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A Gene for Congenital Generalized Lipodystrophy Maps to Human Chromosome 9q34

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ABSTRACT Congenital generalized lipodystrophy (CGL, Berardinelli-Seip Syndrome, OMIM # 269700) is a rare autosomal recessive disorder characterized by near complete absence of adipose tissue from birth. Affected individuals have marked insulin resistance, hypertriglyceridemia and acanthosis nigricans, and develop diabetes mellitus during teenage years. The genetic defect for CGL is unknown. A semi-automated genome-wide scan with a set of highly polymorphic short tandem repeats (STR) was carried out in 17 well-characterized pedigrees and identified a locus for CGL to chromosome 9q34. The maximum two-point lod score obtained was 3.6 at *D9S1818* ($\theta_{max} = 0.05$). There was evidence for genetic heterogeneity ($\alpha = 0.73$) and 2 of the pedigrees were unlinked. Multipoint linkage analysis excluding the 2 unlinked families yielded a peak lod score of 5.4 between loci *D9S1818* and *D9S1826*. The CGL1 critical region harbors a plausible candidate gene encoding the retinoid X receptor α (*RXRA*) that plays a central role in adipocyte differentiation. Identification of the CGL gene(s) will contribute to our understanding of the adipocyte differentiation and elucidation of the mechanisms of insulin resistance in disorders of adipose tissue.

Near total lack of body fat and marked muscularity from birth are hallmarks of the patients with CGL (Fig. 1) (1, 2). Patients have marked insulin resistance, severe hyperinsulinemia, hypertriglyceridemia and low levels of high-density lipoprotein cholesterol (3). Abnormal glucose tolerance and diabetes mellitus appear usually during or after puberty (3-6). Serum leptin concentrations are markedly low (5). Affected individuals show accelerated growth, voracious appetite, increased metabolic rate and advanced bone age during early childhood (3,7). Umbilical hernia seems to be a consistent finding (3-5). Widespread acanthosis nigricans usually appears later (3). Other clinical features include hepatosplenomegaly and slight enlargement of the hands, feet and mandible, resulting in an "acromegaloid appearance". Affected women may have clitoromegaly, hirsutism, oligo-amenorrhea and polycystic ovaries. Successful pregnancy is rare. Affected males, however, have normal reproductive potential. After puberty, focal lytic lesions may appear in the appendicular bones (7,8). Hypertrophic cardiomyopathy has been reported in a few patients (9). Fatty infiltration of the liver occurs early and may lead to cirrhosis and its complications (10,11).

Whole body magnetic resonance imaging (MRI) as well as autopsy findings revealed a near complete absence of metabolically-active adipose tissue from subcutaneous areas, intraabdominal and intrathoracic regions, and bone marrow in CGL (4,11). In contrast, normal amounts of mechanical adipose tissue were present in the orbits, palms, soles, scalp, perineum and peri-articular regions. These findings have led us to propose that the gene defect in CGL interferes selectively with growth and differentiation of metabolically-active adipose tissue (4) and could result from either agenesis of preadipocytes, failure of preadipocytes to differentiate into mature adipocytes, or failure of mature adipocytes to synthesize and/or store triglycerides.

Previous studies, using a candidate gene approach, excluded several genes involved in insulin action, lipid metabolism and adipocyte biology (12-16). However, many other genes involved in adipocyte differentiation, such as CCAAT/enhancer binding proteins (C/EBP) α, β and δ, peroxisome proliferator-activated receptor γ (PPAR γ), retinoid X receptor α (RXR α), sterol responsive element-binding protein 1 (SREBP1), and preadipocyte factor-1 (Pref-1), adipsin, glucose transporter-4 and tumor necrosis factor-α remain as potential candidate genes. Recent reports of 2 transgenic mice exhibiting many features of CGL suggest that altered expression of transcription factors, such as SREBP1c and C/EBP may be involved in the pathogenesis of CGL

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(17,18). We adopted a linkage analysis approach to localize the gene/s altered in CGL.

