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Mapping of a New SGBS Locus to Chromosome Xp22 in a Family with a Severe Form of Simpson-Golabi-Behmel Syndrome

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Summary

Simpson-Golabi-Behmel syndrome (SGBS) is an X-linked overgrowth syndrome with associated visceral and skeletal abnormalities. Alterations in the glypican-3 gene (GPC3), which is located on Xq26, have been implicated in the etiology of relatively milder cases of this disorder. Not all individuals with SGBS have demonstrated disruptions of the GPC3 locus, which raises the possibility that other loci on the X chromosome could be responsible for some cases of this syndrome. We have previously described a large family with a severe form of SGBS that is characterized by multiple anomalies, hydrops fetalis, and death within the first 8 wk of life. Using 25 simple tandem-repeat polymorphism markers spanning the X chromosome, we have localized the gene for this disorder to an ~6-Mb region of Xp22, with a maximum LOD score of 3.31 and with LOD scores < -2.0 for all of Xq. These results demonstrate that neither the GPC3 gene nor other genes on Xq26 are responsible for all cases of SGBS and that a second SGBS locus resides on Xp22.

Introduction

Simpson-Golabi-Behmel syndrome (SGBS; MIM 312870) is an X-linked disorder characterized by pre- and postnatal macrosomia, minor facial anomalies, and variable visceral, skeletal, and neurological abnormalities. Since SGBS was first described by Simpson et al. (1975), a wide range of clinical presentations has been reported. There is great variability in SGBS severity, which ranges from a mild form that is associated with long-term survival to an early lethal form that is associated with multiple congenital anomalies and severe mental retardation. In eight reported families, affected individuals died in infancy. We have previously described a family in which four maternally related male cousins had a severe form of SGBS (Terespolsky et al. 1995). One of these males was voluntarily aborted at 19 wk gestation, after multicystic kidneys were detected on ultrasound. The three live-born males were hydropic at birth and had a combination of craniofacial anomalies, including macrocephaly; apparently low-set, posteriorly angulated ears; hypertelorism; a short, broad nose with anteverted nares; a large mouth with a thin, vermilion upper border; a prominent philtrum; a high-arched or cleft palate; a short neck; redundant skin; hypoplastic nails; skeletal defects involving the upper and lower limbs; and gastrointestinal and genitourinary anomalies. All three patients were hypotonic and neurologically impaired from birth. With the exception of a trilobate left lung in one patient, the cardiorespiratory system was structurally normal. All patients died of multiple complications, including pneumonia and sepsis, within the first 8 wk of life.

SGBS has been mapped to the long arm of the X chromosome in a number of kindreds with moderate expression of the condition (Hughes-Benzie et al. 1992; Ireland et al. 1993; Orth et al. 1994; Xuan et al. 1994). In 1996, Pilia et al. identified, in two female patients with X/autosome translocations, disruptions in the GPC3 locus, on Xq26, which codes for the extracellular proteoglycan glypican-3 (GPC3). Microdeletions in GPC3 were also found to cosegregate with SGBS in three families (Pilia et al. 1996), although GPC3 deletions have not been subsequently detected in all cases of SGBS (Lindsay et al. 1997). Using a single four-generation family segregating an infantile lethal form of SGBS, we have mapped, to chromosome Xp22, a second SGBS locus, which is well removed from the GPC3 locus on Xq26.

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Subject and Methods

Subjects

The subjects in the present study are from a four-generation family with four male cousins affected with an infantile lethal form of SGBS (fig. 1). Informed consent was obtained from all participants, and all study procedures were approved by the ethics committees of the University of Toronto, The Hospital for Sick Children, and Rutgers University. The 15 unaffected members who participated were generally unremarkable phenotypically, with the exception of striking hypertelorism in the carrier females. The clinical features of the four affected males have been described in detail elsewhere (Terespolsky et al. 1995).

Genotyping

DNA was extracted, by standard techniques, from peripheral blood leukocytes, except in the case of family member IV-3, whose DNA was extracted from brain tissue stored in liquid nitrogen. DNA from each subject was genotyped by use of 13 X-chromosome markers (DXS989, DXS1068, DXS6810, DXS1003, DXS7132, DXS6800, DXS6789, DXS6799, DXS6797, DXS6804, DXS1001, DXS1047, and GATA31E08) from the Weber version 6.0 Screening Set as well as 12 additional chromosome-Xp markers (DXS1060, DXS1223, DXS8051, DXS7104, DXS16, DXS8022, DXS207, DXS1053, DXS43, DXS1195, DXS8019, and DXS7107), all obtained from Research Genetics. PCR amplifications were performed in a reaction volume of 12 μl containing 40 ng template DNA; 0.2 mM each of dATP, dGTP, and dTTP; 1.25 μmol dCTP; 25 nM [^{32}P]α-dCTP; 12 pmol each primer; 50 mM KCl; 10 mM Tris-HCl (pH 8.3); 1.9–2.5 mM $MgCl_2$; and 0.12 U Ampli*Taq* Gold DNA polymerase (Perkin-Elmer). Thermocycling was conducted by means of an MJ Research PTC-100 thermocycler, with an initial denaturation step of 15 min at 95°C; then 10 cycles of 15 s at 95°C, 15 s at 55°C, and 15 s at 72°C; an additional 15–20 cycles of 15 s at 89°C, 15 s at 55°C, and 15 s at 72°C; and a final extension step of 10 min at 72°C. PCR products were analyzed by electrophoresis on 6% nondenaturing acrylamide gels, and subsequent autoradiography was done at room temperature with Dupont Reflections film.

