

# Mutations in *Gng3lg* and *AGPAT2* in Berardinelli-Seip Congenital Lipodystrophy and Brunzell Syndrome: Phenotype Variability Suggests Important Modifier Effects

MAO FU, RASA KAZLAUSKAITE, MARIA DE FÁTIMA PAIVA BARACHO, MARIA GORETTI DO NASCIMENTO SANTOS, JOSÉ BRANDÃO-NETO, SANDRA VILLARES, FRANCESCO S. CELI, BERNARDO L. WAJCHENBERG, AND ALAN R. SHULDINER

*Division of Endocrinology, Diabetes and Nutrition (M.F., F.S.C., A.R.S.), University of Maryland School of Medicine, and Geriatrics Research and Education Clinical Center (A.R.S.), Baltimore Veterans Administration Medical Center, Baltimore, Maryland 21201; Division of Endocrinology (R.K.), Cook County Hospital, Chicago, Illinois 60612; Center of Health Sciences (M.d.F.P.B., M.G.D.N.S., J.B.-N.), Federal University of Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil 59010-180; and Endocrine Service (S.V., B.L.W.), Hospital das Clinicas, University of Sao Paulo, Brazil 05403-900*

**Congenital generalized lipodystrophy (CGL) is a rare autosomal recessive disorder caused by mutations in *AGPAT2* and *Gng3lg*. We screened for mutations in *AGPAT2* and *Gng3lg* in 26 families with CGL and one family with Brunzell syndrome. We found mutations in either *AGPAT2* or *Gng3lg* in all but four probands, including three novel mutations in *AGPAT2*, A712T (Lys215X), IVS3-1G→C, and C636A (Phe189X). In three siblings with Brunzell syndrome, we identified a splice site mutation (IVS4-2A→G) in *AGPAT2*, showing that *AGPAT2* mutations can also cause Brunzell syndrome. Eighteen CGL patients from 15 families from the same region of northeastern Brazil were homozygous for a frameshift mutation**

**(669insA of AF05149) in *Gng3lg*. Despite having the same mutation, the subjects had widely divergent clinical manifestations. In our subjects, there did not appear to be any distinguishing clinical characteristics between CGL subjects with *AGPAT2* or *Gng3lg* mutations with the exception of mental retardation in carriers of *Gng3lg*. In summary, mutations in *AGPAT2* and *Gng3lg* are approximately equally represented in CGL; despite harboring the same *Gng3lg* mutation, subjects may have widely divergent clinical manifestations, suggesting modifying influences of other genes and/or environment; and Brunzell syndrome may be caused by a mutation in *AGPAT2*. (*J Clin Endocrinol Metab* 89: 2916–2922, 2004)**

**C**ONGENITAL GENERALIZED lipodystrophy (CGL), or Berardinelli-Seip syndrome (BSCL) [Online Mendelian Inheritance in Man (OMIM) no. 269700], is a rare autosomal recessive disease characterized by near-complete absence of adipose tissue from birth or early infancy (1, 2). Affected individuals have marked insulin resistance, hypertriglyceridemia, and acanthosis nigricans, hyperandrogenism, muscular hypertrophy, hepatomegaly, and altered glucose tolerance or diabetes (3, 4). Plasma leptin concentrations are low (5). Patients have a unique pattern of body fat loss; *i.e.* near-total absence of metabolically active adipose tissue in sc, intraabdominal, intrathoracic, and bone marrow regions but preservation of mechanical fat in the orbits, palms, soles, scalp, perineum, and periarticular regions (6). A related syndrome, Dunnigan-type familial partial lipodystrophy (OMIM no. 151660) is autosomal dominant and due to mutations in the lamin A/C (*LMNA*) gene (7, 8).

Two loci (*BSCL1* and *BSCL2*) linked to CGL have been mapped to chromosomes 9q34 and 11q13, respectively (9, 10). Positional cloning of *BSCL1* revealed mutations in

*AGPAT2*, which encodes the 1-acylglycerol-3-phosphate *O*-acyltransferase 2 (11). This 278-amino-acid protein belongs to the family of acyltransferases and catalyzes the acylation of lysophosphatidic acid to form phosphatidic acid, a key intermediate in the biosynthesis of triacylglycerol and glycerophospholipids (12). Positional cloning of *BSCL2* by Magre *et al.* (10) disclosed mutations in *Gng3lg* (also named *seipin*), which is homologous to the murine guanine nucleotide-binding protein (G protein)  $\gamma$ 3-subunit-linked gene, a gene of unknown function.

