B4GALT1-Congenital Disorders of Glycosylation Presents as a Non-Neurologic Glycosylation Disorder with Hepatointestinal Involvement

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The clinical phenotype of congenital disorders of glycosylation is heterogeneous, mostly including a severe neurological involvement and multisystem disease. We identified a novel patient with a galactosyltransferase deficiency with mild hepatopathy and coagulation anomalies, but normal psychomotor development. The tissue-specific expression of the defective *B4GALT1* gene correlated with the clinical phenotype. *(J Pediatr 2011;159:1041-3)*

Congenital disorders of glycosylation (CDG) represent a large and rapidly growing family of genetic diseases with abnormal glycosylation of proteins, abnormal glycosylation of lipids, or both.[1] In general, CDG presents as multi-system disorder because of the ubiquitous expression of the glycosylation genes. Neurologic involvement is frequent in both CDG-I and CDG-II, and several CDG-II defects show distinct neurologic symptoms such as COG7-CDG[2] and ATP6V0A2-CDG.[3] Additional features in patients with CDG-II include hematologic and hepatic involvement, epilepsy, ataxia, coagulation problems, and dysmorphic features. A growing group of patients with CDG-II, with a wide range of clinical symptoms, have an unsolved genetic etiology.[4] Although certain defects occur relatively frequently (PMM2-CDG or ALG6-CDG), other CDG subtypes have been described in single cases (B4GALT1-CDG, DPM3-CDG), which hampers gene identification on basis of clinical symptoms.

In this paper, we present a tissue-restricted CDG type II defect with an exceptionally benign clinical presentation without neurological involvement.

Clinical Phenotype

Patient 1 was born at term, with age-appropriate growth measures. She was examined for recurrent episodes of diarrhea and mild hepatomegaly (**Table I**). A transient axial hypotonia improved within the first year of life. At age 7 years, she had normal growth and psychomotor development and attended regular school. She had dysmorphic facial features involving hypertelorism, broad nasal bridge, full supra-orbital region, a long philtrum, thin upper lip, low-set ears, and severe myopia (−5 D). With laboratory investigations, abnormal liver test results, abnormal coagulation, and borderline low platelets were shown (**Table I**). Galactosemia was ruled out. At the age of 9 years, she had normal intelligence and completely normal motor performance, including muscle tone and strength, coordination, and deep tendon reflexes.

Patient 2 was reported earlier as having B4GALT1-CDG.[5,6] Severe perinatal complications occurred because of bleeding diathesis. With laboratory investigation, abnormal liver test results, increased levels of creatine kinase, abnormal coagulation, and thrombocytopenia were shown. Hydrocephalus caused by a Dandy-Walker malformation developed, requiring shunt placement. Mild dysmorphic facial features including low-set ears and a broad nose were present. Since the original description of this patient in 2002, his growth curve and psychomotor development normalized. At the age of 11 years, he had no hepatomegaly, diarrhea, or significant neurological symptoms or mental retardation.

Biochemical and Genetic Investigations

Detailed information on biochemical and genetic methods is provided in the **Appendix** (available at www.jpeds.com). Screening for CDG with transferrin isofocusing consistently showed an abnormal type 2 pattern (**Figure**, A). Results of apolipoprotein C-III isofocusing for analysis of mucin O-glycosylation defects were normal (data not shown). Plasma N-glycan profiling with mass spectrometry (**Figure**, B) in control subjects[7] shows fully synthesized N-glycans (such as m/z 2794 and 3606) and lower amounts of the non-completed N-glycans (m/z 2433). In patient 1, the profile

CDG	Congenital disorders of glycosylation
SNP	Single nucleotide polymorphism

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Table I. Clinical features and laboratory findings

Clinical features	Patient 1*	Patient 2*	Remarks
Perinatal bleeding diathesis	–	+	Recurrent
Axial hypotonia	+	+	Transient
Dandy-Walker malformation	–	+	
Dysmorphic facial features	+	+	
Hepatomegaly	+	–	
Episodes of diarrhea	+	–	Recurrent
Growth retardation	–	–	
Myopia	+	+	

Laboratory findings†	Patient 1	Patient 2	Reference range
Activated partial thromboplastin time, seconds	64	74	30-43
Antithrombin III (%)	52	33	80-120
Protein C (%)	40	45	70-140
Protein S (%)	41	46	70-140
Platelets ($\times 10^9$/L)	120	139	120-350
Creatine kinase (IU/L)	320	573	<290
Aspartate aminotransferase (IU/L)	216	270	10-46

*Turkish ancestry.
†The results show the most abnormal values in the course of disease.

was dominated by glycans lacking galactose moieties (such as m/z 1663, 1838, 2083); >80% of the glycans terminate in *N*-acetylglucosamine as opposed to 15% in control subjects. Galactosyltransferase activity in patient fibroblasts was decreased to 5% (**Figure**, C) in control subjects, comparable with B4GALT1-deficient patient 2.

