Gleeson PJ, Wilson V, Cox TE, Sharma SD, Smith-Jackson K, Strain L, Lappin D, McHale T, Kavanagh D, Goodship THJ. [Chromosomal rearrangement – a rare cause of complement factor I associated atypical haemolytic uraemic syndrome](#). *Immunobiology* 2016, 221(10), 1124-1130.

Copyright:

©2016. This manuscript version is made available under the [CC-BY-NC-ND 4.0 license](#)

DOI link to article:

<http://dx.doi.org/10.1016/j.imbio.2016.05.002>

Date deposited:

11/10/2016

Embargo release date:

10 May 2017

This work is licensed under a [Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International licence](#)

Chromosomal rearrangement – a rare cause of *complement factor I* associated atypical haemolytic uraemic syndrome

Patrick J Gleeson^{1*}, Valerie Wilson^{2*}, Thomas E Cox^{3*}, Seema D. Sharma³, Kate Smith-Jackson³, Lisa Strain², David Lappin¹, Teresa McHale¹, David Kavanagh^{3*}, Timothy H J Goodship^{3*},

¹Departments of Nephrology and Histopathology, University Hospital Galway, Galway, Ireland; ²Northern Molecular Genetics Service, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK; ³Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, UK;

Address for correspondence: Professor Tim Goodship
Institute of Genetic Medicine
Newcastle University
Central Parkway
Newcastle upon Tyne
NE1 3BZ
UK
e-mail: t.h.j.goodship@ncl.ac.uk
telephone: 44 191 241 8632

* Contributed equally to this work

Abstract

Chromosomal rearrangements affecting the genes encoding complement factor H and the factor H related proteins have been described in aHUS patients. To date such disorders have not been described in other aHUS associated genes. We describe here a heterozygous 875,324bp deletion encompassing the gene (*CFI*) encoding complement factor I and ten other genes. The index case presented with aHUS and did not recover renal function. No abnormalities were detected on Sanger sequencing of *CFI* but a low factor I level led to a multiplex ligation-dependent probe amplification assay being undertaken. This showed a complete heterozygous deletion of *CFI*. The extent of the deletion and the breakpoint were defined. In the Newcastle aHUS cohort we have identified and report here 32 different *CFI* variants in 56 patients but to date this is the only deletion that we have identified. This finding although rare does suggest that screening for chromosomal rearrangements affecting *CFI* should be undertaken in all aHUS patients particularly if the factor I level is unexplainably low.

Key Words: complement, factor I, atypical haemolytic uraemic syndrome, chromosomal rearrangement, genetic variants

Introduction

Abnormalities affecting the serine protease complement factor I associated with atypical haemolytic uremic syndrome (aHUS) include both mutations (Kavanagh, et al., 2005) and autoantibodies (Kavanagh, et al., 2012). Mutations in *CFI* can lead to either impaired secretion resulting in factor I deficiency or impaired function particularly of the serine protease domain (Kavanagh, et al., 2013, Nilsson, et al., 2010). Mutations associated with factor I deficiency include missense changes, nonsense changes and deletions of a single nucleotide (Bienaime, et al., 2010). Larger deletions encompassing either the whole or part of *CFI* are extremely rare. A homozygous deletion including exon 2 and part of intron 1 was found in a 6 year old boy who presented not with aHUS but with recurrent sepsis including an episode of meningococcal disease (Alba-Dominguez, et al., 2012). We describe here a large heterozygous deletion at 4q25, incorporating 11 genes including *CFI*, which leads to partial factor I deficiency in association with aHUS.

Methods

The study was approved by Newcastle and North Tyneside 1 Research Ethics Committee, and informed consent was obtained in accordance with the Declaration of Helsinki.

Case Report. A 36 year old male, presented with shortness of breath, vomiting and general malaise. There was no history of diarrhoea. He took no regular medication and there was no significant family history. Urinalysis showed +++ blood and ++ protein. Investigations showed creatinine 1122 $\mu\text{mol/L}$, urea 39.4 mmol/L , haemoglobin 8.2 g/dL and platelets $83 \times 10^9/\text{L}$. Blood film revealed occasional schistocytes. Prothrombin time, activated partial thromboplastin time and fibrinogen were within normal limits. Routine autoantibody (ANCA, anti-GBM) and virology (HBV, HCV, HIV) screening was negative. C4 was normal and C3 slightly decreased at 0.71 g/L (0.75-1.86). Subsequent C3 measurements ranged from 0.45 to

0.84 g/L. ADAMTS13 activity was normal. A renal ultrasound showed normal sized hyperechoic kidneys without obstruction.

