J Clin Immunol (2014) 34:1009–1014
DOI 10.1007/s10875-014-0091-7

BRIEF COMMUNICATION

A Novel Mutation in Leukocyte Adhesion Deficiency Type II/CDGIIc

Deniz Cagdas · Mustafa Yılmaz · Nurgün Kandemir · İlhan Tezcan · Amos Etzioni · Özden Sanal

Received: 16 November 2013 / Accepted: 26 August 2014 / Published online: 21 September 2014

Abstract Leukocyte adhesion deficiencies (LAD) are autosomal recessive immunodeficiency syndromes characterized by severe and recurrent bacterial infections, impaired wound healing and leukocytosis. Block in different steps in the leukocyte adhesion cascade causes different types of leukocyte adhesion deficiencies, LAD type I, II and III. In LAD type II, the rolling phase of the leukocyte adhesion cascade is affected due to mutations in the specific fucose transporter GFTP (GDP fucose transporter), causing defect in the biosynthesis of selectin ligands on leukocytes. Thus this syndrome is also called congenital disorder of glycosylation IIc (CGDIIc). LAD II/CGDIIc is very rare and has been diagnosed in nine children to date. Fever, leukocytosis, typical dysmorphic features, growth, psychomotor retardation and the Bombay blood group, are characteristic findings in patients. Here, we describe two Turkish siblings with a novel mutation in GFTP. They both have the characteristic features of the syndrome. The older sibling died of severe bacterial pneumonia at the age of 3 years. The younger sibling, diagnosed at the age of 3 months, responded to high dose oral fucose supplementation. Secundum atrial septal defect which was not described in previously reported patients, but present in both of our patients, may primarily related to the defect in fucosylation.

Keywords Leukocyte adhesion deficiency type II · congenital disorder of glycosylation · psychomotor retardation · atrial septal defect · fucose therapy

Introduction

Leukocyte adhesion deficiencies (LAD) are autosomal recessive immunodeficiency diseases characterized by severe and recurrent bacterial infections, impaired wound healing and neutrophilia [1]. Leukocyte adhesion and movement of leukocytes from blood stream to tissues are crucial for rapid leukocyte accumulation at sites of inflammatory response and tissue injury [2]. Leukocyte adhesion cascade involves several phases. First phase, leukocyte rolling, initiates the loose adhesion to vessel walls and is mediated by members of the selectin family. These include L-selectin (CD62-L) which is expressed on most leukocytes, and E- and P-selectin (CD62E and CD62P), which are expressed on the surface of activated endothelial cells. Second phase is the activation of integrins on the leukocytes by the chemoattractants; chemokines and cytokines. Firm adhesion occurs when the integrins bind to intracellular adhesion molecules (ICAMs) expressed on endothelial vessel. Only then leukocytes can start to transmigrate through the endothelium [3, 4].

Block in different steps in the leukocyte adhesion cascade causes leukocyte adhesion deficiencies. In LADI, the integrin structure and the adhesion of leukocyte to the endothelium is defective due to the mutations in the gene encoding the beta

D. Cagdas · İ. Tezcan · Ö. Sanal (✉)
Section of Pediatric Immunology, Hacettepe University, İhsan Doğramacı Children's Hospital , Ankara, Turkey
e-mail: sanaloz@gmail.com

D. Cagdas
e-mail: deniz.ayvaz@hacettepe.edu.tr

M. Yılmaz
Section of Pediatric Allergy and Immunology, Cukurova University, Adana, Turkey

N. Kandemir
Section of Pediatric Endocrinology, Hacettepe University, İhsan Doğramacı Children's Hospital, Ankara, Turkey

A. Etzioni
B. Rappaport Faculty of Medicine, Rambam Hospital, Technion, Haifa 31096, Israel

(2)-integrin. In LADII/CDGIIc (congenital disorder of glycosylation IIc), the rolling phase of the cascade is affected due to the mutations in the specific fucose transporter causing defect in the biosynthesis of the glycoprotein ligand of selectin. In LADIII, there is defect in activation of all integrins, leading to the adhesion defect and increased bleeding tendency.

