

# Whole exome sequencing aids the diagnosis of Simpson–Golabi–Behmel syndrome in two male fetuses

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## Abstract

**Objective:** To diagnose and explore the genetic aetiology of Simpson–Golabi–Behmel syndrome type I (SGBSI) in two male fetuses.

**Methods:** Prenatal ultrasound scans and further genetic analysis using karyotype analysis, chromosomal microarray analysis, whole exome sequencing (WES) and Sanger sequencing were conducted.

**Results:** Prenatal ultrasound scans of two fetuses showed multiple congenital anomalies and hydramnios. Subsequent to termination of the pregnancies, a novel nonsense variant (c.892G>T, p.E298\*) in the glypican 3 (*GPC3*) gene of the two fetuses was identified by WES and further confirmed by Sanger sequencing. The two fetuses were diagnosed with SGBSI. The mother was heterozygous for the c.892G>T variant.

**Conclusion:** This study describes the prenatal sonographic features of SGBSI, emphasizes the role of WES in the diagnosis of SGBSI and expands the known mutation spectrum of the *GPC3* gene.

## Keywords

Simpson–Golabi–Behmel syndrome type I, *GPC3*, fetal ultrasound findings, whole exome sequencing

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## Introduction

Simpson–Golabi–Behmel syndrome type 1 (SGBS1, OMIM entry no. 312870) is a rare X-linked overgrowth disorder first reported in 1975.<sup>1</sup> SGBS1 is characterized by pre- and postnatal macrosomia, multiple congenital anomalies, and distinctive craniofacial features including macrocephaly, coarse faces, macrostomia, macroglossia and palatal abnormalities.<sup>2–4</sup> Other clinical findings of SGBS1 include supernumerary nipples, congenital heart defects, diaphragmatic hernia, genitourinary defects, skeletal and hand anomalies, mild-to-severe intellectual disability and an increased risk for embryonal tumours.<sup>2–4</sup> In addition, female carriers may have manifestations of SGBS1 such as tall stature and coarse face because of skewed X-chromosome inactivation.<sup>5–7</sup>

Glypican 3 (*GPC3*, OMIM\* 300037) was the first gene reported to be associated with SGBS1 in 1996.<sup>8</sup> *GPC3* is located on chromosome Xq26.2, comprises eight exons and seven introns, and encodes a membrane-associated heparan sulphate proteoglycan of 580 amino acid residues belonging to the glypican family.<sup>9</sup> To date, 86 different mutations of the *GPC3* gene have been reported in patients with SGBS1, including large deletions (34.9%), frameshift mutations (24.4%), nonsense mutations (16.3%), missense mutations (8.1%), large duplications (8.1%), splice site mutations (4.7%), translocations (2.3%) and one in-frame mutation (1.2%).<sup>9</sup> In 2010, a duplication of exons 1–9 of the glypican 4 (*GPC4*) gene, which encodes another member of the glypican family, was identified in a family with SGBS1.<sup>10</sup>

Simpson–Golabi–Behmel syndrome type 1 belongs to a group of overgrowth syndromes and has overlapping clinical features with other overgrowth syndromes, such as Beckwith–Wiedemann syndrome, Weaver syndrome and Perlman syndrome.<sup>4,11</sup> Therefore, diagnosis of SGBS1

based on clinical features alone is difficult. However, this problem could be solved by molecular analysis, which could help to identify the underlying genetic causes, understand genotype–phenotype correlations and aid in diagnosis and treatment.

This current study describes the prenatal ultrasound findings and clinical features of two male fetuses. Further genetic analysis using chromosomal microarray analysis (CMA) and whole exome sequencing (WES) assisted the diagnosis of SGBS1 and revealed a novel nonsense variant in the *GPC3* gene that might be responsible for this disorder.

## Patients and methods

### Patients

This study undertook comprehensive physical examinations and full medical history evaluations in all available members of a family related to two male fetuses that were investigated by the Centre for Reproduction and Genetics, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou, China between June 2016 and December 2018. This study was approved by the Institutional Ethics Committee of the Affiliated Suzhou Hospital of Nanjing Medical University (no. 2016009). Written informed consent was obtained from the parents of the fetuses. A pedigree of the family was created after clinical examination and genetic testing.

### Karyotype analysis

G-banded karyotyping was performed according to the principle of ‘An International System for Human Cytogenetic Nomenclature, ISCN2013’ as described previously.<sup>12</sup>

### Chromosomal microarray analysis

Chromosomal microarray analysis was performed on an Affymetrix CytoScan<sup>®</sup> platform (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's instructions. Briefly, 250 ng of genomic DNA was digested, ligated, amplified using polymerase chain reaction (PCR), purified, fragmented, labelled and hybridized to the Affymetrix CytoScan<sup>®</sup> HD array. After washing, staining and scanning, raw data were analysed using Chromosome Analysis Suite version 3.2 (Affymetrix).

