Short reports

A small interstitial deletion in the GPC3 gene causes Simpson-Golabi-Behmel syndrome in a Dutch-Canadian family

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Abstract

Deletions in the heparan sulphate proteoglycan encoding glypican 3 (GPC3) gene have recently been documented in several Simpson-Golabi-Behmel syndrome (SGBS) families. However, no precisely defined SGBS mutation has been published. We report here a 13 base pair deletion which causes a frameshift and premature termination of the GPC3 gene in the Dutch-Canadian SGBS family in whom the trait was originally mapped. Our analysis shows that a discrete GPC3 disabling mutation is sufficient to cause SGBS. Furthermore, our finding of a GPC3 normal daughter of an SGBS carrier with skeletal abnormalities and Wilms tumour raises the possibility of a trans effect from the maternal carrier in SGBS kindreds.

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Simpson-Golabi-Behmel syndrome (SGBS) is an X linked condition characterised by pre- and postnatal overgrowth, coarse facies, congenital heart defects, cleft lip and palate, enlarged and dysplastic kidneys, skeletal abnormalities, and an increased risk of embryonal tumours.[1] A Dutch-Canadian SGBS family including 13 female carriers and seven affected males with typical SGB syndrome has been previously reported. Three children under the age of 2 developed Wilms tumour in this kindred.[1 2]

Recently, a membrane associated heparan sulphate proteoglycan, glypican 3 (GPC3), has been identified as the SGBS gene.[3] GPC3 is a member of the glypican related integral membrane proteoglycans (GRIPS) which are linked to the cell surface via glycosyl phosphatidylinositol and modulate the interaction between growth factors and receptors.[3–5] GPC3 encodes a protein of 580 amino acids and, despite a comparatively small 2.3 kb transcript, spans more than 500 kb of genomic sequence. Deletions in the GPC3 gene have been found in a number of SGBS kindreds supplying strong evidence that such mutations are responsible for SGBS.[3 6 7] We report here the mutational analysis of the Dutch-Canadian SGBS family. Surprisingly, a family member with the apparent stigmata of SGBS including Wilms tumour appeared not to inherit the SGBS chromosome in a previous linkage study.[2] The definition of the GPC3 mutation has allowed the unequivocal documentation of a normal GPC3 status in this child.

DNA (extracted from peripheral blood and tumour tissues) was obtained from members of the SGBS family and unrelated controls. PCR amplification of exon 2 of the GPC3 gene was performed using the oligonucleotide primers EX2 A (5' gtttgccctgtttgccatg 3') and EX2 B (5' caaataatgatgccactaagc 3') producing a 329 bp fragment in normal subjects.[8] The reactions contained 1 μg of DNA, 50 ng of each primer, 0.2 mmol/l of each of the four dNTPs (dATP, dCTP, dGTP, and dTTP), and 1.2 U of *Taq* DNA polymerase in a total of 25 μl. Reactions were denatured at 94°C for four minutes followed by 30 cycles at 94°C for one minute, 60°C for one minute, 72°C for one minute, and extension at 72°C for 7.5 minutes. The PCR products were cloned (TA cloning kit, Invitrogen) and sequenced using the PRISM™ dye terminator cycle sequencing core kit with AmpliTaq@ DNA polymerase, FS (ABI). The primer used for sequencing was EX2 A. All DNA sequencing was performed on an Applied Biosystems 373A automated DNA sequencer.

A preliminary indication of the mutation site in this Dutch-Canadian family has been shown by a failure of an exon 2 PCR using exonic primers (fig 5, family a in reference 3). To define the molecular lesion fully, we conducted PCR with intronic primers.[8] The affected male was found to generate a smaller band on agarose gel in comparison to the normal control. Sequencing of the subcloned PCR products showed a 13 bp deletion of nucleotides 391-403 in exon 2 (fig 1) predicted to generate a frameshift with a premature stop codon at nt 445-447 (TAA) resulting in a truncated 79 amino acid protein. An *Apa*I restriction site at nt 401-406 was disrupted by the deletion. Digestion of the PCR products with *Apa*I showed 180 and 149 bp bands for normal controls, a single 316 bp fragment for affected subjects, and all three bands for carriers (fig 2).

