

LOCALIZATION OF A GENE FOR FAMILIAL JUVENILE HYPERURICEMIC NEPHROPATHY CAUSING UNDEREXCRETION-TYPE GOUT TO 16p12 BY GENOME-WIDE LINKAGE ANALYSIS OF A LARGE FAMILY

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Objective. Familial juvenile hyperuricemic nephropathy (FJHN, MIM 162000) is an autosomal-dominant disease characterized by underexcretion-type hyperuricemia, gout, and chronic renal failure. No loci responsible for this disease or any underexcretion-type hyperuricemia/gout have ever been identified. The aim of the study was to localize a gene responsible for FJHN by linkage analysis.

Methods. A single large family with at least 20 affected members was analyzed. DNA was obtained from 13 affected and 18 non-affected members after lymphoblastoid cell lines were established. Initially, polymorphic data were obtained for 343 microsatellite loci covering all chromosomes except the X chromosome. Parametric linkage analysis was performed using the obtained data with LINKAGE package software.

Results. Following a genome-wide search using a set of highly polymorphic microsatellite markers, initial evidence for linkage was obtained for a marker on chromosome 16p. We subsequently genotyped the same subjects for 12 additional markers spanning ~30 cM on the short arm of chromosome 16. We obtained a maximum 2-point logarithm of odds (LOD) score of 6.04 at $\theta = 0$ with the marker *D16S401*; multipoint linkage

analysis yielded a maximum LOD score of 6.14 with markers *D16S401* and *D16S3113*, and established a minimum candidate interval of ~9 cM.

Conclusion. A gene for FJHN was localized to a candidate interval of ~9 cM at 16p12. These findings will be useful for the presymptomatic diagnosis of FJHN in some families and for testing genetic heterogeneity of FJHN in general.

Gout is a common disease caused by hyperuricemia. Hyperuricemia is caused by either overproduction or underexcretion of urate. Although some genetic causes of overproduction of urate have been discovered, the genetic causes of underexcretion have not been found (1). In general, a majority of hyperuricemic patients exhibit underexcretion rather than overproduction (1). Although many cases appear to be sporadic, some cases are clearly inherited in an autosomal-dominant manner with a high penetrance (2). Familial juvenile hyperuricemic nephropathy (FJHN: MIM 162000) is one such entity characterized by underexcretion-type hyperuricemia, gout, and chronic renal failure (2-9). Affected family members usually develop hyperuricemia and gout after adolescence, and renal function deteriorates gradually. The impairment of urate excretion starts before puberty. In the present investigation, we performed a genome-wide linkage analysis of a large family with FJHN and localized a responsible gene to 16p12.

SUBJECTS AND METHODS

Subjects. We collected samples from only one family, whose features have been reported previously (6). The pedigree of this family is illustrated in Figure 1. Informed consent was obtained from all subjects. Mononuclear cells were separated from heparinized blood obtained from each individual.

