Characterisation of a *C3* mutation with increased resistance to complement regulation in an individual with recurrent C3GN in a renal transplant.

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Introduction

- C3 glomerulonephritis (C3GN) is characterised by the predominance of C3 deposition.
- Electron microscopy shows sub-endothelial and/or sub-epithelial deposits, an appearance distinct from the laminar dense electron deposits found in dense deposit disease (DDD).
- C3GN and DDD are part of an overall group of diseases called C3 glomerulopathy (C3G) that are thought to be due to disruption of alternative pathway complement regulation, deposition and degradation leading to renal disease[1] (Fig 1).
- The prognosis with individuals with C3GN is variable and recurrence in transplant recipients is common.
- We describe and characterise a genetic variant in *C3* found in a patient with recurrent C3GN in a renal transplant.

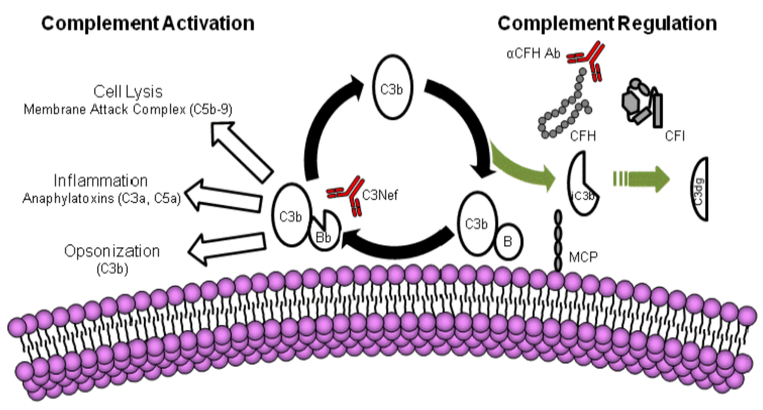

Fig 1. Complement Alternative Pathway (AP). *The AP is a positive feedback amplification loop and generates C3b via the C3 convertase (C3bBb). Autoantibodies which bind C3bBb e.g. C3Nef have been described in C3GN resulting in complement over-activation. Complement regulatory proteins (CFH, CFI, MCP) regulate the turnover of the AP and breakdown C3b into inactive forms. Autoantibodies against and mutations in these regulators have been described in C3GN. Mutations in C3 and FB have also been described.*

Methods

- We reviewed the clinical, serological and pathological characteristics of a boy with recurrent C3GN.
- Screening for rare variants in the complement genes (*C3*, *FB*, *FH*, *FI* and *MCP*) was performed by Sanger sequencing.
- Wildtype (WT) and mutant C3 was expressed in 293T cells.
- C3 was treated with methylamine pH 8 for 1 hour at 37°C prior to binding and functional assays[2].
- Binding of C3 to MCP and FH was determined by
  - Surface plasmon resonance (SPR) and
  - Enzyme-Linked ImmunoSorbent Assay (ELISA)
- Resistance of C3 to FI-mediated cleavage was performed in the fluid phase in the presence of either MCP or FH as a co-factor.

Results

- A 13 year old boy presented with nephrotic syndrome.
- He had low C3 and was negative for C3 nephritic factor.
- A biopsy showed membranoproliferative changes with dominant C3 staining and sub-endothelial and mesangial electron dense deposits.
- He progressed to end-stage renal disease and required dialysis for 9 months before he was transplanted.

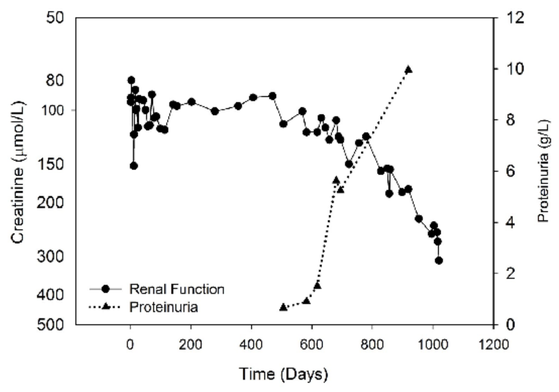

Fig 2. Serial reciprocal creatinine and proteinuria following transplantation. *A consistent downward trend in renal function is noticeable with associated dramatic rise in proteinuria after the first year of transplant.*

- Proteinuria was first evident at 4 months of transplantation and rises significantly alongside a fall in renal function after 12 months (Fig 2).
- C3 remained low and C3 Nephritic factor negative.
- A biopsy showed recurrence of C3GN.
- Mutation screening revealed a heterozygous change c.2363C>T; p.T788M in *C3* (Fig 3).
- There was 1 copy of the MCP_{AAGGT} risk haplotype associated with C3GN and 2 copies of the *FH* H1 haplotype associated with DDD.
- Anti-FH autoantibody was negative.

