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作成上の留意事項

研究成果の刊行に関する一覧表は、別紙４「研究成果の刊行に関する一覧表レイアウト」を参考に作成すること。

(1) 日本工業規格Ａ列４番の用紙を用いること。

(2) 文字の大きさは、１０～１２ポイント程度とする。

〔VI〕

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Clinical report

SOX9 dimerization domain mutation mimicking type 2 collagen disorder phenotype

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ABSTRACT

The classification of bone dysplasia has relied on a clinical/radiographic interpretation and the identification of specific genetic alterations. The clinical presentation of the *SOX9* mutation and type 2 collagen disorders overlap with the Pierre-Robin sequence and talipes equinovarus, but the former is often accompanied by the bent long bones. In its milder form, the *SOX9* mutation is not necessarily associated with the bent long bones. Here, we report a patient with the Pierre-Robin sequence and talipes equinovarus who did not exhibit either bent long bones or scapular hypoplasia; thus, this patient was instead classified as having a type 2 collagen disorder. Despite this phenotypic presentation, the proposita was found to have a *de novo SOX9* mutation. The peculiar location of the mutation within the dimerization domain might account for the relatively mild phenotypic effect of the *SOX9* mutation to a degree that is compatible with a clinical diagnosis of type 2 collagen disorder, except for a developmental delay. We concluded that mutations in *SOX9* can mimic a type 2 collagen disorder-like phenotype.

1. Introduction

The "Nosology and Classification of Genetic Skeletal Disorders" defines each skeletal disorder based on the rather arbitrary combination of morphological assessments and the confirmation of specific genetic alterations [Spranger et al., 2012]. In general, alterations in different molecules involved in the same signaling pathway result in similar phenotypes, defining a specific disease spectrum. Within such spectra, the functional degree of molecular disturbance, rather than the name of the molecule *per se*, can be the major determinant of the patient's phenotype. Accordingly, clinicians often have difficulty identifying the causative gene based on a patient's clinical features alone.

According to the most recent "Nosology and Classification of Genetic Skeletal Disorders," revised in 2010, type 2 collagen disorders include nine disorders [Warman et al., 2011]. Traditionally, clinical features that point to an underlying type 2 collagen disorders include micrognathia, cleft palate, flat midface, visual or hearing impairment, and variable radiographic changes. Achondrogenesis type 2 (OMIM 200610) represents the severe end that is characterized by lethal degree of skeletal undermineralization. Spondyloepiphyseal dysplasia congenita (OMIM 183900) and Kniest dysplasia (OMIM 156550) are characterized by retarded enchondral ossification of the appendicular skeleton and odontoid hypoplasia. Stickler syndrome represents the mild end [Kannu et al., 2010].

The causative gene of these disorders, type 2 collagen gene, *COL2A1*, is directly regulated by *SOX9* [Bell et al., 1997]. The classic presentations of a *SOX9* mutation, campomelic dysplasia, resembles those of type 2 collagen disorders and are often accompanied by severe morphological changes, such as the bending of the long bones. Furthermore, SOX9 plays an essential role in the gonads and in the central nervous system, leading to sex differentiation disorders in male patients and developmental delays when *SOX9* is mutated [Foster et al., 1994; Wagner et al., 1994]. When the molecular defect is less severe, the *SOX9* mutant can manifest as a mild phenotype without the bending of the long bones and is called acampomelic campomelic dysplasia (ACD). In patients with ACD, who lack the characteristic finding of

Abbreviations: ACD, acampomelic campomelic dysplasia; NGS, next generation sequencing.

