

Isoelectric Focusing of Serum Apolipoprotein C-III as a Sensitive Screening Method for the Detection of O-glycosylation Disturbances

**Nina Ondrušková¹, Tomáš Honzík¹, Jitka Kytnarová¹,
Martin Matoulek², Jiří Zeman¹, Hana Hansíková¹**

¹Department of Pediatrics and Adolescent Medicine, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic;

²3rd Department of Medicine – Department of Endocrinology and Metabolism, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic

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Abstract: Apolipoprotein C-III (ApoC-III) is a glycoprotein carrying the most common O-linked glycan structure and is abundantly present in serum, what renders it a suitable marker for analysis of O-glycosylation abnormalities. Isoelectric focusing followed by a Western blot of ApoC-III, using PhastSystem™ Electrophoresis System (GE Healthcare), was introduced as a rather simple and rapid method for screening of certain subtypes of inherited glycosylation disorders. The study's aim was to establish this method in our laboratory, what included performing the analysis in a group of 170 healthy individuals to set the reference range of detected relative amounts of sialylated ApoC-III isoforms and to evaluate the gender- and age-dependent differences. A significant relative increase of asialo-ApoC-III with growing age was found. Secondly, we examined serum from patients with selected metabolic disorders and detected minor O-glycosylation changes

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Mailing Address: RNDr. Hana Hansíková, PhD., Department of Pediatrics and Adolescent Medicine, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Ke Karlovu 2, 128 08 Prague 2, Czech Republic; Phone: +420 224 967 748; e-mail: hana.hansikova@lf1.cuni.cz

in diseases such as Prader-Willi syndrome, PGM1 (phosphoglucomutase 1) or MAN1B (class 1B alpha-1,2-mannosidase) deficiency. Our results show that this method allows for a sensitive detection of ApoC-III O-glycosylation status, however this might be modulated by several factors (i.e. nutrition, medication) whose exact role remains to be determined.

Introduction

Glycosylation is a functionally important and common posttranslational modification, with more than half of all human proteins estimated to be glycosylated (Christiansen et al., 2014). Congenital disorders of glycosylation (CDG) comprise a rapidly expanding family of metabolic diseases resulting from defects in glycosylation-related genes. Depending on which glycoprotein biosynthesis pathway is affected, more than 100 of N- and/or O-glycosylation disorders can be distinguished with a broad spectrum of clinical features (Freeze et al., 2014). In the more common group of N-glycosylation disorders, psychomotor retardation is often present along with hypotonia, cerebellar hypoplasia, dysmorphism, strabismus, atypical fat distribution and coagulopathy; however, various other manifestations such as recurrent infections, gastrointestinal symptoms or deafness may occur. Combined N- and O-glycosylation defects have a similar multi-systemic clinical picture, frequently including skeletal and skin anomalies. In contrast, isolated O-glycosylation disorders usually have a distinct phenotype defined by pronounced involvement of a single organ (brain, muscle) due to tissue-specific character of the enzymes involved in O-glycosylation (Wopereis et al., 2006b). As clinical report and basic laboratory tests are not sufficient for diagnosing CDG, selective screening methods are required to detect and categorize the glycosylation defect. Accordingly, additional experiments are performed (enzyme measurements, glycan structure analysis, immunocytochemical studies, etc.), before diagnosis is confirmed.

For CDG screening, serum N-glycoprotein transferrin (TRF) and O-glycoprotein apolipoprotein C-III (ApoC-III) are used as markers. ApoC-III is a core 1 mucin type plasma protein synthesized predominantly in liver, acting as an inhibitor

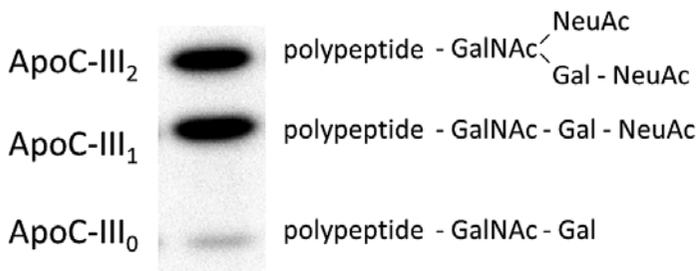


Figure 1 – Structure of ApoC-III O-glycosylated isoforms. Sialylated ApoC-III isoforms separated by isoelectric focusing (left) and the schematic structure of their corresponding glycan chains (right).