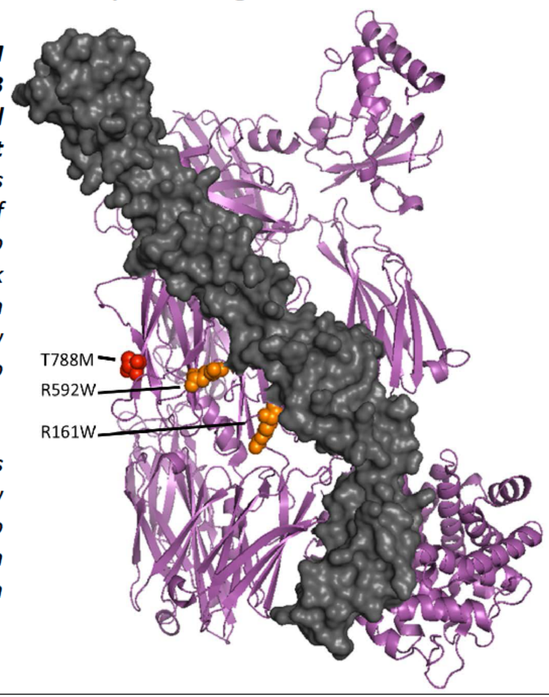

Fig 3. T788M change in C3 modelled in Pymol (Based upon Wu et al[3]). *Red spheres show position of affected amino acid on C3b (pink ribbon) in relation to FH1-4 (Grey spheres). Two neighbouring functionally significant mutants that predominantly affect binding to MCP and function are also shown (orange spheres).*

- Binding of C3 to the co-factors MCP and FH was examined using SPR (Fig 4) and ELISA (Fig 5)
- WT C3 bound with a stronger signal to MCP than T788M in both SPR and ELISA.
- T788M appears to bind with a stronger signal to FH than WT but with evidence of altered association and disassociation using SPR suggesting the mutation has altered the interaction with WT.

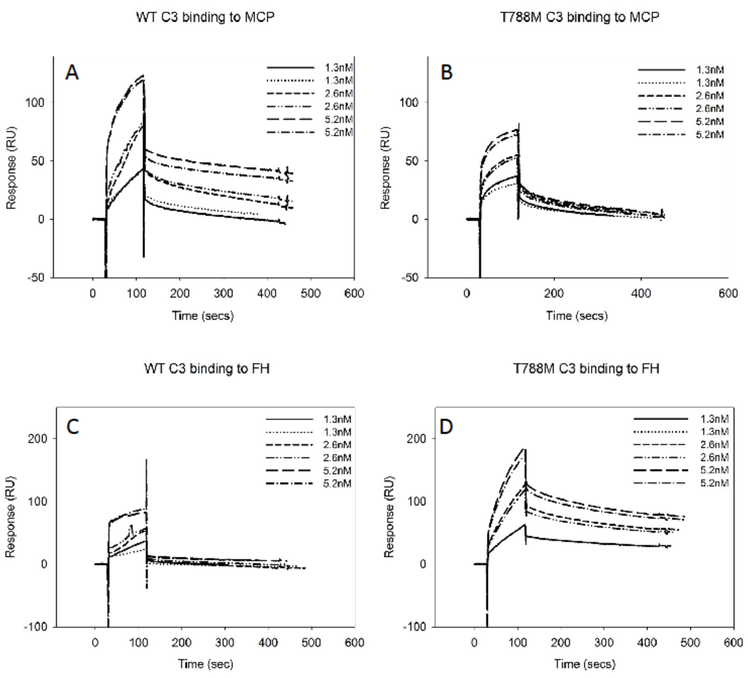

Fig 4. Binding of C3 WT and T788M to MCP and FH by Surface Plasmon Resonance. *MCP and FH were amine-coupled to a CM5 chip in respective experiments. C3 was treated with methylamine before flowing over the chip surface in low-salt conditions (25mM NaCl) at concentrations ranging from 1.3-5.2nM. (A and B) T788M binding to MCP is attenuated compared to WT. (C and D) T788M binding to FH was stronger but has a different kinetic profile compared to WT.*