### Whole exome sequencing and data analysis

Genomic DNA was extracted from fetal cord blood and the peripheral blood of the parents collected in 6 ml ethylenediaminetetra-acetic acid tubes (BD Biosciences, San Jose, CA, USA) and stored at 4°C prior to use. Whole exome sequencing was performed by the WuXi NextCODE Genomics Company (Wuxi, China) using the SureSelect<sup>XT</sup> All Exon Target Enrichment System (Agilent Human All Exon 50-Mb kit; Agilent, Santa Clara, CA, USA) and an Illumina HiSeq X Ten System (Illumina, San Diego, CA, USA). A mean coverage of 87X was obtained. Data analysis was performed using Sentieon Genomics tools version 201611 (Sentieon, Mountain View, CA, USA) and variants were screened by the Clinic Sequence Analyser from WuXi NextCODE. The identified variants were classified according to the Standards and Guidelines for the Interpretation of Sequence Variants released by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology.<sup>13</sup>

### PCR amplification and Sanger sequencing

To confirm the identified variants, exon 3 of the *GPC3* gene was amplified by PCR using the following primers: forward,

5'-TGCTCTTACTGCCAGGGACT-3'; and reverse, 5'- GCTTTCCTGCATTCTTCTGG-3' (Shanghai Generay Biotech Company, Shanghai, China). The PCR reaction was conducted in a total volume of 20 µl containing 0.5 µM each primer, 0.2 mM each dNTP, 1 U FastStart<sup>TM</sup> Taq DNA polymerase (Roche, Basel, Switzerland) and 1 × FastStart<sup>TM</sup> Taq PCR reaction buffer with 2 mM MgCl<sub>2</sub>. The PCR cycling was performed in a GeneAmp 9700 Thermal Cycler (Applied Biosystems, Foster City, CA, USA) with a preliminary denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 45 s, annealing at 56°C for 30 s, and elongation at 72°C for 30 s, followed by a final elongation step at 72°C for 7 min and holding at 4°C. The amplified DNA fragments were purified and sequenced in both directions using an ABI 3130 Genetic Analyzer (Applied Biosystems). The resulting sequences were compared with the reference sequence of *GPC3* (NM\_004484.3) in the NCBI database.<sup>14</sup>

### In silico analysis of variants

The identified variant was searched against the dbSNP database,<sup>15</sup> the Exome Aggregation Consortium database,<sup>16</sup> the Genome Aggregation Database,<sup>16</sup> the 1000 Genomes Project database<sup>17</sup> and the database of Chinese genomes in diseaseDX.<sup>18</sup> The pathogenicity of the variant was predicted by Mutation Taster.<sup>19</sup>

## Results

The two parents investigated in this current study were a healthy, non-consanguineous couple. The woman ('gravid 4, para 0', G4P0) had four pregnancies. Her first pregnancy at the age of 25 was terminated at 24 weeks of gestation due to sonographic evidence of fetal renal anomaly. Her second pregnancy at the age of 26 resulted in a

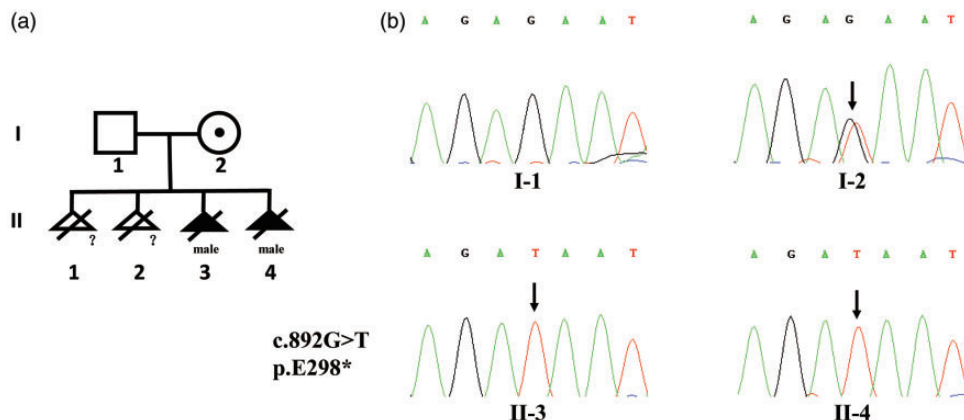


**Figure 1.** Clinical features of a male fetus after termination of the woman's fourth pregnancy at 29 weeks of gestation due to fetal abnormalities identified on a routine ultrasound scan. (a) A frontal photograph shows fetal macrosomia, cleft lip and palate and hypoplasia of external genitalia. (b) Close-up view of the facial phenotype. Note the square face, wide and high forehead, cleft lip and palate, broad nose and alar collapse.

spontaneous abortion at 4 weeks of gestation. No specific analysis was performed at that time. At the age of 27, she was referred to the Centre for Reproduction and Genetics, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou, China during her third pregnancy. A routine mid-trimester fetal ultrasound scan at 25 weeks of gestation suggested complete atrioventricular septal defect, aortic valvular stenosis, hydrops abdominis, hyperechogenic kidneys and hydramnios. The pregnancy was electively terminated and the karyotype of the fetal cord blood was 46,XY. In her fourth pregnancy at the age of 29, cytogenetic analysis of the amniotic fluid was performed at 21 weeks of gestation and revealed a 46, XY karyotype. At 29 weeks of gestation, a fetal ultrasound scan detected cleft lip and palate, small stomach bubble, bilateral hydronephrosis and hydramnios. The pregnancy was

terminated again and a postmortem examination revealed macrosomia with a head circumference of 25 cm and an abdominal circumference of 26 cm, a square face, wide and high forehead, cleft lip and palate, broad nose and alar collapse, and hypoplasia of the external genitalia (Figure 1). Chromosomal microarray analysis of the fetal DNA using an Affymetrix CytoScan<sup>®</sup> HD array revealed a normal male profile.

