Loss-of-function mutations and global rearrangements in \( GPC3 \) in patients with Simpson–Golabi–Behmel syndrome

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Simpson–Golabi–Behmel syndrome type 1 (SGBS1; MIM #312870) is a congenital malformation syndrome characterized by pre/postnatal overgrowth, intellectual disability and distinctive craniofacial features including macrocephaly, coarse facial features, macrostomia, macroglossia and palatal abnormalities.¹,² Diagnosis of SGBS is based on the clinical findings, family history consistent with X-linked inheritance and molecular biological testing of the glypican-3 gene (\( GPC3 \); MIM #300037 [http://omim.org/]) that maps to Xq26. \( GPC3 \), a member of the glypican family, encodes a heparan sulfate proteoglycan that is attached to the cell membrane via a glycosylphosphatidylinositol linkage.³ The gene spans >500 kb of genomic DNA, contains eight exons, and has essential roles in development by modulating cellular responses to growth factors and morphogens. Here, we report the identification of novel \( GPC3 \) mutations: three nucleotide alterations and one global chromosomal rearrangement involving \( GPC3 \).

Patient 1 was a 7-year-old boy with non-consanguineous parents, born at 26 weeks of gestation by emergency cesarean section due to fetal distress. His birth weight, length and occipitofrontal circumference (OFC) were 976 g (>90th centile), 37 cm (75–90th centile) and 24.5 cm (50th centile), respectively, indicating prenatal overgrowth. He was the second child of a mother who had a cleft palate at birth. The patient presented some dysmorphic features, such as macrocephaly, coarse facial features, macrostomia, macroglossia, dental malocclusion, accessory nipples, broad fingers, bilateral planovalgus and genu valgum.

Patient 2 was a 3-month-old male infant born at 32 weeks by cesarean section because of threat of premature labor. His birth weight was 2,560 g and his length was 45 cm, indicating over 90th centile for both. Both his parents were 23 years old and healthy; their first child was also healthy. Before birth, congenital diaphragmatic hernia was noted, and the hernia was surgically repaired at 5 days after birth. Physical examination showed a large tongue and big toes. Abdominal echography showed that both kidneys were on the left side.

Patient 3 was a 10-year-old boy born at 39 weeks of gestation. His birth weight, length and OFC were 4,030 g (>97th centile), 55 cm (>97th centile) and 36 cm (>97th centile), respectively, indicating prenatal overgrowth. Fetal ultrasonography suggested congenital diaphragmatic hernia, which was surgically repaired soon after birth. Lues due to midgut volvulus was noted at 3 months and partial resection of the intestine was required. At present, his height, weight and OFC were 141.9 cm (75–90th centile), 29.8 kg (25–50th centile) and 52.0 cm (10–25th centile), respectively. He exhibited coarse facial features with macroglossia, and bilateral accessory nipples were noted. His developmental milestones were within the normal limit.

Patient 4 was an 18-month-old boy born at 38 weeks of gestation. His birth weight, length and OFC were 4,040 g (>97th centile), 55 cm (>97th centile) and 36 cm (>97th centile), respectively, indicating prenatal overgrowth. He exhibited oculocutaneous hypertelorism, epicanthal folds, upslanting palpebral fissures, a wide nasal bridge, macrostomia (an abnormally large mouth), macroglossia (an abnormally large tongue), dental malocclusion, accessory nipples, broad fingers, bilateral planovalgus and genu valgum.

Loss-of-function mutations and global rearrangements in \( GPC3 \) were identified by modulating cellular responses to growth factors and morphogenesis.
gies, Santa Clara, CA, USA), as described previously. Two microduplications were identified on the X chromosome, one of which was in GPC3 (Supplementary Figure S2). To evaluate the details of these microduplications, a custom array was designed and used, as described previously. The final molecular karyotype was arr Xq26.2 (132,742,336–132,933,597) × 2, QXq26.3 (135,873,188–136,230,169) × 2 (GRCh37/hg19) (Figure 1a), indicating microduplication sizes of 191 and 357 kb. The microduplication in Xq26.2 spans exons 3–6 of GPC3. Patient 5 also harbors two microduplications, which suggests that the two microduplications segregate together. Thus, we suspected the existence of a complicated chromosomal rearrangement involving two regions. To test this hypothesis, we attempted to genotype chromosomal breakpoints and fused regions utilizing various sets of primers. Only one amplicon was successfully generated using the following primer set: primer 1, 5′-GCAACCTCCTTTCCCTGAAG-3′; primer 2, 5′-GCAAGGTCCTGTGGCTGCAA CGG-3′ (Figure 1b). Sequence analysis of this 1,070 bp amplicon included the breakpoints of both microduplications, indicating that the microduplications were fused by a complex chromosomal rearrangement, with an insertion of 16 bp of the unknown sequence (Figure 1c). PCR amplicons were also generated using DNA from patient 5 and the mother, as well as a healthy, maternal aunt of patients 4 and 5. All amplicons were the same size, indicating that the aunt is also a non-symptomatic carrier (Figure 1b).

In general, SGBS is inherited as an X-linked recessive trait; however, patient 5 (female) displayed partial SGBS symptoms (a cleft palate and hepatoblastoma). Thus, the X-chromosome inactivation pattern was analyzed by the human androgen receptor gene assay, as described previously. The mother of patients 4 and 5 showed a skewed X-chromosome inactivation pattern (82% vs. 18%), which was in agreement with her lack of symptoms (Figure 2). In contrast, patient 5 showed a paradoxical X-chromosome inactivation pattern, with the affected allele being predominantly activated.

More than 56 GPC3 mutations have been reported, with most being loss of function. In comparison with those duplications, the microduplication identified in this study is complicated, originating from a distal Xq26.3 region. Furthermore, the mother of patient 4 and patient 5 (the elder sister) had a cleft palate, which is one of the findings of SGBS. Patient 5 also had a hepatoblastoma. Phenotypic...
differences between female carriers appear to depend on the X-chromosome inactivation pattern.12

HGV DATABASE
The relevant data from this Data Report are hosted at the Human Genome Variation Database at http://dx.doi.org/10.6084/m9.figshare.hgv.858, http://dx.doi.org/10.6084/m9.figshare.hgv.861, http://dx.doi.org/10.6084/m9.figshare.hgv.864, http://dx.doi.org/10.6084/m9.figshare.hgv.867 and http://dx.doi.org/10.6084/m9.figshare.hgv.870.

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Supplementary Information for this article can be found on the Human Genome Variation website (http://www.nature.com/hgv)

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COMPETING INTERESTS
The authors declare no conflict of interest.