He commenced haemodialysis and subsequently a renal biopsy was performed which showed a thrombotic microangiopathy with severe concentric fibro intimal expansion (Figure 1). He did not recover renal function and is now undergoing peritoneal dialysis. Mutation screening of *CFH*, *CFI*, *CD46*, *C3*, *CFB* and *DGKE* showed no abnormalities. Factor H autoantibodies were negative and factor H concentration was normal at 0.69 g/l. Factor I concentration, however, was low at 29 mg/l (normal range 38 -58). Multiplex ligation-dependent probe amplification (MLPA) analysis revealed a heterozygous deletion encompassing all exons of *CFI* (Figure 2). Screening of parental samples showed that he (II:1) had inherited this from his father (I:1) who aged 63, is normotensive, has no abnormalities on urinalysis and has an eGFR of 90 ml/min/1.73m².

Measurement of complement components and screening for complement autoantibodies.

C3 and C4 levels were measured by rate nephelometry (Beckman Coulter Array 360). FH and FI levels were measured by radial immunodiffusion (Binding Site). Screening for complement autoantibodies were undertaken using ELISA as described previously (Kavanagh, et al., 2012, Moore, et al., 2010)

Mutation screening of complement genes. Mutation screening of *CFH* (Richards, et al., 2001), *CFI* (Kavanagh, et al., 2005), *CFB* (Kavanagh, et al., 2006), *CD46* (Richards, et al., 2003), *C3* (Fremaux-Bacchi, et al., 2008), and *DGKE* (Lemaire, et al., 2013) was undertaken using Sanger sequencing as previously described. Screening for chromosomal rearrangements affecting *CFH*, *CFHR1*, *CFHR2*, *CFHR3*, and *CFHR5* was undertaken using multiplex ligation-dependent probe amplification (MLPA) as previously described (Challis, et al., 2015).

Genotyping. The *CD46* SNPs -652A>G (rs2796267), -366A>G (rs2796268), IVS9 -78G>A (rs1962149), IVS12 +638G>A (rs859705) and c.4070T>C (rs7144) which define the at-risk *CD46*_{GGAAC} haplotype and the *CFH* SNPs -331C>T (rs3753394), c.184G>A; p.Val62Ile (rs800292), c.1204T>C p.Tyr402His (rs1061170), c.2016A>G; p.Gln672Gln (rs3753396), IVS15 -543G>A intron 15 (rs1410996), c.2808G>T; p.Glu936Asp (rs1065489)) which define the protective *CFH*_{CATAAG} (known as *CFH*-H2) and at-risk *CFH*_{TGTGGT} (known as *CFH*-H3) haplotypes were genotyped using direct sequencing in both patient and parental samples as described previously (Sansbury, et al., 2014).

Screening for chromosomal arrangements. Chromosomal rearrangements affecting *CFI* and *CD46* were screened for by MLPA using a proprietary kit from MRC Holland (SALSA MLPA P296 aHUS, Netherlands). Probes in *CFI* are indicated (Figure 2A)

Defining the extent of the deletion and the breakpoint. To define the extent of the deletion the CytoSure ISCA v2.0 (8x60k) array (Oxford Gene Technology) was used as per the manufacturer's protocol and arranged to human genome reference sequence (GRCh37) and analysed using BlueFuse Multi software v3.3 (Illumina) (Supplementary Figure 1). To further define the deletion microarray testing was performed using the Affymetrix CytoScan 750K SNP array (Affymetrix, Santa Clara, CA). The data was analysed using the Chromosome Analysis Suite v2.0 (Affymetrix) and the results were aligned to GRCh37 (Figures 3 and 4).

To fine map the deletion identified by the CytoScan 750K SNP array, custom single nucleotide polymorphism (SNP) genotyping was undertaken. Common SNPs with an allele frequency of ~0.5 were identified (using UCSC genome browser (<https://genome.ucsc.edu/index.html>)) within the regions identified by the CytoScan 750K SNP array (proximal, Chr4:110,059,471-110,070,698 and distal, Chr4:110,939,335-110,948,618). (Supplementary Tables 1 -3 and Figures 4-5).

