

## A Defect in the Regional Deposition of Adipose Tissue (Partial Lipodystrophy) Is Encoded by a Gene at Chromosome 1q

Stephen N. J. Jackson<sup>1,2</sup> John Pinkney,<sup>3,4</sup> Alex Bargiotta,<sup>4</sup> Colin D. Veal,<sup>1</sup> Trevor A. Howlett,<sup>2</sup> Paul G. McNally,<sup>2</sup> Roger Corral,<sup>3</sup> Andrew Johnson,<sup>3</sup> and Richard C. Trembath<sup>1</sup>

<sup>1</sup>Department of Genetics and Department of Medicine and Therapeutics, University of Leicester, and <sup>2</sup>Department of Diabetes and Endocrinology, Leicester Royal Infirmary National Health Service Trust, Leicester, United Kingdom; and <sup>3</sup>Department of Diabetes and Endocrinology, Southmead Hospital National Health Service Trust, and <sup>4</sup>Department of Diabetes and Endocrinology, Bristol Royal Infirmary National Health Service Trust, Bristol, United Kingdom

### Summary

Partial lipodystrophy (PLD), also known as “Dunnigan-Kobberling syndrome,” is transmitted as a highly penetrant autosomal dominant disorder that is characterized by a dramatic absence of adipose tissue in the limbs and trunk, more evident in females than in males. In contrast, fat is retained on the face, in retro-orbital space, and at perierous sites. Associated metabolic abnormalities, including insulin resistance, hyperinsulinemia, and dyslipidemia, are referred to as “metabolic syndrome X” (Reaven 1988). Despite the intense interest in the genetic determinants underlying fat deposition, the genes involved in the lipodystrophic syndromes have not been identified. We ascertained two multigeneration families, with a combined total of 18 individuals with PLD, and performed a genomewide search. We obtained conclusive evidence for linkage of the PLD locus to microsatellite markers on chromosome 1q21 (*D1S498*, maximum LOD score 6.89 at recombination fraction .00), with no evidence of heterogeneity. Haplotype and multipoint analysis support the location of the PLD locus within a 21.2-cM chromosomal region that is flanked by the markers *D1S2881* and *D1S484*. These data represent an important step in the effort to isolate and characterize the PLD gene. The identification of the gene will have important implications for the understanding of both developmental and metabolic aspects of the adipocyte and may prove useful as a single-gene model for the common metabolic disorder known as “syndrome X.”

### Introduction

Dunnigan et al. (1974) and Kobberling et al. (1975) independently described a familial disorder, partial lipodystrophy (PLD [MIM 151660]), which is characterized by the regional absence of subcutaneous adipose tissue and segregates as an autosomal dominant trait (Jackson et al. 1997b). Anthropometric evaluation and cross-sectional imaging demonstrates the presence of fat in the body cavities and the subcutaneous tissues of the head and neck but its complete absence from the subcutaneous tissues of the trunk and limbs. Of interest, skeletal muscle hypertrophy is also present (Jackson et al. 1997b). A variety of additional features have been reported, including hyperlipidemia, resistance to the glucose-lowering effects of insulin, and systemic hypertension (Davidson and Young 1975; Burn and Baraitser 1986; Kobberling and Dunnigan 1986), all of which are known to be determinants for ischemic heart disease and stroke. In population-based studies, a low ratio of peripheral to central obesity has been associated with an increased risk of cardiovascular disease; this serves to emphasize the importance of fat distribution in the predisposition to common diseases, in developed countries (Kahn et al. 1995).

The adipocyte plays a critical role in human metabolism (Harris and Crabb 1997). Differences in energy turnover, both in the basal state and in response to hormonal stimulation, have been described between subcutaneous and intra-abdominal fat deposits (Bjorntorp 1996). Although the metabolic pathways for energy cycling have been known for many years, little is known of the molecular genetic basis of fat-cell regional distribution.

Attempts to unravel the genetic contribution to common complex traits such as coronary heart disease remain a significant challenge. One promising strategy uses families in which a phenotype of interest is segregating as a Mendelian trait, compatible with a single-gene defect, for gene localization and identification, and the gene's contribution to the familial clustering of common

Received March 6, 1998; accepted for publication May 28, 1998; electronically published June 29, 1998.

