American Journal of Medical Genetics 82:161–165 (1999)

Confirmation of Linkage of Hereditary Partial Lipodystrophy to Chromosome 1q21-22

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Familial lipodystrophy is a genetically heterogeneous set of disorders characterized by a total or partial absence of subcutaneous fat, diabetes mellitus or impaired glucose tolerance, hyperlipidemia, and hypermetabolism [Senior and Gellis, 1964]. One subtype, familial partial lipodystrophy Dunnigan (FPLD), is a rare autosomal dominant trait that results in an gradual loss of subcutaneous fat in the lower trunk and limbs, Type V hyperlipoproteinemia, hypertriglyceridemia, and insulin-resistant diabetes. Previous reports of this condition have been limited to case reports or very small families. Recently, Peters et al. reported on linkage of five families of Western European descent to a 5.3 cM region on chromosome 1q21-22 between the flanking markers D1S305 and D1S1600 [Peters et al., 1998: Nat Genet 18:292–295]. We performed linkage and haplotype analysis using highly polymorphic, microsatellite markers on a large, multigeneration Caucasian kindred of German ancestry. The maximum two-point lod score achieved was 4.96 at $\theta_{max} = 0$ for marker D1S2721. Multipoint analysis gave an overall maximum lod score of 6.27 near marker D1S2721. The results of the haplotype analysis support the minimal candidate region as reported by Peters et al. Am. J. Med. Genet. 82:161–165, 1999.

KEY WORDS: partial lipodystrophy; linkage; chromosome 1; hyperlipidemia

INTRODUCTION

Familial lipodystrophy is a rare causally heterogeneous set of disorders characterized by the total or partial absence of subcutaneous fat, diabetes mellitus or impaired glucose tolerance, hyperlipidemia, and hypermetabolism [Senior and Gellis, 1964]. Lipodystrophy may be expressed as a partial or generalized lipoatrophy with regard to localization of the fat atrophy. When expressed as a partial lipoatrophy, localization may be unique to individuals and/or families. It may be acquired or congenital; the cause of many of the forms is unknown. Two previous family studies have shown an excess of females with partial lipodystrophy, whereby the authors suggested X-linked dominant inheritance with lethality in the hemizygous state [Koberling and Dunnigan, 1986; Wettke-Schafer and Kantner, 1983]. However, since males tend to show greater muscularity in general, the pronounced muscularity characteristic to lipodystrophy is easier to diagnose in females and may be overlooked in males. Diagnosis in children is difficult, particularly before puberty.

One form of hereditary partial lipodystrophy, the Dunnigan variety (FPLD; OMIM 151660), is a rare autosomal dominant subtype that results in a gradual loss of subcutaneous fat in the lower trunk and limbs, Type V hyperlipoproteinemia, hypertriglyceridemia, and insulin-resistant diabetes. Previous reports of this condition have been limited to case reports or very small families. Recently, Peters et al. reported on linkage of five FPLD families of Western European descent to a 5.3 cM region on chromosome region 1q21-22 between the flanking markers D1S305 and D1S1600 [Peters et al., 1998]. We identified and sampled a large, multigeneration Caucasian kindred of German ances-

Contract grant sponsor: National Institutes of Health; Contract grant number: NS23660.

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Received 23 June 1998; Accepted 4 August 1998

try with FPLD characteristics (Fig. 1). The present study confirms linkage of hereditary partial lipodystrophy to a region on chromosome 1q21-22 and presents data supporting the minimal candidate gene region.

MATERIALS AND METHODS

Family Data

The pedigree is shown in Figure 2. The family is a seven-generation Caucasian family of German descent with one consanguineous mating between individuals III-1 and III-2. All participants were examined by an endocrinologist (MK, FQN), a neurologist (WSD, ZM, MAN), and a geneticist (MAN). Laboratory studies in adults included measurements of liver function (AST, ALT, GGT), cholesterol, lipid profile, and hemoglobin A_{1C}. In children, liver functions, cholesterol, and lipids were analyzed.

Clinical diagnosis of partial lipodystrophy was made as follows. (A) The presence of the characteristic distribution of fat present in the neck and upper trunk and absent in the limbs and lower trunk, along with other physical characteristics (acanthosis nigricans, and an excessively muscular appearance in women), together with one of the following: (B) The history of elevated fasting triglycerides and/or cholesterol or presence of either on current laboratory testing (cholesterol >240 mg/dL and/or triglycerides >260 mg/dL; reference values are <200 mg/dL) and/or (C) diabetes, either by history or by the presence of HbA_{1C} greater than 7.5%. Individuals having only one of the findings (A, B, or C) or lacking the characteristic distribution of fat (B and/or C) were diagnosed as probably affected for the purposes of this study.

