B3GAT3-related disorder with craniosynostosis and bone fragility due to a unique mutation

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Purpose: Based on prenatal suspicion of the combination of radioulnar or radiohumeral synostosis and a peculiar shape of the skull suggestive of craniosynostosis, we report on six patients from four unrelated consanguineous families in whom Antley-Bixler syndrome was suspected during the prenatal period without mutation in genes known to be associated with the syndrome.

Methods: Molecular diagnosis involved whole-exome and gene-panel sequencing.

Results:: All sequenced patients showed a unique homozygous mutation of c.667G > A, p.Gly223Ser (NM_012200) in the beta-1,3-glucuronyltransferase 3 (*B3GAT3*) gene known to be involved in linkeropathy syndrome. Linkeropathies correspond to a recently identified group of heterogeneous genetic syndromes along a spectrum of skeletal and connective tissue disorders. These patients

featured mainly craniosynostosis, midface hypoplasia, bilateral radioulnar synostosis, multiple neonatal fractures, dislocated joints, joint contracture, long fingers, foot deformity, and cardiovascular abnormalities. All died before 1 year of age.

Conclusion: We identified a novel *B3GAT3*-related disorder with craniosynostosis and bone fragility, due to a unique homozygous mutation in *B3GAT3*. This syndrome should be considered in the prenatal period in light of the severe outcome and as an alternative diagnosis to Antley-Bixler or Shprintzen-Goldberg syndrome.

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Key Words: Antley-Bixler syndrome; B3GAT3; craniosynostosis; linkeropathy; Shprintzen-Goldberg syndrome

INTRODUCTION

Linkeropathies correspond to a recently identified group of heterogeneous genetic syndromes due to mutations in genes involved in proteoglycan (PG) synthesis pathway. PGs are ubiquitous components of extracellular matrix, subdivided into chondroitin sulfate, dermatan sulfate, and heparan sulfate PGs. They are fundamental for the development of several organs including skeletal muscle, bones, cartilage, retina, and heart.[1] Linkeropathy genes encode for enzymes in charge of branching glycosaminoglycan onto PG via a tetrasaccharide linker. Five genes have been identified so far: two xylo-transferases, namely xylosyltransferase 1 (*XYLT1*, OMIM 608124) and xylosyltransferase 2 (*XYLT2*, OMIM 608125); two galactosyltransferases, namely xylosylprotein 4-beta-galactosyltransferase polypeptide 7 (*B4GALT7*, OMIM

604327) and beta-1,3-galactosyltransferase polypeptide 6 (*B3GALT6*, OMIM 615291); and one glucuronyltransferase, beta-1,3-glucuronyltransferase 3 (*B3GAT3*, OMIM 606374). Mutations affecting these genes are responsible for a spectrum of skeletal and connective tissue disorders combining multiple congenital contracture and/or dislocation, joint laxity, broad digits, brachycephaly, small mouth, prominent eyes due to glaucoma, short or webbed neck, heart malformation, and developmental delay.[2,3] *XYLT1* and *XYLT2* mutations are associated respectively with Desbuquois dysplasia type 2 (OMIM 615777) and with spondylo-ocular syndrome (OMIM 605822). *B4GALT7* mutations are described in a form of Elhers-Danlos syndrome characterized by short stature and limb anomalies (OMIM 130070). *B3GALT6* mutations are involved in two genetic disorders: spondyloepimetaphyseal

dysplasia with joint laxity type 1 (OMIM 271640) and Ehlers-Danlos syndrome progeroid type 2 (OMIM 615349). Finally, *B3GAT3* mutations are reported with multiple joint dislocations, short stature, and craniofacial dysmorphism with or without congenital heart defects (OMIM 245600).

We performed next-generation sequencing for five of six patients from four unrelated families in whom Antley-Bixler syndrome (ABS1, OMIM 201750 and ABS2, OMIM 207410) was suspected during the prenatal period based on the combination of radioulnar or radiohumeral synostosis and a peculiar shape of the skull suggestive of craniosynostosis, where no mutations in known genes involved in ABS were reported.

