# Genetic polymorphisms in CLDN14 (rs219780) and ALP (rs1256328) genes are associated with risk of nephrolithiasis in Egyptian children

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## ABSTRACT

**Objective:** Nephrolithiasis results from metabolic and anatomic abnormalities together with genetic factors. Claudin 14 (CLDN14) is a protein that regulates the passage of small solutes through the kidneys. Alkaline phosphatase (ALPL) hydrolyzes the pyrophosphate to free phosphate, proposing its enabling role in nephrolithiasis development. Solute carrier family 13 member 2 (SLC13A2) encodes Na+-Pi cotransporter 2a, which is responsible for the renal absorption of phosphate. We aimed to detect the association between CLDN14, ALPL, and SLC13A2 genetic variants and susceptibility to nephrolithiasis in the Egyptian pediatric population.

**Material and methods:** We enrolled 204 consecutive pediatric patients with nephrolithiasis, and 126 normal individuals served as controls. Real-time polymerase chain reaction analysis of CLDN14 rs219780, ALPL rs1256328, and SLC34A1 rs11746443 single-nucleotide polymorphisms (SNPs) was performed.

**Results:** We found that individuals carrying the T allele of CLDN14 rs219780 and ALPL rs1256328 SNPs had a significantly higher risk of nephrolithiasis than the controls (p=0.001 and 0.001, respectively). Genetic association analyses identified that CLDN14 rs219780 and ALPL rs1256328SNPs were significantly associated with the nephrolithiasis status (odds ratio [OR] =4.51; 95% confidence interval [CI]=2.758–7.374; p=0.001 and OR=6.088; 95% CI=3.651–10.152; p=0.001, respectively). The sequence variant ALPL rs1256328 T allele had a significant correlation with the increased serum alkaline phosphatase levels in children with nephrolithiasis (p=0.02). No significant association was found between SLC34A1 rs11746443 SNP and the risk of nephrolithiasis (p=0.5).

**Conclusion:** CLDN14 rs219780 and ALPL rs1256328 SNPs might raise the risk of nephrolithiasis in Egyptian children, and might be used as genetic markers in these patients.

**Keywords:** children; CLDN 14; ALPL; SLC13A2; gene polymorphisms; nephrolithiasis; real-time polymerase chain reaction.

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## Introduction

Nephrolithiasis is highly prevalent globally with varying ranges.[1] Because of the increasing rate of recurrence of nephrolithiasis, the recommendations of the American Urological Association[2] and the European Association of Urology[3] were to manage and prevent future recurrences by dietary control and medical treatment. Nephrolithiasis occurs when the urine supersaturates with salts like calcium oxalate or calcium phosphate and when the urinary concentrations of the natural inhibitors of stone formation, such as pyrophosphate, are minimum.[4-7] For instance, studies have elucidated that the family history of nephrolithiasis increases the relative risk of occurrence by 2.57-fold in men and by 32.4% in monozygotic twins.[8-11]

Single-nucleotide polymorphisms (SNPs) play a pivotal role in defining the genes associated with nephrolithiasis that may be employed as future diagnostic markers.[12] Claudin14 (CLDN14) is a member of the claudin family of the membrane proteins, which regulate the paracellular passage of ions and small solutes through the epithelial tight junctions. It is ex-

Turk J Urol 2021; 47(1): 73–80
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pressed in the proximal tubules, loop of Henle, and the epithelia of several other organs, and it has been detected to selectively decrease the permeability of Ca2+ through tight junctions.[13] CLDN14 expression is substantially upregulated by activation of calcium-sensing receptor (CASR) because CASR regulates Ca++ transport through changes in the transepithelial potential and in the paracellular channel permeability. CASR activation increases the gene expression of CLDN14 in the ascending tubules of the loop of Henle. Furthermore, impairment of the regulation of the renal CASR-CLDN14 pathway could lead to nephrolithiasis.[14] A genome-wide association study (GWAS), in which the entire human genome was mapped by Thorleifsson et al.[13] concluded that the CLDN14 SNPs rs219780 and rs219781 in exon 7 were associated with nephrolithiasis in patients from Iceland and the Netherlands.