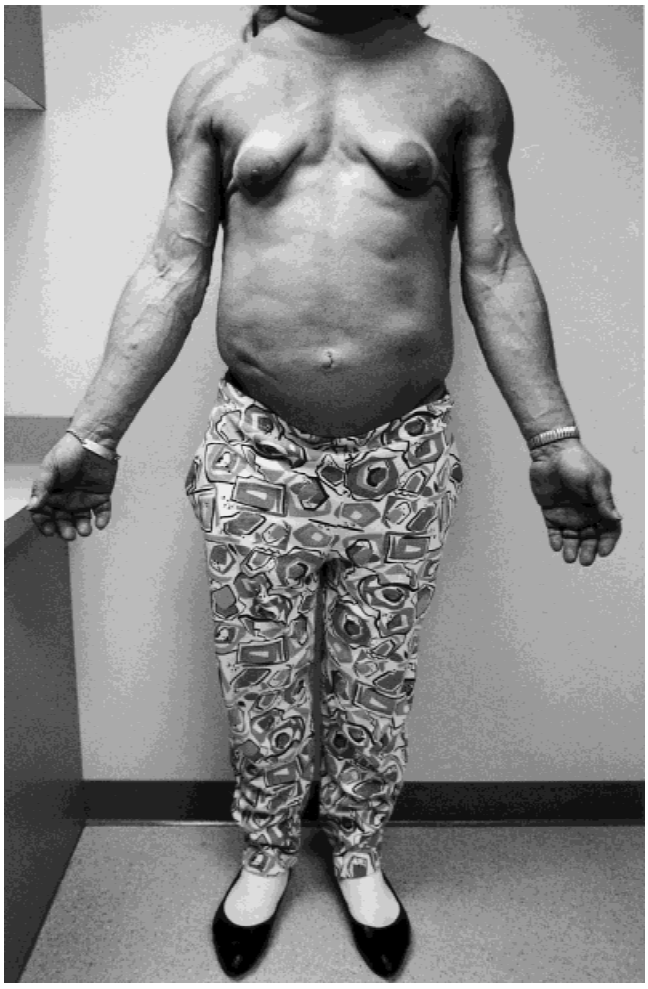

Fig. 1. Forty-year-old female with hereditary partial lipodystrophy. Note prominent muscular outline and veins on arms and shoulders, protuberant abdomen, and loss of subcutaneous fat on limbs and body torso.

Forty-three relatives underwent complete medical history, physical, and neurological exams; 23 of the relatives manifested the physical characteristics of partial lipodystrophy and 15 of these 23 had a cholesterol level over 240mg/dL, triglyceride level over 260mg/dL, or a history of treated diabetes. These 15 individuals met the criteria outlined above and were considered affected in the linkage analysis. Of the remaining eight individuals, five were 10 years of age or younger and three were adults age 30, 34, and 23. These eight individuals were categorized as probably affected in the study. Informed consent and blood samples were obtained from all study participants. The study was approved by the Institutional Review Board at Hennepin County Medical Center.

DNA Analysis

Genomic DNA was extracted from whole blood or transformed lymphocytes using standard procedures [Pericak-Vance et al., 1991]. Microsatellite analysis was performed by means of semiautomated fluorescence imaging (Molecular Dynamics SI Fluorimager, Duke University) [Vance et al., 1996a,b]. The markers chosen for analysis included those previously reported by Peters et al. and additional markers that map to this region (GATA176G01–15–D1S534–6.8–D1S305–1.2–D1S2721–3.0–D1S2624–1.1–D1S1600–1.5–D1S1653–4.1–D1S1167–5.9–D1S1677; map distance in cM) (Généthon). Estimates for population allele frequencies for the markers were generated from a minimum of 100 unrelated Caucasian chromosomes and were compared with the estimates available through the Genome database on the World Wide Web (Genome Database). Genotyping as well as clinical and family history data were databased using the PEDIGENE system [Haynes et al., 1995].

Linkage Analysis

Two-point linkage analysis was performed using the MLINK module of the LINKAGE package [version 5.2; Lathrop et al., 1984] as implemented in the FASTLINK program [Cottingham et al., 1993]. Autosomal dominant inheritance with a disease allele frequency of 1/10,000 for a rare dominant disorder and penetrance of 95% was assumed. Variation of the allele frequency did not change the linkage results (data not shown). Individuals presenting only one of the clinical findings or lacking the characteristic distribution of fat (probably affected) were given an 80% chance of being affected. Unaffected members were assigned a 10% chance of carrying an abnormal lipodystrophy gene.

