



# Primary hyperoxaluria and genetic linkages: an insight into the disease burden from Pakistan

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## Abstract

Autosomal recessive disorders are prevalent in Pakistan, a developing South Asian country where consanguineous marriages are common. This study seeks to determine the prevalence of monogenic causes in children presenting with nephrocalcinosis and nephrolithiasis at a dialysis and transplant center in Karachi, Pakistan. A retrospective analysis was conducted in children aged 1–18 years presenting with nephrocalcinosis, between 2010 and 2019. Demographic information, clinical profile, laboratory parameters and stone analysis were collected, on a pre-designed questionnaire. One hundred and twenty-six children were included, with 11 and 3 diagnosed with renal tubular acidosis and Bartter's syndrome respectively. Next-generation sequencing and Sanger sequencing was performed on 112 children. Eighty-seven patients were diagnosed with primary hyperoxaluria, with mutations in alanine-glyoxylate-aminotransferase gene found in 73, followed by glyoxylate reductase/hydroxy-pyruvate reductase in 13, and 4-hydroxy-2-oxaloglutarate aldolase in 1. Twenty-five patients reported negative for mutations. Sixty-four percent were males, with a statistically significant difference ( $p < 0.05$ ). History of parental consanguineous marriage was found in 98% of the cohort. Fifty-four and 40 patients presented to the clinic with Chronic Kidney Disease Stage 1 and Stage 5, respectively, with a statistically significant difference  $p = 0.007$ . Mutations noted in our cohort are different and more severe than those reported in the developed world. The disease poses a major disease burden in developing world context with the only treatment option of combined liver–kidney transplantation not available in Pakistan.

**Keywords** Primary hyperoxaluria · Nephrocalcinosis · Mutation analysis · Pakistan

## Introduction

The incidence of Pediatric Nephrolithiasis and Nephrocalcinosis (NL/NC) has significantly increased over the last several decades globally [1]. Furthermore, this condition is associated with high morbidity due to episodes of colicky pain, the necessity for surgical intervention and when left

untreated or undetected it may lead to progression in chronic kidney disease (CKD) [1].

NL/NC is common among patients with metabolic conditions, several of which are monogenic diseases including primary hyperoxaluria (PH), renal tubular acidosis (RTA), Bartter's syndrome (BS) and familial hypomagnesemia with hypercalciuria [2]. NL/NC also shares a recognizable degree of heritability. In twin studies, heritability accounted for nearly half of all NL/NC prevalence [3, 4]. Despite the hereditary element, monogenic diseases continue to remain an underestimated cause of CKD thereby creating an unsurmountable disease burden, magnified in developing countries.

A primary cause of NL/NC is primary hyperoxaluria (PH), which are autosomal recessive inherited diseases caused by defects in glyoxylate metabolism. PH Type 1 (PH1) is caused by the deficiency of the liver-specific peroxisomal enzyme, alanine:glyoxylate-aminotransferase (AGXT) which results in increased urinary excretion of both oxalate and glycolate [5]. In PH Type 2 (PH2), the

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deficiency or absence of glyoxylate reductase/hydroxy-pyruvate reductase (GRHPR) leads to elevated urinary excretion of both oxalate and L-glyceric acid [5]. A third type of PH (PH3) was recently found to be caused by an abnormality of enzyme 4-hydroxy-2-oxaloglutarate aldolase (HOGA1) [6].

The spectrum of clinical presentation of PH is broad including recurrent renal stones with or without nephrocalcinosis, hematuria, failure to thrive, dysuria, urinary tract infections or renal colic. Some patients may remain asymptomatic and present with end-stage renal disease as their first presentation while others experience recurrent calcium oxalate urolithiasis with good renal functions in adulthood [7]. PH1 is characterized by an unpredictable age of onset and variable progression to renal failure. Therefore, early diagnosis is mandatory to avoid or postpone renal failure and to prevent damaging effect of systemic oxalate deposition [8, 9].

