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Report

Genetic and Physical Mapping of the Locus for Autosomal Dominant Renal Fanconi Syndrome, on Chromosome 15q15.3

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Autosomal dominant renal Fanconi syndrome is a genetic model for the study of proximal renal tubular transport pathology. We were able to map the locus for this disease to human chromosome 15q15.3 by genotyping a central Wisconsin pedigree with 10 affected individuals. After a whole-genome scan with highly polymorphic simple sequence repeat markers, a maximum LOD score of 3.01 was calculated for marker D15S659 on chromosome 15q15.3. Linkage and haplotype analysis for an additional 24 markers flanking D15S659 narrowed the interval to ~3 cM, with the two highest single-point LOD scores observed being 4.44 and 4.68 (for D15S182 and D15S537, respectively). Subsequently, a complete bacterial artificial chromosome contig was constructed, from the High Throughput Genomic Sequence Database, for the region bounded by D15S182 and D15S143. The identification of the gene and gene product altered in autosomal dominant renal Fanconi syndrome will allow the study of the physiology of proximal renal tubular transport.

Renal Fanconi syndrome (RFS [MIM 134600]) is a consequence of decreased solute reabsorption in the proximal tubule of the kidney. The proximal tubule reabsorbs a very large percentage of the solute filtered by the glomerulus. This solute transfer is a very high energy process. The clinical and laboratory manifestations of patients with RFS are thus a consequence of the failure of solute and water reabsorption in the proximal tubule. The patients have polydipsia and polyuria with phosphaturia, glycosuria, and aminoaciduria. They may develop hypophosphatemic rickets or osteomalacia, acidosis, and a tendency toward dehydration. Some will eventually develop renal insufficiency. Common laboratory abnormalities include glucosuria with a normal serum glucose, hyperaminoaciduria, hypophosphatemia, progressive renal insufficiency, renal sodium and potassium wasting, acidosis, uricosuria, and low–molecular weight proteinuria.

The causes of RFS are quite numerous; they include inborn errors of metabolism, such as cystinosis, galactosemia, fructose intolerance, tyrosinemia, Wilson disease, Lowe syndrome, some of the glycogenoses, and mitochondrial diseases such as cytochrome c oxidase deficiency. Acquired causes include heavy-metal poisoning, glue sniffing, and, most commonly, toxicity from chemotherapeutic drugs such as cisplatin and, particularly, iphosphamide (Foreman 1994). Familial RFS is a genetic model for the study of the pathophysiology of renal tubular transport. Seven families, including the one considered in the present study (Wen et al. 1989), have been described as exhibiting an autosomal dominant mode of inheritance of idiopathic RFS (Tolaymat et al. 1992); autosomal recessive (Illig and Prader 1961) and X-linked inheritance (Niemann et al. 1968) have also been reported. Determination of the gene defect responsible for autosomal dominant RFS would provide fundamental insight into the physiology of normal renal tubular transport. Such insight may lead to better treatment for patients with RFS.

We herein present the results of linkage and haplotype analysis for a large central Wisconsin pedigree with autosomal dominant RFS. The diagnosis of autosomal dominant RFS in this pedigree has been established and

