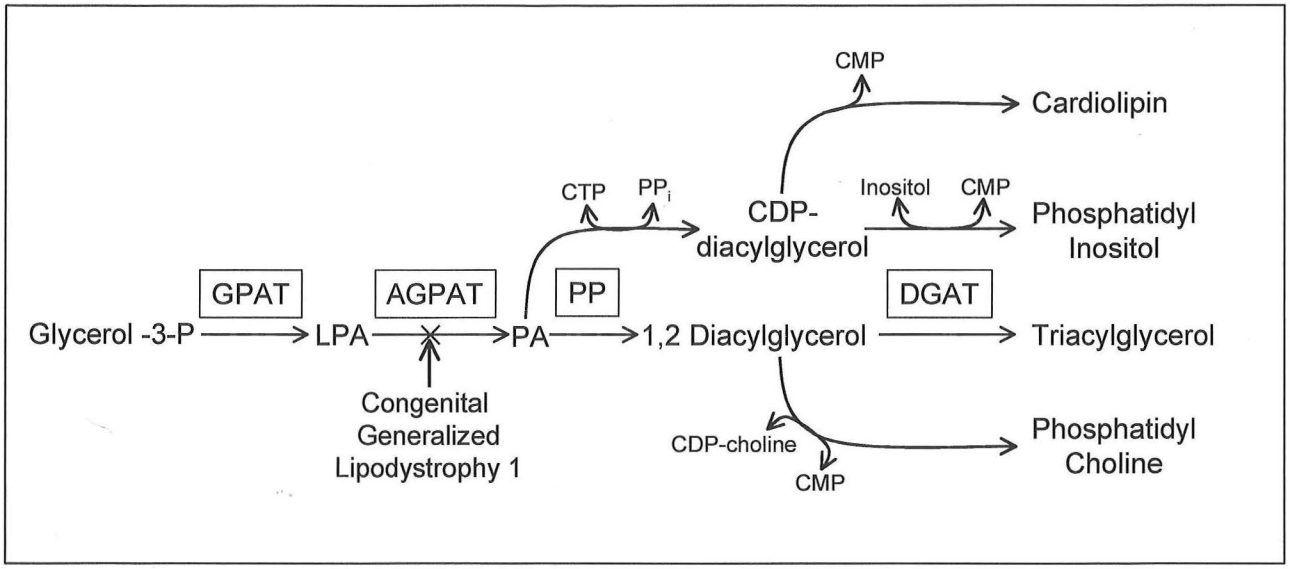
Internal Medicine Grand Rounds
May 16, 2002

MOLECULAR BASIS OF LIPODYSTROPHIES AND OTHER DISORDERS OF ADIPOSE TISSUE



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This is to acknowledge that Abhimanyu Garg, M.D. has disclosed no financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Garg will not be discussing "off-label" uses in his presentation.

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Special Interests:
Lipodystrophies and other disorders of adipose tissue
Lipoprotein disorders in diabetes
Nutrition in patients with diabetes
Regional obesity, insulin resistance and syndrome 'x'

Cover illustration: *Biosynthetic pathway for triacylglycerol and phospholipid from glycerol-3-phosphate in eukaryotes.*
The glycerol-3-phosphate acyltransferase (GPAT) acylates glycerol-3-phosphate at stereospecific number (sn)-1 position to form lysophosphatidic acid (LPA). AGPAT catalyzes acylation of LPA at sn-2 position to form phosphatidic acid (PA) and we have found *AGPAT2* gene to be mutated in patients with congenital generalized lipodystrophy 1, linked to chromosome 9q34. Phosphatidic acid is dephosphorylated by phosphatidate phosphohydrolase (PP) to form 1,2 diacylglycerol, which is acylated to form triacylglycerol using diacylglycerol acyltransferase (DGAT). Synthesis of phosphoglycerols (phospholipids) occurs from PA, e.g. phosphatidyl inositol and cardiolipin (diphosphatidylglycerol), or from 1,2-diacylglycerol, e.g., phosphatidylcholine and phosphatidylethanolamine (not shown).

Acknowledgments: I am indebted to Drs. Anoop Misra, Dali Chen and Vinaya Simha for literature review; Dr. Anil Agarwal for genetic studies; Angela Osborn for illustrations and for management of DNA and cell cultures and the nursing and dietetic services of the General Clinical Research Center for patient care support.

Introduction

My previous grand rounds on this subject dealt mainly with a phenotypic classification of various types of genetic lipodystrophies and other disorders of adipose tissue. Recently, great progress has been made in the understanding the molecular basis of inherited lipodystrophies as well as monogenic syndromes of obesity and in this grand rounds, I will review this progress.

Table 1. Classification of Monogenic Disorders of Adipose Tissue

A. Genetic Lipodystrophies:

1. Congenital generalized lipodystrophy (CGL; Berardinelli-Seip Syndrome)
   a. CGL1: *AGPAT2* (1-acylglycerol-3-phosphate O-acyltransferase 2) mutations
   b. CGL2: *BSCL2* (Berardinelli-Seip congenital lipodystrophy 2) mutations
   c. Other varieties
2. Familial partial lipodystrophy (FPL)
   a. Dunnigan variety (FPLD): *LMNA* (lamin A/C) mutations
   b. FPL1: *PPARG* (peroxisome proliferator-activated receptor-γ) mutations
   c. Kobberling variety
   d. Mandibuloacral dysplasia variety
   e. Other varieties
3. SHORT syndrome
4. Neonatal progeroid syndrome

B. **Multiple Symmetric Lipomatosis**: *MtDNA* mutations

C. **Monogenic Obesity**:

1. *LEP* (leptin) mutations
2. *LEPR* (leptin receptor) mutations
3. *PC1* (prohormone convertase 1) mutations
4. *MC4R* (melanocortin receptor 4) mutations
5. *POMC* (proopiomelanocortin) mutations
6. *ALMS1* mutations (Alstrom syndrome)
7. *SIM1* (single-minded 1) mutation

GENETIC LIPODYSTROPHIES

Congenital Generalized Lipodystrophy (CGL, Berardinelli-Seip syndrome)

Congenital generalized lipodystrophy (Online Mendelian Inheritance in Man [OMIM] # 269700) is an extremely rare autosomal recessive disorder with an estimated prevalence of less than 1 case in 12.5 million people(1). It occurs in subjects of all ethnicities but is more prevalent in populations with consanguinity. The disorder is characterized by a nearly complete absence of adipose tissue from birth, resulting in a marked generalized muscular appearance. During early childhood, they have accelerated growth, voracious appetite, increased basal metabolic rate, and advanced bone age. The final height that is achieved, however, is normal or slightly above normal. Acanthosis nigricans is common and usually appears by 8 years of age. It may be widespread and involve neck, axillae, groin, trunk; it can even cause skin tag formation. Umbilical hernia seems to be a consistent finding. Other clinical features include hepatosplenomegaly and slight enlargement of the hands, feet and

mandible, resulting in an "acromegaloid appearance". Occasional patients may have excessive body hair and hyperhidrosis.

Severe hyperinsulinemia and high serum triglyceride concentrations may be seen even during infancy. As a result, these patients frequently develop chylomicronemia, eruptive xanthomas, and acute pancreatitis. Low concentrations of high-density lipoprotein (HDL) cholesterol are also common. Abnormal glucose tolerance and diabetes usually appear during the pubertal years but sometimes it occurs later in adulthood(2). Severe amyloidosis of the pancreatic islets and a paucity of β cells appear to be the underlying mechanism for diabetes(3). Fatty infiltration of the liver occurs early and may lead to cirrhosis and its complications.

Affected postpubertal women may have clitoromegaly, mild hirsutism, and oligo-amenorrhea. Some women have polycystic ovaries. Successful pregnancy in affected women is rare, but affected men have normal reproductive potential. Postpubertal patients may also develop focal lytic lesions in the appendicular bones(4). A few patients have been reported to have hypertrophic cardiomyopathy(5). Early onset diabetes and dyslipidemia may predispose these patients to accelerated atherosclerosis. During adulthood, they may develop diabetic nephropathy and retinopathy.

Adipose tissue distribution

On the basis of MRI and autopsy studies in patients with congenital generalized lipodystrophy, we have reported that adipose tissue was almost completely absent from most of the subcutaneous areas, intraabdominal and intrathoracic regions, bone marrow and parathyroid glands(6, 7). In contrast, normal amounts of adipose tissue were present in the orbits, crista galli, buccal region, tongue, palms and soles, scalp, perineum, vulva, peri-articular regions, epidural area and pericalyceal regions of the kidney. These findings have led to a new classification of human white adipose tissue into metabolically active adipose tissue, which is almost completely absent in patients with congenital generalized lipodystrophy, and mechanical adipose tissue, which is well preserved.