Studies have investigated the monogenic causes among PH patients who present with NC with or without NL. Hildebrandt et al. [1] a cohort of 143 individuals from Children's Hospital, Boston in United States, aged less than 18 years of age, reported that 16.8% ( $n=24$ ) had a molecular diagnosis, with causative mutations in 14 out of 30 analyzed genes. One hundred and twenty-three patients presented with nephrolithiasis, and 20 had isolated nephrocalcinosis. Another study in 2015 [5], demonstrated that out of 348 patients enrolled in the Rare Kidney Stone Consortium (RKSC) registry of the Mayo Clinic, United States, 34% were diagnosed with nephrocalcinosis, out of which 41% tested positive for PH1.

Pakistan is a South Asian developing country, which has an increasing prevalence of kidney stone diseases. Rizvi et al. [10] in 2016 conducted a literature review prevalence and epidemiology on pediatric urolithiasis in developing countries, and reported the pattern of 21,390 pediatric urolithiasis patients who reported to the outpatient clinic between 1998 and 2015 from within Pakistan. Due to metabolic risk factors and high pattern of consanguineous marriages, NL/NC presents as a significant problem in tertiary healthcare centers, often being diagnosed when the disease has progressed to CKD, and ESKD thereby signifying the hidden burden of this disease.

Talati et al. [11] obtaining data from 2 international registries and a literature review report 217 PH patients of Pakistani origin with clinical evidence, and confirmed genetic analysis. In 150 native Pakistanis, range of onset was 1–15 years with rapid progression to renal failure at adolescence. This study has been useful particularly in terms of its contribution to the broader picture from Pakistan, and those groups from a South Asian descent, however, information on genetic mutations was incomplete for several patients.

The current study aims to determine the prevalence of monogenic causes among pediatric population presenting

with NL/NC at a premier dialysis and transplant center located in the urban mega-center of Karachi, Pakistan. The secondary aim of the study is to identify the etiology and the risk factors which lead to progression of chronic kidney disease in patients present with NL/NC thereby making a valuable contribution to the existing literature.

## Materials and methods

This was a retrospective observational study conducted on a cohort of 126 pediatric patients, aged 1–18 years who reported to the outpatient clinics based at the Pediatric Nephrology and Pediatric Urology Department at Sindh Institute of Urology and Transplantation (SIUT). Patients clinically diagnosed with nephrocalcinosis between 2010 and 2019, with or without renal stones, based on ultrasound and X-ray kidney ureter bladder were included.

Demographic information including sex, ethnicity, family history of kidney disease, history of consanguinity and number of affected siblings (if any) were recorded on a pre-designed questionnaire from the medical records. Clinical profile was also noted on the form. CKD stages were determined at the time of arrival (also recorded in clinical notes) based on creatinine clearance estimated by the Schwartz formula. Children who had normal creatinine clearance which is  $> 95$  mL/min, submitted 24-h urine collection for metabolic study including urinary oxalate, calcium, citrate and uric acid, which was also added to the dataset. Information obtained from routine tests of acid blood gases, serum electrolytes, parathyroid hormone (iPTH), vitamin D levels and renal functions were also recorded. Plasma oxalate and stone analysis were also assessed from the files, where available.

Based on clinical, biochemical and urinary profile, Renal Tubular Acidosis and Bartter's syndrome diagnosis was established. The remaining population was tested for PH gene, including AGXT, GRHPR and HOGA1, after obtaining a separate consent going for mutation analysis.

This study was reviewed and approved by the ethics committee of SIUT.

## Mutation analysis by next-generation sequencing (NGS) and Sanger sequencing

As part of the clinical routine, mutation analysis by next-generation sequencing (NGS) and Sanger sequencing was performed. Detailed description is provided in Supplement 1.

## Statistical analysis

Data were entered and analyzed in Statistical Package for Social Sciences (SPSS) version 26. Categorical variables

including gender, age of presentation, ethnicity, consanguinity, family history of stone disease, affected siblings, stone analysis, nephrocalcinosis, CKD stage, hemodialysis, and genetic mutation were analyzed as descriptive statistics including frequencies and percentages. Chi-square test was used to determine the correlation between any two categorical variables. The  $p < 0.05$  was considered statistically significant for all analyses.

## Results

### Baseline characteristics

One hundred and twenty-six patients diagnosed with nephrocalcinosis were included in the study. Sociodemographic characteristics are shown in Table 1.