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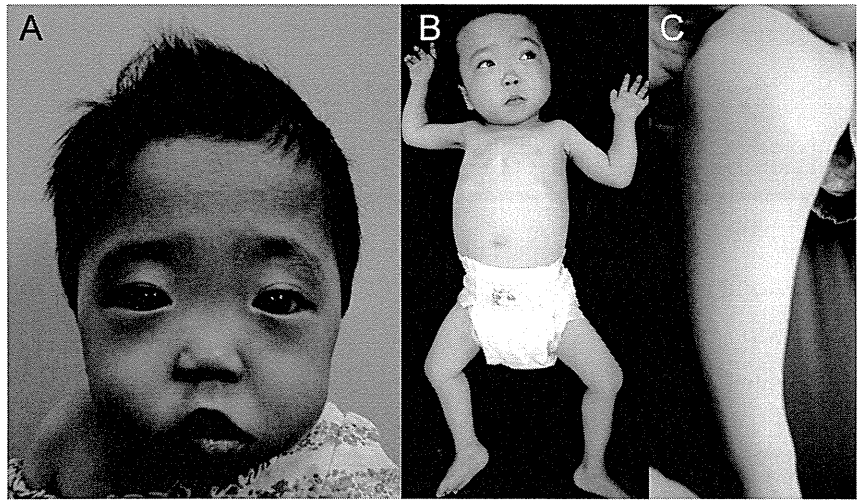

Fig. 1. Morphological characteristics of the proposita. (A) A facial photograph of the proposita at the age of 2 years showing hypertelorism, downslanting palpebral fissures, hypoplasia of the mid-facial structures and micrognathia. Photographs of the propisita's full-length figure (B) and right lower extremity (C) show the absence of bowed tibiae or pretibial dimples.

bent long bones, the presence of scapular hypoplasia is a key diagnostic finding [Glass and Rosenbaum, 1997; Lecointre et al., 2009; Macpherson et al., 1989; Moog et al., 2001; Thong et al., 2000; Wada et al., 2009].

2. Clinical report

The proposita was a product of natural conception by parents with no family history of known genetic conditions. The mother was a 37-year-old primipara woman who had a history of depression and asthma. Her pregnancy was complicated by polyhydramnios, which was first noted at 37 weeks of gestation. The proposita was born at 41 5/7 weeks of gestation via cesarean section because of a non-reassuring fetal status. Her birth weight was 2716 g (−1.5 SD), her length was 44.8 cm (−3.0 SD), and her head circumference was 35 cm (+0.9 SD). The Apgar scores were 8 and 8 at 1 and 5 min, respectively. At birth, her physical examination revealed multiple congenital anomalies including a cleft palate, micrognathia, right talipes equinovarus, thin ribs, absent toenails, and short ulnae. An echocardiogram revealed a patent ductus arteriosus and atrial septal defect, which closed spontaneously.

Soon after birth, she was noted as having severe stridor with retraction and obstructive apnea secondary to glossoptosis and tracheomalacia. She was treated using positive airway pressure support. She was discharged home on a bilevel positive airway pressure and a feeding tube at 4 months of age with a weight of 4881 g (−2.1 SD). After hospital discharge, she experienced multiple episodes of upper airway infections requiring hospitalization. These findings led to a provisional diagnosis of type 2 collagen disorder without molecular confirmation.

During outpatient follow-up examinations, it became apparent that she had a developmental delay. She gained head control at 10 months, rolled over at 12 months, and smiled at 18 months of age. The developmental delay was incompatible with a diagnosis of type 2 collagen disorder. Hence, we performed a mutation analysis for *COL2A1* but did not find a pathologic mutation. An expanded analysis using a custom-designed mutation analysis panel that included *COL2A1, COL11A1, COL11A2, COL9A1, COL9A2*, and *SOX9* revealed a *de novo* missense mutation in the dimerization domain of *SOX9*.

At the age of 2 years and 2 months, she continued to exhibit a severe developmental delay. She sat without support but did not have any meaningful words. Her weight was 7.035 kg (−3.6 SD), her length was 68.8 cm (−5.5 SD), and her head circumference was 48.2 cm (+0.66 SD). A physical examination showed hypertelorism with an inner canthal distance of 3.2 cm, downslanting palpebral fissures, hypoplasia of the mid-facial structures and micrognathia, cleft palate, limitations in the range of joint motions, and normal female external genitalia. A pretibial dimple was not present (Fig. 1). An ophthalmologic examination showed severe myopia, while an audiological evaluation showed severe deafness. Plain radiographs showed a narrow thorax, slender long bones and hip dislocation. Scapular hypoplasia, and bending of the long bones were absent (Fig. 2).

