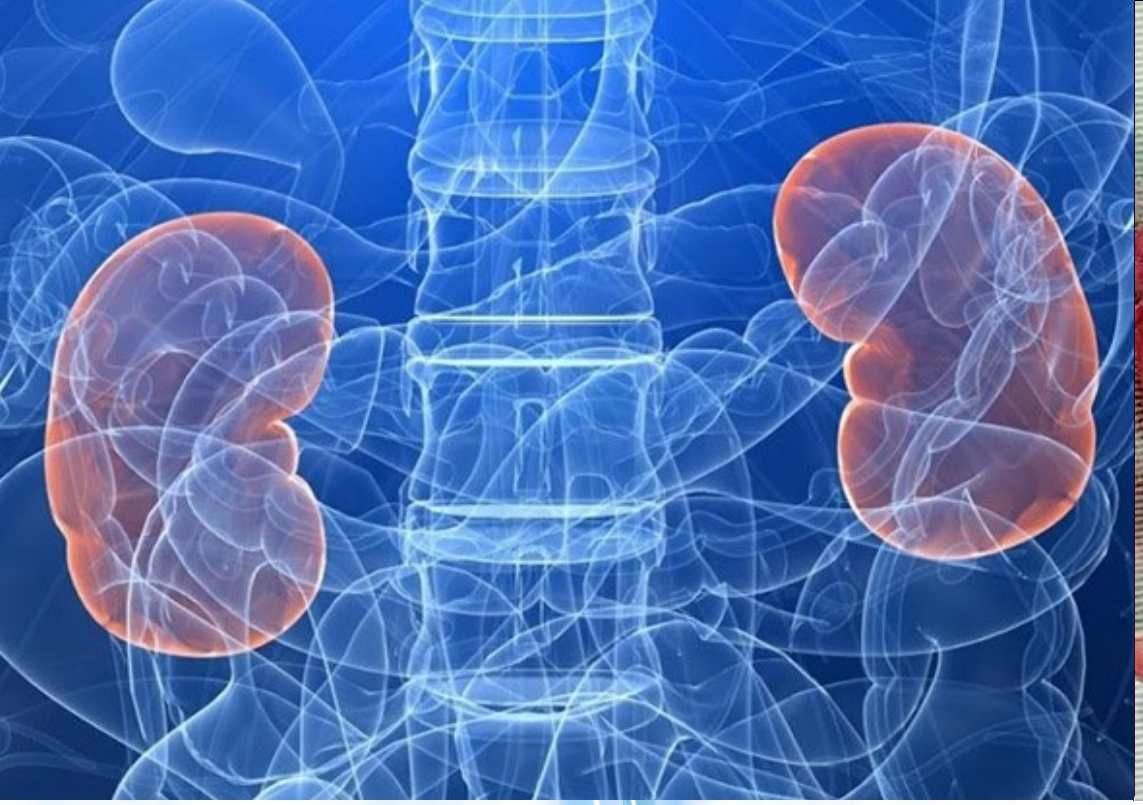
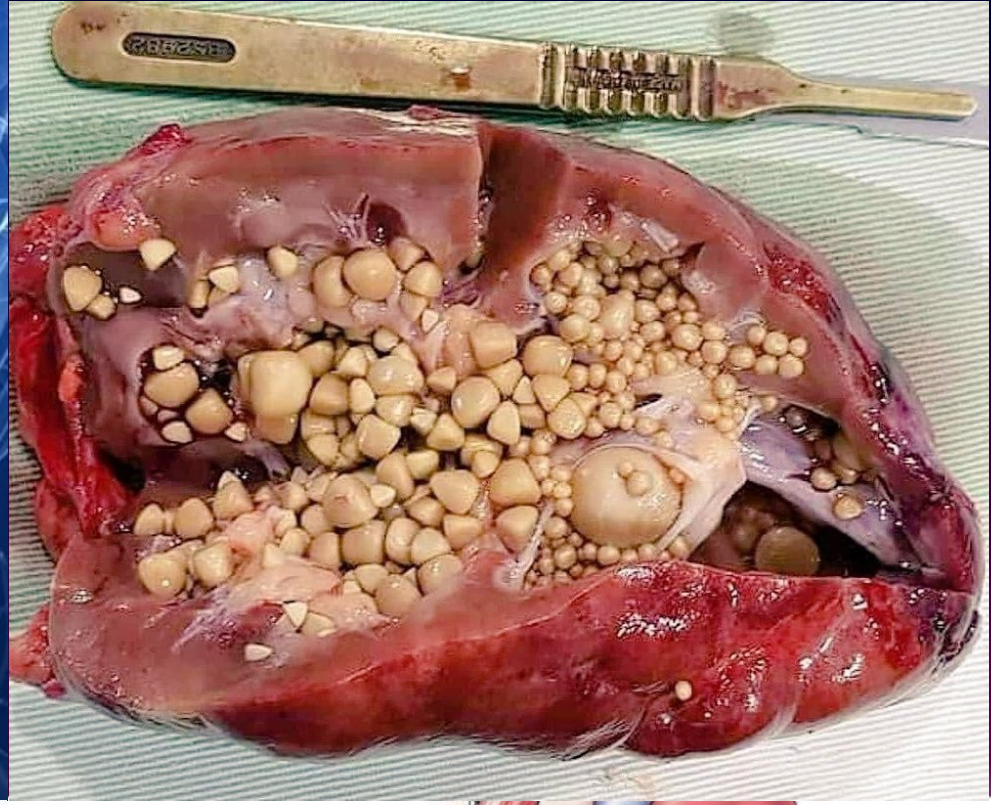
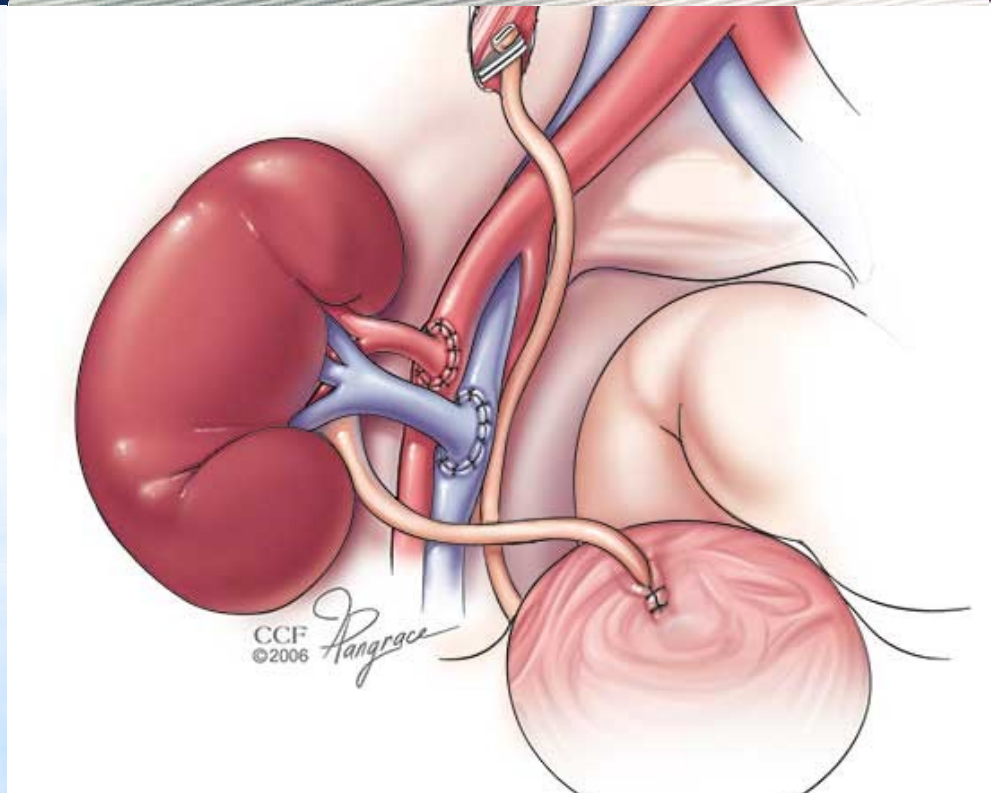





CCF
©2006
Pangrace

Crystal Conundrum: Renal Allograft Failure from Recurrent 2,8-Dihydroxyadenine Nephropathy

[1]Kim Phung L. Nguyen, MD. MS. PGY 5 Nephrology Fellow

[2]Angelina R. Edwards, MD
[3]William F. Glass, MD
[1]Akshta Pai, MD
[1]Aleksandra De Golovine, MD

University of Texas Health Science Center at Houston, McGovern Medical School
[1]Department of Renal Disease and Hypertension
[2]Department of Abdominal Tranpslant
[3]Department of Pathology

No relevant disclosures.

Overview

- Kidney stones in ESRD and renal transplant.
- Case Presentation
- What is 2,8-DHA nephropathy?
- Recurrent 2,8-DHA in renal transplant.
- Treatment of 2,8-DHA in renal transplant.
- Final points on patient case.
- Key points.

Kidney Stone Disease

- Kidney stones 4000 B.C.
- 10-15% of world population, all ages, genders, and races at some stage in their lifetime.
  - Men > women within age 20-49 years.
  - In 2015, worldwide 22.1 million cases with 16,100 deaths from complications.
  - In Middle East 20-25% lifetime risk (hot climates, diet lower in calcium and higher in oxalates).
  - The prevalence of stone disease was 8.4% in U.S, highest among non-Hispanic white individuals, higher among men than women, higher among obese and overweight than normal weight (cross-sectional analysis, data from 2007-2010 NHANES, Scales Jr et al).
- Without prophylactic management, relapse rate 10-23% per year, 50% in 5-10 years, 75% in 20 years (2006).

Victoriano Romero et al. Kidney stones: a global picture of prevalence, incidence, and associated risk factors. Reviews in Urology. Vol. 12. No. 2/3 2010. Vos T et al. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortlity for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 388 (10053): 1459-1544. Charles D. Scales Jr et al. Prevalence of kidney stones in the United States. European Urology 62 (2012) 160-165.

Kidney Stone Risk Factors

Table 2: Risk factors associated with kidney stone formations.

Number	Risk factors	References
1	*Lifestyle habits and dietary/nutritional factors*: such as excessive intake of animal proteins and salt and deficiencies of chelating agents like citrate, fiber, and alkali foods	[9, 13, 19, 45]
2	*Metabolic disorders*: such as hypercalciuria, hypocitraturia, hyperoxaluria, hyperuricosuria, and history of gout (defective metabolism of uric acid)	[38, 46–48]
3	*Hypercalcemic disorders*: primary hyperparathyroidism and other disturbances of calcium metabolism	[49]
4	*Urine composition*: excessive excretion of promoters of urinary crystallization and reduced excretion of inhibitors (urine deficient in inhibitory substances)	[1, 45, 49]
5	*Low urine volume*: inadequate water intake (dehydration and supersaturated urine)	[45, 49, 50]
6	*Recurrent urinary tract infections*: abnormalities of urinary pH and alkalinization of urine by bacterial urease (such as *Proteus mirabilis*)	[38, 49]
7	*Genetic predisposition/inherited disorders*: family history of stones (*genetic* susceptibility); genetic monogenic diseases (single abnormal gene disorders on the autosomes); renal tubular acidosis	[1, 9, 48, 49, 51]
8	*Anatomical abnormalities*: factors such as defects in medullary sponge kidney, ureteropelvic junction stenosis, pyeloureteral duplication, polycystic renal disease, and horseshoe kidney	[1, 48, 49, 52]
9	*Hypertension*	[46]
10	*Obesity*	[46–48]
11	*Climate change* (global warming), occupation, geographic conditions, and seasonal variations (higher in summer than winter)	[1, 49]
12	*Inflammatory bowel disease* and other intestinal malabsorption or associated disease states	[9, 49]
13	Absence of intestinal *oxalate-degrading bacteria*	[53, 54]
14	*Lithogenic drugs*: such as indinavir (Crixivan), a protease inhibitor, sulfonamides (sulfadiazine), uricosuric agents, which have low solubility andpromotes the formation of calculi, and ceftriaxone (high dose on long terms)	[28, 38, 49, 50]

Tilahun Alelign et al. Review Article. Kidney Stone Disease: An Update on Current Concepts. Advances in Urology. Vol 2018.

Kidney Stone Disease: ESRD

- Prevalence has been increasing in the past decades in both developed and developing countries (decreased physical activity, poor dietary habits, warmer climate).
- Studies specifically devoted to assessing the incidence of renal failure, especially ESRD, in patients with urinary stone disease are scarce.
- Annual reports of United States Renal Data System, patients who started renal replacement therapy in the U.S. between 1993-1997.
  - 1.2% had nephrolithiasis as the cause of ESRD, but no precision was given to the type of stone disease.
- ESRD Caused by Nephrolithiasis by Paul Jungers et al. Chart review: overall proportion of nephrolithiasis-related ESRD among the total number of incident ESRD cases was 3.2% (45/1391 patients).

Paul Jungers et al. ESRD caused by nephrolithiasis: prevalence, mechanism and prevention. American Journal of Kidney Disease, Vol 44, No 5 (November), 2004.

Kidney Stone Disease: ESRD

Table 3. Severe Forms of Nephrolithiasis Potentially Leading to ESRD

Hereditary or congenital diseases
Primary hyperoxalurias*
Primary distal tubular acidosis†
Familial hyperparathyroidism
Idiopathic hypercalciuria
Medullary sponge kidney†
Lesch-Nyhan syndrome*
2,8-Dihydroxyadeninuria*
Cystinuria
Dent's disease†
Bartter's syndrome†
Familial hypomagnesemia with hypercalciuria and nephrocalcinosis†
Lowe syndrome†
Other diseases
Enteric hyperoxaluria*
Secondary distal tubular acidosis (Sjögren's syndrome, systemic diseases)†
Primary hyperparathyroidism†
Vitamin D intoxication†
Sarcoidosis†
Uric acid nephrolithiasis
Secondary hyperuricosuria
Infection stones
Anatomic abnormalities
Neurological bladder
Urinary diversion

*Renal parenchymal crystal infiltration.
†Nephrocalcinosis frequently associated with nephrolithiasis.

Paul Jungers et al. ESRD caused by nephrolithiasis: prevalence, mechanism and prevention. American Journal of Kidney Disease, Vol 44, No 5 (November), 2004.

Kidney Stone Disease: Transplant

- Renal transplant lithiasis initially recognized as a complication of renal transplantation in 1975.
- Hospitalized nephrolithiasis after renal transplant by Kevin Abbott et al.
  - Conducted a cohort study of 42,096 renal transplant recipients in the United States Renal Data System between 7/1/1994-6/30/1998.
    - USRDS, indirectly mandated by federal law, incorporates baseline and follow-up demographic and clinical data on all patients receiving ESRD therapy in the U.S. ESRD therapy includes HD, PD, and renal transplantation.
    - They looked at the incidence, risk factors and associated patient survival for only hospitalized cases of nephrolithiasis (based on ICD9 coding) at hospital discharge for nephrolithiasis as the primary discharge diagnosis in renal transplant recipients.

Kevin C. Abbott et al. Hospitalized Nephrolithiasis after Renal Transplantation in the United States. American Journal of Transplantation 2003; 3: 465-470.

Kidney Stone Disease: Transplant

- Hospitalized nephrolithiasis after renal transplant by Kevin Abbott et al.
  - There were 42,096 solitary renal transplant recipients, of whom 39,628 had complete data to calculate survival times.
  - Mean follow up 1.89 +/- 1.15 years (median, 1.91 years).
  - 52 patients were hospitalized with a primary diagnosis of nephrolithiasis.
    - Only a history of renal failure due to stone disease was significantly associated with incident hospitalizations for nephrolithiasis.
  - The rate of nephrolithiasis was higher in females (0.15%) than in male (0.11%) transplant recipients, but not statistically different.