of lipoprotein and hepatic lipases, i.e. VLDL or LDL clearance. It contains one branched oligosaccharide chain (glycan) attached via a hydroxy group to its polypeptide chain (threonine), and three isoforms with different sialic acid content can be detected: ApoC-III₂, ApoC-III₁ and ApoC-III₀ (di-, mono- and asialoform, respectively; Figure 1). In CDG types with impaired mucin O-glycosylation, which occurs exclusively in Golgi as opposed to N-glycosylation localized both in ER (glycan assembly) and Golgi (further glycan modification), a pathological ApoC-III profile is observed characterized by decreased ApoC-III₂ and increased ApoC-III₁ or ApoC-III₀ (relative to total ApoC-III). To this date, aberrant ApoC-III sialylation has been described in glycosylation disorders including the deficiency of SLC35A1 (Wopereis et al., 2007), Conserved Oligomeric Golgi (COG) complex subunits 1, 2, 5, 6, 7 and 8 (Spaapen et al., 2005; Foulquier et al., 2006, 2007; Paesold-Burda et al., 2009; Lübbehusen et al., 2010; Kodera et al., 2015), ATP6V0A2 (Huchtagowder et al., 2009), ATP6V1A (Gardeitchik et al., 2014) and TMEM165 (Zeevaert et al., 2013). Recently, a new type of CDG due to phosphoglucomutase 1 (PGM1) deficiency was identified with an unusual TRF pattern in the CDG screening test, not clearly distinguishing which part of the glycosylation pathway is affected. No hypoglycosylation of ApoC-III was detected in the PGM1-CDG patient described by Pérez et al. (2013). Defect in the class 1B alpha-1,2-mannosidase (*MAN1B1*) gene manifests with a so called type 2 TRF pattern suggesting a disturbance in Golgi glycan modification, however no changes in ApoC-III glycosylation were reported (Van Scherpenzeel et al., 2014). The exact role of *MAN1B1*, originally thought of as an ER-resident mannosidase, has just lately been re-evaluated and it is currently presumed that the protein participates in Golgi-based protein biosynthesis quality control (Pan et al., 2013). Aside from the inborn defects in the glycosylation machinery, there are some pathophysiological factors that can influence the extent of serum ApoC-III sialylation. Documented secondary causes for ApoC-III hypoglycosylation include the acute phase of hemolytic uremic syndrome (HUS), where an enzyme cleaving sialic acid residues (neuraminidase) is released to circulation by *Streptococcus pneumoniae*, and Hutchinson Gilford progeria syndrome (HGSP) due to mutation in *LMNA* gene, possibly altering the biosynthesis of CMP-NeuAc (Wopereis et al., 2007). Other examples are metabolic syndrome (Savinova et al., 2014) and Prader-Willi syndrome (Munce et al., 2010). On the other hand, excess ApoC-III sialylation resulting from CMP-NeuAc overproduction was demonstrated in sialuria, which is caused by defective *GNE* gene (Wopereis et al., 2006a). Increased ApoC-III₂ to ApoC-III₁ ratio was also detected in individuals with chronic renal dysfunction (Holdsworth et al., 1982).

The study's aim was to determine the distribution of ApoC-III sialylated isoforms separated by isoelectric focusing (IEF) in sera from healthy individuals (a reference range) and to evaluate the gender- and age-dependent differences. The second aim was then to use the determined reference range for analysis of potential O-glycosylation abnormalities in patients with selected metabolic disorders.

Material and Methods

Material

The control group consisted of 170 healthy individuals (age interval 1 day–42 years), 89 males and 81 females. To examine O-glycosylation abnormalities possibly arising secondarily to non-CDG etiologies, we analyzed 10 patients with Prader-Willi syndrome (s.), two patients with Rett s. and one individual each from Silver-Russell s., DiGeorge s., Gapo s., Schnitzler s., Marfan s., Stickler s., dyschondrosteosis and unexplained chronic renal dysfunction. The second tested group included 5 selected deficiencies of glycosylation enzymes or proteins (CDG) not directly involved in mucin O-glycosylation due to mutations in *PMM2*

Table 1 – Overview of selected clinical and laboratory parameters in the analyzed group of patients