Despite the identification of these genes, several questions remain. What is the relative prevalence of *AGPAT2* and *Gng3lg* mutations in subjects with CGL? Are there any distinguishing clinical characteristics between CGL subjects with mutations in *AGPAT2* compared with *Gng3lg*? How consistent is the phenotype in CGL subjects with the same mutation? To begin to answer these questions, we studied these two genes in CGL subjects from 26 families. Our findings suggest that mutations in *AGPAT2* and *Gng3lg* are approximately equally represented in CGL. There do not appear to be any obvious distinguishing phenotypic characteristics in subjects with mutations in one gene or the other with the exception that mental retardation appears to be associated with *AGPAT2* mutations but not *Gng3lg* mutations. Furthermore, despite harboring the same *Gng3lg* mutation, subjects may have widely divergent clinical manifes-

Abbreviations: BSCL, Berardinelli-Seip syndrome; CGL, congenital generalized lipodystrophy; OMIM, Online Mendelian Inheritance in Man; UTR, untranslated region.

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tations, suggesting modifying influences of other genes and/or environment. Finally, we identified a mutation in *AGPAT2* in three siblings with Brunzell syndrome (OMIM no. 272500) (13), a related syndrome characterized by generalized lipodystrophy and systemic angiomas. These findings implicate mutations in *AGPAT2* as a cause of Brunzell syndrome.

## Subjects and Methods

### Subjects

We studied 30 affected CGL subjects from 26 families (CGL-F1 to CGL-F26) as well as three affected siblings from a family (B-F1) with Brunzell syndrome. The 18 affected members of families CGL-F1 through CGL-F15 were all from a geographically localized region of Serido, a county of Rio Grande do Norte State in northeastern Brazil. Proband from other families were, to our knowledge, not related to one another. All subjects with CGL had a generalized form of lipodystrophy with near absence of adipose tissue at birth or beginning in early infancy and muscular hypertrophy. Most patients presented with acanthosis nigricans, hepatomegaly, insulin resistance/diabetes, and hypertriglyceridemia. Their clinical characteristics are summarized in Table 1 and Fig. 1. Additional clinical characteristics of the probands from CGL-F16, -F17, -F22, -F25, and -F26, and B-F1 have been reported previously (14–18). This project was approved by the Medical Ethics Committee of the University of Rio Grande do Norte. Written informed consent was obtained from all subjects.

### Mutation screening and genotyping

Genomic DNA was extracted from peripheral blood cells using standard protocols (QIAquick; QIAGEN, Santa Clarita, CA). All exons of *Gng3lg* and *AGPAT2* with adjacent intron-exon junctions were screened for mutations in families CGL-F1, CGL-F2, CGL-F16 through -F26, and B-F1. Exons 1–11 and the surrounding intronic sequences of *Gng3lg* (GenBank accession no. AP001458) were amplified by PCR using previously described primers (10). PCR conditions were 95 C for 2 min, followed by 30 cycles at 95 C for 45 sec, 60 C for 45 sec, and 72 C for 1 min, with a final extension at 72 C for 7 min. Intronic primers for PCR amplification of the six exons and exon-intron boundaries of *AGPAT2* (GenBank accession no. AL590226) were designed using the Primer3 program (primer sequences available from the authors upon request). PCR conditions were 95 C for 2 min, followed by 35 cycles at 95 C for 45 sec, 56 C for 45 sec, and 72 C for 1 min, with a final extension at 72 C for 7 min. All PCR products were sequenced in both directions on an ABI 377 or ABI 3700 DNA sequencer and analyzed with Sequence Analysis 3.2 software (Applied Biosystems Division/PerkinElmer, Foster City, CA).

Once identified by sequence analysis, a rapid genotyping assay was used to screen for the adenosine insertion at codon 669 of exon 4 of *Gng3lg*. Five microliters of exon 4 PCR product, which were generated with upstream primer 5'-TTGTGTGTC AAGGGTCTCA-3' and downstream primer 5'-AAAACAAGACCCACATCA-3', were digested with 7.5 U of *HpaI* restriction endonuclease (New England BioLabs, Beverly, MA) for 3 h at 37 C. The fragments were separated on a 2% agarose gel and visualized after staining with ethidium bromide. The adenosine insertion at codon 669 creates a unique *HpaI* restriction site, which generates two DNA fragments of 178 and 109 bp.

## Results

DNA sequence analysis of exon 4 of *Gng3lg* revealed a homozygous insertion of adenosine at position 669 (669insA, GenBank accession no. AF052149) in three affected subjects from families CGL-F1 and CGL-F2 (Fig. 2A). 669insA predicts a frameshift mutation, resulting in a truncated protein 113 amino acids in length with the last five amino acids mutated (normally *Gng3lg* is 398 amino acids long). Because families CGL-F1 through CGL-F15 were all from a geograph-

ically localized region of Brazil, PCR-RFLP analysis for 669insA was performed in all available family members. All affected members of these 15 nuclear families were found to be homozygous for 669insA, whereas all unaffected members were either heterozygous for 669insA or homozygous for the normal allele (Fig. 2B).