MATERIALS AND METHODS

Clinical data

We identified six patients and obtained written informed consent for genetic testing and DNA sampling for five of them and their relatives. Specific written informed consent for the publication of photos was obtained for three patients. All parents were healthy during pregnancy. They were all natives of Morocco and were first cousins. Follow-up of the six infants occurred at Montpellier and Nîmes hospitals. ABS was suspected from ultrasonography and CT-scan findings. Molecular studies (except for patient 4) of cytochrome P450 oxidoreductase gene (*POR,* OMIM 124015) and fibroblast growth factor receptor 2 gene (*FGFR2*, OMIM 176943) showed no mutations. Clinical data are summarized in Table 1 and full clinical descriptions are in **Supplementary Data S1** online.

Whole-exome sequencing

DNA from patient 1 from family 1 and her mother was analyzed by whole-exome sequencing at the Centre National de Génotypage (Institut de Génomique, CEA, Évry, France). After complete DNA quality control for each sample (duplicate quantification, evaluating DNA integrity/quality, checking for absence of PCR inhibitors), genomic DNA (3 µg) was captured using an in-solution enrichment method (Human All Exon v5—50 Mb, Agilent Technologies, Santa Clara, CA). Library preparation and exome enrichment (~20.000 targeted genes) were performed automatically using NGSx (PerkinElmer, Waltham, MA) and Bravo (Agilent Technologies), respectively, according to the manufacturer's instructions (SureSelect, Agilent Technologies). After normalization and quality control, exome-enriched libraries were sequenced using the Illumina HiSeq2000 system (Illumina, San Diego, CA) as paired-end 100-bp reads. Samples were sequenced as pools of four samples per lane, to obtain an average coverage of 70 to 80 X, with at least 80% of the target nucleotides covered at 30 X. Image analysis and base calling involved use of the Illumina Real Time Analysis Pipeline. Sequence-quality parameters were assessed daily during the 12 days of sequencing. The standard bioinformatics analysis of sequencing data was based on the Illumina pipeline (CASAVA1.8.2) to generate a FASTQ file for each sample.

Gene-panel sequencing

DNA samples from patients 2, 3, and 5 underwent gene-panel sequencing for a constitutional bone-disease gene panel of 49 genes, including linkeropathy genes (*B3GAT3, B3GALT6, B4GALT7, XYLT1, and XYLT2*) and ABS genes (*POR, FGFR2* and *CYP26B1*). Library enrichment was performed with SureSelect[QXT] Target Enrichment for Illumina Multiplexed Sequencing, Version C0, January 2015 (Agilent Technologies), according to the manufacturer's protocol.

Table 1 Clinical features in patients with c.667G > A homozygous B3GAT3 mutation

	Jones et al.[3] n = 1	Family 1 Patient 1	Family 2 Patient 2	Family 3 Patient 3	Family 3 Patient 4	Family 4 Patient 5	Family 4 Patient 6	All patients n = 7
Short stature	+	−	−	nr	nr	nr	−	1/4
Craniofacial features								
Craniosynostosis	nr	−	−	+	+	nr	+[a]	3/6
Midface hypoplasia	+	+	+	+	−	+	+	6/7
Skeletal features								
Kyphoscoliosis	−	−	−	−	−	nr	+	1/6
Radio ulnar synostosis	+	+	+	+	+	+	+	7/7
Dislocated joints	−	+	+	−	−	nr	+	3/6
Multiple fractures	+	+	+	+	+	−	−	5/7
Joints contractures	+	+	+	+	+	+	+	7/7
Femoral bowing	−	−	−	−	−	−	−	0/7
Extremities' features								
Long fingers	+	+	−	−	+	+	+	4/6
Foot deformity	+	+	+	+	+	+	+	7/7
Other features								
Cardiovascular abnormalities	+	+	+	−	−	−	+	4/7

nr, not reported.
[a]Antenatal ultrasonography suspicion.

Enriched library sample sequencing involved the Illumina MiSeq sequencer (Illumina, San Diego, CA) with the MS-103-1002 MiSeq Reagent Micro Kit, v2 (300 cycles). FASTQ files from patient samples were loaded into the MiSeq Reporter (v2.2.31), and reads were mapped against the genomic sequences (human genome version hg19) of the targeted genes. For secondary data analysis, BAM files of samples were loaded into the SeqPilot SeqNext module (v4.0, JSI Medical Systems, Kippenheim, Germany).

