

## Familial DDD associated with a gain-of-function mutation in complement C3.

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Dense Deposit Disease (DDD) is a severe renal disease characterized by accumulation of electron-dense material in the mesangium and the glomerular basement membrane. Previously, DDD has been associated with factor H deficiencies and studies in animal models have linked pathogenesis to the massive C3 activation caused by this deficiency (1-5). We have identified a unique DDD pedigree, including three affected individuals, which illustrate for the first time that mutations in the complement C3 gene are associated with DDD. The DDD-associated C3 mutation described in this case-report is a unique experiment of Nature that provides fundamental insights into both the pathogenic mechanisms underlying DDD and the structural aspects of the activation and regulation of the complement system. All these data significantly advance our understanding of DDD pathogenesis and have important therapeutic implications.

Summary of clinical and histopathology data.

Index case (GN28), a 53y-old woman, presented with hypertension, microscopic hematuria and proteinuria at age 25y, during the third trimester of her first and only identical twin-pregnancy. She had an episode of nephritis at age 7y. After two years of persistent microhematuria, proteinuria rose to 1.5g/day and plasma creatinine (Cr) of 0.9 mg/dL.

A renal biopsy was performed that illustrated segmental mesangial hypercellularity with thickened, brightly eosinophilic segments of basement membrane (Figure 1, 2). There were prominent and diffuse C3 deposits, granular and nodular in some glomerular areas. Transmission electron microscopy (EM) in renal biopsies performed at early stages of the disease demonstrated definitive diagnosis of DDD. These analyses illustrated an electron-dense ribbon-like accumulation along the glomerular basement membrane, and local electron-dense deposits in the mesangium. Presence of a ribbon-like, osmiophilic deposit in the glomerular basement membrane (Figure 3, 4, 5). Mild deposits of C1q, IgA and IgM were also present.

Anti-nuclear (ANA) and anti-DNA antibodies were negative. C3 and C4 levels were within the normal range. During the following 6y her renal function progressively declined with proteinuria reaching a nephrotic range of 7 g/day. In 1991, at age 35y, she started dialysis. In 1992 she received a cadaver kidney allograft that lasted until 1997. Interestingly, from the second month post-transplant the patient presented with hypertension, microhematuria and a progressive proteinuria that reached nephritic range in the fourth year post-transplant. The patient went back to dialysis the following year. In 1998 she received a second cadaver kidney allograft that lasted until 2003, following a similar period of progressive renal insufficiency and proteinuria that this time started three years after transplantation. Biopsies, taken from this and the previous kidney allograft, illustrated similar microscopic findings to those found in the original kidney. In 2006, the patient received a third cadaver kidney allograft that is still functioning. However, the patient presents microhematuria, proteinuria and a progressive renal insufficiency that is accentuating in the third year post-transplant.

First son (III-2), 26y-old, is one of the identical twin sons of GN28. At age 2y, coincident with an episode of fever, he presented with microhematuria. At age 16y (1999), he was admitted to hospital because of hyperuricemia (9.1 mg/dL), showing proteinuria of 1.5 g/day, microhematuria (10-25 erythrocytes per field) and a Cr of 1.4 mg/dL corresponding to a creatinine clearance (CCr) of 73 ml/min. Levels of C3 and C4 were within the normal range. Five years later, at age 21y, his renal function started to decline with a Cr of 2 mg/dL, CCr of 46 ml/min, proteinuria of 1.1 mg/dL and persistent microhematuria. Six months later the Cr and CCr rose to 3 mg/dL and 33 ml/min, respectively. A renal biopsy at this time showed membranoproliferative glomerulonephritis with intense C3 deposits similar to those observed in his mother's kidney biopsies. Nine years later, his renal function deteriorated to ESRD. He is currently on peritoneal dialysis.

