Membranoproliferative glomerulonephritis (MPGN) is a chronic progressive renal disease that is diagnosed on the basis of renal pathologic features. MPGN is subdivided into types I, II, and III on the basis of histologic, immunofluorescence staining, and complement profile differences. All types are characterized by mesangial cell proliferation and capillary wall thickening. Type I is distinguished by subendothelial immune complex deposition and activation of the classic pathway of complement activation. Type II is distinguished by dense deposits within the glomerular basement membrane and type III by deposits in the subepithelial and subendothelial areas. Both types II and III demonstrate activation of the alternative complement pathway. Familial forms of MPGN types I and II have been described, with autosomal dominant and recessive modes of inheritance. To date, this is the only reported family with inherited type III disease; most reported cases are sporadic, with no known family history of MPGN (1).

The epidemiologic features of MPGN type III are poorly understood. However, it is known to be a rare form of glomerulonephritis, accounting for <1% of glomerulonephritis diagnoses in registry data (2). It typically presents with hematuria at an early age, and it progresses to end-stage renal disease by 10 yr after presentation in approximately 50% of cases. Treatment of the condition with steroids and anticoagulants yields variable results (3). Recurrence after transplantation is rare (1,4). Histologic assessments reveal mesangial proliferation and capillary wall thickening resulting from mesangial cell interposition. Electron microscopy reveals subendothelial, mesangial, and subepithelial deposits (5); glomerular basement membrane lamellation has also been described (6). The pathogenesis of the condition is unknown, although West and McAdams (7) proposed that the condition might be the result of perturbation of complement by a slow-acting nephritic factor.

The family described here is unique. There are eight affected members in four generations, suggesting autosomal dominant inheritance. This is the only reported family with an inherited form of MPGN type III. To evaluate the disease in this family, a genome-wide scan was performed with a panel of 402 polymorphic microsatellite markers, defining a grid with an average resolution of 10 cM (centimorgans). Significant evidence for linkage was observed on chromosome 1q31–32, with a maximal logarithm of the odds score of 3.86 at \( \theta = 0.00 \) for microsatellite marker GATA135F02. Recombination events among affected individuals, as detected by haplotype analysis, established a 22-cM minimal candidate region flanked by markers D1S3470 and GATA124F08. The data provide evidence for a gene for familial MPGN on chromosome 1q.
formed consent was obtained from all participants before the start of the study. Evaluation of each individual included the recording of a comprehensive medical history, BP measurements, urinalysis, analysis of a 24-h urine sample for protein levels, and measurement of serum albumin levels, if possible. Because complement is sometimes activated in the sporadic form of this disease, complement studies (C3 and C4) were performed for all available family members considered to be affected or of unknown status. To reduce the possibility of phenocopies, all affected individuals were tested for hepatitis B and hepatitis C and were screened for systemic lupus erythematosus with anti-nuclear antibody. Blood samples were also obtained for DNA extraction. Family members were considered to be affected if they had renal biopsy-proven MPGN type III (in the absence of any causes of secondary disease), were undergoing dialysis, had undergone renal transplantation, or exhibited ≥3+ proteinuria and/or ≥3+ hematuria in qualitative urinalyses (or ≥300 mg protein/24 h) on two occasions. Individuals were categorized as being of unknown status if they exhibited urinary abnormalities less than those defined above and were categorized as being unaffected if they exhibited no detectable urinary abnormalities in qualitative urinalyses or were unrelated married-in spouses.

**DNA Isolation and Genotyping**

Genomic DNA was extracted from whole blood by using a phenol/ethanol extraction protocol. Fluorescence genotyping was performed as described previously (10), with 143 prelabeled multiplex primer sets, comprising 402 microsatellite markers and providing an average grid of 10 cM across the genome. A Hitachi FMBIOII Multiview fluorescent image scanner (MiraiBio Inc., Alameda, CA) was used for detection; BioImage software (RMLuton Inc., Jackson, MI) was used to analyze images, and data were entered into the PEDIGENE database management system (11).