Kevin C. Abbott et al. Hospitalized Nephrolithiasis after Renal Transplantation in the United States. American Journal of Transplantation 2003; 3: 465-470.

Kidney Stone Disease: Transplant

- Renal transplant lithiasis by Konstantinos Stravodimos et al.
  - Retrospective analysis of a consecutive series of 1525 renal transplantations between 1/1983 and 3/2007.
    - 7 patients were found to have allograft lithiasis, incidence of graft lithiasis 0.46% from cadaveric donors.
      - 5 cases with calculi in the renal unit, 2 cases in ureter.
      - 3 patients without symptoms or signs of obstruction, and gross hematuria developed in one patient.
      - Mean time of lithiasis post-transplantation was 3.2 years (2-7 years).
  - Mean time of lithiasis presentation post-transplantation in several series is 1.6 to 3.6 years.

Konstantinos G.Stravodimos et al. Renal Transplant Lithiasis: Analysis of Our Series and Review of the Literature. Journal of Endourology. Volume 26, Number 1, January 2012. He-jia Yuan et al. Minimally invasive treatment of renal transplant nephrolithiasis. World Journal of Urology (2015) 33: 2079-2085.

Kidney Stone Disease: Transplant

Table 2. Selected Series of Graft Lithiasis

Reference	*Type of study*	*Number of transplants*	*Graft lithiasis cases n (%)*	*SWL (n)*	*PCNL (n)*	*URS (n)*	*Open surgery (n)*	*Spontaneous passage (n)*
Abbott et al[7]	Retrospective	42,096	52 (0.13)	1	19	7	0	NA
Rhee et al[6]	Retrospective	1813	8 (0.4)	2	0	1	4	1
Streeter et al[45]	Retrospective	1535	9 (0.6)	1	3	0	2	3
Klingler et al[8]	Retrospective	1179	22 (1)[a]	7	4	5	1	3
El-Mekresh et al[1]	Retrospective	1200	11 (0.9)[a]	3	0	2	0	3
Kim et al[13]	Retrospective	849	15 (1.8)[a]	0	0	0	0	5
Del Pizzo et al[43]	Retrospective	540	14 (2.5)	0	0	14	0	0
Challacombe et al[31]	Retrospective	2085	21 (1.01)	13	3	2	2	2

[a]In these series are also patients included with stones located in the bladder.
SWL = shockwave lithotripsy; PCNL = percutaneous nephrolithotomy; URS = ureteroscopy.

- Renal transplant lithiasis incidence 0.4%-1%, systematic review MEDLINE and PubMed.
- Other sources: 0.2%-6.3% incidence of allograft lithiasis.

Konstantinos G.Stravodimos et al. Renal Transplant Lithiasis: Analysis of Our Series and Review of the Literature. Journal of Endourology. Volume 26, Number 1, January 2012.

Kidney Stone Disease: Transplant

- Overall, nephrolithiasis is reportedly rare after renal transplantation.
  - Careful donor screening for the so-called donor-gifted allograft lithiasis, <1%.
- Several factors predisposed to graft lithiasis:
  - Urinary stasis, reflux, recurrent UTI especially in encrusted retained ureteral stents, renal tubular acidosis, pH changes, supersaturated urine, decreased inhibitor activity, tertiary hyperparathyroidism, hypercalcemia, hypercalciuria, high dietary protein intake, vitamin D.
  - Tertiary hyperparathyroidism represents the inability of the parathyroid glands to restore normal function and resume normal serum calcium levels after kidney transplantation.
    - Elevated urine calcium load was common finding despite cinacalcet use.
  - Cyclosporine is known to produce hyperuricosuria in 50%-60% of patients.

Konstantinos G.Stravodimos et al. Renal Transplant Lithiasis: Analysis of Our Series and Review of the Literature. Journal of Endourology. Volume 26, Number 1, January 2012.

CRYSTAL CONUNDRUM

CASE PRESENTATION

HPI: Transplant Evaluation

- 68 years old Hispanic man, ESRD presumed secondary to hypertension, hemodialysis dependent since 6/2010.
- Past Medical/Surgical History
  - Mitral valve regurgitation s/p prosthetic mitral valve replacement.
  - Left atrial appendage ligation 5/2014.
  - Poorly controlled hypertension.
  - Secondary hyperparathyroidism.
- No prior history of nephrolithiasis, obstructive nephropathy or hydronephrosis.
- Family History:
  - Sister with kidney disease that required dialysis, deceased.
  - Brother deceased unknown medical issues.
  - 45 years old son with hx renal stones.
- Imaging: Retroperitoneal ultrasound
  - Small bilateral renal simple cysts, increased echogenicity.
  - Right kidney 7.7 x 3.6 x 3.7 cm, left kidney 7.7 x 3.7 x 3.7 cm.

Deceased Donor Renal Transplant

- Pt underwent deceased donor renal transplant, right iliac fossa.
  - Cold ischemia time 07:09; Warm ischemia time 00:55.
  - No ureteral stent.
- Donor characteristics:
  - Kidney donor profile index 75%.
  - Cause of death: stroke with intracranial hemorrhage.
- Time Zero Biopsy
  - Mild arterial and arteriolar sclerosis.
  - 9% global sclerosis.
  - 10% interstitial fibrosis with tubular atrophy (IFTA).

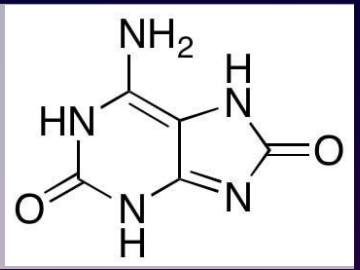
- Blood group compatible, 2A1B1DR HLA mismatch, CMV+/+, EBV+/+.
- Crossmatch negative, panel reactive antibody 89%, no donor specific antibody (DSA).
- Immunosuppression
  - Induction: Thymoglobulin 1.5 mg/kg daily x3 doses.
  - Maintenance:
  - Steroid: solumedrol → prednisone taper.
  - Calcineurin inhibitor: Tacrolimus 0.05 mg/kg, twice daily; titrated for goal level 5-8.
  - Antimetabolite: Mycophenolate mofetil 1000 mg twice daily.

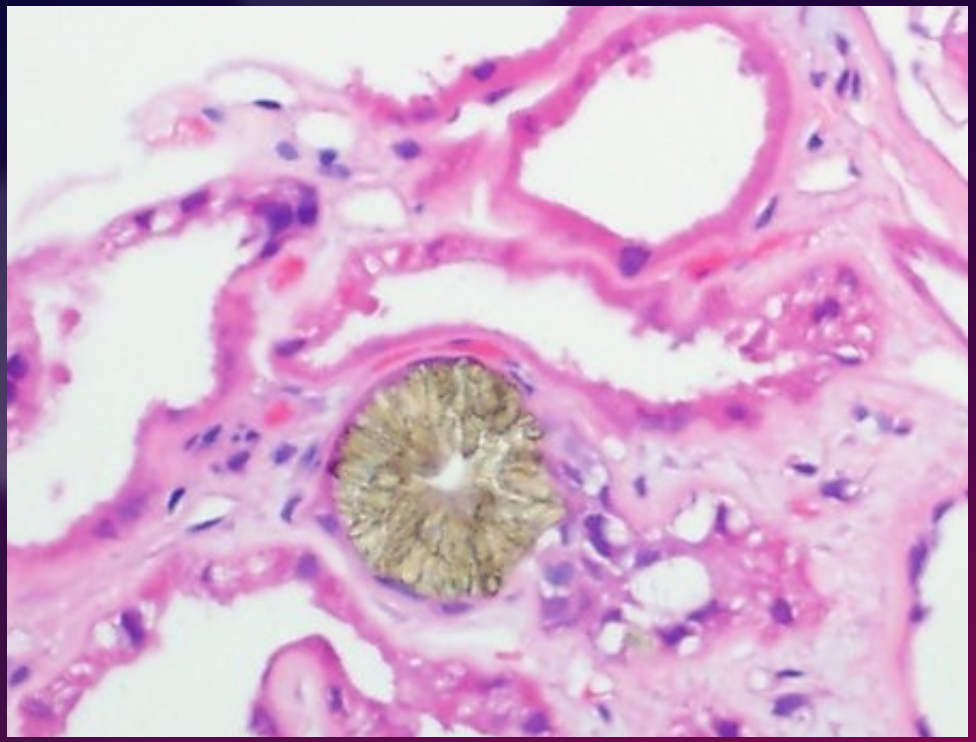
Delayed Graft Function

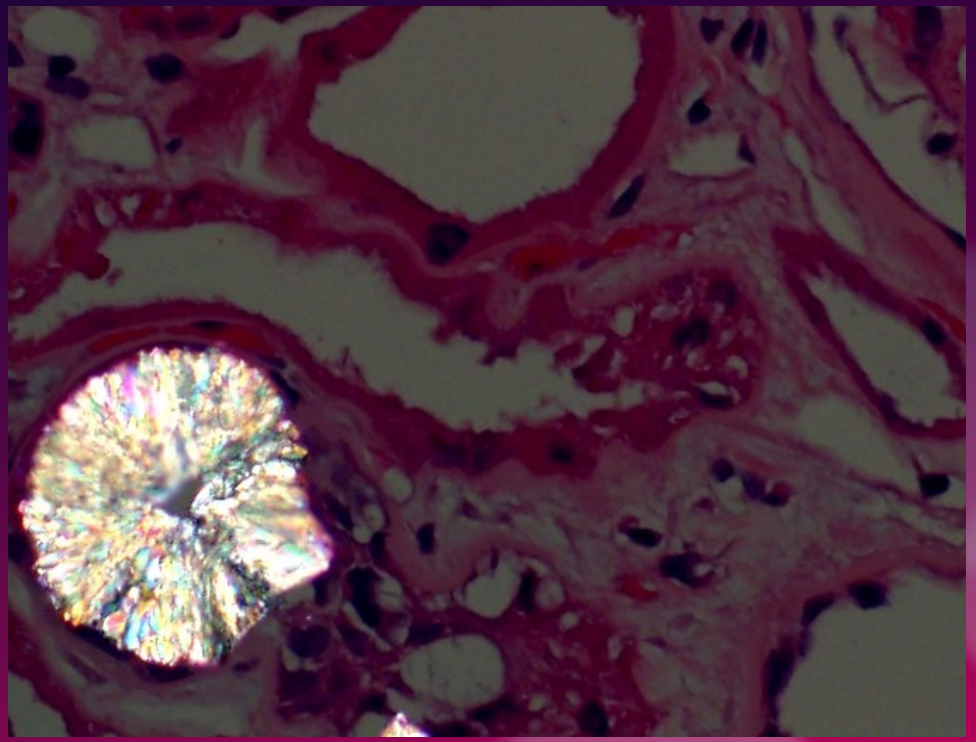
- Transplant renal biopsy 2 weeks post transplant.
  - Acute tubular epithelial injury with numerous calcium oxalate crystals.
  - No evidence of acute antibody or T cell-mediated rejection. Peritubular capillaries negative for C4d.
  - IHC negative for SV40.
  - Arterial and arteriolar sclerosis.
  - Approximately 3% of global glomerulosclerosis and 10% interstitial fibrosis with tubular atrophy.

- Oxalate level ranged 3.4-8.1 uMol/L (range <1.9).
- Hyperoxalosis treatment: HD daily, pyrodoxine 300 mg qday, calcium acetate 667 mg tid.
- 1 week post hyperoxalosis treatment, repeat renal biopsy.
- 4 weeks post transplant – 2,8-DHA nephropathy: allopurinol 100 mg/day, low purine diet, fluid hydration.
- 1 month post 2,8-DHA treatment, Cr 2.56 mg/dL from Cr 7.46 mg/dL.