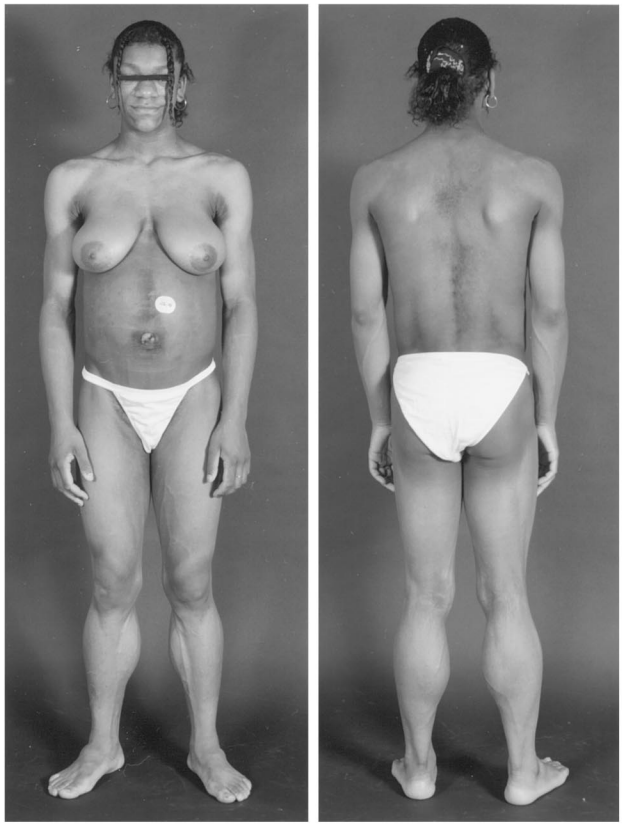

Fig. 1. Clinical features in a 16-year-old girl affected with CGL (CG 600.5). The left and right panels show anterior and posterior view, respectively, demonstrating near total absence of subcutaneous fat from the face, neck, trunk, arms and legs, extreme muscular appearance, acromegaloid features, umbilical hernia and severe acanthosis nigricans in the axillae, trunk and popliteal fosse.

Subjects and Methods

Families

All studies were approved by the appropriate Institutional Review Boards and informed consent was obtained from all subjects. We recruited 17 pedigrees with CGL. Clinical features of affected subjects from 6 families have been previously published (4, 19-23). Pedigrees CG 100 and 3100 are Turkish; CG 300, 700, 2900 and 3300 are Caucasian, CG 400, 800, 900, 3000 and 3200 are African, CG 3500 is Hispanic, CG 1100 is Taiwanese and CG 1300 is Chinese from North America; CG 600 is African and CG 2800 is Caucasian from the United Kingdom; and CG 1000 is from Pakistan. The phenotype was classified as affected or unaffected. This determination was made on the basis of history and physical examination and by review of their medical records. Furthermore, telephone interviews were conducted and photographs of affected subjects were inspected, where available. Generalized lack of body fat and extreme muscularity from birth was the essential criterion for diagnosis. Additional supportive criteria included the presence of acanthosis nigricans, umbilical hernia, acromegaloid features, hirsutism, early-onset diabetes mellitus, hypertriglyceridemia and hyperinsulinemia. In 6 patients, whole body MRI studies provided confirmation of a characteristic body fat distribution previously published by us (4). All phenotypes were assigned prospectively before beginning genotyping.

Genotyping

DNA was extracted from buffy coats or lymphoblastoid cell lines using standard protocols. Genome wide linkage analysis spanning all autosomes and subsequent genotyping of markers flanking locations suggestive of linkage was performed using fluorescently labeled primers from the Weber Version 8 panel of polymerase chain reaction (PCR)-amplifiable polymorphic short tandem repeats (STRs) (Research Genetics, Huntsville, AL) as described (24) using an ABI 377 DNA Sequencer equipped with GeneScan 2.1 and GENOTYPER softwares (Applied Biosystems). Marker order and distances between them were obtained from the database at the Center for Medical Genetics (www.marshmed.org/genetics).