Fever, leukocytosis, typical dysmorphic features, growth and psychomotor retardation are characteristic findings in patients with LADII/CDGIIc. As far as we know there are only nine patients diagnosed as LADII/CDGIIc to date including a recently reported siblings with short stature and developmental delay, but with minimal adhesion defect [5, 6]. Here, we describe two siblings with a novel mutation in GFTP. Secundum atrial septal defect was present in both of the patients in addition to the characteristic features.

Patient I

A 3 year-old male child was admitted with the complaints of recurrent pulmonary infections. He was an offspring of the first degree cousins (pedigree is given in Fig. 1). His paternal twin uncles died in the infantile period. His birth weight was 3,000 g. Past medical history revealed that he had conjunctivitis and pneumonia at the age of 1 month. He had a draining sacral fistule for which he was operated at the age of 1 month. From the age of 7 months persistent leukocytosis (leukocyte count ranged between 46,000 and 89,000/mm^2 with 75–95 % of neutrophils) was noted (Fig. 2). Attempts to transfuse blood for severe anemia were unsuccessful due to severe acute reactions.

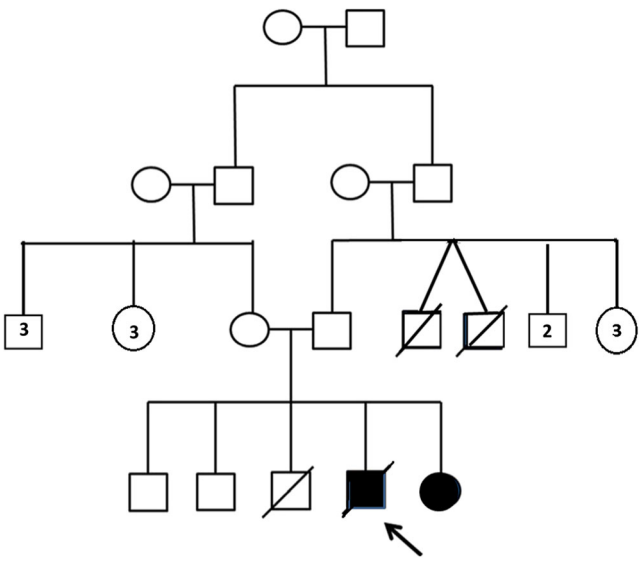

Fig. 1 Pedigree of the family (*numbers* indicate the number of siblings with the same gender)

When examined at the age of 3 years, he was found to suffer from severe growth retardation: weight 5,900 g, length 75 cm, head circumference 43 cm (all below the 3rd percentiles, compatible with 6 months of age). He was asthenic, pale, and tachypneic, had microcephaly, course facial appearence, edematous eyelids, long eyelashes, depressed nasal bridge, big tongue, low-set cup-shaped ears, antihelix anomaly, periodontitis, dry skin, diminished subcutaneous fat tissue, bilateral retro-sourced and short third toes, clubfoot, severe neurodevelopmental delay (Fig. 3). He could stand with support and could not speak at all.

His hemoglobin level was 4.7 g/dl, WBC, 77,200/mm^3; platelet, 931,000/mm^3; peripheral blood smear revealed 92 % PNL, 4 % lymphocytes, 4 % monocytes. Direct Coombs test was positive. He was hospitalized for severe anemia, pneumonia and heart failure, and given intravenous immunoglobulin therapy. The blood group typing showed the absence of H antigen at the surface of leukocytes (Bombay phenotype). Due to the severe anemia leading to heart failure, and since the Bombay blood group is very rare, and compatible donor could not be found; after consent form was signed by the family, 20 ml of blood from his sister was given without any reaction.