The long range deletions observed in some SGBS kindreds, the large size of the GPC3 gene itself, combined with the multisystemic

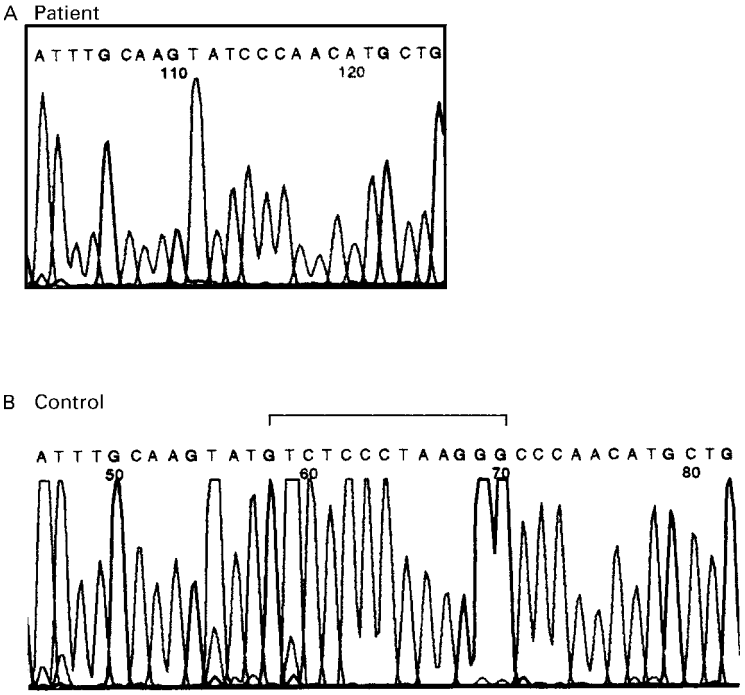

Figure 1 Sequence analysis of the normal and mutant alleles. Both normal and mutant alleles were PCR amplified with EX2 A and EX2 B, cloned with TA cloning kit (Invitrogen), and sequenced with EX2 A. The comparison of the sequences from the patient (A) with normal (B) shows a 13 bp deletion in exon 2.

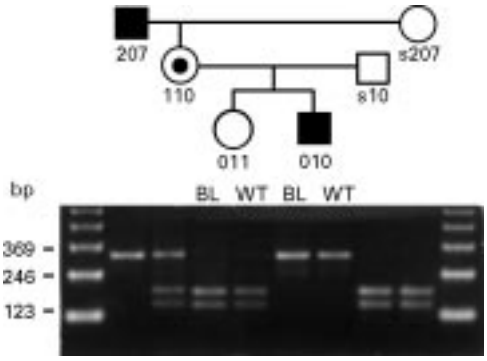

Figure 2 ApaI digestion of PCR products. The top panel is the partial pedigree of the Dutch-Canadian SGBS family. BL: DNA extracted from blood. WT: DNA extracted from Wilms tumour. The patterns of the ApaI digestion are shown below the pedigree. PCR products amplified with EX2 A and EX2 B from affected males, carrier, and control DNA from the family were digested with ApaI and run on a 2% agarose gel.

nature of SGBS have raised the possibility of mutations in contiguous genes contributing to the disease phenotype. The identification of this discrete deletion in a SGBS family strongly suggests that mutations in GPC3 alone are sufficient to cause the disorder.

In our original SGBS mapping paper, a young girl with multiple thoracic hemivertebrae, a Sprengel's deformity of her right shoulder, and Wilms tumour unexpectedly appeared not to carry the SGBS mutation (V.1, fig 1 in reference 2) although her genotype could not be ascertained with certainty. Notwithstanding the phenotype and in contrast to her brother with Wilms tumour, neither leucocyte nor Wilms tumour genomic DNA carry the GPC3 exon 2 deletion in this young girl (see 011 in fig 2). The non-segregation of the GPC3 mutation and Wilms tumour suggests that the somatic overgrowth and the tumorigenesis may be involved in different pathological pathways. It is possible that the risk factors derived from the maternal SGBS carrier contribute to embryonal tumorigenesis during intrauterine life with raised levels of substrates in the maternal circulation normally suppressed by GPC3 crossing the placenta and influencing embryonal development. Consistent with a maternal tumorigenic effect is the observation of an equal incidence of Wilms tumour concordance in both monozygotic and dizygotic twins, underlining the importance of postconception events in tumorigenesis.[9] In this regard, it is noteworthy that high levels of GPC3 are seen in preterm amniotic fluid of normal subjects.[10]

Loss of heterozygosity at chromosome 11p13-15.5, an anomaly that has been implicated in approximately 50% cases of Wilms tumour,[11] was observed in both the GPC3 null and normal Wilms tumour patients in this kindred (data not shown). One model which would link somatic overgrowth to embryonal tumorigenesis in SGBS is a simple increase in mitotic activity leading both to hyperplasia and increased somatic recombination frequency predisposing to 11p loss of heterozygosity. However, the observation of both normal birth weight and growth in the non-SGBS girl with Wilms tumour suggests that, if there is SGBS maternal carrier mediated increase in malignant potential, the mechanism probably involves more than simply increased mitotic activity. Irrespective of the precise mechanism, the data in our report show that a discrete disabling mutation of GPC3 is sufficient to cause SGBS and that such a mutation in a carrier mother may impart features of SGBS including Wilms tumour in offspring with a normal GPC3 gene.

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