A locus for inherited focal segmental glomerulosclerosis maps to chromosome 19q13
Rapid Communication

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A locus for inherited focal segmental glomerulosclerosis maps to chromosome 19q13. *Rapid Communication.* We performed a genome-wide linkage analysis search for a genetic locus responsible for kidney dysfunction in a large family. This inherited condition, characterized by proteinuria, progressive renal insufficiency, and focal segmental glomerulosclerosis, follows autosomal dominant inheritance. We show with a high degree of certainty (maximum 2-point lod score 12.28) that the gene responsible for this condition is located on chromosome 19q13.

Focal segmental glomerulosclerosis (FSGS) represents a pathologic finding in several renal disorders characterized by proteinuria and progressive decline in renal function. Focal segmental glomerulosclerosis occurs in primary (idiopathic) and secondary forms. Observers are increasingly aware of possible genetic contributions to the development of FSGS, and it has been reported in multiple families and sibling pairs [1–4]. Both dominant and recessive forms may exist [1]. Investigators have also noted possible associations between particular HLA alleles and FSGS [5, 6]. FSGS often occurs secondary to other conditions, including conditions with a clear or suspected genetic component such as obesity, Alport's syndrome, and oligomeganephronia [7], raising the possibility that genes involved in inherited forms of FSGS may also be involved in more common causes of FSGS-like secondary renal disease.

As an initial step towards identification of genes involved in the pathogenesis of inherited FSGS, we performed linkage analysis to map the disease gene in a large family with this condition. We show that the FSGS gene in a large family with autosomal dominant inheritance of this condition maps to a region of chromosome 19q13.

METHODS

Clinical studies

The study family, previously identified by B.J.M. and K.E.C. [8], was clinically re-evaluated. At the time of evaluation, a peripheral blood sample was obtained for establishment of lymphoblastoid cell lines, DNA extraction, and serum creatinine measurements. Genotypes were ascertained without knowledge of clinical status. Serum creatinine, urine protein, and urine creatinine were measured by the Brigham and Women's Hospital clinical laboratory. Measurement of urine microalbumin was performed by Nichols Laboratories. Studies were performed after obtaining informed consent in accordance with a protocol approved by the Human Research Committee of the Brigham and Women's Hospital.

DNA analyses

Genomic DNA was extracted from peripheral blood using the SDS-proteinase K method [9]. For the initial genome screen, polymorphic markers were chosen from the Weber 6A set [10]. Short tandem repeat markers within these loci were analyzed by PCR with one end-labeled primer and gel electrophoresis [9]. Other markers used in the chromosome 19q1 region are from the Genethon mapping panel [11], or, in the case of *D19S608, D19S609*, and *D19S610*, from reference [12]. Genotyping was performed precisely as described previously [13] without knowledge of clinical status.

Statistical analyses

Two-point linkage analyses were performed using the MLINK computer program [14, 15]. Logarithm of odds (lod) scores were calculated assuming a disease penetrance of 0.95. Allele frequencies were assumed to be $1/N$, where N is the number of alleles for a given marker.

