# Supplementary information

**S1. Biopsy scoring system**

**Activity index**

The activity index for each element should be combined to give a total score of between 0 to 15.

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| --- | --- | --- |
|  | **Element** | **Score** |
| **1** | Endocapillary hypercellularity (presence of cells in capillary loops with loop occlusion)Each glomerulus is scored for the percentage of the tuft that has endocapillary hypercellularity**Note:** The overall score is % glomeruli with any endocapillary hypercellularity, i.e., % glomeruli with a score of > 0) = 0%, 1–25%, 26–50%, >50% corresponding to scores of 0–3, respectively | 0 = 0%1 = 1–25%2 = 26–50%3 = >50%  |
| **2** | Neutrophils in capillary lumens (each glomerulus is scored)**Note:** The overall score is % of glomeruli with any neutrophils in the capillary lumens. i.e., % of glomeruli with a score > 0) = 0%, 1–25%, 26–50%, >50% corresponding to scores of 0–3, respectively | 0 = 0%1 = 1–25%2 = 26–50%3 = >50%  |
| **3** | Mesangial hypercellularity (more than 4 cells in a mesangial area away from the hilum) Score as percentage glomeruli involved (% glomeruli) | 0 = 0%1 = 1–25%2 = 26–50%3 = >50%  |
| **4** | Necrosis (disruption of the glomerular basement membrane with fibrin exudation and karyorrhexis - at least two of these three lesions need to be present to meet the criteria for necrosis) Score as percentage glomeruli involved (% glomeruli) | 0 = 0%1 = 1–10%2 = 11–25%3 = >25%  |
| **5** | Cellular or fibrocellular crescents Score as percentage glomeruli involved | 0 = 0%1 = 1–10%2 = 11–25%3 = >25%  |

Other features included in the composite activity score:

1. Glomerular C3c staining. This will be scored 0–3 on the standard semiquantitative score.
2. Glomerular macrophage infiltration (count macrophages per glomerulus in a CD68 immunostain). Each glomerulus is scored as 0, 1 (1–3 cells), 2 (4–10 cells), 3 (>10 cells).

Take the median for the overall score.

**For the composite biopsy score**, add the two scores for glomerular C3c staining and glomerular macrophage infiltration to the activity index (described above) to give a score with range of 0 to 21.

**Chronicity index**

Calculated from the sum of the score of the following lesions. The chronicity index is a combination of the scores for each parameter to give a final score between 0 and 12:

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| --- | --- | --- |
|  | Lesion  | Score |
| 1 | Glomerular sclerosis (% of glomeruli with segmental or global sclerosis)  | 0 = <10%, 1 = 10 – 25%2 – 26-50%3 >50% |
| 2 | Fibrous crescents (% of glomeruli with fibrous crescents) | 0 = none1 = <25% of glomeruli affected2 = 26–50% affected3= >50% affected |
| 3 | Tubular atrophy | 0 = <5%1 = 6–25%2 = 26–50%3 = >50% |
| 4 | Interstitial fibrosis | 0 = <5% 1 = 6–25%2 = 26–50%3 = >50% |

**S2. Methodology**

**S2a. Genetic analysis**

Whole blood samples taken from patients at screening were processed to extract DNA for genetic analysis. Targeted sequencing was performed to detect variants on a superset of genes relevant to C3G pathogenesis. Initially, coding regions and splice sites of the following genes were sequenced: *CFH, CFI, MCP (CD46), THBD, C4BPA, C4BPB, CFB, C3, LMNA, DGKE, ADAMTS13, CFHR1, CFHR3, CFHR4* and *CFHR5*. Specific untranslated, intronic and promoter regions known to be associated with aHUS or C3G were also sequenced. The targeted regions were enriched by multiplex PCR and sequenced by massively parallel sequencing. Multiplex ligation-dependent probe amplification (MLPA) was performed to detect deletions and duplications in exons of genes *CFH, CFHR1, CFHR3, CFHR4 and CFHR5*. A bioinformatics pipeline was developed to classify the detected variants according to the criteria developed by the American College of Medical Genetics (ACMG) [1]:

* Only those variants from genes *CFH, CFI, MCP (CD46), THBD, CFB, C3, DGKE, CFHR1* and *CFHR5* were maintained, in accordance with C3G literature recommendations [2].
* Variants with reported allele frequency above 1% in the general population were excluded, as these are automatically classified as Benign by ACMG criteria (ACMG standalone criterion BS1 [1]).
* Synonymous variants with no effects on exon splicing were excluded, as they are predicted to have no functional consequence and no disease associations of this type of variants has been reported (ACMG criterion BP7).
* Rare exonic variants reported as Benign in Clinvar[3]or in Osborne [4] were excluded from final counts.
* Presence of common variants or haplotypes that are not necessarily causal to disease but may have been associated with increased disease risk were not considered in this analysis.
* Multiplex ligation-dependent probe amplification (MLPA) was performed to detect deletions and duplications in exons of genes *CFH, CFHR1 and CFHR5*.

