variations, a missense mutation appeared in a patient suffering from kidney failure on exon 15, which led to a change in the type of protein, in addition to four mutations in the intron before exon 13 in a patient also suffering from kidney failure and thus may have a relationship to pathology.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Ethical Clearance

The Institutional Scientific Committee at the University of Baghdad approved this study (88784 on 29/11/2023) according to the Declaration of Helsinki for human studies, which is consistent with the instructions of the Iraqi Ministry of Health and Environment.

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