RESULTS

Clinical evaluations

A detailed clinical description of this family has been published previously [8]. We re-evaluated all available family members by
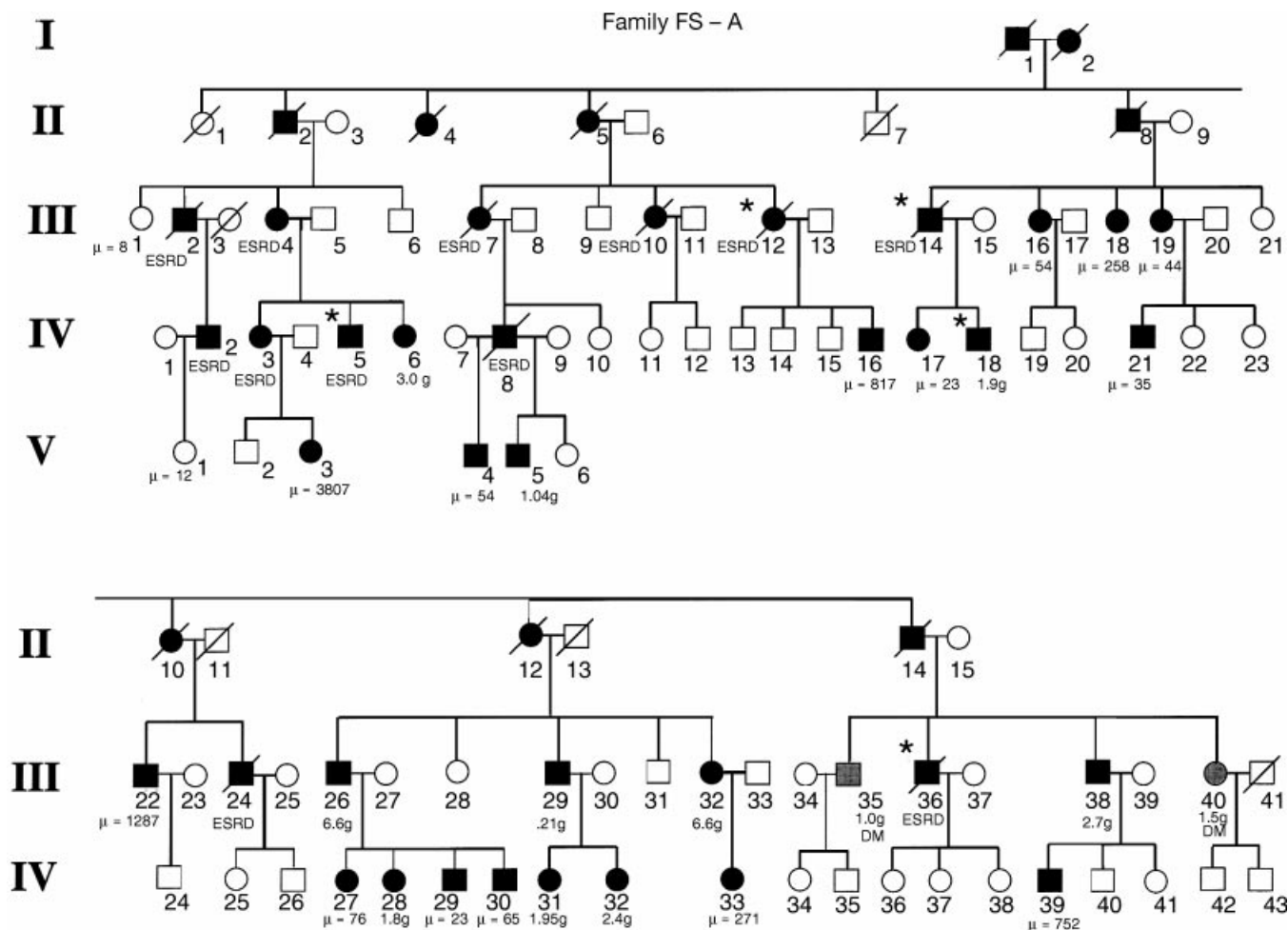
Fig. 1. The pedigree of a large kindred with inherited nephropathy. Individuals with end-stage renal disease are labeled ESRD. Below the symbols of individuals classified as affected are urine protein measurements, either urine microalbumin (expressed as mg microalbumin per gram creatinine) indicated by "μ=" or, total protein in grams per day as estimated by spot urine/creatinine ratio. Asterisks indicate individuals who have undergone renal biopsy. Urine microalbumin values are also indicated for individuals III-1, and V-1, both of whom carry the disease allele but neither of whom has increased microalbumin excretion.

measuring serum creatinine, urine protein, and urine microalbumin excretion. Twenty-four-hour urine protein excretion was estimated from a spot urine protein to creatinine ratio [16]. The pedigree appears in Figure 1 and the chromosome 19 ideogram is shown in Figure 2. An individual was considered affected if he/she had (*1*) biopsy evidence of FSGS; (*2*) end-stage renal disease without another cause; or (*3*) elevated urine microalbumin excretion without another cause (microalbumin > 20 mg/g creatinine). An individual was considered indeterminate if he/she had elevated urine albumin excretion in a random urine sample but another possible cause (such as diabetes). An individual was considered unaffected if he/she had no microalbuminuria. Individuals under the age of 18 were not included in this analysis.