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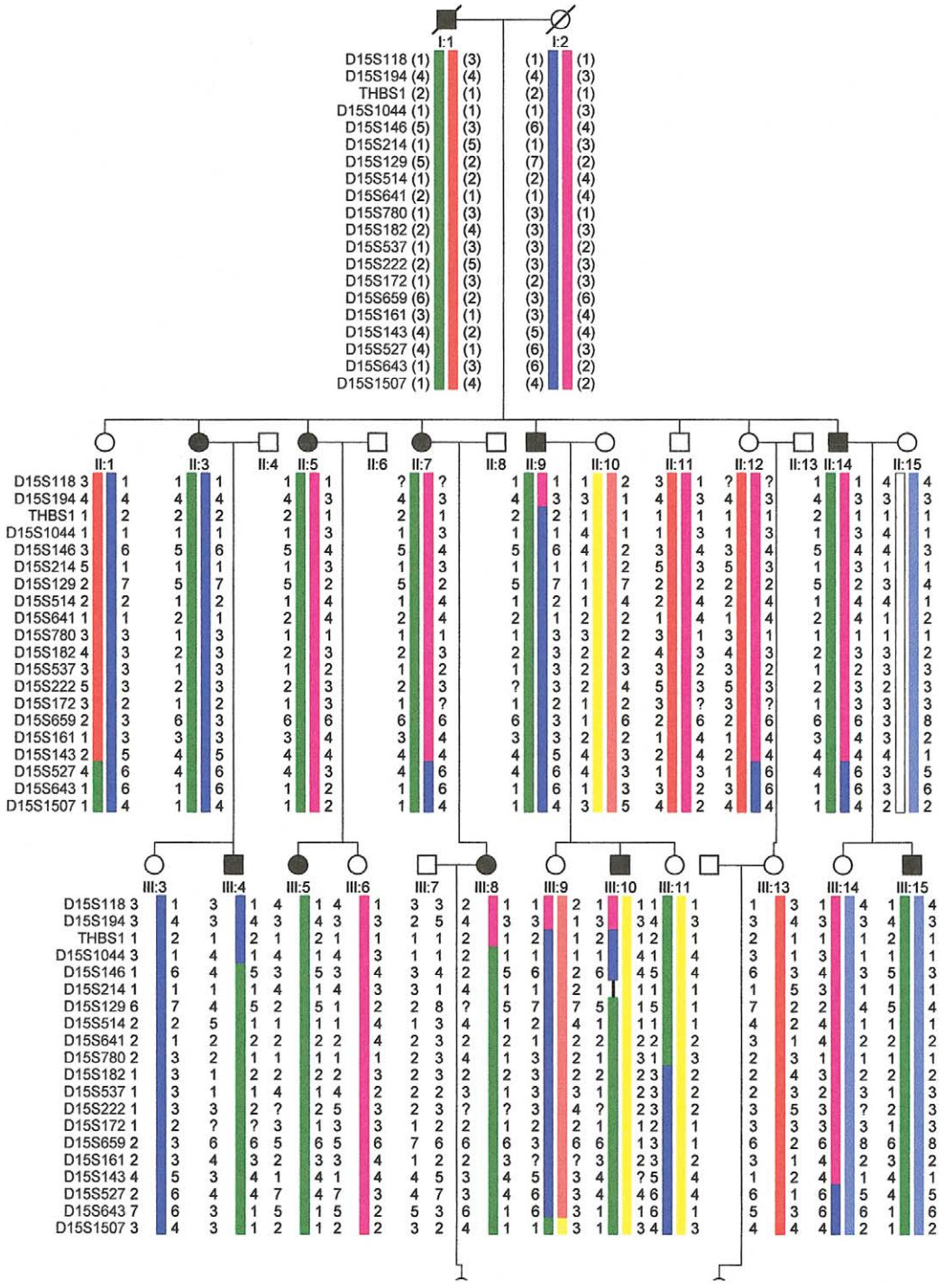

Figure 1 Pedigree of family with autosomal dominant RFS, showing haplotypes constructed for 21 markers from the 15q15-15q21 region in the second and third generations; the haplotypes of the first generation were inferred. Blackened symbols represent affected individuals, unblackened symbols unaffected individuals, and symbols with a question mark indicate individuals whose phenotype cannot be determined because they may be presymptomatic. The RFS haplotype is depicted by a green bar. ? = Unknown alleles.

Table 1

Single-Point LOD Scores for 30 Markers on Chromosome 15q11.2-q24

	Single-Point LOD Score at θ =								
Marker	.00	.01	.05	.10	.20	.30	.40	Z_{max}	θ_{max}[a]
D15S822	−∞	−2.18	−.29	.33	.63	.49	.17	.63	.20
D15S165	−∞	−1.39	−.14	.28	.49	.40	.16	.49	.21
D15S231	−∞	−1.96	−.66	−.19	.11	.13	.05	.14	.26
D15S144	−∞	−1.65	.18	.74	.91	.64	.21	.92	.17
ACTC	−∞	.75	1.86	2.08	1.87	1.29	.50	2.09	.11
D15S118	1.30	1.27	1.15	1.01	.70	.40	.12	1.30	.00
D15S102	−∞	.45	1.58	1.83	1.66	1.15	.43	1.84	.12
D15S221	1.83	1.81	1.73	1.58	1.22	.78	.29	1.83	.00
GATA50C03	−∞	.36	1.49	1.75	1.61	1.11	.42	1.77	.12
D15S194	−∞	−2.32	−.94	−.41	−.04	.03	.01	.03	.31
THBS1	2.32	2.27	2.06	1.80	1.24	.67	.18	2.32	.00
D15S1044	1.19	1.17	1.08	.97	.71	.43	.14	1.19	.00
D15S146	−∞	.75	1.86	2.08	1.87	1.29	.50	2.09	.11
D15S214	3.31	3.25	3.02	2.72	2.05	1.30	.47	3.31	.00
D15S129	−∞	2.57	2.97	2.89	2.35	1.57	.62	2.98	.06
D15S641	−∞	2.66	3.06	2.97	2.41	1.61	.64	3.06	.06
D15S514	−∞	2.01	2.44	2.41	1.96	1.30	.49	2.46	.06
D15S780	−∞	.36	.89	.96	.77	.45	.13	.96	.09
D15S182	4.44	4.36	4.06	3.67	2.81	1.84	.74	4.44	.00
D15S537	4.68	4.60	4.29	3.88	2.98	1.96	.80	4.68	.00
D15S222	−∞	1.19	1.68	1.71	1.41	.91	.31	1.72	.08
D15S172	−∞	−4.82	−2.18	−1.17	−.37	−.09	−.01	.00	NA
D15S659	3.31	3.25	3.02	2.72	2.05	1.30	.47	3.31	.00
D15S1028	2.41	2.36	2.19	1.95	1.44	.87	.27	2.41	.00
D15S161	3.14	3.08	2.86	2.56	1.93	1.21	.42	3.14	.00
D15S143	3.14	3.08	2.85	2.54	1.89	1.17	.40	3.14	.00
D15S527	−∞	2.74	3.14	3.04	2.47	1.66	.69	3.14	.06
D15S643	−∞	2.45	2.86	2.78	2.27	1.52	.61	2.86	.06
D15S1507	−∞	.43	1.56	1.81	1.65	1.14	.44	1.82	.12
D15S818	−∞	−5.54	−2.26	−1.03	−.14	.07	.03	.07	.31