Sequence analysis of *Gng3lg* in an affected female subject from Brazil (CGL-F16) revealed a homozygous insertion of AA at position 645. In an affected subject from family CGL-F17 (unrelated to families CGL-F1-F15), we found a homozygous deletion of C at position 980 in exon 6 of *Gng3lg*. In a subject of Lebanese origin (CGL-F18), we found a homozygous deletion of GTATC at position 659 of *Gng3lg*. All three of these mutations predict frameshifts with premature stop codons.

We also detected five polymorphisms in *Gng3lg* in both affected and unaffected family members: in intron 1 at +62-+64delGGG; in intron 2 at -8C→A; in intron 5 at +69A→G; in the 5' flanking region at -49T→C; and in exon 9 at A1288G (silent variant).

Sequence analysis of *AGPAT2* in both CGL-affected individuals from family CGL-F19 revealed a homozygous A→T substitution at nucleotide 712 in exon 5 (Fig. 3A, GenBank accession no. NM\_006412), generating a TAG termination codon predicting a truncated protein 215 amino acids in length (the full-length *AGPAT2* protein is 278 amino acids long). An affected individual from Brazil (CGL-F20; Table 1) was a compound heterozygote for mutations predicting a premature termination codon (636C→A; Phe189X; Fig. 3B) and a splice-site mutation (IVS3-1G→C; Fig. 3C). Another affected individual from Brazil (CGL-F21) was homozygous for a deletion of exons 3 and 4, predicting a frameshift mutation and premature termination codon. Finally, a homozygous splice-site mutation (IVS4-2A→G) predicting a frameshift and a premature termination codon (Gln196fsX228) was found in *AGPAT2* of an affected female subject (CGL-F22; Table 1) as well as in three affected siblings in a family with Brunzell syndrome (B-F1; Table 1 and Fig. 3D).

We detected five polymorphisms in *AGPAT2* in both affected and unaffected family members: in intron 1 at -58C→G; in the 3' untranslated region (UTR) at 1139C→T; in the 3'UTR at 1321G→C; and in the 3'UTR at 1420 C→T.

We were not able to detect any mutations in *Gng3lg* or *AGPAT2* in four subjects (CGL-F23 through -F26).

## Discussion

Since Berardinelli first described congenital generalized lipodystrophy in 1954 (1), more than 100 patients have been reported. The major clinical characteristics of this rare syndrome include severe insulin resistance and lipodystrophy at birth or in early infancy (3). The high prevalence of parental consanguinity in affected individuals has been well documented, suggesting autosomal recessive transmission. Garg *et al.* (9) undertook a genome-wide scan in 17 well-characterized pedigrees of Turkish, Caucasian, African, Hispanic, and Chinese origins and identified a locus (*BSC11*) on chromosome 9q34 (near marker D9S1818) and also showed at least one other locus in CGL. Recently, they identified homozygous or compound heterozygous mutations in the