Linkage analyses

The PEDMANAGER (Whitehead Institute for Biomedical Research, www.genome.wi.mit.edu) software was used to check for non-Mendelian inheritance of alleles and to estimate allele frequencies (25). The CHROMPIC option of CRIMAP was used to identify double recombinants. An autosomal recessive inheritance and a disease gene frequency of 1 in 1,000 were assumed for conducting the linkage analyses. The penetrance and phenocopy rates were varied between 90-100% and 0-1%, respectively. Parametric analyses, heterogeneity testing and multipoint analyses were performed with GENEHUNTER (26). Two-point lod scores were obtained with the FASTLINK version of LINKAGE (27, 28).

Results

An initial genome-wide linkage scan with 386 polymorphic STRs, spaced an average of 10 cM apart, was undertaken. Seven informative pedigrees (CG 100, 400, 600, 800, 900, 1000 and 1300) that included 5 males and 9 females with CGL and 21 unaffected subjects were analyzed (Fig. 2). Preliminary evidence for linkage was obtained with 5 regions on chromosomes 1p, 1q, 9q, 10q and 13q where two-point lod scores exceeded 1.0. Further exploration with markers from additional loci known to map in these regions provided further evidence of linkage to chromosome 9q34 but did not support linkage to the other regions. Homozygosity was also noted among 2 affected subjects from the consanguineous pedigree CG 1000. However, the other consanguineous pedigree, CG 100, did not appear to be linked to 9q34 since multipoint lod scores for this pedigree were negative across the region (data not shown).

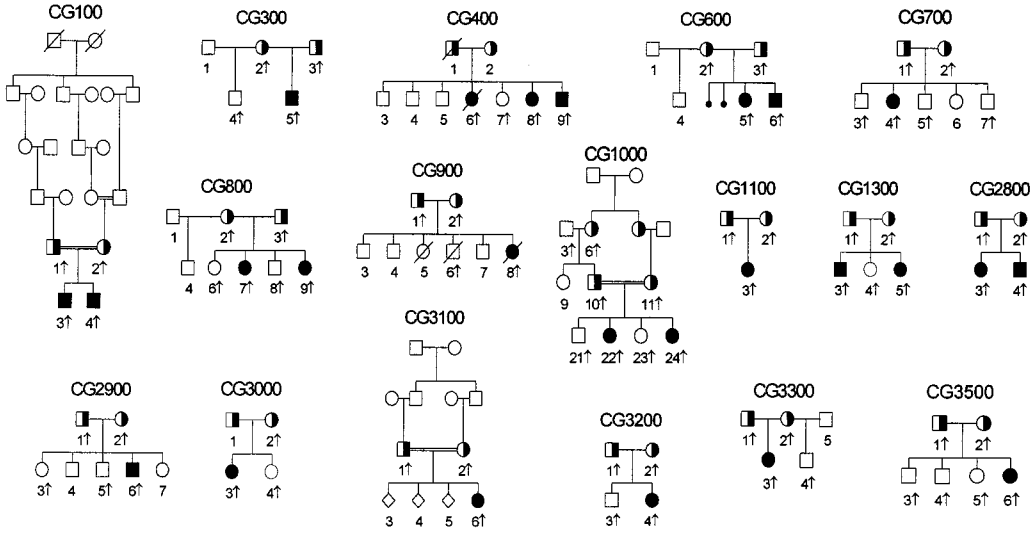

Fig. 2. CGL pedigrees. Individuals for whom DNA was available are indicated by ↑. Affected individuals are shown as filled black symbols, unaffected subjects as unfilled symbols, and parents of affected individuals are shown as half-filled symbols (obligate heterozygotes). Consanguinity is shown as double horizontal lines. Deceased individuals are indicated by a diagonal line, ◊ indicates sex unknown, and • indicates miscarriages.

