CASE REPORT

Novel BSCL2 gene mutation E189X in Chinese congenital generalized lipodystrophy child with early onset diabetes mellitus

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Abstract

Context: Congenital generalized lipodystrophy (CGL) is a rare and heterogeneous disease of autosomal recessive inheritance. Until now, no genetic findings had been reported in Chinese patients with CGL. **Objective:** To analyze Berardinelli-Seip congenital lipodystrophy type 2 (BSCL2) and 1-acylglycerol-3-phosphate O-acyltransferase 2 (AGPAT2) gene variation in a Chinese boy with CGL and his family.

Design, setting, and participants: All exons of BSCL2 and AGPAT2 with adjacent intron–exon junctions were analyzed using direct sequencing.

Main outcome measures: Sequences of each exon and nearby intron of the BSCL2 and AGPAT2 genes of the family members were compared with the gene bank genomic sequences.

Results: DNA sequence analysis of the entire coding regions and surrounding uncoding regions disclosed a novel homozygous G→T mutation at nucleotide 909 in exon 5 of the BSCL2 gene in the affected child. A heterozygous state of the G→T mutation of the BSCL2 gene was also found in other family members. This mutation predicts the substitution of glutamic acid at codon 189 by the stop codon (Glu189X or E189X). No variation was found in the AGPAT2 gene.

Conclusion: E189X is a novel BSCL2 gene mutation that contributes to CGL formation in a family of Chinese origin.

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Introduction

Congenital generalized lipodystrophy (CGL; Online Mendelian Inheritance In Man (OMIM) 269700), originally described in Brazil by Berardinelli in 1954 and reviewed by Seip in 1959, became known as Berardinelli–Seip syndrome. It is a rare autosomal disease characterized by near-complete absence of adipose tissue already apparent at birth or in early infancy, resulting in striking muscular appearance (1). The pattern of body fat loss is unique, i.e., near-total absence of metabolically active adipose tissue in subcutaneous, intra-abdominal, intra-thoracic, and bone marrow regions, but preservation of mechanical fat in the orbits, palms, soles, scalp, perineum, and periarticular regions (2). Even during infancy, patients show extreme hyperinsulinemia, hypertriglyceridemia, and hepatomegaly because of hepatic steatosis. More clinical features include widespread acanthosis nigricans, acromegaloid appearance, umbilical hernia, and, in women, clitoromegaly, hirsutism,

oligo/amenorrhea, and polycystic ovaries. Diabetes mellitus develops mostly during pubertal years and is ketosis resistant (3). Other reported features involve mild mental retardation, hypertrophic cardiomyopathy, nephropathy, and nubecula (4, 5).

Recent studies have reported mutations in two unrelated genes – BSCL2 and AGPAT2 – in patients with CGL linked to human chromosomes 11q13 and 9q34 respectively (3, 6, 7). Other genes (or a single gene) might be inferred from genetic studies of a few families where the disease is not linked to the two identified loci (4, 8). The first gene, AGPAT2, scanned in 1999, encodes 1-acylglycerol-3-phosphate O-acyltransferase 2 that catalyzes acylation of lysophosphatidic acid to phosphatidic acid during the biosynthesis of glycerophospholipids and triglyceride from glycerol-3-phosphate (9, 10). The other affected gene, BSCL2, first identified in 2001, encodes seipin, a 398 amino acid protein (11). Although it might be understood how AGPAT2 mutations result in a lack of body fat (10),

the mechanisms by which *BSCL2* gene defects cause lipodystrophy remain obscure.

BSCL2 mutations were found in patients from Europe, the Middle East, and Japan (12). *AGPAT2* mutations were found predominantly in African ancestry. However, no information is available about putative alterations of these genes in Chinese subjects with CGL. The present study is the first report on gene analysis of CGL in a Chinese family.

Subjects and methods

Study subjects

A 7-year-old Chinese CGL boy was the third child of consanguineous parents from an eastern Chinese province. The first two children had been stillborn. There was no other particular birth history except low birth weight with 2.25 kg after 40-week gestation. Five healthy unrelated children (two males, three females, body mass index (BMI) 16.3–22.3 kg/m²) were recruited as controls. The patient presented with complete lack of generalized subcutaneous fat at birth, accelerated growth, voracious appetite, hypertrophy of all limb muscles, acanthosis nigricans in the neck, groin and outer, enlarged hands and feet, hirsutism in the back, hepatosplenomegaly, crassitude of the penis, nubecula, a cardiac murmur, and mild mental retardation (Fig. 1A–E). He was admitted for gum bleeding repeatedly. The height was 130.5 cm (90–97 percentile) and the weight was 28.0 kg (>90 percentile) with BMI 16.4 kg/m² at the current presentation. Laboratory examinations showed evidence of liver dysfunction (glutamate-pyruvate transaminase 64 U/l and glutamic oxaloacetic transaminase 73 U/l), high levels of bilirubin (total bilirubin 26.6 µmol/l and direct bilirubin 20.7 µmol/l), dyslipidemia with slight increased triglyceride (1.79 mmol/l) but normal cholesterol and lipoprotein. The patient was diagnosed with diabetes mellitus (fasting blood glucose 8.9 mmol/l and haemoglobin A1c 12.5%) and hyperinsulinemia (fasting insulin 153.0 mU/l and fasting C-peptide 11.6 ng/ml). The patient had a normal 46 XY karyotype. Upon echocardiography, a left ventricle hypertrophy was detected and histologic examination of a liver biopsy specimen suggested hepatocirrhosis. Serum leptin levels were not determined. Parents and grandparents were all healthy with no CGL phenotype, their BMI ranging from 20.2 to 21.5 kg/m².