Metabolic derangements

Euglycemic, hyperinsulinemic, glucose clamp studies reveal marked insulin resistance in congenital generalized lipodystrophy. These patients are resistant to ketoacidosis for unclear reasons. Plasma leptin and adiponectin concentrations are low, which is consistent with near total absence of body fat (8).

Molecular Basis

CGL1: *AGPAT2* Mutations

We previously found a CGL locus on chromosome 9q34 by linkage analysis of 17 pedigrees (9). We recently, recruited some additional informative families (CG 3700, 5700 and 7000). All affected members of the CG7000 family were homozygous for 3 alleles at adjacent loci, suggesting that the critical region extended from *D9S1826* to q telomere. The genotyping of the other two new pedigrees (CG3700 and CG5700) supported localization to this region. Our previous genotyping of the two affected siblings belonging to the 9q34-linked CG600 pedigree had shown discordance for the locus *D9S1838*, and we concluded that the critical region was flanked by loci *D9S1826* and *D9S1838*.

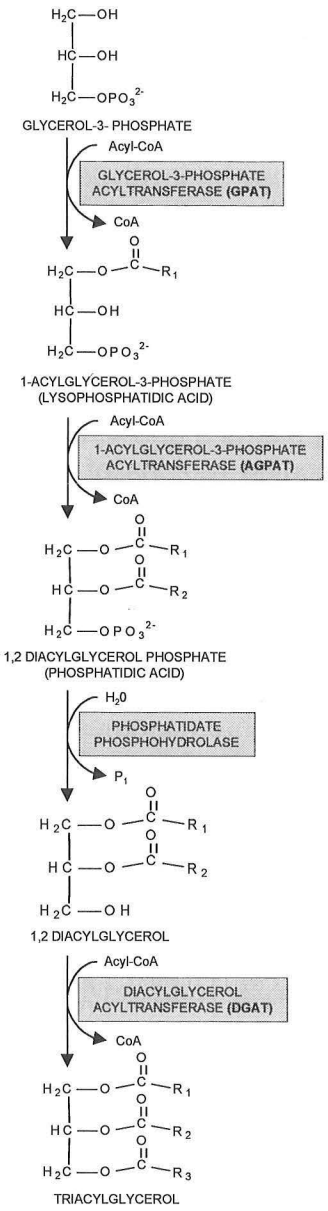
To further define the region, we genotyped 11 single nucleotide polymorphisms (SNPs) and one new polymorphic repeat in the region, in the affected individual CG7000.8 and her

parents to determine the region of homozygosity and in the two affected siblings from the CG600 pedigree for discordance. There was a recombination between the SNPs, *rs174838* and *LHX3* in the patient CG7000.8 and there was discordance for a SNP in *DPP7* between the two affected siblings from CG600. Thus, the fine mapping revealed an approximately 0.86 MB region between the SNPs, *rs1747838,* and in *DPP7* gene. Numerous known genes are located in this region. We initially selected those genes for analysis, which may be involved in adipocyte differentiation, proliferation or involved in apoptosis. These included endothelial differentiation-related factor 1 (*EDF1*), caspase recruitment domain family, member 9 (*CARD9*), Notch homolog 1, translocation-associated (*NOTCH1*), LIM homeobox protein 3 (*LHX3*) and *AGPAT2* (1-acylglycerol-3-phosphate O-acyltransferase 2, also known as lysophosphatidic acid acyltransferase-β), an enzyme involved in glycerophospholipids and triacylglycerol biosynthesis (Fig. 1 and Cover Illustration). Amplification of the exons for *AGPAT2* in all affected subjects from the pedigree CG7000 revealed a deletion spanning exons 3 and 4. Simultaneous sequencing of the entire coding regions of *LHX3*, and partial coding regions of *CARD9* and *EDF1* in 3 affected subjects from the pedigrees 7000, 400 and 600 revealed no substantial molecular alterations. Sequencing of the exons including splice-site junctions for *AGPAT2* located in this region revealed homozygous or compound heterozygous mutations in CGL patients from eleven 9q34-linked pedigrees (Table 1)(2). All affected subjects from a family had the same mutation. All the parents were heterozygous for the mutations and no unaffected sibling possessed two mutant alleles. Sequencing of the exons for 50 unrelated subjects (25 each of European and African origin) revealed no mutations. All the chromosomes carrying the IVS4-2A>G mutation in five families of African origin had the same haplotype for 7 markers extending 33 kb thus supporting a founder effect.

Table 2. Molecular Alterations in *AGPAT2* in patients belonging to CGL pedigrees and their clinical characteristics.

Pedigree	Ethnicity, Origin	Nucleotide alteration(s)	Amino acid change (s)	Status	Pt #	Age/ Sex	Body Fat (%)	Serum Leptin (ng/ml)	DM/ age of onset (y)	Insulin dose (U/d)
CG 400	African-American, US	IVS4-2A>G	Q196fsX228	Hom	6 8 9	23/F 37/F 33/M	NA 4.1* NA	NA 0.6 NA	+/NA +/17 -	- - -
CG 600	Afro-Caribbean, UK	IVS4-2A>G 683T>C	Q196fsX228 L228P	Het	5 6	15/F 13/M	7.8 1.9	0.7 0.7	+/12 -	980 -
CG 700	European descent, US	406G>A 504-505GAdel	G136R V167fsX183	Het	4	42/F	7.9	NA	+/30	-
CG 800	African-American, US	IVS4-2A>G 377-378insT	Q196fsX228 L126fsX146	Het	7 9	31/F 29/F	5.6* 0	0.7	+/13 +/12	750 300
CG 900	African-American, US	IVS4-2A>G	Q196fsX228	Hom	8	23/F	3.3	1.1	+/16	220
CG 3200	Afro-Caribbean Trinidad	IVS4-2A>G 418-420TTCdel	Q196fsX228 F140del	Het	4	3/F	NA	0.2	-	-
CG3300	European descent, US	716C>T 916C>G 3'UTR	A239V Unknown	Het	3	29/F	NA	<0.5	+/10	80
CG 3500	Hispanic, US	IVS5-2A>C	GT221-222del	Hom	6	31/F	NA	0.6	+/27	
CG 3700	European, Belgium	202C>T	R68X	Hom	3 4	17/F 15/F	17.0* 15.0*	1.1 0.8	+/11 +/9	1200 3000
CG 5700	European, Turkey	202C>T	R68X	Hom	3 4	53/F 50/M	11.6* 7.2*	1.3 0.7	+/32 +/40	100 -
CG 7000	European, Portugal	317-588del (ex 3-4 del)	G106fsX188	Hom	1 8 9 10	43/F 1/F 10/F 1/M	NA NA NA NA	NA NA NA NA	- - - -	

Het, compound heterozygote; Hom, homozygous; NA, not available; * Using dual-energy X-ray absorptiometry, Other estimates were made with hydrodensitometry; +, present; -, absent. From Agarwal et al. (2).



AGPAT2, a 278 amino acid protein, belongs to the family of acyltransferases and catalyzes an essential reaction in the biosynthetic pathway of glycerophospholipids and triacylglycerol in eukaryotes(10, 11). To our knowledge, CGL is the first documented human disease due to a genetic defect in this pathway (Fig. 1).

Fig. 1: Biosynthesis of triacylglycerol from glycerol-3- phosphate. The first step involves glycerol-3-phosphate acyltransferase (GPAT) which acylates glycerol-3-phosphate at sn-1 position to form lysophosphatidic acid. AGPAT2 catalyzes acylation of lysophosphatidic acid at sn-2 position to form phosphatidic acid. Dephosphorylation of phosphatidic acid occurs next to form 1,2 diacylglycerol, which is acylated to form triacylglycerol using diacylglycerol acyltransferase (DGAT).

The two well-characterized isoforms of AGPAT, 1 and 2 have 48% identity, the others, 3, 4 and 5 are less homologous (10-12). In these acyltransferases, two motifs, NHX_4D on exon 2, involved in catalytic function, and EGTR on exon 4, involved in substrate binding and recognition, are highly conserved(10, 13). The mutations R68X, G106fsX188 and L126fsX146 affect either one or both motifs. The two splice-site mutations retain these motifs, however, IVS4-2A>G results in an aberrant and truncated protein and IVS5-2A>C deletes two amino acids and may disrupt secondary structure of the protein as may the mutant F140del. The three missense mutations, L228P, A239V and G136R produce nonconservative amino acid changes at positions conserved between the human and mouse proteins.

AGPAT1 is ubiquitously expressed in human tissues with the highest levels of expression in the skeletal muscle(10) whereas AGPAT2 expression is more tissue restricted with high levels in the liver and heart tissues but almost undetectable in the brain(12, 14). Amplification of all *AGPAT* isoforms in normal human omental adipose tissue revealed at least two fold higher *AGPAT2* expression than *AGPAT1* (Fig. 2). *AGPAT2* was expressed at lower level in the liver and much less in the skeletal muscle. Other isoforms were barely detectable.