The human alkaline phosphatase (ALPL) gene located at 1p36.1-34 consists of 12 exons and encodes tissue nonspecific ALPL, which is closely related to the activity of serum alkaine phosphatase enzume (ALP). Mutations in the ALPL gene usually cause typical insufficient mineralization of hard tissue, such as bone and tooth, and increase the level of calcium in the blood. These findings may offer a new insight into the pathogenesis of nephrolithiasis. In addition, it is expressed in the kidney and proximal tubules and hydrolyzes pyrophosphate to free phosphate, proposing its enabling role in nephrolithiasis development.[15]

Solute carrier family 13 member 2 (SLC13A2), which is located on the chromosome 5q35.3, encodes sodium-dependent phosphate transport protein 2A, also known as Na+-Pi cotransporter 2a (NPi2a), which is responsible for renal absorption of phosphate at the apical membrane of the renal proximal tubular cells.[16] Several studies have demonstrated that rare variants, such as SLC34A1, seemed to be correlated with hypophosphatemic nephrolithiasis and osteoporosis in humans.[15]

**Main Points:**

- The T allele of claudin 14 (CLDN14) rs219780 and alkaline phosphatase (ALPL) rs1256328 single-nucleotide polymorphisms (SNPs) had a significantly higher risk of nephrolithiasis in patients than in controls.
- CLDN14 rs219780 and ALPL rs1256328 SNPs were significantly associated with the kidney stone disease status.
- The sequence variant ALPL rs1256328 T allele had a significant correlation with the increased serum ALPL levels in the pediatric population with nephrolithiasis.
- No significant association was found between the solute carrier family 13 member 2 rs11746443 SNP of Na+-Pi cotransporter 2a and risk of nephrolithiasis.
- CLDN14 rs219780 and ALPL rs1256328 SNPs might genetic markers for nephrolithiasis in children.

We aimed to detect some potential causative genes (CLDN14, ALPL, and SLC34A1) to evaluate their associations with the risk of nephrolithiasis in children and determine their relationships with each other to help in the future establishment of molecular targeted therapy. We assessed the relationship of these gene polymorphisms with the biochemical markers of calcium–phosphate metabolism, purine metabolism, kidney function, and acid–base and ion homeostasis in pediatric patients with nephrolithiasis.

## Material and methods

All the participating children's parents gave their informed consent before the blood sampling. Ethical approval for this study was given by the ethical committee of the National Research Centre (NRC), (AR110504), Cairo, Egypt.

### Patients

We enrolled 204 children with nephrolithiasis who presented to the pediatric nephrology unit, Cairo University Children Hospital, Cairo, Egypt. This case-control study was conducted from March 2018 to August 2019. A thorough medical record review was performed for all children to confirm the diagnosis of nephrolithiasis, and a detailed family history suggestive of nephrolithiasis was obtained. Children younger than 18 years and presenting with gross hematuria, renal colic, recurrent urinary tract infection (UTI), spontaneous passage of renal stone, or evidence of stone (>3 mm in diameter) documented by plain X-ray, renal ultrasound, or abdominal computed tomography (CT) without contrast in selected cases were included in the study. The urinary stones were chemically analyzed if they were spontaneously passed or became available after surgery. Nephrolithiasis was defined as a calcium stone when infrared spectrometric analysis reported that it was made of calcium oxalate or calcium phosphate or a mixture of these besides some other elements, such as ammonium and magnesium. The exclusion criteria were patients with asymptomatic nephrolithiasis, only calcification, presence of any extrarenal manifestations, or who were taking any drug affecting electrolyte (steroids, vitamin D, and so on). A recurrent episode was defined as a new stone development occurring at least 6 months after the first stone event.

All controls (n=126) were healthy with no clinical signs and no family history of renal disease as assessed by medical history and clinical examination; furthermore, they were not taking any medicines at the time of the study. All the controls had normal serum creatinine and calcium concentrations. Healthy controls were age-matched and sex-matched to the patients, and they were within the same body mass index (BMI) limits. They were chosen from the pediatric clinic (apart from the centre of excellence) of NRC, which is one of the biggest research centers in Egypt.

**Biochemical parameters**

In patients with nephrolithiasis and healthy controls, we measured the serum concentrations of creatinine, calcium and ALP, urinary calcium/creatinine ratio, urinary oxalate/creatinine ratio, urinary phosphate/creatinine ratio, and urinary uric acid/creatinine ratio.