The study was approved by the ethical committee of Children's Hospital of Fudan University. All subjects and their parents agreed to participate in the study and gave their written consent.

Materials and methods

Venous blood samples from the subjects were collected and genomic DNA was extracted using the proteinase

K and phenol method (13). Eleven primers were designed (Shanghai Shinegene Molecular Biotechnology Co., LTD, Shanghai, China) using online software (<http://scitools.idtdna.com/Primerquest/>) to specifically amplify exons 1–11 and the surrounding intronic sequences of the *BSCL2* gene (NM_632667). PCR was performed at 94 °C for 3 min, followed by 35 cycles of 94 °C for 45 s, and 52–55 °C for 45 s, 72 °C for 45 s, and a final extension at 72 °C for 5 min. The annealing temperature for amplification of each exon is listed in Table 1. Six exons and exon–intron boundaries of *AGPAT2* gene (NM_006412) were amplified as reported earlier (14). Each PCR product was sequenced thrice independently (Shanghai Shinegene Molecular Biotechnology Co., LTD).

Results

DNA sequence analysis of the entire coding regions and surrounding uncoding regions disclosed a novel homozygous G→T mutation at nucleotide 909 in exon 5 of the *BSCL2* gene in the patient (Fig. 2) (4, 7, 15). This mutation predicts a substitution of glutamic acid at codon 189 with a stop codon (Glu189X or E189X). We also found the same mutation in the heterozygous state in the child's parents and grandparents. This is in accordance with the Mendelian autosomal recessive pattern of inheritance of CGL. Because the mutation found by us is predicted to cause a severe alteration of the predicted protein sequence, it is highly likely that this novel mutation is causative for the disease. No alterations were found in the healthy control subjects in the *BSCL2* gene. There was no *AGPAT2* gene mutation in the family affected by CGL nor in the control subjects.

Discussion

Since Berardinelli first described CGL in 1954, more than 200 patients have been reported. The major clinical characteristics of this rare syndrome include severe insulin resistance and lipodystrophy at birth or in early infancy. The well-documented high prevalence of parental consanguinity in affected individuals is suggestive of autosomal recessive transmission.

CGL is a rare autosomal recessive disorder for which mutations in two genes, *AGPAT2* on 9q34 and *BSCL2* on 11q13, have been implicated to play a role in the pathogenesis. Other as yet unknown genes may also be involved in the pathogenesis of the disease. Seipin, coded by *BSCL2*, is homologous to the protein product of the mouse *Gng3lg* gene (localized on mouse chromosome 19) that is orthologous to human 11q (7, 16, 17). It is a predicted protein with 398 amino acids and more than two hydrophobic amino acid stretches, suggesting that it should be a trans-membrane protein. Seipin has no similarity with

Figure 1 Clinical features of the boy with congenital generalized lipodystrophy (CGL) from a Chinese eastern province. Complete lipodystrophy (all panels), acanthosis nigricans (panels A), hepatosplenomegaly (red mark for liver border) (panels B and E), crassitude of the penis (panels B and E, right healthy child of same age), enlarged hands (panels C), muscular hypertrophy (panels D) are depicted.

other known proteins except the product of mouse *Gng3lg* that could be predictive for its function. Therefore, the mechanisms by which *BSCL2* mutations cause lipodystrophy still remain unclear. *BSCL2* mRNA is expressed in most tissues, with the highest expression in the brain and low abundance in adipose

tissue. Based on the high expression of *BSCL2* mRNA in the brain, it was proposed that the lack of body fat in patients with *BSCL2* mutations might be due to hypothalamo-pituitary dysfunction (11). In addition, omental adipose tissue was found to express greater levels of *BSCL2* mRNA than liver and skeletal muscle

Table 1 Oligonucleotide sequences and annealing temperature for PCR amplification of *BSCL2*.