3. Molecular analysis

The present research protocol was approved by an institutional review board. Written consent was obtained from the parents. DNA was extracted from whole blood samples that were obtained from the proposita and her biological parents. Target resequencing using a custom-designed mutation analysis panel (SureSelect XT-Auto;

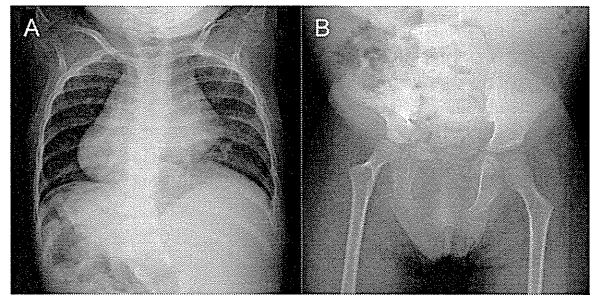

Fig. 2. Skeletal characteristics of the proposita. Anteroposterior radiographs of the chest (A), pelvis and the lower extremities (B) at 2 years and 6 months of age show vertical iliac wings and a right dislocated hip. Neither bent long bones nor scapular hypoplasia was present.

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Agilent Technologies, Santa Clara, CA, USA) was performed. The list of genes (manuscript in preparation) included 100 common genes described in a classic textbook of dysmorphology: Smith's Recognizable Patterns of Human Malformation [Jones, 2006].

This panel was run on a next-generation sequencer (NGS: MiSeq; Illumina, Inc., San Diego, CA, USA). After the sequencing reads were aligned to the reference human genome sequence (hs37d5) using BWA [Li and Durbin, 2009], local realignment around the indels and base quality score recalibration were performed using Genome Analysis Toolkit software [McKenna et al., 2010]. Duplicate reads were removed using Picard (http://picard.sourceforge.net). This trio analysis revealed that the proposita had a *de novo* heterozygous missense mutation, i.e., c.239T>G p.Val80Gly, in exon 1 of *SOX9*. This mutation was located in the dimerization domain of *SOX9* and is a novel variant that is not present in the dbSNP137, 1000 genomes, ESP6500, or our in-house Japanese SNP dataset. We confirmed the mutation detected in the proposita using Sanger sequencing with the following primers: GCGCCTTCCTAAGTGCTC (forward) and AGCGTCCAGTCGTAG CCTTT (reverse). Sex differentiation disorder has been reported in patients with campomelic dysplasia arising from a *SOX9* mutation, but PCR-amplification of the *SRY* region using SRY primers revealed no amplification of these products [Berta et al., 1990].

4. Discussion

Here we report a patient with *de novo SOX9* mutation who exhibited many features of the type 2 collagen disorder including micrognathia, cleft palate, flat midface, and visual and hearing impairment, and a retarded enchondral ossification of the appendicular skeleton during the infantile period. However, the severe developmental delay, which became apparent over time, was rather atypical for a type 2 collagen disorder. A target analysis of the most frequent causative gene for type 2 collagen disorder, i.e., *COL2A1*, failed to detect pathologic mutations, but an expanded mutation analysis using NGS revealed a mutation in *SOX9*, which is an upstream regulator of *COL2A1*.

Lines of existing evidence suggest a causal relationship between the detected mutation in *SOX9* and the patient's phenotype. In general, only single amino acid substitutions occur in the coding region of the genome per generation [Lynch, 2010]. A similar *de novo* amino acid substitution in the dimerization domain was reported in a patient with ACD [Sock et al., 2003]. Although a functional assay would provide more insight into the mechanism, we concluded that the *de novo* amino acid substitution change in the dimerization domain of *SOX9* was responsible for the constellation of short limb dwarfism, Pierre-Robin sequence, and severe developmental delay in the proposita.