Echocardiographic evaluation revealed small secundum ASD and normal systolic functions. He was given antibacterial therapy, fucose treatment was planned, but could not be provided immediately. He died of severe pulmonary infection, respiratory insufficiency and cardiopulmonary arrest. Molecular analysis showed a new homozygous missense mutation (W49R) in the first exon of the SLC35C1 gene, (p. Trp49Arg) and both parents were heterozygous for this mutation.

Patient II

A 3-month-old female infant, sister of patient 1 was diagnosed as leukocyte adhesion deficiency type II. Genetic testing revealed the same mutation as her brother. Her birth weight was 3,000 g. She suffered from a pulmonary infection at the age of 1 month. The laboratory work-up revealed leukocytosis (WBC, 35,700/mm^3; absolute neutrophil count (ANC): 19,400/mm^3) and Bombay blood group. After obtaining a consent form from the parents, L-fucose therapy (L-fucose 125 mg/kg in five doses per day) was started at the age of 5 months as described by Marquardt et al. [7]. After 2 weeks, the leukocyte count and the ANC decreased to 21,700 and 6,400/mm^3 respectively. During the following 36 months, ANCs ranged between 2,900 to 6,400/mm^3, and WBCs ranged between 12,100 to 22,300/mm^3. The anti-H antibody was found to be negative during the follow-up and a hemolytic reaction was not observed. SGOT and SGPT values were normal during the therapy period. On the last follow-up at age of 40 months, she was still taking L-fucose therapy.

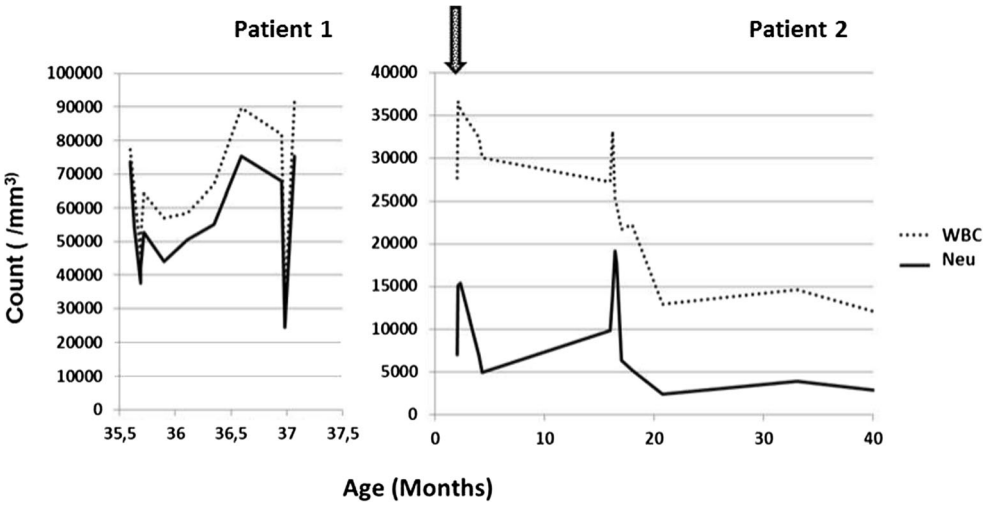

Fig. 2 The white blood cell (*WBC*), and neutrophil (*Neu*) counts of the patients. The *arrow* indicates the beginning of the fucose therapy in patient 2

After fucose therapy, no change was observed in the classical features of LADII she had, similar to his brother. But, the infection susceptibility decreased by the time. She was able to sit without support and walk a few steps, but could not speak at all. Aside from several episodes of tonsillitis, which responded to antibacterial therapy, no infections were recorded. Her weight, length and head circumference continue to be below the 3rd percentile (compatible with 1 year of age). She had stereotypical movements which are considered as autistic signs due to mental retardation. At 30 months of age, she had an episode of atonic convulsion. Echocardiographic evaluation revealed small secundum ASD and normal systolic functions. No abnormalities could be found in echocardiographic evaluation of unaffected siblings, mother and father.

The clinical findings of the patients are given in Table 1.