**TABLE 1.** Mutation and main clinical and biological characteristics of study subjects

DNA mutation	Study subjects	Sex	Ethnicity	Age		BMI (kg/m <sup>2</sup> )	Acanthosis nigricans	Hyper-muscular	Acro-megaloid	Changes in external genitalia	Mental retardation	Hepato-megaly	Diabetes (age at onset; yr)	Insulin (μU/ml) <sup>e</sup>	Trig (mg/dl) <sup>e</sup>	Leptin (ng/dl) <sup>e</sup>
				At study (yr)	Diagnosis of CGL											
<i>Gng3lg</i> <sup>b</sup>																
669insA	CGL-F1	F	C	32	Birth	23.5	Yes	Yes	Yes	Yes	Yes	No	Yes (8)	25.8	175	0.4
669insA	CGL-F1-2	F	C	23	Birth	22.2	Yes	Yes	Yes	Yes	Yes	No	Yes (16)	27.3	384	1.2
669insA	CGL-F2	M	C	13	20 d	20.5	Yes	Yes	Yes	Yes	No	Yes	Yes (2)	25.2	358	1.7
669insA	CGL-F3	F	C	4	Birth	17.1	Yes	Yes	Yes	No	No	Yes	No	4.9	288	1.0
669insA	CGL-F4	M	C	29	Birth	24.2	Yes	Yes	Yes	No	No	No	Yes (15)	18.7	122	0.9
669insA	CGL-F5	M	C	12	Birth	20.3	Yes	Yes	Yes	Yes	Yes	Yes	Yes (6)	37.5	792	2.0
669insA	CGL-F6-1	F	C	14	Birth	23.5	No	Yes	Yes	Yes	Yes	Yes	No	57.6	159	1.6
669insA	CGL-F6-2	F	C	10	Birth	21.2	Yes	No	Yes	Yes	No	Yes	No	30.8	139	2.3
669insA	CGL-F7	F	C	7	Birth	17.3							Yes (7)	273.2	356	0.9
669insA	CGL-F8	F	C	12	Birth	20.0	Yes	Yes	Yes	No	No	No	Yes (5)	29.3	274	1.0
669insA	CGL-F9	M	C	7	30 d	17.3	Yes	Yes	Yes	Yes	No	No	No	8.5	462	0.8
669insA	CGL-F10	F	C	7	Birth	16.1	Yes	Yes	Yes	No	No	No	No	36.2	220	1.0
669insA	CGL-F11	M	C	40	2 yr	26.4	Yes	Yes	Yes	Yes	No	No	Yes (7)	18.5	213	0.9
669insA	CGL-F12-1	M	C	20	15 d	28.7	No	Yes	Yes	Yes	No	No	No	334		
669insA	CGL-F12-2	F	C	7	Birth	19.1	No	Yes	Yes	No	No	No	Yes (2)		489	2.0
669insA	CGL-F13	M	C	16	Birth	21.1	Yes	Yes	Yes	Yes	Yes	Yes	Yes (9)	20.3	288	4.4
669insA	CGL-F14	M	C	16	Birth	19.3	Yes	Yes	Yes	Yes	No	Yes	No	46.4	159	1.2
669insA	CGL-F15	F	C	8	Birth	14.6	Yes	Yes	Yes	Yes	No	Yes	No	24.8	300	2.3
645insAA	CGL-F16	F	C	36	<1 yr	21.0	No	Yes	No	Yes	Yes	Yes	Yes (26)		660	0.8
980delC	CGL-F17	F	CI		<1 yr		Yes				No	No	Yes		>400	<1.0
368delGTATC	CGL-F18	F	C	27												
<i>AGPAT2</i> <sup>c</sup>																
A712T	CGL-F19-1	F	AA	2	Birth	17.7	Yes	Yes	Yes	No	No	Yes	No	28.8	154	0.5
A712T	CGL-F19-2	F	AA	5	Birth	20.1	Yes	Yes	Yes	No	No	Yes	No	11.2	119	1.0
IVS3-1G→C C635A	CGL-F20	F	AA	20	Birth	20.0	Yes	Yes	Yes	Yes	Yes	Yes	Yes (16)		662	1.8
317-588del	CGL-F21	F	C	20	Birth	23.8	Yes	Yes	Yes	No	No	Yes	Yes (15)		2578	1.4
IVS4-2A→G	CGL-F22	F	AA	8	Birth	16.0	Yes	Yes	Yes	No	No	Yes	No	102.0	354	
IVS4-2A→G	B-F1-1	F	AA	14	Birth	18.5	Yes	Yes	Yes	No	No	Yes	No	76.0	420	
IVS4-2A→G	B-F1-2	F	AA	16	20 d		Yes	Yes	Yes	No	No	Yes	Yes	112.0	90	
IVS4-2A→G	B-F1-3	M	AA	11	<5 yr	16.7	No	Yes	No	No	No	Yes	Yes	40.0	560	
Neither																
	CGL-F23	F	C	1	Birth								No	10.7		1.5
	CGL-F24	M	C	30									No			
	CGL-F25	F	C	18	Birth		Yes	No	No	No	No	Yes	Yes (5)		5970	
	CGL-F26	M	C	22	Birth		Yes	Yes	No	No	No	Yes	No	78.0	850	

B, Brunzell syndrome; F, female; M, male; C, Caucasian; AA, African-American; CI, Canadian-Indian; Trig, triglycerides; BMI, body mass index. Cells are empty if the data was not available for a given subject/phenotype.

<sup>a</sup> Insulin, leptin, and triglycerides levels were obtained after an overnight fast.

<sup>b</sup> GenBank accession no. AF052149.

<sup>c</sup> GenBank accession no. NM\_006412.

