Mutation Update of the CLCN5 Gene Responsible for Dent Disease 1

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Key words: *CLCN5*, ClC-5, Dent disease 1, low molecular weight proteinuria, renal failure

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Abstract

Dent disease is a rare X-linked tubulopathy characterised by low molecular weight proteinuria (LMWP), hypercalciuria, nephrocalcinosis and/or nephrolithiasis, progressive renal failure and variable manifestations of other proximal tubule dysfunctions. It often progresses over a few decades to chronic renal insufficiency, and therefore molecular characterization is important to allow appropriate genetic counselling. Two genetic subtypes have been described to date: Dent disease 1 is caused by mutations of the *CLCN5* gene, coding for the chloride/proton exchanger ClC-5; and Dent disease 2 by mutations of the *OCRL* gene, coding for the inositol polyphosphate 5-phosphatase OCRL-1.

Herein, we review previously reported mutations (n= 192) and their associated phenotype in 377 male patients with Dent disease 1 and describe phenotype and novel (n=42) and recurrent mutations (n=24) in a large cohort of 117 Dent disease 1 patients belonging to 90 families. The novel missense and in-frame mutations described were mapped onto a three-dimensional homology model of the ClC-5 protein. This analysis suggests that these mutations affect the dimerization process, helix stability or transport. The phenotype of our cohort patients supports and extends the phenotype that has been reported in smaller studies.

Background

Dent disease (OMIM 300009) is a rare X-linked renal proximal tubulopathy clinically defined by low molecular weight proteinuria (LMWP) associated with hypercalciuria and/or its complications (nephrocalcinosis or nephrolithiasis) and progressive renal failure. LMWP is present in all affected males and in almost all obligate female carriers. Dent disease may also be associated with defective reabsorption of one or several of the following solutes: amino acids, glucose, phosphate, uric acid, potassium and bicarbonate; some patients may also have rickets. Dent disease may also present as a generalized proximal tubular dysfunction (i.e.

renal Fanconi syndrome). The main complication of this disease is progression to chronic kidney disease during the second to the fourth decade of life in 30 to 80% of cases [Devuyst et al. 2010].

Dent disease is genetically heterogeneous: about 50-60% of patients harbour inactivating mutations of the *CLCN5* gene (Dent disease 1) and about 15% harbour inactivating mutations in the *OCRL* gene (Dent disease 2). Other yet unidentified causative genes are likely involved in the disease [Devuyst et al. 2010]

*CLCN5 (*OMIM #300008) maps on chromosome Xp11.22 and encodes a 746 amino-acid electrogenic $2Cl^-/H^+$ antiporter (ClC-5) [Lloyd et al., 1996;Scheel et al., 2005]. The *OCRL* gene maps on chromosome Xq25 and encodes the inositol polyphosphate 5-phosphatase OCRL-1 [Attree et al., 1992].

In the human kidney, ClC-5 is expressed in the proximal tubule, the thick ascending limb and the intercalated cells of the collecting duct. ClC-5 protein is a dimer with two identical subunits, each of which contains a pore responsible for the selective coupling of the Cl^- flux to H^+ counter-transport [Dutzler et al., 2005]. Each subunit is constituted by 18 α-helices in anti-parallel orientation, with two phosphorylation sites and one N-glycosylation site. The carboxy terminus of ClC-5 contains two CBS (cystathionine B-synthase) domains, a PY sorting signal and a potential PDZ protein-binding module [Schwake et al., 2001; Hryciw et al., 2006].

An important number of mutations of the *CLCN5* gene affecting sites throughout the protein have been reported since the identification of the gene in 1996 but only a limited number is described in the databases [HGMD]. *CLCN5* mutations have been grouped into three classes on the basis of functional data: mutations impairing processing and folding inducing retention in the endoplasmic reticulum and degradation of the mutant protein within the cell (class 1); mutations causing a delay in protein processing and reducing stability of ClC-5 (class 2); and

mutations affecting electrical activity but not trafficking of ClC-5 to the cell surface and early endosomes (class 3) [Lourdel et al., 2012]. No correlation between genotype and phenotype has been established.

In this study, we review the published mutations in the *CLCN5* gene (Dent disease 1) as well as their phenotype and report molecular characterisation and clinical data of a cohort of 90 families analysed in our centre. The analysis of this large cohort includes the description of 42 previously non-described mutations and the proposed pathogenic mechanism for novel missense mutations.

Population

The study included 90 unrelated patients diagnosed with Dent disease addressed for genetic analysis to the Genetics Department of the Georges Pompidou European Hospital (Paris, France) from January 2001 to December 2014. The study was approved by the "Comité de Protection des Personnes, Paris-Île de France XI (Ref. 09069)" and informed consent for genetic study was obtained from each proband or, if minor, from their parents. Genetic investigations were usually performed because of the presence of at least two major criteria (LMWP, hypercalciuria, renal failure, familial history of Dent disease) and one minor criterion (mainly one or more defect of proximal reabsorption). In children, the presence of only two major criteria (in most of the cases LMWP and hypercalciuria) or even persistent LMWP of unknown aetiology is sufficient to justify molecular genetic diagnosis. Genetic investigations were extended to relatives in 43 families.

Methods of Mutation Detection and *in Silico* Prediction

DNA was extracted by a saline method or with blood DNA midi kits (Qiagen columns). *CLCN5* exons and the flanking intronic sequences were amplified by PCR and then sequenced using BigDyeTerminator kit v3.1 cycle sequencing kits and run on an ABI Prism 3730XL DNA Analyzer Sequencer (Perkin Elmer Applied Biosystems, Foster City, CA) as previously described [Grand et al., 2009].

DNA mutations were identified using Sequencher software by comparison to the *CLCN5* gene reference sequence: NM_000084.2. Each mutation was confirmed by sequencing a second independent PCR product.

Missense and splicing mutations were interpreted with Alamut V.2.2 software (Interactive Biosoftware, Rouen, France; http://www.interactivebiosoftware.com). Complementary analyses were performed with SIFT (http://www.Blocks.fhcrc.org/sift/SIFT.html), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph/), Mutpred (http://mutpred.mutdb.org/about.html), SNPs&Go (http://snps-and-go.biocomp.unibo.it/snps-and-go/info.htm) and mutation taster (http://www.mutationtaster.org/documentation.html).

VMD software (Humphrey W. et al, J Mol Graph 1996) was used to predict the dimeric structure of the ClC-5; this structure was then optimized by means of energy minimization (12,000 steps) using NAMD2.9 software [Phillips et al., 2005] and the CHARMM27 force field [Mackerell et al., 2004]. The figures were produced with VMD software. The structure of the monomer in the model was built with ICM software [Abagyan et al., 1994], employing the crystallographic data of the StClC channel (PDB ID code 1KPL) as the template.

Mutations

Described Mutations

We searched for *CLCN5* mutations published between February 1996 and October 2014 in PubMed, using the combination of the key words "*CLCN5*", "Dent disease" and "Chloride channel ClC-5". We found 192 different described mutations in the *CLCN5* gene detected in 377 patients belonging to 334 families. These mutations are described in Supp. Table S1, following HGVS nomenclature recommendations and the reference sequence NM_000084.2. To date, functional studies have been performed for 41 mutations (31 missense, 5 nonsense, 4 frameshift or and 1 in-frame), the functional class and references are also quoted in Supp. Table S1. All these mutations have been entered in the Leiden Open Variation Database (http://www.LOVD.nl/CLCN5).

Described mutations include large deletions (4%), nonsense and frameshift mutations that produce premature stop codons (17 and 28% respectively), splice site mutations that interfere or are predicted to interfere with correct splicing (11%), missense and small in-frame deletions affecting conserved amino acid residues (36.5 % and 2.6 % respectively).

Novel and Recurrent Mutations in our population

DNA sequence analysis of the entire coding region of the *CLCN5* gene from one affected member of each of the 90 families revealed 66 different mutations; 42 of the mutations have not previously been described (Table 1 and 2 summarize the novel and recurrent mutations detected). Eleven families of this cohort were previously described [Blanchard et al., 2008, Grand et al., 2009]

Prediction of pathogenicity of novel Mutations (Biological relevance)

The novel mutations include: 14 frameshift, eight splicing, eight nonsense, eight missense, one in-frame mutation and three large deletions. Most of these mutations (25 frameshift nonsense and deletions) are predicted to result in the production of unstable mRNAs, truncated or absent proteins. Six out of the eight splice site mutations disrupt the obligatory

consensus donor or acceptor splice sites and are considered pathogenic. For one mutation located in -8 in the acceptor site of exon 6, MaxEntScan predicts a decreasing of splice site score of 42% and SpliceSiteFinder-like a loss of a splice site and the creation of a cryptic splice site. Finally, the affected nucleotide responsible for synonym p.Glu35Glu change is the last nucleotide of the exon 2 and *in silico* analysis show a significant decreasing of site scores by 36% and 13.2 % for MaxEntScan and SpliceSiteFinder-like respectively. Unfortunately no mRNA was available for analysis of the transcripts for these two patients.

The seven missense and one in-frame mutations we report here for the first time affect highly conserved amino acids and all are predicted to be potentially pathogenic by *in silico* tools (Supp. Table S2). The novel missense and in-frame mutations were mapped onto a three-dimensional homology model of the ClC-5 protein (Figure 1): in-frame (p.Pro258delinsArgAsn) and two missense (p.Thr518Ala and p.Gly530Ser) mutations affect helices involved in the formation of the dimer interface; four missense mutations (p.Lys231Ieu, p.Arg239Pro, p.Gly250Arg and p.Gly470Arg) are near the transporter interface; and the p.Glu267Asp missense mutation is located just before the "proton glutamate" (Glu268), critical for the transport function of ClC $2Cl^-/H^+$ exchangers.