Discussion

Leukocyte adhesion deficiency type II (LADII)/ congenital disorder of glycosylation IIc (CGDIIc) belongs to a group of CDG [8]. In LADII/CGDIIc, the fucosylation of glycoconjugates is affected, and the defect is in the specific GDP-fucose transporter (GFTP) gene [9, 10]. Process of fucosylation by GDP-fucose transporter in the Golgi apparatus has different steps. GFTP translocates GDP-fucose from the cytosol through the Golgi membrane into the Golgi lumen. Fucose is provided via de novo pathway (90 %), synthesis from mannose; or by alternate salvage pathway, (10 %) from exogeneous/dietary fucose [11]. Then, fucosyl transferase transfers fucose to form glycolipids and glycoproteins [12], which are transported to the cell membrane.

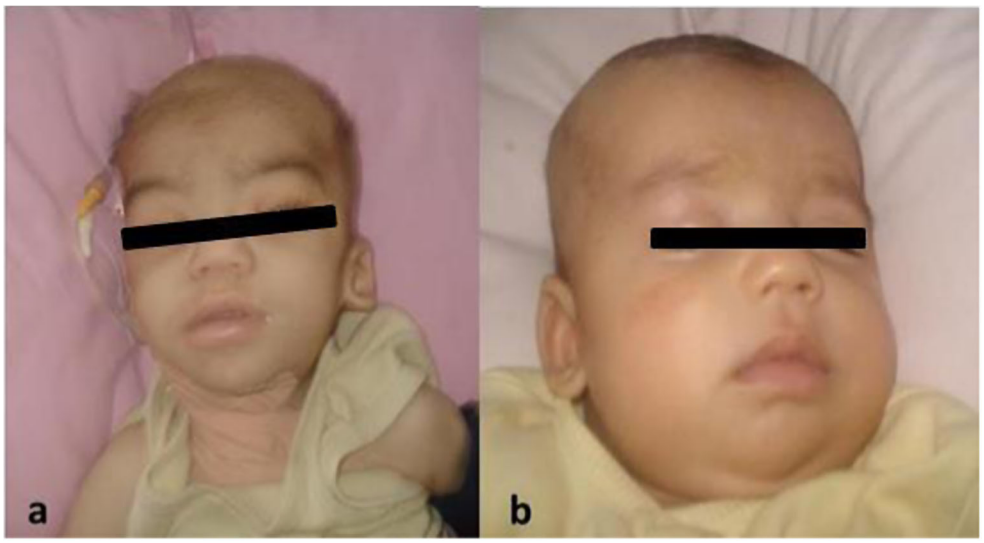

Fig. 3 Patient I (**a**) and patient II (**b**)

Table 1 Clinical findings of the patients

	Patient 1	Patient 2
IUGR	−	−
Growth retardation	+	+
Delayed separation of the umbilical cord	−	−
Omphalitis	−	−
Microcephaly	+	+
Coarse facial appearance	+	Mild
Recurrent infections	+	+
Psychomotor retardation	+	+
Convulsion	−	+
Autistic features	+	+
Atrial septal defect	+	+
Bombay blood group	+	+

Nine different mutations are described in patients with LADII/CGDIIc [5, 6]. Functional studies revealed two types of mutations. In the more common type, the GFTP protein is normally localized in the Golgi apparatus, but its function is impaired due to point mutations. In the second type, in addition to the impaired function, the transporter is improperly located in the endoplasmic reticulum [12]. As our patients' novel missense mutation is in the first exon, it will most probably allow the transporter to be located in the Golgi apparatus, as was described in other cases of LAD II/CDGIIc with point mutations in other exons [13]. The region in the gene where this new mutation W49R exist is highly conserved through evolution, from zebra fish up to humans (UCSC Genome Bioinformatics Site). Using several predictor systems to evaluate the effect of this mutation, we found it to be very deleterious (Mutation Taster = disease causing defect, SIFT/Provean = predicts the mutation to be damaging, PolyPhen2 = Probably damaging with the highest score of 1). Thus, it can be concluded that this mutation caused the clinical symptoms of LAD II observed in our patient.

