Letters to the Editor

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Congenital disorders of glycosylation IIa cause growth retardation, mental retardation, and facial dysmorphism

EDITOR—Congenital disorders of glycosylation (CDG) are a heterogeneous group of autosomal recessive multisystemic conditions causing severe central nervous system and multivisceral disorders resulting from impairment of the glycosylation pathway.[1–3] Two disease causing mechanisms have been identified so far. CDG I is caused by a defect in the assembly of the dolicholpyrophosphate oligosaccharide precursor of N-glycans and its transfer to the peptide chain, while CDG II results from a defect in the processing of N-glycans.[3] CDG I and II have distinct patterns of abnormal glycosylation depending on the reduction of the glycan chain number or its structure. CDG I, the most frequent form, is the result of different enzyme deficiencies: phosphomannomutase (CDG Ia), phosphomannose isomerase (CDG Ib), and glucosyltransferase (CDG Ic).[3–6] CDG IIa is characterised by a defect in N-acetylglucosaminyltransferase II and only two cases have been reported previously.[7–11] Here, we report a new case of CDG IIa sharing a number of clinical features with the two previously reported cases and emphasising the clinical differences from CDG I.

A boy was born at term to unrelated, healthy parents after a normal pregnancy and delivery, birth weight 3050 g, length 48 cm, and OFC 35 cm. At 3 months of age, hypotonia, feeding difficulties, and diarrhoea were noted. A milk protein intolerance was suspected and he was put on a milk free formula until the age of 4 years. He was first referred to our genetic unit at 8 years of age because of mental retardation and facial dysmorphism. On examination, he had severe mental retardation with no speech and an unstable gait. Dysmorphic features included fine hair, large ears, a beaked nose with hypoplastic nasal alae, a long philtrum, thin vermilion border of the upper lip, everted lower lip, large teeth, and gum hypertrophy (fig 1). Long standing feeding difficulties and diarrhoea had resulted in severe growth retardation (height 109.9 cm (−3 SD), weight 20 kg (−2.5 SD), OFC 50.5 cm (−2 SD)). Chromosome analysis was normal and no diagnosis was made at that time. At 11 years of age, dysmorphic features, severe mental retardation, diarrhoea, and growth retardation were still present (height 120.8 cm (−4 SD), weight 23 kg (−2.5 SD), OFC 50.5 cm (−2 SD)). Kyphosis, widely spaced (but not inverted) nipples, and pectus excavatum were also noted. Echocardiography, MRI, and fundoscopy were normal but an electroretinogram was altered with a severe reduction of both cone and rod responses.

Routine laboratory investigations were performed and showed normal serum creatinine, cholesterol, and alkaline phosphatase concentrations but raised ASAT (195 U/l, normal <20 U/l). Coagulation studies were performed before a tooth extraction and showed decreased blood coagulation factors (factor IX 60%, normal 65-160; factor XI 30%, normal 60-160; factor XII 73%, normal 50-160; protein C 30%, normal 70-130; protein S 60%, normal 70-130), abnormal prothrombin time (19 seconds, normal 25 seconds), and activated partial prothromboplastin time (46 seconds, normal 32 ± 8 seconds). The combination of mental retardation, failure to thrive, abnormal electroretinogram, and coagulation abnormalities were highly suggestive of CDG.

Western blot analysis of various serum glycoproteins (transferrin, α_1-antitrypsin, haptoglobin) were performed as previously described[12 13] and showed an abnormal pattern with one single lower band (fig 2A). Similarly, isoelectric focusing of serum transferrin showed a markedly abnormal pattern corresponding to an increase of the disialotransferrin and a nearly complete absence of hexa-, penta-, and tetrasialotransferrins in the patient (fig 2B). These patterns were identical to those previously reported in CDG IIa.[10] Activity of N-acetylglucosaminyltransferase II (MGAT2) was determined at 37°C on cultured skin fibroblasts, according to Tan *et al*,[9] and was profoundly deficient (1.9 ± 0.4 μmol/g protein/h, control 38.9 ± 2.4 μmol/g protein/h).