Address for correspondence and reprints: Dr. Richard C. Trembath, Departments of Genetics, and Medicine and Therapeutics, University of Leicester, University Road, Leicester LE1 7RH, United Kingdom. E-mail: rtrembat@hgmp.mrc.ac.uk

© 1998 by The American Society of Human Genetics. All rights reserved. 0002-9297/98/6302-0029\$02.00

traits is then determined. We have ascertained the largest kindred with PLD thus far investigated and have undertaken a genomewide search, using a panel of highly informative simple tandem repeats (STRs) to localize the PLD gene to chromosome 1q21.

## Material and Methods

### Ascertainment of PLD families

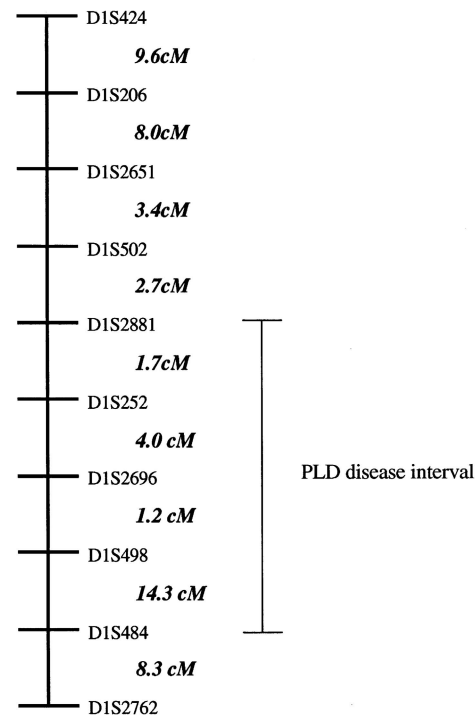
Each kindred was ascertained independently through one of two endocrine clinics (in Bristol and Leicester, United Kingdom). Twenty-three members of PLDFAM1 were examined by S.J., and 30 members of PLDFAM2 were examined by J.P. and A.B. All data, including clinical photographs, were reviewed by S.J. Affected status was assigned according to the presence or absence of subcutaneous fat in the limbs and torso, as determined by clinical assessment of subcutaneous tissue thickness. Eleven lipodystrophic women and seven lipodystrophic men (age range 19–72 years) were ascertained. One 8-year-old boy was excluded from the linkage analysis because his phenotype was unclear. This study was approved by the Leicestershire Ethics Committee.

### DNA STR Polymorphism (STRP) Analysis

Peripheral venous blood (10 ml EDTA) was obtained by venipuncture, and genomic DNA was extracted by use of standard methods. We performed an initial genomewide search, using fluorescence-labeled primers and semiautomated techniques to detect STRPs evenly distributed throughout the genome at a density sufficient to allow >90% confidence of detection of linkage in the primary family-mapping panel. Genotypes were determined as described elsewhere (Trembath et al. 1997). In brief, conditions for PCR were optimized for each primer pair over a range of annealing temperatures (50°C–60°C) and magnesium concentrations (1.0–2.5 mmol MgCl<sub>2</sub>). Amplification reactions were performed with Perkin Elmer 9600 PCR machines.

### Linkage Analysis

Linkage analysis was performed by use of the LINKAGE 5.1 program package (Lathrop et al. 1984) and the FASTLINK computer program (Cottingham et al. 1993). MLINK two-point linkage analysis was performed between the disease and each marker; relative marker positions are shown in figure 1 (Dib et al. 1996). PLD was coded as a fully penetrant autosomal dominant trait with a gene frequency of .00001. Equal recombination frequencies between males and females were assumed. A limited multipoint analysis was performed, under the assumption of a fixed order and distance of