	Gender	Age	BMI	Sleep apnoea	Triacylglycerols in serum (mmol/l)	Metformin therapy
PWS, Patient 1	F	23 y	49.7	Y	3.50	N
PWS, Patient 2	F	27 y	59.1	Y	1.07	Y
PWS, Patient 3	F	20 y	45.8	N	1.69	N
PWS, Patient 4	M	21 y	43.4	N	1.07	Y
PWS, Patient 5	F	33 y	38.1	N	1.05	Y
PWS, Patient 6	M	17 y	41.3	N	0.62	Y
PWS, Patient 7	F	21 y	51.9	N	1.40	N
PWS, Patient 8	F	18 y	27.0	N	1.02	N
PWS, Patient 9	M	27 y	56.1	Y	1.32	N
PWS, Patient 10	M	5 m	14.5	N	NA	N
Chronic renal dysfunction	M	2 y	12.7	N	NA	N
Rett syndrome, Patient 1	F	2 y	13.7	N	1.10	N
Rett syndrome, Patient 2	M	7 y	12.5	N	2.22	N
Silver-Russell syndrome	M	18 y	12.8	N	1.05	N
DiGeorge syndrome	F	12 y	16.5	N	0.46	N
Gapo syndrome	M	18 y	NA	NA	NA	NA
Schnitzler syndrome	M	46 y	NA	NA	NA	NA
Marfan syndrome	M	27 y	NA	N	NA	N
Stickler syndrome	F	32 y	23.1	N	0.80	N
Dyschondrosteosis	F	32 y	32.5	N	0.77	N
PMM2-CDG	F	24 y	23.7	N	1.20	N
EXT1-CDG	F	3 y	NA	N	NA	N
NGBR deficiency	M	11 m	14.7	N	NA	N
PGM1-CDG	M	7 y	21.7	N	0.47	N
MAN1B1-CDG	M	10 y	NA	NA	NA	NA

PWS – Prader-Willi syndrome; F – female; M – male; y – years; m – months; BMI – body mass index; NA – not analyzed/no information

(phosphomannomutase 2), *PGM1*, *EXT1* (exostosin glycosyltransferase 1), *MAN1B1* and *NGBR* (Nogo-B receptor) genes, one patient from each group. Selected clinical and laboratory data of the analyzed patients are shown in Table 1. Serum was separated by centrifugation (630 g, 10 min) and stored at -20°C until tested. Analyses were performed with informed consent from each participant or their parents.

Isoelectric focusing (IEF) and Western blot of ApoC-III

IEF of ApoC-III was carried out as described by Wopereis et al. (2003), with minor modifications. Dry IEF gels (17-0677-01, GE Healthcare) were incubated in a rehydrating solution (8.3 M urea, 4% (V/V) Pharmalyte pH 4.2–4.9 (17-0562-01, GE Healthcare), 2% (V/V) Ampholine pH 3.5–5 (80-1125-89, GE Healthcare)) for 1.5 h. Serum was centrifuged at 6600 g for 2 min and the clear middle fraction (non-diluted) was used as a sample. The separation of samples (approx. 0.5 μl each) by isoelectric focusing was performed on PhastSystem™ Electrophoresis System (GE Healthcare). After the run was completed, gels were briefly washed in transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol) and proteins were blotted onto nitrocellulose membranes (pre-equilibrated in transfer buffer) by diffusion at 60°C for 1 h. The membranes were then blocked in milk solution (5% (w/V) in PBST: PBS with 0.5% (V/V) Tween 20), labelled with primary ApoC-III antibody (0650-1707, Biotrend) diluted 1:1000 in BSA solution (2% (w/V) in PBST) and anti-rabbit secondary antibody conjugated to horseradish peroxidase (A-0545, Sigma; 1:5000 in 2% (w/V) milk in PBST). Each incubation was carried out for 1 h, with 3×10 min washes (PBS with 0.05% (V/V) Tween 20) in between. After the final washing in PBS (2×5 min), blots were visualized by chemiluminescence (SuperSignal West Femto Substrate, Thermo Scientific) using GBOX Imaging System (Syngene). The profiles of ApoC-III isoforms were quantified densitometrically with Quantity One software (Bio-Rad). Examples of both normal and hypoglycosylated ApoC-III profiles are shown in Figure 2. The coefficient of variation for intra-run variability (CV_1) was determined by evaluating 8 profiles of serum from one healthy control analyzed