Finally, direct sequencing of the coding region for the catalytic domain of the *MGAT2* gene identified two distinct point mutations: a missense mutation changing an adenine into a guanine at nt 952 (N318D) and a nonsense mutation at nucleotide 1017 (C339X) leading to a premature stop codon. The father was found to be heterozygous for the N318D mutation and the mother for the C339X mutation.

We report the third observation of CDG IIa in a child with chronic feeding difficulties of early onset, severe mental retardation, and dysmorphic facial features. The two patients reported previously had severe psychomotor retardation with no speech, stereotypic hand washing behaviour, and epilepsy (the last two features were not observed in our case) (table 1). Growth retardation was also consistently observed in all three cases, but major feeding difficulties with chronic diarrhoea were only observed in our case. Dysmorphic features were mentioned in all cases and appeared distinctive with a beaked nose, long philtrum, thin vermilion border of the upper lip, large ears, gum hypertrophy, and thoracic deformity. Ventricular

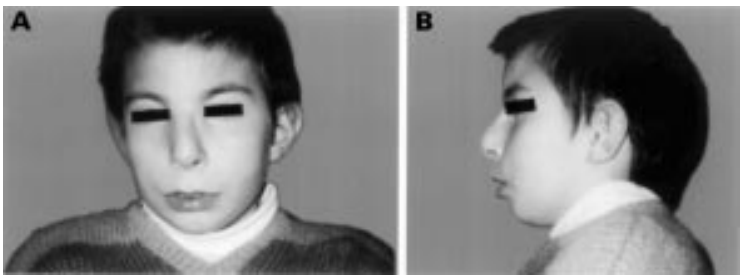

Figure 1 Patient at the age of 11 years. Note the beaked nose with hypoplastic nasal alae, long philtrum, thin vermilion border of the upper lip, everted lower lip, and short neck.

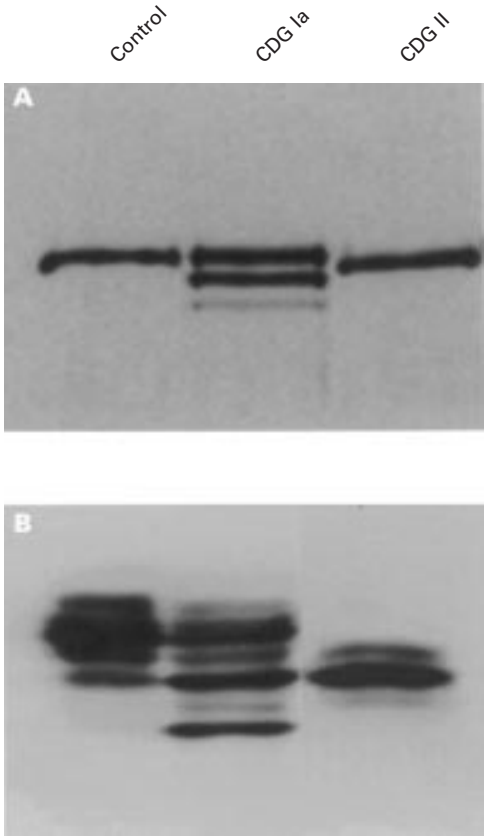

Figure 2 Pattern of serum transferrin on SDS/PAGE (A) and isoelectric focusing (B) from a control, a CDG Ia reference patient, and the CDG IIa patient.

septal defect was observed in 2/3 cases. In one case, MRI showed white matter lesions,[11] but not in the two other cases. Finally, electroretinogram abnormalities affecting both cones and rods were observed in our case. Although CDG Ia and IIa are both multisystemic disorders with major nervous system involvement, they are also characterised by specific dysmorphic features. Inverted nipples, skin lipodystrophy, peripheral neuropathy, and cerebellar hypoplasia have never been observed in CDG IIa and the psychomotor retardation appears to be more severe.