A PCR product spanning the deletion was amplified using REDAccuTaq LA DNA Polymerase (sigma, St Louis). The primers were forward 5'-ACAGTCAGCAGGGAAGAACG-3' and reverse 5'-AGTGCAAACCAGCCATCACT-3'. Products were resolved on a 0.6% agarose gel (Figure 4B). Sanger sequencing of the PCR product was undertaken (Figure 6).

Results

The extent of the deletion. The CytoScan750K Array defined the extent of the deletion at 4q25. At the 5' end of the deletion the last non deleted probe was at position Chr4:110,059,471 with the first deleted probe at position Chr4:110,070,698. At the 3' end of the deletion the last deleted probe was at position Chr4:110,939,335 with the next non deleted probe at position Chr4:110,948,618. The maximum and minimum extent of the deletion as defined by the 750K array are shown in Figure 4A.

The breakpoint. To further delineate the breakpoint, custom genotyping of common SNPs was undertaken as shown on Figures 4A and 5. Informative SNPs (see Supplementary Tables 1-3) further narrowed the region of interest and allowed cross deletion long range PCR using primers rs2704110 (f) and rs17041245 (r). An amplified product was found in the patient's father (I:1) and the patient (II:1) but not the mother (I:2.) (Figure 4B). Sanger sequencing of this product showed the breakpoint to be between Chr4:110,069,101 and Chr4:110,944,425 resulting in a 875,324bp deletion (Figure 6A). This deletion spans 11 genes including factor I (Figure 6B).

Genotyping. *CFH* and *CD46* genotyping in patient and parental samples enabled us to infer that the patient carries *CFH*_{CATAAG} (protective H2 haplotype) on one allele and *CFH*_{TGTAGG} (H7 haplotype) on the other; and *CD46*_{GGAAC} (at risk haplotype) on one allele and *CD46*_{AAGGT} on the other. His father (unaffected carrier of the *CFI* deletion) carries *CFH*_{CGCAGG} (H1

haplotype) on one allele and *CFH*_{TGTAGG} (H7 haplotype) on the other; and *CD46*_{AAGGT} on both alleles.

***CFI* variants in the Newcastle aHUS cohort.** In the Newcastle aHUS cohort we have identified 32 different *CFI* variants in 56 patients that have either not been reported in control populations or have an allele frequency equal to or less than 1% as derived from the data of the Exome Aggregation Consortium (ExAC), Cambridge, MA (URL: <http://exac.broadinstitute.org>) [accessed December 2015] (Table 1). The factor I concentrations for the 26 variants where this is available are shown in Figure 7 along with factor I concentrations for those aHUS patients in whom a variant in *CFI* has not been detected by Sanger sequencing. Twenty-seven of the 32 different variants are non-synonymous and the change in the amino acid is shown for 19 variants in a crystal structure of factor I in Supplementary Figure 2. It was not possible to show the remaining 8 variants because the surrounding structure is flexible in space and as such the coordinates could not be annotated. Within the group of 56 patients who carry a *CFI* variant there are two (17 and 34) who carry more than one rare *CFI* variant. Twenty two of the 56 patients also carry either a rare variant in another complement gene and/or antibodies against factor H. There are three patients (7, 16 and 23) who carry a heterozygous *CFI* variant that leads to a frameshift and/or premature stop codon. Factor I levels are available for two of them and both are low as expected.

Discussion

To our knowledge this is the first description in an aHUS patient of a large deletion that encompasses *CFI* and thus results in factor I deficiency. The deletion includes exons 1-3 of *CO25A1* and 9 other entire genes. For three of these (*CO25A1*, *LRIT3*, *EGF*) a phenotype has been described but only in homozygosity or compound heterozygosity (Table 2). No phenotype has been described for the remaining six. That the father is asymptomatic despite

being factor I deficient is in keeping with the reduced penetrance that is seen for aHUS (Sansbury, et al., 2014). Genotyping of *CFH* and *CD46* showed that he does not carry the at risk *CD46*_{GGAAC} haplotype whereas his affected son does.