**Molecular analysis**

DNA was extracted using the QIAamp DNA Blood Mini Kit-50 (catalog no. 51104) supplied by Qiagen, Hilden, Germany. The DNA concentration was determined using the Nano-Drop 2000c Spectrophotometer (Thermo Fisher). CLDN14, ALPL, and SLC34A1 polymorphisms were detected by polymerase chain reactions (PCRs) using the Rotor-Gene Q PCR system (Qiagen, Germany). CLDN14 (rs219780 C/T), ALPL (rs1256328 C/T), and SLC34A1 (rs11746443 G/A) SNPs were evaluated using the TaqMan genotyping protocol (Applied Bio-systems, Foster City, CA, USA). PCR assays were carried out in a 20-μL reaction volume, including 20–30 ng DNA, 10 μL TaqMan Universal PCR Master Mix, and 0.5 μL of SNP assay in 0.1 real-time PCR tube. The PCR assay was carried out according to the manufacturer's instructions, which included a single step of 10 min at 95°C, 40 cycles of DNA denaturation at 95°C for 15 sec, and annealing/extension at 60°C for 1 min. The final products were analyzed by Rotor-Gene Q Software 2.3.1.49.

**Statistical analysis**

The data were analyzed using the Microsoft Excel 2010 and the Statistical Package for the Social Science version 24.0 for Windows (IBM SPSS Corp.; Armonk, NY, USA). $P<0.05$ was considered statistically significant. Previous data showed that the probability of treatment failure among patients is 0.34, and the correlation coefficient for exposure between matched experimental data was 0.4. If the true odds ratio (OR) for failure in patients is 2.5, we will need to study 102 controls and 204 patients to be able to reject the null hypothesis that this OR equals to 1 with a probability (power) of 0.8. The type I error probability associated with the test of this null hypothesis is 0.05 (power and sample calculation). To compare the means of normally distributed variables between the groups, Student's t-test was performed, Mann–Whitney U test was used for non-normal variables, and the chi-square test or Fisher's exact test was used to determine the distribution of categorical variables between the groups. The Spearman's rank correlation coefficient (r) was determined to show the correlation between different parameters in this study. Effect modifications were evaluated by stratification, and statistical interaction was assessed by including the main effect variables and their product terms in the logistic regression model. The Hardy–Weinberg (H–W) model for SNP genotypes was used for comparing the observed and expected SNP genotypes. SNP genotype frequencies were calculated under the assumption of H–W equilibrium (HWE). Chi-square test was used to compare the observed genotype frequency distributions of the SNPs' antigens with the expected ones under the HWE.

**Haplotype analysis**

The data were analyzed using haplotype analysis software v1.05, which is the software written in visual basic for applications within the Microsoft Excel. The population genetic structure of the population samples (interpopulation analysis) was computed by determining the Nei's minimum genetic distance and genetic differentiation among the populations and contribution of each of them to the total diversity.

## Results

The general characteristics of children with nephrolithiasis and the controls are shown in Table 1. The patients and controls were age matched; the mean age of patients was 5.0±3.0 years and that of controls was 5.0±6.0 years. The disease was more common in men (68.6%). The median duration of disease was 9 months. The most common presenting symptom was renal colic (86 [42.2%]), followed by microscopic or macroscopic hematuria (64 [31.4%]) and anuria (60 [29.4%]). Of the 94 patients, 46.1% (n=21) had a history of recurrent UTI. Approximately 57% of patients had consanguineous parents, and 50% of patients had a family history of nephrolithiasis.

Therefore, the first-line treatment was a low-sodium diet and plenty of fluid intakes for all patients with nephrolithiasis. Potassium citrate and citric acid (Bicitra) (1 mEq/kg/day) were given to 100 patients to alkalinize the urine in terms of calcium oxalate stones and uric acid stones, whereas pyridoxine (5 mg/kg/day) was prescribed for patients with suspected hyperoxaluria (who had high oxalate/creatinine ratio but not confirmed with genetic analysis). Ascorbic acid (500 mg/day) was used for patients with hyperphosphaturia. Specific treatment with thiazide (1.5 mg/kg/day) was used to treat hypercalciuria.

**Biochemical parameters**

Significant differences in the serum biochemical levels were found between patients with nephrolithiasis and controls with regard to serum creatinine 2.1±1.2 mg/dL vs. 0.78±0.35 mg/dL, p=0.01; potassium, 5.2±2.8 mEq/L vs. 3.7±0.8 mEq/L, p=0.04; phosphorous, 5.7±3.1 mg/dL vs. 3.9±0.58 mg/dL, p=0.04; ALP, 250.7±120 IU/L vs. 38.9±1.62 IU/L, p=0.001; hemoglobin, 11.0±1.5 g/dL vs. 14.2±1.5 g/dL, p=0.03; and white blood cells, $14.2\pm1.5\times10^3/mm^3$ vs. 3.57±1.42×103/mm–3, p=0.01. Urinary crystals were found in 102 patients (50%).