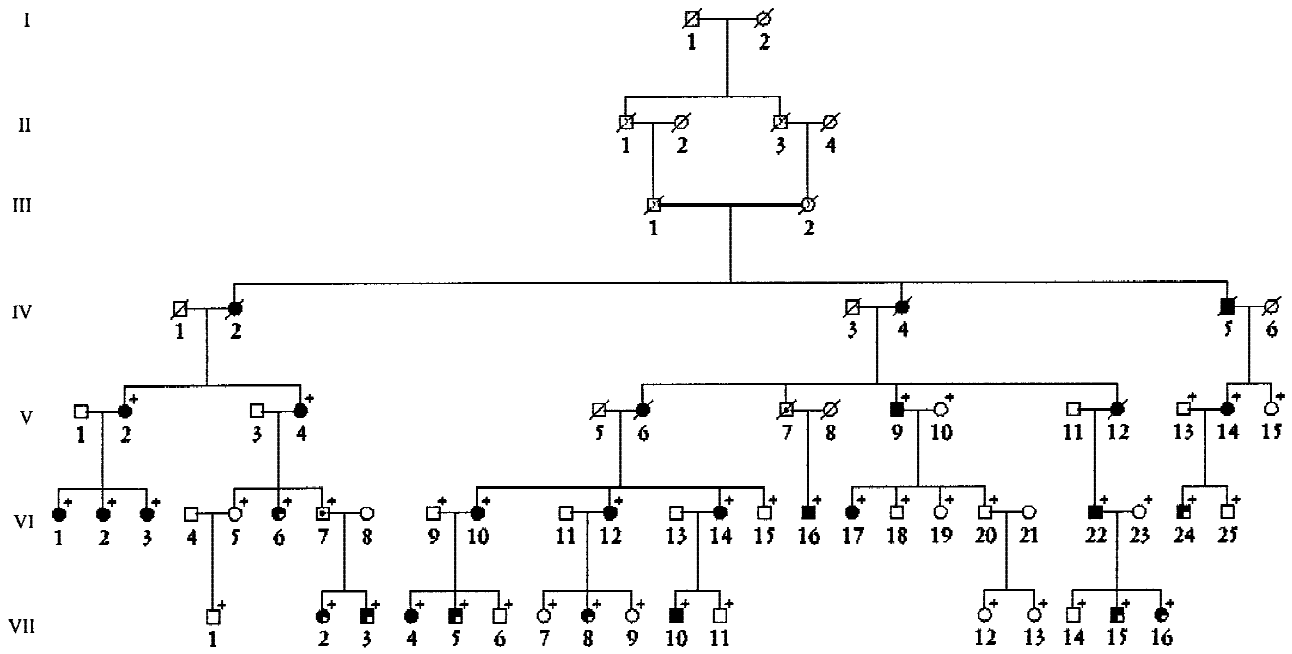

Fig. 2. Pedigree numbers labeled and ages displayed where appropriate. Symbol definitions: □, ○, unaffected; ■, ●, LIPOD; ⊡, ⊙, obligate carrier; ◨, ◑, deceased; ◧+, ◐+, probably affected; ■+, ●+, sampled.

TABLE I. Two-point Analysis Between Partial Lipodystrophy and Chromosome 1q Markers

	Lod Score at θ:							
Marker	0.00	0.05	0.10	0.20	0.30	0.40	Z_{max}/θ_{max}[a]	SI[b]
6G01	−0.871	2.509	2.467	2.006	1.370	0.646	2.52/0.06	
D1S534	3.529	3.541	3.362	2.674	1.721	0.651	3.57/0.03	(0.001,0.22)
D1S305	0.828	0.767	0.676	0.462	0.250	0.084	0.83/0.00	
D1S2721	3.895	3.897	3.728	3.087	2.182	1.109	3.93/0.02	(0.001,0.22)
D1S2624	2.587	2.342	2.038	1.334	0.605	0.044	2.59/0.00	
D1S1600	1.267	1.548	1.620	1.441	1.001	0.421	1.62/0.10	
D1S1653	2.132	2.015	1.864	1.470	0.953	0.377	2.13/0.00	
D1S1167	4.776	4.243	3.661	2.418	1.180	0.189	4.78/0.00	(0.00,0.09)
D1S1677	2.311	2.450	2.338	1.816	1.133	0.468	2.45/0.04	

[a]Z_{max} = maximum two-point lod score; θ_{max} = recombination fraction with maximum lod score.
[b]SI = 1-LOD-unit-down support interval for θ_{max}.