In contrast we have identified 32 different *CFI* variants in 56 patients within the Newcastle aHUS cohort. Three of these variants have previously been reported in aHUS patients in association with low levels of factor I (Gly119Arg (Bienaime, et al., 2010, Maga, et al., 2010), Val152Met (Westra, et al., 2010) and Gly287Arg (Maga, et al., 2010)). Gly119Arg has also been associated with age-related macular degeneration again with low levels of factor I (Kavanagh, et al., 2015). There are 4 patients in the Newcastle cohort who carry Gly119Arg, factor I level is normal in two and low in two. Because factor I is an acute-phase protein the use of absolute levels in individual patients is not sufficiently sensitive to exclude impaired secretion secondary to a heterozygous genetic variant. In the patient that we describe here the factor I level was low and Sanger sequencing of *CFI* did not show an abnormality to explain this. If we had not undertaken MLPA then this individual would have fallen within the group of aHUS patients said to have no identifiable mutation. Such patients are thought to have a lower risk of recurrent disease post-transplant (Zuber, et al., 2011) and as such might not be listed for transplantation with prophylactic eculizumab. Identification of the deletion led to this patient being listed for a transplant with prophylactic eculizumab. In the Newcastle aHUS cohort we have subsequently identified 6 other aHUS patients with a low factor I level who had previously not been found to have any abnormality of *CFI* on Sanger sequencing. We undertook MLPA in all of them and did not find any abnormalities. Thus, a deletion affecting *CFI* appears to be a very rare finding in aHUS. This is in contrast to the genes encoding factor H and the factor H related proteins where deletions secondary to both non-allelic homologous recombination (NAHR) and microhomology-mediated end joining (MMEJ) can result in the formation of aHUS-associated hybrid genes (Francis, et al., 2012, Venables, et al., 2006). The

genomic structure of this region (1q32) is characterised by several large genomic duplications, also known as low copy repeats (LCRs), resulting in a high degree of sequence identity between *CFH* and the genes for the five factor H-related proteins. The resulting areas of macro- and micro-homology predispose to NAHR and MMEJ. In contrast the genomic structure in the vicinity of *CFI* at 4q25 shows little evidence of LCRs and one would, therefore, expect chromosomal rearrangements in this region to be infrequent. However, because such a finding has implications for the clinical management of a patient, particularly with regard to transplantation, we are of the opinion that *CFI* MLPA should be undertaken in aHUS patients, particularly in those with low factor I levels, as part of routine genetic screening.

Acknowledgements

The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007–2013) under grant agreement number 305608 (EURENOMICS). D.K. is a Wellcome Trust Intermediate Clinical Fellow. The authors would like to thank the Exome Aggregation Consortium and the groups that provided exome variant data for comparison. A full list of contributing groups can be found at <http://exac.broadinstitute.org/about>.

Disclosures

Newcastle University has received fees from Alexion Pharmaceuticals for lectures and consultancy undertaken by T.H.J.G. and D.K.