Previous functional investigations in heterologous expression systems demonstrated that *CLCN5* mutations clustering at the dimer interface can result in the retention of ClC-5 in the endoplasmic reticulum and its rapid degradation within the cell [Lourdel et al., 2012]. ClC-5 $2Cl^-/H^+$ exchangers are found as homodimers, leading to the suggestion that the mutant proteins are subjected to early degradation because of the impaired dimerization. This is plausible for the in-frame (p.Pro258delinsArgAsn) and two missense (p.Thr518Ala and p.Gly530Ser) mutations mapping to helices H and P, that are involved in the formation of the dimer interface of ClC-5, as well as helices B, I, O and Q [Dutzler et al., 2002; Dutzler et al., 2003; Wu et al., 2003; Feng et al., 2010].

Four other missense mutations involve residues near the transporter interface: the p.Lys231Ieu mutation is in the loop between helices F and G, and mutations p.Arg239Pro, p.Gly250Arg and p.Gly470Arg are in helices G and N. These mutations may have deleterious consequences for ClC-5 by significantly affecting the stability of the helix and thus preventing appropriate folding of the monomers. Consistent with this, other studies have reported that such mutations result in abnormal ClC-5 proteins that are rapidly degraded [Lourdel et al., 2012].

The p.Glu267Asp mutation maps to helix H, directly adjacent to the "proton glutamate" (Glu268) that is strictly conserved on the internal side of ClC $2Cl^-/H^+$ exchangers. This residue plays a key role in the transport function of ClC $2Cl^-/H^+$ exchangers by acting as an H+ transfer site [Accardi et al., 2005; Neagoeet al., 2010; Zdebik et al., 2008]. Neutralization of the "proton glutamate" in ClC-5 abolishes Cl^- and H^+ flux [Zdebiket al., 2008]. Because the p.Glu267Asp substitution is very close to the H^+ binding site, it may well severely impair the transport cycle of ClC-5.

Recurrent Mutations

Among the 192 mutations described in the literature, 54 different were shared between two or more families (Supp. Table S1). More frequent mutations were: p.Ser244Leu, p. p.Arg637* and p.Arg704*, which were described in 16, 15 and 11 families respectively.

In our cohort, 24 previously described mutations were detected in 43 families (Table 2): the p.Ser244Leu and the p.Arg637* mutations were detected in five different families; the p.Arg347* nonsense mutation was detected in four different families; the p.Arg28* and the p.Arg648* nonsense mutations and a whole gene deletion were detected in probands of three families; the p.Arg704* nonsense mutation, the p.Val523del in-frame mutation and the

splicing mutation c.394-2A>G were found in two different families and finally, eleven missense and two nonsense mutations, all of which had been described previously were detected in only one family each.

Among recurrent mutations, nine nonsense (p.Arg28*, p.Arg34*, p.Trp279*, p.Arg347*, p.Arg467*, p.Arg637*, p.Arg648*, p.Arg704* and p.Arg718*) and three missense (p.Ser244Leu, p.Arg516Trp and p.Ser545Asn) correspond to C>T or G>A transitions arisen in CpG dinucleotides. They have been detected in patients from different geographic origin and can be considered as mutation hotspots.

Figure 2 summarizes novel and described mutations (n=234) by type and their location in cDNA and protein domains. Mutations are scattered along all exons of the gene and protein domains.

Variants of unknown clinical significance

Table 3 summarises variants described in the literature and in our cohort as polymorphisms or variants of unknown clinical significance (VUS). These including: four synonymous changes, one missense variant (p.Ser386Phe) predicted *in silico* as benign and ten non-coding variants. None of intronic variants were predicted to either create or abolish a splicing-related sequence after *in silico* analysis with Alamut software.

Family studies

In our cohort, initial pedigree analysis of the 90 families with Dent disease showed clinical familial history of X-linked disease in only eight families. We were able to get additional information (biochemical and ultrasound evaluation) and perform genetic studies in 134 relatives from 43 families with four (n=2 families), three (n=7), two (n=31) or one (n=3) generation (Supp. Table S3). In these families we found co-segregation of the mutations with

the disease. Thirty-seven of the 39 mothers and four of the six maternal grandmothers analysed were heterozygous for the mutation carried by their son or grandson. In the remaining cases mothers and grandmothers did not harbour the familial mutation for a *de novo* mutation rate of 9%. These results are in accordance with the literature data available for 125 families: at a whole, 12 mothers did not harbour the mutation detected in their sons an in 3 additional cases, without familial antecedents, grandmothers did not harbour the mutation detected in their daughters and grandsons, for a de novo mutation rate of 12%. Diagnosis of Dent disease 1 was confirmed in 28 additional males (brothers, cousins and maternal uncles); in this group, 14 males belonging to 10 families were clinically asymptomatic before the genetic diagnosis, which was performed in seven before the age of 5 years, in three between the ages of 5 and 10 and in the remaining cases between 16 and 32 years (Table 4 and Supp. Table S4). No renal impairment was detected in this group but the genetic diagnosis allowed establishing adequate follow-up by a nephrologist. In our cohort, 68 out of 84 women tested were identified as carriers; such findings are very important for genetic counselling: i.e. early screening of any male children from carrier mother and giving reassurance to non-carrier mothers about the transmission of the disease to their children. The pedigrees of two large families (44 and 66) for which four generations were studied, and the pedigrees of two families (families 11 and 20) for which three generations were studied are given in the Supp. Figure S1.

The geographical distribution available for 332 described families as well as for the additional 79 families of our cohort is described in Supp. Table S5. Dent disease 1 is a worldwide disease. The high number of descriptions in Japan is the result of the detection of LMWP in children as product of nationwide program of urine screening of elementary and junior high school students (Lloyd et al., 1997).

Phenotype (Clinical relevance):

Published clinical data from 377 male patients belonging to 334 families were analysed. Only clinical data from articles published in English were included. We found 57 articles describing the phenotype associated with mutations in the *CLCN5* gene. Phenotype description in papers is very variable from qualitative description of main criteria to detailed description in case reports. Table 4 summarizes and compares global clinical qualitative data from previously described large cohorts, from case-reports and from our cohort (including the phenotypic data of fourteen patients belonging to 11 families previously described [Blanchard et al., 2008, Grand et al., 2009]). Median age at diagnosis was 9 (0.2-67) years in the literature and 7 (0.1-55) in our cohort. Altogether the main manifestations of the disease in our cohort were similar to those described in worldwide literature.

In addition to the main characteristics the following clinical and biological abnormalities have been described in patients with Dent disease 1 (absolute value are given when we only had information of the presence of a given trait on case reports; relative values correspond to the number of positive/total cases when absence or presence of a given trait was clearly described) : micro or macro-haematuria (n=71), polyuria/polydipsia or urinary concentration defect (31/43), failure to thrive including four cases with GH deficiency (n=16), hypouricemia (13/38), proteinuria in the nephrotic range without hypoalbuminemia (n=13) [Lim et al., 2007, Sheffer-Babila et al 2008, Frishberg et al. 2009, Fervenza 2013, Cramer et al. 2014], reduced renal uptake of DMSA (n=7), enuresis (n=5), hypomagnesaemia (4/30), secondary hyperaldosteronism with Bartter-like phenotype (n=3) [Besbas et al. 2005, Bogdanovic et al. 2010, Okamoto et al. 2011] , and night blindness responsive to vitamin A [Sethi et al. 2009].

We retrieved quantitative data on proteinuria, expressed in g/day, in 57 patients; the median value was 1.28 g/24h (min 0.27 – max 4.50) (IQR 0.9-1.92). Renal biopsy was performed in 37 patients as part of diagnosis of proteinuria and/or renal impairment; in addition, renal histologic analysis after necropsy was available in one patient. In three cases light microscopy

analysis was described as normal. In the 35 remaining patients, light microscopy analysis showed mainly tubular atrophy and interstitial fibrosis, tubular or interstitial calcification and nonspecific glomerular changes as sclerosis and periglomerular fibrosis. Twelve of these cases were classified as focal global glomerulosclerosis. These results are summarized in Supp. Table S6.

A considerable intra-familial variability in disease severity has been observed and no genotype-phenotype correlation has been described. For a same mutation, patients from the literature as well as from our cohort have a variable phenotype ranging from renal Fanconi syndrome with or without rickets to the association of LMWP and hypercalciuria (with or without nephrocalcinosis or nephrolithiasis).

Female carriers displayed a milder and variable phenotype, attributed to the differing amounts of inactivation of the X chromosome that harbours the *CLCN5* mutation. We gathered available phenotypic characteristic in 89 heterozygous women described in the literature and data from 6 heterozygous carriers in our cohort: LMWP was found in 60% of female carriers (52/86), 50 times lower than in affected males; median urinary β_2 microglobuline concentration was 1.50 mg/L (IQR 0.74-3.52) mg/l in females and 52.20 (IQR 31-99) mg/l in males. Hypercalciuria was detected in 31% (20/64), nephrolithiasis in 21% (11/51) and nephrocalcinosis in 12% of female carriers (5/40). So far, only 1 female carrier was reported to have end stage renal disease [Wrong 1994], but CKD has been described in 3 additional heterozygous women: CKD stage 3a in one at 56 years [Igarashi 2000] and CKD stage 3b in two at 65 and 78 years [Hoopes 1998, Kelleher 1998] . Supp. Figure S2 shows comparisons of tubular proteinuria and calciuria values in male patients and heterozygous carriers.