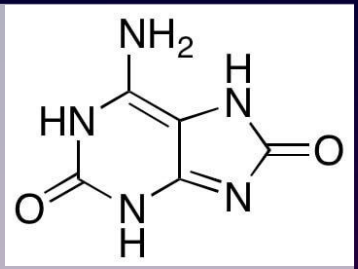
Case Presentation: 2,8-DHA

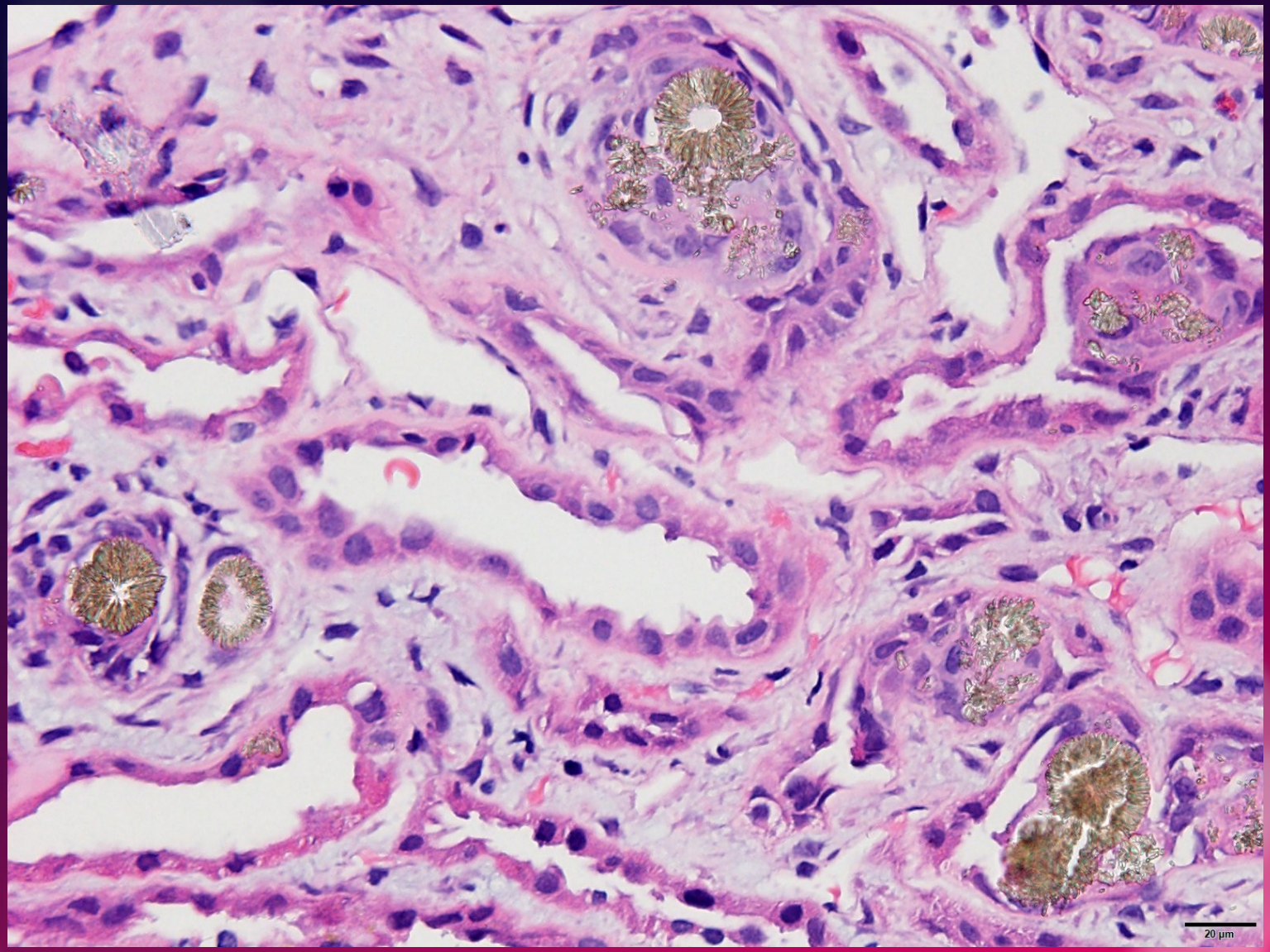




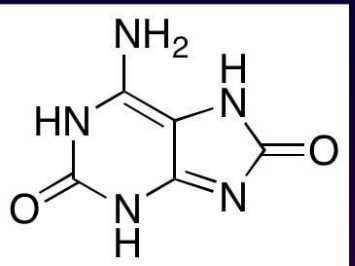


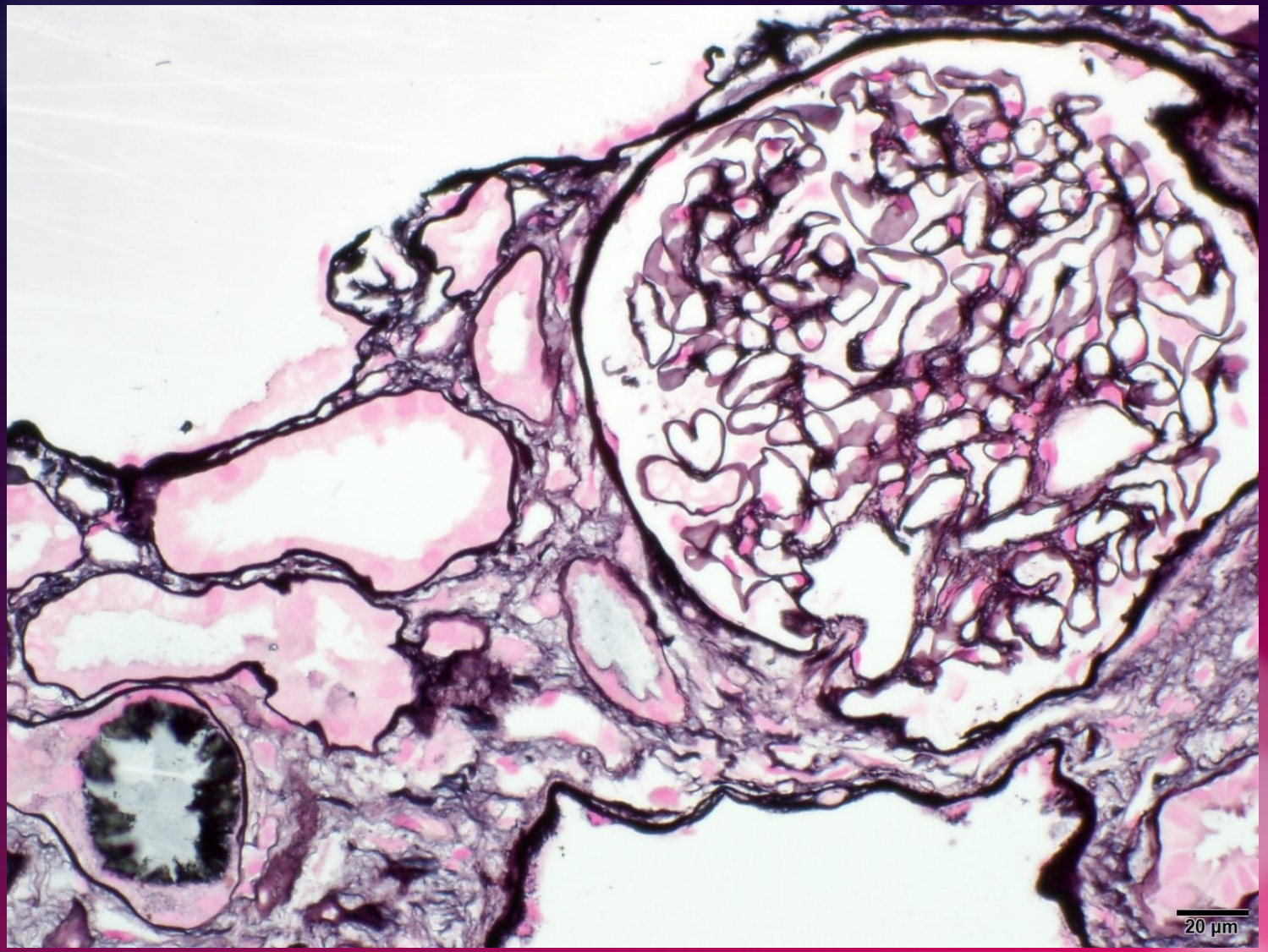
Case Presentation: 2,8-DHA



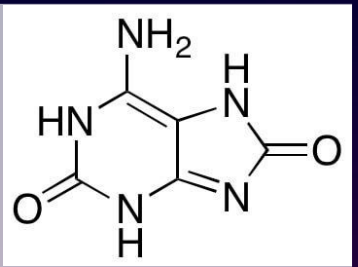


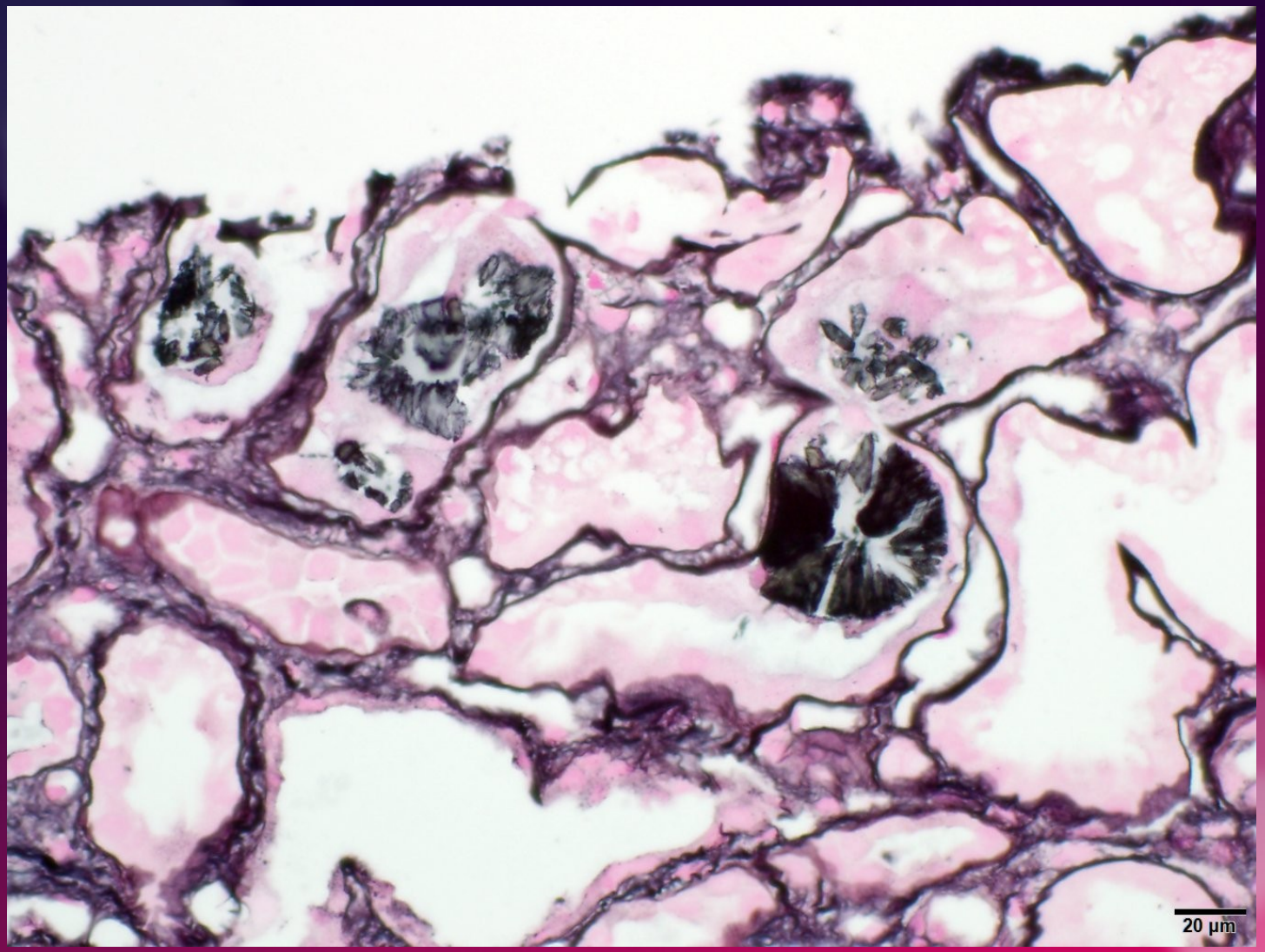
Case Presentation: 2,8-DHA





Case Presentation: 2,8-DHA

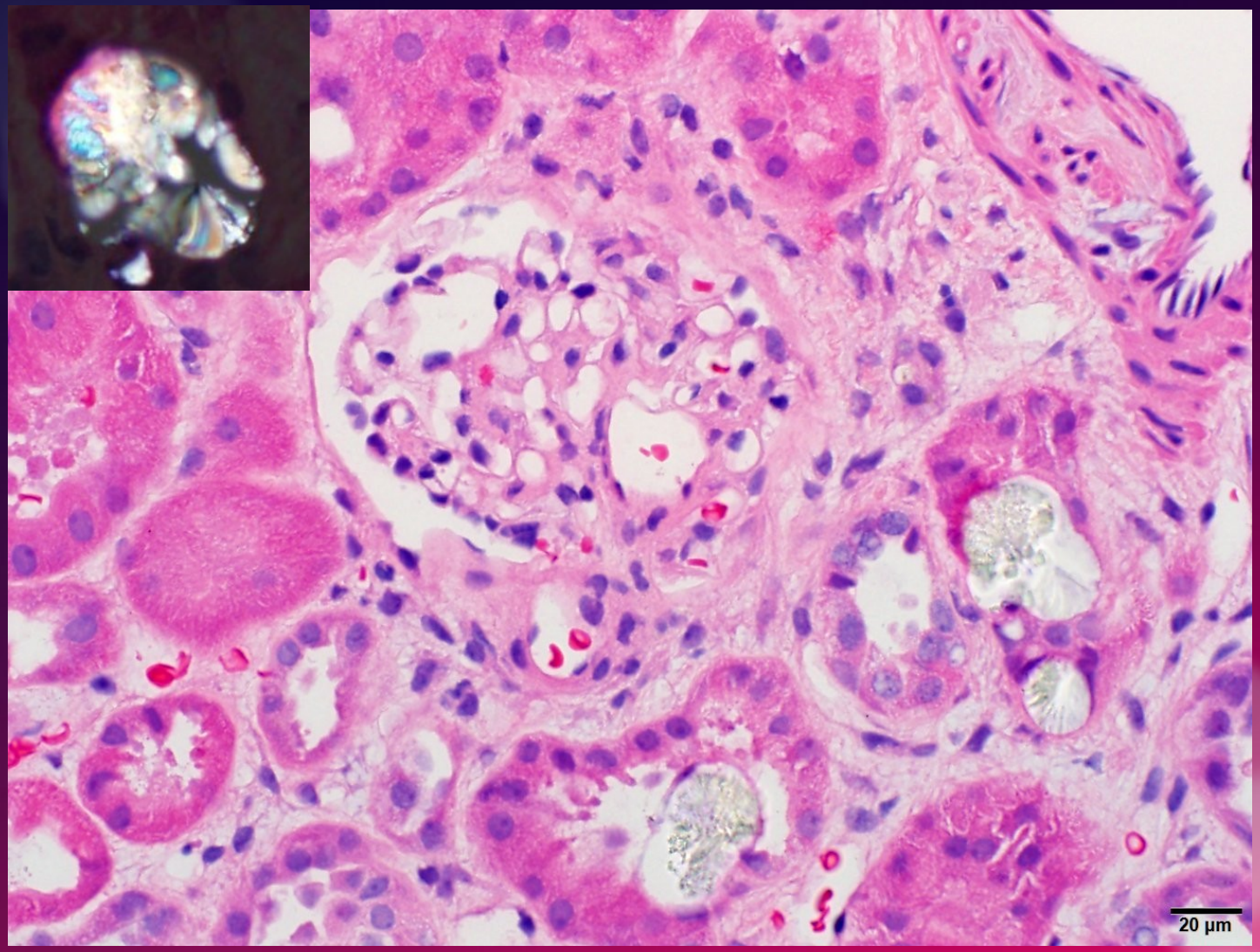




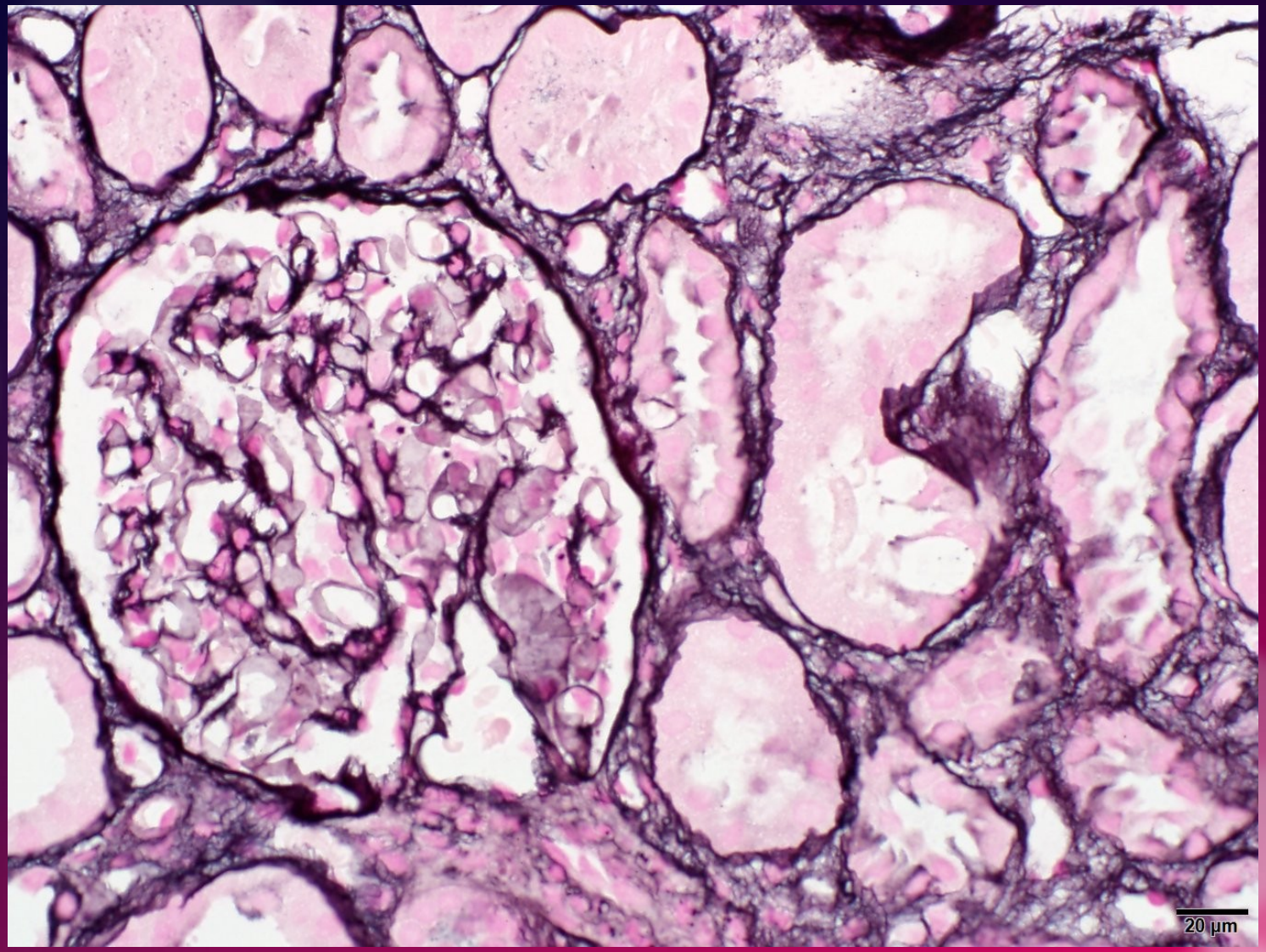
2,8-DHA on Renal Biopsy

- DHA crystals stained brownish-green to brown with the H&E and PAS stains, light blue on TRI and black on Jones Methenamine Silver (JMS).
- They are strongly birefringent under polarized light.
- In the tubular lumina, they are arranged as annular formations of striated crystals or formed fan-like or irregular shapes.
- Within the tubular cell cytoplasm, they appear as small single crystals with rod, needle, rhomboidal or irregular shapes.
- In the interstitium, the single crystals are in annular formations.
- Urinalysis with microscopic examination showed multiple spherical brownish crystals that had a pseudo-Maltese cross appearance under polarized light.
- With oxalate, it can appears yellow in cross polarized light if there are bile crystals.