**SNP selection and genotyping**

The studied genotype frequencies of the 3 polymorphisms in the controls agreed with HWE (p>0.05) (Table 2).

Turk J Urol 2021; 47(1): 73–80
10.5152/tud.2020.20141

**Table 1. General characteristics and biochemical parameters of children with nephrolithiasis and controls**

| | Controls (n=126) | Patients (n=204) | p |
|---|---|---|---|
| Age (years) | 5±6 | 5±3 | 0.11 |
| Sex, M/F | 54 (42.9%)/72(57.1%) | 140 (68.6%)/64 (31.4%) | 0.001* |
| *Duration(months) | / | 9 (2–24) | NA |
| eGFR (mL/min/1.73 $m^2$) | 104±65.2 | 103±60.1 | 0.12 |
| Family history (calculi), n (%) | | 102 (50%) | |
| Creatinine (mg/dL) | 0.78±0.35 | 2.1±1.2 | 0.01* |
| Na (mmol/L) | 137.7±10.6 | 137.6±10.2 | 0.8 |
| K (mmol/L) | 3.7±0.8 | 5.2±2.8 | 0.04* |
| Ca (mg/dL) | 10.91 ±0.4 | 10.2±5.8 | 0.9 |
| Po4 (mg/dL) | 3.9±0.58 | 5.7±3.1 | 0.04* |
| ALP (U/L) | 38.9±1.62 | 250.7±120 | 0.001** |
| Uric acid (mg/dL) | 2.77±1.34 | 3.5±1.7 | 0.09 |
| HB (g/dL) | 14.2±1.5 | 11.0±1.5 | 0.03* |
| WBC ($\times10^3/mm^3$) | 3.57±1.42 | 8.0±2.8 | 0.01* |
| Urinary crystals | | 102 (50%) | |

Data were represented mean±standard deviation, frequency, and percentage or median with interquartile range (25%–75%) as applicable. The T-test was used for statistical analysis. eGFR rate calculated according to the Schwartz-equation (eGFR=0.413 L/Scr). *p<0.01 is significant, **p<0.001 is highly significant. eGFR: estimated glomerular filtration; Na: sodium; K: potassium; Ca: calcium; Po4: phosphate; ALP: alkaline phosphatase; HB: hemoglobin; WBC: white blood cell

**Variant at CLDN14**

We found significant differences in the genotype distribution of CLDN14 rs219780 SNP between the patients and the controls (p<0.05). The frequencies of the CC, CT, and TT genotypes were 40 (19.6%), 164 (80.4%), and 0 (0%) among children with nephrolithiasis and 66 (52.4%), 60 (47.6%), and 0 (0%) among controls, respectively (p=0.001). By logistic regression analysis, when utilizing the CLDN14 rs219780 CC genotype as the reference, the CT (p=0.001, adjusted OR=4.51, 95% confidence interval [CI]=2.758–7.374) genotype was associated with a significantly higher risk of nephrolithiasis than the CC genotype. Moreover, a significantly higher risk was detected in the combined genotypes CT+TT than in the CC genotype (p=0.001, adjusted OR=4.51, 95% CI=2.758–7.374).The rs219780 T allele frequency was 164 (0.402) among the patients and 60 (0.238) among the controls (p=0.001, adjusted OR=2.151, 95% CI=1.514–3.055).

**Variant at ALPL**

We confirmed significant differences in the genotype distribution of ALPL rs1256328 SNP between the patients and the controls (p<0.05). The frequencies of the CC, CT, and TT genotypes were 36 (17.6%), 162 (79.4%), and 6 (2.9%) among children with nephrolithiasis and 69 (54.8%), 51 (40.5%), and 6 (4.8%) among the controls, respectively (p=0.001). By logistic regression analysis, when utilizing the ALPL rs1256328 CC genotype as the reference, the CT (p=0.001, adjusted OR=6.088, 95% CI=3.651–10.152) genotype was associated with a significantly higher risk of nephrolithiasis than the CC genotype. Moreover, a significantly higher risk was detected in the combined genotypes CT+TT than in the CC genotype (p=0.001, adjusted OR=5.649, 95% CI=3.417–9.338). The rs1256328 T allele frequency was 164 (0.402) among the children with nephrolithiasis and 63(0.250) among the controls (p=0.001, adjusted OR=2.231, 95% CI=1.578–3.153).