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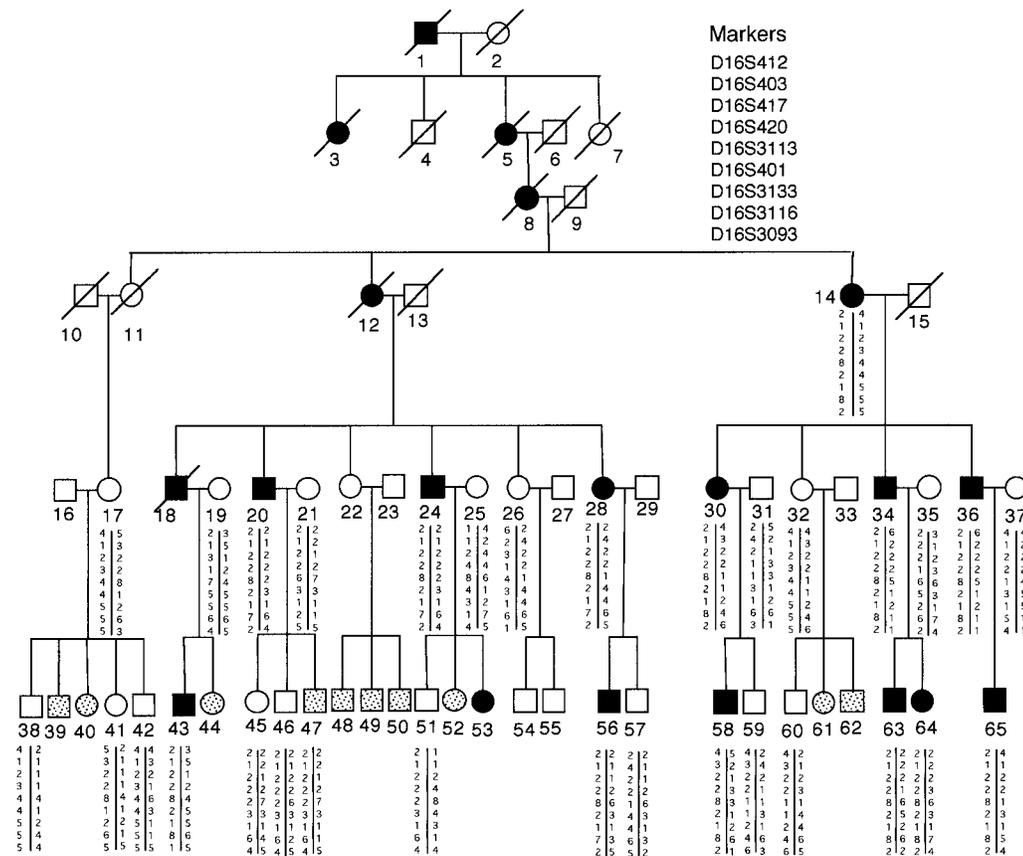


Figure 1. Pedigree of a large family with familial juvenile hyperuricemic nephropathy. Affected individuals are shown with closed squares and circles while unaffected individuals are shown with open squares and circles. Affected status was unclear in individuals with dotted squares and circles. Genotypes for the serial markers on chromosome 16 are shown with allele numbers below each individual from whom a DNA sample was obtained. The markers are *D16S412*, *D16S403*, *D16S417*, *D16S420*, *D16S3113*, *D16S401*, *D16S3133*, *D16S3116*, and *D16S3093* (from top to bottom).

Lymphoblastoid cell lines were established by transformation using Epstein-Barr virus. DNA was extracted from the cells by the phenol extraction method. An individual was judged to be affected if he or she had either definitive severe renal failure or impaired urate excretion as indicated by a fractional urate clearance of <5.5% for men and <7.8% for women. These thresholds were established based on the normal fractional urate clearance values for Japanese (mean ± SD 8.3 ± 1.4% for men versus 12.6 ± 2.4% for women). Previous studies (6) as well as subsequent extensive studies by investigators at our institution have clarified that these thresholds clearly separate unaffected and affected individuals age ≥20 years in the present family. An individual was judged as having definitive severe renal failure only if he or she had undergone renal transplantation, was undergoing hemodialysis, or showed a serum creatinine concentration of >1.5 mg/dl. All the individuals with severe renal failure had experienced either gouty attacks or hyperuricemia.

Genotype analysis. Primers in a linkage mapping set (the first version) for the detection of microsatellite polymorphisms at 358 loci were purchased from Perkin-Elmer Biosystems (Norwalk, CT). Primers for the fine mapping were

designed according to the information from the Internet sites referred to below. The primers were synthesized by Perkin-Elmer Biosystems. Polymerase chain reactions (PCR) were performed in a 5.0-μl reaction mixture containing 20 ng template DNA, 1.65 units *Taq* polymerase (AmpliTaq Gold; Perkin-Elmer Biosystems), 5 μM primer pairs, 1.5–2.5 mM MgCl₂, and 2.5 mM dNTPs in 1× PCR buffer (Perkin-Elmer Biosystems), which was processed in an ABI Prism 877 Labstation (Perkin-Elmer Biosystems). The parameters for PCR were 30 cycles of 15 seconds at 94°C, 15 seconds at 55°C, and 30 seconds at 72°C, with an extra extension reaction at 72°C for 30 minutes. The mixed PCR products were applied to an ABI 377 Prism system, the data were stored as GeneScan files, and the results were analyzed by Genotyper v2.1 (Perkin-Elmer Biosystems).

Two-point linkage analysis. Linkage analyses were performed using the programs UNKNOWN and MLINK contained in the LINKAGE package (10).