Hypofucosylated structures in LADII/CGDIIc include Sialyl Lewis X (SLeX) (CD15a), which is an important ligand for the selectin family and H-antigen at the surface of erythrocytes. Patients have no detectable ABO blood group on their erythrocytes (Bombay blood group) [14].

Owing to the absence of functional fucosylated selectin ligands, such as α-1,3 fucose in sialyl-Lewis X, the interactions of leukocytes with the vascular endothelium are strongly impaired [7, 14]. This leads to an immunodeficiency beginning in early infantile period with persistant leukocyte elevation, which is the dominant feature. The infectious episodes diminish later in life most probably due to the adequate adaptive immune response [15].

Even if a patient with LADII/CGDIIc survives from recurrent infections, he/she suffers from metabolic consequences. Mental and motor retardation in the patients indicates the role of fucose in brain development or synaptogenesis. In fact fucosylated glycans are required in developmental process to guide migration of different group of neuron progenitors in simple organisms [16]. In the study of Fukuda T et al., α-1,6-fucosyltransferase-deficient, Fut (−/−), mice were found to exhibit multiple behavioral abnormalities and have low serotonin levels in both striatum and nucleus accumbens [17]. Thus, the mental and motor retardation in LADII/CGDIIc may be due to the defect in synapse formation, rather than due to the defect in brain development.

Psychomotor retardation with epilepsy are dominant neurological features in patients with LADII/CGDIIc. Other neurological features shared in the reported cases are microcephaly, craniosynostosis, cerebral atrophy, and autistic features [5]. Our patients had microcephaly and psychomotor retardation. Autistic features observed during the follow-up was attributed to mental retardation.

Marquardt et al. showed that oral fucose supplementation has beneficial effect in patients with LADII/CGDIIc [7]. The treatment led to the reappearence of functional selectin ligands concomitently with normalization of neutrophil counts [12] (Table 2). The child treated with L-fucose did not suffer from further infectious episodes and psychomotor condition improved somewhat [7, 18]. Similar laboratory results were seen in a Brazilian patient with LADII [19]. The production of autoantibodies (e.g. anti H antigens) which may lead to autoimmunity (e.g. severe hemolysis due to autoimmune

Table 2 Fucose supplementation in LAD II

Reference	Fucose dose	Normalization of neutrophil counts	Psychomotor improvement	Complications
Marquardt et al. [7]	Starting fucose dose 25 mg/kg (4 divided dose), single doses slowly increased to 492 mg/kg within 9 months.	+	+	−
Etzioni et al. [20]	Loading fucose dose of 5 g (4 divided doses) than 2.5 g—between days 35–70, cessation of fucose—between days 70–105, 1 g/day—between days 105–180.	+	−	Hypoglycemia
Hidalgo et al. [19]	975 mg/kg (4–5 divided doses) between days 0–35, 570 mg/kg—between days 36–260.	+	−	Neutropenia (autoimmune)

hemolytic anemia) was thought as a theoretical adverse effect of fucose supplementation. As fucose therapy may induce the production of autoantibodies due to the appearence of fucosylated neoantigens expressed on cells, it represents a risk for autoimmune phenomena. Autoimmune phenomena related to fucose supplementation was observed in one out of reported four patients treated with fucose. In that patient, autoantibodies against neutrophils are shown to be induced by fucose therapy. On the 36th day of fucose therapy, therapy induced neutropenia was recorded, but the patient never showed clinical or laboratory evidence of autohemolysis. No psychomotor improvement was recorded after therapy, and this was attributed to the limited doses of fucose administration (dose decreased from 975 to 570 mg/kg per day) and limited cerebrospinal fluid fucose concentration. It should be noted that in another patient with LADII/CGDIIc, no improvement was seen after fucose therapy [20]. To explain the differing response in different patients, Luebke et al. suggested that the transporter defect is overcome by the increase in the cytosolic fucose concentrations, and Berninsone et al. suggested that GDP-L-fucose may be imported by means of other carriers in the fucose responsive patient of Marquardt et al. [21, 22].