**Variant at SLC34A1**

We found no significant association between the SLC34A1 rs11746443 SNP of NPi2a and risk of nephrolithiasis (p=0.5).

Gene diversity within each population was 0.417 in controls and 0.461 in children with nephrolithiasis. This result confirmed that the patients and the controls are genetically matched, and it supports our findings about the role of these 2 significant polymorphisms in the risk of occurrence of nephrolithiasis in children.

The only significant association was that the sequence variant ALPL rs1256328 T allele had a significant correlation with increased serum ALP levels (CC vs. CT+TT: 207.0±119.6 vs. 261.3±160.6; p=0.02).

Haplotype association analyses were then carried out to evaluate the effect of the association of the 3 studied SNPs on the risk of nephrolithiasis as shown in Table 3.

**Table 2. Association of the 3 studied SNPs and the risk of nephrolithiasis**

| SNPs | | Controls (n=126) | Nephrolithiasis (n=204) | p | OR | 95% CI | p |
|---|---|---|---|---|---|---|---|
| rs11746443 G/A | AA | 6 (4.8%) | 6 (2.9%) | 0.4 | 1 (Reference) | | |
| | GA | 60 (47.6%) | 94 (46.1%) | 0.8 | 1.733 | 0.535–5.615 | 0.4 |
| | GG | 60 (47.6%) | 104 (51%) | 0.7 | 1.567 | 0.483–5.083 | 0.5 |
| | GA+AA | 66 (52.4%) | 100 (49%) | 0.7 | 1.144 | 0.733–1.784 | 0.6 |
| Allele | A | 72 (0.286) | 106 (0.26) | 0.5 | 1 (Reference) | | |
| | G | 180 (0.714) | 302 (0.74) | 0.7 | 1.14 | 0.802–1.62 | 0.5 |
| rs219780 C/T | CC | 66 (52.4%) | 40 (19.6%) | 0.001** | 1 (Reference) | | |
| | CT | 60 (47.6%) | 164 (80.4%) | 0.001** | 4.51 | 2.758–7.374 | 0.001** |
| | TT | 0 (0%) | 0 (0%) | N.A | - | - | - |
| | CT+TT | 60 (47.6%) | 164 (80.4%) | 0.001** | 4.51 | 2.758–7.374 | 0.001** |
| Allele | C | 192 (0.762) | 244 (0.598) | 0.01* | 1 (Reference) | | |
| | T | 60 (0.238) | 164 (0.402) | 0.001** | 2.151 | 1.514–3.055 | 0.001** |
| rs1256328 C/T | CC | 69 (54.8%) | 36 (17.6%) | 0.001** | 1 (Reference) | | |
| | CT | 51 (40.5%) | 162 (79.4%) | 0.001** | 6.088 | 3.651–10.152 | 0.001** |
| | TT | 6 (4.8%) | 6 (2.9%) | 0.4 | 1.917 | 0.577–6.371 | 0.3 |
| | CT+TT | 57 (45.3%) | 168 (82.3%) | 0.001** | 5.649 | 3.417–9.338 | 0.001** |
| Allele | C | 189 (0.75) | 234 (0.574) | 0.01* | 1 (Reference) | | |
| | T | 63 (0.25) | 174 (0.426) | 0.001** | 2.231 | 1.578–3.153 | 0.001** |

*p≤0.05, significant; **p≤0.01, highly significant. OR: odds ratio; CI: confidence interval; SNP: single-nucleotide polymorphism

**Table 3. Summary of haplotype association analysis in patients with nephrolithiasis and controls**

| | Controls | | Nephrolithiasis | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Haplotype association** | **n** | **%** | **n** | **%** | **p** | **OR** | **95% CI** | **p** |
| GCC | 63 | 50 | 102 | 50 | 0.9 | 1 (reference) | | |
| Specific haplotypes for controls | | | | | | | | |
| GCT | 15 | 11.9 | 8 | 3.9 | 0.001** | 0.329 | 0.132–0.821 | 0.017* |
| ACC | 12 | 9.5 | 5 | 2.5 | 0.001** | 0.257 | 0.087–0.765 | 0.015* |
| ATC | 15 | 11.9 | 6 | 2.9 | 0.001** | 0.247 | 0.091–0.67 | 0.006** |
| Specific haplotypes for nephrolithiasis | | | | | | | | |
| GTT | 7.5 | 6 | 37 | 18.1 | 0.001** | 2.857 | 1.25–6.526 | 0.013* |
| ATT | 3 | 2.4 | 35 | 17.2 | 0.001** | 7.206 | 2.127–24.412 | 0.002** |

*p<0.01 is significant, **p<0.001 is highly significant. The data represented as frequency and percent for the studied single-nucleotide polymorphism associations (haplotypes of 3 investigated polymorphisms: solute carrier family 13 member 2 rs11746443, claudin 14 rs219780 C, and alkaline phosphatase rs1256328).