SOX9 is expressed in many organs including the brain, testis, pancreas, gut, and inner ear [Gordon et al., 2009]. In the downstream signal transduction, a tissue-specific requirement for the dimerization of SOX9 exists. In vitro studies have shown that the dimerization of SOX9 is required in cartilage but that SOX9 binds as a monomer to the downstream regulatory region of the sex-determining gene in the gonads [Bernard et al., 2003]. In the central nervous system, the structural requirement, i.e., the monomerization or dimerization, of SOX9 remains unclear. The severe developmental delay in the proposita could be considered as in vivo evidence that the dimerization of SOX9 is necessary in the developing brain. The presence of a developmental delay in an ACD patient arising from an amino acid substitution in the dimerization domain of *SOX9* further supports this possibility [Sock et al., 2003].

The identification of the causative mutation was rather challenging in the proposita. After the Sanger sequencing of *COL2A1* failed to detect a causative genetic alteration, we resorted to an NGS-based molecular diagnostic approach [Takenouchi et al., 2013]. By using the customly-designed mutation analysis panel covering congenital skeletal disorders, we successfully identified a causative mutation in *SOX9* in the proposita, who did not exhibit campomelia or scapular hypoplasia.

From a radiographic standpoint, she could be classified as having mild end of ACD in that she did not have overt scapular hypoplasia with the molecular diagnosis in mind. According to the most updated 'Nosology and Classification of Genetic Skeletal Disorders', ACD is classified under "bent bone dysplasia" [Warman et al., 2011]. However, the morphological features of the proposita, namely the absence of bent long bones or scapular hypoplasia (which is pathognomonic for ACD), make it unclear whether the proposita should be placed within the group characterized by "bent bone dysplasia". The mere combination of the Pierre-Robin sequence and talipes equinovarus points to a type 2 collagen disorder. Further refinement of the classification of bent bone dysplasia and type 2 collagen disorders may be warranted.

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CLINICAL REPORT

AMERICAN JOURNAL OF medical genetics PART A

Somatic *CTNNB1* Mutation in Hepatoblastoma from a Patient with Simpson–Golabi–Behmel Syndrome and Germline *GPC3* Mutation

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Simpson–Golabi–Behmel syndrome is a rare overgrowth syndrome caused by the *GPC3* mutation at Xq26 and is clinically characterized by multiple congenital abnormalities, intellectual disability, pre/postnatal overgrowth, distinctive craniofacial features, macrocephaly, and organomegaly. Although this syndrome is known to be associated with a risk for embryonal tumors, similar to other overgrowth syndromes, the pathogenetic basis of this mode of tumorigenesis remains largely unknown. Here, we report a boy with Simpson-Golabi–Behmel syndrome who had a germline loss-of function mutation in *GPC3*. At 9 months of age, he developed hepatoblastoma. A comparison of exome analysis results for the germline genome and for the tumor genome revealed a somatic mutation, p.Ile35Ser, within the degradation targeting box of β-catenin. The same somatic mutation in *CTNNB1* has been repeatedly reported in hepatoblastoma and other cancers. This finding suggested that the *CTNNB1* mutation in the tumor tissue represents a driver mutation and that both the *GPC3* and the *CTNNB1* mutations contributed to tumorigenesis in a clearly defined sequential manner in the propositus. The current observation of a somatic *CTNNB1* mutation in a hepatoblastoma from a patient with a germline *GPC3* mutation supports the notion that the mutation in *GPC3* may influence one of the initial steps in tumorigenesis and the progression to hepatoblastoma.

Key words: hepatoblastoma; Simpson–Golabi–Behmel syndrome; CTNNB1; GPC3

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Am J Med Genet Part A 164A:993–997.

