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# Phenotypic and Genetic Heterogeneity in Congenital Generalized Lipodystrophy

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**Congenital generalized lipodystrophy (CGL) is a rare autosomal recessive disorder characterized by near complete absence of adipose tissue from birth. Recently, mutations in 1-acylglycerol-3-phosphate *O*-acyltransferase 2 (*AGPAT2*) and Berardinelli-Seip congenital lipodystrophy 2 (*BSCL2*) genes were reported in pedigrees linked to chromosomes 9q34 and 11q13, respectively. There are limited data regarding phenotypic differences between the various subtypes of CGL. Furthermore, whether there are additional loci for CGL remains unknown. Therefore, we genotyped 45 pedigrees with CGL for *AGPAT2* and *BSCL2* loci and compared the phenotypes in the various subtypes. Twenty-six pedigrees harbored mutations, including seven novel variants, in the *AGPAT2* gene, and 11 pedigrees harbored mutations in the *BSCL2* gene, including five novel variants. Eight pedigrees had no substantial alterations in either gene. Of these, three informative pedigrees showed no linkage to markers spanning the *AGPAT2* and *BSCL2* loci, and in six of the affected subjects, the transcripts of *AGPAT2* and *BSCL2* were normal. All subtypes of CGL showed high prevalence of diabetes, hypertriglyceridemia, and acanthosis nigricans. However, patients with *BSCL2* mutations had lower serum leptin levels, an earlier onset of diabetes, and higher prevalence of mild mental retardation compared with other subtypes. We conclude that besides *AGPAT2* and *BSCL2*, there may be additional loci for CGL. The genetic heterogeneity in CGL patients is accompanied by phenotypic heterogeneity. (*J Clin Endocrinol Metab* 88: 4840–4847, 2003)**

CONGENITAL GENERALIZED LIPODYSTROPHY [CGL; Berardinelli-Seip syndrome, Online Mendelian Inheritance in Man (OMIM) no. 269700; http://www.ncbi.nlm.nih.gov/Omim/] is a rare autosomal recessive disorder characterized by near complete absence of adipose tissue since birth resulting in a marked generalized muscular appearance. Patients have accelerated growth, voracious appetite, and advanced bone age during early childhood. Affected individuals have severe hyperinsulinemia, hypertriglyceridemia, low concentrations of high-density lipoprotein cholesterol, and often widespread acanthosis nigricans (1, 2). Diabetes mellitus usually appears during the pubertal years (3, 4). Fatty infiltration of the liver occurs early and may lead to cirrhosis and its complications (5, 6). Umbilical hernia or prominence seems to be a consistent finding (2). Other clinical features include slight enlargement of the hands, feet, and mandible, resulting in an acromegaloid appearance, clitoromegaly, mild hirsutism, and hyperhidrosis. Postpubertal patients may also develop focal lytic lesions in the appendicular bones (7–9). A few patients have been reported to have hypertrophic cardiomyopathy (10–12) and mental retardation (1, 3, 4).

Recent studies have revealed two loci for CGL, and mutations in 1-acylglycerol-3-phosphate *O*-acyltransferase 2 (*AGPAT2*) were reported in pedigrees linked to chromosome 9q34 (3), and in the Berardinelli-Seip congenital lipodystrophy 2 (*BSCL2*) gene in pedigrees linked to chromosome 11q13 (13). Whether there are additional loci for CGL remains to be established. Furthermore, the phenotypic differences between CGL patients with *AGPAT2* or *BSCL2* mutations or those without mutations in either of these two genes have not been well characterized (3, 4). Therefore, we conducted mutational analysis of 45 pedigrees with CGL, of various eth-

Abbreviations: *AGPAT2*, 1-Acylglycerol-3-phosphate *O*-acyltransferase 2; *BSCL2*, Berardinelli-Seip congenital lipodystrophy 2; CGL, congenital generalized lipodystrophy; SNP, single nucleotide polymorphism.



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nicities ascertained from all over the world, for mutations in *AGPAT2* and *BSCL2* genes and linkage to these loci, and evaluated the phenotypic differences between the various subtypes of CGL.

## Subjects and Methods

### *Sample collection*

A total of 45 pedigrees have been ascertained by us from all over the world. Pedigrees of the 11 CGL families with *AGPAT2* mutations were recently published by us (3). The remaining 34 pedigrees are shown in Figs. 1–3. The protocol was approved by the appropriate Institutional Review Boards, and all subjects gave informed consent. The phenotype was classified as affected or unaffected on the basis of history, physical examination, review of medical records, responses to a written questionnaire, and inspection of photographs where available. Generalized lack of body fat since birth was considered the essential criterion for diagnosis. Additional supportive criteria included the presence of acanthosis nigricans, diabetes mellitus, hypertriglyceridemia, or low serum high-density lipoprotein cholesterol levels. In some patients, generalized lack of body fat on whole body magnetic resonance imaging provided confirmation of the diagnosis. Patients with acquired generalized lipodystrophy were excluded on the basis of their normal appearance at birth with gradual onset of loss of fat subsequently (14), whereas those with Wiedemann-Rautenstrauch syndrome were excluded based on the presence of supragluteal fat pads and other somatic anomalies (2).

