Clinical History

Family 1

II:2 had presented aged 10yrs with walking difficulty and pex cavus (figure 1a). A motor axonal neuropathy was found and a diagnosis of Charcot-Marie-Tooth was made. Age 17 she presented with proteinuria (12g/l) and a creatinine of 1141umol/l. There was no evidence of thrombotic microangiopathy at the time of presentation. A renal biopsy demonstrated end-stage changes with diffuse global sclerosis (figure 2 c). She had haemodialysis for 2 years when she had a renal transplant which lasted for 6 years. After a further 5 years on dialysis she had another renal transplant. After 5 years she presented with worsening renal function, low platelets and low haptoglobins. A renal biopsy demonstrated thrombotic microangiopathy (figure 2,d ,e).

The proposita's brother (III:1), 3 aunts (II:1, II:3; II:4) and the maternal grandmother (I:2) do not carry the p.V102D mutation. II:1, II:3; II:4 and III:1 are all heterozygous for the CD46 aHUS risk haplotype. I:2 does not carry this risk factor. I:2, II:1, II:3; II:4 are all heterozygous for the CFH-H3 haplotype while III:1 is homozygous. They do not have proteinuria, or haematuria and have normal renal function. There is no neurological phenotype. There is no information on the maternal grandfather and no DNA was available to test.

Family 2

III:1 presented aged 27 with a creatinine of 172 µmol/l and hypertension. At presentation the haemoglobin was 14.2 g/dl and the platelet count 271×10^9/L. The proteinuria at presentation was quantified at 5.8g/24hrs. Within 11 months of presentation she reached ESRF and commenced RRT (figure 2 f, g).

She had a living related transplant from her mother 2 years later and there was delayed graft function due to intra and post operative hypotension. A biopsy at day 6 demonstrated features of acute tubular necrosis. By day 16 her creatinine had fallen to 220 µmol/L . At day 23 she represented with hypertension and fluid overload. She was anaemic (Hb 6.0g/dl), thrombocytopenic (platelet count 96×10^9/L) with a blood film demonstrating microangiopathic haemolytic anaemia. Her tacrolimus levels were always within the normal range. A renal transplant biopsy was undertaken demonstrating severe thrombotic microangiopathy and she started plasma exchange (figure 2 h, i) . Over the course of the next months she received daily plasma exchange with fresh frozen plasma and a further 2 transplant biopsies demonstrated on-going TMA. Her renal function continued to deteriorate and she recommenced haemodialysis.

The father of III:1, II:2 developed ESRF and underwent renal replacement therapy. He died aged 25. No renal biopsy or further clinical information is available.

III:2 presented age 36 with a creatinine of 492 µmol/l and hypertension. At presentation the haemoglobin was 13.5 g/dl and the platelet count 390×10^9/L The proteinuria was quantified at 4.1g/24hrs. Within 7 months of presentation he reached ESRF and commenced RRT. A renal biopsy demonstrated a subacute arterial TMA with fibroproliferative intimal thickening and luminal obliteration in small arteries and arterioles (figure 2 j, k). 12/16 glomeruli were globally sclerosed and one demonstrated segmental sclerosis. The sclerosed glomeruli were hypercellular and showed tubularisation of Bowman's space.

He had a deceased heart beating donor aged 39. The immunosuppressive regime was Cyclosporin, prednisolone and MMF. A renal biopsy was undertaken after 4 years due to a decline in the graft function demonstrating end stage changes with fibrous

obliteration of arteries compatible with a diagnosis of chronic arterial thrombotic microangiopathy or chronic transplant vasculopathy (figure 2 l).

III:2's mother (II:2) was an obligate carrier who died aged 42 of unknown cause. No historical record or DNA is available. III:2's daughter (IV:1) and brother (III:3) do not carry the p.R177H variant and have normal renal function. IV:1 has one copy of aHUS *FH-H3* risk haplotype but has no copies of the $CD46_{GGAAC}$ risk haplotype. III:3 has one copy of aHUS *FH-H3* risk haplotype but has no copies of the $CD46_{GGAAC}$ risk haplotype

Supplemental Table 1 Complement Antigenic Levels

	Fm1 II:1	Fm1 I:2	Fm2 III:1	Fm2 III:2
INF 2 mutation	p.V102D	p.V102D	p.R177H	p.R177H
C3 g/L (0.68-1.38 g/l)	0.56	0.66	0.97	0.76
C4 g/L (0.18-0.60 g/l)	0.19	0.29	0.26	0.30
FH g/L (0.35-0.59 g/l)	0.39	0.47	0.50	0.52
FI mg/L (38-58 mg/L)	47	61	60	51
FH auto Ab	Negative	Negative	Negative	Negative

Supplemental Table 2 Exome raw data

		Fm1		Fm2- II:2
		I:2	II:1	
Total number of reads received		63413530	70865504	61141134
Percentage of reads mapped to the reference genome (after removal of duplicates)		75.3	85.8	96.3
Total mean coverage (x)		57.9	67.6	62.4
Percentage of exome Covered	1x	99.0	99.1	98.3
	5x	95.9	96.7	95.5
	10x	89.5	91.7	90.6
	20x	70.9	76.9	80.2
	40x	36.2	44.4	55.3

Supplemental Table 3 Whole exome sequencing filtering results

Step	Number of variants het in both
Total	230854
1. Only exonic (and splicing)	17365
2. Rare	1924
3. Segregates	571
4. Deleterious	113
5. ≥0.1% 1000g ESP6500	49
6. <2 GERP++ <0.5 PhyloP removed	34

Deleterious in at least one of 6 predictor programs (Polyphen-2 HDIV and HVAR, Mutation Taster, Mutation Assessor, FATHMM and RadialSVM). The prediction score thresholds used, were based on what has been previously described in the literature (Li *et al.*, 2014; Dong *et al.*, 2015).