INTRODUCTION

Simpson–Golabi–Behmel syndrome (SGBS, OMIM312870) represents an overgrowth syndrome associated with organomegaly and macroglossia accompanied by characteristic external features, such as supernumerary nipples, supernumerary ribs, hypospadias, and cryptorchidism, as well as internal malformations, such as cardiac defects, diaphragmatic hernias, and cystic dysplasia of the kidneys [Cottereau et al., 2013]. SGBS is caused by loss-of-function mutations in the heparan sulphate proteoglycan, glypican 3 gene (*GPC3*) at chromosome Xq26 [Pilia et al., 1996]. The *GPC3* gene encodes an extracellular matrix protein that is expressed during development and that regulates cell proliferation and apoptosis during

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development through the modulation of growth factor action, including that of IGF2 [Gonzalez et al., 1998; Pellegrini et al., 1998].

Patients with SGBS are at an increased risk for the development of embryonal tumors, such as Wilms tumor [Xuan et al., 1994; Hughes-Benzie et al., 1996; Lindsay et al., 1997] and hepatoblastoma [Lapunzina et al., 1998; Li et al., 2001; Buonuomo et al., 2005; Mateos et al., 2013]. In a recent article published in this journal, Mateos et al. [2013] documented a patient with SGBS and a *GPC3* duplication who developed a hepatoblastoma. The pathogenetic basis of the triggering and progression of embryonal tumors in the absence of a functional *GPC3* is currently unknown. Here, we document an infant with a *GPC3* mutation who developed a hepatoblastoma in which the tissue was shown to harbour a *CTNNB1* mutation using exome sequencing. This observation sheds new insight on the stepwise progression of hepatoblastoma.

CLINICAL REPORT

The propositus was born at 41 weeks of gestation as the first child of nonconsanguineous parents. He was delivered by cesarean section. His mother was 35 years old, had a height of 165 cm (+1.3 SD), and had coarse facial features. The father was 54 years old and was healthy. The birth weight of the propositus was 4,068 g (+2.65 SD), his length was 55 cm (+2.8 SD), and his head circumference was 37.5 cm (+2.66 SD). He had a ventricular septal defect that was repaired at the age of 1 month.

At the age of 4 months, his weight was 8.55 kg (+1.61 SD), his length was 68.8 cm (+1.71 SD), and his head circumference was 43.8 cm (+1.6 SD). He had an upturned bulbous nose, a wide nasal bridge, apparent hypertelorism, macrostomia, macroglossia, a midline grooved tongue, a right accessory nipple, and a short webbed neck. His hands were broad, and he had right index fingernail hypoplasia. Based on these clinical features, he was diagnosed as having SGBS (Fig. 1A). Regular surveillance was started to screen for the possible development of abdominal tumors, including hepatoblastoma and Wilms tumor. A cystic lesion was detected in the hepatic parenchyma at 9 months during an abdominal ultrasound examination. An abdominal CT scan revealed a 45 mm × 35 mm × 35 mm heterogeneously enhancing mass localized in S4 that was classified as PRETEXT stage III (Fig 1B,C). The patient's serum α-fetoprotein was elevated to 658 ng/ml. A fine needle biopsy led to a pathological diagnosis of hepatoblastoma. After chemotherapy with cisplatin and tetrahydropyranyladriamycin, the residual mass was surgically removed at the age of 14 months. At the age of 2 years, he continued to demonstrate overgrowth, with a weight of 17.1 kg (+4.58 SD) and a length of 95.7 cm (+3.4 SD).