Forty-three percent ( $n = 54$ ) presented to the clinic with CKD Stage 1, followed by CKD Stage 5 in 32% ( $n = 40$ ) of the patients. Thirty-two percent ( $n = 41$ ) required dialysis at the time of presentation. Mean serum creatinine at presentation was 3.53 (SD 4.13). Plasma oxalate was available in 59 patients with a mean of 48.7  $\mu\text{mol/L}$  (SD 61.65).

All patients underwent kidney imaging including ultrasound kidney ureter bladder (KUB) and X-ray KUB. Computed tomography (CT) was indicated and performed in 19% of the patients. Urological procedures of stone removal were performed in this sub-cohort.

Stone analysis was available for 19% ( $n = 24$ ) patients who underwent a urological procedure. Out of this subset, 95% ( $n = 23$ ) showed presence of calcium oxalate monohydrate (COM). One patient showed presence of uric acid and calcium oxalate dihydrate (COD).

Sixty-three percent ( $n = 76$ ) presented with renal stones along with bilateral medullary nephrocalcinosis. Cortical nephrocalcinosis was found in two patients. Medullary nephrocalcinosis was present in 36% of patients ( $n = 45$ ).

### Mutation analysis for primary hyperoxaluria

Based on clinical history and phenotype, Renal Tubular Acidosis (RTA) was diagnosed in 11 patients, out of which 9 were Type 1, and remaining were Type 3. Three patients were diagnosed with Bartter's syndrome.

Screening for mutation analysis for PH Type 1, 2 and 3 was performed on 112 patients. Molecular diagnosis of PH was made in 69% of patients ( $n = 87$ ), whereas 25 patients screened negative for PH.

Refer to Fig. 1 showing flow chart of the study patients.

**Table 1** Sociodemographic characteristics frequencies and association with genetic mutations

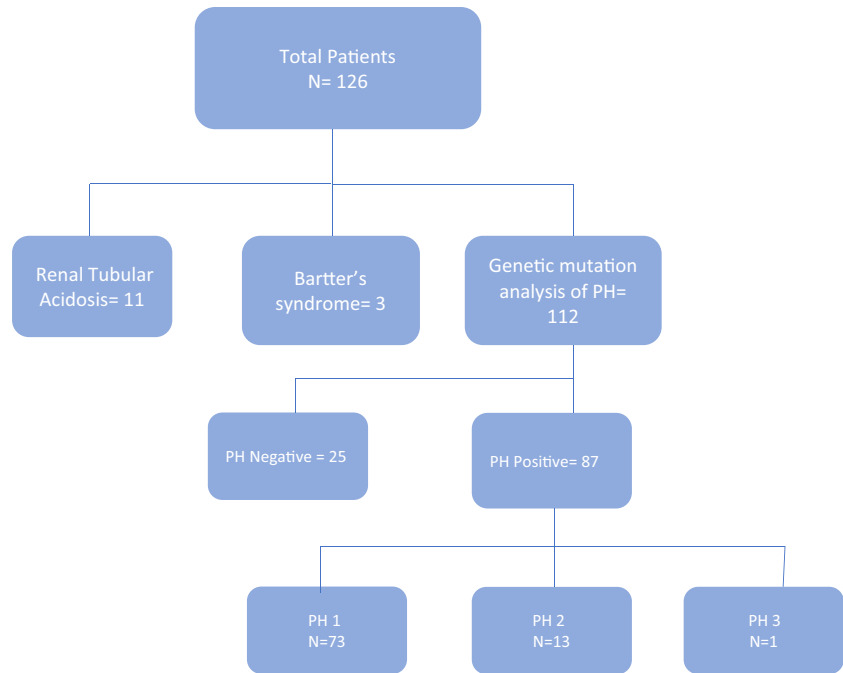
Characteristics	Frequency (%)	<i>p</i> value
Age		
< 5 years	56 (36.5%)	$p = 0.459$
5–10 years	47 (37.3%)	
11–15 years	28 (22.2%)	
> 15 years	5 (4.0%)	
Age median	1.94	
Age mean	2	
Sex		
Male	80 (63.5%)	$p = 0.003^*$
Female	46 (36.5%)	
Ethnicity		
Sindhi	54 (42.9%)	$p = 0.095^*$
Urdu speaking	35 (27.8%)	
Punjabi	18 (14.3%)	
Pashtun	19 (15.1%)	
Consanguinity		
Positive	124 (98.4%)	$p = 0.0558$
Negative	2 (1.6%)	
Family history of stone disease		
Positive	43 (34.1%)	$p = 0.002^*$
Negative	83 (65.9%)	
Affected siblings		
Affected	33 (26.2%)	$p = 0.003^*$
Non-affected	93 (73.8%)	
Nephrocalcinosis		
Cortical	2 (1.6%)	$p = 0.000$
Medullary	45 (35.7%)	
Medullary with stones	79 (62.7%)	
Chronic kidney disease stages		
Stage 1	54 (42.9%)	$p = 0.007^*$
Stage 2	16 (12.7%)	
Stage 3	8 (6.3%)	
Stage 4	8 (6.3%)	
Stage 5	40 (31.7%)	

