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

Susan Lindsay, Maggie Ireland, Ottie O’Brien, Jill Clayton-Smith, Jane A Hurst, Jillian Mann, Trevor Cole, Julian Sampson, Sarah Slaney, David Schlessinger, John Burn, Giuseppe Pilia

Abstract

Aims of the study—To identify the type and proportion of deletions present in the gene which is responsible for Simpson-Golabi-Behmel syndrome (SGBS). 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 suggests 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 proportion of deletions found in some other disorders involving large genes (for example, in the dystrophin gene in patients with Duchenne muscular dystrophy).

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, SGBS-19 and 20 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
Large deletions in GPC3 may account for some cases of Simpson-Golabi-Behmel syndrome

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. These are intragenic 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’ gcttattatcctgaagatctc 3’; (reverse) 5’ tctttggaatagctcgg 3’; exon 7 (forward) 5’ tgaagaacctgc-tatgc 3’; (reverse) 5’ cggtgatgatgaagatga 3’. For each exon, one primer was end labelled with γ32P ATP using T4 polynucleotide kinase (MBI Fermentas) using the manufacturer’s buffer and protocol. PCR was carried out in reaction volumes of 10 μl containing standard manufacturer’s buffer, 200 μmol/l dNTPs, 0.5 μmol/l forward and reverse primers, 100 ng of genomic DNA, and 1 unit of TBr polymerase (MBI Fermentas). 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 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-9, in exons 6, 7, and 8 in SGBS-7, in exon 1 in SGB-25, and in exon 8 in SGBS-5. See text for further discussion.
SGBS-27. SGBS-4, 25, and 27 are isolated cases while there are two affected subjects in each of SGBS-5 and SGBS-7. Deletions of exons 6 and 8 had already been reported in SGBS-7. This study shows that exon 7 is also deleted in both affected subjects in the family (table 2 and data not shown). The deletion of exons 4 and 5 was present in both affected subjects in SGBS-5 (data not shown). No deletions were detected in any of the other cases, which includes SGBS-20 the family originally reported by Professor Behmel and her colleagues.

Discussion

Five deletions have been found in 18 SGBS patients screened with exon primer sets for all eight exons of the GPC3 gene. Seventeen of these patients (all but SGBS-5) were clearly identified as having SGBS because their phenotypes were characteristic of this disorder and included pre- and postnatal overgrowth and the characteristic facial features. In addition to these patients, DNA samples from a further 13 patients with overgrowth disorders, but without the characteristic facial features of SGBS, were also screened for deletions in the GPC3 gene. Of these, a deletion was only detected in SGBS-5 (table 2 and data not shown).

With primer sets for exons 1, 2, 3, 5, 6, and 8, an earlier study of the GPC3 gene found deletions in three of six patients tested. One had a deletion of exon 2 (family a) and the other two were both deleted for exons 6 and 8 (families b and c (SGBS-7)). The same group have recently reported finding a further four independent deletions.

We have identified five different deletions in SGBS-4, 5, 7, 25, and 27. Although the affected subjects in SGBS-7, 25, and 27 have similar phenotypes (table 1) they have very different deletions: in SGBS-7 the deletion is of exons 6 to 8, in SGBS-25 it is exon 1, while in SGBS-27 it is of exon 8. There are subtle differences in the phenotypes, however; for example, the affected subjects in SGBS-7 and 27 have renal tract abnormalities while in SGBS-25 the affected person does not. In addition to the features shown in table 1, SGBS-25 also had a submucous cleft palate which was repaired. The younger of the two affected males in SGBS-7 developed hydrocephalus and has required the insertion of a ventriculoperitoneal shunt. This complication has not previously been described in SGBS, although a male in SGBS-12 is similarly affected. The significance of these differences is unclear at present.

The affected male in SGBS-4 (deleted for exons 1 and 2) had originally been diagnosed as having BWS. He was ascertained in a follow up study of UK cases of BWS. His facial features, which included a broad midface, upward slanting palpebral fissures, grooved tongue, and notch in the lower lip, are much more characteristic of SGBS. In contrast to most SGBS patients, however, he had moderate/mild psychomotor retardation. At the age of 7 he had a Wilms' tumour removed but despite chemotherapy he died from a recurrence. Affected subjects in a previously reported family with a deletion of exon 2 also had a number of embryonal tumours but were of normal intelligence. The affected subject in SGBS-25, who is also of normal intelligence, is deleted for exon 1. SGBS-4 is deleted for both exons 1 and 2 and, in contrast, has significant

<table>
<thead>
<tr>
<th>Table 2 Results of deletion screening in 18 European families</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon No</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>8</td>
</tr>
</tbody>
</table>
Large deletions in GPC3 may account for some cases of Simpson-Golabi-Behmel syndrome

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. 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. 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 BWS′ and there is now evidence to suggest that there is an interaction between GPC3 and IGF2. Evidence from mouse mutants also indicates that abnormalities of IGF2 expression affect growth. 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.

We are grateful to the following clinical geneticists and paediatric oncologists for supplying us with samples from patients and their families: Dr Annemarie Behmel, Dr Louise Bruton, Dr Brian Coppin, Professor Alan Craft, Dr Nick Dennis, Dr Amanda Collins, Dr Heather Fletcher, Dr Judith Goodship, Dr Eli Hatchwell, Dr Raoul Hennekam, Shirley Hodgson, Dr Rainer Konig, Alison Lasbrow, Dr Ana Medeiros, Dr Ann Sla- vovicsek, Dr Sabine Stengel-Rutkovsky, and Professor Ian Young. We would also like to thank Mr Charles Stilller and the Childhood Cancer Research Group (Oxford). In addition, SL would like to acknowledge the support of the Wellcome Trust and SL, JB, and MI thank the North East Children′s Cancer Research Fund for their initial support. GP would like to thank Italian Telethon (award No E.357) for research support.