Diabetes mellitus was diagnosed on the basis of previous history, use of hypoglycemic medications, or whether fasting serum glucose concentration exceeded 7.0 mmol/liter on two or more occasions (15). Hypertriglyceridemia was diagnosed on the basis of previous history, use of hypolipidemic medication, or whether the fasting serum triglyceride concentration exceeded 2.26 mmol/liter (16). Diagnosis of mental retardation was based on historical information and assessment by referring physicians, whereas the diagnosis of cardiomyopathy was based on documentation of ventricular hypertrophy on electrocardiogram or a transthoracic echocardiogram. Skeletal roentgenograhic surveys were obtained in eight patients evaluated at University of Texas Southwestern Medical Center (Dallas, Texas).

Blood samples were also obtained from 50 unaffected subjects (25 each of European and African origin) to screen for mutations in the *AGPAT2* gene.

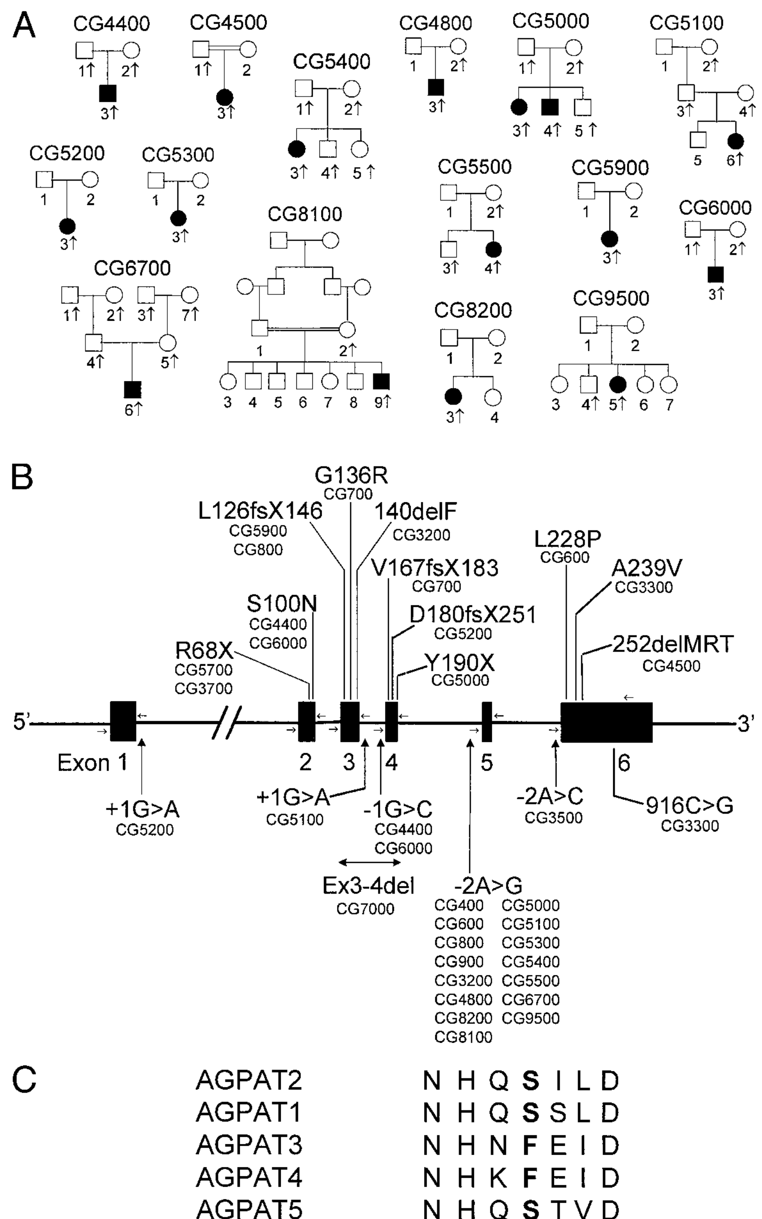


FIG. 1. A, Pedigrees of CGL patients with mutations in the *AGPAT2* gene. The pedigrees and each member are numbered for identification. *Squares* and *circles* indicate males and females, respectively; *filled symbols*, affected subjects; and *open symbols*, unaffected subjects. The *vertical arrows* indicate subjects for whom DNA was available. B, The gene structure of *AGPAT2* and the orientation of the gene are shown. The *filled boxes* show the exons, and the *in-between lines* show the introns. The various mutations observed in patients with CGL are marked. *Arrows* close to the exons represent the location of the primers used for sequencing. The pedigree numbers under the mutation indicate the families in which the mutation was seen. C, Amino acid residues in the $NHX_4D$ motif of the different AGPAT isoforms. Residues corresponding to Serine 100, where mutations were found in pedigrees CG4400 and CG6000, are shown in **bold**.