TABLE II. Two-Point Affecteds-Only Analysis Between Partial Lipodystrophy and Chromosome 1q Markers

	Lod Score at θ:							
Marker	0.00	0.05	0.10	0.20	0.30	0.40	Z_{max}/θ_{max}[a]	SI[b]
6G01	−1.358	1.362	1.496	1.322	0.935	0.473	1.50/0.11	
D1S534	4.517	4.050	3.563	2.551	1.519	0.528	4.52/0.00	(0.00,0.10)
D1S305	1.284	1.096	0.912	0.576	0.304	0.114	1.28/0.00	
D1S2721	4.956	4.499	4.023	3.029	2.002	0.972	4.96/0.00	(0.00,0.11)
D1S2624	1.778	1.465	1.154	0.580	0.148	−0.060	1.78/0.00	
D1S1600	3.212	2.874	2.526	1.814	1.092	0.411	3.21/0.00	(0.00,0.14)
D1S1653	2.560	2.261	1.950	1.325	0.743	0.265	2.56/0.00	
D1S1167	2.966	2.501	2.041	1.177	0.466	0.012	2.97/0.00	
D1S1677	1.786	1.727	1.551	1.094	0.636	0.258	1.79/0.01	

[a]Z_{max} = maximum two-point lodscore; θ_{max} = recombination fraction with maximum lod score.
[b]SI = 1-LOD-unit-down support interval for θ_{max}.

	6g01	D1S534	D1S305	D1S2721	D1S2624	D1S1600	D1S1653	D1S1167	D1S1677	Phase Known or Inferred
Known Affected										
V-2	○	●	●	●	●	●	●	●	●	Phase Inferred
Probably Affected										
VII-5	□	○	●	●	□	●	□	●	●	Phase Inferred
VII-4	□	●	●	●	□	●	□	●	○	Phase Inferred
VII-8	○	●	□	●	□	○	○	□	○	Phase Inferred

Fig. 3. ●: disease haplotype inherited, ○: nondisease haplotype inherited, and □ uninformative at the marker locus.

Additionally, a conservative low-penetrance, "affecteds-only" two-point analysis was performed, which used phenotype information only from the affected and probably affected individuals. In preparation for multipoint analysis, the consanguineous mating in the pedigree was removed and the VITESSE program package was used to perform two-point analyses for comparison to the LINKAGE results [O'Connell and Weeks, 1995]. For all two-point lod scores greater than 3.0, a 1-lod-unit-down support interval was determined to approximate a 95% confidence interval [Ott, 1991].

Haplotype analysis was performed (both manually and using SIMWALK2) to define and narrow the candidate interval in our data set [Sobel and Lange, 1996]. To construct haplotypes, phase was inferred as to minimize the number of recombination events. Breakpoints were established using information exclusively from affected individuals and fully informative meioses.

Multipoint linkage analysis was also performed using the VITESSE program. The markers used in the multipoint analysis included the most informative marker, D1S2721, and the flanking markers determined by the haplotype analysis, D1S534 and D1S1600.

RESULTS

Linkage Analysis

Two-point lod scores are given in Tables I and II. D1S2721 yielded the highest lod score, clearly establishing linkage in this pedigree to this region, with a maximum lod score of 3.93 in the full pedigree analysis and 4.96 in the affecteds-only analysis.

Comparison of two-point scores before and after the consanguineous loop was removed did not result in a significant loss (<10%) of linkage information (data not shown).

Multipoint analysis of the map D1S534-D1S2721-D1S1600 gave an overall maximum lod score of 6.27 near the position of marker D1S2721 and confirmed evidence for linkage (data not shown).

Haplotype Analysis

Haplotype analysis of the GATA176G01-D1S1677 interval revealed an affected individual, V-2, with a crossover between GATA176G01 and D1S534, defining one boundary on the p arm of chromosome 1 (Fig. 3). The other recombinant individuals are probably affected. Individual VII-5 displays a crossover and defines the proximal boundary of the candidate interval as D1S534. The distal boundary is defined by a recombination event exhibited by individual VII-8 between D1S1600 and D1S2721. The interval defined by these two probably affected individuals is approximately 12.1 cM.