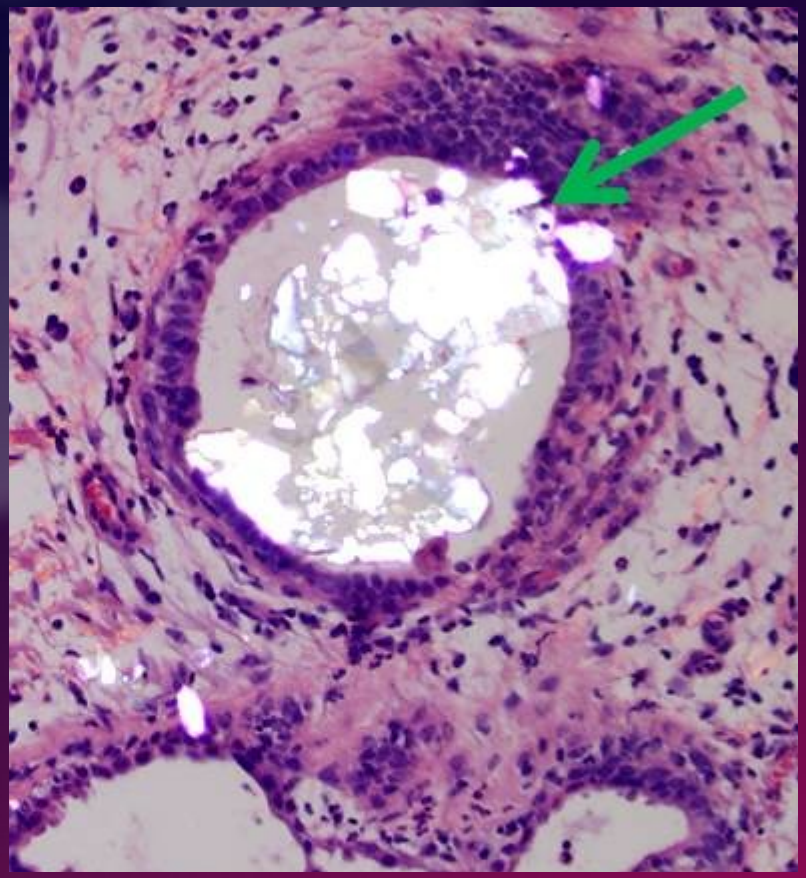
Oxalate Under Microscope

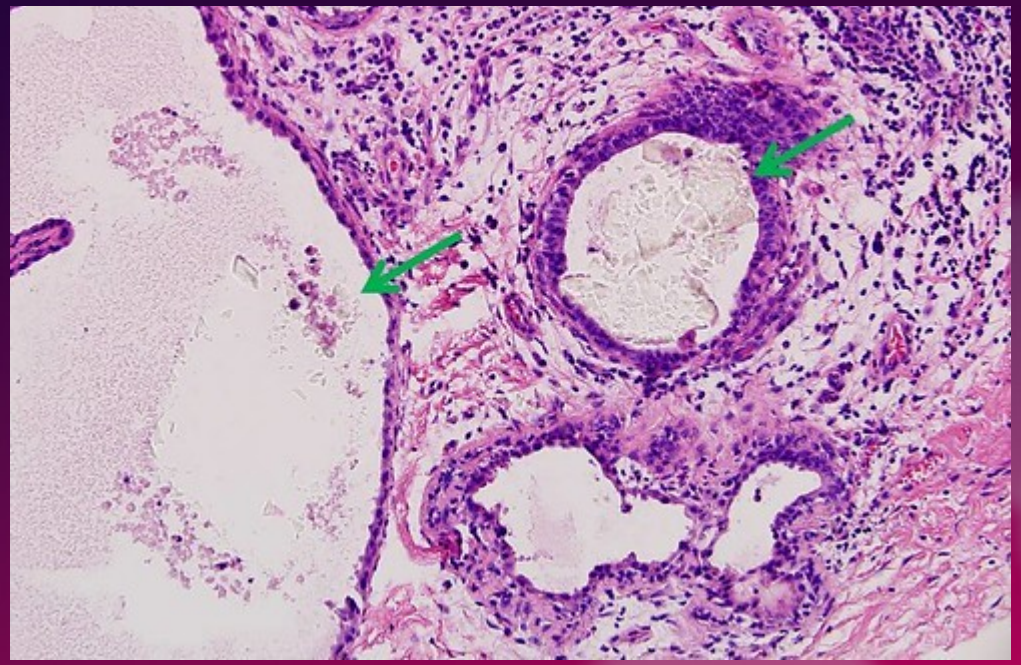


Oxalate Under Microscope



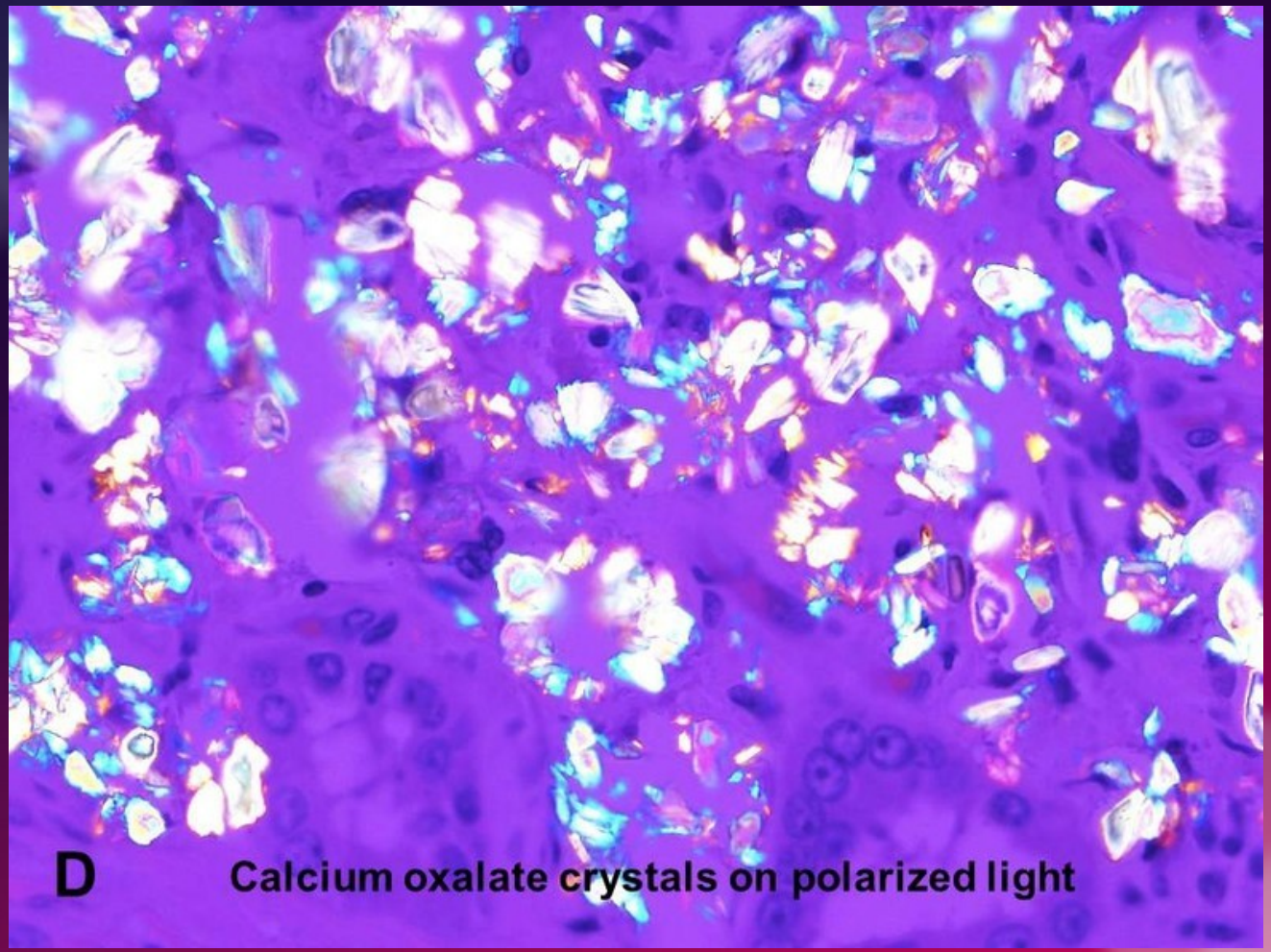
Oxalate Under Microscope





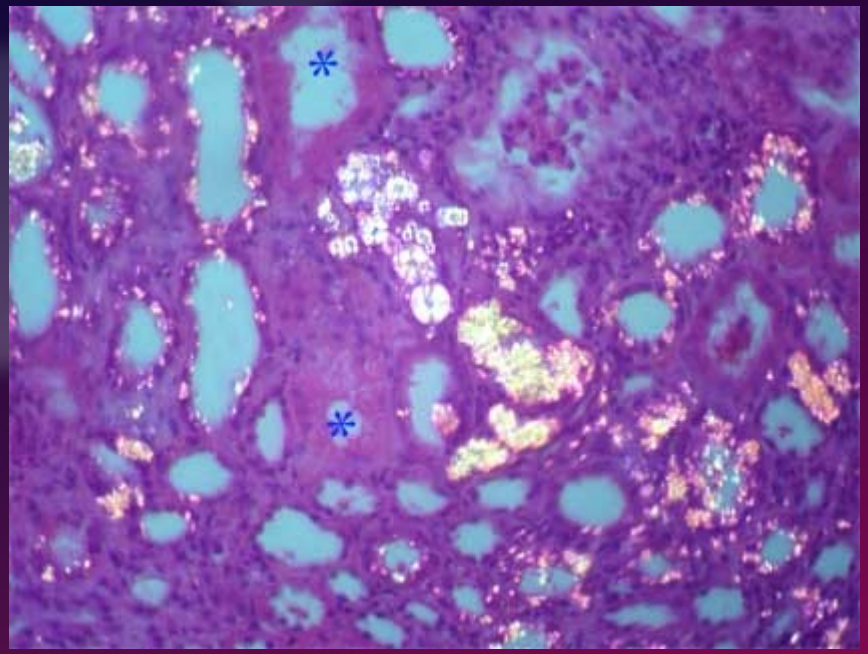
https://farm3.static.flickr.com/2160/13113951614_b6ef82b756_b.jpg, https://c1.staticflickr.com/4/3722/13113948664_af26ecdbef.jpg

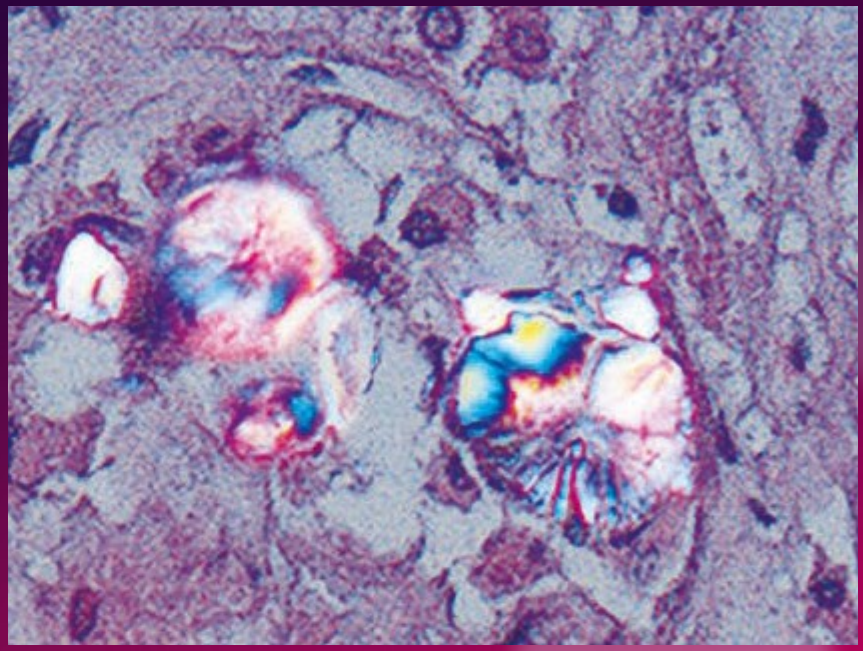
Oxalate Under Microscope



https://www.auanet.org/images/education/pathology/kidney-carcinomas/acquired_cystic-figureD_Big.jpg

