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Genotype and Phenotype Correlation in Patients With Dent's Disease Type 1-Clearing Muddy Waters

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Dent disease is a rare X-linked recessive disorder characterized by various degrees of proximal tubular dysfunction, nephrocalcinosis or nephrolithiasis, and slowly progressive loss of kidney function resulting in end-stage kidney disease in the third to fifth decade in 30% to 80% of affected males. The hallmark of Dent disease is low-molecular-weight proteinuria, which is invariably present (Figure 1). Although proteinuria is in the nephrotic range in about one-half of the patients, serum albumin levels are always normal, which distinguishes tubular protein loss from the glomerular disease.[1,2] The clinical picture is age-dependent; whereas hypercalciuria is more frequent in children, kidney dysfunction, nephrolithiasis, and nephrocalcinosis are more prevalent in adults.[3] Based on a French registry, the prevalence is at least 1 in 500,000,[4] however, that may be higher because of the variable phenotype and insidious course of the disease.

In about two-thirds of cases, Dent disease is caused by variants in the *CLCN5* gene (named Dent disease 1), which is *de novo* in about 10%.[4] With the advent of molecular testing, it has become clear that the diagnoses "X-linked recessive nephrolithiasis," "X-linked recessive hypercalciuric hypophosphatemic rickets," and "low molecular proteinuria with hypercalciuria and nephrocalcinosis" share the same genetic basis as Dent disease 1. In about 15%, disease-causing variants are detected in *OCRL* (named Dent disease 2), the gene which also causes Lowe oculocerebrorenal syndrome. In another 25%, no disease-causing variants in either of these genes can be demonstrated.[2]

Treatment of Dent disease is largely symptomatic, aiming at the correction of electrolyte losses and metabolic acidosis.[3] The slowly progressive course poses a major obstacle to test potential interventions aiming to preserve kidney function. Although thiazide diuretics have been shown to reduce calcium excretion in clinical trials, their effect on kidney function has not been studied. This also applies to angiotensin-converting-enzyme inhibition, which does not consistently reduce proteinuria and bears the risk of prerenal renal failure in this salt-losing condition. Citrate supplements have been shown to have a beneficial effect on interstitial calcium deposits and loss of kidney function in a knock-out mouse model; however, they have not been tested prospectively in humans.

CLCN5 encodes the electrogenic Cl^-/H^+ antiporter chloride channel 5 (ClC-5). It is expressed in proximal tubular cells, in the thick ascending loop of Henle, in collecting duct α-intercalated cells[5] and podocytes.[2] In the proximal tubule, ClC-5 is mainly located in the intracellular subapical endosomes, where it is coexpressed with the proton pump (V-type H^+ ATPase). ClC-5 plays a crucial role in the acidification of early endosomes, which is necessary for the reabsorption of low-molecular-weight proteinuria via the receptors, megalin and cubulin (Figure 1). Although the effect of ClC-5 dysfunction on low-molecular-weight absorption is well-understood, the mechanisms underlying the other proximal tubular dysfunctions and progressive renal failure are largely elusive. Recently, Durán *et al.*[6] demonstrated disturbances in the expression of genes involved in kidney development, anion homeostasis, organic acid transport, extracellular matrix organization, and cell-migration biologic processes in tubular cell-lines with *CLCN5* mutants.[6] In addition, the

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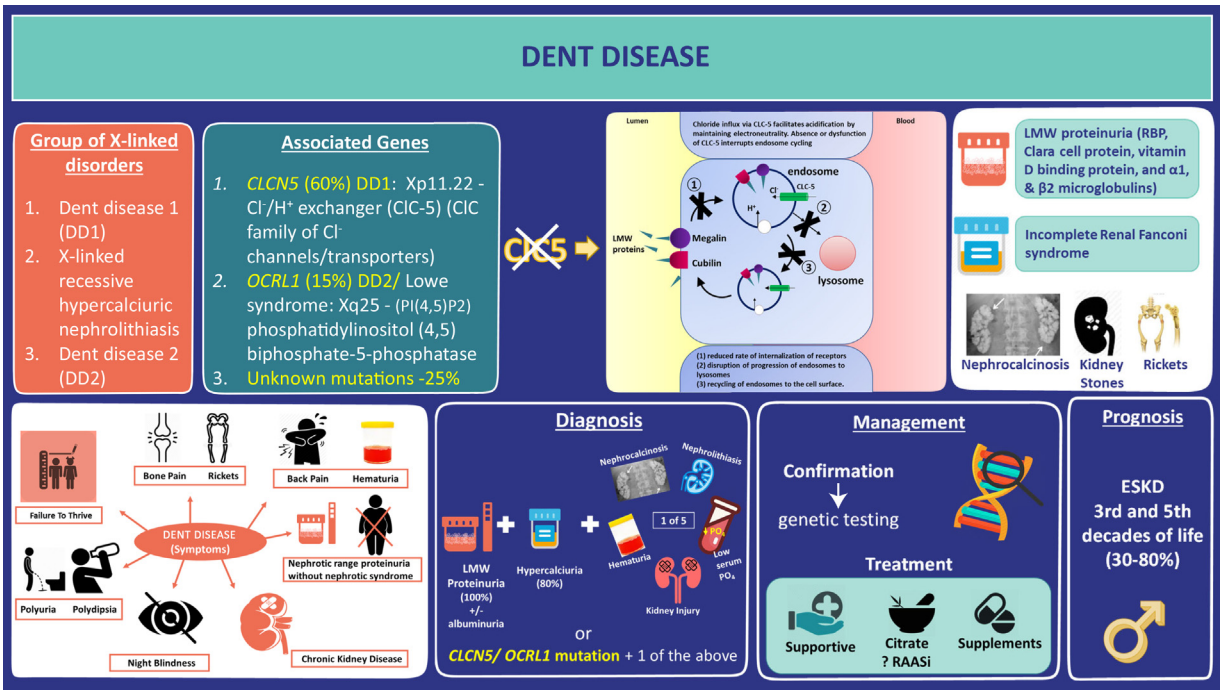