MOLECULAR INVESTIGATION

Informed consent from the parents and approval from the institutional review board were obtained for the molecular studies. We first performed Sanger sequencing of the *GPC3* gene using DNA obtained from a peripheral blood sample of the propositus. A c.1159C > T, p.Arg387X mutation was identified, confirming the diagnosis of SGBS. Next, we obtained DNA from the hepatoblastoma tissue resected at the time of biopsy. A matched non-tumor

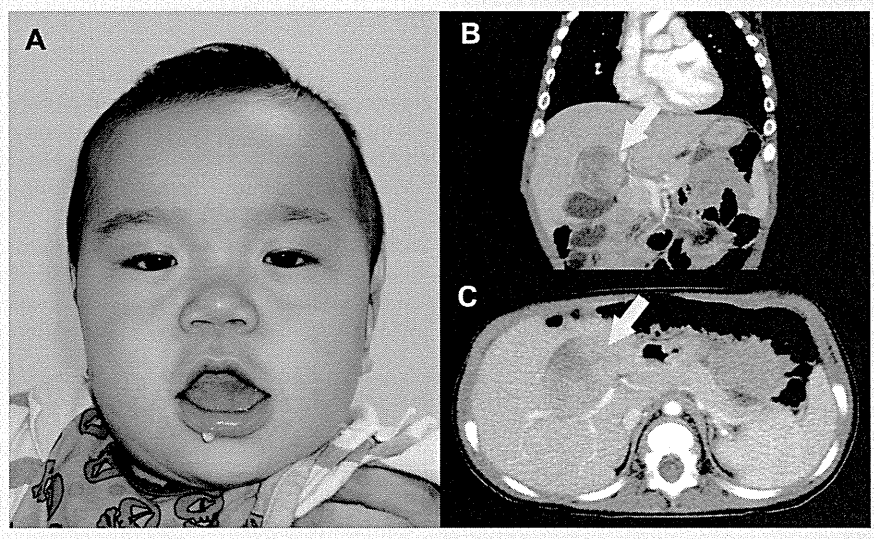

FIG. 1. The characteristic facial features and hepatoblastoma in the propositus. A: Note that the facial features of the propositus included upturned bulbous nose, a wide nasal bridge, apparent hypertelorism, macrostomia, macroglossia, and a midline grooved tongue. B and C: Coronal (B) and axial (C) slices of magnetic resonance imaging at 9 months of age showed a well-demarcated heterogeneously enhancing mass, measuring 45 mm × 35 mm × 35 mm, in S4 of the liver (yellow arrows).

peripheral blood DNA sample was also obtained. Whole-exome sequencing was performed for both DNA samples. Massive parallel sequencing on an Illumina HiSEQ platform yielded ~11 gigabases per sample, with a mean coverage of 114-fold across 54 Mb of targeted coding regions (SureSelectXT2 Human All Exon V4; Agilent Technologies, Santa Clara, CA) for each sample. The sequence reads were aligned to the reference genome assemblies (hg19) using BWA [Li and Durbin, 2009]. Local realignment around the insertions/deletions and base quality score recalibration were performed using the Genome Analysis Tool Kit software [McKenna et al., 2010], with duplicate reads removed using Picard. On average, 73% of the coding bases were covered in sufficient depth in both the tumor and the matched normal samples to allow for confident mutation detection.

MuTect version 1.14 [Cibulskis et al., 2013] was used for comparison of the exome data derived from hepatoblastoma and that derived from the peripheral blood. The default parameters were used except that max_alt_alleles_in_normal_count and minimum_mutation_cell_fraction were set to 0 and 0.1, respectively. The Mutect program detected seventy mutations as a somatic change. These 70 mutations were annotated by the program SnpEff [Cingolani et al., 2012] and classified into the following classes of mutations: non-synonymous coding, non-synonymous start, splice site acceptor, splice site donor, start lost, stop gained, and stop lost. A mutation c.104T > G, p.Ile35Ser (NM_00904) was identified at exon 3 of the *CTNNB1* that encodes β-catenin, and was the only remaining somatic mutation through the filtering process described above. This alteration was confirmed using Sanger sequencing (Fig. 2). An analysis of the reads at the mutant position after the removal of duplicated reads revealed that 72 out of 171 reads were mutant.