The [\*] illustrates statistically significant difference

### Genetic description of primary hyperoxaluric patients

PH Type I was found in 83% patients ( $n = 73$ ). Within PH Type 1, Gly350Asp was the most common mutation, identified in eight patients, followed by Cys173His174delinsTrpAsn in eight and Gly82Glu in seven.

PH Type II was found in 15% ( $n = 13$ ) patients. Most common mutations included L6F in three, Trp138Arg also in three patients, followed by Arg124Cys in two patients. Gly165Asp, Val213Ala, Ala115Glu, Arg99Ter and

**Fig. 1** Flow chart of the study patients**Table 2** Genotype–phenotype relationship

Type of gene (n)	Zygoty state (n)	Sex (n)	Age (n)	Presence of stones (n)	CKD Stage
AGXT (73)	Homozygous (68)	Males (54)	<5 (30)	Yes (57)	1 (27)
			5–10 (36)	No (16)	2 (5)
	Heterozygous (1)	Females (19)	10–15 (3)		3 (4)
			> 15 (4)		4 (3)
					5 (34)
GRHPR (13)	Homozygous (12)	Male (7)	< 5 (2)		1 (4)
			5–10 (5)	Yes (13)	2 (6)
	Heterozygous (1)	Female (6)	10–15 (6)		3 (1)
					4 (1)
HOGA1 (1)	Homozygous (1)	Male (1)	5–10 (1)	Yes (1)	5 (1)
					4 (1)

Glu311AsnfsX39 were identified in remaining individual patients.

One patient was diagnosed with Type III PH. The identified mutation was Val239Ile.

Table 2 shows the genotype–phenotype relationship between the identified genes and clinical profile of patients. Eighty-one patients were homozygous. Thirty-four patients from PH1 presented with CKD Stage 5 requiring dialysis, which was a statistically significant relationship ( $p < 0.05$ ).

Supplement 2 describes the genetic mutation analysis of 35 PH patients presenting with CKD Stage 5, who required dialysis.

Novel mutations were identified among six patients, out of which four were PH1. Table 3 provides details about the types of novel mutation.

**Table 3** Novel mutations for PH patients

Gene (protein) analyzed	Amino-acid change (novel mutation)
AGXT	p.Gly109Glu p.Arg233His p.Leu276Glu p.Leu276Glu
GRHPR	p.Glu311AsnfsX39 p.Val213Ala

Refer to Supplement 3 for a detailed description of genetic analysis of 87 children presenting with nephrocalcinosis.

## Correlation between socio-demographic characteristics with molecular genetic results

Chi-square test was run to find out the relationship between socio-demographic characteristics (including sex, age, ethnicity, history of consanguinity, history of stone disease, affected siblings, type of nephrocalcinosis and stage of kidney disease) with molecular genetic results.

Refer to Table 1 for more information.

## Patient outcomes

Two PH1 patients out of 87 received live-related liver and kidney transplantation, and their grafts are functioning well. Another one received isolated liver transplantation with serum creatinine of 0.8 mg/dl. Remaining patients were continued either on dialysis or CKD medical management, as clinically indicated.