Other forms of Dent disease:

Dent disease is genetically heterogeneous: in published cohorts about 50-60% of patients have Dent disease 1 caused by mutations of the *CLCN5* gene and about 15% have Dent disease 2 caused by mutations in the *OCRL* gene. One case of co-inheritance of mutations in both genes has been described in a patient with a phenotype close to Lowe syndrome [Addis et al., 2013]. In our cohort of 150 distinct families, Dent disease 1 represents 61 % and Dent disease 2 represents 7% of the cases; most of these cases have been previously described [Hichri et al., 2011]. Accordingly, 25 to 35% of cases with Dent disease still remain without molecular identification. *CLCN4*, *CFL1*, *SLC9A6* and *TMEM27* genes coding for ClC-4, Cofilin-1, NHE6 and collectrin have been excluded as cause of Dent disease [Ludwig and Utsch 2004, Hoopes R et al 2005, Wu et al., 2009 and Tosetto et al., 2009].

Animal models:

Two independent strains of ClC-5 knock-out (KO) mice have been generated [Piwon et al. 2000, Wang et al. 2000]. These two strains recapitulated the major features of Dent disease 1, including LMW proteinuria and other manifestations of proximal tubule dysfunction. Nevertheless, as in human, there is a phenotype variability: Piwon's model showed no hypercalciuria, increased urinary levels of PTH and reduced levels of $1.25(OH)_2$-vitamin D_3 and Wang's model showed hypercalciuria and elevated levels of $1.25(OH)_2$-vitamin D_3. *In vitro* experiments showed defective acidification of vesicles isolated from ClC-5 KO mice, supporting a role for ClC-5 in acidification of early endosomes [Günther et al. 2003, Novarino et al. 2010]. After the discovery in 2005 that ClC-5 was in fact an electrogenic, 2Cl-/H+ exchanger, instead of being a simple Cl- channel [Picollo & Pusch, 2005, Scheel et al. 2005], a knock-in (KI) mouse model carrying a point mutation p.Glu211Ala was generated. This change affects a glutamate residue that is essential for the gating of the ClC exchangers converting ClC-5 into a pure uncoupled Cl- conductor [Novarino et al. 2010]. The E211A mutant ClC-5 did not affect endosomal acidification; however, the KI mice showed the same

renal phenotype than KO mice and patients with Dent disease, including LMW proteinuria, glycosuria, hyperphosphaturia and hypercalciuria. Furthermore, both the KI and KO mouse showed a similar impairment in PT endocytosis, with reduced levels of the endocytic receptors megalin and cubilin and internalization of the sodium-phosphate cotransporter NaPi-2a indicating a trafficking defect [Novarino et al. 2010].

Conclusion

In this paper, we have compiled, previously reported and novel mutations of the *CLCN5* gene responsible for Dent disease 1 and described the phenotype associated. We also describe the characterization of 42 novel mutations of the *CLCN5* gene and propose mechanisms of pathogenicity for the novel missense and in-frame mutations. By familial screening, we identified 67 carrier females and 14 asymptomatic affected males, highlighting the importance of genotyping the probands. Further studies are necessary to confirm the value of early diagnosis and follow-up, as well as to understand the phenotypic variability and to identify the molecular causes in the about 30% of the Dent disease patients for whom the aetiology is unclear.

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The authors have no conflict of interest to declare.

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Figure Legends

Figure 1.

Three-dimensional model of ClC-5 based on homology with the structure of StClC [Dutzler et al., 2002] showing the location of the ClC-5 mutations. Views from the side of the membrane with the extracellular medium at the top (A) and from a direction rotated by 90º from the A monomer (B). The view in (B) shows the interface between the two subunits of the dimeric protein. Mutated residues are shown as red spheres, representing the alpha-carbon atom of each residue. The helices involved in the formation of the dimer interface are shown in yellow.

Figure 2.

CLCN5 previously described and novel mutations. A: Exon structure of the *CLCN5* gene with geometric shapes indicating relative positions of different types of mutations. B: ClC-5 domains. C: percentages of mutations by type.

Table 1. Novel mutations detected in 47 patients with Dent disease 1 (42 mutations)

Patient	Type of mutation	Nucleotide*	Protein	Exon/intron
Dent 8-1	Large deletion	c.1-?_105+?del (E2del)	p.Met1_Glu35del (in-frame)	2
Dent 15-1	Large deletion	c.1-?_205+?del (E2-3del)	p.Met1_Ser68del (frameshift)	2-3
Dent 26-1	Large deletion	c.1-?_393+?del (E2-4del)	p.Met1_Glu131del (in-frame)	2-4
Dent 164-1	Frameshift	c.166del	p.Ser56Profs*5	3
Dent 36-1	Frameshift	c.403_404del	p.Ala135Leufs*37	5
Dent 22-1	Frameshift	c.410dup	p.Val138Serfs*35	5
Dent 111-1	Frameshift	c.597_598dup	p.Leu200Argfs*8	6
Dent 70-1	Frameshift	c.681_682del	p.His227Glnfs*9	6
Dent 176-1	Frameshift	c.699del	p.Lys234Argfs*20	6
Dent 21-1	Frameshift	c.780del	p.Gly261Glufs*98	7
Dent 89-1	Nonsense	c.1536del	p.Val514*	10
Dent 148-1	Frameshift	c.1571dup	p.Met525Asnfs*3	10
Dent 5-1	Frameshift	c.1574del	p.Met525Serfs*4	10
Dent 149-1	Frameshift	c.1747del	p.Met583Trpfs*3	10
Dent 9-1	Frameshift	c.1751del	p.Asp584Valfs*2	10
Dent 170-1	Frameshift	c.1893dup	p.Val632Cysfs*14	10
Dent 43-1	Frameshift	c.1962del	p.Val655Leufs*23	11
Dent 66-1 Dent 50-1 Dent 191-1	Frameshift	c.2079_2080insG	p.Thr694Aspfs*48	11
Dent 65-1	Nonsense	c.2184del	p.Leu729*	12
Dent 69-1	Nonsense	c.308G>A	p.Trp103*	4
Dent 173-1	Nonsense	c.309G>A	p.Trp103*	4
Dent 91-1	Nonsense	c.438G>A	p.Trp146*	5
Dent 80-1	Nonsense	c.721G>T	p.Glu241*	7
Dent 103-1	Nonsense	c.1210G>T	p.Glu404*	8
Dent 83-1	Nonsense	c.1288C>T	p.Gln430*	8
Dent 87-1	Splicing	c.105G>A (exon 2 last nucleotide)	p.Glu35Glu splicing effect[1]	2
Dent 182-1	Splicing	c.206-1G>C	Loss of splice acceptor site	
Dent 11-4	Splicing	c.517-8A>G	p.? [2]	6
Dent 136-1	Splicing	c.724-1G>A	Loss of splice acceptor site	7
Dent 12-3 Dent 154-1	Splicing	c.724-2A>G	Loss of splice acceptor site	7
Dent 113-1	Splicing	c.1348-2del	Loss of splice acceptor site	9
Dent 116-1	Splicing	c.1535-1G>T	Loss of splice acceptor site	10
Dent 44-1 Dent 197-1	Splicing	c.2150+1G>A	Loss of splice donor site	11
Dent 139-1	In-frame	c.773delinsGGAA	p.Pro258delinsArgAsn	7
Dent 155-1	Missense	c.692A>T	p.Lys231Ile	6
Dent 105-1	Missense	c.716G>C	p.Arg239Pro	6
Dent 158-1	Missense	c.748G>C	p.Gly250Arg	7
Dent 162-1	Missense	c.801A>C	p.Glu267Asp	7
Dent 134-1 Dent 159-1	Missense	c.1408G>A	p.Gly470Arg	9
Dent 119-1	Missense	c.1552A>G	p.Thr518Ala	10
Dent 106-1	Missense	c.1588G>A	p.Gly530Ser	10
Dent 38-1	Missense	c.810C>G	p.Ser270Arg	8

* Numbering is according to the cDNA sequence (GenBank entry NM 000084.2). The A of the ATG of the Methionine initiation codon is defined as nucleotide 1.[1]Splice site scores are decreased by 36% for MaxEntScan (10.1 to 6.4) and 13.2 % for SpliceSiteFinder-like (91.6 to 79.5). [2]Splice site score is decreased by 42% forMaxEntScan (11.4 to 4.8).SpliceSiteFinder-like predicts the loss of a splice site and the creation of a cryptic splice site. No mRNA was available for analysis of the transcripts for these patients.