Figure 1. Etiopathogenesis, clinical features, diagnosis, management, and prognosis of Dent disease. ESKD, end-stage kidney disease; LMW, low molecular weight.

pathogenesis of tubulointerstitial fibrosis and glomerulosclerosis causing progressive loss of kidney function is still poorly understood. Although glomerulosclerosis has been considered as secondary to tubulointerstitial disease, the presence of nephrotic proteinuria in combination with glomerulosclerosis and evidence of *CLCN-5* expression in podocytes points toward a genuine role of the glomeruli in the pathogenesis.[2]

The *CLCN5* gene spans approximately 170 kb on chromosome Xp11.23/p11.22 and comprises 17 exons. ClC-5 has a large intra cytoplasmatic terminus containing 2 cystathionine beta-synthase domains and an energy-sensing domain for the allosteric control mediated by ATP.[7] ClC-5 comprises 18 helices with an internally repeated pattern forming dimers that span the membrane in opposite (antiparallel) orientations, thus creating a chloride selective pore. The following 2 glutamic acids are crucial to proper ClC-5 function: the "gating glutamate" Glu211 and the "proton glutamate" Glu268. ClC-5 possesses a unique N-glycosylation site at Asn408.[2]

Based on the presumed mechanism leading to ClC-5 dysfunction, disease-causing variants can be classified into 3 classes. Class 1 comprises variants affecting protein processing in the endoplasmic reticulum, leading to defective trafficking to the cell surface and/or the early endosomes. In class 2 variants, ClC-5 protein is present in the early endosomes, although with defective electrical activity because of abnormal protein synthesis and stability. Class 3 variants are characterized by normal protein targeting and folding yet reduced or abolished chloride current.[2] However, these effects do not seem to correlate with the phenotypic heterogeneity seen in patients with Dent disease 1.[2]

To date, more than 300 distinct disease-causing variants in *CLCN5* have been identified. There are no clear mutation hotspots because only a small number of recurrent variants has been reported in different geographic areas.[4] It has been held that there is no correlation between genotype and phenotype in Dent disease 1,[8] which was supported by large European registry studies.[1,4] Comparing severe variants (large deletions, frameshift, nonsense, and slice-site) with missense variants, Blanchard *et al.*[1] found no difference in age at diagnosis, estimated glomerular filtration rate, proteinuria, or hypercalciuria. In addition, several case reports documented different phenotypes in affected members of the same family.[2] However, a recent analysis of 163 European patients performed by Burballa *et al.*,[3] using a functional classification of variants (i.e., early stop variants, variants affecting the pore, variants affecting the cystathionine beta-synthase domain, and others) showed a higher frequency of decreased kidney function in mutations affecting the pore or the cystathionine beta-synthase domain compared to early stop mutations.[3] Of note, analysis by severity of mutation (nonsense,

frameshift, large deletion, or splice site vs. missense and in-frame variants) revealed no difference with respect to age at diagnosis, disease manifestations, and estimated glomerular filtration rate at last follow-up.

In this issue of *Kidney International Reports*, Arnous *et al.*[9] addressed genotype-phenotype correlation in Dent disease 1 in a cohort of 162 patients from 121 different families enrolled in the Rare Kidney Stone Consortium Dent disease registry. The clinical and genetic factors of 110 patients with 51 different truncating variants were compared with 52 patients with 31 different nontruncating variants. Mutations were separated by severity as follows: canonical splicing, nonsense and frameshifting duplications, multiexon deletions, and copy number variant deletions were classified as truncating mutations, whereas missense, in-frame, noncanonical splicing variants, and stop-loss variants were classified as milder, nontruncating mutations. Similar to the population studied by Burballa *et al.*,[3] median age at last follow-up was 17 years. At this age, about 50% of patients with Dent disease 1 are expected to have chronic kidney disease (CKD) stage 2, which limits the power to detect differences in progression of renal failure. In line with the European registry, Arnous *et al.*[9] found no difference in low-molecular-weight proteinuria (97% in truncating variants vs. 96% in nontruncating variants), hypercalciuria (81% vs. 78%), nephrocalcinosis (67% vs. 55%), kidney stone events (22% vs. 18%), and kidney failure at the last available follow-up visit (10% vs. 4%).