[a] Maximum-likelihood estimate of θ. NA = not applicable.

reported elsewhere and has excluded both acquired causes and inherited metabolic disease causes (Wen et al. 1989). In this previous publication, clinical data for three pedigree members (subjects II:3, II:5, and III:4; fig. 1) were reported. In the present study, diagnosis of Fanconi syndrome was established or excluded by clinical laboratory evaluation in all but two genotyped pedigree members (two descendants of healthy individuals were not evaluated by laboratory tests). The clinical laboratory values used as diagnostic criteria for RFS were a tubular reabsorption of phosphorus (calculated as maximum rate of tubular absorption of phosphate/glomerular filtration rate) <2.5 mg/dl (Wen et al. 1989) and aminoaciduria and glucosuria in patients with normal serum glucose (Foreman 1994). Subjects were considered normal if they had normal laboratory values. Most of the affected family members developed polyuria and loss of proximal tubular function during the 2d decade of life and demonstrated significant renal insufficiency by the 3d decade. By our diagnostic criteria, 10 of the related family members whose genomes were analyzed had RFS and 14 did not. This study was approved by the institutional review board of Marshfield Clinic and St. Joseph's Hospital. Genomic DNA was isolated from peripheral blood cells, as described by Woo et al. (1983).

Initially, a whole-genome scan was performed using the polymorphic markers of Weber screening sets 8 and 9 (Yuan et al. 1997; Center for Medical Genetics Web site) to genotype the DNA of 27 pedigree members. The average spacing of these markers is reported to be one marker every 10 cM throughout the human genome. The sets included 12 markers for chromosome 15, which, according to the GeneMap'99 Database, is 110 cM in length. In nine cases the distance between markers was in the 8–12-cM range, and in three cases the distance between markers was <8 or >12 cM (range 4–17 cM). Fluorescently labeled products were generated by incorporation of dyes into DNA fragments via PCR. The PCR products were resolved on denaturing polyacrylamide gels and were detected by a scanning fluorescence detector (SCAFUD) (Yuan et al. 1997). Linkage analysis was performed using the programs MLINK and ILINK in the LINKAGE package, version 5.1 (Lathrop et al. 1984). Pairwise LOD scores were calculated under an

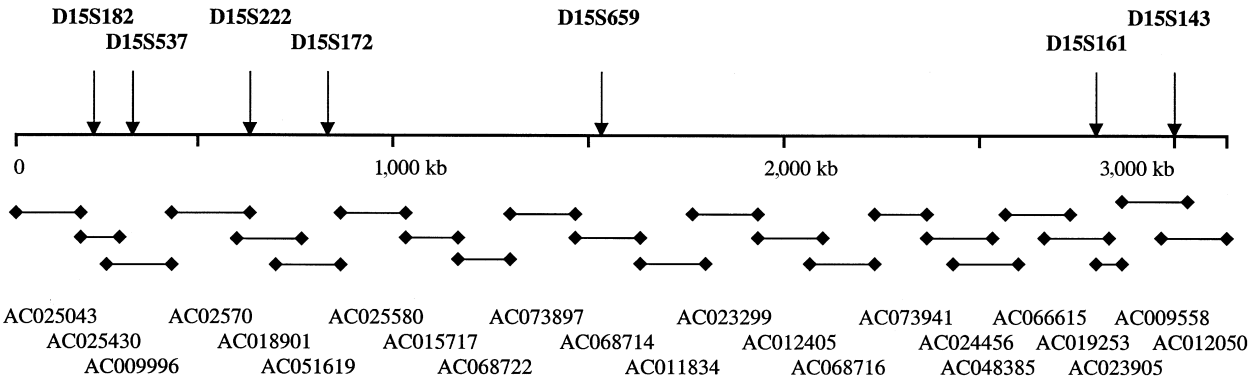

Figure 2 Diagram of the BAC clone contig of the critical region for RFS. The markers in the region are shown in boldface above the distance line for the contig. The marker positions are shown with regard to distances within the contig (0 = telomeric end of the contig) and indicate the BAC recombinant on which each marker resides. The GenBank accession numbers for the BAC clone sequences are represented below the BAC-clone insert symbol (blackened diamonds connected by a line), in a tiling fashion, from left to right.

autosomal dominant, completely penetrant model with disease allele frequency .0001. Analysis of the results of the genomewide scan yielded a maximum LOD score (Z_{max}) of 3.01 at marker D15S659 on the long arm of chromosome 15. A LOD score of $\geq$3.0 was considered significant for the establishment of linkage.