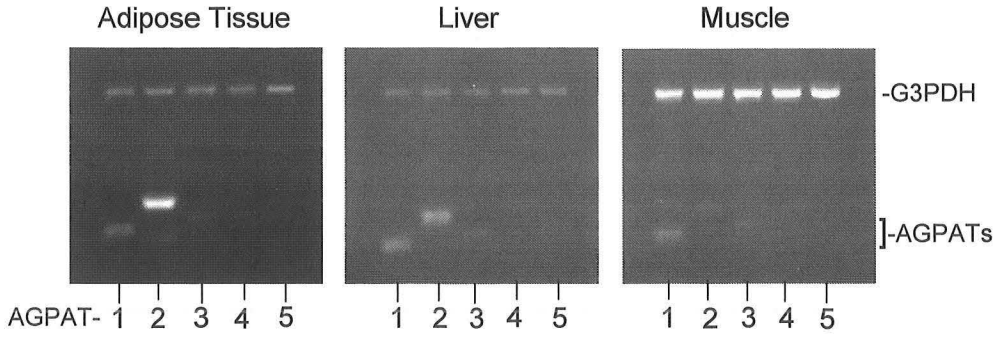


Fig. 2 Expression of *AGPATs* in human tissues. Gel images show that in the adipose tissue, *AGPAT2* was expressed 2-fold more than *AGPAT1*. In the liver, the expression of both *AGPAT1* and *2* was the same and in the skeletal muscle, *AGPAT1* was expressed 1.8-fold more than *AGPAT2*. The other isoforms, *AGPAT3, 4*, and *5* were barely detectable. The results were normalized to the signal generated from *G3PDH*.

The expression pattern of various *AGPATs* suggests that the aberrant AGPAT2 enzyme is more likely to affect triacylglycerol synthesis in the adipose tissue and may cause lipodystrophy by resulting in "triglyceride-depleted adipocytes". It is also likely that reduced AGPAT2 activity could increase tissue levels of lysophosphatidic acid, which may affect adipocyte functions. Lysophosphatidic acid is a ligand for G protein coupled receptors and may have a role in preadipocyte proliferation and adipogenesis (15). Decreased AGPAT2 activity could also lead to reduced bioavailability of phosphatidic acid and phosphoglycerols (phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine), which are important in intracellular signaling and could affect adipocyte functions(16).

CGL2: *BSCL2* Mutations

Our previous linkage study had provided evidence for at least two CGL loci: CGL1 on human chromosome 9q34, and one other, CGL2, as yet unmapped. Recently, Magre and colleagues (17) mapped CGL2 locus to chromosome 11q13. They found that the gene *BSCL2* was mutated in patients with CGL. Various mutations including, major deletions, small insertions and deletions and single nucleotide substitutions were described in the CGL families. Several CGL patients of the Lebanese descent had a homozygous five-nucleotide deletion in exon 4 (659-663delGTATC resulting in F105fsX111) and several Norwegian patients had a homozygous missense mutation (G978C resulting in A212P). This gene encodes a protein, seipin, of 398 amino acid of unknown function with a high homology with the mouse guanine nucleotide-binding protein γ3 subunit-linked gene (Gng3lg). *BSCL2* is expressed variably in many tissues, with highest expression in the brain and testis. Based on high expression of *BSCL2* in the brain and weak expression in adipocytes, a primary defect in the hypothalamic-pituitary axis has been suggested. We have found BSCL2 mutations in seven CGL pedigrees. Interestingly, all CGL patients of Lebanese descent show the same homozygous mutation suggesting a strong founder effect. We found the same mutation (669insA) in a CGL patient of Portuguese origin as described earlier. The phenotypic differences between the patients with AGPAT2 and BSCL2 mutations remain to be elucidated. However, CGL patients with *BSCL2* mutations seem to have mild mental retardation and cardiomyopathy, which are not seen in our patients with *AGPAT2* mutations.

Table 3. Molecular Alterations in *BSCL2* in patients belonging to CGL pedigrees and their clinical characteristics.

Pedigree	Ethnicity, Origin	Nucleotide alteration(s)	Amino acid change (s)	Status	Pt #	Age/ Sex	Body Fat (%)	Serum Leptin (ng/ml)	DM/ age of onset (y)	Insulin dose (U/d)
CG 1000	Pakistani, Pakistan	500insTT	F53fsX93	Hom	22 24	15/F 5/F	NA NA	NA NA	+/11 -	-
CG 1100	Chinese, US	1126insG unidentified	G271fsX283	Het	3	5/F	NA	0.38	-	
CG 3800	White, Portugal	669insA	F108fsX113	Hom	5	10/F	NA	0.05	+/30	-
CG 4100	Lebanese, US	659delGTATC	F105fsX111	Hom	4	19/M	NA	0.12	+/	-
CG 4700	Lebanese, US	659delGTATC	F105fsX111	Hom	12 17 18	32/M 4/F 5/M	NA	NA	- - -	
CG 6800	Lebanese, US	659delGTATC	F105fsX111	Hom	3	18/M	3.6	0.12	+/9	90
CG 6900	Lebanese, US	659delGTATC	F105fsX111	Hom	3	19/M	NA	0.11	NA	

Other CGL subtypes:

Out of a total of 44 pedigrees of various ethnicities, we have identified disease-causing mutations in 26 with *AGPAT2* mutations and 7 with *BSCL2* mutations. Eleven pedigrees do not show mutations in any of the two genes and therefore it is very likely that CGL may be caused by disruption of at least one or more genes. These observations suggest that at least two distinct mechanisms may underlie extreme lack of adipose tissue in CGL patients.

Familial Partial Lipodystrophy, Dunnigan Variety (FPLD)

This autosomal dominant disorder (OMIM # 308980) has an estimated prevalence of less than 1 in 15 million. Most of the affected subjects are of European origin, although we have described an Asian Indian and an African American pedigree.

Clinical features:

All affected subjects have normal adipose tissue distribution during childhood. With the onset of puberty, subcutaneous adipose tissue is lost from the extremities, giving rise to the characteristic appearance of "increased muscularity". A variable loss of fat occurs from the trunk. Subsequently, patients may develop a double chin, excess supraclavicular fat, and a round face. Acanthosis nigricans, hirsutism, menstrual abnormalities, and polycystic ovaries are observed infrequently. Although the phenotype can be recognized easily in affected women, affected men have been previously under-reported because of difficulty in recognizing the "increased muscularity" phenotype. Furthermore, men may not be as severely affected with metabolic complications as women, thus escaping recognition.

Adipose tissue distribution

MRI studies have reported a similar body fat distribution in both sexes, characterized by a marked paucity of subcutaneous fat in all extremities but an apparent increase in intermuscular adipose tissue(18). The loss of subcutaneous truncal fat was more evident anteriorly than posteriorly. Excess fat was evident in the neck, face, and submental areas. Intra-abdominal and intra-thoracic fat was not reduced. Bone marrow fat and mechanical adipose tissue were present in normal amounts.

Metabolic derangements

Affected patients develop diabetes, usually after age 20. Patients have high serum triglycerides and low serum HDL cholesterol concentrations. As a result, they are predisposed to develop chylomicronemia and acute pancreatitis. Elevation of fasting plasma free fatty acid concentrations and lack of adequate suppression after an oral glucose load have been reported. The insulin-mediated glucose disposal rate is markedly reduced (19).

There is gender dimorphism in disease severity(20). We found that compared to the affected men, women had higher prevalence of diabetes (18% and 50%, respectively; P=0.05) and atherosclerotic vascular disease (12% and 45%, respectively; P=0.04); and had higher serum triglycerides (median values 2.27 and 4.25 mmol/L, respectively; P=0.02) and lower high-density lipoprotein cholesterol concentrations (age-adjusted means 0.70 and 0.94 mmol/L, respectively; P=0.04). The prevalence of hypertension, and fasting serum insulin

concentrations were similar. Thus, women with FPLD are more severely affected with metabolic complications of insulin resistance than men.

Molecular Basis

Using genome-wide linkage analysis in 5 well-characterized families, we were the first to find a locus for FPLD on chromosome 1q21-22 (21). Cao and Hegele(22) found a mis sense mutation in lamin A/C gene (*LMNA*), R482Q, in five Canadian probands. Subsequently, our group and others reported missense mutations in the gene in affected subjects, both sporadic and belonging to various pedigrees(23, 24). Most of the mutations (G465D, R482Q, R482W, R482L and K486N) lie within exon 8 of *LMNA* gene and affect both lamin A and C splice forms.