Second son (III-1), 26y-old, is the identical twin brother of III-2. Despite microhematuria being evident in occasional follow up visits, he was not available for assessment until 2006, aged 23y. In his first visit to the nephrologist he showed hyperuricemia of 8.5 mg/dL, Cr of 1.4 mg/dL with a CCr of 105 ml/min, proteinuria of 0.4 mg/dL and microhematuria (12 erythrocytes per field). Levels of C3 and C4 were within the normal range. These values were similar in February 2009. Very recently, in April 2010, this

second son accepted to submit a kidney biopsy that showed focal glomerular lesions similar to those found in his mother and brother. The main histopathology characteristic of the glomerular lesion is a mesangial segmental increase with widening of some glomerular capillary wall. Positive nodules of C3 deposits were demonstrated by IF.

## Conclusions

The histopathology glomerular characteristic of this family cases affecting three members (mother and two sons) is a Dense Deposit Disease (DDD) also called Membranoproliferative disease type II (MPGNII). In contraposition of MPGN I, there is no constant glomerular finding by light microscopy with a variation of microscopic pattern (6). The main light microscopic characteristics in this family were a segmental and global glomerular affection with proliferative glomerulonephritis and presence of leucocytes. The mesangium displayed different degrees of matrix increase with segmental accentuated nodular-lobular pattern. Related with this segmental lesions, there was capillary wall thickening. This thickening was caused by irregular PAS + intramembranous deposits. The DDD takes its name from the characteristic ultrastructural appearance. The hallmark is the presence of dense osmiophilic deposits into GBM, and sometimes in basal membrane of the Bowman capsule and tubules. The elongated deposits in the GBM usually affect long stretches of many capillary loops. In the first mother's biopsy ultrastructural study only a mild involvement was demonstrated with some microtubules deposits and granular variant of dense deposit. This complicated the diagnosis at that time.

The Immunofluorescence microscopy reported C3 deposition usually diffuse, global and intense as our cases. The mesangial deposits were coarse granular in the most of our studies.

DDD almost invariably recurs in the renal allograft. It is easy to recognize, because in our cases all recurrences had similar histopathology features to the original native kidney biopsy and similar to the sons biopsies, with glomerular hypercellularity and PAS deposits in mesangium and capillary wall. The IF studies the most intense deposit was of C3. The mother was transplanted for three times and in the different allograft biopsies the similar histologic DDD features was demonstrated.

The C3<sub>923ADG</sub> mutant protein was structurally and functionally characterized using a battery of approaches that includes: protein purification techniques, mass spectrometry, single particle electron microscopy, surface plasmon resonance and functional complement assays. These analyses provided a pathogenic mechanism that explains the development of DDD in our patients: C3<sub>923ADG</sub> circulates in the plasma of the patients at stable and high levels and constantly produces activated C3 molecules by the "tick-over" mechanism (or through non-complement mediated proteolysis) which cannot be inactivated by fH in plasma. In turn, the activated mutant C3 molecules generate active AP C3 convertases which cannot be regulated by fH, resulting in complement dysregulation in the fluid phase and substantial consumption of the wild type C3 protein and fB in these heterozygote DDD patients (7).

## References

1. Pickering MC, Cook HT. Translational mini-review series on complement factor H: renal diseases associated with complement factor H: novel insights from humans and animals. *Clin Exp Immunol* 2008; 151: 210-230.
2. Appel GB, Cook HT, Hagerman G et al. Membranoproliferative glomerulonephritis type II (dense deposit disease): an update. *J Am Soc Nephrol* 2005; 16: 1392-1403.
3. Licht C, Schlotzer-Schrehardt U, Kirschfink M et al. MPGN II – genetically determined by defective complement regulation? *Pediatr Nephrol* 2007; 22: 2-9.
4. Abrera-Abeleda MA, Nishimura C, Smith JL et al. Variations in the complement regulatory genes factor H (CHF) and factor H related 5 (CFHR5) are associated with membranoproliferative glomerulonephritis type II (dense deposit disease). *J Med Genet* 2006; 43: 582-589.
5. Pickering MC, Cook HT, Warren J et al. Uncontrolled C3 activation causes membranoproliferative glomerulonephritis in mice deficient in complement factor H. *Nat Genet* 2002; 31: 424-428.
6. Walker PD. Dense deposit disease: new insights. *Curr Opin Nephrol Hypertens* 2007; 16: 204-212.
7. de Cordoba SR, de Jorge EG. Translational minireview series on complement factor H: genetic and disease associations of human complement factor H. *Clin Exp Immunom* 2008; 151: 1-13.