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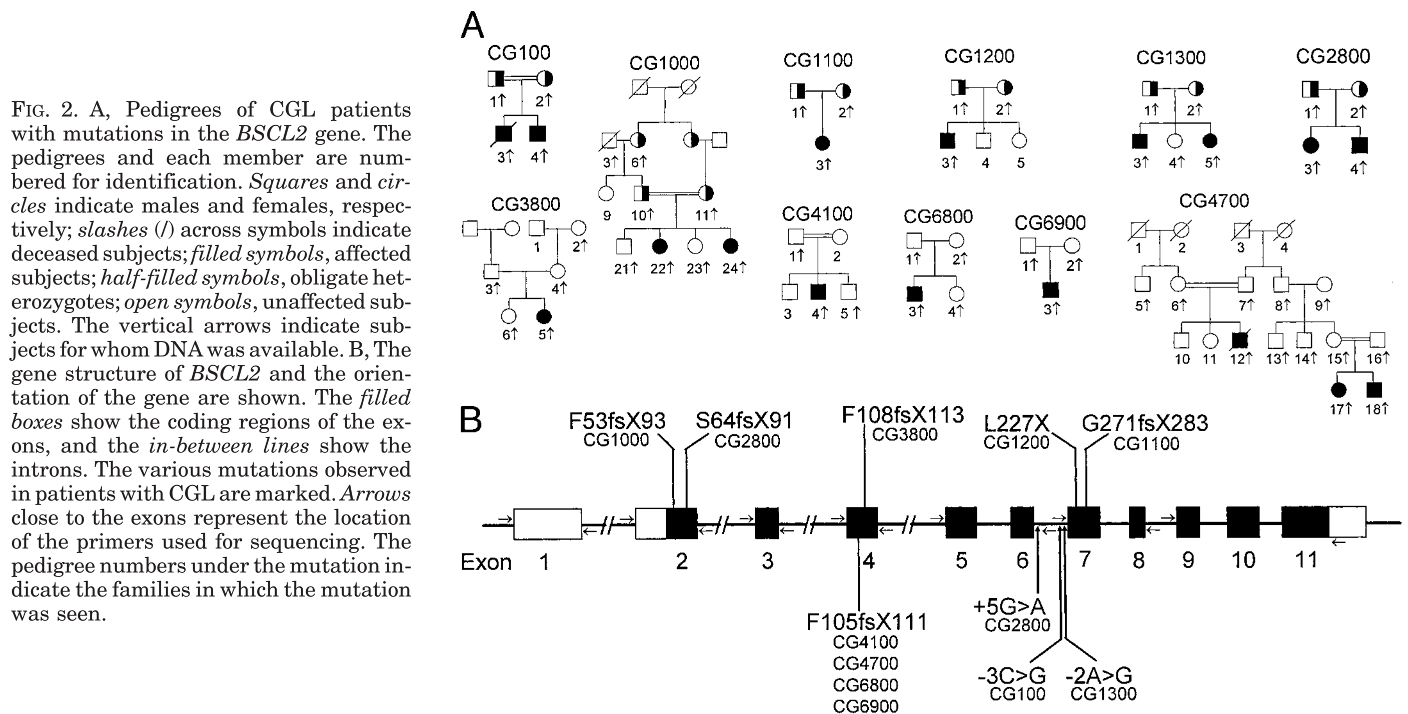


FIG. 2. A, Pedigrees of CGL patients with mutations in the *BSCL2* gene. The pedigrees and each member are numbered for identification. *Squares* and *circles* indicate males and females, respectively; *slashes* (/) across symbols indicate deceased subjects; *filled symbols*, affected subjects; *half-filled symbols*, obligate heterozygotes; *open symbols*, unaffected subjects. The vertical arrows indicate subjects for whom DNA was available. B, The gene structure of *BSCL2* and the orientation of the gene are shown. The *filled boxes* show the coding regions of the exons, and the *in-between lines* show the introns. The various mutations observed in patients with CGL are marked. *Arrows* close to the exons represent the location of the primers used for sequencing. The pedigree numbers under the mutation indicate the families in which the mutation was seen.

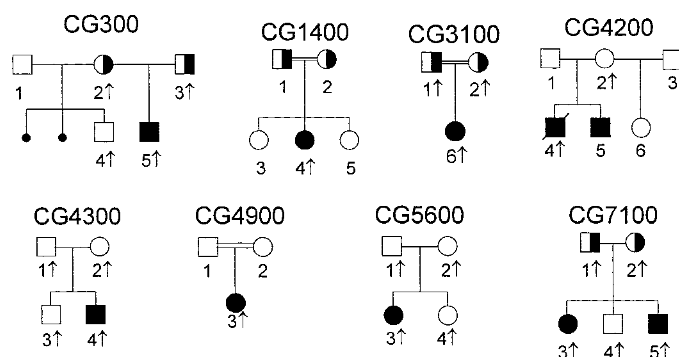


FIG. 3. Pedigrees of CGL patients lacking mutations in either the *AGPAT2* or the *BSCL2* gene. The pedigrees and each member are numbered for identification. *Squares* and *circles* indicate males and females, respectively; *slashes* (/) across symbols indicate deceased subjects; *filled symbols*, affected subjects; *half-filled symbols*, obligate heterozygotes; *open symbols*, unaffected subjects. The *vertical arrows* indicate subjects for whom DNA was available.

### *Mutational analysis*

Lymphoblastoid cell lines were established by Epstein-Barr virus transformation of peripheral blood lymphocytes, and genomic DNA was isolated from these cells or from buffy coat using DNAzol (Life Technologies, Inc., Rockville, MD) according to the manufacturer's protocol. The coding regions and the splice site junctions of the *AGPAT2* and *BSCL2* genes were amplified by using gene-specific primers (available on request). PCR was assembled as described earlier (3). The PCR product was purified to remove primers and deoxynucleotide triphosphate and sequenced using ABI Prism 3100 (Applied Biosystems, Foster City, CA).

### *Linkage analysis*

To study linkage to the *AGPAT2* and *BSCL2* loci, we chose the dinucleotide repeat markers *D9S1826, D9S158, D9S905* and *D9S1838* flanking the *AGPAT2* gene in the 9q34 region, and *D11S2006, D11S2016, D11S913* and *D11S2363* flanking the *BSCL2* gene in the 11q13 region. The PCR products were analyzed on ABI 377, and linkage analysis was performed manually.

