Abstract. Membranoproliferative glomerulonephritis (MPGN) type III is a chronic progressive renal disease of unknown cause. The diagnosis is based on renal pathologic features (specifically immunofluorescence staining patterns and ultrastructural appearance). Mesangial cell proliferation and subendothelial and subepithelial deposits characterize the renal disease. Although the actual prevalence of this disease is not known, the disease is rare and usually sporadic. The clinical features of MPGN include the nephrotic syndrome and hematuria, with renal dysfunction occurring in approximately 50% of patients. Progression to end-stage renal disease is variable, and some patients exhibit stabilization or even improvement. Here is presented an Irish family in which 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-
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 individuals. Individuals who were not available for examination were considered to be of unknown status. Brief clinical descriptions 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; electron microscopic evaluation was not performed. There was no evidence of hepatitis B, hepatitis C, or systemic lupus erythematosus 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 members 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 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 contained LOD scores of >2.0 (Figure 2).

To further define the minimal candidate region, haplotypes were constructed by using nine microsatellite markers, spanning the region from 202 cM to 240 cM on chromosome 1 (Figure 3). The recombinations observed among affected individuals 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 obtained 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 process. 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 dominant inheritance, certain family members might exhibit non-penetrance, 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 estimate of 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>51</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 nephritic 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

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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</th>
<th>Z Values at θ</th>
<th>1 – Unit Support Interval for θ</th>
</tr>
</thead>
<tbody>
<tr>
<td>192.05</td>
<td>D1S1589</td>
<td>−2.54, 0.64, 1.11, 1.28, 1.30, 1.08, 0.63</td>
<td>1.30, 0.30</td>
<td>NA</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>
<td>2.49, 1.49</td>
<td>0.05 to 0.30</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>
<td>1.00, 0.00</td>
<td>NA</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>
<td>3.01, 2.01</td>
<td>0.05 to 0.24</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>
<td>1.68, 0.68</td>
<td>0.00 to 0.31</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>
<td>3.86, 2.86</td>
<td>0.00 to 0.19</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>
<td>2.57, 1.57</td>
<td>0.00 to 0.23</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>
<td>0.11, −0.89</td>
<td>NA</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>
<td>2.07, 1.07</td>
<td>0.10 to 0.34</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>
<td>1.01, 0.01</td>
<td>NA</td>
</tr>
</tbody>
</table>

a LOD, logarithm of the odds; NA, not available.

Figure 2. Two-point logarithm of the odds (LOD) score histogram. A histogram of all positive two-point LOD scores was generated for the entire genome. No LOD scores were >2.00 except in the region of linkage on chromosome 1.

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).

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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


Figure 3. Haplotype analysis. Recombinations observed among affected individuals established a minimal candidate interval of 22 cM, between markers D1S3470 and GATA124F08. Only affected individuals and spouses of affected individuals are presented, for clarity. Individual III:16 is unaffected but exhibits the affected haplotype (see text). Alleles in parentheses are inferred, and alleles surrounded by question marks have unknown phase.