The renal biopsy from individual IV-18 is shown in Figure 3. Biopsies from individuals III-14 and III-36 were originally read as diffuse glomerulosclerosis and membranous nephropathy, respectively. However, review of biopsy III-36 showed glomeruli with focal and segmental sclerosing lesions and no subepithelial or intramembranous electron-dense deposits were seen by electron microscopy, suggesting that this individual in fact had FSGS. Biopsy III-14 was not available for review.

Variable expression of the gene defect in this family was demonstrated by the fact that, whereas some family members developed ESRD by the fourth decade or had severe proteinuria, others demonstrated only mild microalbuminuria, including one individual whose two daughters were severely affected (individual III-29).

Genetic analysis

All of the family members in this study were genotyped using 160 polymorphic genetic markers from the CHLC markers (Weber set 6) spaced throughout the genome [10]. Sufficient information was typically obtained to exclude linkage within 10 to 20 centiMorgans (cM) flanking each locus (lod score < −2). Analysis of marker *D19S589* suggested that the disease gene might be located on chromosome 19. The maximum two point lod score with *D19S589* was 1.63 at $\theta = 0.25$. To clarify the location of the disease locus, analyses were performed with other loci from
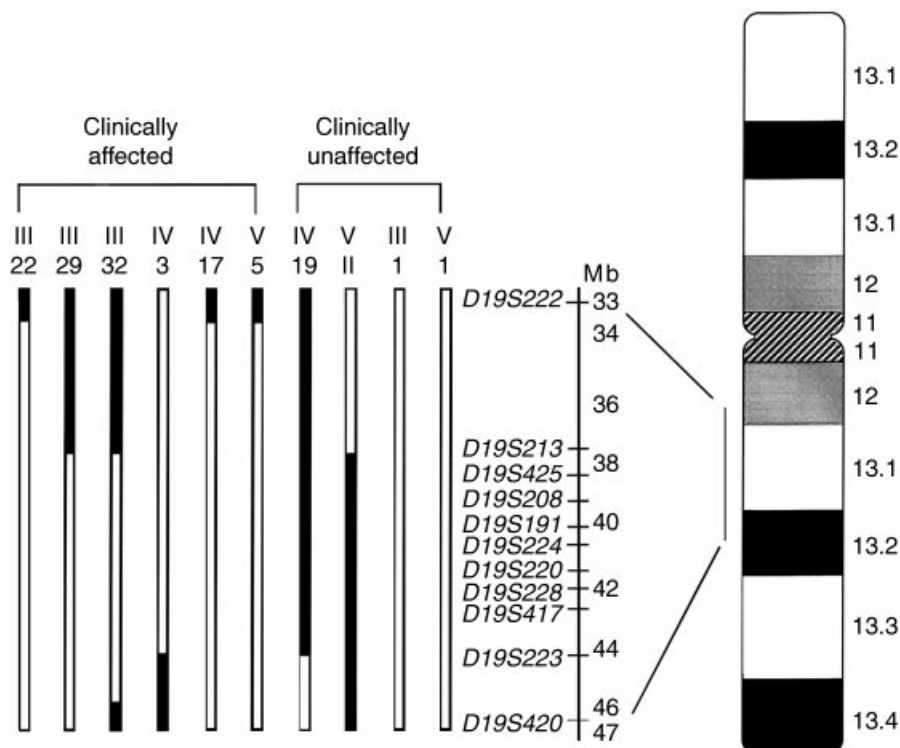
Fig. 2. Chromosome 19 ideogram. Shown is a linear representation of recombination events occurring in the study family. Open bars represent the disease-associated haplotype. Closed bars represent a non disease-associated haplotype. Individuals III-1 and V-1 are clinically unaffected but carry the disease allele; these individuals are presumed to be examples of non-penetrance. Recombination events in affected individuals place the disease gene interval within the region flanked by loci *D19S213* and *D19S223*. The physical distances indicated to the left of the ideogram are in accordance with the Lawrence Livermore National Laboratory chromosome 19 data.