Both CLDN14 rs219780 and ALPL rs1256328 SNPs were positively correlated with recurrent UTI (r=0.209, p=0.003 and r=0.180, p=0.01, respectively) and ureteric stones (r=0.145, p=0.038 and r=0.151, p=0.031, respectively). The 3 studied SNPs (CLDN14 rs219780, ALPL rs1256328, and SLC34A1 rs11746443) were correlated in the presence of crystals in the urine (r=0.148, p=0.034; r=0.161, p=0.022; and r=0.179, p=0.011, respectively). A positive correlation was found between CLDN14 rs219780 SNP and sex (r=0.241, p=0.0001).

Turk J Urol 2021; 47(1): 73-80
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## Discussion

Several procedures are used for the surgical treatment of nephrolithiasis. Treatment strategies are mainly based on stone location and size and the patient's comorbidities and preferences. Recognizing the genetic predisposition of nephrolithiasis allows the clinicians to decide which individuals would require rigorous evaluation and screening and may potentially alter nephrolithiasis management in the future.[1]

Candidate gene association studies have attempted to assess the role of several genes involved in calcium homeostasis on nephrolithiasis.[12] In this study, we identified the intronic SNPs of the genes CLDN14 rs219780 and ALPL rs1256328 to be significantly associated with nephrolithiasis in Egyptian children. Our haplotype analysis further confirmed the association of the SNPs with the risk of nephrolithiasis; individuals with ATT>T haplotype had approximately 7-fold increased risk of nephrolithiasis. These results supported the relevance of the minor allele T at CLDN14 rs219780 and ALPL rs1256328 for nephrolithiasis risk in children.

Calcium nephrolithiasis is one of the most prevalent uronephrologic disorders worldwide, and its treatment is considered to be of high cost by urologists.[1] It is a heterogeneous disease owing to multiple genetic or environmental factors that regulate calcium salt precipitation in the urinary system. Idiopathic hypercalciuria is the most common risk factor.[1] Both nephrolithiasis and hypercalciuria can be caused by mutations in a single gene required for renal calcium excretion.[1]

Several genetic markers, including the polymorphisms of genes coding for the CASR and CLDN14,[15] have been investigated as a risk factor of nephrolithiasis. Hypercalciuria owing to alteration of renal regulations of calcium excretions was the major risk factor for the development of calcium-containing stone. The overexpression of CLDN14 because of the activating mutation of the CASR gene could cause hypomagnesemia, hypercalciuria,[17-21] and nephrocalcinosis.[15] Polymorphisms of this gene (rs219778, rs219779, rs219780, and rs219781) were identified in a study conducted by Thorleifsson et al.[13] in European patients with nephrolithiasis. Furthermore, Guha et al.[19] confirmed this in patients from the Eastern Part of India.

Recently, Oddsson et al.[15] performed a GWAS of 28.3 million sequence variants through whole-genome sequencing of DNA from 2,636 Icelanders and identified the sequence variants that were strongly associated with nephrolithiasis at ALPL rs1256328 T allele, (OR=1.21, p=5.8×10-10). Mutations in the ALPL gene caused typical insufficient mineralization of the hard tissues, such as bone and tooth, and increased the level of calcium in the blood that could cause nephrolithiasis.[22,23]

We found no significant association between SLC34A1 rs11746443 SNP of NPi2a and risk of nephrolithiasis (p=0.5). Urabe et al.[24] reported that the risk allele of ALPL rs11746443 SNP was associated with the reduction of estimated glomerular filtration rate, a marker of renal function, suggesting that variations in this region could regulate renal function and subsequently affect the risk of nephrolithiasis.

For establishment of the genotype and phenotype correlation, we could identify significant signals between both CLDN14 rs219780 and ALPL rs1256328 SNPs and relevant nephrolithiasis clinical characteristics within patients. Both CLDN14 rs219780 and ALPL rs1256328 SNPs were positively correlated with recurrent UTI and ureteric stones. The 3 studied SNPs (CLDN14 rs219780, ALPL rs1256328, and SLC34A1 rs11746443) were correlated in the presence of crystals in the urine. A positive correlation was found between the CLDN14 rs219780 SNP and sex that may prove the need for assessment of the genetic component of nephrolithiasis for men and women separately.