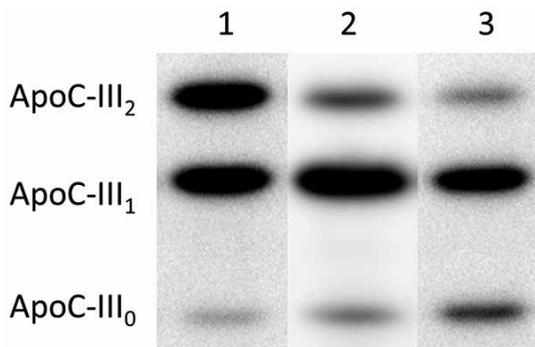


Figure 2 – Comparison of normal vs. hypoglycosylated ApoC-III IEF profiles. The picture shows an example of a physiological ApoC-III profile (1) and pathological profiles characterized by a relative decrease of ApoC-III₂ with relatively increased ApoC-III₁ (2) or ApoC-III₀ (3). The ratios vary in different syndromes and patients.

within one gel/run, and the same control sample was analyzed in 35 different gels/runs for the calculation of the coefficient of variation for inter-run variability (CV₂). CV₁: 8.4, 6.8, 22.1; CV₂: 6.7, 4.9, 23.5 (for ApoC-III₂, ApoC-III₁ and ApoC-III₀, respectively).

Statistical analysis

Because of the non-normal distribution of the data, the reference range was calculated using 2.5% and 97.5% sample quantiles. Gender-determined variations in given age categories were tested with non-parametric Wilcoxon test. Differences between the age categories were tested for both genders using Kruskal-Wallis test. A statistically significant difference was set as p<0.05.

Results

Individuals from the analyzed control group were divided into three separate categories based on their age: 1) less than 2 years old, 2) 2–6 years old, and 3) 7–42 years old. The statistically evaluated distribution of ApoC-III sialylated isoforms – including the median, mean values and reference range – determined for the whole group is shown in Figure 3.

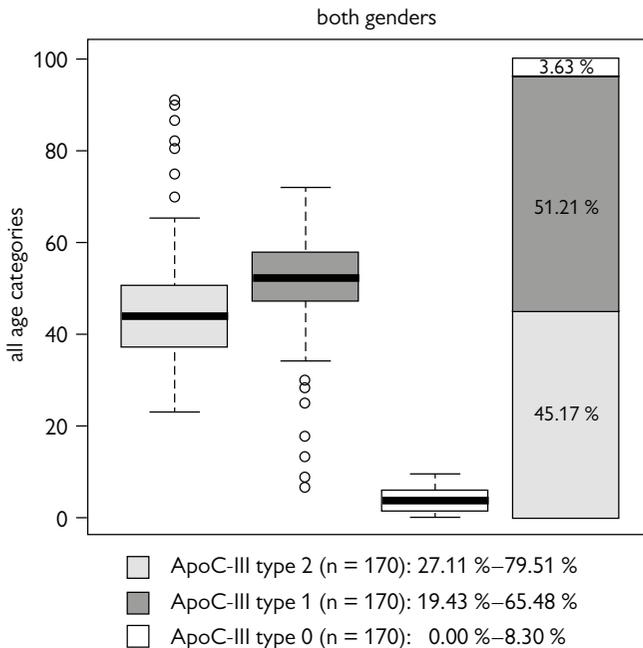


Figure 3 – Distribution of ApoC-III sialylated isoforms separated by isoelectric focusing of serum samples, determined in a group of healthy individuals (n=170) of both genders in the age interval 1 day–42 years. The bold line inside box-plot represents median, the column graph on the right shows mean values. Intervals below are the reference range.