Figure 1. Widening of mesangial matrix with PAS positive deposits (arrow).

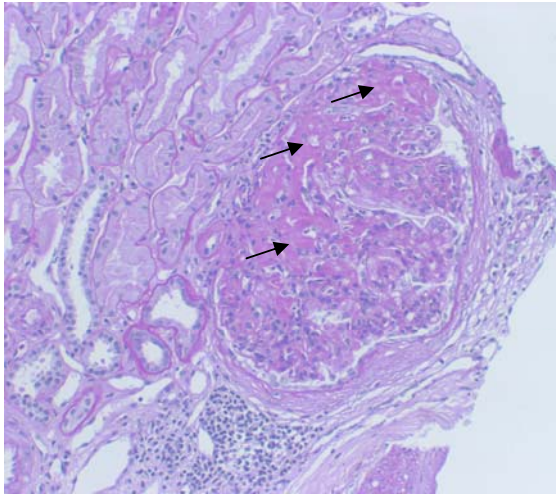


Figure 2. Lobular glomerular pattern with increase of mesangial, matrix (HE) (arrow).

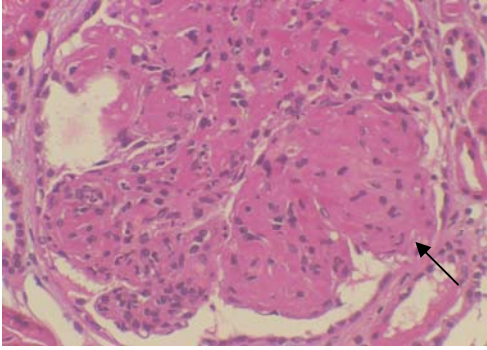


Figure 3. Trichromic positive material and lobular pattern (Masson Trichromic) (arrow).

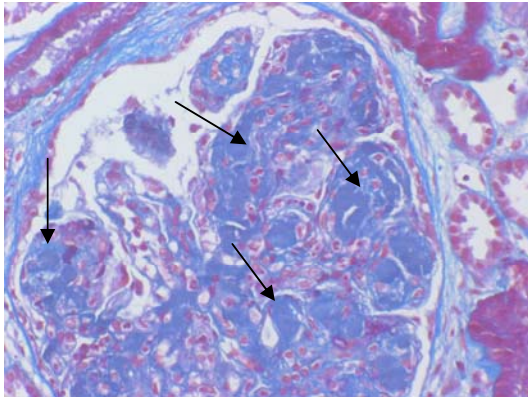


Figure 4. Continuous electron-dense deposits (EDD) along of glomerular basement membrane (arrow).

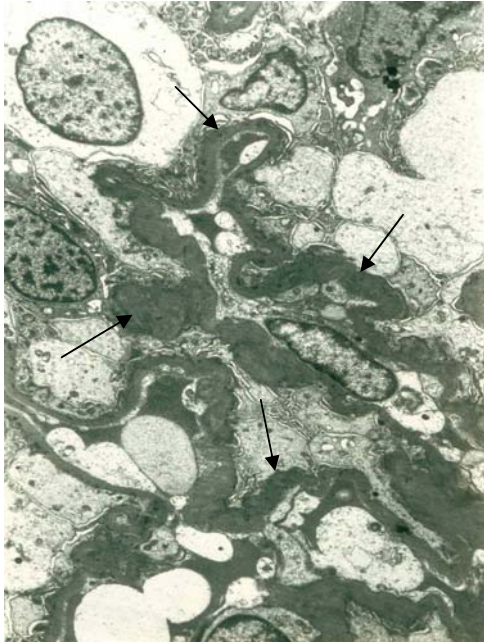


Figure 5. EDD in the basement membrane and mesangial nodules (double arrow).