DISCUSSION

These data confirm that partial lipodystrophy in this family is an autosomal dominant condition, and that the gene responsible for the disease is linked to chromosome region 1q21-22 as reported previously by Peters et al. [1998]. Haplotyping defined the candidate interval as a region spanning approximately 12.1 cM and bounded proximally by D1S534 and distally by D1S1600 when the two individuals (VII-5 and VII-8) diagnosed as probably affected are considered. Individual VII-5 was 23 years old and had a serum cholesterol level of 279mg/dL and a triglyceride level of 698mg/dL. Due to his large size (190.5cm, 113 kg), he did not overtly present with the abnormal body fat distribution observed in this family. Individual VII-8 was 10 years old. She clearly did present with abnormal body fat distribution and had a serum cholesterol level of 208mg/dL and a triglyceride level of 217mg/dL. When these individuals are not included, the minimum candidate region is bound by GATA176G01, which overlaps with that of Peters et al. [1998].

Examining the individual haplotypes resulted in the identification of a few individuals designated as unaffected who carry the disease segregating haplotype. These included two obligate gene carriers (VI-7 and V-7) and two unaffected children (VII-7 and VII-9). Both obligate carriers were male; one was 28 years old and the other was deceased. Neither manifested nor harbored a history of the clinical symptoms. The two children (age 7 and 14) who carried the disease haplotype and lacked the definitive characteristics of lipodystrophy should be reexamined after puberty.

ACKNOWLEDGMENTS

The authors would like to thank the family for their participation, which made this project possible. We also thank Dr. Marcy Speer, Dr. William Scott, Jill Barker, and Stephen Brown for discussion and assistance; Helen Harbett and Peggy Pate for data entry and management; and the personnel of the Center for Human Genetics at Duke University Medical Center.

NOTE ADDED IN PROOF

Jackson et al. [1998] have also reported linkage of FPLD to the region of chromosome 1q21 flanked by markers D152881 and D15484, in two additional families.

REFERENCES

Cottingham RW Jr, Indury RM, Schaffer AA. 1993. Faster sequential genetic linkage computation. Am J Hum Genet 53:252–263.

Généthon. CEPH-Généthon Map Data. Available via INTERNET at http://www.genethon.fr/genethon_en.html.

Genome Database. Available via INTERNET at http://gdbwww.gdb.org/.

Haynes C, Speer MC, Peedin M, Roses AD, Haines JL, Vance JM, Pericak-Vance MA. 1995. PEDIGENE: A comprehensive data management system to facilitate efficient and rapid disease gene mapping. Am J Hum Genet 57:A193.

Koberling J, Dunnigan MG. 1986. Familial partial lipodystrophy: two types of an X-linked dominant syndrome, lethal in the hemizygous state. J Med Genet 23:120–127.

Lathrop GM, Lalouel JM, Julier C, Ott J. 1984. Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 8:3443–3446

O'Connell JR, Weeks DE. 1995. The VITESSE algorithm for rapid exact multilocus linkage analysis data via genotype set-recording and fuzzy inheritance. Nat Genet 11:402–408.

Ott J. 1991. Analysis of human genetic linkage. Rev. ed. Baltimore: Johns Hopkins University Press.

Pericak-Vance MA, Bebout JL, Gaskell PC, Yamaoka LH, Hung W-Y, Alberts MJ, Walker AP, Bartlett RJ, Haynes CA, Welsh KA, Earl NL, Heyman A, Clark CM, Roses AD. 1991. Linkage studies in familial Alzheimer disease: evidence for chromosome 19 linkage. Am J Hum Genet 48:1034–1050.

Peters JM, Barnes R, Bennett L, Gitomer WM, Bowcock AM, Garg A. 1998. Localization of the gene for familial partial lipodystrophy (Dunnigan variety) to chromosome 1q21-22. Nat Genet 18:292–295.

Senior B, Gellis SS. 1964. The syndromes of total lipodystrophy and of partial lipodystrophy. Pediatrics 33:593–612.

Sobel E, Lange K. 1996. Descent graphs in pedigree analysis: applications to haplotyping, location scores, and marker sharing statistics. Am J Hum Genet 58:1323–1337.

Vance JM, Jonasson F, Lennon F, Sarrica J, Damji KF, Stauffer J, Pericak-Vance MA, Klintworth GK. 1996a. Linkage of a gene for macular corneal dystrophy to chromosome 16. Am J Hum Genet 58:757–762.

Vance JM, Slotterbeck B, Yamaoka L, Haynes C, Roses AD, Pericak-Vance MA. 1996b. A fluorescent genotyping system for multiple users, flexibility and high output. Am J Hum Genet 59:A239.

Wettke-Schafer R, Kantner G. 1983. X-linked dominant inherited diseases with lethality in hemizygous males. Hum Genet 64:1–23.