**References:**

1. Richards S, *et al*. Genet Med 2015;17(5):405–24.

2. Goodship T, *et al*. Kidney Int 2017;91(3):539–551.

3. Landrum M, *et al*. Nucleic Acids Research 2018;46(D1):D1062–D1067.

4. Osbourne A, *et al*. J Immunol 2018;200(7):2464–2478.

**S2b. Autoantibody identification**

Presence of C3NeF was evaluated in a functional microplate assay in which IgG recovered from patient serum was assessed for stabilization of co-assembled AP C3 convertase (C3bBbP) against FH-mediated decay. Briefly, pre-adsorbed C3b was incubated with FB, FD, and properdin in GVB++ buffer in the presence or absence of IgG purified from patient serum. Assembled convertase was subjected to a 20-minute decay period with factor H, after which residual C3 convertase was supplied with C3 substrate and the generation of soluble C3a product assessed by commercial ELISA (MIcrovue, Quidel). Stabilizing activity was evaluated relative to positive and negative control IgG samples.

The presence of C3NeF was further evaluated in a binding assay in which IgG binding was measured by a custom ELISA adapted from Paixão-Cavalcante et al, 2012 [1]. Ni++- and properdin-stabilized C3 convertase was preformed from purified proteins on immobilized C3b protein and incubated with patient serum for 30 min. After washing, bound IgG was detected with polyclonal anti-IgG antibody conjugated with horseradish peroxidase (Abcam). Binding activity was assessed relative to positive to negative and positive serum samples.

Presence of anti-FH autoantibody was identified in patient serum using a custom ELISA assayin which IgG binding was measured following incubation of patient serum diluted 1:50 with immobilized FH for 1 hour. After washing, bound IgG was detected with polyclonal anti-IgG antibody conjugated with horseradish peroxidase (Life Tech). Binding activity was assessed relative to positive and negative samples.

**References**

1. Paixão-Cavalcante D, *et al*. Kidney Int 2012;82:1084–1092.

**S3. Institutional Review Board/Independent Ethics Committee Approval Information**

|  |  |  |
| --- | --- | --- |
| **Country** | **Ethics Committee Address** | **Approval Number** |
| ***Study 204*** |
| United Kingdom | London-City & East Research Ethics CommitteeBristol Research Ethics Committee CenterWhitefriars, Level 3, Block B, Lewins MeadBristol, UKBS1 2NT | 235423 |
| United States | John Hopkins Medicine Office of Human Subjects Research1620 McElderry StReed Hall B130Baltimore, MDUSA 21205 | 20180406 |
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| United States | Columbia University Medical Center IRB154 Haven Avenue, 1st FloorNew York, NYUSA 10032 | 20180406 |
| United States | Western Institutional Review Board (WIRB)1019 39th Avenue SE Suite 120Puyallup, WashingtonUS 98374-2115 | 20180406 |
| ***Study 205*** |
| Australia | Melbourne Health Human Research Ethics CommitteeOffice for Research,Royal Melbourne Hospital (City Campus)Level 2, South West, 300 Grattan StreetParkville, Victoria 3050Australia | HREC/18/MH/3 |
| Belgium | Commissie voor Medische Ethiek ZNAKoningin Paola Kinderziekenhuis (P4-Route 34)Lindendreef 1Antwerpen, AntwerpenBelgium2020 | 5098 |
| Italy | Comitato Etico di BergamoASST Papa Giovanni XXIIIPiazza Organizzazione Mondiale della Sanita, 1Bergamo, BGItaly24127 | 112/18 |
| Netherlands | Commissie Mensgebonden Onderzoek, regio Arnhem-NijmegenRadboud University Medical CenterHuispost 348Postbus 91016500 HB NijmegenNetherlands | 2018-4479 |
| Netherlands | Medisch-Ethische toetsingscommissieLeiden Den Haag Delft (METC LDD)Secretariaat METC-LDD Postzone P5-P Postbus 9600 2300 RC LeidenNetherlands | 2018-4479 |
| United States | University of Pennsylvania Institutional Review Board3600 Civic Center BoulevardPhiladelphia, PAUSA19104 | 20181141 |
| United States | Western Institutional Review Board1019 39th Ave. SE Suite 120Puyallup, WAUSA98374-2115 | 20181141 |
| United States | Yale University Human Research Protection Program25 Science Park, 3rd Floor150 Munson St, PO Box 208327New Haven, CTUSA06520-8327 | 20181141 |
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| United States | Ann & Robert H. Lurie Children’s Hospital of Chicago Institutional Review Board225E. Chicago Ave., MB# 205Chicago, ILUSA60611 | 20181141 |