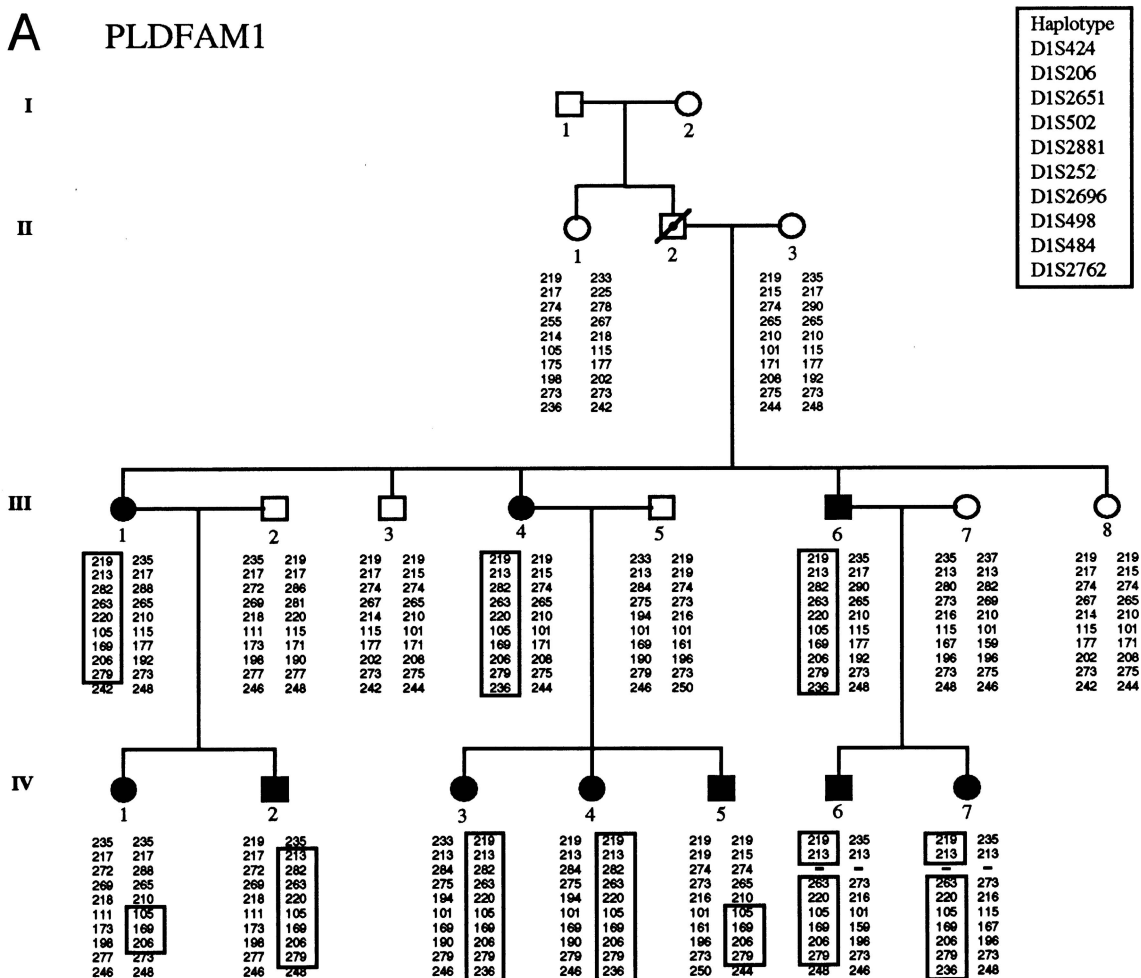
**Figure 1** Genetic map showing the order of markers and the location of the PLD disease gene. Recombination intervals are in centimorgans and are derived from the Génethon map (Dib et al. 1996).

three loci from the region, by means of the program VITESSE (O'Connell and Weeks 1995).

## Results

### Two-Point Linkage Analysis

We undertook an initial search for linkage, using the unambiguously affected and married-in members of pedigree PLDFAM1 only (fig. 2A). From the panel of 351 STR markers, with an average intermarker separation of 10 cM, only the marker *DIS498* gave a suggestive linkage to PLD (LOD score 3.59 at recombination fraction [ $\theta$ ] .00). Both families PLDFAM1 and PLDFAM2 were then used to test for linkage with a higher map of additional markers spanning the region flanked by *DIS424* and *DIS2762* (fig. 2A and B). Two-point LOD-score analysis supported a locus for PLD in the region containing markers *DIS498* and *DIS252*. Two-point LOD scores for these and the additional markers from that region of chromosome 1 are summarized in table 1. A maximum combined LOD score ( $Z_{\max}$ ) of 6.89 at  $\theta = .00$  was generated. Examination of the two-point LOD scores for each family gave no evidence of heterogeneity; that is, each family showed evidence for linkage to this region (see table 1). None of the other ge-



**Figure 2** Pedigrees of PLD kindreds. A, PLDFAM1 (above); B, PLDFAM2 (facing page). Blackened symbols represent affected individuals; unblackened symbols represent unaffected individuals. Genotypes for microsatellite markers from chromosome 1 are shown below each symbol. Shared haplotypes between affected individuals are boxed.

nomic regions tested for linkage gave evidence suggestive of linkage in the primary mapping panel of patients.