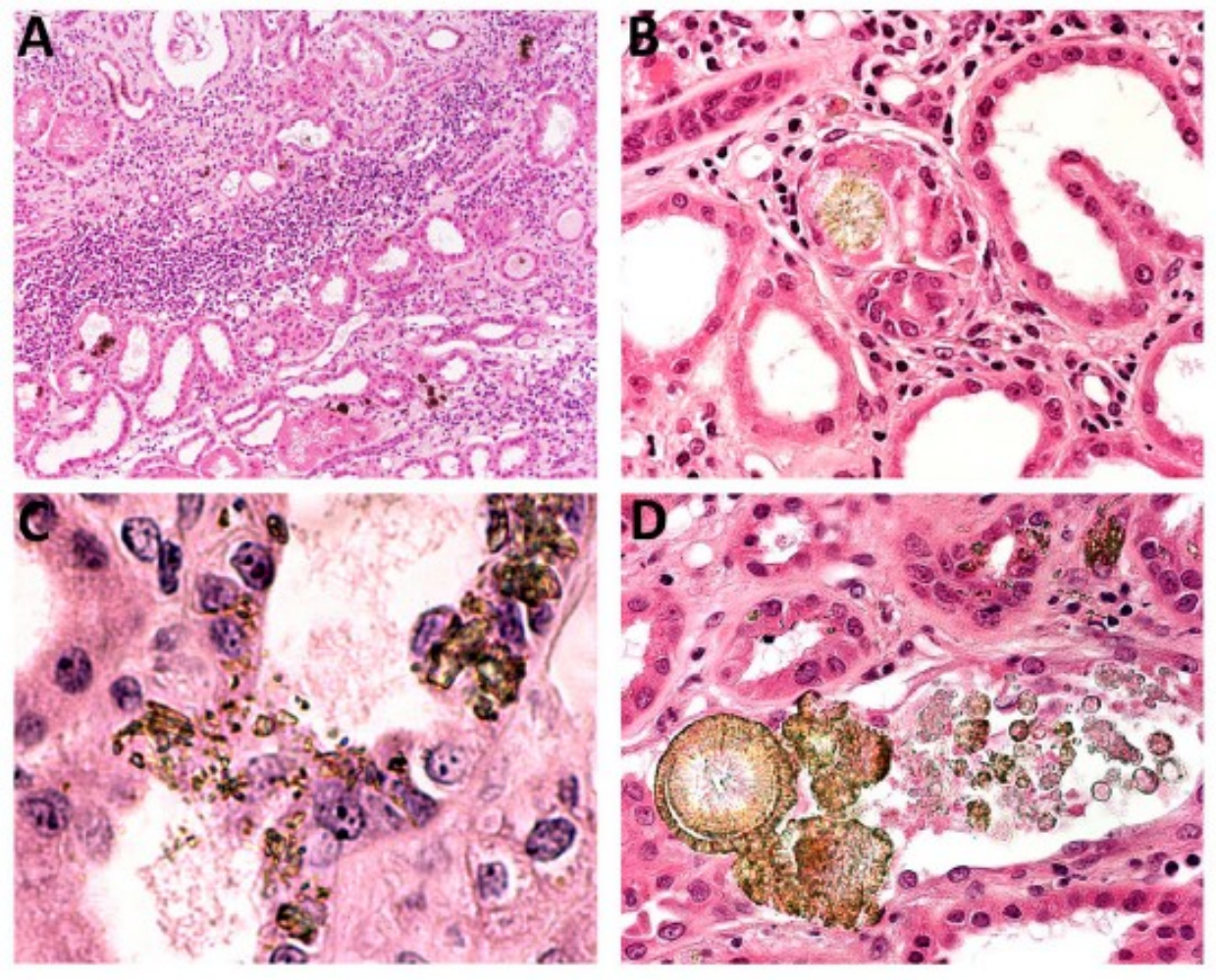
Oxalate Under Microscope





http://www.kidneypathology.com/Imagenes/Diabetes/Oxalato.4.w.jpg, https://www.omicsgroup.org/articles-admin/disease-images/hyperoxaluria-and-oxalosis-72550.jpg

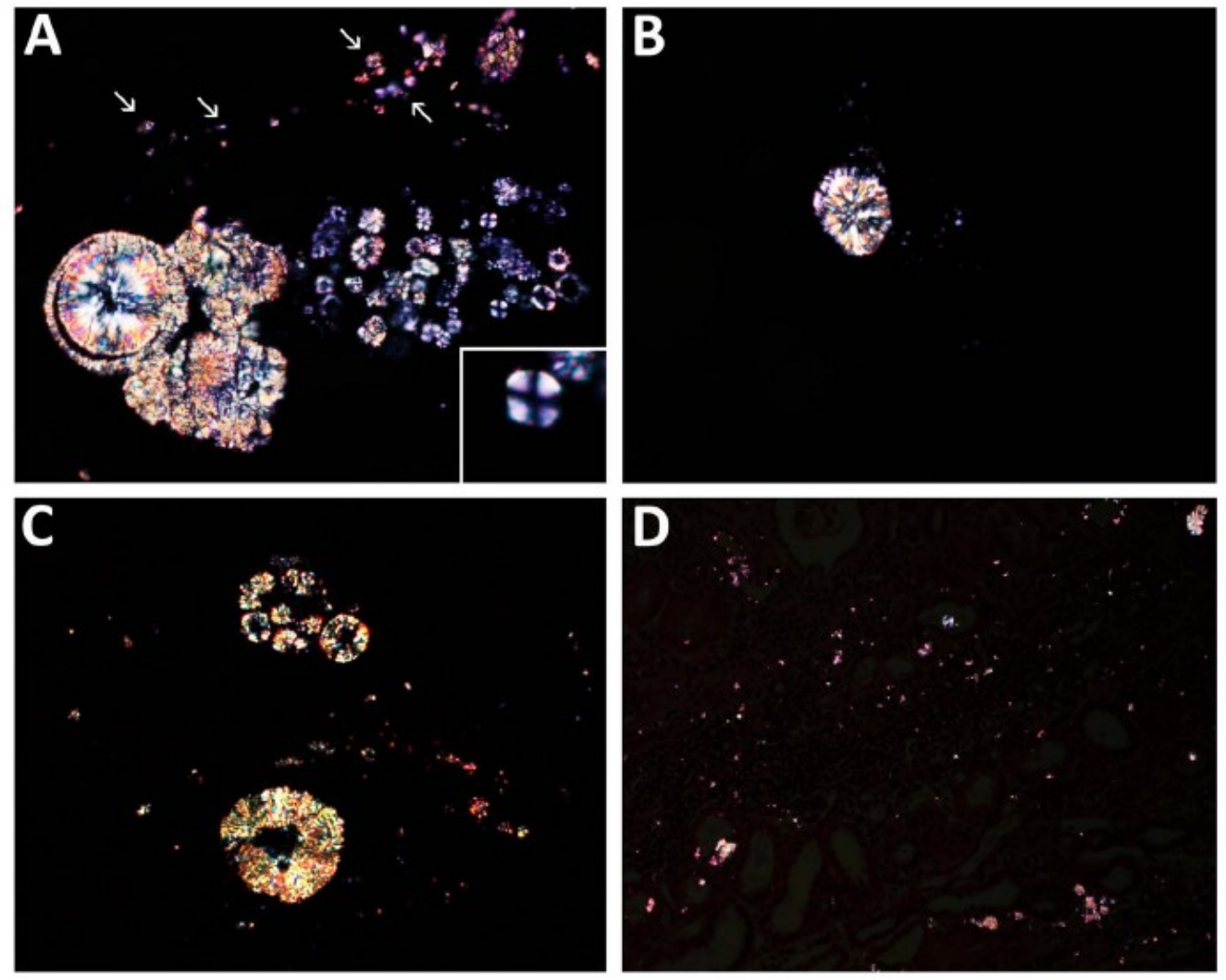
Other 2,8-DHA Case Reports



- Low magnification view. Focal deposition of crystals in the allograft parenchyma with diffuse inflammatory interstitial infiltrates and varying degrees of fibrosis and tubular atrophy.
- Deposits of 2,8-DHA crystals within tubular lumens forming spherical aggregates, causing tubular obstruction with foreign-body type reaction.
- Small needle-shaped and irregular crystals located within the tubular epithelial cells.
- Spherical crystal aggregates in the tubular lumen and within tubular epithelial cells.

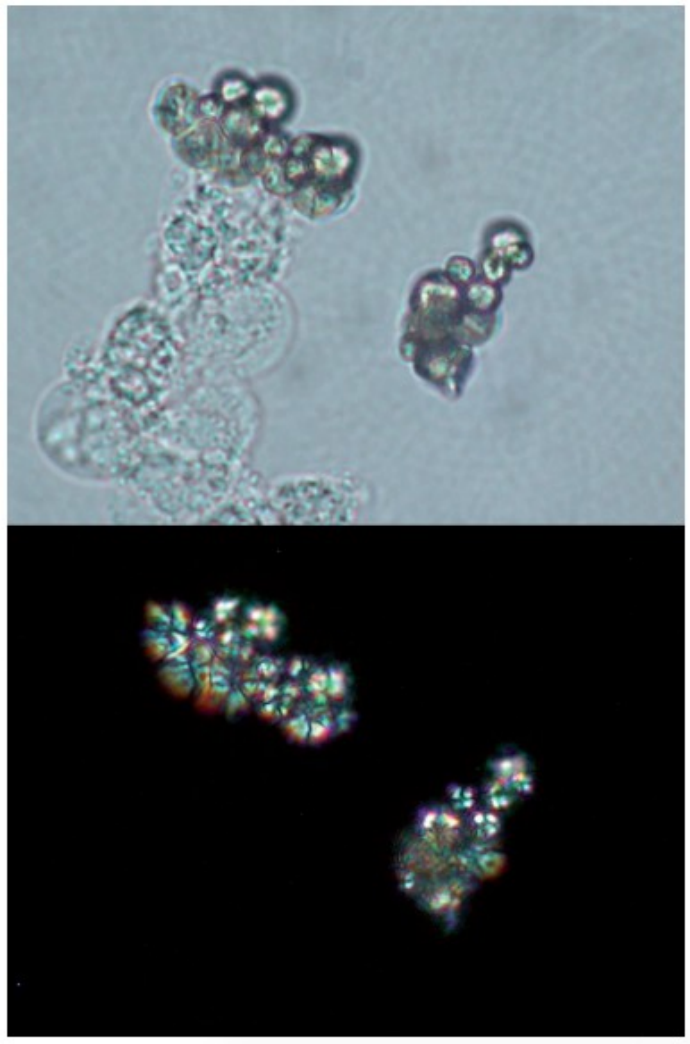
Zaidan M. et al. Recurrent 2,8-dihydroxyadenine nephropathy: a rare but preventable cause of renal allograft failure. American Journal of Transplantation 2014; 14: 2623-2632.

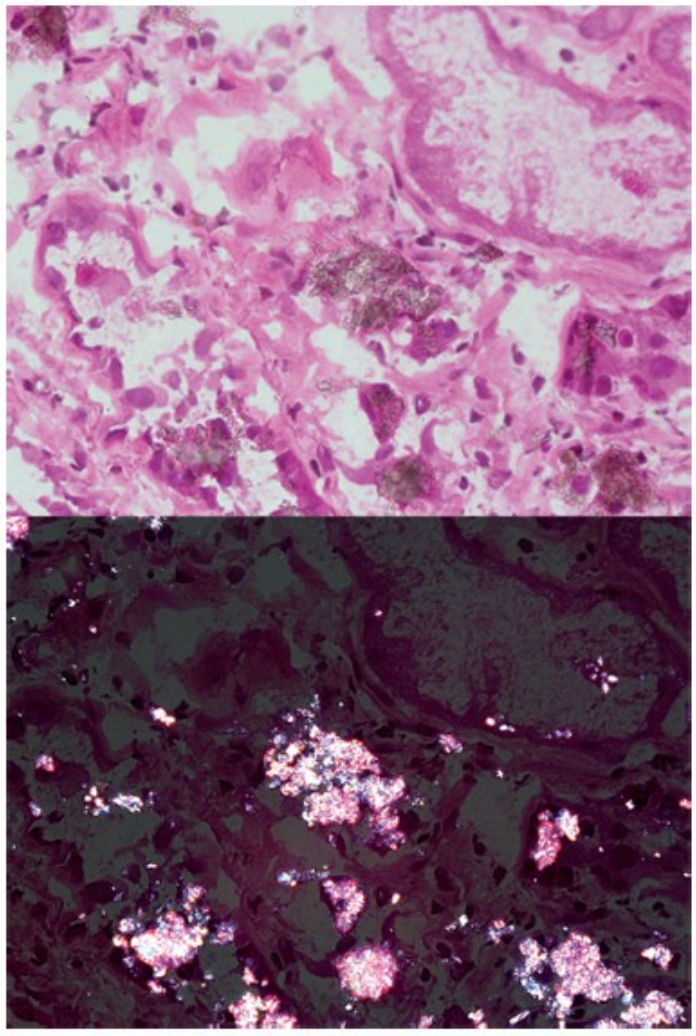
Other 2,8-DHA Case Reports



Zaidan M. et al. Recurrent 2,8-dihydroxyadenine nephropathy: a rare but preventable cause of renal allograft failure. American Journal of Transplantation 2014; 14: 2623-2632.

Other 2,8-DHA Case Reports

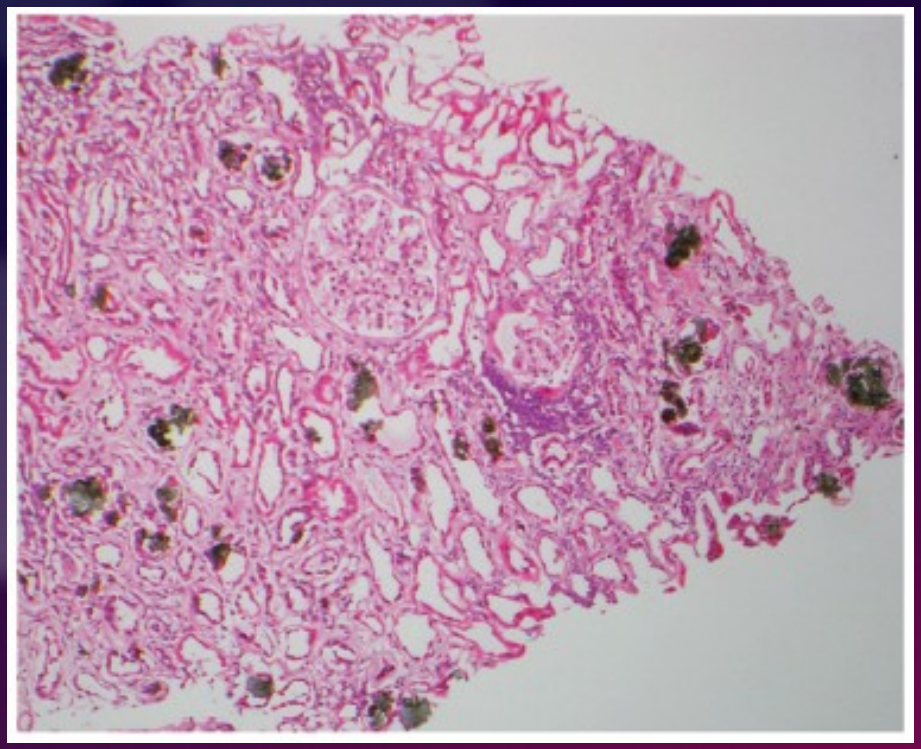


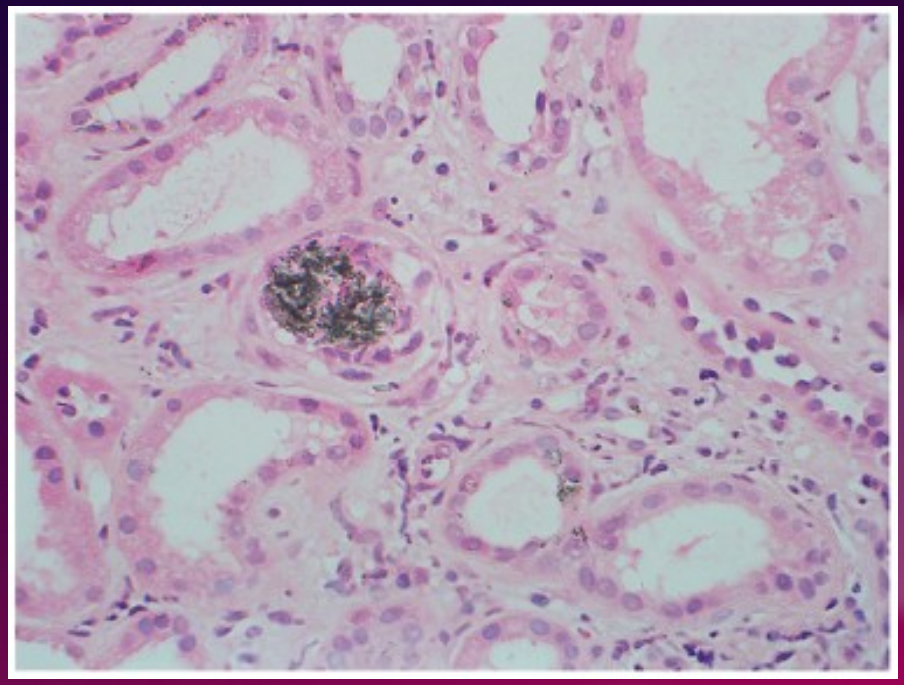


- Urine microscopy. Urinary sediments with spherical brownish crystals that have a birefringent and Maltose cross appearance under polarized light.
- Renal biposy. H&E staining showed interstitial inflammation surrounding multiple intra-tubular round brownish crystals that are birefringent under polarized light.