## Discussion

This study presents a genetic analysis of the largest cohort of pediatric patients with nephrocalcinosis from a single center in Pakistan. Another study from the same country taking data from five different tertiary care hospitals has reported a diverse cohort of stone formers families. Out of 229 probands, only 32% were less than 20 years of age, with molecular diagnosis made only in 7% of 4 different NL genes [12]. Therefore, the current study reflects the single largest cohort of pediatric patients. Within pediatric population, genetic and/or metabolic disorders are considered the main reason for nephrolithiasis and nephrocalcinosis, in contrast to the adult population where environmental factors play a major role [13].

Among the 126 children whose data was reviewed over a period of 9 years, 69% were diagnosed with primary hyperoxaluria based on molecular genetic testing. A small percentage of patients with non-PH causes for nephrocalcinosis in our cohort could be because the center is primarily a renal center, and non-PH causes can be dealt by other clinical specialties.

This is the highest positivity rate ever reported from any center in the world, according to the best of the authors' knowledge. A study reporting data from 3 renal stone clinics from United States and Europe found PH positive only among 16.8% of 143 individuals [1]. Similarly, Halbritter (2015) reported molecular diagnosis only 14.9% of 106 pediatric patients from an international cohort [14]. A large number from our study site indicates a disease burden that may not be presented in the developed world, but has implications on populations from South Asian origin or descent.

Our study also reported a high percentage of consanguinity, a norm in Pakistani setting where cousin marriages across generations are extremely common. Moreover, the number of people suffering from genetic diseases in the country is estimated between 14 and 16 million, with 1.6 million mutations found in the country, with a high number of children suffering from genetic disorders [15–18].

Consanguinity is similarly reported from Egypt and Tunisia [7, 20]. Other evidence is contributed by another study conducted in Israel among 56 patients of Arab origin, and were result of consanguineous mating, with marriage of 1st degree cousins being the most common pattern [9]. In populations of North Africa and South Asia (especially Pakistan, Bangladesh and South India), consanguineous marriages are culturally and socially favored and constitute 20–50% of all marriages [15, 16], without considering its negative impact, in terms of increased genetic risk to the offspring as opposed to the potential social and economic benefits.

While this observation was not statistically significant ( $p$  value = 0.558), the high percentage of consanguinity among patients with NL/NC indicates a potential relationship. However, since data pertaining to consanguinity was not normally distributed ( $p < 0.05$ ), the association between these two variables could not be determined.

Our data also revealed that more male children report with positive PH mutations, with a statistically significant difference. This is consistent with previous study [13]. Another possible explanation in our cohort for this trend could also be socio-cultural factors that influence seeking medical treatment [15–18]. Since there is a general preference for male children within many sections of the Pakistani society since boys carry on the family name and become breadwinners in the future, it is likely that more male children present to the clinic in the first place. It has also been reported from elsewhere that medical care is sought more for sons than for daughters [18, 19].

Frequency statistics also showed that positive mutations were found in majority of children who were less than 10 years of age. This is also in line with the findings presented by Halbritter, who found that all individuals with infantile manifestation of NL/NC in his cohort harbored causative mutations in recessive rather than dominant monogenic genes [14]. This association, however, was not statistically significant in our cohort which was likely because our dataset only included pediatric population.

Ethnicity data revealed that majority of the patients were arriving from rural Sindh as well as the urban center of Karachi, with a statistically significant relationship with genetic mutations. This area requires further exploration in a prospective fashion since it could give an insight whether specific ethnicities from within the country are more prone to these gene mutations. This association cannot be conclusively established because a large portion of patients



reporting to the clinic may come to our center due to close proximity to our center.

Another important insight into the risk factors for NL/NC among pediatric population could be history of stone disease in the family and whether there are other siblings in the family with renal complications. A study reporting on experiences from our center illustrates that stone diseases run in families with multiple siblings affected [15, 16]. This was an area that could not be conclusively established from the findings of our study. While both associations were statistically significant with genetic mutations, the direction of the relationship could not be reasonably ascertained. This may have occurred because this was self-reported data from families, and genetic testing was not performed on siblings of the affected patients. This is another potential area for exploration in prospective studies, where genetic testing could be performed on the entire nuclear family unit.