Sanger sequencing

Exon 3 of *B3GAT3* gene underwent direct Sanger sequencing to confirm next-generation sequencing results as described (BDT3.1 technology in a ABI3130XL sequencer; Barat-Houari et al., 2015[4]) on a DNA sample from probands and their relatives, except for patient 4, because neither DNA samples nor informed consent were available.

RESULTS

Clinical data

Ultrasonography at the second trimester of pregnancy revealed several malformations in our six patients, namely restricted hand movement ($n = 4$), equinovarus position of feet ($n = 4$), hypoplasia of nasal bone ($n = 3$), ventricular septal defect ($n = 2$), overlapping fingers ($n = 2$) and suspicion of bilateral radioulnar synostosis ($n = 2$) (**Figure 1**).

In the postnatal period, patients mainly presented bilateral radioulnar synostosis (6/6), joint contracture (6/6), foot deformity (6/6), midface hypoplasia (5/6), multiple neonatal fractures (4/6), abnormally long fingers (4/6), dislocated joints (3/5), craniosynostosis (3/5), and cardiovascular abnormalities (3/6) (**Figure 2**). All patients died before the age of one year. Clinical features are fully described in **Table 1** and **Supplementary data S1**.

Exome sequencing

Post-natal exome analysis of patient 1 revealed a potentially pathogenic homozygous variation, c.667G > A, p.Gly223Ser, in exon 4 of *B3GAT3* (NM_012200). The homozygous c.667G > A mutation was confirmed by Sanger sequencing in patient 1 and segregation analysis revealed that both parents were heterozygous for this variation. According to the ExAC database (http://exac.broadinstitute.org), this mutation was already reported in 1/119,290 alleles, with a frequency of 0.000008. This variant in ExAC was reported in a European (non-Finnish) population. Nucleotide and amino-acid multiple alignments among different animal species revealed that p.Gly223 is highly conserved. In silico variant prediction tools with wANNOVAR[5] included SIFT T (0.07), PolyPhen2 HDIV D (0.999), and Combined Annotation Dependent Depletion (phred score 33). No genetic variation was observed in known genes involved in ABS or an ABS-like phenotype, nor in the other linkeropathy genes. This variant was reported once in the literature for one patient with a linkeropathy diagnosis.[3]

Gene-panel sequencing

Analysis with a gene panel approach for three other probands, patients 2, 3, and 5, revealed the same homozygous mutation, c.667G > A, p.Gly223Ser, in *B3GAT3* (NM_012200) identified by whole-exome sequencing in patient 1. Sanger sequencing confirmed that affected patients were indeed homozygous for the mutation. Segregation analysis revealed that all parents were heterozygous for the same mutation.