Subsequently, 10 additional pedigrees (CG 300, 700, 1100, 2800, 2900, 3000, 3100, 3200, 3300 and 3500) were genotyped for markers spanning a 41 cM region of chromosome 9q34 (Fig. 2). Table 1 shows the total pairwise lod score data for loci which provided the highest lod scores. The maximum total two-point lod score, summed over all 17 families was 3.6 at recombination fraction (θ_{max}) = 0.05 with *D9S1818* (100% penetrance and 0% phenocopy rate). The admixture test was significant when tested against multipoint lod score data, with the proportion of linked families, $\alpha = 0.73$. In addition to CG 100, pedigree CG 2800 did not appear to be linked to the critical region (all multipoint lod scores for this pedigree were negative). There were, however, no phenotypic or clinical differences between the affected subjects from the 2 unlinked and other pedigrees. The maximum total two-point lod score excluding the unlinked pedigrees was 4.1 with *D9S1793* and *D9S1818 at* $\theta_{max} = 0.04$ and 0.03, respectively. Using equal allele frequencies and those from the CEPH database revealed maximum total two-point lod scores of 3.64 and 3.82, respectively at $\theta_{max} = 0.05$, with *D9S1818*. Assuming reduced penetrance of 90% and a phenocopy rate of 1% made little difference to the maximum lod score or the placement of the CGL locus (data not shown).

TABLE 1. Pairwise total lod scores between polymorphic microsatellite containing loci on chromosome 9q34 and CGL

	θ						
Locus	0.00	0.01	0.05	0.1	0.2	Zmax	θmax
D9S1793	-∞	1.85	3.13	3.18	2.35	3.23	0.08
D9S1818	-∞	2.97	3.64	3.35	2.28	3.64	0.05
D9S1826	-∞	0.76	1.66	1.67	1.20	1.71	0.07
D9S158	-∞	1.59	2.43	2.34	1.64	2.44	0.06

All 17 pedigrees were analyzed. Lod scores were generated with a recessive mode of inheritance, a penetrance of 100% and no phenotypic a phenocopy rate of 0. Locus order from centromere to q telomere is *D9S1793, D9S1818, D9S1826* and *D9S158*.

Multipoint analysis over a 41 cM region of chromosome 9q34 assuming genetic homogeneity in all 17 pedigrees revealed negative lod scores (LOD17) over much of the region and reached a maximum of 2.0 between loci *D9S1818* and *D9S1826*. Assuming genetic heterogeneity, a maximum lod score (HLOD17) of 3.6 was attained (Fig. 3). Multipoint analysis excluding the 2 unlinked families yielded a maximum lod score (LOD15) of 5.4 between loci *D9S1818* and *D9S1826* (Fig. 3).

Within the linked 9q34 region are genes for the human Surfeit locus 1 (SURF1), progesterone-associated endometrial protein (PAEP), carboxyl ester lipase (CEL), fucosyltransferase 7 (FUT7), ribosomal protein 7A (RPL7A), type V collagen, alpha 1 (COL5A1), ficolin

(collagen/fibrinogen domain-containing) 1 (FCN1) and RXRα. Of these, the gene for RXRα is a strong candidate gene for CGL because of its role in adipocyte differentiation. Mutational analysis is underway to determine if *RXRA* is defective in affected subjects from the pedigrees linked to 9q34. In the 2 pedigrees that were unlinked to the 9q34 region (CG 100 and 2800), direct sequencing of *PPARγ* exons in affected members failed to reveal any alterations in this gene (results not shown).