Table 2. Recurrent mutations detected in 43 patients with Dent disease 1 (24 mutations)

Patient	Type of mutation	cDNA	Protein	Exon/ Intron	Reference
Dent 23-1 Dent 74-1 Dent180-1	Large deletion	c.(?_-30)_(*220_?)del	Whole gene deletion	1-12	Akuta et al., 1997
Dent 18-1	Frameshift	c.2108delT	p.Phe703Serfs*27	11	Blanchard et al., 2008
Dent 133-1 Dent 46-1 Dent 59-1	Nonsense	c.82C>T	p.Arg28*	2	Hoopes et al., 1998
Dent 13-1	Nonsense	c.608 C>A	p.Ser203*	6	Blanchard et al., 2008
Dent 146-1 Dent 16-1 Dent 2-1 Dent 27-1	Nonsense	c.1039C>T	p.Arg347*	8	Akuta et al., 1997 Besbas et al., 2005
Dent 171-1	Nonsense	c.1399C>T	p.Arg467*	9	Ludwig et al., 2005 Li et al., 2009
Dent 17-1 Dent 40-1 Dent 202-1 Dent 203-1 Dent 210-1	Nonsense	c.1909C>T	p.Arg637*	10	Takemura et al., 2001
Dent 31-1 Dent 128-1 Dent 181-1	Nonsense	c.1942C>T	p.Arg648*	11	Lloyd et al., 1996 Igarashi et al., 1998
Dent 126-1 Dent196-1	Nonsense	c.2110C>T	p.Arg704*	11	Lloyd et al., 1996 Nakazato et al., 1999
Dent 53-1	Nonsense	c.2152C>T	p.Arg718*	12	Carballo-Trujillo et al., 2003 Grand et al., 2009
Dent 35-1 Dent 37-1	Splicing	c.394-2A>G	Loss of splice acceptor site	4	Zhu et al., 2010
Dent 33-1 Dent 144-1	Inframe	c.1566_1568del	p.Val523del	10	Wu F et al., 2009
Dent 56-1	Missense	c.536G>A	p.Gly179Asp	6	Grand et al., 2009
Dent 96-1	Missense	c.608C>T	p.Ser203Leu	6	Grand et al., 2009
Dent 47-1	Missense	c.635G>C	p.Gly212Ala	6	Grand et al., 2009
Dent 124-1	Missense	c.661T>C	p.Cys221Arg	6	Hoopes et al., 2004 Grand et al., 2009
Dent 71-1 Dent 97-1 Dent 20-1 Dent 25-1 Dent 62-1	Missense	c.731C>T	p.Ser244Leu	7	Lloyd et al., 1996
Dent 208-1	Missense	c.781G>A	p.Gly261Arg	7	Tosetto et al., 2009
Dent 45-1	Missense	c.1406T>C	p.Leu469Pro	9	Grand et al., 2009
Dent 195-1	Missense	c.1535G>A	p.Gly512Asp	10	Tosetto et al., 2009
Dent 179-1	Missense	c.1546C>T	p.Arg516Trp	10	Akuta et al., 1997
Dent 76-1	Missense	c.1556T>A	p.Val519Asp	10	Tosetto et al., 2009
Dent 104-1	Missense	c.1636A>G	p.Lys546Glu	10	Hoopes et al., 2004
Dent 163-1	Missense	c.1639T>G	p.Trp547Gly	10	Ramos-Trujillo et al., 2007

* Numbering is according to the cDNA sequence (GenBank entry NM 000084.2). The A of the ATG of the Methionine initiation codon is defined as nucleotide 1.

Table 3. Summary of polymorphisms and variants of unknown clinical significance (VUS)

	Nucleotide	Protein	Location	RefSNP	MAF*	Geographic origin	Reference
Polymorphisms	c.993G>C	p.Leu331Leu	Exon 8	rs34122840	EA 0.06%; AA 14.84%	Dent 132-1: France Dent 165-1: Senegal Dent 200-1: Algeria	This study
	c.1452C>T	p.Thr484Thr	Exon 9			Japan	Akuta et al., 1997
	c.1704C>T	p.Pro568Pro	Exon 10	rs34173954	EA 0.07%; AA 20.65%	North America[1] Cape Verde Dent 010-1: Comoros Dent 117-1: France	Hoopes et al., 2004 Tosetto et al., 2006 This study
	c.1839T>C	p.Ser 613Ser	Exon 10			Korea	Cheong et al., 2005
VUS	c.1157C>T	p.Ser386Phe	Exon 8			Italy[1]	Tosetto et al., 2009
	c.-47-433G>A		5'UTR			Italy (Sardinia)	Tosetto et al., 2009
	c.106-17T>G		Intron 2	rs56117808	EA 0.7%; AA 0.05%	NA	Forino et al., 2004
	c.206-6A>T		Intron 3	rs6651602	EA 0.04%; AA 5.37%	Dent 040-1: France[1]	This study
	c.724-35T>G		Intron 6			Turkey[1]	Ludwig et al., 2006
	c.1348-14G>C		Intron 8	rs7063765	EA (C) 0.02%; AA (G) 47%	Dent 117-1: France Dent 132-1: France Dent 153-1:Guadeloupe[1] Dent 165-1: Senegal Dent 200-1: Algeria	This study
	c.1535-53G>A		Intron 9			Dent 118-1: Vietnam	This study
	c.1934-60G>A		Intron 10	rs150713901	NA	Dent 153-1:Guadeloupe[1]	This study
	c.2151-67C>T		Intron 11	rs56041343	NA	Dent 017-1: France[1] Dent 040-1: France[1] Dent 110-1: France	This study
	c.2151-18A >G		Intron 11			Spain[1]	Ramos-Trujillo et al., 2013
	c.*22A>G		3'UTR			Dent 100-1: France	This study

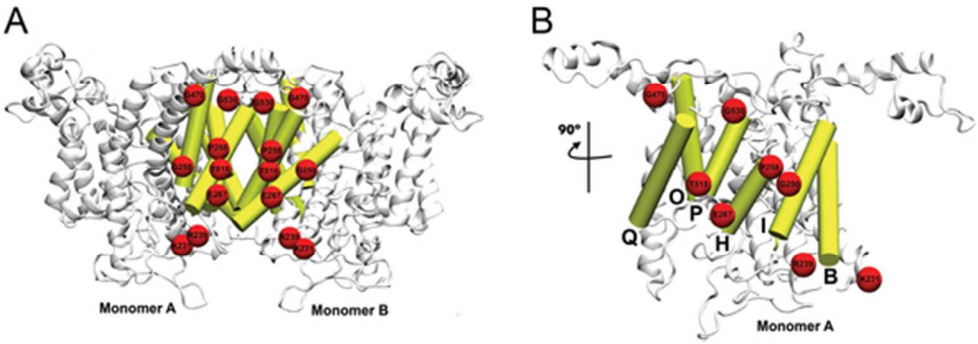
*MAF: minor allele frequency from exome sequencing project (ESP6500SIV2); EA: European American; AA: African American.
[1]These patients harbour a *CLCN5* pathogenic mutation; NA: not available

Table 4. Phenotype of male patients with Dent disease 1 in the literature and in our cohort

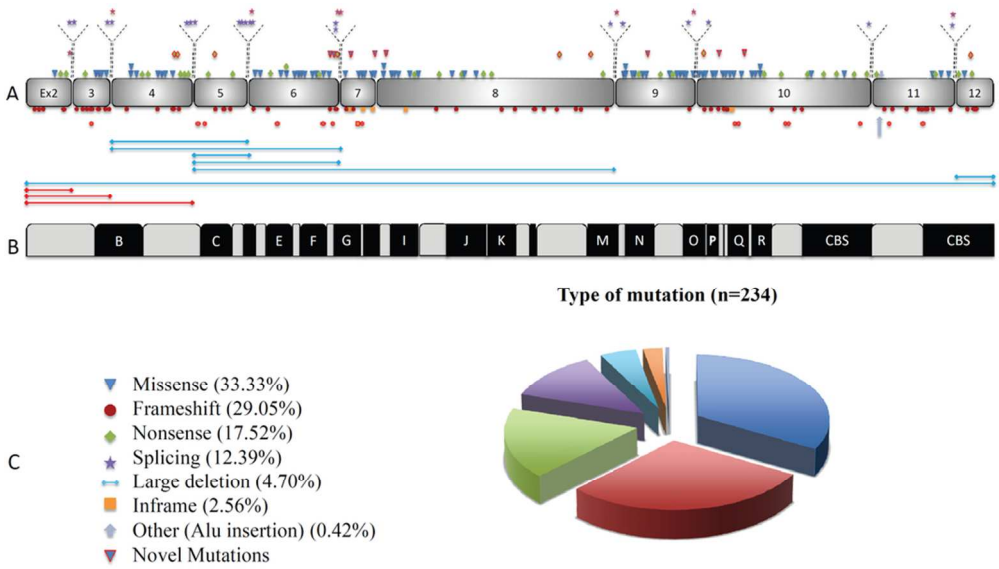
Phenotype	Large published cohorts			Global literature Analysis	This study		
	Europe and North America*	Japan (Sekine et al., 2014)	Bökenkamp et al., 2009 (include literature data)	57 articles (n=377)	Patients with clinical diagnosis of Dent disease (n=103)	Patients with screening after diagnosis in a relative (n=14)	Total (n=117)
Age at diagnosis (years)				9 (0.2-67)** (n=311)	7 (0.1-55)**§ (n=89)	5.5 (0.2-32)** (n=14)	7 (0.1-55)** (n=103)
LMW proteinuria	100%	100%	(212/212) 100%	(365/365) 100%	(99/99) 100%	11/12	(110/111) 99%
Hypercalciuria	95%	89%	(180/200) 90%	(287/359) 80%	(89/95) 94%	6/11	(87/99) 88%
Nephrocalcinosis	74%	76%	(137/182) 75%	(156/282) 55%#	(59/91) 65%	3/11	(62/102) 61%
Aminoaciduria	76%	-	(31/75) 41%	(45/93) 48%	(23/34) 67%	1/5	(24/39) 61%
Renal Insufficiency	64%	42%	(60/203) 30%	(92/347) 26.5%	(52/97) 53%	0/13	(52/110) 47%
Hypophosphatemia of renal origin	50%	-	(35/156) 22%	(72/200) 36%	(39/65) 60%	2/11	(41/76) 54%
Hypokalemia of renal origin	35%	-	(10/67) 15%	(22/60) 37%	(33/75) 44%	1/11	(34/86) 39%
Lithiasis	49%	-	ND	(41/235) 17%#	(27/81) 33%	3/10	(30/91) 33%
Rickets	30%	33%	ND	(53/247) 21%	(14/81) 17%	0/12	(14/93) 15%
Metabolic Acidosis	-	-	(2/68) 3%	(6/66) 9%	(9/60) 15%	0/8	(9/68) 13%
Glycosuria	54%	-	(18/108) 17%	(27/105) 26%	(27/62) 43%	1/8	(28/70) 40%