DISCUSSION

Using exome and gene-panel sequencing, we characterized a unique homozygous mutation, c.667G > A, p.Gly223Ser, in *B3GAT3* in five of our six patients. Recently, a Mexican boy with this same mutation was described.[3] The association of a similar phenotype for this patient, namely, radioulnar or radiohumeral synostosis, midface hypoplasia, and joint
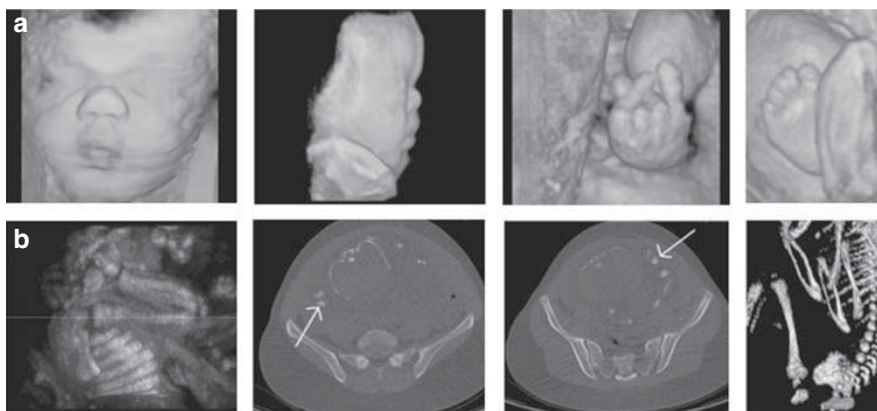
Figure 1 Prenatal investigation. (**a**) 3D ultrasonography reconstruction in patients 3 (front view) and 4 (lateral view, hand and feet). Note midface hypoplasia, clenched hand and varus feet. (**b**) Prenatal ultrasonography of patient 1, computed tomography scan, and 3D reconstruction showing bilateral proximal radioulnar synostosis (arrows).
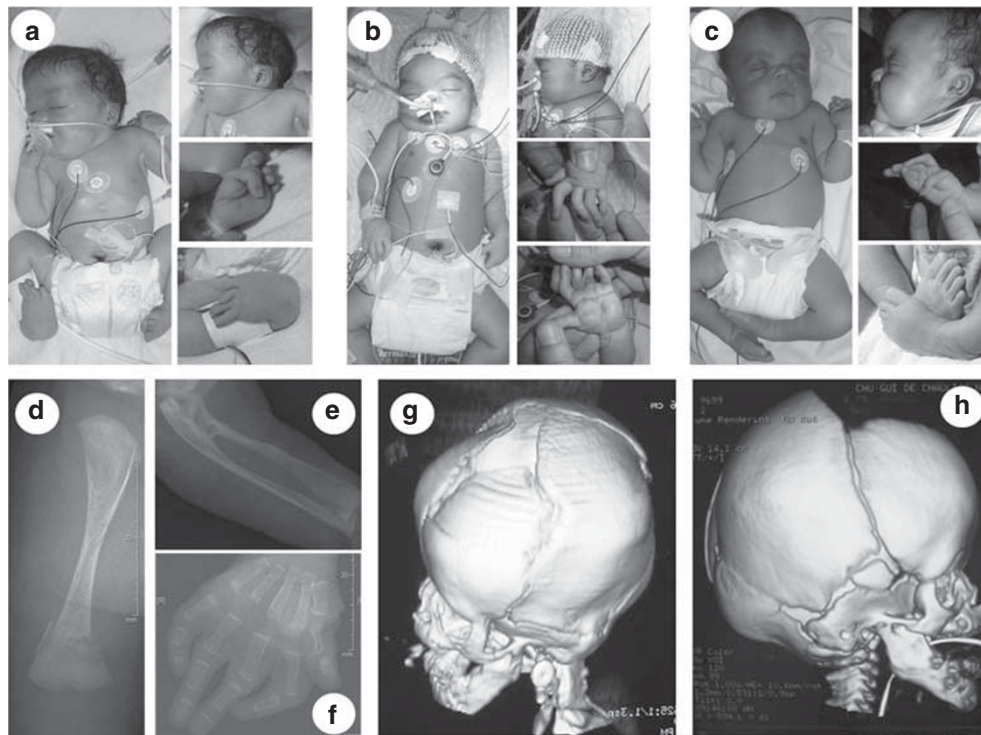
Figure 2 Clinical and radiological features in reported patients. Postnatal clinical data: front, lateral and extremities view in patients 1 (**a**), 5 (**b**), and 6 (**c**). Note clenched hands, varus feet, arthrogryposis, midface hypoplasia, and brachycephaly, as well as ocular proptosis in some patients. Radiological features: skeletal X-ray of patient 2 showing (**d**) fracture of right femur, (**e**) radioulnar synostosis, and (**f**) finger deformations. 3D reconstruction and computed tomography scan showing coronal and lambdoid craniosynostosis of patients 3 (**g**) and 4 (**h**).

contracture, led us to consider *B3GAT3* as the gene responsible for the phenotype in our patients. In contrast to other linkeropathy genes encoding for xylosyltransferase or galactosyltransferase, *B3GAT3* is the only one to encode for a glucuronosyltransferase, an enzyme involved in posttranslational modification and assembly of PG. Conversely, this enzyme links glycosaminoglycans to the core protein of PGs by the addition of glucuronic acid, the last component of the linker region between glycosaminoglycans and PGs. Gulberti et al.[6] demonstrated that substituting an alanine residue for Gly222 or Gly223 reduced the glucuronosyltransferase 1 activity of recombinant human glucuronosyltransferase 1 in yeast cells. The Gly223 mutation is localized in the catalytic acceptor substrate-binding subdomain (residues 198–308, Pedersen et al.[7]). Baasenjav et al.[8] described patients with a mutation in this subdomain and *B3GAT3* expression shown in mice hearts, bones, aortas, and osteoblasts. The clinical features they share with our patients suggest that p. Gly223Ser mutations are pathogenic. **Table 1** summarizes the clinical manifestations of patients with homozygous *B3GAT3* c.667G > A mutation from the literature and our patients. We contemplated a unique common ancestor for all patients, but the fact that the patients with the same rare homozygous mutation were from different countries and continents led us to consider a "hotspot" mutation in *B3GAT3*. We suggest that this *B3GAT3*-related disorder with craniosynostosis and bone fragility presentation should be considered as a differential