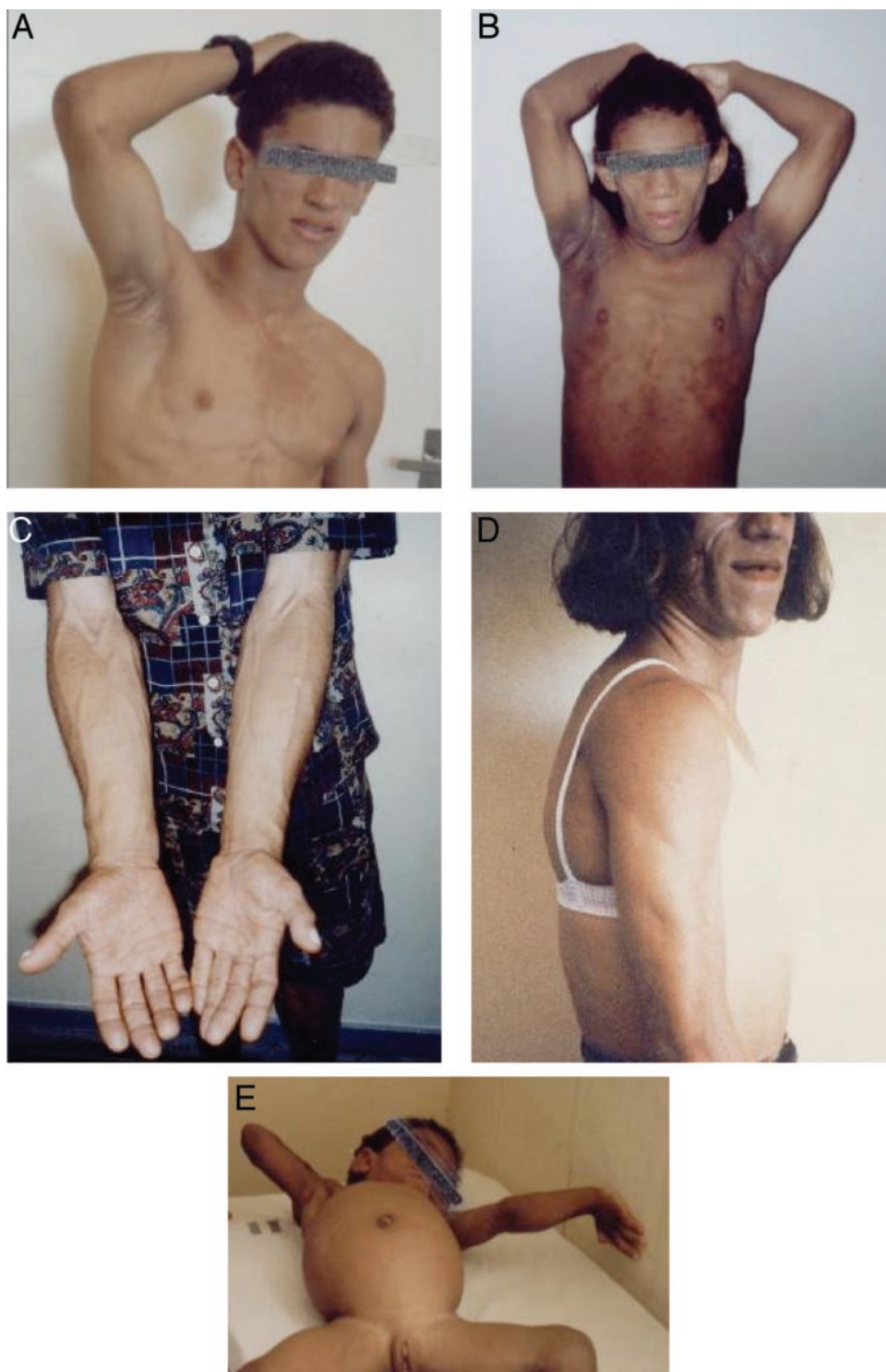


FIG. 1. Characteristic clinical features of patients with congenital generalized lipodystrophy from a geographically localized region of Serido, a county of Rio Grande do Norte State in Northeastern Brazil including complete lipodystrophy (all panels), acanthosis nigricans (panels A and B), muscle hypertrophy (panels A, B, and C), acromegaloid features (panels C and D), and clitoromegaly (panel E).

*AGPAT2* gene among CGL-affected individuals showing linkage to 9q34 (11). Magre *et al.* (10) studied 29 families and 17 additional patients from Turkey, Norway, Italy, United Kingdom, Brazil, France, Lebanon, Portugal, and India and identified a locus (*BSCL2*) within the 2.5-Mb interval flanked by markers D11S4076 and D11S480 on chromosome 11q13 using a genome-wide analysis. Sequence analysis of genes located in the 11q13 interval disclosed mutations in a gene

homologous to the murine guanine nucleotide-binding protein (G protein),  $\gamma$ -subunit-linked gene (*Gng3lg*) in all *BSCL2*-linked families (10).