*Adapted from Scheiman SJ, in Genetic diseases of the kidney

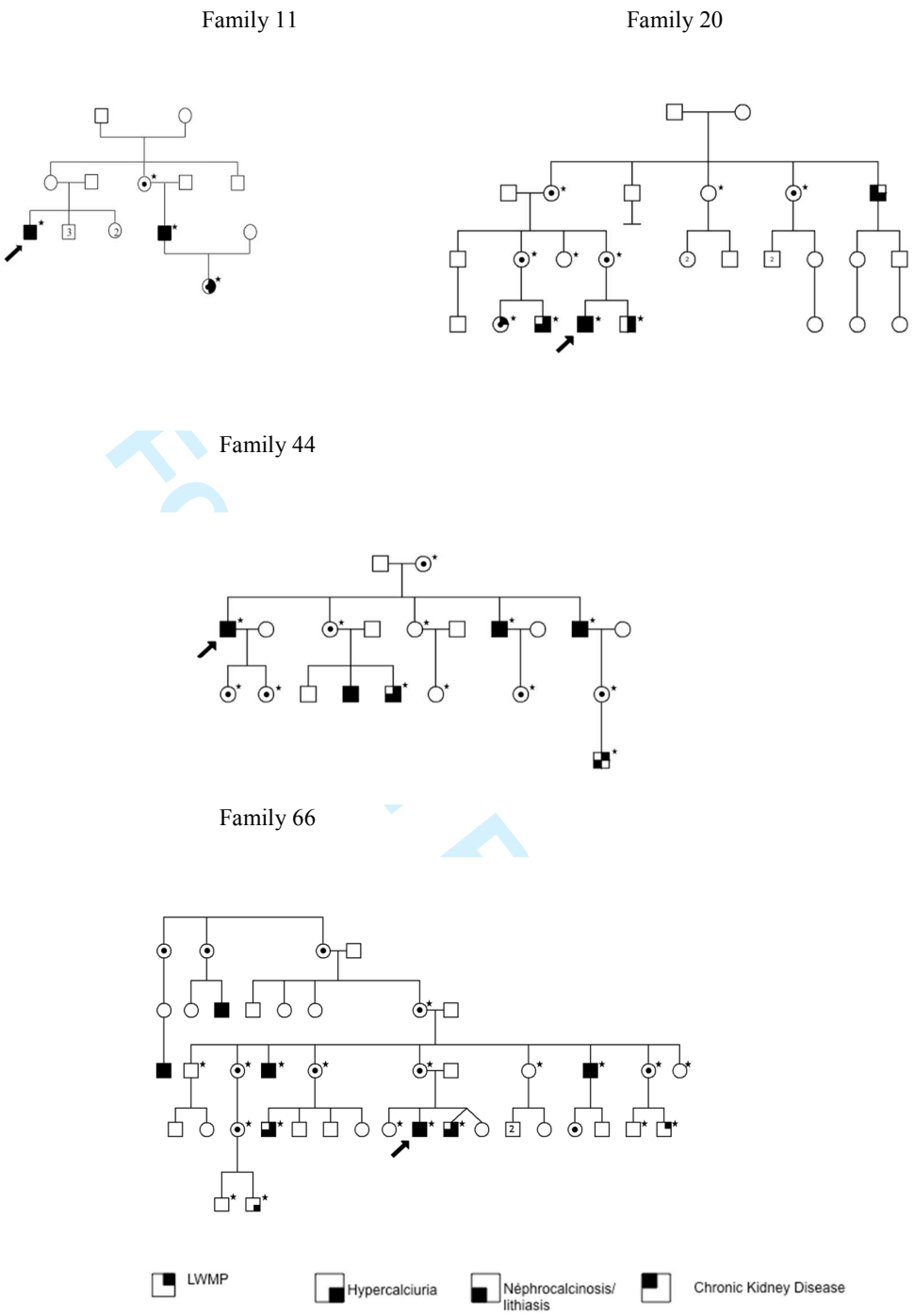
#72 additional cases were described as nephrocalcinosis or nephrolithiasis. Including these case the value for nephrocalcinosis/nephrolithiasis is 232/354 (65.5%); **Median (min-max); §for 9 probands the precise age was not available but the proximal tubulopathy was diagnosed in childhood.



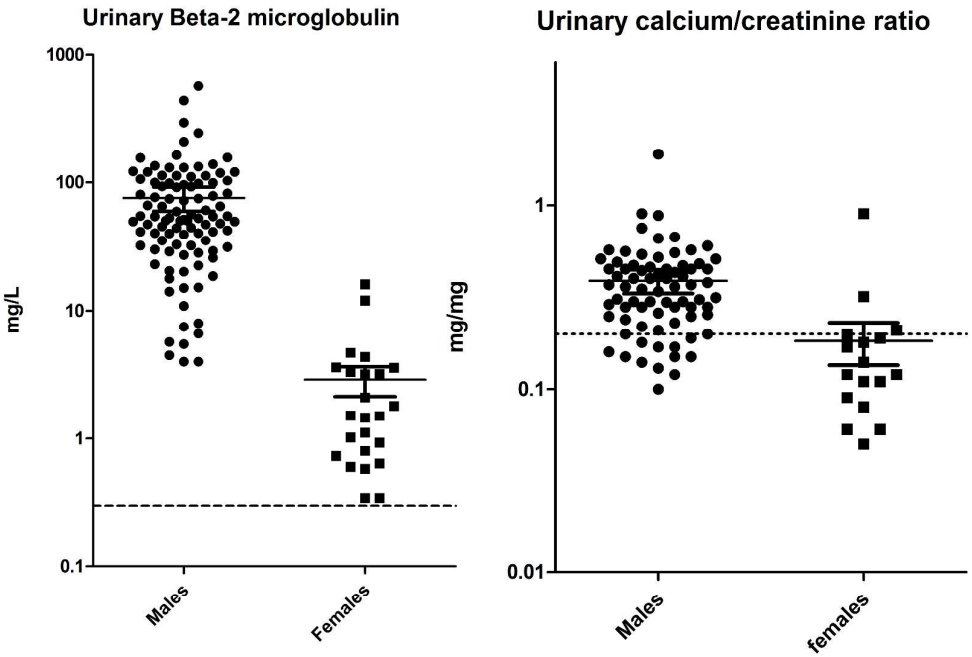
Three-dimensional model of ClC-5 based on homology with the structure of StClC [Dutzler et al., 2002] showing the location of the ClC-5 mutations. Views from the side of the membrane with the extracellular medium at the top (A) and from a direction rotated by 90º from the A monomer (B). The view in (B) shows the interface between the two subunits of the dimeric protein. Mutated residues are shown as red spheres, representing the alpha-carbon atom of each residue. The helices involved in the formation of the dimer interface are shown in yellow.
51x17mm (300 x 300 DPI)



CLCN5 previously described and novel mutations. A: Exon structure of the CLCN5 gene with geometric shapes indicating relative positions of different types of mutations. B: ClC-5 domains. C: percentages of mutations by type.
90x51mm (300 x 300 DPI)



Supp. Figure S1. Pedigrees of four families with X-linked Dent disease 1: the arrow indicates the proband in each case; the asterisks indicate individuals for whom DNA sequencing analysis was performed. In families 11 and 44, father-to-daughter transmission was observed for one and three daughters, respectively.



Supp. Figure S2. Urinary Beta-2 microglobulin (left panel) and urinary calcium/creatinine ratio (right panel) values in male patients with Dent disease 1 compared with values in female carriers. Dotted lines indicate normal upper values.