LMNA gene:

This gene encodes lamins A and C, which are components of the nuclear lamina; a polymeric structure intercalated between chromatin and the inner membrane of the nuclear envelope. The coding region spans ~ 24 kilobases and contains 12 exons (Fig. 3). Alternative splicing within exon 10 gives rise to two different mRNAs that code for prelamin A and lamin C(25). Human lamins A and C are identical for the first 566 amino acids(26). Lamin C has 6 unique carboxy-terminal amino acids and lamin A has 80 unique carboxy-terminal amino acids. Usually, alternative splicing produces approximately equal amounts of the two respective mRNAs within the same cell. However, different splice variants of lamin A may be expressed at different levels depending on the cell types(27). Prelamin A has a CAAX box at the carboxy terminus, which undergoes isoprenylation, specifically farnesylation, which is, required for conversion of prelamin A to lamin A. In contrast, lamin C does not undergo isoprenylation. A specific prelamin A endoprotease (zinc metalloproteinase STE24; ZMPSTE24) cleaves carboxy terminus 18 amino acids to from lamin A from prelamin A(28).

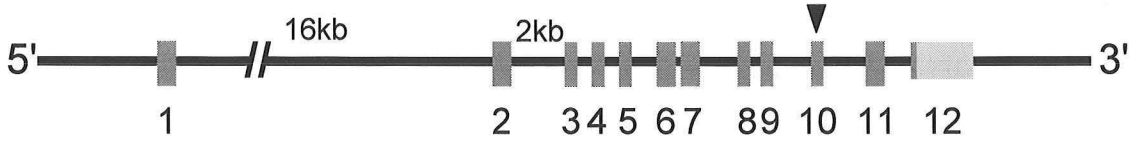


Fig. 2 Lamin A/C (*LMNA*) gene structure. Numbers under the boxes denote the exons and the arrow indicates the site at which alternate splicing occurs to form Lamin A or C.

LMNA is a member of the intermediate filament multigene family and lamins A and C have primary and secondary structures similar to cytoplasmic intermediate filament proteins. Two other lamins, B1 and B2 are products of two different genes. These proteins have a central α-helical rod, an amino-terminal head, and carboxy terminal tail domains(26). They dimerize through their rod domain to form 10-nm diameter filaments and bind and assemble on the surface of mitotic chromosomes at specific sites on the rod(26, 29). Both homodimers and heterodimers can form between various lamins i.e. A, B1, B2 and C(30). Lamins B1 and B2 are expressed widely in tissues whereas lamins A and C are expressed mainly in differentiated non-proliferating cells. Lamins A and C bind to DNA, histones and retinoblastoma gene product (which controls cell cycle and gene expression) and thus play a role in organization of DNA transcription in cells. Lamins B bind to nuclear membrane and are associated with replicating chromatin in mammalian cells. Lamins A and C also associate with integral proteins of the nuclear envelope such as lamin associated polypeptides (LAPs) 1A and 1B and emerin whereas lamins B associate with LAP1 and LAP2 and lamin B receptor(31).

Genotype-Phenotype Relationship

We found mutations within exon 8 of *LMNA* (R482Q, R482W and G465D) in 12 families with typical FPLD and in exon 11 (R582H) in one atypical family(23). To investigate phenotypic heterogeneity, we compared body fat distribution using anthropometry and whole body magnetic resonance imaging, and metabolic parameters in women with atypical and typical FPLD. Compared to women with typical FPLD, the two sisters with atypical FPLD had less severe loss of sc fat from all the extremities and trunk, and particularly from the gluteal region and medial parts of proximal thighs. Both types had similar excess of fat deposition in the neck, face, intra-abdominal and intermuscular regions. Women with atypical FPLD tended to have lower serum triglyceride and higher high-density lipoprotein cholesterol concentrations(32). Since, exon 11 of *LMNA* does not comprise part of the lamin C coding region; R582H mutation can affect lamin A protein only. Therefore, we proposed that the unique phenotype of atypical FPLD with exon 11 mutation may result from disrupted interaction of lamin A with other proteins and chromatin compared to typical FPLD, in which interaction of both lamins A and C may be disrupted.

Interestingly, other mutations in *LMNA*, mostly missense, have been reported in patients with dilated cardiomyopathy and conduction-system disease (Mendelian Inheritance in Man # 115200) (33, 34)and in patients with autosomal dominant varieties of muscular dystrophies: autosomal dominant Emery-Dreifuss muscular dystrophy (Mendelian Inheritance in Man # 181350) and limb girdle muscular dystrophy (Mendelian Inheritance in Man # 159001)(35-38). In patients with dilated cardiomyopathy and conduction-system disease, the missense mutations affect the rod domain of lamin A/C(33, 34), whereas patients with autosomal dominant Emery-Dreifuss muscular dystrophy harbor different missense alterations mostly in the globular C-terminal portion. Mutations in patients with limb girdle muscular dystrophy affect both the rod and globular C-terminal tail region.

We have recently identified two novel missense mutations in exon 1 of the lamin A/C gene in two pedigrees with FPLD(39). One mutation, R28W (CGG→TGG), affected the amino terminal head domain and the other, R62G (CGC→GGC), affected the α-helical rod domain. Affected subjects from both the families had an increased prevalence of cardiac manifestations such as atrioventricular conduction defects, atrial fibrillation, pacemaker implantation and congestive heart failure due to ventricular dilatation. Our findings, therefore, in these two families with FPLD who also had cardiac conduction system defects and other manifestations related to cardiomyopathy and possibly mild muscular dystrophy support the existence of a multisystem dystrophy syndrome in patients with mutations in lamin A/C gene (Fig. 4). The severity and age of onset of these manifestations may differ depending upon the site of missense mutations. For example, in our patients with FPLD, cardiac manifestations occurred late and muscular involvement, if anything was mild. Similarly, only mild muscular manifestations were reported in patients with cardiomyopathy due to *LMNA* mutations. It is also possible that patients with *LMNA* mutations and cardiomyopathy or muscular dystrophies develop lipodystrophy but it may be mild, occurs later in life, and thus may not be recognized clinically. In future, systematic studies of body fat distribution and prevalence of metabolic disorders related to insulin resistance in patients with cardiomyopathy and muscular dystrophies and *LMNA* mutations may provide insights into the occurrence of lipodystrophy.

Table 4. Comparison of clinical characteristics and prevalence of coronary heart disease, its risk factors and cardiac conduction defects and other manifestations related to cardiomyopathy in affected adults with familial partial lipodystrophy, Dunnigan type due to exon 1 and 8 mutations of the *LMNA* gene.

	LMNA exon 1 Mutations Pedigrees = 2		LMNA Exon 8 Mutations Pedigrees = 8		P value
	N	%	N	%	
M/F	6/6		14/23		0.29
Age, y, (Mean ± SD)	48 ± 14		42 ± 16		0.48
Body mass index, kg/m^2 (Mean ± SD)	23.1 ± 2.7		25.3 ± 3.6		0.33
Coronary heart disease	4/9	44	7/37	19	0.26
Diabetes Mellitus	2/9	22	14/37	38	0.42
Lipid Lowering Drugs	2/8	25	11/37	30	0.69
Hypertension	4/8	50	13/37	35	0.59
Acanthosis nigricans	2/8	25	12/34	34	0.59
Alcohol	1/8	12.5	15/35	43	0.68
Smoking	1/8	12.5	16/35	46	0.51
Conduction system disease	5/11	45	0/37	0	0.003
Atrial Fibrillation	5/11	45	0/37	0	0.003
Pacemaker Implantation	4/12	33	0/37	0	0.003
Congestive Heart Failure	5/10	50	1/37	3	0.02

P values are from Wilcoxon rank sum test comparing families or from analysis of variance adjusting for clustering within families. From Garg et al. (39).

How LMNA mutations cause multisystem dystrophy?

Several questions remain to be answered about how autosomal dominant *LMNA* variants cause FPLD. Recently, the structure of C-terminal domain was recently identified as a new variant of immunoglobulin fold and FPLD mutations in exon 8 were show to segregate to a localized surface of this domain (40). However, this observation fails to explain how mutations in exons 1 and 11 cause FPLD. Interestingly, expression of mutant alleles of *LMNA* associated with EDMD and CMD1A in HeLa cells and in fibroblasts derived from *Lmna* null mice disrupt nuclear architecture and lamina organization, and redistribute emerin to the endoplasmic reticulum(41). However, mutation associated with FPLD had no such effects. Thus, disruption of ER function and cell death due to nuclear membrane breakdown may contribute to EDMD and CMD1A phenotype and possibly to FPLD phenotype as well. Vigoroux et al. (42) however showed several structural changes in nuclear architecture such as abnormal blebbing nuclei and disorganized peripheral meshwork formed with lamin A/C in fibroblasts from patients harboring R482Q and R482W mutations . Another group reported that upon transfection of C2C12 myoblasts with cDNA encoding mutant *LMNA* cause aberrant localization of lamin A/C, partially disrupt the endogenous lamina and alter emerin localization. However, LMNA mutation associated with FPLD did not cause these changes (43).