*Haplotype and Multipoint Analysis*

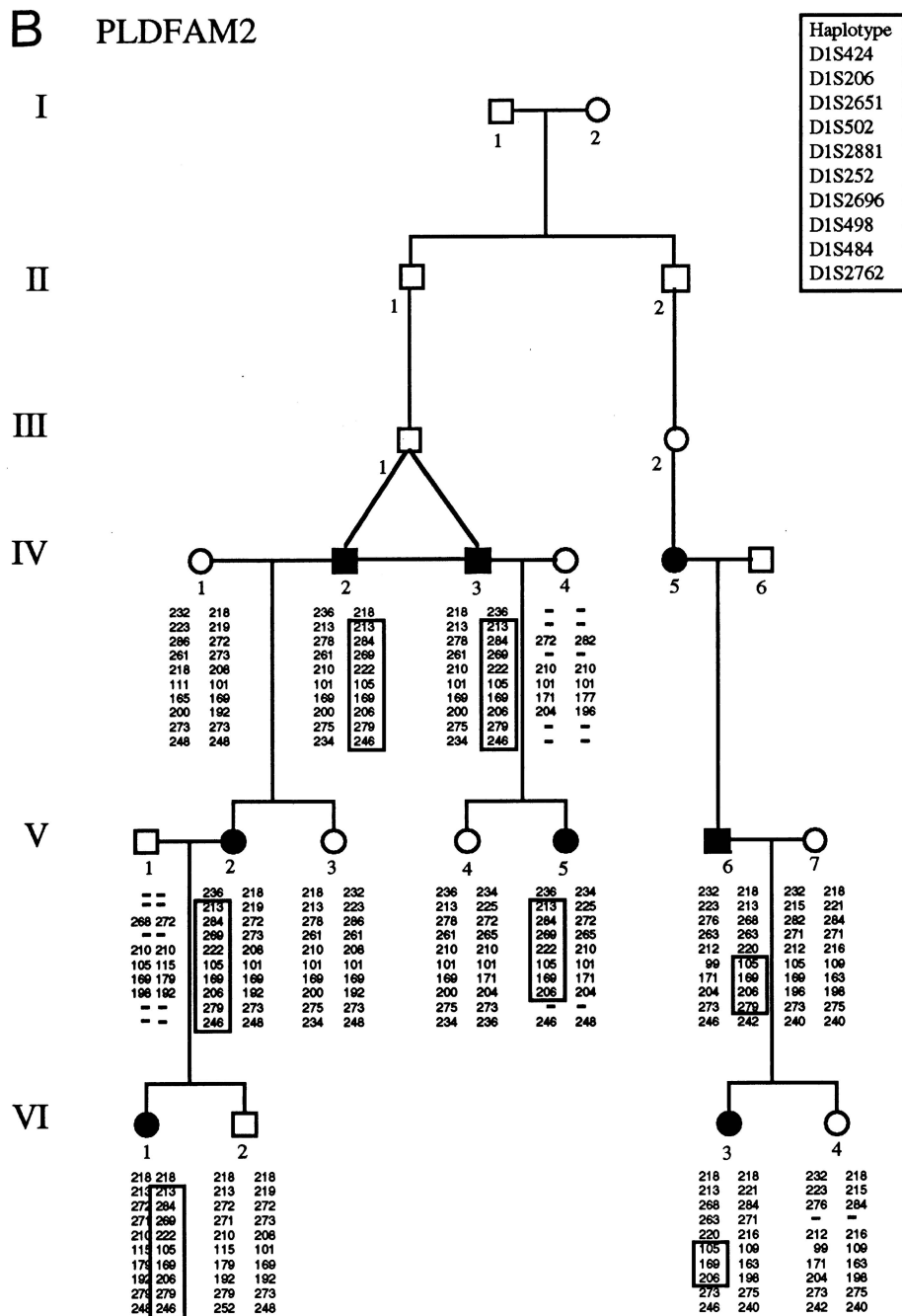
To define the minimum interval containing the PLD gene, haplotypes were constructed for each family and were examined for evidence of recombination events (fig. 2A and B). The minimum disease-gene interval, as defined by recombination between chromosome 1q21 markers and the PLD locus for each family studied, is shown in figure 2. Obligatory recombination events placed the disease-gene location proximal to *D1S484* and distal to *D1S2881*, a genetic distance of 21.2 cM (fig. 1). Using multipoint linkage analysis of three markers genotyped from the region, we obtained a maximum LOD score of 6.6 at *D1S498*. The 95% confidence in-