Supp. Table S1. Mutations described in the *CLCN5* gene

Type of Mutation	Nucleotide	Protein	Exon/Intron	Reference
Large deletion	c.206?_516+?del	Codons 69-172 deleted	4-5	Sekine et al., 2014
Large deletion	c.205+3_725del	p.Gly69Alafs*12	4-6	Igarashi et al., 2000
Large deletion	c.394-532_516+3delins49	Codons132-172 deleted	5	Brakemeier et al., 2004
Large deletion	c.394?_723+?del	Codons 132-241 deleted	5-6	Lloyd et al., 1996
Large deletion	c.394?_1347+?del	Codons 132-449 deleted	5-8	Morimoto et al., 1998
Large deletion	c.2151?_2241+?del	Codons 717 -746 deleted	12	Park et al., 2014
Large deletion	c.(?_-30)_(*220_?)del	Whole gene deletion	1-12	Lloyd et al., 1996, Akuta et al., 1997
Promoter	c.-47-433G>A		5'UTR	Tosetto et al., 2009
Frameshift	c.16del	p.Glu6Serfs*6	2	Tosetto et al., 2006
Frameshift	c.36delG	p.Thr13Profs*	2	Zhang et al., 2014
Frameshift	c.86_88dup	p.Asp29_Arg30insHis	2	Lloyd et al., 1997
Frameshift	c.92delA	p.Asp31Valfs*17	2	Fervenza et al., 2013
Frameshift	c.118del	p.Ser40Alafs*8	3	Hoopes et al., 1998
Frameshift	c.132del	p.Trp45Glyfs*3	3	Hoopes et al., 1998
Frameshift	c.161dup	p.Ser56Phefs*42	3	Dinour et al., 2009
Frameshift	c.165_169del	p.Phe55Leufs*41	3	Sekine et al., 2014
Frameshift	c.172_173del	p.Trp58Valfs*39	3	Tosetto et al., 2006
Frameshift	c.191del	p.Ile64Metfs*7	3	Sekine et al., 2014
Frameshift	c.200dup	p.Leu67Phefs*31	3	Park et al., 2014
Frameshift	c.258del	p.Glu87Lysfs*51	4	Cox et al., 1999, Dinour et al., 2009
Frameshift	c.299dup	p.His100Glnfs*7	4	Matsuyama et al., 2004
Frameshift	c.346del	p.Cys116Valfs*22	4	Nakazato et al., 1999
Frameshift	c.445dup	p.Leu149Profs*24	5	Cho et al., 2008, Lee et al., 2009
Frameshift	c.470_471del	p.Leu157Argfs*15	5	Cox et al., 1999
Frameshift	c.485del	p.Ala162Glyfs*12	5	Park et al., 2014
Frameshift	c.564del	p.Val192Leufs*15	6	Tosetto et al., 2009
Frameshift	c.687delinsTA	p.Asn230Lysfs*7	6	Akuta et al., 1997
Frameshift	c.722_723del	p.Glu241Glyfs*26	6	Cox et al., 1999
Frameshift	c.744_745ins CAGC	p.Ala249Glnfs*20	7	Addis et al., 2013
Frameshift	c.746_752del	p.Ala249Aspfs*3	7	Sekine et al., 2014
Frameshift	c.930_931delinsA	p.Phe310Leufs*49	8	Wu et al., 2009
Frameshift	c.977del	p.Gly326Alafs*33	8	Ramos-Trujillo et al., 2007
Frameshift	Not described	p.Val363fs	8	Cramer et al., 2014
Frameshift	c.1126delT	p.Tyr376Thrfs*4	8	Park et al., 2014
Frameshift	c.1157C>T/1160_1161dup	p.Ser386Phe/p.Leu388Serfs*47	8	Tosetto et al., 2009
Frameshift	c.1175_1176del	p.Cys392Trpfs*10	8	Yamamoto et al., 2000
Frameshift	c.1182del	p.Leu395Trpfs*39	8	Yamamoto et al., 2000
Frameshift	c.1208del	p.Tyr403Leufs*31	8	Nakazato et al., 1997
Frameshift	c.1245del	p.Pro416Leufs*18	8	Dinour et al., 2009
Frameshift	c.1248delT	p.P416fsX*17	8	Ramos-Trujillo et al., 2013
Frameshift	c.1331del	p.Phe444Serfs*5	8	Tosetto et al., 2006
Frameshift	c.1526del	p.Ala509Alafs*3	9	Sekine et al., 2014
Frameshift	c.1537del	p.Gly513Glyfs*2	10	Sekine et al., 2014
Frameshift	c.1540dup	p.Val514Glyfs*14	10	Nakazato et al., 1999
Frameshift	c.1550dup	p.Met517Ilefs*11[a]	10	Ludwig et al., 2005[a]
Frameshift	c.1558dup	p.Ser520Phefs*8	10	Nakazato et al., 1997, Park et al., 2014
Frameshift	c.1562_1563del	p.Leu521Argfs*6[a,e]	10	Ludwig et al., 2005 [a,e]
Frameshift	c.1668del	p.Gly556Glyfs*30	10	Sekine et al., 2014
Frameshift	c.1765dup	p.Arg589Profs*4	10	Tosetto et al., 2009
Frameshift	c.1782del	p.Leu594Phefs*2	10	Li et al., 2009, Zhu et al., 2010
Frameshift	c.1974del	p.Ile659Serfs*19	11	Yamamoto et al., 2000
Frameshift	c.2061del	p.Phe688Serfs*3	11	Hoopes et al., 2004
Frameshift	c.2079dup	p.Thr694Tyrfs*48	11	Hoopes et al., 2004
Frameshift	c.2080dup	p.Thr694Asnfs*48	11	Park et al., 2014
Frameshift	c.2081_2082insC	p.Thr694Thrfs*48	11	Sekine et al., 2014

Frameshift	c.2108del	p.Phe703Serfs*27	11	Blanchard et al., 2008
Frameshift	c.2142dup	p.His715Thrfs*27	11	Cho et al., 2008
Frameshift	c.2085del	p.Met696Trpfs*4	11	Lloyd et al., 1997
Frameshift	c.2179del	p.Asp727Metfs*3	12	Frishberg et al., 2009
Frameshift	c.2183_2205del	p.Val728Glyfs*6[d]	12	Yamamoto et al., 2000 [d]
Complex	c.[320A >C,324_330del]	p.[p.His107Pro,p.Thr109Lysfs*27]	4	Ramos-Trujillo et al., 2013
Nonsense	c.82C>T	p.Arg28*	2	Hoopes et al., 1998, Matsuyama et al., 2004, Sekine et al., 2004, Dinour et al., 2009, Cramer et al., 2014
Nonsense	c.100C>T	p.Arg34*	2	Langlois et al., 1998, Hoopes et al., 1998, Cox et al., 1999, Ludwig et al., 2006, Tosetto et al., 2006, Sekine et al., 2014, Ramos-Trujillo et al., 2013
Nonsense	c.134G>A	p.Trp45*	3	Hoopes et al., 2004, Ludwig et al., 2005
Nonsense	c.277G>T	p.Gly93*	4	Sekine et al., 2014
Nonsense	c.285G>A	p.Trp95*	4	Ludwig et al., 2006, Cho et al., 2008, Park et al., 2014
Nonsense	c.352G>T	p.Glu118*	4	Morimoto et al., 1998
Nonsense	c.366G>A	p.Trp122*	4	Frishberg et al., 2009
Nonsense	c.370C>T	p.Gln124*	4	Sekine et al., 2014
Nonsense	c.420T>G	p.Tyr140*	5	Ramos-Trujillo et al., 2007
Nonsense	c.492T >G	p.Tyr164*	5	Ramos-Trujillo et al., 2013
Nonsense	c.566G>A	p.Trp189*	6	Sekine et al., 2014
Nonsense	c.608C>A	p.Ser203*	6	Blanchard et al., 2008
Nonsense	c.663T>A	p.Cys221*	6	Hoopes et al., 2004
Nonsense	c.837G>A	p.Trp279*[d]	8	Lloyd et al., 1996 [d], Akuta et al., 1997, Sekine et al., 2014
Nonsense	c.942G>A	p.Trp314*	8	Ramos-Trujillo et al., 2007
Nonsense	c.996G>A	p.Trp332*	8	Ludwig et al., 2005, Ludwig et al., 2006, Tosetto et al., 2006
Nonsense	c.1028G>A	p.Trp343*	8	Lloyd et al., 1997, Hoopes et al., 1998
Nonsense	c.1039C>T	p.Arg347*[a]	8	Akuta et al.,. 1997, Morimoto 1998, Hoopes et al., 2004, Ludwig et al., 2005 [a], Besbas et al., 2005, Tosetto et al., 2006, Wu et al., 2009, Sekine et al., 2014
Nonsense	c.1298T>G	p.Leu433*	8	Nakazato et al., 1997, Carballo-Trujillo et al., 2003
Nonsense	c.1399C>T	p.Arg467*	9	Ludwig et al., 2005, Tosetto et al., 2006, Ramos-Trujillo et al., 2007, Dinour et al., 2009, Li et al., 2009, Zhu et al., 2010
Nonsense	c.1467G>A	p.Trp489*	9	Sekine et al., 2014
Nonsense	c.1506T>A	p.Tyr502*	9	Ramos-Trujillo et al., 2007
Nonsense	c.1641G>A	p.Trp547*	10	Tosetto et al., 2006
Nonsense	c.1701C>(A/G ?)	p.Tyr567*	10	Lee et al., 2009,

				Okamoto et al., 2012
Nonsense	c.1798C>T	p.Gln600*	10	Tosetto et al., 2006
Nonsense	c.1825G>T	p.Glu609*	10	Cheong et al., 2005, Cho et al., 2008
Nonsense	c.1851C>A	p.Tyr617*[c]	10	Yamamoto et al., 2000, Mo et al. 2004 [c]
Nonsense	c.1885C>T	p.Gln629*	10	Sekine et al., 2014
Nonsense	c.1909C>T	p.Arg637*	10	Takemura et al., 2001, Cheong et al., 2005 Ludwig et al., 2005, Tosetto et al., 2006, Cho et al., 2008, Dinour et al., 2009, Li et al., 2009, Lee et al., 2009, Zhu et al., 2010, Sekine et al., 2014
Nonsense	c.1942C>T	p.Arg648* [c]	11	Lloyd et al., 1996 [c] and 1997, Igarashi et al., 1998, Bosio et al., 1999, Cox et al., 1999, Tosetto et al., 2006, Blanchard et al., 2008, Frishberg et al., 2009, Sekine et al., 2014, Mo et al., 2004 [c]
Nonsense	c.2110C>T	p.Arg704* [c]	11	Lloyd et al., 1996 [c], Langlois et al., 1998, Igarashi et al., 1998, Nakazato et al., 1999, Cox et al., 1999, Mo et al., 2004 c, Ramos-Trujillo et al., 2007, Ramos-Trujillo et al., 2013, Sekine et al., 2014, Cramer et al., 2014
Nonsense	c.2152C>T	p.Arg718* [a]	12	Carballo-Trujillo et al., 2003, Hoopes et al., 2004, Wu et al., 2009, Grand et al., 2009[a]
Nonsense	c.2186T>G	p.Leu729*	12	Sethi et al., 2009
Splicing	c.105+2T>C	skipping of exon 3, frameshift	intron 2	Wu et al., 2009
Splicing	c.106-2A>G	skipping of exon 3, frameshift	intron 2	Ramos-Trujillo et al., 2007
Splicing	c.205+2T>C	skipping of exon 3, frameshift	intron 3	Tosetto et al., 2006, Ji et al., 2014
Splicing	c.206-1G>A	p.?	intron 3	Tosetto et al., 2006, Sekine et al., 2014
Splicing	c.393+1G>A	p.?	intron 4	Sekine et al., 2014
Splicing	c.393+4A>G	p.?	intron 4	Tosetto et al., 2009
Splicing	c.394-2A>C	p.?	intron 4	Sekine et al., 2014
Splicing	c.394-2A>G	p.?	intron 4	Zhu et al., 2010, Sekine et al., 2014
Splicing	c.516+5G>T	skipping of exon 5	intron 5	Tosetto et al., 2006
Splicing	c.516+2T>G	p.?	intron 5	Lloyd et al., 1996
Splicing	c.516+1G>A	p.?	intron 5	Lloyd et al., 1996, Sekine et al., 2014
Splicing	c.517-2A>G	skipping of exon 6	intron 5	Igarashi et al., 2000
Splicing	c.517-3C>A	skipping of exon 6	intron 5	Cox et al., 1999
Splicing	c.723+1G >T	p.?	6	Ramos-Trujillo et al., 2013
Splicing	c.723+1G >A	p.?	6	Cramer et al., 2014
Splicing	c.1347+1G>T	loss of 290 bp of exon 8, frameshift	intron 8	Tosetto et al., 2009, Sekine et al., 2014
Splicing	c.1534+1G>T	p.?	9	Park et al., 2014
Splicing	c.1535-1G>A	p.?	9	Cramer et al., 2014
Splicing	c.1535-2A>C	p.?	9	Park et al., 2014