The mode of inheritance was set as autosomal dominant, since it was clear from the data in Figure 1. The penetrance of this disease seems to be almost 100%, at least among the adults in this family. Thus, the penetrance was set at

Table 1. Logarithm of odds scores obtained by 2-point analysis

| Locus* | Distance, cM | θ | | | | | | |
|-----------------|--------------|-----------|-------|-------|-------|-------|-------|-------|
| | | 0.000 | 0.025 | 0.050 | 0.100 | 0.200 | 0.300 | 0.400 |
| <i>D16S405</i> | | –infinity | –3.30 | –2.09 | –1.00 | –0.29 | –0.19 | –0.18 |
| <i>D16S501</i> | 5.92 | –infinity | –0.70 | 0.04 | 0.63 | 0.88 | 0.70 | 0.33 |
| <i>D16S499</i> | 0.00 | –infinity | 1.77 | 1.67 | 1.46 | 1.05 | 0.66 | 0.30 |
| <i>D16S410</i> | 1.22 | –infinity | –0.78 | –0.32 | 0.06 | 0.23 | 0.16 | 0.05 |
| <i>D16S3045</i> | 5.21 | –infinity | –4.76 | –3.49 | –2.16 | –0.91 | –0.34 | –0.08 |
| <i>D16S412</i> | 2.16 | –infinity | 1.97 | 2.11 | 2.10 | 1.72 | 1.16 | 0.51 |
| <i>D16S403</i> | 1.08 | –infinity | 0.75 | 0.94 | 1.01 | 0.82 | 0.49 | 0.17 |
| <i>D16S417</i> | 0.00 | 0.49 | 0.46 | 0.42 | 0.36 | 0.23 | 0.13 | 0.04 |
| <i>D16S420</i> | 0.56 | 1.81 | 1.71 | 1.62 | 1.42 | 1.00 | 0.58 | 0.19 |
| <i>D16S3113</i> | 1.11 | 5.73 | 5.51 | 5.29 | 4.82 | 3.78 | 2.61 | 1.28 |
| <i>D16S401</i> | 1.38 | 6.04 | 5.79 | 5.54 | 5.01 | 3.88 | 2.63 | 1.25 |
| <i>D16S3133</i> | 0.00 | 2.29 | 2.21 | 2.11 | 1.91 | 1.43 | 0.91 | 0.38 |
| <i>D16S3116</i> | 3.66 | –infinity | –2.07 | –0.76 | 0.34 | 0.97 | 0.89 | 0.48 |
| <i>D16S3093</i> | 1.66 | –infinity | 2.96 | 3.17 | 3.19 | 2.70 | 1.89 | 0.90 |
| <i>D16S409</i> | 6.20 | –infinity | 0.28 | 0.74 | 1.06 | 1.07 | 0.79 | 0.36 |
| <i>D16S411</i> | 1.22 | –infinity | –1.31 | –0.53 | 0.11 | 0.48 | 0.46 | 0.28 |

* Markers are listed in order from telomere (top) to centromere (bottom).

0.95 or 1. The frequencies of marker alleles were calculated from the data for a separate Japanese population ($n = 60$), but when the frequency of an allele related to this family was <0.02 , it was set at 0.02, since underestimation of an allele frequency sometimes results in overestimation of the logarithm of odds (LOD) scores. The frequency of the disease gene was assumed to be 0.01 in the general population. After finding high LOD scores, we performed extensive analysis on such loci by changing various parameters, i.e., the penetrance and the frequencies of marker and disease alleles. We found that the high LOD scores obtained were stable, and the changes in them were minimal.

Multipoint linkage analysis. Multipoint analyses were performed using the LINKMAP program (10). Mapping information was obtained from the database (GeneMap99, Genetic Maps at Marshfield Medical Research Foundation, http://www.marshmed.org/genetics/Map_Markers/data/Maps_inquiry_date_7/2/99). However, since the calculation time was too long when using the programs in the original LINKAGE package, the LINKMAP and UNKNOWN programs were replaced by those in the FASTLINK package, version 3.0P (11).