### *Transcript analysis*

To detect any mutations in the noncoding regions (introns, proximal regulatory regions, and 3′ untranslated regions) of the *AGPAT2* and *BSCL2* genes, we performed transcript analysis on patients whose transformed lymphocytes were available. Transformed lymphoblasts of the affected subjects were spun down to remove the culture medium and lysed with RNA-Stat-60 (Tel-Test, Friendswood, TX). Total RNA was extracted according to the manufacturer's protocol. Approximately 5 $\mu$l (2.5–5 $\mu$g) of total RNA were used for the reverse-transcriptase reaction using Thermoscript RT-PCR system (Life Technologies, Inc.) in a 20-$\mu$l reaction volume according to the manufacturer's protocol with minor modification. Briefly, DNase I was added to the total RNA to remove residual genomic DNA and heat-inactivated at 65 C for 10 min. The reverse transcriptase reaction was carried out for 50 min at 50 C, followed by inactivation at 85 C for 5 min. Residual RNA was removed by treating the reaction with 2 U of RNase H for 20 min at 37 C. The cDNA was used for PCR using primer pair AGPAT2_F and AGPAT_6Ra, spanning the entire coding region of both the genes. PCR conditions were the same as mentioned above for exon amplification.

### *Biochemical analysis*

Serum leptin was measured using a commercial RIA for human leptin (Linco Research, Inc., St. Charles, MO). Serum glucose and triglyceride levels were determined according to standard methods using automated equipment (Beckman Instruments, Fullerton, CA).

## Results

### *Mutational analysis*

Of the 45 pedigrees studied, 26 harbored mutations in the *AGPAT2* gene, 11 in the *BSCL2* gene, and eight in neither of these two genes. The mutations found in *AGPAT2* and *BSCL2* in the affected subjects segregated in their parents and unaffected siblings in accordance with Mendelian autosomal recessive inheritance.

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*Mutations in the AGPAT2 gene*

We found mutations in the *AGPAT2* gene in affected patients from a total of 26 pedigrees, including 11 pedigrees that have been reported previously (3). In affected individuals from families CG4800, 5000, 5100, 5300, 5400, 5500, 6700, 8100, and 8200 of African origin, previously described IVS4–2A>G mutation (Table 1 and Fig. 1A) was detected. The pedigrees CG4800, 5300, 5400, 5500, 6700, 8100 and 8200 had the homozygous IVS4-2A>G mutation. Affected subjects from pedigrees CG5000 and 5100 were compound heterozygotes for novel mutations, 570C>A, and a splice site mutation, IVS3 + 1G>A, respectively. Interestingly, the affected subjects from the Hispanic pedigree CG9500 also had the same (IVS4–2A>G) homozygous mutation. We had reported earlier that the IVS4–2A>G mutation in subjects of African origin had a common ancestral origin and that the chromosome carrying the mutation in these pedigrees shared a common haplotype spanning 33 kb around the mutation, including dinucleotide repeat markers and single nucleotide polymorphisms (SNPs). We therefore compared the haplotype generated from the known SNPs within the *AGPAT2* gene in the pedigree CG9500 with the previously reported haplotype for the African pedigrees (3). Indeed the IVS4–2A>G mutation in CG9500 was associated with a different haplotype than that noted in the African pedigrees (Table 2), indicating that it originated by a separate mutational event. Although there was no evidence of consanguinity, the chromosome carrying the mutation in both the parents shared the same haplotype.

An affected individual from another pedigree of African-American origin (CG5900) was homozygous for a frameshift, L126fsX146, mutation. The L126fsX146 mutation was previously reported by us in affected individuals from the CG800 pedigree who were compound heterozygotes with IVS4–2A>G mutation. An affected individual from pedigree CG5200 carried compound heterozygous mutations, a novel splice site mutation, IVS1+1G>A and a single guanine nucleotide deletion, 538Gdel. The amino acid change in AGPAT2 protein due to IVS+1G>A mutation remains unknown. The 538Gdel causes a frameshift and a premature termination codon resulting in a truncated protein, D180fsX251. Affected patients from two pedigrees from Argentina (CG 4400 and 6000) were homozygous for two novel mutations, a splice site mutation, IVS3–1G>C, and a missense mutation, S100N. The impact of the IVS3–1G>C mutation at the protein level remains unclear, but it most likely leads to skipping of exon 4 resulting in an inactive truncated protein. An affected subject from CG4500, of Pakistani origin, was homozygous for a novel nine nucleotide deletion mutation resulting in a truncated protein 252delMRT.

Sequencing of all exons of the *AGPAT2* gene in 50 unaffected individuals revealed no mutations.

*Mutations in the BSCL2 gene*

Mutations in the *BSCL2* gene were found in affected patients from 11 pedigrees (Table 3 and Fig. 1B). In the pedigrees CG 4100, 4700, 6800, and 6900, which were of Lebanese descent, we observed a homozygous five-nucleotide deletion in exon 4 (659delGTATC) leading to frameshift and trun-