250K single nucleotide polymorphism (SNP) array showed a large homozygous stretch on chromosome 9, including the *B4GALT1* gene, which encodes UDP-Gal:*N*-acetylglucosamine β-1,4-galactosyltransferase I (B4GALT1; EC 2.4.1.22). A homozygous insertion in exon 5 was identified (c.1031-1032insC), heterozygous in both parents. The mutation is identical to the previously described mutation in patient 2,[5] leading to a premature stop codon with loss of the 50 C-terminal amino acids. To find an explanation for the tissue-restricted phenotype, expression analysis was performed of the *B4GALT1* and *B4GALT2* genes in human adult and fetal tissues (**Figure**, D). Additional information are presented in the **Appendix** and **Table II** (available at www.jpeds.com). *B4GALT1* was widely expressed in all tissues,

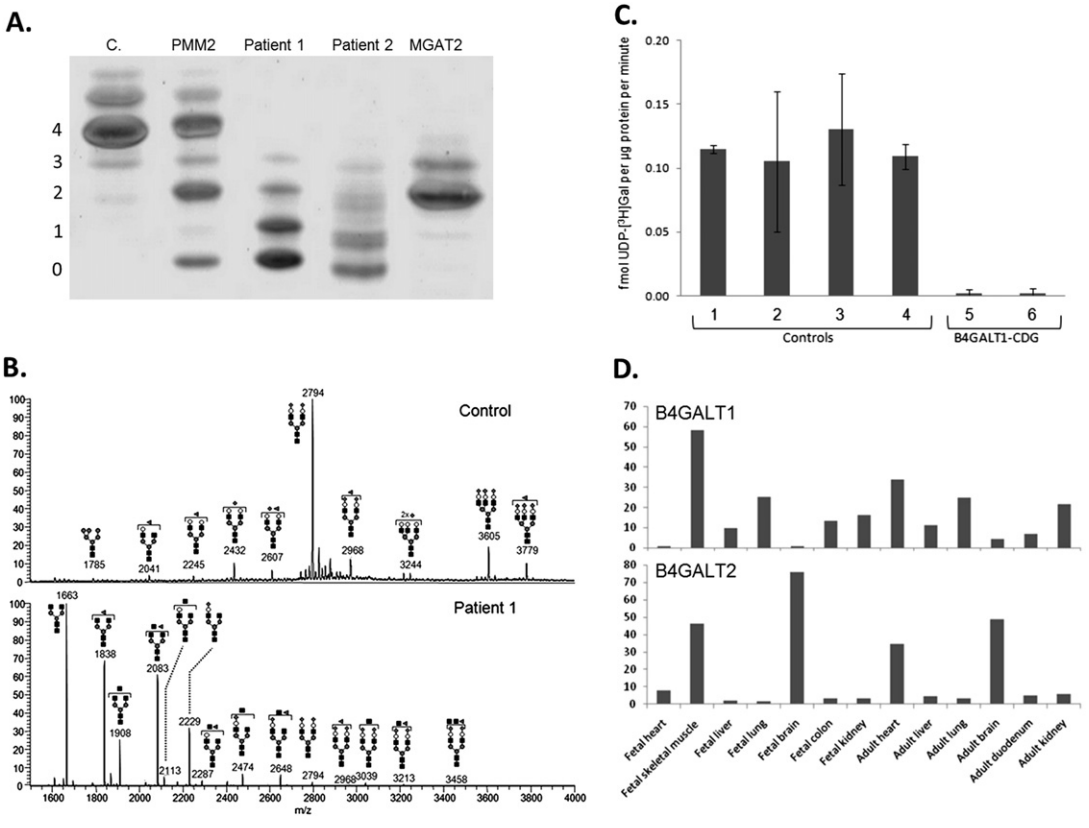

Figure. **A,** Serum transferrin isoelectric focusing profiles, digits on the left show the total number of sialic acids on transferrin glycans. *C,* control; *PMM2/MGAT2,* CDG-Ia and CDG-IIa defects; *patient 1 and 2*, CDG type 2 profiles of both patients. **B,** matrix-assisted laser desorption ionisation-MS profile of serum N-glycans of a control subjects and patient 1. ■ = N-acetyl glucosamine, ● = mannose, ○ = galactose, ◄ = fucose, ◆ = sialic acid. **C,** Transfer of UDP[^{3}H]-Gal to *Para*-nitrophenyl-*N*-acetyl-β-D-glucosamine in fibroblasts from control subjects (1-4), patient 1 (5), and patient 2 (6). **D,** Expression of *B4GALT1* and *B4GALT2* in different human fetal and adult tissues. Relative expression levels are given as the fold change in comparison with the tissue or area with the lowest expression level. All fetal tissues are from 20- or 21-week-old embryos after gestation.

with the exception of the fetal heart and brain and adult brain. In contrast, *B4GALT2* is very specifically expressed in fetal skeletal muscle, fetal brain, adult heart, and adult brain.