The PH-positive patients were subdivided into three groups, cortical nephrocalcinosis (1.1%), medullary nephrocalcinosis without kidney stones (19.5%) and medullary nephrocalcinosis with kidney stones (79.3%) with a statistically significant relationship. This is in comparison with the data presented by Milliner in 2015, where only 24% of the patients with PH showed nephrocalcinosis with stones, 10% with nephrocalcinosis only and 47% with kidney stones only at the time of first imaging [5]. It was noted that the decline in glomerular filtration rate (GFR) was more pronounced in the group which has both nephrocalcinosis and stones in our cohort. This is in line with findings in previous studies [5].

This has broader clinical implications since individuals presenting with medullary nephrocalcinosis with kidney stones have a higher chance of recurrence and, therefore, may not be viable candidates for renal transplantation, thereby emphasizing importance of genetic screening prior to transplantation. Such patients may have transplant failures in the future which is a significant cause of concern for the transplantation community, pronounced more in developing countries with significant resource constraints.

Among 87 patients diagnosed with PH, Type 1 was most common, followed by Type 2. Only one patient reported with Type 3 gene. PH1 patients have more severe long-term clinical course than those with PH2 and PH3. Eighty-one patients were homozygous expected in societies where consanguinity is widespread, as noted in our populations [9]. The most common mutations within the PH1 category identified in our study were Gly350Asp and Gly82Glu patients in contrast to Gly170Arg and Ile244Thr which were reported by Harambat [21] in 2010. Novel mutations were also identified in our analysis; therefore, mutations in our cohort, therefore, are different from those reported in the West. The frequency and types of these mutations varies between different ethnic groups.

Moreover, mutations found in our group tend to have a more aggressive course of disease, made clinically evident since children diagnosed with these genetic mutations required dialysis at the time of presentation. Thirty-four patients from PH1 group also presented in CKD Stage 5. This is a very high percentage compared to other studies [21] and reflect the fact that these children reported late to the clinic and, therefore, were diagnosed late.

PH1 patients who present with nephrocalcinosis may eventually require combined liver–kidney transplantation, which is not only expensive but is also not a viable option for developing countries like Pakistan.

Against this backdrop, genetic screening has wider implications for patients and their family members since it can aid with management, prognosis, genetic counseling and screening for at-risk family members. In the Pakistani setup, where renal transplantation is reliant on live-related donation, genetic screening becomes even more important.

The current retrospective analysis, therefore, presents insight into the NL/NC disease burden among the pediatric population, which has not been performed before among the South Asian population. The findings of our study provide guidance for nephrologists working in countries where consanguineous marriages are widespread, and also in the developed part of the world where populations of South Asian, particularly Pakistani descent, reside.

Despite providing an overview of PH patients among pediatric population for the first time in Pakistan while also establishing the genetic linkages, there are several limitations of the study. Since data were obtained retrospectively, several key areas could not be ascertained conclusively including, for instance, family history of stone disease, and number of affected siblings. This is also a single-center experience from a public sector hospital which tends to attract patients from low socioeconomic backgrounds, and obtaining data from another center could have been useful to provide a comparison of patients presenting with NL/NC.

## Conclusion

The incidence of pediatric nephrolithiasis and nephrocalcinosis has significantly increased over the last several decades [1]. More insight into the disease burden through determination of monogenic causes in Pakistan is pivotal to improving therapeutic strategies for this devastating disease, especially with new promising therapeutic modalities under development [22–26]. Moreover, genetic screening if practiced routinely can lead to increased knowledge of the molecular diagnosis which may change plans for prophylaxis and treatment. Moreover, given the unavailability of combined liver–kidney transplantation for most children in the country,

a targeted screening program should be implemented, with an additional component of pre-marital counseling.

Our study has some limitations. Since our institute is primarily a tertiary care renal hospital, it cannot reflect the magnitude of other etiologies like distal renal tubular acidosis and Bartter's syndrome. We were also unable to perform genetic testing (other than for oxalosis) in non-PH patients and patients with tubular disorders.

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**Author contributions** SH: conceptualized, designed the study, collected data, and wrote the manuscript. AA: performed genetic testing. SS: helped in data collection. SS: revised and edited the paper. AL: helped in data collection. MN Zafar: supervised the study.

**Data availability** The data underlying this article cannot be shared publicly to protect confidentiality of individual participants but will be shared on reasonable request to the corresponding author.

## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All the authors have seen the manuscript and approved its submission to your journal. This study was approved by our institutional review board and was performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki and its later amendments.

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