Table 1. Pairwise lod scores reflecting linkage between chromosome 19 loci and a focal segmental glomerulosclerosis locus

Locus	Recombination fraction (θ)							
	0.00	0.01	0.05	0.10	0.15	0.20	0.30	0.40
D19S414	−7.44	−4.14	−1.51	−0.11	0.63	1.01	1.11	0.68
D19S714	−2.59	−2.29	−0.81	0.62	1.53	1.99	2.01	1.25
D19S222	0.97	3.04	4.28	4.61	4.52	4.20	3.12	1.62
D19S213	−3.09	−1.79	−0.64	0.59	1.21	1.47	1.49	0.75
D19S425	7.61	8.70	9.19	8.87	8.21	7.34	5.17	2.54
D19S208	8.54	8.53	8.22	7.60	6.83	5.98	4.04	1.91
D19S609	6.12	6.07	5.76	5.24	4.66	4.03	2.66	1.16
D19S610	7.38	7.31	6.94	6.32	5.61	4.85	3.24	1.53
D19S608	8.42	10.63	10.94	10.43	9.59	8.55	6.04	3.05
D19S191	12.28	12.19	11.66	10.73	9.64	8.43	5.73	2.77
D19S224	2.52	4.98	6.04	6.15	5.83	5.28	3.76	1.82
D19S220	7.75	7.70	7.34	6.77	6.10	5.35	3.68	1.82
D19S228	3.61	6.30	6.43	5.99	5.40	4.73	3.23	1.56
D19S417	7.17	7.11	6.76	6.20	5.55	4.85	3.28	1.55
D19S223	3.99	9.59	10.06	9.69	8.96	8.02	5.71	2.90
D19S420	0.34	6.64	8.55	8.77	8.37	7.65	5.59	2.91
D19S589	−13.79	−4.07	−1.09	0.53	1.30	1.61	1.45	0.78

Lod scores were calculated at various recombination fractions as described in the **Methods** section.

chromosome 19q (Table 1). Among affected individuals, no recombinants were seen between kidney dysfunction and all loci examined in the region between *D19S213* and *D19S417*. Two point lod scores between disease and these loci in this region were all highly significant (Table 1). The maximum two point lod score was 12.28 at *D19S191* at genetic distance $\theta = 0.0$, equivalent to a less than 1 in 10^{12} chance that the cosegregation of *D19S191*

alleles and nephropathy was a chance association. Although these calculations were performed using allele frequencies of $1/N$, where N is the number of alleles, the result was insensitive to changes in allele frequencies. We also calculated lod scores excluding the individuals whose diagnoses are most likely to be in error (because of either non-penetrance or proteinuria of another cause). If all clinically unaffected individuals are excluded, then
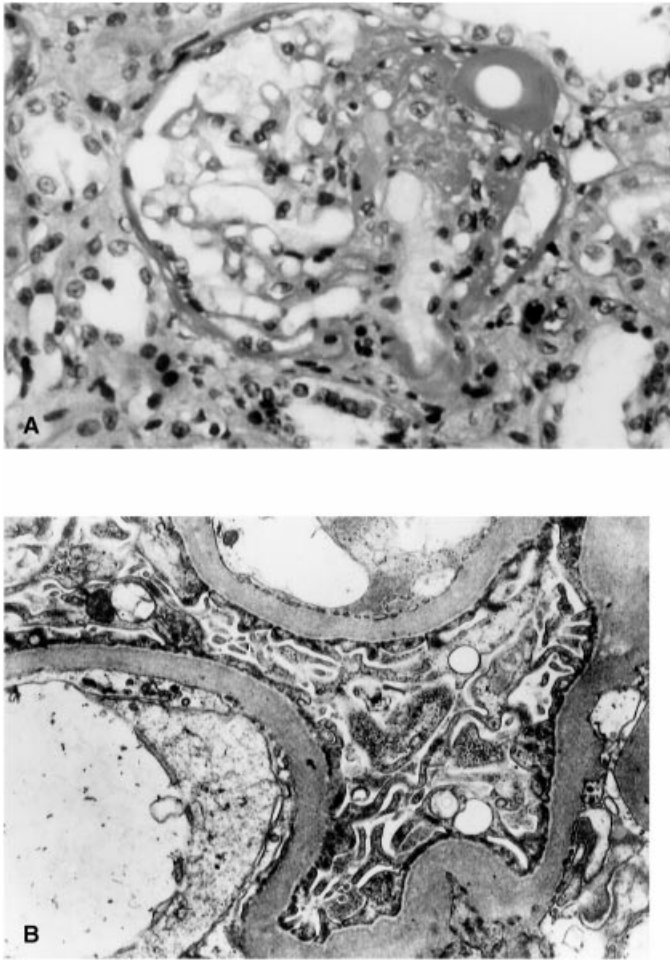
Fig. 3. (*A*) **Glomerulus from patient IV-18 showing perihilar segmental sclerosis, capillary collapse, and hyalinosis.** (*B.*) Electron microscopy shows a mild increase in mesangial matrix and areas of epithelial foot process effacement. There is no evidence of an immune complex disorder or a primary basement membrane defect.