Our studies in 33 subjects from 26 CGL families and one Brunzell syndrome family represents one of the largest studies of CGL reported to date. We found four mutations in *AGPAT2* and five mutations in *Gng3lg*, which explained the CGL phenotype in all but four subjects. Three of the muta-

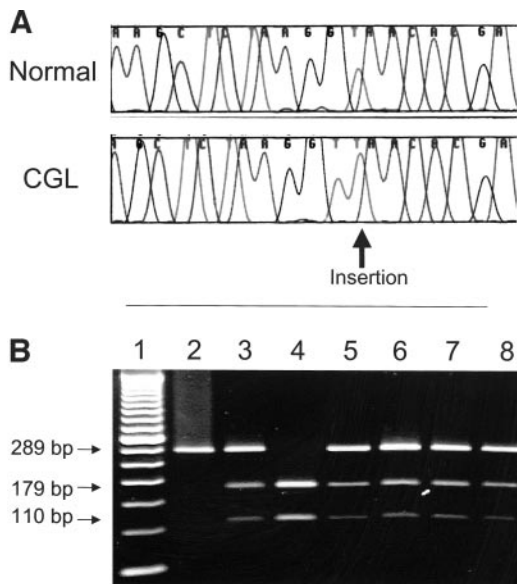


FIG. 2. Identification of 669insA in exon 4 of *Gng3lg*. A, DNA sequence analysis of subject CGL-F1. Sequencing of the opposite strand showing the insertion of a T is shown. The subject was homozygous for the insertion. B, PCR-RFLP of 669insA in affected individuals and family members. The insertion, 669insA, introduces a *HpaI* restriction site. Lane 1, Fifty-base-pair DNA ladder; lane 2, homozygous normal (unaffected); lane 4, homozygous for 669insA (affected); lanes 3 and 5–8, heterozygous for 669insA (unaffected).

tions we found in *AGPAT2* are novel, two of which predict premature chain termination and a truncated protein: A712T (Lys215X) and 636C→A (Phe189X). Figure 4 summarizes all mutations in *AGPAT2* (A) and *Gng3lg* (B) identified to date.

Eighteen affected individuals from 15 Caucasian families (CGL-F1 through -F15; Table 1) who lived in a geographically localized region of Serido county of Rio Grande do Norte State in northeastern Brazil harbored the same homozygous mutation in *Gng3lg* (669insA). The same mutation was described previously by Magre *et al.* (10) in a subject of Portuguese origin in South Africa. In this region of Brazil, the vast majority of Caucasians are known to have originated from Portugal, and thus it is likely that 669insA in *Gng3lg* arose from a single founder of Portuguese origin. Indeed, affected individuals were found to be homozygous for the same flanking short tandem repeat markers within this region of chromosome 11q13 (data not shown). Although all 18 individuals homozygous for this mutation had several of the cardinal manifestations of congenital generalized lipodystrophy, including lipoatrophy (body mass index, mean  $\pm$  SD = 20.4  $\pm$  3.5 kg/m<sup>2</sup>), acromegaloid dysmorphism, and muscular hypertrophy, other features were present in some but not all of the subjects [*i.e.* hypertriglyceridemia (89% of subjects), acanthosis nigricans (82% of subjects), hyperinsulinemia (75% of subjects), external genitalia enlargement (69% of subjects), umbilical hernia (60% of subjects), low plasma leptin concentration (59% of subjects), diabetes (56% of subjects; age at onset of diabetes, 2–16 yr), hepatomegaly (50% of subjects), mental retardation (29% of subjects), splenomegaly (12% of subjects), and hirsutism (10% of female subjects)]. Thus, despite harboring the same mutation, subjects