References

- Alba-Dominguez, M., Lopez-Lera, A., Garrido, S., Nozal, P., Gonzalez-Granado, I., Melero, J., Soler-Palacin, P., Camara, C., Lopez-Trascasa, M. 2012. Complement factor I deficiency: a not so rare immune defect: characterization of new mutations and the first large gene deletion. *Orphanet J Rare Dis* 7, 42.
- Bienaimé, F., Dragon-Durey, M.A., Regnier, C.H., Nilsson, S.C., Kwan, W.H., Blouin, J., Jablonski, M., Renault, N., Rameix-Welti, M.A., Loirat, C., Sautes-Fridman, C., Villoutreix, B.O., Blom, A.M., Fremeaux-Bacchi, V. 2010. Mutations in components of complement influence the outcome of Factor I-associated atypical hemolytic uremic syndrome. *Kidney International* 77, 339-49.
- Challis, R.C., Araujo, G.S.R., Wong, E.K.S., Anderson, H.E., Awan, A., Dorman, A.M., Waldron, M., Wilson, V., Brocklebank, V., Strain, L., Morgan, B.P., Harris, C.L., Marchbank, K.J., Goodship, T.H.J., Kavanagh, D. 2015. A de novo deletion in the regulators of complement activation cluster producing a hybrid complement *Factor H/Complement Factor H-Related 3 Gene* in atypical hemolytic uremic syndrome. *Journal of the American Society of Nephrology*.
- Francis, N.J., McNicholas, B., Awan, A., Waldron, M., Reddan, D., Sadlier, D., Kavanagh, D., Strain, L., Marchbank, K.J., Harris, C.L., Goodship, T.H. 2012. A novel hybrid CFH/CFHR3 gene generated by a microhomology-mediated deletion in familial atypical hemolytic uremic syndrome. *Blood* 119, 591-601.
- Fremeaux-Bacchi, V., Miller, E.C., Liszewski, M.K., Strain, L., Blouin, J., Brown, A.L., Moghal, N., Kaplan, B.S., Weiss, R.A., Lhotta, K., Kapur, G., Mattoo, T., Nivet, H., Wong, W., Gie, S., Hurault de Ligny, B., Fischbach, M., Gupta, R., Hauhart, R., Meunier, V., Loirat, C., Dragon-Durey, M.A., Fridman, W.H., Janssen, B.J., Goodship, T.H.J., Atkinson, J.P. 2008. Mutations in complement C3 predispose to development of atypical hemolytic uremic syndrome. *Blood* 112, 4948-52.
- Kavanagh, D., Goodship, T.H., Richards, A. 2013. Atypical hemolytic uremic syndrome. *Seminars in Nephrology* 33, 508-30.
- Kavanagh, D., Kemp, E.J., Mayland, E., Winney, R.J., Duffield, J.S., Warwick, G., Richards, A., Ward, R., Goodship, J.A., Goodship, T.H. 2005. Mutations in complement factor I predispose to development of atypical hemolytic uremic syndrome. *Journal of the American Society of Nephrology* 16, 2150-5.
- Kavanagh, D., Kemp, E.J., Richards, A., Burgess, R.M., Mayland, E., Goodship, J.A., Goodship, T.H. 2006. Does complement factor B have a role in the pathogenesis of atypical HUS? *Molecular Immunology* 43, 856-9.
- Kavanagh, D., Pappworth, I.Y., Anderson, H., Hayes, C.M., Moore, I., Hunze, E.M., Bennaceur, K., Roversi, P., Lea, S., Strain, L., Ward, R., Plant, N., Nailescu, C., Goodship, T.H., Marchbank, K.J. 2012. Factor I autoantibodies in patients with atypical hemolytic uremic syndrome: disease-associated or an epiphenomenon? *Clinical Journal of the American Society of Nephrology* 7, 417-26.

Kavanagh, D., Yu, Y., Schramm, E.C., Triebwasser, M., Wagner, E.K., Raychaudhuri, S., Daly, M.J., Atkinson, J.P., Seddon, J.M. 2015. Rare genetic variants in the CFI gene are associated with advanced age-related macular degeneration and commonly result in reduced serum factor I levels. *Human Molecular Genetics* 24, 3861-70.

Lemaire, M., Fremeaux-Bacchi, V., Schaefer, F., Choi, M., Tang, W.H., Le Quintrec, M., Fakhouri, F., Taque, S., Nobili, F., Martinez, F., Ji, W., Overton, J.D., Mane, S.M., Nurnberg, G., Altmuller, J., Thiele, H., Morin, D., Deschenes, G., Baudouin, V., Llanas, B., Collard, L., Majid, M.A., Simkova, E., Nurnberg, P., Rioux-Leclerc, N., Moeckel, G.W., Gubler, M.C., Hwa, J., Loirat, C., Lifton, R.P. 2013. Recessive mutations in DGKE cause atypical hemolytic-uremic syndrome. *Nature Genetics* 45, 531-6.

Maga, T.K., Nishimura, C.J., Weaver, A.E., Frees, K.L., Smith, R.J. 2010. Mutations in alternative pathway complement proteins in American patients with atypical hemolytic uremic syndrome. *Human Mutation* 31, E1445-60.

Moore, I., Strain, L., Pappworth, I., Kavanagh, D., Barlow, P.N., Herbert, A.P., Schmidt, C.Q., Staniforth, S.J., Holmes, L.V., Ward, R., Morgan, L., Goodship, T.H.J., Marchbank, K.J. 2010. Association of factor H autoantibodies with deletions of CFHR1, CFHR3, CFHR4 and with mutations in CFH, CFI, CD46, and C3 in patients with atypical haemolytic uraemic syndrome. *Blood* 115, 379-87.

Nilsson, S.C., Kalchishkova, N., Trouw, L.A., Fremeaux-Bacchi, V., Villoutreix, B.O., Blom, A.M. 2010. Mutations in complement factor I as found in atypical hemolytic uremic syndrome lead to either altered secretion or altered function of factor I. *European Journal of Immunology* 40, 172-85.