In this study, the sequence variant ALPL rs1256328 T allele had a significant association with increased serum ALP levels in children with nephrolithiasis. Moreover, 2 of the identified genes are involved in phosphate homeostasis (ALPL and SLC34A1), and the other gene plays a key role in the renal handling of calcium (CLDN14).[25] Oddsson et al.[15] screened the nephrolithiasis-associated variants for their association with the serum levels of biochemical traits and detected the association of variants at ALPL with ALP levels. They reported that the observed variants at specific loci can influence the serum levels of biochemical traits but do not associate with the disease in all cases. This was an example of allelic heterogeneity that is particularly interesting in the context of the relationship between metabolic bone disease and nephrolithiasis.[15]

Daga et al.[26] established whole-exome sequencing as an efficient approach toward a molecular-genetic diagnosis in individuals with nephrolithiasis/nephrocalcinosis who manifest before the age of 25 years. A mutation in 7 recessive genes (AGXT, ATP6V1B1, CLDN16, CLDN19, GRHPR, SLC3A1, and SLC12A1), in 1 dominant gene (SLC9A3R1), and in 1 gene (SLC34A1) with both recessive and dominant inheritance was detected. Ure et al.[27] suggested that children with the insulinoma-associated 1 binding site within the CLDN14 risk haplotype have a higher likelihood of hypercalciuria and kidney stones. Enhanced CLDN14 expression may play a role in the pathophysiology of their hypercalciuria.

Some limitations of this study should be noted. First, the sample size was small, which limited the statistical power of the analysis. Second, the lack of detailed information on nephrolithiasis

risk factors, such as diet, BMI, and serum parathormone hormone, further limited evaluating the associations between these factors and nephrolithiasis risk. Moreover, our study was a case-control study so that the inherent selection bias could not be entirely excluded. However, all the genotype frequencies of the 2 polymorphisms that occurred in patients and controls in this study agreed with HWE, suggesting that the selection bias was unlikely to be substantial.

In conclusion, CLDN14 rs219780 and ALPL rs1256328 polymorphisms might increase the risk of nephrolithiasis in Egyptian children. The sequence variant ALPL rs1256328 had a significant association with increased serum ALP levels in the pediatric population with nephrolithiasis. Further epidemiological studies with a larger sample size and more environmental and risk factors are needed to confirm our findings.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the ethics committee of National Research Centre (AR110504).

**Informed Consent:** Written informed consent was obtained from participating children's parents who participated in this study.