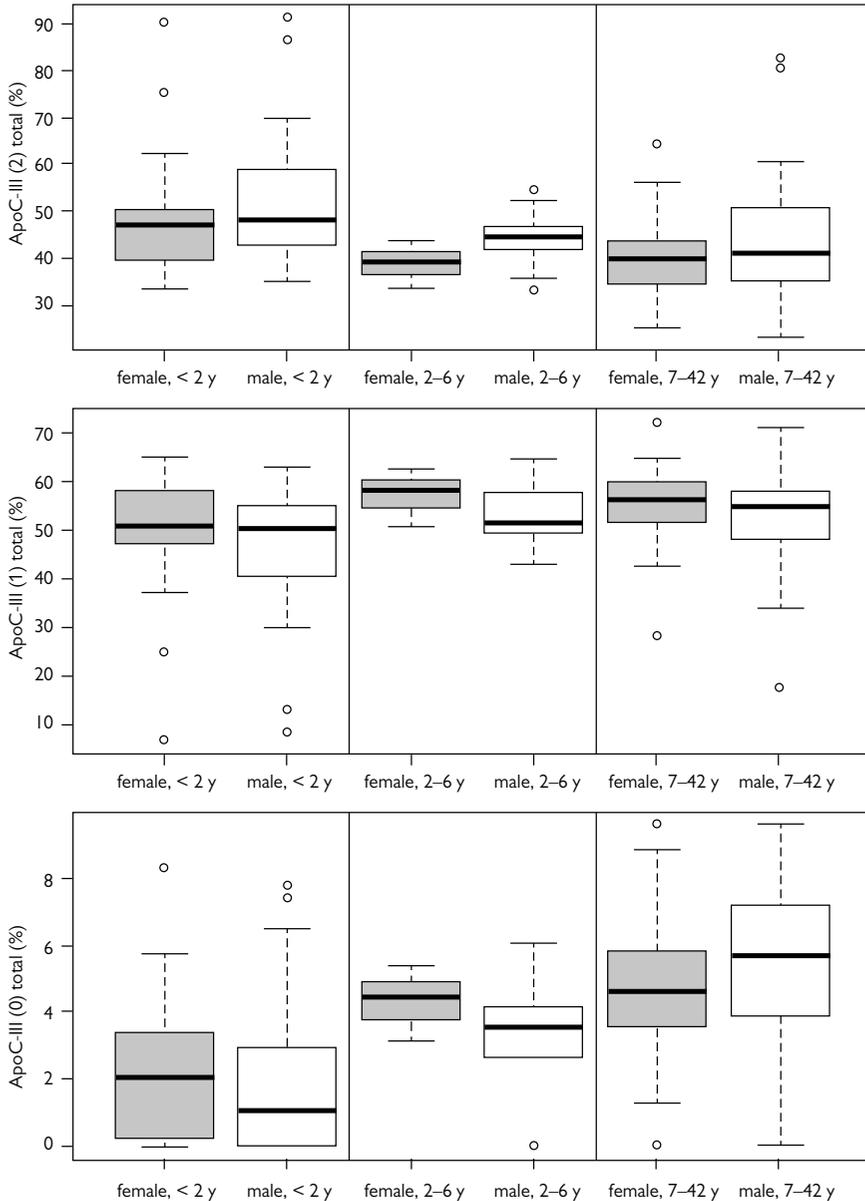


Figure 4 – Evaluation of gender- and age-dependent differences in the distribution of ApoC-III sialylated isoforms separated by isoelectric focusing of serum samples, determined in a group of healthy individuals ($n=170$) of both genders (89 males, 81 females) in three different age categories: 1) < 2 y ($n=70$), 2) 2–6 y ($n=16$) and 3) 7–42 y ($n=84$) (y – years). The bold line inside box-plots represents median. 1) Wilcoxon test results (female vs. male) for a) ApoC-III₂: $p=0.238$ (< 2 y); 0.157 (2–6 y); 0.345 (7–42 y), b) ApoC-III₁: $p=0.226$ (< 2 y); 0.281 (2–6 y); 0.181 (7–42 y) and c) ApoC-III₀: $p=0.522$ (< 2 y); 0.380 (2–6 y); 0.165 (7–42 y). 2) Kruskal-Wallis test results (age dependent differences) for a) ApoC-III₂: $p=0.002$ (female – F); 0.008 (male – M), b) ApoC-III₁: $p=0.041$ (F); 0.110 (M) and c) ApoC-III₀: $p<0.001$ (F); < 0.001 (M).