Fabrice MW et al. 2,8-dihydroxyadeninuria-induced progressive renal failure. Nephrology Dialysis Transplantation Plus (2008) 6: 437-439.

Other 2,8-DHA Case Reports

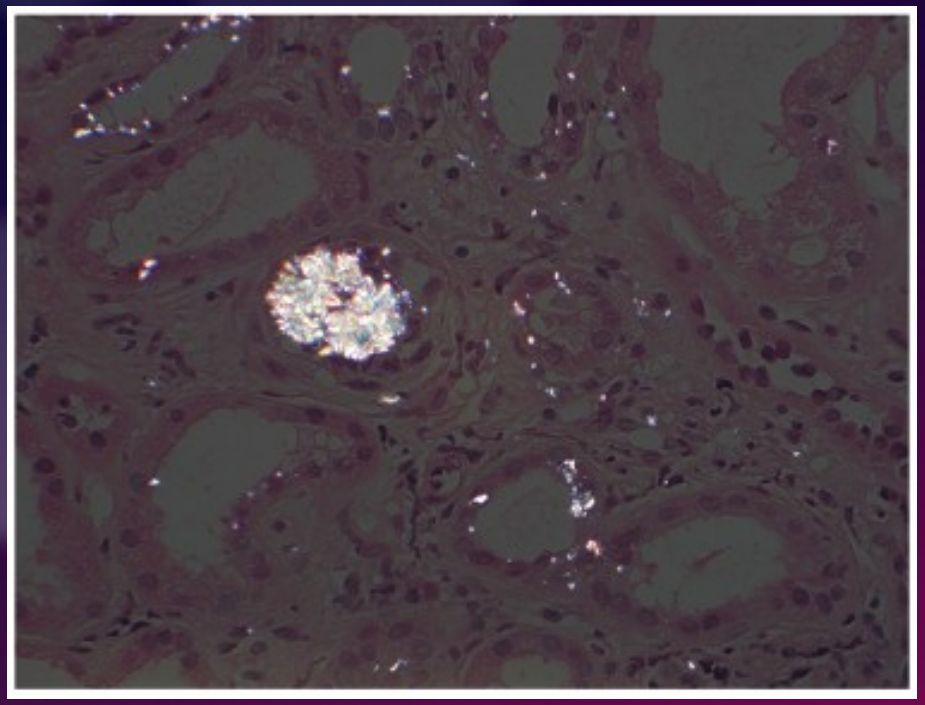


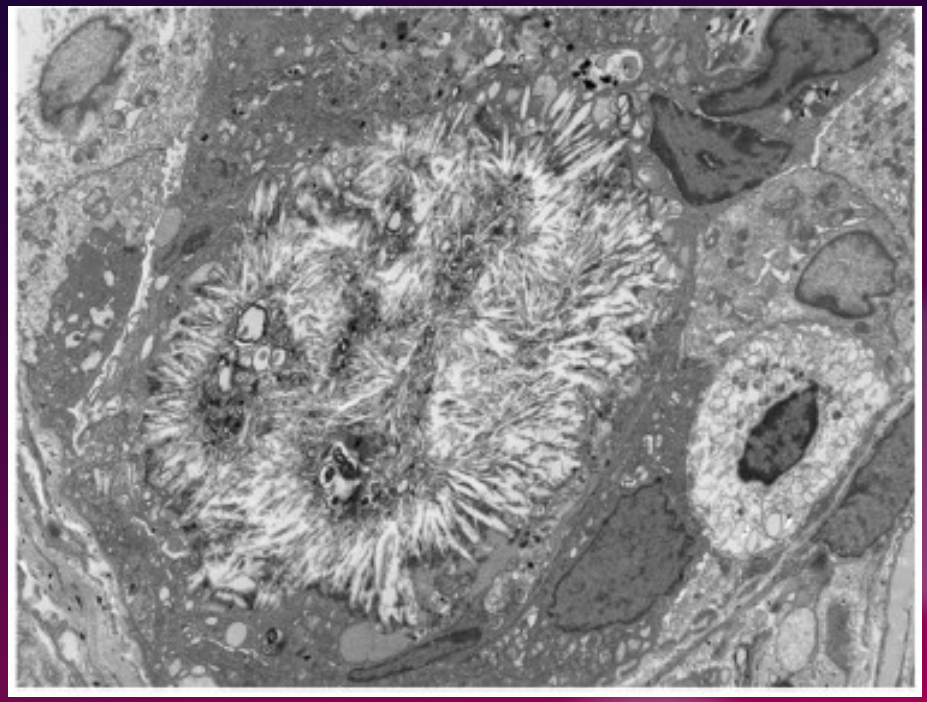


- Low power view showed diffuse tubular changes with numerous intraluminal tubular DHA crystals. Focal interstitial inflammation, moderate interstitial fibrosis.
- At high magnification, intraluminal globular aggregates and rod-shaped intracytoplasmic brownish-green DHA crystals.

Samih H.N. et al. Crystalline nephropathy due to 2,8-dihydroxyadeninuria: an under-recognized cause of irreversible renal failure. Nephrology Dialysis Transplant (2010) 25: 1909-1915.

Other 2,8-DHA Case Reports

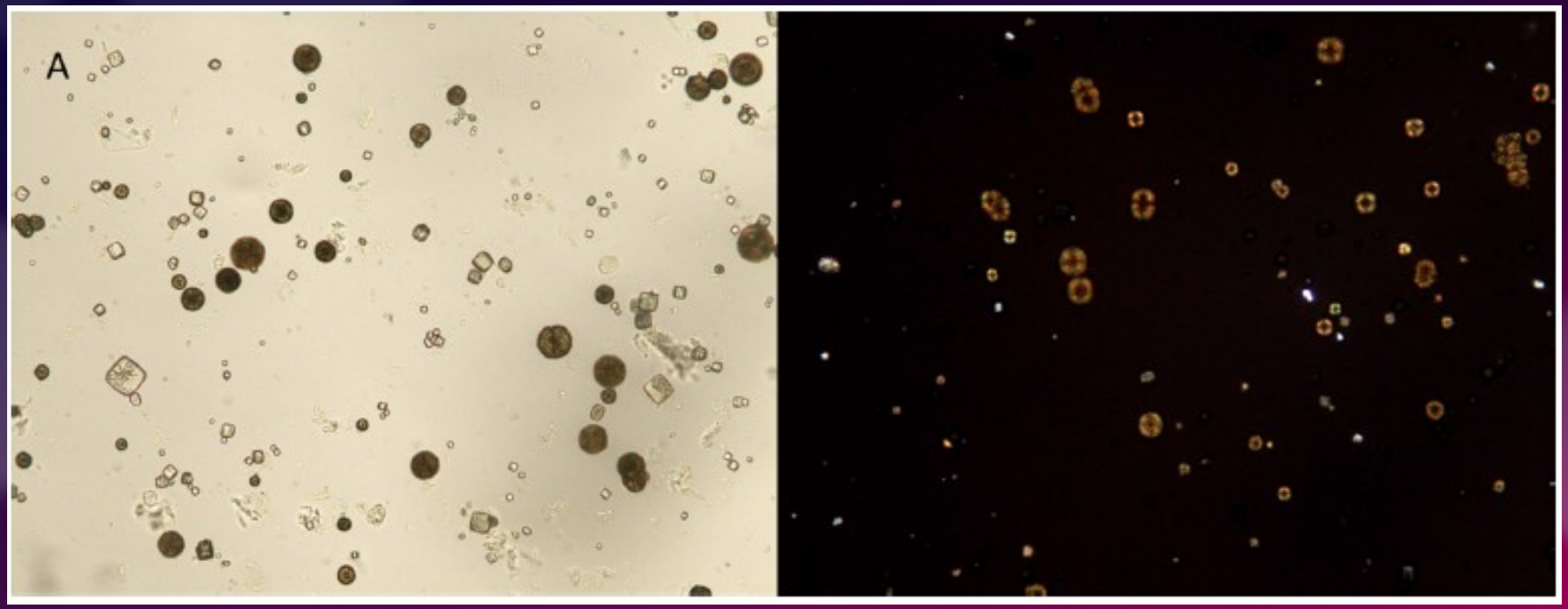




- At high magnification, same field as previously, but under polarized light. The intraluminal globular aggregates and rod-shaped intracytoplasmic brownish-green DHA crystals are strongly birefringent.
- EM, the intraluminal DHA deposits form radially-oriented clusters of needle-shaped, electronlucent crystals.

Samih H.N. et al. Crystalline nephropathy due to 2,8-dihydroxyadeninuria: an under-recognized cause of irreversible renal failure. Nephrology Dialysis Transplant (2010) 25: 1909-1915.

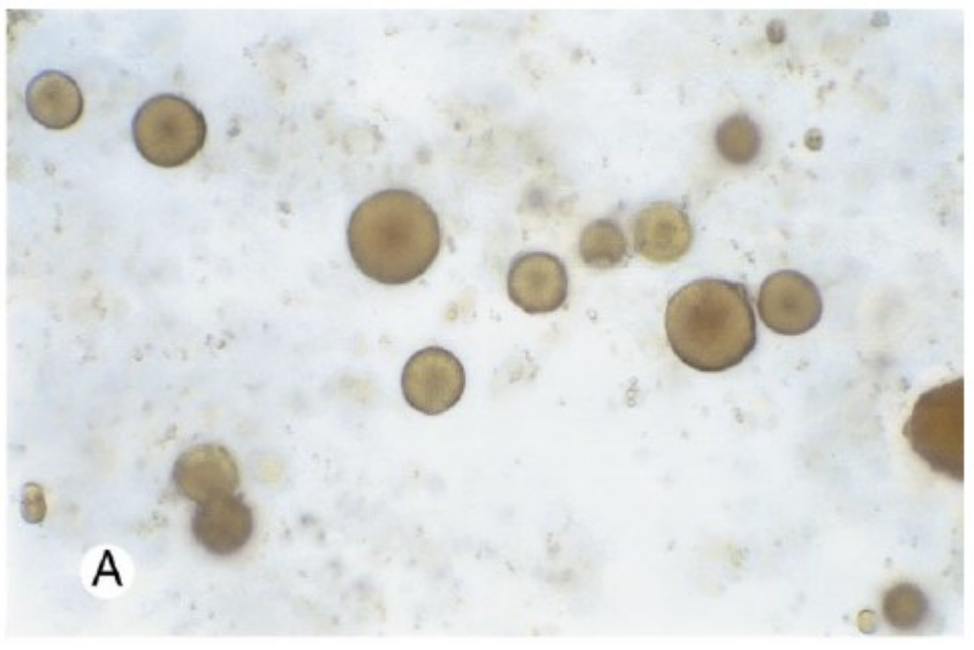
Other 2,8-DHA Case Reports

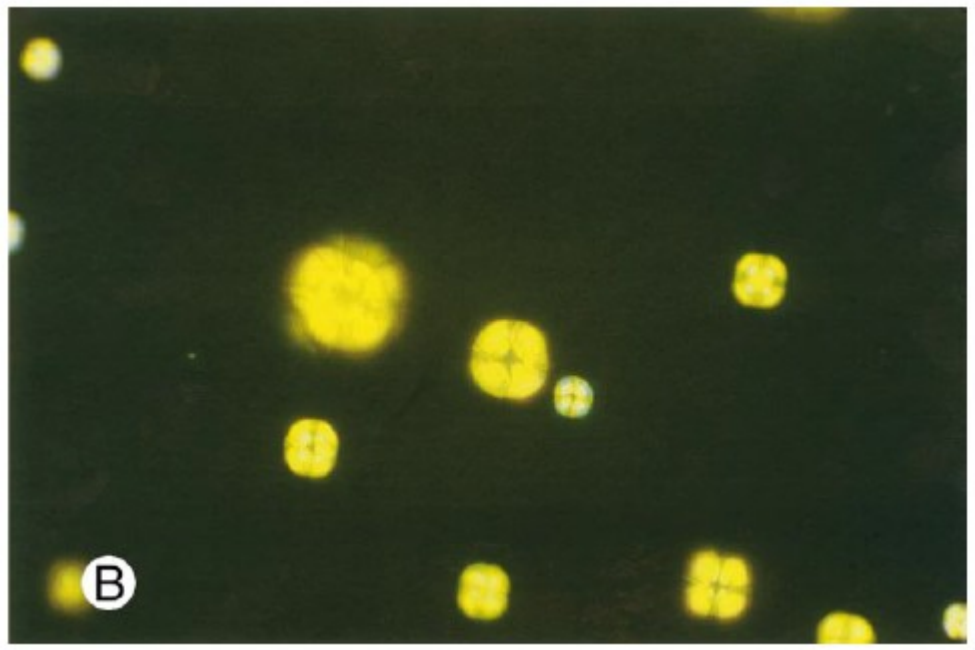


- Light microscopy of urinary sediment.
  - Medium-sized crystals are brown with dark edges and central spicules.
  - Polarized light microscopic view showed small- and medium-sized crystals appear yellow and produce a central Maltese cross pattern.

Hrafnhildur L.R. et al. Kidney disease in adenine phosphoribosyltransferase deficiency. American Journal Kidney Disease (AJKD). 2016; 67 (3): 431-438.

Other 2,8-DHA Case Reports





- Light microscopy of urinary sediment.
  - Reddish-brown crystals with dark edges and central spicules.
  - Polarized light microscopic view showed small- and medium-sized crystals appear yellow and produce a central Maltese cross pattern.

Edvardsson V. et al. Clinical features and genotype of adenine phosphoribosyltransferase deficiency in Iceland. American Journal of Kidney Diseases (AJKD), Vol 38, No 3 (September), 2001: 473-480.

2,8-DHA Nephropathy Findings

- **Renal allograft biopsy findings:**
  - Varying degrees of interstitial fibrosis, tubular atrophy, acute tubular necrosis, chronic interstitial nephritis.
  - Interstitial inflammatory infiltrates with lymphocytes, eosinophils, macrophages, histiocytes, multinucleated giant cells surrounding birefringent crystals.
  - Crystal depositions usually in cortex involving proximal and distal tubules.
  - No obvious glomerular lesions, no acute or chronic allograft rejection, no drug toxicity.

Fabrice MW et al. 2,8-dihydroxyadeninuria-induced progressive renal failure. Nephrology Dialysis Transplantation Plus (2008) 6: 437-439.

2,8-DHA Nephropathy Findings

- **DHA Features:**
  - DHA crystals stained brownish-green to brown with the H&E and PAS stains, light blue on TRI and black on JMS.
  - They are strongly birefringent under polarized light.
  - In the tubular lumina, they are arranged as annular formations of striated crystals or formed fan-like or irregular shapes.
  - Within the tubular cell cytoplasm, they appear as small single crystals with rod, needle, rhomboidal or irregular shapes.
  - In the interstitium, the single crystals are in annular formations.