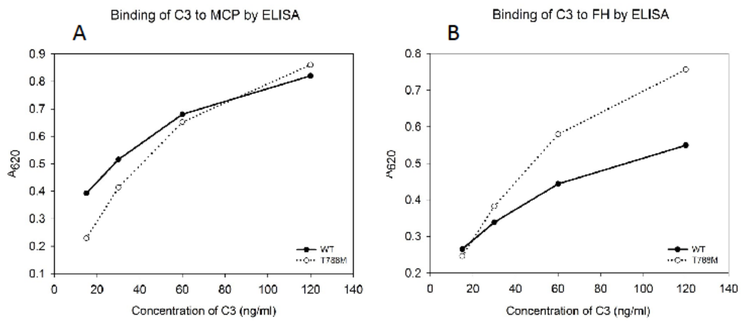

Fig 5. Binding of C3 WT and T788M to MCP and FH by ELISA. *MCP or FH were coated onto the ELISA plate before adding methylamine treated C3 (WT or T788M) at 15-120ng/ml in low salt conditions (25mM NaCl). Binding was detected using polyclonal goat anti-Human C3 Ig. (A) There was decreased binding of T788M to MCP compared to WT. (B) T788M binding to FH was stronger compared to WT.*

- Functional activity of methylamine treated C3 was tested in fluid-phase co-factor assays.
- C3 T788M was more resistant than WT to inactivation by FI in the presence MCP (Fig 6A).
- Despite the stronger binding signal of C3 T788M to FH, C3 T788M, in the presence of FH was also more resistant than WT to inactivation by FI.

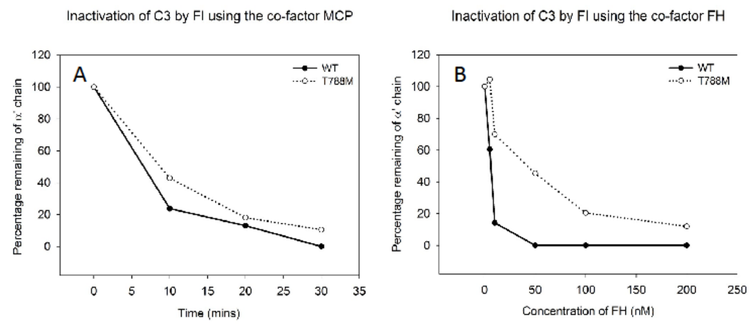

Fig 6. Inactivation of C3 by FI in the presence of MCP or FH. *5 ng of methylamine treated WT and T788M C3 was incubated with either 10 ng FI and 100 ng MCP over 0 to 30 min or 20 ng FI for 20 min using 5-200 ng FH at physiological ionic conditions (150mM NaCl) at 37°C. The samples were then separated by SDS PAGE gels under reducing conditions and transferred to nitrocellulose membrane. Following development with a goat anti human C3 antibody, densitometry was used to determine the percentage of α' chain remaining. Inactivation of methylamine treated C3 by FI-mediated cleavage resulting in the loss of α' chain was perturbed in the presence of (A) MCP over a time course and (B) FH over a concentration range.*

Conclusions

- We present a patient with recurrent C3GN with persistently low C3 in a renal transplant.
- We suspected an underlying complement abnormality and repeatedly found C3 nephritic factor was negative .
- We screened the complement genes and identified a rare genetic variant in *C3*.
- Functional analysis suggests that the mutant C3 is responsible for over activation of the complement system via increased resistance to FI-mediated inactivation via its co-factors, MCP and FH.
- In patients with a diagnosis of C3GN, the presence of a consistently low C3 level, the absence of a C3Nef should prompt genetic analysis. This will highlight those at risk of recurrent disease.

References

- 1 Pickering et al, *Kidney International* (2013) 84, 1079–1089
- 2 Mitchell et al, *J Immunol Methods* (2008)337(1):49-54
- 3 Wu et al, *Nat Immunol* (2009) 10(7):728-33

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