It is likely that mutations causing FPLD may disrupt interaction of lamin A with other proteins. Recently, lamin A was found to interact with sterol regulatory element binding proteins (SREBP) 1 and 2 (43a). It was postulated that reduced activity of SREBP1 in adipocytes may be responsible for causing FPLD. The mechanisms underlying the regional differences in fat loss in FPLD are not well understood. Lelliott et al. recently studied mRNA expression of lamin A and C in adipocytes from omental, sc abdominal and neck sites and found no differences in depot-specific expression (44).

The underlying mechanisms by which *LMNA* mutations result in multisystem dystrophy syndrome remain to be elucidated. It is possible that haploinsufficiency or a dominant negative effect caused by mutations in lamin A/C can alter the nuclear lamina and the nuclear

envelope, eventually resulting in cell death (45). Lamin organization may be important for the elongation phase of DNA replication (46), which may be disrupted due to gene alterations. Although type A lamins are present in the nuclear envelope of most somatic cells, it seems that tissues derived from mesenchymal stem cells such as cardiac and skeletal muscle, fat and tendons are affected in those with mutations in lamin A/C gene (31). Whether multisystem dystrophy syndrome involves other mesenchymally derived tissue such as bone and cartilage is not known. Mice with a targeted homozygous deletion of *Lmna* gene, extending from exon 8 to middle of exon 11 show evidence of multisystem dystrophy (47). They developed myocardial and muscular dystrophy and lacked white adipose tissue. However, recent data suggests that neither homozygous nor heterozygous *Lmna* ablated mice exhibit aspects of the phenotype seen in humans with FPLD(48). The adipose tissue deficiency in homozygous *Lmna* -/- mice is probably due to their muscular dystrophy and reduced energy intake.

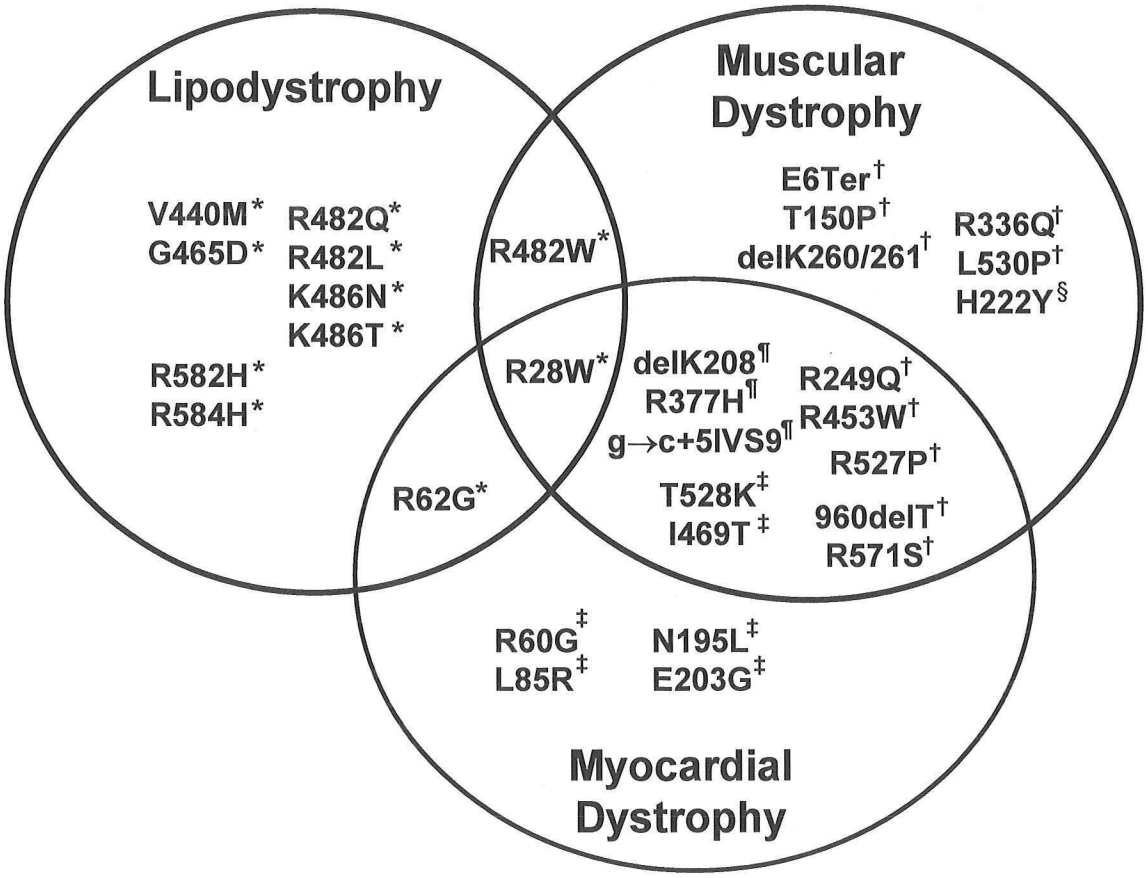


Fig. 4 Evidence of multisystem dystrophy involving adipose tissue, myocardium and skeletal muscle in patients with mutations in *LMNA* gene. Patients shown were initially ascertained as either *familial partial lipodystrophy, Dunnigan type, ‡cardiomyopathy, †autosomal dominant Emery-Dreifuss muscular dystrophy, ¶limb girdle muscular dystrophy or §congenital muscular dystrophy. As shown, in several affected subjects there is evidence of multisystem dystrophy. Many patients with autosomal dominant Emery-Dreifuss muscular dystrophy and limb girdle muscular dystrophy have cardiac manifestations due to myocardial dystrophy and some patients with cardiomyopathy have mild muscular dystrophy. Both of our pedigrees with familial partial lipodystrophy, Dunnigan type with R28W and R62G mutations had evidence of myocardial dystrophy, with mild muscular dystrophy in one of them. From Garg et al.(39)

Familial Partial Lipodystrophy (*PPARG* mutation)

Recently, we reported a heterozygous mutation C to T at nucleotide 1273 in exon 6 of *PPARG* in a patient with familial partial lipodystrophy (FX200.21) (Fig. 5)(49). This patient had no mutation in the coding region of *LMNA*. This mutation changes codon CGC to TGC causing an arginine to cysteine (R425C) substitution. DNA samples of four unaffected family members did not reveal this mutation (Fig. 5); although two of them, FX200.9 and FX200.17, had diabetes mellitus but no hypertriglyceridemia. This mutation was absent from 96 normal alleles.

The proband is a 64-year-old woman who developed diabetes mellitus and hypertriglyceridemia at the age of 32 years. She received oral hypoglycemic drugs for 10 years and subsequently insulin therapy. She noted gradual loss of sc fat from the arms and legs resulting in prominence of sc veins in the extremities at the age of 50 years. She also noted increased body hair. She had borderline hypertension. She had irregular menstruation for several years after menarche at age 18 years but subsequently had regular menstruation till menopause at age 51 years. She did not desire pregnancy and had no children. Physical examination revealed excess terminal hair on the arms, forearms, legs, upper lip and chin. She had no acanthosis nigricans or virilization. She had marked loss of sc fat from the upper and lower extremities and the face but truncal region, palms and soles were spared. She had no excess fat deposition in the neck.

Skinfold thickness measurements revealed marked loss of sc fat from the peripheral sites (biceps, triceps, forearm, thigh and calf skinfolds; all $\leq$ 6 mm) but excess sc fat at the truncal sites (mid-axillary, abdominal, subscapular, and suprailiac skinfolds range 24-38 mm). Whole body MRI revealed loss of sc fat in the upper and lower extremities, particularly distally in the forearms and calves. She had hypertriglyceridemia, low high-density lipoprotein cholesterol but normal serum leptin levels.

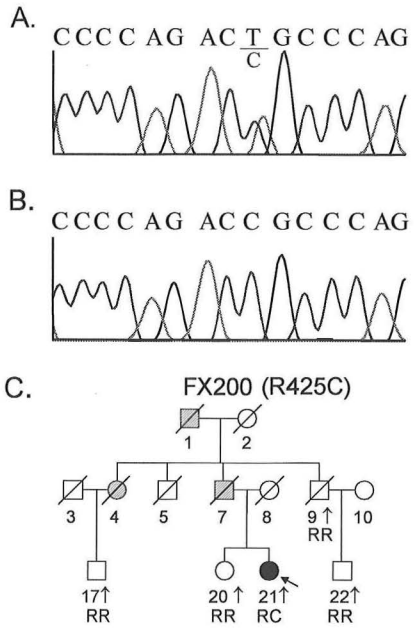


Fig. 5. A. Sequence analysis of the *PPARG* gene illustrating a heterozygous C to T mutation at nucleotide 1273 in the patient FX200.21. B. Sequence of a healthy subject showing presence of a homozygous C at nucleotide 1273. A, C, G, and T are the different nucleotides. C. FX200 Pedigree. Each member is numbered for identification. Squares and circles indicate males and females, respectively. The proband (affected subject) is shown as filled black symbol and by an inclined arrow, unaffected subjects as unfilled symbols and those with uncertain phenotype as gray symbols. Deceased individuals are indicated by a diagonal line and individuals for whom DNA was available by ↑. Patient with R425C mutation is shown as RC under the symbol whereas the wild type is shown as RR.