Discussion

We identified a novel patient with a CDG-II and c.1031-1032insC mutation in *B4GALT1*, with a mild hepatopathy, recurrent episodes of diarrhea, and coagulation abnormalities without neurological involvement. The previously reported patient (patient 2) with an identical mutation[5] showed progressive hydrocephalus because of a Dandy-Walker malformation, hypotonia, elevated creatine kinase levels, and a transient cholestatic syndrome. After shunt positioning and extensive rehabilitation, he had a benign neurological course. At 11 years, he had normal cognitive development, and his motor performance was only slightly abnormal. The common features of the children are dysmorphic features, hypotonia, and laboratory findings of disturbed coagulation factors, and abnormal liver enzymes.

Dandy-Walker malformation is a relatively common congenital anomaly that can be associated with numerous other malformations of the brain and extra-cranial abnormalities, whether or not in the context of specific syndromes. When not diagnosed with prenatal ultrasound scanning, the Dandy-Walker malformation generally presents with head enlargement in the first year of life. Its enormously heterogeneous origin might explain its equally variable prognosis: patients may sustain severe neurological deficits, despite early and correct neurosurgical management. However, as many as half the patients are reported to have normal cognitive development.

The 7 known β-1,4-galactosyltransferases differ in kinetics, tissue expression, and oligosaccharide acceptor specificity.[8] B4GALT1 and B4GALT2 are considered most important in galactosylation of N-glycans.[9] B4GALT1 mouse knock-out models[10] were mainly characterized by growth retardation and early death. There was no evidence for neurological deficits. Other symptoms, including transient skin lesions or puffy faces and pituitary insufficiency, were not observed in our patients. Abnormal villi and enhanced proliferation of epithelial cells were observed in the small intestine of these mice, which may correlate with the diarrhea in patient 1. In contrast, B4GALT2 knock-out mice showed motor-learning retardation and impaired motor coordination.[11] Our expression data indicate brain-specific expression of *B4GALT2*, in which the expression of *B4GALT1* is low. This is in line with the absence of neurological symptoms in our novel B4GALT1-CDG patient.

In this study, we describe the phenotype of B4GALT1-CDG including inherited coagulation disturbances with hepatopathy, mild hypotonia, and dysmorphic facial features and a variable presentation of diarrhea, hepatomegaly, and myopia. We propose to screen patients for CDG who have mild gastrointestinal symptoms, bleeding disorders, or both with hepatopathy, also in the absence of neurologic symptoms. ■

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Appendix

Plasma samples were collected from EDTA or heparin blood with centrifugation and stored at −20°C. Unless stated otherwise, chemical reagents were acquired from Sigma Aldrich (St Louis, Missouri). Isoelectric focusing of serum transferrin and apolipoprotein C-III was performed essentially as described.[1,2] Presence of sialidase in plasma as a secondary cause of undersialylation, as seen in patients with hemolytic uremic syndrome with a similar transferrin isoelectric focusing profile, was excluded in patient 1. Plasma N-glycan profiling of both patients was performed with matrix-assisted laser desorption ionisation-linear ion trap mass spectrometry as described,[1] with 10 μL of serum.