Splicing	c.1933+2_1933+3insTGGT	p.?	Intron 10	Sekine et al., 2014
Splicing	c.2150+1G>T	skipping of exon 11, frameshift	intron 11	Tosetto et al., 2009
Inframe	c.782_784	p.Gly261del	7	Tosetto et al., 2009
Inframe	c.800_802del	p.Glu268del	7	Tosetto et al., 2009, Sekine et al., 2014
Inframe	c.830_832dup	p.Thr277_Leu278insSer	8	Tosetto et al., 2009
Inframe	c.1566_1568del	p.V523del[d]	10	Wu et al., 2009[d], Ramos-Trujillo et al., 2013, Sekine et al., 2014, Park et al., 2014
Missense	c.64T>G	p.Trp22Gly[c]	2	Morimoto et al., 1998[c], Ramos-Trujillo et al., 2013
Missense	c.170G>T	p.Gly57Val[d]	3	Lloyd et al., 1997[d], Smith et al., 2009 [d]
Missense	c.173G>T	p.Trp58Leu	3	Tosetto et al., 2009
Missense	c.174G>C	p.Trp58Cys	3	Sethi et al., 2009
Missense	c.193G >A	p.Gly65Arg	3	Ramos-Trujillo et al., 2013
Missense	c.263G>A	p.Gly88Asp	4	Ludwig et al., 2006
Missense	c.263G>T	p.Gly88Val	4	Sekine et al., 2014
Missense	c.270C>G	p.Cys90Trp	4	Sekine et al., 2014
Missense	c.299A>G	p.His100Arg	4	Park et al., 2014
Missense	c.302G>A	p.Cys101Tyr	4	Cho et al., 2008, Lee et al., 2009
Missense	c.307T>C	p.Trp103Arg	4	Sekine et al., 2014
Missense	c.527T>A	p.Ile176Asn	6	Sekine et al., 2014
Missense	c.536G>A	p.Gly179Asp[a]	6	Grand T et al., 2009[a]
Missense	c.599T>G	p.Leu200Arg[a]	6	Lloyd et al., 1996[a], Grand et al., 2009[a]
Missense	c.608C>T	p.Ser203Leu[a]	6	Grand et al., 2009[a]
				Sekine et al., 2014, Park et al., 2014
Missense	c.631G>C	p.Glu211Gln	6	Sekine et al., 2014
Missense	c.634G>A	p.Gly212Ser	6	Sekine et al., 2014
Missense	c.635G>C	p.Gly212Ala[c]	6	Grand et al., 2009[c]
Missense	c.638C>T	p.Pro213Leu	6	Sekine et al., 2014
Missense	c.655T>C	p.Cys219Arg[a]	6	Ramos-Trujillo et al., 2007, Grand et al., 2009[a]
Missense	c.661T>C	p.Cys221Arg[a]	6	Hoopes et al., 2004, Ludwig et al., 2005[a], D'Antonio et al., 2013 [a]
Missense	c.674T>C	p.Leu225Pro [a]	6	Ramos-Trujillo et al., 2007, Grand et al., 2011[a]
Missense	c.731C>T	p.Ser244Leu[c]	7	Lloyd et al., 1996[c], Oudet et al.,.1997, Kelleher et al., 1998, Hoopes et al., 1998 and 2004, Tosetto et al., 2006, Ludwig et al., 2006, Anglani et al., 2006, Blanchard et al., 2008, Wu et al., 2009, Zhu et al., 2010, Samardzic et al., 2011, Sekine et al., 2014, Grand et al., 2011[c]
Missense	c.779G>T	p.Gly260Val[b]	7	Tosetto et al., 2006, Anglani et al., 2006, Grand et al., 2011[b]
Missense	c.781G>A	p.Gly261Arg	7	Tosetto et al., 2009
Missense	c.782G>A	p.Gly261Glu	7	Bogdanović et al., 2010
Missense	c.796C>G	p.Leu266Val	7	Sekine et al., 2014
Missense	c.800A>C	p.Glu267Ala	7	Hoopes et al., 2004
Missense	c.808A>C	p.Ser270Arg[a]	8	Igarashi T et al., 1998[a],

				Smith et al., 2009[a]
Missense	c.808A>G	p.Ser270Gly	8	Hoopes et al., 2004
Missense	c.814T>A	p.Tyr272Asn	8	Sekine et al., 2014
Missense	c.815A>G	p.Tyr272Cys[c]	8	Tosetto et al., 2006, Sekine et al., 2014, Grand et al., 2011[c]
Missense	c.817T>C	p.Phe273Leu	8	Ramos-Trujillo E et al., 2007
Missense	c.834G>C	p.Leu278Phe[b]	8	Igarashi T et al., 1998, Sekine et al., 2014, Grand et al., 2011[b]
Missense	c.839G>C	p.Arg280Pro[d]	8	Lloyd et al., 1997[d]
Missense	c.971T>G	p.Leu324Arg[a]	8	Ludwig et al., 2005[a]
Missense	c.997G>A	p.Gly333Arg[a]	8	Tanuma et al., 2007[a]
Missense	c.1020C>G	p.Asn340Lys[a]	8	Tosetto et al., 2006, Grand et al., 2011[a]
Missense	c.1384G >A	p.Gly462Ser	9	Ramos-Trujillo et al., 2013
Missense	c.1385G>A	p.Gly462Asp	9	Hoopes et al., 2004
Missense	c.1385G>T	p.Gly462Val[a]	9	Ludwig et al., 2005[a]
Missense	c.1396G>C	p.Gly466Arg	9	Valina et al., 2012
Missense	c.1397G >A	p.Gly466Asp	9	Ramos-Trujillo et al., 2013
Missense	c.1403T>C	p.Leu468Pro	9	Sekine et al., 2014
Missense	c.1406T>C	p.Leu469Pro[a]	9	Grand et al., 2009[a], Lee et al., 2009
Missense	c.1505A>G	p.Tyr502Cys	9	Sekine et al., 2014
Missense	c.1511T>A	p.Met504Lys	9	Sethi et al., 2009
Missense	c.1514T>G	p.Val505Gly	9	Zhu et al., 2010
Missense	c.1516G>A	p.Gly506Arg	9	Matsuyama et al., 2004, Sekine et al., 2014
Missense	c.1517G>A	p.Gly506Glu[a]	9	Lloyd et al., 1996[a], Scheinman et al., 2000
Missense	c.1534G>C	p.Gly512Arg[d]	9	Lloyd et al., 1997[d]
Missense	c.1535G>A	p.Gly512Asp	10	Tosetto et al., 2009
Missense	c.1537G>A	p.Gly513Arg[a]	10	Hoopes et al., 2004, Sekine et al., 2014, Grand et al., 2011[a]
Missense	c.1538G>A	p.Gly513Glu[a]	10	Akuta et al., 1997, Smith et al., 2009[a]
Missense	c.1546C>T	p.Arg516Trp[a]	10	Akuta et al., 1997, Hoopes et al., 2004, Ludwig et al., 2005[a], Ludwig et al., 2006, Wu et al., 2009, Sekine et al., 2014, Park et al., 2014, Smith et al., 2009[a]
Missense	c.1547G>A	p.Arg516Gln	10	Sekine et al., 2014
Missense	c.1556T>A	p.Val519Asp	10	Tosetto et al., 2009
Missense	c.1558T>C	p.Ser520Pro[c]	10	Lloyd et al., 1996[c]
Missense	c.1561C>T	p.Leu521Phe	10	Cramer et al., 2014
Missense	c.1566_1568del	p.Val522del	10	Sekine et al., 2014
Missense	c.1571T>A	p.Ile524Lys[a]	10	Takemura et al., 2001, Yanagida et al., 2004, Sekine et al., 2014, Smith et al., 2009[a]
Missense	c.1581A>T	p.Glu527Asp[b]	10	Lloyd et al., 1997[b], Smith et al., 2009[b]
Missense	c.1634G>A	p.Ser545Asn	10	Hoopes et al., 2004,Cho et al.,. 2008, Lee et al., 2009
Missense	c.1636A>G	p.Lys546Glu[b]	10	Hoopes et al., 2004, Grand et al., 2011[b]
Missense/splicing	c.1639T>G	p.Trp547Gly[b] (skipping of exons 10 and 11)	10	Ramos-Trujillo et al., 2007, Grand et al., 2011[b]