The transmission is consistent with autosomal dominant inheritance although the mutation may have appeared *de novo* in the proband. However, we could not confirm it since DNA from our subject's parents was not available. The *PPARG* arginine 425 is a highly conserved residue between species. To elucidate the functional aspects of this mutation, we examined the X-ray crystallographic structure of PPARγ. The crystal structure of PPARγ1 ligand binding domain consists of 13 alpha helices and a small four-stranded beta-sheet. Helices H4, 5, and 8 are tightly packed between helices H1, 3, 7 and 10 at top half of the ligand-binding domain. The residue arginine 425 (corresponding to residue 397 of PPARγ1) lies in the loop 9-10 and likely forms a salt bridge with glutamic acid at position 352 (residue 324 of PPARγ1) located in helix 6 that

keeps PPARγ protein in proper configuration. This tertiary configuration is lost when arginine 425 is mutated to cysteine.

Previously, besides the polymorphisms, P12A and H449H, in *PPARG* gene (50) only three missense mutations, P115Q, P467L and V290M have been described (51, 52). Ristow *et al.* (51) described P115Q mutation in three markedly obese men with diabetes and one obese female. Barroso *et al.* (52) reported *PPARG* P467L mutation in a 55-year-old woman and her 31-year-old son and V290M mutation in a 15-year-old girl. All three had insulin resistance, diabetes and hypertension. Although, the authors stated that these patients did not have any evidence of lipoatrophy or abnormal body fat distribution (52), on subsequent MRI studies some adipose tissue depots were found to be attenuated.

Our patient with *PPARG* R425C mutation had loss of sc fat not only from the extremities but also from the face and neck. The patients described by Kobberling *et al.* (53, 54) were characterized by complete absence of sc fat from the arms and legs. In contrast, sc fat was normal on the face and some patients showed moderate truncal obesity. The index case was a 24-year-old woman with diabetes mellitus since age 11 years and marked hyperlipidemia and hepatomegaly (53). The onset of lipodystrophy occurred during childhood. Her 54-year-old mother and 21-year-old sister were reported to have body habitus identical to her but had normal glucose tolerance and only mild hypertriglyceridemia. Two other women, 69- and 59-year-old with diabetes mellitus, hyperlipidemia and mild hepatomegaly were also reported (54). Subsequently, a 64-year-old woman with diabetes mellitus and severe hypertriglyceridemia and her daughter were reported to have similar type of lipodystrophy(55). Thus, our patient may have a unique type of FPL, distinct from FPLD and Kobberling variety.

PPARγ has been shown to be essential for adipogenesis (56). A PPARγ null pup recovered through tetraploid rescue exhibited striking absence of all types of adipose tissue and a severely fatty liver (57). Heterozygous PPARγ-deficient mice have been reported to have smaller size of adipocytes and reduced fat mass(58, 59). These knockout mouse models support our results that *PPARG* R425C mutation could be the molecular basis for one of the FPL phenotypes. The mechanisms by which the *PPARG* mutation causes loss of peripheral sc fat but spares truncal fat are still unclear.

Familial Partial Lipodystrophy, Köbberling variety

In this variety, the loss of adipose tissue is restricted to the extremities(53-55). Patients have normal amounts of facial fat and may even have excessive amounts of subcutaneous truncal fat. Previously published reports are limited to a few affected women from 2 small pedigrees and 4 sporadic cases. Most of the affected patients had hypertriglyceridemia and diabetes mellitus. The age of onset, mode of inheritance and diagnostic features of adipose tissue distribution are not known. Although, all patients described thus far are women, it cannot be concluded that men are exempt. Whether the Köbberling variety is a distinct entity or a variant of the Dunnigan phenotype is unknown.

Familial Partial Lipodystrophy, Mandibuloacral Dysplasia variety

Mandibuloacral dysplasia (OMIM # 248370) is an autosomal recessive condition characterized by mandibular and clavicular hypoplasia, acroosteolysis, delayed closure of cranial sutures, joint contractures, mottled cutaneous pigmentation, short stature, high pitched voice, dental abnormalities and ectodermal defects, such as skin atrophy, alopecia, and nail dysplasia. We have recently reported that it is associated with two distinct types of

lipodystrophies(60). Three of our four subjects had loss of sc fat from the extremities with normal or slight excess in the neck and truncal regions (termed Type A lipodystrophy pattern). In contrast, one patient had generalized loss of sc fat involving the face, trunk and extremities (Type B lipodystrophy pattern). All the patients had normal glucose tolerance but had fasting and postprandial hyperinsulinemia suggestive of insulin resistance. Elevated serum triglycerides with low high-density lipoprotein cholesterol levels were noted in three subjects. Thus, both types of lipodystrophies associated with MAD accompanied by insulin resistance and its metabolic complications.

Table 5. Clinical features of our patients with MAD and previously reported cases.

Features	Patient ID				Previous cases (n = 34)¶
	100.3	100.4	300.4	400.5	
Age (y)	20	16	12	25	25.5 ± 16.5†
Sex	F	F	M	M	21 M, 12 F*
BMI (kg/m^2)	18.6	21.1	22.9	16.8	17.1 ± 4†‡
Age of onset (y)	5	4	ND	7	4.3 ± 2.5†
Growth retardation	+	+	-	+	25/28
Micrognathia	+	+	+	+	28/29
Bird like facies	+	+	-	+	26/29
Clavicular hypoplasia	+	+	+	-	25/30
Acroosteolysis	+	+	+	-	27/30
Wide cranial sutures	-	-	ND	ND	22/24
Skin atrophy	+	+	-	+	24/26
Mottled hyperpigmentation	+	+	+	+	22/26
Joint contractures	+	+	+	-	22/28
Peripheral fat loss	+	+	+	+	15/16
Excess fat over the neck	+	+	+	-	3/15

+, present; -, absent; ND, not determined; BMI, body mass index; M, male; F, female; † Mean ± SD; * one case report (18) did not mention sex of the patient; ‡ n = 22 subjects; ¶ numerator signifies number of subjects with the clinical feature out of total number of subjects shown in denominator. From Simha and Garg (60).

Familial Partial Lipodystrophies, other varieties

There is a possibility of other unique types of familial lipodystrophies. For example, an autosomal dominant variety of generalized lipodystrophy with acromegalic features and onset after 18 years of age was recently reported in a pedigree from Brazil (61). In another pedigree under evaluation by me, the proband had marked loss of subcutaneous fat from the extremities, face, palms and soles, but had excessive subcutaneous fat in the neck and trunk. Her father and paternal uncle also have loss of fat from the extremities.

SHORT Syndrome associated Lipodystrophy

The SHORT syndrome with the clinical manifestations of ***s***hort stature, ***h***yperextensibility of joints and/or inguinal hernia, ***o***cular depression, ***R***eiger anomaly and ***t***eething delay was described by Gorlin et al.(62) and Sensenbrenner et al. (63) (OMIM # 269880 and 151680). Reiger anomaly constitutes eye abnormalities such as hypoplasia of iris stroma, prominent Schwalbe ring, iridocorneal synechiae, micro or megalo cornea, strabismus, and predisposition to glaucoma; and tooth abnormalities such as hypodontia, microdontia, enamel hypoplasia and atypically shaped and positioned teeth. Other clinical features may include intrauterine growth retardation with slow post natal weight gain, delayed speech development with normal intellect, frequent childhood illnesses, small head circumference, bilateral clinodactyly and sensorineural hearing loss. Patients also have

distinct facial abnormalities like disproportionately small and triangular face, micrognathia, sunken eyes, wide nasal bridge with hypoplastic ala nasi, chin dimple, prominent pinnae, inguinal hernia, systolic ejection murmur and partial lipodystrophy. Infrequently, patients may have bilateral symmetrical lens opacities and insulin resistant diabetes mellitus. To date, about 22 patients with SHORT syndrome have been reported in the literature.