No statistically significant changes in ApoC-III sialylated isoforms' relative amounts were found between males and females ($p > 0.05$, Figure 4). However, regarding age-dependent differences, we noted a relative increase of ApoC-III₀ with growing age, accompanied by a relative decrease of the ApoC-III₂ isoform. This trend was calculated as statistically significant, for both genders (the lowest value $p < 0.001$ was obtained when comparing ApoC-III₀ content between groups < 2 years and 7–42 years; Figure 4).

Pathological ApoC-III profile was defined as a combination of relatively decreased ApoC-III₂ and relatively increased ApoC-III₁ or ApoC-III₀ compared to controls. Out of the tested non-CDG patients, all showed physiological ApoC-III profile except for three individuals with Prader-Willi s. (PWS; Patients 1, 3 and 7 in Table 1) and a boy with chronic renal dysfunction. As expected, normal O-glycosylation of ApoC-III was found in patients with genetic defects in *PMM2*, *EXT1* and *NGBR*. Multiple serum samples were analyzed from a boy with *PGM1* deficiency, displaying from borderline to pathological pattern, and a slight ApoC-III hypoglycosylation was also seen in patient with *MAN1B1* defect. The abnormal ApoC-III profiles are depicted in Figure 5 and the quantification of all analyzed ApoC-III profiles is shown in Table 2.

Discussion

To establish a screening assay for detecting O-glycosylation abnormalities in serum, we performed separation of ApoC-III sialylated isoforms by isoelectric focusing, followed by a Western blot detection – a method developed by Wopereis et al. (2003) – and determined a reference range of their relative amounts in our control group of 170 healthy subjects. While we found no variations between genders, there was a statistically significant relative decrease of ApoC-III₂ and increase of ApoC-III₀ with growing age. It underlines the need to consider the patient's age when evaluating their profile. This finding was in agreement with previous

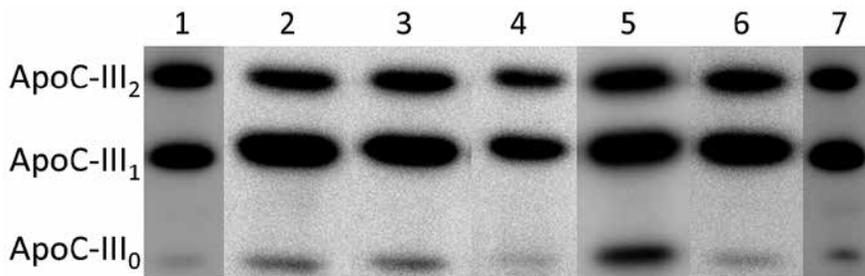


Figure 5 – ApoC-III hypoglycosylation detected in the analyzed group of patients. Serum samples were separated by isoelectric focusing, followed by a Western blot detection of ApoC-III. Relatively decreased ApoC-III₂ and increased ApoC-III₁ and/or ApoC-III₀ compared to a control (lane 1) were seen in three patients with Prader-Willi syndrome (2–4), unexplained chronic renal dysfunction (5), *PGM1* (6) and *MAN1B1* (7) deficiency.

Table 2 – Relative amounts of sialylated ApoC-III isoforms in the analyzed group of patients

	Age	Percentage of total ApoC-III (%)		
		Disialo-(2)	Monosialo-(1)	Asialo-(0)
Reference range	1 d–42 y	27.1–79.5	19.4–65.5	0–8.3
PWS, Patient 1 (lane 2 in Figure 5)	23 y	24.2	73.3	2.5
PWS, Patient 2	27 y	39.9	51.1	9.0
PWS, Patient 3 (lane 3)	20 y	27.3	70.3	2.4
PWS, Patient 4	21 y	37.2	57.4	5.4
PWS, Patient 5	33 y	48.4	48.8	2.9
PWS, Patient 6	17 y	42.6	57.5	ND
PWS, Patient 7 (lane 4)	21 y	28.2	71.9	ND
PWS, Patient 8	18 y	39.2	58.8	2.0
PWS, Patient 9	27 y	35.7	61.3	3.1
PWS, Patient 10	5 m	46.8	51.3	1.9
Chronic renal dysfunction (lane 5)	2 y	25.0	62.3	12.7
Rett syndrome, Patient 1	2 y	40.8	55.2	4.0
Rett syndrome, Patient 2	7 y	34.1	59.4	6.5
Silver-Russell syndrome	18 y	50.8	47.4	1.8
DiGeorge syndrome	12 y	46.6	51.9	1.6
Gapo syndrome	18 y	43.8	51.1	5.2
Schnitzler syndrome	46 y	39.9	57.2	2.9
Marfan syndrome	27 y	55.9	37.7	6.4
Stickler syndrome	32 y	37.2	60.0	2.8
Dyschondrosteosis	32 y	39.0	60.0	1.0
PMM2-CDG	24 y	34.8	59.8	5.4
EXT1-CDG	3 y	59.0	39.2	1.9
NGBR deficiency	11 m	43.1	54.4	2.5
PGM1-CDG (lane 6)	7 y	27.4	71.7	1.0
MAN1B1-CDG (lane 7)	10 y	27.9	69.3	2.8