**TABLE 1.** 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 | Patient no. | Age (yr)/sex | Serum leptin (ng/ml) | DM/age of onset (yr) | Mental retardation | Cardiomyopathy |
|---|---|---|---|---|---|---|---|---|---|---|
| CG 4400 | European, Argentina | 299G>A<br>IVS3-1G>C | S100N<br>Not known | Hom | 3 | 3/M | NA | – | NA | NA |
| CG 4500 | Pakistani, United Kingdom | 755TGAGGACC A del | 252delMRT | Hom | 3 | 5/F | 2.0 | +/3 | – | + |
| CG 4800 | African-American, United States | IVS4-2A>G | Q196fsX228 | Hom | 3 | 10/M | 1.3 | +/7 | – | – |
| CG 5000 | African-American, United States | IVS4-2A>G<br>570C>A | Q196fsX228<br>Y190X | Het | 3 | 18/F | 2.5 | +/14 | – | – |
| | | | | | 4 | 20/M | 0.7 | – | – | – |
| CG 5100 | African-American, United States | IVS4-2A>G<br>IVS3+1G>A | Q196fsX228<br>Not known | Het | 6 | 1/F | 1.57 | – | NA | NA |
| CG 5200 | European, Germany | IVS1+1G>A<br>538Gdel | Not known<br>D180fsX251 | Het | 3 | 9/F | 0.6 | – | – | NA |
| CG 5300 | African, South Africa | IVS4-2A>G | Q196fsX228 | Hom | 3 | 7/F | NA | – | NA | NA |
| CG 5400 | African-American, United States | IVS4-2A>G | Q196fsX228 | Hom | 3 | 23/F | NA | +/NA | NA | NA |
| CG 5500 | African-American, United States | IVS4-2A>G | Q196fsX228 | Hom | 4 | 41/F | 1.4 | +/NA | NA | NA |
| CG 5900 | African-American, United States | 377insT | L126fsX146 | Hom | 3 | 2/F | 0.31 | – | NA | – |
| CG 6000 | European, Argentina | 299G>A<br>IVS3-1G>C | S100N<br>Not known | Hom | 3 | 1/M | NA | – | NA | NA |
| CG 6700 | African-American, United States | IVS4-2A>G | Q196fsX228 | Hom | 6 | 1/M | 0.05 | – | NA | NA |
| CG 8100 | African, United Arab Emirates | IVS4-2A>G | Q196fsX228 | Hom | 9 | 7/M | NA | – | – | NA |
| CG 8200 | African-American, United States | IVS4-2A>G | Q196fsX228 | Hom | 3 | 35/F | 1.07 | +/10 | – | – |
| CG 9500 | European, Mexico | IVS4-2A>G | Q196fsX228 | Hom | 4 | 29/M | 0.15 | +/27 | NA | NA |
| | | | | | 5 | 27/F | 0.7 | +/NA | NA | NA |

Het, Compound heterozygote; Hom, homozygous; NA, not available; +, present; −, absent; DM, diabetes mellitus; F, female; M, male.

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**TABLE 2.** Haplotypes surrounding the IVS4-2A>G mutation in the *AGPAT2* gene in CGL families of African and Hispanic origin

| | SNP 994A>G | SNP 891G>T | SNP IVS5+121C>T | IVS4-2A>G mutation | SNP −60IVS1C>G |
|---|---|---|---|---|---|
| African haplotype | A | G | C | G | C |
| Hispanic haplotype (CG 9500) | G | G | T | G | G |
| Physical distance from the mutation (kb) | 1.2 | 1.1 | 0.1 | 0 | 2.8 |

cated protein, F105fsX111. Magre *et al.* (13) also observed the same mutation in CGL pedigrees ascertained from Lebanon. An affected subject from a Portuguese pedigree (CG3800) had a homozygous insertion of a nucleotide, 669insA in exon 4, resulting in a frameshift and truncated protein, F108fsX113. We detected a novel mutation, a dinucleotide insertion, 500insTT, in exon 2 in CG1000 pedigree from Pakistan, which leads to frameshift and premature stop, F53fsX93. The affected subject from pedigree CG1100 of Chinese descent had a heterozygous single nucleotide, guanine insertion at position 1126 in exon 7 (1126insG), resulting in frameshift and a truncated protein of 283 residues, G271fsX283. We were unable to find another mutation in the coding region, splice site junctions, as well as in the 1.5-kb 5′ proximal region of the *BSCL2* gene in this subject. A novel homozygous splice site mutation IVS6−3C>G was observed in a Turkish pedigree (CG100), whereas affected patients from CG1300, a pedigree of Chinese origin showed a homozygous IVS6–2A>G mutation. On analysis of the transcripts, we found that both of these mutations resulted in a frameshift and a truncated protein of 288 residues, F224fsX288. A novel, homozygous null mutation L227X was detected in the affected subject from pedigree CG1200. Affected individuals from CG2800, of British origin, were found to be compound heterozygotes with a splice site mutation IVS6+5G>A and a 537delCinsGGA mutation, both of which resulted in a frameshift and truncated proteins of 225 and 91 residues, respectively.

Sequencing of the *BSCL2* gene in 96 Center d'Etude du Polymorphisme Humain (CEPH) family founders by Magre *et al.* (13) revealed none of the five novel variants described above.

### *Pedigrees lacking mutations in AGPAT2 or BSCL2 genes (other CGL types)*

We identified eight CGL pedigrees (Fig. 3) in which the affected patients showed no substantial alterations in the coding regions of the *AGPAT2* and *BSCL2* genes by direct sequencing. Of these eight pedigrees, three (CG3100, 5600, and 7100) had enough power for linkage analysis. We further analyzed these pedigrees using polymorphic microsatellite markers in the 9q34 and 11q13 regions flanking the *AGPAT2* and *BSCL2* genes, respectively. Pedigrees 5600 and 7100 showed linkage to 9q34 region, but transcript analysis did not reveal any mutation in *AGPAT2* gene. Pedigree CG3100 showed linkage to 11q13, and in CG5600, linkage data were inconclusive for this region. However, the transcript analysis of *BSCL2* was normal in affected individuals from the CG3100 and CG5600 pedigrees. Of the remaining five pedigrees, which were not included in the linkage analysis due to lack of power, lymphoblastoid cell lines were only available in affected subjects from three pedigrees (CG 300, 4200, and 4300). Transcript analysis in affected subjects from these pedigrees was normal for *BSCL2* and *AGPAT2* genes (Table 4). Linkage and transcript analysis could not be performed in pedigrees CG1400 and CG4900.