All the CDG cases share common biological features, namely liver abnormalities and decreased coagulation factors. All cases result from an alteration of the N-glycosylation pathway through distinct mechanisms. In CDG IIa, N-acetylglucosaminyltransferase II deficiency hampers transfer of the N-acetylglucosaminyl residue, the first residue of the antennae, to its substrate. The lack of one glycoprotein antenna causes a molecular weight loss and a reduction in electrical charge.[10] While the mutations identified in our patient are different from those previously reported, they all occur in the C-terminal end of the catalytic domain of the protein. This domain is highly conserved between rat and humans.[9] MGAT2, which is present in the trans Golgi apparatus, appears to be an essential enzymatic step for the biosynthesis of complex Asn linked glycans. The observation of severe multisystemic developmental anomalies in CDG IIa patients is suggestive of a crucial role of complex N-glycans in human development and particularly in the nervous system.

No treatment is available for CDG IIa at present. However, the identification of the enzyme defect and the disease causing gene make prenatal diagnosis feasible in this rare but underdiagnosed autosomal recessive disorder.

Although all types of CDG share common features, the clinical manifestations of CDG IIa differ from the typical features of CDG I. In two cases (including ours), the diagnosis was fortuitous (coagulation testing) and made only after the age of 8 years. We therefore suggest giving consideration to the diagnosis of CDG IIa when dealing with the association of developmental delay, dysmorphic features, and growth retardation.

Table 1 Clinical profile of our patient compared to the previously reported cases

	Jaeken et al[8]	*Ramaekers et al*[11]	*This case*
Ethnic origin	Belgian	Iranian Consanguineous parents	French
Birth weight	3250 g	?	3050 g
Birth length	50 cm		48 cm
Head circumference	35 cm		35 cm
Presenting symptom	Hypotonia at birth	Developmental delay	Feeding difficulties Diarrhoea at 3 months
Method of diagnosis	Investigation of the coagulation	?	Investigation of the coagulation
Age at diagnosis	9.5 y	3 y	11 y
Neurological symptoms			
Developmental delay	Severe	Severe	Severe
	A few steps without support Monotonous sounds	Generalised hypotonia	No speech at 11 Unstable gait
Epilepsy	Yes, at 6	Yes	No
Abnormal behaviour	Stereotypic	Hand washing movement	No
Neuropathy	No	No	No
Cerebellar hypoplasia	No	No	No
Failure to thrive	+ After 2 y	+	+
	L 3rd centile	L 3rd–10th centile	L<−4 SD
	W 3rd centile	W 3rd–10th centile	W<−2.5 SD
Gastrointestinal problems	Volvulus of the stomach	−	Chronic diarrhoea
Dysmorphic features		Coarse face	
Nose	Hooked	−	Beaked
Lips	Thin	−	Thin upper lip, Everted lower lip
Ears	Large, dysplastic	Large, low set	Large
Gums	Hypertrophy	−	Hypertrophy
Teeth	Large	−	Large
Mandible	Prognathism	−	−
Neck	Short	−	Short
Thorax	−	−	Pectus excavatum
	−	Widely spaced nipples	Widely spaced nipples
	Kyphoscoliosis	−	Kyphosis
Cardiac defect	Ventricular septal defect	Ventricular septal defect	−
ERG	?	?	Altered
Mutation	A1467G/A1467G	C1551T/C1551T	A952G/T1017A

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Characterisation of six large deletions in *TSC2* identified using long range PCR suggests diverse mechanisms including Alu mediated recombination