terval (-1 LOD) would place the PLD locus within a 21-cM interval flanked by *D1S2881* and *D1S484*.

**Discussion**

We have localized a gene for PLD to a 21.2-cM interval at chromosome 1q21 (*D1S498*;  $Z_{max} = 3.59$  at  $\theta = .00$ ). Analysis of a second PLD kindred also gave support for linkage to this region, with a combined two-point LOD score of 6.89 at  $\theta = .00$  (*D1S498*). Of interest, detailed haplotype analysis identified a shared disease haplotype between the two families, which suggests the segregation of a common founder mutation for PLD.

The two families investigated in this study were ascertained independently, with PLDFAM1 residing in the



eastern Midlands and PLDFAM2 in southwest England. Despite detailed questioning, no common ancestor has been identified, but it is of interest that both kindreds are known to have originated in Ireland. Studies of additional families with PLD will be required, to confirm genetic homogeneity for the disease locus and to explore the possibility of allelic association. Of importance, de-

lineation of the minimum shared interval for the disease haplotype places the PLD gene between the markers *D1S2881* and *D1S484*. This region extends into the centromere of chromosome 1 and forms the boundary between a gene-dense area and the gene-sparse heterochromatic region (Craig and Bickmore 1994).

Although findings for both of these families support

**Table 1**

**Two-Point Linkage Analysis between STRs from Chromosome 1 and PLD, for Kindreds PLDFAM1 and PLDFAM2 and for Both Families Combined**

MARKER AND FAMILIES	LOD SCORE AT $\theta =$							$Z_{\max} : \theta$
	.00	.01	.05	.1	.2	.3	.4	
<i>D1S424</i>								
PLDFAM1	-4.56	-1.16	-.50	-.24	-.03	.05	.05	.03 : .4
PLDFAM2	-9.19	-3.23	-1.51	-.82	-.28	-.08	-.02	
Combined	-13.74	-4.39	-2.01	-1.06	-.30	-.04	.03	
<i>D1S206</i>								
PLDFAM1	-8.11	-.46	.72	1.05	1.06	.77	.36	2.41 : .1
PLDFAM2	1.82	1.77	1.59	1.36	.92	.53	.22	
Combined	-6.29	1.31	2.31	2.41	1.98	1.30	.58	
<i>D1S2651</i>								
PLDFAM1	-8.27	-.88	.33	.68	.77	.56	.24	2.23 : .1
PLDFAM2	.15	1.07	1.52	1.54	1.28	.86	.40	
Combined	-8.12	.19	1.84	2.23	2.04	1.42	.64	
<i>D1S502</i>								
PLDFAM1	-3.30	-.46	.72	1.05	1.06	.77	.36	2.12 : .1
PLDFAM2	-.46	.53	1.01	1.07	.89	.58	.25	
Combined	-3.76	.07	1.73	2.12	1.96	1.35	.60	
<i>D1S2881</i>								
PLDFAM1	-3.30	-.46	.72	1.05	1.06	.77	.36	2.59 : .1
PLDFAM2	.15	1.07	1.52	1.54	1.28	.86	.4	
Combined	-3.16	.61	2.24	2.59	2.34	1.63	.75	
<i>D1S252</i>								
PLDFAM1	2.85	2.83	2.71	2.52	2.02	1.40	.68	5.63 : .0
PLDFAM2	2.78	2.72	2.46	2.14	1.48	.87	.34	
Combined	5.63	5.54	5.17	4.65	3.50	2.26	1.02	
<i>D1S2696</i>								
PLDFAM1	3.59	3.53	3.28	2.96	2.26	1.51	.71	5.26 : .0
PLDFAM2	1.67	1.63	1.47	1.27	.88	.54	.25	
Combined	5.26	5.16	4.75	4.23	3.15	2.05	.95	
<i>D1S498</i>								
PLDFAM1	3.59	3.53	3.28	2.96	2.27	1.51	.71	6.89 : .0
PLDFAM2	3.30	3.23	2.96	2.62	1.92	1.21	.54	
Combined	6.89	6.76	6.25	5.58	4.18	2.72	1.25	
<i>D1S484</i>								
PLDFAM1	-2.15	1.53	2.00	2.00	1.66	1.14	.53	2.77 : .1
PLDFAM2	-2.61	.16	.68	.76	.64	.42	.19	
Combined	-4.76	1.69	2.68	2.77	2.30	1.56	.72	
<i>D1S2762</i>								
PLDFAM1	-9.24	-4.93	-3.41	-2.16	-.95	-.40	-.13	.38 : .2
PLDFAM2	2.56	2.50	2.25	1.94	1.33	.75	.27	
Combined	-6.68	-2.44	-1.15	-.22	.38	.35	.14	