Linkage Analysis

Two-point linkage analyses were performed by use of the MLINK program of the LINKAGE package, version 5.1 (Lathrop et al. 1984), and multipoint analyses were conducted by use of the LINKMAP program of the FASTLINK package, version 4.0P (Cottingham et al. 1993; Schäffer et al. 1994). All analyses were conducted by use of an X-linked model, with a disease-allele frequency of .0001 and penetrances of 1.0 for disease-allele hemizygotes and homozygotes and with penetrances of .0 for all other genotypes. All 25 markers were used in the two-point analyses. Multipoint analyses were conducted by use of a set of 23 markers. Marker order and recombination fractions for 10 Xp markers distal to DXS989 were taken from the Généthon genetic map (Dib et al. 1996), and the remaining markers and recombination fractions were taken from the map provided with the Weber version 6.0 Screening Set. The two maps were joined at marker DXS989, which is present in both maps. The final map used was as follows: DXS1060-.036-DXS1223-.020-DXS8051-.037-DXS7104-.047-DXS8022-.015-DXS1053-.026-DXS1195-.001-DXS8019-.036-DXS7101-.049-DXS1052-.070-DXS989-.160-DXS1068-.060-DXS6810-.130-DXS1003-.090-DXS7132-.100-DXS6800-.140-DXS6789-.050-DXS6799-.060-DXS6797-.060-

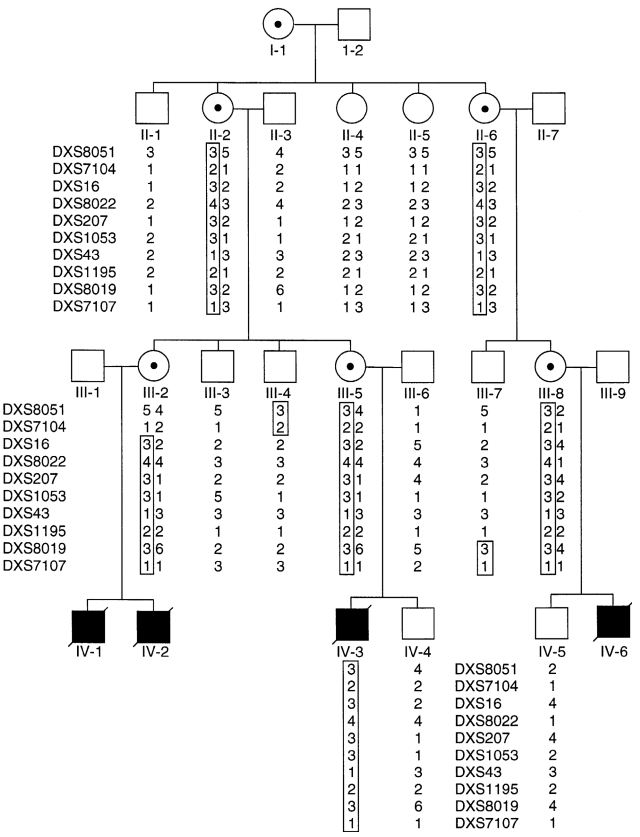

Figure 1 Pedigree of a family with an infantile lethal variant of SGBS, with genotypes of markers from Xp22. Affected individuals are denoted by a blackened symbol. Obligate carrier females are designated by a black dot within an unblackened circle. The haplotype segregating with SGBS is boxed. Flanking recombinants occur between the loci DXS7104 and DXS16 in individuals III-2 and III-4 and between the loci DXS1195 and DXS8019 in individual III-7.

DXS6804-.140-DXS1001-.120-DXS1047-.110-GATA31E08. Overlapping sets of five-point analyses were conducted and assembled to produce the chromosome-wide multipoint analysis. Recombination fractions (θ) were converted to map distances by use of the Kosambi mapping function. Haplotypes were assembled for markers from Xp22, according to the physical order in the map constructed by Ferrero et al. (1995). This map was also used to estimate the physical distances between these markers.