EDITOR—Tuberous sclerosis complex (TSC) is an autosomal dominant familial tumour syndrome (OMIM 19110 and 191092, http://www.ncbi.nlm.nih.gov/omim/). It is characterised by the development of benign tumours (hamartomas), most frequently in the brain, skin, and kidneys. It is highly penetrant although with variable expression. In the majority of cases, there is significant neurological morbidity as seizures and mental retardation are common. Two causative genes for TSC have been identified, *TSC1* and *TSC2*.[1 2] Reports on mutation analysis in TSC show over 300 unique mutations with a varied spectrum. In cases where a mutation can be identified, approximately 80% have a *TSC2* mutation and 20% have a *TSC1* mutation. All reported *TSC1* mutations are small point mutations causing nonsense changes or splice site changes, or small insertions/deletions causing frameshift mutations. In *TSC2*, the majority (approximately 85%) are small mutations (point mutations causing splice, nonsense, or missense changes, or small insertion/deletions). The remaining 15% of reported *TSC2* mutations are large deletions (ranging in size from 1 kb to 1 Mb). Other large rearrangements (inversions, insertions, translocations) have also been reported, but these account for <1% of reported *TSC2* mutations (http://zk.bwh.harvard.edu/ts).[1–12]

Because TSC is often a devastating disorder with a high frequency of sporadic cases, there is significant demand for genetic testing. Much progress has been made in detecting small mutations in *TSC1* and *TSC2* using a variety of techniques, such as heteroduplex (HD) analysis, single stranded conformation analysis (SSCP), protein truncation test (PTT), denaturing gradient gel electrophoresis (DGGE), and most recently denaturing high performance liquid chromatography (DHPLC).[3 5 6 8–11 13 14] Although it is important for improving the overall mutation detection rate in TSC patients, there has been less effort to develop new techniques for identifying large deletions in *TSC2*, which make up a small but significant percentage of *TSC2* mutations. Although screening for small mutations is the best initial strategy for detecting mutations in unknown cases, if a small mutation cannot be detected, the next approach should be screening for large *TSC2* deletions. Southern blotting is currently the standard approach but unfortunately it has the disadvantage of requiring substantial quantities of DNA. Cytogenetics and fluorescence in situ hybridisation (FISH) are also standard techniques for detecting large deletions, but require either a fresh blood sample or cultured lymphocytes, and have other limitations.

Table 1 TSC2 long PCR primers

Base number position	*Location*	*Sequence 5'>3'*
Forward primers		
4672F	5'UTR	cattccttagctacaaaggcactactcctcc
8506F	5'UTR	tctttttctttcttggctcactacaacctcc
16432F	5'UTR	cctgagtacatagcaaagattgtcacgtcc
20805F	5'UTR	gagtggagagggctatttaaaacccatctg
25118F	5'UTR	gctgtagttgagttctcccagggagtg
28891F	Intron 2	agagtattgtcaatgagacaaaggaggtgagag
33093F	Intron 6	gtggagatgtagctcagggtggatgac
36910F	Intron 9	gtcgtcctggttttatagtgatgagctgc
39178F	Exon 12	cctccctcctgaacctgatctcctatagag
42770F	Intron 15	agcttgagaacctcctgagcataccagtag
46954F	Intron 15	ggttgggttttacttttgctgctgtg
49327F	Intron 19	ttcacctcacattcctggtgtgttacttg
52733F	Intron 22	ccccttctcatctcaggtttaatcagtacatc
55586F	Intron 25	acgcctgttgggtctttccgag
60883F	Intron 32	gttctctttgggatggtccttctagtcg
63753F	Intron 37	ctgagtgtctgtcaggagtaactggcaag
Reverse primers		
21647R	5'UTR	tgtagatgaccaaacatacccaaaccagac
25460R	Intron 1	ctagcctagcaaagacacaggtagctcactc
33058R	Intron 6	gactcctgaggctcagagagaccgag
38185R	Intron 10	gagtagccacaactacaagcctttcttgc
42469R	Intron 15	aggaaggttctgctgcctgctgag
45965R	Intron 15	tatgacataaaagcaacatccctttcctcg
49637R	Exon 20	gtaagagattaatgctgtcagcactggaacc
54565R	Intron 25	atgcaacctttccacccctcgtc
60911R	Intron 32	cgactagaaaggaccatcccaaagagaac
65432R	3'UTR	cgcaccaagcagacaaagtcaataaaagag
74454R	3'UTR	tgattctaagaggtgggttccctagagaaac
78956R	3'UTR	gtaaactacatcgtcatgctgacatgtgc