Region	Primer sequence	Annealing temperature (°C)
Exon 1	F: 5'-ACTCTGACACAGCACTTAGCACCT-3' R: 5'-TAAAGTCCTTCAGAGGCAAGGCCA-3'	56
Exon 2	F: 5'-TGGCAGAGTGGCACAATCATTTGG-3' R: 5'-TGTGAAAGTTGAGAGGCCCTGGAA-3'	55
Exon 3	F: 5'-GAAGGGTGCCTGTTCTGA-3' R: 5'-GTCTCGAACTCCCAACCT-3'	52
Exon 4	F: 5'-ACAGATTGAAAGGCCCGTTAGGCT-3' R: 5'-ACTCAACCTCCCAGGTTTAAGCGA-3'	54
Exon 5	F: 5'-TGGAATGGTTGGATGAGACTGAGG-3' R: 5'-GTTAGCCCCCGTGAAGAGTT-3'	55
Exon 6	F: 5'-AAGCCGCCTTCATAGACT-3' R: 5'-GGAGGGAAGATGAACAGG-3'	52
Exon 7-8	F: 5'-TTTCTGGGACATGAGAAGGCTGGT-3' R: 5'-TCACCTTCCTCGCCTTTCCTTTGA-3'	54
Exon 9-10	F: 5'-TCAAAGGAAAGGCGAGGAAGGTGA-3' R: 5'-TGGGAAAGTGCTGGAATGTGAGGA-3'	55
Exon 11	F: 5'-AAGATGCAGCTTTGCTGACGGA-3' R: 5'-TCCCTCCTTGGACTTCCTAGGCTTAT-3'	55

(18). Thus, an alternate hypothesis is that lipodystrophy is due to the direct disruption of adipocyte differentiation or function. The differentiation of preadipocytes in humans to white adipocytes can potentially proceed towards either metabolically active or mechanical adipocytes (1). Although the exact gene expression pattern of these two types of adipocytes is unknown, it seems logical to speculate that *BSCL2* gene defects could affect the process of differentiation more proximally compared with *AGPAT2* gene defects, which mainly affect triglyceride and/or glycerophospholipid biosynthesis, which is probably a late event in adipocyte differentiation and function (11).

According to Agarwal's analysis of the phenotypic and genetic heterogeneity in CGL from 45 pedigrees, all subtypes had extreme paucity of subcutaneous adipose tissue already at birth and markedly decreased

serum leptin levels (14). Other characteristic features of CGL such as acanthosis nigricans and umbilical prominence were also noted in almost all subjects. There was a uniformly high prevalence of diabetes mellitus and hypertriglyceridemia in all individuals with CGL. A higher proportion of female subjects with *AGPAT2* mutations has also been reported. However, patients with *BSCL2* mutations had an earlier onset of diabetes compared with those with *AGPAT2* mutations. 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. The prevalence of cardiomyopathy was also more than three times higher in patients with *BSCL2* mutations compared to those with *AGPAT2* mutations. Serum leptin levels were lower in patients with *BSCL2* mutations compared to those with *AGPAT2* mutations. Simha *et al.* report that CGL patients with *BSCL2* mutations had a more severe lack of body fat, which affected both metabolically active and mechanical adipose tissue, as compared with patients with mutations in the *AGPAT2* gene (19). In summary, the genetic heterogeneity in CGL patients is accompanied by phenotypic heterogeneity. These observations suggest that the protein product of the *BSCL2* gene may turn out to have less restricted functions and/or a less limited expression pattern than that of the *AGPAT2* gene product. The findings also emphasize the importance of attempting to make a molecular diagnosis of the syndromes of CGL as this may aid in both prognostic and genetic counseling.

As reported, patients with CGL have either homozygous or compound-heterozygous mutations of the *BSCL2* gene, most of which are nonsense or frameshift mutations that are expected to cause loss of function of the protein. Individuals that are heterozygous for these mutations, such as the parents and siblings of

Figure 2 Sequence of the *BSCL2* gene and various mutations found in the affected individuals with CGL. A novel homozygous G to T mutation at nucleotide 909 (arrow) in exon 5 in the CGL patient.

the affected CGL subjects, do not reveal any phenotype. Our studies found that the Chinese patient with the homozygous 909 G → C mutation showed typical clinical signs of CGL and metabolic abnormalities including insulin resistance and dyslipidemia. Early onset of diabetes, cardiomyopathy, and mild mental retardation were characteristic in our subject. This is in accordance with findings reported in CGL subjects with *BSCL2* mutations from other ethnic groups. *BSCL2* mutations, in the meantime, have also been identified in American–Taiwanese (CG 1100), American–Chinese (CG 1300), and Japanese patients (6, 12). We did not find any *AGPAT2* mutations in our Chinese patient, suggesting that *AGPAT2* has a minor causative role, if any.

In summary, CGL is one of the human diseases to be caused by a dysfunction of adipogenesis or adipocyte differentiation. It has prompted interest in physiology and pathophysiology of adipose tissue disorders in the human. Future functional studies of *BSCL2* and *AGPAT2* and their protein products will provide greater insights into adipose tissue biology, and the clinical spectrum of obesity and lipodystrophy.

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