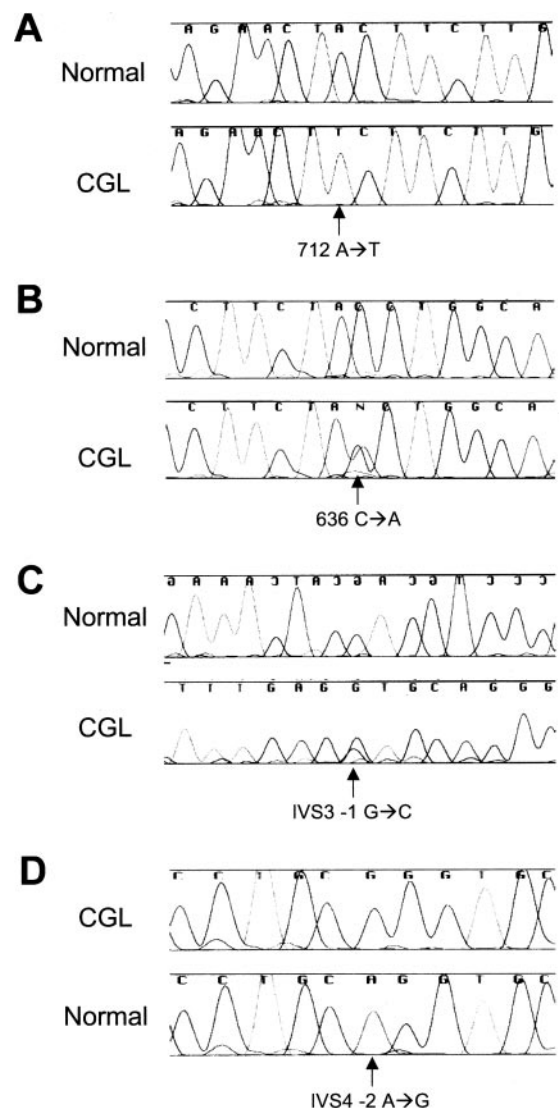


FIG. 3. Identification of mutations in *AGPAT2* in subjects with CGL. A, Homozygous mutation of 712 A→T in subject CGL-F19, which predicts a nonsense mutation. B, Heterozygous mutation of 636 C→A in subject CGL-F20, which predicts a premature termination codon (Phe189X). C, Heterozygous mutation of IVS3-1G→C in subject CGL-F20, and premature termination codon (Asn164fsX249). Note subject CGL-F20 is a compound heterozygote. D, Homozygous mutation (IVS4-2A→G), which eradicates a consensus splice site, in a patient with Brunzell syndrome.

had widely divergent clinical manifestations, suggesting modifying influences of other genes and/or environment.

A Brazilian female (CGL-F16) with CGL and severe insulin resistance (17), unrelated to Brazilian families CGL-F1-F15, had a *Gng3lg* mutation (645insAA). The same mutation was found in another Brazilian case by Magre *et al.* (10). Subject CGL-F17 lived in Canada, was of Indian origin, and had 980delC, which was previously described in a CGL case from India (10). The study subject CGL-F18 carried the diagnosis of acquired generalized lipodystrophy. This Lebanese subject had a 368delGTATC mutation in *Gng3lg* previously described in subjects with lipodystrophy of Lebanese origin (10). Thus, these findings are more likely to reflect a founder

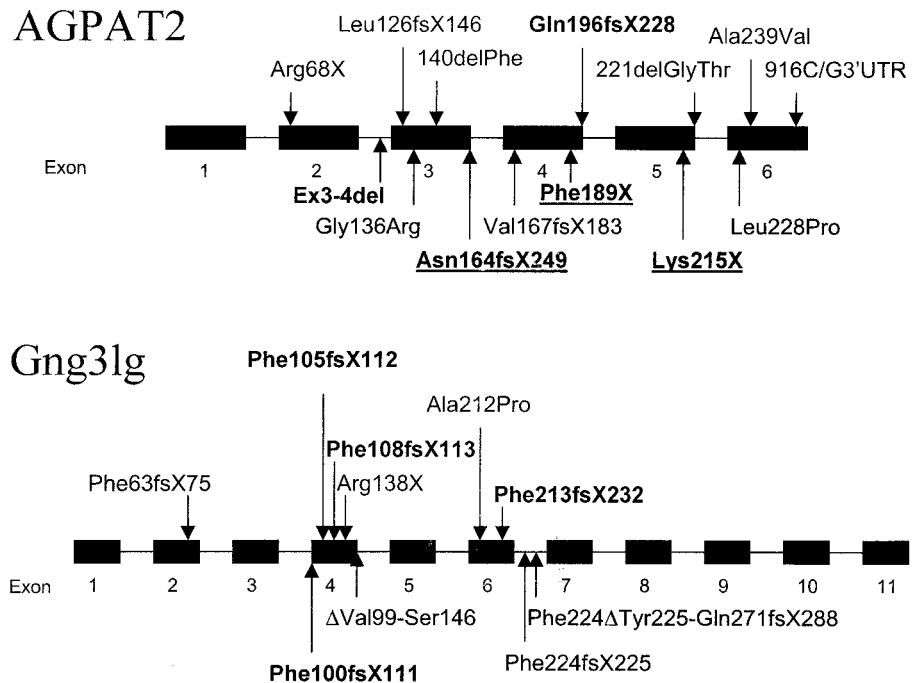


FIG. 4. Schematic of mutations in *Gng3lg* (A) and *AGPAT2* (B). Rectangles represent exons and lines between rectangles represent introns (not drawn to scale). Mutations that are *underlined* and in *bold* are new mutations identified in this study, mutations that are *bold only* were identified in this study as well as by others, and mutations that are neither *bold* nor *underlined* were identified by others.

effect, although we cannot rule out the possibility of *de novo* mutations in *Gng3lg*.

