

Large scale deletions in the GPC3 gene may account for a minority of cases of Simpson-Golabi-Behmel syndrome

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Abstract

Aims of the study—To identify the proportion and type of deletions present in the glypican 3 (GPC3) gene in a group of patients with Simpson-Golabi-Behmel syndrome (SGBS).

Subjects and methods—PCR analysis using primer pairs which amplify fragments from each of the eight exons of the GPC3 gene was carried out in a series of 18 families with SGBS (approximately half of reported cases).

Results—Deletions were detected in only five families (one reported previously). We found deletions in all exons of the gene except exon 3.

Conclusions—Our results suggest that large scale deletions may be less common in SGBS than was originally thought. One patient, with an exon 4 and 5 deletion, lacked the characteristic facial dysmorphic features. This raises the possibility of involvement of GPC3 gene defects in a wider range of overgrowth disorders.

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Keywords: Simpson-Golabi-Behmel syndrome; glypican 3; X linked; overgrowth disorder

Simpson-Golabi-Behmel syndrome (SGBS) is an X linked overgrowth syndrome associated with characteristic facial features that are usually described as being coarse in quality. Although early motor milestones and speech are delayed, most affected males are of normal intelligence. Abnormalities of the skeletal system, heart, central nervous system, kidney, and gastrointestinal tract may also be present.¹⁻⁴ In addition, some affected subjects develop embryonal tumours.⁵ Recently, the gene responsible for SGBS has been cloned and shown to be glypican 3 (GPC3),⁶ a member of the glypican family of heparin sulphate proteoglycans. The expression pattern of GPC3 suggests that it may play an important role in growth control in embryonic mesoderm and endoderm derived tissues. Initial ligand and western blotting experiments have suggested that GPC3 may interact with insulin-like growth factor 2 (IGF2), which may be significant given the overlap in phenotype between Beckwith-Wiedemann syndrome (BWS) and SGBS patients.⁷

The known sequence of the GPC3 transcript is 2130 bp and spans approximately 500 kb of genomic DNA. Initial studies, examining six of the eight exons, identified deletions in three of the six patients tested.⁶ This suggested that large scale deletions might be responsible for a significant proportion of cases of SGBS. This would not be unexpected given the large region of genomic DNA covered by the GPC3 gene and the high proportion of deletions found in some other disorders involving other large genes (for example, in the dystrophin gene in patients with Duchenne muscular dystrophy).

Aims of the study

The aims of the study were to identify the proportion and type of deletions present in the eight exons of the GPC3 gene in SGBS patients from 18 families.

Patients and methods

PATIENTS

Blood samples were collected from patients and their families and DNA prepared following standard methods. Table 1 shows the main clinical features in the 18 index cases studied. Family 7 was reported as family C in reference 6. Of the 18 cases, five are familial (SGBS-1, 5, 7, 19, and 20) and 13 appear to be sporadic cases. With the exception of SGBS-2, 9, 19, and 20, all the cases were ascertained by contacting clinical geneticists and paediatric oncologists in the UK. The patient and his family in SGBS-2 are from Angola (of Portuguese ancestry), while those in SGBS-9 are from Germany,⁸ SGBS-19 and 20³ from Austria, and SGBS-17 from The Netherlands. The patient in SGBS-4 is Afro-Caribbean and those in SGBS-5 are of Lithuanian-Italian extraction.

The characteristic facial features of SGBS and their development with age are illustrated in fig 1 which shows a male from family SGBS-1 at birth, 3 years, and 7 years of age. The characteristic facial features, which are all present at 7 years of age, include a broad nose, prominent jaw, upward slanting eyes, longitudinal groove in the tongue, and central groove in the lower lip. Note that the face appears to develop with age; at birth the face looks relatively normal, but by 3 years the nose is broad and the eyes upward slanting but the jaw is still of normal size. In contrast, one of the patients in SGBS-5 is shown in fig 2. In this



Figure 1 One of the affected males in family SGBS-1 at birth, 3 years, and 7 years of age. See text for further discussion. (All photographs reproduced with permission.)



Figure 2 One of the affected males in SGBS-5. See text for further discussion.

family the facial features are less striking, in particular the broad midface is absent (see below for further discussion).

