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SHORT REPORT

Congenital generalized lipodystrophy in an Indian patient with a novel mutation in *BSCL2* gene

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Summary Congenital generalized lipodystrophy (CGL) is an autosomal recessive metabolic syndrome with involvement of multiple organs. Mutations in *BSCL2* are known to be associated with a severe form of CGL and mental retardation (MR). The genetic heterogeneity in CGL patients is accompanied by phenotypic heterogeneity in different ethnic groups. Studies in the Indian context are very few in this regard. We report here a detailed clinical analysis of a CGL case from infancy to adult hood. Interestingly, the patient was found to be homozygous for a novel *BSCL2* mutation, but with normal intellectual development contrasting with the MR associated with *BSCL2* mutation in CGL patients. The biochemical investigations at the time of diagnosis (9 months) included total cholesterol, total lipids, triglycerides, phospholipids, β-lipoprotein and free fatty acids, which were above normal limits. The clinical phenotype, viz. lack of subcutaneous fat, hepatosplenomegaly, cardiomegaly, and advanced bone age was also documented. The patient was found to be insulin resistant and diabetes mellitus was diagnosed by age 13 years. Ultrasonography of the ovaries at age 22 showed polycystic features with elevated levels of gonadotropins and negligible levels of serum leptin. For genetic analysis, direct DNA sequencing of *BSCL2* was carried out and disclosed an 11-base-pair deletion in exon 6 (H217fsX272) resulting in a truncated protein. This is a novel mutation that contributes to CGL formation in a family of Indian origin and adds to the array of variants reported in this disorder. Moreover, the novel mutation is found to be associated with normal intellectual ability.

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Abbreviations

AGPAT	1-acylglycerol-3-phosphate *O*-acyltransferase 2
BSCL	Berardinelli–Seip congenital lipodystrophy 2

CGL	congenital generalized lipodystrophy
FSH	follicle-stimulating hormone
LDL	low-density lipoprotein
LH	leutinizing hormone

Introduction

Congenital generalized lipodystrophy (CGL; OMIM 269700) is also known as Berardinelli–Seip syndrome. It is a rare metabolic disorder inherited as an autosomal recessive trait (Garg 2000). 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 (Garg et al 1992). Even during infancy patients show hyperinsulinaemia, hypertriglyceridaemia and hepatomegaly because of hepatic steatosis. More clinical features include widespread acanthosis nigricans, acromegaloid appearance, umbilical hernia and in women, cliteromegaly, hirsutism, oligo/amenorrhoea and polycystic ovaries. Diabetes mellitus develops mostly during the pubertal years and is ketosis resistant. Other reported features involve mild mental retardation, hypertrophic cardiomyopathy, nephropathy, and nubecula (Agarwal et al 2002; Van Maldergem et al 2002).

CGL is classified into three types, viz. BSCL-1, BSCL-2 and BSCL-X based on the phenotype and the genotype. One of the first genes identified to be associated with CGL phenotype was *AGPAT2* linked to 9q34. Subsequently, Magre and colleagues (Magre et al 2001) studied 29 families of CGL and identified a second locus on chromosome 11 within the 2.5 Mb interval flanked by markers D11S4076 and D11S480. This locus had the gene called *BSCL2*. Subsequently, a number of homozygous and heterozygous mutations in the *BSCL2* gene were identified (van Maldergem et al 2002). Since, cases harbouring *BSCL2* mutations had a phenotype different from those having *AGPAT2* mutations, CGL was subclassified as CGL: BSCL-2.

Mutations in *BSCL2* gene have been reported from Europe, the Middle East and Japan and recently in Chinese subject with CGL (Magre et al 2001). While the clinical features of cases of CGL from the Indian subcontinent have been reported, the genetic alterations that may occur in these patients have not been investigated in most cases (Kher et al 1990; Mandal et al 2006). Moreover, the changes in phenotype that occur at puberty in CGL have not been reported. In the present study, we report the clinical phenotype of a patient with CGL from infancy to adulthood. We also report the presence of a novel mutation in the *BSCL2* gene of this patient.

Case presentation and analysis

Case report/clinical phenotype

The female patient was referred to the Genetic Research Centre of our Institute at the age of 9 months for genetic evaluation. She was born at term to non-consanguineous Hindu parents. On physical examination at 9 months, her head circumference was 43 cm (>50th centile) and height was 76 cm (>90th centile). Her forehead was small with a mop of hair and she had peculiar pinched facies (Figs. 1, 2). A lack of subcutaneous fat and hyperpigmentation with acanthosis nigricans around the neck and axilla was seen. The body was hirsute with prominent musculature and phlebomegaly. Biochemical investigations at the time of diagnosis revealed that serum was lipaemic with cholesterol 7.254 mmol/L (279 mg/dl), total lipids 7.55 mmol/L (755 mg/dl), triglycerides 1.936 mmol/L (176 mg/dl) and phospholipids 3.00 mmol/L (300 mg/dl). Her β-lipoprotein was 1.75 mmol/L (67.6 mg/dl) free fatty acids were 1730 mmol/L (173 mg/dl). A skin biopsy showed absence of adipose tissue. Systemic examination also revealed hepatosplenomegaly and mild cardiomegaly. Her bone age was advanced (2.5 years). Examinations of gastrointestinal system and renal systems were unremarkable.