the lod score at $\theta = 0.0$ is 7.44; if those individuals with urine albumin excretion less than 500 mg/g creatinine are excluded as well, the lod score at $\theta = 0.0$ is 5.41.

Haplotype analysis defined recombinant events between *D19S213* and *D19S425* in two affected individuals, between *D19S222* and *D19S213* in two affected individuals, between *D19S223* and *D19S420* in one affected individual, and between *D19S420* and *D19S223* in one affected individual. Recombinant events in two clinically unaffected individuals were also observed (Fig. 2). These recombinant events suggest that the gene defect responsible for microalbuminuria and FSGS in this family is located in the 7 Mb region between *D19S223* and *D19S213*.

DISCUSSION

We have presented evidence that the gene for renal disease in a large pedigree is located within an approximately 7 centiMorgan region on chromosome 19. Of particular interest is the fact that this region includes the 1Mb region harboring the gene for congenital nephrotic syndrome of the Finnish type (CNF) [17, 18]. Thus, it is a plausible hypothesis that these two very distinct forms

of nephrotic syndrome and kidney failure may represent different mutations in the same gene. The region of chromosome 19 we have defined is syntenic to a portion of mouse chromosome 7 [19]. We are unaware of any mouse mutants with FSGS-like phenotypes that are the result of genetic defects in this region.

Recombination events suggest that this FSGS region is flanked by loci *D19S223* and *D19S213*. It is possible that this precise location is incorrect if the diagnoses of some individuals with these critical crossovers are incorrect if, for example, the affected individuals have proteinuria from another cause, or the unaffected individuals have the disease allele but are non-penetrant. We feel this is unlikely, however, as these conclusions are based on crossovers observed in several individuals. The extremely high lod scores that persist even when all those individuals with the least certain diagnoses are excluded from the analysis make it certain that the disease gene is located in or near this region. Individuals III-1 and V-1, who are both clinically unaffected, carry the disease-associated haplotype over the entire region of chromosome 19 indicated, and they are presumed to be examples of non-penetrance.

It is important to emphasize the great variability in the phenotypic expression of the disease gene. Although we have termed the pathologic condition in this family inherited FSGS, this may be misleading. Some family members carrying the FSGS allele exhibit end-stage renal failure at a relatively young age, whereas others show only microalbuminuria, or in the case of individuals III-1 and V-1, no abnormalities at all. This suggests that there are other factors involved in the pathogenesis of renal failure in these patients. These factors may be genetic, environmental, or both.

Focal segmental glomerulosclerosis is seen not typically observed as an inherited entity; much more frequently it presents as an idiopathic lesion or as a consequence of other underlying disease such as intravenous drug use, vesico-ureteral reflux, or HIV infection. This raises the possibility that this FSGS gene may also be involved in the susceptibility to so-called secondary renal disease. It should now be possible for investigators studying genes involved in renal failure progression (for example, from diabetes) to test this hypothesis utilizing genetic markers in this region. It remains to be determined in other families with inherited FSGS whether the disease gene maps to this same locus, or, alternatively, if familial FSGS is genetically heterogeneous. Patients with idiopathic (primary) FSGS may also have a genetic disease: some fraction of patients with primary FSGS may in fact have sporadic mutations in this (or another) FSGS gene. Testing this hypothesis will await identification of the FSGS gene(s).

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