Mutations within a targeting box are known to lead to the accumulation of intracytoplasmic and nuclear β-catenin protein [Koch et al., 1999; Purcell et al., 2011]. The catalog of somatic mutations in cancer (COSMIC) version 64 database contained 28 instances of samples containing the somatic mutation p.Ile35Ser in *CTNNB1* under the query conditions "confirmed somatic" or "previously reported"; "tumor sample, not cultured"; and "not reported as polymorphism in the 1,000 genome projects". Out of the 29 samples, 21 originated from the liver, 2 from soft tissue, and 1 each from the endometrium, pituitary, thymus, central nervous system, and lung. Hence, most of, if not all, the samples with p.Ile35Ser were derived from the liver. Among the 21 samples, 4 samples were specifically labeled as hepatoblastoma samples; in the remaining samples, the patient's age was not mentioned, and the clinical distinction between hepatocellular carcinoma versus hepatoblastoma was not mentioned. Furthermore, a literature review on *CTNNB1* mutation analyses in hepatoblastomas in patients without multiple malformation syndromes indicated that at least five patients carried the c.104T > G, p.Ile35Ser mutation [Takayasu et al., 2001; Cairo et al., 2008; Lopez-Terrada et al., 2009; Purcell et al., 2011; Chavan et al., 2012]. The article by Takayasu et al. was not catalogued in the COSMIC database.

DISCUSSION

Through Bayesian comparison of the exome data between the germline genome and the tumor genome, we identified a somatic *CTNNB1* mutation, p.Ile35Ser, within the degradation targeting box of β-catenin in the hepatoblastoma tissue of a patient with an overgrowth syndrome, SGBS, who had a loss-of-function mutation in the *GPC3* gene.

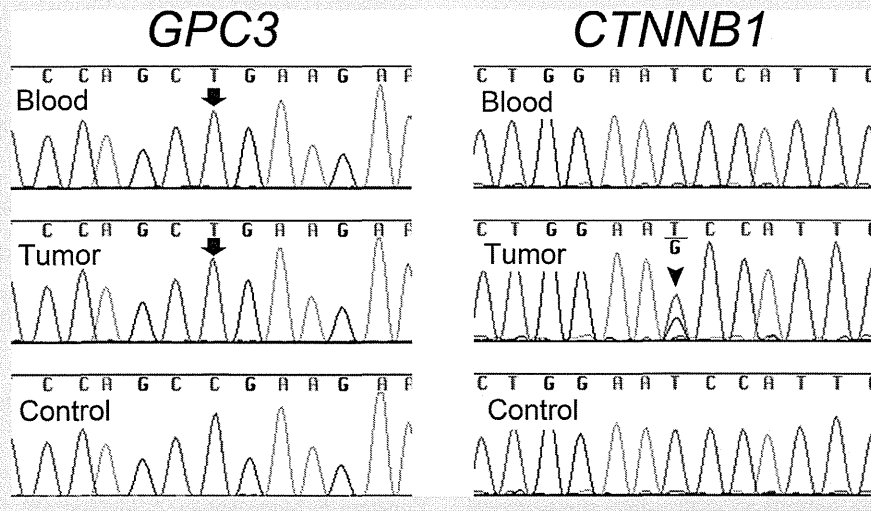

FIG. 2. Partial DNA sequences, including the sequences containing the mutations in the *GPC3* and *CTNNB1* genes. In a blood sample, a hemizygous mutation, c.1159C > T (top arrow), was identified in *GPC3*, but no mutations were identified in *CTNNB1*. In tumor tissue, a hemizygous mutation, c.1159C > T (bottom arrow), was identified in *GPC3* and a heterozygous mutation, c.104T > G (arrowhead), was identified in *CTNNB1*. The control shows a normal peripheral blood sample from a normal individual.