Fabrice MW et al. 2,8-dihydroxyadeninuria-induced progressive renal failure. Nephrology Dialysis Transplantation Plus (2008) 6: 437-439.

2,8-DHA Nephropathy Findings

- **Urinalysis findings:**
  - Hematuria, low grade proteinturia, granular casts, amorphous crystals.
  - Urinalysis with microscopic examination showed multiple spherical brownish crystals that had a pseudo-Maltese cross appearance under polarized light.
- **Imaging findings:**
  - Imaging (CT scan, CTA, renal ultrasound) revealed kidneys of normal size or small atrophic kidneys without any kidney stones, no nephrocalcinosis.
  - Mass in kidney on renal ultrasound, not able to delineate further with CT, MRI or renal angiography. Nephrectomy proved mass to be large renal stone within a benign cyst.

Fabrice MW et al. 2,8-dihydroxyadeninuria-induced progressive renal failure. Nephrology Dialysis Transplantation Plus (2008) 6: 437-439.

2,8-DHA Nephropathy Globally

- Cases reported in PubMed
  - 222 search results for 2,8-dihydroxyadenine
  - 184 search results for 2,8-dihydroxyadenine, human
  - 21 searchh results for 2,8-dihydroxyadenine, pediatric
  - 83 search results for 2,8-dihydroxyadenine, adult
  - 55 search results for 2,8-dihydroxyadenine, adult, kidney
  - 10 search results for 2,8-dihydroxyadenine, acute kidney injury
  - 44 search results for 2,8-dihydroxyadenine, chronic kidney disease
  - 20 search results for 2,8-dihydroxyadenine, renal transplant (1998')

- More than 40 pathogenic mutations of APRT have been identified in more than 400 affected people from more than 25 countries, most of whom are from France, Iceland, and Japan. Only 15 cases originated from the U.S.
  - ~ 60% affected individuals are adults.
  - Kidney failure requiring RRT is the presenting features in 15% of adult cases.
  - Recognized only after renal transplant after transplant dysfunction occurs.
  - Asymptomatic.

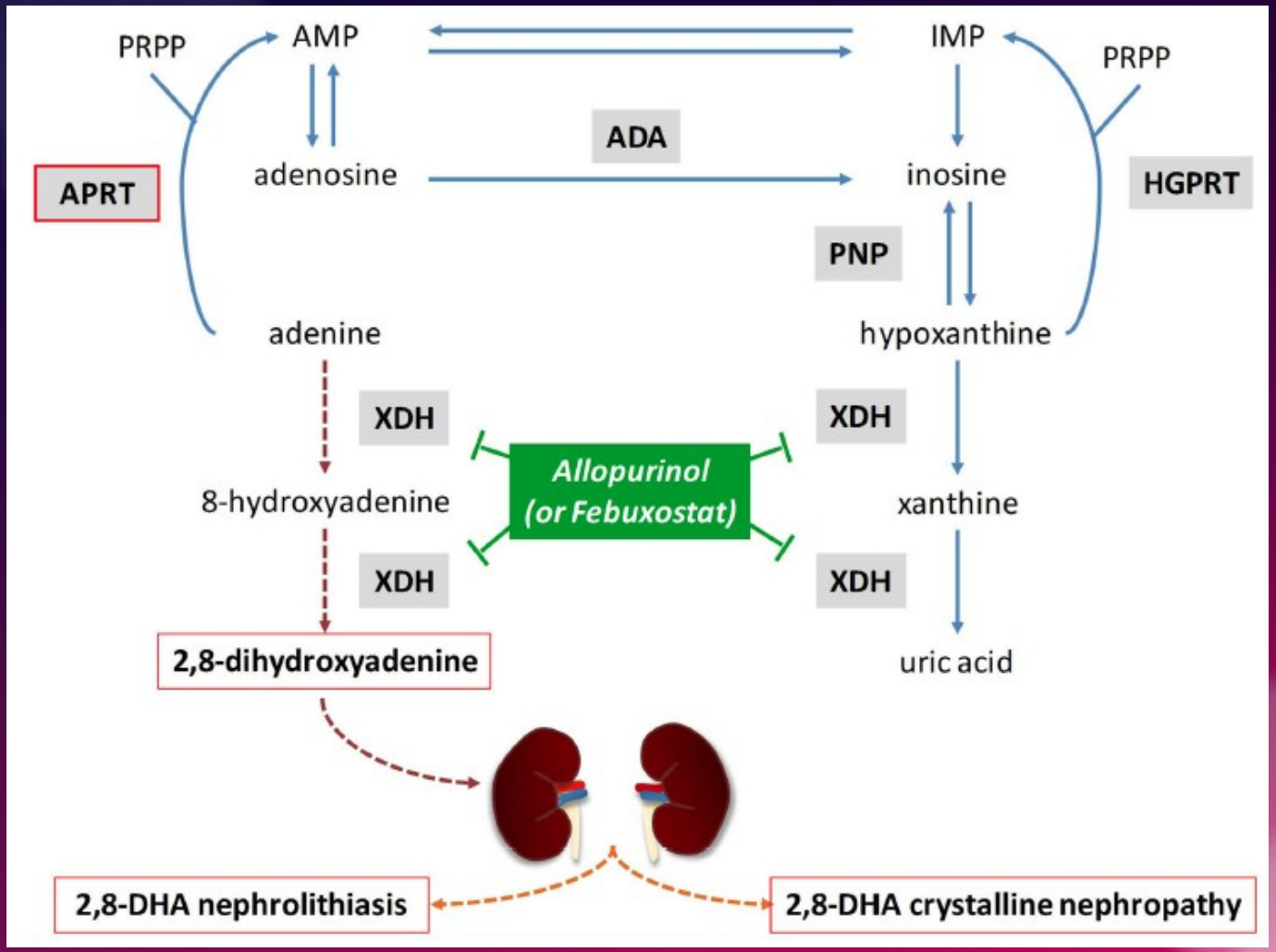
Hrafnhildur L.R. et al. Kidney disease in adenine phosphoribosyltransferase deficiency. American Journal Kidney Disease (AJKD). 2016; 67 (3): 431-438.

2,8-DHA Nephropathy

- In 1968, Kelly et al described partial adenine phosphoribosyltransferase (APRT) deficiency in four asymptomatic patients.
- The association of complete APRT deficiency and 2,8-dihydroxyadenine (2,8-DHA) renal stones was first described by Cartier and Hamet in 1974.
- There are two types:
  - Type 1 defect: complete absence of APRT in RBC extracts/lysates or intact RBCs. In Caucasians.
  - Type 2 defect: variable APRT activity in cell extracts, but no activity in intact cells or in vivo. Found only in Japan.
  - Both inherited in autosomal recessive manner.
  - Likely that most DHA stones that do occur in North American populations are misdiagnosed as uric acid or calcium oxalate stones.

Kelly W.N. et al. Adenine phosphoribosyltransferase deficiency: a previously undescribed genetic defect in man. Journal of Clinical Investigation. 1968; 47: 2261-2289. Kenneth H.F. et al. Adenine Phosphoribosyltransferase deficiency with renal deposition of 2,8-dihydroxyadenine leading to nephrolithiasis and chronic renal failure.

2,8-DHA Pathway



Zaidan M. et al. Recurrent 2,8-dihydroxyadenine nephropathy: a rare but preventable cause of renal allograft failure. American Journal of Transplantation 2014; 14: 2623-2632.

2,8-DHA Disease: Mechanisms

- Disorder of purine metabolism.
- Adenine phosphoribosyltransferase converts adenine into adenylate monophosphate (AMP).
- With APRT deficiency, adenine is oxidized by xanthine oxidase first into 8-hydroxyadenine and then into 2,8-DHA, eliminated by the kidneys through tubular secretion.
- DHA relatively insoluble at any pH, significant concentrations (>40 mg/L) of urinary DHA can lead to urolithiasis.
- Patients may have repeated kidney stones or no prior history of renal stones.
- 15% asymptomatic throughout life due to individual differences in the ability to supersaturate the urine with DHA. DHA crystals do not deposit in other tissues.

Fabrice MW et al. 2,8-dihydroxyadeninuria-induced progressive renal failure. Nephrology Dialysis Transplantation Plus (2008) 6: 437-439. Kenneth H.F. et al. Adenine Phosphoribosyltransferase deficiency with renal deposition of 2,8-dihydroxyadenine leading to nephrolithiasis and chronic renal failure.

2,8-DHA vs Primary Hyperoxaluria

	Primary hyperoxaluria	2,8-dihydroxyadeninuria
Defected enzyme	AGT (in type I) GRHPR (in type II)	APRT
Mode of inheritance	Autosomal recessive	Autosomal recessive
Onset of clinical symptoms	In childhood in most patients	In adulthood in most patients
Presenting features	Nephrolithiasis in children Chronic renal failure in infants	Nephrolithiasis Rarely chronic renal failure
Extrarenal manifestations	Cardiac conduction defects, vasculopathy, anemia, osteopathy, retinopathy	None known
Gold standard diagnostic test	Determining AGT and GRHPR activity by liver biopsy	Determining APRT activity in red blood cells
Treatment	Low oxalate diet, high fluid intake, pyridoxine, orthophosphate, combined liver–kidney transplant	Low-purine diet, high fluid intake, allopurinol
Rate of recurrence after renal transplant and graft loss due to recurrence	High	High

AGT, alanine/glyoxalate aminotransferase; GRHPR, glyoxylate reductase/hydroxypyruvate reductase; APRT, adenine phosphoribosyltransferase.

Samih H.N. et al. Crystalline nephropathy due to 2,8-dihydroxyadeninuria: an under-recognized cause of irreversible renal failure. Nephrology Dialysis Transplant (2010) 25: 1909-1915.

Metabolic Disease: Nepropathy

	Oxalate	Urate	Cystine	2,8-dihydroxyadenine
Birefringence	Yes	Yes in alcohol fixed tissue (usually dissolve with formalin fixation/processing)	Yes in alcohol fixed tissue (dissolve with formalin fixation/processing)	Yes
Staining characteristics	Usually transparent (deep blue if admixed with calcium)	Transparent or pale blue (deep blue if admixed with calcium)	Difficult to see (transparent)	Brownish-green or brown with the H&E and PAS stains, TRI-light blue, JMS-black
Shapes and arrangements	Rhomboid shapes often with cluster or rosette-like arrangement	Elongated, rectangular or amorphous shapes, usually in large collections	Rectangular or needle shapes, present singly or in small collections	Needle, rod or rhomboid shapes, present singly, as annular formations of striated crystals or as fan-like or irregular clusters
Distribution	Mainly in tubular lumina and cytoplasm, sometimes in the interstitium (more abundant in the cortex)	Mainly in collecting ducts lumina and interstitium in the medulla (not in tubular cells)	Mainly in interstitium, sometimes in tubular cells and podocytes	Mainly in tubular lumina and cytoplasm, sometimes in the interstitium (more abundant in the cortex)
Giant cell reaction to crystals	Sometimes in interstitium	Common in tubules and interstitium	Multinucleated podocytes	Sometimes in interstitium and tubules

Samih H.N. et al. Crystalline nephropathy due to 2,8-dihydroxyadeninuria: an under-recognized cause of irreversible renal failure. Nephrology Dialysis Transplant (2010) 25: 1909-1915.

2,8-DHA Progression in Kidneys

- Observational cohort study in Iceland.
- Studied the kidney stone recurrence in APRT deficiency.
- All patients enrolled in the APRT Deficiency Registry of the Rare Kidney Stone Consortium.
  - Total 53 patients (from 42 families):
    - From Iceland 33; U.S. 13; Austria 2; Italy 2; UK 1; India 1; Norway of Turkish descent 1.
- Median duration of follow-up was 10.3 years (range, 0.0-31.5 years).

Hrafnhildur L.R. et al. Kidney disease in adenine phosphoribosyltransferase deficiency. American Journal Kidney Disease (AJKD). 2016; 67 (3): 431-438.

2,8-DHA Progression in Kidneys

Clinical characteristics at diagnosis of APRT deficiency

Characteristic	Value
Female sex	30 (57)
Age	
Median (range), y	37.0 (0.6–67.9)
<18 y	14 (26)
≥18 y	39 (74)
Kidney stones	29 (55)
Chronic kidney disease stages 3–5	20 (38)
Renal replacement therapy	8 (15)
Acute kidney injury	16 (30)
Lower urinary tract symptoms	15 (28)
Reddish-brown diaper stain in infancy	11 (21)
2,8-DHA crystalluria	34 (64)
Asymptomatic	5 (9)
Family screening	3
Incidental finding	2

Note: N=53. Unless otherwise indicated, values are given as number or number (percentage).

Abbreviations: APRT, adenine phosphoribosyltransferase; DHA, dihydroxyadenine.

- 50 patients were white, 1 African American, 1 Indian, and 1 Japanese..
- Median age of the 20 patients who had developed CKD stages 3-5 at diagnosis was 44.5 years.
- The diagnosis of APRT deficiency was initially suggested by detection of urinary 2,8-DHA crystals in 34 cases, histologic findings of crystal nephropathy in 9, and kidney stone analysis in 8 cases. Two cases were dignosed postmortem by review of autopsy findings that were consistent with 2,8-DHA crystal nephropathy.
- In 52/53 cases, the diagnosis of APRT deficiency was confirmed by genetic testing (n=45) and/or absence of APRT activity in RBC lysates (n=11).

Hrafnhildur L.R. et al. Kidney disease in adenine phosphoribosyltransferase deficiency. American Journal Kidney Disease (AJKD). 2016; 67 (3): 431-438.

2,8-DHA Progression in Kidneys

Kidney stones in patients with APRT deficiency

Variable	Value
Total no. of patients with kidney stones	33 (62)
At time of diagnosis	29 (55)
During follow-up	4 (8)
Age	
At first kidney stone event, y	26.4 (0.3–56.4)
≥18 y at first episode	26 (49)
Kidney stones before diagnosis of APRT deficiency	20 (38)
Delay from first clinical stone event to diagnosis, y	10.5 (0.8–47.9)
Kidney stone recurrence	18 (34)
Off XDH inhibitor treatment	2 (4)
On XDH inhibitor treatment	16 (30)
Allopurinol dosage, mg	200 (100–600)
Kidney stone events	
1	14 at diagnosis; 7 during f/u
2–3	10 at diagnosis; 8 during f; u
4–5	1 at diagnosis; 2 during f; u
>5	3 at diagnosis; 0 during f; u
Asymptomatic stones	5 at diagnosis; 2 during f; u
Urologic procedures	
extracorporeal shockwave lithotripsy	4 at diagnosis; 6 during f; u
Endoscopic surgery	6 at diagnosis; 9 during f; u
Open or percutaneous surgery	4 at diagnosis; 3 during f; u

- A median delay in diagnosis of 7.5 years occurred in 37 patients following their first symptomatic stone event or detection of elevated serum Cr level.
- Misidentification of radiolucent kidney stone as uric acid calculi in 4 patients and confusion of renal histopathologic findings with other forms of crystal nephropathy in 6 patients contributed to diagnostic delay.
- Urinary 2,8-DHA crystals not correctly identified in 17 cases.
- 18 patients had 35 clinical stone events while receiving allopurinol treatment.

Hrafnhildur L.R. et al. Kidney disease in adenine phosphoribosyltransferase deficiency. American Journal Kidney Disease (AJKD). 2016; 67 (3): 431-438.