Polyphen2 scores of ≥0.453 or ≥0.447, in HDIV and HVAR respectively, were labelled damaging. A score of ≥0.5 was classed as deleterious. Mutation Assessor scores of ≥0.785, were classed as high impact and therefore deleterious. FATHMM scores of ≥0.453, were predicted to be deleterious. A RADIAL SVM score of ≥0.5, was classed as deleterious.

Supplemental Table 4 Phenotypic Interogation

Chr	Position	AAChange	dbSNP138	GeneName	Renal Phenotype (Dominant)	Neuro Phenotype (Dominant)
chr16	16267146	G928S	rs142470921	*ABCC6*		
chr5	131308482	E325K	rs145869312	*ACSL6*		
chr17	42226378	G403R	rs143555103	*C17orf53*		
chr1	7724048	M481V	rs142884344	*CAMTA1*		●
chr13	111294792	R498Q		*CARS2*		
chr5	177673296	P458S	rs200786621	*COL23A1*		
chr17	6684053	F289S	rs200774821	*FBXO39*		
chr1	27942346	A692T	rs2231878	*FGR*		
chr9	14807956	P1024T		*FREM1*		
chr16	20325981	A368V		*GP2*		
chr3	128780764	N61S	rs5030764	*GP9*		
chr14	105168007	V102D		*INF2*	●	●
chr12	26774085	Q1145K		*ITPR2*		
chr3	134338075	R188C	rs377549995	*KY*		
chr17	44408485	I1281T	rs1054818	*LRRC37A*		
chr17	45299776	Q173R	rs373421058	*MYL4*		
chr7	45007287	T567A	rs61745695	*MYO1G*		
chr17	40695858	S612G	rs148881970	*NAGLU*		
chr3	27233566	N132I	rs35493524	*NEK10*		
chr4	47850293	D875N	rs201648191	*NFXL1*		
chr6	72006495	Q223K		*OGFRL1*		
chr1	248059744	N286H		*OR2W3*		
chr7	148716271	Q144H		*PDIA4*		
chr7	148718104	F123S		*PDIA4*		
chr4	25260763	Q287H	rs143048917	*PI4K2B*		
chr1	20964372	P142L		*PINK1*		●
chr1	14109023	P1377L	rs148083107	*PRDM2*		
chr12	15734706	W55G		*PTPRO**		
chr16	24580389	R660H	rs371006887	*RBBP6*		
chr22	44385092	T183A		*SAMM50*		
chr5	145435759	R513Q	rs141349885	*SH3RF2*		
chr10	90676486	A152V	rs140820995	*STAMBPL1*		
chr17	42092230	C31G	rs139186093	*TMEM101*		
chr7	100371479	R1923P	rs71555302	*ZAN*		

Supplemental Table 5 *INF2* primers and conditions

Exon	Forward	Reverse	PCR product size (bp)	Annealing temperature (°C)
2	TGGTGGCCAGGAGGACA	CTGAAGCCTCATACCAGGTC	524	55
3/4	TGCACAGGCATGGGAAG	ATGCACTGGCCAAGAGGC	466	55
5.1	CTCTGGGTGGCAGAAGTGA	CCTTGTTCTAGTGGGCCTG	570	63
5.2	GGACGCAAGATGGAGACA	ACCTGAATGAACTGCATG	550	55
6	TGGTACACTGGCGCTGAC	CCCAGCCTCTGGACCTCA	300	60
7	CACAGTCCTTAGTCCACCA	GACCTCAGTGTCTGCCCA	260	60
8.1	CTTCTCCCTTGACCCTGGA	CCAGGCCTGGCAGGGGTG	448	60
8.2	TAAGGCCCTCCCAACAGCA	TCTCCCCAGCAGAGACCAG	448	60
9/10	ACATCCTTTAGACCCTCT	CCCAAGTCAGAAGGCTCA	464	55
11	CTCGACTGTTCTGTGTCC	CCTGTGGCTCACGGTTCA	377	60
12	TGAACCGTGAGCCACAGG	TTGGCACAGCCTCTTCTC	305	55
13	CAGAGCCCTGTGCTGAGT	CAGCCATTATGATACTGGC	284	55
14/15	GCTCCGAACCAAGAGCCC	ATGGGGGTATGGCCTCTAC	379	60
16	GACCTTAATTGCTAAGGG	TGCGCTGCAGCTGTGAGA	282	55
17	TCAGAGTACAGCCTGCAGG	AGACAGCACAGCCACTCG	320	60
18	CTCTCACGGGACTGTCA	AAAGGTGCCTCTGCCCAT	291	60
19/20	CGACACAGCCATGTGGGC	GACCATGAGGAGGCAGTG	546	60
21.1	GGGCGTTCAGGTAGACAG	CTCGTCCTCGTCCTCATC	582	55
21.2	GAGGCCGACAGCACAAGTG	TCCTGGGTGCCGCTGGAAC	371	55
22	GAGTCAGGGTTGCTTCTG	AGAAGCCAGGTTAGTGTC	217	55

Dong, C., Wei, P., Jian, X., Gibbs, R., Boerwinkle, E., Wang, K. and Liu, X. (2015) 'Comparison and integration of deleteriousness prediction methods for nonsynonymous SNVs in whole exome sequencing studies', *Hum Mol Genet*, 24(8), pp. 2125-37.
Li, Q., Liu, X., Gibbs, R.A., Boerwinkle, E., Polychronakos, C. and Qu, H.-Q. (2014) 'Gene-Specific Function Prediction for Non-Synonymous Mutations in Monogenic Diabetes Genes', *PLoS One*, 9(8), p. e104452.