Methods

PCR was carried out using the six sets of primer pairs previously defined.⁶ These are intraexonic primers for exons 1, 2, 3, 5, 6, and 8. Primers for exons 4 and 7 have since been defined and are as follows: exon 4 (forward) 5' gcttattacctgaagatctc 3'; (reverse) 5' ttctttggaat-aggtcgg 3'; exon 7 (forward) 5' tgagaacctatgctc-tatgc 3'; (reverse) 5' cggtgatgatgaagatga 3'.

For each exon, one primer was end labelled with $\gamma^{32}P$ ATP using T4 polynucleotide kinase (MBI Fermentas) using the manufacturer's buffer and protocol. PCR was carried out in reaction volumes of 10 μ l containing standard manufacturer's buffer, 200 μ mol/l dNTPs, 0.5 μ mol/l forward and reverse primers, 100 ng of genomic DNA, and 1 unit of TBr polymerase

(NBL). The reaction cycles were as follows: 94°C for one minute, annealing temperature for one minute, 72°C for two minutes for 32 cycles. This was preceded by a three minute incubation at 94°C and followed by a 10 minute incubation at 72°C. The annealing temperatures were exon 1 60°C, exon 2 53°C, exons 3, 4, 5, and 7 55°C, exons 6 and 8 57°C. The reaction products were electrophoresed on denaturing acrylamide gels which were dried down and exposed to XAR5 autoradiographic film at -80°C overnight.

Results

A summary of the deletions identified is given in table 2, while fig 3 shows representative results obtained for exons 2, 6, and 8. From table 2 it can be seen that deletions were detected in exons 1 and 2 in SGBS-4, in exons 4 and 5 in SGBS-5, in exons 6, 7, and 8 in SGBS-7, in exon 1 in SGB-25, and in exon 8 in

Table 1 Clinical features of patients with SGBS

	SGB Family No																	
	1	2	3	4	5	6	7	8	9	10	11	12	16	17	19	20	25	27
Prenatal overgrowth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
Postnatal overgrowth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Delayed speech	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+
Delayed motor milestones	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
CNS abnormalities	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-
Cardiac malformation	+	-	-	-	+	-	+	-	+	-	+	+	-	-	+	+	-	-
Characteristic facial features	+	+	+	+	+/-	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tract abnormalities	+	+	-	+	+	-	+	-	-	+	-	-	-	-	-	-	-	+
Embryonal tumours	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Supernumerary nipples	+	+	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Skeletal abnormalities	+	+	-	-	+	-	-	+	+	+	+	-	-	-	-	+	+/-	-
Hernias	-	-	-	+	-	+	-	+	-	+	-	-	-	-	+	+	+	-

psychomotor retardation. The deletion in this case may extend 5' to the GPC3 gene to involve other genes. To investigate this possibility, the extent of the deletion in the patient in SGBS-4 is currently being characterised. In addition, the results from this study and that of Hughes-Benzie *et al*⁶ suggest that mutations in the 5' end of the gene may be involved in tumour development in SGBS patients. The affected subject in SGBS-2, however, also had a Wilms' tumour (table 1) and no deletion was detected in exons 1 or 2 (or any other exon) (table 2). However, it remains to be seen whether there is another type of mutation in either of these exons in this family and also what the true frequency of embryonal tumours is in SGBS.

The facial features of the two males in SGB-5 are not typical of SGBS (fig 2). They lack the broad midface but the older of the two has a rather prominent jaw and upward slanting palpebral fissures. However, there were pre- and postnatal overgrowth, delayed motor milestones, and other features suggestive of SGBS (table 1). Interestingly, in this case exons 4 and 5 were deleted and this was not seen in any of the more classical SGBS cases. It may be advisable, therefore, to extend the analysis of the GPC3 gene to patients with pre- and postnatal overgrowth who have abnormalities in other organ systems, but who lack the characteristic facial features of SGBS.

There is significant overlap in phenotype between SGBS and BWS.¹⁰ Indeed, this and the fact that SGBS has only relatively recently been delineated as a single entity probably means that SGBS is underdiagnosed. The IGF2 gene (which maps to 11p15.5) has been implicated in the underlying molecular pathology of BWS⁷ and there is now evidence to suggest that there is an interaction between GPC3 and IGF2.⁶ Evidence from mouse mutants also indicates that abnormalities of IGF2 expres-

sion affect growth.^{11 12} It may, therefore, also be valuable to look for mutations in the GPC3 gene in male BWS patients, for whom there is no indication of chromosome 11 involvement.

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