linkage to chromosome 1q21, genetic heterogeneity for PLD may still exist. The original families described by Kobberling appeared to have a more severe degree of hyperlipidemia that was associated with occlusive vascular disease and subsequent premature death. Linkage analysis of surviving relatives of these and other PLD kindreds will be required, to determine the degree of genetic heterogeneity.

Two genes have recently been demonstrated to be genetic determinants for obesity in humans (Jackson et al. 1997a; Montague et al. 1997). The endopeptidase prohormone convertase 1 (PC1) is a member of a family of precursor endoproteases that cause preferential cleavage of both prohormones and proneuropeptides in acidic

secretory vesicles. The mechanism by which reduced activity of PC1 leads to obesity in humans remains unclear. Leptin is produced exclusively by adipocytes and acts on the hypothalamic leptin receptors to both suppress food intake and increase energy expenditure. Both in mice and in humans, homozygous leptin mutations result in profound obesity due primarily to excessive food intake. However, leptin does not appear to be required for the normal intrauterine development of fat stores, since, in leptin-deficient mice and humans, birth weights are normal (Coleman 1979).

The reduction of subcutaneous fat stores in PLD is apparent only after the neonatal period, which suggests the presence of normal regional fat development during

intrauterine life. Genes involved in the differentiation of preadipocytes into adipocytes or in the maintenance of adipocyte integrity are candidates for the causative mutation in this disorder. The gene encoding cellular retinoic acid-binding protein 2 is within the critical region and would be expected to play a role in adipocyte differentiation (Elder et al. 1992). Its gene product is an intracellular binding protein with specificity for retinoic acid and has been demonstrated to be expressed in 3T3-L1 cells (a murine committed-preadipocyte cell line). The role of retinoic acid is complex, and it appears to have both permissive and inhibitory activity at different stages of differentiation, but it has been shown to block adipogenesis of 3T3-L1 cells induced to differentiate by ectopic expression of the transcription factors PPAR $\gamma$  and C/EBP $\alpha$  (Dani et al. 1997; Schwarz et al. 1997).

Peters et al. (1998) have recently independently reported linkage of the PLD gene to 1q21-22. Positional cloning of the PLD gene will require the further refinement of the minimum linkage interval. However, localization of the disease to chromosomal region 1q21 represents an important step toward identification of a gene critical in the maintenance of regional fat distribution. The eventual characterization of the PLD gene will permit assessment of its contribution to the major cardiovascular risk factors—namely, insulin resistance, hyperlipidemia, hypertension, and obesity.

## Acknowledgments

We acknowledge a training fellowship from the Leicester Royal Infirmary NHS Trust Charitable Fund (to S.N.J.J.) and equipment support from the British Diabetic Association (R.C.T.). We thank the families for their support and encouragement and Prof. S. O'Rahilly for helpful discussions. The assistance of Drs. M. Rhodes, A. Dearlove, F. Visser, and A. Thompson (Human Genome Mapping Project Resource Centre, Hinxton, United Kingdom) is gratefully acknowledged. Dr. A. C. Burden has been responsible for the metabolic management of a number of members of PLDFAM1.