As the two affected fetuses were males, X-linked recessive inheritance was suspected and WES was performed with DNA from the two probands and their parents. Ultimately, a heterozygous variant (c.892G>T, p.E298\*) in exon 3 of the *GPC3* gene was identified. Direct Sanger sequencing validated the variant in both fetuses and revealed that the mother was heterozygous for the c.892G>T variant (Figure 2). A pedigree of this family is



**Figure 2.** Genetic analysis of the family related to the male fetus shown in Figure 1. (a) The pedigree of the family showing the father (I-1) and mother (I-2) with their four offspring numbered II-1–4. Individuals marked with a question mark (?) were not analysed for the glypican 3 (*GPC3*) gene. (b) Sanger sequencing chromatographs of the *GPC3* gene in available family members revealed a mutation in the two probands and their mother. Mutations are indicated by black arrows. The colour version of this figure is available at: <http://imr.sagepub.com>.

shown in Figure 2. The c.892G>T variant was not recorded in the dbSNP database, the Exome Aggregation Consortium database, the Genome Aggregation Database, the 1000 Genomes Project database or the database of Chinese genomes in diseaseDX. The c.892G>T variant causes a premature stop codon (p.E298\*) and was predicted to be disease causing by Mutation Taster with a probability value of 1.0. According to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology variant classification guideline,<sup>13</sup> the c.892G>T variant could be classified as pathogenic (i) with 1 very strong (PVS1), 1 moderate (PM2) and 1 supporting (PP1) evidence.

## Discussion

Previously, most patients with SGBS1 were diagnosed postnatally and mutations of the *GPC3* gene were identified by a targeted analysis after diagnosis of SGBS1.<sup>9</sup> With the recent advent of chromosomal microarray analysis and next-generation

sequencing, *GPC3* variants can be detected prenatally in fetuses with abnormal ultrasound findings before the diagnosis of SGBS1.<sup>20–25</sup> Recently, four *GPC3* variants were identified by WES in four families without a preliminary clinical diagnosis of SGBS1.<sup>11,21,25</sup> Among these, three variants were detected in fetuses with abnormal prenatal ultrasound findings such as fetal overgrowth, diaphragmatic hernia, enlarged kidneys and hydramnios, which are not pathognomonic for SGBS1.<sup>21,25</sup> One variant was detected postnatally in a 6-year old male patient with unknown overgrowth syndrome.<sup>11</sup> In this current study, two male fetuses were diagnosed with SGBS1 after identification of a loss-of-function mutation in the *GPC3* gene by WES.

Prenatal abnormal findings among fetuses diagnosed with SGBS1 include elevated maternal serum alpha-fetoprotein, increased nuchal translucency, craniofacial anomalies, macrosomia, polyhydramnios, renal anomaly and cardiac malformation.<sup>20–27</sup> In this current study, the woman missed the maternal serum screening and



first-trimester ultrasound screening, hence the data for maternal serum alpha-fetoprotein level and fetal nuchal translucency thickness were unavailable. A mid-trimester ultrasound examination of the two affected fetuses revealed cleft lip and palate, congenital heart defect, renal anomaly, and hydramnios, which was consistent with previous reports.<sup>20–26</sup>

As a member of the glypican family, the human GPC3 protein is attached to the exocyttoplasmic surface of the plasma membrane through a covalent glycosylphosphatidylinositol anchor.<sup>9</sup> GPC3 was reported to regulate cell proliferation negatively by inhibiting soluble hedgehog activity<sup>28</sup> and promote the growth of hepatocellular carcinoma by stimulating Wnt signaling.<sup>29</sup> Until now, 86 different *GPC3* mutations have been identified, which are dispersed along all the coding regions with no obvious mutation hotspots, and the majority of *GPC3* mutations lead to a premature stop codon (49/86).<sup>9</sup> In this current study, a novel c.892G>T variant was identified in the *GPC3* gene of the two fetuses, which was inherited from the mother. The c.892G>T variant created a premature stop codon and resulted in a truncated protein (p.E298\*), which is predicted to be disease causing.

In conclusion, this current report describes the diagnosis of two male fetuses with SGBS1 by a combination of ultrasound scan and genetic analysis; and the identification of a novel nonsense variant in the *GPC3* gene of the two fetuses. These current findings demonstrated the potential of WES in the diagnosis of SGBS1 and broaden the mutation spectrum of *GPC3* in SGBS1.

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### Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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