**Analyses**

Two-point and multipoint logarithm of the odds (LOD) scores were calculated by using the VITESSE statistical program (12). Two autosomal dominant models were analyzed, i.e., (1) a "full pedigree" model, with 99.5% penetrance and a 0.5% phenocopy rate, and (2) an "affecteds-only" model, in which only affected individuals contributed to the LOD score; information from other pedigree members was used only to establish linkage phase. For each model, a disease allele frequency of 0.001 was assumed. Significant evidence in favor of linkage was declared when the LOD values at any recombination were ≥3. One-unit support intervals for the maximal-likelihood estimate of θ were calculated from two-point LOD scores by means of the one-unit-down method (13). Marker allele frequencies were calculated by using 100 chromosomes from unrelated Caucasian subjects (http://www.chg.mc.duke.edu/index.html). Map distances for the marker loci were obtained from published data (http://research.marshfieldclinic.org/genetics/). The maximal attainable LOD scores for the pedigree, using the full pedigree and affecteds-only models, were calculated via computer simulation with the SIMLINK 4.1 program (14). With the assumption of a four-allele system with frequencies of 0.4, 0.3, 0.2, and 0.1, simulations used a marker heterozygosity of 0.7 and 5% recombination with the disease locus. Haplotype analysis was performed as described previously (15), to identify critical recombination events. The analysis was performed via visual inspection and was confirmed by using SIMWALK software (16). A candidate interval was considered excluded when two affected individuals within the pedigree inherited different haplotypes.

**Results**

**Family Data**

Family data have been described previously (1) and are presented in Figure 1. Briefly, this is a four-generation, 51-

Figure 1. Autosomal dominant family with membroproliferative glomerulonephritis type III. The family is a four-generation, 51-member kindred from southern Ireland. Ages (in years) are indicated below the generation:individual numbers. Genders have been concealed for privacy. There was male-to-male transmission of the disease.
member kindred from southern Ireland. A total of 39 blood
samples were obtained for DNA extraction, including samples
from eight affected, three unknown, and 28 unaffected indi-
viduals. Individuals who were not available for examination
were considered to be of unknown status. Brief clinical de-
scriptions are provided in Table 1. The mean age at the time
of diagnosis of renal disease was 27.3 yr (range, 4 to 51 yr). Five
of the eight affected individuals had biopsy-proven MPGN
type III. For individual III:5, MPGN (without subclassification)
had been diagnosed via light microscopy 25 yr earlier; elec-
tron microscopic evaluation was not performed. There was
no evidence of hepatitis B, hepatitis C, or systemic lupus
erthematous for any of the affected individuals. Two of the
affected individuals received renal transplants. One affected
individual developed recurrence of the disease in the allograft
and subsequently underwent a second transplant. Screening of
the family revealed one new case, which was subsequently
confirmed via renal biopsy, and two additional family mem-
bers who were probably affected. Hypocomplementemia was
observed only transiently in the index case at the time of
presentation and was not noted for other family members who
underwent complement studies.

**Linkage Analysis**

Power calculations established that the maximal attainable
LOD score for the full pedigree model was 6.50, with a mean
LOD score of 4.10. Simulation results for the affecteds-only
model indicated a maximal LOD score of 2.66 and a mean
LOD score of 4.10. Simulation results for the affecteds-only
model included a maximal LOD score of 6.50, with a mean
LOD score for the full pedigree model was 2.10 at marker GATA135F02. No other regions of the genome con-
tained LOD scores of >2.0 (Figure 2).

To further define the minimal candidate region, haplotypes
were constructed by using nine microsatellite markers, span-
ning the region from 202 cm to 240 cm on chromosome 1
(Figure 3). The recombinations observed among affected indi-
viduals established a minimal candidate interval of 22 cm,
between markers D1S3470 and GATA124F08. It is noteworthy
that one individual classified as unaffected demonstrated the
disease haplotype.

**Discussion**

In this study, we demonstrated linkage of familial MPGN
type III to chromosome 1q in a family with an autosomal
dominant form of the disease. The maximal LOD score ob-
tained was 3.86 at \( \theta = 0.00 \) for microsatellite marker
GATA135F02. Haplotype analysis revealed D1S3470 and
GATA124F08 as flanking markers, yielding a 22-cM minimal
candidate region.