The patient was evaluated every year and diagnosis of insulin resistance and diabetes mellitus was made through her follow-up at the age of 13 years. Hence she was kept on strict low-fat diet and supplementation of fish oil was started at age 13 years. This fish oil treatment improved her skin texture and improvement in hirsutism was seen with disappearance of acanthosis nigricans. The lipaemia of the serum was also reduced.

At 22 years of age (Fig. 3B) during her follow-up, ultrasonography of the ovaries showed polycystic features. Sex hormones at the age of 22 years showed elevated FSH (32 mIU/ml), LH (12.8 mIU/ml) and negligible leptin levels (0.03 ng/ml). Serum 17-OH-progesterone level was normal. She did not show any signs of mental retardation and studied up to

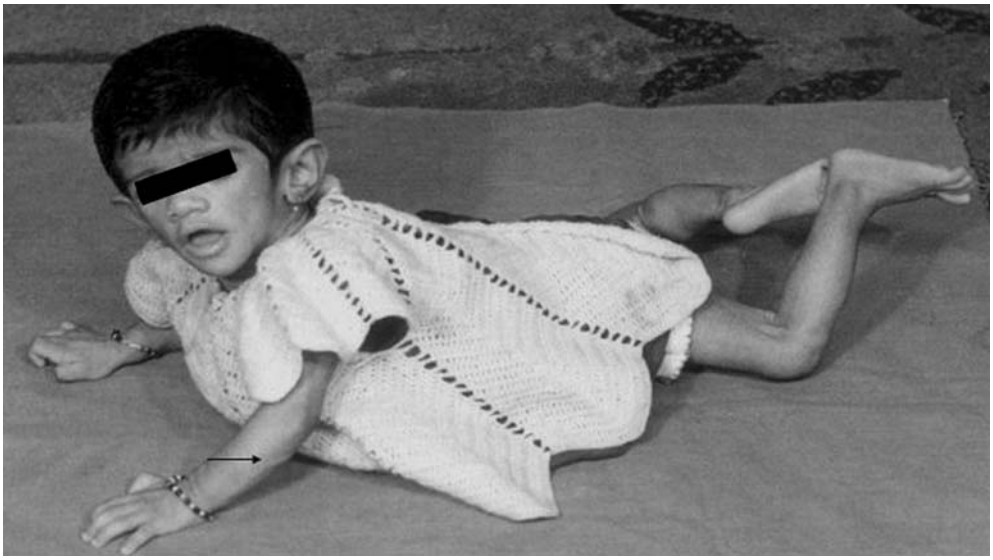

Fig. 1 Patient at 9 months of age

graduation level with average performance. Her mid-parental height was 1.55 m, whereas the measured height at adulthood was 1.67 m. The patient died at the age of 29 years due to renal failure.

Genetic analysis

For the genetic analysis, blood was collected at age 22 years with informed consent. DNA was extracted