In general, the mutations identified in tumor tissue can be classified into two groups [Burgess, 2013]: "Driver mutations" that are directly involved in tumorigenesis followed by tumor progression, and "passenger mutations" that are not responsible for tumorigenesis or tumor progression but are by-products of genomic instability in tumor cells and are biologically neutral. A distinguishing feature of driver mutations is the recurrent appearance of the same somatic mutation in different individuals. Since the p.Ile35Ser mutation has been reported at least five times in hepatoblastomas [Takayasu et al., 2001; Cairo et al., 2008; Lopez-Terrada et al., 2009; Purcell et al., 2011; Chavan et al., 2012] and 17 times in samples from non-hepatoblastoma liver tumors, including hepatocellular carcinoma, it is reasonable to assume that the p.Ile35Ser *CTNNB1* mutation in the tumor tissue from the propositus represents a driver mutation.

The software MuTect has been shown to be efficient at detecting somatic mutations in a relatively small percentage (i.e., <10%) of tumor cells in a normal tissue background. Hence, the chance of missing mutations in other genes that are present in a subset of the cells in the tumor tissue is unlikely to be very high. Nevertheless, the classes of mutations that have been missed could include but are not limited to: (1) mutations in low coverage areas; (2) mutations in non-coding portions of the genome, such as in non-coding RNAs or regulatory elements; and (3) epigenetic changes that are undetectable using exome sequencing.

The identification of the *CTNNB1* mutation in a patient with SGBS sheds new light on the pathogenesis of hepatoblastoma: *CTNNB1* mutations within a targeting box, in which the propositus' p.Ile35Ser mutation resided, are known to lead to the accumulation of intracytoplasmic and nuclear β-catenin protein and to potentiate canonical Wnt/β-catenin signaling [Koch et al., 1999; Purcell et al., 2011]. Of note, the loss of Gpc3 leads to the activation of canonical Wnt/β-catenin signaling in *Gpc3*-knockout mice [Song et al., 2005]. If this finding is extrapolated to humans, the *GPC3* loss-of-function mutation could have exerted an additive effect on the potentiation of canonical Wnt/β-catenin signaling by the *CTNNB1* mutation. Given the fact that the propositus harbored a germline *GPC3* mutation and that the tumor harbored a somatic *CTNNB1* mutation together with the *GPC3* mutation, *GPC3* and *CTNNB1* apparently contributed to tumorigenesis in a clearly defined sequential manner, at least in the propositus. Whether mutations in *GPC3* and *CTNNB1* must occur in this specific sequence, and not vice versa, remains uncertain. Somatic loss-of-function mutations in *GPC3* have been reported in tumor tissues with various origins, including the lung (6/18), kidney (3/18), endometrium (3/18), large intestine (2/18), breast (1/18), prostate (1/18), and skin (2/18), but not in the liver according to the COSMIC database, version 66 [Forbes et al., 2011], and a search performed under the query conditions "confirmed somatic" or "previously reported"; "tumor sample, not cultured"; and "not reported" as polymorphism in the 1,000 genome projects. Hence, mutations in *GPC3* are unlikely to yield a liver-tumor-specific susceptibility to tumorigenesis or tumor progression.

From an etiological standpoint, SGBS and another prototypic overgrowth syndrome, Beckwith–Wiedemann syndrome (BWS, OMIM130650), share a key fetal growth accelerator, IGF2: the overproduction of IGF2 in BWS and the lack of an anchoring action of IGF2 by the extracellular matrix protein GPC3 in SGBS both promote fetal growth. Patients with BWS are known to have an increased susceptibility to hepatoblastoma, similar to patients with SGBS [Fukuzawa et al., 2003]. Further elucidation of the role of the *CTNNB1* mutation in hepatoblastomas in patients with BWS is warranted. Similarly, the likely role of *CTNNB1* mutation in the pathogenesis of Wilms tumor in both SGBS and BWS should be explored, together with the potential role of *GPC3* mutation in isolated hepatoblastomas.

In summary, we here document a somatic *CTNNB1* mutation in a hepatoblastoma from a patient with SGBS and a germline *GPC3* mutation. The current observation supports the notion that a mutation in *GPC3* may represent an initial step in the tumorigenesis and progression of hepatoblastoma.

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