### *Comparison of CGL phenotypes*

Affected individuals from all 45 pedigrees had extreme paucity of sc adipose tissue from birth and markedly low serum leptin levels. Other characteristic features of CGL like acanthosis nigricans and umbilical prominence were also noted in almost all subjects. We noted a higher proportion of female subjects with *AGPAT2* mutations (Table 5). There was a uniformly high prevalence of diabetes mellitus and hypertriglyceridemia in all of the CGL subtypes. However, patients with *BSCL2* mutation had an earlier onset of diabetes at a median age of 10 yr, compared with those with *AGPAT2* mutation who had onset of diabetes at a median age of 12.5 yr ($P = 0.04$). Although half of the patients with *BSCL2* mutations were documented to have mental retardation, none with *AGPAT2* mutations were noted to have impaired mental functions ($P < 0.0001$). The prevalence of cardiomyopathy was also over three times higher in those with *BSCL2* mutations compared with those with *AGPAT2* mutations, and approached statistical significance ($P = 0.057$). Serum leptin levels were lower in patients with *BSCL2* mutations compared with those with *AGPAT2* mutations. On gender-specific comparisons, females with *BSCL2* mutation had significantly lower serum leptin levels than those with *AGPAT2* mutation ($0.48 \pm 0.32$ *vs.* $1.08 \pm 0.78$ ng/ml, respectively; $P < 0.05$), whereas serum leptin levels in males with *AGPAT2* and *BSCL2* mutation were comparable ($0.36 \pm 0.22$ and $0.56 \pm 0.43$ ng/ml, respectively; $P = 0.3$).

Affected patients unlinked to the *AGPAT2* and *BSCL2* genes also showed the typical features of CGL such as generalized fat loss from birth, low leptin levels, acanthosis nigricans, and a high prevalence of diabetes mellitus and hypertriglyceridemia. Patients from pedigree CG7100 also had congenital myopathy and developmental delay.

Skeletal survey revealed that lytic bone lesions in the appendicular skeleton were almost exclusively limited to affected adult subjects with the *AGPAT2* mutations. We had previously reported focal osteolytic lesions in long bones such as the femur, tibia, and humerus in affected subjects from pedigrees CG800 and CG900 (9) who have mutations in *AGPAT2* (3). We found similar lesions in affected individuals from pedigrees CG400, 600, and 8200, all with *AGPAT2* mutations. Skeletal surveys in affected subjects CG1300.3, 1300.5, 4700.12, and 6800.3, who have mutations in *BSCL2*, were essentially normal except for small lytic lesions in the humerus in patient CG4700.12.

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**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 | Patient no. | Age (yr)/sex | Serum leptin (ng/ml) | DM/age of onset (yr) | Mental retardation | Cardio-myopathy |
|---|---|---|---|---|---|---|---|---|---|---|
| CG 100 | Turkish, Turkey | IVS6-3C>G | F224ΔY225-Q271fsX288 | Hom | 3 | 4/M[a] | 0.29 | − | + | − |
| | | | | | 4 | 6/M | 0.12 | − | + | − |
| CG 1000 | Pakistani, Pakistan | 500insTT | F53fsX93 | Hom | 22 | 15/F | 0.07 | +/11 | − | NA |
| | | | | | 24 | 5/F | 0.14 | − | + | NA |
| CG 1100 | Chinese, United States | 1126insG | G271fsX283 | Het | 3 | 9/F | 0.38 | +/8 | + | − |
| CG 1200 | Caucasian/Native Indian, United States | 1028C>G | L227X | Hom | 3 | 4/M | 0.32 | − | − | − |
| CG 1300 | Chinese, United States | IVS6-2A>G | F224ΔY225-Q271fsX288 | Hom | 3 | 17/M | 0.68 | +/13 | − | + |
| | | | | | 5 | 11/F | 0.19 | +/9 | + | + |
| CG 2800 | Caucasian, United Kingdom | 537delCinsGGA | S64fsX91 | Het | 3 | 16/M | 0.9 | − | − | − |
| | | IVS6+5G>A | F224fsX225 | | 4 | 12/F | 0.8 | +/12 | − | − |
| CG 3800 | Caucasian, Portugal | 669insA | F108fsX113 | Hom | 5 | 10/F | 0.64 | +/30 | − | − |
| CG 4100 | Lebanese, United States | 659delGTATC | F105fsX111 | Hom | 4 | 19/M | 0.12 | +/NA | NA | + |
| CG 4700 | Lebanese, United States | 659delGTATC | F105fsX111 | Hom | 12 | 32/M | 0.5 | − | + | − |
| | | | | | 17 | 4/F | 0.7 | − | − | + |
| | | | | | 18 | 5/M | 0.6 | − | − | − |
| CG 6800 | Lebanese, United States | 659delGTATC | F105fsX111 | Hom | 3 | 19/M | 0.12 | +/9 | + | − |
| CG 6900 | Lebanese, United States | 659delGTATC | F105fsX111 | Hom | 3 | 18/M | <0.5 | +/11 | + | + |

Het, Compound heterozygote; Hom, homozygous; NA, not available; +, present; −, absent; DM, diabetes mellitus. Patient numbers are the same as shown in Fig. 2A.
[a] Sudden death at age 4 yr.