diagnosis in the prenatal period for ABS and in the postnatal period for Shprintzen-Goldberg syndrome (SGS, OMIM 182212). Indeed ABS is suspected during pregnancy, especially during the second and third trimester, if ultrasonography provokes a strong suspicion of craniofacial deformities, based on the suspicion of craniosynostosis (a hallmark of ABS), midface hypoplasia, bilateral radiohumeral or radioulnar synostosis, and femoral bowing.[9–11] We reviewed the literature for ABS patients and found a patient[12] without a molecular diagnosis but with a strikingly similar phenotype: craniosynostosis, midface hypoplasia with proptosis, long tapered fingers, elbow joint contracture due to radioulnar synostosis, and equinovarus position of feet. Without mutation of the *FGFR2* gene on molecular analysis, we suggest searching for the *B3GAT3* c.667G > A mutation in this patient. In the postnatal period, SGS could also be suspected, based on the association of craniosynostosis and arachnodactyly. Indeed, SGS is characterized by craniosynostosis and distinctive craniofacial features, skeletal anomalies (arachnodactyly, dolichostenomelia, camptodactyly, pes planus, pectus excavatum or carinatum, scoliosis, joint hypermobility or contractures, and C1/C2 spine malformation), cardiovascular anomalies, intellectual deficiency, neurologic and brain anomalies (hydrocephalus, dilatation of the lateral ventricles, and Chiari 1 malformation). The SKI proto-oncogene (*SKI*, OMIM 182212) is the only gene related to SGS known to date.[13]

Table 2 B3GAT3 genotype–phenotype association from literature and this study's patients

Phenotype	Alazami et al.;[16] *n* = 1	Budde et al.;[15] *n* = 8	Baasanjav et al.,[8] Von Oetttingen et al.;[14] *n* = 6	Job et al.;[17] *n* = 1	Jones et al.[3] and our patients; *n* = 7
	GO-like	Larsen-like		Intermediate	AB/SG-like
Short stature	+	8/8	6/6	−	1/4
Craniofacial features					
Craniosynostosis	nr	nr	nr	+	3/6
Midface hypoplasia	nr	8/8	6/6	+	7/7
Skeletal features					
Kyphoscoliosis	nr	4/8	0/6	+	1/6
Radio ulnar synostosis	nr	2/2	1/1	−	7/7
Dislocated joints	nr	8/8 (elbow)	6/6 (elbow)	+(hips)	3/6 (hips)
Multiple fractures	+	nr	0/6	+	5/7
Joints contractures	nr	4/8	5/5	−	7/7
Extremities' features					
Long fingers	nr	nr	nr	+	4/6
Foot deformity	nr	6/8	6/6	−	7/7
Other features					
Cardiovascular abnormalities	nr	0/3	6/6	+	4/7
B3GAT3 homozygous or compound heterozygous variant	Exon 1 c.245C > T p.Pro82Leu	Exon 3 c.419C > T p.Pro140Leu	Exon 4 c.830G > A p.Arg277Gln	Exon 4 c.671T > A p.Leu224Gln Exon 1 c.1A > G p.Met1?	Exon 4 c.667G > A p.Gly223Ser