Lipodystrophy is another common feature of this syndrome, and typically affects the face, upper extremities and sometimes the trunk, with relative sparing of the lower extremities. This syndrome was initially described as an autosomal recessive condition, but Aarskog et al (64) described a family with four affected members spread over three generations suggestive of autosomal dominant inheritance. These patients differed from other typical patients with the SHORT syndrome by not having joint hyperextensibility. Further the pattern of fat loss was different, involving only the face and patchy areas of the buttock and elbows. Similar features and mode of inheritance were noted in an Australian family described by Bankier et al(65). However, Sorge et al.(66) reported a 9-year-old boy with the typical features of SHORT syndrome including hyperextensibility and lipodystrophy of the trunk and upper extremities with an autosomal dominant mode of inheritance. The genetic basis remains to be determined.

Neonatal progeroid syndrome (Wiedemann-Rautenstrauch syndrome)

This syndrome was first described by Rautenstrauch and co-workers in 1977 (67) in two sisters with congenital malformations reminiscent of progeria. In 1979, Wiedemann defined a new progeroid syndrome based on his two personal observations and the earlier report (68). To date, nine other cases with this rare syndrome have been reported.

The syndrome is characterized by a progeroid face (triangular, old-looking face with relatively large skull, prominent veins especially of the scalp, sparse scalp hair, large anterior fontanelle) and nearly total absence of sc fat (giving the clinical appearance of prominent veins and muscles). SC fat, however, is evident in the gluteal area. These features are apparent at birth and therefore this syndrome needs to be differentiated from congenital generalized lipodystrophy. Inheritance seems to be autosomal recessive.

MULTIPLE SYMMETRIC LIPOMATOSIS (mtDNA mutations)

This syndrome is characterized by a symmetrical, progressive growth of nonencapsulated sc adipose tissue primarily in the neck (bull neck with buffalo hump and double chin), supraclavicular and shoulder regions. Fat may also accumulate in the trunk and proximal limbs but the distal arms and legs are spared. Laryngeal, tracheal or vena caval compression may occur rarely by deep lipomatous infiltration in the neck and mediastinum. Many patients also have peripheral neuropathy. Only some patients have hypertriglyceridemia and hyperuricemia. Serum HDL cholesterol levels are usually elevated and diabetes mellitus has not been reported.

Although most of the patients have a preceding history of heavy ethanol intake, an inherited condition has been described in a few patients with MERRF (myoclonic epilepsy with ragged red fibers). Besides myoclonic epilepsy and myopathy characterized by the presence of ragged red fibers, this disease is associated with a wide variety of other features such as action myoclonus, ataxia, deafness, optic atrophy, peripheral neuropathy and dementia. The affected patients had mitochondrial DNA mutations A8344G and G8363A and deletions in the $tRNA^{Lys}$. However, most of these patients had multiple, discrete and encapsulated lipomas distributed symmetrically over the nape of the neck, the interscapular

and suprascapular area (69-75). This is unlike typical patients of multiple symmetric lipomatosis in whom adipose tissue growth is not encapsulated and insinuates along fascial planes. Recently, a patient with MSL and the A8344G mutation was reported to have Wernicke's encephalopathy, suggesting that mitochondrial abnormalities may call for higher thiamine requirements to compensate for the impairment in energy production machinery(76).

MONOGENIC OBESITY

Several single gene mutations underlying obesity syndromes in humans have been identified recently (Table 6). In all reported cases gross abnormalities of brain structures were excluded.

Leptin (*LEP*) gene mutations

An 8-year-old girl and her 2-year-old male cousin from a highly consanguineous family of Pakistani origin presented with early onset (at 4 and 3 months, respectively) severe obesity and hyperphagia(77). Serum leptin levels were very low. Fasting plasma glucose concentration was normal in both, but fasting plasma insulin level was elevated in the girl. Slightly elevated TSH levels were noted in both patients, whereas gonadotropin and gonadal hormone levels were appropriate for prepubertal age. Both patients were homozygous for a single nucleotide deletion at position 398 of the leptin gene. This mutation resulted in a frame-shift of the leptin-coding region after Gly132 and a premature termination of the peptide synthesis. All of their parents and one of the four siblings were heterozygous for this mutation. The mothers were moderately obese with borderline low serum leptin levels. The other three siblings had normal leptin alleles. This study established relevance of leptin in regulating energy balance in humans.

A homozygous single nucleotide transversion in leptin gene resulting in Arg→Trp substitution in the mature peptide and low serum leptin levels has also been discovered in three other morbidly obese individuals, including three adults, from a highly consanguineous family (78). They were hyperinsulinemic, and an affected woman was hyperglycemic. The affected woman also had primary amenorrhea. Other clinical features include hypothalamic hypogonadism, alterations of growth hormone and parathyroid hormone and calcium homeostasis, dysfunction of the sympathetic nervous system, amenorrhea and frequent infections(79). These findings suggest a role of leptin in the initiation of puberty and reproduction in humans. The other family members studied were either heterozygous for the mutation or had normal alleles.

In addition, heterozygous missense leptin gene mutations have been reported in two unrelated obese Finnish men, both of whom had low serum leptin levels(80). Since relevant information regarding inheritance of the genotypes and phenotypes are not available, whether these mutations were the cause of obesity in these men is unclear. Interestingly, the heterozygotes for leptin gene mutation from the first two pedigrees all had normal leptin levels for their body fat mass (77, 78). Thus, whether heterozygous mutations in leptin gene can cause obesity in humans remains to be determined.

Leptin receptor (*LEPR*) mutation

Three morbidly obese sisters (age 13-19 y) from a consanguineous family were noted to have markedly high serum leptin levels (81). They had developed hyperphagia and

severe obesity within a few months after birth. Emotional lability and social disability, but not mental retardation, were also noted. These patients also had hypogonadotropic hypogonadism and failure of pubertal development, hypothalamic hypothyroidism, mild growth delay and subnormal growth hormone response to hypoglycemia.

The three subjects were homozygous for a single nucleotide substitution at a splice site of exon 16 of the *LEPR* gene. This mutation produced a protein of 831 amino acids (OB-Rhd), lacking both the transmembrane and intracellular domains. Both parents and 4 siblings who were heterozygous for the mutation had normal or slightly increased body fat, suggesting autosomal recessive inheritance for this disorder. Unlike *db/db* mice, however, the patients with *LEPR* mutation had normal fasting and postprandial blood glucose, insulin and lipids levels and normal hypothalamic-pituitary-adrenal axis.

3. **Prohormone convertase 1 (*PC1*) gene mutation**

The proband for *PC1* gene mutation was a 43-year-old, moderately obese woman with a history of severe childhood obesity (82). She also had impaired glucose tolerance, postprandial hypoglycemia, hypogonadotropic hypogonadism, hypocortisolism, elevated plasma proinsulin and POMC concentrations and very low plasma insulin concentration. Serum leptin concentration was appropriate for body mass index.

She was a compound heterozygote for two mutations in PC1 gene. A G→A substitution in exon 13 resulted in a Gly→Arg missense mutation at position 483 in the PC1 peptide. This mutation abolished the autocatalytic cleavage ability of PC1 in the endoplasmic reticulum, resulting in reduced production of mature and functional PC1. The second mutation, an A→C substitution, occurred at a donor splice site in intron 5 of PC1 gene resulting in a frame shift and premature termination in the catalytic domain of PC1. All of her four children were heterozygous for one of the two mutations, but they were clinically normal, suggesting an autosomal recessive inheritance. Processing of POMC by PC1 enables the formation of melanocortins, including α-MSH. Thus, α-MSH deficiency due to reduced production may be the cause of obesity in this patient. The other clinical features may be related to diminished processing of proinsulin, POMC and precursor of gonadotropin-releasing hormone.

4. **Proopiomelanocortin (*POMC*) gene mutation**

Mutations in the *POMC* gene have been described in a 5-year-old girl and a 5-year-old boy from unrelated families (83). Both patients developed early onset obesity with hyperphagia. They also had red hair pigmentation and ACTH deficiency diagnosed during infancy. Both patients had undetectable serum ACTH levels after corticotropin-releasing hormone (CRH) stimulation. In response to TRH stimulation, α-MSH level was undetectable in the girl and only low normal in the boy. The older brother of the girl died at age 7 months with underlying adrenal hypoplasia suggestive of secondary adrenal insufficiency.

Two compound heterozygous mutations in exon 3 of POMC coding region were discovered in the girl and her older brother. The G→T transversion in the paternal allele at nucleotide position 7013 resulted in a premature termination at codon 79 and hence the complete loss of synthesis of α-MSH and ACTH. The second mutation was a single nucleotide deletion at position 7133 in the maternal allele, resulting in a frame-shift and disruption of the receptor-binding core motif of α-MSH and ACTH and a premature termination at codon 131. The mutation in the 5-year-old boy was a homozygous C→A substitution in exon 2 of POMC gene at nucleotide position 3804 in the 5' untranslated region.

This mutation created an out-of-frame initiation codon that interfered with the normal initiation of the wild-type peptide translation.