In our patient, the infection susceptibility decreased by the time. However fucose supplement may have reduced the infection susceptibility, this may well be due to classical course of the disease [5]. The number of WBC and neutrophil counts decreased with therapy and her appetite increased. Although weight, height and head circumference were still lower than 3rd percentiles, they seemed better than her brother's at the same age. This difference in growth may be at least partly due to the decrease in infectious episodes.

Both of our patients had secundum atrial septal defect (ASD) as a concomitant feature. The secundum atrial septal defects account for about 10 % of congenital heart diseases, and there is a relatively high prevalence of familial occurrence of secundum ASD (10 % of all ASD patients) [23]. An autosomal dominant mode of inheritance for familial ASD has been described with incomplete penetrance and variable expressivity. No abnormality could be detected in our patients' unaffected siblings and parents by echocardiographic assessment, however, familial ASD could not be excluded in this family. Conotruncal heart defects and cardiomyopathy have been reported in patients with CDGIa [24]. Fucose and fucosylation were shown to have effects on cardiac development. In the study with farm animals, fucosyl transferase activity is shown in heart in addition to liver, lung and brain [25]. Additionally Shier et al. showed that for nodal (a member of the TGF-β superfamily) signalling, which plays essential roles in the embryonic development of vertebrates, including mesoderm formation and the generation of left-right asymmetry, threonine that carries fucose is required [26]. These findings may indicate that ASD in our patients may primarily be related to the defect in fucosylation.

We suggest echocardiographic assessment of patients with LADII/CGDIIc and to start fucose supplementation once the diagnosis is made, as it may affect the prognosis at least in some of the patients.

References

1. Etzioni A, Sturla L, Antonellis A, Green ED, Gershoni-Baruch R, Berninsone PM, et al. Leukocyte adhesion deficiency (LAD) type II/carbohydrate deficient glycoprotein (CDG) IIc founder effect and genotype/phenotype correlation. Am J Med Genet. 2002;110:131–5.
2. Hanna S, Etzioni A. Leukocyte adhesion deficiencies. Ann N Y Acad Sci. 2012;1250:50–5.
3. van de Vijver E, van den Berg TK, Kuijpers TW. Leukocyte adhesion deficiencies. Hematol Oncol Clin N Am. 2013;27:101–16.
4. van den Berg JM, Kuijpers TW. Educational paper: defects in number and function of neutrophilic granulocytes causing primary immunodeficiency. Eur J Pediatr. 2011;170:1369–76.
5. Gazit Y, Mory A, Etzioni A, Frydman M, Scheuerman O, Gershoni-Baruch R, et al. Leukocyte adhesion deficiency type II: long-term follow-up and review of the literature. J Clin Immunol. 2010;30:308–13.
6. Dauber A, Ercan A, Lee J, James P, Jacobs PP, Ashline DJ, et al. Congenital disorder of fucosylation type 2c (LADII) presenting with short stature and developmental delay with minimal adhesion defect. Hum Mol Genet. 2014;23:2880–7.
7. Marquardt T, Lühn K, Srikrishna G, Freeze HH, Harms E, Vestweber D. Correction of leukocyte adhesion deficiency type II with oral fucose. Blood. 1999;94:3976–85.
8. Moloney DJ, Panin VM, Johnston SH, Chen J, Shao L, Wilson R, et al. Fringe is a glycosyltransferase that modifies Notch. Nature. 2000;406:369–75.
9. Lühn K, Wild MK, Eckhardt M, Gerardy-Schahn R, Vestweber D. The gene defective in leukocyte adhesion deficiency II encodes a putative GDP-fucose transporter. Nat Genet. 2001;28:69–72.
10. Puglielli L, Hirschberg CB. Reconstitution, identification, and purification of the rat liver golgi membrane GDP-fucose transporter. J Biol Chem. 1999;274:35596–600.
11. Schachter H. The search for glycan function: fucosylation of the TGF-beta1 receptor is required for receptor activation. Proc Natl Acad Sci U S A. 2005;102:15721–2.
12. Helmus Y, Denecke J, Yakubenia S, Robinson P, Lühn K, Watson DL, et al. Leukocyte adhesion deficiency II patients with a dual defect of the GDP-fucose transporter. Blood. 2006;107:3959–66.
13. Yakubenia S, Wild MK. Leukocyte adhesion deficiency II. Advances and open questions. FEBS J. 2006;273:4390–8.
14. Etzioni A, Frydman M, Pollack S, Avidor I, Phillips ML, Paulson JC, et al. Brief report: recurrent severe infections caused by a novel leukocyte adhesion deficiency. N Engl J Med. 1992;327:1789–92.
15. Etzioni A, Gershoni-Baruch R, Pollack S, Shehadeh N. Leukocyte adhesion deficiency type II: long term follow-up. J Allergy Clin Immunol. 1998;102:323–4.
16. Ohata S, Kinoshita S, Aoki R, Tanaka H, Wada H, Tsuruoka-Kinoshita S, et al. Neuroepithelial cells require fucosylated glycans to guide the migration of vagus motor neuron progenitors in the developing zebrafish hindbrain. Development. 2009;136:1653–63.
17. Fukuda T, Hashimoto H, Okayasu N, Kameyama A, Onogi H, Nakagawasai O, et al. Alpha1,6-fucosyltransferase-deficient mice