AB, Antley-Bixler syndrome; GO, geroderma osteodysplatica; nr, not reported; SG, Shprintzen-Goldberg syndrome.
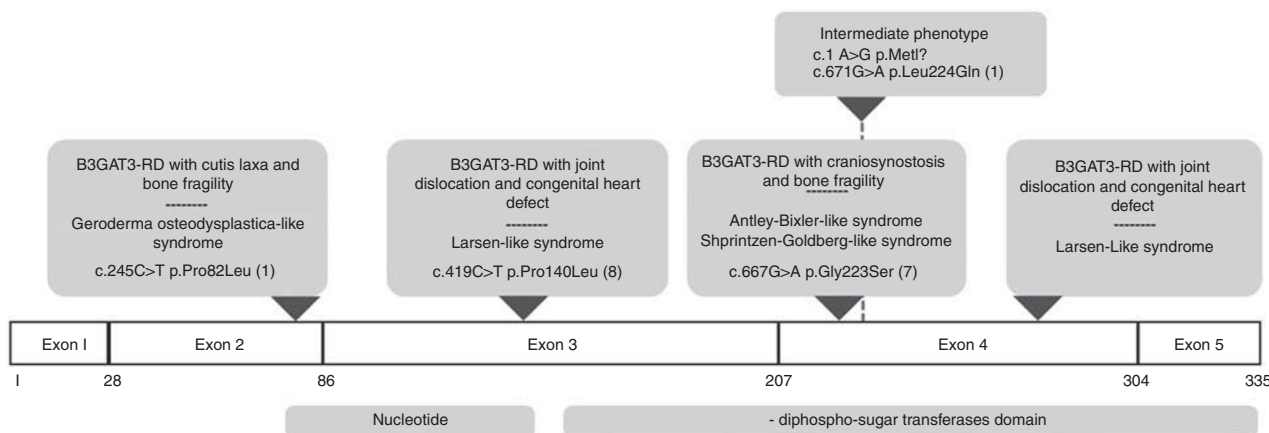
Figure 3 *B3GAT3* **linked phenotypes.** Mutation and protein domains were localized using the SCOP (structural classification of proteins) database[18] and the Protein Data Bank.[19]

B3GAT3 is involved in a spectrum of several connective tissue and skeletal disorders. **Table 2** summarizes the clinical features in all patients with the *B3GAT3* mutation. Among the different *B3GAT3*-related disorders, a Larsen-like phenotype characterized by joint dislocation and congenital heart defect was reported in three different studies. Von Oettingen et al.[14] reported an undetermined syndrome in a 5-year-old boy presenting skeletal dysplasia with bilateral radioulnar synostosis, brachycephalia, global developmental delay, and multiple congenital anomalies. Baasanjav et al.[8] described patients with short stature, radioulnar synostosis, exophthalmia, cardiac abnormalities, and brachycephaly. All patients had the same homozygous missense mutation in *B3GAT3* (c.830G > A, p.Arg277Gln). In a second large consanguineous

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Indonesian family, Budde et al.[15] described eight patients with bone dysplasia and short stature. The patients also had dislocated joints with short upper arms, midface hypoplasia, and broad fingers and toes. No cardiac involvement was described. All patients had the same homozygous missense mutation in *B3GAT3* (c.419C > T, p.Pro140Leu).

Alazami et al.[16] reported a gerodermia osteodysplatica–like syndrome in a patient with spondyloepimetaphyseal dysplasia, cutis laxa, osteoporosis, fracture, multiple bony chondromas, and short stature. The patient had a homozygous missense mutation in *B3GAT3* (c.245C > T, p.Pro82Leu). Phenotypes of the linkeropathy gene *B3GAT3* are heterogeneous but precise *B3GAT3*-related disorder (*B3GAT3*-RD) phenotype seems to be related to specific mutations (**Figure 3**).

Besides the observation of a presumed well-separated phenotype, a phenotypic continuum could be considered in light of the description of a 6-year-old boy[17] who presented hip-joint dislocation due to severe ligamentous laxity, osteoporosis, kyphoscoliosis, multiple fractures, and cardiac abnormalities (patent foramen ovale, bicuspid aortic valve, mild aortic root enlargement, and ascending aorta dilation). The patient had a compound mutation in *B3GAT3* (c.1A > G, p.Met1?; c.671T > A, p.Leu224Gln). Existence of an intermediate phenotype with compound mutation led us to discuss also the possibility of a continuum phenotype classification for *B3GAT3*-RD.

Here we report a novel *B3GAT3*-RD with craniosynostosis and bone fragility. We describe an expanded craniofacial phenotype of linkeropathy syndromes, especially craniosynostosis, and six patients with c.667G > A *B3GAT3* homozygous mutation. This gene should be screened, along with *POR*, *FGFR2*, and *CYP26B1* (OMIM 605207) in patients with suspected ABS during the prenatal period, because of the lethal severity of symptoms in the early months of life. This *B3GAT3*-RD should be suspected in the postnatal period as an alternative diagnosis to ABS or SGS.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

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