Truncating mutations were distributed throughout the *CLCN5* gene, whereas nontruncating mutations were largely clustered in the middle exons encoding the voltage chloride-channel domain. This stresses the importance of this domain, which is not unexpected, considering the role of ClC-5 in endosomal function. The missense variant p.Ser314Leu was the most abundant and observed in 9 families. This variant has been demonstrated to cause loss of ClC-5 current in cultured mammalian cells and Xenopus oocytes.[9] Burballa *et al.*,[3] too, observed a clustering of missense mutations in the regions encoding the selectivity filter and pore (amino acids numbers 237–283 and 455–550), whereas other mutations were scattered along the gene, although with a clustering of nonsense mutations in the 2 cystathionine beta-synthase domains.

In the total group, Arnous *et al.*[9] found a correlation between calcium excretion and lifetime stone events but no correlation between proteinuria and CKD progression, similar to the study by Blanchard *et al.*[1] However, subanalysis in truncating mutations revealed a correlation between proteinuria and CKD progression and between lifetime stone events and CKD progression, suggesting a more severe phenotype, which is obscured when analyzing the entire group. In addition, patients with truncating variants had a higher albumin excretion and experienced symptomatic stone passages at a younger age. It might be hypothesized that the truncating group represents a more homogeneous population among whom ClC-5 protein function is largely abolished whereas the nontruncating group consists of a mixture of patients with absent or poorly functioning protein and those with significant residual activity.

Survival analysis failed to demonstrate a difference in age at detection of nephrocalcinosis or diagnosis of CKD3 or CKD5 between truncating and nontruncating variants. However, 11 of 13 patients reaching end-stage kidney disease had a truncating mutation and 2 had a missense mutation severely impairing ClC-5 current.

In conclusion, 2 recent retrospective series found evidence of a genotype-phenotype correlation in Dent disease 1, which had not been recognized before. Still, statistical power is limited, and the registry-based data sets are incomplete. Considering that the *CLCN5* gene responsible for Dent disease was only discovered in 1996, the number of well-characterized patients with documented *CLCN5* mutations reaching the age when severe CKD is seen is still low. This will change in the future and allow for a more detailed and reliable analysis, which may form the basis for counseling, identification of risk factors, and the design of therapeutic studies. In the meantime, the authors might consider pooling their data sets and performing an in-depth analysis of genotype-phenotype correlation using both the functional and the severity-of-mutation based classification of variants.

DISCLOSURE

All the authors declared no competing interests.

REFERENCES

1. Blanchard A, Curis E, Guyon-Roger T, et al. Observations of a large Dent disease cohort. *Kidney Int.* 2016;90:430–439. https://doi.org/10.1016/j.kint.2016.04.022
2. Gianesello L, Del Prete D, Anglani F, Calò LA. Genetics and phenotypic heterogeneity of Dent disease: the dark side of the moon. *Hum Genet.* 2021;140:401–421. https://doi.org/10.1007/s00439-020-02219-2
3. Burballa C, Cantero-Recasens G, Prikhodina L, et al. Clinical and genetic characteristics of Dent's disease type 1 in Europe. *Nephrol Dial Transplant.* 2022. https://doi.org/10.1093/ndt/gfac310

4. Mansour-Hendili L, Blanchard A, Le Pottier N, et al. Mutation update of the CLCN5 gene responsible for Dent disease 1. *Hum Mutat*. 2015;36:743–752. https://doi.org/10.1002/humu.22804
5. Devuyst O, Christie PT, Courtoy PJ, Beauwens R, Thakker RV. Intra-renal and subcellular distribution of the human chloride channel, CLC-5, reveals a pathophysiological basis for Dent's disease. *Hum Mol Genet*. 1999;8:247–257. https://doi.org/10.1093/hmg/8.2.247
6. Durán M, Burballa C, Cantero-Recasens G, et al. Novel Dent disease 1 cellular models reveal biological processes underlying ClC-5 loss-of-function. *Hum Mol Genet*. 2021;30:1413–1428. https://doi.org/10.1093/hmg/ddab131
7. Wu F, Roche P, Christie PT, et al. Modeling study of human renal chloride channel (hCLC-5) mutations suggests a structural-functional relationship. *Kidney Int*. 2003;63:1426–1432. https://doi.org/10.1046/j.1523-1755.2003.00859.x
8. Tosetto E, Ghiggeri GM, Emma F, et al. Phenotypic and genetic heterogeneity in Dent's disease-the results of an Italian collaborative study. *Nephrol Dial Transplant*. 2006;21:2452–2463. https://doi.org/10.1093/ndt/gfl274
9. Arnous MG, Arroyo J, Cogal AG, et al. The site and type of CLCN5 genetic variation impact the resulting Dent disease-1 phenotype. *Kidney International Reports*. 2023;8:1220–1230. https://doi.org/10.1016/j.ekir.2023.03.012