PWS – Prader-Willi syndrome; d – day; y – years; m – months; ND – not detected

report by Wopereis et al. (2007), who, in addition, found higher relative levels of ApoC-III₂ in premature neonates than in full-term newborns. The observed age-dependent changes of ApoC-III sialylation are, in our opinion, likely downstream consequences of alterations in lipid metabolism associated with development/aging. It is unknown whether they directly hold any implications for the individual's health status, however in a study done by Tertov et al. (1996), it was determined that the circulating immune complexes containing low density lipoprotein (CIC-LDL), present in the blood of patients with coronary atherosclerosis, have considerably (2.1-fold) lower sialic acid content than native LDL.

It has been long known that increased ApoC-III level is associated with elevated triacylglycerols (TAG) in plasma (Kashyap et al., 1981), and is considered a valuable

marker for the risk of coronary heart disease (Sacks et al., 2000). Overexpressing ApoC-III in transgenic mice elevated TAG levels (Ito et al., 1990), while disruption of ApoC-III gene caused hypotriglyceridemia (Maeda et al., 1994). Vice versa, high total plasma TAG and VLDL-TAG in hypertriglyceridemia lead to accumulation of ApoC-III in triglyceride rich lipoproteins (TRL), resulting from both increased transfer of ApoC-III to TRL and increased hepatic production of VLDL ApoC-III (Marcoux et al., 2001). Recently, Savinova et al. (2014) found an increase of total ApoC-III (approx. 1.4-fold) together with its slightly reduced glycosylation in 56 subjects with metabolic syndrome, compared to controls. In their study, VLDL fraction of the patients contained 33% of ApoC-III₂, 58% of ApoC-III₁ and 9% of ApoC-III₀, whereas the controls had 40%, 52% and 8%, respectively. They point out that ApoC-III sialylation is responsive to prescription of metformin, as well as of omega-3 fatty acids and niacin (author's note: these medications are known to lower plasma TAG). Interestingly, some experiments in rats also documented alterations in the relative amounts of ApoC-III sialylated isoforms under different dietary conditions, where carbohydrate-rich diet, accompanied by a rise in plasma TAG levels, induced an increase of plasma ApoC-III₀ (Holdsworth et al., 1982). All the data above suggest a direct relationship between TAG levels and ApoC-III hyposialylation, however the underlying mechanism is currently not clear. At this point we can only speculate whether this is caused by impaired ApoC-III catabolism and consequent rises in relative amounts of its hyposialylated isoforms, or perhaps due to the increased production rate of new ApoC-III molecules and their secretion through Golgi apparatus (where sialylation occurs), the glycosylation machinery is overwhelmed and fails to fully modify all the passing glycoproteins. We confirm an observation of pathological ApoC-III profiles with decreased ApoC-III₂ and increased ApoC-III₁ (> 70%) in 3 individuals (positive) and a borderline profile with increased ApoC-III₀ (9%) in one individual (Patient 2 in Table 1), out of 10 analyzed patients with confirmed PWS. While Munce et al. (2010) found a correlation between abnormal ApoC-III profiles in PWS patients and sleep abnormalities, in our study only one positive patient suffered from a sleep apnoea. They reasoned that the observed glycosylation abnormality could be caused by deficiency in regulating protein degradation, based on their finding of two genes with putative roles as E3 ubiquitin ligases in the common deleted region. No relationship between aberrant ApoC-III and BMI was detected by Munce et al. (2010) or us, however we noted higher TAG (≥ 1.4 mmol/l) in all positive PWS patients compared to the others, supporting our assumption that ApoC-III hyposialylation in PWS is attributable to increased plasma TAG levels. We believe that drug treatments could have affected the observed results in our study, i.e. normalized the possible hyposialylation of ApoC-III through lowering plasma TAG.