Richards, A., Buddles, M.R., Donne, R.L., Kaplan, B.S., Kirk, E., Venning, M.C., Tielemans, C.L., Goodship, J.A., Goodship, T.H.J. 2001. Factor H mutations in hemolytic uremic syndrome cluster in exons 18-20, a domain important for host cell recognition. *American Journal of Human Genetics* 68, 485-90.

Richards, A., Kemp, E.J., Liszewski, M.K., Goodship, J.A., Lampe, A.K., Decorte, R., Muslumanoglu, M.H., Kavukcu, S., Filler, G., Pirson, Y., Wen, L.S., Atkinson, J.P., Goodship, T.H.J. 2003. Mutations in human complement regulator, membrane cofactor protein (CD46), predispose to development of familial hemolytic uremic syndrome. *Proceedings of the National Academy of Sciences of the United States of America* 100, 12966-71.

Sansbury, F.H., Cordell, H.J., Bingham, C., Bromilow, G., Nicholls, A., Powell, R., Shields, B., Smyth, L., Warwicker, P., Strain, L., Wilson, V., Goodship, J.A., Goodship, T.H., Turnpenny, P.D. 2014. Factors determining penetrance in familial atypical haemolytic uraemic syndrome. *Journal of Medical Genetics* 51, 756-64.

Venables, J.P., Strain, L., Routledge, D., Bourn, D., Powell, H.M., Warwicker, P., Diaz-Torres, M.L., Sampson, A., Mead, P., Webb, M., Pirson, Y., Jackson, M.S., Hughes, A., Wood, K.M., Goodship, J.A., Goodship, T.H. 2006. Atypical haemolytic uraemic syndrome associated with a hybrid complement gene. *PLoS Medicine* 3, e431.

Westra, D., Volokhina, E., van der Heijden, E., Vos, A., Huigen, M., Jansen, J., van Kaauwen, E., van der Velden, T., van de Kar, N., van den Heuvel, L. 2010. Genetic disorders in

complement (regulating) genes in patients with atypical haemolytic uraemic syndrome (aHUS). *Nephrology Dialysis Transplantation* 25, 2195-202.

Zuber, J., Le Quintrec, M., Sberro-Soussan, R., Loirat, C., Fremeaux-Bacchi, V., Legendre, C. 2011. New insights into postrenal transplant hemolytic uremic syndrome. *Nature Reviews Nephrology* 7, 23-35.

Legends to Figures

Figure 1. Photomicrographs of renal biopsy A) H&E stain (x40) shows vascular luminal obliteration by sub-intimal expansion with fragmented red blood cells, and without fibrinoid necrosis. B) The same vessel as in panel A stained with trichrome (x10) shows myxoid sub-intimal expansion. C) Electron micrograph shows sub-endothelial electron lucent expansion typical of acute thrombotic microangiopathy.

Figure 2. A) Representation of the *CFI* gene structure. The MLPA probes in *CFI* are denoted by arrows and the distance between the probes shown. B) Multiplex ligation-dependent probe amplification of *CFI* demonstrating the copy number ratio. The dosage quotient of ~0.5 for I:1 (father) and II:1 (patient) is consistent with a deletion of one allele of *CFI*.

Figure 3. CytoScan 750K SNP array results. Microarray-based copy number analysis performed using the CytoScan 750K SNP array and visualised using the Chromosome Analysis Suite v2.0. A 8.69 Mb deletion was identified in II.1. The last non-deleted probes are located at Chr4:110,059,471 and Chr4:110,948,617. The first deleted probes are located at Chr4:110,070,698 and Chr4:110,939,335.

Figure 4. A) A CytoScan750K Array was performed to define the extent of the deletion in chromosome 4. At the 5' end of the deletion the last non deleted probe was at position Chr4:110,059,471 with the first deleted probe at position Chr4:110,070,698. At the 3' end of the deletion the last deleted probe was at position Chr4:110,939,335 with the next non deleted probe at position Chr4:110,948,618. The maximum and minimum extent of the deletion as defined by the 750K array are highlighted in the diagram. To further delineate the breakpoint, custom SNP genotyping of common SNPs was undertaken as highlighted. Informative SNPs (see supplementary data) further narrowed the region of interest and allowed cross deletion long range PCR using primers rs2704110 (f) and rs17041245 (r). B) The amplified product

was run on a 0.6% agarose gel demonstrating amplified product in I:1 (father) and II:1 (patient) but not I:2 (mother).