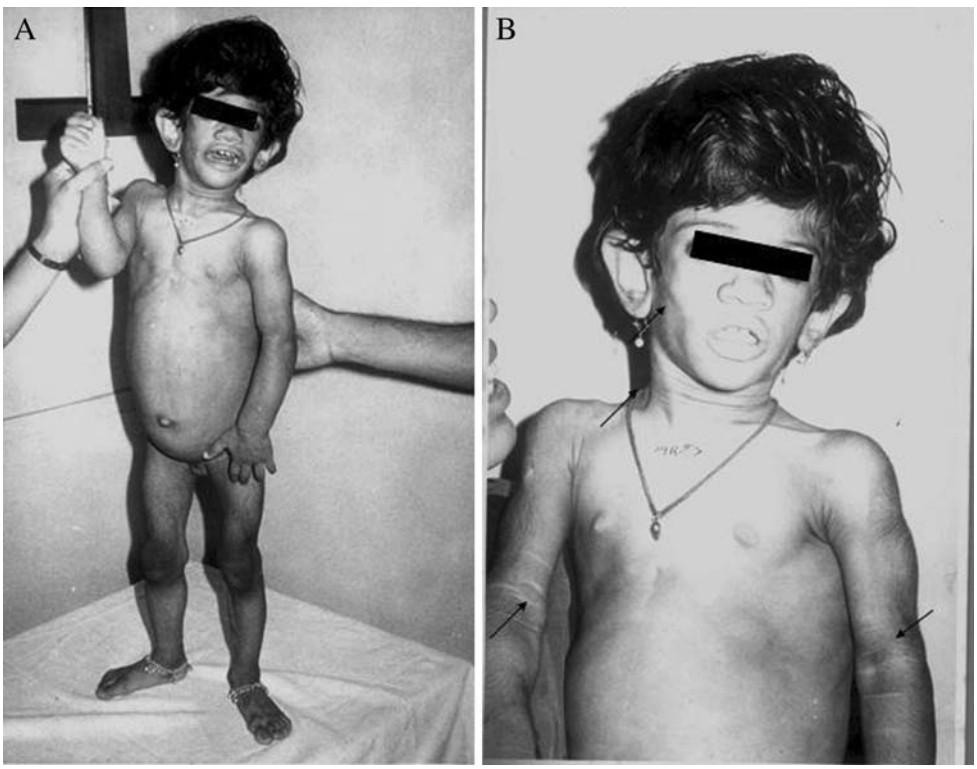

Fig. 2 (**A**) Patient at 2 years of age. The forehead is small and head circumference is >50th centile. The body is bony and hirsute with prominent musculature and the patient requires support to stand. (**B**) Closer (facial) view at 2 years of age. Acanthosis nigricans clearly visible at the axillary region (indicated by arrow). The head is big with a mop of hair and typical pinched facies

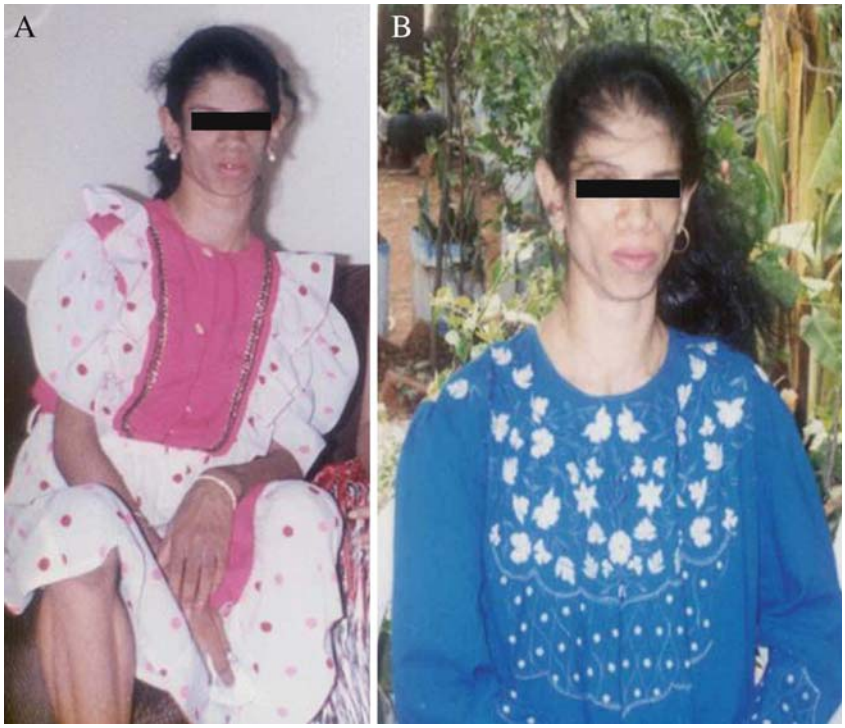

Fig. 3 (**A**) The patient at 16 years of age. (**B**) The patient at 22 years of age. The fish oil treatment improved her skin texture and improvement in hirsutism was seen with disappearance of acanthosis nigricans

from peripheral blood leukocytes using Gene Elute extraction protocol (Sigma, St Louis, MO, USA). The extracted DNA was analysed both qualitatively as well as quantitatively. A part of this extracted DNA was sent to the laboratory of Dr L. Van Maldergem, Belgium, for analysis of *BSCL2* gene.

The analysis of *BSCL2* gene revealed a homozygous 11 bp deletion in exon 6 (Fig. 4B). This deletion was