**TABLE 4.** Clinical features and linkage analysis of the 9q34 and 11q13 region and analysis of the transcripts for *AGPAT2* and *BSCL2* genes in affected subjects without *AGPAT2* or *BSCL2* mutations

| Pedigrees | Ethnicity, origin | Linkage to 9q34 region | Transcript analysis for *AGPAT2* | Linkage to 11q13 region | Transcript analysis for *BSCL2* | Patient no. | Age (yr)/sex | Serum leptin (ng/ml) | DM/age of onset (yr) | Mental retardation | Cardiomyopathy |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CG 300 | Caucasian, United States | Not done | Normal | Not done | Normal | 3 | 14/M | 1.29 | +/birth | − | NA |
| CG 1400 | Arab, Israel | Not done | Not done | Not done | Not done | 4 | 9/F | NA | +/6 | − | + |
| CG 3100 | Turkish, Turkey | Unlinked | Not done | Linked | Normal | 6 | 8/F | NA | − | − | − |
| CG 4200 | Caucasian, United States | Not done | Normal | Not done | Normal | 4 | 22/M[a] | 0.04 | +/NA | − | NA |
| | | | | | | 5 | 23/M | | | | |
| CG 4300 | Caucasian, Germany | Not done | Normal | Not done | Normal | 4 | 2/M | 0.02 | +/birth | NA | + |
| CG 4900 | South Asian, United Kingdom | Not done | Not done | Not done | Not done | 3 | | NA | NA | NA | NA |
| CG 5600 | Hispanic, Chile | Linked | Normal | Not determined | Normal | 3 | 29/F | 0.51 | +/NA | − | NA |
| CG 7100 | Hispanic, Mexico | Linked | Normal | Unlinked | Normal | 3 | 7/F | 0.07 | − | + | + |
| | | | | | | 5 | 1.5/M | 0.27 | − | + | + |

NA, Not available; +, present; −, absent; DM, diabetes mellitus. Patient numbers are the same as shown in Fig. 3.
[a] Died at age 22 yr.

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**TABLE 5.** Comparison of clinical features of CGL patients with mutations in *AGPAT2*, *BSCL2*, or unknown genes

| Variable | *AGPAT2* mutations | *BSCL2* mutations | Others |
|---|---|---|---|
| Age (yr) | 19.6 ± 15.2 (38) | 11.9 ± 7.7[a] (17) | 11.9 ± 9.5 (9) |
| Sex (M/F) | 11/27 (38) | 9/8 (17) | 4/5 (9) |
| BMI (kg/m$^2$) | 20.4 ± 3.3 (17) | 20.6 ± 4.7 (13) | 21.3 ± 5.8 (2) |
| Onset of diabetes (yr) | 16.8 ± 10.1 (18) | 9.5 ± 3.1[a] (8) | 2.1 ± 3.4 (3) |
| Serum leptin (ng/ml) | 0.96 ± 0.74 (31) | 0.42 ± 0.27[a] (17) | 0.37 ± 0.49 (6) |
| Diabetes mellitus (%) | 63.9 (36) | 52.9 (17) | 62.5 (8) |
| Hypertriglyceridemia (%) | 71 (31) | 62.5 (16) | 75 (8) |
| Acanthosis nigricans (%) | 68.2 (22) | 73.3 (15) | 57.1 (7) |
| Mental retardation (%) | 0 (20) | 50[b] (16) | 28.6 (7) |
| Cardiomyopathy (%) | 13 (23) | 42.9 (14) | 80 (5) |

Numbers in *parentheses* represent patients in the group on whom data for the particular variable were available. Data for the first five variables (except sex) are expressed as mean ± SD. BMI, Body mass index.

[a] $P < 0.05$, [b] $P < 0.001$ for comparison between patients with *AGPAT2* and *BSCL2* mutations.

## Discussion

CGL is a rare autosomal recessive disorder for which mutations in two genes, *AGPAT2* on 9q34 and *BSCL2* on 11q13, discovered via positional cloning, have been implicated in the pathogenesis. We have assembled CGL pedigrees from all over the world. In our cohort, mutations in the *AGPAT2* gene were about two times more frequent than in the *BSCL2* gene. Interestingly, all patients of African origin in our study had *AGPAT2* mutations. On the other hand, all patients of Lebanese origin had *BSCL2* mutations. Patients of European and Asian origin revealed mutations in both the *AGPAT2* and *BSCL2* genes. The IVS4–2A>G mutation of *AGPAT2* seems to be highly prevalent in patients of African origin, and all except one of these patients were either homozygous or compound heterozygous for this mutation. We also found seven novel mutations in the *AGPAT2* gene.

In one of the Hispanic pedigrees, we observed the homozygous mutation IVS4–2A>G on a haplotype different from that observed in the pedigrees of African origin. This suggests that this mutation may have occurred as a separate event. The occurrence of two homozygous mutations in the affected subjects from Buenos Aires, Argentina (CG4400 and CG6000), raises the question about which of the two mutations is likely to be disease causing. The Serine residue at position 100 in AGPAT2 lies within the conserved acyltransferase motif NHX$_4$D (N, Asn; H, His; X, any amino acid; D, Asp) and may influence the enzymatic activity (Fig. 1C). However, the alignment of this motif in all the known human AGPATs reveals that this amino acid is not conserved between the different isoforms of AGPAT, although it is preserved in isoforms 1, 2, and 5 (Fig. 1C). Blast search, however, does reveal that this residue is conserved in known AGPAT2s across various species. Thus, the role of Serine 100 residue is uncertain; nevertheless, this mutation can influence the kinetic properties of the enzyme. On the other hand, the splice site mutation, IVS3–1G>C, is likely to skip exon 4 and thus cause an inactive truncated protein because exon 4 contains the EGTR (E, Glu; G, Gly; T, Thr; R, Arg) motif involved in catalytic activity of the enzyme. It is therefore likely that IVS3–1G>C is the disease-causing mutation. Interestingly, both families shared the same haplotype consisting of similar intragenic SNPs.