Missense	c.1639T>C	p.Trp547Arg	10	Tosetto et al., 2009
Missense/splicing	c.1802A>T	p.Asp601Val ; (loss of 133 bp of exon 10), p.Asp601Lysfs*33	10	Yamamoto et al., 2000
Missense	c.1862C>T	p.Pro621Leu	10	Tosetto et al., 2009
Missense	c.2108T>C	p.Phe703Ser	11	Sekine et al., 2014
Missense	c.2117T>C	p.Leu706Pro	11	Sekine et al., 2014
Missense	c.2133C>G	p.Cys711Trp	11	Sekine et al., 2014
Missense	c.2173A>G	p.Lys725Glu	12	Ludwig et al., 2006, Sekine et al., 2014
	c.1948insAlu(345pb)		11	Claverie Martin et al., 2003 and 2005

* Numbering is according to the cDNA sequence (GenBank entry NM 000084.2). The A of the ATG of the Methionine initiation codon is defined as nucleotide 1. Superscript letters indicate mutations in vitro expressed, their reference and classification as follow: [a]class 1 mutations inducing defective protein processing; [b]class 2 mutations inducing delayed protein processing and lower stability of the mature protein; [c]class 3 mutations inducing altered conduction without any change in subcellular distribution; [d]mutations needing further investigation to allow its classification; [f]unctionally tested as L521R

Supp.Table S2. *In silico* pathogenicity predictions for new in-frame and missense mutations

Nucleotide change	Amino acid change	Exon	PolyPhen-2	Sift	MutationTaster	SNPs&Go	MutPred
c.692A>T	p.Lys231Ile	6	Probably damaging	Deleterious Score 0.01	Disease causing p=1	Disease ri=8	g=0.689; loss of methylation (p=0.0196); gain of helix (p=0.0496)
c.716G>C	p.Arg239Pro	6	Benign	Deleterious Score 0.02	Disease causing p=0.99	Disease ri=9	g=0.762
c.748G>C	p.Gly250Arg	7	Probably damaging p=0.993	Deleterious Score 0	Disease causing p=1	Disease ri=9	g=0.894; gain on methylation (p=0.0078)
c.801A>C	p.Glu267Asp	7	Probably damaging p=1	Deleterious Score 0	Disease causing p=1	Disease ri=9	g=0.974; loss of catalytic residue (p=0.0138); gain of methylation
c.1408G>C	p.Gly470Arg	9	Probably damaging p=1	Deleterious Score 0.01	Disease causing p=0.99	Disease ri=9	g=0.914
c.1552A>G	p.Thr518Ala	10	Probably damaging p=1	Deleterious Score 0	Disease causing p=1	Disease ri=6	g=0.854
c.1588G>A	p.Gly530Ser	10	Probably damaging p=1	Deleterious Score 0	Disease causing p=1	Disease ri=7	g=0.768
c.773delinsGGAA	p.Pro258delinsArgAsn	7	NA	NA	Disease causing	NA	NA

For SIFT: a change is predicted to be deleterious if the score <0.05
For MutationTaster: a change is predicted to be disease causing if the score p>0.5,
For SNPs&GO: a change is predicted to be disease causing if the reliability index (ri)>5,
For MutPred: a change is predicted to be deleterious if the general score g>0.75; in addition this tool gives scores (*p*) for 5 structural and functional properties. Combinations of high values of general scores and low values of property scores are referred to as hypotheses: scores with $g>0.5$ and $p<0.05$ are referred to as actionable hypotheses; scores with $g>0.75$ and $p<0.05$ are referred to as confident hypotheses and scores with $g>0.75$ and $p<0.01$ are referred to as very confident hypotheses.

Supp. Table S3. Genotype of 134 relatives analysed in 43 families.

Relative	Genotype	Number of generations (number of families)				
		1 (n=3)	2 (n=31)	3 (n=7)**	4 (n=2)***	Total
Mother	Carrier	-	29	6	2	37
	Wt	-	2	0	0	2
Grandmother	Carrier	-	-	3	1	4
	Wt	-	-	2	0	2
Daughter of affected male	Carrier	-	0	1	4	5
Other female	Carrier	0	8	9	5	22
	Wt	2	5	0	5	12
Other males*	Hemizygous	2	12	4	9	28
	Wt	0	9	1	2	13
Father	Wt		7		1	8
Maternal grandfather	Hemizygous	-	-	0	0	0
	Wt	-	-	1	0	1
Total						134

* Sons of carrier mothers or brothers in sporadic cases (probands are not included).
** In two families, the third generation is the generation after that of the proband.
*** The fourth generation is one or two generations after that of the proband.

Supp. Table S4. Phenotype of patients clinically asymptomatic before the genetic diagnosis

Patient	2-4	20-2	20-4	31-2	33-3	33-4	37-2	44-13	45-2	66-6	66-8	66-9	134-2	136-5
Age at diagnosis (years)	4.0	8.0	2.0	16.0	3.0	3.5	23.0	0.5	7.0	19.0	32.0	1.3	8.0	0.2
LMW proteinuria	+	+	+	+	+	+	NA	+	+	+	NA	-	+	+
Hypercalciuria	+	-	+	+	+	+	+	-	-	-	NA	NA	NA	-
Nephrocalcinosis	-	NA	+	-	+	-	+	-	-	NA	-	-	NA	-
Aminoaciduria	NA	+	NA	NA	-	-	NA	-	-	NA	NA	NA	NA	NA
Renal Insufficiency	-	-	-	-	-	-	-	-	-	-	-	-	NA	-
Hypophosphatemia of renal origin	-	-	-	-	+	-	NA	-	+	-	-	-	NA	NA
Hypokalaemia of renal origin	-	-	-	-	+	-	NA	-	-	-	-	-	NA	NA
Lithiasis	+	-	-	-	NA	-	NA	+	-	NA	+	-	NA	-
Rickets	-	-	-	-	-	-	NA	-	-	-	-	-	NA	-
Metabolic Acidosis	-	-	-	-	-	NA	NA	-	NA	NA	NA	-	NA	-
Glycosuria	-	-	NA	NA	NA	-	NA	-	-	-	NA	-	NA	+

+, present; -, absent; NA, not available

Supp. Table S5. Geographical Distribution

		Described families	This paper	Total	
Continent	Country	Number	Number		Percentage
Asia	Japan	96		96	37.46%
	Korea	32		32	
	Chine	11		11	
	Israel	11		11	
	India	4		4	
Europe	Italy	44	2	46	50.60%
	Spain	25		25	
	Germany	19		19	
	North Europe	13		13	
	Unit Kingdom	10		10	
	France	9	65	74	
	Turkey	5		5	
	Finland	3		3	
	Austria	2		2	
	Belgium	1	2	3	
	Montenegro	1		1	
	Portugal	1		1	
	Serbia	1	1	2	
	Switzerland	-	3	3	
	Ukraine	-	1	1	
North America	United States of America	33	1	34	10.21%
	North America	7		7	
	Canada	1		1	
South America	Bolivia	1		1	0.73%
	Uruguay	1		1	
	Argentina	-	1	1	
North Africa		1	3	4	0.97%
Total		332	79	411	

Supp. Table S6. Data of renal biopsy

Reference	Wrong et al., 1994 Lloyd et al., 1996			Langlois et al., 1998		Hoopes et al., 1998		Igarashi et al., 1998					Takemura et al., 2001		Yanagida et al., 2004	Brakemeier et al., 2004	Cheong et al., 2005	Anglani et al., 2006	Lim et al., 2007	Copelovitch et al., 2007		Sheffer Babila et al., 2008	
Age at biopsy (years)	63 *	62	13	5	6.5	4	2	14	6	8	9	13	13	16	2	9	9	15	3	12	9	8	11.5
Minimal alteration in glomeruli and interstitium													+		+				+				
Periglomerular fibrosis	+	+	+	+	+																+		
Interstitial fibrosis	+	+	+		+	+	+											+		+	+		
tubular atrophy		+	+		+	+	+			+	+	+				+				+	+		
tubular or interstitial calcification		+	+	+	+			+	+	+	+			+			+				+		
Sclerosed glomeruli				+	+ 10/50	+	+		+	+	+	+				+				+ 9/37	+ 2/6	+ 3/18	
Glomerular hyalinosis	+	+	+													+		+		+			
Mesangial proliferation														+		+			C1q (++)				
FGGS[2]																				+	+	+	+
EM				N	N													N	EFP	EFP	EFP		

Reference	Frishberg et al., 2009			Li et al., 2009	Valina et al., 2012	Okamoto et al., 2011	Ramos-Trujillo et al., 2013		Fervenza et al., 2013	Cramer et al., 2014					
Age at biopsy (years)	8	7	9	11	7	14	12		18	3	6	7	9	6	4
Minimal alteration in glomeruli and interstitium									+						
Periglomerular fibrosis					+										+
Interstitial fibrosis			+	+	+	+			+			+			+
tubular atrophy			+		+										
tubular or interstitial calcification									+						
Sclerosed glomeruli	+ 2/50	+ 5/50	+ 4/24		+ 8/23	+	+	+	+ 4/21		+			+	
Glomerular hyalinosis															
Mesangial proliferation	+										+			+	
FGGS[2]	+	+	+		+				+	+				+	+
EM		EFP								10% EFP	30-40% EFP	10% EFP	Minimal EFP		

*Necropsy; FGGS =: focal global glomerulosclerosis; EM: electron microscopy; N: normal; EFP: effacement of the foot processes