We found three novel missense mutations in *AGPAT2*, two in a Brazilian female (CGL-F20), who was found to be a compound heterozygote (IVS3-1G→C and C636A, Phe189X), and one in two affected female siblings from family CGL-F19 (A712T, Lys215X). Brazilian subject CGL-F21 was a female who had a 317–588del of *AGPAT2*, which also is most likely to have arisen in Portugal because this same mutation was described in a subject from Portugal, and European settlers from this region of Brazil are known to have originated from Portugal (11).

We did not find mutations in either *Gng3lg* or *AGPAT2* in four subjects. Affected members of family CGL-F23, in addition to lipodystrophy, had neurodegenerative disorder and congenital cataracts. The proband from consanguineous pedigree CGL-F24 also had idiopathic pulmonary fibrosis and premature puberty. An 18-yr-old white female (CGL-F25) with lipodystrophy, diabetes, multiple xanthomas, bilateral cataracts, hemorrhagic pancreatitis, and cardiomyopathy also did not have mutations in *Gng3lg* or *AGPAT2*. Van Maldergem *et al.* (19) also identified three families from a total 44 families for which they did not find a mutation in either *Gng3lg* or *AGPAT2*. It is possible that these subjects may have mutations in regions of *Gng3lg* or *AGPAT2* not studied (*i.e.* regulatory elements in flanking regions or introns) or may have mutations in yet-to-be identified genes that cause related lipodystrophic syndromes.

Reports of cases with generalized lipodystrophy indicate phenotypic variation. Subjects with Brunzell syndrome typically have congenital generalized lipodystrophy with cystic angiomas of the long bones (13). It has been suggested that Brunzell syndrome might have the same genetic etiology as BSCL (<http://www.ncbi.nlm.nih.gov/entrez/dispmim.cgi?id=272500>). However, others have suggested that Brun-

zell syndrome could be a separate entity (20). Two sisters (subjects B-F1-1 and B-F1-2) from an African-American pedigree had congenital generalized lipodystrophy, cystic angiomas of long bones, and primary amenorrhea, attributed to polycystic ovaries. The brother (subject B-F1-3), in whom systemic angiomas was not detected, and both affected sisters carried the same splice site mutation (IVS4-2A→G) in *AGPAT2*, showing directly that Brunzell syndrome is a clinical and genetic variant of CGL. Van Maldergem *et al.* (19) also recently reported a mutation in *Gng3lg* in a subject with Brunzell syndrome.

Our studies of a relatively large number of CGL subjects with mutations in both *AGPAT2* and *Gng3lg* provided the opportunity to compare clinical characteristics of subjects with each genetic etiology. Although definitive conclusions cannot be made, it appears that the two genetic etiologies of CGL have very similar, albeit variable, clinical characteristics. One notable exception is that mental retardation appears to be associated with *Gng3lg* mutations but not *AGPAT2* mutations (Table 1). A similar observation was made by Van Maldergem *et al.* (19), suggesting that *Gng3lg* plays a role in brain development or function, although we cannot rule out the possibility that the mental retardation was due to consanguinity and homozygosity for one or more mutations at other loci.

In summary, these studies have led to several novel findings. First, in our relatively large sample set, mutations in *AGPAT2* and *Gng3lg* were approximately equally prevalent in patients with CGL. Second, there do not appear to be any major distinguishing phenotypic characteristics between subjects with *AGPAT2* or *Gng3lg* mutations, with the possible exception of mental retardation, which appears to be associated with *Gng3lg* mutations but not *AGPAT2* mutations. Third, studies of 18 subjects from the same locale in Brazil with the same mutation in *Gng3lg* show that there is great

phenotypic variability in clinical manifestations, suggesting modifying effects of other genes or environmental factors. Finally, we show that Brunzell syndrome may be caused by mutations in *AGPAT2*. Future functional studies of *Gng3lg* and *AGPAT2* will undoubtedly unveil greater insights into the clinical spectrum of lipodystrophy disorders as well as adipose tissue biology.

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Address all correspondence and requests for reprints to: Mao Fu, Division of Endocrinology, Diabetes and Nutrition, University of Maryland School of Medicine, 660 West Redwood Street, Baltimore, Maryland 21201. E-mail: mfu@medicine.umaryland.edu.

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