As regards the *BSCL2* gene, the Lebanese and Norwegian mutations have been shown to be of ancestral origin, and our data in four Lebanese pedigrees further supports the previous findings. In addition, we report five novel mutations in the *BSCL2* gene. Because all of these variants found by us are predicted to cause severe alteration of the predicted protein sequence, these are highly likely to be disease-causing mutations. The affected subject from pedigree CG1100 revealed only one heterozygous mutation in *BSCL2*. We were unable to locate the second *BSCL2* mutation or any mutations in the *AGPAT2* gene in this patient.

Among the 45 pedigrees we screened for *AGPAT2* or *BSCL2* mutations, eight affected pedigrees did not have any substantial alterations in either of these two genes. Patients who showed linkage to polymorphic microsatellite markers in either the 9q34 and 11q13 regions had normal transcript analysis of the *AGPAT2* and *BSCL2* genes. Our observations that some patients with CGL do not have mutations in either *AGPAT2* or *BSCL2* suggest additional genetic loci for CGL. Affected individuals from one of these pedigrees (CG7100) also had congenital myopathy. The high prevalence of cardiomyopathy in affected subjects from four pedigrees in this group is also interesting. It is likely that the genetic defect in these patients affects the development of both adipocytes and myocytes. Heathcote *et al.* (17) also reported two Omanese families with CGL who did not map to either the 9q34 or the 11q13 loci. However, affected patients from these pedigrees seem to have a different phenotype than that observed in typical patients with CGL, such as the presence of infantile pyloric stenosis and percussion myoedema. Furthermore, affected subjects from these pedigrees did not have diabetes or hyperinsulinemia.

Although patients with both *AGPAT2* and *BSCL2* mutations have similar metabolic abnormalities, there were many phenotypic differences between the two patient subgroups. We found a high preponderance of females to males among patients with *AGPAT2* mutations but not in the other subtypes. Whether this reflects a sampling bias, or is due to the less severe fat loss in patients with *AGPAT2* mutations that may cause the diagnosis to be missed in many males is not clear. We noticed significantly lower leptin levels in patients with *BSCL2* mutation compared with *AGPAT2* mutation, which may reflect more severe fat loss. We have noted that *BSCL2* patients have loss of fat from their palms and soles, and from other areas where predominantly mechanical adipose tissue is present, whereas this is well preserved in

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patients with *AGPAT2* mutation (18). However, in the present study, patients with *BSCL2* mutations were younger, which could also account for their lower leptin levels. Other phenotypic differences between the two groups include the higher prevalence of mental retardation and cardiomyopathy in patients with *BSCL2* mutation and the presence of lytic lesions in the appendicular skeleton in patients with *AGPAT2* mutation. Van Maldergem *et al*. (4) also reported significantly higher prevalence of mental retardation in patients with *BSCL2* mutations. It seems that high expression of *BSCL2* in the brain may be related to increased prevalence of mental retardation in CGL patients with *BSCL2* mutations.

AGPAT2 catalyzes the acylation of the lysophosphatidic acid at the sn-2 position to form phosphatidic acid, a key intermediate in the biosynthesis of triacylglycerol and glycerophospholipids (19). High expression of *AGPAT2* mRNA in adipose tissue compared with other isoforms suggests that the mutations might affect the adipose tissue the most (3). Although the precise mechanisms by which *AGPAT2* mutations cause lipodystrophy remain unclear, the lack of triglyceride synthesis in the adipocytes or reduced bioavailability of phosphatidic acid and glycerophospholipids such as, phosphatidylinositol, phosphatidylcholine, and phosphatidylethanolamine, which are essential components of cell membranes and play an important in intracellular signaling, could be responsible (20).

The mechanisms by which *BSCL2* mutations cause lipodystrophy, however, still remain unclear. Based on high expression of *BSCL2* mRNA in the brain, a primary defect in hypothalamic-pituitary axis has been suggested to cause lipodystrophy (13). However, a recent semiquantitative RT-PCR of *BSCL2* in our laboratory revealed twice as much expression in the human omental adipose tissue compared with liver, whereas skeletal muscle expression was very poor (21). Thus, besides a central nervous system defect, a primary abnormality in adipose tissue could cause lipodystrophy in patients with *BSCL2* mutations.

It is quite likely that mutations in additional isoforms of AGPATs, as well as in the other enzymes of the pathway, such as glycerol-3-phosphate acyltransferases, phosphatidic acid phosphatases, and diacylglycerol acyltransferases may also cause CGL. Both mitochondrial glycerol-3-phosphate acyltransferase and the two microsomal isoforms of diacylglycerol acyltransferase are known to be highly expressed in the adipose tissue (20, 22, 23) and remain candidate genes for CGL patients without *AGPAT2* or *BSCL2* mutations.

In summary, CGL is caused by mutations in *BSCL2*, *AGPAT2*, and other as yet unmapped genes. This genetic heterogeneity is also accompanied by phenotypic heterogeneity.

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