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## References

1. Sorokin I, Mamoulakis C, Miyazawa K, Rodgers A, Talati J, Lotan Y. Epidemiology of stone disease across the world. World J Urol 2017;35:1301-20. [Crossref]
2. Pearle MS, Goldfarb DS, Assimos DG, Curhan G, Denu-Ciocca CJ, Matlaga BR, et al. Medical management of kidney stones: AUA guideline. J Urol 2014;192:316-24. [Crossref]
3. Türk C, Petrík A, Sarica K, Seitz C, Skolarikos A, Straub M, et al. EAU guidelines on diagnosis and conservative management of urolithiasis. Eur Urol 2016;69:468-74. [Crossref]
4. Mosharafa AA. Prevalence of renal cysts in a Middle-Eastern population: an evaluation of characteristics and risk factors. BJU Int 2008;101:736-8. [Crossref]
5. Scales Jr. CD, Smith AC, Hanley JM, Saigal C.S. Prevalence of kidney stones in the United States. Eur Urol 2012;62:160-5. [Crossref]
6. Moochhala SH, Sayer JA, Carr G, Simmons NL. Renal calcium stones: insights from the control of bone mineralization. Exp Physiol 2008;93:43-9. [Crossref]
7. Coe FL, Evan A, Worcester E. Kidney stone disease. J Clin Invest 2005;115:2598-608. [Crossref]
8. Monico CG, Milliner DS. Genetic determinants of urolithiasis. Nat Rev Nephrol 2011;8:151-62. [Crossref]
9. Khan SR, Pearle MS, Robertson WG, Gambaro G5, Canales BK, Doizi S, et al. Kidney stones. Nat Rev Dis Prim 2016;2:16008. [Crossref]
10. Curhan GC, Willett WC, Rimm EB, Stampfer MJ. Family history and risk of kidney stones. J Am Soc Nephrol 1997;8:1568-73.
11. Goldfarb DS, Fischer ME, Keich Y, Goldberg J. A twin study of genetic and dietary influences on nephrolithiasis: a report from the Vietnam Era Twin (VET) Registry. Kidney Int 2005;67:1053-61. [Crossref]
12. Yasui T, Okada A, Hamamoto S, Ando R, Taguchi K, Tozawa K, et al. Pathophysiology-based treatment of urolithiasis. Int J Urol 2017;24:32-8. [Crossref]
13. Thorleifsson G, Holm H, Edvardsson V, Walters GB, Styrkarsdottir U, Gudbjartsson DF, et al. Sequence variants in the CLDN14 gene associate with kidney stones and bone mineral density. Nat Genet 2009;41:926-30. [Crossref]
14. Guha M, Bankura B, Ghosh S, Pattanayak AK, Ghosh S, Pal DK, et al. Polymorphisms inCaSRandCLDN14 genes associated with increased risk of kidney stone disease in patients from the eastern part of India. PLoS One 2015;10:1-14. [Crossref]
15. Oddsson A, Sulem P, Helgason H, Edvardsson VO, Thorleifsson G, Sveinbjörnsson G, et al. Common and rare variants associated with kidney stones and biochemical traits. Nat Commun 2015;6:7975. [Crossref]
16. Yasui T, Okada A, Urabe Y, Usami M, Mizuno K, Kubota Y, et al. A replication study for three nephrolithiasis loci at 5q35.3, 7p14.3 and 13q14.1 in the Japanese population. J Hum Genet 2013;58:588-93. [Crossref]
17. Tzou DT, Taguchi K, Chi T, Stoller ML. Animal models of urinary stone disease. Int J Surg 2016;36:596-606. [Crossref]
18. Edvardsson VO, Indridason OS, Haraldsson G, Kjartansson O, Palsson, R. Temporal trends in the incidence of kidney stone disease. Kidney Int 2013;83:146-52. [Crossref]
19. Guha M, Bankura B, Ghosh S, Pattanayak AK, Ghosh S, Pal DK, et al. Polymorphisms in CaSR and CLDN14 Genes Associated with Increased Risk of Kidney StoneDisease in Patients from the Eastern Part of India. PLoS One 2015;10:e0130790. [Crossref]
20. Krause G, Winkler L, Mueller SL, Haseloff RF, Piontek J, Blasig IE. Structure and function of claudins. Biochim Biophys Acta 2008;1778:631-45. [Crossref]
21. Gong Y, Hou J. Claudin-14 underlies $Ca^{++}$-sensing receptor-mediated $Ca^{++}$ metabolism via NFAT-microRNA-based mechanisms. J Am Soc Nephrol 2014;25:745-60. [Crossref]
22. Smith M, Weiss MJ, Griffin CA, Murray JC, Buetow KH, Emanuel BS, et al. Regional assignment of the gene for human liver/bone/kidney alkaline phosphatase to chromosome 1p36.p1–p34. Genomics 1988;2:139-43. [Crossref]

Turk J Urol 2021; 47(1): 73-80
10.5152/tud.2020.20141

23. Ozono K .Genetic basis for skeletal disease. Clinical characteristics of hypophosphatasia and its treatment. Clin Calcium 2010;20:1220-7.
24. Urabe Y, Tanikawa C, Takahashi A, Okada Y, Morizono T, Tsunoda T, et al. A genome-wide association study of nephrolithiasis in the Japanese population identifies novel susceptible loci at 5q35.3, 7p14.3, and 13q14.1. PLoS Genet 2012;8:e1002541. [Crossref]
25. Monico CG, Milliner DS. Genetic determinants of urolithiasis. Nat Rev Nephrol 2012;8:151-62. [Crossref]
26. Daga A, Majmundar AJ, Braun DA, Gee HY, Lawson JA, Shril S, et al. Whole exome sequencing frequently detects a monogenic cause in early onset nephrolithiasis and nephrocalcinosis. Kidney Int 2018;93:204-13. [Crossref]
27. Ure ME, Heydari E, Pan W, Ramesh A, Rehman S, Morgan C, et al. A variant in a cis-regulatory element enhances claudin-14 expression and is associated with pediatric-onset hypercalciuria and kidney stones. Hum Mutat 2017;38:649-57. [Crossref]