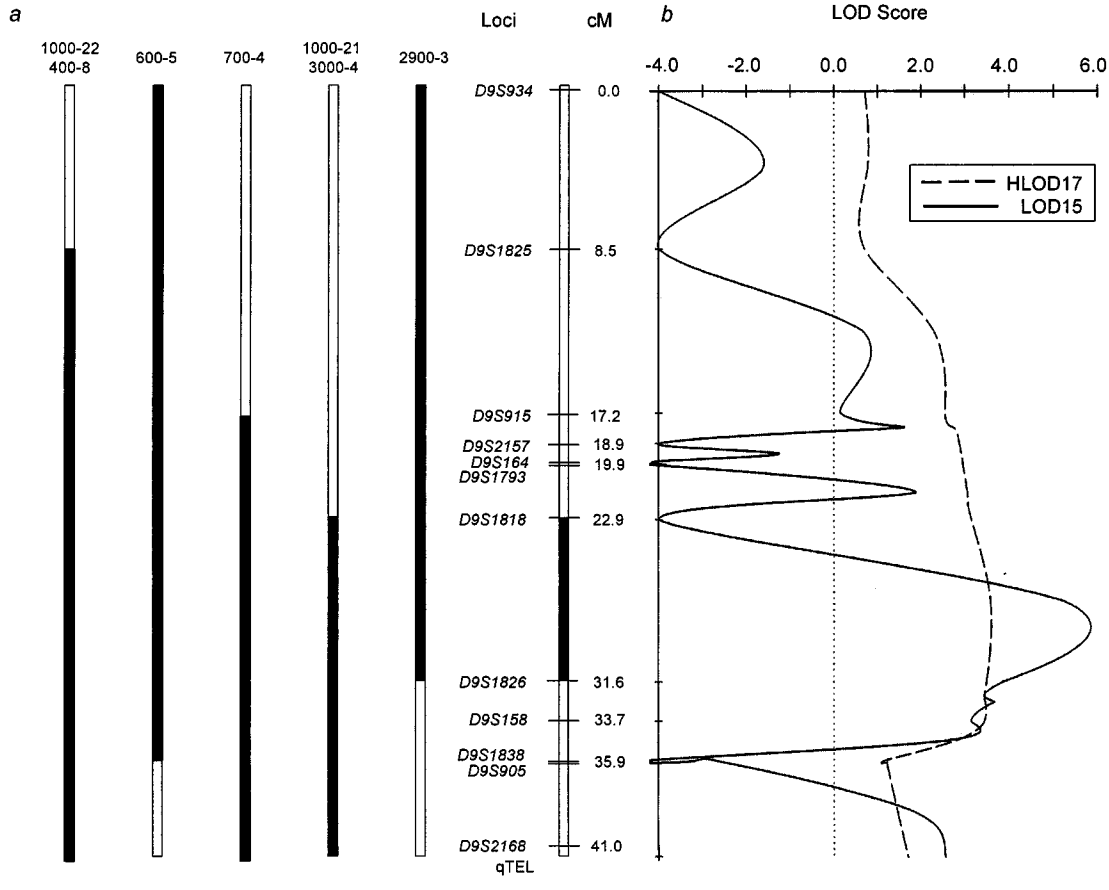

Fig. 3. Localization of a gene for CGL. ***a,*** Recombinants from the linked pedigrees that define the critical region. Filled portion of the vertical bar indicates the interval likely to harbor the *CGL1* gene based on the haplotypes (The individuals are designated as shown in Fig. 2). Recombinants localize the defective gene to 8.7 cM region between loci *D9S1818* and *D9S1826*, shown as the filled region of the locus bar. The disease-associated haplotypes differed for each family. The location of the q telomere is indicated by "qTEL". ***b,*** Multipoint analysis: The position of *D9S934* was arbitrarily set at 0.0-cM and the positions of the other loci were fixed according to the sex-averaged distance determined with CEPH pedigree data. The total distance between *D9S934* and *D9S2168* is 41 cM. Multipoint lod scores on the x-axis are plotted against chromosome 9 loci on the y-axis. HLOD17 (interrupted line) shows the results of the LOD score analysis under the heterogeneity hypothesis, when all families were considered. LOD15 (solid, uninterrupted line) was obtained under the hypothesis of homogeneity in 15 pedigrees, excluding the 2 unlinked pedigrees (CG 100 and 2800).

Discussion

Our data provide evidence for at least two CGL loci: CGL1 on human chromosome 9q34, within a 8.7 cM interval spanned by the loci *D9S1818* and *D9S1826* and one other, CGL2, as yet unmapped. Cloning of CGL genes will lead to a better understanding of the primary pathogenetic mechanisms involved in the loss of adipose tissue and may provide insights into embryonic differentiation of preadipocytes into metabolically active or mechanical adipocytes. Furthermore, it may lead to elucidation of mechanisms by which adipose tissue disorders such as lipodystrophies and common forms of regional adiposity lead to insulin resistance, diabetes mellitus and dyslipidemia.

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