exhibit multiple behavioral abnormalities associated with a schizophrenia-like phenotype: importance of the balance between the dopamine and serotonin systems. J Biol Chem. 2011;286: 18434–43.
18. Wild MK, Lühn K, Marquardt T, Vestweber D. Leukocyte adhesion deficiency II: therapy and genetic defect. Cells Tissues Organs. 2002;172:161–73.
19. Hidalgo A, Ma S, Peired AJ, Weiss LA, Cunningham-Rundles C, Frenette PS. Insights into leukocyte adhesion deficiency type 2 from a novel mutation in the GDP-fucose transporter gene. Blood. 2003;101:1705–12.
20. Etzioni A, Tonetti M. Fucose supplementation in leukocyte adhesion deficiency type II. Blood. 2000;95:3641–3.
21. Lübke T, Marquardt T, von Figura K, Körner C. A new type of carbohydrate-deficient glycoprotein syndrome due to a decreased import of GDP-fucose into the golgi. J Biol Chem. 1999;274: 25986–9.
22. Berninsone P, Hwang HY, Zemtseva I, Horvitz HR, Hirschberg CB. SQV-7, a protein involved in Caenorhabditis elegans epithelial invagination and early embryogenesis, transports UDP-glucuronic acid, UDP-N-acetylgalactosamine, and UDP-galactose. Proc Natl Acad Sci U S A. 2001;98:3738–43.
23. Gelernter-Yaniv L, Lorber A. The familial form of atrial septal defect. Acta Paediatr. 2007;96:726–30.
24. Romano S, Bajolle F, Valayannopoulos V, Lyonnet S, Colomb V, de Baracé C, et al. Conotruncal heart defects in three patients with congenital disorder of glycosylation type Ia (CDG Ia). J Med Genet. 2009;46:287–8.
25. Struppe E, Staudacher E. Occurence of GDP-L-fucose: beta-N-acetylglucosamine (Fuc to asn-linked GlcNAc) alpha 1,6-fucosyltransferases in porcine, sheep, bovine, rabbit and chicken tissues. Biochim Biophys Acta. 2000;1475:360–8.
26. Schier AF. Nodal signaling in vertebrate development. Annu Rev Cell Dev Biol. 2003;19:589–621.