MPGN type III has been noted for the insidious nature of the
condition, with up to 65% of cases being diagnosed after
chance discovery of hematuria and/or proteinuria (17), as well
as the wide range of ages at onset. In fact, the disease did not
present in two family members until they were >50 yr of age,
and four individuals were diagnosed during the screening pro-
cess. This variability highlights the difficulty of assessing
penetrance values and affection status in MPGN type III.
Inasmuch as data for this family suggest an autosomal domi-
nant inheritance, certain family members might exhibit non-
penetration, possessing the characteristic genotype but not the
phenotype. Interestingly, one 48-yr-old individual exhibits the
disease haplotype but was classified as unaffected on the basis
of urinalysis results. This person might be a presymptomatic
carrier, or the disease might be nonpenetrant in this individual.
The presence of this carrier might suggest that our original
date for 99.5% penetrance is too high. Eight of nine carriers
(88%) of the haplotype at this locus are affected, suggesting
that a lower or age-dependent penetrance model might be
appropriate for MPGN.

**Table 1. Clinical details for affected individuals**

<table>
<thead>
<tr>
<th>Individual Number</th>
<th>Age at Diagnosis (yr)</th>
<th>ESRD</th>
<th>Urinary Protein Level (g/24 h)</th>
<th>Hematuria</th>
<th>Serum Albumin Level (g/L)</th>
<th>C3</th>
<th>C4</th>
<th>Biopsy Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>III:5</td>
<td>25</td>
<td>Yes</td>
<td>&gt;3.0</td>
<td>+++</td>
<td>30</td>
<td>Normal</td>
<td>Normal</td>
<td>MPGN</td>
</tr>
<tr>
<td>III:8</td>
<td>31</td>
<td>No</td>
<td>3.06</td>
<td>+</td>
<td>34</td>
<td>Normal</td>
<td>Normal</td>
<td>MPGN III</td>
</tr>
<tr>
<td>III:13</td>
<td>51</td>
<td>No</td>
<td>++++</td>
<td>+++</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>IV:7</td>
<td>22</td>
<td>No</td>
<td>++++</td>
<td>Trace</td>
<td>40</td>
<td>Low</td>
<td>Normal</td>
<td>Insufficient</td>
</tr>
<tr>
<td>IV:13</td>
<td>28</td>
<td>No</td>
<td>3.77</td>
<td>+</td>
<td>26</td>
<td>Normal</td>
<td>Normal</td>
<td>MPGN III</td>
</tr>
<tr>
<td>IV:14</td>
<td>4</td>
<td>Yes</td>
<td>5.0</td>
<td>Negative</td>
<td>21</td>
<td>Low</td>
<td>Low normal</td>
<td>MPGN III</td>
</tr>
<tr>
<td>IV:15</td>
<td>21</td>
<td>No</td>
<td>3.13</td>
<td>+</td>
<td>28</td>
<td>Normal</td>
<td>Normal</td>
<td>MPGN III</td>
</tr>
<tr>
<td>IV:24</td>
<td>16</td>
<td>No</td>
<td>0.3</td>
<td>+++</td>
<td>40</td>
<td>Normal</td>
<td>Normal</td>
<td>MPGN III</td>
</tr>
</tbody>
</table>

*ESRD, end-stage renal disease; MPGN, membranoproliferative glomerulonephritis; ND, not done.
*Only qualitative urinalysis data available.
The course of the disease is uncertain, but up to 50% of patients are expected to develop end-stage renal disease by 10 yr after presentation (2). A comparison with MPGN type I has demonstrated that type III disease is more likely to exhibit deterioration in renal function despite treatment with steroids, more likely to demonstrate persisting urinary and complement abnormalities, and more likely to relapse. Nephrotic syndrome at presentation is also a poor prognostic indicator (18).