RESULTS

A genome-wide linkage analysis was undertaken to determine the locus of the gene causing FJHN in a single large family (Figure 1). Since all the samples used were from a single family and the disease is inherited in an autosomal-dominant manner, the responsible genetic locus and the responsible mutation should be the same. Such homogeneity is beneficial for the present study, since one can avoid the heterogeneity in expression that is seen between families and that may cause ambiguity in judging the affected status of individuals. In fact,

McBride et al stated that there was considerable heterogeneity in expression of FJHN between families (12).

We used the DNA from 18 men and 14 women. Of these, 9 men and 4 women were affected, 8 men and 10 women were unaffected, and the phenotype was interpreted as uncertain in 1 man because his age was <20 years. We initially screened with 343 microsatellite markers scattered on all the chromosomes except for the X chromosome, and the data were analyzed by MLINK with the assumption that the disease was inherited in an autosomal-dominant mode. A 2-point analysis by setting the penetrance at 0.95 showed that the LOD score was the highest (5.60) for *D16S401* at $\theta = 0$. All the other markers had maximum LOD scores of <1.7 (data are not presented here, but the genotype data for all chromosomes [except for the X chromosome] will be sent on request). Finally, we examined 12 additional markers known to map in this region.

Table 1 shows the 2-point linkage analysis data obtained using 16 markers in this region. This time, the mode of inheritance was assumed to be dominant, and the penetrance was set at 1. The single family yielded a maximum 2-point LOD score of 6.04 at $\theta = 0$ with *D16S401*, and a LOD score of 5.73 at $\theta = 0$ with the adjacent marker *D16S3113* (Table 1). When the penetrance was set at 0.95, the LOD scores at $\theta = 0$ were 5.76 and 5.45 for *D16S401* and *D16S3113*, respectively. Multipoint analysis incorporating the data from 3 marker loci simultaneously in this region of chromosome 16 yielded a maximum LOD score of 6.14 at *D16S401* and

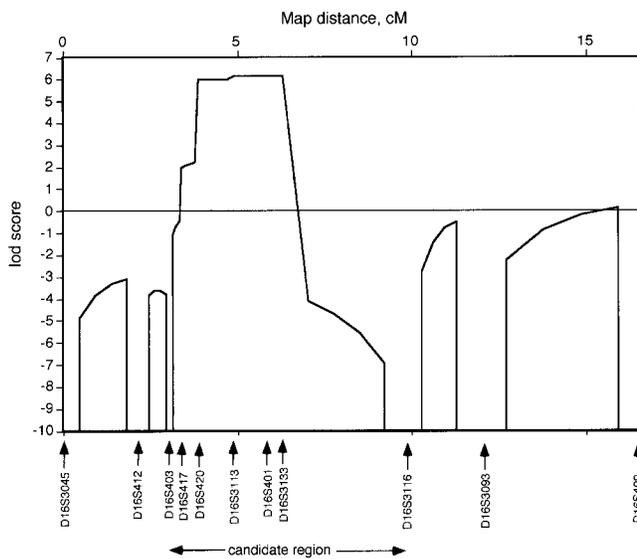


Figure 2. Results of multipoint analysis using the data from 3-marker loci and the affection status locus simultaneously. Penetrances for a homozygote without the disease allele, a heterozygote, and a homozygote with the disease allele were set at 0, 1, and 1, respectively. Markers are ordered with the telomere at the left and the centromere at the right. The candidate region is indicated. lod = logarithm of odds.

D16S3113 (Figure 2). Figure 1 shows the data of genotypes (by allele numbers) for 9 serial markers from *D16S412* (top) to *D16S3093* (bottom). The data indicate that a dominant haplotype 2-2-8-2-1 (*D16S417-D16S420-D16S3113-D16S401-D16S3133*) is shared by all the affected individuals in this family. This is in accord with the data in Table 1 indicating that the LOD scores for the 5 markers were not $-\infty$ under the conditions of $\theta = 0$ and penetrance = 1. This means that there was no evidence of recombination between the disease locus and any of the 5 marker loci.

We therefore propose the interval between *D16S403* and *D16S3116*, which includes the above 5 markers, as the region to search for the candidate disease-responsible gene (Figure 2). Multipoint analysis incorporating the data from 4 marker loci was not possible, since the calculation time required was too long.