Figure 5. Extent of deletion as defined by custom SNP genotyping. Ampicon 8 (rs10021804) covers a deleted region. Amplicons 3 (rs2704110) and 9 (rs17041245) cover non-deleted regions. The maximum size of the deletion is 878740bp and the minimum size of the deletion is 871899bp. The distance between amplicons 8 and 9 is 1931bp.

Figure 6. Sanger sequence trace of the breakpoint. A) This highlights a 875,324bp deletion between Chr4:110,069,101 and 110,944,425. B) This deletion results in loss of exons 1-3 of *COL25A1*, *SEC24B-AS1*, *SEC24B*, *CCDC109B*, *CASP6*, *PLA2G12A*, *CFI*, *GARI*, *RRH*, *LRIT3* and *EGF*.

Figure 7. Serum FI levels by rare CFI variant in the Newcastle aHUS cohort. The lower limit of normal (38 mg/l) is demonstrated by a dotted line. ^ represents a homozygous variant.

Patient	FI concentration (mg/l)	Zygoty		Nucleotide change	Amino acid change	ExAC allele frequency (%)	Additional variants/antibodies
1	35	Heterozygous	Sporadic	148C>G	Pro50Ala	0.011	
2	40	Heterozygous	Sporadic	355G>A	Gly119Arg	0.053	
3	63	Heterozygous	Sporadic	355G>A	Gly119Arg	0.053	<i>CFH</i> (c.2596+1G>C)
4	34	Heterozygous	Sporadic	355G>A	Gly119Arg	0.053	
5	63	Heterozygous	Sporadic	355G>A	Gly119Arg	0.053	FH Ab
6	68	Heterozygous	Sporadic	414G>T	Met138Ile	0.0008	
7	29	Heterozygous	Sporadic	436G>A	Trp145*	0	
8	34	Heterozygous	Sporadic	436A>C	Ser146Arg	0.0016	<i>CD46</i> Homozygous (c.469T>C; p. Cys157Arg), FH Ab
9	36	Heterozygous	Sporadic	454G>A	Val152Met	0.003	
10	79	Heterozygous	Sporadic	482+6C>T		1.292	
11	n/a	Heterozygous	Sporadic	482+6C>T		1.292	FH Ab
12	73	Heterozygous	Sporadic	482+6C>T		1.292	
13	n/a	Heterozygous	Sporadic	548A>G	His183Arg	0.063	
14	n/a	Heterozygous	Sporadic	560G>A	Arg187Gln	0.012	
15	94	Heterozygous	Sporadic	612G>A	Met204Ile	0	
16	n/a	Heterozygous	Sporadic	651delG	Ala219fs	0	
17	54	Heterozygous	Sporadic	667A>G	Met223Val	0	FH Ab
17	54	Homozygous	Sporadic	1315G>A	Gly439Arg	0.027	FH Ab
18	n/a	Heterozygous	Sporadic	782G>A	Gly261Asp	0.133	<i>CFH</i> (c.2808G>T; p.Glu936Asp)
19	n/a	Heterozygous	Sporadic	782G>A	Gly261Asp	0.133	<i>CFH</i> (c.3628C>T; p.Arg1210Cys)
20	37	Heterozygous	Sporadic	859G>A	Gly287Arg	0.003	FH Ab
21	55	Heterozygous	Sporadic	859G>A	Gly287Arg	0.003	
22	54	Heterozygous	Sporadic	859G>A	Gly287Arg	0.003	
23	29	Heterozygous	Familial	893delC	Ser298Leufs*10	0	