2,8-DHA Progression in Kidneys

Stages of CKD and RRT in patients with APRT deficiency

	At diagnosis	At last follow-up
CKD stage		
1	19	19
2	14	12
3A	2	2
3B	2	3
4	5	3
5	11	14
RRT		
Functioning allograft	3	7
Dialysis	5	2

- Of the 14 patients with CKD stage 5 at the last follow up, 12 had initiated RRT, of whom 11 had undergone kidney transplant and received total of 15 transplants. Three patients died with a functioning transplant at a median age of 43.2 years.
- None of the 11 patients who had developed CKD 5 by the time of diagnosis received XDH inhibitor therapy.
- Initiated daily dose of allopurinol varies from 100-300 mg qday.
- Prescribed dose of allopurinol increased over time, up to 400-600 mg/day regardless of kidney function.
- Allopurinol therapy was discontinued in 7 patients after a median of 7.1 years of treatment due to presumed adverse reactions (eg. itching, hair loss, severe ocular symptoms such as pain, photosensitivity and blurred vision). Febuxostat, 80mg/day, was substituted.

Hrafnhildur L.R. et al. Kidney disease in adenine phosphoribosyltransferase deficiency. American Journal Kidney Disease (AJKD). 2016; 67 (3): 431-438.

2,8-DHA Nephropathy

- Important points:
  - The most commonly observed clinical manifestations were nephrolithiasis, AKI, progressive CKD, eventually requiring dialysis or kidney transplantation in a significant proportion of patients.
  - The absence of nephrolithiasis in many of the patients with CKD stages 3 to 5.
  - Patients who progressed to kidney failure almost invariably had not received XDH inhibitor treatment, emphasized the importance of correct early diagnosis and initiation of therapy.
  - Recurrent stones occurred despite the use of allopurinol, questionable therapeutic doses and adherence. The XDH inhibitor dose required to adequately reduce urinary 2,8-DHA excretion has not been defined.
  - Limitations of the study: small sample size (rare disease), use of observational registry data, duration of observation varied, scope of laboratory evaluation varied, large proportion of patients from Iceland limiting generalization.

Hrafnhildur L.R. et al. Kidney disease in adenine phosphoribosyltransferase deficiency. American Journal Kidney Disease (AJKD). 2016; 67 (3): 431-438.

2,8-DHA UnderDiagnosis

- The frequency of heterozygosity at APRT locus ~0.4-1.1% in Caucasians, which would suggest a homozygosity rate 1/50,000-1/100,000. Given population of ~300 million, 3000-6000 people in the U.S. may be at risk of developing DHA disease.
  - Asymptomatic homozygotes, up to 15%.
  - Misdiagnosed as uric acid stones or oxalate stones.
    - DHA stones misdiagnosed as uric acid stones but successfully treated with allopurinol and low purine diet.
- 2,8-DHA nephropathy should be considered in the differential diagnosis of radiolucent kidney stones or unexplained CKD and in cases of kidney transplant dysfunction of unclear cause since APRT deficiency is a preventable cause of CKD.

Samih H.N. et al. Crystalline nephropathy due to 2,8-dihydroxyadeninuria: an under-recognized cause of irreversible renal failure. Nephrology Dialysis Transplant (2010) 25: 1909-1915.

ID'ing 2,8-DHA Nephropathy

- In infants, reddish-brown diaper stains.
- Radiolucent stones.
- 2,8-DHA stones - wet friable reddish brown or dry chalky grayish crystals.
- **Crystalluria sensitivity and specificity close to 100%.**
  - Pitfalls: requires experienced operator.
  - Differentiate between uric acid stones vs 2,8-DHA in urinalysis.
  - Absence of urinary 2,8-DHA crystals was generally considered indicative of adequate drug dosing.
  - Assessment of crystalluria not systematically performed.
- **Renal biopsy very high sensitivity and specificity.**
  - Pitfalls: may not characterize very small crystals <12 micrometer.
  - Differentiate between oxalate crystals vs 2,8-DHA on renal biopsies.

Kenneth H.F. et al. Adenine Phosphoribosyltransferase deficiency with renal deposition of 2,8-dihydroxyadenine leading to nephrolithiasis and chronic renal failure. Samih H.N. et al. Crystalline nephropathy due to 2,8-dihydroxyadeninuria: an under-recognized cause of irreversible renal failure. Nephrology Dialysis Transplant (2010) 25: 1909-1915.

ID'ing 2,8-DHA Nephropathy

- **Lysates of RBCs would show no APRT enzyme activity. Sensitivity and specificity of 100%. Pitfalls: availability.**
- X-ray diffraction analysis can identify DHA stones in renal tissue.
- High performance liquid chromatography (HPLC) can detect the presence of adenine, 8-hydroxyadenine, and DHA in urine.
- PCR can detect DNA/APRT gene isolated from blood.
- **Genetic testing. Identification of 80-90% of APRT gene mutations. Pitfalls: cost.**

Kenneth H.F. et al. Adenine Phosphoribosyltransferase deficiency with renal deposition of 2,8-dihydroxyadenine leading to nephrolithiasis and chronic renal failure. Samih H.N. et al. Crystalline nephropathy due to 2,8-dihydroxyadeninuria: an under-recognized cause of irreversible renal failure. Nephrology Dialysis Transplant (2010) 25: 1909-1915.

Therapy: 2,8-DHA Nephropathy

- The likelihood of complete regression of crystal deposition and recovery of allograft function depends on the extent of kidney damage at treatment initiation.
- Xanthine oxidase inhibitor
  - Start treatment early.
  - Allopurinol at 5-10 mg/kg body weight (maximum daily dose 800 mg/d).
    - Adenine more soluble than DHA.
  - Dosing of allopurinol largely empiric.
  - Alternatively with febuxostat 80 mg/d.
- Allopurinol or febuxostat for at least 6 weeks prior to transplant surgery.
- Lifelong therapy to prevent recurrent DHA nephropathy in transplanted kidney. The allopurinol dose should not be reduced in affected individuals who have impaired renal function.
- Low purine diet and high fluid regimen. Most metabolized adenine is not dietary in origin.
- Family members should also be screened.

Kenneth H.F. et al. Adenine Phosphoribosyltransferase deficiency with renal deposition of 2,8-dihydroxyadenine leading to nephrolithiasis and chronic renal failure.

FOLLOW UP

PATIENT CASE

APRT Genetic Testing

Adenine Phosphoribosyltransferase Deficiency
APRT Sequence Analysis

79801-2950566

RESULTS:

An apparently homozygous c.400+2dupT pathogenic variant was detected.

Pathogenic Variant(s)/ Mutation(s)

Nucleotide Change	Amino Acid Change	Location	Zygosity	Reference(s) / Comment(s)
c.400+2dupT		intron 4	homozygous	PMID: 3680503

INTERPRETATION:

Test results should be interpreted in the context of this individual's clinical and family history. It is our understanding that this individual has clinical symptoms suggesting adenine phosphoribosyltransferase (APRT) deficiency, including DHA crystals in the kidneys and a failed kidney transplant. Additionally, this individual has a positive family history for kidney disease. For the purpose of this analysis, we assume that this information is accurate.

An apparently homozygous c.400+2dupT pathogenic variant was detected. This variant affects the normal splicing of intron 4, causing skipping of exon 4 and leading to deficiency in APRT enzyme activity (PMID: 3680503).

Although these results are consistent with a diagnosis of APRT deficiency, targeted sequence analysis (test code 2826, performed on a fee-for-service basis) is recommended for this individual's biological parents in order to confirm that each is heterozygous for this variant, as opposed to the variant being in one allele with failure of the other allele to amplify or give a signal, or for other genetic reasons.

Clinical correlation and genetic counseling are recommended. Targeted sequence analysis (test code 2826) is available to at-risk relatives.

METHOD:

The coding regions of the APRT (NM_000485.2) gene are PCR amplified and then sequenced in the forward and reverse directions using automated fluorescent dideoxy sequencing methods. Nucleotide 1 corresponds to the A of the start codon ATG. Variants detected in exons and in introns within up to 20bp of the exon/intron boundaries are reported. This analysis will not detect large heterozygous deletions or duplications, inversions, or mutations within the promoter or deep intronic regions.

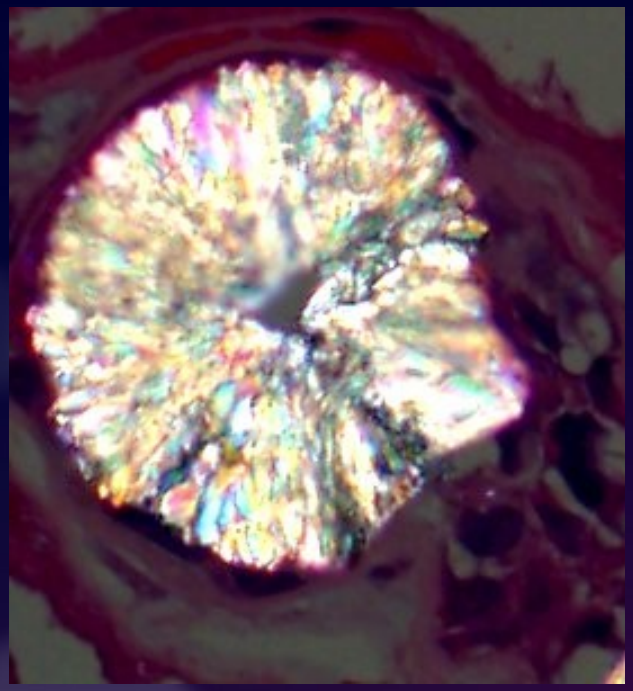
NOTES: The interpretation of nucleotide changes is based on our current understanding of the specific gene. This interpretation may change over time as more information about this gene becomes available. Possible diagnostic errors include sample mix-ups, genetic variants that interfere with analysis, incorrect assignment of biological parentage, and other sources. Please contact a genetic counselor at the Medical Genetics Laboratories if there is reason to suspect one of these sources of error.

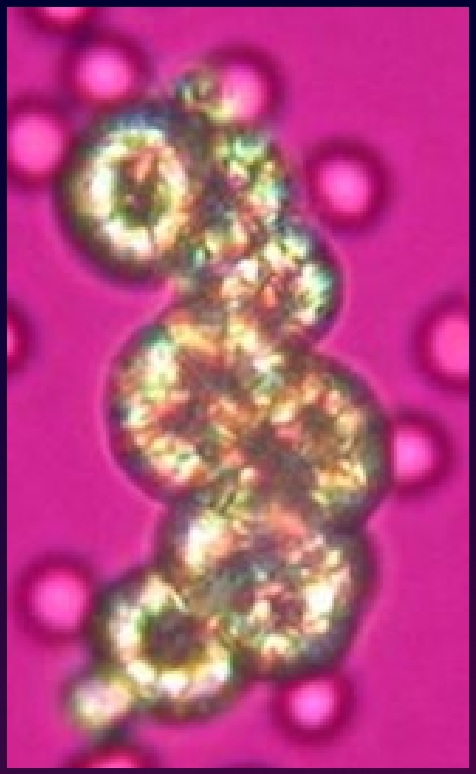
His Clinical Course

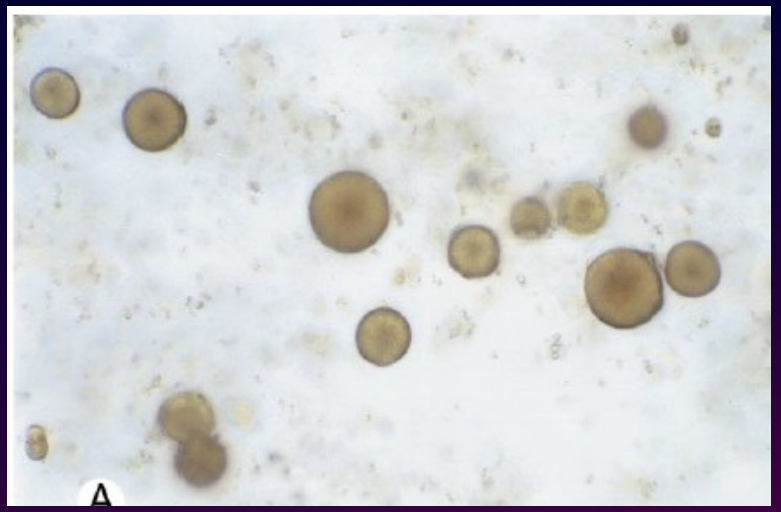
- Multiple infections (Sphingomonas paucimobilis and enterococcal bacteremia, UTI with mucopurulent urethritis/balanitis with Pseudomonas, enterobacter, and corynebacterium)
- Right perinephric urinoma/lymphocele.
- Transplant nephrectomy 5 months after transplant. Furosemide renal scan GFR 11 mL/min.
- Septic shock, possible disseminated fungal infection.
- Toxic/metabolic/infectious encephalopathy.
- Bilateral pulmonary cavitary lesions/HCAP.
- Aortic root abscess with vegetations on bioprosthetic valve.
- Subacute embolic strokes.
- Comfort care.
- Expired 5 months after renal transplant.

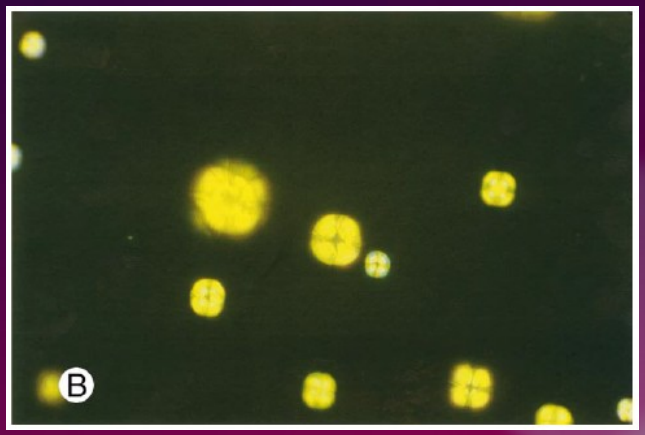
Key Points

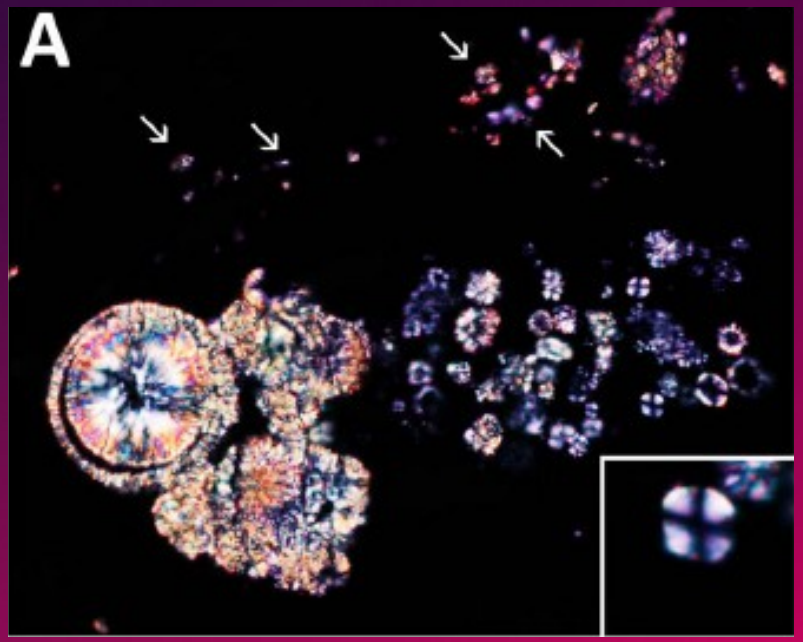
- Underdiagnosed and misdiagnosed
- Variable timing and presentation
- High rate of recurrence but also preventable
- Early aggressive therapy
- Renal biopsy, urinalysis, ARPT activity in lysates, APRT genetic testing











Thank you.

Questions?