Ooi et al. (2011) studied ApoC-III metabolism in subjects with moderate chronic kidney disease and found that they had significantly higher plasma ApoC-III concentrations, and the selected isoform for analysis, ApoC-III₁, had

significantly lower fractional catabolic rate. They hypothesize that elevated ApoC-III sialylation documented by Holdsworth et al. (1982) could render ApoC-III-containing TRL particles less suitable for lipolytic degradation. Moreover, the delayed ApoC-III catabolism could be due to impaired renal function as kidney is partly involved in the removal of ApoC-III from plasma. This contradicts our finding of a hypoglycosylated ApoC-III pattern (\downarrow ApoC-III₂, \uparrow ApoC-III₀) in a patient with chronic renal dysfunction as the predominant symptom. We cannot exclude the potential effect of elevated TAG level as it was not measured in the analyzed serum. Interestingly, hypoglycosylation linked to renal dysfunction is well documented in IgA nephropathy (IgAN), characterized by specific undergalactosylation of IgA1 O-glycans (Narita and Gejyo, 2008). They suggest that there is no primary abnormality in O-galactosylation pathway in B lymphocytes, but the IgA1 glycosylation defect is rather secondary to aberrant immunological reaction with IgA1 overproduction. In addition, recent studies have found decreased expression of *GALNT2* (the corresponding enzyme polypeptide N-acetylgalactosaminyltransferase initiates mucin type O-glycosylation) modulated by miRNA in IgA1-producing cells of IgAN patients (Serino et al., 2015). The finding is intriguing, since decreased *GALNT2* expression was also detected in circulating blood cells of obese diabetic patients, probably triggered by their hyperglycemia (Marucci et al., 2013). Moreover, certain SNPs in *GALNT2* gene were found to be associated with plasma TAG and cholesterol levels (Kathiresan et al., 2008). Thus, altered *GALNT2* activity might be linked to various conditions of metabolic derangement. While our patient did not present with features of IgAN, the observed ApoC-III hypoglycosylation could be secondary to yet unrecognized metabolic factor associated with his severe phenotype.

PGM1 deficiency indirectly lowers the intracellular concentration of UDP-Gal (Tegtmeyer et al., 2014), one of the substrates needed for mucin type 1 O-glycosylation, what could possibly explain our finding of reduced ApoC-III sialylation in the analyzed patient. However, the extent of detected hypoglycosylation was variable in serum samples withdrawn at different periods and sometimes the abnormal changes were rather subtle. Xia et al. (2013) performed LC/MS-MS analysis to quantify selected plasma O-glycan structures in various CDG subtypes and, interestingly, their PGM1-CDG patient had increased T/ST-antigen ratio (Gal-GalNAc/Sia-Gal-GalNAc) similar to those reported in galactosemia. The other analyzed PGM1-CDG patient who was on galactose treatment showed normal pattern, supporting the idea that the observed glycosylation disturbances in PGM1-CDG result from reduced UDP-Gal pool, which was also reported in galactosemic patients (Ng et al., 1989). Borderline ApoC-III hyposialylation was detected in our patient with pathogenic mutation in *MAN1B1* gene. Its deficiency disturbs Golgi morphology and impairs Golgi anterograde trafficking as studied in *MAN1B1*-depleted cells (Péanne et al., 2014), likely affecting both N- and O-glycosylation.

Conclusion

We determined a reference range and assessed gender- and age-dependent differences of the distribution of serum apolipoprotein C-III sialylated isoforms detected by Western blot after isoelectric focusing. Besides finding a mild ApoC-III hypoglycosylation in some individuals with Prader-Willi syndrome what had already been documented elsewhere, we suggest it is linked to plasma triacylglycerol levels. We also show, for the first time, slightly reduced O-glycosylation of ApoC-III in patients with PGM1 and MAN1B1 deficiency. Thus, we conclude the established method is sufficiently sensitive and suitable for screening of mucin type 1 O-glycosylation disturbances. Our results also demonstrate the importance of multiple analyses in the same individual. Further studies should be done to elucidate how patients' medication and diet might influence the outcome of the analysis.

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