24	66	Heterozygous	Sporadic	916A>G	Ile306Val	0.048	<i>CFB</i> (c.1598A>G; p.Lys533Arg)
25	82	Heterozygous	Sporadic	1019T>C	Ile340Thr	0.004	
26	70	Heterozygous	Sporadic	1166G>A	Arg389His	0.002	
27	41	Heterozygous	Sporadic	1216C>T	Arg406Cys	0.006	FH Ab
28	63	Heterozygous	Sporadic	1217G>A	Arg406His	0.006	
29	51	Heterozygous	Sporadic	1217G>A	Arg406His	0.006	
30	30	Heterozygous	Sporadic	1246A>C	Ile416Leu	0.111	
31	n/a	Heterozygous	Sporadic	1246A>C	Ile416Leu	0.111	
32	n/a	Heterozygous	Sporadic	1253A>T	His418Leu	0.001	<i>CD46</i> (c.390-2A>G)
33	48	Heterozygous	Sporadic	1268C>A	Ala423Glu	0.002	<i>CFB</i> (c.1598A>G; p.Lys533Arg)
34	33	Heterozygous	Sporadic	1402A>G	Ile468Val	0	<i>CD46</i> homozygous (c.100G>A; p.Ala34Thr)
34	33	Heterozygous	Sporadic	1642G>C	Glu548Gln	0.072	<i>CD46</i> homozygous (c.100G>A; p.Ala34Thr)
35	41	Homozygous	Sporadic	1456T>C	Trp486Arg	0	FH Ab
36	56	Heterozygous	Sporadic	1534+5G>T		0.893	
37	90	Heterozygous	Sporadic	1534+5G>T		0.893	<i>C3</i> (c.485C>G; p.Thr162Arg, c.2203C>T; p.Arg753Trp)
38	68	Heterozygous	Sporadic	1534+5G>T		0.893	FH Ab
39	67	Heterozygous	Sporadic	1534+5G>T		0.893	<i>CFH</i> (c.3616C>T; p.Arg1206Cys), <i>CFB</i> (c.724A>C; p. Ile242Leu)
40	53	Heterozygous	Sporadic	1534+5G>T		0.893	<i>CFH</i> (c.351-4dup)
41	90	Heterozygous	Sporadic	1534+5G>T		0.893	
42	n/a	Heterozygous	Sporadic	1534+5G>T		0.893	
43	69	Heterozygous	Sporadic	1534+5G>T		0.893	
44	68	Heterozygous	Sporadic	1534+5G>T		0.893	
45	56	Heterozygous	Sporadic	1534+5G>T		0.893	<i>C3</i> (c.485C>G; p.Thr162Arg, c.2203C>T; p.Arg753Trp)
46	30	Heterozygous	Sporadic	1534+5G>T		0.893	
47	48	Heterozygous	Sporadic	1534+5G>T		0.893	
48	52	Heterozygous	Sporadic	1534+5G>T		0.893	

49	70	Heterozygous	Sporadic	1534+5G>T		0.893	<i>CFH</i> (c.3398C>G; p.Ser1133*)
50	85	Heterozygous	Sporadic	1555G>A	Asp519Asn	0.002	
51	26	Heterozygous	Sporadic	1635T>A	Ser545Arg	0	<i>CFHR1/CFH</i> hybrid
52	60	Heterozygous	Sporadic	1657C>T	Pro553Ser	0.127	
53	63	Heterozygous	Sporadic	1657C>T	Pro553Ser	0.127	
54	86	Heterozygous	Sporadic	1657C>T	Pro553Ser	0.127	
55	89	Heterozygous	Familial	1657C>T	Pro553Ser	0.127	<i>CFH</i> (c.3648dupA;Trp1157fs)
56	41	Heterozygous	Sporadic	1733T>C	Ile578Thr	0.003	

Table 1 *CFI* variants in the Newcastle aHUS cohort. The ExAC allele frequency is taken from <http://exac.broadinstitute.org/>. Normal range for FI concentration is (38-58 mg/l). n/a not available.

Gene	Gene MIM	Phenotype	Phenotype MIM	Inheritance
<i>COL25A1</i>	61004	FIBROSIS OF EXTRAOCULAR MUSCLES, CONGENITAL, 5; CFEOM5	616219	Recessive
<i>SEC24B-AS1 and SEC24B</i>	607184	None described.		
<i>CCDC109B</i>	n/a	None described		
<i>CASP6</i>	601532	None described		
<i>PLA2G12A</i>	611652	None described		
<i>GAR1</i>	606468	None described		
<i>RRH</i>	605224	None described		
<i>LRIT3</i>	615004	NIGHT BLINDNESS, CONGENITAL STATIONARY, TYPE 1F; CSNB1F	615058	Recessive
<i>EGF</i>	131530	HYPOMAGNESEMIA 4, RENAL; HOMG4	611718	Recessive

Table 2. Genes within the deletion and associated phenotype.

Figure 1

Figure 2

A)

B)

Figure 3

A)

B)

Figure 4

Figure 5

(a)

Chr4:110,069,101-110,944,425

(b)