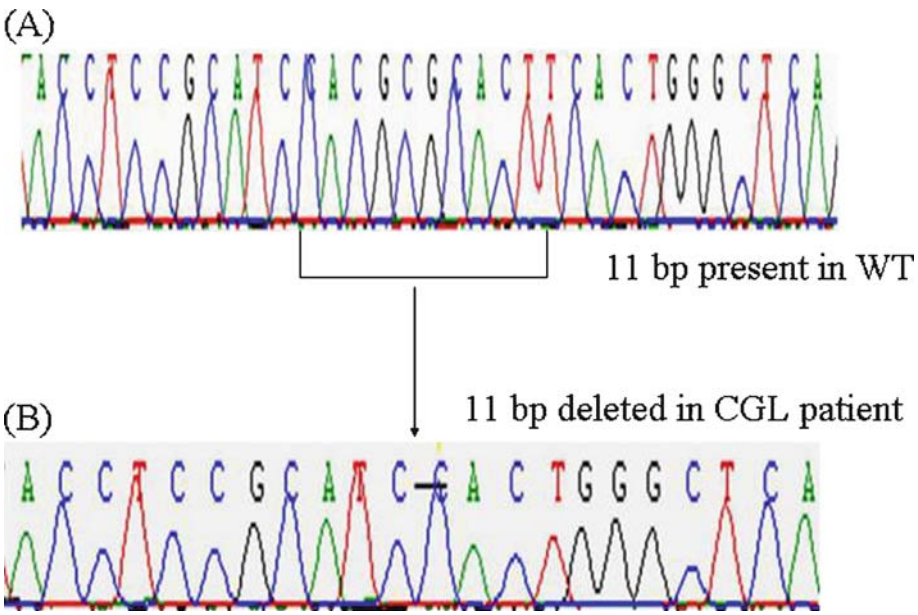

Fig. 4 (**A**) Wild-type partial sequence of exon 6 of *BSCL2* gene from a control subject. (**B**) Partial sequence of exon 6 of *BSCL2* gene of the CGL patient, showing 11 bp deletion

predicted to result in a frameshift giving a truncated protein that would lack 130 amino acids from its C-terminal end. To the best of our knowledge this mutation H217fsX272 in *BSCL2* has not previously been described in any case with CGL.

Discussion

The CGL case described here has all the clinical features classically described by Maldergem and colleagues (Van Maldergem 2001) except mental retardation, and this is the first report of a novel 11 bp deletion in the *BSCL2* gene in CGL patients.

CGL is a rare autosomal recessive condition with involvement of multiple organs such as brain, pancreas, spleen, liver, adipose tissue, bones and cardiac system (Garg 2004). The patient reported here displayed at birth the classical features of CGL, mainly absence of subcutaneous fat with acanthosis nigricans and advanced bone age. Her biochemical status of hyperlipidaemia was evident from the elevated levels of lipids. The occurrence of diabetes mellitus was evident around the age of puberty. The patient suffered from oligoamenorrhoea and had typical signs of polycystic ovary syndrome (PCOS). All these features have been described for CGL (Garg 2004). Interestingly, the physical features of acanthosis nigricans and hyperpigmentation including hirsutism could be controlled by treatment with fish oil and low-fat diet, suggesting that the monitoring of dietary regimes and inclusion of essential supplements could form part of the management of this congenital disorder.

Two main genes have been implicated in the aetiology of CGL. These include *AGPAT2* and *BSCL2* (Downes et al 1998; Magre et al 2001; Simha and Garg 2003). However, the mutations observed in these two genes show distinct ethnic and racial distribution. While *AGPAT2* mutations have been prevalent in African and American CGL cases, *BSCL2* mutations have been observed in families from European, Afro-Asian and Middle-Eastern countries (Ebihara et al 2004). In keeping with these observations, in the present study we identified a novel 11 bp deletion in the *BSCL2* gene of this patient. To date, 19 mutations have been identified in *BSCL2* gene, most of which have an inactivating effect (Agarwal et al 2003). The mutation reported here would also be of inactivating type because it induces premature stop codon, thereby removing 130 amino acids from the C-terminal end. From the Indian subcontinent, Mandal and colleagues have reported three different mutations in the *BSCL2* gene of CGL patients from India (Mandal et al 2006). The mutation reported here adds to the *BSCL2* variants reported in this disorder.

One clinical feature that distinguishes CGL patients harbouring *BSCL2* mutations from those with *AGPAT2* mutations is the occurrence of mental retardation. It has been reported that cases with *BSCL2* mutations generally have mental retardation whereas those with *AGPAT2* mutations are intellectually normal. In contrast to this generalization, the patient we reported harbours a *BSCL2* mutation but is mentally normal. Beyond the general social ability to perform, this patient was also intellectually competent as she successfully completed her graduation at an age expected for the average Indian student (22–23 years). This observation is striking since most cases (≈80%) harbouring *BSCL2* mutations are known to have mental retardation. However, it is important to note that such normal intellectual ability has been reported in an Indian patient (Van Maldergem et al 2002) and Japanese patients with CGL and *BSCL2* mutations (Ebihara et al 2004). These observations indicate that mental retardation may not be associated with *BSCL2* mutation but may be a result of modifying environmental and other genetic influences (Fu et al 2004)

In summary, the CGL patient reported here harbours a novel 11 bp deletion mutations in exon 6 of *BSCL2* gene that is not associated with mental retardation, emphasizing the phenotypic heterogeneity of this metabolic disorder. Functional studies of *BSCL2* gene and its protein product will provide greater insights into the mechanism of pathogenesis of this disorder.

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