The red hair pigmentation and obesity are thought to be due to deficiency of α-MSH. ACTH deficiency causes secondary adrenal insufficiency. Normal phenotype of the heterozygous parents in both families suggests that this syndrome has a recessive mode of inheritance.

5. Melanocortin receptor 4 (*MC4R*) mutation

Recently, obesity resulting from *MC4R* mutations has been reported in several individuals and families, confirming an important role of MC4R in energy balance in humans. In the first report, a heterozygous mutation of 4-nucleotide deletion at codon 211 of *MC4R* gene was identified in 1 of 63 individuals with early onset obesity (84). This mutation resulted in a frame-shift that introduced 5 aberrant amino acids and caused premature termination of MC4R in the fifth transmembrane domain, a critical site for G-protein binding and activation. The index patient was a 4-year-old boy who had normal birth weight but suffered progressive weight gain and obesity with hyperphagia at the age of 4 months. There was no evidence of other endocrine dysfunction, and serum leptin level was appropriate for BMI. The father of the boy, who was heterozygous for this mutation, also had early onset morbid obesity, whereas the mother was unaffected. The same group reported *MC4R* heterozygous mutations in 4% of 209 morbidly obese patients but none in 254 controls (85).

Another proband, a 35-year-old woman, with *MC4R* mutation was identified as a result of screening 43 individuals with early onset severe obesity (86). Six severely obese members over four generations in her family carried a heterozygous 4-nucleotide insertion mutation at nucleotide position 732 (codon 244) of the *MC4R* gene. This mutation was not found in 275 nonobese control subjects. In the mutant receptor, the sixth transmembrane domain and the rest of the C-terminal structure were replaced by a short and aberrant peptide. The proband had normal blood glucose, lipid and leptin levels. She had normal pubertal development, and three of other affected women had normal reproductive function. This group also investigated 243 patients with severe and early-onset obesity and reported mutations in 7 subjects (87). Two subjects had a novel two-base pair GT insertion in codon 279 and five subjects had four novel missense mutations N62S, R165Q, V253I, C271Y and one previously reported mutation (T112M)).

More recently, *MC4R* mutations were identified in 10 probands as a result of screening 461 individuals, of whom 52 were normal-weight, 25 were underweight but otherwise healthy, 306 were severely obese, 51 had anorexia nervosa and 27 had bulimia nervosa (88). All but one of the individuals with *MC4R* mutations was obese. A 19-year-old female proband and her mother, both of whom were obese, were found to have the same 4-bp deletion at codon 211 as described above. Two other obese female index subjects, age 10 and 16 years old, respectively, were noted to have combined nonsense mutation (Tyr35stop in the extracellular domain of MC4R) and a missense mutation (Asp37Val in the 3' untranslated region of MC4R). These mutations were inherited from their respective mothers, who were also obese. One 15-year-old obese male had double missense mutations (Ser30Phe near the N-terminal and Gly252Ser in the sixth transmembrane domain, respectively). Four other male probands, age 9, 13, 11, and 19 years old, respectively, were noted to harbor 4 different missense mutations, respectively. These mutations included Pro78Leu in the first intracellular loop, Thr112Met in the first extracellular loop, Arg165Trp in the second intracellular loop, and Ile317Thr in the C-terminal of MC4R, respectively. A silent mutation (C579T and Val193Val) in the second extracellular loop of MC4R was found in a underweight healthy man. In

addition, two polymorphisms, Val103Ile and Ile251Leu, were detected in the survey of the entire coding region of the *MC4R* gene. This group described one more haploinsufficiency mutation caused by the nonsense mutation at codon 35 of *MC4R* gene in further studies on 186 obese individuals (89). This carrier, as ascertained later, was second-degree cousin of two index patients described previously (88, 89). Studies on the phenotype and genotype of 43 family members of total of four index cases from the two studies conducted by the group revealed 19 carriers with morbid obesity but moderate obesity was also observed (88, 89). These studies have confirmed the previous findings related to *MC4R* mutation, a characteristic dominant inheritance and that there are no apparent phenotypic abnormalities besides obesity. This dominant inheritance is thought to be due to haplo-insufficiency mechanism commonly seen in the mutations of the G-protein coupled receptors. In another study on 190 obese individuals, three allelic variants were identified. One of the novel variant mutations Ile137Thr in an extremely obese proband was reported to be severely impaired in ligand binding and signaling (90). Five dominantly inherited heterozygous missense mutations (Val50Met, ser58Cys, Ile102Ser, Ile170Val and N274S) in 4 unrelated children (91) and in an obese female patient (92) have also been reported.

Collectively, 51 individuals with various *MC4R* gene mutations have been described till now. According to two reports (85, 87), mutations in *MC4R* gene occur at an approximate frequency of 3-4% among morbidly obese subjects and appear to be the most common monogenic mutations causing early-onset obesity in humans.

6. Single Minded homolog 1(Drosophilia) (*SIM 1)* gene mutation

The proband for *SIM1* gene mutation was girl who had accelerated growth and obesity during childhood (93). By 25 months she weighed 19.8 kg (+5.2 SD) and was 96 cm tall (+3.1 SD). There were no dysmorphic features but she had slightly elevated serum insulin and depressed cortisol concentrations. Serum leptin concentrations were appropriate for age. At 67 months of age she had a voracious appetite and consumed approximately 5000 kJ/day. Karyotyping revealed a balanced translocation between chromosomes 1p22.1 and 6q16.2 which apparently did not affect transcription unit on chromosome 1p. On chromosome 6q, it disrupted *SIM1* gene. This gene encodes for the transcription factor involved in the formation of supraoptic, paraventricular and anterior periventricular nuclei of hypothalamus in newborn mice. MC4R receptors are located in paraventricular and closely regulate inhibition of food intake. It is postulated but unproven that hyperphagia and weight gain in *SIM1* gene mutation might be related to satiety and feeding pathways in the hypothalamus and responsible for severe obesity in the proband. Mutation of *SIM 1* gene appears to be a rare cause of early-onset obesity. SSCP analysis and limited DNA sequencing of 45 markedly obese children did not reveal any coding and splice site mutation(93).

7. *ALMS 1* gene mutation

Alstrom syndrome is an autosomal recessive disorder where predominant manifestations are childhood obesity, diabetes mellitus, retinal degeneration and sensory deafness. Additional features include dilated cardiomyopathy, liver dysfunction, hypogonadism in males, short stature and developmental delay. Recently, two groups independently identified mutations in the *ALMS1* gene in 21 subjects in 13 unrelated families(94, 95). This gene does not have sequence homology with any other reported gene. It is postulated the *ALMS1* mutation in the hypothalamus increases appetite and obesity, and additionally when mutated in the pancreas may lead to hyperglycemia.

Table 6. Clinical features of human obesity syndromes with single gene mutations

	Genes with mutations							
	LEP	*LEPR*	*PC1*	*POMC*	*PPARG*	*MC4RR*	*SIM1*	*ALMS1*
Subjects (N)	6	3	1	3	4	51	1	21
Inheritance	AR	AR	AR	AR	?	AD	?	AR
Onset of obesity (mo)	3-4	4-5	<36	4-5	?	4-6	3	6
Age at report (y)	2–34	13-19	43	5	32-74	4 – 78	18	?
Early hyperphagia	+	+	?	+	?	+	+	+
Serum leptin	low	high	normal	?	?	normal	Normal	?
Diabetes /IGT	–	–	–	–	+	–	–	+
Serum insulin	high	high	low	?	low	normal	Slightly high	High
Hypogonadotropic hypogonadism	+	+	+	?	?	–	?	+
ACTH deficiency	–	–	+	+	?	–	?	–
Other features	↑ TSH	Emotional problems, growth delay, hypothalamic hypothyroidism, sympathetic dysfunction	Postprandial hypoglycemia, autoimmune thyroiditis, ↑ proinsulin	Red hair, ↓ □-MSH	?	–	Slightly depressed cortisol	Retinal degeneration, neurogenic deafness, cardiomyopathy, short stature etc

ACTH, adrenocorticotropin. AR, autosomal recessive. AD, autosomal dominant (due to haplo-insufficiency), IGT, impaired glucose tolerance. TSH, thyrotropin. +, presence. –, absence. ?, unknown. Modified from Chen and Garg (96).

CONCLUSIONS

In the last few years, there has been an explosion of knowledge regarding the molecular basis of monogenic disorders of adipose tissue, including obesity and lipodystrophies. This knowledge seems like the tip of the iceberg considering many questions that still remain to be answered about the genetic and molecular basis of common forms of obesity and abnormalities in body fat distribution that predispose to metabolic complications such as type 2 diabetes, dyslipidemia and hypertension. Certainly, the recent knowledge has led to interesting novel therapies for patients with lipodystrophies such as leptin replacement therapy(97). It is hoped that precise understanding of underlying molecular defects will help us relate adipocyte physiology to common human diseases.

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