The pathogenesis of MPGN type III is poorly understood. Secondary forms of MPGN types I and II have been associated with infections, connective tissue disease, and neoplastic conditions (19). However, secondary forms of MPGN type III are not observed. Complement perturbation is usually noted for sporadic cases of the condition and is thought to be related to a slow-acting nphritc factor, which stabilizes a properdin-dependent C3-convertase (20). The deposits observed in renal biopsies seem to be temporally related to C3-convertase activity (21). Interestingly, the area of linkage described above encompasses the regulators of complement cluster on chromosome 1 (22). These genes code for a highly homologous group of soluble and membrane-associated proteins that regulate C3-convertase activity, including complement receptor-1 (CR-1), membrane cofactor protein, decay-accelerating factor, and factor H. Abnormalities in any of these proteins might cause excess circulating C3b, thus mimicking the action of convertase stabilization by a nephritic factor. West et al. (23) postulated that low CR-1 levels might increase the severity of glomerular disease in the presence of a nephritic factor, and glomerular CR-1 activity has been demonstrated to be absent in areas of complement deposition (24). Decay-accelerating factor is upregulated in the mesangium in renal disease and has been observed to be correlated with C3 deposition (25), which suggests that this factor might also play a role in protecting the kidney against the products of complement activation. Paramesangial subepithelial deposits are observed in MPGN types II and III and are thought to be related to excess circulating C3b and its breakdown products. Factor H abnormalities are also associated with excess C3b and paramesangial deposits, although the associated nephritis is usually mild and hypocomplementemia is usually severe (7). Factor H abnormalities have been observed in a variety of renal diseases, including familial hemolytic uremic syndrome (26), collagen III nephropathy (27), and atypical MPGN type II (28).

In summary, we have mapped a gene for MPGN to chromosome 1q31–32. This represents the first genetic locus established for MPGN type III and confirms a familial form of this disease.

**Acknowledgments**

We thank all family members for participating in this study. We thank the support staff of the Center for Human Genetics, Duke University Medical Center, for technical assistance. Grant support was provided in part by the Beaumont Foundation (to Dr. Conlon) and in part by the National Kidney Foundation (to Dr. Winn), with core support from the Duke University Medical Center Faculty Development Fund. To facilitate further work in this area, we have founded an International Collaboration to Investigate Genetic Forms of Membranoproliferative Glomerulonephritis, based at Beaumont Hospital (Dublin, Ireland). We welcome collaboration with other physicians who care for patients with sporadic or

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**Table 2. Full-pedigree two-point LOD score analysis**

<table>
<thead>
<tr>
<th>Location (cM)</th>
<th>Marker</th>
<th>Two-Point LOD Score at Recombination Fraction (θ) of Z Values at θ (1 – \text{Unit Support Interval for } θ)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.00 0.05 0.10 0.15 0.20 0.30 0.40 Z(\text{max})</td>
</tr>
<tr>
<td>192.05</td>
<td>D1S1589</td>
<td>-2.54 0.64 1.11 1.28 1.30 1.08 0.63 1.30 0.30 0.63</td>
</tr>
<tr>
<td>202.19</td>
<td>D1S518</td>
<td>2.28 2.49 2.45 2.31 2.09 1.49 0.74</td>
</tr>
<tr>
<td>204.51</td>
<td>D1S3470</td>
<td>-0.98 0.45 0.84 0.98 1.00 0.80 0.40</td>
</tr>
<tr>
<td>205.40</td>
<td>D1S1189</td>
<td>3.00 3.01 2.88 2.65 2.37 1.67 0.81</td>
</tr>
<tr>
<td>206.15</td>
<td>D1S2625</td>
<td>1.68 1.53 1.37 1.22 1.06 0.72 0.36</td>
</tr>
<tr>
<td>210.47</td>
<td>GATA135F02</td>
<td>3.86 3.73 3.47 3.15 2.78 1.92 0.93</td>
</tr>
<tr>
<td>212.44</td>
<td>D1S1660</td>
<td>2.57 2.36 2.15 1.93 1.69 1.20 0.64</td>
</tr>
<tr>
<td>218.46</td>
<td>D1S1678</td>
<td>0.11 0.09 0.08 0.06 0.04 0.02 0.01</td>
</tr>
<tr>
<td>226.16</td>
<td>GATA124F08</td>
<td>1.00 1.97 2.07 1.98 1.81 1.29 0.61</td>
</tr>
<tr>
<td>239.66</td>
<td>D1S549</td>
<td>-8.22 0.19 0.73 0.95 1.01 0.86 0.47</td>
</tr>
</tbody>
</table>

* LOD, logarithm of the odds; NA, not available.
familial forms of MPGN type I, II, or III. Please address correspondence to Dr. P. J. Conlon, Beaumont Hospital, Dublin 9, Ireland (E-mail: peter.conlon@beaumont.ie).

References