DISCUSSION

Our data demonstrate that a gene for FJHN resides on chromosome 16 (at 16p12), most likely within the 9-cM interval flanked by the loci *D16S403* and *D16S3116* (Figure 2). This is the first genetic map

position for familial gout and hyperuricemia of the underexcretion type (renal gout). Regarding the overproduction-type gout, the loci for both hypoxanthine phosphoribosyltransferase deficiency (13) and 5-phosphoribosyl-1-pyrophosphate synthetase superactivity (1) were shown to be on the X chromosome. Those loci were not identified from a linkage study, but by the classical method, i.e., by identification of the defective protein and then the locus coding for that protein. Cloning of the gene responsible for gout in the present family will lead to an understanding of the mechanisms involved in the underexcretion of urate from the kidney. In fact, the primary defect in FJHN is not known, but underexcretion of urate seems to precede renal function impairment (14). Various possibilities have been suggested, including a metabolic defect resulting in increased availability of a counter ion which stimulates urate reabsorption, a genetic defect in this exchanger, altered function of the voltage-sensitive pathway, and a functional modification in the basolateral transport (12).

The sodium channel, nonvoltage-gated 1, β gene (a defect of which causes Liddle's syndrome) has been localized on 16p12 (GeneMap99, <http://www.ncbi.nlm.nih.gov/genemap99/>, inquiry date 8/9/99). In addition, the γ gene has also been localized in this region (GeneMap99). The features of Liddle's syndrome, however, are quite different from those of FJHN. Except for these loci, no likely candidate genes have been located in this region (GeneMap99).

The present results are likely to benefit affected families, since treatment of affected individuals in the early stage of the disease is considered to prevent, at least in part, the progression of renal failure. If the 5 markers *D16S417*, *D16S420*, *D16S3113*, *D16S401*, and *D16S3133* are used, children who will develop hyperuricemia and renal failure can be correctly diagnosed in the present family, and possibly in other families with FJHN as well. The question of whether the same locus is responsible in other FJHN families, or of whether FJHN is genetically heterogeneous, should be answered by further studies. The gene map information described herein will be useful for testing genetic heterogeneity.

An association with hyperuricemia and/or gout has been reported for both polycystic kidney disease type 1 (PKD1) (9) and an autosomal-dominant form of nephronophthisis-medullary cystic disease. Previous studies have excluded association of the gene for FJHN with either juvenile nephronophthisis on chromosome 2p13 or the autosomal-dominant PKD1 on chromosome 16p13 (15). The present study confirmed that there is at least 1 locus responsible for FJHN independent of the

loci for the other 2 diseases. Although the locus for PKD1 is close to that for FJHN (both are on 16p), the locus for PKD1 has been confirmed to be distal to *D16S418*. Since the locus for FJHN is considered to be proximal to *D16S417* (GeneMap99), the 5 marker loci we examined, i.e., *D16S407*, *D16S405*, *D16S410*, *D16S3045*, and *D16S412*, are between the locus for PKD1 and that for FJHN. The LOD scores between such marker loci were very low, as shown by the linkage analyses (Table 1, Figure 2, and unpublished observations). This indicates that the 2 diseases are different entities from the viewpoint of the genetic position.

Further studies should be aimed at cloning the gene responsible for FJHN. However, hundreds of genes, many of which have not been identified, are expected to be localized in the candidate region.

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Errata

In the article by Bredholt et al published in the December 1999 issue of *Arthritis & Rheumatism* (pp 2583–2592), there was an error in the second full sentence in the right column on page 2590. The sentence should have read, “Extrapolating from these results, it could be that an antigen-presenting cell (APC), when simultaneously presenting self and non-self peptides to T cells, can subsequently activate self-specific T cells when non-self-specific T cells are proliferating in the same microenvironment.”

In the article by Généreau et al published in the December 1999 issue (pp 2674–2681), the address for reprint requests was incorrect. Reprint requests should be sent to Loïc P. Guillevin, MD, Hôpital Avicenne, 125 Rue de Stalingrad, 93000 Bobigny, France. It should also have been noted that Hôpital Avicenne is the coordinating center for the French Vasculitis Study Group.

In the table of contents of the March 2000 issue, the title of the Clinical Image by Erkan et al (p 714) was listed incorrectly. The title should have been listed as “Ehlers-Danlos Syndrome Presenting as Olecranon Bursitis.”

We regret the errors.