Results

Two-point analyses produced predominantly negative results throughout the length of the X chromosome, with the exception of the markers centered around Xp22 (table 1). With markers DXS16 and DXS207, the maximum two-point LOD score was 3.31 at $\theta = 0$; four additional markers from Xp produced somewhat lower maximum LOD scores at $\theta = 0$. The maximum multipoint LOD score was 3.31 for the entire 4.1-cM region from DXS8022 to DXS1195 (fig. 2), with a 1-LOD-unit support interval spanning the 9-cM region from DXS7104 to DXS8019. Multipoint analysis produced LOD scores <-2.0 for the region of Xp distal to DXS7104 and proximal to DXS1068, as well as for all of Xq, including the region containing the GPC3 locus.

Table 1

Pairwise LOD Score with Chromosome-X Markers

	LOD Score at θ =						
Locus	.0	.01	.05	.1	.2	.3	.4
DXS1060	−∞	−.73	.49	.85	.95	.75	.41
DXS1223	−∞	−.73	.49	.85	.95	.75	.41
DXS8051	−∞	−1.47	−.18	.29	.57	.55	.35
DXS7104	−∞	−1.63	−.37	.05	.27	.23	.11
DXS16	3.31	3.26	3.04	2.76	2.16	1.49	.76
DXS8022	2.71	2.67	2.49	2.25	1.75	1.19	.06
DXS207	3.31	3.26	3.04	2.76	2.16	1.49	.76
DXS1053	3.01	2.96	2.74	2.47	1.89	1.28	.66
DXS43	3.01	2.96	2.74	2.47	1.88	1.26	.62
DXS1195	1.69	1.67	1.57	1.45	1.17	.84	.45
DXS8019	−∞	1.26	1.77	1.81	1.55	1.12	.59
DXS7101	−∞	−.33	.3	.5	.57	.47	.28
DXS989	−∞	1.26	1.77	1.81	1.55	1.12	.59
DXS1068	−∞	−3.95	−1.92	−1.11	−.4	−.09	.02
DXS6810	−∞	−.81	−.16	.07	.21	.22	.14
DXS1003	−∞	−5.33	−2.62	−1.53	−.58	−.16	.01
DXS7132	−∞	−10.71	−5.88	−3.87	−1.97	−.97	−.36
DXS6800	−∞	−3.19	−1.8	−1.2	−.62	−.31	−.12
DXS6789	−∞	−6.05	−3.39	−2.35	−1.38	−.78	−.32
DXS6799	−∞	−5.91	−3.16	−2.03	−.98	−.45	−.15
DXS6797	−∞	−5.93	−3.18	−2.04	−.99	−.45	−.15
DXS6804	−∞	−3.61	−1.61	−.82	−.17	.07	.11
DXS1001	−∞	−6.71	−3.33	−1.96	−.76	−.23	−.01
DXS1047	−∞	−10.71	−5.88	−3.87	−1.97	−.97	−.36
GATA31E08	−∞	−3.91	−1.88	−1.08	−.38	−.08	.03

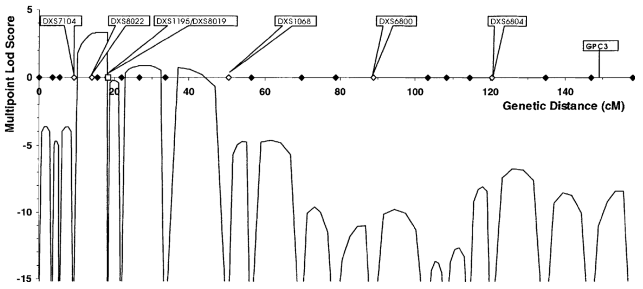

Figure 2 Multipoint linkage analysis of SGBS and X-chromosome markers. The order of the markers is DXS1060, DXS1223, DXS8051, *DXS7104*, *DXS8022*, DXS1053, *DXS1195, DXS8019*, DXS7101, DXS1052, DXS989, *DXS1068*, DXS6810, DXS1003, DXS7132, *DXS6800*, DXS6789, DXS6799, DXS6797, *DXS6804*, DXS1001, DXS1047, and GATA31E08. For orientation, the positions of the italicized markers are plotted as unblackened symbols on the *X*-axis, and their names are listed on the figure. The remainder of the single marker positions are plotted as blackened diamonds. Because of their proximity, DXS1195 and DXS8019 appear together and are plotted as an unblackened square. The locus GPC3 was not used in the multipoint analysis, but it is plotted for reference.

Haplotypes constructed from 10 Xp markers revealed flanking recombination events between the adjacent markers DXS7104 and DXS16 and between adjacent markers DXS1195 and DXS8019 (fig. 1). This confines the linked locus to a region of ~5.7–6.5 Mb on chromosome Xp22.