To further narrow the region of interest, the DNA of 23 individuals in the pedigree was analyzed with 24 additional simple sequence repeat (SSR) markers flanking marker D15S659 (table 1 and fig. 1). The markers were chosen on the basis of information available from the Web sites of the Center for Medical Genetics, The Genome Database, and the Genetics Location Database. These genotypes were determined using GENESCAN (version 2.1.1) software and an ABI 377XL DNA Sequencer (PE Biosystems). SSR-marker primers were purchased from Research Genetics or were synthesized by MWG Biotech, and PCRs were performed for 25 cycles as recommended by the supplier. Genomic DNA from CEPH individuals 1331-01 and 1331-02, with known allele sizes, was typed, as a control, with each marker.

Linkage analysis employing the data for the additional 24 markers from the interval D15S165–D15S643 mapped the locus for autosomal dominant RFS to 15q15.3, with the two highest Z_{max} values being 4.44 and 4.68, for markers D15S182 and D15S537, respectively (table 1). Genotype data for the total of 36 markers analyzed on chromosome 15 were used to form haplotypes. Different databases and publications (Fougerousse et al. 1994; Broman et al. 1998; GeneMap'99; Genetics Location Database) have suggested different orders for these markers. BLAST searches of the High Throughput Genomic Sequence Database, using the sequences of the 36 markers, detected bacterial artificial chromosome (BAC) clones containing the sequence for each of the 36 markers searched. Additional BLAST searches employing segments of BAC insert sequences revealed markers ACTC and D15S1232 to reside on the same BAC insert; markers D15S1044, THBS1, D15S146, D15S214, and D15S129 to reside on six overlapping BAC inserts; and marker D15S641 to be at the TYR03 locus (Allamand et al. 1995). A complete BAC contig, 3 Mb in length, could be constructed for the region flanked by the markers D15S182 and D15S143 (fig. 2). In figure 2, the GenBank accession number is provided for each of the recombinants contributing to the minimum tiling path for the region. The recombinant sequence depth in the 3-Mb region ranged from two- to sixfold, allowing all fragments of the draft-quality BAC sequences to be ordered with a high degree of confidence. The resulting contig represents a nearly complete and ordered sequence of the region flanked by markers D15S182 and D15143. Construction of the contig established the order and proximity of the seven markers in the critical region for RFS, which was consistent with that published by Allamand et al. (1995) for the region between markers THBS1 and D15S143. For the remaining markers, we applied the order determined by Broman et al. (1998), which is based on the analysis of eight CEPH families.

Haplotype analysis revealed two critical recombination events (individuals II:1 and III:11; fig. 1). A recombination event between markers D15S780 and D15S182 in individual III:11 establishes the centromeric boundary of the region of interest, and a recombination between markers D15S143 and D15S527 in individual II:1 establishes the telomeric boundary. These recombination events narrow the critical region to the interval between markers D15S182 and D15S143, which Allamand and Beckmann (1997) estimated as spanning 3 cM and which, by our results, was shown to represent a physical distance of 3 Mb (fig. 2). Candidate genes

from the region of interest are under investigation for the presence of segregating mutations.

The isolation of the gene and gene product that are altered in autosomal dominant RFS will allow study of the physiology of proximal renal tubular transport, thereby contributing to the understanding of both hereditary and acquired forms of RFS. Such studies should, consequently, lead to a better treatment of these entities.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Center for Medical Genetics, http://research.marshfieldclinic.org/genetics/

GeneMap'99, http://www.ncbi.nlm.nih.gov/genemap99/

Genetic Location Database, http://cedar.genetics.soton.ac.uk/public_html/ldb.html

Genome Database, The, http://www.gdb.org/

High Throughput Genomic Sequence Database, http://www.ncbi.nlm.nih.gov/HTGS/ (for markers [accession numbers AC025043, AC025430, AC009996, AC02570, AC018901, AC051519, AC025580, AC015717, AC068722, AC073897, AC068714, AC01134, AC023299, AC012405, AC068716, AC073941, AC024456, AC048385, AC066615, AC019253, AC023905, AC009558, and AC012050])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for autosomal dominant RFS [MIM 134600])

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