## References

- Bjorntorp P (1996) The regulation of adipose tissue distribution in humans. *Int J Obes Relat Metab Disord* 20: 291–302
- Burn J, Baraitser M (1986) Partial lipodystrophy with insulin resistant diabetes and hyperlipidaemia (Dunnigan syndrome). *J Med Genet* 23:128–130
- Coleman DL (1979) Obesity genes: beneficial effects in heterozygous mice. *Science* 203:663–665
- Cottingham RW, Idury RM, Schaffer AA (1993) Faster sequential genetic linkage computations. *Am J Hum Genet* 53: 252–263
- Craig JM, Bickmore WA (1994) The distribution of CpG islands in mammalian chromosomes. *Nat Genet* 7:376–382
- Dani C, Smith AG, Dessolin S, Leroy P, Staccini L, Villageois P, Darimont C, et al (1997) Differentiation of embryonic stem cells into adipocytes in vitro. *J Cell Sci* 110:1279–1285
- Davidson MB, Young RT (1975) Metabolic studies in familial partial lipodystrophy of the lower trunk and extremities. *Diabetologia* 11:561–568
- Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 380: 152–154
- Dunnigan MG, Cochrane MA, Kelly A, Scott JW (1974) Familial lipodystrophic diabetes with dominant transmission: a new syndrome. *Q J Med* 43:33–48
- Elder JT, Astrom A, Pettersson U, Voorhees JJ, Trent JM (1992) Assignment of human CRABP-II gene to chromosome 1q21 by nonisotopic in situ hybridization. *Hum Genet* 89: 487–490
- Harris RA, Crabb DW (1997) Metabolic interrelationships. In: Devlin TM (ed) *Textbook of biochemistry*. Wiley-Liss, New York, pp 525–562
- Jackson RS, Creemers JW, Ohagi S, Raffin-Sanson ML, Sanders L, Montague CT, Hutton JC, et al (1997a) Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. *Nat Genet* 16: 303–306
- Jackson SNJ, Howlett TA, McNally PG, O'Rahilly S, Trembath RC (1997b) Dunnigan-Kobberling syndrome: an autosomal dominant form of partial lipodystrophy. *Q J Med* 90:27–36
- Kahn SE, Leonetti DL, Prigeon RL, Boyko EJ, Bergstrom RW, Fujimoto WJ (1995) Relationship of proinsulin and insulin with noninsulin-dependent diabetes mellitus and coronary heart disease in Japanese-American men: impact of obesity—clinical research center study. *J Clin Endocrinol Metab* 80:1399–1406
- Kobberling 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
- Kobberling J, Willms B, Kattermann R, Creutzfeldt W (1975) Lipodystrophy of the extremities: a dominantly inherited syndrome associated with lipodystrophic diabetes. *Human-genetik* 29:111–120
- Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multilocus linkage analysis in humans. *Proc Natl Acad Sci USA* 81:3443–3446
- Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, Wareham NJ, Sewter CP, et al (1997) Congenital leptin deficiency is associated with severe early onset obesity in humans. *Nature* 387:903–908
- O'Connell JR, Weeks DE (1995) The VITESSE algorithm for rapid exact multilocus linkage analysis via genotype set-recording and fuzzy inheritance. *Nat Genet* 11:402–408
- 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
- Reaven GM (1988) Role of insulin resistance in human disease. *Diabetes* 37:1595–1607
- Schwarz EJ, Reginato MJ, Shao D, Krakow SL, Lazar MA

(1997) Retinoic acid blocks adipogenesis by inhibiting C/EBPbeta-mediated transcription. *Mol Cell Biol* 17: 1552–1561  
Trembath RC, Clough RL, Rosbotham JL, Jones AB, Camp

RDR, Frodsham A, Browne J, et al (1997) Identification of a major susceptibility locus on chromosome 6p and evidence for further disease loci revealed by a two stage genome-wide search in psoriasis. *Hum Mol Genet* 6:813–820