Galactosyltransferase activity was measured in fibroblast homogenates with UDP-[^{3}H]Gal as donor and *p*-nitrophenyl-*N*-acetyl-β-D-glucosamine as acceptor, by using a procedure modified from literature.[3] To a pellet of 10 million fibroblasts, 75 μL lysis buffer was added (0.05% v/v Triton X-100, 150 mM NaCl and protease inhibitor [Roche Diagnostics, Basel, Switzerland]) in 50 mM Tris-HCl pH 8.8) before 3 rounds of sonication (10 seconds, on ice). Protein concentration was determined with 2D Quant (GE Healthcare, Buckinghamshire, United Kingdom). Galactosyltransferase activity was measured by incubating 3 μg of proteins from the cell homogenate with 20 μL buffer A (10 mM Tris-HCl pH 7.7, 250 mM sucrose, 0.5% v/v Triton X-100, 1 mM EDTA) and 30 μL buffer B (5.2 μL 0.5 M Tris-HCl (pH 7.4), 1.5 μL Triton X-100, 4.4 μL 0.2 M $MnCl_2$, 15.4 μL H_2O, 2.5 μL 1 M *p*-nitrophenyl-*N*-acetyl-β-D-glucosamine in H_2O, 0.44 μL 0.2 M ATP, 0.25 μL 1 mM UDP-galactose, 0.22 μL 0.2 μCi/μL UDP-[^{3}H]Gal (American Radiolabeled Chemicals, St Louis, Missouri) for 2 hours at 37°C. After addition of 500 μL ultrapure water, the reaction volume was purified on Sep-Pak Vac C18 cartridges (Waters, Milford, Massachusetts). The cartridge was washed with 5 mL ultrapure water before elution with 2 mL 10% aqueous methanol. 900 μL of eluent was dissolved in 10 mL Ultima Gold XR counting fluid (Perkin Elmer, Waltham, Massachusetts), and the incorporated radioactive galactose was counted in the Liquid Scintillation Analyzer Tricarb 2810TR (Perkin Elmer). After substraction of blancs (identical procedure without protein from cell homogenate), the data were expressed as fmol UDP-[^{3}H]Gal incorporated per μg protein per minute.

Genomic DNA was extracted and genotyped with Affymetrix Genome-Wide Human SNP 250K Nsp1 arrays. Copy number analysis and exporting of SNPs were performed on the raw data using the Affymetrix Genotyping Console 3.0. Homozygosity mapping on a 250K SNP array was performed on the genomes of patient 1, her parents, and a healthy sibling to restrict the number of candidate genes for mutation analysis. The genotyped SNPs were analyzed for homozygous stretches with in house algorithms. The longest homozygous stretch, not homozygous in parents or unaffected sibling, was detected on chromosome 9, spanning 63 Mb from 9p21.1 to 9q22.32 and included the *B4GALT1* gene, which encodes UDP-Gal:*N*-acetylglucosamine β-1,4-galactosyltransferase I (B4GALT1; EC 2.4.1.22). *B4GALT1* is a 6-exon gene located on chromosome 9p13, encoding a 398 amino acid protein. DNA analysis was performed on genomic DNA. The *B4GALT1* gene (ENST00000379731) was amplified in 6 fragments (exon 1-6). Fragments included both DNA sequences of the individual exons and splice donor and splice acceptor sites. Oligonucleotide primers were designed from the human *B4GALT1* gene sequence (primer sequences are available on request). The nucleotide numbering follows complementary DNA numbering with +1 corresponding to the A of the ATG translation codon in the reference sequence. The initiation codon is codon 1. In DNA from 100 healthy control individuals with similar ethnicity and in the patient's healthy sibling, no mutation was identified.

SYBR Green-based real-time quantitative polymerase chain reaction expression analysis was performed on a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, California) by using Power SYBR Green PCR Master Mix (Applied Biosystems) according to the manufacturer's instructions. Primers were developed with the primer3 program (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and validated as described before.[4] Primer sequences are given in **Table II**. *GUSB* and *PPIB* were used as reference genes. Total RNA from different human adult and fetal tissues was ordered from Stratagene Europe (Amsterdam, The Netherlands). Total RNA was transcribed into complementary DNA by using the iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, California) according to the manufacturer's protocol. Complementary DNA was purified by using the NucleoSpin extract II kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol. Quantitative polymerase chain reaction quantifications were performed in duplicate on the equivalent of 12.5 ng total RNA input. Experimental threshold cycles (Ct) values were within the range of complementary DNA dilutions used to validate the primers. The melt curves of all polymerase chain reaction products showed a single polymerase chain reaction product. Results of all water controls were negative. Differences in expression of a gene of interest in two samples were calculated by the comparative Ct or $2^{\Delta\Delta Ct}$ method.[5,6] Data are expressed relative to the tissue with lowest expression and a Ct value below 32, set at 1.

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Table II. Primer sequences for the quantitative polymerase chain reaction analysis of *B4GALT1-2*

Gene	Gene ID	Forward 5′->3′	Reverse 5′->3′
GUSB	NM_000181.1	agagtggtgctgaggattgg	ccctcatgctctagcgtgtc
PPIB	NM_000942.4	cggaaagactgttccaaaaac	gattacacgatggaatttgctg
B4GALT1	NM_001497.3	ctatatctcgcccaaatgctg	gtgcaattcggtcaaacctc
B4GALT2	NM_001005417.1	cgcgacaagcataacgaac	agacctggtaccgcactgac