Discussion

We have used linkage analysis to map to Xp22 a second locus for SGBS, a locus that is clearly distinct from the GPC3 gene on Xq26. Cases of SGBS that are as severe as those seen in the family reported in the present study are rare, and we are unaware of any similar families that are available for linkage analysis. Despite the small size of this family, we were able to obtain a significant LOD score of 3.31 and to define a candidate region of ~6 cM between markers DXS8022 and DXS1195. The candidate region cannot be narrowed further, since, in this family, there are no other informative polymorphic markers that map to this region.

The finding, in this family, of a new locus for SGBS adds an additional gene to the constellation of players acting in the molecular pathway of SGBS. The phenotypic overlap between SGBS and the related overgrowth disorder Beckwith-Wiedemann syndrome (BWS) has previously suggested that there is a final, common metabolic pathway for these two conditions. For BWS, genetic heterogeneity is already well documented, in that the genes for insulin-like growth factor 2 (IGF2), the cyclin-dependent kinase inhibitor $p57^{KIP2}$, the untran-

slated transcript H19, and the potassium-channel component KVLQT1 have all been implicated (Li et al. 1998). Increased activity of IGF2 appears to play a central role in the development of the BWS phenotype, and it is hypothesized that other genes achieve their effects through modulation of IGF2 expression or function. A similar role has been proposed for GPC3, and this idea is supported by data from mouse experiments in which targeted deletions of $p57^{KIP2}$ or GPC3 and overexpression of IGF2 produce phenotypes overlapping both BWS and SGBS (Li et al. 1998).

There are still a number of etiologic and nosological issues to be resolved with respect to SGBS. The wide clinical variation among cases of SGBS has resulted in extensive discussion about possible genetic heterogeneity (Opitz 1984; Opitz et al. 1988; Neri et al. 1998). To date, only translocations or deletions in the GPC3 gene have been described. However, in studies of individuals with SGBS who have normal karyotypes, only 30% of these individuals have deletions in GPC3 (Lindsay et al. 1997). Other types of mutations have not yet been identified in this gene, even within families in which SGBS is linked to a locus at Xq26. DNA sequencing may eventually identify point mutations in GPC3 in these patients. Although the glypican-4 (GPC4) gene, which is located adjacent to the GPC3 gene on Xq26, could also be a candidate for SGBS, all deletions of GPC4 that have been reported to date also involve GPC3 (Huber et al. 1998; Veugelers et al. 1998).

For some families and individuals with less severe forms of SGBS, it is possible that causative mutations are present in this new, Xp22 locus for SGBS. Further data will be needed to determine whether this is true or whether all Xp22-associated cases of SGBS will exhibit a severe phenotype, which would suggest that the disorder caused by the Xp22 locus should be classified as a distinct genetic disorder. Final resolution of this dilemma will have to be deferred until the SGBS gene at Xp22 is identified and until mutations can be defined in the relevant candidate genes on Xp22 and Xq26, for a full spectrum of patients with SGBS.

The SGBS region identified in the present study contains no known genes that can be obviously implicated in the etiology of SGBS. The region is known to contain the genes for phosphoribosyl pyrophosphate synthetase-2, the neuronal membrane glycoprotein M6B, the α-2 subunit of the glycine receptor, phosphatidylinositol glycan class A, the nonreceptor tyrosine kinase BMX, the gastrin-releasing peptide receptor, the U2 small-nuclear ribonucleoprotein auxiliary factor small subunit 2, and the vitamin D–dependent calcium-binding protein calbindin3 (Ferrero et al. 1995). In addition, sequences are present that have high homology to the murine genes for ubiquitin-conjugating enzyme UbcM3 and clatharin coat-assembly protein AP19, as well as to the human genes for SET (suppressor of variegation, enhancer of zeste and Trithorax) protein, CTP (cytidine 5′-triphosphate) synthetase, and ribosomal protein L6. It is of interest that the genes of known function in this region include such signaling molecules as BMX and the receptor for the growth-factor gastrin-releasing peptide. There is no indication that any of these genes represent a good candidate gene for this second SGBS locus. However, since the candidate region of 6 Mb is expected to contain >100 genes, it is not surprising that the small percentage of genes identified in this area to date do not contain a likely candidate.

The linkage of SGBS to Xp22 will facilitate the positional cloning of this gene. Ultimately, identification of a second SGBS gene will lead to the elucidation of its biological role and will contribute to our understanding of the genes that underlie human overgrowth syndromes.

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Electronic-Database Information

The accession number and URLs for data in this article are as follows:

Généthon, http://www.genethon.fr
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for SGBS [MIM 312870])

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